# PHARMACOLOGICAL EFFECTS OF METHANOLIC EXTRACT OF *GARCINIA KOLA* (HECKEL) SEED ON THE REPRODUCTIVE PROFILES OF SOME MALE EXPERIMENTAL ANIMALS

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## DECLARATION

I hereby declare that this work is the product of my own research efforts, undertaken under the supervision of Prof. (Mrs.) Mary O. Uguru with Prof. F.K. Okwuasaba as co-supervisor and has not been presented elsewhere for the award of a degree or certificate. All sources have been duly distinguished and appropriately acknowledged.

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## CERTIFICATION

This is to certify that the research work for this thesis and subsequent preparation of this thesis by Bukata Bayero Bukar (PGPH/UJ/13765/02) was carried out under my supervision with Prof. Okwuasaba as a co-supervisor.

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## DEDICATION

This work is dedicated to the glory of God, the provider of knowledge and to all males suffering from infertility.

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## ABSTRACT

*Garcinia kola* is commonly consumed in Nigeria in social ceremonies and for leisure purposes. It is speculated to enhance sexual performance in males. The effects of methanolic extract of *Garcinia kola* seed on the male reproductive profiles of rats were determined at oral doses of 125, 250 and 500 mg/kg for 20 and 60 days respectively. The animals were administered the extract orally for either 20 or 60 days after which analysis of the parameters were carried out using known standard procedures. The acute toxicity test revealed that the $LD_{50}$ of the methanolic extract is 3125 ± 52.68 mg/. The extract caused significant increase in luteinizing hormone and follicle stimulating hormone ($P$<0.05), but not so in testosterone compared to control ($P$>0.05). However, at 500 mg/kg there was decreased in the parameters especially in groups treated for 60 days. The results show that the extract did not significantly decrease sperm concentration and motility (slow progression). However, there was a significant decrease in rapid progression at all dose levels for both durations of treatment. Similarly there was no significant increase in death of sperm cells in treated groups compared to control ($P$>0.05). Results of the histological examinations of the testes, epididymis anterior pituitary and liver showed that the extract caused no visible serious morphological alterations on cells in rats treated with 125 and 250 mg/kg. The extract caused significant increase in testicular weights of rats in a dose-dependent manner ($P$<0.05). The fertility and reproductive performance tests showed that the extract at 250 mg/kg significantly increased the frequency of mounting, intromission and ejaculation ($P$<0.05). Similarly, the number of litters per female paired with male rats treated with the extract was found to be highest with the dose of 250 mg/kg for both the 20 and 60 days and lowest with 500 mg/kg. The extract caused a dose-dependent relaxation of an isolated corpus cavernosum smooth muscles of the rabbit. Results of

the sperm viability analysis showed that the extract of *G. kola* did not cause significant decrease in viability compared with control. The antithrombotic test showed that the extract caused significant decrease in bleeding and clotting time in a dose-dependent manner in treated rats compared with control ($P<0.05$). Similarly the extract caused significant decrease in platelet counts in treated groups compared to control but not in any particular manner. The extract caused decrease in both the onset and duration of action in phenobarbitone-induced sleep in treated rats. Results of the effect of the extract on blood pressures showed that the extract caused a dose-dependent decrease in blood pressure. The phytochemical analysis of the extract revealed the presence of alkaloids, glycosides, saponins, tannins, flavonoids, carbohydrates and steroids while the elemental analysis revealed the presence of copper, iron, zinc, magnessium, potassium and sodium. The results when considered together suggest that the methanolic extract of *Garcinia kola* seed can improve male sexual performance and fertility at 125 and 250 mg/kg. However, consumption of it continuously for a long period and in high quantity of 500 mg/kg should be discouraged to avoid deleterious effects on spermatogenesis and sperm cells.

# CHAPTER ONE
# INTRODUCTION

## 1.1 BACKGROUND TO THE STUDY

Reproduction is central to the continued existence of mankind on earth. In sexual animals including humans, reproduction is important for both males and females for life to continue. In humans, the importance of reproduction to married couples can hardly be over emphasized. Procreation is achieved by an act of sexual intercourse which commences the process of fertilization. For this process to be successful, the reproductive system of both the male and female must be in their normal conditions. Failure or inability to perform this important natural act by either partner will inevitably result in reproductive failure and this will raise many questions bordering on continuity of life and sometimes inheritance.

The desire for mankind to solve health problems dates back to pre-historic periods. One of such problems involves that associated with reproductive failure sometimes manifesting as infertility. Consequently, infertility, whether in the males or females remains both a medical and social problem. Generally speaking, infertility has been addressed more in females than in males especially in developing countries due to some traditional, cultural and religious reasons (Toppary *et al*., 1996). Paradoxically, there have been aggressive pursuits for medicinal products that will address decreased sexual performance experience by the males. The fact remains that infertility occurs in both males and females in equal proportion. This therefore underscores the importance of addressing the issue of male reproductive health problems which sometimes manifest as infertility. According to the World Health Organisation (WHO), infertility is defined as ‘the inability of a couple to achieve pregnancy following 24 months of unprotected intercourse’ (WHO, 1975; Larson, 2005). Infertility could be primary

(when the couple has had no pregnancy) or secondary (following a previous pregnancy). When infertility is due to a male factor, it is regarded as 'male infertility'. Male fertility disorders are said to account for 30 % of infertility globally. This has continued to elicit interest in the area of male fertility drugs, including those of plant-based. Problems of male reproductive health are grossly underestimated, but the reality is that male factors contribute significantly to infertility outcome and this is observed to be on the increase (Ikechebelu *et al*., 2003). On the other hand, sexual violence consequent to abnormally high sexual urge (aphrodisiomania) sometimes following the use of chemical agents (aphrodisiacs and fertility drugs) is on the increase. The use of herbal medicines to increase the sexual performance of the male-folks can be a contributing factor to this social violence.

It is therefore apparent that the inability for a man to bear children becomes a tragedy which brings a sense of loss, failure and exclusion. A demographic health and survey report by Rustein and Iqbal (2004) indicated that infertility also has demographic and health implications.

Knowledge of male reproductive health lags behind that of the female, perhaps for the fact that male infertility is basically expressed in the female and many factors could affect this expression. According to Feng (2003), a large number of infertile men are unable to impregnate their female counterparts because of lack of sperm (azoospermia), too little sperm (oligospermia), abnormal sperm morphology (tetratozoospermia) or insufficient sperm motility (athenozoospermia).

Serious alarm has been raised from studies on decreased or low sperm count over a period of 50 years (Carlsen *et al*., 1992; Swan *et al*., 2000). This has however attracted attention and debates among scientists (Jouanet *et al*., 2001). This therefore appears to remain a serious concern in contemporary studies. Toppari *et al*., (1996)

observed that male reproductive health has declined progressively since the Second World War as a result of changes in environmental or lifestyle factors. He further confirmed that the most fundamental change has been the striking decline in sperm counts in the ejaculates of normal men.

Eede (1995) had also revealed that more than 90 % of male infertility cases are due to low sperm counts, poor sperm quality or both, while Stevens *et al*., (1996) suggested that in 30-40 % of cases of sperm abnormalities, the cause remained largely unknown. However, Sinclair (2000) revealed that between 40% and 90% of male infertility is due to deficient sperm production, while Adeniji *et al*., (2003) reported that abnormal semen quality remained a significant contributor to overall infertility in men. According to Olayemi (2010), the factors responsible for male infertility could be inexhaustible. More worrisome for him are those factors that appear safe which include the use of medicinal plants. He admitted that the use of medicinal plants for male fertility disorders is on the increase, but posited that this could also be responsible for same disorders, manifesting as infertility. In a similar review, Amadi *et al*., (2011) corroborated his observation and cautioned on the public and reproductive health hazards from ingestion of herbs. Notwithstanding, as part of solution for male fertility disorders, drugs including those of plant-based are being employed to addresse such issues. This has shifted interest to medicinal plants for possible pharmacological interventions to the problems. Some of such medicinal plants are being evaluated for possible aphrodisiac and/or fertility properties especially at pre- clinical level in different laboratories all over the world. Many of such medicinal plants have been traditionally used for long by different communities. Examples of such medicinal plants include *Pausinystalia yohimbine, Tribulus terrestris, Lepidium meyenii, Fadogia*

*agrestis, Annona senegalensis, Garcinia kola, Sesamun* i*ndicum* among numerous others.

The plant, *Garcinia kola*, popularly called "bitter kola'' is one of such herbs being consumed purportedly to increase male sexual performance or to address the issue of male infertility among other disorders. This is due to a traditional claim that it possesses strong aphrodisiac or fertility property which has not been scientifically proved (Azija, 1998: personal communication).

## 1.2 AIM OF THE STUDY

The aim of this study is to investigate the pharmacological effects of *Garcinia kola* on the reproductive system and some other organs of male experimental animals in order to verify the traditional claims that it possesses aphrodisiac and/or fertility property.

## 1.3 SPECIFIC OBJECTIVES

The objectives of the study are to determine the effects of methanolic extract of *Garcinia kola* seed on male reproductive profiles and whether or not the effects vary with the dose and/or duration. Findings from the study will contribute to the pharmacological knowledge of *Garcinia kola* on the male reproductive system and also bring to the fore the often silent issues of male reproductive health disorders.

## 1.4 RESEARCH QUESTIONS

i). What are the pharmacological benefits of chronic ingestion of *Garcinia kola* on the male reproductive system?

ii). Does the seed extract of *Garcinia kola* posses any significant pharmacological effect on the measured parameters of the male reproductive system

## 1.5 STATEMENT OF PROBLEMS

The chronic consumption of *Garcinia kola* has for long been traditionally claimed to improve sexual performance in men among other benefits. The search for drugs that can address male fertility disorders, which account for 30 % of global infertility among couples, has elicited interest in research on medicinal plants as possible alternative source to current pharmacological interventions. This is coupled with the fact that infertility due to male factor is expressed in the female and is usually underestimated for some erroneous traditional, cultural and religious reasons.

## 1.6 SCOPE OF THE STUDY

The study essentially involves the pharmacological investigation of the methanolic extract of *Garcinia kola* seeds on the male reproductive system of experimental animals. Since the reproductive system is not a single entity, few other systems that are directly or indirectly linked to it are involved in the study to broaden our understanding of any observed effects

## 1.7 RESEARCH HYPOTHESIS

**$A_1$: NULL HYPOTHESIS:** Any observed pharmacological effect of *Garcinia kola* on any parameter of the male reproductive system or other biological systems of the experimental animals could be a chance occurrence and not real.

**$A_2$: ALTERNATIVE HYPOTHESIS:** Any observed effect of *Garcinia kola* on any parameter of the male reproductive system or other biological system could be real and not a chance occurrence.

## 1.8 RESEARCH FRAME WORK/DESIGN

The study will involve the chronic and non-chronic oral administration of the seed extract of *Garcinia kola* to the experimental animals at pre-determined doses

following standard procedures. In all cases the effects in treated groups are compared with that of control for appropriate decisions to be taken

# CHAPTER TWO
# LITERATURE REVIEW

## 2.1 RESEARCH ON MEDICINAL PLANTS

Plants and other living organisms are regarded as great potentials for drugs that could solve human intractable health problems. For a very long time, scientists especially those in the biomedical fields have identified two distinct types of biomedical researches that seek to develop each potential.

One type of the research is said to explore the value of medicinal plants as traditionally used. This type is said to constitute the only available medicines for most people in developing economies. The other type of research uses bioassays. This could produce useful lead compounds for the development of drugs.

According to the World Health Organization (WHO, 1977), a medicinal plant is "any plant which in one or more of its organs contains substances that can be used for the therapeutic purposes or which are precursors for the synthesis of useful drugs". Anonymous (2007) described the term "herbal medicine" as that part(s) of a plant used for preparing medicine.

Research on the abundant and potentially useful medicinal plants in Nigeria and most other developing countries are observed as critical in their economy and health system (Shellard, 1979; Idu, 2010). However, as once observed by Lambo (1980), "the continued existence of a fragile and irrelevant infrastructure is a common feature of many developing countries which has made it difficult for a truly national ideology to take place". It is my opinion that this ideology is needed more in the areas of research on medicinal plants. This is more curious given the present and perhaps future global socioeconomic and political intricacies which have rendered the developing countries entirely dependent on their developed counterparts for their drug needs which usually

come at high prices. Since the demand for drugs are said to be inelastic, this will always result in perpetual economic burden on such countries.

In a report titled" Bringing down drug cost: The Sri-Lankan example", Gunaratne (1980) suggested how developing countries can improve their health care system which should include the introduction of cost effective measures in the procurement of drugs such as the development and adoption of essential drug list (EDL) together with effective monitoring of drug distribution. To prove the urgent need and significance of this step the same report showed that there was an increase in pharmaceutical products marketed into developing countries most of which were without due consideration to the health need of such countries. The success of this indiscriminate market strategy was due to the lack of cost effective measures and the non introduction of the EDL by developing countries. Of equal importance as a reason was the aggressive but effective commercial promotion of such products. The commercial promotion of these drugs especially the new ones usually involve amounts that are more than the budgetary allocation to the health sector of these developing countries. This was observed to be the case in 1977 which resulted in high sale of pharmaceutical products to the developing countries amounting to the tune of seventy five billion dollars (Gunaratne, 1980). Despite these disturbing scenarios, there was still a wide difference in effective economic demand for pharmaceuticals between the developed and developing nations. For example, Gunaratne (1980) revealed that the amount spent per person annually on drugs in Germany was then $53.4, Japan ($38.5) and USA ($35.10), while in developing countries, such as Nigeria it was then as low as $1.2, India ($0.75) and Sri-Lanka ($0.58). This sharp difference was not easy to explain since the health expenditure in most developing countries were sometimes said to represent a sizeable proportion of the budgetary allocation to the health sector.

In order to check this ugly trend rational and cost effective approach for procurement, distribution and consumption of drugs was put into consideration. This has improved the situation to some extent in some of the developing countries, thus resulting in reduction of the overall drug expenditures. Some of the developing countries that could not check such ugly trends recorded high expenditures as was the case with Bangladesh with a drug expenditure of 63%, Nepal (44.3%), India (18.8%), Thailand (30.5%) and Burma (24.5%) (Gunaratne, 1980). It was perhaps with this scenario in mind that developing nations were advised not only to learn and adopt the meaning of self- reliance, but to also put it in practice by full implementation of their health policies. This was considered very imperative since majority of the population in such developing countries are said to patronize traditional medicine rather than orthodox, mainly for reasons of cultural beliefs, cost and availability (Chiwuzie *et al*., 1987). In most of developing countries, prices of drugs are usually so high that the liberty for choice by majority of the people is always restricted or even curtailed entirely. This scenario seems to have become a permanent feature to the extent that the larger population for the same reason of poverty depends on government health facilities for their medical needs. This is more so since the private health institutions are usually associated with high cost of health services not affordable by the majority poor. This could also explain why there are also shortages of essential drugs in government health institutions. This has consequently given birth to the circulation of fake or sub-standard pharmaceuticals by unscrupulous agents (Anonymous, 1988), all to the detriment of the large unsuspecting consumers. This has been complicated by the fact that most of the developing nations depend on the importation of drugs in their finished forms from the usually lean budgetary allocations to the health sector which in most

cases hardly meet the 5% requirement of the total budget of such countries as recommended by World Health Organization (WHO).

## 2.2 CHALLENGES ON PLANTS DRUG RESEARCH

For developing countries to fully realize their objective of affordable drugs for their teeming populations, scientists in such countries need to identify and maintain proper systems of drug procurement and distributions. This is usually made more complicated by deep conflict of interest in areas of priorities, sometimes resulting in involving the wrong personnel with little or no experience in the international drug market. The rich and influential who do easily afford to purchase the limited quality of imported drugs at any price usually question the rationale of research on our medicinal plants, while the policy makers do consider it too expensive and time consuming. Quality control mechanisms are also lacking and where available, are inefficient due to overuse thereby giving room to importation and subsequent consumption of fake and sub-standard pharmaceuticals at high costs and risk to lives (Anonymous, 1988). Moreover, issuances of import licenses for such pharmaceuticals are usually characterized with fraud whereby the licenses are wrongly issued to non-trained personnel.

In developing countries, it has been observed that while most of the researches are conducted by the Universities and Research Institutes, such are usually controlled by International donors and policy makers (Gunaratne, 1980). Findings from such studies are usually unlikely to be credited to the researcher or brought to recognition as such. In most cases the research techniques are regarded as sub-standard or outdated due to obsolete equipments and low quality laboratory animals, making reproducing of results in higher animals unpredictable and risky (Farnsworth & Moris, 1984).

The use of medicinal plants by traditional healers based on mere observations of signs and vague description of a disease condition by the patient or relatives, together with absence of records of such treatments can only be discouraged or eliminated through meaningful research on medicinal plants. Indeed, roughly 70% of adults using commonly consumed herbs do not do so in accordance with evidenced-based indications (Bardia *et al.,* 2007). Most government policies on health are not stable or sustainable since the government of such countries are themselves not stable or ideologically independent. This always creates unfavorable climates for investors to support independent researches on medicinal plants. Government at all levels in such countries have vital role to play in encouraging research on medicinal plants in order to boost the production of plant- based medicines from the abundant natural products. When this is fully realized, I believe our socio-economic profile will improve beyond its current, status since drugs are central to the health system of any given society and as the saying goes "Health is Wealth".

## 2.3 MEDICINAL PLANT RESEARCH

Medicinal plant research should always be a priority to scientists involved in drug development, especially for developing countries. Policy makers and health administrators alike are expected to make such researches national priorities in their countries (Weniger, 1991). This is suggested to be done through sustained motivation by adequate funding; the results of such research findings should be directly translated into fully developed drugs since the raw material will certainly almost be totally sourced locally. While this will ensure meeting most of our drug needs it could also lead to industrial revolution (Balandrin *et al*., I985). It is also believed that such research findings will be more relevant to the peculiar health needs of such countries. When fully realized, this is expected to ultimately reduce and perhaps eliminate the

heavy dependence on importation of all sorts of pharmaceuticals, some of which are not relevant to the health need of the population. Each country ought to have its national drug policy (WHO, 2004). According to Farnsworth (1991), a drug policy that has as a guiding principle the fact that most health problems in developing countries are different from those of their developed counterparts will certainly be most relevant and motivational. However, such a drug policy can be hardly complete, acceptable and cheap without considering the abundant medicinal plants found in such developing countries as their natural habitats. This becomes even true if the practice is socially blended and accepted by the indigenous people (Sofowora, 1982). The examples of China and India where medicinal plants have become intergraded part of their formal health system and are used in over 40 % of cases at primary health care level is certainly encouraging (Akerele, 1990). The continued deteriorating health problems in developing countries remain unresolved. Gunaratne (1980) submitted that factors such as disease pattern within a given geographical area, economic condition of the countries, strong political will, co-operation between the scientific community and policy makers or health administrators and reduction in over dependence on imported finished pharmaceuticals are important in addressing our drug demands.

Although accurate statistical data and the value and extent of use of medicinal plants and their products as pharmaceuticals are not readily available and appears lacking, WHO as at 1990 estimated that about 80% of the world's population was relying on traditional medicine providers who employ the use of medicine plants in most of their therapies. To support this revelation, Farnsworth and Soejarto (1985) reported that even in developed countries, plant- based drugs were highly in use. The same report indicated that in 1980, consumers of pharmaceutical products in the USA spent more than 800 million dollars on prescriptions containing active principles of

medicinal plants origin. Interestingly, there was an earlier indication of this observation in another report by Farnsworth and Morris (1984), which showed that from 1959 – 1980, 25 % of prescriptions in the USA contained plant extracts or their active principles. It was also revealed that 119 distinct chemical substances derived from 91 species of medicinal plants were in use as important drugs in various countries while about 62 distinct therapeutic categories were distinguished from such principles (Farnsworth & Moris, 1984; Farnsworth & Soejarta, 1985). Yet in another report, Farnsworth (1989) reported that of 76 compounds from higher plants usually found in USA prescriptions, only 7% were commercially produced by total synthesis, indicating the urgent need for cultivation and research on such plants. This was indeed corroborated by the report of Principe (1989), which showed that imports of medicinal plants and their products world-wide was estimated to be 355 million dollars as at 1976 which increased to 551 million dollars in 1989. Nonetheless, consumers may be misled by vendors' claim that herbal products can treat, prevent, diagnose or cure specific diseases (Astin, 1998; Morris & Avon, 2003).

Murray *et al*., (1994) reported that in 1989, the world spent close to 1.7 trillion dollars on health in poorer countries and yet health provisions in such countries were still inadequate especially in the area of drug supply. To address this disturbing challenge, especially through advocacy and motivation on research on our medicinal plants, Bisset (1991), stated that "while most of the medicinal plants have been in use for long , their early use in most cases were as poisons", a view earlier held by Farnsworth (1989). With this knowledge in mind, plants exhibiting poisonous effects should not be abandoned in research since the beneficial and toxic effects are in most cases said to be does–dependent. Considering the fact that most of the medicinal plants are presently obtained in developing countries, often at cheaper rates, but

paradoxically, most of the developing countries import 80-90 % of the raw materials (Elisabetsky, 1991), the need for intensive research on medicinal plants to first check the excessive loss due to foreign exchange and secondly to improve the health and socioeconomic conditions of the people need to be taken seriously by scientists and health administrators in developing countries. The urgent need for this call was indicated in a UNIDO 1986 statistics report which revealed that as at 1976, among the top 20 world medicinal markets, none was from Africa and only two (Venezuela & India) were from developing countries. The situation remained the same as at 1985 when it was discovered that USA topped the list in both year with sales of 7.96 billion dollars (18.3 % of the total word market in 1976) and 26.45 billion dollars (28.1% in 1985).

No doubt, relevant research on medicinal plants in developing countries may turn out to be a significant health and economic contribution such developing countries can make to the advancement of their citizens and the world in general (Tonkins & Work, 1945). This is even true, given the fact that transportation of medicine resulting from importation from countries with different climatic conditions have been associated with excessive lose of stability and potency (Abu-Reid, 1990). Therefore, the need for the establishment and maintenance of a pharmacopeia in respect of medicinal plants that are common to developing countries is timely. Such a pharmacopeia will contain relevant information such as the botanical and chemical names, pharmacological and therapeutic details etc. Such a record should also define clearly the methods of isolation of the active principles and how to prepare a stable formulation as well as cultivation method with requirement conditions to sustain production and availability of the plants.

## 2.4 GLOBAL ESSENTIAL MEDICINE CONSUMPTION

Statistical report showed that a large segment of the world population in developing countries does not have access to basic and essential drugs (Antezana, 1981; WHO, 2001; 2004; 2011). This is because these drugs are either unavailable in their respective countries, inadequately distributed or too expensive due to high import cost (Greenhalgh, 1986). This was clearly revealed in a consumption profile of pharmaceutical products between the developed and developing nations. It was shown that 77 % of the world population (then at 5.3 billion) were living in developing countries with a consumption capacity of only 21 % of the world's total medicines produced. This could have been due to high cost of the drugs or scarcity following lack of procurement as a result of high importation cost. Curiously, on the other hand, the developed countries with then a population of 1.2 billion (23 %) consumed nearly 95 billion dollars worth of drugs as against only 25 billion dollars for developing countries (Fazal, 1983; Reich, 1987; Ann.Stat., 1990). The same report indicated that, of the world's total population, 1.5 billion people in developing countries had little or no access to essential drugs. This was in sharp contrast to the situation obtained in developed countries where most, if not all of the 1.2 billion people were said to have regular and adequate access to essential drugs. These were the disturbing trends associated with poor health care delivery system of the developing countries that had earlier prompted the World Health Assembly (WHA) in 1973 to pass a resolution (WHA, 1975) directing WHO to develop means of selection and procurement of medicines according to the national needs of developing countries. In compliance, a first model list to that effect was first published by WHO in 1977. However, because the first measure could not give the desired impact, another resolution (WHA, 1979) was passed which saw the establishment of the Essential Drug (and vaccines) List

(EDL) in 1981 which to some extent and for the first time, appeared to have addressed the issue of irrational use of pharmaceutical products. Nevertheless, these resolutions, good as they were, could not adequately improve the poor health care delivery system of the developing countries. This was against the efforts of some of such countries of establishing their national drug policy, as was the case for Nigeria in 1990. This again could explain why majority of the people living in these countries, especially the low and medium income groups do not always have access to essential drugs relevant to tropical diseases. This negative trend seems to continue unabated since all necessary health measures do prove ineffective. A good and perhaps reliable solution for this intractable problem could lie in our vast but unexploited medicinal plants (Shellard, 1979; Sofowora, 1982). This could be true given the fact that some of these medicinal plants have been used for years to treat disease conditions by the indigenous people. According to Owolabi *et al.*, (2007), over 80% of the world's population relies on plant-based medicines. Large populated countries such as China and India rely solely on traditional medicine practice such as Ayuveda and Unani which employ the use of herbal drugs. Okigbo *et al*., (2008) underscored the use of medicinal plants which naturally have active constituents that are capable of cure of diseases or ailments. The medicinal properties of plants are diverse as investigations into their healing properties continue (Adesokan *et al*., 2008). Consequently, medicinal plants such as *Garcinia kola* could serve as a sure source of variety of drugs.

## 2.5 THE ECONOMIC VALUES OF MEDICINAL PLANTS

According to a report by Pearce and Moran (1994), ascribing economic value to medicinal plants can be done on two bases:

(i) The one that relates to the existing value which in turn are for commercial drugs and for traditional medicine.

(ii) The one that relates to the option value of plants i.e. the extent to which conservation is required to protect the future use value.

In all, plant-based medicines are said to be comparatively less expensive than synthetic drugs. The introduction of a single synthetic drug into the market is said to take about 10-15 years of time and about 100-300 million dollars in expense (Abelson, 1990). Plant species are used for medicinal purposes in two ways; both of which have economic values:

**(A)** As traditional medicines singly or in formulations such as those prepared and dispensed by traditional medicine practitioners (TMP), which may or may not attract a market price.

**(B)** As commercial products dispensed by prescription or as over-the-counter (OTC) sales such as patented or licensed medicinal products of allopathy or traditional system of medicine.

