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Full Length Research Paper

The effect of Yoyo cleanser bitters on the cerebellum of adult male wistar rat

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Yoyo cleanser bitters, a polyherbal drug is widely used by Nigerians with the purpose of purifying the entire system of the body. This study was aimed at determining the effect of the drug on the morphology of the cerebellum of wistar rats. 16 rats divided into four groups; a control group, an Low dose of 0.05 ml group, a normal dose of 0.10 ml group and an High dose of 0.20 ml group. The weight of the wistar rats was between 150 – 230 g. The rats were fed and administered these amounts of drugs daily with their weights recorded after one day interval for 2 weeks. The result of the weights of the animals, and cerebellum of each of the experimental groups does not reveal any significant effect morphologically. The plates picked at random for all the groups revealed a marked change when compared with the control. The over dose group showed that the granular layer of the cerebellar cortex is hypertrophied with a corresponding increase in granular cells. It is deduced that high consumption of yoyo cleanser bitters for long period of time will have a significant effect on the histology of the cerebellum of wistar rats which may lead to cerebellar dysfunction.

Key words: Yoyo cleanser bitters, wistar rats, cerebellum, hypertrophied, granular cells.

INTRODUCTION

There has been extensive research into the structure and working of the cerebellum. It is one part of the vertebrate brain that is well understood in various aspects. Scientists think the cerebellum helps in physical co-ordination. But looking at the functional imaging studies of the brain MRI, researchers have also seen activity in the cerebellum when mental topics are being processed (Singh, 2006). Here, the anatomy of the cerebellum will be examined once again with the use of Yoyo cleanser bitters, one among the class of bitters.

The cerebellum is the largest part of the hindbrain, which is dorsal to the pons and medulla oblongata. Its median region is separated from the pons and medulla by the fourth ventricle. The cerebellum lies in the posterior cranial fossa of the skull and is covered by dura mater called Tentorium Cerebelli. It (cerebellum) is roughly spherical in shape, but somewhat constricted in its median region which is flattened with the greatest diameter being transverse.

It has been estimated that the surface area of the cerebellar cortex is about half or 50% of the area of the cerebral cortex. Just like the cerebrum, the cerebellum has a superficial layer of grey matter “Cerebellar Cortex”. This covers the cortical core of the white matter. Cerebellum, being one of the interesting structures of the CNS, constitutes 10% of the entire brain weight, but contains 50% of the neurons (Abllat, 2009)

Yoyo cleanser bitters is one among the class of bitters produced by Abllat Nigeria Company Limited, an indigenous health care product provider. Is a botanic medicine launched into the Nigerian market in the year 2003. Since its introduction into the Nigerian drug market, it has received wide acceptance and use by the general

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populace (Ganong, 2003). The drug is certified by National Agency for Food, Drugs and Control (NAFDAC) as the first real bitters without alcohol, colouring or artificial preservatives produced in Nigeria (Ganong, 2003). The ingredient contained in yoyo cleanser bitters include, Aloe, Acinos Arvensis, Citrus Aurantifolla, Chenopodium Murale, Cinnamomum Arromaticum (Ganong, 2003).

Yoyo cleanser bitters is a powerful blend of some premium quality herbs well formulated to reduce free radical damage and removal of harmful toxins in the body, thereby supporting the immune system and the body's ability to resist disease. They help the following systems in the body (Ganong, 2003) :-

i.) Urinary and Excretory system; dissolve existing kidney stones and prevent the formation of new ones; prevent kidney and bladder infection and normalizes the functions of the intestine (reduction of gastric activity, enhancing G I tolerability and prevents gastric ulcer).
ii.) Circulatory System; enhances blood circulation, facilitate blood pressure control through arterial dilation, assists in the elimination of cholesterol, sugar, triglycerides, creatinine and uric acid.
iii.) Nervous system; beneficial in the treatment of such disorders as insomnia, stress, and depression.
iv.) Hardening of tissues; dissolves any encased toxic materials in the body and enhances cell formation and growth.
v.) Weight control; reduces excess body fat; enhances healthy weight loss.
vi.) It enhances bodies' immunity.

