TOXICITIES AND SEDATIVE EFFECTS OF TOLOACHE, *DATURA INNOXIA* MILLER, ON THE AFRICAN CATFISH *CLARIAS GARIEPINUS* BURCHELL, FINGERLINGS

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CERTIFICATION

This is to certify that this research work for this thesis and the subsequent preparation of this thesis by Victoria Ogeh Ayuba (PGNS/UJ/10254/97) were carried out under our supervision:

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DEDICATION

TO MY CHILDREN:

JOHN, STEFANNIE AND AKATIKI

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ABSTRACT

Studies on toxicological and sedative effects of *Datura innoxia* plant parts (leaf, seed, stem, pod and root) on *Clarias gariepinus* fingerlings were conducted under laboratory conditions using the static bioassays and continuous aeration. The aim was to develop an effective anaesthetic from an indigenous plant material that will be available at low cost to aquaculturists and which would be non-toxic to the fish and consumers. The active ingredients and their compositions in the plant parts as well as their 96 hours acute toxicity (LC_{50}) on *C. gariepinus* fingerlings were investigated. The effects of 12 weeks sublethal concentrations of the leaf, seed, stem, pod and root of the plant on growth, tissue composition, blood parameters, energy reserves, gills, gonad, kidney and liver of *C. gariepinus* were evaluated. Data obtained from this investigation were subjected to simple percentages and analysis of variance (ANOVA) at 0.05 level of significance. The phytochemical screening of the plant (*D. innoxia*) showed that the leaf, seed, stem, pod and root had alkaloids, atropine, hyoscyamine, scopolamine, flavonoids, essential oils, tannins and saponins. Similarly proximate analysis showed the presence of protein in all the five morphological parts examined. The Amino acid analysis indicated the presence of essential and non-essential amino acids at various levels in the leaf, seed, stem, pod and root. The *D. innoxia* water extracts resulted in a number of physiological impairment of *C. gariepinus* fingerlings. The mean LC_{50} range from 120.23 to 208.93 mg/l. Weight gain by *C gariepinus* exposed to sublethal concentrations of *D. innoxia* extracts ranged from 30.66 to 70.03%. *Clarias gariepinus* fingerlings exposed to various extracts of *D.*

innoxia passed sequentially through the various stages of anaesthesia. The behavioural responses were excess mucus secretion and apathy, air gulping, distension of the mouth and opercula, erratic swimming and loss of balance. The crude extract of *D. innoxia* leaf was able to induce fish to loose reactivity to stimuli at concentration 2.50g/l within 40.33 (2.63) minutes, while at concentration 3.00g/l. the same effect was observed in 28.2 (2.06) minutes. No such observations were made at concentrations less than 2.50 g/l. Similar results were obtained from fish exposed to unseparated chloroform-methanol extract and lipid fraction of chloroform-methanol extract though the time taken to reach anaesthesia in the alcoholic extract was significantly ($P < 0.05$) shorter. The time required for *C. gariepinus* to succumb to the anaesthetic effects of the seed, stem, pod and root ranged from 1.04 (0.38) in the seed to 3.60 (1.08) minutes in the root for fish to loose reactivity to stimuli in unseparated chloroform-methanol and lipid fraction of chloroform-methanol extracts respectively. This study has shown that *D. innoxia* is a highly effective, cost efficient and safe anaesthetic for *C. gariepinus* use in aquaculture and laboratory research settings at concentrations not more than 3g/l.

CHAPTER ONE

INTRODUCTION

1.1 GENERAL INTRODUCTION

Datura innoxia belongs to the family Solanaceae. This family, which is of great economic importance, is one of the largest flowering plant families with about 2,300 species, the largest genus, *Solanum*, contains over 1000 species. Solanaceae is of great value for its food plant. Perhaps the most important of the plants is the potato (*Solanum tiberosum)* and related species, which lies fourth in world food production after wheat, maize and rice. Other well known food plants are the tomato (*Lycopersion exculentum),* various chilli peppers (*Capsicum species*), the Bringal, eggplant (*Solanum melongenii)* and the scarlet eggplant (*S. aethiopicum).* There are many other important Solanaceous fruits such as pepine (*S. muricatum*) lulo *(S. quitoctise*) hush tomato (*Physalis* species) and the tree tomato (*Cyptomadra betacea)* as documented by Hawkes *et al., (1991).*

The family is also extremely important as a source of drugs in medicine, pharmacology but many are poisonous when used in excess.

The first written reference of Solanaceae plants in prevention and relief of medical disorders is about 5000 years ago in Egypt, Mesopotamia, India and China (possibly others) (Shah 1981). In addition, members of the Solanaceae with narcotic or hallucinogenic properties (e.g. *Nicoliana, Brug, Mansia, Datura spp.*) have figured prominently in rituals, magic and superstitions associated with healing in the ancient civilizations of both old and new world (Sheleiffer, 1973, 1979, Lockwood 1979, Schultes 1979).

It is interesting to note that certain Solanaceae plants have been used widely as basis for biotechnological research especially in the fields of tissue culture, protoplasts fusion, somaclonal variation, DNA studies and other culture such as “vegetable guinea pigs” include tomato, potato, petunia and tobacco (Hawkes *et al., 1991)*.

The compounds from the Solanaceae that play such a prominent role as therapeutic drugs are all types of tropane alkaloids. A large number of tropane alkaloids are known, the pharmacologically important alkaloids in which the nitrogenous base is esterified with tropic acid (or its derivative) are apparently unique in this family (Romeike 1978). However, only three compounds have attained worldwide eminence as chemotherapeutic agents viz: L-hyoscyamine, L-hyoscine (Scopolamine) and atropine. Hyoscyamine and hyoscine are the principal, naturally occurring forms. Other related but more recently discovered compounds, which are assuming growing importance, include anisodomine (6-β-hyoxyhyoscamine) and anisodine (daturamine). Shoot tissues are the main sources of tropane alkaloids, although roots of *Atropa* and *Scopolia spp* and seeds of *Datura* species are used.

Most plants, including food plants, contain low levels of natural plant toxins. A chemist measures the levels of toxins in plant tissues to evaluate their safety for animal feed and drug. Effect of naturally-occurring plant-made toxins found at low levels in many foods and drugs that we take, on humans and animals, is based on laboratory tests using toxin

concentrations much higher than the concentrations normally found in food and drug.

Plants are chemical factories. Unlike animals having the luxury of teeth and claws and legs to help them get out of a tight spot, most plants spend their lives in one place and have evolved to rely upon elaborate chemical defenses to ward off unwanted predators. For this reason, plants have in their arsenal an amazing array of thousands of chemicals noxious or toxic to bacteria, fungi, insects, herbivores, and even humans. Fortunately, this chemical diversity also includes many compounds that are beneficial to humans: vitamins, nutrients, antioxidants, anticarcinogens, and many other compounds with medicinal value (Novak and Haslberger, 2000).

Most plant species in the world are not edible, many because of the toxins they produce. The process of domestication has gradually reduced the levels of these compounds over the millennia so that plant foods are far less toxic than their wild relatives. Many of these toxins evolved as a way to fight off predators, not surprisingly, modern food plants are much more susceptible to disease.

Toxin concentrations in a plant can vary tremendously, often by 100 times or more, and can be dramatically affected by environmental stress on the plant (drought, heat/cold, mineral deficiencies, etc) and disease. Different varieties of the same plant species can also have different levels of toxins and nutritional value (D'Mello, 2000).

Examples of toxins, their occurrence in plants and their effects on humans and animals include; cyanogenic glycosides which occur in sweet potatoes, stone fruits and lima beans can cause gastrointestinal inflammation, inhibition of cellular respiration (Novak and Haslberger, 2000). Glucosinolates rape (canola), found in mustard, radish, cabbage, peanut, soybean, and onion cause goiter, impaire metabolism and decrease protein digestion. Glycoalkaloids found in Potato and tomato can cause depressed central nervous system, kidney inflammation, carcinogenic and birth defects. Gossypol is available in cottonseed, its effects include reduced iron uptake, spermicidal; carcinogenic. Lectins are available in most cereals, soybeans, other beans and potatoes. They can cause intestinal inflammation, decreased nutrient uptake/absorption. Oxalate is found in spinach, rhubarb and tomato, which reduce solubility of calcium, iron, and zinc. Phenols are available in most fruits and vegetables, cereals, soybean, potato, tea, and coffee. Phenols have been reported to destroy thiamine, raise cholesterol level and mimic estrogen. Coumarins are found in celery, parsley, parsnips, and figs. David and Caffoor (1998) have documented them to cause light-activated carcinogens and skin irritation.

Rendering fish quiet (sedation) or unconscious (anaesthesia) is crucial to several aspects of fish handling during aquacultural operations such as stripping for gametes, sorting, weighing, treatment for and against diseases, transportation etc. Ross *et al.* (1993) reported that administration of anesthetics reduced the effect of stress during handling and hauling of

fish. Different handling procedures demand different anesthetic approaches. For instance light anaesthesia (sedation), which is defined as reduced activity and reactions to external stimuli, is sufficient for procedures such as transport or weighing of fish. Full anaesthesia can be defined as loss of consciousness and reduced sensing of pain, loss of muscular tonus and reflexes and is needed when surgical procedures are applied (Farland 1959).

Chung (1980) classified anaesthetic into four different stages: first stage is where fish is normal, reacts to external stimuli normally, swimming and opercular movements are normal. The second stage is where the fish is in a state of light anaesthesia, it becomes sluggish, has weak equilibrium, it swims partially and opercular movement is also partial while the third stage is where the fish is in a stage of deep anaesthesia, exhibits loss of movement and very weak equilibrium with partial movement of the opercular. The 4^{th} stage, which is characterized by total loss of equilibrium, movement of opercular, this in a few minutes leads to heart failure. The second and third stages are of great relevance as the fish is then insensitive to pain.

The choice of anaesthetics for fish must be based on species, the size of fish involved and the duration of operation in question. Water temperature and chemistry also have to be taken into consideration when choosing the method since the work involving anaesthetics often has to be done under uncontrolled field conditions without accurate control of concentrations and exposure time, an anaesthetic with a good safety

margin between effective anaesthesia and irrevocable collapse is essential (Lemm, 1993).

Biologically the African catfish, *Clarias gariepinus*, is undoubtedly the most ideal aquaculture species. It is widely distributed, not only in African countries but also in the Netherlands, thrieves in diverse environments, temperate to tropical *(*Hecht *et al*., 1996). It is hardy and adaptable principally as a consequence of its air breathing ability, feeds on a wide array of natural prey under diverse conditions, is able to withstand adverse environmental conditions, is highly resistant to diseases, is highly fecund and easily spawned under captive conditions. It has a wide tolerance of relatively poor water quality and possibly the most exciting feature of the species is its potential for highly intensive culture without prerequisite pond aeration or high water exchange rates and its excellent meat quality. Recent work in Rwanda has shown that strains of the species can also be grown successfully at lower temperatures and at high attitudes (Hecht *et al. 1996).*

1.2 JUSTIFICATION

The prohibitive cost (eg 200 US Dollars per 500g MS222) and non availability of synthetic anaesthesias is a good reason why aquaculturists must look inward for available indigenous plant materials that can be used as fish sedatives to reduce cost.

Although some scientific investigations have been done on *Datura* species, there are no available data on the possible use of this plant (*Datura innoxia*) as fish sedatives.

The study is basically important in fisheries development especially in the handling of live fish during tagging, stripping for gametes, weighing and in transport. It is hoped this study would provide solutions to frequent fish losses resulting from excessive struggle during aquacultural operations and transportation.

Clarias gariepinus is a common fauna in the tropical freshwaters where it is widely used in aquaculture in several African countries, hence its choice for this investigation.

1.3 OBJECTIVES OF STUDY

The objectives of this study are: To determine the active ingredients and their concentrations in the various parts of the plant *Datura innoxia* i.e. leaf, seed, stem, pod and root.

To determine the 96-hour LC_{50} of water extract of the (acute toxicity levels) various parts of the plant on *Clarias gariepinus.*

To evaluate the effects of sublethal concentrations of the various parts of the plant on growth and tissue composition of *Clarias gariepinus.*

To investigate the effects of sublethal concentrations of *Datura innoxia* on blood parameters and energy reserves of *Clarias gariepinus*.

To assess the effects of sublethal concentrations of the various parts of the plant, *Datura innoxia* on target organs (gills, liver, kidney, operculum and tail beat frequency) of *Clarias gariepinus.*

To characterize the efficiency (potency) of the different parts of the plant, *Datura innoxia,* as a sedative and anaesthesia for *Clarias gariepinus.*

1.4 LITERATURE REVIEW

1.4.1 *Importance of plants to the animal kingdom*

Plants are a common sight and so generally taken for granted. They live everywhere on earth, in many forms, from cacti in the desert to the oaks of the forest and the seaweed of the coastal waters. They are in many forms from the microscopic bacteria to the giant sequoias. All together they constitute the earth's vegetation. Yet few people fully realize what an important part of the environment plants are. Their influence on man has been and will always be very direct and profound.

(a) As source of food for animals

Green plants are most significant in their role as producers of food. From them directly or indirectly come animal foods. Protein, carbohydrates and fats as well as assessory nutrients such as vitamins and minerals are all made available to man and other animals through green plants. Fields and orchards yield such important staples as apples, grapes, cereal, grains, potatoes, tomatoes, beans and sugars.

The chief staple food of over half the people on earth is rice (Friend 1967), whereas wheat and corn serve similarly for almost all the remainder. These plants are grasses. When other grasses such as rye, oats, sugarcane and the pasture grasses for animal feeding are added, the importance of this group of plants can only be visualized. Valuable food oils are obtained from oil palm, olives, peanuts, corn, cottonseeds and soyabeans. Large quantities of cotton seeds and soyabeans oils are converted to solid fat by hydrogenation and used in the manufacture of margarine.

(b) As source of raw materials for industry

The utilization of plant materials for industrial purposes stands next to its importance as food. Wood is still our (man) chief structural material for building, *Chlorophora exelse* (Iroko tree), and the fir trees furnish about a quarter of the saw timber and a large amount is also derived from pine, various hard wood, e.g. bitch, maple, mahogany rosewoods and others. In recent years the amount of hard wood converted to plywood has increased due to the development of better gluing and bonding methods. The material and chemical conversion of wood to pulp for the manufacture of paper and certain synthetic textile consumes enormous quantities of timber**.**

The distillation of wood yields a great variety of valuable chemicals such as methanol and acetic acid. Turpentine and resin, linseed oil, tanning materials, rubber and cork are just a few of the many other industrial raw materials as documented by Ladeji (1991).

Many Species of plants yield fibres for the manufacture of fabrics. Cotton appears to be the most important in the world. Besides its prominence in the textile industry, cotton is used to make cording for automobile tyres and light duty rope. The oil and protein extracted from cotton are used for animals' feeds. Other fibres such as hemp is used for rug manufacture while Manila is used for heavy duty ropes and twines and along with sisal are used in making fishing gears. In some parts of Nigeria, Delep palm (*Borassus flabelli*), bamboo and raphia palm are used for making mats, straw hats, trays for sieving, cane chairs, tables, baskets and roofing materials as documented by Nengel (1990).

The great stores of energy represented by the deposits of coal, petroleum and natural gas were laid down mostly during the carboniferous period. These fossils are principal energy sources (Schorr, 1963). In many parts of Nigeria wood is still the principal source or fuel because of the affordability and availability.

Medicinal plants are the sources of many important scientific drugs of the modern world. According to Myers (1982) almost 80% of present day drugs are directly or indirectly derived from plants.

Since 1960, the medicinal plants that have been identified and used as sources of modern drug have increased to a large extent. Some examples of such drugs are shown below (Table 1).

Table 1: Some Common Drugs Derived from Plant Sources and Their Uses

DRUG	PLANT SOURCE	USES
Acacia (Anabin) gum	*Acacia Senegal*	Emulsifying agents
Penicillin	*Penicillium griseofulvum*	Antibiotics
Atropine hyoscine	*Atropa, belladonna Datura*	Antispasmodic
Caffeine	*Theasinensia*	CNS stimulant
Quinin	*Circhona sussirubra*	Biltertonic
Cocaine	*Erythroxylon coca*	Local anaesthetic
Ephedrine	*Ephedra sinice*	For the relief or asthma and hay fever
Ergometrine	*Claviceps purpurea*	Uterine stimulant
Nicotine	*Nicotiana fobaceri*	Destruction and Aptuids
Morphine B. Codeire	*Papava somniferum*	Narcotic Analgesic
Berberine	*Beriberis aristala*	Antidiarrhoeal

Source: Pharmacopia Codex (1973).

(c) ***Importance of the family Solanaceae in medicine and drug therapy***

Members of the Solanaceae have been used by man for the prevention and relief of medical disorders since the dawn of civilization (James, 1991). Today, solanaceous plants continue to figure prominently in traditional medicine especially in developing countries. In more developed nations, purified antimuscarinic drugs based on tropane alkaloids e.g. *Dubolisia*, *Scopolia*, *Datura spp* are important in the treatment of a wide variety of ailments. In contrast, *Nicotiana tabacum* (L.) exerts a negative impact on the human health on a global scale (Hawkes *et al., 1991).*

(d) Modern drugs from Solanaceae

The compounds from the Solanaceae that play such a prominent role as therapeutic drugs are all types of tropane alkaloids. A large number of tropane alkaloids are known, the pharmacologically important alkaloids in which the nitrogenous base is esterified with tropic acid (or a derivative) are apparently unique in this family (Romeike, 1978). However, only three compounds have attained worldwide eminence as chemotherapeutic agents viz: L-hyoscyamine, L-hyoscryne (scopolamine) and atropine. Hyoscyamine and hyoscine are the principal, naturally occurring forms. Other related but more recently discovered compounds, which are assuming growing importance, include anisodamine (6-β-hyoxyhyosyamine) and anisodine (daturamine). Shoot tissues are the main

sources of tropane alkaloids, although roots of *Atropa* and *Scopolia sp*. are used and occasionally seed of *Datura species*.

The solanaceous antimuscarinic drugs are well established in the treatment of a wide variety of medical disorders (Martindale 1982, Weiner, 1985) and have been claimed to be components of a greater number of medicinal preparations than any other medicinal plant compounds, (Bailey 1977). Susceptible organs or systems show differential sensitivity to these compounds and while small doses (around 0.5 mg) effects reduce glandular secretions on the eye, gastrointestinal tract require progressively larger doses. Treatment of certain conditions may therefore be accompanied by side effects, although these are normally more inconvenient than dangerous. The fatal dose of tropane alkaloids is variable and uncertain but 10mg of atropine would probably be toxic to adults and possibly fatal to children (Weiner 1985). The most important chemotherapeutic uses of antimuscarinic tropane alkaloids are shown in Table 2.

(e) The Solanaceae in traditional medicine

Primitive and folk medicine have long been associated with superstition, magic and ritual (Watt, 1979) and diagnosis of particularly difficult conditions often necessitated communication with higher spirits through the medium of hallucinogenic plants. The Solanaceous tropane alkaloids especially hyoscine are considered, after the indole alkaloids, to be the most important hallucinogenic compounds (Schultes and Hoffmann, 1979, 1980). Particularly important in ritualistic medicine in South America

are: *Methysticodendron amerisanum, Lochroma fuchsiodes, Latua pubifora* and various species of *Datura*.

Different plants assume different importance in different societies as can be shown on Table 2, but there can be no disputing that Solanaceae plants occupy a prominent position and are higher valued in medicinal practices and rites of most of the major ethnic groups. In South and Central America, for example, where the greatest concentration of solanaceous plants exists, a wide range of species is used, although *Datura specie*s and *Solanum species* are particularly important (Ayensu, 1981). Traditional medicine in Africa and India probably call on fewer solanaceous genera, although species of *Solanum* figure prominently in healing practices (Jain, 1981, and Jain and Borthakur, 1986).

(f) Solanaceous plants as precursors and pharmaceutical steroids

Solanaceae is an abundant and useful source of steroidal compounds. The steroids in question are alkaloids which have no therapeutic value in their own right but can be used as precursors for the production of pharmaceutically important steroids such as corticosteroids (for rheumatoid arthritis, inflammatory conditions, skin disorders). Sex steroids (for disorders of reproductive system and contraception) and anabolic steroids (to aid muscle disposition) are now the most prescribed type of medicinal compounds (Fowler 1984) and are one of the cornerstones of modern population control measures.

Table 2: Some Selected Solanaceous Plants in Traditional Medicine

COUNTRY/CONTINENT	SPECIES	USES
South/Central America	*Brugmansia*	Rheumatism, wound emetic, carminative erysipelas
	Brunfelsia	Syphilis, rheumatism, diuesis, emetic, purgative abortifacient, fever
	Cestrum latifolium	Skin disorders
	Datura	Anaesthetic, wounds, and bruises, arthritis, ulcers, prolapse haemorrhoids, neuralgia, fever, asthma, flu, headache and tumours
Africa	*Capsicum annuum*	Stomachache, diuretic, stimulant, haemorrhoids
	Datura fastuosa	Abortifacient
	D. Stramonium	Ear problems, rheumatism
	D. innoxia	and joint disorders, asthma, cough, headache, sedative, anaesthetic
China	*Atropa belladonna*	Convulsions, analgesic
	Datura metel	Cough, asthma, analgesic
	Hyosyamus niger	Convulsion, analgesic
	Lycium barbarum	Tonic, roborant, impotence
India	*Capsicum frutescens*	Headache, bronchitis, colds, sores, crocodile bite.
	Datura metel	Headache, asthma, leprosy, sores, epilepsy, convulsion, veneral disease, mumps.
	Solanum spirale	Intestinal worms
	S. nigrum	Gonorrhoea, stomachache

Source: James (1991).

The principal source of steroid raw material remains the sapogenin from tubers of *Dioscorea species* of Mexico, Central America and China, but problems in the supply of tubers have resulted in various countries moving towards other plant sources, which contain similar compounds and fewer cultivation problems. The most promising species are those of the genus *Solanum* which accumulate the steroid alkaloid solasodine, a nitrogen analogue of diosgenin which can be readily cleaved chemically to yield, with similar or even higher efficiency, the intermediate 16-dehydropregnenolene from which various medicinal steroids are derived. Even cheaper sources of steroid precursors (e.g. soya oil products) are becoming available.

(g) Deliterious effects of Solanaceous plants on human health

Certain tropane alkaloid and steroid alkaloid containing plants make significant and positive contributions to the fight against diseases, the smoking of tobacco (*Nicotiana tabacum*) causes serious pathological conditions and premature death. The practice is so widespread (hundreds of millions of people consume 6.7 million tones of tobacco per annum IARC, 1986) and the burden it places on health care facilities is so immense that it cannot reasonably be excluded from any assessment of the impact of the Solanaceae on human health.

A link between tobacco smoking and cancer was first suggested towards the end of the 18th century, but not clinically established until the 1950s (IARC, 1986). Correlations have been demonstrated between

smoking and carcinoma of a number of organs e.g. lip, tongue, larynx, bronchus, lung, oesophagus, stomach, pancreas, kidney, liver, uterus, with the strongest by far being that of the lung (IARC 1986). Lung cancer is now the most common form of cancer and in 1984 accounted for almost 1 million deaths world wide, of which nearly 90% were estimated to be directly due to tobacco smoking (Doll, 1986). Causal links between smoking and other diseases and conditions such as chronic bronchitis, peripheral vascular disease, peptic ulceration and increased prenatal mortality were subsequently demonstrated (Clee and Clark 1982). In Europe, 85% of deaths due to chronic obstructive lung disease (e.g. bronchitis, emphysema) and aortic aneurisms and 25% of fatalities due to ischaemic heart disease are attributed to smoking (IARC, 1986). The effects of smoking are complex and may be aggravated by other factors such as alcohol and stress.

1.4.2 Toxins

Toxins are effective and specific poisons produced by living organisms. They usually consist of an amino acid chain, which can vary, in molecular weight between a couple of hundred (peptides) and one hundred thousand (proteins). They may also be low-molecular organic compounds. Toxins are produced by numerous organisms, e.g. bacteria, fungi, algae and higher plants. Many of them are extremely poisonous, with a toxicity that is in several orders of magnitude greater than the nerve agents.

Plant toxins, in many cases, the poisonous substances are merely by-products or wastes from the essential functions of the plant. Often, toxic compounds or at least stuff that is unpleasant to taste or smell has evolved in plants to discourage or repel vegetarian insects or grazing animals. Plant toxins don't bother the plant that produces them, and often, they don't bother many other living things. Many plant components have the potential to precipitate adverse effects on the productivity of farm animals (D'Mello, 2000). These compounds are present in the foliage and/or seeds of virtually every plant that is used in practical feeding.

1.4.3 *Toxicity*

This refers to the degree at which a substance is being harmful, destructive or poisonous to life (Boyd and Lichtkoppler, 1979)

There are a number of terms that are associated with toxicity among them is acute, this measure can be used to reflect the levels of toxicity. Two of such that may sometimes be confused are LC_{50} and LD_{50}. The former refers to that concentration of a chemical or toxicant that kills 50% of a sample population. This measure is generally used when exposure to a chemical is through the animal breathing in, while the latter refers to the dose of chemical or toxicant which kills 50% of a sample population when the exposure is by swallowing, through skin contact or by injection.

The toxicity of most plant extracts varies depending on the type and the animal species involved. This is due in part to the phytochemical

composition of the extract and also due to the very great variation in susceptibility between individual animals.

Studies on acute toxicity of cassava leaf extracts on the African catfish *Clarias gariepinus* indicated that mortality occurred after 96 hours in graded concentrations of 100, 80 and 60 % of the graded extract (Aguigwo 1998). Similarly, an acute toxicity of tobacco (*Nicotiana tobacum*) leaf dust was carried out on *Oreochromis niloticus* by Agbon *et al.,* (2002). The extract was found to be toxic with 48 hour LC_{50} value of 109.6mg/l. Oti (2002) reported the 96 hour LC_{50} of cassava mill effluent on African catfish Heteroclarias, hybrid of *Heterobranchus bidorsallis* (male) and *Clarias gariepinus* (female) to be 50.19mg/l. Several other authors, Konar, (1970), Walden and Howard, (1971), Ufodike and Omoregie (1994) and Saha and Kaviraj (1996) have also investigated the acute toxicity of certain plant extracts on various fish species.

(a) Sublethal effects

A sublethal effect of a substance will not cause death but may cause stress or injury (Boyd and Lichtkoppler 1979). Many times there are sublethal effects and are not immediately noticed, as the organism so affected does not die. There are, however, consequences of the impending impairment of the health of the organism, its behaviour, physiology (metabolism), and life cycle. For example, some heavy metals such as lead interfere with biochemical activities at the cellular level in many organisms. While not directly leading to death, in many cases this

impairment can lead to poor health and subject the animals to infections and other equally debilitating illnesses. Omoregie *et al.* (1994) observed that sub-lethal concentrations of toxicants in the aquatic environment did not result in mortality of the aquatic organisms, however they had significant effects, which resulted in several physiological dysfunctions. Results from various investigations revealed that fish exposed to sub-lethal concentrations of toxicants grew significantly less and had poorer food conversion compared to their counterparts, which were not exposed to the toxicant.

Ade-Serrano (1982), Omoregie and Okpanachi (1992) and Oluwole and Bolarinwa, (1995) noted growth reduction and disruptive properties in animals exposed to sub-lethal concentrations of some plants (*Jatropha curcas and Azadirachta indica*) extracts.

Onusiriuka (2002) observed a significant difference in weight gained by *Clarias gariepinus* exposed to sub-lethal concentrations of formalin, noting that as the concentration of the toxicant increased so did the weight gain decreased. Similarly a dose-dependent impairment in erythrocyte count and plasma glucose of *Oreochromis niloticus* exposed to sub-lethal concentrations of formalin for 12 weeks, were observed by Omoregie *et al.* (1994). A similar observation was made by Sampath *et al.* (1993) when *Oreochromis niloticus* was exposed to sub-lethal concentrations of Organophosphorus pesticide, Ekalux.I t produced a time and dose dependent decrease in red blood corpuscle (RBC), haemoglobin (Hb) content, and mean corpuscular volume (MCV) leading to microcytic anaemia and an increase in the total leucocyte (TLC) owing to the rising granulocytes. Haematological changes in *Clarias*

gariepinus exposed to sub-lethal concentrations of copper and lead observed by Annune and Ahuma (1998) indicated a decrease in haematocrit, red blood cell count, mean corpuscular haemoglobin concentration, mean corpuscular volume and an increase in white blood cell count.

Other investigations into the effect of sub-lethal concentrations of toxicants on the haematology of *Tilapia zilli* was observed by Omoregie *et al.* (2002) to have caused anaemia due to significant decrease in erythrocytes, haemaglobin and haematocrit. A similar observation was made by Agbon *et al.* (2002) when they exposed *Oreochromis niloticus* to sub-lethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust to have caused significant difference in the values of red blood cell count and haemoglobin compared to the control groups. The histopathological examination of the gills, liver, and kidney of *Oreochromis niloticus* exposed to sub-lethal concentrations of Actelic 25 EC for 10 weeks indicated necrosis in the liver and kidney while the gills, which were the most damaged organs, resulted in respiratory impairment of the fish (Omoregie and Ufodike, 1991).

1.4.4 Datura

Datura is a well-known genus in the Solanaceae as a genus of drug plant with several weedy species. Because of the far-reaching distribution of *Datura* species across the planet there is some dispute concerning their origin. The greatest variety of species occurs in Mexico and Central America which has led some Botanist to believe that the explorers of the New World had been responsible for bringing *Datura* back to Europe,

along with other members of the nightshade family. Other sources suggest that their original home could be found somewhere in the vicinity of the Capsian Sea from where it spread south to Africa and east to Asia, eventually arriving in Europe, supposedly with the gypsies sometime during the middle Ages (Emboden, 1979).

It appears that *Datura* have always played a significant role as “culture plants” and evidence regarding their uses both in Asia and the New world dates back at least 3000 years. In both hemispheres *Daturas* were regarded as sacred and especially valued for their power to induce visionary dreams, to see the future and to reveal the causes of disease and misfortune.

All over the new world, from the southwestern corner of the North America, throughout Mexico as well as in Central and South America the historical and contemporary uses of the local *Datura* species (*D. innoxia, D. tatula, D. ferox, D. ceratocaula and D. discolor*) by the indigenous population is well documented. From historical accounts numerous sacred and medicinal plants were several types of *Datura species.* One of these *Daturas*, called Toloache, *D. innoxia*, was used as a painkiller in certain initiation rituals and given as narcotic to the ritual sacrifices. For this purpose, the preferred method of administration was enema or rolled up leaf suppository, which reduces some of the less pleasant side effects of the drug.

Another type of *Datura (D. ceratocaula*) called Atlinan by the Aztess, enjoyed a particularly sacred status, as another hallucinogenic plant.

These plants are so sacred that only the priests were allowed to use them. With their help they held counsel with the Gods–divining the outcome of future events, discovering the whereabouts of lost or stolen objects and prognosticating the causes of diseases, especially if the black magic was suspected. As a medicinal remedy they prepared an ointment for cracked soles and injured feet, made plasters for ulcers, pustules and infected wounds and skin sores and used it for poultices to treat rheumatic aches and pains (Emboden, 1979).

The use of *Datura* as a magical plant was and probably still is also common in the Caribbean. There it is known as ’herbe aux sorciers’ (herb of the sorcerers) and ‘concombre - zombi’ (Zombie–Cucumber). This name refers to a rather sinister use of the plant - literally zombification. Deliquents in particular became the victims of this practice. Criminals who did not seem to improve their records upon other means of punishment sometimes were turned into zombies. A strong herbal brew containing among other plants, *Datura* combined with the extremely potent extract of puffer – fish poison (*D. tubucurine*) was given to the criminal. The effect of the brew was to stupefy the convict to the point of pseudo comma and to numb his physical sensations. The zombie – to – be was declared dead and placed into a coffin with an attached air tube and a funeral ceremony was conducted. After 3 days or so the coffin was retrieved from the ground and the zombie was given another dose of *Datura* followed by an initiation into ‘another life, in which he was brainwashed in accordance with the rules of the new order. From that day on he was given regular doses of the

Datura concoction to maintain the hypnotic state. The spirit of the victim was thus literally forced to get out and stay out of the body and the zombie lost all sense of self or ego identity.

Throughout the middle Ages *Datura* flowers were commonly sold for their aphrodistic qualities all over central and southern Europe. They had the reputation of breaking down any resistance to sexual approaches. Pimps in particular knew how to use the herb to their best advantage. An indignant German writer aptly documents this common use of *Datura*, as a tool of brothel keepers, wicked seducers of girls, depraved courtesans and shameless lechers, (Emboden, 1979).

Evidently *Datura* did not enjoy such degree of reverence and reputation by the people of Europe as it received in Asia or the Americas. The difference of attitude among the Church- fathers on either side of the Atlantic, who in Europe had been largely responsible for sullying the reputation of *Datura*, is epitomiticed in the image ‘Santo Toloache’, the patron Saint of *Datura* found in Mexico. In the New World the Catholic Church was forced to sanctify the old pre Christian deity. Santo Toloache helps those who wish for reciprocated love. The faithful worshipers who pray to him make offerings of *Datura* flowers and take a tea of *Datura* leaves as a special sacrament. Here too the ancient use of as a powerful aphrodisiac is clearly implied.

Datura preparations can have very powerful effects, depending not only on the type of species and dose used, but also very much dependent on the set and setting ‘ of the person using it. There are so many different

species in the *Datura* genus. Probably the two most well known species are *Datura innoxia* and *Datura stramonium* (Jimson's weed or Thornapple). Other species include: *Datura arborea, D. aurea, D. candida, D. discolor, D. ceratocaula, D. dolichocarpa, D. fatuosa, D. ferox, D. indica, D. metel, D. metoloides, D. sanguinea, D. suaveolens, D. tatula, D. vulcanicola, D. willemsi*.

All species of *Datura* are leafy green plants with bright pink to white flowers. The flowers are all fragrant. *Datura* grows all over the world. The seeds are found in a small fruit which are completely covered with short, sharp spines (hence the name "Thornapple"). The stalks are bristly and somewhat thin in comparison to the rest of the plant. The leaves are flat mostly featureless and can either be multi-edged (with between 4 and 15 points) or basically ovoid.

Native peoples of the Southwestern U.S. have long used all species of *Datura* in puberty and other ceremonies, because of their hallucinogenic alkaloids. People trying to imitate Native Americans have often poisoned themselves, sometimes fatally, as all the plant parts contain various amounts of chemicals.

Daturas main effects on human beings, which can last anywhere from 12 to 24 hours, if not longer, include: stimulation and or anxiety, extreme nausea, dilated pupil, blurred or fixed-focus vision, rapid heartbeat, extreme disorientation, loss of memory. Others are loss of time, delirium, profound sensitivity to light and noise, seamless crossover into a variety of dream states, extreme lack of coordination, loss of body control,

extreme audio, visual and tactile hallucinations, apparent astral travel to familiar places, interaction with friends, relatives and other random people who are not physically present; extreme drying and irritation of the mouth, throat, eyes, urinary tract, and other mucous membranes; potential for uncontrollably emotional or violent activity; and inability to recall anything. Emboden (1979) noted that perhaps no more diverse practices exist with respect to hallucination than is found with *Datura*.

Datura innoxia, which is a native of Central America, has been naturalised in the Pelion area. *D. innoxia's* common names are Angel's trumpet or Thorn apple, it is also known as 'loco weed because of it's effect on cattle.

The Chumash people of California use *D. innoxia* medically as an anaesthetic for setting bones, to treat bad bruises and wounds, to 'freshen the blood' and to treat haemorrhoids. The anaesthetic and narcotic properties of the plant would numb the pain receptors thereby reducing stress and tension in the patient, which in turn speeds up the healing process.

D. innoxia is listed as one of the poisonous houseplants (Dereck, 2002) as well as a sedative. In Nigeria 'Zakami' (*D. innoxia),* has been reported as the latest nacortic drug of abuse in Benue State (Okeke, 1998).

The following have been listed as constituents for *Datura*: alkaloids, hyoscyamine, oleic acid, palmitic acid, scopolamine, acetic acid, acetone,

acotonic acid, aesculetin, atropine, caffeic acid, chlorogenic acid, hyoscine, scopoline, pyridine, tannin and succinic acid (Prabhakar *et al*., 1971).

Phytochemical constituents of this plant have been reported to include active components such as: scopolamine, hyoscamine, meteoidine, caffeic acid, coumaricacid, daturadiol, atropine (Pagani, 1992).

Datura innoxia is an erect annual, it can reach a height of up to 2m (Plate 1).It has small green ovoid leaves. The surface is not waxy or sticky. There are no fluids in them when broken**.** They are fairly plastic and don't break easily unless one is trying to break them. They dry into a brownish colour and curl up (Plate 2) Upon bisection, the fruit appears to have a structure similar to that of bell pepper. Many seeds grow inside and eventually the fruit breaks open and dries up releasing the seeds on the ground. The fruit is normally basically a sphere with spines, which are about 0.25 inches long covering the entire fruit. The spines are not sharp enough to pierce skin under normal conditions (Plate 3). The seeds of *D. innoxia* range in size depending on maturity, from miniscule to 0.25 x 0.25 x 0.5 inches. When the seeds are young (the fruit is still green and the shell is intact) they are yellow or off-white. They have an unpleasant taste and mildly bitter. It may be that the growth of the seeds causes the fruit to crack (Plate 4). The flowers are when closed, cylindrical but twisted. The outer ends are a light pink to purple colour. It has a fragrant distinct aroma, very hard to mistake with any other plant. It has been reported that people have become intoxicated from the aroma of the plant (Plate 5). *Datura* seems to have a 'strain' for every place in the world. It grows in a humid to

somewhat dry environment (not very dry). It grows in landfills. For this reason, the Native American Indians gave it the name “White mans Weed”. It grows well in rainforest. It can grow on very poor soil and is generally regarded as a pest.

