ANALYSES OF THE EFFECTS OF SWEDISH BITTERS ON SELECTED METABOLIC PROFILES

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ABSTRACT

Swedish Bitters® is a traditional herbal tonic that has detoxification potential and has been claimed to contribute to weight loss and normal functioning of body organs. In this study Swedish Bitters was analyzed for its phytochemical, proximate and antioxidant constituents as well as a measurement of the effects of daily administration of 3.5 ml of the extract to rats for three weeks on tissues and organs of rats *in vivo*. The presence of Phenol and Tannins at levels of 418.61 and 817.27 µg GAE / mg respectively were obtained. The antioxidant assay of the extract showed that it had its highest level of DPPH radical scavenging activity (22.6 %) at 100 % and total reducing antioxidant power was highest (0.87) at 25 %. The proximate analysis of the extract showed that the moisture content was the highest at 80 %. There was a noted reduction in blood cholesterol levels and an increase in the level of triglycerides relative to the control. The antioxidant enzyme assays which were carried out on the sera of the rats showed that there was a reduction in the levels of SOD and CAT in the test group compared to the control. There was also a slight decrease in the level of GSH in the test group compared to the control, while the level of MDA in the test group increased when compared with the control which suggests increased levels of lipid peroxidation. However these results were not statistically significant. The results obtained from the liver function tests did not suggest any form of damage caused by the administration. There was also no statistical difference in the blood glucose levels of the treated rats relative to the control. From the study, it can be concluded that short-term administration of Swedish bitters had no marked end-organ damage, but rather helped reduce the level of cholesterol *in vivo*.

Keywords: Phytochemicals, Antioxidants, Liver function test, Lipid peroxidation, Cholesterol.

INTRODUCTION

Bitters are a diverse group of chemical compounds that share the common characteristic of a bitter taste or bittersweet taste and have been flavored with herbal essences. Bitters can be used to strengthen and improve the whole digestive system in the body as well as the nervous system. Bitters also act to increase the vital energy centers in the body. Because they have such a broad effect on the entire physiology, tone, and function of the body bitters are principles that can be used to treat the body as a whole. There are numerous brands of bitters that were formerly marketed as patent medicines but are now considered to be digest ifs (drinks which are served at the end of meals to aid digestion, although modern medicine discredits this supposed aid in digestion), rather than medicines. They can also serve as flavoring in cocktails. Bitters are prepared by infusion or distillation, using aromatic herbs, bark, roots and / or fruit for their flavor and medicinal potentials. Some of the typical contents of bitters formulas include; angelica root, artichoke leaf, bitter orange peel, blessed thistle leaves, gentian root, goldenseal rhizome, wormwood leaves and yarrow flowers¹. All over the world, some people are constantly taking one herbal preparation or the other to alleviate digestion difficulties. Swedish Bitters is one of such preparations taken by many. Swedish Bitters is reportedly composed of Aloe, Myrrh, Saffron, Senna Leaf, Camphor, Rhubarb Root, Zedoary, Manna, Theriaca Venezian, Carline Root and Angelica Root in definite concentrations, all of which, aid respiratory health, normal digestion, joint mobility, healthy bladder and helps the skin eliminate toxins. It therefore has become necessary to investigate the potentials of Swedish bitters in the management of conditions like diabetes and obesity by performing chemical and antioxidant studies on the Swedish bitters extract in order to study its effects on the body organs and tissues. This is because the ingredients used in preparing the bitters are plant-based and have been shown to have some medicinal use. Also, there are claims that Swedish bitters aid

digestion, detoxify the body and may be marketed as a weight loss aid due to the claims about being a detoxifier. Due to the fact that Swedish bitters is quite common in most countries and easily affordable, any scientific evidence that suggests that it would help in the management of diabetes and obesity will go a long way in saving millions of lives and improve the financial standings of individuals and nations worldwide as they get to save money on these expensive health disorders. Also, it will help to improve the importance of bitters globally so that more emphasis could be placed on its research thereby creating an avenue for obtaining a greater insight on the mechanism of action of these herbal tonics². The objectives of this study were therefore designed to understand the changes that would occur in the body of albino rats after three weeks of administration of Swedish bitters and to document the proximate and phytochemical constituents in this herbal tonic.

MATERIALS AND METHODS

All equipment and chemicals used were of analytical grade and obtained from reputable companies, Sigma and Merck, without further purification.