The aims and objectives are:

-To identify and analyze the effect of Yoyo cleanser bitters on the cerebellum of wistar rats morphologically and histological.
-To deduce the areas of usefulness of this work that would be of benefit to subsequent experiments involving wistar rats and other laboratory animals and as applicable to human.

MATERIALS AND METHODS

Animals

The number of wistar rats used was sixteen (16), these rats were divided into four (4) groups as follows;
Control group
Low dose group
Normal dose group and
High dose group.

They were all fed with vital feeds (grower) from grand cereal, with the test groups being administered with different doses of Yoyo cleanser bitters accompanied with weight recordings. The four (4) groups of the wistar rats were fed with equal amount of vital feed (grower) daily along with water for two weeks. The vital feed was always mixed with some quantity of water and molded in form of a ball (prepared at feeding period), which makes it starchy. The Yoyo bitters was administered to all the experimental groups of the wistar rats daily with 24 h interval using 1 ml syringe Oropharyngeal tube, with the exception of the control group. These modes of administration were as follows;

Low dose = 0.05 ml
Normal dose = 0.10 ml
High dose = 0.20 ml

The drug was administered 1 h after they were fed. The weight of the animals was between 150 – 230 g before feeding and drug administration. Each of the animals were weighed with two (2) days interval before daily feeding and administering drugs for two (2) weeks using weighing balance. This was carried out for all the groups.

At the end of two weeks, all the wistar rats were sacrificed by cervical dislocation method with each of the head obtained and processed using decalcifier for the duration of one (1) week. Subsequently, at the end of one (1) week, the cerebellum was obtained from each rat and preserved in formaldehyde fixative for tissue processing. The method employed to process the cerebellar tissue of all the groups was the paraffin wax method.

The following steps are involved:

1) Decalcification of the rats head using decalcifier. The reason for decalcification is to;
a. Remove calcium component of bone, hence makes it softer.
b. For easy removal of the brain from the skull.
2) Fixation using 10% formaldehyde. The reason for fixation are as follows;
a. To coagulate blood protein.
b. It facilitates staining.
c. It hardens the tissue.
d. Helps to prevent autolysis.
e. Helps to prevent putrefaction.
3) Rinsing, that is, excess fixative removal with water.
4) Dehydration (in increasing alcohol e.g. ethanol from 70% to absolute alcohol) for 2 h each. This helps to remove water from the tissue.
5) Clearing (to replace ethanol with a solvent miscible with both ethanol and paraffin wax) with xylene used for 2 h.
6) Embedding (impregnation of tissue in molten paraffin wax and subsequently hardening by cooling). The tissue may have microscopic holes which are filled by the paraffin wax. This helps to harden tissues for easy sectioning.
7) Sectioning (slicing the wax-impregnated tissue on a microtome). It aids easy fixing of tissue on glass slide.

8) A fixing section of glass slide (usually with egg albumin). The albumin helps to hold tissue on glass slide.
9) The prepared slide is dewaxed using xylene for 5mins.
10) The tissue is then hydrated with decreasing grades of alcohol (e.g. from absolute alcohol to 70%) for 5mins each.
11) The tissue is transferred to Distilled H_2O for 1 min.
12) Staining of tissue is done using haematoxylin stain for 7mins. The staining is most widely used and important in general-purpose stain combination. Haematoxylin is a basic nuclear stain.
13) After staining with Haematoxylin, the tissue is washed in Distilled H_2O for 2 mins.
14) Differentiate in 1% acid alcohol for 20 s to remove excess stain that water cannot remove.
15) Wash in water for 1 min.
16) Blue with Scott's Tap Water until it turns blue within 5mins. This helps to increase the intensity of the stain (Haematoxylin) and make the tissue blue.
17) Counter stain with Eosin for 12 mins. Eosin is the acidic cytoplasmic counter stain which helps to stain the cytoplasm, as the Haematoxylin helps to stain the nucleus.
18) Wash in tap water for 2 mins to remove excess counter stain.
19) Dehydrate in increasing grade of alcohol (e.g. 70%, 80% to absolute alcohol) for 5 mins each. This enable the removal of excess stain and water, because stain contains water and water is an agent of microorganism.
20) Clear in xylene for 5mins to remove or replace alcohol.
21) Mount with dipropanyl xylene (DPX) with cover slip which helps tissue to stick to slide, and allowed to dry overnight.
22) Finally it is observed under the light microscope.
Phytochemical analysis for major biochemical constituent of the Yoyo bitters was undertaken using standard qualitative method, which was analyzed for the presence of biochemically active compounds like flavonoids, alkaloids, tannins, saponins, steroids and balsam. This was conducted in the Biochemistry laboratory of University of Jos.