The classification is given as follows:

Phylum: Plantae

Superclass: Tracheophyta

Class: Angiosperm

Subclass: Dicotyledoneae

Order: Polemoniales

Family: Solanaceae

Genus: *Datura*

Species: *innoxia* Miller, (1768).

Common English name: Toloache

Hausa name: Zakami

1.4.5 Anaesthetic

Fish respond to handling as a stress. Their stress response is practically speaking, like human beings upon perception of the stress by the nervous system, adrenaline is released into the bloodstream. Induction of deep anaesthesia, some of those most widely used, have an excitatory effect during initial absorption by the fish which largely defeats the purpose of calming fish which are to remain conscious. Many established fish anaesthetics are central nervous system depressants.

The different types of anaesthesia include:

***(a) Physical Sedation*.**

Physical sedation can be obtained by lowering temperature or by electric shock. The former method is mainly applicable for transportation. Cold water adapted fish species and marine fish require lower temperatures for sedation than warm water species and freshwater fish (Chung, 1980). Water-cooling can also be used in conjunction with other anaesthetics (e.g. Benzocaine) but the dosage must be reduced by about 30% as documented by Ross and Ross (1983). Electroanaesthesia has a number of advantages such as rapid immobilization of fish, no need for chemicals, rapid return to consciousness and low costs (Madden and Houston 1976, Gunstrom and Bethers 1985, Cowx and Lamarque, 1990, Cowx, 1990). But these are outweighed by the fact that the method cannot be used in saline water, and the danger of using inappropriate voltage levels, which may give severe physiological stress response in

experimental fish (Shreck *et al.,* 1976). Hovda and Linley (2000) noted that fish (adult pink salmon) were anaesthesized using hypothermia under experimental conditions. This they observed was effective for short time anaesthesia of Pacific salmon, *Oncorhynchus sp.* for spawning but noted that it's application for iteroparous or freshwater stenohaline species was problematic because of the physiological effect induced by cold shock and exposure to high salinity. Using water of moderate salinity to mitigate stressful procedures with fish is effective regardless of whether the fish are coming from salt or fresh water, and likewise independent of the destination salinity. In cases where fish will be returned to fresh water, it will be beneficial if they can be given a recovery period of one to two days in brackish water.

(b) Chemical sedation and anaesthesia

Chemical sedation is given to fish in liquid dilutions of varying strengths depending on the agent used. The sedative is inhaled by the fish and diffused across the gill epithelia. In minor quantities it can also diffuse into the fish via the skin. The skin may be a particularly significant route in scaleless fish with well vascularised skins since these chemicals are absorbed and excreted predominantly via the gills. Fish with a large surface area of gill epithelium for a given body weight (e.g. salmonoids) require lower doses of anaesthetics than fish (e.g. eels) with relatively smaller epithelial surfaces (Ross and Ross 1983). Other factors affecting the absorption and excretion of chemical are the relationship between the

surface of the gill epithelium and the body volume, thickness of epithelium, type of anaesthetic, dosage and temperature.

Widely used anaesthetics of the barbiturate group in fishery science are:

i. MS 222 – Tricaine methane sulphonate. (Chemical name: ethyl-amino benzoate methane sulphonate).

MS 222 is probably the most widely used fish anaesthetic worldwide and there are numerous studies on the physiological effects of this agent on fish. It is a crystalline powder, which is easily dissolved in freshwater and seawater. The recommended dosage for anaesthesia is 50-100mg/l (Shreck *et al.,* 1976). It is observed that MS 222 becomes toxic in seawater exposed to sun. MS 222 gives acid solution and a dose of $75mgl^{-1}$ can cause the pH to drop to 4.0 in soft water (Wedemeyer, 1970). The effect can however be mediated by adding 5-6ml saturated (10%) solution of $NaHC0_3$ to 1 litre of $100mgl^{-1}$ solution of MS 222.

ii *Benzocaine (Ethyl-ρ-aminobenzoate)*

Benzocaine whose chemical name is Ethyl-ρ-aminobenzoate is also very widely used in fish anaesthesia. It is chemically close to MS 222 both being derivatives of ρ-aminobenzioc acid. Benzocaine is a white crystalline powder, which is insoluble in water and has to be dissolved in ethanol in a 'master solution' of $l\ gl^{-1}$ 96% alcohol. The recommended dosage is 2.5ml of this master solution to 10l of aerated water. According to Wedemeyer (1970) comparison between Benzocaine and MS222 as anaesthetics for salmonoids

was slightly in favour of Benzocaine as less metabolic changes was observed. More recent studies by Savio and Huhti (1977) showed few differences between the two, both caused hyperglycemia. However benzocaine caused somewhat less hyperglycemia than MS-222.

iii. Chlorobutanol *(*chemical name *Chlorobutanol-Chlorbutol-Chorethone –Acetochloroform*).

Although classified as a safe anaesthetic for fish, it has not been widely used outside Scandinavia due to health hazards to humans connected with its use. Inhalation of larger quantities may cause unconsciousness in human; it can also irritate skin and eyes. Chlorobutanol is a crystalline colourless powder that has to be dissolved in ethanol. The usual base solution is 30g to 100ml 96% ethanol and the dose 10ml base solution to 10 litres aerated water. Chlorobutanol is considered a safe anaesthetic for fish, although a study showed an 87% reduction in return rates of Atlantic salmon (*Salmo solar*) smolts anaesthesia before release in comparison with untreated fish (David and Caffoor, 1998).

iv. *Methomidate* (Methomidate chloride)

Methomidate (Methomidate chloride) is hypnotic (sleeping agent) and not a barbiturate. It therefore causes less depression of respiration than MS-222 or Benzocaine. This may lead to fewer and less serious side effects. Methomidate is water-soluble. Gilderhus

and Marking (1987) showed that there is a relatively long wakeup time and also some mortality after treatment with methomidate.

v. *Quinaldine*

This is not easily soluble in water and is irritating to human skin and mucus membranes. Quinaldine-sulphate does not have these negative effects but gives an acid solution and most therefore be buffered with sodium bicarbonate.

vi. *Propanidide*

Propanidide has few physiological side effects and can be used both for short and long duration anaesthesia. Ross and Ross (1983) reported that the blood circulation could also remain unaffected when fish are treated with propanidide.

vii. *Clove oil*

Clove oil whose chemical name is eugenol (4-allyl-z-methoxy-phenol), is an effective anesthetics for both juvenile and adult rainbow trout (*Onchorhynchus mykiss*) as MS 222. Clove oil does not affect swimming performance and it also provides swift induction and recovery from anaesthesia.

vii. *Etomidate and Metomidate*

Many established fish anaesthetics are central nervous system depressants, which contribute to the transient stress during induction. A few fish anaesthetics, however, are classed as hypnotics, and do not have an excitatory impact. These are etomidate and its analogue, metomidate. Initial studies with these

compounds indicated their suitability for use in fish based on wide safety margins, lack of electrolyte disturbance, and minimal elevation of cortisol in striped bass and channel catfish.

Kreiberg and Powell, (1991) assessed the value of metomidate for cushioning handling impact on chinook salmon, a species which can suffer loss of health and condition after seemingly minor handling procedures. Quietly introducing stock solution of metomidate to give a sedating dose in the fishes' home tank, was found that blood cortisol response to stress of mild crowding and dipnetting in air was negligible relative to untreated control fish.

(c) Plant extracts Anaesthetics

The use of plant extract as fish anaesthetics is being widely researched upon. Mgbenka and Ejiofor (1998) while working on extracts of dried leaves of *Erythrophleum suaveolers* noted that *Clarias gariepinus* and *Heterobranchus longifillis* were anaesthetized when exposed to up to 3.5g/l crude extract recovered in fresh water. However all the fingerlings exposed to 4g/l did not recover from the anaesthesia effect.

In a preliminary test conducted by Ebah (1998) on the leaf of *D. innoxia*, it was observed that crude extract (10 ml. of extract per litre of water) tranquilized *C. gariepinus* within 33.5 minutes.

1.5 MORPHOLOGY, DISTRIBUTION AND CLASSIFICATION OF *CLARIAS GARIEPINUS*

Clarias gariepinus (Plate 6) has the following features: The body is elongated with a large head, depressed and bony with small eyes. It has a narrow and angular occipital process, wide gill openings, air-breathing labyrinthine organ arising from gill arches with first gill arch having 24 to 110 gill rackers, number of gill rackers increasing with size. It has a pointed cleithrum, narrow with longitudinal ridges and with sharpness. The mouth is large and terminal with four pairs of barbles, nasal barbles are shorter than the head, while maxillary barbles are rarely shorter and somewhat longer than head, reaching to a point midway between the origin of the dorsal fin and the insertion of the pelvic fins. The outer mandibular barbel is longer than inner pair. *Clarias gariepinus* has long dorsal and anal fins without spines and adipose fin. The dorsal spines (total) have 0-0;dorsal softrays 61-80; anal spines 0-0;and anal softrays 45-65. The vertebrae are 56-63.The pectoral fin spine is serrated only on its outer side i.e. anterior edge, with rounded caudal fin. It is generally dark, grayish-black above, creamy-white below with a fairly distinct black longitudinal band on each side of the ventral surface of head. The young of less than 9 cm long have a similar colour pattern except that the black bands on head are absent. The larger young of 10-30 cm are mottled and grey-khaki above. It attains a maximum size of 150cm (Standard Length) and maximum weight of 60 kg (Reed *et al.,* 1967).

Clarias gariepinus which is found in fresh water of the Niger River in Africa, also extending to Southern Africa in the Lipopo, Orange-Vaal, Okavango and Israel, Jordan, Lebanon and Syria. It has been widely introduced to other parts of Africa, Europe and Asia (FAO 2000). *Clarias gariepinus* is widely tolerant of extreme environmental conditions. It is a bottom feeder, which occasionally feeds at the surface. It is omnivorous, feeds on insects, crabs, plankton, snails and fish but also takes young birds, rotting flesh, plants and fruits. *C. gariepinus* is marketed fresh, frozen, eaten boiled, fried and baked (FAO 2000) .

Classification

Kingdom:	Animalia
Phylum:	Chordata
Class:	Actnopterygii (ray-finned fishes)
Order:	Clariidae (airbreathing catfishes)
Genus:	*Clarias*
Species:	*gariepinus* Burchell (1822).
Common English name:	Mudfish
Hausa name:	Tarwada

CHAPTER TWO

MATERIALS AND METHODS

2.1 PHYTOCHEMICAL SCREENING OF *DATURA INNOXIA* LEAF, SEED, STEM, POD STEM AND ROOT.

The screening test for the presence of alkaloids, saponins, tanins, atropine, scopolamine and hyosyamine were performed according to the procedures of Wagner *et al.* (1984).

2.1.1 *Preparation of D. innoxia Leaf, Seed, Stem, Pod and Root for Phytochemical Analysis*

Fresh samples of parts of *D. innoxia* plant (leaf, seed, stem, pod and root) were collected from University of Agriculture, Makurdi. The samples were separated into leaves, seeds, stems, pods and roots. These samples were air dried to constant weight under laboratory conditions Temperature 22^{o}C) and ground to fine powder in a clean laboratory mortar. The resultant powder was sieved (0.2mm) and stored in airtight wide mouthed bottles for analysis.

2.1.2*. Preparation of TLC Plates*

A 55.5g of silical gel (60 F_{254}) was thoroughly mixed with 4.5 g of starch (cornstarch) in a dry beaker, 172 ml of distilled water was slowly added. While stirring, the mixture was heated on a steam bath to 70^{0}C. The thickened mixture was cooled under tap water to about 32^{0}C and

carefully with a spreader, the cooled mixture was spread on washed and airdried 20 cm x 20 cm glass plates.

2.1.3. *Identification of Alkaloids in Leaf, Seed, stem, Pod and Root*

A quantity of 1g of each of the stored samples (leaf, seed, stem, pod and root) was thoroughly mixed with 1ml of 10% ammonia solution then extracted by shaking for about 5 minutes with 5ml methanol at 60^{o}C (water bath). The filtrate was cooled and concentrated, 100μl was applied to the TLC plate using a micropipette.

The plate was then put in a chromatograph tank containing a mixture of Acetone - Water - conc. Ammonia (90:7:3) after allowing the tank to saturate for 30 minutes in an air conditioned room (18^{o}C). The tank was allowed to stand in the air-conditioned room until the solvent rose to a distance of 15cm for development. Identification was done based on the similarity of colour intensity and zone size between standard solutions and the plant extract. Inspection was done under uv-254nm and chromatogram was analyzed for presence of alkaloid by spraying with dragendorrf s reagent.

2.1.4. *Test for atropine*

One gramme of each of the stored samples was moistened with 1ml of 10% ammonia solution, then extracted by shaking for 15 minutes at

60^{o}C (water bath) with 5ml methanol, 20μl of the filtrate was applied to the chromatogram. The plate was put in a pre-saturated tank containing ethylacetate - methanol - water (100:13.5:10). The tank was allowed to stand in a room temperature of 18^{o}C (air conditioned) until the solvent rose to a distance of 15cm identification was done based on similarity of colour intensity and zone size between standard solutions and the plant extract. Inspection of chromatogram was done in UV 365nm and each chromatogram was analysed for the presence of atropine by spraying with dragendorrff reagent.

2.1.5*. Identification of coumarins*

One gramme each of the stored samples was extracted by heating under reflux for 15 minutes with 10cm Dichloromethane (DCM). Each filtrate was evaporated to dryness, and each residue was dissolved in 1ml Toluene. A total of 50μl of this toluene solution was applied to the chromatogram and each was put in a pre-saturated tank containing toluene ethyl acetate and left in an air-conditioned room (18^{0}C) for the solvent to rise to a distance of 15cm identification was done based on similarity of colour intensity and zone size between standard solutions and the plant extract. Inspection of each chromatogram was done in UV-254nm and analyzed for the presence of coumarins after spraying with vanillin-sulphuric acid reagent (vs) and photographs taken using digital camera (model CDS 4100).

2.1.6. *Identification of saponins*

One gramme of each of the stored samples was moistened with 1ml of 10% ammonia solution and then extracted by shaking for 15 minutes at 60^0C (water bath) with 5ml methanol. This was evaporated to 1ml mixed with 0.5ml water, then extracted with 3ml butanol. A total of 20μl of the butanol phase was applied to the chromatogram. The plate was put in a pre-saturated tank containing chloroform – methanol – water (64:50:10) and allowed to stand in a room (temperature 18^0C) until the solvent rose to a distance of 15cm. Identification was done based on similarity of colour intensity and zone size between standard solutions and the plant extract. Inspection was done under uv-365nm and chromatogram was analyzed for presence of saponins by spraying with vanillin–sulphuric acid reagent.

2.1.7. *Identification of phenol carboxylic acids*

A known weight of 1g each of the stored samples was extracted by heating under reflux for 15 minutes with 10ml Dichloromethane ((DCM). Each filtrate was evaporated to dryness, and each residue was dissolved in 1ml toluene. 50μl of this toluene solution was applied to the chromatogram and each was put in a pre-saturated tank containing toluene ethyl acetate and left in an air-conditioned room (18^0C) for the solvent to rise to a distance of 15cm identification was done based on similarity of colour intensity and zone size between standard solutions and the plant extract. Inspection of each chromatogram was done in UV-254nm and analyzed for the presence of phenol carboxylic acids after

spraying with fast blue salt reagent and photographs taken using digital camera.

2.1.8. *Test for hyosyamine*

One gramme of 1g of each of the stored samples was moistened with 1ml. of 10% ammonia solution, then extracted by shaking for 15 minutes at 60^{o}C (water bath) with 5ml methanol, 20μl of the filtrate was applied to the chromatogram. The plate was put in a pre-saturated tank containing ethylacetate - methanol -water (100:13.5:10). The tank was allowed to stand in a room temperature of 18^{o}C (air conditioned) until the solvent rose to a distance of 15cm identification was done based on similarity of colour intensity and zone size between standard solutions and the plant extract.

Inspection of chromatogram was done in uv-365nm and each chromatogram was analysed for the presence of hyosyamine by spraying with dragendorrff reagent.

2.1.9. *Identification of flavonoids*

One gramme of 1g each of the stored samples was extracted with 10ml methanol for 5 minutes on a water bath at about 60^{0}C. 20μl of the filtrate was applied to the chromatogram. This was put in a pre-saturated tank containing Ethyl acetate-methanol-water (100:23.5:20) at temperature of 18^{0}C until the solvent rose to a distance of 15cm. Identification was

done based on similarity of colour intensity and zone size between standard solutions and the plant extract. Inspection of each chromatogram was done in UV-365nm and analyzed for the presence of flavonoids after spraying with natural products polyethyl eneglycol reagent (NP/PEC) and photographs taken using digital camera.

2.1.10. *Identification of essentials oils*

A weight of 1g each of the stored samples was extracted by heating under reflux for 15 minutes with 10ml Dichloromethane ((DCM). Each filtrate was evaporated to dryness, and each residue was dissolved in 1ml toluene. 50μl of this toluene solution was applied to the chromatogram and each was put in a pre-saturated tank containing toluene ethyl acetate and left in an air-conditioned room (18^0C) for the solvent to rise to a distance of 15cm. Identification was done based on similarity of colour intensity and zone size between standard solutions and the plant extract. Inspection of each chromatogram was done in UV-254nm and analyzed for the presence of essential oils after spraying with Komarowsky reagent and photographs taken using digital camera.

2.1.11. *Identification of cardiac glycosides*

A known weight of 1g each of the stored samples was mixed with 5ml of 50% methanol and 10ml of 10% lead (ii) acetate solution, then heated for 10 minutes on a water bath and allowed to cool. Each cooled filtrate was extracted with two separate 10ml quantities of dichloromethane

(DCM) each combined DCM extracts were completely evaporated each residue was dissolved in DCM-methanol (1:1) and 100μl of this solution was applied to the chromatogram. Each plate was put in a pre-saturated tank containing ethyl acetate methanol-water (100:13.5:10) and left in an air-conditioned room (18^{o}**C**) for the solvent to rise to a distance of 15cm identification was done based on similarity of colour intensity and zone size between standard solutions and the plant extract. Inspection and detection of each chromatogram was done in UV-365nm and analyzed for cardiac glycosides after spraying with Kedde reagent and photograph were taken using digital camera.

2.1.12. *Identification of valepotriates*

A known weight of 1g each of the stored samples was extracted by heating under reflux for 15 minutes with 10cm Dichloromethane ((DCM). Each filtrate was evaporated to dryness, and each residue was dissolved in 1ml toluene. 50μl of this toluene solution was applied to the chromatogram and each was put in a pre-saturated tank containing toluene ethyl acetate and left in an air-conditioned room (18^{0}C) for the solvent to rise to a distance of 15cm identification was done based on similarity of colour intensity and zone size between standard solutions and the plant extract. Inspection of each chromatogram was done in UV-254nm and analyzed for the presence valepotriates after spraying with conc. Hydrochloric acid–glacial acetic acid reagent and photographs taken using digital camera.

2.1.13. *Identification of tannin*

Tannin was determined using standard methods described by AOAC (1980). A total of 2g of each stored sample was defatted for 2 hours using Soxhlet extraction apparatus. Each residue was oven dried for 1 hour at 100^0C, boiled with 300ml of distilled water, diluted to 500ml in standard volumetric flask and filtered through non-absorbent cotton wool. A volume of 25ml of the infusion was measured into 2 litre porcelin dish and titrated with 0.1N potassium permanganate against 0.1N oxalic acid until blue solution changed green, then few drops of 0.1N potassium permanganate was added. The difference between the two titration was multiplied by 0.006235 to obtain the amount of tannin in the sample.

0.1N oxalic acid = 0.0066235g tannin.

2.1.14. *Test for scopolamine*

A weight of 1g of each of the stored samples was moistened with 1ml of 10% ammonia solution, then extracted by shaking for 15 minutes at 60^0C (water bath) with 5ml methanol, 20μl of the filtrate was applied to the chromatogram. The plate was put in a pre-saturated tank containing ethylacetate - methanol -water (100:13.5:10). The tank was allowed to stand in a room temperature of 18^0C (air-conditioned room) until the solvent rose to a distance of 15cm Identification was done based on similarity of colour intensity and zone size between standard solutions and

the plant extract. Identification was done based on similarity of colour intensity and zone size between standard solutions and the plant extract.

Inspection of chromatogram was done in uv-365nm and each chromatogram was analysed for the presence of scopolamine by spraying ith dragendorrff reagent.

2.2 DETERMINATION OF MINERAL COMPOSITION OF *D. INNOXIA* LEAF, SEED, STEM, POD AND ROOT.

Determination of Sulphur, Phosphorus, calcium, magnesium, potassium, sodium, iron, lead, cadmium and chromium in *D. innoxia*, leaf, stem**,** pod *and* root was carried out using the method described by AOAC(1980*)*

2.2.1. Determination of Sulphur

From the stored samples 1g of each sample was weighed into separate beakers of 100ml, Aquaregia (10ml HNO_3 and 30ml HCL) was added and beakers were put on a hot plate to dry. The samples were then moistened with distilled water and a few drops of hydrochloric acid was added and put back on the hot plate to dry again. Water and ammonium chloride (75ml) was added to each sample and boiled. The solutions were precipited by adding ammonia solution. Each beaker's precipitet was washed several times (5 times) with hot distilled water. The fiterate was made up to 100ml in volumtric flasks. Using a pipette, 50ml of each filterate was measured into 100ml volumtric flasks and 5ml of conditioning reagent was added and a little (1g) of Bariumchloride was added and vigorously shaken for at least 2 minutes. This was then allowed to stand

for 30 minutes. The samples were then ran with standards in uv visible. Spectrophotometer (model SP6-450-uv/vis Pye-Unican) at 420 nm wavelength.

2.2.2. Determination of Phosphorus

From the dried stored samples 1g of each sample was weighed into separate test tubes, 0.5g of Dipotassium Disulphite VI heptaoxide ($K_2S_2O_7$) added and mixed by rotating test-tubes. The samples were fused by heating, and heating continued for 1 minute after fusion using burnsen burner flame. The test tubes were removed and rotated so that melts made thin on sides of the test tubes and cooled. Thereafter 5ml of 4M nitric acid was added to each sample and placed in a water bath (60^oC) to digest the melt. Each sample was cooled, diluted with distilled water to 10ml mark. This was then mix thoroughly and allowed to settle. Using clean test tubes, 2ml of each sample solution was put in separate test tubes and 2ml of Vanada Molybdate reagent was added and diluted to 10ml with distilled water. The test tubes were stoppered and shaken thoroughly to mix. They were allowed to stand for 30 minutes thereafter. The samples were then ran with standards in uv visible spectrophotometer at wavelength of 470nm.

2.2.3. *Determination of calcium, magnesium, potassium, sodium, iron, lead, cadmium and chromium*

A total of 20g of each dried sample was weighed into sepeate pocelain crucibles and ashed at 950^{o}C for 2 hours in a furnance (Galenkamp muffle furnace size 2) and cooled. Out of the ashed samples, 2g of each was weighed into 250ml separate beakers and digested using Aguaregia. After which 75ml of distilled water was added to each sample and boiled for ten minutes. Each sample was then filtered into 100ml volumetric flask and allowed to cool. The volume then made up to the 100ml mark using distilled water. Each sample was then read using an Atomic Absorption Spedtrophotometer using their standard and castlock lamps of each element for calibrations as shown below.

2.3 PREPARATION OF EXPERIMENTAL DIET

Diet fed to experimental fish was prepared using the composition of Ita (1994) the ingredients (fish, soyabean, bone meal, ricebran, starch) were ground separately using a manual grinding machine (Molino Victoria model), sieved to fine consistency. Each ingredient was weighed to the formulation (Table 4) and thoroughly mixed together to form a homogeneous mass. The mixture was added to boiling water and mixed further to form thick dough. The dough was extruded as semi moist pellet using a pelleting machine (FOCT 4025-83 model). The pellets were later spread out to dry in the sun.

Table 3: Wavelength and Lamp Current of Calcium, Magnesium, Potassium, Sodium, Iron,Lead, Cadmium and Chromium

Element	Wavelength	Lamp current
	(nm)	(MA)
Calcium	422.7	10
Magnesium	285.2	4
Iron	248.3	15
Lead	217.0	6
Cadmium	228.8	6
Chromium	357.9	10
Potassium	766.5	8
Sodium	589.0	8

2.4 DETERMINATION OF AMINIO ACID COMPOSITION OF *D. INNOXIA* LEAF, SEED, STEM, POD AND ROOT

Determination of amino acid composition of *D. innoxia* leaf, seed, stem, pod and root was done using the methods described by Spackman *et. al.* (1958). A weight of 0.175g each of the stored powered samples (leaf, seed, stem, pod and root) were weighed into tubes and 5ml of 6M-hydrochloric acid added. A stream of nitrogen was passed into each tube and the tubes sealed. The content of each tube was hydrolyzed at 120^{0}C for 18 hours. Each resultant hydrolysate was quantitatively transferred into a 100ml flask and evaporated in vacuo in a rotary film evaporator. 5ml portion of distilled water was added to each flask to resuspend the residue and the hydrolysate evaporated in vacuo. The process was repeated with a further 5ml portion of distilled water. This procedure removed all traces of acid in the samples. The de-acidified residues were then taken up in 1ml of 0.2M acetate buffer, pH 4.0, for amino acid analysis.

Amino acid analysis was carried out for each residue (leaf, seed, stem, pod and root) by the method of Spakeman *et al*. (1958) on a Technicon TSM-1 amino acid analyzer. The area under each peak was calculated using nor leucine as an internal standard and molecular weight of each amino acid relative to the standard and percentage nitrogen in the sample, the concentration of each amino acid was calculated. Since acid hydrolysis destroys tryptophan and cysteine, these two amino acids were determined directly by the acid ninhydrin method. Tryptophan was

determined by the method of Gaitonde and Dovey (1970), while cysteine was determined by the method of Gaitonde (1967). Determination was replicated twice.

2.5. DETERMINATION OF PROXIMATE COMPOSITION OF *D. INNOXIA* LEAF, SEED, STEM, POD, ROOT AND EXPERIMENTAL DIET

Determination of proximate composition of *D. innoxia* leaf, seed, stem, root, pod and experimental diet were done using the methods described by AOAC (1980).

2.5.1. *Determination of moisture content*

Weighed airdried sample (10g) was oven dried to constant weight at 105^{O}C for 24 hours. The sample was allowed to cool in a desiccator and reweighed. The loss in weight was recorded as the moisture content, which was further, expressed as a percentages of the original weight. Determination of moisture composition was replicated.

2.5.2. Determination of crude lipid content

A known weight (2g) of each sample was weighed and placed in an extractor thimble. The lipid content of each sample was then extracted with a chloroform- methanol mixture (2:1) in a Soxhlet apparatus for 8 hours. The mean loss in weight of the extractor thimble and sample, and gain in weight of the extraction flask was recorded as the Lipid content which was

further expressed as a percentage of the original weight. The determination of crude lipid content was replicated.

2.5.3. *Determination of crude protein content*

A known weight of 0.2g of each of the fat-free samples of the leaf, seed, stem, pod and root was first wrapped in Whatman filter paper (No. 1) and put in a Kjeldhal digestion flask. 10ml of concentrated sulphuric and acid was added. 1g of catalyst (a mixture of sodium sulphate (Na_2SO_4O) copper sulphate ($CuSO_4$) and selenium oxide (SeO_2), ratio 10:5:1 was added into the flask to facilitate digestion. Four pieces of antibumping granules were added. The flasks were then put on Gallenkamp digestion apparatus for 2 hours until liquid turned light green. The digested samples were cooled and diluted with distilled water to 100ml in standard volumetric flask. A 10ml aliquot of each diluted solution with 10ml each of 45% sodium hydroxide were put into the Manhan distillation apparatus and distilled into 10ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70ml of distillate was collected. Each distillate was then titrated with standardized 0.01N hydrochloric acid to grey coloured end point to obtain nitrogen. The percentage crude protein in the original sample was calculated using the formula.

$$\text{Crude protein (\%)} = \frac{(a-b) \times 0.01 \times 14.01 \times C}{d \times e} \times 100 \times 6.25$$

Where:

a = Titre value of the digested sample

b = Titre value of the blank sample

c = Volume to which the digested sample was made up to with distilled water (100ml)

d = Volume of sodium hydroxide used for distillation (10ml)

e = Weight of dried fat-free sample

2.5.4. *Determination of the crude fibre content*

A known weight (2g) of each defatted sample was transferred into 250ml. quickfit flask. Tetraoxosulphate (vi) acid (1.25%), 5 pieces of anti-bumping granules and 5 drops of octanol were added. This was refluxed for 30 minutes and filtered through a Whatman filter paper (No. 4). The residue was later refluxed in 1.25% sodium hydroxide for 30 minutes, filtered and dried in an oven at 100^0C for 12 hours. It was later weighed and ashed at 600^0C for 24 hours. The ash was weighed on Mettler digital analytical balance (Mettler AE 100). The difference in weight between the crucible plus sample and crucible plus ash was recorded as the fibre content. The determination of the crude fibre content was duplicated.

2.5.5. *Determination of ash content*

A known weight (2g) of each stored sample was weighed into pre-dried porcelain ashing crucible and properly covered and put in a Gallenkamp Muffle furnace (Model FR 614) at 600^0C for 24 hours. The final weight of the each sample was recorded as the ash content, which was further, expressed as percentage of the original weight. The determination of Ash content was replicated.

2.6 COLLECTION AND ACCLIMATION OF EXPERIMENTAL FISH (*CLARIAS GARIEPINUS)*

The fish*, Clarias gariepinus*, fingerlings weighing 10.3 ± 0.35g were collected from Rockwater fish farm, Jos, Plateau State. The fish were transported in oxygenated bags to the Fisheries and Hydrobiology Research Laboratory, University of Jos. They were kept in glass aquaria tanks 60 x 40 x 80cm (115.2L capacity) where they were acclimated to laboratory conditions for two weeks prior to exposure. During that period the fish were fed 4% of their body weight once daily (0900 hours) with laboratory formulated fish feed (Table 4). Each aquarium was supplied with dechlorinated, well-aerated tap water, which was changed once daily. After the acclimation period, the fish were randomly selected and stocked 10 fish per aquarium in 14 glass aquaria for the experimental runs.

2.7 PREPARATION OF *DATURA INNOXIA* LEAF, SEED, STEM AND ROOT EXTRACTS FOR BIOASSAYS

A known weight of 500g of each stored sample was dissolved in 2 litres each of distilled water at room temperature (23 ± 0.3^0C) for 24 hours (Omoregie and Onuogwu, 2000). The settled aqueous portion was then decanted and filtered through a Whatman filter paper (No. 1) using a vacuum pump. The filtrates were freeze dried and stored in a refrigerator (10^0C) for use.

Table 4: Ingredient and Proximate Composition of Diet Fed to *Clarias gariepinus*

INGREDIENT(%)	**DIET**
Fish Meal	30
Soyabean Meal	35
Bone Meal	10
Rice Bran	15
Corn Oil	3
*Vitamin & Mineral Mix	5
Starch	2
PROXIMATE COMPOSITION (% DM)	
Crude Protein	44.5
Crude Fat	8.6
Crude Fibre	5.1
Ash	16.4
NFE	25.5

***Vitamin and mineral compositions are shown in Appendix 1.

2.8 DETERMINATION OF ACUTE TOXICITIES AND SUBLETHAL TESTS

Preliminary experimental tests were carried out to determine suitable concentration of the various parts of *D innoxia* that will not result in outright mortality of fish. Static bioassays were employed during which periods the set-up were continuously aerated. For each batch of experiment fourteen (14) glass aquaria (60 x 40 x 80cm i.e. 115.2L capacity) were used. Six different concentrations 400.00, 350.00, 300.00, 250.00, 200.00 and 150.00 mg/l for the pod, stem and root, while 200.00, 180.00, 160.00, 140.00, 120.00 and 100.00 mg/l were used for the leaf and seed. Each concentration had a replicate. Two glass aquaria without the extract served as the control experiment. For the sublethal bioassays, the following concentrations were used: 100.00, 50.00, 25.00, 12.50 and 6.25 mg/l for the leaf, seed, stem, pod and root. Fresh preparations were introduced into the experimental media on a daily basis.

The water physio-chemical parameters of the various experimental media were monitored every 24 hours. The parameters determined were:

i. Temperature (^{0}C)
ii. pH
iii. Free carbon-dioxide (mg/l)
iv. Dissolved oxygen (mg/l)
v. Total alkalinity (mg/l)
vi. Biological Oxygen Demand
vii. Ammonia

For each batch of experiment, 10 fingerlings of *Clarias gariepinus* (mean wt 10.3 ± 0.38g) were introduced into each aquarium. The fish were fed with 4% diets once daily (Table 4). The following experiments were then conducted to determine:

1. Effect of acute concentrations of *D. innoxia* leaf, seed, stem, pod and root extracts on:
    a. Mortality rate of the fish
    b. The opercular ventilation rate of the fish
    c. The tail fin beat frequency of the fish.
2. Effect of sublethal concentrations of *D. innoxia* leaf, seed, stem, pod and root extracts on:
    a. The growth rate of the fish
    b. Some blood parameters

Methods of conducting acute bioassays as described in UNEP (1989) report were employed for the acute toxicity investigation. The exposure period lasted 96 hours (4 days) during which period the fish were not fed. The fish were observed at 3 hours intervals for the higher concentrations until complete mortality occurred. At lower concentration they were observed every 6 hours. Dead fish were removed immediately from the experimental set-up. Raw mortality data were analyzed by probit analysis (Barr *et al*., 1979) to determine the concentration of *D. innoxia* extract that will result in 50% mortality of fish in each aquarium. The 96-hour LC_{so} was determined as a graphical summary of the percentage mortality data.

The opercular ventilation rate and tail beat frequencies per minute were read at the start of the exposure period and 24 hours thereafter. The sublethal bioassays lasted 12 weeks. The mean weight gain of the fish was obtained on a weekly basis.

Haematological examinations were conducted for the following parameters

Haemoglobin (Hb)

Red blood cells (RBC)

White blood cells (WBC)

Packed cell volume (PVC)

Erythrocyte sedimentation rate (ESR)

Mean corpuscular haemoglobin (MCH)

Mean corpuscular volume (MCV)

Mean corpuscular haemoglobin concentration (MCHC)

2.9 ADMINISTRATION OF *D. INNOXIA* AS ANAESTHETICS TO *C. GARIEPINUS* FINGERLINGS

Administrations of 24-hour water extract and chloroform-methanol extract of *D. innoxia* to *C. gariepinus* fingerlings were done through emerson of the fish in the extracts' bath.

2.9.1 Administration of 24 Hour Water extract of D. innoxia to C. gariepinus fingerlings

Graded series of the stored leaf of *D. innoxia* were soaked in fresh declorinated tap water (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 g/l) for 24 hours at room

temperature (28^{o}C), after which the mixture was filtered. Before sedation, fish were fasted 24 hours and aeration was provided to anaesthetic bath. Ten healthy actively swimming *C. gariepinus* fingerlings (mean weight 10.08 $\pm$0.02g) were individually immensed into the anaesthetic solutions and behavioural responses carefully monitored and classified according to Ross and Ross (1999) for a period of 180minutes at 28^{o}C. Each concentration had three replicates. If anaesthesia was attained, each fish was then placed in a 60l aquarium containing 40l of aerated dechlorinated tap (fresh) water for recovery and time taken to recover noted. Immediately following recovery blood was drawn from the caudal peduncule with heparinized syringe from nine fish at each concentration (three from each replicate) of *D. innoxia* leaf extract. Glucose level, red blood cell count, white blood cell count, packed cell volume, haemoglobin, erythrocyte sedimentation rate, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and mean corpuscular volume were calculated.

Also histopathological analysis of the gills, gonad, kidney and liver were done. Control values were obtained for fish not exposed to *D innoxia* leaf extracts, the remaining fish were observed at an interval of 12 hours for the next 7 days for mortalities. The experiment was repeated for *D. innoxia* seed, stem, pod and root.

2.9.2 *Administration of chloroform-methanol Extract of D. innoxia to C. gariepinus* fingerlings

Fifty grammes of the stored air dried sample of *D. innoxia* leaf sample were macerated twice in 240ml chloroform-methanol (2:1) mixture for 24 hours and filtered through No. 1 Whatman filter paper. The filtrate was used as the unseperated extract. To separate this extract into different fractions, the filtrate was mixed with 0.2 volume of distilled water and seperated into lipid and non-lipid layers in a separating funnel. The layers were dried over a heating block and weighed and the concentrations calculated. The lipid layer was dissolved with ethanol and the non-lipid layer dissolved in water. To test the anaesthetic effects of these on the fingerlings, *C*. *gariepinus* were exposed to (1) the unseparated methanol-chloroform extract, (2) the lipid and (3) non- lipid fractions of the alcohol extracts. Ten fingerlings were individually exposed to dosages of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0g/l of serially diluted solutions and the fish monitored as in the crude aqueous filtrate tests above.