The economic values of medicinal plants are usually classified into the following:

1. Certain plant species are used in large number of formulations. The use of particular specie with reference to the number of therapeutic effects it exerts or the number of formulations in which it is an ingredient is expressed as the "therapeutic index" and "frequency index" respectively. Consequently a higher index reflects a higher economic value attributable to a particular specie, such species are referred to as "elite species" e.g *Azadirachta indica*.
2. Certain species are of great importance in the treatment of particular diseases as they happened to be the only specie (sometimes among very few) with that therapeutic potential e.g. alkaloids of *Catharanthus roseus* in the treatment of leukemia. Since the importance of the disease is also a factor, such species attract high economic value.

3. Some species have a narrow distribution or do occur in small population. Such species also command higher economic value, e.g. *Trichpis zeylanicus* found in India and use for improving standard, sex draft, bursting the immune system etc.
4. Certain species of medicinal plants e.g. *Rauwolfia serpentina* and *Saraca asoca* are over exploited and are becoming scarce in nature. While it is difficult to cultivate *R. serpentina* it is rather easy to propagate *S. asoca.*
5. There are synthetic substitutes for several original plant- derived products e.g. Clove oil, which affect their economic values. However, for others e.g. Digoxin and digitoxin, which, have not been produced synthetically, their values remained high. Some like vinblastine, vincristine and opiates etc that have been synthesized have proven to be less efficient than the natural products.
6. Time also affects the economic value of particular specie of medicinal plant. An effective synthetic substitute or the discovery of a better natural alternative or the disuse of the specie or product over a period of time may deplete the species of its value, eg. till sulphonamides come into use, Sandalwood oil was the most widely use effective antiseptic. However, sandalwood oil (*Santalum album*) has fallen into disuse as an antiseptic, but it has other uses with higher economic values.
7. The cost involved in isolation and purification of an active principle usually involve several considerations e.g. it requires about a tone of leaves of *C. roseus* to obtain 1 g of the alkaloid, vincristine, essentially needed to treat leukaemia. Vincristine is one of the expensive plant products costing about 24,000 dollars per gramme. Again, vinblastine, another alkaloid from the same

species use to treat Hodgkin's disease is present in quantity thousand times more than vincristine. It costs much lower, about 6,800 dollars per gramme.

## 2.6 PLANTS THAT IMPROVE MALE LIBIDO

For centuries, natural herbs and plants have been used to increase libido in men. These herbs can achieve this effect by different mechanisms such as acting directly on testosterone and sex hormones levels or by combining directly with neurotransmitters (e.g serotonin, noradrenaline) linked to sexual arousal. Though medicinal plant supplements are commonly used as libido enhancers, there seems to be lack of clinical evidence supporting such claims in most cases especially in developing countries.

There are however, numerous number of herbs claimed to improve male libido (Patel *et al*., 2011). Identifying such plants is crucial because nowadays more and more men are becoming interested in using natural herbs to enhance their sexual life. There are several herbs that can be used to activate sexual desire, promote erection and maintain prostate and at the same time increase virility. Some of these plants include.

### 2.6.1 Yohimbine

This is derived from the tree called *Pausinystallia yohimbine*. It is commonly referred to as "eternity medicine" It is discovered to increase nor-adrenaline level by up to 100% (Grossman, 1993). It appears to stimulate the brains sex centre in the hypothalamus, thereby increasing sexual arousal, an effect that is different from that of sildenafil (viagra$^{TM}$). It however assists in erectile dysfunction (Roland *et al*., 1997)

### 2.6.2 Tribulus

This is obtained from *Tribulus terrestris*, a plant found in tropical climate. It is sometimes referred to as the "Ayuvedic cure" because it is commonly found in both Chinese and Indian traditional medicines. It is used to combat low libido and physical stamina. It is said to stimulate the pituitary glands which releases LH leading to

increased testosterone levels in men and hence increase sex drive. A study showed that it increases sexual activity and prostate weight in treated rats as compared to those of control (Martino-Andrade *et al*., 2010)

### 2.6.3 Damiana

This is a native of South America. It is an aphrodisiac or libido enhancer dating back to the Mayan civilization. It also has effect on female libido. Zava *et al*., (1998) reported that Damiana herb contains properties that are similar to the effect of progesterone, possibly by their compatibility to bind with progesterone receptors

### 2.6.4 Horny Goat Weeds (Epimedium)

This is a native of South China. It is reported that it was first observed by a herder who saw an increase in sexual activity in his flock when they ate the plant. It is found to support the neurotransmitters in the brain which stimulate sexual arousal.

### 2.6.5 Tongkat Ali

This is a flowering plant native to Indonesia and Malaysia. It is believed to have a direct effect on increasing testosterone level in men. It is commonly used to treat both decreased sexual drive and erectile dysfunction

### 2.6.6 Panax Ginseng

This is a strain of ginseng commonly referred to as Korean ginseng. It is found to increase libido and circulation leading to erectile capability (Hong *et al*., 2002). It is also said to increase sperm count as well as motility. Red ginseng has also been investigated for treatment of erectile dysfunction (Dai-ja *et al*., 2008).

### 2.6.7 Maca (Peruvian Ginseng)

This is obtained from the plant *Lepidium meyenii*. This has long been used for sexual enhancement (Gonzales *et al*., 2002; Bustos-Obregon *et al.*, 2008).

### 2.6.8 Saw Palmetto

This is claimed to promote prostate health and has been used for such since the 19th century. It is believe to balance testosterone levels in the male's body and reduces prostate swelling that impedes urine flow. Blood flow may also be restricted due to prostate enlargement which can also affect erection.

### 2.6.9 Muira Puama

This is also known as potency wood. It increases libido and desire. It is effective in erection dysfunction and fatigue.

### 2.6.10 *Laudatia Phragmitiodes*

This is a pampas –like grass. It is a perennial plant that grows between 200-400 high. It is found in tropical Africa. It bears fruits with adherent pericarps (Clayton *et al*., 2006). In Nigeria, they are turned into arrow shafts and in Gabon it is used to make whistle. It is called "tsitsinyar maza" by the *Hausa* and "Erapo" by the *Yorubas*. The Hausa name covers several grasses of their type which are sold in markets as aphrodisiacs, but is not yet scientifically established whether it possesses this property (Dalziel, 1937; Burkill, 1985).

### 2.6.11 *Combretum Molle* (Family: Combretaceae)

This is a shrub or small, graceful deciduous tree 3-13 m high. It may be evergreen or deciduous and it yields gum. The leaves are soft. It is widely distributed in tropical Africa often occurring on ant-hills. It is a native of many Central African countries. It has several uses. In medicine, it is used to induce abortion and treat constipation, leprosy, headache, stomach pains, fevers and dysentery. The leaves and roots together are believed to be an anti-dote for snake bite. No mention of its claimed aphrodisiac property is documented (Beentje, 1994; Bekele-Tesemma *et al*., 1993).

### 2.6.12 *Cyperus Esculentus* (Tigernut) (Family: Cyperaceae)

According to Daniel and Maria (2000*), Cyperus esculentus* is found in sub-tropical regions of the northern hemisphere and often cultivated for its edible tubes (tiger nuts). It is described as an annual or perennial plant growing to 90 cm tall. It is commonly grown in Ghana, Nigeria, Burkina Faso, and Mali. It is said to have high commercial value. It is claimed to possess rich mineral content especially phosphorus and potassium. It is also claimed to possess aphrodisiac property as well as carminative, digestive, diuretic, stimulant and tonic properties (Chopra *et al*., 1986; Chavallier, 1996).

### 2.6.13 *Fadogia Agrestis* (Family: Rubiaceae)

This is indigenous to Nigeria and is said to have aphrodisiac property (Yakubu *et al*., 2005; 2008).

### 2.6.14 *Asparagus Africanus* (Family: Liliaceae)

This is an evergreen but perennial thorny plant growing as a climbing plant. It grows up to 5 ft high. It is widely distributed in tropical Africa. In Nigeria it is called "tarkon bera" (Hausa) and "Aluku" (Yoruba) (Daziel, 1956). It is found to be useful in some treatments (Hassan *et al*., 2008; Diesta, 1999).

### 2.6.15 *Borassus Aethiopum* (Family: Arecaceae)

This is specie of Borassus palm indigenous to tropical Africa. It is commonly referred to as African fan palm or Africa palmyra palm. In Nigeria, the *Igbos* called it "Ubiri" *Hausa* – "Giginya', *Yoruba* – Agbon Oludu" and the *Kanuris* –'Kenelutu'. It has many uses, the fruits being edible as well as the fresh roots. It grows up to 25 m high. Almost all parts of the plant are used, producing food, oil, timber, dyes, fibre, wine and raw materials for mats and baskets. The roots are claimed to be effective in the treatment of stomach parasites, bronchitis, sore throat and asthma as well as being

used for a mouth wash. The leaves are said to have strong aphrodisiac property. It is also reported as having significant anabolic effect (Akinniyi *et al*., 2010).

**2.6.16 *Annona Senegalensis* (Family: Annonoceae)**

This is commonly called African custard apple. It is either a shrub or tree. It is native to tropical Africa but can also be found in subtropical Africa. Its primary use is as foodstuff. The flowers, leaves and fruits are edible and culinary. According to Chhabra *et al*., (1987) and Kokworo (2009) the plant has many medicinal properties including aphrodisiac attributed to its Zinc content (Ogunlesi *et al*., 2009). It is also used for the treatment of venereal diseases (Sofowora, 1993).

**2.6.17 *Syzygium Guineense* (Family: Myrtacene)**

This is a forest tree found in many parts of Africa either as wild or domesticated. Its fruits and leaves are both edible. It is sometimes called "water berry". It grows to a height of between 10 and 15 m but some species can be as tall as 25 m. The bark is reported to possess a purgative property, according to a documentary report by Idu *et al*., (2010). Its use to enhance male sexual performance has not been established but a variety of it *S. aromaticum* has been reported as having aphrodisiac and fertility properties (Pallavi *et al*., 2011).

**2.6.18 *Acacia Sieberiana* (Family: Fabaceae)**

This is a perennial tree native to Africa. It grows 3- 25 m high. It is used as forage, medicine and wood. It is claimed to be effective in the treatment of urinary tract infections and many other indications.

**2.6.19 *Dichrostachys Cinera* (Family: Mimosacese)**

This is a legume. It is a native of Africa, but can be found in other places such as India, the Caribbean and parts of South–East Asia. It is a semi deciduous tree. It typically grows up to 7 m high and has strong alternative thorns. It is common in rain

forest zones at altitude of up to 2000 m. Its leaves and fruits are edible to animals. It is claimed to be effective in the treatment of headache, dysentery, toothache, leprosy, syphilis etc. It also serves as a laxative with antihelmintic property. The tender shoots of the plant are said to be effective when applied in cases of ophthalmic disorders. The root is astringent and is used in rheumatism. Jayakumari *et al*., (2011) reported that the plant has significant anti-urolithiatic activity thereby supporting the folkloric claim of its use in urinary calculi.

### 2.6.20 *Sesamum Indicum* (Family: Pedaliaceae)

This is commonly known as beniseed. It is a flowering plant indigenous to tropical Africa and some parts of India, but is widely cultivated in most of tropical regions of the world. It is one of the oldest plants to mankind. Its leaves and seeds are edible. Its seeds have high oil content and have made the plant a highly commercial item worldwide. It grows to a height of 50-100 cm. It is reported as having many health benefits (Kamal-Eldin *et al*., 2011; Anila and Vijayalakshmi, 2000). It has been investigated for its anti-lipidemic effect (Biwash *et al*., 2010), wound-healing property (Shenoy *et al*., 2011), fertility in male rats (Ashamu *et al*., 2010), anti-artherogenic activities (Visavadiya *et al*., 2009), vasorelaxant activity (Swesh-Kamar *et al*., 2008) and antimicrobial activity (Costa *et al*., 2007). Pamplona-Roger (2004) and Palavi *et al*., (2011) documented it among several other plants as having aphrodisiac property.

### 2.6.21 *Garcinia kola* (Family: Guttifereae)

This is a plant indigenous to most rain forest of tropical Africa. It grows to a height of about 10-13 m. It grows natural and is also cultivated in most of West and central Africa (Dalziel, 1937; 1956). The seeds are commonly chewed almost in an addictive manner by both men and women, but mainly by men in Nigeria and other West African countries. It is mainly chewed during recreation or when on heavy duty

and/or long distant journeys. The seeds possess strong bitter taste and are commonly called “bitter kola” by both its consumers and non-consumers. Local names for the seed include”Namijin goro” (Hausa), “Abilu” (Igbo) and “Orogbo” (Yoruba). The seeds are resinous and white in colour but covered with a dark–brown dusk. The seeds have been reported as possessing anti-infective and ulcerogenic effects (Oluwole *et al*., 1990; Hussein & Ogbowey, 1982). The seeds have also been reported to possess anti-hepatotoxic and antitithrombotic effects (Iwu 1985; Olajide, 1999; Akantonwa & Essein, 1990; Braide, 1991; Olatunde, 2000). According to traditional claims the seeds have been traditionally used for a very long time for sexual stimulation (Azija, 1998: personal communication). This claim has been investigated through some studies (Ajibola & Satake 1992, Ralebona *et al*., 2012, Uko *et al*., 2001). Arguably, Chilaka *et al*., (2009) reported that *G. kola* seeds produced decreased serum testosterone levels, decreased semen counts and decreased semen motility. The active constituents of the seeds have been isolated and identified as kolavirone which has been reported as possessing some pharmacological activities (Braide, 1990; Olatunde 2000; Adaramoye *et al*., 2005; Adaramola & Akinloye, 2000; Iwu *et al*., 1990; Tita *et al*., 2001, Adegboye *et al*, 2008). It is also reported as having anti-oxidant property (Olatunde *et al*., 2004), appetite suppressant property (Uko *et al*., 2001), erythropoietic effect (Esomonu *et al*., 2005), anti-artherogenic effect (Adaramoye *et al*., 2005, Ajani *et al*., 2008), antiinflamatory activity (Braide, 1993), antitrypansomial effect (Ogbadeyi *et al*., 2011) anticholesterolemia (Ahumobe & Braide, 2009) and brochodilator effect (Orie & Ekon, 1993). According to a report by Eze and Eze (2007), *G. kola* has a high economic value as a raw material in pharmaceutical and food industries; with great demand in many countries such UK, USA, Japan, Germany, China, France, India, and

Italy. It is reported that a kilogram of the seed commands an international price of 17 dollars and a local price of 200 NGN.

A recent study of the seed extract in animal showed increased in activities of the enzyme lactate dehydrogenase and glucose-6-phosphate dehydrogenase (Olajide & Adeniyi, 2011). An ethanolic extract of the seed has been found to enhance growth of catfish (Dada & Ikuerowo, 2009). Adesuyi *et al*., (2012) reported the importance *of G. kola* as a source of carbohydrate and protein with presence of some essential trace elements. Nigeria is said to produce over 70% of bitter kola in the whole world and farmers valuation of *Garcinia kola* include; medicinal (41.8%),food/social value (31.7%), fuel wood (10%) and cash value (8.4%) (Aiyelaagbe *et al*., 2003). Indeed, *Garcinia kola* is said to be one of the many non-timber forest products that are of high socioeconomic importance (Adebisi, 2004).

## 2.7 THE MALE REPRODUCTIVE SYSTEM

The male reproductive system basically consists of the following components, not in any particular order:

### 2.7.1 A Pair of Testes

These are enclosed by the scrotum and each testis consists of several lobules that are composed of germinal epithelial cells supported by sertoli cells. These together form the seminiferous tubules and in between the tubules are the interstitial or Leydig cells. The anterior pituitary hormone, follicle stimulating hormone (FSH) stimulates the sertoli cells to produce the germ cells (spermatozoa) while another pituitary hormone, leutenising hormone (LH) stimulates the Leydig cells to produce testosterone (Lipsett, 1980; Plant & Marshal, 2001).

### 2.7.2 A Pair of Epididymis

These are convoluted tubules joined from the seminiferous tubules which eventually coalesced to form the vas deferens duct. The primary function of the epididymis is for maturation and storage of the spermatozoa (Turner, 1979; Jones, 1999).

### 2.7.3 A pair of Seminal Vesicles

These produce seminal fluids which mix with the sperms to form the semen.

### 2.7.4 A pair of Spermatic Cords

These are said to suspend the testes in the scrotum which enables it to decent outside the body. They eventually joined the ductus as vas deferens to form the ejaculatory ducts.

### 2.7.5 A pair of Ejaculatory Ducts

These ensure the safe and complete passage of the semen during ejaculation.

### 2.7.6 A pair of Prostate Glands

These are found within the pelvic cavity and they produce secretions that form part of the seminal fluid during ejaculation.

### 2.7.7 The Penis

This consists of three elongated masses of erectile tissues that are very rich in blood vessels. Two of the three tissues are called corpora carvernosa and are on the upper part of the penis while the third is called corpus spongiosum which expands beneath the other two to form the penis. The corpora carvernosa are essential for penile erection when they are relaxed which allow flow of blood into the arteries

Of all the components of the male reproductive system, it is only the testes that are considered as primary sex organs while the others are regarded as accessories or secondary.

## 2.8 FUNCTIONS OF THE MALE REPRODUCTIVE SYSTEM

The basic function of the male reproductive system is production of spermatozoa which has been identified to involve essentially three phases (Roosen-Runge, 1962; Clermont, 1966; Huckins, 1971; Kluin *et al*., 1982; de Rooij and Russell, 2000).

**(a) Phase I- Stem cell renewal**

This is sometimes otherwise referred to as spermatocytogenesis and is basically mitotic. This phase guarantees the continuous availability of undiminishing number of undifferentiated germ cells for subsequent step of spermatogenesis (Sanderson, 2006). Stem cells at this stage are said to be of two types:

I. Dark- type A (AP), which rarely divide under normal conditions.

II. Pale-type A (AP), which actively divide.

**(b) Phase II- Germ cell proliferation**

This is essentially meiotic and it commences when 50% of the daughter cells divide by replicating themselves. There are about 13 steps involved in this phase and it terminates with the formation of primary spermatids.

**(c) Phase III- Spermiogenesis**

This essentially involves the morphological changes in the immature or primary spermatids that results in the generation of a highly differentiated and motile spermatozoa.

This cycle is said to take between 63 and 74 days to complete. The sperm, which is the end product, is considered the main reproductive cell in males. The sperms differ in that each carries with it a set of chromosomes dividing each into either a male (Y-gene) or female (X-gene) sperm.

While the primary roles of FSH and LH on spermatogenesis have been firmly established, the relative importance of FSH on the initiation and maintenance of the process has been debatable over the years. Plants and Marshall (2001) submitted that FSH may not be required for the initiation of spermatogenesis in primates, but this could not necessarily imply that LH is obligatory for the initiation of spermatogenesis.

## 2.9 HORMONAL REGULATION OF THE MALE REPRODUCTIVE SYSTEM

Hormones that have been identified as having control over the male reproductive system play vital roles on the integrity of the system.

The gonadotrophin-releasing hormone (GnRH) is secreted by the hypothalamus into the anterior pituitary gland which eventually releases the gonadotrophic hormones, FSH and LH. The LH stimulates the Leydig cells in the testes leading to the production of testosterone while the FSH, under the influence of testosterone stimulates the sertoli cells to produce spermatozoa. Testosterone is therefore commonly called the "male hormone" or "androgen". This is more so because it is responsible for all the male physical manifestations (secondary characters) beginning at puberty.

It is therefore obvious that the primary regulator of the male reproductive system is the hypothalamic-pituitary-gonadal (HPG) axis (IPCS, 2002; Kronenberg, 2008). In addition, according to the report, the hypothalamic-pituitary-adrenal (HPA) axis, which consists of neurons in the hypothalamus, complements it by release of corticotrophin-releasing hormones (CRH) into the blood which stimulates the synthesis and secretion of adrenocorticotropic hormones (ACTH) that play important roles in nutrient metabolism, anti-inflammatory effect and stress responses. The HPA also affects the male reproductive system through adrenal gland secretions of weak androgens which include dehydroepiandrosterone (DHEA), androstenedione and

DHEA-sulphate which are peripherally converted to more potent androgens (testosterone and dihydrotestosterone) in target tissues (Kronenberg, 2008).

Another regulator of the male reproductive system is the hypothalamic-pituitary-thyroid (HPT) axis which secretes thyrotrophic-releasing hormone (TRH) into the blood leading to the synthesis and release of thyroid-stimulating hormone (TSH) into the general circulation. It is reported that during the pre-pubertal stage circulating thyroid hormones cause the transformation of sertoli cells from immature, proliferative cells to mature, non-dividing forms (Jannini *et al*., 1995). Fluctuations in the levels of thyroid hormones is said to directly affect testicular weight and sperm production (Holberger, 2005)

## 2.10 PENILE ERECTION

The erection of the penis is critical on the integrity of any man. The erection of the penis is said to depend on a complex interaction of psychological, neural, vascular and endocrine factors. Erection is said to usually occur when the corpora carvenosa become filled with blood. This largely depends on the vasodilatation induced by parasympathetic nerves. This may result from any physiological stimuli. Penile erection usually results from sexual stimulation and/or arousal, but can also occur by such causes as full bladder or during wet dreams. Erection is said to be associated with the development of sexual desire (libido), but not necessary with fertility. Erectile dysfunction (impotence), on the other hand inhibits erection. This is common in old age but can also have a physical cause such as injury, disease or side effects of some drugs. It is observed that any disorder that impairs blood flow in the penis or cause injury to the nerves has the potential to cause erectile dysfunction with failure of penile erection.

## 2.11 DISORDERS OF THE MALE REPRODUCTIVE SYSTEM

A healthy male reproductive system is the desire of all including the women. In many cultures and especially in ancient Roman law all citizens are determined on purely patrilineal basis, just as the modern inheritance of surnames. In the Bible (and perhaps books of other religions) the line of descendants for monarchs and main personalities is almost exclusively through the main male personality. Again it is said that in cultural anthropology, a patrilineage is a consanguineal male and female kin groups each of which is related to the common ancestors through male forebears.

Disorders of the male reproductive system which in most cases manifest as male infertility, sub-fertility or decreased sexual performance remain a social or medical challenge. The disorder could simply refer to any abnormality (Carlsen *et al*., 1992). As a result, there has been a rise on the interest and concern in all the factors potentially affecting male infertility since several studies support progressive decline in male fertility over the last few decades. However, this has not been as simple as it appears as the debate goes on. This is even more so as it has been observed that a good portion of apparently normal males are unable to impregnate a woman, even when the woman is also considered to be normal. According to Shaban (2007), there are various causes of male fertility disorders some of which could be pre-testicular, testicular or post-testicular.

### 2.11.1 Pre-Testicular Causes

#### (a) Hypothalamic Disease

This is associated with deficiency of the gonadotropins (FSH & LH) and is collectively called Kallmann's syndrome. It is however said to be uncommon.

**(b) Pituitary Disease**

This is said to result due to pituitary insufficiency either as a result of tumor, infections, or iatrogenic causes like surgery or radiation. It can result from hyperprolactinemia and hemochromatosis**.**

### 2.11.2 Testicular Causes

These are said to include chromosomal abnormalities such as Klinefelter's syndrome (presence of an extra X chromosome), Noonan and Turner's syndromes (genetic congenital disorders), myotonic dystrophy (a multi-systemic disorder), bilateral anorchia (vanishing testes syndrome), sertoli-cell only syndrome (germ cell aplasia), gonadotoxins (drugs, radiations), orchitis, trauma, systemic diseases (e.g. renal failure, hepatic diseases, sickle cell disease), defective androgen synthesis or action, cryptorchidism and varicocele.

### 2.11.3 Post-testicular Causes

This essentially involves disorders of sperm transport, motility or function and sexual dysfunctions. Sperm transport disorder can be congenital and is usually associated with azoospermia due to absence of the major portion of the epididymis, vas deferens and seminal vesicles. It is said to also be acquired due to bacterial infections which may involve the epididymis with subsequent scarring and obstruction. It can also be due to functional obstruction following neuropathic insults like injuries to the sympathetic nerves resulting in lack of emission and failure of the bladder to close at the time of ejaculation leading to retrograde ejaculation. Diabetic males with autonomic neuropathy are usually associated with this failure. Spinal cord injury can result in paraplegia or quadriplegia with resultant erectile dysfunction and lack of emission or ejaculation. Many medications such as tranquillizers, antidepressants, and anti-hypertensives that interfere with the sympathetic nervous system affect sperm transport.

Disorder of sperm motility or function can also result from congenital defects of the sperm tail, maturation defects or immunological defects. In some cases, the sperm count can be normal, but motility can be reduced or even absent. Epididymal dysfunctions can result in maturation defects of the sperm. Sperm antibodies and infections have also been implicated in a number of male fertility disorders. Sexual dysfunction has been reported in quite a number of infertile men. Sexual dysfunction presents as (1) decreased sexual drive, (2) erectile dysfunction, (3) premature ejaculation and (4) failure of intromission. Decreased libido and erectile dysfunction may reflect low serum testosterone levels, with perhaps an organic cause. Performance anxiety is also often a cause, but can be abated with reassurance.

## 2.12 FACTORS THAT AFFECT MALE FERTILITY

Many factors that predispose an individual male to infertility have been identified. It has been reported that vasectomy (56%), varicocele (14%), absence of sperm (6%) and unknown reasons (8%) account for most of the infertility in the males. Notwithstanding, the following factors have been associated with infertility in the males.

i. **Age:** Though, there is no clear correlation between age and infertility as in women, reduction in sperm count and quality has been reported especially in men above 50 years. Also, genetic defects in sperm have been noted with advances in age (Fieldman *et al*, 1994; Hassan & Killick, 2003; Araujo *et al*, 2004). However, this seems to remain controversial as findings by Priskorn *et al*., (2014) revealed that there is no convincing effect of age on a man's semen quality.

ii. **Lifestyle:** Several lifestyle factor are observed to decrease sperm counts, some of which include:

**(a)** **Emotional Stress:** This is said to interfere mainly with GnRH and other sperm pathologies (Senbel & Taylor, 1982; Collodel *et al*., 2008).

**(b)** **Sexual Issues:** These include impotence, premature ejaculation, psychological/relationship issues (McGrady, 1984).Use of condoms with lubricants, spermicides, oils and vaseline affect fertility.

**(c)** Testicular over heating e.g. due to high fever, saunas, and hot tubs can lower sperm counts. It has also been observed that driving for only 2 hours every day can increase temperature in the scrotum and this can reduce sperm counts.

**(d)** Substance abuse e.g. cocaine and marijuana appear to temporarily reduce the number and quality of sperm s by as much as 50%.Sperms are said to be receptors for some compounds in marijuana that may impair the sperm's ability to swim and also inhibit their ability to penetrate the ovum. Chronic alcoholism is said to affect fertility in men (Muthusami & Chinaswamy, 2005).

