Evaluation of the Effects of Super Bitters on Albino Rats

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ABSTRACT

This study evaluated the effects of super bitters on albino rats. Super bitters were administered using an oro-gastric gavage to different sets of male albino Wistar rats, after which the biochemical and haematological indices and the histopathological status of the bitters fed rats as well as the acute toxicity (LD_{50}) and subchronic toxicity of the super bitters on the rats were investigated using standard laboratory procedures. During the 28 days of feeding with the super bitters there was 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. The super bitters were well tolerated as they improved rather than adversely affected the appetite of the rats. 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 super bitters-fed rats compared to same in the control rats, was indicative that super bitters preserved the functions of these organs. There was a non-significant (P>0.05) increase in the CD_{4} count. The decrease in the level of the fasting blood glucose in the super 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 Super bitters was significant (P<0.05). Super bitters-fed rats had a significant decrease (P<0.05) in their serum malondialdehyde (MDA) levels and a significant increase (P<0.05) in some serum antioxidant enzymes namely catalase, and glutathione peroxidase, when compared with that of the control rats. The results from this study indicate that super bitters may be said to be possess hypolipidaemic, hypoglycaemic, immunity-boosting, choleretic, hepatoprotective, antihypertensive, as well as having antioxidant properties and protective against cardiovascular diseases.

Keywords: Super bitters, haematology, serum, antioxidant, histopathology, rat
INTRODUCTION

Modern pharmacology had its origin in these medicinal plants and till date some drugs are products of active components from plants [1 and 2]. 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 and 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".

The term “bitters” as it is used presently, is a beverage, often alcoholic, flavoured with herbal essences that gives it a bitter or bittersweet flavour. The generic term applies to all bitter liquors and herbal bitters. 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 aid in digestion and as flavouring in cocktails. 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 and 4].

The product to be studied is Super Bitters, a western Nigeria product, composed of four (4) herbal constituents. “Bitters” generally are 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.

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 have also been said to ensure good digestion of fats and oils, and proper functioning of the liver in excretion, reduce accumulated fat (triglycerides) and cholesterol levels thereby conferring 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 [2 and 4].

MATERIALS AND METHODS

MATERIALS

Super bitters was purchased from reputable pharmaceutical stores opposite the University of Benin Teaching Hospital (UBTH), Ugbowo Lagos Road, Benin City, Edo State, Nigeria. The super bitters was 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 [5].

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 Miller and Tainter [6] as adapted by Randhawa [7] 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 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 oro-gastric gavage [6, 7, 8]. 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 1$^{st}$ dose that caused the death of the 2 rats in each subgroup was noted; these doses were used to determine the range to be used in the LD$_{50}$ determination for the super bitters [6, 7, 8].

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 [6], 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-LD$_{50}$ 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 [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 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 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 [6] and Randhawa (7).
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 super 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)

\[
\text{Xml} = \frac{40\text{ml}}{70,000\text{g}} \times 162\text{g} = 0.093\text{ml} \quad \text{(approximately 0.1ml)}
\]

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), [8]. 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), [8].

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, [9, 10].

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 [11], 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 blood was used [12, 13, 14].

Assay of Fasting Blood Glucose
The blood glucose was assayed using the glucose-oxidase method (14), 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 [15, 16].

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 [17, 18, 19, 20, 21].

Assessing the Kidney Function Status
The parameters assayed are the electrolytes- Na⁺, K⁺ using the Flame Photometer [22]; Cl⁻ using the mercurumetric (titrimetric) method [23]; HCO₃⁻ using the titrimetric method [24]; urea- using the Berthelots reaction method [25]; and creatinine- using the spectrophotometric method [26].

Assessing the Cardiac Function Status
The cardiac enzymes assessed are creatine kinase - using the UV method [27] and lactate dehydrogenase- using the UV method [28].

Assessing the Antioxidant Status and Lipid Peroxidation Effect
The parameters assessed in vivo and the methodology employed are-malondialdehyde (MDA) level [29]; vitamin E [30]; vitamin C [31, 32]; catalase (CAT) [33]; superoxide dismutase (SOD) [34]; glutathione peroxidase (GPx) [35].

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

Table 1: The LD_{50} of the various bitters

<table>
<thead>
<tr>
<th></th>
<th>Super bitters</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD_{50} (ml/kg)</td>
<td>164.00±63.00</td>
</tr>
<tr>
<td>LD_{50} (mg/kg x10^{3})</td>
<td>164.00±63.00</td>
</tr>
<tr>
<td>LD_{50} (g/kg)</td>
<td>164.00±63.00</td>
</tr>
</tbody>
</table>

The values were expressed as mean±S.E.M.