Test for saponins

1 ml of Yoyo bitters was added to 4mls of distilled water and shaken vigorously, the formation of froth in the mixture indicates the presence of saponnins.

Test for tannins

2 mls of Yoyo bitters was added to few drops of 10% ferric chloride to give a mixture of a dip blue or green colour for the presence of tannins.

Test for alkaloids

2 mls of Yoyo bitters was added to few drops of Dragendorf reagent to give a mixture an orange colour for the presence of Alkaloids.

Test for flavonoids

2 mls of Yoyo bitters was added to few drops of 10% lead acetate, a cream or light yellow colour mixture is a positive result.

Test for steroids

1 ml of Yoyo bitters was added to 2 mls of concentrated sulphoric acid along the side of test tube, formation of reddish-brown ring at the interphase within the mixture indicates the presence of steroids.

Test for Balsam

3 drops of alcoholic ferric chloride is added to 2mls of Yoyo bitters, a dark green colouration of the mixture shows the presence of balsam.

RESULTS

Result of the phytochemical analysis of Yoyo bitters

The result of the phytochemical analyses for major biochemical constituent of the Yoyo bitters as earlier mentioned in the methodology, which was analyzed for the presence of biochemically active compounds like flavonoids, alkaloids, tannins, saponins, steroids and balsam is seen in Table 1.

With reference to Table 1, the result revealed that all the biochemically active compounds tested for are all negative.

The weights of the wistar rats

The weight of the rats before daily feeding and drug administration was 150 – 230 g. Below is a table representing the weight of Wistar rats measured after one day interval during the experiment for both the control and tests groups, it lasted for 2 weeks. In each group contained four rats labelled head, neck, back and tail, to be able to differentiate them from each other within each group.

The summary of histological results

i.) a section of the Cerebellum showing the layers of Cerebellar Cortex (Table 1 and Figure 1)
ii.) Section of the Cerebellar Cortex showing the Cerebellar cells (Table 2 and Figure 2) present in layer as

Table 1. Weights of wistar rats for control group.

Head(g)	Neck(g)	Back(g)	Tail(g)
165	150	155	150
185	190	180	170
195	190	180	175
200	200	200	170
210	210	205	190
225	220	220	220
230	225	230	200

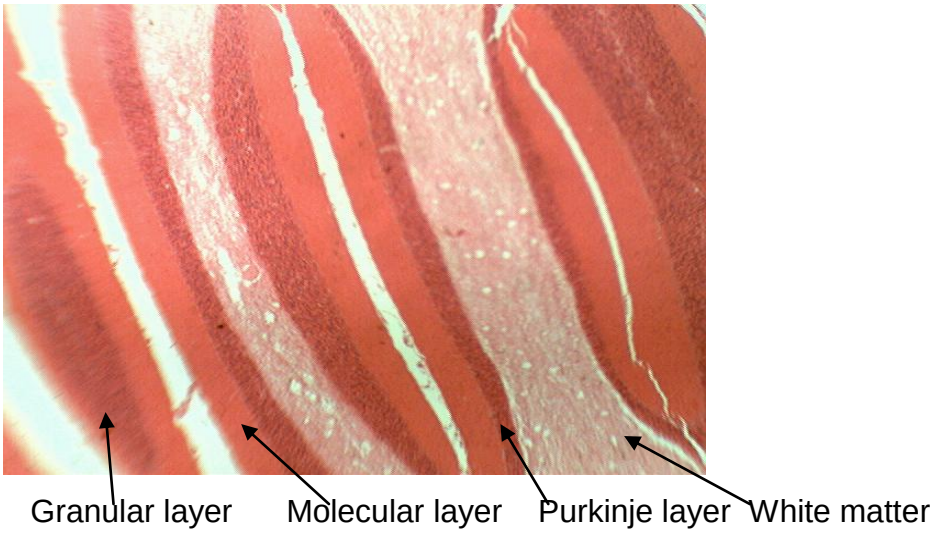

Figure 1. Light micrographic section of cerebellum showing the layers of cerebellar cortex (Control group).
(Control): Magnification X100 Stain: H & E.