**(e)** **Smoking:** This is said to affect sperm motility, reduces sperm life span and may cause genetic changes that may affect the offspring (Vine *et al*, 1994).

**(f)** **Malnutrition and Nutrient Deficiency**: Deficiency in protein and certain nutrients such as vitamin C, selenium, zinc and folate are suspected as risk factors for male infertility (Smith *et al*., 1975). Obesity in men has been associated with infertility, though debatable.

iii. **Genetic factors:** Defective genetic materials such as damaged DNA as well as problems in the acrosome can affect fertility in males. Other factors may

include cystic fibrosis, Klinefelter syndrome, kartagener syndrome and polycystic kidney disease (Kaplan *et al.*, 1968; Vecchi *et al.*, 2002).

iv. **Environmental Assaults:** Exposure to environmental toxins, chemicals or infections are said to likely cause reduction in sperm counts either directly (on testicular function) or indirectly (by altering hormone system).Though, this is said to be controversial; experts have attributed a general worldwide decline in male infertility to this. Free radicals (oxidants) are said to be the primary suspects in the relationship between environmental assaults and infertility. Sperms are particularly vulnerable to the damaging effects of oxidation processes (Sharma & Agarwal, 1996). It has been noted that significant levels of oxidation occur in the semen of about 25% of infertile men. Over exposure to estrogen and hormonal disrupting chemicals such as bisphenol A, phthalates, pesticides, industrial chemicals (e.g. benzene, toluene, xylene etc) as well as heavy metals such as lead, cadmium etc in males have been reported to reduce the number of sertoli cells (Thonneau *et al.*, 1999). Radiation treatments such as x-rays and other forms of radiations affect any rapidly dividing cells. Indeed, sperm cells are quite sensitive to radiation damage.

v. **Low Semen Levels:** This can result from structural abnormalities especially in the tubes transporting the sperm. A normal ejaculate volume is from 2.5-5ml (Kidd, 2001). However, finding by Priskorn *et al.,* (2014), showed that both mother's and father's age have minimal effect on semen quality in men.

vi. **Varicoceles:** These are abnormally dilated or enlarged testicular veins (pampiniform plexus) in the scrotum. Varicoceles are found in 15-20% of all men and in 25-40% of infertile men. Vaicoceles are suspected to obstruct the passage through which sperms pass, elevate temperature of the testicles or

produce high levels of nitric oxide which might be beneficial to blood flow but harmful to sperm cells (Peng *et al.*, 1990; Gorelick & Goldstein, 1993).

vii. **Testosterone Deficiency (Hypogonadism):** Severe deficiency in GnRH, the primary hormone that signals the process leading to the synthesis and release of testosterone and other gonadotrophins is a major cause of infertility in the males. Indeed, low level of testosterone from any cause may result in defective sperm production.

viii. **Auto-antibodies:** Though the role of auto-antibodies in male infertility has been controversial for sometimes, the antibodies are believe to target the sperm cells which they perceived as foreign instead of protecting them. This can result in sperm agglutination, failure of interaction with cervical mucosa and inability to penetrate the egg. Genital infections or injuries are also linked with anti-sperm antibodies (Mahi-Brown, 1994; Bronson & Fusi, 1994; Gleicher, 1998).

ix. **Retrograde Ejaculation:** This occurs when muscles of the urethra do not pump properly during orgasm and sperms are forced backward into the bladder, thereby reducing the volume. This can occur due to surgery of the bladder neck or prostate, diabetes, exstrophy/epispadias, spina bifida, multiple sclerosis, spinal cord injury, side effects of drugs such as some anti-hypertensives e.g. phenoxybenzamine, clonidine etc. (Merber, 1974; Thomas Jr, 1983; Master & Turek, 2001).

Treatment of retrograde ejaculation is related to its cause, e.g. if it is drug induced, the said medication can be withdrawn if desirable. If it is caused by other factors, then drugs can be tried to close the bladder neck and avoid entry of the semen into the bladder during ejaculation. Drugs such as pseudoephedrine, imipramine, phenylephrine and chlorpheniramine are said to be effective in this case (Master & Turek, 2001)

## 2.13 PHARMACOLOGICAL INTERVENTIONS FOR MALE FERTILITY DISORDERS

According to Haidl *et al*., (2000), apart from surgery, drug treatment remains an active domain in the therapy of male fertility disorders. The report showed that a careful diagnostic work-up with elucidation of the underlying disease is essential to achieve a successful therapy.

As Haidl *et al*., (2000) had shown, pharmacological interventions for male fertility disorders may include:

### 2.13.1 Antibiotics

These are said to be effective when the underlying cause is infection especially in prostatitis. Antibiotics such as tetracycline, doxycycline, erythromycin, ciprofloxacin etc have been found effective.

### 2.13.2 Antiphlogistic Agents

These agents are known for their effectiveness in reducing inflammation or fever. They are said to be effective when the epididymis is affected in male fertility disorder. Examples include diclofenac, indomethacine, aspirin etc. This is supported by the finding of Barkay *et al*., (1984) which showed that such agents are effective in male fertility disorders.

### 2.13.3 Kallikreins

Kallikrein is a protein that has proteolytic activity, cleaving kininogen to produce kinins such as bradykinin and kallidin that act locally in the inflammatory response. The kallikrein-kinin system has activity in the regulation of sperm motility. Saitoh *et al*., (1987) and Schill (1982) reported the usefulness of kallikrein in the treatment of male infertility .This is because of its importance in sperm motility and metabolism.

### 2.13.4 Mast Cell Blockers

It is reported that mast cells are sometimes increased in the testicular tissues of infertile men, where they appear to affect the production of testosterone (Fijak & Meinhardt, 2006). It is therefore, postulated that restriction of mast cells activation in the testes could be beneficial during treatment of inflammatory conditions and treatment with mast cell blockers such as ebastine, tranilastine etc has proved beneficial in some types of male fertility disorders (Yamamoto *et al*., 1995; Matsuki *et al*., 2000).

### 2.13.5 Zinc Salts

Several reports have supported the role of zinc as an essential trace element for spermatogenesis as well as in other biological activities (El-Tawil, 2003; Abbasi *et al*., 1979; Yamaguchi *et al*., 2009). However, whether administration of exogenous Zn in the absence of Zn deficiency is beneficial remains debatable. Therefore Zn salts are approved for treatment of male infertility particularly for patients with testicular Zn deficiency (Marmar *et al*., 1975; Colagar *et al*., 2009).

### 2.13.6 Corticosteroids

These are said to be useful in the management of anti-sperm antibodies, autoimmune orchitis, etc, that are usually associated with infertility. De-Almeida *et al*., (1985) however reported that prednisolone treatment had no significant effect, compared to placebo on fertility. This was corroborated by the report of Bals-Pratsch (1992). Nonetheless, Hendy *et al*., (1990) had reported a significant improvement on fertility as compared to placebo.

### 2.13.7 Hormone Preparations

These are useful in empiric treatment of fertility disorders due to hypogonadotropic hypogonadism. It is the most common form of treatment in replacement therapy. Agents such as GnRH, testosterone, HCG, FSH, anti-estrogens

(e.g tamoxifen, clomiphene), aromatase inhibitors which block the conversion of testosterone to estradiol (such as anastrazole, testolactone etc), antioxidants (vitamins A, C and E), growth hormone etc have successfully been employed especially in deficiency disorders. However, Solar *et al*., (2007) warned that the use of tamoxifen and clomiphine should be with cautions especially in empiric treatment to avoid their detrimental effects. The same finding showed that though aromatase inhibitors are found to increase sperm concentration, they have no improvement in sperm motility.

### 2.13.8 Pentoxifylline

This is a derivative of methylxanthine. It has been reported as improving sperm motility and number. It is among the early drugs used in male fertility disorders (Fuse *et al*., 1993; Oliva *et al*., 2009).

### 2.13.9 Alpha-Sympathomimetics and Anticholinergics

Following observations that retrograde ejaculation compromises male infertility, midodrine (an alpha sympathomimetic) and imipramine (an antidepressant with peripheral anticholinergic effect) were found useful in cases of retrograde ejaculation or transport aspermia due to emission failure. However, these drugs are said to be of limited efficacy following poor results from some findings (Ochsenkuhn *et al*., 1999; Sanchez *et al*., 2000).

### 2.13.10 Phosphodiesterase-5 Inhibitors

These are not fertility drugs, but are employed as adjuncts for the therapeutic management of male infertility. PDE – 5 inhibitors include sildenafil (viagra$^{TM}$), tadalafil (cialis$^{TM}$) and verdenafil (levitra$^{TM}$). They are popularly known for their effectiveness in the management of erectile dysfunction. However, Dimitriadis *et al*., (2009) suggested that these groups of agents enhance the leydig cell secretory function and could play a role in the regulation of the tunica albuginea and the epididymis. In

addition the report suggested that these agents could increase the prostatic secretory function that results in an improvement in sperm motility. Also some earlier report by Lenzi *et al*., (2003) and Jannini *et al*., (2004) suggested that sildenafil was effective in encouraging compliance of male patients facing infertile couple management procedures and is used to improve some sperm parameters. Further insight into the effectiveness of these agents is given by the study of Goyal (2011) which showed that verdanafil and sildenafil enhance leyding cell secretory function and various seminal parameters in infertile men.

## 2.14 SUMMARY OF LITERATURE REVIEW

A summary of the literatures used to justify any gap that this study would fill was made and presented as references.

# CHAPTER THREE
# MATERIALS AND METHODS

## 3.1 ETHNOBOTANICAL SURVEY

A survey of some medicinal plants that are claimed traditionally to have aphrodisiac property was carried out in 2007 by the assistance of an herbalist, Mrs. Azamya Sule of Naraguta Village, near Jos, Plateau State. Some of such plants were collected, photographed, identified by Mr A.I. Kareem of Federal College of Forestry; Jos and voucher specimen numbers ETAP01-11/007 were made and deposited in their herbarium.

## 3.2 ANIMALS

About 10 week-old male in-bred Wistar rats weighing between 200-260 g were obtained from the Animal House Unit, University of Jos at different periods as needed according to each experiment described below. Male cats and rabbits were bought from a market in Jos metropolis and kept at the Animal House unit to acclimatize before the experiment. All the rats were housed in stainless steel cages and handled under conventional conditions and standard guidelines for the use and care of laboratory animals. They were fed freely with standard pellets and water *ad libitum* until the commencement of the experiment. For each experiment, 20 rats were randomly divided into 4 groups of 5 animals at the commencement of the experiment.

## 3.3 PURCHASE OF *GARCINIA KOLA* SEEDS

The seeds of *Garcinia kola* (Heckel) were purchased from reputable dealers in the markets in Jos metropolis at different periods between November, 2012 and June, 2014. The leaves were collected and identified by Mr A.I. Kareem of Federal College of Forestry, Jos and a specimen voucher numbered GCL0153/04 was prepared and deposited.

## 3.4 CHEMICALS AND REAGENTS

All chemicals and reagents used for this study were of analytical grades with high purity. These include:

- Normal saline 0.9% (Juhel)
- Formalin 10% (M&B)
- Heamatoxylin &Eosin (Abbey)
- Methanol (M&B)
- Methylated spirit (Vconnect)
- Boar's solution (Vconnect)
- ELISA Kit (Syntron)
- *G. kola* extract
- Phenobarbitone (Zeneca)
- HCL 1% (A&R)
- Wagner's Reagent (Sigma)
- Dragendoff Reagent (Sigma)
- Picric acid (A&R)
- Ferric chloride(Sigma-Aldrich)
- Dil. Ammomium solution (BDH).
- Dil. Sulphuric acid (M&B)
- Potassium hydroxide (Sigma)
- Fehling's solution (M&B)
- Conc. $H_2SO_4$ (10%) (M&B)
- Lead subacetate solution (Sigma).
- Acetone (AR)
- Glacial Acetic acid (M&B)
- Molisch's Reagent
- Urethane (M&B)
- Inj. Heparin (Pfizer)
- Acetylcholine (Sigma)
- Atropine (Sigma)
- Standard pellets (Agrofeed)
- Iced water
- Tyrode Solution
- Phenylephrine (SKB)
- Conc. Trioxonitrate (IV) acid (Sigma)
- Perchloric acid (Sigma)

## 3.5 EQUIPMENT

- Soxhlet extractor (M&G Co)
- Stainless steel cages
- Syringes & Needles
- Feeding bottles
- Digital Camera (Panasonic)
- Slides & pins

- Mircotomes (Radical Instruments) - Light microscope (Canon)
- Sterile scissors - Separating funnel
- Analytical Weighing balance (Mettler)- Stopped watch
- Dissecting kits (DRI) - Haematocytometres
- Round bottom flasks - Lancet
- Filter paper - Cotton wool
- Neubauer counting machine (Sigma) - Conical flask
- Non-heparinised tubes - Test tubes
- Atomic absorption spectrophotometer (GBC). - Kymograph (PSL)
- Tracing papers - Canula
- Dessicator (Gallenkamp)

### 3.6 PREPARATION OF EXRACT

The seeds were washed, dehusked and cut into small pieces. They were then dried under the shade for 18 days. Thereafter, they were grounded to powder with mortar and pestle.

150 g of the powder was weighed and soaked with 500 ml methanol. The extraction was carried out in a soxhlet extractor at 62$^{o}$C for 72 hours. The extract was evaporated to dryness in a vacuum evaporator at 40$^{o}$C and a constant yield following repeated weighing was found to be 57.9 g, (38.6%), indicating the complete removal of methanol from the extract. The extract was stored in a refrigerator at -65$^{o}$C until use for the experiments. The extract was reconstituted in distilled water for the oral administration to the animals designated for such treatment.

## 3.7 ACUTE TOXICITY TEST

The $LD_{50}$ dose was determined using the methods of Miller and Tainter (1944). 25 male rats were divided into 5 groups of 5 animals each. Predetermined doses of the seed extract of *Garcinia kola* ranging from 1000-5000 mg/kg of body weight were administered in animals in respective groups. The animals were then observed for 24 hours and the number of death recorded for each group. The % death and corresponding probit values were calculated for each group and the $LD_{50}$ determined.

## 3.8 SPERM VIABILITY/MOTILITY TESTS

The method described by Bavister and Andrews, (1988) was used. 40 male rats were obtained and divided into 8 groups of 5 rats each. The first four groups were treated for 20 days with normal saline 1 ml/100g (control) and the other three groups with *G. kola* seed extract by oral gavages at doses of 125, 250 and 500 mg/kg. The last four groups were similarly treated with normal saline or the extract but for 60 days. After the last dose for each group the animals were allowed to rest for 24 hours. Thereafter, they were anaesthetized with urethane (1.8 mg/kg) and the distal cauda epididymis was dissected and placed in 2ml saline at 37ºC and pH 7.2. A needle was used to release the sperm cells from the Cauda epididymis into the saline solution which was diluted with the saline solution to give a final concentration of $2 \times 10^6$/ml for the sperm motility assay. The solution was then placed under a light microscope and observed at ×400 and the motility (both rapid and slow), viability and % death of sperms were also determined at 4 hours and recorded for each group.

## 3.9 DETERMINATION OF SPERM COUNTS AND HISTOLOGY OF THE EPIDIYMIS

The methods described by Freund and Carol (1965) and that by Robb *et al.*, (1978) were used. 40 male rats were obtained and divided as described for sperm

concentration/motility. They were similarly treated. At the end of each treatment, the animals were anaesthetized with urethane (1.8 mg/kg) and the testes carefully removed. The caudal epididymis from each rat was isolated and placed in 2ml of saline maintained at 37ºC and pH 7.2. Small sections were made where semen from the caudal epididymis was released and the sperm cells suspended in a saline solution. 2ml of the suspension was diluted with 8 ml of saline and kept for 2 hours at about 4$^{o}$C. The suspension was then filtered with a Whatman filter paper. A few drops were transferred into the chambers of Neubauer hemocytometer using a glass pipette and was used for sperm count. Sections of the testes and epididymis of a rat in each group were cut with microtome, smeared and stained with H & E and then examined under a light microscope at ×400 for the histology of the epididymal cells.

### 3.10 EFFECTS ON GONADOTROPHINS

The microwell enzyme-linked immunosorbent assay (ELISA) method which is based on competitive binding of the Gonadotrophins on immobilized specific antibody as described by Braide (2003) and that by Gan and Patel (2013) was used. This allows detection of very small quantities of antigens such as proteins, peptides, hormones etc in a fluid sample. It utilizes enzyme-labeled antigens and antibodies to detect the biological molecules. The antigen is allowed to bind a specific antibody which is itself subsequently detected by a secondary enzyme-coupled antibody. A chromogenic substrate for the enzyme yields visible color change indicating the presence of an antigen which allows for quantitative or qualitative measurement assessed based on such colometric reading.

Following the oral administration of the extract (125, 250 and 500 mg/kg) or normal saline (1 ml/100kg) for 20 and 60 days to each animal (n=5) as described above under the determination of sperm concentration, the animals were anaesthetized using

chloroform and blood was obtained through cardiac puncture. The blood sample from each animal was collected in a non-heparinised tube and allowed to stand for 3 hours in iced water to enable separation of the serum (rats blood haemolysis on standing, thus, ice water will prevent haemolysis). It was then centrifuged for 10 minutes and the serum was collected and stored for 2 days to allow for a complete separation. Bioassay was carried out each for LH, FSH and Testosterone. The mean values were calculated and recorded for each group.

## 3.11 FERTILITY ASSESSMENT TESTS

### 3.11.1 Reproductive Performance

Twenty male rats were divided into 4 groups of 5 rats each. The first group (control) was administered normal saline 1 ml/100g daily and groups 2, 3 and 4 administered daily doses of 125, 250 and 500 mg/kg respectively for 20 days. After the last day, they were allowed to stay for 24 hours and thereafter 1 male rat was randomly selected from each group and matched with 2 untreated virgin female rats in a stainless steel cage in accordance with the natural mating method described by Wong *et al*., (1987). They were kept together and fed *ad libitum* under conventional conditions. The frequency of mount, ejaculation and intromission were observed for the first and last 30 minutes of 48 hours. The males were then allowed to continue staying with the females for the next 48 hours after which they were separated. Thereafter, the females were observed daily for pregnancy and subsequent littering. The first female to litter was removed with its litters into a separate cage. At the end, the number of litters for each female was recorded and the mean for both calculated in each group. The same procedure was used for the 60 days administration of the extract and results obtained were similarly recorded.

### 3.11.2 Effect of the Extract on Corpus Cavernosum

The methods described by Zhongcheng *et al*., (2001) and that by Regadas *et al*., (2010) were used.

A matured male rabbit weighing about 3.5 kg was purchased from a market in Jos Metropolis. The animal was anaesthetized with sodium pentobarbital (40 mg/kg) and exsanguinated through the carotid artery. The penis was removed and the corpus cavernosum tissues were carefully dissected to separate them from the surrounding tissues of the tunica albuginea. A section of the corpus cavernosum measuring about 3 x 3 x 8mm size was made and mounted in a 50ml organ chamber containing tyrode solution maintained at $37^{o}C$ by constant aeration with air (95% $O_2$, 5% $CO_2$). The tissue was then allowed to equilibrate for 90 minutes. During this interval, it was washed with tyrode solution at intervals of 15 minutes. The resting tension was set at 1g. After the equilibration period, a contractile response was obtained with phenylephrine ($4 \times 10^{-6}$g/ml) and thereafter, a solution of the extract was administered in increasing concentrations from 0.4 – 2.0 mg/ml. The responses were recorded on a tracing paper driven by a kymograph machine. The procedure was repeated three times each using fresh sections of the corpus cavernosum muscles. Contractile responses following each administration of phenylephrine were recorded as well as the cumulative effect of the extract on the observed contraction. The contractions were measured and expressed as mean ± SEM and statistically analysed using the Student's *t* – test with a *p* value of 0.05.

## 3.12 EFFECT OF EXTRACT ON THE HISTOLOGY OF THE TESTES

### 3.12.1 Effect on Animals Treated with Extract for 20 Days

20 male rats were randomly divided into 4 groups of 5 rats each. Animals in the first group were orally administered normal saline 1 ml/100kg daily for the period of study. Animals in groups 2, 3 and 4 were administered with the extract by oral route at predetermined daily doses of 125, 250 and 500 mg/kg respectively for a period of 20 days. After the last dose, the animals were starved for 24 hours and thereafter sacrificed by decapitation. The testes were removed, weighed and fixed in 10% formalin solution. The weights of the testes were individually measured and the mean weighed calculated. For each group the testes of a selected rat was cut and a cross-section of about 5um were cut with a microtome and slides made from them for histological examination were stained with haematoxylin and eosin (H & E) and viewed under a light microscope at a magnification of ×400 and photographed.

### 3.12.2 Effect on Animals Treated with Extract for 60 Days

A similar procedure with another set of 20 rats divided into 4 groups was followed as in 20 day treatment explained above except that animals in this group were orally administered the extract and normal saline daily for 60 days.

## 3.13 EFFECT ON HISTOLOGY OF THE ANTERIOR PITUITARY

The brain of a randomly selected rat was cut open and the anterior pituitary tissue identified and removed. It was put into a saline solution with decalcifier solution which allows for ease of cutting and fixing the sections. It was kept for 5 days to allow it become softer. About 5um was sliced with microtome and fixed with 10% formalin, stained with H & E then viewed under a light microscope at a magnificent of 400X and photographed. This procedure was repeated for those treated for 60 days.

## 3.14 EFFECT ON HISTOLOGY OF THE LIVER

The oral administration of the extract and normal saline was same as described earlier for both the 20 and 60 days treatment. At the end of each treatment and after sacrifice by decapitation, slices were made from a cross section of the liver and fixed in 10% formalin for 24 hours and embedded in paraffin. About 5um section was cut and stained with H & E and assessed in a light microscope (x400) and photographed.

## 3.15 ANTI-THROMBOTIC EFFECT OF THE EXTRACT

### 3.15.1 Effect on Bleeding Time (*in vivo*)

Following oral administration of the extract as described above, each treated animal was placed in a restrainer and the tail passed out from one of the openings. The tail was then disinfected with methylated spirit solution and then pricked with a lancet about 4 mm deep and the timing of blood flow was started immediately. The blood was cleaned with filter paper at 15 second interval until it stopped. The procedure was repeated four times on each rat to get the average bleeding time in a minute. This was done for each rat in a group and the mean bleeding time was calculated.

### 3.15.2 Effect on Clotting Time (*in vitro*)

The clotting time was determined by restraining the animal and disinfecting the tip of the tail and then clipping it with sterile scissors. Drops of blood were collected on a clean slide and a clean pin was used to gradually lift up the blood on the slide at 15sec interval until coagulation was noted. The time was recorded. This was repeated 4 times to get the total time per minute. The same was done for each animal and the mean clotting time was calculated and recorded.

### 3.15.3 Effect on Platelet Counts

The dilution and microscopy method was used. The animal was restrained and its tail cleaned and massaged. The tip of the tail was disinfected with methylated spirit

and cut with a sterile pair of scissors and then pressed for blood to flow out. 0.5 ml of the blood was drawn into a white blood cell pipette and diluted with 0.5 ml of Boar's solution containing EDTA. 9 ml of diluting solution was added to give a volume of 10 ml and this was mixed well for 3 min. 2 drops of the mixture was put into the Nabeaur counting chamber and allowed to settle for 20 mins undisturbed. It was then placed under a light microscope and viewed at a magnification of x10 and x40 with the ruling of the grid at the centre. Platelets were counted in 5 large central square and the readings were recorded. This was repeated for each of the 5 rats in each group and the mean values calculated.

### 3.16 EFFECT OF EXTRACT ON BLOOD PRESSURE

Cats were purchased from a market in Jos metropolis and housed at the Animal House, University of Jos. At commencement of the experiment, a selected cat was anaesthetized with urethane (1.8 mg/kg) administered through the intraperitonial route. The femoral vein was identified and cannulated to allow for iv injection of reference drug, and the extract. The carotid artery was also cannulated and connected to a pressure transducer and physiograph for the recording of blood pressure. Heparin (1000 IU/kg) was injected through the femoral vein to prevent clot formation. The trachea was exposed and also cannulated to allow for artificial respiration. The animal was allowed to equilibrate for about 30 min and thereafter, the baseline blood pressure was recorded, followed by IV administration of normal saline (1 ml/100kg), acetylcholine ($5 \times 10^{-2}$ -$5 \times 10^{-5}$ mg/ml) and various concentrations of extract (5-30 mg/kg) and alone and in the presence of increasing concentrations of atropine ($2\times10^{-5}$ - $2\times10^{-3}$ mg/kg). The time cycle used for the administration of the drugs and extract was maintained at 2 minutes.

## 3.17 EFFECT OF THE EXTRACT ON CHEMICALLY-INDUCED SLEEP

Following daily oral administration of the extract or normal saline for 20 days and 60 days respectively and after 24 hours of the last dose for each group, each rat was administered phenobarbitone (40 mg/kg) by intraperitonial route. The onset and duration of sleep was recorded for each animal and the mean values, were calculated.

## 3.18 PRELIMINARY PHYTOCHEMICAL ANALYSIS

### 3.18.1 Test for Alkaloids

The method described by Trease and Evans (1983) was used. 0.5g of the extract was weighed and mixed with 3 ml of 1% aqueous hydrochloric acid (HCl) on a stream bath and stirred very well. Few drops of Wagner reagent, Dragendoff reagent and picric acid solutions were separately treated with 1ml each of the filtrate. They were then repeatedly observed for precipitation.

### 3.18.2 Test for Saponins

This test was carried out by the method of Wall *et al*., (1952) 0.5g for the extract was carefully weighted and dissolved in 5ml of distilled water in a test-tube. This was then shaken very well and warmed for 5 min. the formation and persistence of frothing was observed for preliminary evidence for the presence of saponins.

### 3.18.3 Test for Tannins

0.5g of the extract was weighed and dissolved in 1ml of distilled water. It was then filtered and mixed with 5% ferric chloride reagent. The solution was then observed for a blue-green precipitate as evidence for the presence of tannins (Trease & Evans, 1983).

### 3.18.4 Test for Anthraquinones

0.5g of the extract was weighed in a test tube. 5ml of chloroform was added into it and shaken for 5min. The solution was then filtered and the filtrate mixed with

equal volume of 10% ammonium solution. The mixture was observed for red colouration in the ammonical layer for the presence of anthraquinones (Trease & Evans, 1993).