The experiment was repeated for the seed, stem, pod and root.

2.10 HAEMATOLOGICAL ANALYSIS

The caudal peduncle of exposed *C. gariepinus* was cut with a sharp blade and the blood collected in a watch glass containing EDTA solution, an anticoagulant (6% Ethylene Diamine Tetra Acetic acid). Owing to insufficient amount of blood, the haematocrit determination for each experimental schedule was done on pooled samples in triplicate (from 2

fishes each) in sterile heparanized vials. Each triplicate was duplicated thus obtaining a total of six samples per treatment. Blood-filled heparanized microhaematocrit capillary tubes were centrifuged at 12000 for 5 minutes using a microhaematocrit centrifuge (Hermle model Z320) and the haematocrit (Hct) values were read directly. The haemoglobin concentration was measured by the cyanmethaemoglobin method (Blaxhall and Daisley, 1973) at a wavelength of 540nm. Concurrently, the Total Red Blood Cell (RBC) White Blood Cells (WBC) and Mean Corpuscular Volume (MCV) were obtained by employing a Coulter-model T540 cell Counter. The Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated using the methods described by Dacie and Lewis (1968). MCH was calculated in picograms/cell = (Hb/RBC) x 10 and MCHC = (Hb 100mg blood/Hct) x 100. The erythrocyte sedimentation rate (ESR), the rate at which erythrocytes settle out of unclotted blood in one hour, was determined by diluting blood (4:1) with anticoagulant (EDTA). The diluted blood was put in Wintrobe tube (100mm) and allowed to stand for one hour, at the end of which the distance from the miniscus to the top was recorded as the ESR. Similar procedures were adopted for the control (untreated) groups belonging to the respective treatment periods. Blood sampling was completed in less than 2 minutes; the entire auopsy procedure was performed in less than 4 minutes to minimize the risk of stressful condition.

2.11 DETERMINATION OF MUSCLE AND LIVER GLYCOGEN

A known weight of muscle and liver from the fish (approximately 0.15-2g) were dissected from the exposed fish and immediately transferred into a test tube containing 2ml of 30% potassium hydroxide. The tube was then heated in a water bath, while stirring gently for 20 minutes and cooled. A bout 0.5ml of saturated sodium sulphate solution and 3.5ml of 95% ethanol were carefully added and boiled for 5 minutes. The mixture was then cooled and centrifuged. The supernatant was discarded. The glycogen was then dissolved in 2ml of distilled water and reprecipitated with 2.5ml of 95%ethanol, 2ml of 5M hydrochloric acid was added and the mixture was cooled and neutralised with 0.5M sodium hydroxide, using one drop of phenol red as indicator. The mixture was then diluted to 100ml using distilled water. Out of the neutralised sample 5ml was then transferred into a test tube, 10ml of anthrone solution was carefully added and the absorbance read from a colorimeter at 620nm wavelength. Distilled water was used as blank and 5ml of glucose standard solution as standard. The milligram glycogen per 1g of liver or muscle was read from the calibrated standard curve.

2.12 DETERMINATION OF WATER QUALITY PARAMETERS.

Methods employed in the determination of the various water quality parameters were those described by APHA/AWWA/WPCF (1980).

2.13 HISTOPATHOLOGICAL EXAMINATION

The gills, gonads, liver and kidney of exposed *C. gariepinus* fingerlings to acute, sublethal and anaesthetic tests were dissected out of the fish. The organs were fixed in 10% normal saline solution. They were prepared for histological presentation using the routine histological methods and haematoxiline aesin staining techniques described by Drury and Wallington (1967) and modified by Bucke (1972). The automatic duplex processor, standon and Southern (Model: C. 35H) was used to process the organs. Graded alcohol (70%, 90%, absolute I, II, III) and graded chloroform (I, II, III) were used in dehydrating and cleaning respectively. The organs were infiltrated and embedded in molten paraffin wax. The Cambridge Rock Microtome (Model: M 64) was used to section the organs to the desired thickness (0.2mm) and finally transferred into a clear slide.

During the staining procedure, the sections were de-waxed in xylem and hydrated in graded alcohol (95%, 80%, 70% and 50%) respectively. The sections were stained in haematoxylin for 5 minutes, differential in 1% acid alcohol (hydrochloric acid and 70% alcohol), washed with tap water and counter-stained in 5% aqueous eosin for 5 minutes. The section was thoroughly washed and dehydrated in graded alcohol and chloroform and further de-parafined in xylene. The section was fully mounted in Canada Balsam and carefully covered with slide cover slip.

Photographs of the prepared sections were taken using a mounted photo-microscopic camera.

CHAPTER THREE

RESULTS

3.1 INGREDIENT AND PROXIMATE COMPOSITION OF DIET FED TO C*. GARIEPINUS*

The ingredients and proximate composition of diet fed to *C. gariepinus* is shown on Table 4. This table shows that the diet fed to the experimental fish had 44.5% crude protein, 25.5% nitrogen free extracts (NFE), low crude fibre content of 5.1 % while fat and ash contents were 8.6 and 16.4% respectively.

3.2 PHYTOCHEMICAL SCREENING OF *DATURA INNOXIA* LEAF, SEED, STEM, POD AND ROOT

The presence of clear zones after spraying with reagents inferred the presence of the various compounds as shown below.

3.2.1. Determination of Alkaloids

The production of deep orange zones immediately after spraying the chromatograms with Dragendorrff reagent (Rf value 0.11) indicated the presence of alkaloids in the leaf, seed, stem, pod and root of *D. innoxia* (Plate 7).

3.2.2 Determination of Atropine

The appearance of brown zones immediately after spraying the chromatograms with Dragendorrff reagent (Rf 0.19) indicated the presence of atropine in the leaf, seed, stem, pod and root of *D. innoxia* (Plate 8).

3.2.3 *Determination of Scopolamine*

The appearance of light orange zones immediately after spraying the chromatograms with Dragendorff reagent indicated the presence of scopolamine in the leaf, seed, stem, pod and root of *D. innoxia*.(Plate 9) .

3.2.4 *Determination of Saponins*

The production of dark brown zones in the Rf range of 0.2 – 0.37 after spraying the chromatograms with vanillin– sulphuric acid reagent indicated the presence of saponins in the leaf, seed, stem, pod and root of *D. innoxia*. (Plate 10).

3.2.5 *Determination of Flavonoids*

The presence of yellow brown zones in the Rf range of 0.6 – 0.75 after spraying the chromatograms with vanillin – sulphuric acid reagent indicated the presence of flavonoids in the leaf, seed, stem, pod and root of *D. innoxia*. (Plate 11).

3.2.6 *Determination of Essential Oils*

An intense blue light was produced when chromatogram was sprayed with vanillin – sulphuric acid reagent (Rf range 0.32 - 0.42) which indicated the presence of essential oils in the leaf, seed, stem, pod and root of *D. innoxia* (Plate12).

3.2.7 *Determination of Cardiac Glycosides*

The production of pink colour after spraying with Kedde reagent indicated the presence of cardiac glycosides in the leaf, seed, stem, pod and root of *D. innoxia*. (Plate 13). The colour faded after a few minutes but was regained by repeated spraying.

3.2.8 *Determination of Phenols*

There was no distinct colour observed when chromatograms were sprayed with fast blue salt reagent which indicated the absence of phenols in the leaf, seed, stem, pod and root of *D. innoxia*

3.2.9 *Determination of Coumarins*

There was no distinct colour observed when chromatograms were sprayed with potassium hydroxide reagent and inspected under UV – 365 nm light which indicated the absence of coumarins in the leaf, seed, stem, pod and root of *D. innoxia*.

3.2.10 *Determination of Carboxylic Acid*

There was no distinct colour observed when chromatograms were sprayed with fast blue salt reagent which indicated the absence of carboxylic acid in the leaf, seed, stem, pod and root of *D. innoxia*.

3.2.11 *Determination of Valepotriates*

There was no distinct colour observed when chromatograms were sprayed with Dinotrophenylhydrazine (DNPH) – acetic acid reagent which indicated the absence of valepotriates in the leaf, seed, stem, pod and root of *D. innoxia*.

3.3 COMPARATIVE MINERAL COMPOSTION OF *D. INNOXIA* IN LEAF, SEED, STEM, POD AND ROOT.

The result of the mineral composition of the five morphological parts of *D. innoxia* is presented in Table 5. All the ten minerals analysed were present in the leaf, seed, stem, pod and root except cadmium, which was not detectable in the leaf, pod and root. Mineral composition varied significantly ($P < 0.05$) with plant part e.g. the stem had the highest composition of calcium (11500ppm), sodium, (2400ppm) and phosphorus (4700ppm) while they were the least in the seed (460, 6300 and 32ppm respectively). The pod had the highest concentration of potassium (6100ppm) with the seed having the least potassium concentration of 6300ppm. Iron (630ppm) magnesium (1950ppm) chromium (1.283ppm) sulphur (0.1570ppm) and lead (4.38ppm) were found to be highest in the leaf, these metals were least in the seed except iron and lead which were found to be the least in root (38.38ppm) and stem (1.096ppm) respectively. Cadmium detected in the seed (0.025ppm) was found to be higher than that of the stem (0.018ppm). Statistical analysis showed that there was significant difference ($P < 0.05$) between the minerals of the leaf, seed, stem, pod and root except between magnesium of the seed and root, and calcium of the leaf, pod and root.

Table 5: Comparative Mineral Composition of *D. innoxia* Leaf, Seed, Stem, Pod and Root.

Mineral (ppm)	Plant Parts				
	Leaf	Seed	Stem	Pod	Root
Ca	10900	460	11500	1400	2600
Mg	1950	680	1700	920	680
K	37200	6300	37800	61000	37200
Na	1900	360	2400	680	1600
Fe	630	55.92	137.87	156.12	38.38
Pb	4.38	2.49	1.096	1.898	2.897
Cd	N.D.	0.125	0.018	N.D.	N.D.
Cr	1.283	0.156	0.448	0.654	0.296
P	4500	32.00	4700	3000	1800
S	0.157	0.022	0.154	0.124	0.099

ND: Not Detectable.

3.4 COMPARATIVE PROXIMATE COMPOSITION OF *D. INNOXIA* IN LEAF, SEED, STEM, POD AND ROOT

The comparative proximate composition of *D. innoxia* in leaf, seed, stem, pod and root is presented in Table 6. The crude protein of the leaf, stem and pod were found to be 17.21, 6.16 and 2.12% respectively. The moisture content in the plant also varied with the root having the lowest value (3.5%) while the stem had the highest value of 15%. The seed, pod and leaf had 10.00, 8.50 and 7.50% moisture content respectively. The crude lipid content followed the same pattern with the seed having the highest value of 15.52% and the root had the least value (6.00%.) The pod, stem and leaf had 9.00, 8.50 and 7.50% respectively. The value of crude fibre was highest in the root (31.72%) followed by the stem (29.66%), the pod, leaf and seed had 16.13, 8.95 and 6.55% respectively.

Table 6: Comparative Proximate Composition of *D. innoxia* in Leaf, Seed, Stem, Pod and Root

	Concentration (%DM)				
Component	Leaf	Seed	Stem	Pod	Root
Moisture	7.50	10.00	15.00	8.50	3.50
Total ash	16.59	8.26	19.80	16.28	25.71
Crude lipid	7.50	15.52	8.50	9.00	6.00
Crude fibre	8.95	6.55	29.66	16.13	31.72
Crude protein	17.21	13.90	6.16	2.12	2.09
Nitrogen free extract (NFE)	42.25	46.67	20.88	47.97	30.58

3.5 COMPARATIVE AMINO ACID COMPOSITION OF *D. INNOXIA* IN LEAF, SEED, STEM, POD AND ROOT

The comparative amino acid composition of *D innoxia* in leaf, seed, stem, pod and root is presented in Table 7.The five morphological parts of *D. innoxia* examined contain all amino acids essential for animal nutrition. The total amino acid was highest in the leaf (74.34%), followed by the seed (58.75%), stem (51.01%), pod (42.74%) and root (28.51%).

Table 7: Comparative Amino Acids of *D. innoxia* Leaf, Seed, Stem, Pod and Root.

Amino acid	Leaf	Seed	Stem (%)	Pod	Root
Histidine	2.07	0.73	1.06	1.08	0.4
Ammonia	1.54	3.47	4.47	2.39	1.68
Arginine	5.38	4.11	1.89	8.32	2.86
Aspartic	8.15	5.46	1.61	4.82	3.32
Serine	3.12	2.44	2.29	1.08	0.74
Glutamic	11.56	7.05	10.03	6.71	7.80
Proline	6.98	4.48	4.52	0.94	0.91
Glycine	3.70	3.13	2.74	1.14	0.52
Alanine	2.27	3.38	2.54	1.60	2.09
Cysteine	1.61	0.61	0.76	0.22	0.84
Tyrosine	3.56	1.44	2.01	1.54	1.24
Isoleucine	3.37	3.21	1.92	1.44	1.51
Leucine	1.54	2.75	1.87	2.37	1.27
Nor leucine	4.04	2.70	2.04	1.42	1.54
Phenylanine	4.2	4.32	2.70	1.20	0.31
Methionine	1.10	0.94	0.66	0.52	0.37
Threonine	2.82	1.64	2.07	2.11	0.74
Valine	7.33	6.89	5.73	3.84	0.65
Total Amino Acid	***74.34***	***58.75***	***51.01***	***42.74***	***28.51***

3.6 ACUTE TOXICITY OF *D. INNOXIA* LEAF EXTRACT TO *C. GARIEPINUS* FINGERLINGS

The results of the mortality rates of the fish *C. gariepinus* exposed to *D. innoxia* leaf extract are presented in Appendices 2a and 2b respectively. The mean mortality values are presented in Table 8. The logarithmic-probability curve of the mean mortality rates is presented in Figure 1. It was observed during the exposure that fish placed in media devoid of the extract survived the 96-hour exposure period. No mortality was observed in the group exposed to 140 mg/l within the first 24 hours of exposure. The 96-hour LC_{50} of *C. gariepinus* exposed to various concentrations of *D. innoxia* leaf extract was 120.23 mg/l with lower and upper confidence limits of 96.18 and 150.29 mg/l respectively.

The regression equation of the relationship was calculated to be Probit y = -4.302 + 4.463 log Conc. x and an R square value (r^2) of 0.7501. These expressions i.e. the regression equation r^2 value indicated that mortality rate of the test fish and concentrations of leaf extract are positively correlated. This shows that the mortality rate of the fish increased with increase in the concentrations of *D. innoxia* leaf extract.

During the exposure period, the test fish exhibited various behavioural patterns before death occurred. Restlessness, loss of balance (i.e. overturning), air gulping, and convulsion were frequently observed. However, these observations were minimal in the groups of fish exposed to 100 mg/l of the leaf extract. These observations were not noticed in the groups of fish exposed to the media devoid of *D. innoxia* leaf extract.

The opercular ventilation rate and tail beat frequencies per minute are not presented because all the test fish exhibited irregular opercular ventilation rate and tail beat frequency. This may not be unconnected with the presence of assessory organs, which enables it breathe in atmospheric oxygen other than dissolved oxygen.

During the exposure period of the acute toxicity test, it was observed that the mean values of the water quality parameters were not significantly different ($P > 0.05$) for temperature and pH. Eventhough dissolved oxygen, free carbondioxide and total alkalinity values differ significantly ($P < 0.05$) compared to the control values, they were still within acceptable limits (Mackereth, 1963). The result of the mean values is presented in Appendix 3.

Table 8: Mortality Rate* of *C. gariepinus* Fingerlings Exposed to Acute Concentrations of *D. innoxia* Leaf Extract

Concentrations (mg/1)	Log	Time(h) for 50% Mortality	Mean total mortality (%)	Mean Probit Value
200.00	2.30	36 (0.00)	90 (10.00)	6.28 (3.72)
180.00	2.25	36 (0.00)	80 (0.00)	5.84 (0.00)
160.00	2.20	48 (0.00)	55 (5.05)	5.13 (3.35)
140.00	2.15	54 (6.00)	50 (0.00)	5.00 (0.00)
120.00	2.08	90 (0.00)	50 (10.00)	5.00 (3.72)
100.00	2.00	-	25.(0.05)	4.32 (3.35)
0.00	0.00	-	-	-

***** Mean values with Standard Error in parentheses

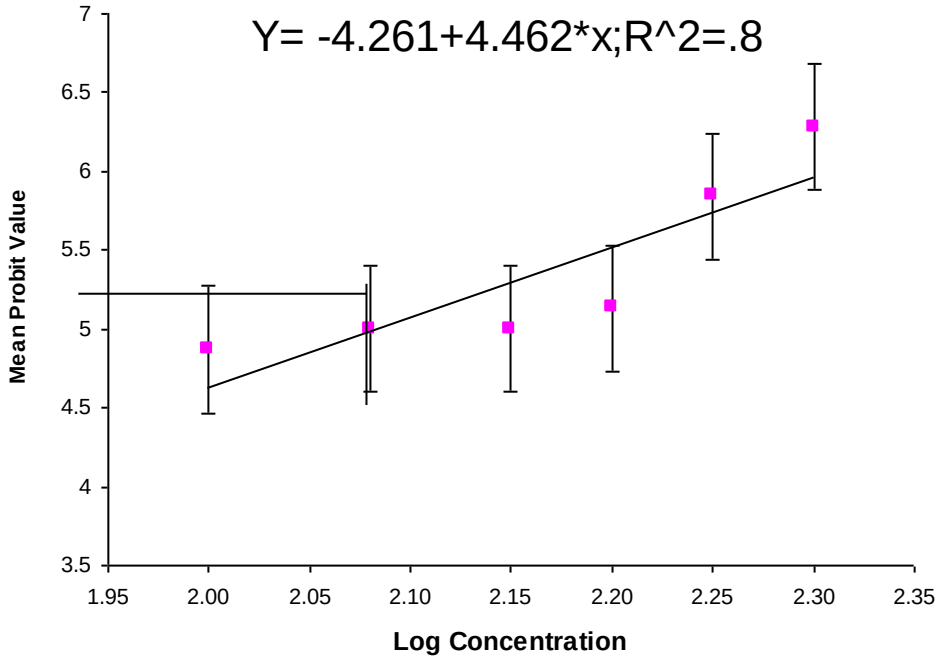

Fig.1 : Linear Relationship between Mean Probit Mortality and Log Concentration of *D. innoxia* Leaf Extract Exposed to *C gariepinus* for 96 Hours

3.7 ACUTE TOXICITY OF *D. INNOXIA* SEED EXTRACT TO *C. GARIEPINUS* FINGERLINGS

The results of the mortality rates of the fish *C. gariepinus* exposed to *D. innoxia* seed extracts are presented in Appendices 4a and 4b, respectively. The mean mortality values are presented in Table 9. The logarithmic- probability curves of the mean mortality rates is presented in Figure 2. It was observed during the exposure that fish placed in media devoid of the extract survived the 96-hour exposure period. No mortality was observed in the group exposed to 140 mg/l within the first 36 hours of exposure. The 96-hour LC_{50} of *C. gariepinus* exposed to various concentrations of *D. innoxia* seed extract was 138.04 mg/l with lower and upper confidence limits of 123.25 and 154.61 mg/l respectively.

The regression equation of the relationship was calculated to be Probit y = -12.012 + 7.948 log Conc. x and an R square value (r^2) of 0.9592. This expression i.e. the regression equation r^2 value indicated that mortality rate of the test fish and concentrations of seed extract are positively correlated. This shows that the mortality rate of the fish increased with increase in the concentrations of *D. innoxia seed* extract.

During the exposure period, the test fish exhibited various behavioural patterns before death occurred. Restlessness, loss of balance (i.e. overturning), air gulping, and convulsion were frequently observed. However, these observations were minimal in the groups of fish exposed to 100 mg/l of the seed extract. These observations were not noticed in the groups of fish exposed to the media devoid of *D. innoxia* seed extract.

The opercular ventilation rate and tail beat frequencies per minute are not presented because all the test fish exhibited irregular opercular ventilation rate and tail beat frequency. This may not be unconnected with the presence of assessory organs, which enables it breathe in atmospheric oxygen other than dissolved oxygen.

During the exposure period of the acute toxicity test, it was observed that the mean values of the water quality parameters were not significantly different ($P > 0.05$) for temperature and pH. Eventhough dissolved oxygen, free carbondioxide and total alkalinity values differ significantly ($P < 0.05$) compared to the control values, they were still within acceptable limits (Mackereth, 1963). The result of the mean values is presented in Appendix 5.

Table 9: Mortality Rate* of *C. gariepinus* Fingerlings Exposed to Acute Concentrations of *D. innoxia* Seed Extract

Concentrations (mg/1)	Log	Time(h) for 50% Mortality	Mean total mortality (%)	Mean Probit Value
200.00	2.30	36	70 (0.00)	5.52 (0.00)
180.00	2.25	48	65 (5.05)	5.39 (3.35)
160.00	2.20	60	55 (5.05)	5.13 (3.35).
140.00	2.15	72	50 (0.00)	5.00 (0.00)
120.00	2.08	96	40 (0.00)	4.72 (0.00)
100.00	2.00	-	20 (0.00)	4.16 (0.00)
0.00	-	-	-	-

***** Mean values with Standard Error in parentheses

3.8 ACUTE TOXICITY OF *D. INNOXIA* STEM EXTRACT TO *C. GARIEPINUS* FINGERLINGS

The results of the mortality rates of the fish *C. gariepinus* exposed to *D. innoxia* stem extract are presented in Appendices 6a and 6b respectively. The mean mortality values are presented in Table 10. The logarithmic-probability curves of the mean mortality rates is presented in Figure 3. It was observed during the exposure that fish placed in media devoid of the extract survived the 96-hour exposure period. No mortality was observed in the group exposed to 200 mg/l within the first 48 hours of exposure. The 96-hour LC_{50} of *C. gariepinus* exposed to various concentrations of *D. innoxia* stem extract was 199.53 mg/l with lower and upper confidence limits of 172.10 and 231.46 mg/l respectively.

The regression equation of the relationship was calculated to be Probit y = -12.348 + 7.536 log Conc. x and an R square value (r^2) of 0.6340. These expressions i.e. the regression equation r^2 value indicated that mortality rate of the test fish and concentrations of *D. innoxia* stem extract are positively correlated. This shows that the mortality rate of the fish increased with increase in the concentrations of *D. innoxia* stem extract.

During the exposure period, the test fish exhibited various behavioural responses before death occurred. Restlessness, losses of balance (i.e. overturning) air gulping, and convulsion was frequently observed. However, these observations were minimal in the groups of fish exposed to $150mgl^{-1}$ of the stem extract. These observations were not

noticed in the groups of fish exposed to the media devoid of *D. innoxia* stem extract.

During the exposure period of the acute toxicity test, it was observed that the mean values of the water quality parameters were not significantly different ($P > 0.05$) for temperature and pH. Eventhough dissolved oxygen, free carbondioxide and total alkalinity values differ significantly ($P < 0.05$) compared to the control values, they were still within acceptable limits (Mackereth, 1963). The result of the mean values is presented in appendix 7.

Table 10: Mortality Rate* of *C. gariepinus* Fingerlings Exposed to Acute Concentrations of *D. innoxia* Stem Extracts.

Concentrations (mg/1)	Log	Time(h) for 50% Mortality	Mean total mortality (%)	Mean Probit Value
400.00	2.60	12 (0.00)	85 (0.05)	6.04 (0.00)
350.00	2.54	24 (0.00)	80 (0.00)	5.84 (3.35)
300.00	2.48	42 (6.02)	70 (0.00)	5.52 (0.00)
250.00	2.40	66 (6.02)	55 (5.05)	5.13 (3.35)
200.00	2.30	-	45 (5.05)	4.87 (3.35)
150.00	2.18	-	40 (0.00)	4.75 (0.00)
0.00	-	-	-	-

***** Mean values with Standard Error in parentheses

3.9 ACUTE TOXICITY OF *D. INNOXIA* POD EXTRACT TO *C. GARIEPINUS* FINGERLINGS

The results of the mortality rates of the fish *C. gariepinus* exposed to *D. innoxia* pod extract are presented in Appendices 8a and 8b respectively. The mean mortality values are presented in Table 10b. The logarithmic- probability curves of the mean mortality rates is presented in Figure 4. It was observed during the exposure that fish placed in media devoid of the extract survived the 96-hour exposure period. No mortality was observed in the group exposed to 200 mg/l within the first 36 hours of exposure. The 96-hour LC_{50} of *C. gariepinus* exposed to various concentrations of *D. innoxia* pod extract was 181.97 mg/l with a lower and upper confidence limits of 156.87 and 211.08 mg/l respectively.

The regression equation of the relationship was calculated to be Probit y = 11.994 + 7.421 log. Conc. x and an R square value (r^2) of 0.7664. This expression i.e. the regression equation r^2 value indicated that mortality rate of the test fish and concentrations of *D. innoxia* pod extract are positively correlated. This shows that the mortality rate of the fish increased with increase in the concentrations of *D. innoxia* pod extract.

During the exposure period, the test fish exhibited various behavioural patterns before death occurred. Restlessness, loss of balance (i.e. overturning), air gulping, and convulsion were frequently observed. However, these observations were minimal in the groups of fish exposed to 150mg/l of the pod extract. These observations were not noticed in the groups of fish exposed to the media devoid of *D. innoxia* pod extract.

During the exposure period of the acute toxicity test, it was observed that the mean values of the water quality parameters were not significantly different ($P > 0.05$) for temperature and pH. Eventhough dissolved oxygen, free carbondioxide and total alkalinity values differ significantly ($P < 0.05$) compared to the control values, they were still within acceptable limits (Mackereth, 1963). The result of the mean values is presented in appendix 9.

Table 10b: Mortality Rate* of *C. gariepinus* Fingerlings Exposed to Acute Concentrations of *D. innoxia* Pod Extract

Concentrations (mg/l)	Log	Time(h) for 50% Mortality	Mean total mortality (%)	Mean Probit Value
400.00	2.60	12(0.00)	90(0.00)	6.28(0.00)
350.00	2.54	24(0.00)	85(5.05)	6.04(0.00)
300.00	2.48	24(0.00)	65(5.05)	5.52(0.00)
250.00	2.40	42(6.02)	60(0.00)	5.39(0.00)
200.00	2.30	60(0.00)	55(5.05)	5.13(3.35)
150.00	2.18	96(0.00)	40(0.00)	4.75(0.00)
0.00	-	-	-	-

Y= -11.994+7.421*X;R^2=.643

***** Mean values with Standard Error in parentheses

3.10 ACUTE TOXICITY OF *D. INNOXIA* ROOT EXTRACT TO *C. GARIEPINUS* FINGERLINGS.

The results of the mortality rates of the fish *C. gariepinus* exposed to *D. innoxia* root extract are presented in appendices 10a and 10b respectively. The mean mortality values are presented in Table 11. The logarithmic- probability curves of the mean mortality rates is presented in Figure 5. It was observed during the exposure that fish placed in media devoid of the extract survived the 96 - hour exposure period. No mortality was observed in the group exposed to 200mgl^{-1} within the first 60 hours of exposure. The 96 - hour LC_{50} of *C. gariepinus* exposed to various concentrations of *D. innoxia* root extract was 208.93 mg/l with a lower and upper confidence limits of 184.89 and 236.09 mg/l respectively.

The regression equation of the relationship was calculated to be Probit y = -14.939 + 8.567 log. Conc. x and an R square value (r^2) of 0.7171. This expression i.e. the regression equation r^2 value indicated that mortality rate of the test fish and concentrations of *D. innoxia* root extract are positively correlated. This shows that the mortality rate of the fish increased with increase in the concentrations of *D. innoxia* root extract.

During the exposure period, the test fish exhibited various behavioural patterns before death occurred. Restlessness, loss of balance (i.e. overturning) airs gulping, and convulsion was frequently observed. However, these observations were minimal in the groups of fish exposed to 200 and 150mgl^{-1} of the root extract. These observations were not noticed in the groups of fish exposed to the media devoid of *D. innoxia* root extract. The opercular ventilation rate and tail beat frequencies per minute

are not presented because all the test fish exhibited irregular opercular ventilation rate and tail beat frequency. This may not be unconnected with the presence of assessory organs, which enables it breathe in atmospheric oxygen other than dissolved oxygen.

During the exposure period of the acute toxicity test, it was observed that the mean values of the water quality parameters were not significantly different ($P > 0.05$) for temperature and pH. Eventhough dissolved oxygen, free carbondioxide and total alkalinity values differ significantly ($P < 0.05$) compared to the control values, they were still within acceptable limits (Mackereth, 1963). The result of the mean values is presented in appendix 11.

Table 11: Mortality Rate* of *C. gariepinus* Fingerlings Exposed to Acute Concentrations of *D. innoxia* Root Extract.

Concentrations (mg/1)	Log	Time(h) for 50% Mortality	Mean total mortality (%)	Mean Probit Value
400.00	2.60	12(0.00)	90(0.00	6.28(0.00)
350.00	2.54	30(6.100)	75(5.05)	5.68(3.35)
300.00	2.48	42(6.00)	60(5.05)	5.25(3.35)
250.00	2.40	54(6.00)	50(0.00)	5.22 (0.00)
200.00	2.30	-	45(5.05)	4.87(3.35)
150.00	2.18	-	20(0.00)	4.72 (0.00)
0.00	-	-	-	-

* Mean values with Standard Error in arentheses

3.11 EFFECTS OF SUBLETHAL CONCENTRATIONS OF *D. INNOXIA* LEAF EXTRACT ON GROWTH PARAMETERS OF *C. GARIEPINUS* FINGERLINGS.

Weight gain by the exposed fish to the various concentrations of *D. innoxia* leaf extract is presented in Appendix 12 and graphically represented as Fig. 6.

The highest concentration (100.00 mg/l) gave the least weight gain values (30.66%). Statistical analysis showed that the test fish exposed to the various sublethal concentrations of *D. innoxia* leaf extract had significantly ($P < 0.05$) lower weight gain compared to the group of fish placed in water devoid of the leaf extract.

Specific growth rate of *C. gariepinus* exposed to the various sublethal concentrations of *D. innoxia* leaf extract is presented in Appendix 13 and graphically in Fig. 7. The highest specific growth rate (0.63) was observed in the concentration devoid of *D. innoxia* leaf extract. While the highest concentration (100 mg/l) had the least specific growth rate value (0.32). Statistical analysis showed that the test fish exposed to the various sublethal concentrations of *D. innoxia* leaf extract had significantly ($P > 0.05$) lower specific growth rate compared to the group of fish placed in water devoid of the leaf extract.

During the exposure period of the acute toxicity test, it was observed that the mean values of the water quality parameters were not significantly different ($P < 0.05$) for temperature and pH. Eventhough, dissolved oxygen, free carbondioxide and total alkalinity values differ significantly ($P < 0.05$) compared to the control values, they were still within acceptable

limits (Mackereth, 1963). The result of the mean values is presented in Appendix 14.

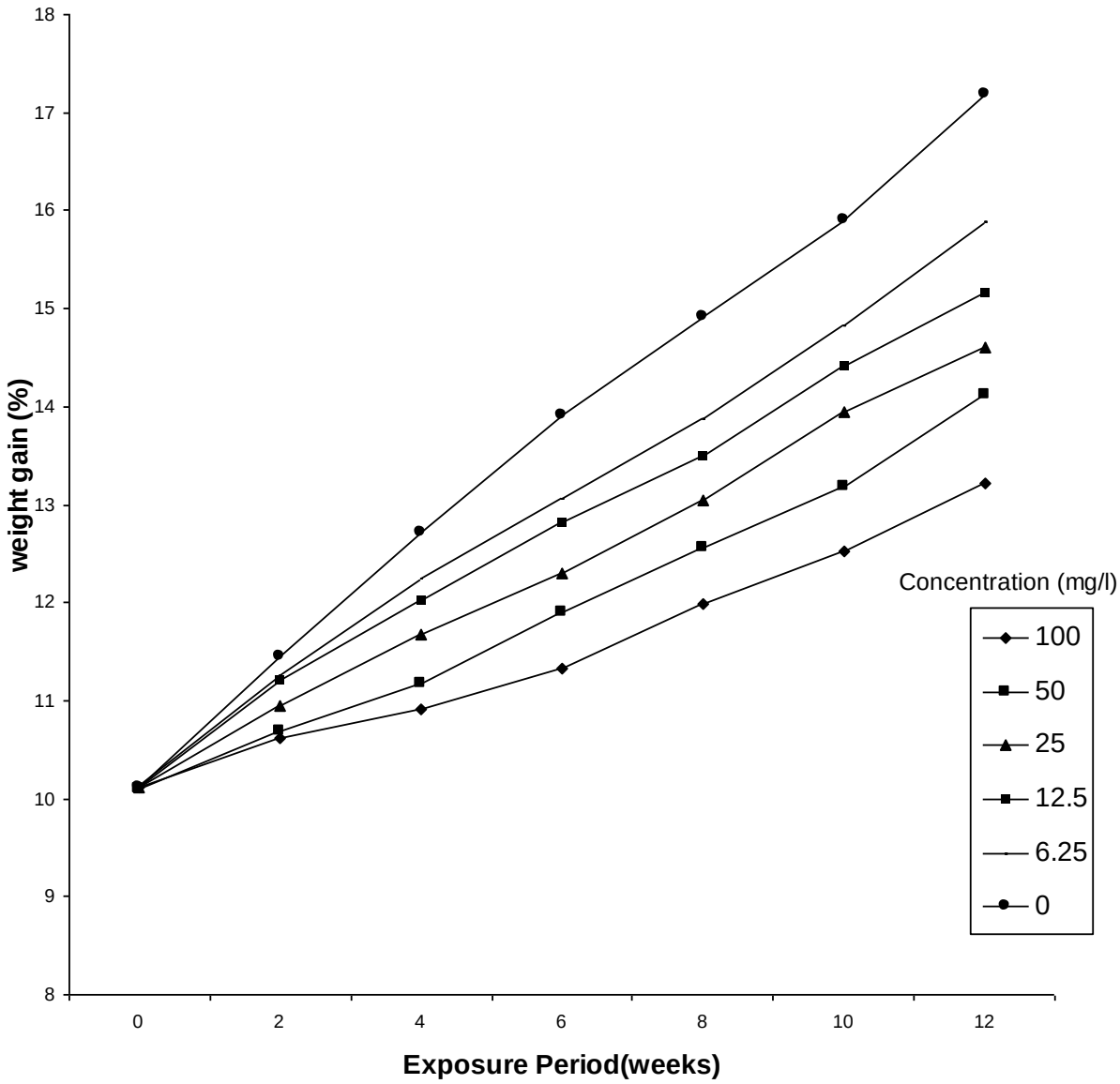

Fig. 6: Mean Weight Gain by *C. gariepinus* Fingerlings Exposed to Sublethal Concentrations of *D. innoxia* Leaf Extract for 12 Weeks.

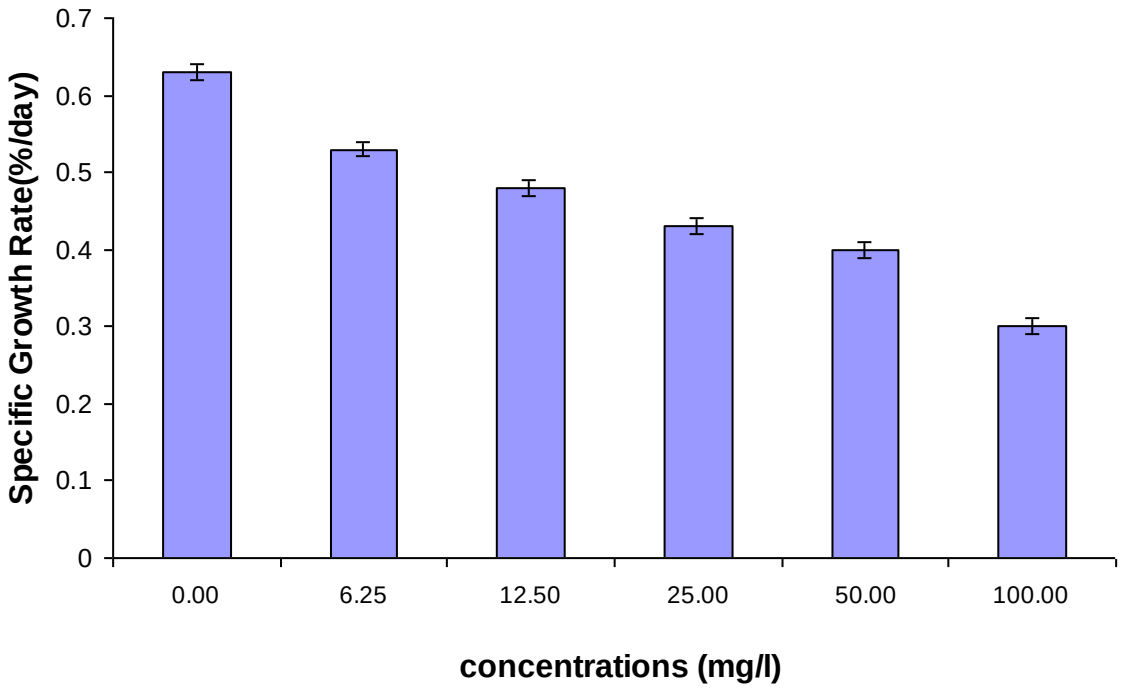

Fig 7: Mean Specific Growth Rate of *C. gariepinus* Fingerlings Exposed to Sublethal Concentrations of *D. innoxia* Leaf extract for 12 weeks

3.12 EFFECTS OF SUBLETHAL CONCENTRATIONS OF *D. INNOXIA* SEED EXTRACT ON GROWTH OF *C. GARIEPINUS* FINGERLINGS.

The weight gain by the exposed fish to the various concentrations of *D. innoxia* seed extract is presented in Appendix 15 and graphically represented as Fig. 8. It was observed that the highest concentration (100.00 mg/l) gave the least weight gain (30.62%). Statistical analysis showed that the test fish exposed to the various sublethal concentrations of *D. innoxia* seed extract had significantly ($P < 0.05$) lower weight gain compared to the group of fish placed in water devoid of the seed extract.