Table 2: Mean body weight (g) of rats per group per week

<table>
<thead>
<tr>
<th></th>
<th>Week 0</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>161.63±11.00a</td>
<td>168.26±10.66a</td>
<td>176.65±11.01a</td>
<td>180.78±10.98a</td>
<td>192.69±10.20a</td>
</tr>
<tr>
<td>Super</td>
<td>161.75±11.41a</td>
<td>173.83±9.89a</td>
<td>178.63±9.57a</td>
<td>183.33±10.24a</td>
<td>185.14±9.78a</td>
</tr>
</tbody>
</table>

Values were expressed as Mean±SEM. Statistical evaluation shows that for each week and throughout the entire four weeks of the research there was no significant difference (P>0.05) in the body weights of the super bitters fed rat when compared with that of the control.

Table 3: Mean of feed consumed per day weekly (g)

<table>
<thead>
<tr>
<th></th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19.34±1.49a</td>
<td>17.76±0.87a</td>
<td>16.92±0.87a</td>
<td>19.38±2.1a</td>
</tr>
<tr>
<td>Super</td>
<td>17.00±1.36a</td>
<td>15.57±0.45a</td>
<td>18.36±1.41a</td>
<td>14.77±1.52b</td>
</tr>
</tbody>
</table>

Values were expressed as Mean±SEM. Results in table 3 indicates that when compared to control, the feed consumed per day weekly in the first three weeks is lesser in Super
bitters, statistical evaluation however shows that there was no significant difference (P>0.05) in the mean of the feed consumed.

### Table 4: Pack Cell Volume (PCV), Haemoglobin (Hb) Conc. and Blood Cell Counts of Control and Test Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>PCV (%)</th>
<th>Hb. conc. (g/dL)</th>
<th>RBC count (x10⁶/µL)</th>
<th>WBC count (%)</th>
<th>Platelet count(x10³/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>41.56±3.80ᵃ</td>
<td>14.88±1.19ᵃ</td>
<td>6.39±0.64ᵃ</td>
<td>5.79±1.22ᵃ</td>
<td>72.63±11.58ᵃ</td>
</tr>
<tr>
<td>Super</td>
<td>42.63±3.51ᵃ</td>
<td>14.28±1.20ᵃ</td>
<td>6.95±0.64ᵃ</td>
<td>6.60±0.63ᵃ</td>
<td>85.75±4.51ᵃ</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. The PCV, WBC and Platelet counts in rats fed with Super bitters were increased, compared to that of the control, but this increase was not statistically significant (P>0.05).

### Table 5: White Blood Cell Differentials and CD₄ Count of the Test and Control Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lymphocyte count (%)</th>
<th>Neutrophil count (%)</th>
<th>Monocyte count (%)</th>
<th>CD₄ count (/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>65.60±2.19ᵃ</td>
<td>23.43±1.90ᵃ</td>
<td>11.39±1.34ᵃ</td>
<td>186.00±7.63ᵃ</td>
</tr>
<tr>
<td>Super</td>
<td>67.76±3.45ᵃ</td>
<td>20.25±4.87ᵃ</td>
<td>12.00±2.15ᵃ</td>
<td>194.25±12.84ᵃ</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. The lymphocyte count and the CD₄ count show a non-significant (P>0.05) increase.

### Table 6: Red Blood Cell Indices of the test and control groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>48.98±0.99ᵃ</td>
<td>36.79±0.70ᵃ</td>
</tr>
<tr>
<td>Super</td>
<td>48.15±1.43ᵃ</td>
<td>36.58±0.49ᵃ</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. Though there are differences in the MCV, MCH and MCHC of the control and super bitters fed rats, these differences are minimal and statistical evaluation shows that there is no significant differences (P>0.05) between them.

### Table 7: Fasting blood glucose (FBG) level (mg/dl) of control and test groups
Values are expressed as Mean±SEM. The fasting blood sugar in rats fed the super bitters were all reduced compared to that of the control with the reduction being statistically significant (P<0.05).

### Table 8: Lipid profile (mg/dl) of control and test groups

<table>
<thead>
<tr>
<th>Research groups</th>
<th>Cholesterol (mg/dL)</th>
<th>Triacylglycerol (mg/dL)</th>
<th>HDL-chol. (mg/dL)</th>
<th>LDL-chol. (mg/dL)</th>
<th>VLDL-chol. (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>98.63±3.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.00±2.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.88±2.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.15±1.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.60±0.55&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Super</td>
<td>93.75±1.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.63±1.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.50±3.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51.98±1.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.77±0.30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM. Super bitters caused a non-significant (P>0.05) decrease in rat blood cholesterol level and a significant (P<0.05) decrease in the LDL-cholesterol compared to that in the control rats. The bitters also caused a significant increase in HDL-cholesterol.

