

Evaluation of the Effects of Swedish Bitters on Albino Rats

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ABSTRACT

The study was designed to evaluate the effects of swedish bitters on albino rats. The Swedish bitters composed of thirteen herbal constituents was fed using an oro-gastric gavage, to different sets of male albino wistar rats, after which the acute toxicity (LD₅₀), biochemical and haematological indices as well as the subchronic toxicity and histological status of the herbal bitters on the rats were investigated using standard laboratory procedures. The high acute toxicity (LD₅₀) of the bitters of 24ml/kg body weight (equivalent to 24g/kg body weight) indicates it has low-lethality. The minimal and non-significant differences (P>0.05) in all the indices used to assess, the liver, kidney and cardiac function statuses of the bitters-fed rats compared to same in the control rats, was indicative that the bitters preserved the functions of these organs. The increase in the CD₄₊ count and decrease in the level of the fasting blood glucose in the bitters-fed rats when compared to that of the control rats was significant (P<0.05). The decrease in serum triacylglycerol and LDL-cholesterol levels and the increase in HDL-cholesterol caused by the bitters was significant (P<0.05). The bitters-fed rats had a significant decrease (P<0.05) in their serum malondialdehyde (MDA) levels and a significant increase (P<0.05) in their serum vitamin C and E levels and activities of antioxidant enzymes namely superoxide dismutase, catalase, and glutathione peroxidase, when compared with that of the control rats. The results from this study indicate that swedish bitters may be said to be hypolipidaemic, hypoglycaemic, immunity-boosting, choleric, hepatoprotective, antihypertensive, as well as having antioxidant properties and protective against cardiovascular diseases.

Keywords: Swedish bitters, haematology, serum, antioxidant and rat.

INTRODUCTION

Medicinal plants have been defined as those plants which contain in one or more of their organs, substances that can be used for the synthesis of useful drugs. Modern science may have widened for some time the differences in terms of medication between orthodox and unorthodox/traditional medicine, this gap seems to be closing fast as the current trend is that they are both adopting practices from each other [1], [2]. This has led to the resurgence of an ancient remedy for digestive problems in the repackaging of "herbal bitters" and products like it in an "orthodox way".

Bitters have been claimed to help heal piles/haemorrhoids and improve sexual function. Enhance blood circulation, purification of blood by the kidneys, blood pressure regulation through arterial dilatation and prevent formation of kidney stones, cleanse the colon of impurities and have also been said to possess anti-tumour properties and especially protects against, colo-rectal cancers. They are also said to have anti-inflammatory, antibiotic and antifungal properties. Bitters are made up of numerous groups of chemical compounds extracted from the herbs and roots

(medicinal plants) that have the common characteristic of a bitter taste and act to increase the vital energy centres in the body [3], [4], [5]. Unorthodox traditional medicine practice which employ the use of herbs (medicinal plants) in treatment of ailment have gained much publicity and recognition, seemingly elusive to the system of orthodox medical practice.

It is often summarized that bitters stimulate digestive secretions and metabolism as a whole and in so doing increase appetite, relieve constipation, and generally ease the heavy glumness of sluggish digestion. But, this is really too simple and cursory a summation, and a deeper look into the actions of bitters is not only theoretically insightful but practically invaluable [6]. Bitters are produced from herb and root extracts, from the narcotic components of (primarily) tropical and subtropical plants and spices. They are usually dark in colour and valued for their ability to promote appetite and digestion hence their use as patent medicine and as digestive aids and as flavouring in cocktails. Bitters are made up of numerous groups of chemical compounds extracted from the herbs and roots that have the common characteristic of a bitter taste and act to increase the vital energy centres in the body [7]. Since they act upon the bitter receptors of the mouth, thereby producing the bitter taste in the mouth, their stimulation do not produce any electrical changes on the surface of the cell. Instead, the bitter molecules bring about intracellular biochemical changes by acting on the cell membrane receptors. This facilitates an increase in calcium concentrates within the cell and signals the gustatory nerve [8], [9]. This is their possible mechanism of action. As soon as the bitter taste bud is stimulated, it causes the release of the gastrointestinal hormone gastrin.