### 3.18.5 Test for Glycosides

100mg of the extract was weighed and transferred into a test-tube. 2.5 ml of a diluted sulphoric acid ($H_2SO_4$) was added to it and boiled in a water bath for 15 min. It was allowed to cool and thereafter neutralized with 2 ml of 20% KOH solution 5ml of Fehling's solutions A & B was added and boiled for 3min. It was then observed for a brick-red precipitate as evidence of reducing sugars (glycosides).

### 3.18.6 Salkowski Test for Steroids

100mg of the extract was dissolved in 2ml of chloroform and conc. $H_2SO_4$ was added to form a layer. This was then observed for a brown coloration as evidence for presence of steroids (Sofowora, 1982).

### 3.18.7 Test for Flavonoids

2g of the extract was dissolved with acetone, and the acetone was then evaporated and the residence was extracted in warm water. The mixture was filtered while hot and 5 ml of filtrate was mixed with lead sub acetate solution. It was then observed for yellow precipitate as preliminary indication for presence of flavonoids.

### 3.18.8 Test for Cardiac Glycosides

100mg of the extract was dissolved in 3 ml of glacial acetic acid containing 2 drops of ferric chloride solution. This was mixed with 2ml of conc. $H_2SO_4$. It was then observed for the development of brown ring precipitate as evidence for the presence of cardiac glycosides (Trease & Evans, 1993).

### 3.18.9 Test for Cyanogenetic Glycosides

100mg of the extract was weighed and moistened with distilled water in a test tube. A sodium pirate paper was placed at the tip of the test-tube using a stopper. This was then heated in a water bath for between 30 mins to 1 hour. The picric paper was then removed and observed for brown colouration was indication for the presence of cyanogenetic glycosides.

### 3.18.10 Test for Carbohydrates

100mg of the extract was dissolved in 3ml of distilled water in an inclined test-tube and then mixed with 2-3 drops of molisch reagent 1ml of conc. $H_2SO_4$ was carefully added down the side of the tube so that the acid forms a layer beneath the aqueous solution without mixing or shaking. This was then observed for a reddish ring precipitation at the junction of the liquid as evidence for the presence of carbohydrates (Trease & Evans, 1983).

## 3.19 ELEMENTAL ANALYSIS OF *GARCINIA KOLA* SEED EXTRACT

The elemental analysis of the *Garcinia kola* seed extract was carried out by the atomic absorption spectroscopy (AAS) via the use of an atomic absorption spectrometer at the Metallurgical Centre, Jos using the method described by Olutayo *et al.*, (2012). The atomic absorption spectroscopy is a spectroanalytical procedure for the quantitative determination of chemical elements using the absorption of light by free atoms in the gaseous state (Koirtyohann, 1991).

5g was dried in an oven and taken samples of it weighed. They were then placed in a hot furnace and ashed at 600ºC for 3 hours. The furnace was cooled to 120ºC and the samples were then removed and placed in a dessicator for 1 hour to cool before weighing. The weighing process was repeated until a constant weight was obtained. 0.5 g of the ashed samples were weighed and transferred into digestive tubes.

5 ml each of distilled water, concentrated trioxonitrate (IV) acid and perchloric acid were added and the content mixed properly. The tubes were placed into digestive blocks inside a fume cupboard and the temperature was set at 150ºC and digested for 90 minutes. The temperature was then increased to 230ºC and digested for another 30 minutes and thereafter the temperature reduced back to 150ºC. 1 ml of hydrochloric acid was then added to the tube within 5 minutes and the digest was not allowed to cool to room temperature to prevent formation of insoluble precipitates such as potassium percholate. More water was added to the tube to give a convenient volume and the content was mixed and filtered and the resulting solution was used for the elemental analysis at appropriate wavelength, temperature and current for the different elements.

### 3.20 STATISTICAL ANALYSIS

Results obtained were expressed as the mean ± S.E.M for each group where applicable. The mean values were analyzed using one-way analysis of variance (ANOVA) and in some cases complemented with student's *t* test. The validity of differences in means was taken at 5% significant level and values of $p<0.05$ were considered statistically significant as described by Dawson and Trapp, (2004); Mahajan (1987); and Zar (1999).

# CHAPTER FOUR
# RESULTS

## 4.1 ETHNOBOTANICAL SURVEY

Some medicinal plants traditionally claimed as having aphrodisiac effects were identified in Jos North Local Government Area of Plateau State, collected and documented by the assistance of a herbalist, Mrs Azamya Sule of Babale village in Jos North LGA, Plateau state. Some examples of such plants are presented in Appendix A. *Garcinia kola,* though not one of the plants collected, is popular for its traditional use as a powerful aphrodisiac agent among indigenous and non-indigenous populations. The seeds of *Garcinia kola* were chosen for this study because it is commonly consumed by many people in Nigeria and indeed West Africa for the sole purpose of increasing sexual performance especially among the males.

## 4.2 $LD_{50}$ OF METHANOLIC EXTRACT OF *GARCINIA KOLA* SEED

Result of the acute toxicity test of the methanolic extract of *Garcinia kola* seed revealed that the $LD_{50}$ is about 3,215 ± 52.68 mg/kg.

## 4.3 EFFECT OF METHANOLIC EXTRACT OF *GARCINIA KOLA* SEED ON SOME SPERM PARAMETERS OF RATS

From the results, the methanolic extract of *Garcinia kola* seed did not cause any significant decrease in sperm counts, slow and rapid progressions and death percentages in rats that received 125 mg/kg for both 20 and 60 days compared to control ($P>0.05$). However, in groups that received 250 and 500 mg/kg for 60 days, the extract caused significant decrease in sperm counts and slow progression ($P<0.05$). Also in rats that received 500 mg/kg for 60 days the extract caused significant increase in death of sperm cells compared with control ($P<0.05$). (Figure 1-4). Results of the sperm viability test showed that the extract did not cause significant decrease in sperm viability in rats at all doses used for 20 days compared with control ($P>0.05$). However,

the extract caused a significant decrease in viability at the dose of 125 mg/kg for 60 days compared with control ($P$<0.05) (Table 1).

## 4.4 EFFECT OF METHANOLIC EXTRACT OF *GARCINIA KOLA* SEED ON SOME ANTERIOR PITUITARY GONADOTROPHINS

The extract produced an increase in the level of LH ($P$>0.05) compared to control following the administration of the extract (125 and 250 mg/kg) for 20 day while a decrease at 500 mg/kg. Following 60 days of administration, the extract decreased LH level at all doses ($P$>0.05) although, not in a dose-dependent manner (Figure 5). Comparatively, the decrease was observed to be prominent on duration of administration rather than on doses, as result data obtained from the 60 days of administration were respectively lower than those of 20 days.

The extract was observed to cause an increase on the level of FSH at 125 and 250 mg/kg after 20 and 60 days treatments compared to control (Figure 6). However, at a dose of 500 mg/kg (20 and 60 days treatment), the extract caused a decrease in FSH compared to control ($P$>0.05).

Studies on testosterone revealed a non-significant but dose-dependent decrease on the level of the hormone following 20 days administration ($P$>0.05). However, in groups treated with the extract for 60 days, there was increase on the level of the hormone at 125 and 250 mg/kg, but a decrease at 500 mg/kg (Figure 7).

## 4.5 EFFECT OF METHANOLIC EXTRACT OF *GARCINIA KOLA* SEED ON SEXUAL BEHAVIOURS OF MALE RATS

Results of the fertility/reproductive performance test as presented on Table 2 showed that *Garcinia kola* seed extract significantly increased the frequency of mounting, intromission and ejaculation for male rats treated with 250 mg/kg for both 20 and 60 days compared with control ($P$<0.05). Similarly, the number of litters per

female rat paired with the treated males was highest in the group that received the dose of 250 mg/kg for both 20 and 60 days, although this was not significantly different compared with control (Tables 2-5). On the other hand, results from animals in the group treated with 500 mg/kg for both 20 and 60 days showed that the extract caused decreased in frequency of mount, intromission and ejaculation, though not significant compared with control ($P$>0.05). In the same vein, the number of litters per female rat paired with the male treated with 500 mg/kg for both 20 and 60 days significantly reduced compared with control ($P$<0.05)

## 4.6 EFFECT OF METHANOLIC EXTRACT OF *GARCINIA KOLA* SEED ON CORPUS CAVERNOSUM

The results of the effect of *Garcinia kola* seed extract on corpus cavernosum smooth muscles of the rabbit are presented on Figures 8, 9 and 10.

The results showed that phenylephrine caused a sustained but dose-dependent contractions of the corpus cavernosum (Figure 8A) while the extract induced a dose-dependent relaxation in the basal equilibrium state of the corpus cavernosum muscle strip (Figure 8B). The extract caused a dose – dependent relaxation of the pheylephrine pre – contracted corpus cavernosum in a dose dependent manner (Figure 9 & 10). The relaxation significant ($P$< 0.05) at concentrations from 1.6 – 2.0 mg/ml while at lower concentrations from 0.4 – 1.2 mg/ml, the relaxation action was not significant ($p$>0.05) compared with the maximum contraction induced by phenylephrine.

## 4.7 EFFECT OF METHANOLIC EXTRACTS OF *GARCINIA KOLA* SEED ON WEIGHTS OF RATS' TESTES

Results of the effect of the methanolic extract of *Garcinia kola* seed are presented on tables 6 and 7. The results showed that the extract caused increased in testicular weights of treated rats for both 20 and 60 days. The increase was found to be significantly different compared to control in groups treated with 500 mg/kg ($P$<0.05).

The results also showed that that the testicular weights increased correspondingly with increased in duration fro 20 days to 60 days of treatment with the extract.

## 4.8 EFFECT OF METHANOLIC EXTRACT OF *GARCINIA KOLA SEED* ON HISTOLOGY OF TESTES OF RATS

The content of the seminiferous tubules were clear. Similarly, the sustentacular cells were normal and appeared numerous in number. The spermatogonia and dividing spermatogonia were many and appeared normal in size. The spermatocytes and spermatids were present in large numbers. The spermatozoa were present in large numbers in the centers of all the seminiferous tubules (Plates I-IV).

## 4.9. EFFECT OF METHANOLIC EXTRACT OF *GARCINIA KOLA* SEED ON HISTOLOGY OF EPIDIDYMIS OF RATS

Results revealed that the extract caused disruption of the testicular cells. The morphology of the testicular cells of rats treated with the extract at 125 and 250 mg/kg appeared normal compared to that of control but 500 mg/kg produced some abnormality. The epithelial cells of control group were observed to be densely packed (Plates V-VIII), compared to those from treated groups especially with 500 mg/kg. The epithelial cells were observed to be loosed spared or even absent.

## 4.10 EFFECT OF METHANOLIC EXTRACT OF *GARCINIA KOLA* SEED ON HISTOLOGY OF ANTERIOR PITUITARY OF MALE RATS

The results showed that the extract at doses of 125 and 250 mg/kg did not cause any significant morphological change on the cellular integrity compared to control. However at 500 mg/kg for both durations, there were morphological disruptions (Plates IX-XII)

## 4.11 EFFECT OF METHANOLIC EXTRACT OF *GARCINIA KOLA* SEED ON HISTOLOGY OF THE LIVER OF MALE RATS (60 DAYS)

The results showed that the extract at 125 and 250 mg/kg did not cause any significant change on the cellular integrity compared to that of control. However, at the dose of 500 mg/kg for the duration of 60 days, there were some morphological disruptions of the cells. The hepatocytes had radial disbursement cells and limited sinusoids (Plates XIII & XIV).

## 4.12 ANTI-THROMBOTIC EFFECT OF METHANOLIC EXTRACT OF *GARCINIA KOLA* SEED IN MALE RATS

Results of the anti-thrombotic effect of the methanolic extract of *Garcinia kola* seed showed that the extract caused significant and dose-dependent increase in bleeding and clotting time in rats treated with the extract at 250 and 500 mg/kg for both 20 and 60 days compared with control ($P<0.05$) (Figures 10 & 11). On the other hand the results on platelet count showed a significant decrease in platelet counts in rats treated with the extract at 250 and 500 mg/kg for both 20 and 60 days compared with control ($P<0.05$) (Figure 12). Similarly, the bleeding and clotting time were significantly shorter in rats treated for 60 days compared with those treated for 20 days ($P<0.05$). On the other hand platelet counts were lower in rats treated for 60 days compared with those treated for 20 days ($P>0.05$).

## 4.13 EFFECT OF METHANOLIC EXTRACT OF *GARCINIA KOLA* SEED ON BLOOD PRESSURE OF A MALE CAT

The effect of the extract on blood pressure as presented on plate XV showed that the extract produced a dose-dependent decrease on blood pressure of male cat. This effect was similar compared with that of acetylcholine. The blood pressure decreasing effect of the extract was blocked by atropine.

## 4.14 EFFECT OF METHANOLIC EXTRACT OF *GARCINIA KOLA SEED* ON ONSET AND DURATION OF SLEEP IN MALE RATS

The results of the effect of the extract on onset and duration of sleep are presented on tables 8 and 9.

The results showed that the extract did not cause significant increase in onset of action in rats treated for 20 days compared with control ($P>0.05$). However there was significant increase in onset of action in rats treated 125 and 250 mg/kg for 60 days compared with control ($P<0.05$).

Results of the effect of the extract on duration of sleep showed a significant decrease in duration of sleep in rats treated with 500 mg/kg for 20 days compared with control group ($P<0.05$) but not so in groups treated with 125 and 250 mg/kg ($P>0.05$). On the other hand, there was significant decrease in groups treated with all the doses used for 60 days compared with control group ($P<0.05$). Also the duration of sleep significantly decreased in all groups treated for 60 days compared with those treated for 20 days ($P<0.05$).

## 4.15 PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACT OF *GARCINIA KOLA* SEED

Results of the phytochemical screening revealed that the extract contains alkaloids, glycosides, saponins, tannins, flavonoids, carbohydrates and steroids. The extract does not contain anthraquinones (Table 10).

## 4.16 ELEMENTAL ANALYSIS OF METHANOLIC EXTRACT OF *GARCINIA KOLA* SEED

The elemental analysis of the methanolic extract of *Garcinia kola* seed revealed the presence of the following essential elements: copper (Cu), iron (Fe), zinc (Zn), magnessium (Mg), potassium (K) and sodium (Na) but does not contain selenium (Table 11).

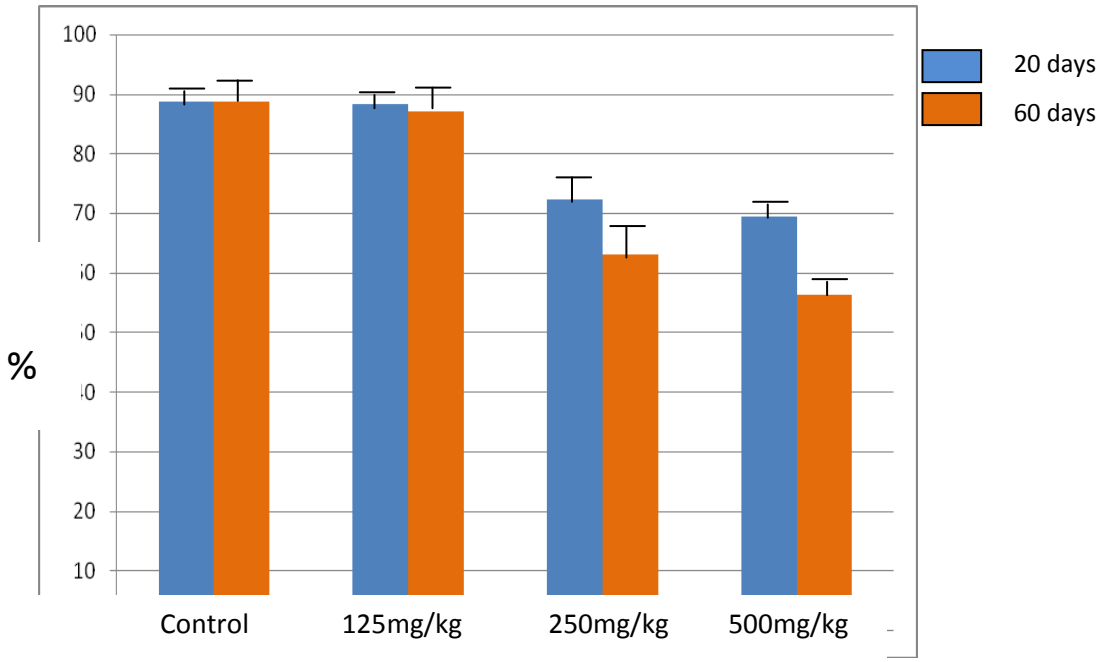



**Figure 1: Histogram of the Effect of Methanolic Extract of *Garcinia kola* Seed on Sperm Counts (percentage) in Rats**

***P*<0.05**

**n=5**

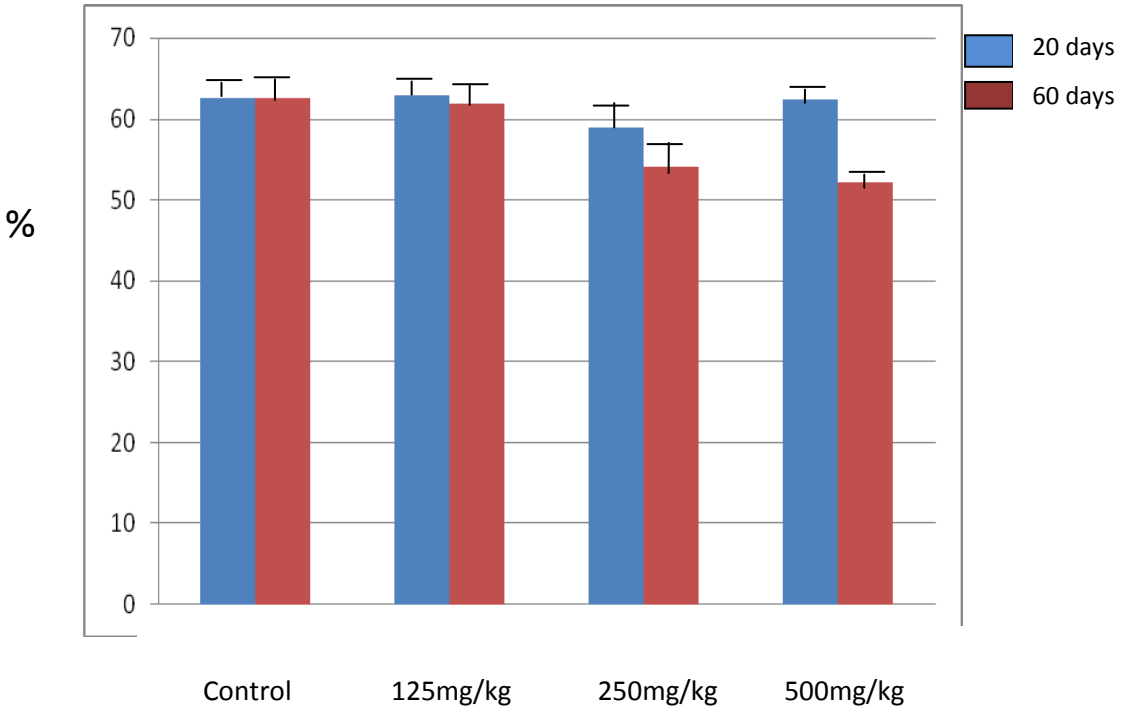



**Figure 2: Histogram of the Effect of Methanolic Extract of *Garcinia kola* Seed on Sperm Motility (Slow Progression) in Rats**

***P*<0.05**
**n=5**

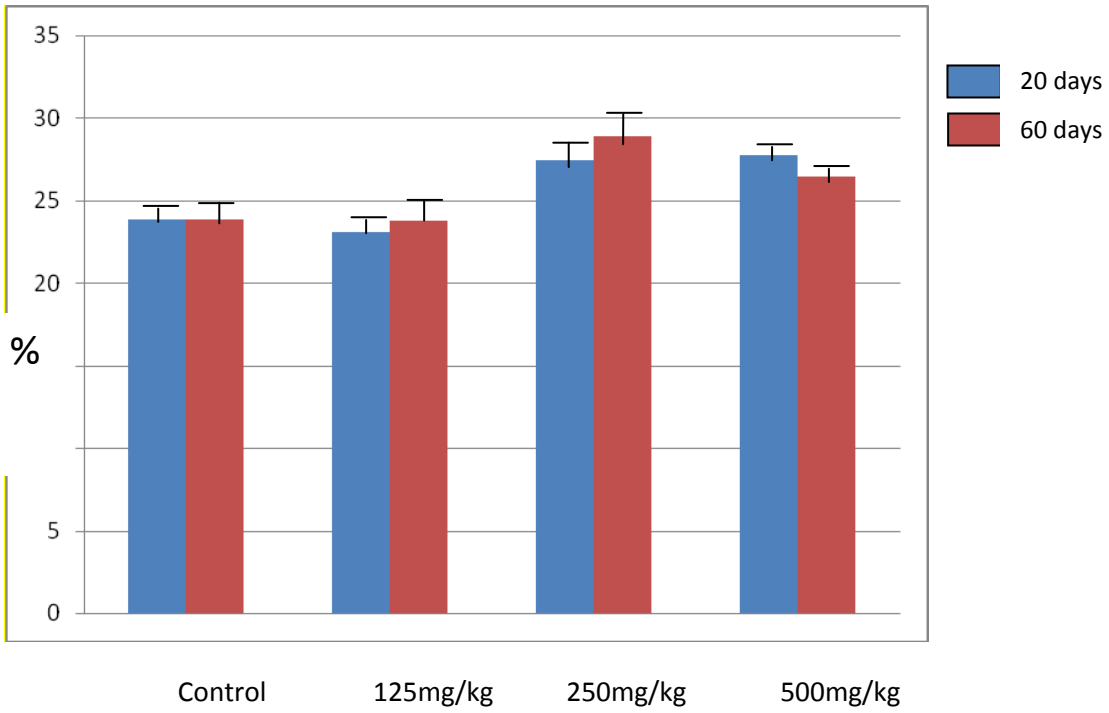



**Figure 3: Histogram of the Effect of Methanolic Extract of *Garcinia kola* Seed Extract on Sperm Motility (Rapid Progression) of Rats**

***P*<0.05**
**n=5**

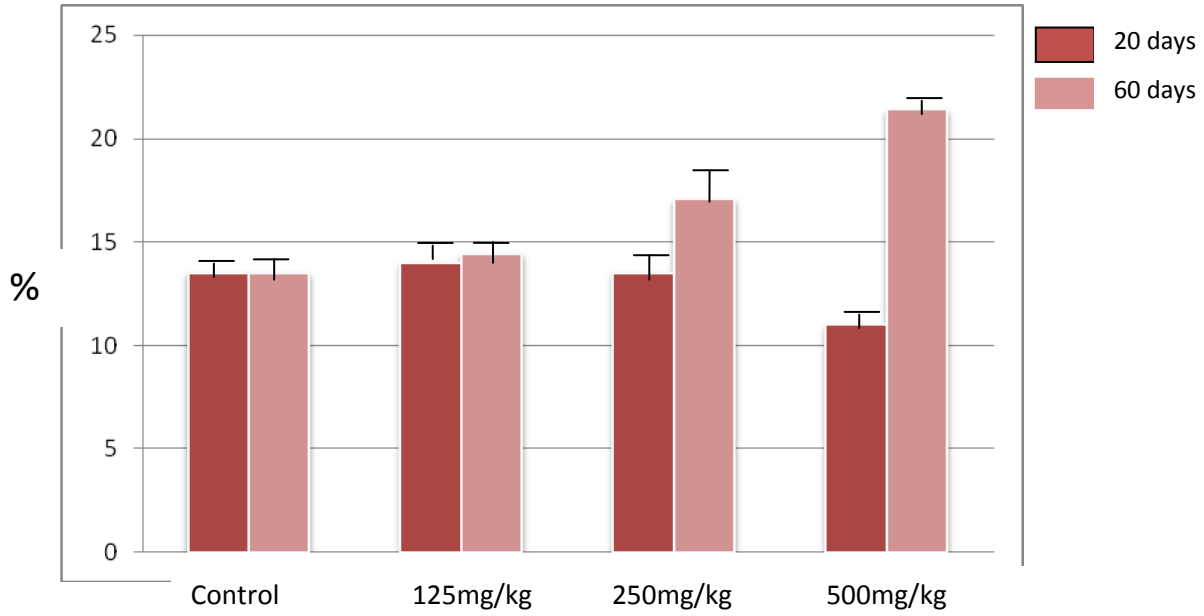



**Figure 4: Histogram of the Effect of Methanolic Extract of *Garcinia kol*a Seed on Death of Sperm Cells in Rats**

***P*<0.05**
**n=5**

**Table 1: Effect of Methanolic Extract of *Garcinia kola* Seed on Sperm Viability in Rats Treated for 20 and 60 Days**

| Dose (mg/kg) | % viability 20 Days | % viability 60 Days |
|---|---|---|
| **Control** | **81.72±4.81** | **86.60±1.15** |
| **125** | **73.18±2.61** | **79.93±1.90*** |
| **250** | **84.86±3.37** | **79.47±4.83** |
| **500** | **71.92±4.73** | **86.53±5.62** |