Drug Administration

Swedish Bitters^R, which was purchased at Health plus Pharmacy in Ikeja, Lagos, Nigeria. In this study, fourteen female albino rats were allowed to acclimatize for a period of two weeks during which they were fed *ad-libitum* with standard rodents feed (rat chow) and tap water. Then, the rats were equally divided (7 rats / group) into two groups; the test and the control group. The weights of the rats in the test group were measured and used to calculate the dosage of Swedish bitters to be administered to each rat throughout the three weeks of treatment. Administration was done via the oral route with the aid of an oral cannula. The dosage of Swedish bitters for an adult human being (70 kg) is 5 ml of Swedish bitters in 1 cup (100 ml) of water. From this stock

solution, the volume that would be given to a rat weighing X kg is; $(X \text{ kg} \times 100 \text{ ml}) \div 5 \text{ ml}$. (In this study, the rats in the test group weighed 175 g, hence 3.5 ml of the diluted Swedish bitters was administered to each rat). During the administration phase, freshly prepared Swedish bitters extract was administered daily to the rats in the test group *ad libitum* with their chow and tap water, while the rats in the control group were fed with chow and tap water only. After three weeks of administration, blood was collected from the rats into labeled lithium-heparin, fluoride and EDTA bottles via retro-orbital sinus technique. The rats were then sacrificed by cervical dislocation and their livers were collected into plain bottles and placed in ice. The plasma and livers were then analyzed for various parameters.

Phytochemical Screening

Chemical tests were carried out on Swedish bitters using standard procedures to identify the constituents as described previously^{1,3}. A diluted solution was made by mixing 2 ml of sample with 20 ml of distilled water.

Test for Tannins

0.5 ml of the diluted solution of the extract was put in a test tube and few drops of 0.1 % ferric chloride were added and observed for brownish green or a blue-black coloration which is an evidence of tannin presence.

Test for Phlobatannins

0.5 ml of diluted solution of the sample was mixed with few drops of 1 % aqueous hydrochloric acid and boiled. Deposition of a red precipitate confirms presence of phlobatannins.

Test for Flavonoids

Few drops of 1 % aluminum solution were added to 0.5 ml of diluted solution of the extract. A yellow coloration indicates the presence of flavonoids.

Test for Cardiac Glycosides (Keller-Killani test)

5 ml of the sample was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout a thin layer.

Test for Steroids

2 ml of acetic anhydride was added to 0.5 ml of diluted solution of the sample with 2 ml H_2SO_4 . A colour change from violet to blue or green indicates the presence of steroids.

Test for Saponin

1 ml of the prepared extract was mixed with 2.5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for Phenol

2 ml of the extract was mixed with 2 ml of ferric chloride. The formation of a dark blue coloration is indicative of phenol.

Quantitative Determination of the Phytochemical Constituency

0.2 g of the sample was weighed and dissolved in 100 ml of water. Various volumes were taken from this extract for the analysis as follows:

Tannin Determination

0.1 ml of the prepared solution of the sample was measured into a boiling tube and mixed with; 1.6 ml of distilled water, 1 ml of 0.1M FeCl_3 in 0.1N HCl and 1 ml 0.008 M potassium ferrocyanide. The absorbance was measured at 720 nm.⁴

Determination of the Total Phenolic Content

1 ml of the prepared extract above was added to 1.5 ml of 20 % sodium carbonate, 0.5 ml Folin-Ciocalteu reagent, and diluted with 10 ml of distilled water. The mixture was allowed to stand for two (2) hours before the absorbance was read at 765 nm.⁵

Antioxidant Assay of Swedish Bitters

0.2 g of the tonic was weighed and diluted with 100 ml of water. From this solution, 20 ml solution with concentrations of; 100, 75, 50 and 25 were obtained and labeled appropriately.

Reducing Power Capacity

1 ml of the solution prepared above was mixed with 2.5 ml of phosphate buffer and 2.5 ml of potassium ferrocyanide and incubated at 50°C for 30 minutes. Since no precipitate was formed, 2.5 ml of the mixture was added 0.5 ml of freshly prepared 0.1 % ferric chloride. The absorbance was measured at 700 nm.

DPPH Radical Scavenging Assay

The hydrogen donating activity of GOEE was examined in the presence of DPPH stable radical⁶. 1 ml of each fraction of the solution made above was added 3 ml of methanol, 2 ml of DPPH and placed in the dark for 30 minutes after which the absorbance was read at 514 nm. Mixture of 4 ml of methanol and 2 ml of DPPH was used as negative control and Ascorbic acid as the positive control. 2 ml of methanol was used as blank.

