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Evaluation of the Effects of Alomo Bitters on Albino Rats

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ABSTRACT

This study was designed to evaluate the effects of Alomo bitters on albino rats. The Alomo bitters was fed using an oro-gastric gavage, to different sets of male albino wistar rats, after which the biochemical and haematological indices and histopathological status of the organs of the bitters -fed rats, as well as the acute toxicity (LD_{50}) and subchronic toxicity of the Alomo bitters on the rats were investigated using standard laboratory procedures. The Alomo bitters were well tolerated and not toxic to the rat organs and blood cells, during the 28-day duration of the study. The acute toxicity (LD_{50}) of 29 x 10^3 mg/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 Alomo-fed rats compared to same in the control rats, was indicative that the Alomo preserved the functions of these organs. The increase in the CD_{4+}-count and decrease in the level of the fasting blood glucose in the Alomo-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 Alomo-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.

Keywords: Alomo bitters, haematology, serum, antioxidant, histopathology, rat.

INTRODUCTION

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 [1,2, 3]. 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. 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 [2, 4]. 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

They are also said to have anti-inflammatory, antibiotic and antifungal properties. Bitters have also been said to ensure good digestion of fats and oils, and proper functioning of the excretory functions of the liver, reduce accumulated fat (triglycerides) and

cholesterol levels thereby confering on it hypolipidaemic properties. They are said to reduce excess body fat and promote healthy weight loss, act as a liver tonic and body detoxifier; being hepatoprotective and enhancing its functions generally and helping in body detoxification. Bitters act on the pancreas and liver, help in cell division and growth of the pancreas thereby helping to normalizing blood sugar and promote the production and release of pancreatic enzymes. Some are even said to have hypoglycaemic properties. In modern herbal medicine, "bitter principles" occupy a central place in herbal therapeutics, bearing the acrid constituents. Most people consuming herbal medicines complain about the bitterness of the medicines prescribed. This is the only defining attribute of herbal medicine and the only feature to set it apart from other therapies [1, 3]. In times past our traditional diets were not devoid of bitter foods as is presently the case in most modern diets, hence [5] desires that we see the medicinal side of bitters in an entirely different light in other that we use it to prevent what he termed the "Bitter Deficiency Syndrome" of our era, which in his opinion is the predisposing factor to many ailments of our time [5, 3]. All these make the study of the constituent and pharmacological effects of present day bitters desirable. Alomo Bitters is a herbal alcoholic product (42% alcohol) made from carefully chosen tropical plant extracts and very well known for its proven medicinal values. Alomo Bitters, a

Ghanaian product (composed of seven (7) herbal constituents). It has its roots in the traditional herbal industry that is meticulously researched by the Centre for Scientific Research into Plant Medicine a World Health Organization affiliate, based at Mampong Akuapem in the Eastern region of Ghana. As an aphrodisiac, it enhances sexual function particularly on erectile stamina. It also takes care of back pains, pile, improving digestion and hence prevents diseases associated with carbohydrate metabolism (diabetes) and lipid metabolism (hyperlipidaemia)] and strengthening the immune system.

The manufacturers claim they are non-FDA reviewed or approved, natural alternatives, to use for heart disease, chronic fatigue, cancers, hypertension and obesity. Uses vary, but may include eliminating body odour and alleviating eating disorders and sluggish digestion, fighting infection and increasing white blood cells. Other uses include cleansing skin and moisturizing skin. Alomo works without any perceivable stress on the body if taken as prescribed, that is it does not work by increasing the heart rate or by increasing blood pressure. The Alomo bitters in summary are generally claimed to be effective in curing all allergic, metabolic and immunological conditions where the diagnosis points to a fault in the digestive process, improves immunity, help in anaemia, wound healing, and blood clotting by increasing the population in tissues, of red blood cells, white blood cells and platelets, help with inflammatory conditions of the gastrointestinal tract (Colitis, Crohn's disease, nonspecific inflammation). In addition to the action of bitters on digestive secretions aiding good digestion, they also strengthen the tone of tissues throughout the digestive tract, as well as aid in the healing of damaged mucous membranes. They are generally said to regenerate and heal mucosal lining of the G.I.T especially duodenal and gastric ulcers. This helps resolve conditions ranging from gastroesophageal reflux to ulcers to leaky gut syndrome.

