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EFFECT OF ORAL ADMINISTRATION OF BITTER EXTRA ON THE HISTOMORPHOLOGY OF THE CEREBELLUM

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Abstract

Background: Bitter Extra is a brand of herbal product adjudged to be efficacious in the treatment of various ailments. This study is aimed at investigating the effect of Bitter Extra on the histomorphology of the cerebellum.
Method: Sixteen adult male Wistar Rats with weight between 158-230g were used for this study and were divided into four groups (1,2,3 and 4) with four (4) rats in each group. Group 1 served as the control while groups 2, 3 and 4 were taken as the test groups. Group 2 was administered 1.35ml/kg (low dose), group 3 was administered 2.7ml/kg (Medium dose), Group 4 was administered 5.4ml/kg (High dose) of Bitter Extra through an orogastric tube for a period of four weeks while group 1 was given water and feed only. The rats were fed and administered these amounts of drugs daily with their weights recorded after a week interval for four weeks.
Results: There were no significant changes ($P>0.05$) in the mean body weights of the rats in the test groups administered with Bitter Extra. However, in the histology of the cerebellum, rats in Group 4 showed increased proliferation of the Purkinje cell bodies into the molecular layer. Group 3 showed focal increase in Purkinje cell proliferation and hypertrophy of the Purkinje cell layer with pushing of the molecular layer, while Group 2 showed increased hypertrophy of Purkinje cell bodies and hyperplasia of cells in the granular layer.
Conclusion: These finding suggest that consumption of Bitter Extra does not in any way affect the body weight. High dosage consumption of Bitter Extra may cause hyperplasia, hypertrophy of the cells of the cerebellum which may lead to observable cerebellar dysfunction features.

Keywords: *Bitter Extra, Body Weight, Cerebellum, Rats, Cerebellar Dysfunction*

Introduction

According to World Health Organization (WHO) herbal medicine is defined as any part of the plant that can be used for therapeutic purposes or as precursors for the synthesis of important drugs[1]. Based on the information from WHO, the use of herbal medicine worldwide has surpassed the use of conventional therapies by two to three times[2]. Plants, (herbs or ethno botanicals) have been used from the beginning of human race and are still used throughout the world for promotion of health and treatment of diseases[3]. Plants and herbs form the basis of today's modern medicine and have contributed enormously to the commercial drug preparations manufactured today[4]. It has been discovered that about 25% of the drugs prescribed worldwide are synthesized from plants[2]. In most developing countries, herbs rather than conventional drugs are often used in health care services. For some individuals, herbal medicine is the preferred method of treatment, while for others; herbs are used as adjunct to therapy with conventional pharmaceuticals. However, in most developing countries, traditional medicine of which herbal medicine is a core part is the only system of health care that can be assessed and is affordable[5].

Herbal medicine has been reportedly used by about 80% of the world population both in the developing and developed countries where modern medicines are predominant[6,7]. A study by Ibrahim[8] showed that more than 60% of the surveyed population claimed to have used some herbal mixture either alone or in combination with other medicines. The rising popularity of phytomedicines could be attributed to the alleged advantages of being efficacious and also a more affordable source of medical care. In contrast, there is growing disillusion with modern medicines coupled with the misconception that herbal supplements might be devoid of adverse and toxic effects, which are associated with conventional and allopathic medicines. But recent reports have raised concerns that indiscriminate use of packaged herbal bitters may have a toxic effect on the spleen, pancreas, heart and other structures of the body[9].

In addition, herbal supplements are administered in most clinical conditions over a long period of time, without taking cognizance of their toxic effects which might result from a prolonged usage[10]. In most cases, these herbal products are not often prescribed by a physician and neither were they dispensed by a pharmacist. The individual reports of any potential adverse effect are mostly absent or inaccurate[11]. Therefore, the danger associated with the potential toxicity of many of these herbal products of which Bitter Extra is a brand and other herbal therapies, which are being used over long period of time demands that the practitioners and even the general public be kept abreast of the reported incidence of any tissue toxicities.