The specific growth rate of *C. gariepinus* exposed to the various sublethal concentrations of *D. innoxia* seed extract is presented in appendix 16 and graphically in figure 9. The highest specific growth rate (0.63) was observed in the concentration devoid of *D. innoxia* seed extract, while the highest concentration (100 mg/l) had the least specific growth rate (0.32). Statistical analysis showed that the test fish exposed to the various sublethal concentrations of *D. innoxia* seed extract had significantly ($P < 0.05$) lower specific growth rate compared to the group of fish placed in water devoid of the seed extract.

During the exposure period of the acute toxicity test, it was observed that the mean values of the water quality parameters were not significantly different ($P > 0.05$) for temperature and pH. Eventhough, dissolved oxygen, free carbondioxide and total alkalinity values differ significantly ($P < 0.05$) compared to the control values, they were still within acceptable limits (Mackereth, 1963). The result of the mean values is presented in Appendix 17.

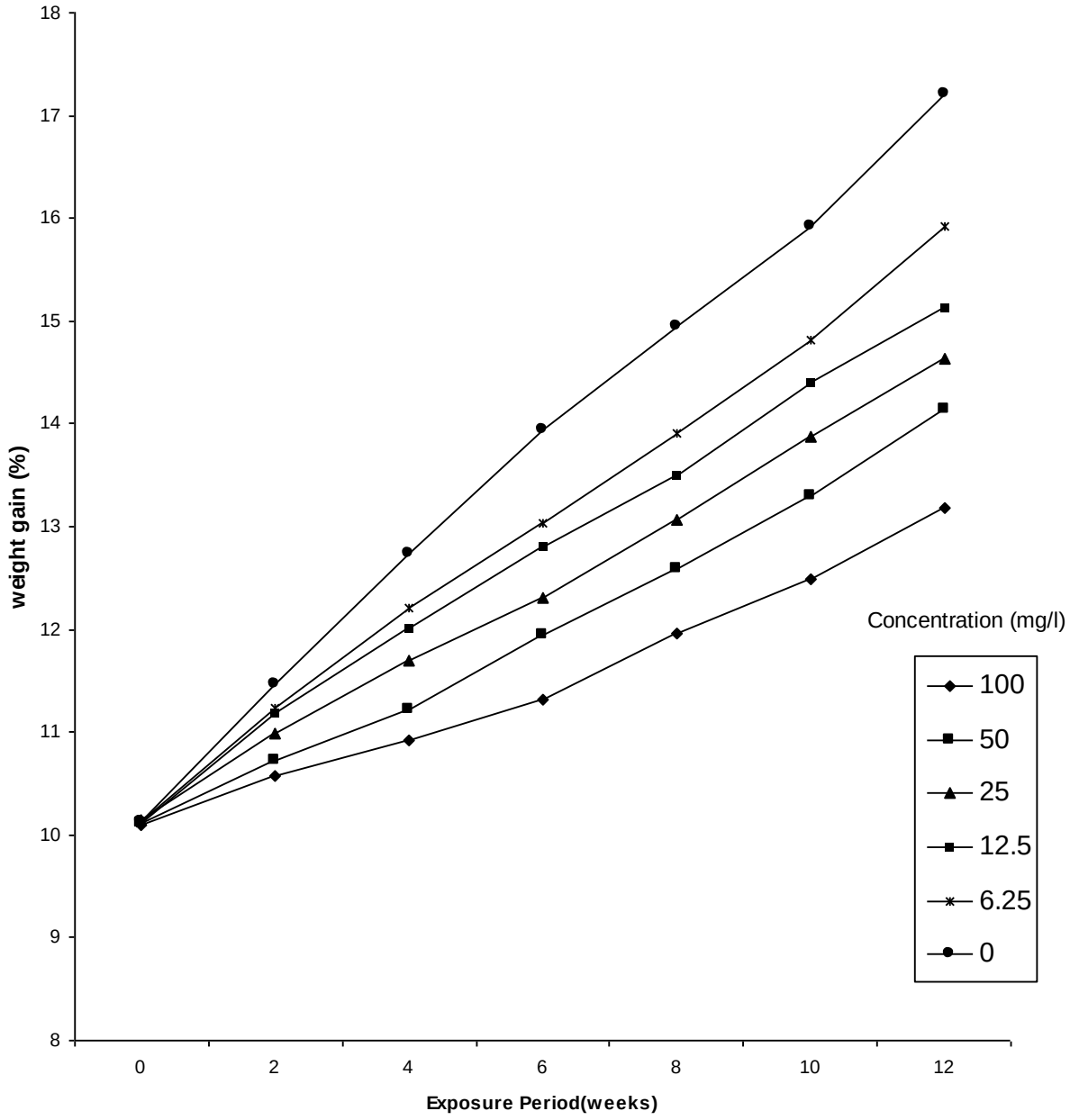

Fig 8 : Weight Gain by *C. gariepinus* Fingerlings Exposed to *D. innoxia* Seed Extract for 12 Weeks.

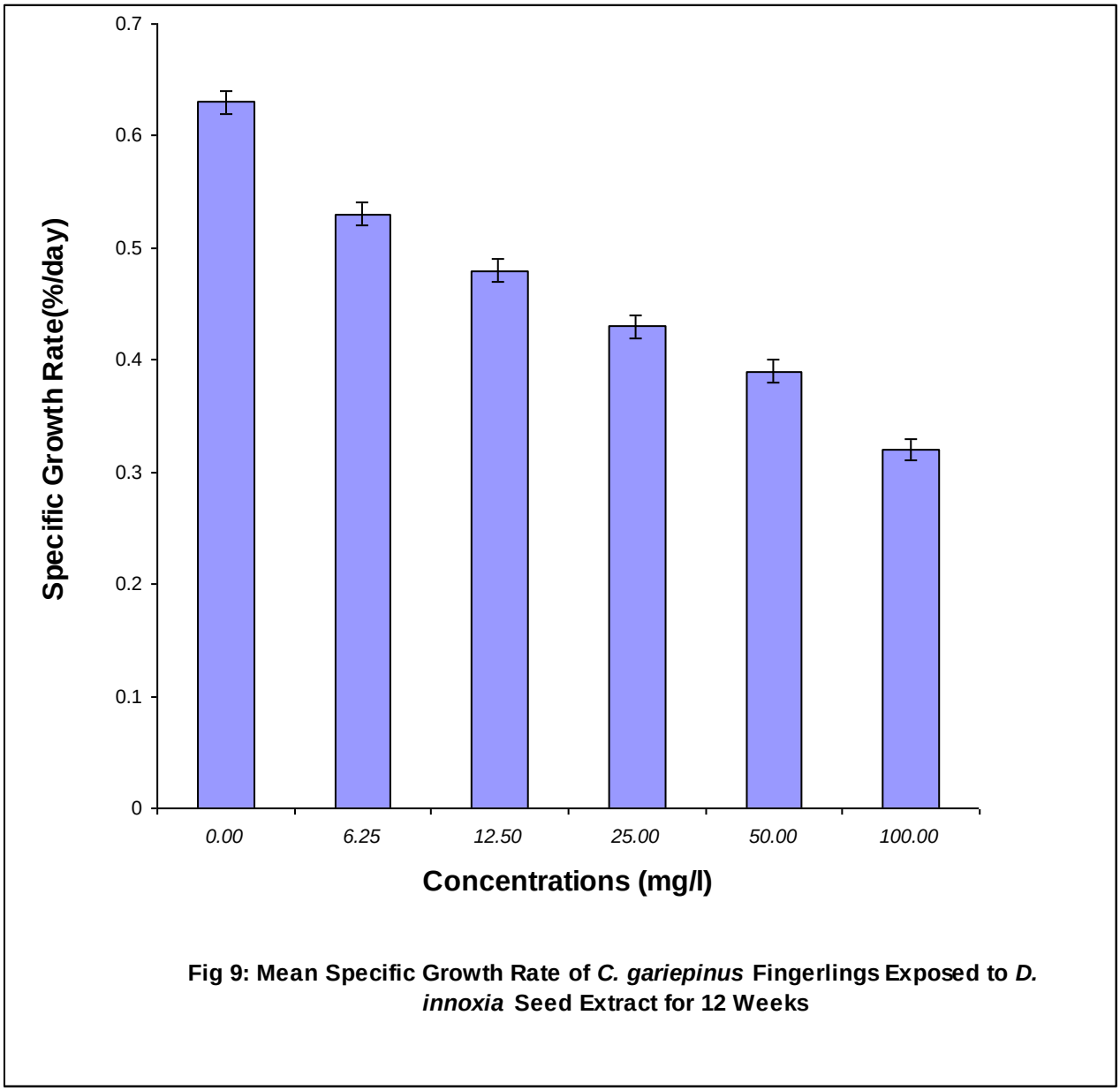

Fig 9: Mean Specific Growth Rate of *C. gariepinus* Fingerlings Exposed to *D. innoxia* Seed Extract for 12 Weeks

3.13 EFFECTS OF SUBLETHAL CONCENTRATIONS OF *D. INNOXIA* STEM EXTRACT ON GROWTH OF *C. GARIEPINUS* FINGERLINGS

The weight gain by the exposed fish to the various concentrations of *D. innoxia* stem extract is presented in Appendix 18 and graphically represented as Fig. 10. The highest concentration (100.00 mg/l) gave the least weight gain (30.66%). Statistical analysis showed that the test fish exposed to the various sublethal concentrations of *D. innoxia* stem extract had significantly ($P > 0.05$) lower weight gain compared to the group of fish placed in water devoid of the stem extract.

Specific growth rate of *C. gariepinus* exposed to the various sublethal concentrations of *D. innoxia* stem extract is presented in appendix 19 and graphically in Fig. 11. The highest specific growth rate (0.64) was observed in the concentration devoid of *D. innoxia* stem extract. While the highest concentration (100 mg/l) had the least specific growth rate (0.32) Statistical analysis showed that the test fish exposed to the various sublethal concentrations of *D. innoxia* stem extract had significantly ($P < 0.05$) lower specific growth rate compared to the group of fish placed in water devoid of the stem extract.

During the exposure period of the acute toxicity test, it was observed that the mean values of the water quality parameters were not significantly different ($P > 0.05$) for temperature and pH. Eventhough, dissolved oxygen, free carbondioxide and total alkalinity values differ significantly ($P < 0.05$) compared to the control values, they were still within acceptable limits (Mackereth, 1963). The result of the mean values is presented in Appendix 20

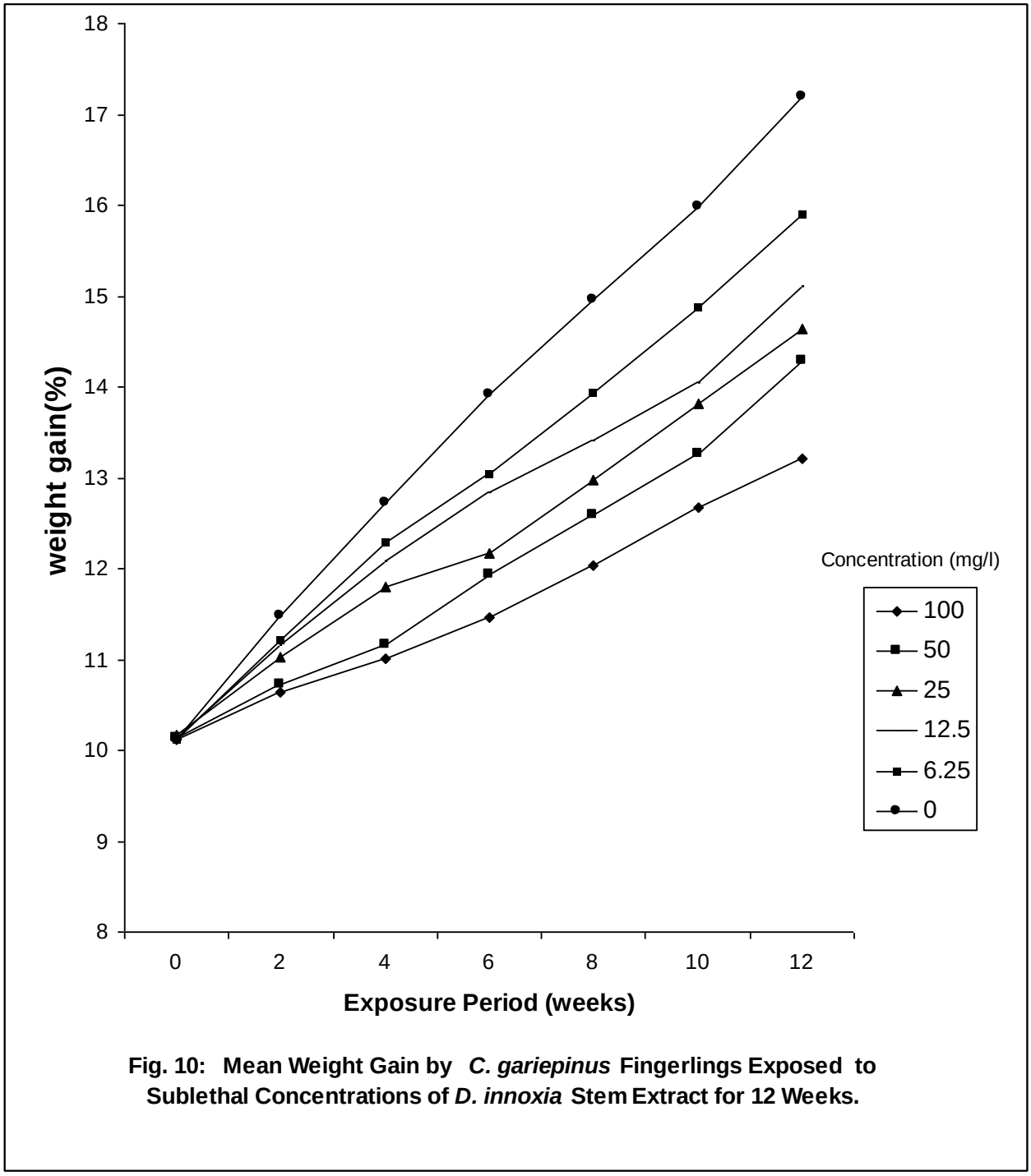

Fig. 10: Mean Weight Gain by *C. gariepinus* Fingerlings Exposed to Sublethal Concentrations of *D. innoxia* Stem Extract for 12 Weeks.

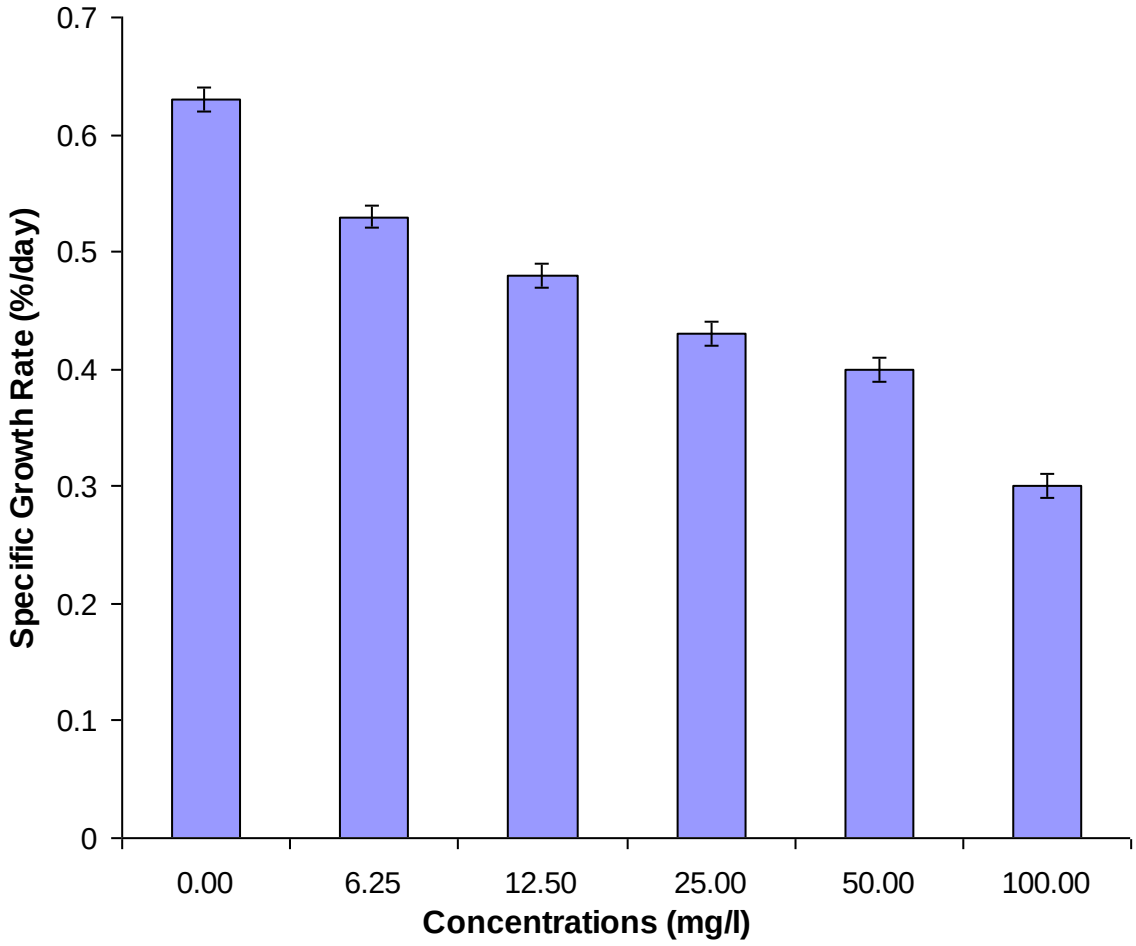

Fig 11: Mean Specific Growth Rate of *C. gariepinus* Fingerlings Exposed to Sublethal Concentrations of *D. innoxia* Stem Extract for 12 Weeks

3.14 EFFECTS OF SUBLETHAL CONCENTRATIONS OF *D. INNOXIA* POD EXTRACT ON GROWTH OF *C. GARIEPINUS* FINGERLINGS.

The weight gain by the exposed fish to the various concentrations of *Datura innoxia* pod extract is presented in Appendix 21 and graphically represented as Fig. 12 .The highest concentration (100.00 mg/l) gave the least weight gain (30.35%). Statistical analysis showed that the test fish exposed to the various sublethal concentrations of *D. innoxia* pod extract had significantly ($P < 0.05$) lower weight gain compared to the group of fish placed in water devoid of the pod extract.

Specific growth rate of *C. gariepinus* exposed to the various sublethal concentrations of *D. innoxia* pod extract is presented in Appendix 22 and graphically in Fig. 13. The highest specific growth rate (0.63) was observed in the concentration devoid of *D. innoxia* pod extract. While the highest concentration (100mg/l) had the least specific growth rate (0.31) Statistical analysis showed that the test fish exposed to the various sublethal concentrations of *D. innoxia* leaf extract had significantly ($P < 0.05$) lower specific growth rate compared to the group of fish placed in water devoid of the pod extract.

During the exposure period of the acute toxicity test, it was observed that the mean values of the water quality parameters were not significantly different ($P > 0.05$) for temperature and pH. Eventhough, dissolved oxygen, free carbondioxide and total alkalinity values differ significantly ($P < 0.05$) compared to the control values, they were still within acceptable limits (Mackereth, 1963). The result of the mean values is presented in Appendix 23.

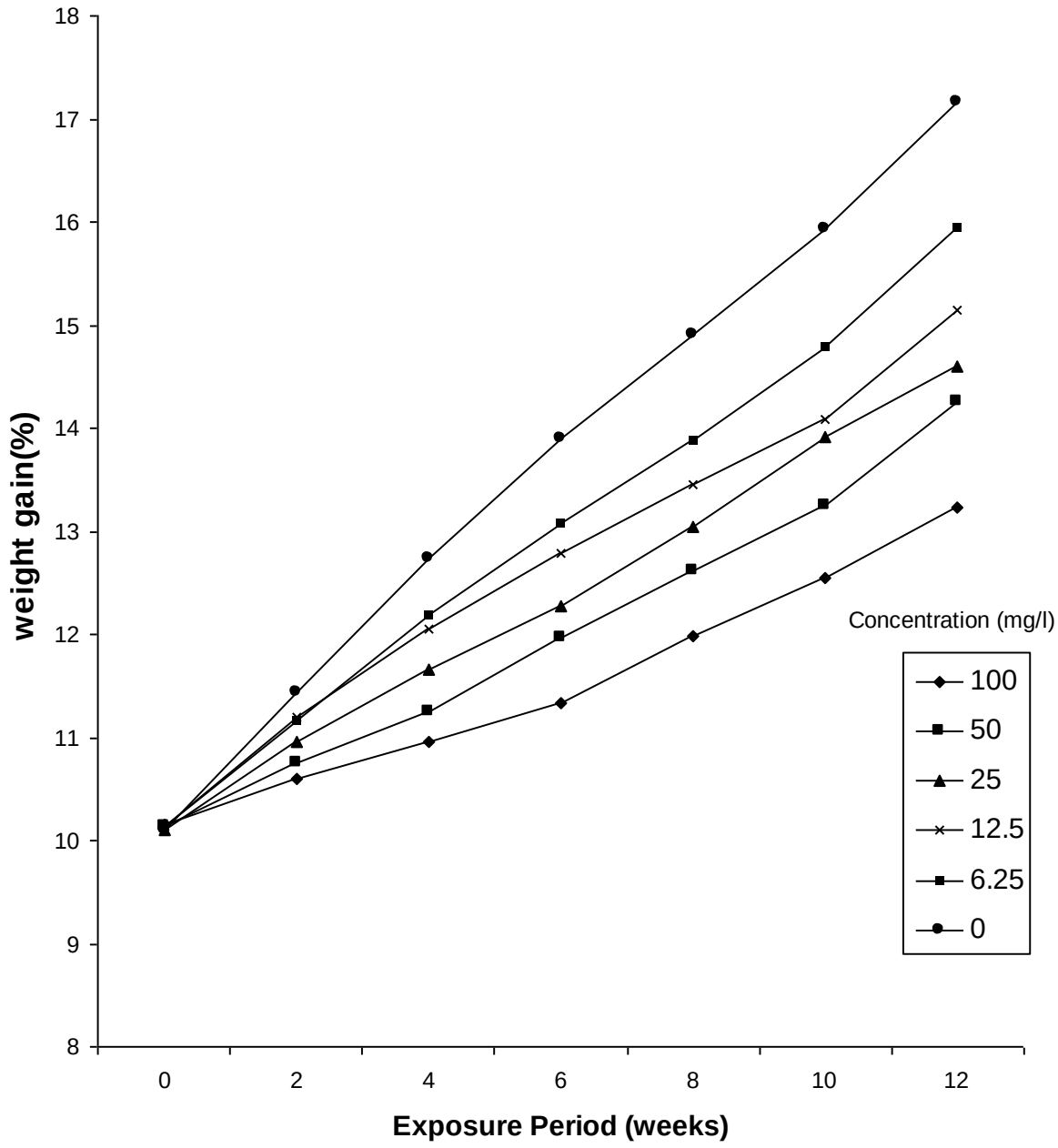

Fig 12 : Weight Gain by *C. gariepinus* Fingerlings Exposed to Sublethal Concentrations of *D. innoxia* Pod Extract for 12 Weeks

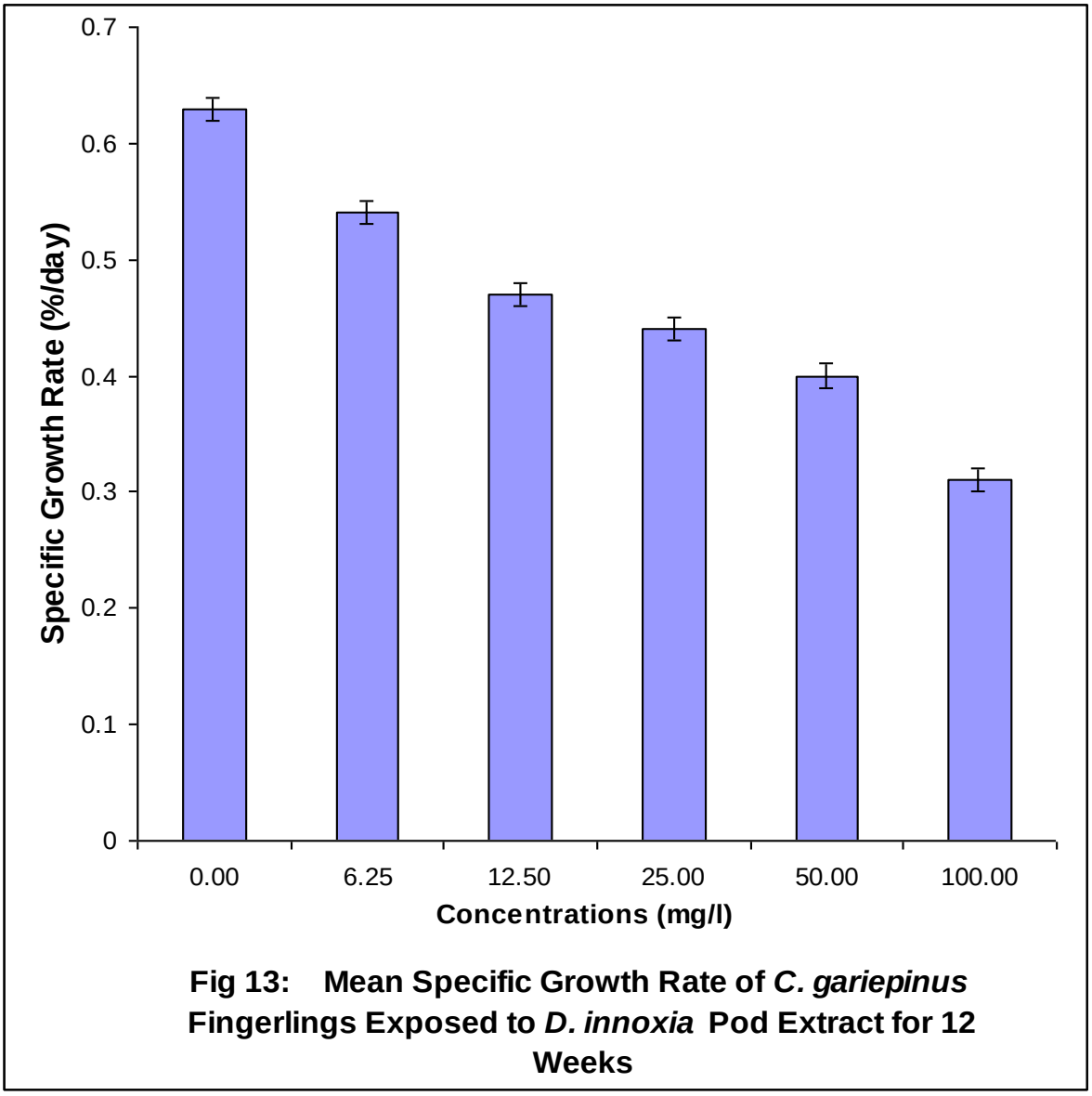

Fig 13: Mean Specific Growth Rate of *C. gariepinus* Fingerlings Exposed to *D. innoxia* Pod Extract for 12 Weeks

3.15 EFFECTS OF SUBLETHAL CONCENTRATIONS OF *D. INNOXIA* ROOT EXTRACT ON GROWTH OF *C. GARIEPINUS* FINGERLINGS

The weight gain by the exposed fish to the various concentrations of *D. innoxia* root extract is presented in Appendix 24 and graphically represented as Fig. 14. The highest concentration (100.00 mg/l) gave the least weight gain (31.42%). Statistical analysis showed that the test fish exposed to the various sublethal concentrations of *D. innoxia* root extract had significantly ($P < 0.05$) lower weight gain compared to the group of fish placed in water devoid of the root extract

Specific growth rate of *C. gariepinus* exposed to the various sublethal concentrations of *D. innoxia* root extract is presented in Appendix 25 and graphically in Fig. 15. The highest specific growth rate (0.62) was observed in the concentration devoid of *D. innoxia* root extract. While the highest concentration (100 mg/l) had the least specific growth rate (0.33). Statistical analysis showed that the test fish exposed to the various sublethal concentrations of *D. innoxia* root extract had significantly ($P < 0.05$) lower specific growth rate compared to the group of fish placed in water devoid of the root extract.

During the exposure period of the acute toxicity test, it was observed that the mean values of the water quality parameters were not significantly different ($P > 0.05$) for temperature and pH. Eventhough, dissolved oxygen, free carbondioxide and total alkalinity values differ significantly ($P < 0.05$) compared to the control values, they were still within acceptable limits (Mackereth, 1963). The result of the mean values is presented in Appendix 26.

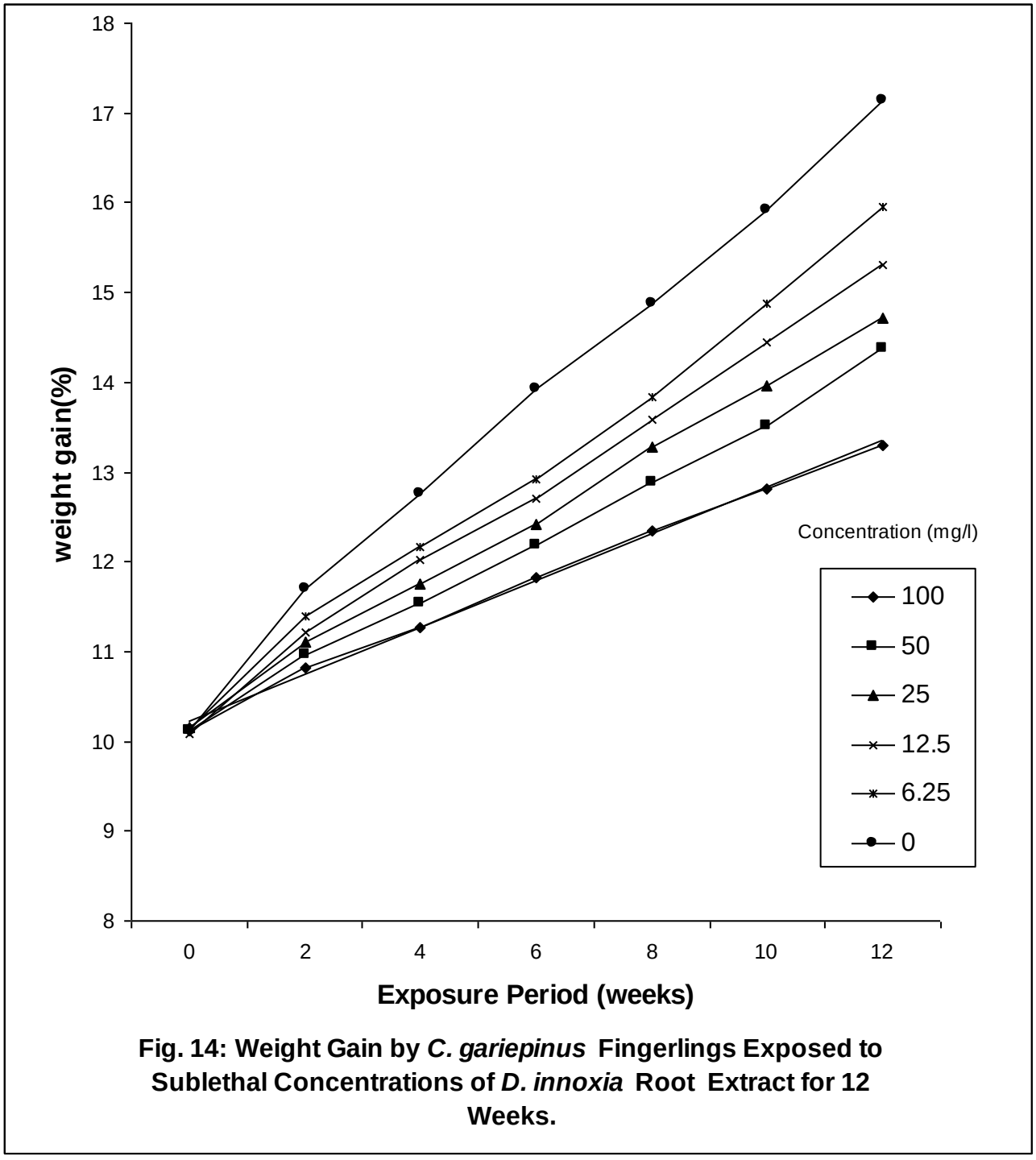

Fig. 14: Weight Gain by *C. gariepinus* Fingerlings Exposed to Sublethal Concentrations of *D. innoxia* Root Extract for 12 Weeks.

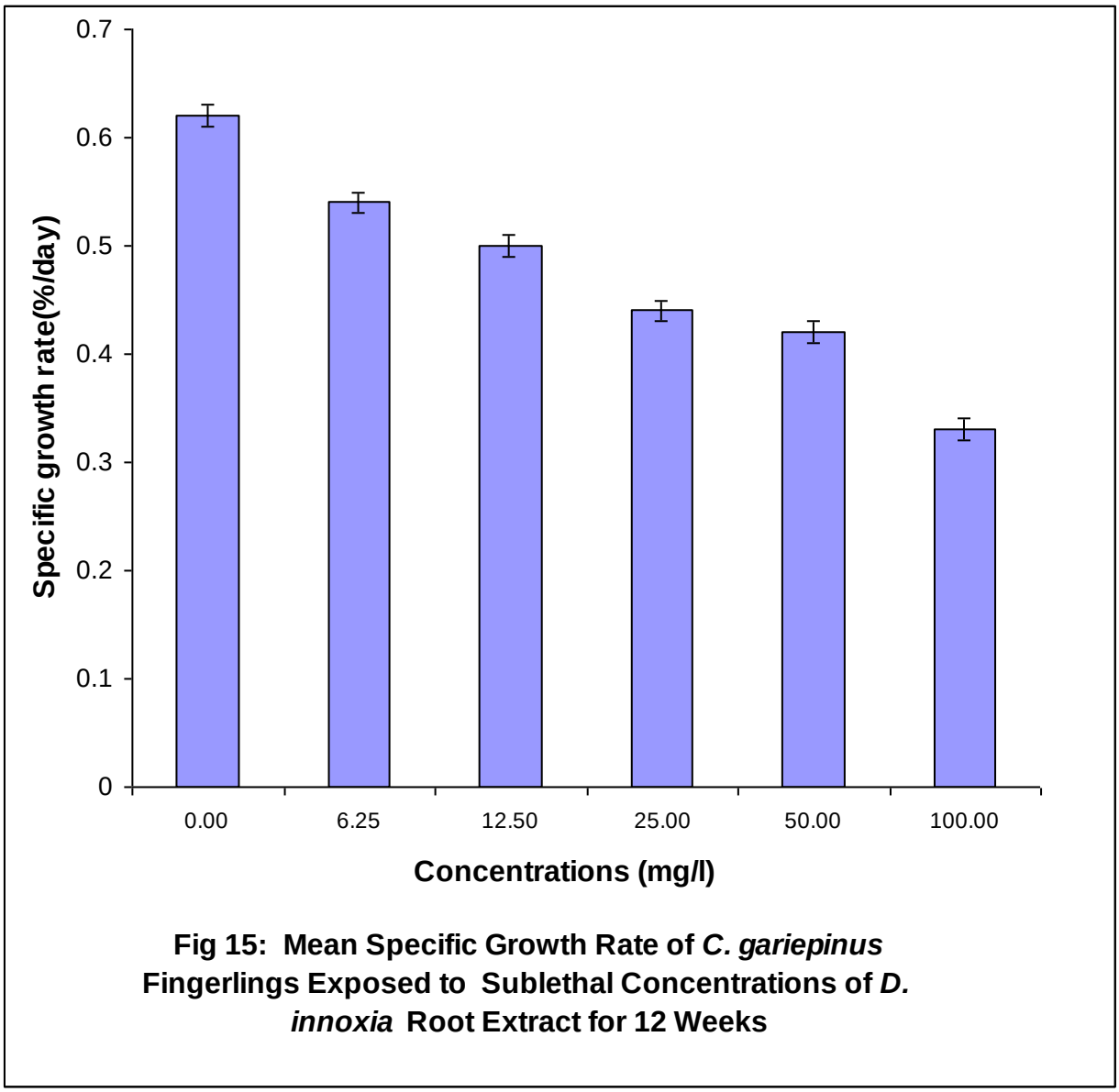

Fig 15: Mean Specific Growth Rate of *C. gariepinus* Fingerlings Exposed to Sublethal Concentrations of *D. innoxia* Root Extract for 12 Weeks

3.16 EFFECTS OF SUBLETHAL CONCENTRATIONS OF *D.INNOXIA* EXTRACT ON HAEMATOLOGICAL PARAMETERS OF *C.GARIEPINUS.*

The comparative haemoglobin concentration of *C. gariepinus* exposed to the various sublethal concentrations of *D. innoxia* extract is presented in Table 12. Haemoglobin concentration decreased with increase in *D. innoxia* root extract concentrations. Statistical analysis showed that the test fish exposed to the various sublethal concentrations of *D. innoxia* extracts had significantly ($P > 0.05$) lower haemoglobin concentration compared to the group of fish placed in water devoid of the *D. innoxia* extracts.