MATERIALS AND METHODS

Materials

Alomo bitters was purchased from reputable pharmaceutical stores opposite the University of Benin Teaching Hospital (UBTH), Ugbowo Lagos Road, Benin City, Edo State, Nigeria. The alomo bitters was bought as liquid formulations and stored at room temperature (30-36^{o}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 [6]. 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^{0}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 Miller and Tainter [7] as adapted by Randhawa [8] 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 LD_{50} 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 preceeding 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 oro-gastric gavage [7, 8, 9]. 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 was noted; these doses were used to determine the range to be used in the LD_{50} determination for the herbal bitters [7, 8, 9].

Phase II Acute Toxicity Study: Experimental Design/Protocol

This was for the determination of the LD_{50}. After 14 days of acclimatization, the 50 experimental animals for the determination of the LD_{50}, using the Miller and Tainter method [7], 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 preceeding one following the range as determined from pre-LD_{50} determiation 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 [6, 7, 8]. 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 were 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 % Mortality Formula for 0 and 100% [8]

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 LD_{50}: 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 LD_{50}. Other calculations were made according to the method described by Miller and Tainter [7] and Randhawa[8].

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 Alomo 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 of were done to determine the initial equivalent doses of the bitters (distilled water in the case 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) Xml = 40ml x 162g = 0.093ml (approximately 0.1ml)

70,000g

0.1ml for a 162g rat means a dose of 0.1ml/162g = approx. 6.2 x10^{-4}ml/g 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 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), [9].

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), [9].

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, [10, 11].

Blood Sample Collection and Preparation

Two specimen bottles were used for collection of blood from each animal. Anticoagulant bottles containing K$_2$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 K_2 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 K_2 EDTA anticoagulant bottles were immediately sent for automated analysis for full/complete blood count and CD_{4+} 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 [12], while for the CD_{4+}- T-Lymphocyte count, CYFLOW SL- GREEN, an automated portable flow cytometer for the enumeration of CD_{4+} - T-Lymphocyte cells in the whole blood was used [13, 14].

Assay of Fasting Blood Glucose:The blood glucose was assayed using the glucoseoxidase method [15], as outlined in the glucose kit by Randox lab. UK.

Assay of Serum Lipid Profile

The parameters assayed are total cholesterol, triaclglycerol, HDL-cholesterol, LDLcholesterol and VLDL-cholesterol using Randox kit (Randox lab. UK) and following the standard procedures as described by the manufacturers [16, 17].

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 [18, 19, 20, 21, 22].

Assessing the Kidney Function Status

The parameters assayed are the electrolytes-Na^+, K^+- using the Flame Photometer [23]; Cl^- - using the mercurumetric (titrimetric) method [24]; HCO_3^- - using the titrimetric method [25]; urea- using the Berthelots reaction method [26]; and creatinine- using the spectrophotometric method [27].

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 LD_{50} of Alomo bitters was 24.00±5.10 ml/kg (expressed in other units considering the specific gravity/density of the bitters, the LD_{50} (mg/kg x10^3) is approximately 24.00±5.10 and the LD_{50} (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 Alomo bitters for four weeks

Groups	Week 1	Week2	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]
Alomo	16.69±0.63[a]	15.43±0.51[b]	15.15±0.34[a]	13.74±1.39[b]

Values are expressed as Mean±SEM. Values in the same column with different superscript letters differ significantly (P<0.05) from one another. The feed consumed by the bitters-fed rats were consistently lower in all the weeks of the study and this decrease was significantly different in weeks 2 and 4 (P<0.05) from that consumed by the control.

Acute toxicity: determination of the LD_{50} of the various bitters

Table 2: The LD_{50} of the various bitters

Bitters	Alomo
LD_{50}(ml/kg)	29.00±7.00
	29.00±7.00
LD_{50} (mg/kg x10^3)	
LD_{50} (g/kg)	29.00±7.00

The values were expressed as mean±S.E.M.
For the conversion from ml/kg to g/kg the density/specific gravity (weight/ml) of the various bitters ranging
rom 1.0004g/ml (for Alomo bitters) was used in multiplying the ml/kg value.

Table 3: Leucocyte Count , Leucocyte Differentials, CD_4 Count, and Platelet Count of rats fed with Alomo bitters

Groups	Leucocyte Count(x10^3/ µL)	Lyphocyte Count (%)	Monocyte Count (%)	Neutrophil Count (%)	CD_4 Count (/µL)	Platelet Count x 10^3/ µL)
Control	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 ±11.58[a]
Alomo	4.80±0.25[a]	62.38±5.32[a]	9.80±1.61[a]	27.95±4.88[a]	197.25±7.65[a]	71.75 ± 6.00[a]

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, monocyte and platelet counts in rats fed Alomo bitters were

slightly decreased compared to that of the control, statistical evaluation show that there were no significant difference (P>0.05) between them. The CD_4^+ lymphocyte count and neutrophil counts on the other hand show a non-significant (P>0.05) elevation.