Table 2. Weights of wistar rats for under-dose group.

Head (g)	Neck (g)	Back(g)	Tail (g)
175	175	155	125
175	175	175	125
190	180	180	130
200	180	180	135
205	190	190	145
205	200	190	155
205	200	200	150

follows:
-The Granule cells are more obvious, small in size, numerous in numbers, densely stained and closely packed as compared to other layers, contained in the Granular layer.
-Purkinje cells are less obvious, bigger in size, lesser in numbers, less dense and sparse in their arrangements as contained in the Purkinje layer.
-Basket cells are obvious, bigger in size than the granule cells, less in number, faintly dense and widely spaced in their arrangements as contained in the molecular layer.
-The White matter combinations of axons of purkinje cells and other fibres.
iii.) Observation; the granular and molecular layers are of equal width, with the granular cells more prominent (as stained deep); the purkinje cells are less obvious as

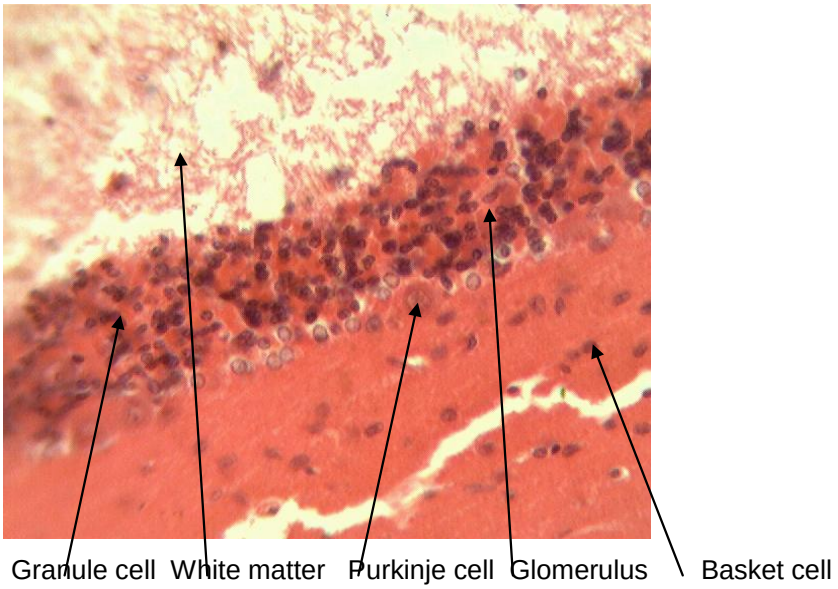

Figure 2. Light micrograph section of cerebellum showing the cells of cerebellar cortex (control group).
(Control): Magnification X400 Stain H & E.

Table 3. Weights of wistar rats for normal-dose group.

Head(g)	Neck(g)	Back(g)	Tail(g)
150	150	175	155
180	165	180	160
170	165	190	170
175	180	185	175
195	190	200	185
215	200	210	200
215	210	210	200

contained in the purkinje layer occupying a small width; the basket cells arranged sparsely in the molecular layer is equally prominent, and width of the white matter is reduced (Table 3 and Figure 3)
iv.) Observation; the width of granular layer is reduced with the granule cells less dense; the molecular layer is also reduced in width with Basket cells less dense; the purkinje cells becomes prominent and the width of white matter is increased when compared with C above (Table 4 and Figure 4).
v.) Observation; the width of granular layer is increased with granule cells been less dense and wide spaces appear between them; so also the width of the molecular layer is reduced with basket cells been so faint likewise the purkinje cells and the width of white matter been more prominent when compared to C above (Table 5 and Figure 5).
vi.) Observation; the width of granular layer is drastically increased as a result of increase of granule cells which becomes more dense, while the white matter, the purkinje and molecular layers becomes absent as compared to C (control) (Table 6 and Figure 6).