The comparative red blood cell count (RBC) of *C. gariepinus* exposed to the various sublethal concentrations of *D. innoxia* extract is presented in Table 13. RBC count decreased with increase in *D. innoxia* extract concentrations. Statistical analysis showed that the test fish exposed to the various sublethal concentrations of *D. innoxia* extracts had significantly ($P < 0.05$) lower RBC concentration compared to the group of fish placed in water devoid of the *D. innoxia* extracts

Table 14 shows the comparative mean corpuscular haemoglobin concentration (MCHC) of *C. gariepinus* exposed to the various sublethal concentrations of *D. innoxia* extracts. Mean corpuscular Haemoglobin concentration decreased with increase in *D. innoxia* extract concentrations.. Statistical analysis showed that the test fish exposed to the various sublethal concentrations of *D. innoxia* extracts had significantly ($P < 0.05$) lower haemoglobin concentration compared to the group of fish placed in water devoid of the *D. innoxia* extracts.

Table 12: Comparative Haemoglobin Changes* in *C. gariepinus* Fingerlings Exposed to Sublethal Concentrations of *D. innoxia* Extracts for 12 Weeks.

Concentration (mg/l)	Haemoglobin (mg/l)				
	Leaf	Seed	Stem	Pod	root
0.00	8.87 (0.63)	8.90 (0.55)	9.16 (0.51)	9.05 (0.91)	8.88 (1.06)
6.25	8.07 (0.72)	8.61 (0.80)	9.03 (0.55)	8.94 (0.88)	8.81 (0.07)
12.50	7.96 (0.86)	8.44 (0.75)	8.97 (0.80)	8.90 (0.63)	8.77 (0.83)
25.00	7.81 (0.75)	8.13 (0.62)	8.92 (0.75)	8.87 (0.43)	8.69 (0.80)
50.00	7.75 (0.89)	7.96 (0.83)	8.86 (0.63)	8.79 (0.41)	8.62 (0.75)
100.00	7.55 (0.86)	7.92 (0.51)	8.88 (0.70)	8.76 (0.85)	8.57 (0.83)

* Mean values with Standard Error in parentheses

Table 13: Comparative Red Blood Cell Count* (RBC) in *C. gariepinus* Fingerlings Exposed to Sublethal Concentrations of *D. innoxia* Extracts for 12 Weeks.

Concentration (mg/l)	RBC count (x $10^6 mm^{-3}$)				
	Leaf	Seed	Stem	Pod	root
0.00	2.25 (0.85)	2.17 (0.59)	2.36 (0.50)	2.35 (0.84)	2.28 (0.86)
6.25	2.20 (0.82)	2.10 (0.44)	2.31 (0.87)	2.29 (0.60)	2.23 (0.92)
12.50	2.18 (0.44)	2.04 (0.57)	2.25 (0.60)	2.22 (0.87)	2.16 (0.71)
25.00	2.11 (0.67)	1.96 (0.48)	2.20 (0.76)	2.16 (0.74)	2.07 (0.53)
50.00	2.06 (0.91)	1.91 (0.63)	2.13 (0.64)	2.11 (0.69)	2.01 (0.64)
100.00	1.98 (0.62)	1.84 (0.70)	2.08 (0.64)	2.03 (0.84)	1.93 (0.91)

* Mean values with Standard Error in parentheses

Table 14: Comparative Mean Corpuscular Haemoglobin Concentration (MCHC) (with Standard Error in Parentheses) in *C. gariepinus* Fingerlings Exposed to Sublethal Concentrations of *D. innoxia* Extracts for 12 Weeks.

Concentration (mg/l)	Mean Corpuscular Haemoglobin Concentration (g/e)				
	Leaf	Seed	Stem	Pod	Root
0.0	24.75	24.78	25.93	26.22	25.87
	(0.25)	(0.11)	(0.25)	(0.23)	(0.26)
6.25	23.66	24.43	26.51	26.15	26.05
	(0.26)	(0.34)	(0.25)	(0.12)	(0.22)
12.50	24.63	24.24	26.61	26.58	26.49
	(0.23)	(0.29)	(0.21)	(0.33)	(0.22)
25.00	24.76	24.08	27.51	27.10	26.38
	(0.24)	(0.28)	(0.23)	(0.19)	(0.26)
50.00	25.68	23.95	28.51	27.96	27.09
	(0.19)	(0.23)	(0.30)	(0.26)	(0.24)
100.00	25.83	25.34	29.34	28.44	28.44
	(0.23)	(0.31)	(0.22)	(0.29)	(0.19)

Table 15 shows the comparative packed cell volume of *C. gariepinus* exposed to the various sublethal concentrations of *D. innoxia* extract. As the concentrations of *D. innoxia* increased packed cell volume decreased. Statistical analysis showed that the test fish exposed to the various sublethal concentrations of *D. innoxia* extracts had significantly ($P < 0.05$) lower packed cell volume compared to the group of fish placed in water devoid of the *D. innoxia* extracts.

The comparative mean corpuscular volume of *C. gariepinus* exposed to the various sublethal concentrations of *D. innoxia* extract is presented in Table 16. Statistical analysis showed that the test fish exposed to the various sublethal concentrations of *D. innoxia* extracts had significantly ($P < 0.05$) lower mean corpuscular volume, which decreased with increase in *D. innoxia* extract concentrations, compared to the group of fish placed in water devoid of the *D. innoxia* extracts.

Table 15: Comparative Packed Cell Volume* (PCV) in *C. gariepinus* Fingerlings Exposed to Sublethal Concentrations of *D. innoxia* Extracts for 12 Weeks.

Concentration (mg/l)	PCV (%)				
	Leaf	Seed	Stem	Pod	Root
0.00	44.14 (0.11)	45.25 (0.21)	45.80 (0.17)	44.24 (0.13)	44.98 (0.16)
6.25	42.07 (0.09)	43.80 (0.13)	43.11 (0.12)	43.62 (0.11)	43.33 (0.17)
12.50	40.19 (0.16)	41.98 (0.18)	42.00 (0.16)	42.18 (0.10)	42.26 (0.10)
25.00	39.14 (0.19)	40.06 (0.16)	40.32 (0.15)	40.93 (0.12)	41.08 (0.19)
50.00	38.00 (0.12)	38.71 (0.18)	38.69 (0.16)	38.84 (0.13)	39.00 (0.22)
100.00	36.55 (0.10)	37.06 (0.18)	37.32 (0.19)	37.56 (0.14)	38.12 (0.16)

* Mean values with Standard Error in parentheses

Table 16: Comparative Mean Corpuscular Volume (MCV) (with Standard Error in Parentheses) in *C. gariepinus* Fingerlings Exposed to Sublethal Concentrations of *D. innoxia* Extracts for 12 Weeks.

Concentration (mg/l)	Mean Corpusular Volume (f/l)				
	Leaf	Seed	Stem	Pod	Root
0.00	196.18 (0.14)	208.53 (0.17)	191.02 (0.09)	188.26 (0.14)	197.11 (0.11)
6.25	191.23 (0.19)	208.57 (0.15)	186.62 (0.14)	190.48 (0.08)	194.30 (0.14)
12.50	184.36 (0.11)	205.78 (0.14)	186.67 (0.15)	190.00 (0.17)	195.65 (0.18)
25.00	185.50 (0.16)	204.39 (0.12)	183.27 (0.18)	189.49 (0.13)	198.45 (0.16)
50.00	184.47 (0.13)	202.67 (0.17)	181.64 (0.16)	184.08 (0.15)	194.03 (0.10)
100.00	184.60 (0.17)	201.41 (0.18)	179.42 (0.11)	185.02 (0.16)	197.51 (0.13)

The comparative erythrocyte sedimentation rate of *C. gariepinus* exposed to the various sublethal concentrations of *D. innoxia* extract is presented in Table 17. Mean erythrocyte sedimentation rate decreased with increase in *D. innoxia* extract concentrations. Statistical analysis showed that the test fish exposed to the various sublethal concentrations of *D. innoxia* extracts had significantly ($P < 0.05$) lower erythrocyte sedimentation rate compared to the group of fish placed in water devoid of the *D. innoxia* extracts.

The comparative mean white blood cell of *C. gariepinus* exposed to the various sublethal concentrations of *D. innoxia* extract is presented in Table 18. Mean white blood cell increased with increase in *D. innoxia* root extract concentrations. Statistical analysis showed that the test fish exposed to the various sublethal concentrations of *D. innoxia* extracts had significantly ($P < 0.05$) higher mean white blood cell compared to the group of fish placed in water devoid of the *D. innoxia* extracts.

The comperative mean liver glycogen, muscle glycogen and plasma glocuse reserves of *C. gariepinus* exposed to sublethel concentration of *D. innoxia* extracts are presented in Tables 21, 22 and 23 respectively. There was a reduction, in muscles and liver glycogen contents of the fish as the concentration of the extracts increased. While the plasma glucose increased with increase in *D. innoxia* concentration.

Table 17: Comparative Erythrocyte Sedimentation Rates* (ESR) in *C. gariepinus* Fingerlings Exposed to Sublethal Concentrations of *D. innoxia* Extracts for 12 Weeks

Concentration (mg/l)	ESR (mm/h)				
	Leaf	Seed	Stem	Pod	Root
0.00	2.20 (0.29)	2.60 (0.58)	2.40 (0.10)	2.17 (0.58)	2.35 (0.30)
6.25	2.20 (0.28)	2.70 (0.30)	2.40 (0.44)	2.19 (0.36)	2.40 (0.27)
12.50	2.70 (0.28)	2.80 (0.30)	2.49 (0.44)	2.20 (0.36)	2.44 (0.27)
25.00	2.95 (0.50)	2.80 (0.29)	2.53 (0.68)	2.25 (0.40)	2.45 (0.33)
50.00	3.05 (0.58)	2.95 (0.67)	2.58 (0.77)	2.28 (0.45)	2.52 (0.26)
100.00	3.12 (0.38)	3.09 (0.56)	2.65 (0.45)	2.33 (0.60)	2.63 (0.21)

***** Mean values with Standard Error in parentheses

Table 18: Comparative Mean White Blood Cell Count (with Standard Error in Parentheses) in *C. gariepinus* Fingerlings Exposed to Sublethal Concentrations of *D. innoxia* Extracts for 12 Weeks.

Concentration (mg/l)	WBC count (x $10^3 mm^{-3}$)				
	Leaf	Seed	Stem	Pod	Root
0.00	38.80 (0.56)	38.50 (0.75)	38.75 (0.40)	38.65 (0.86)	38.85 (0.70)
6.25	39.00 (0.83)	38.70 (0.54)	38.40 (0.84)	38.85 (0.54)	39.10 (0.63)
12.50	39.55 (0.45)	38.95 (0.89)	38.65 (0.63)	39.20 (0.70)	39.25 (0.41)
25.00	39.80 (0.50)	39.15 (0.82)	39.05 (0.41)	39.55 (0.32)	39.40 (0.32)
50.00	39.95 (0.82)	39.80 (0.90)	39.35 (0.96)	39.80 (0.63)	39.50 (0.61)
100.00	40.90 (0.89)	40.25 (0.30)	39.95 (0.30)	40.05 (0.98)	39.75 (0.86)

Table 19: Comparative Mean Liver Glycogen Reserve (with Standard Error in Parentheses) of *C. gariepinus* Fingerlings Exposed to Sublethel Concentrations of *D innoxia* Extracts

	Plant parts				
Concentration (g/l)	Leaf	Seed	Stem	Pod	Root
0.00	0.35	0.36	0.34	0.36	0.35
	(0.04)	(0.06)	(0.11)	(0.13)	(0.11)
6.25	0.37	0.37	0.35	0.36	0.35
	(0.08)	(0.05)	(0.16)	(0.08)	(0.13)
12.50	0.40	0.39	0.37	0.36	0.36
	(0.03)	(0.08)	(0.18)	(0.09)	(0.14)
25.00	0.41	0.40	0.38	0.37	0.37
	(0.02)	(0.09)	(0.11)	(0.16)	(0.11)
50.00	0.45	0.42	0.40	0.38	0.37
	(0.09)	(0.07)	(0.12)	(0.09)	(0.07)
100.00	0.48	0.43	0.41	0.38	0.37
	(0.10)	(0.14)	(0.16)	(0.13)	(0.09)

Table 20: Comparative Mean Muscle glycogen (with Standard Error in Parentheses) of *C. gariepunis* Fingerlings Exposed to Sublethel Concentrations of *D. innoxia* Extracts.

	Plant parts				
Concentration (g/l)	Leaf	Seed	Stem	Pod	Root
0.00	0.47	0.46	0.47	1.48	1.47
	(0.02)	(0.02)	(0.02)	(0.03)	(0.05)
6.25	1.40	1.42	1.44	1.47	1.47
	(0.05)	(0.06)	(0.07)	(0.08)	(0.03)
12.50	1.38	1.39	1.40	1.42	1.45
	(0.09)	(0.08)	(0.14)	(0.20)	(0.13)
25.00	1.36	1.36	1.37	1.39	1.41
	(0.04)	(0.13)	(0.10)	(0.16)	(0.09)
50.00	1.31	1.33	1.35	1.36	1.40
	(0.11)	(0.14)	(0.16)	(0.18)	(0.11)
100.00	1.24	1.28	1.30	1.32	1.38
	(0.12)	(0.13)	(0.13)	(0.11)	(0.14)

Table 21: Mean Plasma Glucose (with Standard Error in parentheses) of C. *gariepinus* Fingerlings Exposed to Sublethel Concentration of *D. innoxia* Extracts.

	Plant parts				
Concentration (g/l)	Leaf	Seed	Stem	Pod	Root
0.00	1.60	1.59	1.60	1.60	1.60
	(0.16)	(0.10)	(0.11)	(0.11)	(0.13)
6.25	1.58	1.58	1.59	1.60	1.60
	(0.11)	(0.14)	(0.17)	(0.09)	(0.05)
12.50	1.55	1.56	1.59	1.59	1.60
	(0.19)	(0.1)	(0.16)	(0.13)	(0.14)
25.00	1.54	1.55	1.57	1.59	1.58
	(0.11)	(0.08)	(0.09)	(0.13)	(0.12)
50.00	1.52	1.53	1.55	1.57	1.57
	(0.10)	(0.10)	(0.08)	(0.20)	(0.19)
100.00	1.50	1.51	1.53	1.56	1.57
	(0.12)	(0.09)	(0.11)	(0.18)	(0.16)

3.17 ADMINISTRATION OF *D. INNOXIA* EXTRACTS AS ANESTHESIA TO *C. GARIEPINUS* FINGERLINGS

Clarias gariepinus fingerlings exposed to various extracts of *D. innoxia* passed sequentially through the various stages of anaesthesia. The behavioural responses were excess mucus secretion and apathy, air gulping, distension of the mouth and opercula, erratic swimming and loss of balance. Table 22 shows that the crude extract of *D. innoxia* leaf was able to induce fish to loose reactivity to stimuli at concentration 2.50g/l within 40.33 (2.63) minutes, while at concentration 3.00g/l. The same effect was observed in 28.2 (2.06) minutes. No such observations were made at concentrations less than 2.50 (i.e. 2.00, 1.50, 1.00, 0.50 and 0.00 g/l). This extract (crude) at 3.00g/l concentration was able to induce loss of equilibrium within 54.34 (2.85) minutes. The fingerlings were able to recover within five minutes of immersion into fresh aerated water.

Similar results were obtained from fish exposed to unseparated chloroform-methanol extract and lipid fraction of chloroform-methanol extract though the time taken to reach anaesthesia in the alcoholic extract was significantly ($P < 0.05$) shorter. To induce fish to a total loss of equilibrium, a dosage of 0.50 g/l was required within 1.16 (0.63) minutes. However, at this dosage, a relatively longer time (1.62 minutes) was needed to induce the minimal opercula movement. To induce fish to minimal opercula movement needed for surgical anaesthetic state within 30 seconds, a higher dosage of 3.00 g/l is required. Recovery time increased with increase in concentration of the extract (Table 22). The non-lipid fraction was able to induce loss of reactivity in *C. gariepinus*

within 48.78 and 35.96 minutes at 2.50 and 3.00 g/l respectively. The control had no observable anaesthetic effect on the fish which swam actively and behaved normally without reaching any stage or anaesthesia during the period of observation which lasted 180 minutes.

The time required for *C. gariepinus* to succumb to the anaesthetic effects of the seed, stem, pod and root is presented in tables 23, 24, 25 and 26 respectively. Induction time ranged from 1.04 (0.38) in the seed to 3.60 (1.08) minutes in the root for fish to loose reactivity to stimuli in unseparated chloroform-methanol and lipid fraction of chloroform-methanol extracts respectively. While loss of equilibrium time varied from 1.16 (0.95) in seed to 8.89 (1.13) minutes in the root respectively. To induce fish to loose total equilibrium it took 1.27 (0.46) minutes for the seed, 10.25 (1.09) minutes for the stem, 8.03 (0.47) minutes for the pod and 12.00 (1.25) minutes for the root at concentration 0.50 g/l of the unseperated chloroform-methanol extract.

The unseparated and lipid fractions of chloroform-methanol extracts induced effective and minimal opercula movements of *C. gariepinus* fingerlings at 0.59 and 0.68 minutes for the seed, 12.48 and 13.75 minutes respectively in the stem, 11.66 and 13.28 minutes respectively in the pod and 15.92 and 16.07 minutes respectively in the root at concentration 3.00 g/l. Fish soon recovered from anaesthesia on immersion into recovery tank, without adverse response such as darting, jumping, twitching and cramp-like convulsions observed during sedation.

The crude extract of the seed was only able to induce loss of reactivity to stimuli at concentration 3.00 after 60 minutes of exposure. The crude extract of the stem, pod and root, their respective non-lipid fractions as well as the control had no observable sedative effects throughout the period of exposure (180 minutes). No mortality was observed in any of the experimental aquaria throughout the exposure period and after the exposure time (21 days).

Haematological values with standard errors obtained for *C. gariepinus* fingerlings exposed to *D. innoxia* sedations are presented in Tables 27-33. The mean comparative haemoglobin concentration (Table 27), Red blood cell count (Table 28) and white blood cell count (Table 29) showed no significant difference ($P > 0.05$) between treatment and within treatment for all the extracts (crude, unseparated chloroform-methanol, lipid fraction and non-lipid fractions of the chloroform-methanol). Similarly, comparative mean Erythocyte sedimention rates (Table 30), Packed cell volume (Table 31), Mean corpusalar Haemoglobin concentration (Table 32), Mean compuscular Volume (Table 33) and Mean Corpusalar haemoglobin (Table 34) had no significant difference ($P < 0.05$) between the control groups and the various concentrations of the crude, unseparated chloroform-methanol, lipid and non-lipid fractions of the chloroform-methanol extracts of the leaf, seed, stem, pod and root extracts. The comperative mean liver glycogen, muscle glycogen and plasma glocuse reserves of *C. gariepinus* exposed to sadetion of *D. innoxia* extracts are presented in Tables 35, 36 and 37 respectively. There

was no significant difference ($P > 0.05$) between the muscle and liver glycogen and plasma glucose contents of the fish for the various concentrations and their respective controls.

Table 22: Behavioural Responses* of *C. gariepinus* Fingerlings Exposed to various Concentrations of *D. innoxia* Leaf Extracts.

	Concentration (g/l)	Behavioural Responses (minutes)					
		Loss of Reactivity To stimuli	Loss of equilibrium	Total loss of equilibrium	Reduced opercular movement	Minimal opercular movement	Recovery of equilibrium
Crude extract	0.5	-	-	-	-	-	-
	1.00	-	-	-	-	-	-
	1.50	-	-	-	-	-	-
	2.00	-	-	-	-	-	-
	2.50	40.33 (2.63)	-	-	-	-	2.67 (0.47)
	3.00	28.21 (2.06)	54.34 (2.55)	-	-	-	4.20 (0.47)
Unseperqated chloroform-methanol extract	0.50	0.90 (0.55)	1.07 (0.47)	1.16 (0.63)	1.28 (0.34)	1.62 (0.44)	5.92 (0.77)
	1.00	0.77 (0.68)	0.93 (0.38)	1.10 (0.82)	1.39 (0.66)	1.51 (0.29)	8.22 (1.01)
	1.50	0.62 (0.85)	0.71 (0.48)	0.93 (0.49)	1.12 (0.90)	1.40 (0.63)	10.80 (0.96)
	2.00	0.44 (0.69)	0.59 (0.26)	0.77 (0.53)	1.03 (0.67)	1.28 (0.81)	14.43 (1.18)
	2.50	0.28 (0.70)	0.38 (0.30)	0.46 (0.39)	0.61 (0.38)	0.76 (0.39)	20.67 (1.13)
	3.00	0.19 (0.63)	0.24 (0.39)	0.29 (0.73)	0.37 (0.61)	0.47 (0.42)	23.00 (1.66)
Lipid fraction of chloroform-methanol extract	0.50	0.98 (0.52)	1.18 (0.44)	1.53 (0.41)	1.88 (0.81)	2.43 (1.44)	4.60 (1.20)
	1.00	0.79 (0.61)	1.03 (0.31)	1.24 (0.33)	1.62 (0.74)	2.11 (0.39)	6.11 (0.81)
	1.50	0.68 (0.80)	0.75 (0.44)	0.99 (0.71)	1.49 (0.28)	1.89 (0.66)	9.50 (0.88)
	2.00	0.46 (0.71)	0.66 (0.24)	0.89 (0.92)	0.34 (0.38)	1.46 (0.28)	12.14 (0.46)
	2.50	0.31 (0.77)	0.41 (0.31)	0.55 (0.81)	0.70 (0.28)	0.83 (0.48)	18.00 (0.28)
	3.00	0.23 (0.66)	0.30 (0.33)	0.36 (0.69)	0.48 (0.66)	0.66 (0.33)	21.11 (0.56)
Non- lipid fraction of chloroform-methanol extract	0.50	-	-	-	-	-	-
	1.00	-	-	-	-	-	-
	1.50	-	-	-	-	-	-
	2.00	-	-	-	-	-	-
	2.50	48.78	-	-	-	-	1.55 (1.01)
	3.00	35.96	-	-	-	-	3.50 (1.22)

* Mean values with Standard Error in parentheses

Table 23: Behavioural Responses* of *C. gariepinus* Fingerlings Exposed to various Concentrations of *D. innoxia* Seed Extracts.

	Conceration (g/l)	Behavoural Responses (minutes)					
		Loss of Reactivity to stimuli	Loss of equilibrium	Total loss of equilibrium	Reduced opercular movement	Minimal opercular movement	Recovery of equilibrium
Crude extract	0.5	-	-	-	-	-	-
	1.00	-	-	-	-	-	-
	1.50	-	-	-	-	-	-
	2.00	-	-	-	-	-	-
	2.50	-	-	-	-	-	-
	3.00	58.50 (0.95)	-	-	-	-	1.50 (0.71)
Unseperqated chloroform-methanol extract	0.50	1.04 (0.38)	1.16 (0.95)	1.27 (0.46)	1.39 (0.52)	1.69 (0.73	5.11 (1.18)
	1.00	0.89 (0.09)	1.04 (0.39)	1.15 (0.51)	1.25 (0.29)	1.45 (0.79)	6.13 (1.09)
	1.50	0.72 (0.31)	0.88 (0.50)	0.98 (0.38)	1.19 (0.36)	1.42 (0.48)	11.65 (2.02)
	2.00	0.54 (0.37)	0.70 (0.11)	0.79 (0.15)	0.82 (0.44)	1.19 (0.60)	15.50 (2.11)
	2.50	0.36 (0.06)	0.51 (0.29)	0.60 (0.12)	0.70 (0.19)	0.88 (0.24)	19.15 (1.68)
	3.00	0.25 (0.09)	0.39 (0.22)	0.44 (0.09)	0.51 (0.14)	0.59 (0.21)	24.03 (1.91)
Lipid fraction of chloroform-methanol extract	0.50	1.11 (0.44)	1.23 (0.73)	1.54 (0.66)	1.90 (0.81)	2.48 (1.01)	5.09 (1.37)
	1.00	0.95 (0.26)	1.07 (0.86)	1.29 (0.41)	1.68 (0.74)	2.16 (0.99)	7.03 (1.22)
	1.50	0.82 (0.19)	0.91 (0.27)	1.07 (0.70)	1.51 (0.69)	1.94 (0.46)	12.10 (1.38)
	2.00	0.58 (0.11)	0.62 (0.44)	0.94 (0.11)	1.36 (0.72)	1.55 (0.29)	16.12 (0.98)
	2.50	0.42 (0.26)	0.49 (0.29)	0.58 (0.09)	0.81 (0.33)	0.93 (0.11)	20.00 (1.11)
	3.00	0.35 (0.38)	0.40 (0.18)	0.46 (0.08)	0.55 (0.18)	0.68 (0.14)	22.66 (1.02)
Non lipid fraction of chloroform-methanol extract	0.50	-	-	-	-	-	-
	1.00	-	-	-	-	-	-
	1.50	-	-	-	-	-	-
	2.00	-	-	-	-	-	-
	2.50	-	-	-	-	-	-
	3.00	52.35 (3.94)	-	-	-	-	2.66 (3.42)

* Mean values with Standard Error in parentheses

Table 24: Behavioural Responses* of *C. gariepinus* Fingerlings Exposed to various Concentrations of *D. innoxia* Stem Extract.

	Concentration (g/l)	Behavoural Responses (minutes)					
		Loss of Reactivity To stimuli	Loss of equilibrium	Total loss of equilibrium	Reduced opercular movement	Minimal opercular movement	Recovery of equilibrium
Crude extract	0.5	-	-	-	-	-	-
	1.00	-	-	-	-	-	-
	1.50	-	-	-	-	-	-
	2.00	-	-	-	-	-	-
	2.50	-	-	-	-	-	-
	3.00	-	-	-	-	-	-
Unseperqated methanol chloroform-methanol extract	0.50	2.55 (0.67)	7.99 (0.91)	10.25 (1.09)	15.88 (1.44)	18.26 (2.13)	3.84 (1.00)
	1.00	2.39 (0.91)	7.63 (0.72)	9.74 (1.25)	14.19 (2.03)	15.93 (2.29)	4.11 (1.19)
	1.50	2.26 (0.63)	7.29 (0.78)	9.13 (1.22)	13.76 (2.14)	14.74 (2.00)	4.28 (1.22)
	2.00	2.08 (0.84)	7.11 (0.62)	8.95 (1.10)	12.88 (2.06))	14.19 (1.69)	4.50 (1.05)
	2.50	1.95 (0.49)	7.00 (0.55)	8.32 (1.65)	12.09 (1.68)	13.03 (2.18)	4.96 (1.20)
	3.00	1.84 (0.88)	6.83 (0.83)	7.96 (1.28)	11.81 (1.35)	12.48 (2.14)	5.26 (1.12)
Lipid fraction of chloroform-methanol extract	0.50	2.70 (0.88)	8.01 (0.72)	11.10 (0.88)	16.31 (1.68))	19.00 (2.02)	3.72 (1.10)
	1.00	2.63 (0.39)	7.84 (0.83)	10.53 (1.13)	15.12 (1.68)	16.66 (2.42)	4.06 (0.89)
	1.50	2.54 (0.76)	7.29 (0.90)	10.08 (0.96)	14.69 (1.93)	15.00 (2.19)	4.18 (1.40)
	2.00	2.38 (0.44)	7.10 (0.78)	9.66 (1.18)	13.99 (1.77")	14.91 (2.08)	4.30 (1.33)
	2.50	2.16 (0.58)	6.89 (0.93)	9.32 (1.23)	13.10 (1.39)	14.04 (2.44)	4.86 (1.00)
	3.00	2.01 (0.76)	6.53 (0.85)	9.17 (0.94)	12.86 (1.94)	13.75 (2.13)	4.99 (1.21)
Non lipid fraction of chloroform-methanol extract	0.50	-	-	-	-	-	-
	1.00	-	-	-	-	-	-
	1.50	-	-	-	-	-	-
	2.00	-	-	-	-	-	-
	2.50	-	-	-	-	-	-
	3.00	59.00 (2.24)	-	-	-	-	1.05 (1.02)

* Mean values with Standard Error in parentheses

Table 25: Behavioural Responses* of *C. gariepinus* Fingerlings Exposed to various Concentrations of *D. innoxia* Pod Extract.

	Concentration (g/l)	Behavioural Responses (minutes)					
		Loss of Reactivity to stimuli	Loss of equilibrium	Total loss of equilibrium	Reduced opercular movement	Minimal opercular movement	Recovery of equilibrium
Crude extract	0.05	-	-	-	-	-	-
	1.00	-	-	-	-	-	-
	1.50	-	-	-	-	-	-
	2.00	-	-	-	-	-	-
	2.50	-	-	-	-	-	-
	3.00	-	-	-	-	-	-
Unseperated choroform methano Extract	0.50	2.35 (0.63)	6.18 (0.47)	8.03 (0.47)	12.68 (0.26)	14.10 (1.94)	4.10 (0.88)
	1.00	2.21 (0.74)	6.04 (1.13)	7.58 (1.70)	11.11 (0.63)	13.52 (1.50)	4.28 (0.92)
	1.50	2.08 (0.94)	5.29 (0.98)	7.12 (1.25)	10.72 (0.86)	12.72 (1.22)	4.94 (1.25)
	2.00	1.89 (0.71)	5.10 (0.67)	6.95 (0.47)	10.01 (0.74)	12.18 (1.28)	5.50 (1.52)
	2.50	1.71 (0.95)	4.88 (1.21)	6.14 (0.87)	9.78 (0.79)	11.98 (1.11)	5.50 (1.12)
	3.00	1.53 (0.67)	4.65 (1.09)	5.96 (1.25)	9.24 (0.93)	11.66 (1.36)	6.26 (0.92)
Lipid fraction of chloroform-methal extract	0.50	2.42 (0.26)	6.22 (1.38)	8.09 (0.68)	12.76 (1.12)	15.00 (2.01)	4.10 (0.93)
	1.00	2.38 (0.63)	6.02 (1.79)	7.40 (0.84)	11.20 (1.02)	14.63 (2.00)	4.96 (1.12)
	1.50	2.20 (0.34)	5.25 (1.25)	7.19 (0.49)	10.88 1.22)	14.32 (2.16)	4.99 (1.00)
	2.00	1.97 (0.67)	5.08 (0.98)	6.83 (0.80)	9.99 (1.31)	14.01 (2.11)	5.15 (1.16)
	2.50	1.84 (0.79)	4.81 (0.71)	6.31 (0.93)	9.73 (1.22)	13.55 (2.09)	5.58 (0.98)
	3.00	1.65 (0.54)	4.43 (0.97)	5.97 (0.78)	8.87 (1.20)	13.28 (2.14)	6.30 (1.14)
Non-lipid fraction of chloroform-methal extract	0.50	-	-	-	-		-
	1.00						
	1.50						
	2.00						
	2.50						
	3.00	57.55 (1.13)	-	-	-		1.60 (0.66)

* Mean values with Standard Error in parentheses

Table 26: Behavioural Responses* of *C. gariepinus* Fingerlings Exposed to various Concentrations of *D. innoxia* Root Extract.

		Behavioural Responses (minutes)					
	Concentration (g/l)	Loss of reactivity to stimuli	Loss of equilibrium	Total loss of equilibrium	Reduced opercular movement	Minimal opercular movment	Recovery of equilibrium
Crude Extract	0.50	-	-	-	-	-	-
	1.00	-	-	-	-	-	-
	1.50	-	-	-	-	-	-
	2.00	-	-	-	-	-	-
	2.50	-	-	-	-	-	-
	3.00	-	-	-	-	-	-
Unexected chloroform-methanol extract	0.50	3.12 (1.06)	8.56 (1.41)	12.00 (1.25)	15.90 (1.38)	19.44 (1.41)	4.00 (0.60)
	1.00	2.90 (1.63)	8.31 (1.47)	11.78 (1.82)	15.14 (1.16)	19.18 (1.16)	4.35 (0.93)
	1.50	2.68 (1.03)	8.12 (1.25)	11.63 (1.35)	14.82 (1.41)	18.49 (2.06)	4.96 (0.99)
	2.00	2.43 (0.47)	8.00 (1.94)	11.51 (1.95)	14.26 (1.89)	17.74 (2.05)	5.61 (0.77)
	2.50	2.21 (0.47)	7.85 (1.53)	11.37 (1.89)	13.76 (1.94)	16.83 (2.09)	5.73 (0.94)
	3.00	2.08 (0.49)	7.73 (1.08)	11.11 (1.26)	13.55 (1.82)	15.92 (1.49)	6.51 (0.78)
Lipid fraction of chloroform methanol extract	0.50	3.60 (1.08)	8.89 (1.13)	12.69 (1.22)	16.80 (1.04)	20.12 (2.23)	4.11 (0.11)
	1.00	3.42 (1.16)	8.64 (1.28)	12.44 (1.60)	16.49 (1.41)	19.47 (1.94)	4.36 (0.43)
	1.50	3.28 (1.24)	8.32 (1.41)	12.30 (1.14)	16.00 (1.19)	18.59 (2.09)	4.90 (0.58)
	2.00	3.11 (1.53)	8.11 (1.33)	12.16 (1.92)	15.40 (0.98)	17.72 (2.66)	5.66 (0.39)
	2.50	2.90 (0.41)	7.98 (1.01)	12.02 (1.11)	14.77 (1.08)	16.58 (1.99)	5.78 (0.19)
	3.00	2.75 (0.86)	7.86 (0.77)	11.83 (1.71)	14.13 (1.26)	16.07 (1.76)	6.55 (0.66)
Non-lipid fraction of chloroform-methanol extract	0.50	-	-	-	-	-	-
	1.00	-	-	-	-	-	-
	1.50	-	-	-	-	-	-
	2.00	-	-	-	-	-	-
	2.50	-	-	-	-	-	-
	3.00	60.00 (0.38)	-	-	-	-	1.00 (0.22)

* Mean values with Standard Error in parentheses

Table: 27 Comparative Mean Haemoglobin Concentration (with Standard Error in Parentheses) in *C. gariepinus* Fingerlings Exposed to *D. innoxia* Sedation.