Table 4: Red Blood Cell (RBC) Count, Haemoglobin Concentration, Packed Cell Volume (PCV) and Red Cell Indices of rats fed with alomo bitters

Groups	Red Blood Cell, RBC Count ($x10^6$)	Haemoglobin Concentration (g/dl) (%)	Packed Cell Volume, PCV	Mean Corpuscular (fl) Volume, MCV	Mean Corpuscular Haemoglobin MCH (pg)	Mean Corpuscular Haemoglobin Concentration, MCHC
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]
Alomo	5.79±0.43[a]	21. 79±2.40[a]	52.25±2.62[b]	51.93±1.2.15[a]	18.90±0.78[a]	36.59±1.10[a]

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, MCV, MCH and MCHC in rats fed alomo bitters when compared to that of the control, statistical evaluation shows that these differences were not significant (P>0.05). The PCV of Alomo fed rats however show a significant increase.

Table 5: The effect of alomo bitters on fasting blood glucose (FBG) level of rats.

Groups	FBG (mg/dl)
Control	91.63±8.57[a]
Alomo	48.38±4.84[b]

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 alomo bitters were significantly (P<0.05) reduced compared to that of the control.

Table 6: The effect of alomo bitters on lipid profile of wistar rats

Research Groups	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]
Alomo	95.38±1.74[a]	55.50±2.21[a]	31.88±4.44[b]	57.28±1.73[b]	11.10±0.44[a]

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 LDL-cholesterol was statistically significant (P<0.05), same also with the elevation in HDL-cholesterol.

Table 7: liver function indices of rats administered with alomo bitters

Analytes	Control	Alomo
Total Bilirubin (mg/dl)	0.28±0.03[a]	0.33±0.04[a]
Conjugated Bilirubin (mg/dl)	0.16±0.02[a]	0.18±0.03[a]
Aspartate Transaminase (IU/L)	28.25±2.97[a]	29.00±1.89[a]
Alanine Transaminase (IU/L)	3.88±0.30[a]	3.13±0.13[a]
Alkaline Phosphatase (IU/L)	10.13±0.61[a]	12.00±1.13[a]
Total Protein (mg/dl)	4.93±0.10[a]	5.08±0.12[a]
Albumin (mg/dl)	3.11±0.10[a]	3.11±0.06[a]
γ-Glutamyl Transpeptidase (IU/L)	2.75±0.16[a]	3.13±0.35[a]

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 8: Kidney Function Indices of rats administered with Alomo bitters

Analytes	Control	Alomo
Na^+ (mmol/L)	137.13±2.43[a]	138.00±3.39[a]
K_+ (mmol/L)	14.05±1.08[a]	13.16±0.90[a]
Cl^- (mmol/L)	108.63±3.39[a]	103.88±13.51[a]
HCO^{-3} (mmol/L)	5.13±0.97[a]	5.50±0.65[a]
Urea (mg/dL)	35.38±2.43[a]	40.37±3.23[a]
Creatinine (mg/dL)	1.15±0.12[a]	0.98±0.05[a]

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 bittersfed rats, these differences are minimal and statistical evaluation shows that there are no significant difference (P>0.05) between them.

Table 9: Cardiac Function Enzymes of the Control and Test Groups

Analytes	Control	Alomo
Creatine Kinase (U/L)	40.89±5.98[a]	38.41±2.14[a,c]
Lactate Dehydrogenase (U/L)	125.50±0.82[a]	125.63±1.15[a,c]

Values are expressed as Mean±SEM. The values in the same column with different superscript letters differ significantly (P<0.05) from one another. Though there are variations in the levels of the enzymes used to assess the cardiac function status 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 10: The Effect on Lipid Peroxidation (MDA) and Antioxidant Status of rats administered with Alomo 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]
Alomo	1.28±0.1d[b]	1.21±0.06[b]	2.29±0.32[b]	3.58±0.85[a]	1.85±0.20[b]	0.89±0.05[b]

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 Alomo 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 by increase of appetite, relief of constipation, and generally ease the heavy glumness of sluggish digestion. Action 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 [1,3]. 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. During the 28 days of feeding with the various bitters, there was no mortality, apart from a slight increase in activity for a few minutes especially after dosing with Alomo (possibly from alcoholic euphoria from their high alcoholic contents) 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 Alomo was well tolerated as they did not adversely affect the appetite of the rats, there were materials that were part of its stool suggestive of increased excretion of epithelial cells of the G.I.T which is in keeping with the claimed effect of Alomo causing general toning of the G.I.T and increase in turnover of its epithelial cells that makes it an ulcer preventive and healing agent.