Discussion

The result of the micrographs for all the experimental groups compared with the control group revealed that there are marked changes with the width of the granular layer as a point of reference. Considering Figure 3 (Control), the width of the granular layer and the molecular layer are of equal size with the purkinje layer been indistinct. In Figure 4 (Low dose) compared to Figure 3, the width of the granular layer is reduced with the granule cells less stained, while in Figure 5 (Normal dose), the width of the granular layer is slightly increased with the granule cells faintly dense compared to Figures 4 and 3. The width of the granular layer in Figure 6 (High dose) as compared to Figures 3, 4 and 5, is drastically increased (Hypertrophic) with complete absence of

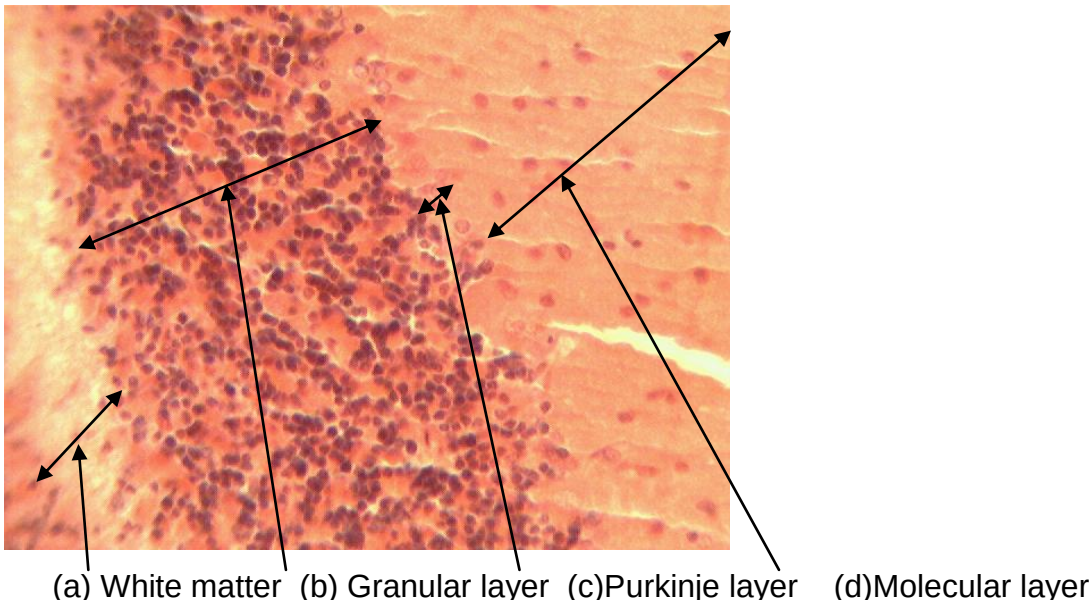

Figure 3. Light micrographic section of cerebellar layer for control group. (Control): Magnification X400, Stain: H & E.

Table 4. Weights of wistar rats for high-dose group.

Head(g)	Neck(g)	Back(g)	Tail(g)
150	175	175	155
175	175	200	185
165	180	210	200
175	175	215	200
180	190	220	205
190	200	230	215
180	190	225	220

The mean of the weights of wistar rats was then calculated and tabulated as shown below.

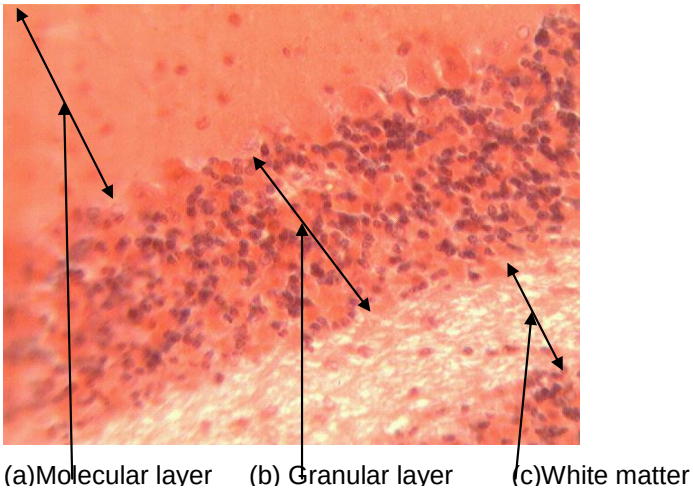

Figure 4. Light micrographic section of cerebellar layer for under-dose group.
(Under dose): Magnification X400, Stain: H & E.