Bitter Extra as a brand of bitters contains complex carbohydrates and alkaloids. Alkaloids are groups of naturally occurring chemical compounds that mostly contained basic nitrogen atoms. It also contains weak acidic properties[12]. The alkaloids have a wide range of pharmacological activities including antimalaria (e.g. quinine), antiasthma (e.g. ephedrine), anticancer (e.g. homoharringtonine)[13]. It also has other activities like cholinomimetic (e.g. galantamine)[14], vasodilatory (e.g. vincamine), antiarrhythimic (e.g. quinidine), analgesic (e.g. morphine)[12], antibacterial (e.g. chelerythrine)[15], and antihyperglycemic activities (e.g. piperine)[16]. It is mostly used as a cleanser drug for purifying the entire body system.

The cerebellum is a vital part of the brain that plays a cognitive role and in balanced

movements. Any distortion in the histo-architecture or morphology of the cerebellum may lead to cerebellar dysfunction resulting in uncoordinated movements and other neurological consequences. In a related study on the effect of Yoyo Cleanser Bitters on the cerebellum of adult male Wistar Rats by Shugaba[11]), marked changes with the width of the granular layer was observed.

There are growing incidences of many chronic diseases which affect some vital organs of the body coupled with high consumption rate of bitter products in our locality, it however, becomes imperative to carry out a study on the possible effect that may occur in the cerebellum following Bitter Extra administration.

Materials And Methods

Drugs

The herbal product, Bitter Extra was purchased from a registered pharmaceutical shop, in Abakaliki, Ebonyi State, South-East Nigeria. The Bitter Extra was ascertained to have been registered with the National Agency for Food, Drug Administration and Control (NAFDAC). The manufacture and expiry date of the product were inspected and all were confirmed not to have been expired. The manufacturer's seal was also inspected to ascertain that its originality was intact. Each bottle contains 200 ml of the content.

Experimental animals

A total number of sixteen (16) adult male Wistar rats with their initial weight ranging from 155g-230g were procured from the Animal House of the Department of Veterinary Medicine, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The animals were handled in accordance with the guide for use of experimental animals by the Faculty of Medicine Pre-Clinical Research and Ethics Committee of Ebonyi State University, Abakaliki, Ebonyi State, Nigeria with ethical code number: MPC/1704/02/001. They were kept in well ventilated poly-ethylene cages in the animal house. The animal house was kept properly ventilated, cleaned and disinfected at an interval of 3 days to ensure a healthy environment. Also, they were kept in a suitable experimental condition within room temperature and a normal light cycle (12 hour light and 12 hour dark) during the period of the experiment. The animals were allowed free feeding with a standard diet and drinking water *ad libitum*.

Experimental design

Grouping of animals

The animals were divided into four (4) experimental groups, each consisting of four rats and treated for a period of four (4) weeks as follows: Group 1 was given distilled water (Control), Group 2 was administered with 1.35ml/kg of Bitter Extra (Low dose), Group 3 was administered with 2.7ml/kg of Bitter Extra (Medium dose) whereas Group 4 took 5.4ml/kg of Bitter Extra (High dose). The graded daily doses gave the opportunity of studying the effect of the low, medium and higher doses of Bitter Extra. They were all weighed daily with BRECKNELL EPB500 Pocket Balance Digital Scale with its calibrations in grams and their weights recorded.

Drug administration

The Bitter Extra was administered to the animals through an orogastric tube for a period of four weeks (28 days). The dosage of bitter extra was as per the human recommended dose of 0.45 ml/kg body weight.

Collection of experimental specimen and histological analysis

The animals were sacrificed by anesthetizing them in a jar containing cotton wool soaked in diethyl ether 24 hours after the last dose administration of their respective treatment and their various cerebellum harvested. The tissues were blotted dry using blotting paper. They were further subjected to normal routine histological procedures, stained with Hematoxylin-Eosin and examined using the light microscope. The main significant histological changes were noted and recorded.

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 rat's 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.

Statistical analysis

Students' t-test was employed and the mean was presented in Mean ±SD. P values less than 0.05 were considered to be statistically significant.

Result

Mean body weight

The mean body weight of the animals in the test groups were measured and compared with that of the control group to ascertain the changes associated with their body weights as shown in Table 1. The mean body weights were not significant (P>0.05) among and within the test groups and the control. The histological results obtained are discussed and are based on the comparison between the histological features of the control group and those of the Bitter Extra treated groups.