MATERIALS AND METHODS

A Swedish bitter was purchased from reputable pharmaceutical stores opposite the University of Benin Teaching Hospital (UBTH), Ugbowo Lagos Road, Benin City, Edo State, Nigeria. The Swedish bitter was

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The Swedish bitters composed of thirteen herbal constituents, *Angelica archangelica* (33mg), *Curcuma zedoaria* (33mg), Aloe Vera leaf juice (33mg), *Cassia angustifolia* (33mg), *Rheum palmatum* (33mg), *Bamboo manna* (58mg), *Commiphora molmo* (16mg), *Carlina acaulis* (16mg), *Cinnamomum camphora* (8.3mg), *Crocus sativa* (0.6mg) and Theriak Venez.S. Opio (33mg). Our ancestors commonly consumed a diet that included ample bitter herbs and vegetables, which helped them maintain efficient digestive systems. Despite having four different taste buds on the surface of our tongues to discern taste (sour, sweet, salty and bitter) today's modern diet is virtually void of bitter substances. They are ideal to use before meals to stimulate digestion or after meals to lessen feelings of fullness or gas. The Swedish bitters are used as; traditionally used in herbal medicine as a digestive tonic and mild laxative stimulates the liver to produce more bile for enhanced digestion, helps stimulate the stomach and gall bladder. The salivary glands are activated to increase secretion, Eases constipation and regulates bowel movements, Reduces bloating and gas, Supports healthy circulation and also promotes physical and mental energy, You may use it to clean wounds as an antiseptic, Clear away all blemishes or scars from your body and sooth any burns, Fight toothache, improve digestion, fight off insomnia and skin allergies, as well as treat many more ailments. Swedish Bitters is also extremely helpful for pregnant women and helps fight morning sickness. It is excellent for breast-feeding moms as it works effectively in reducing the inflammation of the nipples. Even people suffering from epilepsy, eye infections, haemorrhoids, frostbites, eye diseases etc have shown great improvement by consuming this tonic.

bought as liquid formulations and stored at room temperature (30-36°C) throughout the period of the experiment. Reagent kits and other reagents used were of standard quality and were purchased

from qualified/accredited dealers/suppliers or their manufacturers' representative in Nigeria. All the experimental animals for all stages of this study were handled in strict compliance with international guidelines as prescribed by the Canadian Council on the Care and Use of Laboratory Animals in Biomedical Research, 1984 edition [10]. Male albino rats of the Wistar strain were obtained from the Anatomy Department, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria. The rats were housed in a well ventilated room in the animal house of the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria, with the room temperature ranging from 30-36°C. They were allowed the diurnal cycle, which is the recommended 12-hr light and dark cycle. The rats were fed ad-libitum with standard pelleted mash and clean tap water for an acclimatization period of two weeks.

Acute Toxicity Study The method of [11] as adapted by [12] was used for the acute toxicity study. It was done in two phases.

Phase I Acute Toxicity Study:

Experimental Design/Protocol

This was done using the "staircase method" for the determination of the lethal dose and dose range prior to the actual LD₅₀ determination. After 14 days of acclimatization, the 10 experimental animals for the determination were divided into 5 groups of two rats each with each set of 2 rats given a dose of the bitters higher than the preceding one to determine which dose will cause zero death and which one will cause 100% death after 72hrs of oral dose of the bitters using an pro-gastric gavages [13], [14], [15]. The Animals were observed for signs of toxicity and mortality. At the end of the 3 days, for each group, the dose(s) that caused no death and the 1st dose that caused the death of the 2 rats in each sub-group were noted; these doses were used to determine the range to be used in the LD₅₀ determination for the herbal bitters [16], [17], [18].

Phase II Acute Toxicity Study:

Experimental Design/Protocol

This was for the determination of the LD₅₀. After 14 days of acclimatization, the 50 experimental animals for the determination of the LD₅₀, using the Miller and Tainter method [19], were weighed and divided into 5 groups of 10 rats each according to their weight range, making sure that the distribution was in such a way that the average weight per group was about 162g. Each group of 10 rats was given a dose of the bitters higher than the preceding one following the range as determined from pre-LD₅₀ determination study (phase I). This was to determine which dose will cause death ranging from 0 to 100%, after 72hrs of oral dose of the bitters [19], [20], [21]. The animals were observed for the first 2 hours, and then at the 6th, 24th, 36th, 48th, 60th and 72nd hours for any toxic symptoms. After 72hrs, the number of deceased rats was counted in each group and percentage of mortality calculated and tabulated. The percentage of dead rats for 0 and 100 was corrected before the determination of probits as shown:

Corrected % Formula for 0 and 100% Mortality (7)

For 0% dead = $100(0.25/n)$

For 100% dead = $100(n-0.25)/n$; where n = 10, their values were 2.5 and 97.5 respectively.