Table 5. Average weight of each Wistar rat in grams (g).

	Head(g)	Neck(g)	Back(g)	Tail(g)
Control	201	198	196	182
Under dose	216	180	193	178
Normal	174	184	211	197
High dose	194	185	181	138

Average weight of each group, Control -194g, Low dose -192g, Normal dose-192g, High dose -175g.

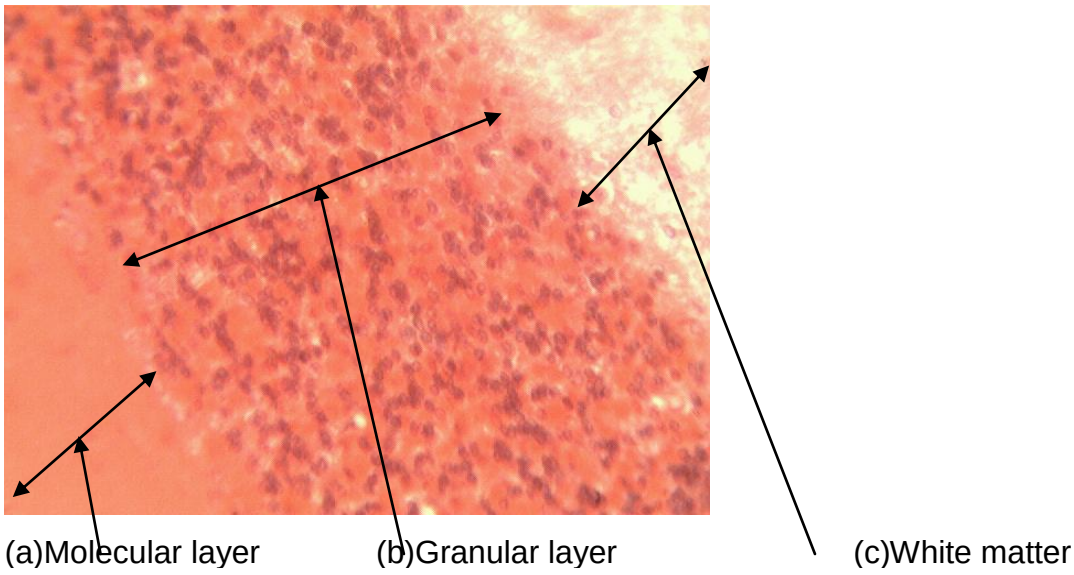

Figure 5. Light micrographic section of cerebellar layer normal-dose group. (Normal Dose): Magnification X400, Stain: H & E.

Table 6. Showing the weights of cerebellum measured in grams.

	Bottle 1	Bottle 2	Bottle 3	Bottle 4
Control	1.46	1.86	1.38	1.49
Under dose	1.61	1.45	1.53	1.52
Normal	1.41	1.49	1.53	1.37
High dose	1.43	1.27	1.51	1.56

The average weight of cerebellum for each group: Control = 1.54g, Low dose =1.53g, Normal = 1.45 g
High dose = 1.44 g.

Molecular layer and the White matter, and the granule cells increased (Hyperplastic), are dense, adequately arranged with some evenly observed spaces called Cerebellar Islands. These inlands occupied by special synaptic structures called Glomeruli (Abllat, 2009). The climbing fiber inputs exert a strong excitatory effect on a single Purkinje cells, whereas mossy fiber inputs exert a weak excitatory effect on many Purkinje cells via the granule cells. Also the basket and stellate cells are also excited by these same granule cells via the parallel fibers, and their output inhibits Purkinje cell discharge, feed-back inhibition (Aniagu et al., 2004). Therefore, the hyperplastic nature of the granule cells will exert a strong excitatory effect on the purkinje cell and this will cause a further increase in the excitation of the stellate and basket cells which will in return cause the hypotrophy of the purkinje cells as reflected in Figure 6, disappearance of purkinje layer. The output of purkinje cells which is in