*** *p*< .0.05**
**n=5**

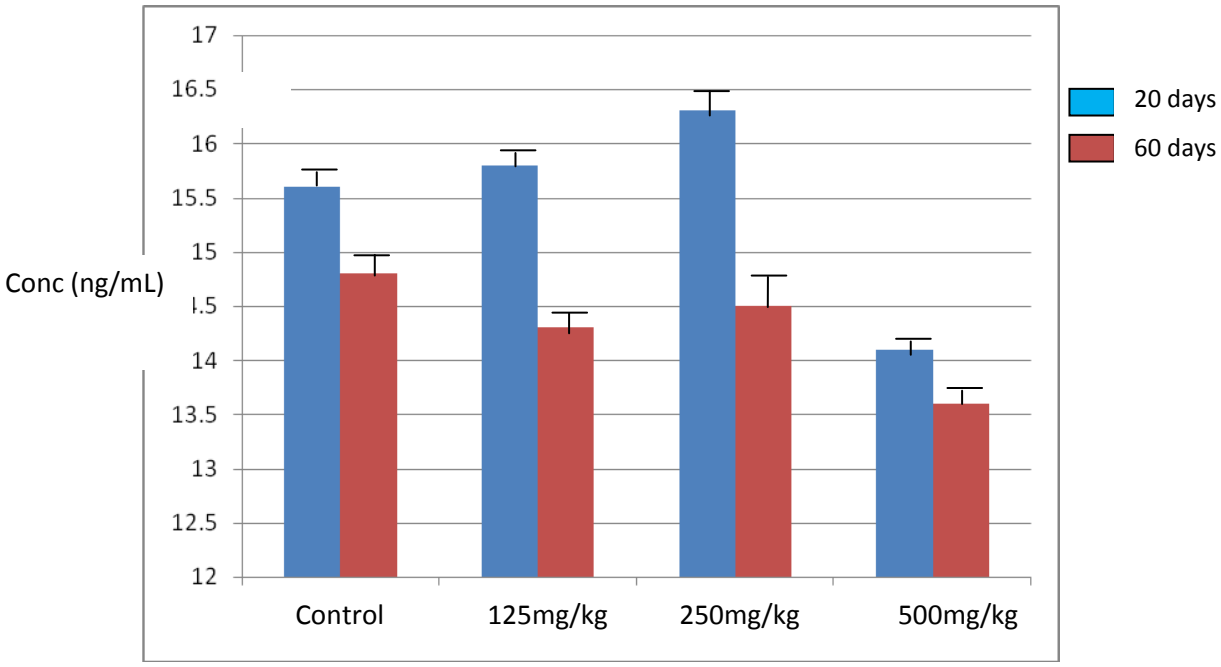



**Figure 5: Histogram of the Effect of Methanolic Extract of *Garcinia kola* Seed on Luteinizing Hormone (LH) Levels in Male Rats**

***P*<0.05**
**n=5**

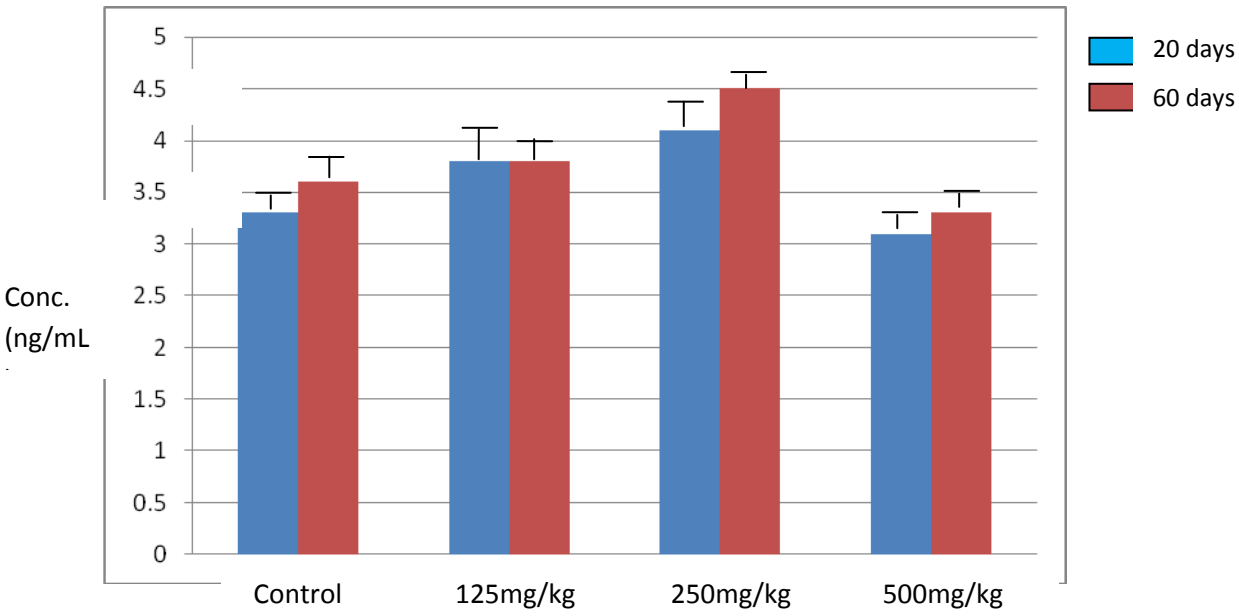



**Figure 6: Histogram of the Effect of Methanolic Extract of *Garcinia kola* Seed on FSH Concentration in Male Rats**

***P*<0.05**
**n=5**

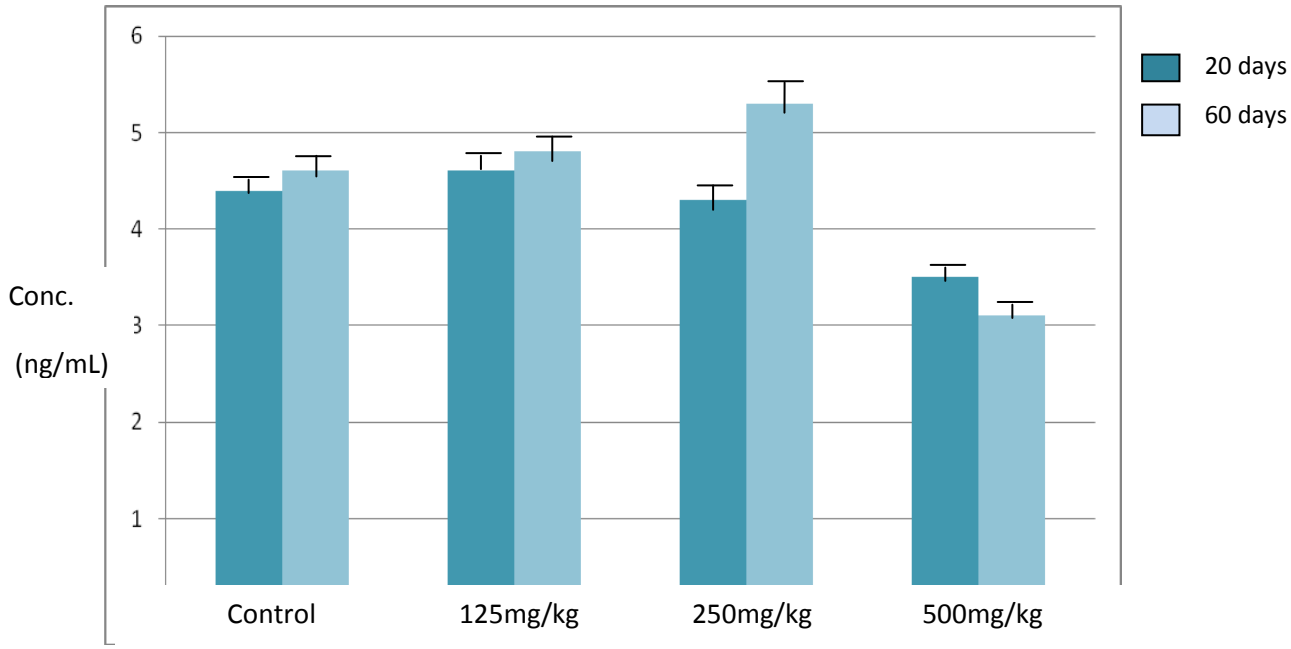



**Figure 7: Histogram of the Effect of Methanolic Extract of *Garcinia kola* Seed on Testosterone Levels in Male Rats**

***P*<0.05**
**n=5**

**Table 2: Effect of Methanolic Extract of *Garcinia kola* Seed on Sexual Behaviours in Male Rats for 20 Days**

| Dose(mg/kg) | Mount | Intromission | Ejaculation |
|---|---|---|---|
| Control | 14.2± 5.8 | 8.3 ±2.4 | 3.8 ±1.1 |
| 125 | 20.5± 5.6$^{+}$ | 15.1 ±6.3$^{+}$ | 3.6 ±1.2$^{+}$ |
| 250 | 27.0± 8.2* | 18.7 ±3.1* | 5.1 ±1.7$^{+}$ |
| 500 | 19.1± 6.8$^{+}$ | 11.6 ±6.9$^{+}$ | 3.2 ±2.8$^{+}$ |

***P*< 0.05**
**$^{+}$ *P*> 0.05**
**n=5**

**Table 3: Effect of Methanolic Extract of *Garcinia kola* Seed on Sexual Behaviours in Male Rats 60 Days**

| Dose(mg/kg) | Mount | Intromission | Ejaculation |
|---|---|---|---|
| Control | 15.8± 3.6 | 10.4 ±2.2 | 5.1 ±1.2 |
| 125 | 23.5± 4.6* | 14.2 ±3.2$^{+}$ | 7.3 ±2.6$^{+}$ |
| 250 | 25.1± 5.4* | 19.7 ±6.5* | 10.4 ± 2.7* |
| 500 | 16.9± 3.6$^{+}$ | 13.5 ±3.6$^{+}$ | 6.9 ±1.1$^{+}$ |

***$p$< 0.05**

**$^{+}p$> 0.05**

**n=5**

**Table 4: Effect of Methanolic Extract of *Garcinia kola* Seed on Reproductive Performance in Male Rats (20 Days)**

| (mg/kg) | M1 | | M2 | | M3 | | M4 | | M5 | | MEAN NL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | PR | NL | PR | NL | PR | NL | PR | NL | PR | NL | |
| Control | 2 | 15 | 2 | 14 | 2 | 15 | 2 | 12 | 2 | 15 | 7.20 ±0.68 |
| 125 | 2 | 13 | 2 | 18 | 2 | 13 | 2 | 10 | 2 | 16 | 7.00 ± 1.38 |
| 250 | 2 | 16 | 2 | 13 | 2 | 21 | 2 | 19 | 2 | 20 | 8.90 ± 1.46 |
| 500 | 2 | 10 | 2 | 12 | 1 | 08 | 1 | 06 | 2 | 10 | 4.6 ± 1.02 |

**M=Male Rat PR= Pregnant Rat NL=No. of Litters**
**n=5**
***P*<0.05**

**Table 5: Effect of Methanolic Extract of *Garcinia kola* Seed on Reproductive Performance (60 Days)**

| Mg/kg | M1 | | M2 | | M3 | | M4 | | M5 | | MEAN NL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | PR | NL | PR | NL | PR | NL | PR | NL | PR | NL | |
| Control | 2 | 16 | 2 | 18 | 2 | 17 | 2 | 15 | 2 | 18 | 8.00 ± .581 |
| 125 | 2 | 15 | 2 | 18 | 2 | 20 | 2 | 18 | 2 | 19 | 9.00 ± 0.84 |
| 250 | 2 | 19 | 2 | 21 | 2 | 19 | 2 | 20 | 2 | 21 | 10.00 ±0.44 |
| 500 | 2 | 12 | 1 | 10 | 2 | 14 | 2 | 11 | 1 | 09 | 5.60 ± 0.86 |

**M=Male Rat PR= Pregnant Rat NL=No. of Litters**
**n=5**
***P*<0.05**

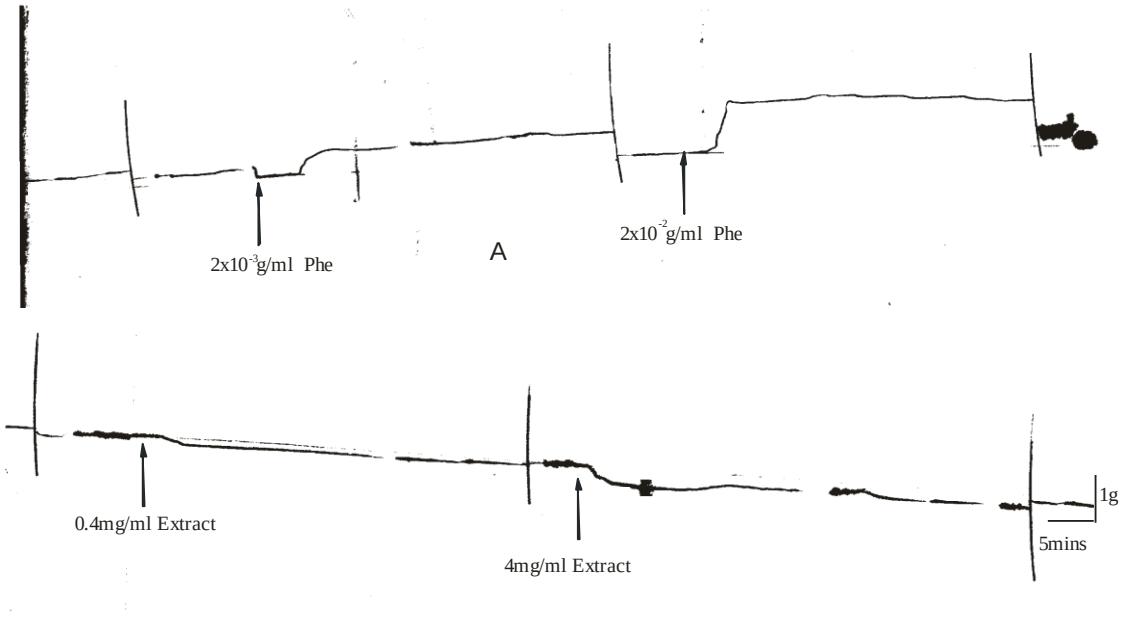



**Figure 8: Effect of Phenylephrine (A) and the Extract (B) Alone on Corpus Cavernosum**

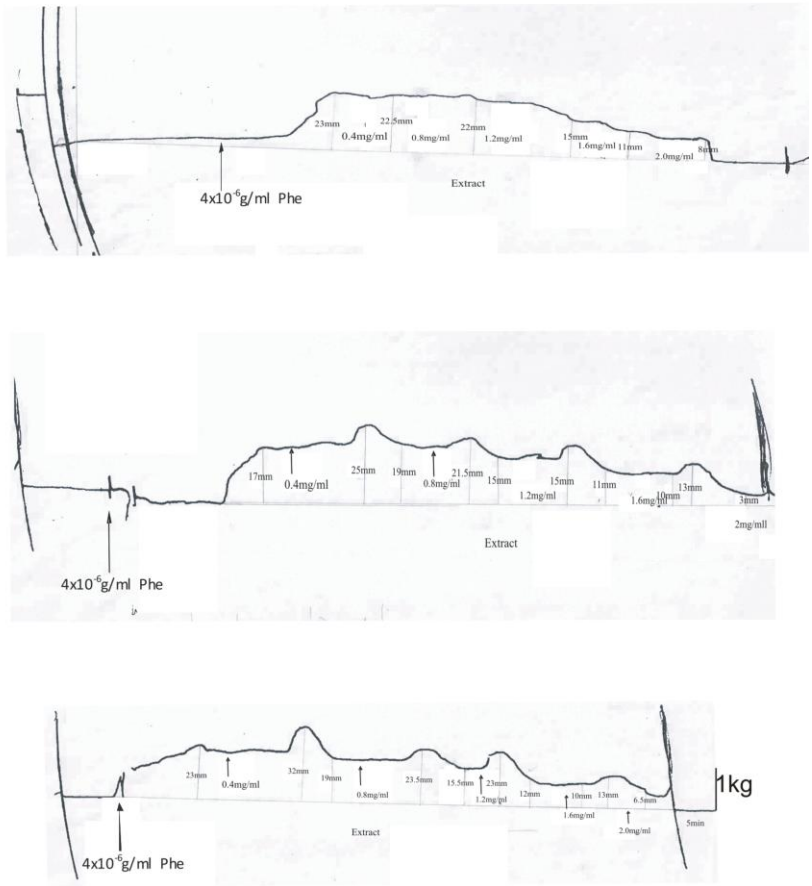



**Figure 9: Effect of the Extract on Phenylephrine Contracted Corpus Cavernosum**

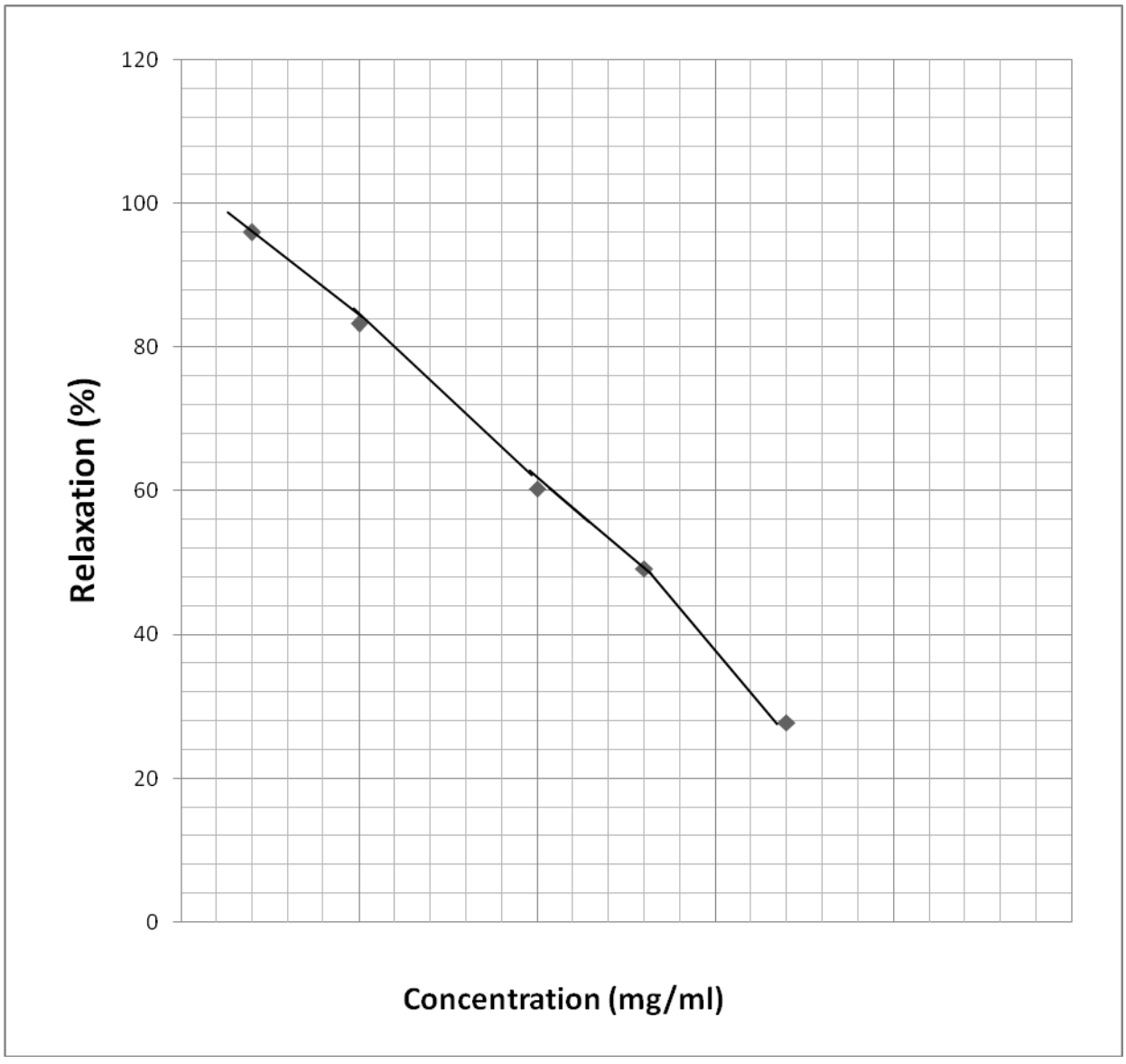



**Figure 10: Relaxation Effect (%) of the Extract on Phenylephrine Contracted Corpus Cavernosum**

**Table 6: Effect of Methanolic Extract of *Garcinia kola* Seed on Weight of Testes After 20 Days**

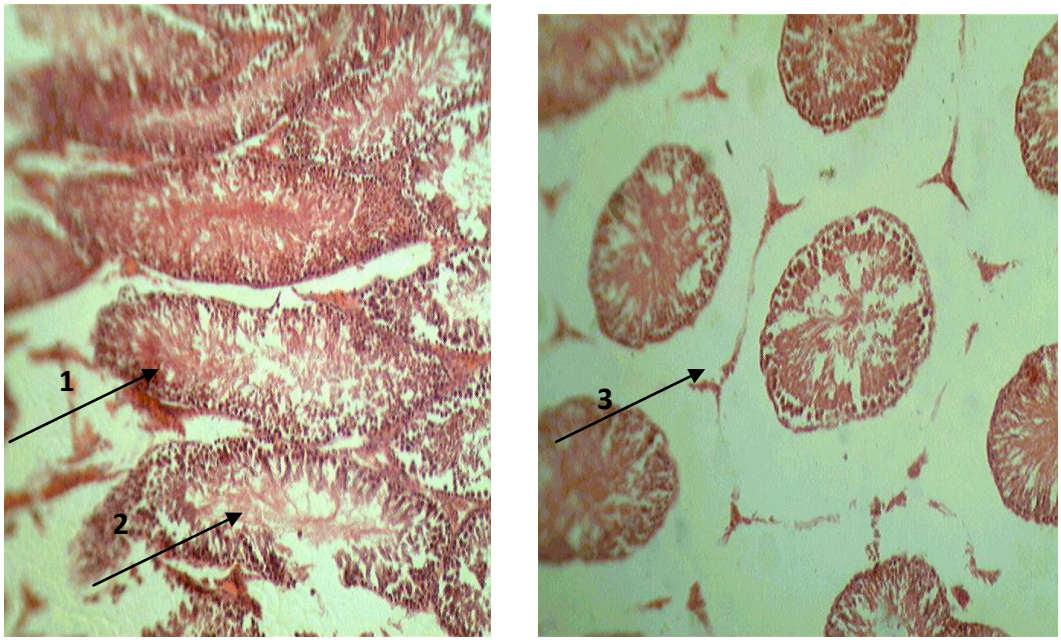
| Treatment (mg/kg) | Mean wet weight of Testes (g/100g) body weight |
|---|---|
| **Control** | **0.81±0.02** |
| **125** | **0.80±0.03** |
| **250** | **0.88±0.96** |
| **500** | **1.10±0.06*** |

*** *P*<0.05**
**n=5**

**Table 7: Effect of Methanolic Extract of *Garcinia kola* Seed on Weight of Testes After 60 Days**

| Treatment (mg/kg) | Mean wet weight of Testes (g/100g) body weight |
|---|---|
| **Control** | **1.55±0.19** |
| **125** | **1.64±0.18** |
| **250** | **1.40±0.31** |
| **500** | **1.96±0.03*** |

***P*<0.05**
**n=5**

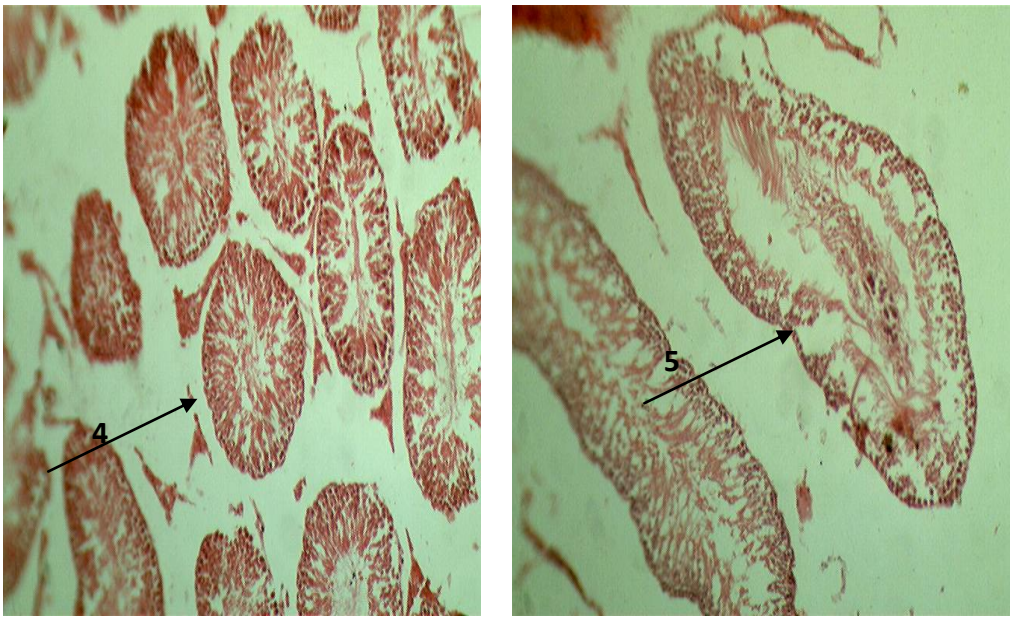



**A** **B**

**Plate I: Photomicrograph of the Effect of Methanolic Extract of *Garcinia kola* Seed on Testes of Rats Treated for 20 Days (x400)**

**A= Normal Saline** **B= 125mg/kg**

**1, 2, 3 = Sperm cells from seminiferous tubules**

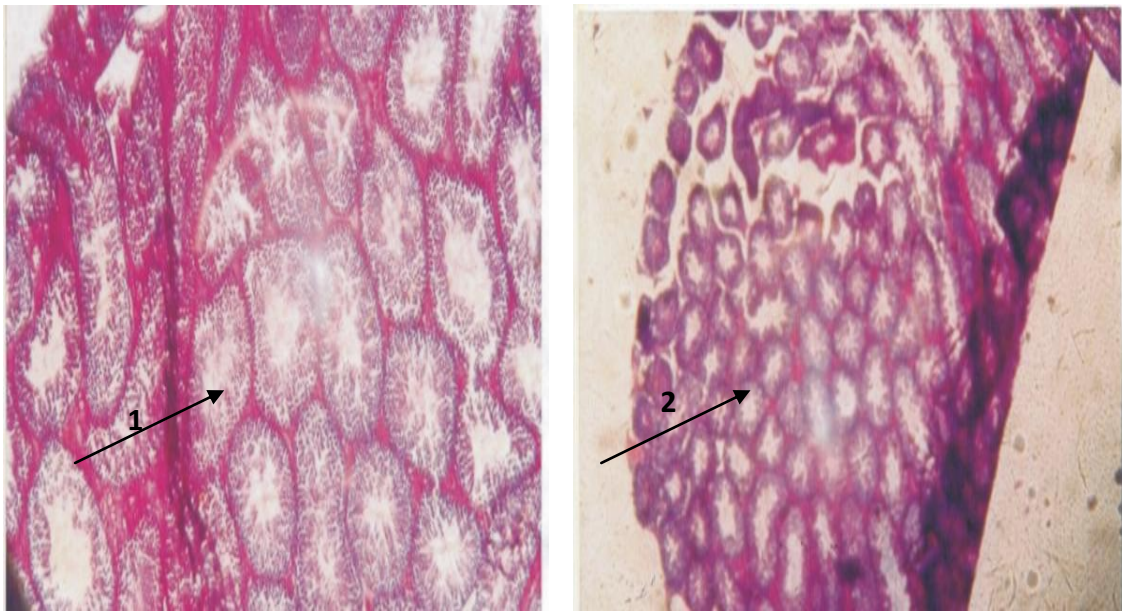



**C** **D**

**Plate II: Photomicrograph of the Effect of Methanolic Extract of *Garcinia kola* Seed on Testes Of Rats Treated for 20 Days (x400)**