Determination of the LD₅₀: The Probit values were plotted against log-doses and then the dose corresponding to probit 5, that is 50%, was extrapolated, the value identified and noted as the LD₅₀. Other calculations were made according to the method described by [22] [23].

Subchronic Toxicity Study

Animal for study: Sixteen (16) male albino rats of the wistar strain weighing between 110-210g, Average weight per group approximately 162g. Grouping of the animals: After 14 days acclimatization, the 16 animals were weighed and divided into two (2) groups A and B, of eight (8) rats each, making sure that the weights of those in a group were representative of the weight range of all the rats, such that the average weight of all the groups at the

onset of the experimental period was 162g.

Feeding regime and care of the animals:

The rats were fed ad-libitum on standard pelleted mash and clean tap-water during the entire course of the 28-day study and allowed the recommended 12-hr light and dark cycle. Care was taken to determine the quantity of feed consumed daily. The rats were housed in wooden cages with a tiny-wire meshed/iron gauze flooring to allow the rat-excreta to be collected into another steel tray receptacle below covered with a bedding material. The cages, their surroundings, the receptacle tray below with its bedding, were cleaned and disinfected daily.

Experimental procedure: In addition to the feed and clean pipe-borne water, the rats in group B were given orally, the swedish bitters, using an oro-gastric gavage, according to the equivalent dose (to the weight of the rats for that week) of the effective dose already prescribed for man. An equivalent volume of distilled water was given to the control group which was group A. The animals were observed for signs of toxicity and mortality.

Dosage regimen: An adult man was expected to consume on the average 40ml of herbal bitters daily. Appropriate calculations were done to determine the initial equivalent doses of the bitters (distilled water in the case of the control group) in ml/g mean body weight of the rats to be given in each group. As the initial mean weights of rats in each group at the beginning of the study was 162g, the equivalent volume [in millilitres-(ml)] of the bitters/distilled water that was given to the rats was as calculated:

If 40ml was consumed by a 70,000g man (70kg)

How many ml was a 162g rat expected to consume? (Xml)

$$X_{ml} = 40ml \times 162g = 0.093ml$$

(approximately 0.1ml)

70,000g

0.1ml for a 162g rat means a dose of

$$0.1ml/162g = \text{approx. } 6.2 \times 10^{-4} ml/g \text{ of rat.}$$

The rats were weighed weekly and the weight used to calculate the equivalent doses/volume to be administered for each group of rats for that week. The

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relationship between this weight and the quantity of feed consumed and appetite of the rats was also investigated.

Weekly Body Weight: The body weight of each rat was assessed using a sensitive balance during the acclimatization period, once before commencement of dosing (day 1), once weekly during the dosing period, (day 7, 14 and 21) and once on the day of sacrifice (day 29), [24].

Weekly Quantity of Feed Consumed: The quantity of feed given to each group of rats daily was determined by subtracting the quantity of feed left the next morning from that given the day earlier. From the results the average quantity consumed weekly by the rats was determined. This quantity of feed consumed by each rat was assessed using a sensitive balance from the commencement of dosing (day 1), until the day of sacrifice (day 29), [25].

Clinical Signs and Mortality: The animals were observed for signs of weakness, increased or decreased appetite, weight loss and other physiological changes including mortality.

Clinical signs to be assessed before dosing, immediately and 4hrs after dosing, include level of sedation, restlessness, changes in nature of stool, urine and eye colour, excretion of worms, diarrhoea, haematuria, uncoordinated muscle movements, etc. The animals will be observed for toxic symptoms such as weakness or aggressiveness, food refusal, loss of weight, diarrhoea, discharges from the eyes and ears, noisy breathing and mortality, [26] [27].

Blood Sample Collection and Preparation

Two specimen bottles were used for collection of blood from each animal. Anticoagulant bottles containing K₂ EDTA for haematological tests and lithium heparin bottles for assay of other parameters were used for initial collection of blood from all animals. The last dose of the bitters was administered on the morning of the 28th day. All meals were stopped by 7pm on the 28th day. After an overnight fast and following chloroform anaesthesia and opening up of the animals, blood samples were collected

from the animals using syringes and needles via the inferior vena cava and cardiac puncture, into already labelled K₂ EDTA and lithium heparin bottles without undue pressure to either the arm or the plunger of the syringe. The samples were then mixed by gentle inversion. The samples in the K₂ EDTA anticoagulant bottles were immediately sent for automated analysis for full/complete blood count and CD₄⁺ T-Lymphocyte count. The samples in the lithium heparin bottles were centrifuged at 4000r/min for 10mins to obtain plasma. The plasma supernatants were then separated into sterile plain bottles and were used for assay of the required parameters.