		Haemoglobin (mg/l)				
	Concentration (g/l)	Leaf	Seed	Stem	Pod	Root
Crude Extract	0.50	8.86 (0.61)	8.86 (0.61)	8.87 (0.55)	8.85 (0.86)	8.85 (0.72)
	1.00	8.85 (0.75)	8.90 (0.55)	8.88 (0.92)	8.88 (0.77)	8.87 (0.86)
	1.50	8.86 (0.80)	8.91 (0.82)	8.88 (0.80)	8.88 (0.83)	8.85 (0.80)
	2.00	8.88 (0.97)	8.87 (0.86)	8.89 (0.95)	8.88 (0.77)	8.91 (0.51)
	2.50	8.89 (0.89)	8.88 (0.75)	8.87 (0.64)	8.91 (0.86)	8.88 (0.43)
	3.00	8.89 (0.88)	8.85 (0.89)	8.86 (0.72)	8.90 (0.75)	8.87 (0.80)
Unexected chloroform-methanol extract	0.00	8.87 (0.66)	8.86 (0.86)	8.86 (0.99)	8.87 (0.85)	8.87 (0.80)
	0.50	8.88 (0.55)	8.87 (0.89)	8.88 (0.81)	8.90 (0.41)	8.88 (0.75)
	1.00	8.87 (0.20)	8.88 (0.55)	8.89 (0.63)	8.86 (0.58)	8.87 (0.28)
	1.50	8.86 (0.29)	8.89 (0.27)	8.88 (0.48)	8.86 (0.44)	8.86 (0.48)
	2.00	8.86 (0.28)	8.88 (0.30)	8.88 (0.72)	8.85 (0.78)	8.88 (0.57)
	2.50	8.88 (0.27)	8.89 (0.25)	8.87 (0.76)	8.85 (0.60)	8.85 (0.64)
	3.00	8.89 (0.59)	8.90 (0.35)	8.89 (0.49)	8.87 (0.29)	8.88 (0.54)
Lipid fraction of chloroform methanol extract	0.00	8.86 (0.50)	8.87 (0.39)	8.86 (0.88)	8.86 (0.53)	8.86 (0.56)
	0.50	8.88 (0.35)	8.88 (0.33)	8.86 (0.86)	8.86 (0.64)	8.86 (0.70)
	1.00	8.86 (0.31)	8.85 (0.63)	8.88 (0.51)	8.87 (0.48)	8.88 (0.61)
	1.50	8.87 (0.38)	8.86 (0.45)	8.87 (0.83)	8.87 (0.64)	8.87 (0.49)
	2.00	8.85 (0.56)	8.86 (0.24)	8.88 (0.84)	8.87 (0.70)	8.88 (0.70)
	2.50	8.88 (0.45)	8.87 (0.29)	8.87 (0.70)	8.86 (0.62)	8.86 (0.71)
	3.00	8.88 (0.41)	8.88 (0.20)	8.85 (0.41)	8.86 (0.39)	8.87 (0.66)
Non-lipid fraction of chloroform-methanol extract	0.00	8.86 (0.39)	8.88 (0.55)	8.87 (0.43)	8.88 (0.48)	8.86 (0.39)
	0.50	8.87 (0.67)	8.87 (0.29)	8.88 (0.62)	8.85 (0.57)	8.86 (0.39)
	1.50	8.87 (0.67)	8.88 (0.35)	8.88 (0.29)	8.87 (0.44)	8.85 (0.71)
	2.00	8.88 (0.68)	8.87 (0.52)	8.86 (0.25)	8.86 (0.59)	8.85 (0.48)
	2.50	8.89 (0.30)	8.85 (0.63)	8.86 (0.30)	8.86 (0.50)	8.86 (0.63)
	3.00	8.89 (0.56)	8.87 (0.42)	8.86 (0.30)	8.86 (0.50)	8.86 (0.63)

Table 28: Comparative Mean Red Blood Cell Count (with Standard Error in parentheses) in *C. gariepinus* Fingerlings Exposed to *D. innoxia* Sedation

		RBC Count ($x10^6 mm^{-3}$)				
	Concentration (g/l)	Leaf	Seed	Stem	Pod	Root
Crude Extract	0.00	2.25 (0.31)	2.25 (0.33)	2.26 (0.39)	2.25 (0.17)	2.26 (0.16)
	0.50	2.24 (0.25)	2.25 (0.38)	2.27 (0.46)	2.26 (0.24)	2.27 (0.17)
	1.00	2.26 (0.32)	2.25 (0.26)	2.26 (0.25)	2.27 (0.19)	2.27 (0.24)
	1.50	2.25 (0.55)	2.26 (0.42)	2.26 (0.34)	2.26 (0.30)	2.26 (0.22)
	2.00	2.25 (0.20)	2.27 (0.29)	2.27 (0.38)	2.26 (0.20)	2.27 (0.27)
	2.50	2.26 (0.26)	2.27 (0.30)	2.27 (0.30)	2.26 (0.17)	2.27 (0.19)
	3.00	2.26 (0.38)	2.27 (0.50)	2.27 (0.23)	2.27 (0.26)	2.26 (0.24)
Unexected chloroform-methanol extract	0.00	2.25 (0.27)	2.25 (0.26)	2.25 (0.58)	2.26 (0.19)	2.26 (0.29)
	0.50	2.26 (0.20)	2.26 (0.30)	2.27 (0.22)	2.25 (0.22)	2.25 (0.19)
	1.00	2.25 (0.15)	2.27 (0.28)	2.26 (0.28)	2.26 (0.28)	2.25 (0.26)
	1.50	2.27 (0.26)	2.27 (0.27)	2.26 (0.20)	2.26 (0.23)	2.26 (0.24)
	2.00	2.25 (0.31)	2.25 (0.24)	2.27 (0.27)	2.26 (0.18)	2.26 (0.11)
	2.50	2.25 (0.15)	2.26 (0.33)	2.26 (0.39)	2.26 (0.11)	2.25 (0.19)
	3.00	2.25 (0.22)	2.26 (0.25)	2.26 (0.36)	2.25 (0.20)	2.26 (0.22)
Lipid fraction of chloroform methanol extract	0.00	2.25 (0.18)	2.26 (0.20)	2.25 (0.44)	2.25 (0.17)	2.26 (0.16)
	0.50	2.25 (0.15)	2.25 (0.18)	2.26 (0.28)	2.25 (0.33)	2.25 (0.17)
	1.00	2.26 (0.29)	2.26 (0.13)	2.26 (0.23)	2.26 (0.29)	2.26 (0.23)
	1.50	2.26 (0.22)	2.26 (0.26)	2.25 (0.19)	2.25 (0.18)	2.26 (0.18)
	2.00	2.25 (0.27)	2.25 (0.28)	2.25 (0.15)	2.25 (0.25)	2.26 (0.16)
	2.50	2.25 (0.25)	2.25 (0.11)	2.25 (0.28)	2.25 (0.37)	2.26 (0.25)
	3.00	2.26 (0.29)	2.26 (0.33)	2.25 (0.23)	2.25 (0.21)	2.27 (0.31)
Non-lipid fraction of chloroform-methanol extract	0.00	2.25 (0.18)	2.26 (0.28)	2.26 (0.11)	2.26 (0.17)	2.26 (0.19)
	0.50	2.26 (0.23)	2.26 (0.23)	2.26 (0.26)	2.25 (0.23)	2.25 (0.22)
	1.00	2.26 (0.25)	2.26 (0.19)	2.25 (0.50)	2.26 (0.30)	2.25 (0.20)
	1.50	2.27 (0.29)	2.26 (0.22)	2.25 (0.34)	2.26 (0.21)	2.25 (0.19)
	2.00	2.26 (0.23)	2.26 (0.25)	2.25 (0.29)	2.26 (0.27)	2.26 (0.22)
	2.50	2.26 (0.28)	2.26 (0.19)	2.25 (0.30)	2.26 (0.25)	2.25 (0.31)
	3.00	2.26 (0.21)	2.26 (0.20)	2.25 (0.28)	2.26 (0.29)	2.25 (0.38)

Table 29: Comparative Mean White Blood Cell Count(with Standard Error in Parentheses) in *C. gariepinus* Fingerlings Exposed to *D. innoxia* Sedation.

		WBC Count (X 10^3 mm^{-3})				
	Concentration g/l	Leaf	Seed	Stem	Pod	Root
Crude Extract	0.00	38.40	38.42	38.41	38.40	38.40
		(0.65)	(0.57)	(0.63)	(0.39)	(0.58)
	0.50	38.41	38.41	38.40	38.42	38.40
		(0.40)	(0.54)	(0.55)	(0.40)	(0.44)
	1.00	38.40	38.42	38.41	38.41	38.42
		(0.86)	(0.38)	(0.80)	(0.67)	(0.32)
	1.50	38.42	38.41	38.41	38.42	38.42
		(0.70)	(0.91)	(0.82)	(0.45)	(0.59)
	2.00	38.41	38.43	38.42	38.42	38.41
		(0.75)	(0.29)	(0.32)	(0.60)	(0.77)
	2.50	38.41	38.42	38.41	38.41	38.42
		(0.38)	(0.40)	(0.90)	(0.33)	(0.29)
	3.00	38.42	38.43	38.42	38.42	38.41
		(0.54)	(0.25)	(0.41)	(0.80)	(0.52)
Unexpected chloroform methanol extract	0.00	38.40	38.40	38.42	38.40	38.41
		(0.89)	(0.30)	(0.86)	(0.36)	(0.39)
	0.50	38.42	38.42	38.41	38.40	38.42
		(0.27)	(0.70)	(0.98)	(0.27)	(0.20)
	1.00	38.42	38.42	38.41	38.42	38.42
		(0.56)	(0.61)	(0.64)	(0.30)	(0.49)
	1.50	38.42	38.41	38.41	38.40	38.41
		(0.39)	(0.58)	(0.36)	(0.29)	(0.54)
	2.00	38.42	38.42	38.42	38.42	38.41
		(0.45)	(0.40)	(0.57)	(0.57)	(0.29)
	2.50	38.43	38.41	38.41	38.41	38.42
		(0.50)	(0.45)	(0.70)	(0.70)	(0.32)
	3.00	38.43	38.42	38.41	38.41	38.42
		(0.75)	(0.28)	(0.63)	(0.63)	(0.60)
Lipid fraction of chloroform methanol extract	0.00	38.40	38.41	38.40	38.40	38.40
		(0.40)	(0.50)	(0.30)	(0.30)	(0.45)
	0.50	38.41	38.41	38.41	38.41	38.40
		(0.54)	(0.25)	(0.41)	(0.41)	(0.70)
	1.00	38.41	38.41	38.41	38.41	38.42
		(0.44)	(0.38)	(0.21)	(0.51)	(0.38)
	1.50	38.40	38.41	38.41	38.41	38.41
		(0.28)	(0.41)	(0.32)	(0.32)	(0.33)
	2.00	38.41	38.42	38.40	38.40	38.41
		(0.30)	(0.32)	(0.50)	(0.50)	(0.80)
	2.50	38.42	38.42	38.40	38.40	38.42
		(0.31)	(0.36)	(0.68)	(0.68)	(0.42)
	3.00	38.43	38.42	38.42	38.42	38.42
		(0.61)	(0.70)	(0.26)	(0.26)	(0.39)
Non-lipid fraction of chloroform-methanol extract	0.00	38.41	38.41	38.41	38.41	38.40
		(0.32)	(0.40)	(0.39)	(0.39)	(0.31)
	0.50	38.41	38.41	38.41	38.41	38.40
		(0.41)	(0.39)	(0.30)	(0.30)	(0.54)
	1.00	38.41	38.42	38.40	38.40	38.41
		(0.63)	(0.28)	(0.25)	(0.25)	(0.68)
	1.50	38.40	38.41	38.41	38.41	38.41
		(0.40)	(0.35)	(0.22)	(0.22)	(0.32)
	2.00	38.41	38.41	38.41	38.41	38.42
		(0.25)	(0.30)	(0.59)	(0.59)	(0.42)
	2.50	38.41	38.42	38.41	38.41	38.41
		(0.54)	(0.33)	(0.50)	(0.50)	(0.55)
	3.00	38.42	38.42	38.42	38.42	38.41
		(0.57)	(0.29)	(0.40)	(0.40)	(0.73)

Table 30: Comparative Mean Erythrocyte Sedimentation Rates (with Standard Error in parentheses) *in C. gariepinus* Fingerlings Exposed to *D. innoxia* Sedation.

		ESR (mm/h)				
	Concentration (g/l)	Leaf	Seed	Stem	Pod	Root
Crude Extract	0.00	2.35 (0.12)	2.34 (0.19)	2.34 (0.18)	2.34 (0.20)	3.33 (0.11)
	0.50	2.35 (0.11)	2.33 (0.16)	2.34 (0.22)	2.34 (0.15)	2.33 (0.18)
	1.00	2.35 (0.20)	2.34 (0.28)	2.33 (0.25)	2.34 (0.24)	2.33 (0.12)
	1.50	2.35 (0.17)	2.35 (0.16)	2.36 (0.18)	2.33 (0.13)	2.33 (0.23)
	2.00	2.36 (0.16)	2.35 (0.29)	2.35 (0.12)	2.33 (0.11)	2.34 (0.21)
	2.50	2.36 (0.20)	2.35 (0.19)	2.35 (0.22)	2.33 (0.16)	2.34 (0.25)
	3.00	2.37 (0.15)	2.35 (0.20)	2.35 (0.24)	2.33 (0.29)	2.34 (0.28)
Unexpected chloroform methanol extract	0.00	2.35 (0.23)	2.35 (0.22)	2.34 (0.19)	2.33 (0.29)	2.33 (0.24)
	0.50	2.35 (0.11)	2.35 (0.18)	2.34 (0.26)	2.34 (0.22)	2.34 (0.21)
	1.00	2.34 (0.09)	2.35 (0.21)	2.35 (0.22)	2.34 (0.19)	2.34 (0.21)
	1.50	2.36 (0.14)	2.36 (0.23)	2.36 (0.19)	2.34 (0.29)	2.34 (0.23)
	2.00	2.36 (0.21)	2.35 (0.25)	2.34 (0.14)	2.34 (0.16)	2.33 (0.24)
	2.50	2.36 (0.29)	2.34 (0.24)	2.35 (0.21)	2.34 (0.20)	2.33 (0.22)
	3.00	2.36 (0.12)	2.36 (0.20)	2.36 (0.23)	2.34 (0.28)	2.34 (0.28)
Lipid fraction of chloroform methanol extract	0.00	2.35 (0.24)	2.34 (0.20)	2.34 (0.25)	2.34 (0.25)	2.33 (0.29)
	0.50	2.35 (0.19)	2.34 (0.23)	2.34 (0.27)	2.34 (0.19)	2.33 (0.23)
	1.00	2.35 (0.22)	2.35 (0.19)	2.35 (0.22)	2.34 (0.20)	2.33 (0.20)
	1.50	2.36 (0.15)	2.35 (0.20)	2.34 (0.24)	2.34 (0.28)	2.34 (0.24)
	2.00	2.36 (0.27)	2.34 (0.21)	2.36 (0.26)	2.34 (0.28)	2.34 (0.21)
	2.50	2.36 (0.23)	2.34 (0.24)	2.35 (0.21)	2.34 (0.25)	2.34 (0.19)
	3.00	2.36 (0.29)	2.34 (0.24)	2.35 (0.20)	2.35 (0.17)	2.34 (0.16)
Non-lipid fraction of chloroform-methanol extract	0.00	2.36 (0.25)	2.35 (0.22)	2.35 (0.20)	2.34 (0.13)	2.33 (0.18)
	0.50	2.36 (0.14)	2.35 (0.25)	2.35 (0.24)	2.35 (0.28)	2.34 (0.11)
	1.00	2.35 (0.21)	2.35 (0.28)	2.34 (0.25)	2.35 (0.24)	2.34 (0.13)
	1.50	2.35 (0.26)	2.36 (0.22)	2.35 (0.21)	2.35 (0.11)	2.35 (0.13)
	2.00	2.35 (0.23)	2.35 (0.21)	2.34 (0.15)	2.35 (0.13)	2.34 (0.22)
	2.50	2.36 (0.26)	2.35 (0.20)	2.35 (0.19)	2.35 (0.18)	2.34 (0.16)
	3.00	2.36 (0.23)	2.35 (0.12)	2.35 (0.15)	2.35 (0.11)	2.34 (0.14)

Table 31: Comparative Mean Packed Cell Volume (with Standard Error in parentheses) in *C. gariepinus* Fingerlings Exposed to *D. innoxia* Sedation.

		PCV (%)				
	Concentration (g/l)	Leaf	Seed	Stem	Pod	Root
Crude extract	0.00	45.30 (0.25)	45.30 (0.19)	45.29 (0.16)	45.30 (0.12)	45.30 (0.17)
	0.50	45.30 (0.11)	45.30 (0.16)	45.29 (0.10)	45.30 (0.13)	45.30 (0.13)
	1.00	45.30 (0.21)	45.29 (0.15)	45.29 (0.13)	45.30 (0.10)	45.30 (0.14)
	1.50	45.29 (0.17)	45.29 (0.16)	45.29 (0.15)	45.29 (0.16)	45.30 (0.14)
	2.00	45.29 (0.11)	45.28 (0.12)	45.30 (0.18)	45.29 (0.15)	45.30 (0.11)
	2.50	45.30 (0.13)	45.28 (0.12)	45.30 (0.22)	45.29 (0.17)	45.30 (0.11)
	3.00	45.30 (0.18)	45.28 (0.17)	45.30 (0.13)	45.28 (0.19)	45.30 (0.13)
Unexpected chloroform methanol extract	0.00	45.30 (0.15)	45.30 (0.13)	45.30 (0.14)	45.30 (0.14)	45.30 (0.20)
	0.50	45.30 (0.12)	45.30 (0.11)	45.30 (0.11)	45.30 (0.18)	45.30 (0.18)
	1.00	45.30 (0.12)	45.30 (0.10)	45.30 (0.19)	45.29 (0.14)	45.29 (0.11)
	1.50	45.30 (0.10)	45.29 (0.12)	45.30 (0.16)	45.29 (0.12)	45.29 (0.10)
	2.00	45.30 (0.19)	45.29 (0.13)	45.30 (0.11)	45.29 (0.19)	45.29 (0.13)
	2.50	45.29 (0.11)	45.29 (0.14)	45.30 (0.15)	45.29 (0.11)	45.29 (0.17)
	3.00	45.29 (0.19)	45.29 (0.22)	45.30 (0.10)	45.29 (0.17)	45.29 (0.19)
Lipid fraction of chloroform methanol extract	0.00	45.30 (0.19)	45.30 (0.10)	45.29 (0.22)	45.30 (0.20)	45.30 (0.17)
	0.50	45.30 (0.13)	45.30 (0.16)	45.30 (0.17)	45.30 (0.11)	45.30 (0.11)
	1.00	45.30 (0.11)	45.30 (0.11)	45.30 (0.14)	45.30 (0.09)	45.30 (0.18)
	1.50	45.30 (0.18)	45.30 (0.20)	45.30 (0.12)	45.30 (0.11)	45.29 (0.13)
	2.00	45.30 (0.20)	45.29 (0.19)	45.30 (0.13)	45.30 (0.20)	45.29 (0.11)
	2.50	45.30 (0.22)	45.29 (0.21)	45.30 (0.21)	45.30 (0.21)	45.29 (0.17)
	3.00	45.30 (0.18)	45.30 (0.17)	45.30 (0.19)	45.30 (0.19)	45.29 (0.16)
Non-lipid fraction of chloroform-methanol extract	0.00	45.30 (0.11)	45.30 (0.16)	45.30 (0.16)	45.30 (0.14)	45.30 (0.17)
	0.50	45.29 (0.09)	45.30 (0.21)	45.30 (0.17)	45.30 (0.11)	45.30 (0.13)
	1.00	45.29 (0.19)	45.30 (0.19)	45.30 (0.11)	45.30 (0.14)	45.30 (0.11)
	1.50	45.29 (0.22)	45.30 (0.15)	45.30 (0.18)	45.30 (0.09)	45.29 (0.20)
	2.00	45.29 (0.19)	45.29 (0.13)	45.30 (0.16)	45.30 (0.21)	45.29 (0.22)
	2.50	45.30 (0.17)	45.29 (0.22)	45.30 (0.13)	45.30 (0.17)	45.29 (0.18)
	3.00	45.29 (0.19)	45.29 (0.12)	45.30 (0.13)	45.30 (0.17)	45.28 (0.28)

Table 32: Comparative Mean Corpuscular Haemoglobin Concentration (with Standard Error in parentheses) in *C. gariepinus* Fingerlings Exposed to *D. innoxia* Sedation

	Concentration (g/l)	MCHC (g/e)				
		Leaf	Seed	Stem	Pod	Root
Crude extract	0.00	19.56	19.56	19.63	19.54	19.54
	0.50	(0.16) 19.55	19.56	19.62	19.55	19.56
	1.00	19.54	19.63	19.61	19.60	19.58
	1.50	19.56	19.67	19.61	19.61	19.54
	2.00	19.61	19.59 (0.16)	19.62	19.67	19.65
	2.50	19.63	19.61	19.58	19.63	19.54
	3.00	19.63	19.55 (0.16)	19.56	19.66	19.60
Unexpected chloroform methanol extract	0.00	19.58	19.56	19.56	19.58	19.58
	0.50	19.60	19.58	19.60 (0.16)	19.62	19.60
	1.00	19.58	19.60	19.62	19.56	19.58
	1.50	19.56	19.63	19.60	19.56	19.56
	2.00	19.56	19.61	19.60 (0.16)	19.54	19.61
	2.50	19.61	19.63	19.58	19.54	19.54 (0.16)
	3.00	19.63	19.65	19.63	19.58	19.61
Lipid fraction of chloroform methanol extract	0.00	19.56	19.56	19.56	19.56	19.56
	0.50	19.58	19.58	19.58	19.58	19.60 (0.16)
	1.00	19.60	19.58	19.56	19.56	19.56
	1.50	19.56	19.60	19.60	19.58 (0.16)	19.58
	2.00	19.58	19.61	19.58	19.58	19.58
	2.50	19.54	19.54 (0.16)	19.60	19.58	19.61
	3.00	19.60	19.56	19.58	19.58	19.56
Non-lipid fraction of chloroform-methanol extract	0.00	19.56 (0.16)	19.56	19.54	19.56	19.56
	0.50	19.58	19.58	19.58	19.60	19.58
	1.00	19.58	19.58	19.58	19.58	19.58
	1.50	19.58 (0.16)	19.60	19.60	19.58	19.54
	2.00	19.61	19.58	19.60	19.58	19.55
	2.50	19.65	19.54	19.56 (0.16)	19.56	19.54
	3.00	19.65 (0.16)	19.58	19.56	19.56	19.56

Table 33: Comparative Mean Corpuscular Volume (with Standard Error in parentheses) in *C. gariepinus* Fingerlings Exposed to *D. innoxia* Sedation

		MCV (f/l)				
	Concentration (g/l)	Leaf	Seed	Stem	Pod	Root
Crude Extract	0.00	201.33 (0.22)	201.33 (0.27)	200.40 (0.16)	201.33 (0.12)	200.44 (0.28)
	0.50	201.33 (0.42)	201.33 (0.36)	199.52 (0.32)	200.44 (0.29)	199.56 (0.23)
	1.00	202.23 (0.51)	201.29 (0.42)	200.40 (0.29)	199.56 (0.52)	199.56 (0.28)
	1.50	200.44 (0.29)	200.40 (0.52)	200.40 (0.34)	203.40 (0.56)	200.44 (0.47)
	2.00	201.28 (0.32)	199.47 (0.39)	199.56 (0.53)	203.40 (0.49)	199.51 (0.69)
	2.50	201.20 (0.39)	200.35 (0.46)	199.56 (0.29)	203.40 (0.22)	199.56 (0.16)
	3.00	200.35 (0.28)	199.56 (0.39)	199.56 (0.39)	200.35 (0.47)	200.44 (0.49)
Unexpected chloroform-methanol extract	0.00	201.33 (0.39)	201.33 (0.22)	201.33 (0.32)	201.33 (0.56)	200.44 (0.34)
	0.50	200.44 (0.29)	200.44 (0.44)	199.60 (0.28)	201.33 (0.32)	201.33 (0.46)
	1.00	201.33 (0.22)	199.56 (0.32)	200.44 (0.16)	200.27 (0.27)	201.33 (0.20)
	1.50	199.56 (0.24)	200.40 (0.41)	200.44 (0.62)	200.27 (0.52)	201.29 (0.32)
	2.00	201.33 (0.32)	199.52 (0.22)	199.56 (0.46)	200.40 (0.58)	201.29 (0.43)
	2.50	201.28 (0.23)	200.39 (0.16)	200.40 (0.42)	200.40 (0.52)	200.40 (0.44)
	3.00	199.56 (0.35)	200.40 (0.54)	200.40 (0.52)	200.44 (0.51)	200.44 (0.60)
Lipid fraction of chloroform-methanol extract	0.00	201.33 (0.44)	200.44 (0.16)	201.29 (0.42)	200.44 (0.62)	201.33 (0.22)
	0.50	201.33 (0.42)	199.56 (0.61)	200.44 (0.52)	200.44 (0.47)	201.33 (0.49)
	1.00	200.35 (0.67)	199.56 (0.55)	200.44 (0.52)	200.44 (0.48)	201.33 (0.52)
	1.50	200.44 (0.43)	200.44 (0.71)	201.29 (0.16)	201.33 (0.42)	200.40 (0.40)
	2.00	201.33 (0.46)	201.29 (0.46)	201.29 (0.41)	201.33 (0.39)	200.40 (0.33)
	2.50	201.33 (0.42)	200.40 (0.41)	201.29 (0.63)	201.33 (0.42)	200.40 (0.53)
	3.00	201.23 (0.46)	200.40 (0.40)	201.29 (0.16)	201.35 (0.32)	200.44 (0.42)
Non-lipid fraction of chloroform-methanol extract	0.00	201.33 (0.28)	200.44 (0.46)	201.29 (0.54)	201.35 (0.47)	200.44 (0.52)
	0.50	202.23 (0.42)	200.40 (0.16)	200.44 (0.29)	201.33 (0.46)	199.56 (0.76)
	1.00	200.40 (0.46)	200.44 (0.52)	200.44 (0.56)	200.44 (0.16)	200.44 (0.36)
	1.50	199.52 (0.42)	200.44 (0.63)	201.29 (0.42)	200.44 (0.47)	201.33 (0.44)
	2.00	203.40 (0.52)	199.52 (0.62)	201.29 (0.52)	200.44 (0.59)	200.40 (0.47)
	2.50	200.40 (0.16)	200.40 (0.54)	201.29 (0.46)	200.44 (0.16)	201.29 (0.52)
	3.00	200.40 (0.33)	200.40 (0.42)	201.40 (0.39)	200.44 (0.20)	201.29 (0.52)

Table 34: Comparative Mean Corpuscular Haemoglobin (with Standard Error in parentheses) in *C*. *gariepinus* Fingerlings Exposed to *D. innoxia* Sedation.

		MCH (pg)				
	Concentration (g/l)	Leaf	Seed	Stem	Pod	Root
Crude extract	0.00	39.39	39.37	39.33	39.33	39.15
	0.50	39.34	39.55	39.29	39.11	39.07
	1.00	39.16	39.42	39.29	39.29	39.15
	1.50	39.38	39.07	39.16	39.42	39.20
	2.00	39.47	39.22	39.07	39.38	39.98
	2.50	39.34	38.90	39.03	39.38	39.29
	3.00	39.34	39.04	39.34	39.29	39.30
Unseperated chloroform methanol extract	0.00	39.42	39.37	39.37	39.42	39.24
	0.50	39.29	39.24	39.11	39.51	39.46
	1.00	39.42	39.11	39.33	39.20	39.42
	1.50	39.03	39.16	39.29	39.20	39.20
	2.00	39.38	39.46	39.11	39.15	39.29
	2.50	39.47	39.36	39.24	39.15	39.33
	3.00	39.16	39.38	39.33	39.42	39.29
Lipid fraction of chloroform-methanol extract	0.00	39.37	39.20	39.37	39.37	39.20
	0.50	39.37	39.20	39.37	39.37	39.20
	1.00	39.29	39.24	39.20	39.20	39.29
	1.50	39.37	39.29	39.46	39.42	39.29
	2.00	39.27	39.46	39.42	39.42	39.42
	2.50	39.33	39.33	39.46	39.42	39.29
	3.00	39.29	39.20	39.42	39.37	39.03
Non-lipid fraction of chloroform-methanol extract	0.00	39.37	39.20	39.46	39.20	39.20
	0.50	39.24	39.24	39.42	39.46	39.42
	1.00	39.08	39.29	39.47	39.95	39.94
	1.50	39.07	39.29	39.46	39.15	39.37
	2.00	39.29	39.07	39.46	39.24	39.15
	2.50	39.33	39.15	39.37	39.20	39.33
	3.00	39.33	39.24	39.37	39.20	39.37

3.18 HISTOLOGICAL SECTIONS

The histopathological sections of the gills, gonad, kidney and liver of *C. gariepinus* fingerlings exposed to *D. innoxia* for twelve weeks, those of the control and after recovery from anaesthetic effects are presented in Plates XIV - XX. Examination of the gill hemibranchs from the control fish speciments indicate that they consist of long thin filaments, the primary lamellae, which project from the arch, like the teeth of a comb. The surface area of the primary lamella is further increased by the formation of regular semi lunar folds across its dorsal and ventral surface by the secondary lamellae. The gills, liver and kidney of the fish exposed to 100mg/l of the leaf extracts had more pathological signs than those of the seed, stem, pod and root of the same concentration. The most common pathological signs observed on the liver are necrosis of the hepatocytes and hepatocyte vacuolation. No apparent significant histological changes were observed in the gills, gonad, liver and kidney of fish exposed to anaesthetics and those of the control tanks.

CHAPTER FOUR

DISCUSSION

4.1 DISCUSSION OF RESULTS

Phytochemical analysis of *D. innoxia* leaf, seed, stem, pod and root revealed the presence of alkaloids, scopolamine, hyoscine, essential oil, saponin, tannin, atropine and hyoscyamine. In solvent system, hyoscyamine/atropine migrate in the lower Rf range (Rf ca. 0.1- 0.15), while scopolamine is found in the upper region of the chromatogram (Rf ca. 0.8).

The presence of atropine, scopolamine, hysocyamine and hyoscine in *D. innoxia* is in line with the findings of Karpenko (1985), Prabhakar *et al.* (1971), Zielinska-Sowicka and Szepczynska (1972), Pagani (1982), Avriette (1998), Whitewolf (2002).

Atropine, scopolamine, hyoscine, and hyoscyamine have all been used in mainly two ways: opthamologically and systemically. Atropine (dl-hyosciamine) a plant alkaloid, when used systematically in adequate doses, produces an initial transitory central nervous stimulant action before the blocking effect is manifested. The findings of the phytochemical analysis of this research indicated the presence of some active drug compounds (Atropine, scopolamine, hyoscine, and hyoscyamine), which have sedative, anaesthetic as well as medicinal potency as evidenced in the various uses of *D. innoxia.* Okeke, (1998) reported the use of *D. innoxia* fruit extract in Benue state of Nigeria by young men to sedate girls. James (1991) reported that the Chumash people of California use *D.*

innoxia medically as an anaesthetic for setting bones, to treat bad bruises and wounds, to ‘freshen the blood’ and to treat haemorrhoids. This according to the author is because they believe the anaesthetic and narcotic properties of the plant would numb the pain receptors thereby reducing stress and tension in the patient, which in turn speeds up the healing process (James 1991). Zagari, (1992) reported that the plant is used for respiratory decongestion and wound healing in Iran. Its use in the stimulation of the central nervous system has been documented (Manandhar, 1995). It has been reported that the indigenes of Makurdi in Nigeria use the plant to feed goats and fowls as a medicinal means of preventing these animals from falling sick and dying (Okeke, 1998). Nuhu and Ghani (2002) reported the presence of hyoscyamine, hyoscine, atropine, ligloidine and metaliodine in the leaves of three Nigerian *Datura* species (*D. innocia, D. metal and D. stramonium).* According to these authors, total alkaloid content was determined in the plants to be 1.75, 1.22 and 1.29% in *D. innoxia, D. metal and D. stramonium* respectively. The total alkaloid in an official drug to be considered to be a standard drug is 0.2- 0.5%. They therefore observed that these three Nigeria species are good quality drugs that could be used as sources of these important alkoloids.

The mineral analysis of *D. innoxia* leaf, seed, stem, pod and root revealed the presence of both metals and non-metalic minerals at various levels in the plant parts. According to Martin and Coughtrey, (1982) metals are natural constituents of the environment and are found in varying levels

in the soil, ground and surface waters. Some are essential, required for normal metabolism of organisms, while others are non- essential and play no significant biological roles (Rainbow and White, 1989). For instance, some metals are naturally found in the body and are essential to human health e.g. iron prevents anaemia, zinc is a cofactor in over 100 enzyme reactions. They normally occur at low concentrations and are known as trace elements. In high doses, they may be toxic to the body or produce deficiencies in other trace metals, e.g. high zinc level can result in a deficiency of copper. In addition to their natural occurence, metals may enter and conterminate the environment from five different general sources namely geological weathering, industrial processing of metals and ores, the use of metals and their compounds, leaching of metals from municipal and solid waste dumps especially mine dumps and animal and human excretions (Rainbow and Whiteman 1989). Birch *et al.,* 1996 observed that metals are pesistent and tend to accumulate in the environment especially in the sediments.The chemical characteristics of metal are responsible for the fact that all metals ultimately become toxic at some elevated concentration (Rainbow 1985). Heavy metals have no function in the body and can be highly toxic. These toxic metals are stable elements that cannot be metabolized by the body and so bioaccumulative (passed up the food chain to humans). These include lead, cadmium, mercury, copper, arsenic nickel. Abnormally high concentrations can cause the inabilty of organism to excrete, sequester or otherwise detoxify themselves especially in the case of non essential metals (Thorp *et al*., 1979). They have also

been reported to become strongly enriched in the food chain, through a process of biomagnification (Forstner and Muller 1976). Organisms can accumulate metals to levels above those, which are required for normal physiological functioning.

Coetzee *et al* (2002) reported that fish, unlike most terrestrial animals, can absorb some minerals (inorangic elements) not only from their diets but also from their environment. Calcium (Ca), magnesium (Mg), Sodium (Na), Potassium (K), Iron (Fe), Copper (Cu), and Selenium (Se) are generally derived from the water to satisfy part of the nutritional requirements of fish.

Hamre *et al (*2004) observed that inorganic elements are required for normal life processes of fish. Their main functions include the formation of skeletal structure, electron transfer, regulation of acid-base equilibrium and osmoregulation. Minerals are also important components of hormones and enzymes, and they activate enzymes. The electrolytes, $Na^{+,}$ K^{+}, Mg^{2+}, Ca^{2+} and HCO_3 play a major role in the osmotic and ionic regulation of extra and intracellular fluids in fish (Ashley 1972).

Calcium and phosphorus are directly involved in the development and maintenance of the skeletal system and participate in several physiological processes. Fish scales are also an important site of calcium metabolism and deposition. In addition to its structural functions, calcium plays an important role in muscle contraction, blood clot formation, nerve impulse transmission, the maintenance of cell integrity and acid-base equilibrium and activation of several important enzymes.

According to Ogino and Takeda (1978) fish absorb calcium from their environment and rely on calcium present in water during dietary calcium deprivation. The calcium requirement of fish is affected by the water chemistry and species difference.

Onishi *et al* (1981) observed that Phosphorus was an important constituent of nucleic acids and cell membranes and is indirectly involved in all energy producing cellular reactions. The role of phosphorus in carbohydrate, lipid and amino acid metabolism, as well as in various metabolic processes involving buffers in body fluids is also well established (Onishi *et al* 1981). In most fish, the main phosphorus deficiency signs include poor growth, feed efficiency and bone mineralization. Other signs of deficiency in carp include increase in the activity of certain gluconeogenic enzyme in liver, increase in carcass fat with decrease in carcass water content, reduced blood phosphate levels, deformed head and deformed vertebrae. (Ogino and Takeda, 1976, Onishi *et al* 1981, Takeuchi and Nakazoe, 1981and Hamre *et al*, (2004)). A reduction in haematocrit level has been reported in catfish deficient of phosphorus (Andrews *et al* 1973). A low phosphorus intake by red sea bream was reported by Sakamoto and Yone (1980) to have caused curved, enlarged vertebrae, increase serum alkaline phosphatese activity, higher lipid depostiion in muscle, liver and vertebrae and reduction in liver glycogen content. Lall (1989) reported the phosphorus requirement of *C. gariepinus* to be 0.45% in calcium free water.

Magnesium has been reported by Houston (1985) to be an essential cofactor in many enzymatic reactions in intermediary metabolism. The author observed that magnesium plays an important role in respiratory adaptation of

freshwater fish and is required in skeletal tissue metabolism, osmoregulation and neuromuscular transmission. The quantitative magnesium requirement of rainbow trout (Shearer 1989), Carp (Ogino and Chiou 1976) Channel Catfish (Gatlin et al 1982), eel (Nose and Arai 1979) and guppy (Shim and Ng 1988) have been estimated to range from 0.04 to 0.06 percent of the diet. A dietary magnesium of 0.06 to 0.08 percent was required for tilapia (Dabrowska *et al* 1989) and 0.04% for *Clarias gariepinus (*Lall, 1989).

Magnesium deficiency has been observed to cause anorexia, reduced growth, lethargy, and reduced tissue magnesium content in fish (Onino *et al* 1987). In rainbow trout, magnesium deficiency is known to have caused calcinosis of the kidney, vertebrae deformity and degeneration of muscle fibres and epithelial cells of the pyloric ceacum and gill filaments (Cowey *et al.*, 1977). It has been observed by Knox *et al* (1981a); that rainbow trout fed magnesium deficient diets showed flaccied appearance of their muscle and carp maintained on a low magnesium diet also developed convulsions and cataracts (Ogino and Chiou 1976).

Salman and Eddy (1988) reported that Sodium, Potassium and Chloride are the most abundant electrolytes in the body. Sodium is the principal

cation in the extracellular fluid whereas, Potassium is the major monovalent intracellular cation.

The signs of Potassium deficiency in Chinook Salmo included anorexia, Convulsions, lethargy and death (Shearer 1989). Iron has been reported by Walker and Fromm (1976) to be an essential element in the cellular respiratory process through its oxidation-reduction activity and electron transfer. It is found in the body mainly in the complex form bound to proteins, such as heame compounds (haemoglobin and myoglobin) haeme enzymes (Cytochromes catalase, peroxidase etc) and nonhaeme compounds (transferring, ferritin, and iron-containing flavo-proteins (ferrodoxins, hydrogenases).

The iron requirements have been reported for catfish *Clarias gariepinus* Atlantic salmo (Lall and Hines 1987) and eel (Nose and Arai 1979), (Lall 1989), to be 30, 60 and 170mg/kg of diet respectively. Iron deficiencies have been reported to cause characteristic microcytic anaemia in brook trout (Kawatsu 1972), red sea bream, (Lall 1989) yellowtail eel (Nose and Arai (1979) and Carp (Sakamoto and Yone 1978b). These authors noted that in most cases growth was not influenced by iron deficiency but the normal liver colour changed to yellowish white during iron deficiency. Dietary iron toxicity signs developed in rainbow trout fed more than 1380mgFe/kg (Desjardins *et al* 1987). The major effects of iron toxicity included reduced growth, increased mortality, diarrhea and histopathological damage of the liver cells. Since the fish will not be

exposed to *D. innoxia* extracts for a long period of time. The accumulation of these metals in the body of the fish is not envisaged or feared.