**C= 250mg/kg** **D= 500mg/kg**

**4, 5 = sperm cells from seminiferous tubules**

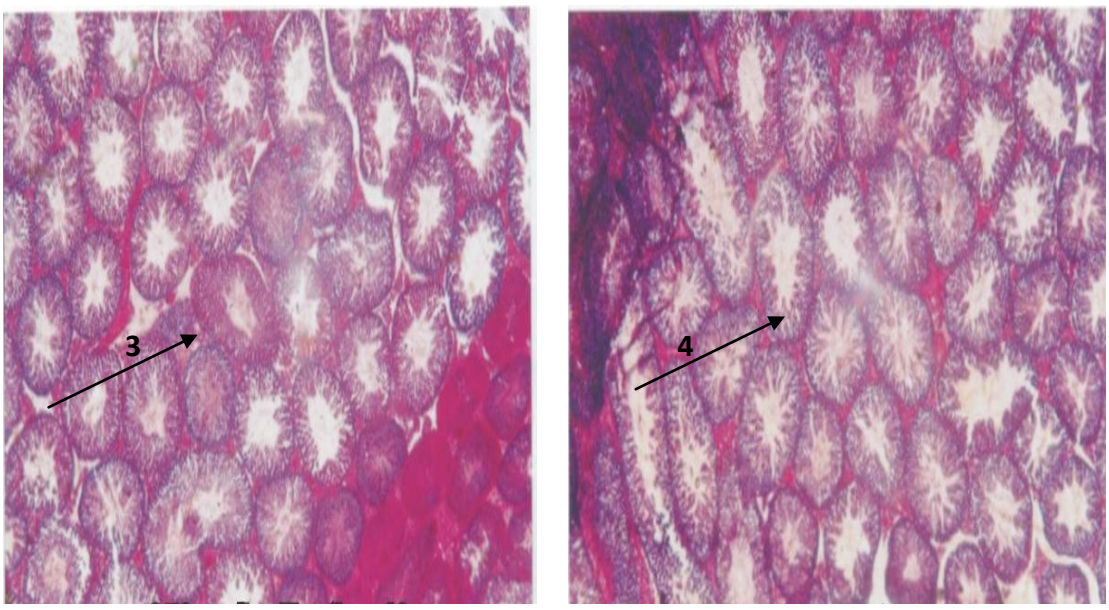



**A** **B**

**Plate III: Photomicrograph of the Effect of Methanolic Extract of *Garcinia kola* Seed on Testes of Rats Treated for 60 Days (x400)**

**A= Normal Saline** **B= 125mg/kg**

**1, 2 = Sperm cells from seminiferous tubules**

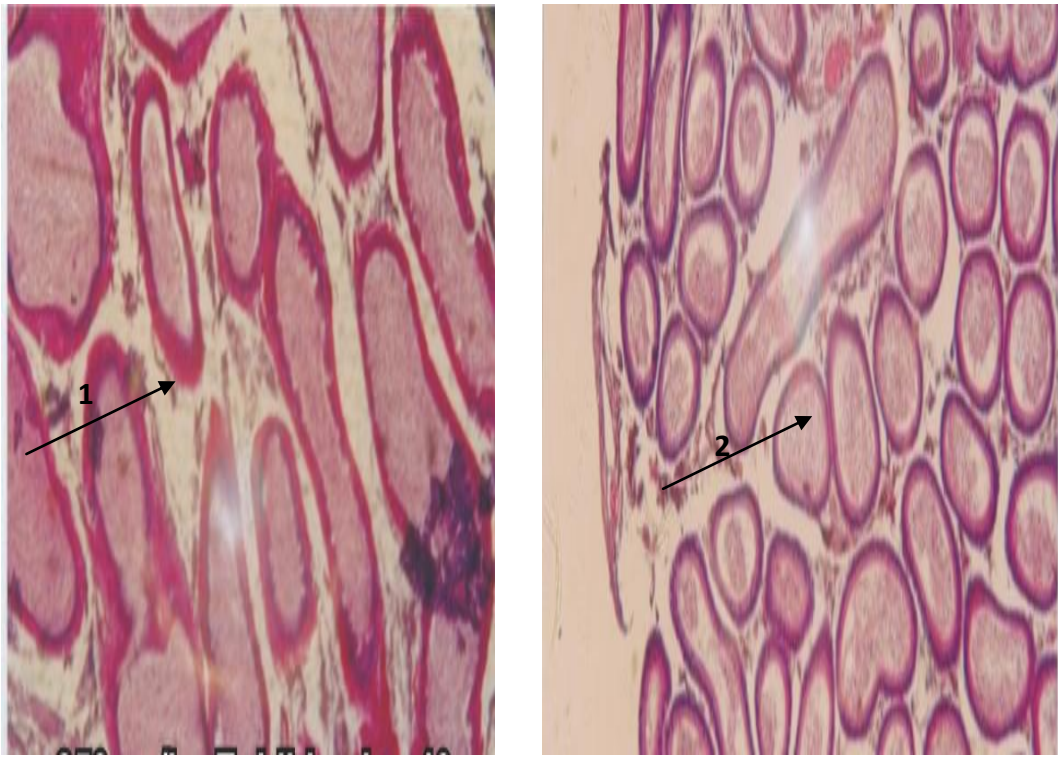



**C** **D**

**Plate IV: Photomicrograph of the Effect of Methanolic Extract of *Garcinia kola* Seed on Testes of Rats Treated for 60 Days (x400)**

**C= 250mg/kg** **D= 500mg/kg**

**3, 4 = Sperm cells from seminiferous tubules**

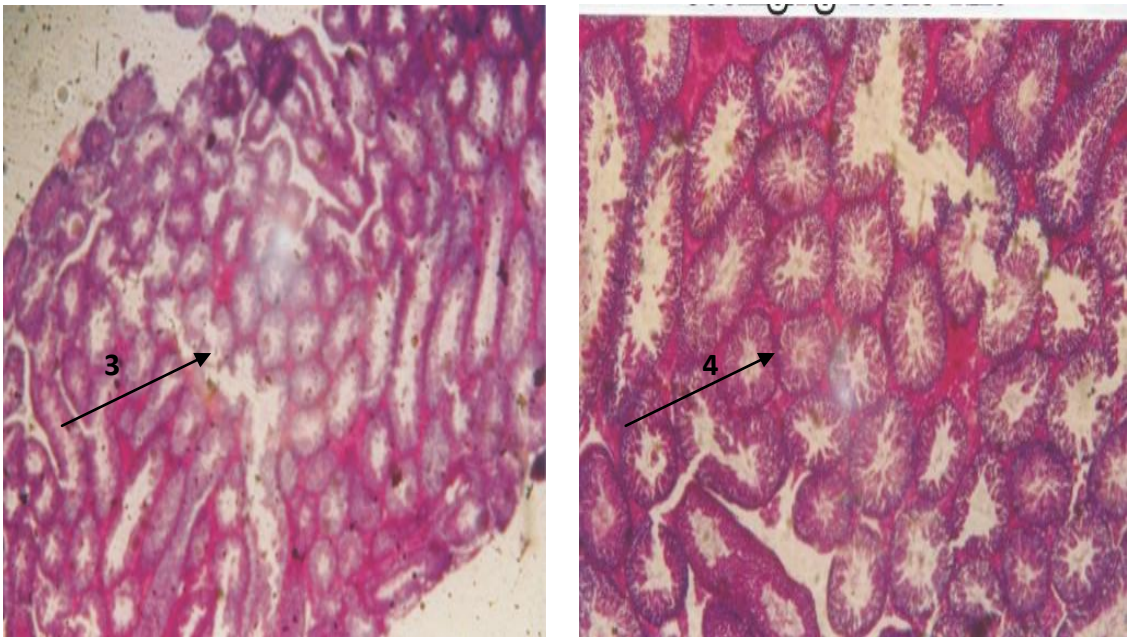



**A** **B**

**Plate V: Photomicrograph of the Effect of Methanolic Extract of *Garcinia kola* Seed on Epididymis of Rats Treated for 20 Days(x400)**

**A= Normal Saline;** **B= 125mg/kg;**

**1, 2 = Sperm cells from epididymal ducts**

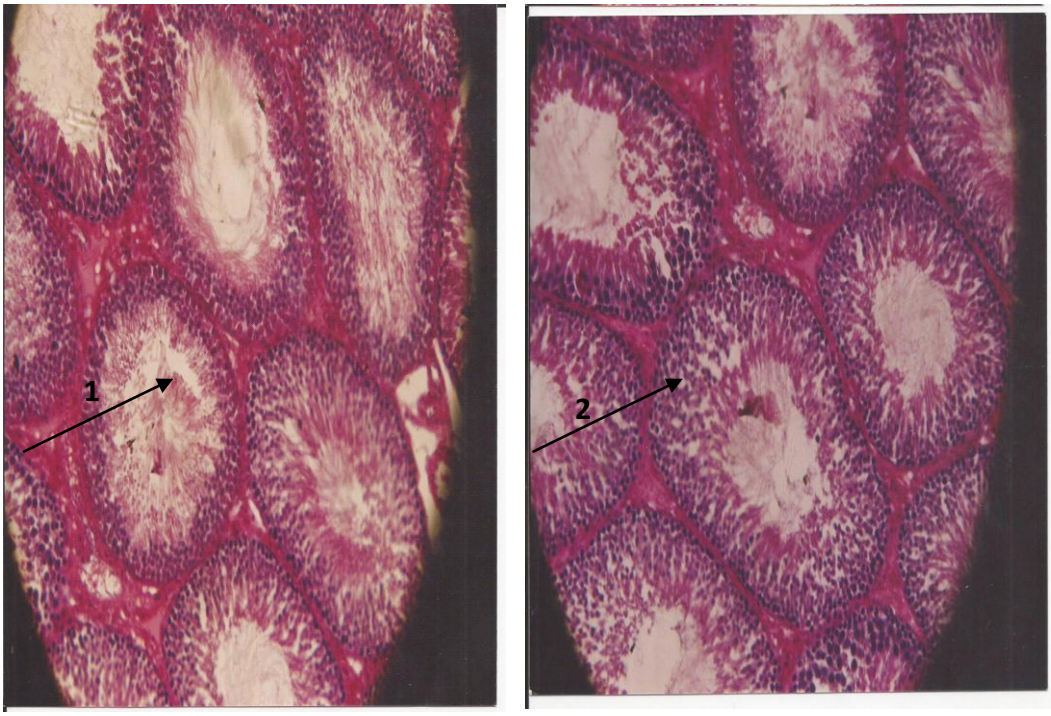



**C** **D**

**Plate VI: Photomicrograph of the Effect of Methanolic Extract of *Garcinia kola* Seed on Epididymis of Rats Treated for 20 Days (x400)**

**C= 250mg/kg;** **D= 500mg/kg**

**3, 4 = Sperm cells from epididymal ducts**

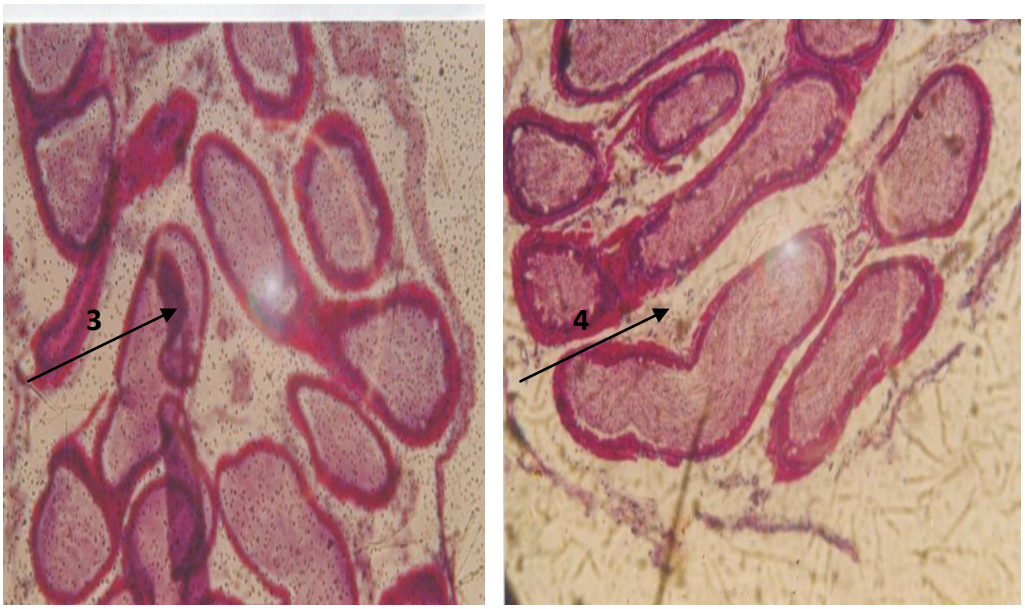



A B

**Plate VII: Photomicrograph of the Effect of Methanolic Extract of *Garcinia kola* Seed on Epididymis of Rats Treated for 60 Days(x400)**

**A=Control B=125mg/kg**

**1, 2 =Sperm cells from epididymal cells**

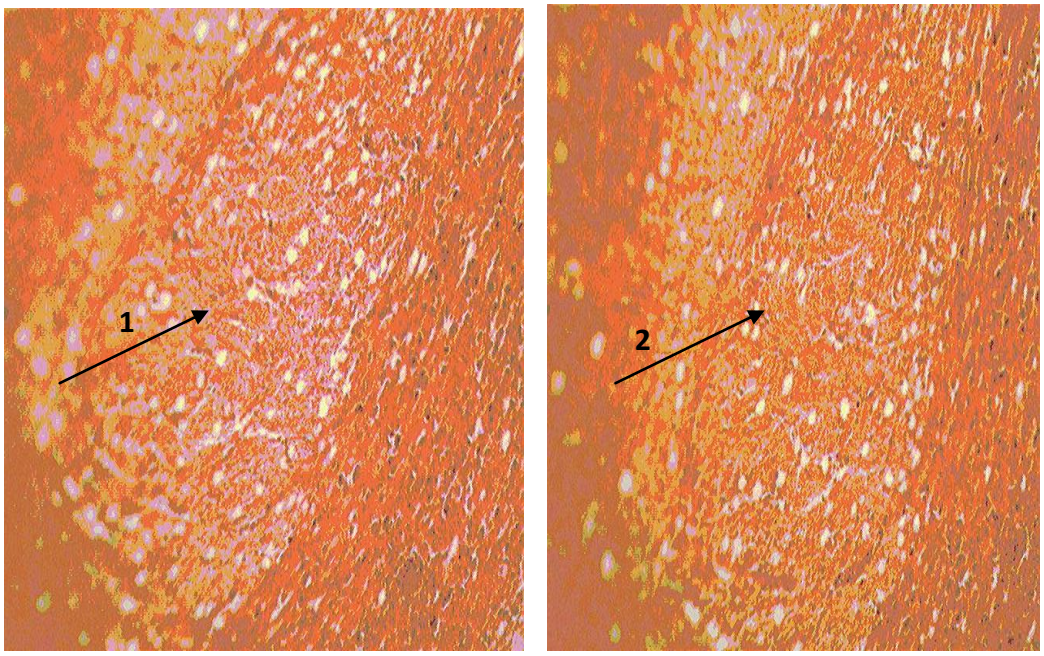



C D

**Plate VIII: Photomicrograph of the Effect of Methanolic Extract of *Garcinia kola* Seed on Epididymis of Rats Treated for 60 Days (x400)**

**C=250mg/kg D=500mg/kg**

**3, 4 = Sperm cells from epididymal ducts**

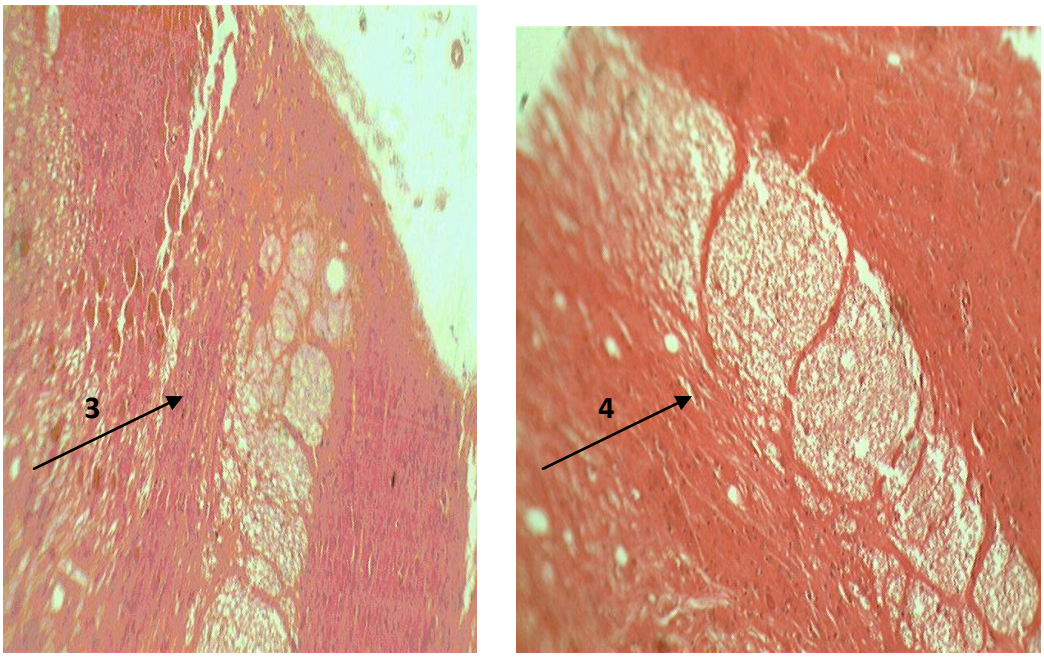



**A** **B**

**Plate IX: Photomicrograph of the Effect of Methanolic Extract of *Garcinia kola* Seed on Anterior Pituitary of Male Rats (20 Days) (x400)**

**A= Normal Saline** **B= 125mg/kg**

**1, 2 = Parenchymal cells of the pituitary**

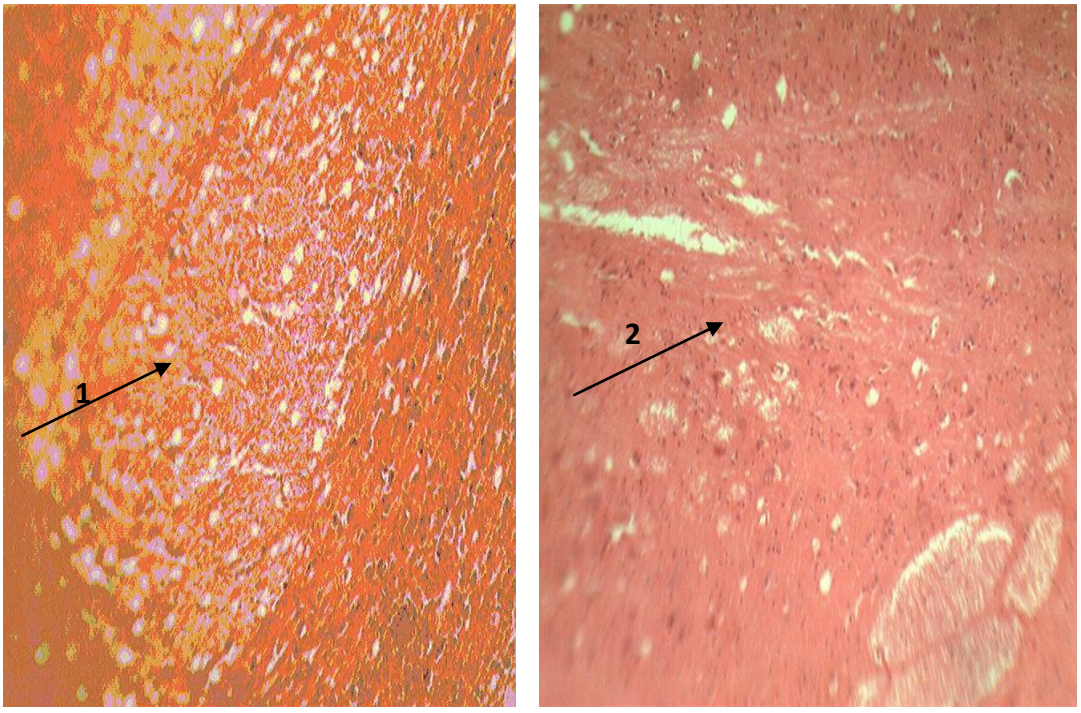



**C** **D**

**Plate X: Photomicrograph of the Effect of Methanolic Extract of *Garcinia kola* Seed on Anterior Pituitary of Male Rats (20 Days) (x400)**

**C= 250mg/kg** **D= 500mg/kg**

**3, 4 = Parenchymal cells of the pituitary**

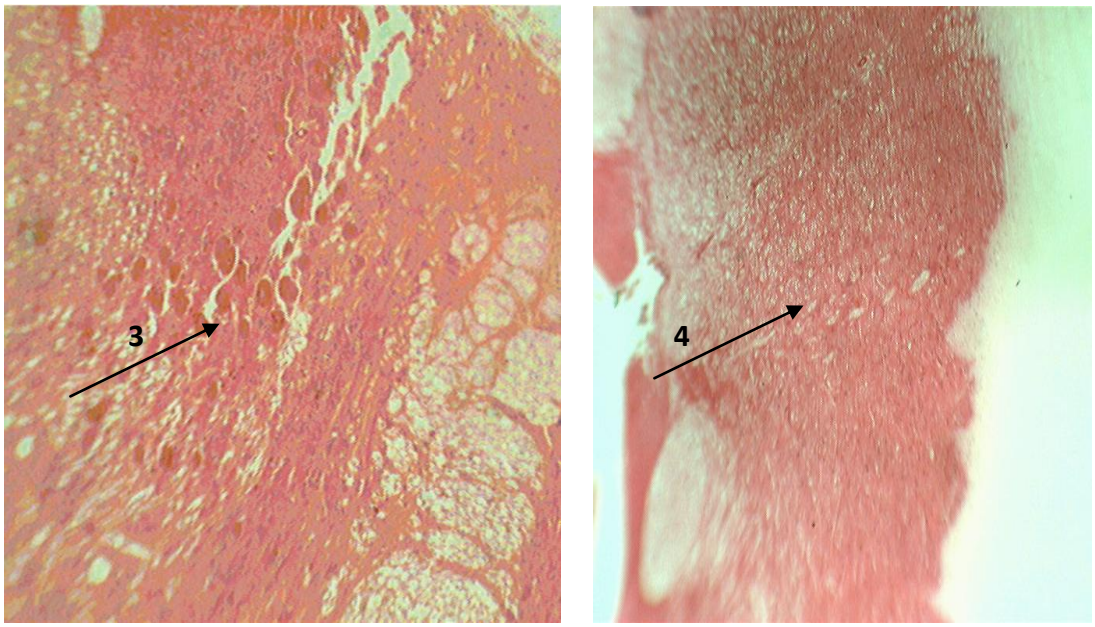



A B

**Plate XI: Photomicrograph of the Effect of Methanolic Extract of *Garcinia kola* Seed on Anterior Pituitary of Male Rats (60 Days) (x400)**

**A= Normal Saline** **B= 125mg/kg**

**1, 2 = Parenchymal cells of the pituitary**

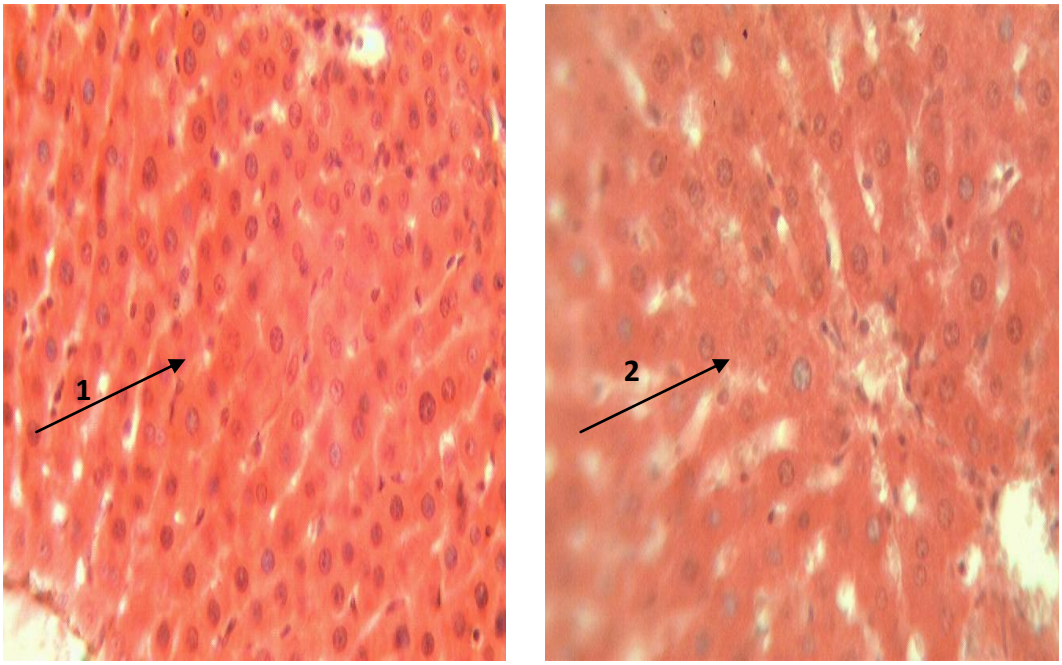



**C** **D**

**Plate XII: Photomicrograph of the Effect of Methanolic Extract of *Garcinia kola* Seed on Anterior Pituitary of Male Rats (60 Days) (x400)**