Assay of Haematological Indices

These were determined following the instructions of the manufacturers of the automated instrument:

The full/complete blood count, was determined using a KX-21N, an automated blood cell count analyser [28], while for the CD₄⁺ T-Lymphocyte count, CYFLOW SL-GREEN, an automated portable flow cytometer for the enumeration of CD₄⁺ T-Lymphocyte cells in the whole blood⁴⁺ was used [29], [30].

Assay of Fasting Blood Glucose

The blood glucose was assayed using the glucose-oxidase method [31], as outlined in the glucose kit by Randox lab. UK.

Assay of Serum Lipid Profile

The parameters assayed are total cholesterol, triacylglycerol, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol using Randox kit (Randox lab. UK) and following the standard procedures as described by the

manufacturers [32], [33].

Assessing the Liver Function Status

The parameters assayed are total protein, albumin, total bilirubin, conjugated bilirubin, alanine transaminase, aspartate transaminase, alkaline phosphatase and gamma-glutamyl transferase, using Randox kit (Randox lab. UK) and following the standard procedures as described by the manufacturers [34], [35], [36], [37], [38].

Assessing the Kidney Function Status

The parameters assayed are the electrolytes-Na⁺, K⁺ using the Flame Photometer [39]; Cl⁻ using the mercurimetric (titrimetric) method [40]; HCO₃⁻ using the titrimetric method [41]; urea- using the Berthelots reaction method [42]; and creatinine- using the spectrophotometric method [43].

Assessing the Cardiac Function Status

The cardiac enzymes assessed are creatine kinase - using the UV method [28] and lactate dehydrogenase- using the UV method [29].

Assessing the Antioxidant Status and Lipid Peroxidation Effect

The parameters assessed in vivo and the methodology employed are-malondialdehyde (MDA) level [30]; vitamin E [31]; vitamin C [32], [33]; catalase (CAT) [34]; superoxide dismutase (SOD) [35]; glutathione peroxidase (GPx) [36].

Statistical Analysis

Data was subjected to appropriate statistical analysis using the students paired t-test from the computerized statistical package for the social sciences, edition 17 (SPSS 17). P<0.05 was considered significant. The results were expressed as Mean ± SEM.

RESULTS

Clinical Signs and Symptoms and Mortality

During the 28 days of feeding with the bitters, there was no mortality, apart from a slight increase in activity for a few minutes, (possibly from alcoholic euphoria) no other adverse clinical manifestations were observed (no sedation, no changes in nature of stool, urine and eye colour, no discharge from the eyes and ears, no haematuria, no diarrhoea and no uncoordinated muscle

movements, etc). The bitters was well tolerated as it improved rather than adversely affecting the appetite of the rats; there were materials that were part of the stool suggestive of increased excretion of epithelial cells of the G.I.T which is in keeping with the claimed effect of bitters causing general toning of the G.I.T and increase in turnover of its epithelial cells that makes it possibly an ulcer preventive and healing agent.

Acute Toxicity: The LD₅₀ of swedish bitters was 24.00±5.10 ml/kg (expressed in other units considering the specific gravity/density of the bitters, the LD₅₀ (mg/kg x10³) is approximately 24.00±5.10

Anionye and Onyeneke and the LD₅₀ (g/kg) is approximately 24.00±5.10) Subchronic Toxicity and Pharmacological/Biochemical Effects of Herbal Bitters on Rats

Table 1: Feed consumed by rats after administration of swedish bitters for four weeks

Groups	Week 1	Week 2	Week 3	Week 4
Control	19.34±1.49 ^a	17.76±0.87 ^a	16.92±0.87 ^a	19.38±2.10 ^a
Swedish	20.44±1.26 ^{a,c}	21.38±1.07 ^{b,c}	18.58±0.83 ^{a,c}	17.04±1.49 ^{b,c}

Values are expressed as Mean±SEM. Values in the same column with different superscript letters differ significantly (P<0.05) from one another. Though the feed consumed by the bitters-fed rats were

consistently higher, apart from the 3rd week, the feed consumed were not significantly different (P>0.05) from that consumed by the control.