The results of this study show that the feed consumed per day in the 4 weeks of this study is lesser in rats fed-Alomo bitters, when compared with that of the control; statistical evaluation however showed that there was no significant difference (P>0.05) in the mean of the feed consumed. The findings of this study is also in agreement with the findings of [9] and as with Nature Cure bitters they worked with, we can say that the diets were well accepted by the Alomo-fed rats, suggesting that the Alomo did not possibly cause any drastic alterations in the carbohydrate, protein or fat metabolism in these experimental animals in such a way as

to prevent a weight gain expected of animals that are continually supplied with food and water *ad libitum* [9]. 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 toxicty 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 maintainance 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, 37, 38,39;]. The results of this study indicates that the Alomo 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 Alomo bitters compared to the control. The increase in the CD_4^+ count in rats fed with Alomo 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 [38]. The results of the study of the lipid profile of rats fed with the Alomo bitters compared with that of the control reveal that generally, the Alomo 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. 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 Aniagu, *et al*[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 [40]. 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 [40]. 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 [40]. 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 [40]. The minimal increase seen in the level of Gamma-glutamyl transpeptidase in plasma of all the 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 [40].

The result of this study also indicates that in some of the parameters used to assess the kidney function status of the control and Alomo-fed rats, there were differences which are minimal but statistical evaluation shows that those differences were not significant ($P>0.05$). The reduced levels of sodium and creatinine probably indicate that Alomo bitters did not interfere with the renal capacity to excrete these metabolites. The lack of significant difference between the metabolites of the control and Alomo-fed groups used in assessing the kidney function status may also be a reflection of the preserved renal integrity of the treated rats [9]. Hence, Alomo bitters can be said not to have a reno-toxic effect on the kidneys of the Alomo-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 Alomo-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 (creatine 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 all the 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 Alomo-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 Alomo did not cause any cardio-cellular damage to the heart of the rats [40]. 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 [41]. 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 [42]. Reactive oxygen species may be beneficial as they are used by the immune system as a way to attract and

kill pathogens [42]. Excessive oxidative stress particularly at unwanted places (e.g vascular lining, blood brain barrier) will damage the defence system. The result of this study also indicates that the MDA levels in all the Alomo bitters-fed rats were reduced, when compared to the MDA level in the control. Malondialdehyde (MDA) is a product of lipid peroxidation that can be easily measured and the results of this research shows the herbal bitters prevented the lipid peroxidation of the membranes of tissues and cells in the rats; this is an antioxidant effect, meaning the bitters have antioxidant constituents. The results of this study indicate that the Vitamin C levels of the Alomo-fed rats were increased significantly ($P<0.05$) when compared to the control. As for vitamin E, its level in the Alomo bitters-fed rats were increased significantly ($P<0.05$). This can be said to be as a result of the Alomo adding to and preserving the immediate 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 indigenious antioxidants [43;33]. Generally the superoxide dismutase activity, the Catalase activity and Glutathione Peroxidase activity of the rats fed with Alomo bitters were significantly ($P<0.05$) increased compared to the activities of these same enzymes in the control 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 Alomo bitters contain the herbaceous plants and species that are harmless sources for obtaining the natural antioxidants that may not only anticarcinogenic but may also be protective against cardiovascular diseases.

The described changes in the histopathological studies (photomicrographs not shown) done on the tissues of the heart, kidneys, liver, pancreas, small intestine and colon of Alomo bitters- fed rats did not reveal adverse differences when compared to those of control organs.

CONCLUSION

The results of this study showed that the Alomo 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-lethality at doses they were likely to be consumed. The biochemical and haematological assay results of this study showed that the Alomo bitters of this study may be said to have the potential or possibility of having the following pharmacological properties - hypocholesterolaemic, hypoglycaemic, anti-anaemic and anti-inflammatory, immuno-modulatory, hepatoprotective, *invivo* and *invitro* antioxidant capacity and by extension anticarcinogenic, vasodilatory and antihypertensive properties and the ability to protect against/prevent coronary artery disease and cardiovascular diseases generally.

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