Proximate Analysis of Swedish Bitters

Ash Content Determination

Ash is the mineral component of a sample determined by burning the sample in a muffle furnace at 600°C for 2 h. A clean dry porcelain dish was weighed on an analytical balance. 2 g of sample was weighed into the dishes, it was dried in an oven at 100°C for 3 h and after that was removed from the oven. The dish was heated over a Bunsen flame to initiate the destruction of carbon. It was heated gently until the content turned black and then the dish was placed including the content into a muffle furnace. It was heated at 560°C for 7 h until greyish white residue was obtained to indicate the destruction of carbonaceous portion of the sample. The ash obtained was moistened with some drops of water to expose any un-ashed carbon still present. Again, it was dried in the oven for 3 h, re-ashed at 560°C in a muffle furnace for 45 minutes, was removed and cooled; it was placed in a desiccator to cool completely and for traces of moisture present to be absorbed by a suitable desiccant. The dish was weighed with the content and the level of ash was determined.

Moisture Content Determination

The principle of the air oven method determination of moisture content is that a sample subjected to a temperature of 100°C for 17 h or 105°C for 14 h in the oven becomes dried and the loss in weight of the sample after drying is taken as the moisture content. The weight of the moisture dish with its lid was taken for each sample. 2 g of sample (wet weight) was weighed into each dish respectively. The sample was dried in the dish without the lid in an oven maintained at 100°C for 17 h. After drying, the sample was covered with the lid and removed from the oven. The dish was placed in a desiccator containing silica gel as the moisture absorbing material (desiccant). The sample was cooled in the desiccator to room temperature and weighed. The process of drying, cooling and weighing continued until a constant weight was obtained.

Lipid Content Determination

The lipid content was determined using previously described methods⁷. 1 g of the extract was weighed and mixed with 10 ml of chloroform methanol 2:1. The resultant solution was subjected to centrifugation, when generally 3 layers are seen; a clear lower layer of chloroform containing all the lipids, a coloured aqueous layer of methanol and a thick pasty interface was seen. The methanol layer was discarded and the lower layer was carefully collected free of interface by filtration. This was put into a separating funnel and 5 ml of sodium chloride was added to the solution, subsequently forming two layers. The lower layer was decanted into an evaporating dish of known weight and placed on an electric burner.

Protein Content Determination

Crude protein is determined by the Kjeldahl or Dumas combustion technique which determines the amino nitrogen of a sample and also multiplying the % nitrogen by a protein conversion factor which is usually 6.25. The principle involves the use of concentrated tetraoxosulphate (VI) acid (H_2SO_4) in the presence of a suitable catalyst to digest the sample and the nitrogen from the protein and other constituents of the sample is converted to ammonium tetraoxosulphate (VI) (NH_4)₂SO₄. The latter is rendered alkaline and its NH_3 which is distilled off is trapped into a standard boric acid solution and the distillate is titrated against a standard acid. The percentage nitrogen calculated from a titre value is converted to food protein by multiplying with a protein conversion factor depending on the food product being analyzed.

Crude Fibre Determination

Crude fibre is that portion of the moisture and fat free sample that remains after digestion with weak acid and base. It is of low digestibility and composed of cellulose, hemi-cellulose and some lignins. The defatted sample is treated with boiling H_2SO_4 and later with boiling NaOH, and the residue left after subtraction of the ash is taken as the fibre.

Carbohydrate Content determination

The sum of all the above proximate determinations was subtracted from a 100 % to obtain the proximate carbohydrate composition of the sample.

Haematological Assay

A complete blood count was carried out on the blood of the sacrificed rats using an Automated Analyzer. The levels of;

WBC (white blood cell), RBC (red blood cell), HB (haemoglobin), HCT (hematocrit), MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), MCHC (mean corpuscular haemoglobin concentration), Neutrophils and Lymphocytes. Also, the level of total bilirubin was analysed and a lipid profile was done to determine the levels of: HDL (High density lipoproteins), LDL (Low density lipoproteins), Total Cholesterol and Triglycerides.

Liver Function Test

Liver function test was carried out on livers of the rats to determine the levels of; AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), Creatinine, Albumin, Alkaline Phosphatase, Total protein, Serum Glucose and Urea³.

Antioxidant Enzymes Assay

The following antioxidant enzymes activities were determined spectrometrically as follows:

Determination of Superoxide Dismutase (SOD) Activity

Superoxide Dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480 nm as described⁸. The reaction mixture (3 ml) contained 2.95 ml 0.05 M sodium carbonate buffer pH 10.2, 0.02 ml of liver homogenate and 0.03 ml of epinephrine in 0.005 N HCL was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate (epinephrine) and 0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 minutes. $\Sigma = 4020\text{M}^{-1}\text{cm}^{-1}$

Determination of Catalase Activity

This was determined adopting previously described methods⁹. Hydrogen peroxide was prepared with phosphate buffer; 0.2 ml of sample was added to 1.8 ml of 30 mM of hydrogen peroxide (H_2O_2) substrate in a 2 ml curvette. The phosphate buffers were used as a blank. The absorbance for the test sample, blank and standard was read against a blank at 240 nm at 30s interval for 1 minute. The enzyme activity was calculated using the molar extinction coefficient of 40.00 $\text{M}^{-1}\text{cm}^{-1}$ expressed as unit / mg protein.