Table 2: Leucocyte Count , Leucocyte Differentials, CD₄ Count, and Platelet Count of rats fed with swedish bitters

Groups	Leucocyte Count (x 10 ³ / μL)	Lyphocyte Count (%)	Monocyte Count (%)	Neutrophil Count (%)	CD ₄ Count (/μL)	Platelet Count (x 10 ³ / μL)
Control ±11.58 ^a	5.79±1.22 ^a	65.60±2.19 ^a	11.39±1.34 ^a	23.43±1.90 ^a	186.00±7.63 ^a	72.63
Swedish ± 5.46 ^{a,c}	4.20±0.39 ^{a,c}	64.81±2.43 ^{a,c}	12.36±1.21 ^{a,d}	22.83±3.09 ^{a,c}	204.88±5.98 ^{a,c}	67.63

Values are expressed as Mean±SEM. Values in the same column with different superscript letters differ significantly (P<0.05) from one another. Though the total white blood cell count, the lymphocyte and platelet counts in rats fed swedish bitters were increased compared to that of the control, statistical

evaluation show that there were no significant difference (P>0.05) between them. The CD₄ lymphocyte count on the other hand was significantly (P<0.05) elevated, while the monocyte and neutrophil counts show a non-significant (P>0.05) decrease.

Table 3: Red Blood Cell (RBC) Count, Haemoglobin Concentration, Packed Cell Volume (PCV) and Red Cell Indices of rats fed with swedish bitters

Groups	Red Blood Cell, RBC count (x10 ⁶)	Haemoglobin concentration (g/dl)	Packed Cell volume, PCV (%)	Mean Corpuscular Volume, MCV (fl)	Mean Corpuscular Haemoglobin MCH (pg)	Mean Corpuscular Haemoglobin concentration MCHC (g/dl)
Control	6.39±0.64 ^a	14.88±1.19 ^a	41.56±3.80 ^a	48.98±0.99 ^a	19.48±0.45 ^a	36.79±0.70 ^a
Swedish	5.42±0.47 ^{a,c}	17.09±0.46 ^{a,c}	50.75±1.72 ^{b,c}	47.83±1.34 ^{a,c}	18.53±0.55 ^{a,c}	37.01±0.53 ^{a,c}

Values are expressed as Mean±SEM. Values in the same column with different superscript letters differ significantly (P<0.05) from one another. Though there were differences in the RBC count,

haemoglobin concentration, PCV, MCV, MCH and MCHC in rats fed swedish bitters when compared to that of the control, statistical evaluation shows that these differences were not significant (P>0.05).

Table 4: The effect of swedish bitters on fasting blood glucose (FBG) level of rats.

Groups	FBG (mg/dl)
Control	91.63±8.57 ^a
Swedish	85.50±1.35 ^{a,d}

Values are expressed as Mean ± SEM. Values in the same column with different superscript letters differ significantly (P<0.05) from one another. Statistical

evaluation indicates that the fasting blood glucose levels in rats fed swedish bitters were significantly (P<0.05) reduced compared to that of the control.

Table 5: The effect of swedish bitters on lipid profile of wistar rats

Research group	Cholesterol (mg/dL)	Triacylglycerol (mg/dL)	HDL-Chol (mg/dL)	LDL-Chol (mg/dL)	VLDL-Chol (mg/dL)
Control	98.63±3.02 ^a	58.00±2.74 ^a	18.88±2.14 ^a	68.15±1.75 ^a	11.60±0.55 ^a
Swedish	93.50±1.72 ^{a,c}	60.88±3.63 ^{a,d}	26.88±1.55 ^{b,c}	54.53±2.53 ^{b,c}	12.1±0.75 ^{a,d}

Values are expressed as Mean ± SEM. Values in the same column with different superscript letters differ significantly (P<0.05) from one another. The bitters caused a reduction in rat blood cholesterol, triacylglycerol, LDL-cholesterol and VLDL-cholesterol levels

and an increase in the HDL-cholesterol when compared to that of the control. The reduction in triacylglycerol and LDL cholesterol was statistically significant (P<0.05), same also with the elevation in HDL-cholesterol.