Table 1: Changes in Mean Body Weight of the Rats

		N	Minimum	Maximum	Mean± SD	*P*-value
Week 1	G1	4	149.20	160.70	154.70±4.74	0.12
	G2	4	163.90	233.30	189.18±30.35	
	G3	4	149.60	198.00	169.98±20.44	
	G4	4	157.20	230.00	196.55±32.68	
	Total	16	149.20	233.30	177.60±27.80	
Week 2	G1	4	157.60	163.60	161.30±2.61	0.33
	G2	4	154.90	235.30	189.33±33.51	
	G3	4	138.90	202.90	172.63±26.30	
	G4	4	157.30	237.80	195.05±34.96	
	Total	16	138.90	237.80	179.58±28.28	
Week 3	G1	4	155.50	172.80	162.38±7.83	0.11
	G2	4	178.60	233.50	201.57±28.53	
	G3	4	182.60	203.10	190.67±10.93	
	G4	4	160.20	230.10	198.53±29.46	
	Total	16	155.50	233.50	187.16±25.25	
Week 4	G1	4	155.90	175.20	163.83±8.14	0.30
	G2	4	176.30	238.20	198.20±34.69	
	G3	4	173.20	199.60	184.10±13.79	
	G4	4	149.90	217.40	189.20±28.87	
	Total	16	149.90	238.20	182.79±24.51	

Histological features

The normal cerebellum has a smooth molecular layer and a rough granular layer separated by the Purkinje layer. The control, Group 1 has a smooth molecular layer while other groups have some degree of hypertrophy and hyperplasia. In Group 4 (Plate 4), increased stimulation of proliferation of the Purkinje cell bodies into the molecular layer was observed. Focal increase in Purkinje cells proliferation into the molecular layer and hypertrophy coupled with spreading of the molecular layer was observed in Plate 3 of Group 3 administered with normal dose. It was observed in Plate 2 of Group 2 an increased hypertrophy of Purkinje cell bodies within the molecular layer and hyperplasia of cells in the granular layer.

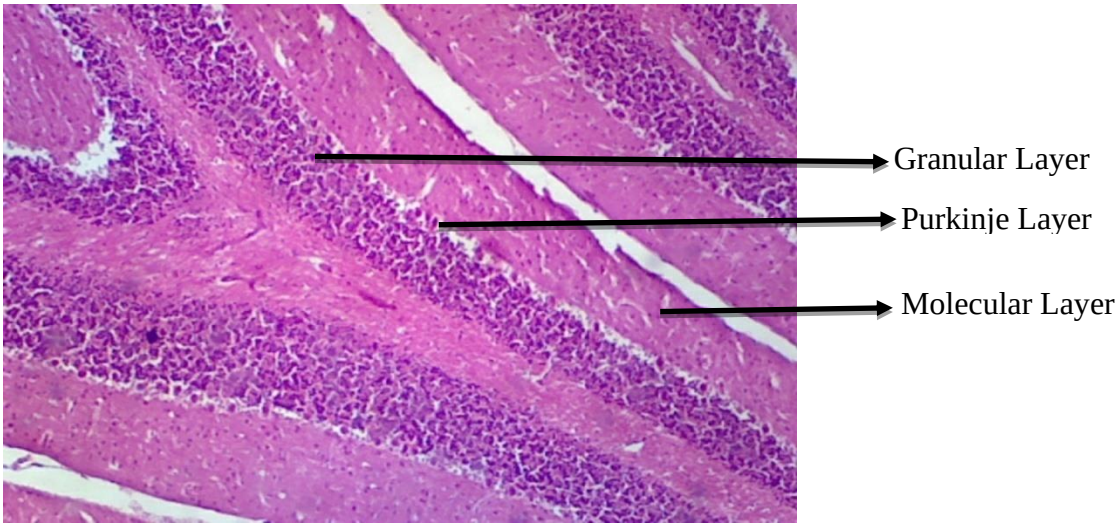

Figure 1: A section of cerebellum of rat (control) showing smooth Molecular Layer, Purkinje Layer and rough Granular Layer. Stain: **H & E, X100**