**C= 250mg/kg** **D= 500mg/kg**

**3, 4 = Parenchymal cells of the pituitary**

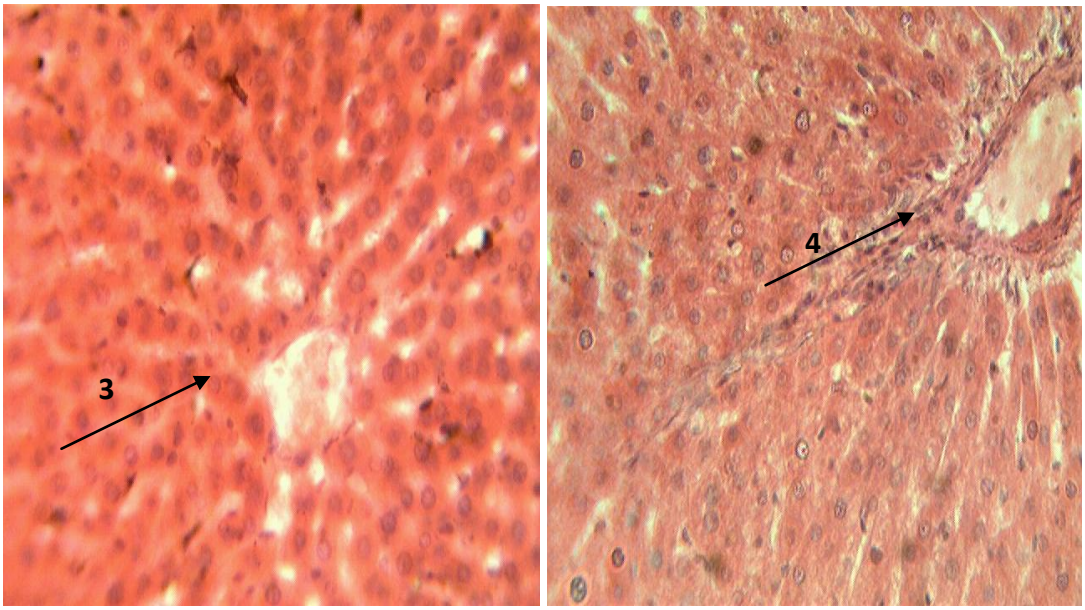



A B

**Plate XIII: Photomicrograph of the Effect of Methanolic Extract of *Garcinia kola* Seed on Liver of Male Rats (60 Days) (x400)**

**A= Normal Saline** **B= 125mg/kg**

**1, 2 = Parenchymal liver cells (hepatocytes)**




**C** **D**

**Plate XIV: Photomicrograph of the Effect of Methanolic Extract of *Garcinia kola* Seed on Liver of Male Rats (60 Days) (x400)**

**C= 250mg/kg** **D= 500mg/kg**

**3, 4 = Parenchymal liver cells (hepatocytes)**

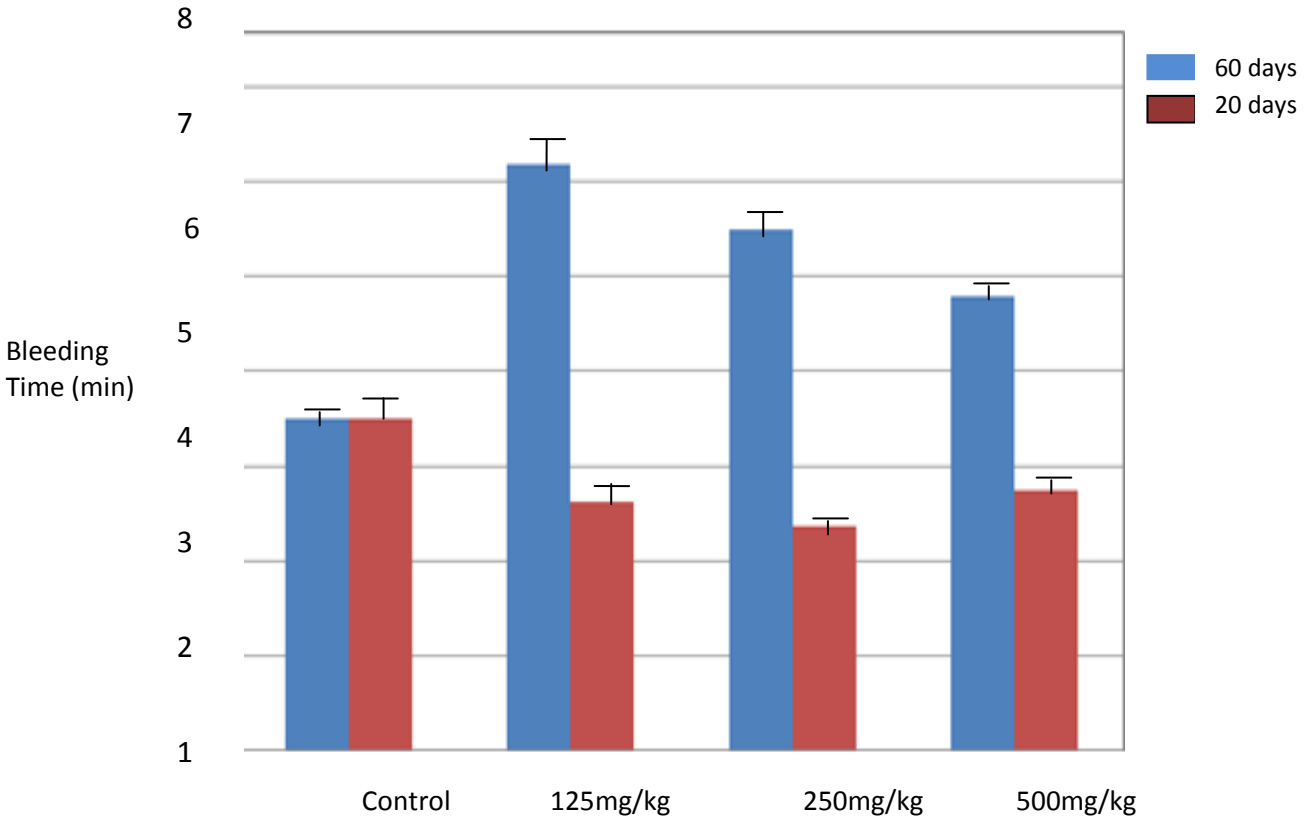



**Figure 11**: **Histogram of the Effect of Methanolic Extract of *Garcinia kola* Seed on Bleeding Time in Male Rats**

***P*<0.05**
**n=5**

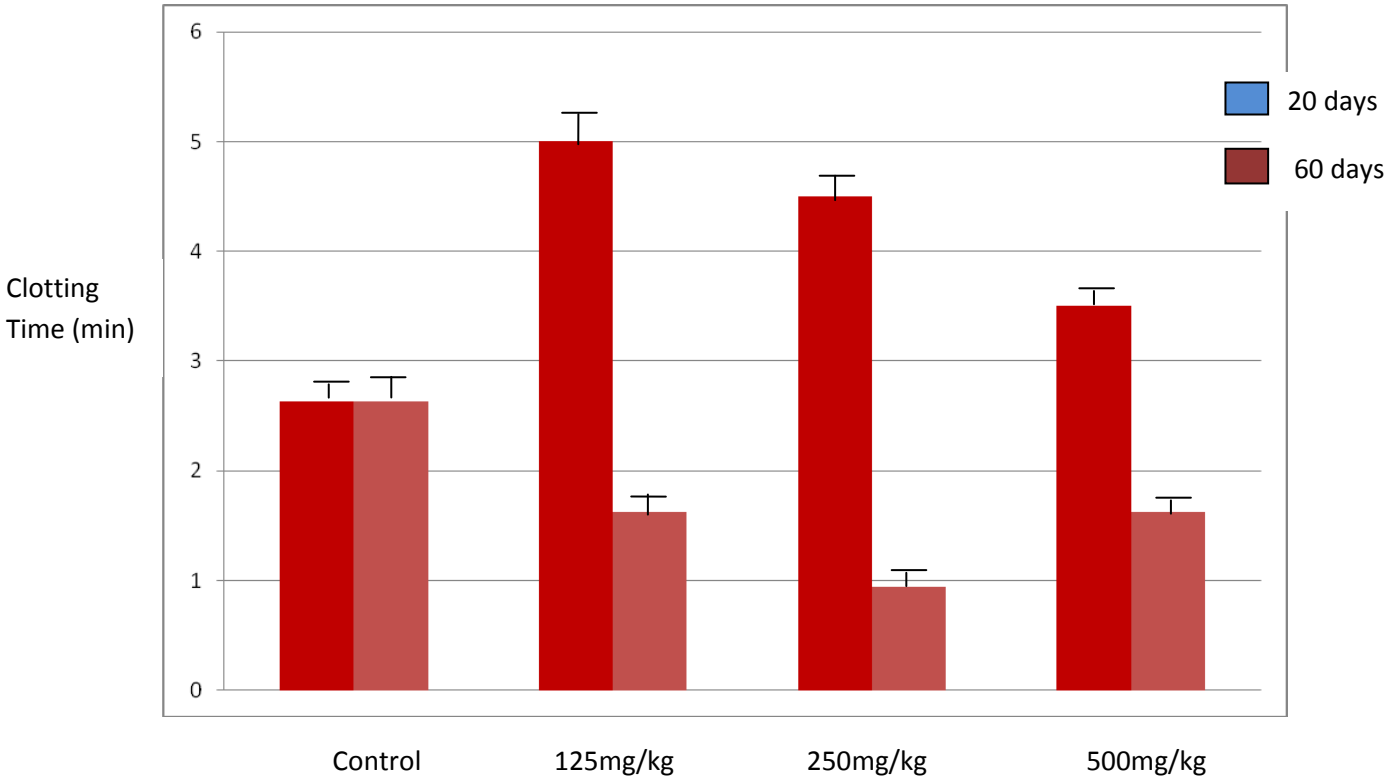



**Figure 12: Histogram of the Effect of Methanolic Extract of *Garcinia kola* Seed on Clotting Time in Male Rats**

******P*<0.05**
**n=5**

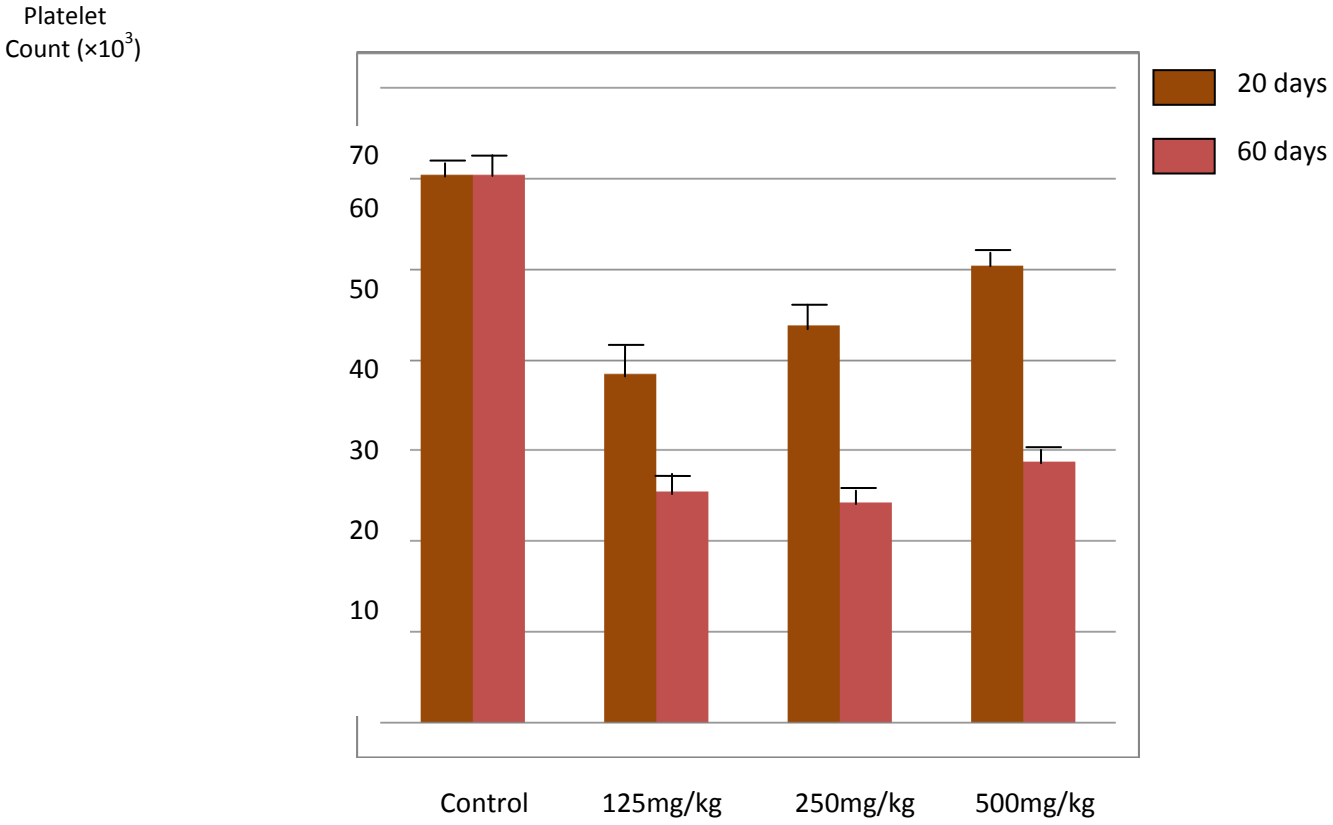
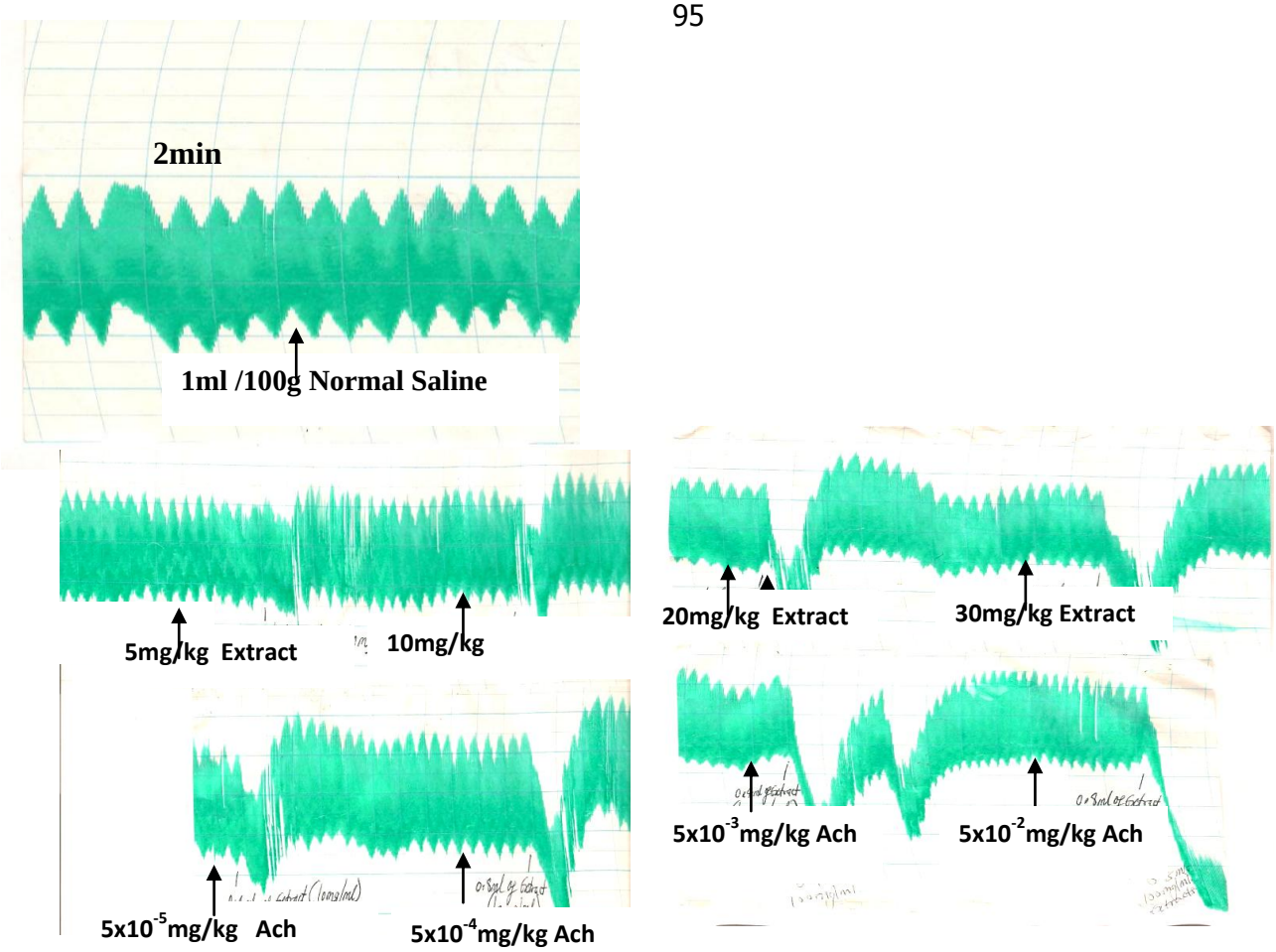



**Figure 13: Histogram of the Effect of Methanolic Extract of *Garcinia kola* Seed on Platelet Counts in Male Rat**

***P*<0.05**
**n=20**



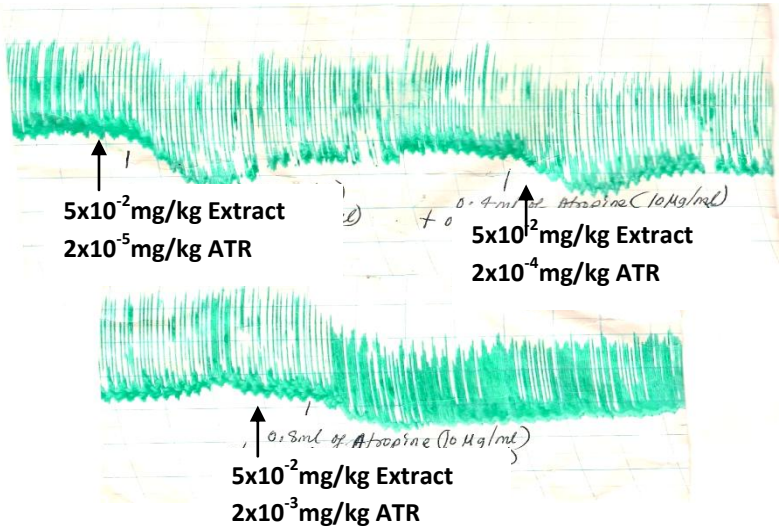




**Plate XV: Effects of Methanolic Extract of *Garcinia kola* Seed on Blood Pressure of a Male Cat**

**Table 8: Effect of Methanolic Extract of *Garcinia kola* Seed on Onset and Duration of Sleep in Male Rats for 20 Days**

| Dose (mg/kg) | Onset of Action (Mins) mean ± S.E.M | Sleep Duration (Mins) mean ± S.E.M |
|---|---|---|
| Control | 32.00±3.85 | 177.80±11.50 |
| 125 | 23.00±0.63 | 170.80±5.40 |
| 250 | 26.20±0.80 | 165.80±11.60 |
| 500 | 32.60±2.70 | 160.80±10.90 |

******P*<0.05

n=5

**Table 9: Effect of Methanolic Extract of *Garcinia kola* Seed on Onset and Duration of Sleep in Male Rats for 60 Days**

| Dose (mg/kg) | Onset of Action (Mins) mean ± S.E.M | Sleep Duration (Mins) mean ± S.E.M |
|---|---|---|
| Control | 31.90±2.00 | 158.70±3.22 |
| 125 | 36.70±1.60* | 143.20±3.50* |
| 250 | 33.10±2.70$^{+}$ | 124.20±3.10* |
| 500 | 35.50±1.90* | 137.10±4.60* |

***P*<0.05**

**n=5**

**Table 10: Phytochemical Screening of Methanolic Extract of *Garcinia kola* Seed**

| CONSTITUENT | REMARK |
|---|---|
| Alkaloids | + |
| Glycosides | + |
| Saponins | ++ |
| Tannins | ++ |
| Flavonoids | + |
| Anthraquinones | - |
| Carbohydrates | + |
| Steroids | + |

**(+) = Present** **(-) = Absent**

**Table 11: Elemental Analysis of Methanolic Extract of *Garcinia kola* Seed**

| ELEMENT | CONCENTRATION (mg/L) |
|---|---|
| **Se** | **Nil** |
| **Zn** | **4.5801** |
| **Cu** | **0.6792** |
| **Fe** | **3.3585** |
| **Ca** | **3.0799** |
| **Mg** | **23.2992** |
| **K** | **16.5245** |
| **Na** | **96.8325** |

# CHAPTER FIVE
# DISCUSSIONS AND CONCLUSION

## 5.1 DISCUSSIONS

The investigation of the effect of different doses of the methanolic extract of *Garcinia kola* seed and duration of administration on sperm concentration showed that the extract caused a dose-dependent decrease on sperm concentration. The decrease was especially significant at 250 and 500 mg/kg for both the 20 and 60 days of administration compared to control ($P<0.05$). Interestingly, it was observed that even at the highest dose of 500 mg/kg, the sperm concentration remained above 50%. This however put questions on the safety of the extract at the doses used and may not support its chronic use by its consumers. The motility of the sperm cells was assessed within 4 hours of release. Sperms were generally observed to be in slow progression than rapid progression. The proportion of rapid progression in this work was lower at all dose levels, but lowest at 125 mg/kg, with 23.08 ± 1.86%. The high proportion of slow progression may be attributed to the period of exposure (4 hours) before the counting since sperms become sluggish with increase exposure (Makler *et al*., 1979).

Sperm motility test is a routine procedure for fertility test in men because poor sperm motility is one of the major causes of male infertility. It is reported that the rat seminal vesicle secretion possesses both motility promoting as well as inhibitory protein factors (Peitz, 1988; Jeng *et al.,* 1993). The determination of overall motility and the proportion of motile sperm in relation to speed of progression from ineffective vibrating and circling motions are said to be important (Amelar *et al*., 1980). Indeed, sperm motility is one of the biological characteristics of the spermatozoa. This is said to be more significant than the concentration.

According to WHO, sperm motility is graded from a-d as follows:

a. Fast or rapid progression, which swim forward in a straight line.

b. Slow progressions which swim forward but in a curved or crooked line or slowly.

c. Non-progression which move their tails but do not move forward

d. Immotile which do not move at all.

Given the fact that at all given time, the proportion of rapid progression is usually lower; the results obtained may serve as experimental evidence that methanolic extract of *Garcinia kola* seed may be beneficial for sperm motility. Similarly, sperm viability test showed that *Garcinia kola* seed extract significantly reduced the viability of the cells at 125 and 250 mg/kg compared to control. Interestingly, at all the doses, the viability was greater than 70% indicating that *Garcinia kola* may not have significant deleterious effects on the total viability of the cells. It can be recalled that following release, sperm cells can sustain their reproductive viability for several days especially as they are meant to interact with hostile acidic environment of the vaginal secretions. Sperm viability which is closely linked with sperm metabolism deals essentially with the sperm cells having the energy to keep moving in a straight line (rapid progression). This is also an important integral part of male fertility health. After ejaculation, a sperm cell is said to live for 3-5 days in the fallopian tube, but when outside the body, it can survive for only about 20 minutes to an hour, depending on how expose the semen is to air and other environmental factors. Indeed it is reported that non-motile sperms can however, be viable as described by Jeyendran *et al*., (1984) and such are said to be useful in intracytoplasmic sperm injection (ICSI) in *in-vitro* fertilization procedures.

Consequently, the results of this investigation could serve as experimental evidence that the extract of *Garcinia kola* seed may provide sustainability of the metabolic function of the sperm cells which could improve fertility in the males. This finding is in agreement with that of the sperm motility and concentration tests. The results of the effect of the extract on the survival of the sperm cells expressed as proportion death following the administration in male rats showed that the death of sperm cells was highest at 500 mg/kg after 60 days of administration but lowest with the same dose following 20 days of administration. Indeed, while the death decreased with increased dose of extract for 20 days treatment, the reverse case was observed in the groups that received the extract for 60 days, the death increasing with increase in the dose. Interestingly, death at all the doses used for both durations of treatment were less than 50% with the highest being 21.38±3.66% at 500 mg/kg after 60 days. This may indicate toxic effect of the extract when given at the dose of 500 mg/kg continuously for that long period. In general, the extract could be said to offer little energizing or protective effect on the sperm cells especially at 500 mg/kg. Sperm degeneration and death of sperms after ejaculation has been reported as one of the causes of human male infertility (Wilton *et al*., 1988).

*Garcinia kola* seed extract was found to cause increase in the level of LH following the administration of extract for 20 days at 125 and 250 mg/kg while a decreased value was observed at 500 mg/kg. Following the 60 days of administration, it was found that the extract also decreased LH level at all doses ($P>0.05$) but not in a dose dependent mechanism. Comparatively, the decrease was observed to be prominent on duration of administration rather than on doses, as values obtained from the 60 days of administration were respectively lower than those of 20 days. This may reflect its toxic effects. LH is a hormone produced by gonadotropin cells in the anterior pituitary

gland. In males it stimulates the leydig cells to produced testosterone which controls spermatogenesis. Therefore, results obtained from this investigation could provide an experimental evidence that *Garcinia kola* seed extract can be beneficial on spermatogenesis at 125 and 250 mg/kg but not so at 500 mg/kg. This may also justify its traditional claim that it has aphrodisiac property.

The extract was observed to cause an increase on the level of FSH at 125 and 250 mg/kg after both the 20 and 60 days treatments compared to control. However, at a dose of 500 mg/kg for both the duration of administration, the extract caused a non-significant decrease on the level of the hormone compared to control. FSH, like LH, is a polypeptide hormone synthesized and secreted by gonadotropins-releasing hormones or cells of the anterior pituitary gland. In males, it stimulates the testes through the sertoli cells to produce germ cells for spermatogenesis. Therefore, it can be suggested that the extract of *Garcinia kola* could be beneficial in spermatogenesis but the exact mechanism remains to be determined (Sanderson, 2006).