Table 6: liver function indices of rats administered with Swedish bitters

Analytes	Control	Swedish
Total Bilirubin (mg/dl)	0.28±0.03 ^a	0.34±0.03 ^{a,c}
Conjugated Bilirubin (mg/dl)	0.16±0.02 ^a	0.19±0.02 ^{a,c}
Aspartate Transaminase (IU/L)	28.25±2.97 ^a	29.13±4.26 ^{a,c}
Alanine Transaminase (IU/L)	3.88±0.30 ^a	4.63±0.50 ^{a,c}
Alkaline Phosphatase (IU/L)	10.13±0.61 ^a	14.00±1.22 ^{a,c}
Total Protein (mg/dl)	4.93±0.10 ^a	5.00±0.06 ^{a,c}
Albumin (mg/dl)	3.11±0.10 ^a	3.25±0.07 ^{a,c}
γ-Glutamyl Transpeptidase (IU/L)	2.75±0.16 ^a	3.63±0.42 ^{a,c}

Values are expressed as Mean ± SEM. Values in the same row with different superscript letters differ significantly (P<0.05) from one another. Though there are differences in the liver function

status indices of the control and bitters-fed rats, these differences are minimal and statistical evaluation shows that there are no significant difference (P>0.05) between them.

Table 7: Kidney Function Indices of rats administered with swedish bitters

Analytes	Control	Swedish
Na ⁺ (mmol/L)	137.13±2.43 ^a	136.75±1.85 ^{a,c}
K ⁺ (mmol/L)	14.05±1.08 ^a	13.25±1.12 ^{a,c}
Cl ⁻ (mmol/L)	108.63±3.39 ^a	107.38±3.08 ^{a,c}
HCO ⁻³ (mmol/L)	5.13±0.97 ^a	4.25±0.75 ^{a,c}
Urea (mg/dL)	35.38±2.43 ^a	38.88±1.72 ^{a,c}
Creatinine (mg/dL)	1.15±0.12 ^a	1.04±0.06 ^{a,c}

Values are expressed as Mean ± SEM. The values in the same row with different superscript letters differ significantly (P<0.05) from one another. Though there are differences in the kidney function

status indices of the control and bitters-fed rats, these differences are minimal and statistical evaluation shows that there are no significant difference (P>0.05) between them.

Table 9: The Effect on Lipid Peroxidation (MDA) and Antioxidant Status of rats administered with swedish bitters

Groups	Malondialdehyde (MDA) (U/mg protein x10 ⁻⁴)	Vitamin C (g/100ml)	Vitamin E (mMoles)	Superoxide dismutase (SOD) (U/mg protein x10 ⁻²)	Catalase (CAT) (U/mg protein)	Glutathione peroxidase (GPx) (U/ml)
Control	2.98±0.29 ^a	0.87±0.07 ^a	0.79±0.05 ^a	3.90±0.52 ^a	0.35±0.08 ^a	0.53±0.08 ^a
Swedish	1.37±0.11 ^{b,d}	1.78±0.06 ^{a,d}	1.56±0.16 ^{b,d}	4.04±0.71 ^{a,c}	4.27±0.08 ^{b,c}	0.78±0.07 ^{b,d}

Values are expressed as Mean±SEM. Values in the same column with different superscript letters differ significantly (P<0.05) from one another. The parameters used to measure the level of lipid peroxidation and antioxidant status show

general statistically significant (P<0.05) increase in their level or activity in the Paxherbal bitters-fed rats when compared with the control, with the exception of SOD whose increase was not statistically significant (P>0.05).

DISCUSSION

It is often summarized that bitters stimulate digestive secretions and the metabolism as a whole and in so doing increase appetite, relieve constipation, and generally ease the heavy glumness of sluggish digestion. But, this is really too simple and cursory a summation, and a deeper look into the actions of bitters is not only theoretically insightful but practically invaluable, especially as some plant products have been known to be toxic to the human system[3, 4]. Their composition as they are presently constituted have never been ascertained neither has their numerous pharmacological claims being subjected

to proper scientific scrutiny yet the use of bitters is getting popular.

The LD₅₀ of the bitters in this study are high, meaning that one may have to consume them more than 10 times their normal therapeutic dose before one gets a lethal/toxic effect. While it is not so important to calculate the LD₅₀ exactly for substances that are so highly toxic at 1mg/kg, LD₅₀ values greater than 5,000mg/kg are of no practical interest because such substances have low-lethality at doses they are likely to be consumed. The results of this study reveal that all the bitters have LD₅₀ values greater than 5,000mg/kg which indicates

that they have low-lethality at doses they are likely to be consumed, this is in agreement with the findings of [37].