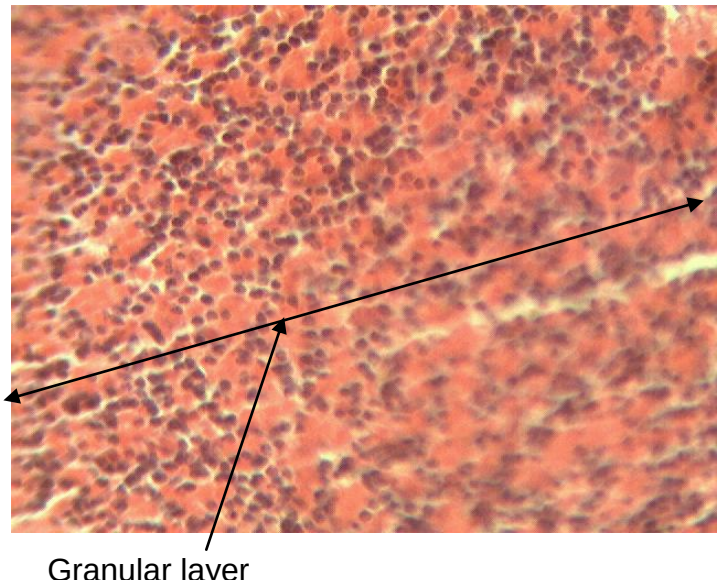

Figure 6. Light micrographic section of cerebellar layer for high-dose group. (Over Dose): Magnification X400, Stain H & E.

turn inhibitory to the deep cerebellar nuclei (Aniagu et al., 2004) make these nuclei to be continuously excited by inputs via the collaterals from the mossy and climbing fibers due to the hypotrophy of the purkinje cells. It is interesting, in view of the inhibitory purkinje cell input, that the output of the deep cerebellar nuclei to the brain stem and thalamus is always excitatory (Aniagu et al, 2004), hence the deep cerebellar nuclei remains excitatory to the brain stem and thalamus via the following fibers:

i.) Cerebello-rubral fibers
ii.) Cerebello-thalamic fibers
iii.) Cerebello-reticular fibers
iv.) Cerebello-olivary fibers
v.) Cerebello-nuclear fibers (Abllat, 2009).

In a study using Nature Cure Bitters (NCB) to feed Wistar rats the preliminary results associated with the toxicity studies did not produce severe toxicological effects on organ weights, haematological and biochemical indices as given at normal therapeutic doses. The graded doses of NCB were administered daily (100, 200 and 400 mg/kg) to rats for 28 days and the effects on body weight, organ weight, clinical signs, gross pathology, haematology, histology and serum biochemical parameters were evaluated. The relative weights of the heart, liver and testes of treated rats were unaffected in contrast to a significant increase in the relative weights of the lungs, kidneys and spleen (Niiho et al., 2005) Physiologically, the packed cell volume and haemoglobin concentrations were significantly reduced, whereas total leucocyte counts and glucose levels were remarkably increased. The calculated therapeutic index was >37.5 and histological findings did not reveal any treatment related effects (Niiho et al., 2005).

Also the effects of feeding of four vegetables commonly consumed in Thailand, namely, flowers of the neem tree (Azadirachta indica var. siamensis), fruits of Thai and the Chinese bitter gourd (Momordica charantia Linn.) and leaves of sweet basil (Ocimum basilicum Linn) on the levels of phase I enzymes, which include cytochrome P450 (P450), aniline hydroxylase (ANH) and aminopyrine-N-demethylase (AMD) as well as the capacity to activate the mutagenicities of aflatoxin B1 (AFB1) and benzo[a]pyrene (BaP), and to induce the phase II enzymes [i.e. glutathione S-transferase (GST)] in rat liver. It was found that feeding of the diets containing 12.5% neem flowers and Thai bitter gourd fruits for 2 weeks strongly enhanced GST activity, 2.7- and 1.6- fold of the pair-fed control values, respectively, while resulting in a marked reduction of the levels of most phase I reactions (Ogbonnia et al., 2008).

The results in the study clearly demonstrated that Neem flowers and Thai bitter gourd fruits contain monofunctional phase II enzyme inducers and compounds capable of repressing some monooxygenases, especially those involved in the metabolic activation of chemical carcinogens.

1. Sweet basil leaves contain compounds, probably bifunctional inducers, capable of inducing both phase I and phase II enzymes.
2. Chinese bitter gourd fruits contain only compounds capable of repressing some monooxygenases (Ogbonnia et al., 2008).