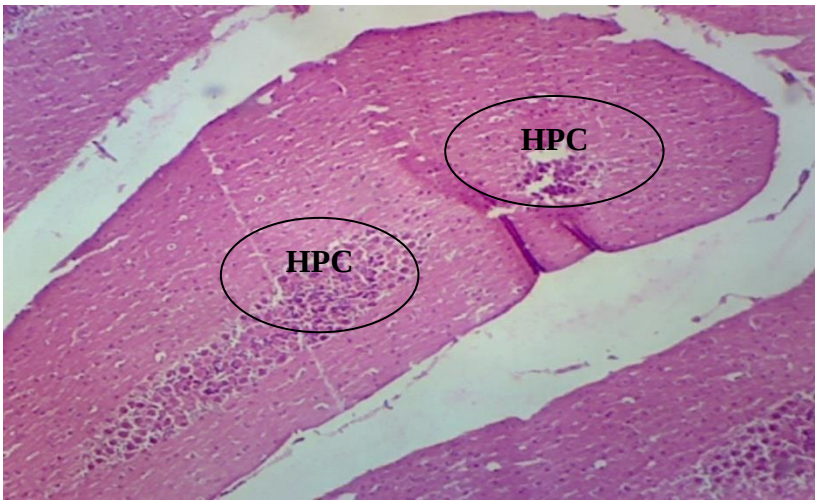

Figure 2: A section of cerebellum of rat treated with 5.4ml/kg (High dose) of Bitter Extra. Showing increased Hypertrophy of Purkinje Cell (HPC) bodies and hyperplasia of cells in the Granular Layer. Stain: **H & E, X100.**

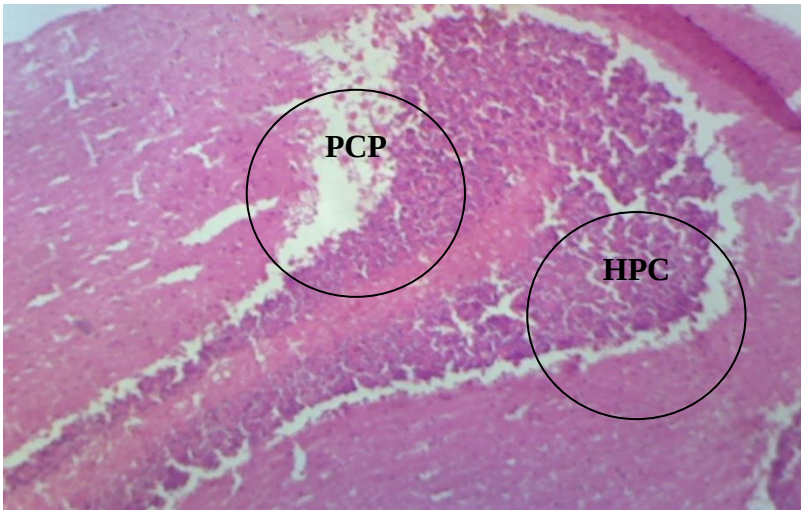

Figure 3: A section of cerebellum of rat treated with 2.7ml/kg (Normal dose) of Bitter Extra. Showing focal increase in Purkinje Cells Proliferation (PCP) and Hypertrophy of the Purkinje Cell (HPC) layer with pushing of the Molecular Layer. Stain: **H &E, X100**