The proximate analysis of *D. innoxia* indicated that as usual in plant parts, there was a variation in crude protein content which ranged from 17.21% in the leaf to 2.09% in the root. The crude protein of the seed, stem and pod were found to be 13.90, 6.16 and 2.12% respectively. According to the NRC (1993), crude protein of less than 20% indicates low protein content of that feed stuff. These crude protein results are however comparable with the result of some tropical plant seeds analysed by Ezeagu *et al*. (2000), who reported that *Diospyno mespiliformis* and *Entandrophrgma angolense* had crude protein contents of 3.46 and 12.34% respectively.

The moisture content which measures the amount of water in the plant also varied with the root having the lowest (3.5%) moisture content while the stem had the highest of 15%. The seed, pod and leaf had 10.00, 8.50 and 7.5% moisture content respectively. According to NRC (1993), moisture content of 5-20% (DM) is regarded as high. This indicates that the moisture content of the leaf, seed, stem and pod were high. The result of this investigation is comparable with those obtained by Ezeogu *et al*. (2000) for *Gliricidia sepium* 6.77%, *Albizu zygia* 7.8%, *Doneillia ogea* 9.86% and *Diospyros mespiliformis* 8.99%.

The crude lipid content followed the same pattern with the seed having the highest lipid content of 15.52% and the root had the least (6%). The pod, stem and leaf had 9.0, 8.50 and 7.50% respectively. Crude fibre,

which measures the fibrous component (cellulose, hemicellulose and lignin) was highest in the root 31.72% followed by the stem 29.66%, the pod, leaf and seed had 16.13%, 8.95 and 6.55% respectively. Crude fibre content was low in the seed, leaf and pod while stem and root had high crude fibre (NCR 1993).

Total ash was highest in the root (25.71%) while it was lowest in the seed (8.26%). The leaf, stem and pod had 21.59%,19.50% and 16.28% respectively. The Nitrogen free extract (NFE) was highest in the pod (47.97%) and least in stem (20.88%). The seed, leaf and root had 46.67%, 42.25% and 30.58% respectively, total ash was highest in the root (25.71%) while it was lowest in the seed (8.26%). The leaf, stem and pod had 21.59%, 19.50 and 16.28% respectively. The Nitrogen free extract (NFE) was highest in the pod (47.97%) and least in the stem (20.88%), The seed, leaf and the root had 45.67%, 42.25% and 30.58% respectively.

Comparison of the amino acid composition of *D. innoxia* with FAO *et al*., (1985) recommended values for human beings indicated that the isoleucine, which is an essential aminoacid needed for the production of haemoglobin, required for pre-school children (2 - 5 years) and school children (10-12yr) as 28mg/g protein, while for adults (over 18 years) as 13mg/g protein, leucine 66, 44 and 19mg/g protein for pre-school, school children and adults respectively. Lysine as 58, 44, and 16 mg/g protein for pre-school, school children and adults respectively. Methionine (an essential aminoacid needed to breakdown fats and to detoxify the system from heavy metals) and cysteine (aids in detoxification and protects the

body against radiation) as 25, 22 and 17 mg/g protein for pre-school, school children and adults respectively. Phenylanine and tyrosine as 63, 22 and 19 mg/g protein for pre-school, school children and adults respectively. Threonine as 34, 28 and 7 mg/g protein for pre-school, school children and adults respectively. Tryptophan as11, 9 and 5 mg/g protein for pre-school, school children and adults respectively. Valine as 35, 25 and 13 mg/g protein for pre-school, school children and adults respectively. The result of amino acid from this investigation is comparable with those obtained by Ladeji *et. al.* (1994) for the leaf of fluted pumpkin (*Telferia occidentalis*).

Amino acids are the building blocks of proteins. The amino acid content of proteins, particularly feed proteins may differ markedly. Some, such as gelatin (a mixture of proteins derived from collagen) or zein (a protein from maize gluten), are largely deficient in one or more amino acids. (Mambrini and Kaushik, 1995). Others such as fishmeal have balanced amino acids that more closely meet the requirements of fish (Mambrini and Kaushik, 1995).

It has been established that all telost fish require ten essential amino acids (EAA) namely arginine, histidine, isoleucine, leucine, lysine, methionine threonine, tryptophan, phenyalanine and valine. Along with these 10 essential amino acids, fish require a source of nitrogen (amino group) for the synthesis of non-essential amino acids (Mambrini and Guillaume, 1999).

Pedro and Bureau (2004) stated that quantitative requirements for all ten essential amino acids has been established for a number of species and that given the similarity of amino acid composition and metabolism of most fish species, careful generalisation of these values to most fish species may be warranted. A report by NRC (1993) stated that juvenile chun Salmo (*Salmon solar)* required 2.6% arginine, 0.7% histidine, 1.0% isoleucine, 0.3% tryptophan, 1.2% valine, 1.5% leucine, 1.9% lysine, 1.2% methionine and cystine, 2.5% phenylalnine and tryosine, 1.2% threonine, 0.3% tryptophan as percentage of dry diet. Similar observations were made by Coloso *et al* (2004) when graded levels of tryptophan were fed to juvenile Asian sea bass *Lates calcarifer* for 12 weeks. According to these authors, on the basis of break-point analysis of the growth response, the dietary tryptophan requirement of *lates calcarifer* was 0.41% of dietary protein.

Similarly, the amino acid requirement of juvenile gilthead saebream have been reported to include arginine (1.7%), lysine (1.7%), methionine (1.4%) and tryptophan (0.2%), as percentage of dry diet. (NRC 1993). Juvenile lake trout have been reported to require isoleucine (0.3 – 0.7%) leucine (1.0 – 1.3%), valine (0.6 – 0.8%) as percentage of dry diet (NRC 1993). Imtiaz *et al.,* (2004) recommended that the diet for the Indian major carp, *Cirrhinus myrigala* should contain 4.50g/100g diet protein for uptimum growth and efficient food utilization.

Rodehutscord *et al* (1997) reported that a deficiency of indispensable amino acid creates poor utilization of dietary protein and

hence growth retardation, poor live weight gain, and feed efficiency. In severe cases, deficiency reduces the ability to resist diseases and lowers the effectiveness of the immune response mechanism.

Mambrini and Guillaume (1999) reported that for most indispensable amino acids, deficiency is manifest as a reduction in weight-gain. In certain species of fish, however, a deficiency of methionine or tryptophan leads to pathologies, because these amino acids are not only incorporated into proteins but also used for the synthesis of other essential compounds.

Salmonds, including rainbow trout, Atlantic salmon (*Solar solar*), and lake trout (*Salvelinus nanaycush*) suffer from cataracts when given a diet deficient in methionine (Mambrini and Kaushik, 1995). The lens begins to become opaque after 2 to 3 months, depending on the extent to which the fish are deficient in sulfur amino acids. As the deficiency increases, lens opacity gradually progresses, causing a large reduction in light transmission. Cataracts also occur as a consequence of tryptophan deficiency in rainbow trout (Mambrini and Kaushik, 1995, NRC 1993). According to these authors, the developmental pattern of the cataracts is similar to that occurring in methionine deficiency.

Tryptophan deficiency leads to scoliosis (lateral curvature of the vertebral column) and to a derangement of mineral metabolism in certain Salmonds, including rainbow trout, Sockeye Salmo (*Oncorhynchus nerka*) (Halver and Shanks, 1960), and Chum Salmon (*Oncochynchus keta*) (Akiyama *et al*, 1986). Restoring tryptophan to normal concentrations in the diet may reverse scoliosis in Chum Salmo. The condition may be

related to a decline in levels of the brain neurotransmiter serotonin, which is formed from tryptophan. Thus, inclusion of serotonin. Deficiency of lysine have been reported to cause fin erosion and mortality.

In the light of the result of the present study, *D. innoxia*, especially the leaf, is rich in both essential and non-essential amino acids that could satisfy the amino acid requirement of Chun Salmo and some other fish species.

The LC_{50} values of 120.23 mg/l (upper limit of 150.29 mg/l and lower limit of 96.18 mg/l) for the leaf, 138.04 mg/l (upper limit of 154.61 mg/l and lower limit of 123.25 mg/l) for the seed, 199.53 mg/l (upper confidence limit 231.46 mg/l and lower confidence limit of 172.10 mg/l) for the stem, 181.97 mg/l (upper limit of 211.09 mg/l and lower limit of 156.87 mg/l) for the pod and 208.93 mg/l, (upper limit of 236.09 mg/l and lower limit of 184.89 mg/l) for the root was reported for this investigation. The 96 hour LC_{50} had earlier been reported by Onusiriuka and Ufodike, (1991) for the African catfish, *C. gariepinus* to be 25.71, 26.92 and 8.3 mg/l exposed to water extracts of floral parts of Akee apple (*Blighia sapida*), the bark of sausage plant (*Kigelia africana*) and the bark of *B. sapida* respectively. While Oronsaye and Ogbebo (1995) reported the LC_{50} (96 hours) of copper sulphate to be 0.4 mg/l, unionised ammonia was observed to have LC_{50} toxicity values of 6.5 and 9.10 mg/l for *C. gariepinus* and the *Heterobranchus* x *C. gariepinus* hybrid respectively.

The difference reported in this investigation and those of other workers could be attributed to the difference in toxicant and the age of the

experimental fish used. The restlessness, loss of balance, air gulping and convulsion observed in these investigations are in agreement with earlier reports of De-Silva and Ranasinghe (1989), Ufodike and Omoregie (1990), Okwuosa and Omoregie (1995) and Omoregie (2002) when they exposed fish to concentrations of various toxicants. These behavioural responses are indications of death due to nervous disorders and insufficient gaseous exchange across the gill epithelia.

Results from this investigation revealed that *C. gariepinus* exposed for 12 weeks to various sublethal concentrations of *D. innoxia* Leaf, seed, stem, pod and root extracts grew significantly less than unexposed fish (control). Weight increase over the 12-week period for the leaf was 30.66, 39.84.44.37, 49.70, 56.61 and 70.03% with concentrations 100.00, 50.00, 25.00, 12.50, 6.25 and 0.00 mg/l respectively. The seed was 30.62, 40.51, 44.38, 49.65, 57.16 and 69.79% with concentrations 100.00, 50.00, 25.00, 12.50, 6.25 and 0.00 mg/l. respectively. The stem was 30.66, 40.97, 44.10, 57.27and 69.86% with concentrations 100.00, 50.00, 25.00, 12.50, 6.25mg/l and 0.00mg/l. respectively. The pod was 30.35, 40.63, 44.56, 49.46, 57.30 and 69.60% with concentrations 100.00, 50.00, 25.00, 12.50, 6.25m and 0.00mg/l. respectively. Those of the root were 31.42, 42.14, 45.03, 51.64, 57.51 and 69.27% with concentrations 100.00, 50.00, 25.00, 12.50, 6.25 and 0.00mg/l. respectively. Results from this investigation indicated that fish exposed to sublethal concentrations of *D. innoxia* extracts grew significantly less and had poorer food conversion ratio than their counterparts in the media devoid of the extracts. It was observed that

the feed given to the fish was of high nutritional quality with a mean crude protein value of 44.5%. Observations also revealed that the water physico–chemical parameters in the various test tanks all had values that suggested tolerance range (Boyd, 1979). These observations showed that the food and water quality could not be the limiting factors for the poor growth and food conversion reported in this study. Earlier report by Oluwole and Bolarinwa (1995), observed that plant extracts contain several bioagents that are implicated in impaired physiology of laboratory animals. These authors documented that extracts of the plant, *Jatropha curcas* (family Euphorbiaceae), lead to the suppression of leucopoietic in rats. Oladimeji and Ologunmeta (1987), when they exposed Oreochromis niloticus made a similar observation to sublethal concentrations of lead. Similarly by Ufodike and Omoregie (1990), when *Oreochronis niloticus* were exposed to Gammalin 20 and Acetellic 25 EC, Omoregie and Okpanachi (1992) when they exposed *Tilapia zilli* to sublethal concentrations of crude extracts of *Azadirachta indica,* Omoregie *et al*., (1998), Omoregie and Onuogu, (2000), and Aguigwo (2002), when they exposed *Oreochromis niloticus*, *Aphyosemion gairdneri, Labeo senegalensis* and *C. gariepinus* to sublethal concentrations of various toxicants respectively. Those workers also reported the dose - dependent reduction in weight reported in the present investigations. The inhibition in growth reported in this study may therefore be due to impaired physiology and depleted food reserves hence becoming stressed as the exposure period increased.

Exposure of *C. gariepinus* fingerlings to sublethal concentrations of *D. innoxia* leaf, seed, stem, pod and root extracts for 12 weeks caused a significant ($P < 0.05$) decrease in Red Blood Corpuscle, Haemoglobin, Haematokrit and Mean Corpuscular Volume values. A similar result was obtained in *Oreochromis mossambicus* exposed to Ekalux (Sampath *et al.*, 1993). The significant reduction in Red Blood Corpuscle and Haemoglobin content on exposure to sublethal concentrations of *D. innoxia* extracts caused anaemia in *C. gariepinus* fingerlings, the decline of Mean Corpuscular Volume with increase in *D. innoxia* concentrations suggest that anaemic effect could be attributed to the destruction of the erythrocytes or inhibition of erythrocyte production. Similar trends in Red Blood Corpuscle in fishes exposed to various toxicants have been observed and reported by other workers (Koyama and Ozaki 1984, Srivastava and Narain 1985, Van Der Merwe 1992, Sampath *et al.*, 1993, Musa and Omoregie 1999, Bhagwant and Bhikajee 2000). Saponin, which is known to haemolyse RBC, was found to be present in *D. innoxia* (Eltohami 2002, George and Pamplona Roger 1998) which might explain the decline in haemoglobin content. Possibly the toxicants' (*D. innoxia* extracts) stress in the present study could have caused the anaemic condition in *C. gariepinus* fingerlings by haemolysis of the Red Blood Cells and thus reducing the Red Blood Cells count.

Clarias gariepinus white blood cells showed concentration dependent on increased toxicant. Ezeri (2001) Musa and Omoregie (1999) have also reported such an increase. The increase in white blood cells

count in the present study suggests that the immune mechanism of the experimental fish species become stimulated under *D. innoxia* extracts stress to fight against the pollutant in the environment. An increase in ESR value suggests tissue damage (Sampath *et al.,* 1993).

The fluctuation in the mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) in the present study, clearly indicates that the concentration of haemoglobin in the red blood cells were much lower in the exposed fish than in the control fish, depicting aneamic condition. Bhagwant and Bhikajee (2000) observed similar fluctuations. The Mean Corpuscular Haemoglobin Concentration is a good indicator of red blood cell swelling (Wepener *et al.,* 1992), the Mean Corpuscular Haemoglobin Concentration, which is the ratio of haemoglobin concentration as opposed to the haemotocrit, is not influenced by the blood volume for the number of cells in the blood, but can be interpreted incorrectly only when the new cells, with a different haemoglobin concentration, are released into blood circulation (Savio and Nikinma 1981). This investigation has revealed that changes occur in the physiological status of the fish when they are exposed to sublethal concentrations of *D. innoxia,* which is reflected in their haematological profile.

Omoregie *et al.* (1990) reported that sublethal concentration of toxicants in the aquatic environment would not necessarily result in outright mortality of aquatic organisms but may have significant effects, which can result in several physiological dysfunctions in the fish.

Studies have shown that when water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of the haematological parameters (Van Vuren, 1986). Thus, water quality is one of the major factors, responsible for individual variations in fish haematology, since they live in close association with their environment and are sensitive to slight fluctuation that may occur within their internal milicu (Cassilas and Smith, 1977). In the light of the present study, the non-significant change in the water quality parameters of the various experimental aquaria indicates that the sublethal concentration of *D. innoxia* extracts did not adversely lead to reduction in water quality as the values observed were all within tolerance range.

The dose-dependent reduction in the muscle and liver glycogen contents of the fish exposed to *D. innoxia* extracts was slight. The depleted muscle and liver glycogen contents indicated that fish were slightly stressed and that metabolism of carbohydrate was almost impaired. As earlier noted, fish increased their metabolic rates to metabolise and excrete toxicants and consequently, allocate more energy to haemostratic maintenance than storage, hence reduction in stored energy food as reserves. James et al (1996); Hontela (1997) and Heinimaa (2003) have documented increase in metabolic rate response to the presence of toxicant.

Increase in metabolic rate in response to toxicant was also documented by Olaifa *et al.* (2003) who observed that lead exerted toxic stress on *C. gariepinus* fingerlings. Roman and Dixon (1996) also noted

the same increase in the hepatic lipid of rainbow trout (Oncorhynchus mykiss) exposed to chronic toxicity of waterborne thiocyonate. The accumulation of *D. innoxia* by fish and excessive hepatocyte necrosis and vacuolation reported in *C. gariepnus* also accounted for the reduction in muscle and liver reserves in these group of fish. This is in agreement with the reports of Bleau *et al* (1996), Laflamme *et al* (2000) and Heinimaa (2003) who reported that one of the secondary tissue responses that reflect the effects of the stressors include depletion of liver glycogen. Vijayan and Moon (1992) and Vijayan *et al* (1997) reported that liver glocogen depletion might result fron the activation of enzymatic activities in the liver such as action of phosphorylase. Olademeji and Ologunmeta (1987) also reported that depletion of muscle and liver glycogen in fish might be due to insufficient absorption of soluble glucose from the intestine as well as a result of stress.

The progressive accumulation of plasma glucose reported in this study indicates hyperglycermic response in *C. gariepinus* when exposed to toxicant. This response was also observed to be dose-dependent. This hyperglycermic response was more pronounced in the highest concentrations of *D. innoxia* leaf than that of the lowest concentration. Levesque *et al* (2002) documented that fish can show marked hyperglycermic response in stress fill environment. Increase in plasma glucose according to Levesque *et al.* (2002) and Olaifa *et al* (2003) was due to the depletion of stored glycogen by the effect of the stress hormone catecholamine. The glucose will consequently be mobilized into the blood

stream. This result is in line with those obtained by Savio and Hunti (1977), Sampath *et al.* (1993) Knoph (1995) and Hovda and Linley (2000).

The crude aqueous leaf extract of *D. innoxia* was found to be a potent anaesthetic for *C. geriepinus* fingerlings at 3.00g/L within 28.21 (2.06) minutes. To be able to attain loss of reactivity to stimuli and to loose equilibrium, it took 28.21 (2.06) and 54.34 (2.85) minutes respectfully. At lower concentrations (< 2.50g/l) no observable changes in the fish took place. The efficacy of *D. innoxia* leaf extract was influenced by the dissolution of its active ingredients in chloroform-methanol (2:1) as evidenced by the significantly ($P < 0.05$) shorter time (0.90 minutes) and lower concentration (0.50 g/l) of the unseparated chloroform-methanol extract for *C. gariepinus* to loose reactivity to stimuli. In the crude aqueous extract, *D. innoxia* leaf was unable to induce *C. gariepinus* to a total loss of equilibrium, reduced opercular movement and minimal opercular movement. These behavioural events were however achieved in 1.16 (0.63), 1.28 (0.34) and 1.62 (0.44) minutes respectively at concentration 050g/l. Similar high efficacy was achieved using the lipid fraction of the chloroform-methanol extract where minimal opercular movement was attained at concentration 0.50 g/l within 2.43 (0.44) minutes of exposure.

Fish anaethesized with unseparated chloroform-methanol extract and the lipid fraction of the chloroform-methanol extract, however took longer time (23.00 and 21.11 minutes respectively at 3.00g/l) to recover than fish anaesthesized with crude aqueous extract of *D. innoxia* leaf extract at the same concentration. Induction time also varied significantly

($P<0.05$) with concentration of the extracts, the higher the concentration the shorter the anaesthetic time.

The results obtained for crude aqueous seed extract indicated that only loss of reactivity to stimuli could be attained. This took place 58.50 minutes of exposure. No reaction was observed at lower concentrations throughout the experimentation period. On the other hand, unseparated chloroform-methanol and the lipid fraction of the chloroform-methanol extract produced similar results to those of the leaf. The various concentrations were able to induce fish anaesthetisias ranging from loss of reactivity to stimuli to minimal opercula movement stage within the experimental period. The control did not produce any observable effects of indication of anaesthesia.

The crude aqueous extracts and the control of the stem, pod and root were not able to induce any anaesthetic effects on *C. gaeriepinus* fingerlings throughout the period of the experiment. The results of the unseparated chloroform-methanol and the lipid fraction of the chloroform-methanol extracts, on the hand, produced similar effects as in the leaf and seed though the anaesthetic time was significantly ($P<0.05$) longer, with the root having the longest anaesthetic time.

It was observed that anaesthetic time was influenced by dose concentration, plant part (leaf, seed, stem, pod and root) and extraction medium (aqueous, unseparated chloroform-methanol, lipid and non-lipid fractions of chloroform-methanol extracts). However, these effects and variations are not unlike the results of other anaesthetic used on fish as

observed by Jennings and Looney (1998), Kaiser and Vine (1998), Smith *et al*. (1999), Ross and Ross (1999), Edwards *et al.* (2000), Prince and Powell (2000), Hovda and Linley (2000), Roubach *et al*. (2001), Gomes *et al*. (2001), Kazun and Siwicki (2001), Sandodden *et al*. (2001), Browser (2001), Ortuno *et al*. (2002), Walsh and Pease (2002), Woody *et al*. (2002) and Wagner *et al*. (2003),

In general, the lower the dose the longer the time for the anaesthesia, but the faster the recovery. For the crude and non-lipid fractions of chloroform-methanol extracts of the leaf, seed, stem, pod and root no fish were anaesthetized within 30 minutes at 2g/l but were anaesthetized in unseparated chloroform-methanol and lipid fractions of chloroform-methanol extracts of the leaf, seed, stem, pod and root at 2g/l within 18 minutes. Although recovery was much longer in the unseparated chloroform-methanol and lipid fraction of chloroform-methanol extract, all fish recovered within 25 minutes of immersion into fresh aerated water. The recovery from anaesthesia seems to be extremely rapid as evidence in the carbohydrate values which were not significantly different from those of their controls. Thus indicating that the fish recovered fully from anaesthetic effect of *D. innoxia* which suggests minimal stress during the exposure period. This also indicates that external stimuli influeunce was minimized. Stress response has been reported with different anaesthetic drugs, such as MS-222 and quinaldine (Ross and Ross 1999) and normally is associated with exposure to high anaesthetic dosages or a prolonged exposure. Such result was not observed with *D. innoxia*

extracts, where blood plasma glucose was similar (P>0.05) for all dosages. Mean plasma glucose values of nine fish for each concentration was 72.80 (6.16) mg/l for 2.0 g/l dosage, 78.26 (0.40) mg/l for the highest dose (3.00g/l) of the unseparated chloroform-methanol and lipid fraction of the chloroform-methanol extracts of the leaf, seed, stem, pod and root and a value of 71.60 (4.28) mg/l for the control. The result of this research is in line with the findings of Le Brass (1982), Barham and Schoonbee (1990) and Knoph (1995).

In earlier work, Eze (1991) reported that *C. gariepinus* were anaesthetized in 4g/l aqueous extract of air-dried ground leaves of suaveolens, recovered and behaved normally after 24 hours. Mgbenka and Ejiofor (1998) reported that *C. gariepinus* and *Heterobranches longifilis* fingerlings were anaesthetized in up to 3.5g/l. Crude extract of air dried Erythrophleum suaveolens and recovered in fresh water. According to these authors, the two clariids, when exposed to pure unseparated extract were anaesthetized in significantly ($P < 0.05$) shorter time than in the crude extract.

The susceptibility of *C. gariepinus* (the species) to the anaesthetic could be reflected in the induction times, the shorter the induction time, the less the stress and hence faster time.

D. innoxia (leaf, seed, stem, pod and root) anaesthesia was not toxic to *C. gariepinus* tissue at concentration 3g/l and below within 180 minutes as indicated by absence of pathological, haematological changes and mortality. In all the tests, *C. gariepinus* fingerlings recovered and exhibited

no abnormal behaviour, swam and fed actively after recovery. Similar observations were made by Anderson *et al*. (1997), Bernier and Randall (1998) and Stone and Tostin (1999) when they exposed fish to clove oil, carbondioxide and clove bud oil respectively under laboratory conditions. Studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of the haematological parameters (Van Vuren 1986). Thus, water quality is one of the major factors, responsible for individual variations in fish haematology since they live in close association with their environment and are sensitve to slight fluuctuation that occur within their internal millieu (Cassilas and Smith 1977). In the light of the present study, there was no significant difference in ($P > 0.05$) between the water quality parameters of the control and the anaesthetic tanks and they were within tolerance range.

Woody *et al* (2002), reported that clove oil anaesthesized adult Sockeye Salmon more with increase in concentration and recommended 50mg/l of clove oil for anaesthetizing Sockeye Salmon from 400 to 550mm in length.

The histopathological sections of the gills, gonad, kidney and liver of *C. gariepinus* fingerlings exposed to sublettal concentrations of *D. innoxia* extracts showed that the groups of fish exposed to the highest concentrations had more pathological alternations compared to those exposed to the lowest concentrations.

The gills were the organs mostly affected by the toxicant. Hyperplasia at the base of the primary and secondary gill lamellae, odema at the tips of the primary lamelae and tenlangietasis at the base of the secondary lamellae were observed. Histopathology of the gill lamellae revealed dialation of the lamellar capillary and pooling of the blood. These changes according to the report of Penrice and Eddy (1993), Grizzle and Kinyen (1993) Al-Yuosuf *et al* (2000), Marty *et al* (1999), lead to impairment in gaseous exchange efficiency of the gills, thereby affecting metabolic rate of the fish. Necrosis and vacuolation of the hepatocytes reported in this work have also been reported by Omoregie and Ufodike (1991), Ericson *et al* (1998) Koponen *et al* (2001) Coetzee *et al* (2002) and Wenbin (2003) when fish were exposed to various toxicants. Necrosis of the hepatocytes in fish is due to the excessive work required by the fish to get rid of the effluent from the system during detoxification process of the liver. Necrosis could also be attributed to the inability of the fish to regenerate new hepatocytes as a result of constant presence of *D. innoxia* extract in the environment. Necrosis of the hepatocytes eventually results in prolonged sodium pump failure (Roberts 1978). The failure leads to hepatocyte vacuoles, hence the vacuolation of the hepatocytes of the fish with the highest concentration of *D. innoxia* extract.

4.2 CONCLUSIONS

The study has shown that *D.innoxia* is a highly effective, cost efficient and safe anaesthetic for *C. gariepinus* fingerlings for use in aquaculture and laboratory research setings.

Clarias gariepinus fingerlings were sufficiently sadated for normal sampling (length, weight scale sampling) in crude aqueous form of the leaf extract at concentration 3g/l (Temp. 22-28^{o}C). However, for surgical procedures, the fish require either the unseparated chloroform-methanol extract or lipid fraction of chloroform-methanol extract. *Datura innoxia*, being a plant, is biodegradable and so the amount of residues likely to be ingested by the consumer is very low and of no toxicological concern, besides the fish are unlikely to be consumed during treatment.

Datura innoxia is worthy of further study and evaluation, which should include residue and metabolite analysis. More safety data are needed on other fish species. In addition registration is needed with the National Agancy for Food, Drug Administration and Control (NAFDAC) before *D.innoxia* can be used in this country. It is hoped that this study will encourage others to continue additional testing.

4.3 SUMMARY OF THE RESULTS

Summary of the results of this research findings are:

The phytochemical screening of the plant (*D. innoxia*) revealed the presence of alkaloids, atropine, hyoscyamine, scopolamine, flavonoids, essential oils, tannins and saponins in the leaf, seed, stem, pod and root. Mineral analysis indicated the presence of calcium, magnesium, potassium, sodium, iron, lead, cadmium, chromium phosphorus and sulphur while amino acid analysis indicated the presence of essential and non-essential amino acids at various levels in the plant parts.

The mean LC_{50} for the leaf, seed, stem, pod and root were 120.23, 138.04, 123.25, 182.97, and 184.89 mg/1 respectively.

Weight gain by *C gariepinus* exposed to *D. innoxia* extracts ranged from 30.66 to 70.03% while muscle and liver glycogen decreased with increase in *D. innoxia* dose to *C. gariepinus* fingerlings

Fish exposed to 100 mg/l concentrations exhibited anaemic conditions as evidenced by the lower erythrocyte, haematocrite, haemoglobin and mean haemoglobin volume. Evidence of physiological stress as indicated by decrease in plasma glucose, due to the effects of *D. innoxia* extracts was observed to be higher with increase in concentrations, this was however not noticed in the control groups.

More pathological signs were observed in the gills, gonad, kidney and liver of *C. gariepinus* fingerlings exposed to *D. innoxia* leaf extract than those of the seed, stem, pod and root of the same concentration.

Aquoeus extract of *D. innoxia* leaf was found to sedate *C. gariepinus* to loose equilibrium while unseperated chloroform–methanol extract and the lipid fraction of chloroform-methanol extract of the leaf, seed, stem, pod and root induced *C. gariepinus* to pass through the various stages of anaesthesia depending on the concentration of the extracts.

4.4 THE CONTRIBUTIONS TO KNOWLEDGE BY THE FINDINGS OF THIS RESEARCH ARE:

1. *Datura innoxia* has been found to contain atropine, hyoscianine and scopolamine which are active drug ingredients
2. Mineral analysis indicated the presence of calcium, magnesium, potassium, sodium, iron, lead, cadmium, chromium phosphorus and sulphur
3. Amino acid analysis indicated the presence of essential and non-essential amino acids at various levels in the plant parts.
4. The mean LC_{50} for the leaf, seed, stem, pod and root were 120.23, 138.04, 123.25, 182.97, and 184.89 mg/l respectively.
5. Aquoeus extract of *D. innoxia* leaf was found to sedate *C. gariepinus* to loose equilibrium while unseperated chloroform–methanol extract and the lipid fraction of chloroform-methanol extract of the leaf, seed, stem, pod and root induced *C. gariepinus* to pass through the various stages of anaesthesia depending on the concentration of the extracts.

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Appendix 1: Vitamin and Mineral Composition of Diet Fed to *Clarias gariepinus* fingerlings.

Vitamin/mineral	Specification/100g
Vitamin A	15,000,000iu
Vitamin D3	4,400,000iu
Vitamin E	1,350iu
Vitamin K	4,350mg
Vitamin B2	4,350mg
Vitamin B6	2,350mg
Vitamin B12	11,350mcg
Vitamin C	1,000mg
Nicotinic Acid	16,700mg
Panothetic Acid	5,350mg
Potassium Chloride	87,000mg
Sodium Sulphate	212,000mg
Sodium Chloride	50,000mg
Magnessium Sulphate	12,000mg
Copper Sulphate	12,000mg
Zinc sulphate	12,000mg
Manganese Sulphate	12,000mg
Lysine	15,000mg
Methionine	10,000mg
Lactose Q. S.	1,000g

Appendix 2a: Mortality Rates of *C. gariepinus* Fingerlings Exposed to Various Acute Concentrations of *D. innoxia* Leaf Extract for 96 Hours. (Replicate1)

Conc. (mgl^{-1})	**Log Conc. (mgl^{-1})**	**Mortality rates (hours)**								**Mean mortality (%)**	**Mean Probit mortality**
		12	24	36	48	60	72	84	96		
200.00	2.30	-	4	5	1	-	-	-	-	100	8.72
180.00	2.25	-	4	2	1	-	-	-	-	70	5.52
160.00	2.20	-	2	1	1	1	1	-	-	60	5.25
140.00	2.15			1	2	2	-	-	-	50	5.00
120.00	2.08						1	1	3	50	5.00
100.00	2.00	-	-	-	-	-	-	1	2	30	4.48
0.00	0.00	-	-	-	-	-	-	-	-	-	-

Appendix 2b: Mortality Rates of *C. gariepinus* Fingerlings Exposed to Various Acute Concentrations of *D. innoxia* Leaf Extract for 96 Hours. (Replicate2)

Conc. (mgl^{-1})	**Log Conc. (mgl^{-1})**	**Mortality rates (hours)**								**Mean mortality (%)**	**Mean Probit mortality**
		12	24	36	48	60	72	84	96		
200.00	2.30		3	6	1	-	-	-	-	100	8.72
180.00	2.25		4	4	-	1	-	-	-	90	6.28
160.00	2.20	-	2	2	2	1	1	-	-	80	5.84
140.00	2.15			-	2	2	-	-	-	50	5.00
120.00	2.08							3	2	50	5.00
100.00	2.00	-	-	-	-	-	-	-	3	30	4.48
0.00	0.00	-	-	-	-	-	-	-	-	-	-

Appendix 3: Mean Water Quality Parameters obtained during the Exposure of *C. gariepinus* Fingerlings to Acute Concentrations of *D. innoxia* Leaf Extract for 96 Hours.