Furthermore, Gentianae Radix, the dried root and rhizome of Gentiana lutea L. (Gentianaceae), has long

been used as a remedy for liver and stomach inflammation, eye troubles, etc. Here the gastro protective effects of the methanol extract of Gentian root (GM) were studied using different gastric lesion models. In pylorus-ligated rats, administration of GM in the duodenum suppressed gastric juice secretion and total acid output in a dose-dependent manner (Ekong et al., 2008)

Oral or duodenum administration of GM showed significant protection against acute gastric ulcer induced by aspirin plus pylorus ligation, water-immersion restraint stress-induced ulcers, and gastric mucosal injury induced by ethanol (Ekong et al., 2008).

Antimicrobial evaluation of acute and subchronic toxicity studies in rodents, of a Nigerian polyherbal formulation called Leon Bitters. Leon Bitters is prepared with Gongronema latifolia (climbing stem), Cocos nucifera (coconut) roots and Parinari curatellifolia seeds. Toxicity of the polyherbal preparation was evaluated in Swiss albino mice by administering to the animals oral graded doses of the lyophilized drug in the ranges of 1.0 to 20.0 g/kg body weight and observed continuously for the first 4 h and hourly for the next 12 h, then 6 hourly for 56 h (72 h, acute toxicity). Wistar rats were also fed with different doses of the lyophilized drug for 30 days and the effects of the drug on some tissues - heart, liver, kidney and testes - were microscopically examined. Also the effects on the biochemical and haematological parameters were evaluated (sub-chronic toxicity model). The median acute toxicity value (LD_{50}) of the polyherbal medicine was determined to be 7.2 g/kg body weight. No significant increase in the body weight was observed in the groups treated with the drug compared to the control. The drug significantly reduced ($p \leq 0.05$) triglyceride (TG) level while low density lipoprotein (LDL)-cholesterol level was not altered, but led to increase in high density lipoprotein (HDL)–cholesterol in the treated groups compared to the control. There was no significant change in the mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration compared to the control. The study showed that the drug exhibited hypolipidemic activity and good reducing effects on cardiovascular factors.

Since, most of the work carried out is on bitters associated to the digestive system. Here the work is extended beyond the digestive system to include the Nervous system, a part called Cerebellum.

Finally, the results revealed that the administration of Yoyo Cleanser Bitters to the experimental groups has no significant effect on the weights of the animals as compared to the control and so also the weight of the cerebellum. Histologically, the results of the experimental groups compared with control for the plates revealed that the granular layer in the over dose group is inflamed, while the granule layer is reduced in the under dose group which confirms that Yoyo cleanser bitter drug has a great effect on the cerebellum. And when yoyo bitters is consumed excessively it can lead to observable cerebellar damage with time like; ataxia, dysmetria, dysdiadochokinesia, scanning speech, intention tremor and nystagmus, and possible Neocerebellar Syndrome and cerebellar dysfunctional features (Abllat, 2009; Kusamran et al.,1998).

Conclusion

The result of this study reveals that the Yoyo Bitters Cleanser has no significant effect on the weight of wistar rats, while histologically it is deduced that high consumption of Yoyo Bitters for a long period of time has significant effect which may lead to any of the cerebellar dysfunctions as listed above.

Therefore, it is advised that high consumption of Yoyo Bitters should be discouraged as it can affect the nervous system considering the fact that it is a cleanser drug for purifying the entire body system. Following the outcome of this study (effect of Yoyo Cleanser Bitters on cerebellum of adult wistar rats) we recommend that more research should be carried out on this subject on other systems of the body in addition to the nervous system.

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APPENDIX

SHOWING CONTENTS OF PELLET SEED GROWERS FEED

Vital Feed (Pellet Seed Growers Feed.)
Crude Protien----------14.50%
Fat----------7.00%
Crude fibre----------7.20%
Calcium ----------0.80%
Available Phosphorus----------0.40%
MetabolisableEnergy---------- 2500kcal/kg.
Vital feed contain the following ingredients,
Cereals/grains,
Animal protein,
Vegetable protein,
Minerals, salt,
Essential amino acids,
Antibiotics,
Antioxidant and
Vitamin pre-mix.