### Table 9: Liver function indices results for the control and test groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Super</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Bilirubin (mg/dl)</td>
<td>0.28±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.29±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Conjugated Bilirubin (mg/dl)</td>
<td>0.16±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.19±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aspartate Transaminase (IU/L)</td>
<td>28.25±2.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.63±1.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alanine Transaminase (IU/L)</td>
<td>3.88±0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.63±0.42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Values are expressed as Mean±SEM. Though there are differences in the liver function test parameters of the control and Super bitters fed rats, these differences are minimal and statistical evaluation shows that there is no significant difference (P>0.05) between them.

Table 10: Kidney function indices results for the control and test groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Super</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Na⁺ (mmol/L)</strong></td>
<td>137.13±2.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134.63±2.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>K⁺ (mmol/L)</strong></td>
<td>14.05±1.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.74±0.48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Cl⁻ (mmol/L)</strong></td>
<td>108.63±3.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>111.13±2.27&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>HCO₃⁻ (mmol/L)</strong></td>
<td>5.13±0.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.88±0.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Urea (mg/dL)</strong></td>
<td>35.38±2.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.63±2.88&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Creatinine (mg/dL)</strong></td>
<td>1.15±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.89±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM. Though there are differences in some of the parameters used to assess the kidney function status of the control and bitters fed rats,
these differences are minimal and statistical evaluation shows that there is no significant
difference (P>0.05) between them.

### Table 11: Cardiac function indices results for the control and test groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Super</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine Kinase (U/L)</td>
<td>40.89±5.98a</td>
<td>40.01±0.75a</td>
</tr>
<tr>
<td>Lactate (U/L)</td>
<td>125.50±0.82a</td>
<td>125.13±1.42a</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM. Though there are differences in some of the parameters used to assess the Cardiac function status of the control and bitters fed rats, these differences are minimal and statistical evaluation shows that there is no significant difference (P>0.05) between them.

### Table 12: Test Results of the Antioxidant Status and Effect on Lipid Peroxidation

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (U/mg protein x10^4)</th>
<th>Vit.C (g/100 ml)</th>
<th>Vitamin E (mMoles)</th>
<th>SOD activity (U/mg protein x10^-2)</th>
<th>Catalase activity (U/mg protein)</th>
<th>GPx activity (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.98±0.29a</td>
<td>0.87±0.07a</td>
<td>0.79±0.05a</td>
<td>3.90±0.52a</td>
<td>0.52±0.08a</td>
<td>0.53±0.08a</td>
</tr>
<tr>
<td>Super</td>
<td>1.48±0.23b</td>
<td>1.02±0.03a</td>
<td>0.99±0.06a</td>
<td>3.53±0.64a</td>
<td>0.94±0.11b</td>
<td>1.26±0.2b</td>
</tr>
</tbody>
</table>
Values are expressed as Mean±SEM. MDA values of the Super bitters-fed rats were reduced and statistical evaluation indicates that this reduction was significant (P<0.05) when compared to the MDA level in the control. The Vitamin C and Vitamin E levels of the Super bitters-fed rats increased but statistical evaluation however indicates that this increases were not significant (P>0.05). The superoxide dismutase activity of the rats fed the super bitters were generally increased compared to control, statistical evaluation however indicates that this increased activity was not significant (P>0.05).

The Catalase activity and Glutathione peroxidase (GPx) activities of the rats fed with Super bitters were generally increased compared to control, statistical evaluation also indicates that this increased activity was significant (P<0.05).

**DISCUSSION**

Researchers and endeavours are geared towards discovery of new therapeutic agents or newer and richer sources of known drugs of natural origin, and the basic goal of such drug discovery efforts always hinges on developing new products with enhanced therapeutic benefits, that is, higher efficacy and low toxicity profile [36, 37]. The LD$_{50}$ of the super 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.

The weight changes between the control and Super bitters fed groups was not statistically significant (P>0.05), all the groups progressively gained weight, though at different rates throughout the 4 weeks period of this study.

The results of this study show that the feed consumed per day in the 4 weeks of this study is higher in rats fed with super 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. These changes however gives the impression that even if super bitters were generally well tolerated and did not lead to a drastic reduction in food consumption, some of them causes an increase in appetite more than others, leading to more food being consumed and giving credence to the claim that some bitters increase appetite [1]. The findings of this study is also in agreement with the findings of Aniagu et al [8] and as with Nature Cure bitters they worked with, we can say that the diets were well accepted by the bitters fed rats, suggesting that the bitters 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* [8].