Even if the weight changes between the control and bitters fed groups was not statistically significant ($P>0.05$), all the groups progressively gained weight, though at different rates throughout the 4 week period of this study, with the increase in rats fed Swedish bitters when compared to the control rats. The rats fed with Swedish bitters seemed to have had a lesser change in weight when compared to that of the control, but because these differences were not statistically significant ($P>0.05$) it cannot be said if the bitters were really useful as agents of weight loss/weight gain.

If a herb or herbal tonic is toxic, this can be reflected in a reduction in some or all of the haematological parameters measured in a full/complete blood count because of direct toxicity to or lysis of the cells in the blood. If however it is non toxic or actually nourishing and immunity boosting, this will reflect in the maintenance or increase in levels of some of the haematological parameters and cells especially those implicated as imparting immunity, though this increase will not be as high as the increase seen in a pathological state. The cells implicated as contributing especially to natural immunity are maintained at normal levels or raised to normal levels or a little above normal levels by herbs. Herbs have been shown to be more involved in imparting natural immunity than acquired immunity, though it can enhance acquired immunity when necessary [9, 38]. The results of this study indicate that the Swedish bitters did not exhibit any form of haematological toxicity, as statistical evaluation did not show any significant difference ($P>0.05$) between the values of the haematological parameters studied in the rats fed herbal bitters compared to the control. The increase in the CD₄ Count in rats fed Swedish bitters could be an attestation to the claim that bitters improve body immunity as it may be arising from the fact that the bitters may

contain biologically active principles that have the ability to boost the immune system through increasing the population of defensive white blood cells [9].

The manufacturers of the bitters used in this study made claims ranging from assertions that bitters help to increase the activity of the pancreas by bringing sugar level under control, assist in the elimination of sugar, to the assertion that it helps in the prevention of diabetes. The results of this study seem to provide some form of evidence to this claim as the fasting blood sugar in rats fed the bitters were reduced compared to that of the control. The bitters of this study show they possibly have as part of their constituents, some of these anti-diabetic plants or constituents in these plants that are said to have anti-diabetic properties.

The results of the study of the lipid profile of rats fed with the Swedish bitters compared with that of the control reveal that the Swedish bitters relatively have hypo-cholesterolaemic and hypo-triacylglycerolaemic effects, while decreasing the LDL-cholesterol (bad cholesterol) and VLDL-cholesterol levels and increasing the HDL-cholesterol (good cholesterol) level. This result seems to give credence to the claim by bitters manufacturers that they have hypo-lipidaemic effect. The results of this research on the serum lipid profile give positive evidence that the herbal bitters have the potential of being a lipid-lowering supplement/drug in mixed hyperlipidaemic states. There is evidence that a salient relationship exists between high serum cholesterol levels and the incidence of atherosclerosis and cardiovascular diseases [9], the observed hypocholesterolaemic effect of these herbal bitters is therefore a desired positive effect.

Liver cell damage is characterised by a rise in plasma enzymes (AST, ALT, LDH etc). From the results of this study AST concentrations were consistently higher than the ALT level, which is to be expected since body cells contain more AST than ALT, this is in agreement with the findings of [9]. But since AST is more

intracellular than ALT which is localised primarily in the cytosol of hepatocytes, ALT is a more sensitive marker of hepatocellular damage than AST. Thus the minimal and non-significant differences ($P>0.05$) in the AST and ALT levels in the bitters-fed rats compared to that of the control rats of this study is indicative that the bitters did not cause any hepatocellular damage to the liver of the rats [39].

The minimal and non-significant differences ($P>0.05$) in the ALP, total bilirubin and conjugated bilirubin levels in the bitters-fed rats compared to that of the control of this study is indicative that the bitters did not cause any form of cholestasis, excessive haemolysis, nor did it impair the capacity of the liver to excrete bilirubin. Cholestatic liver disease is characterised by an elevation in the plasma level of alkaline phosphatase (ALP), while hyperbilirubinaemia is seen in conditions causing excessive haemolysis and hepatic liver diseases that impair the excretion of bilirubin [9]. The minimal and non-significant differences ($P>0.05$) in the serum albumin and total protein levels in the bitters-fed rats compared to that of the control of this study is indicative that the bitters did not cause any dysfunction in the synthetic function of the liver [3]. Increased synthesis of Gamma-glutamyl transpeptidase in the liver resulting from microsomal enzyme induction by some drugs and alcohol (in chronic drinkers) produces increased plasma level [2]. The minimal increase seen in the level of Gamma-glutamyl transpeptidase in plasma of the Swedish bitters fed rats of this study may well be as a result of their "high" alcohol content this increase however did not result in a level of Gamma-glutamyl transpeptidase that is significantly different ($P>0.05$) from that of the control, so this increase is not associated with any hepatocellular damage [11].