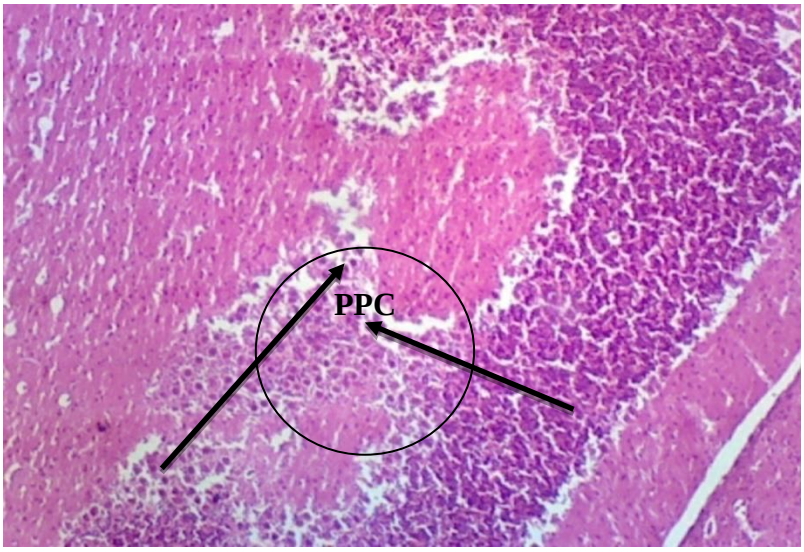

Figure 4: A section of cerebellum of rat treated with 1.35ml/kg (Low dose) of Bitter Extra. Showing increased Proliferation of Purkinje Cell (PPC) bodies into the Molecular Layer.

Stain: **H & E, X100.**

Discussion

Globally and indeed in Nigeria, local medicinal herbs are employed in the management of various diseases. The effect of Bitter Extra on the cerebellum and body weight of Wistar rats has been investigated in this study. The body weights of rats administered with Bitter Extra was not statistically significant when compared with the control, an indication that Bitter Extra has no effect on the body weight. This is in agreement with Shugaba[11] on the effect of Yoyo Cleanser bitters on the cerebellum of adult Wistar rats which revealed that overdose group showed hypertrophy of the granular layer of the cerebella cortex with a corresponding increase in granular cells. In this study, the result of the experimental groups when compared with the control group revealed a proliferation of purkinje cell bodies within the molecular layer which became more pronounced as the administered dose increases, a hypertrophy of the purkinje cell layer coupled with hyperplasia of the cells within the granular layer. The hypertrophy observed suggests increase in the size of the purkinje cells which may affect their proper functioning, hence, delays in sending inhibitory projections to the deep cerebellar nuclei and also affect the output of all motor coordination in the cerebellar cortex. The hyperplasia as seen in the granular cells is the increase in the number of cells of the granular layer which is an indication of a compromised function in the area of processing visual and motor information to learning and memory. In addition, when Bitter Extra is consumed excessively as observed in the overdose group, and possibly for a long period of time, it can lead to an observable cerebellar damage and cerebellar dysfunction features. This is in line with the findings that bitters can cause cerebellar damages and dysfunctions[17].

In a similar study using Nature Cure Bitters (NCB), the preliminary results associated with the toxicity studies did not produce severe toxicological effects on organ weights, biochemical and haematological indices as given at normal therapeutic doses. The graded doses of NCB were administered on a daily basis (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[18]. 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[18].

Bitter Extra facilitates digestion, and therefore too much consumption can lead to poor absorption of vitamin B12 which is the main cause of pernicious anemia[19]. The deficiency of vitamin B12 has neurological consequences as it is an essential vitamin for the proper functioning and development of the brain and nerve cells[20]. Vitamin B12 plays an important role in the maintenance of the myelin sheaths that cover and protect the nerves of the central nervous system.

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 P_{450} (P_{450}), 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[21]. 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. Sweet basil leaves contain compounds, probably bifunctional inducers, capable of inducing both phase I and phase II enzymes. Chinese bitter gourd fruits contain only compounds capable of repressing some monooxygenases[21].

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[22]. 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[22]. 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 Bitter Extra to the experimental groups has no significant effect on the weights of the animals as compared to the control. Histologically, the results of the experimental groups compared with control for the plates revealed that the cells

in the granular layer in the over dose group is increased, while the cells in the granule layer is reduced in the under dose group which confirms that Bitter Extra drug has a great effect on the cerebellum. And when Bitter Extra 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[23,24].

Conclusion

The result of this study reveals that Bitter Extra has no significant effect on the weight of Wistar rats, while histologically it is observed that consumption of Bitter Extra in high doses may have a significant effect on the morphology of the cerebellum. Therefore, it is advised that consumption of Bitter Extra in high doses 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.

Conflict Of Interest

Authors declared they have no competing interest.

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