A toxic herb or herbal tonic is will naturally cause 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 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 [38; 39; 40]. The results of this study indicates that the Super herbal 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 with Super herbal bitters compared to the control. The increase in the CD+ Count in rats fed with Super bitters could be an attestation to the claim that it improves 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 results of the study of the lipid profile of rats fed with Super bitters compared with that of the control revealed that Super bitters relatively have hypo-cholesterolaemic and hypo-triacylglycerolaemic effects, while significantly (P<0.05) decreasing the LDL-cholesterol (bad cholesterol) and significantly (P<0.05) increasing the HDL-cholesterol
(good cholesterol) level. This result seems to give credence to the claim by Super bitters manufacturers that they have hypo-lipidaemic effect. Super bitters act on both the pancreas and liver/gall bladder, helping to promote the production and release of the pancreatic enzyme lipase and bile, which ensure good digestion of fats and oils and proper functioning of the excretory functions of the liver thereby conferring on it hypolipidaemic properties. It acts as a liver tonic, being hepatoprotective and enhancing its functions. A healthy flow of bile helps rid the liver of waste products, prevents the formation of gallstones, and emulsifies lipids, which the pancreatic enzymes then breakdown along with proteins and carbohydrates for absorption in the small intestine. The results of this research on the serum lipid profile give positive evidence that the Super 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 disease [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) [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 Super bitters-fed rats compared to that of the control rats of this study is indicative that Super bitters did not cause any hepatocellular damage to the liver of the rats [41].

The minimal and non-significant differences (P>0.05) in the ALP, total bilirubin and conjugated bilirubin levels in the super bitters-fed rats compared to that of the control of this study is indicative that super 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 [41].

The minimal and non-significant differences (P>0.05) in the serum albumin and total protein levels in the super bitters-fed rats compared to that of the control of this study is indicative that super bitters did not cause any dysfunction in the synthetic function of
the liver [41]. 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 [41]. The minimal increase seen in the level of Gamma-glutamyl transpeptidase in plasma of all the super 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 [41].

The result of this study indicates that in some of the parameters used to assess the kidney function status of the control and super 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 super bitters did not interfere with the renal capacity to excrete these metabolites. The lack of significant difference between the metabolites of the control and super bitters fed groups used in assessing the kidney function status may also be a reflection of the preserved renal integrity of the treated rats [8]. Hence, super 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 Super 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 (creatine kinase, LDH etc), from the results of this study, there was no significant increase (P>0.05) in either creatine kinase nor LDH. Thus the minimal and non- significant differences (P>0.05) in the creatine kinase and LDH levels in the super 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 super bitters did not cause any cardio-cellular damage to the heart of the rats [41].

Oxidative Stress represents an imbalance in production and clearance of reactive oxygen species/free radicals in biological systems [42]. 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 [43]. Reactive oxygen species may be beneficial as they are used by the immune system as a way to attract and kill pathogens [43]. Excessive oxidative stress particularly at unwanted places (e.g. vascular lining, blood brain barrier) will damage the defence system. The results of this study indicate that the MDA levels in the Super 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 super 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. This aligns with the known fact that antioxidant constituents can delay or inhibit the oxidation of lipids and other compounds by inhibiting the propagation of oxidation chain reaction [44].

The results of this study indicate that the Vitamin C and Vitamin E levels of Super bitters-fed rats were increased though not significantly (P>0.05) when compared to the control. This can be said to be as a result of the Super bitters 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 indigenous antioxidants [45; 33]. Herbaceous plants and species like those used as constituents of bitters are harmless sources for obtaining natural antioxidants. Vitamins are important and their deficiencies cause adverse effects on the metabolism of the human body and even in a trace amount, they are very essential for the body metabolism [33].

Generally the Catalase activity and Glutathione Peroxidase activity of the rats fed with Super 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 super 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 these 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 [45; 33].
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 the Super bitters-fed rats did not reveal adverse differences when compared to those of control organs.

Conclusion

The results of this study showed that Super bitters could be safe for consumption, as the acute toxicity (LD$_{50}$) of Super bitters, indicated a relatively high LD$_{50}$ as a result they have low-lethality at doses likely consumed. The biochemical and haematological assay results of this study suggest that Super bitters may have the following pharmacological properties: hypolipidaemic, hypoglycaemic and immunity-boosting/immuno-modulatory, choleretic/hepatoprotective and \textit{in vivo} and \textit{in vitro} antioxidant capacity and by extension antitumour as well as diuretic/vasodilatory properties and the ability to protect against/prevent diseases of the cardiovascular system.

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