The result of this study indicates that in some of the parameters used to assess the kidney function status of the control and bitters fed rats, there are differences

which are minimal but statistical evaluation shows that there is no significant difference ($P>0.05$) between them. The reduced levels of sodium and creatinine probably indicate that the bitters did not interfere with the renal capacity to excrete these metabolites. The lack of significant difference between the metabolites of the control and bitters fed groups used in assessing the kidney function status may also be a reflection of the preserved renal integrity of the treated rats [20]. Hence the bitters can be said not to have a reno-toxic effect on the kidneys of the bitters fed rats as they preserved its renal integrity and did not affect its capacity to excrete metabolites. There are minimal differences in the parameters used to assess the cardiac function status of the control and bitters fed rats but statistical evaluation shows that there is no significant difference ($P>0.05$) between them. Though other tissue damage may lead to a rise in our metabolites of interest, cardiac cell/muscle damage is characterised by a combination of a rise in plasma enzymes (creatinine kinase, LDH etc), from the results of this study, there was no significant increase ($P>0.05$) in either creatine kinase nor LDH, infact the creatine kinase level was consistently lower in the Swedish bitters fed rats compared to the control suggesting some form of cardio-protectivity. Thus the minimal and non-significant differences ($P>0.05$) in the creatine kinase and LDH levels in the bitters-fed rats compared to that of the control of this study is not just a reflection of the preserved cardiac integrity of the treated rats but indicative that the bitters did not cause any cardio-cellular damage to the heart of the rats [17]. Hence the bitters can be said not to have a cardio-toxic effect on the heart of the rats as they preserved its cardiac integrity.

Oxidative Stress represents an imbalance in production and clearance of reactive oxygen species/free radicals in biological systems [40]. Disturbances in the normal redox state of tissues can cause toxic effects through the production of

peroxides and free radicals that damage all components of the cell, including protein, lipid and DNA, hence in humans; oxidative stress has been identified as one of the causal factors in many diseases [41]. Reactive oxygen species may be beneficial as they are used by the immune system as a way to attract and kill pathogens [41]. Excessive oxidative stress particularly at unwanted places (e.g vascular lining, blood brain barrier) will damage the defence system.

The result of this study indicates that the MDA levels in the Swedish bitters-fed rats were reduced, when compared to the MDA level in the control. The results of this study indicate that the Vitamin C levels of the Swedish bitters-fed rats were decreased significantly ($P<0.05$) when compared to the control. As for vitamin E, its level in the Swedish bitters-fed rats were increased significantly ($P<0.05$). This can be said to be as a result of the bitters adding to and preserving the immediate

The results of this study showed that the Swedish bitters were safe for consumption, as the acute toxicity (LD_{50}) of the bitters, indicated they have a relatively high LD_{50} and so they will have a low-lethality⁵⁰ at doses they were likely to be consumed. The biochemical and haematological assay results of this study with inferences derived from some of the already established effects of some of the phytochemical and mineral constituents of the bitters, gave some evidence that the herbal bitters of this study may be said to have the potential or possibility of having

use of these vitamins in the rats as its inherent antioxidant capacity act as firstline antioxidants as well as protects the rats from excessive use of its indigenous antioxidants [33], [24]. Generally the superoxide dismutase activity, the Catalase activity and Glutathione Peroxidase activity of the rats fed Swedish bitters were significantly ($P<0.05$) increased compared to the activities of these same enzymes in the control rats. This further confirms the antioxidant improving capacity of herbal bitters generally. The enzymes are all antioxidant enzymes that battle oxidants and free radicals implicated in causing many diseases especially cardiovascular diseases and cancer. The results of this study imply that the Swedish bitters contain the herbaceous plants and species that are harmless sources for obtaining the natural antioxidants that may not only anticarcinogenic but may also protective against cardiovascular diseases.

CONCLUSION

the following pharmacological properties hypolipidaemic/hypocholesterolaemic, hypoglycaemic, anti-anaemic and anti-inflammatory, stimulant and immunity-boosting/immuno-modulatory, antimutagenic/antitumour as well as diuretic/vasodilatory and antihypertensive properties and the ability to protect against/prevent coronary artery disease and cardiovascular diseases generally. The conclusive biochemical finding from this study indicated that at the prescribed dosage of the various bitters, they exert some positive pharmacological effects.

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