Parameters	Concentrations (mg/l)						
	200.00	180.00	160.00	140.00	120.00	100.00	0.00
Temperature (^{o}C)	22.**97**	22.98	22.96	22.94	22.92	22.90	22.90
	(0.65)	(0.60)	(0.65)	(0.66)	(0.68)	(0.63)	(0.65)
Dissolved oxygen (mg/l)	5.24	5.62	6.24	6.26	6.30	6.34	6.94
	(0.27)	(0.21)	(0.10)	(0.13)	(0.19)	(0.28)	(0.34)
Free carbondioxide (mg/l)	3.80	3.58	3.32	3.18	2.88	2.14	1.48
	(0.27)	(0.76)	(0.62)	(0.64)	(0.73)	(0.49)	(0.58)
Total alkalinity (mg/l)	32.60	31.80	30.50	27.00	25.80	22.80	19.88
	(1.72)	(2.01)	(2.01)	(0.89)	(0.91)	(1.62)	(0.37)
$_{P}$H	6.94	7.00	7.00	7.06	7.06	7.02	7.00
	(0.05)	(0.04)	(0.04)	(0.05)	(0.08)	(0.04)	(0.07)
Ammonia	0.23	0.22	0.22	0.22	0.22	0.22	0.22
(unionized NH_3)	(0.03)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)

Appendix 4a: Mortality Rates of *C. gariepinus* Fingerlings Exposed to Various Acute Concentrations of *D. innoxia* Seed Extract for 96 Hours. (Replicate1)

Conc. (mgl^{-1})	**Log Conc. (mgl^{-1})**	**Mortality rates (hours)**								**Mean mortality (%)**	**Mean Probit mortality**
		12	24	36	48	60	72	84	96		
200.00	2.30	-	3	4	-	-	-	-	-	70	5.52
180.00	2.25	-	1	2	2	1	-	-	-	70	5.52
160.00	2.20	-	1	1	2	2	-	-	-	60	5.25
140.00	2.15	-	-	-	1	1	2	-	-	40	4.72
120.00	2.08	-	-	-	-	-	2	1	1	40	4.72
100.00	2.00	-	-	-	-	-	-	-	-	-	-
0.00	0.00	-	-	-	-	-	-	-	-	-	-

Appendix 4b: Mortality Rates of *C. gariepinus* Fingerlings Exposed to Various Acute Concentrations of *D. innoxia* Seed Extract for 96 Hours. (Replicate2)

Conc. (mgl^{-1})	**Log Conc. (mgl^{-1})**	**Mortality rates (hours)**								**Mean mortality (%)**	**Mean Probit mortality**
		12	24	36	48	60	72	84	96		
200.00	2.30	-	2	5	1	-	-	-	-	80	5.84
180.00	2.25	-	2	2	3	-	-	-	-	70	5.52
160.00	2.20	-	1	1	1	2	-	-	-	50	5.00
140.00	2.15	-	-	-	2	2	2	-	-	60	5.25
120.00	2.08	-	-	-	-	-	1	1	2	40	4.72
100.00	2.00	-	-	-	-	-	-	-	-	-	-
0.00	0.00	-	-	-	-	-	-	-	-	-	-

Appendix 5: Mean Water Quality Parameters obtained During Exposure of *C. gariepinus* Fingerlings to Acute Concentrations of *D. innoxia* Seed Extract for 96 Hours

Parameters	Concentrations (mg/l)						
	200.00	180.00	160.00	140.00	120.00	100.00	0.00
Temperature (^{o}C)	20.19	20.22	20.20	20.19	20.23	20.20	20.21
	(0.20)	(0.10)	(0.20	(0.26)	(0.23)	(0.13)	(0.15)
Dissolved oxygen (mg/l)	4.08	4.42	4.68	5.06	5.64	6.34	7.12
	(0.32)	(0.33)	(0.35)	(0.33)	(0.21)	(0.16)	(0.32)
Free carbondioxide (mg/l)	7.70	7.04	5.54	4.80	4.05	3.64	3.456
	(0.99)	(0.82)	(0.62)	(0.71)	(0.54)	(0.52)	(0.47)
Total alkalinity (mg/l)	29.60	28.20	25.80	24.00	23.00	21.20	20.20
	(0.75)	(1.24)	(1.68)	(1.09)	(1.14)	(1.06)	(1.07)
$_{P}$H	7.004	7.00	7.06	7.06	7.04	7.02	7.01
	(0.06)	(0.04)	(0.03)	(0.03)	(0.03)	(0.02)	(0.01)
Ammonia	0.22	0.22	0.22	0.22	0.22	0.22	0.22
(Unionized NH_3)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)

Appendix 6a: Mortality Rates of *C. gariepinus* Fingerlings Exposed to Various Acute Concentrations of *D. innoxia* Stem Extract for 96 Hours. (Replicate1)

Conc. (mgl^{-1})	**Log Conc. (mgl^{-1})**	**Mortality rates (hours)**								**Mean mortality (%)**	**Mean Probit mortality**
		12	24	36	48	60	72	84	96		
400.00	2.60	8	-	1	-	-	1	-	-	100	8.72
350.00	2.54	3	5	1	-	-	-	-	-	90	6.28
300.00	2.48	1	3	1	2	-	-	-	-	70	5.52
250.00	2.40	-	1	1	1	2	1	-	-	60	5.25
200.00	2.30	-	-	-	-	2	2	1	1	60	5.25
150.00	2.18	-	-	-	-	-	1	1	2	40	4.75
0.00	0.00	-	-	-	-	-	-	-	-	-	-

Appendix 6b: Mortality Rates of *C. gariepinus* Fingerlings Exposed to Various Acute Concentrations of *D. innoxia* Stem Extract for 96 Hours. (Replicate2)

Conc. (mgl^{-1})	Log Conc. (mgl^{-1})	Mortality rates (hours)								Mean mortality (%)	Mean Probit mortality
		12	24	36	48	60	72	84	96		
400.00	2.60	9	-	1	-	-	-	-	-	100	8.72
350.00	2.54	4	4	-	-	-	-	-	-	80	6.28
300.00	2.48	2	2	2	1	-	-	-	-	70	5.52
250.00	2.40	-	2	1	1	1	1	-	-	60	5.52
200.00	2.30	-	-	-	-	1	2	1	1	50	5.00
150.00	2.18	-	-	-	-	-	2	1	1	40	4.75
0.00	0.00	-	-	-	-	-	-	-	-	-	-

Appendix 7: Mean Water Quality Parameters Obtained During Exposure of C. *gariepinus* Fingerlings to Acute Concentrations of *D. innoxia* Stem Extract for 96 Hours

Parameters	Concentrations (mg/l)						
	400.00	350.00	300.00	250.00	200.00	150.00	0.00
Temperature (^{o}C)	22.25	21.21	21.19	21.19	21.18	21.16	21.13
	(0.36)	(0.41)	(0.43)	(0.46)	(0.48)	(0.43)	(0.46)
Dissolved oxygen (mg/l)	6.75	6.80	6.85	7.35	7.58	7.87	8.32
	(0.36)	(0.55)	(0.43)	(0.43)	(0.80)	(0.79)	(0.88)
Free carbondioxide (mg/l)	6.37	6.20	5.50	5.03	3.70	3.50	2.99
	(0.27)	(0.23)	(029)	(0.31)	(0.35)	(0.27)	(0.20)
Total alkalinity (mg/l)	33.67	30.00	27.00	26.00	24.33	23.67	20.36
	(0.58)	(3.22)	(2.08)	(3.00)	(1.34)	(1.60)	(0.38)
$_{P}$H	6.94	7.03	7.07	7.06	7.10	7.02	7.07
	(0.07)	(0.07)	(0.04)	(0.05)	(0.05)	(0.05)	(0.04)
Ammonia	0.23	0.23	0.23	0.22	0.22	0.22	0.22
(Unionized NH_3)	(0.02)	(0.02)	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)

Appendix 8a: Mortality Rates of *C. gariepinus* Fingerlings Exposed to Various Acute Concentrations of *D. innoxia* Pod Extract for 96 Hours. (Replicate1)

Conc. (mgl^{-1})	**Log Conc. (mgl^{-1})**	**Mortality rates (hours)**								**Mean mortality (%)**	**Mean Probit mortality**
		12	24	36	48	60	72	84	96		
400.00	2.60	9	1	-	-	-	-	-	-	100	8.72
350.00	2.54	6	2	-	1	-	-	-	-	90	6.28
300.00	2.48	2	2	-	1	-	1	-	-	60	5.25
250.00	2.40	2	1	1	-	-	1	1	-	60	5.25
200.00	2.30	-	-	-	2	1	1	-	1	50	5.00
150.00	2.18	-	-	-	-	-	2	1	1	40	4.72
0.00	0.00	-	-	-	-	-	-	-	-	-	-

Appendix 8b: Mortality Rates of *C. gariepinus* Fingerlings exposed to various acute Concentrations of D. *innoxia* Pod Extract for 96 Hours. (Replicate2)

Conc. (mgl^{-1})	**Log Conc. (mgl^{-1})**	**Mortality rates (hours)**								**Mean mortality (%)**	**Mean Probit mortality**
		12	24	36	48	60	72	84	96		
400.00	2.60	9	-	-	1	-	-	-	-	100	8.72
350.00	2.54	6	2	-	-	1	-	-	-	90	6.28
300.00	2.48	4	1	1	-	1	-	-	-	70	5.52
250.00	2.40	1	1	1	2	-	-	-	1	60	5.25
200.00	2.30	-	-	-	3	1	-	-	1	50	5.00
150.00	2.18	-	-	-	-	-	1	1	2	40	4.72
0.00	0.00	-	-	-	-	-	-	-	-	-	-

Appendix 9 : Mean Water Quality Parameters Obtained During Exposure of *C. gariepinus* Fingerlings to Acute Concentrations of *D. innoxia* Pod Extract for 96 Hours

Parameters	Concentrations (mg/l)						
	400.00	350.00	300.00	250.00	200.00	150.00	0.00
Temperature (^{o}C)	20.39	20.34	20.31	20.30	20.18	20.18	20.13
	(0.20)	(0.22)	(0.21)	(0.20)	(0.19)	(0.16)	(0.14)
Dissolved oxygen (mg/l)	4.34	4.70	5.26	5.52	6.00	6.52	7.12
	(0.26)	(0.29)	(0.41)	(0.37)	(0.33)	(0.34)	(0.32)
Free carbondioxide (mg/l)	7.06	6.64	6.22	5.80	5.14	4.28	3.70
	(1.22)	(1.17)	(1.14)	(1.12)	(0.98)	(0.94)	(0.80)
Total alkalinity (mg/l)	31.20	29.40	27.80	26.00	23.20	21.40	20.40
	(1.02)	(1.16)	(0.86)	(1.14)	(1.39)	(1.29)	(0.81)
$_{p}$H	6.90	6.98	7.02	7.04	7.06	7.06	7.01
	(0.03)	(0.04)	(0.04)	(0.03)	(0.03)	(0.03)	(0.02)
Ammonia	0.22	0.21	0.21	0.21	0.21	0.21	0.21
(Unionized NH_3)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)

Appendix 10a: Mortality Rates of *C. gariepinus* Fingerlings Exposed to Various Acute Concentrations of *D. innoxia* Root Extract for 96 Hours. (Replicate1)

Conc. (mgl^{-1})	**Log Conc. (mgl^{-1})**	**Mortality rates (hours)**								**Mean mortality (%)**	**Mean Probit mortality**
		12	24	36	48	60	72	84	96		
400.00	2.60	9	1	-	-	-	-	-	-	100	8.72
350.00	2.54	1	2	4	-	-	1	-	-	70	5.52
300.00	2.48	2	1	2	1	-	-	-	-	60	5.25
250.00	2.40	-	-	2	3	-	-	-	1	50	5.00
200.00	2.30	-	-	-	-	-	2	1	1	40	4.72
150.00	2.18	-	-	-	-	-	-	-	2	20	4.16
0.00	0.00	-	-	-	-	-	-	-	-	-	-

Appendix 10b: Mortality Rates of *C. gariepinus* Fingerlings Exposed to Various Acute Concentrations of *D. innoxia* Root Extract for 96 Hours. (Replicate2)

Conc. (mgl^{-1})	Log Conc. (mgl^{-1})	Mortality rates (hours)								Mean mortality (%)	Mean Probit mortality
		12	24	36	48	60	72	84	96		
400.00	2.60	9	1	-	-	-	-	-	-	100	8.72
350.00	2.54	2	5	-	1	-	-	-	-	80	5.84
300.00	2.48	2	2	1	-	-	-	-	1	60	5.25
250.00	2.40	-	-	2	2	1	-	-	-	50	5.00
200.00	2.30	-	-	-	-	1	2	1	1	50	5.00
150.00	2.18	-	-	-	-	-	-	1	1	20	4.16
0.00	0.00	-	-	-	-	-	-	-	-	-	-

Appendix 11 : Mean Water Quality Parameters Obtained During Exposure of *C. gariepinus* Fingerlings to Acute Concentrations of *D. innoxia* Root Extract for 96 Hours.

Parameters	Concentrations (mg/l)						
	400.00	350.00	300.00	250.00	200.00	150.00	0.00
Temperature (^{o}C)	21.75	21.62	21.47	21.39	21.32	21.30	21.24
	(0.20)	(0.41)	(0.43)	(0.42)	(0.28)	(0.42)	(0.22)
Dissolved oxygen (mg/l)	6.83	6.98	7.05	7.21	7.71	7.82	8.08
	(0.83)	(0.90)	(0.83)	(0.56)	(0.90)	(0.27)	(0.48)
Free carbondioxide (mg/l)	5.28	5.23	5.08	4.93	3.83	2.78	2.58
	(0.51)	(0.48)	(044)	(0.45)	(0.23)	(0.84)	(0.35)
Total alkalinity (mg/l)	31.15	30.45	30.45	30.31	30.00	29.05	29.00
	(3.54)	(3.77)	(3.71)	(335)	(3.56)	(3.32)	(3.32)
$_{p}$H	7.00	7.00	7.00	7.06	7.08	7.03	7.03
	(0.07)	(0.07)	(0.07)	(0.03)	(0.04)	(0.03)	(0.03)
Ammonia (Unionized)	0.22	0.21	0.21	0.21	0.21	0.21	0.21
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)

Appendix 12: Mean Weight Gain by *C. gariepinus* Exposed to Sublethal Concentrations of *D. innoxia* Leaf Extract for 12 Weeks

Concentrations (mg/l)	Exposure period(weeks)						
	0	2	4	6	8	10	12
100.00	10.11	10.61	10.91	11.33	11.98	12.52	13.21
	(0.37)	(0.50)	(0.51)	(0.53)	(0.57)	(0.46)	(0.57)
50.00	10.09	10.68	11.17	11.90	12.55	13.18	14.11
	(0.27)	(0.58)	(0.59)	(0.63)	(0.49)	(0.44)	(0.48)
25.00	10.12	10.95	11.68	12.29	13.04	13.95	14.61
	(0.36)	(0.33)	(0.38)	(0.39)	(0.31)	(0.38)	(0.42)
12.50	10.12	11.20	12.02	12.82	13.50	14.42	15.15
	(0.33)	(0.48)	(0.45)	(0.53)	(0.32)	(0.40)	(0.48)
6.25	10.14	11.25	12.24	13.06	13.88	14.83	15.88
	(0.37)	(0.35)	(0.40)	(0.46)	(0.42)	(0.55)	(0.51)
0.00	10.11	11.45	12.71	13.91	14.92	15.91	17.19
	(0.29)	(0.32)	(0.24)	(0.20)	(0.28)	(0.33)	(0.38)

Appendix 13: Specific Growth Rate (SGR), Food Conversion Ratio (FCR) and Survival of *C gariepinus* Fingerlings Exposed to Sublethal Concentrations of *D. innoxia* Leaf Extract for 12 Weeks.

Parameters	**Concentrations (mg/l)**					
	100.00	50.00	25.00	12.50	6.25	0.00
Initial weight(g)	10.11	10.09	10.12	10.12	10.14	10.11
	(0.02)	(0.03)	(0.02)	(0.02)	(0.03)	(0.03)
Final weight (g)	13.21	14.11	14.61	15.15	15.88	17.19
	(0.04)	(0.04)	(0.05)	(0.05)	(0.05)	(0.05)
Weight gain (g)	3.1	4.02	4.49	5.03	5.74	7.08
	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)
Weight gain (%)	30.66	39.84	44.37	49.70	56.61	70.03
	(0.07)	(0.07)	(0.07)	(0.08)	(0.07)	(0.08)
SGR($\log_e W_2$-$\log_e W_1 x100/T_2-T_1$)	0.32	0.42	0.44	0.47	0.53	0.63
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
FCR(feed intake/weight gain)	1.28	1.05	0.98	0.91	0.83	0.73
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
Survival(%)	100	100	100	100	100	100
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)

Appendix 14: Mean Water Quality Parameters obtained During Exposure of *C. gariepinus* Fingerlings to Sublethal Concentrations of *D. innoxia* Leaf Extract for 12 Weeks

Parameters	Concentrations (mg/l)					
	100.00	50.00	25.00	12.50	6.25	0.00
Temperature (^{o}C)	20.54	20.50	20.50	20.50	20.50	20.50
	(0.34)	(0.34)	(0.34)	(0.34)	(0.34)	(0.34)
Dissolved oxygen (mg/l)	6.95	7.16	7.90	8.09	8.64	9.13
	(0.46)	(0.46)	(0.46)	(0.61)	(0.72)	(0.83)
Free carbondioxide (mg/l)	5.49	5.08	3.74	3.07	2.61	2.55
	(0.34)	(0.35)	(0.24)	(0.28)	(0.24)	(0.21)
Total alkalinity (mg/l)	28.63	27.33	26.72	26.72	25.61	25.6
	(1.73)	(1.59)	(1.76)	(1.78)	(1.65)	(1.65)
$_{P}$H	7.03	7.08	7.06	7.06	7.06	7.05
	(0.05)	(0.03)	(0.02)	(0.02)	(0.02)	(0.02)
Ammonia (Unionized)	0.21	0.21	0.21	0.21	0.21	0.21
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)

Appendix 15: Mean Weight Gain by *C. gariepinus* Exposed to Sublethal Concentrations of *D. innoxia* Seed Extract for 12 weeks

Concentrations (mg/l)	Exposure period(weeks)						
	0	2	4	6	8	10	12
100.00	10.09	10.57	10.92	11.31	11.96	12.49	13.18
	(0.56)	(0.53)	(0.57)	(0.55)	(0.53)	(0.56)	(0.58)
50.00	10.12	10.72	11.21	11.94	12.59	13.21	14.22
	(0.60)	(0.59)	(0.63)	(0.61)	(0.58)	(0.64)	(0.62)
25.00	10.14	10.98	11.69	12.30	13.06	13.88	14.64
	(0.47)	(0.49)	(0.46)	(0.48)	(0.53)	(0.44)	(0.50)
12.50	10.11	11.18	12.01	12.80	13.49	14.41	15.13
	(0.40)	(0.48)	(0.95)	(0.66)	(0.58)	(0.28)	(0.32)
6.25	10.13	11.24	12.21	13.01	13.90	14.81	15.92
	(0.62)	(0.56)	(0.60)	(0.55)	(0.45)	(0.54)	(0.59)
0.00	10.13	11.47	12.73	13.94	14.95	15.92	17.20
	(0.99)	(0.90)	(0.96)	(0.94)	(0.98)	(0.85)	(0.83)

Appendix 16: Specific Growth Rate (SGR), Food Conversion Ratio (FCR) and Survival of *C gariepinus* Fingerlings Exposed to Sublethal Concentrations of *D. innoxia* Seed Extract for 12 Weeks

Parameters	Concentrations (mg/l)					
	100.00	50.00	25.00	12.50	6.25	0.00
Initial weight(g)	10.09	10.12	10.14	10.11	10.13	10.13
	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)
Final weight (g)	13.18	14.22	14.64	15.13	15.92	17.20
	(0.04)	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)
Weight gain (g)	3.09	4.10	4.50	5.02	5.79	7.07
	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)
Weight gain (%)	30.62	40.51	44.38	49.65	57.16	69.79
	(0.06)	(0.07)	(0.07)	(0.06)	(0.06)	(0.07)
SGR($\log_e W_2$-$\log_e W_1$ x100/ T_2-T_1)	0.32	0.41	0.44	0.48	0.54	0.63
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
FCR (feed intake/weight gain)	1.28	1.04	0.98	0.90	0.83	0.73
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
Survival(%)	100	100	100	100	100	100
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)

Appendix 17: Mean Water Quality Parameters obtained During Exposure of *C. gariepinus* Fingerlings to Sublethal Concentrations of *D. innoxia* Seed Extract for 12 Weeks

Parameters	Concentrations (mg/l)					
	100.00	50.00	25.00	12.50	6.25	0.00
Temperature (^{o}C)	21.64	21.64	21.64	21.64	21.64	21.64
	(0.44)	(0.44)	(0.44)	(0.44)	(0.44)	(0.44)
Dissolved oxygen (mg/l)	6.76	7.01	7.18	7.71	7.98	8.11
	(0.48)	(0.46)	(0.42)	(0.48)	(0.66)	(0.62)
Free carbondioxide	5.13	4.93	4.42	4.13	4.04	3.86
(mg/l)	(0.55)	(0.25)	(0.21)	(0.20)	(0.34)	(0.42)
Total alkalinity (mg/l)	28.30	26.28	25.11	24.33	23.60	23.40
	(1.56)	(0.88)	(0.82)	(0.69)	(0.65)	(0.64)
$_{p}$H	7.08	0.78	0.78	0.79	7.1	7.1
	(0.03)	(0.03)	(0.03)	(0.03)	(0.02)	(0.02)
Ammonia (Unionized)	0.21	0.21	0.21	0.21	0.21	0.21
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)

Appendix 18:Mean Weight Gain by *C. gariepinus* Exposed to Sublethal Concentrations of *D. innoxia* Stem Extract for 12 Weeks

Concentrations (mg/l)	Exposure period(weeks)						
	0	2	4	6	8	10	12
100.00	10.11	10.64	11.01	11.47	12.03	12.67	13.21
	(0.68)	(0.69)	(0.66)	(0.65)	(0.67)	(0.68)	(0.64)
50.00	10.13	10.72	11.16	11.94	12.59	13.26	14.28
	(0.65)	(0.68)	(0.65)	(0.66)	(0.64)	(0.67)	(0.66)
25.00	10.16	11.02	11.70	12.16	12.97	13.81	14.64
	(0.47)	(0.43)	(0.42)	(0.44)	(0.49)	(0.46)	(0.41)
12.50	10.14	11.16	12.09	12.84	13.42	14.05	15.11
	(0.86)	(0.82)	(0.80)	(0.84)	(0.83)	(0.85)	(0.88)
6.25	10.11	11.21	12.28	13.05	13.94	14.88	15.90
	(0.80)	(0.88)	(0.86)	(0.84)	(0.78)	(0.79)	(0.83)
0.00	10.12	11.48	12.72	13.91	14.95	15.98	17.19
	(0.66)	(0.67)	(0.61)	(0.62)	(0.60)	(0.62)	(0.67)

Appendix 19: Specific Growth Rate (SGR), Food Conversion Ratio (FCR) and Survival of *C gariepinus* Fingerlings Exposed to Sublethal Concentrations of *D. innoxia* Stem Extract for 12 Weeks

Parameters	Concentrations (mg/l)					
	100.00	50.00	25.00	12.50	6.25	0.00
Initial weight(g)	10.11	10.13	10.16	10.14	10.11	10.12
	(0.02)	(0.02)	(0.03)	(0.03)	(0.02)	(0.02)
Final weight (g)	13.21	14.28	14.64	15.11	15.90	17.19
	(0.05)	(0.05)	(0.06)	(0.06)	(0.05)	(0.06)
Weight gain (g)	3.1	4.15	4.48	4.9799	5.79	7.07
	(0.02)	(0.02)	(0.02)	(0.02)	(0.03)	(0.03)
Weight gain (%)	30.66	40.97	44.10	49.01	57.27	69.86
	(0.07)	(0.07)	(0.07)	(0.08)	(0.08)	(0.07)
SGR($\log_e W_2$-$\log_e W_1$ x100/T2 T_2-T_1)	0.32	0.41	0.44	0.48	0.54	0.63
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
FCR (feed intake / weight gain)	1.28	1.03	0.98	0.91	0.82	0.73
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
Survival(%)	100	100	100	100	100	100
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)

Appendix 20: Mean Water Quality Parameters Obtained During Exposure of *C. gariepinus* Fingerlings to Sublethal Concentrations of *D. innoxia* Stem Extract for 12 Weeks

Parameters	Concentrations (mg/l)					
	100.00	50.00	25.00	12.50	6.25	0.00
Temperature (^{o}C)	20.50	20.50	20.50	20.50	20.50	20.50
	(0.34)	(0.34)	(0.34)	(0.34)	(0.34)	(0.34)
Dissolved oxygen (mg/l)	6.89	7.58	7.83	8.11	8.84	9.13
	(0.42)	(0.52)	(0.60)	(0.60)	(0.77)	(0.82)
Free carbondioxide (mg/l)	5.68	4.98	3.71	3.26	2.62	2.53
	(0.31)	(0.19)	(0.23)	(0.29)	(0.24)	(0.22)
Total alkalinity (mg/l)	28.28	26.02	26.63	25.98	25.6	25.45
	(1.73)	(1.91)	(1.82)	(1.78)	(1.65)	(1.68)
$_{p}$H	7.04	7.08	7.07	7.05	7.06	7.05
	(0.04)	(0.03)	(0.03)	(0.02)	(0.02)	(0.02)
Ammonia (Unionized)	0.21	0.21	0.21	0.21	0.21	0.21
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)

Appendix 21: Mean Weight Gain by *C. gariepinus* Exposed to Sublethal Concentrations of *D. innoxia* Pod Extract for 12 Weeks.

Concentrations (mg/l)	Exposure period(weeks)						
	0	2	4	6	8	10	12
100.00	10.15	10.59	10.96	11.34	11.99	12.54	13.23
	(0.18)	(0.20)	(0.24)	(0.19)	(0.25)	(0.22)	(0.21)
50.00	10.14	10.75	11.25	11.97	12.62	13.25	14.26
	(0.22)	(0.23)	(0.26)	(0.24)	(0.31)	(0.25)	(0.31)
25.00	10.10	10.95	11.66	12.27	13.04	13.92	14.60
	(0.34)	(0.33)	(0.38)	(0.36)	(0.31)	(0.29)	(0.28)
12.50	10.13	11.20	12.05	12.79	13.06	14.08	15.14
	(0.17)	(0.21)	(0.24)	(0.19)	(0.18)	(0.20)	(0.22)
6.25	10.14	11.17	12.19	13.07	13.88	14.79	15.95
	(0.27)	(0.26)	(0.28)	(0.23)	(0.21)	(0.29)	(0.22)
0.00	10.11	11.43	12.74	13.90	14.91	15.93	17.16
	(0.19)	(0.24)	(0.17)	(0.27)	(0.25)	(0.23)	(0.26)

Appendix 22: Specific Growth Rate (SGR), Food Conversion Ratio (FCR) and Survival of *C gariepinus* Fingerlings Exposed to Sublethal Concentrations of *D. innoxia* Pod Extract for 12 Weeks.

Parameters	Concentrations (mg/l)					
	100.00	50.00	25.00	12.50	6.25	0.00
Initial weight(g)	10.15	10.14	10.10	10.13	10.14	10.11
	(0.03)	(0.03)	(0.02)	(0.03)	(0.03)	(0.02)
Final weight (g)	13.23	14.26	14.60	15.14	15.95	17.16
	(0.04)	(0.06)	(0.07)	(0.07)	(0.08)	(0.06)
Weight gain (g)	3.08	4.12	4.50	5.01	5.81	7.05
	(0.02)	(0.02)	(0.02)	(0.03)	(0.03)	(0.02)
Weight gain (%)	30.35	40.63	44.56	49.46	57.30	69.60
	(0.07)	(0.08)	(0.08)	(0.07)	(0.08)	(0.08)
SGR($\log_e W_2$-$\log_e W_1$ x100/ T_2-T_1)	0.32	0.41	0.44	0.48	0.54	0.63
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
FCR (feed intake/ weight gain)	1.29	1.04	0.97	0.91	0.83	0.73
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
Survival(%)	100	100	100	100	100	100
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)

Appendix 23: Mean Water Quality Parameters obtained During Exposure of *C. gariepinus* Fingerlings to Sublethal Concentrations of *D. innoxia* Pod Extract for 12 Weeks

Parameters	Concentrations (mg/l)					
	100.00	50.00	25.00	12.50	6.25	0.00
Temperature (^{o}C)	21.42	21.42	21.42	21.42	21.42	21.42
	(0.36)	(0.36)	(0.36)	(0.36)	(0.36)	(0.36)
Dissolved oxygen (mg/l)	4.23	4.5	4.92	5.15	5.37	6.34
	(0.19)	(0.26)	(0.26)	(0.15)	(0.20)	(0.09)
Free carbondioxide (mg/l)	7.65	6.72	6.12	5.88	4.63	4.03
	(0.57)	(0.52)	(0.29)	(0.30)	(0.32)	(0.28)
Total alkalinity (mg/l)	29.54	27.03	26.00	24.58	23.08	22.42
	(0.42)	(0.36)	(0.52)	(0.66)	(0.60)	(0.59)
$_{p}$H	7.04	7.06	7.07	7.01	7.01	7.01
	(0.02)	(0.03)	(0.02)	(0.02)	(0.02)	(0.02)
Ammonia (Unionized)	0.21	0.21	0.21	0.21	0.21	0.21
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)

Appendix 24:Mean Weight Gain by *C. gariepinus* Exposed to Sublethal Concentrations of *D. innoxia* Root Extract for 12 Weeks

Concentrations (mg/l)	Exposure period(weeks)						
	0	2	4	6	8	10	12
100.00	10.12	10.82	11.27	11.82	12.34	12.82	13.30
	(0.23)	(0.21)	(0.38)	(0.50)	(0.56)	(0.44)	(0.42)
50.00	10.11	10.97	11.53	12.18	12.88	13.52	14.37
	(0.46)	(0.34)	(0.37)	(0.39)	(0.31)	(0.42)	(0.48)
25.00	10.15	11.10	11.76	12.42	13.27	13.96	14.72
	(0.36)	(0.39)	(0.31)	(0.46)	(0.28)	(0.40)	(0.42)
12.50	10.09	11.22	12.03	12.71	13.59	14.44	15.30
	(0.36)	(0.32)	(0.33)	(0.29)	(0.26)	(0.34)	(0.28)
6.25	10.13	11.40	12.17	12.92	13.83	14.88	15.95
	(0.27)	(0.34)	(0.28)	(0.26)	(0.38)	(0.23)	(0.25)
0.00	10.12	11.69	12.76	13.93	14.88	15.91	17.13
	(0.31)	(0.33)	(0.29)	(0.26)	(0.23)	(0.21)	(0.24)

Appendix 25: Specific Growth Rate (SGR), Food Conversion Ratio (FCR) and Survival of *C gariepinus* Fingerlings Exposed to Sublethal Concentrations of *D. innoxia* Root Extract for 12 Weeks

Parameters	Concentrations (mg/l)					
	100.00	50.00	25.00	12.50	6.25	0.00
Initial weight(g)	10.12	10.11	10.15	10.09	10.13	10.12
	(0.02)	(0.03)	(0.02)	(0.02)	(0.03)	(0.03)
Final weight (g)	13.30	14.37	14.72	15.30	15.95	17.13
	(0.04)	(0.05)	(0.05)	(0.06)	(0.05)	(0.04)
Weight gain (g)	3.18	4.26	4.57	5.21	5.82	7.01
	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)
Weight gain (%)	31.42	42.14	45.03	51.64	57.51	69.27
	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)
SGR($\log_e W_2$-$\log_e W_1$x100/ T_2-T_1)	0.33	0.42	0.44	0.50	0.54	0.63
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
FCR (feed intake/weight gain)	1.26	1.01	0.97	0.88	0.82	0.73
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
Survival(%)	100	100	100	100	100	100
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)

Appendix 26: Mean Water Quality Parameters obtained During Exposure of *C. gariepinus* Fingerlings to Sublethal Concentrations of *D. innoxia* Root Extract for 12 Weeks

Parameters	Concentrations (mg/l)					
	100.00	50.00	25.00	12.50	6.25	0.00
Temperature (^{o}C)	20.13	20.13	20.13	20.13	20.13	20.13
	(0.26)	(0.26)	(0.26)	(0.26)	(0.26)	(0.26)
Dissolved oxygen (mg/l)	6.32	6.49	6.96	7.20	7.52	7.88
	(0.26)	(0.30)	(0.43)	(0.44)	(0.36)	(0.58)
Free carbondioxide (mg/l)	5.29	5.14	4.83	4.38	3.87	3.46
	(0.34)	(0.35)	(0.34)	(0.33)	(0.31)	(0.24)
Total alkalinity (mg/l)	30.33	29.25	28.08	27.08	26.25	26.08
	(1.12)	(1.09)	(1.32)	(1.40)	(1.30)	(1.27)
$_{P}$H	6.98	7.01	7.05	7.05	7.04	7.03
	(0.04)	(0.04)	(0.02)	(0.02)	(0.02)	(0.02)
Ammonia (Unionized)	0.21	0.21	0.21	0.21	0.21	0.21
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)

Appendix 27: Comparative Mean Weight Gain (%) by *C. gariepinus* Exposed to Sublethal Concentrations of *D. innoxia* Extracts for 12 Weeks

Concentrations (mg/l)	Plant Parts				
	Leaf	Seed	Stem	Pod	Root
100.00	30.66	30.62	30.66	30.35	31.42
50.00	39.84	40.51	40.97	40.63	42.14
25.00	44.37	44.38	44.10	44.56	45.03
12.50	49.70	49.65	49.01	49.46	51.51
6.25	56.61	57.16	57.27	57.3	57.51
0.00	70.03	69.79	69.86	69.8	69.27

Appendix 28: Comparative Mean Food Conversion Ratio by *C. gariepinus* Exposed to Sublethal Concentrations of *D. innoxia* Extracts for 12 Weeks

Concentrations (mg/l)	Plant Parts				
	Leaf	Seed	Stem	Pod	Root
100.00	1.23	1.28	1.28	1.29	1.26
50.00	1.05	1.04	1.03	1.04	1.01
25.00	0.98	0.98	0.98	0.97	0.97
12.50	0.81	0.90	0.91	0.91	0.88
6.25	0.83	0.83	0.82	0.83	0.82
0.00	0.73	0.73	0.73	0.73	0.73

Appendix 29 Mean Water Quality Parameters obtained during the Exposure of *C. gariepinus* Fingerlings to Sedation of *D. innoxia* Leaf Extract.

Parameters	Concentrations (g/l)						
	3.00	2.50	2.00	1.50	1.00	0.50	0.00
Temperature (^{o}C)	22.90	22.90	22.90	22.92	22.92	22.90	22.90
	(0.60)	(0.60)	(0.65)	(0.61	(0.61	(0.63)	(0.65)
Dissolved oxygen (mg/l)	624	662	6.24	6.26	6.30	6.30	6.94
	(0.27)	(0.21)	(0.10)	(0.13)	(0.19)	(0.28)	(0.34)
Free carbondioxide (mg/l)	1.80	1.58	1.32	1.48	1.68	1.59	1.48
	(0.27)	(0.76)	(0.62)	(0.64)	(0.73)	(0.49)	(0.58)
Total alkalinity (mg/l)	23.60	31.80	30.50	23.00	23.80	22.80	22.80
	(1.70)	(2.00)	(2.01)	(0.89)	(0.96)	(0.62)	(0.37)
$_{P}$H	7.00	7.00	7.00	7.06	7.06	7.02	7.00
	(0.05)	(0.04)	(0.04)	(0.05)	(0.08)	(0.04)	(0.07)
Ammonia	0.23	0.22	0.22	0.22	0.22	0.22	0.22
(unionized NH_3)	(0.03)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)

Appendix 30 Mean Water Quality Parameters obtained during the Exposure of *C. gariepinus* Fingerlings to Sedation of *D. innoxia* Seed Extract.

Parameters	Concentrations (g/l)						
	3.00	2.50	2.00	1.50	1.00	0.50	0.00
Temperature (^{o}C)	22.90	22.90	22.90	22.92	22.90	22.90	22.90
	(0.60)	(0.60)	(0.65)	(0.61	(0.61	(0.63)	(0.65)
Dissolved oxygen (mg/l)	624	662	6.29	6.27	6.30	6.30	6.94
	(0.27)	(0.21)	(0.10)	(0.13)	(0.19)	(0.28)	(0.34)
Free carbondioxide (mg/l)	1.80	1.58	1.32	1.48	1.68	1.59	1.48
	(0.27)	(0.76)	(0.62)	(0.64)	(0.73)	(0.49)	(0.58)
Total alkalinity (mg/l)	23.60	31.80	30.50	23.00	23.80	22.80	22.80
	(1.70)	(2.00)	(2.01)	(0.55)	(0.96)	(0.62)	(0.37)
$_{P}$H	7.00	7.00	7.00	7.06	7.06	7.02	7.00
	(0.05)	(0.04)	(0.04)	(0.05)	(0.08)	(0.04)	(0.07)
Ammonia	0.23	0.22	0.22	0.22	0.22	0.22	0.22
(unionized NH_3)	(0.03)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)

Appendix 31 Mean Water Quality Parameters obtained during the Exposure of *C. gariepinus* Fingerlings to Sedation of *D. innoxia* Stem Extract.

Parameters	Concentrations (g/l)						
	3.00	2.50	2.00	1.50	1.00	0.50	0.00
Temperature (^{o}C)	22.50	22.31	22.59	22.58	22.18	22.58	22.50
	(0.30)	(0.44)	(0.43)	(0.40)	(0.42)	(0.43)	(0.60)
Dissolved oxygen (mg/l)	6.75	6.80	6.85	6.85	6.55	6.87	6.77
	(0.16)	(0.15)	(0.13)	(0.13)	(0.20)	(0.17)	(2.28)
Free carbondioxide (mg/l)	1.37	1.29	1.50	1.63	1.70	1.50	1.19
	(0.17)	(0.13)	(0. 20)	(0.10)	(0.15)	(0.20)	(0.21)
Total alkalinity (mg/l)	23.67	23.00	23.50	23.21	23.33	23.60	20.36
	(0.28)	(0.12)	(0.08)	(1.00)	(0.34)	(0.60)	(0.38)
pH	7.01	7.03	7.07	7.06	7.10	7.02	7.07
	(0.07)	(0.07)	(0.04)	(0.05)	(0.05)	(0.05)	(0.04)
Ammonia	0.23	0.22	0.22	0.22	0.22	0.22	0.22
(unionized NH_3)	(0.03)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)

Appendix 32 Mean Water Quality Parameters obtained during the Exposure of *C. gariepinus* Fingerlings to Sedation of *D. innoxia* Pod Extract.

Parameters	Concentrations (g/l)						
	3.00	2.50	2.00	1.50	1.00	0.50	0.00
Temperature (^{o}C)	22.39	22.34	22.31	22.30	22.18	22.18	22.13
	(0.21)	(0.60)	(0.65)	(0.61	(0.61	(0.63)	(0.65)
Dissolved oxygen (mg/l)	6.34	6.70	6.26	6.52	6.00	6.52	6.72
	(0.27)	(0.21)	(0.10)	(0.13)	(0.19)	(0.28)	(2.94)
Free carbondioxide (mg/l)	1.06	1.64	1.22	1.80	1.14	1.28	1.16
	(0.27)	(0.76)	(0.62)	(0.64)	(0.73)	(0.49)	(0.58)
Total alkalinity (mg/l)	23.60	31.80	30.50	23.00	23.80	22.80	22.80
	(1.20)	(1.17)	(1.14)	(1.12)	(0.98)	(0.94)	(0.80)
$_{P}$H	7.00	7.00	7.00	7.06	7.06	7.02	7.00
	(0.05)	(0.04)	(0.04)	(0.05)	(0.08)	(0.04)	(0.07)
Ammonia	0.23	0.22	0.22	0.22	0.22	0.22	0.22
(unionized NH_3)	(0.03)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)

Appendix 33 Mean Water Quality Parameters obtained during the Exposure of *C. gariepinus* Fingerlings to Sedation of *D. innoxia* Root Extract.

Parameters	Concentrations (g/l)						
	3.00	2.50	2.00	1.50	1.00	0.50	0.00
Temperature (^{o}C)	22.75	22.62	22.47	22.39	22.32	22.30	22.42
	(0.20)	(0.41)	(0.40)	(0.42)	(0.28)	(0.42)	(0.24)
Dissolved oxygen (mg/l)	6.83	6.98	6.05	6.21	6.71	6.82	6.08
	(0.83))	(0.90)	(0.83)	(0.56)	(0.90)	(0.27)	(0..48)
Free carbondioxide (mg/l)	1.28	5.23	1.08	1.93	1.83	1.78	1.58
	(0.55)	(0.48)	(0.40)	(0.45)	(0.29)	(0.14)	(0.35)
Total alkalinity (mg/l)	23.15	23.45	23.45	20.31	23.00	23.65	23.81
	(0.54)	(0.77)	(1.71)	(0.35)	(0.53)	(0.32)	(0.80)
$_{P}$H	7.00	7.00	7.00	7.06	7.08	7.03	7.03
	(0.07)	(0.07)	(0.07)	(0.06)	(0.08)	(0.03)	(0.03)
Ammonia	0.23	0.22	0.22	0.22	0.22	0.22	0.22
(unionized NH_3)	(0.03)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)