The effect of the *Garcinia kola* seed extract on testosterone did not follow any consistent pattern when compared with that of LH. Testosterone production is directly dependent on LH. Given the fact that *Garcinia kola* is eaten by its consumers almost in an addictive manner, it has been reported that the use of some recreational substances have been associated with decreased serum testosterone and sperm concentration (Nudell *et al.,* 2002; Braken *et al*., 1990). Nonetheless, it can be suggested that the extract may be beneficial in the production of testosterone at 125 and 250 mg/kg. This finding also suggests that the extract may possess an androgenic property. When taken together, the results suggest that the administration of *Garcinia kola* may play roles in spermatogenesis. Except for testosterone, this beneficial effect appears lost at a higher dose of 500 mg/kg and this may indicate toxicity and a warning signal against the high

and indiscriminate intake of *Garcinia kola* for long periods. Sperm production is essentially controlled by FSH in the seminiferous tubules, while testosterone production is controlled by LH (Plant & Marshall, 2001)

Results of the fertility/reproductive performance tests showed that *Garcinia kola* seed extract at a dose of 250 mg/kg for 20 days increased the frequency of mounting, intromission and ejaculation. Similarly, the number of litters per female rat paired with the treated males was highest with that which received the dose of 250 mg/kg. These parameters are important in fertility assessment of males but they are also said to be psychogenic. Infertility following faulty intromission with attendant failures of ejaculation has been observed occasionally in some couples due to decreased sexual arousal. Male sexual performance consists of a complex processes involving both central and peripheral mechanisms. These results could be closely linked to the fact that the extract also increased the level of testosterone in some dose levels especially with increased sexual performance including intromission and ejaculation and therefore this observation could be linked to the increased in testosterone level or perhaps any other possible mechanism. Given the fact that increased sexual performance usually correlates with fertility (Bitran & Hull, 1987; Abdulwahab *et al.*, 2007), it can be suggested based on the result that *Garcinia kola* has a positive modulatory effect on motivation to engage in sexual function. This finding indeed corroborates that of Ralebona *et al*., (2012) who had carried out a similar investigation and submitted that an ethanolic extract of *Garcinia kola* at 200 and 400 mg/kg for 28 days enhanced libido and potency in male rats with increased testosterone secretion. However, the exact mechanism for this effect remains to be determined but it is a known fact that androgens enhance sexual performance by improving libido which results in increased intromission and ejaculation. These effects could be centrally or peripherally mediated

(Anderson, 2001; Fabbri, 2001; Mills *et al*., 1996). However drugs that affect libido are said to usually act centrally and those that interfere with the autonomic system will have negative effect on erection, intromission and ejaculation. An extract of the medicinal plant, *Lipidium meyenii* has been investigated for a similar property and this could further underscore the importance of result obtained from this study on *Garcina kola* (Gonzales *et al*., 2001; Zheng *et al*., 2000).

The results of the effect of G*arcinia kola* seed extract on a phenylephrine precontracted corpus cavernosum smooth muscles of the rabbit obtained from three repeated tests.

The results showed phenylephrine caused sustained but dose-dependent contractions of the corpus cavernosum while the extract produced a dose-dependent relaxation in the basal equilibrium state of the corpus cavernosum muscle strip. The extract caused a dose – dependent relaxation of the phenylephrine precontracted corpus cavernosum. This perhaps suggests a competitive mechanism. A relaxation of 72.24% was obtained at a concentration of 2.0 mg/ml of the extract while a relaxation of 4.00% was obtained with a concentration of 0.4 mg/ml (Figure 10). The relaxation action was significant at concentration of 1.6 and 2.0 mg/ml ($P<0.05$) while at lower concentrations of 0.4, 0.8 and 1.2 mg/ml, it was not significant ($P>0.05$) compared with the maximum contraction caused by phenylephrine. However, the relaxation actions of the lower doses were preceded by brief contractions which may explain why the relaxations at these concentrations were not significant.

From these results, it can be deduced that *Garcinia kola* seed extract may possess active compounds that induce relaxation of the corpus cavernosum. Notwithstanding the observation that the relaxation was preceded by contraction with lower concentrations may suggest the presence of compounds that induce contraction as

well. Plant extract are known to contain many active principles which may act synergistically or antagonistically. Thus multiple mechanisms may be involved in the relaxation of corpus cavernosum by the extract. This may need further investigation. Essentially, the results may also suggest that *Garcinia kola* perhaps possesses an effect on penile erection that could be beneficial in erectile dysfunction. This supports part of the findings of this study which revealed that *Garcinia kola* increased the frequency of mounting, intromission and ejaculation (Table 2 and 3).

Basically, pharmacological agents that induce relaxation of the corpus cavernosum are believed to cause penile erection through vasodilation that increases blood flow and pressure of the cavernosal arteries. Such agents have formed the basis for pharmacological interventions in erectile dysfunction (Andeson, 2011). Different pharmacological mechanisms are reported to be involved in penile tumescence which is associated with complex processes. The cavernosal smooth muscles are said to receive a rich adrenergic innervations that maintain the integrity of the penis. Indeed, the penis is said to receive sympathetic, parasympathetic, somatic and sensory innervations (Dail, 1993). For this reason, it is generally accepted that the penis is kept in the erectile state through a relaxation effect on the corpus cavesnosa while the flaccid state is maintained by vasoconstriction which reduces blood flow into the cavernosum arteries.

Noradrenaline is reported as one of the main factors that maintain the corpus cavernosum muscles tone through the stimulation of α-adrenergic receptors, particularly post – junctional $\alpha_1$ which is said to be the predominant subtype in the penile erectile tissues (Priesto, 2008). Indeed, studies have revealed that α1 adrenoceptor agonists that contract such smooth muscles do so through an α-1 isoform (α-1L), though α-1a seems predominant. This is because such agonists are said to

display low affinity for α1 antagonists such as prazosin or tamsulosin (α-1a) (Daplane & Galzim, 1996; Sironi *et al.*, 2000; Morton *et al.*, 2007). However, according to Rudner *et al.,* (1999), distribution of the α-1 isoforms may not be the same in different animal models. Consequently, the choice of phenylephrine, an $\alpha_1$ specific agonist as a carvernosal smooth muscle contractile agent in studies of this nature is perhaps informed by this property.

It is therefore my view that the relaxation action of the extract could be mediated by peripheral mechanism, perhaps through competitive antagonism of the $\alpha_1$ receptors. As part of this study, the extract was shown to reduced blood pressure of an anaesthetized cat which was blocked by atropine (Plate XIV). This could suggest a parasympathetic mechanism. This is by no means conclusive as other mechanisms could be involved. Some of such mechanisms that have been used to produce specific agents that have proven useful in erectile dysfunction include; blockage of phosphodiesterase type 5 enzymes (sildenafil, tadanafil etc.), $\alpha_2$–adrenergic antagonists (Yohimbine, though its action may not be peripheral since $\alpha_1$ is the predominant subtype in penile erection), non–selective α- antagonism (Phentolamine), stimulation of guanylyl cyclase that liberates endothelium derived relaxing factor (EDRF) or nitric oxide (Nitroglycerin) and potassium channel openers(cromakalin, pinacidil etc) (Anderson, 2001)

Notwithstanding, given the observation that the extract induced relaxation of the phenylephrine precontracted cavernosal smooth muscles preceded by brief contraction at lower concentrations it could be inferred that the extract could be acting through different mechanisms. It can be recalled that mechanisms such as hyperpolarisation and secretion of EDRF (nitric oxide) cause smooth muscle relaxation

preceded by contraction at low concentrations, while on the other hand mechanisms that increase intracellular calcium sequestration are said to cause only relaxation without preceding contractions. Based on these results, it can be submitted that *Garcinia kola* possesses a relaxation action on the corpus cavernosum. This could be useful in erectile dysfunction therapy and may perhaps explain its traditional use as an aphrodisiac (Azija, 1998, personal communication)

The effect of the *Garcinia kola* seed extract on the histology of the testes and epididymis revealed that the extract caused disruption on the histology of the testicular cells. This observation, perhaps indicate that the extract at the said doses level may likely interfere with the function of the epididymis on sperm maturation. Given the fact that the testes are the primary sites for sperm production and the epididymis the organ for sperm maturation and storage, it can be inferred that *Garcinia kola* could affect sperm quality. This seems to correlate with the result on sperm viability and percent death. Though, the exact mechanism for this histological observations remains to be determined it is known that androgens such as testosterone in high doses do cause negative feed-back on spermatogenesis. Also it can be noted that the close morphological association between sertoli cells and germ cells at different stages of their developments (e.g. spermatogonia, spermatocytes, primary and secondary spermatids) is sometimes only visible in the seminiferous tubules. As a result of such morphological intimacy between the sertoli cells and germ cells, it is conceivable that extensive interactions and communications take place between these cells in the testes throughout spermatogenesis both at the biochemical and molecular levels (Robaire *et al*., 1995).

Results of the effect of *Garcinia kola* seed extract on the histology of the anterior pituitary showed that the extract at 125 and 250 mg/kg did not cause any

significant morphological change on the cellular integrity. However, at higher doses, there was clear morphological disruption of the cells. Curiously a similar pattern of results was obtained with the histological examination of the liver, a vital organ in the body. There was no visible disruption of the cellular architecture at doses of 125 and 250 mg/kg compared to control. However, at 500 mg/kg the hepatocytes appeared abnormal with irregular cells and limited sinusoids. This effect may perhaps have some clinical implications on the integrity of the testes. It has been reported that hepatocyte disruption such as in cirrhosis of the liver is usually associated with testicular atrophy and reduced free testosterone without decreased gonadotrophins (Rather, 1947; Mowat *et al.*, 1976). However, given the observation that the *Garcinia kola* seed extract caused increased in the testicular weight of treated rats, it can be inferred that the extract may be beneficial in cirrhotic conditions, though the pathogenesis of endocrine change in cirrhotic men is said to be multifactorial which may involved decreased hepatic clearance of some estrogenic compounds and autoimmune mediated primary testicular defects (Green, 1977).

The anterior pituitary is known for its production of the gonadotropins such as LH and FSH which are the principal hormones that initiate the process of spermatogenesis in the males. Therefore this experimental finding may indicate the aphrodisiac or fertility beneficial effect of *Garcinia kola* at the doses of 125 and 250 mg/kg while at the same time revealing its toxic effect at 500 mg/kg especially when administered for a long period of time. This is typical of many drugs. This finding correlates with the results obtained on effect of the extract on LH, FSH and testosterone. The liver on the other hand is an important organ in the body system that controls the function of many other organs. How important the finding of this investigation directly related with sexual performance of the male rats in this

investigation will be of interest in future studies. Nonetheless it is of interest to suggest that the findings reveal that the extract of *Garcinia kola* could enhance the production of LH and FSH from the anterior pituitary gland at 125 and 250 mg/kg.

The extract caused a dose-dependent increase of testicular weights of treated animals. It is a known fact that increased in testicular weight is usually associated with increased in germ cells and spermatogenesis (Sharpe *et al*., 1995). However, in this case there is no specific conclusion on the cellular components accountable for the increase due to the dose increase of the extract and the lateral increase due to the duration. Mechanisms such as decreased apoptosis or increased cellular proliferation are sometimes associated with increased testicular weight. On the other hand and in pathology, germ cell tumors can contribute to increase testicular weight. This can be cancerous or non-cancerous. It has also been observed that increase in testicular weight especially following hemicastration has been associated with increase in daily sperm production. This is said to be due to a compensatory mechanism (Bergh *et al*., 1982). This finding could, therefore serve as an experimental evidence that the intake of *Garcinia kola* may improve spermatogenesis especially at the initial stage as suggested by Le Blend & Clermont (1952). This finding is in agreement with that of Ralebona *et al*., (2012) and a further scientific support to the traditional claim that *Garcinia kola* has aphrodisiac property.

There was significant increase in bleeding time of animals treated with extract for 20 days in all the groups compared with those of control. However, as the dose of extract increased the bleeding time decreased, though this remained higher than that of the control group. This perhaps signifies that the effect could be dose-dependent. The group that received the extract at 125 mg/kg for a period of 20 days had a mean bleeding time of 6.20 ± 0.57 minutes while the group that received a dose of 500 mg/kg

for the same period had a mean bleeding time of 4.80 ± 0.8 minutes. These values were both significantly higher than that of the control group with a mean of 3.50 ± 0.41 minutes ($P$<0.05). On the contrary, the bleeding time was found to be significantly shorter in groups that were administered the extract for a period of 60 days as compared to the control group ($P$<0.05). In general, the extract increased the bleeding time of the treated male rats compared to control following daily administration for 20 days while this appears compromised in groups that received the corresponding daily doses for 60 days.

The extract caused increase in the clotting time of treated rats after daily administration for 20 days at all the dose levels. This was lower compared to that of control at 60 days. This is consistent when compared with the findings on bleeding time since both the bleeding and clotting time are functions of the coagulability factor.

Similarly, the effects of the extract on platelet counts following administration for 20 and 60 days in all doses used were significantly lower compared to control ($P$<0.05). However, the results did not follow any consistent pattern. In general, the extract significantly decreased platelet concentration in dose and duration-dependent manners. This is expectedly in agreement with the results obtained on bleeding and clotting times. This is not surprising since the principal function of platelets is to prevent bleeding through clot formation during thrombotic processes. This suggests that *Garcinia kola* may possess anti-platelet as well as anti-thrombotic property. The anti-thrombotic effect of *Garcinia kola* has also been reported by Ohadoma *et al*., (2011). This property may be relevant to the traditional claim that *Garcinia kola* possesses aphrodisiac and may justify its traditional use. Venous thrombosis and other similar disorders associated with hyperhomocysteinemia have been associated with oligozoospermia in male patients (Rees & Rodgers, 1993; Undas *et al*., 2005; Varghese

& Asif, 2013). Equally, varicocele (a major risk factor in male infertility), though essentially caused by valvular incompetence, can sometimes be due to renal vein thrombosis, since the left testicular vein connects to it. This is common in idiopathic varicoceles (Kleinclauss *et al.*, 2001).

The extract produced a dose-dependent decrease on blood pressure in cats. This effect was blocked by atropine, an antimuscarinic agent. This therefore suggests that *Garcinia kola* may possess a blood pressure regulatory property that is sensitive to atropine and thereby mediated by the cholinergic mechanism. Five subtypes of muscarinic receptors have been identified and designated $M_1$-$M_5$.Of these subtypes, $M_1$, $M_3$ and $M_5$ are said to coupled with a G-protein that generates phospholipace C (PLC) and subsequently inositol triphosphate (IP3).While the $M_3$ is said to generally cause contraction of smooth muscles, however, with respect to vascular sooth muscles, it specifically causes increased in nitric oxide (NO) which diffuses to the vascular smooth muscles and causes relaxation (Walch *et al*., 2001). Indeed, dilatation of vascular beds by exogenously administered acetylcholine is said to be specifically due to $M_3$ receptor subtype (Caulfield & Birdsall, 1998). Penile erection has been associated with agents that decrease blood pressure through the central regulatory mechanism. The role of acetylcholine in the regulation of penile erection has been inferred from some limited neuropharmacological studies that involved muscarinic agonists and antagonists (Anderson 2001, Maeda *et al*., 1990; 1994a). These findings suggested that cholinergic mechanism may have a regulatory role in penile erectile function. It is conceivable that the inhibitory effect of atropine could partly explain the effect of *Garcinia kola* on erectile function observed in this study. Acetylcholine has been reported to cause relaxation of strips of penile muscles suggesting that acetylcholine through muscarinic receptors plays a role in penile erection (Irwin, 1992). Similarly, Anderson (1998)

reported that disturbance of the cholinergic nervous system function is associated with some neurological system disorders including erectile dysfunction.

The effect of the methanolic extract of *Garcinia kola* seed on phenobarbitone – induced sleep, suggests that *Garcinia kola* could possess some stimulatory or anti-hypnotic effects. It is indeed reported that sleep disturbances e.g. narcolepsy are usually associated with sexual dysfunction in men (Karacan, 1986; 1992). Loss of libido secondary to sleepiness has been reported. While acute sleep deprivation produces hyper sexuality, chronic sleep loss on the other hand correlates with loss of sexual interest. Similarly it has been reported that psychostimulant agents such as amphetamine can reverse sexual dysfunctions in men by augmenting sexual functioning though when used chronically the same drug inhibits sexual function (Bartlik *et al*., 1995; Montejo *et al*., 2001; Sigman, 2007). Many stimulant users have reported that such drugs are strongly associated with sex. Male users in particular have reported that stimulant drugs increase their libido and decrease sexual inhibition (Washton & Zweben, 2009). These effects may explain the chronic use of *Garcinia kola* for recreational purposes and by long distance drivers and travelers in order to avoid sleeping. This result serves as experimental evidence that *Garcinia kola* could possess stimulatory effect that may enhance sexual function, although the exact mechanism is not known.

The phytochemical analysis revealed that the seed extract of *Garcinia kola* contains alkaloids, glycosides, saponins, tannins, flavonoids, carbohydrates and steroids. This is similar to the findings of Monago and Akhidue, (2002). Among these phytochemicals, alkaloids, saponins and flavonoids, have been reported to have some critical function in male fertility. Udoh *et al*., (2009) reported the presence of an alkaloid of the extract of carcicapryl-99, inhibiting serum level of testosterone which

results in male infertility. However, Olayemi and Raji (2011) reported that quinolizidine alkaloid possesses reversible male anti-fertility effects. It can be recalled that yohimbine, a compound extracted from the plant, *Pausinyastalia yohimbe* is an alkaloid that is popularly beneficial for erectile dysfunction in male infertility.

A report by Salvati *et al*., (1996) showed that saponins in *Panax ginseng* are found to have an effect at different levels of the hypothalamic-pituitary-testes (HPT) axis. Indeed, other studies have shown that saponin components of plants enhance aphrodisiac properties due to the stimulatory effect of androgen production (Gauthaman *et al*., 2002; Koumanov *et al*., 1982). The presence of flavonoids, specifically kolavirone, a biflavonoid in *Garcinia kola* and some of its pharmacological activities have been reported (Hussein *et al*., 1982; Olatunde, 2000; Adaramoye *et al*., 2005). Flavonoids are known for their actions as antioxidants (Pietta 2000, Jovanovic *et al*., 1994, Terao, 2009). This property could therefore be beneficial in the treatment of male infertility, although this has not been conclusive as it remains controversial (Agarwal & Sekhan, 2010; Agarwal, 2004; Garrido *et al*., 2004).

Based on the aforementioned it can be speculated that *Garcinia kola* could process some beneficial effects on male fertility based on the presence of some of these phytochemical constituents. This therefore serves as a further experimental evidence and support for its traditional use as an aphrodisiac.

The elemental analysis of the methanolic extract of *Garcinia kola* seed extract revealed the presence of the following trace elements: copper (Cu), iron (Fe), zinc (Zn), magnessium (Mg), potassium (K) and sodium (Na). It showed that the extract does not contain selenium (Se), an element also of interest for this investigation. The finding that the extract contains Zn, among other elements appears interesting. According to Slivkova *et al*., (2009) trace elements are said to be important components of semen.

Many studies have shown that Zn is a critical element in spermatogenesis (Prasad, 1991; Miller *et al*., 1958; Underwood, 1977; Endre *et al*., 1990; Kvist, 1980; Capino *et al*., 1998; Yuyan *et al*., 2008). Indeed, Zn is said to possess an anti-oxidative property and this could play an important role in protecting sperm cells from scavenging oxidative elements (Colagar *et al*., 2009). Also the presence of trace elements such as Mg, Cu, Fe, Na and K are equally of interest since these elements are reported as being critical in seminal plasma following semen analysis (Gavella & Lipovec, 1998; Colagar *et al*., 2009; Wong *et al*., 2001; Shinohara and Watanabe, 1996; Lukac *et al*., 2009; Aydemir *et al*., 2006). Notwithstanding, other reports have confirmed the negative effects of high concentrations of some of the trace elements e.g. Cu in semen on spermatozoa motility profile and subsequent reproductive alteration in male sexual function (Roychoudhury & Massanyi, 2008). Trace elements are said to be essential for the functions of various enzymes and other proteins. The effects of trace elements biochemistry and physiology on parameters of fertility for some of these elements revealed in the extract of *Garcinia kola* have been reported (Leonard–Marek, 2000). This result therefore, provides experimental evidence that *Garcinia kola* contains trace element that could be vital on semen quality and this could justify its use in traditional medicine practice to improve male fertility and sexual function.

## 5.2 SUMMARY OF RESULTS

1. An acute toxicity test revealed that the methanolic extract of *Garcinia kola* has an $LD_{50}$ of 3215 mg/kg. This means the extract is generally non toxic.
2. There was increased in LH and FSH at 125 and 250 mg/kg. This suggests benefit in spermatogenesis.

3. The sperm motility test indicates that the methanolic extract of *Garcinia kola* has no adverse effect on motility of sperm cells. However, there was a general decrease in rapid progression.
4. The results on death of sperm cells revealed that the extract could be protective on sperm cells since the % death was less than 50 in all the doses used in this study.
5. The results on testosterone levels of animals in treated groups did not follow any consistent pattern, but the increased may suggest androgenic effect of the methanolic extract of *Garcinia kola.*
6. The fertility tests showed that the methanolic extract increased sexual behavior and this can be beneficial in enhancing reproductive capacity.
7. The effect of the extract on an isolated corpus cavernosum muscle strip indicate relaxation suggesting it could be useful in the therapy of penile erectile dysfunction
8. The histological examinations of the testes, epididymis and the anterior pituitary showed that the extract did not cause morphological alteration on sperm cells tissues at 125 and 250 mg/kg. This may suggest benefit in spermatogenesis.
9. The effect of the methanolic extract on the histology of the liver showed no alteration in hepatocyte architecture. This can be beneficial in therapy of oligozoospermia associated with cirrhosis of the liver.
10. The methanolic extract of *Garcinia kola* was found to increase the mean testicular wet weights of male rats. This could mean germ cell proliferation and support for spermatogenesis.
11. The Sperm viability test revealed that the extract of *G. kola* did not adversely affect the viability.

12. The Anti-thrombotic investigation showed that the extract decreased bleeding and clotting times. Similarly the extract caused increase in platelet counts. This suggests that the extract could be beneficial in treatment of male infertility due to varicocele and also in venous thrombosis associated with hyperhomocysteinemia which can cause oligozoospermia.

13. The extract produced decreased in blood pressure of an anaesthetized cat. Though the exact mechanism remains unknown, it can be speculated that if this is mediated through the $M_3$ receptor subtype coupled with a G-protein that results from the release of nitric oxide from endothelial cells in vascular beds, it may be beneficial in therapy of erectile dysfunction.

14. The effect on induced sleep showed that the extract increased the onset but decreased the duration of sleep. This may be useful in sleep disorders such as narcolepsy which are usually associated with sexual dysfunction and loss of libido in men.

15. The preliminary phytochemical analysis of methanolic extract of *Garcinia kola* revealed the presence of Alkaloids, Glycosides, Saponins, Tannins, Flavonoids, carbohydrates and steroids. Flavonoids are known for their antioxidant properties which are beneficial to sperm cells.

16. The elemental analysis revealed the presence of copper, iron, zinc, magnessium, potassium and sodium. The presence of zinc may suggest benefit in spermatogenesis while the presence of sodium and potassium may suggest benefit in semen quality.

## 5.3 CONCLUSION

Based on the results of this study, it can be submitted that the methanolic extract of *Garcinia kola* seed has significant pharmacological effects on the male reproductive profiles at some doses that could be beneficial in male fertility disorder therapy. These observed effects were not due to chance variations and hence the null hypothesis is rejected.

Sexual reproductive health problem is one of the health challenges that have remained intractable in both medical and social circles. Of interest and concern is the issue of male sexual health problems manifesting ultimately as infertility. This is usually under-reported due to many reasons including but not limited to culture, traditional beliefs and assumed supremacy of the male over the female. The burden of proof for male fertility or otherwise usually lies on the female since these are usually expressed in the female. Notwithstanding, the quest for interventions in male infertility remain high in any cultural setting.

The significance of the present work lies on the fact that it could serve as a holistic and novel scientific evidence in justifying the traditional use of *Garcinia kola* as an aphrodisiac to solve some male reproductive problems. Results obtained could therefore serve as templates or leads for further studies with the ultimate benefit tailored towards a cost-effective and better pharmacological intervention in male infertility, in addition to existing ones that appear ineffective yet costly to the larger population of infertile couples.

## 5.4 RECOMMENDATIONS

1. Long term and high dose consumption of *Garcinia kola* as is the current practice by its consumers should be discouraged as this has the potential of suppressing spermatogenesis.

2. The results of this study can be used as templates or leads for further studies to widen the horizon of pharmacological knowledge of *Garcinia kola* on the subject matter.
3. Government should encourage the cultivation of indigenous medicinal plants such as *Garcinia kola* which have high economic and medicinal values.

## 5.5 SUGGESTIONS FOR FURTHER RESEARCH

1. A similar study of the effect of *Garcinia kola* seed extract on the reproductive profiles of female animals.
2. Studies of the effect of *Garcinia kola* seed extract on other biological systems.
3. Studies on molecular mechanism(s) of *Garcinia kola* seed.

## 5.6 CONTRIBUTION TO KNOWLEDGE

The results scientifically support and justify the traditional use of *Garcinia kola* seeds as aphrodisiac or fertility agents in males as there was a positive correlation between its intake and increased reproductive capacity at 125 and 250 mg/kg. It also revealed that long term and high dose (500 mg/kg) intake as being practiced currently is deleterious on spermatogenesis.

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# APPENDIX A

## PLANTS TRADITIONALLY USED AS APHRODISIACS

**Plate A1: Leaves of *Loudetia phragmitoides* (Peter C.E. Hubb*)***

The leaves are traditionally used for aphrodisiac effect

**Plate A2: *Combretum molle* (R.Br. ex G. Don)**

The leaves are traditionally used for aphrodisiac effect

**Plate A3: *Cyperus esculentus* (L)**

(Roots)

**Plate A4: *Fadogia agrestis* (Schweint ex Hiern)**

(Leaves and Stem)

**Plate A5: *Asparagus africanus* (Lam)**

(Stem)

**Plate A6*: Borassus aethiopum* (Mart)**
(Leaves and Fruits)

**Plate A7**: ***Annona senegalensis*** **(Pers)**
(Leaves, stem and fruits)

**Plate A8: *Syzygium guineense* (Wall)**
(Leaves and Fruits)

**Plate A9:** ***Acacia sieberiana*** **(Var.Woodii)**
(Leaves and Fruits)

**Plate A10**: ***Dichrostachys cinerea*** **(Wight et Arn)**
(Roots and leaves)

**Plate A11**: ***Sesamum indicum*** **(L)**
(The seeds)