Determination of Reduced Glutathione

The reduced glutathione (GSH) content of liver tissue as non-protein sulphhydryls was estimated¹⁰. To the homogenate 10 % TCA was added, centrifuged. 1.0 ml of supernatant was treated with 0.5 ml of Ellmans reagent (19.8 mg of 5, 5-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1 % sodium nitrate) and 3.0 ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412 nm. $\Sigma = 1.34 \times 10^4 \text{M}^{-1}\text{cm}^{-1}$

Determination of Lipid Peroxidation

Malondialdehyde (MDA) an index of lipid peroxidation was determined using previously described methods¹¹. 1.0 ml of the supernatant was added to 2 ml of (1:1:1 ratio) TCA-TBA-HCl reagent (thiobarbituric acid 0.37 %, 0.24 N HCl and 15 % TCA) tricarboxylic acid- thiobarbituric acid-hydrochloric acid reagent boiled at 100°C for 15 minutes and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 minutes. The supernatant was removed and the absorbance read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA- complex of $1.56 \times 10^5 \text{M}^{-1}\text{cm}^{-1}$.

Determination of Total Protein

This was determined using Biuret method¹². 5.0 ml of blank Biuret reagent prepared by dissolving $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ crystal in 500 ml of distilled water was added to sample blank. These were mixed well and allowed to stand for 20 minutes at room

temperature 25 - 27°C. Absorbance was read for one test and standard against a blank at 540 nm. The concentration of protein was calculated using: optical density for standard $\times$ concentration of standard.

Table 1: Phytochemical Analysis of Swedish Bitters

Phytochemical Test	Result
Tannin	+
Phlobatanin	-
Saponins	-
Flavonoids	-
Cardiac glycosides	-
Alkaloids	-
Phenol	+

+ stands for present; - stands for absent

Figure 1: Quantitative Estimation of the level of Phytochemicals in Swedish Bitters

Figure 2A: Total Reducing Antioxidant Power of Swedish Bitters

The graph shows that the Total reducing antioxidant power of Swedish bitters was highest at 25 %, Followed by 75 %, then 100 %, with the lowest being at 50 %.

Figure 2B: DPPH Radical Scavenging Activity of Swedish bitters

From the graph, the DPPH free radical scavenging activity of Swedish bitters increased with increasing concentration.

Figure 3: Proximate analysis of Swedish bitters

Figure 4A: Result for Haematological Analysis of the Treated Rats Compared to the Control

Figure 4B: Level of PLT in the test group compared to the control

From the graph(s), there were reductions in the values of; WBC, LYMP %, NEUT ABS and PLT while there was an increase in; RBC, HB, HCT, MCV, MCH, MCHC and NEUT %, in the test group relative to the control.

Figure 5: Liver Function Test of Treated Rats Compared to the Control

From the graph, there were reductions in the values of; AST (Aspartate transaminase), ALT (Alanine transaminase), Creatinine, Urea, Albumin and ALP (Alkaline phosphatase), while there was an increase in the value of T.PRO, in the test group relative to the control.

Figure 6: Blood Glucose Level in Treated Rats Compared to the Control

The graph shows that there was a slight reduction in the blood glucose level of the test group relative to the control.

Figure 7: Result for Determination of the Lipid Profile of the Treated Rats Compared to the Control

From the graph, the level of; HDL, LDL, Total Cholesterol and Total Bilirubin in the test group reduced when compared with the control while the level of Triglycerides in the test group increased relative to the control.

Figure 8A: Level of Antioxidant Enzymes (GSH, SOD and Catalase) in the Treated Rats Compared to the Control

Figure 8B: Level of MDA in the Treated Rats Compared to the Control

From the graph(s), the levels of; GSH (Reduced glutathione), SOD (Superoxide dismutase) and Catalase, in the test group reduced relative to the control while the level of MDA (Malondialdehyde) in the test group was higher when compared with the control.

RESULTS

Qualitative Phytochemical Screening of Swedish Bitters

The extract was analyzed for its phytochemical constituents and the presence of tannins and phenols was detected as shown in Table 1.

Quantitative Estimation of the Phytochemical Constituents

The quantity of the identified phytochemicals was determined with phenol and tannins at levels of 418.61 and 817.27 μg GAE / mg respectively (Figure 1).

Antioxidant Assay of Swedish Bitters

The extract was analysed to determine its Total Reducing Antioxidant power (TRAP) and the DPPH radical scavenging activity and the results are presented in the Figure 2A and B.

Proximate Analysis of Swedish Bitters

The result of the proximate analysis of the extract is shown in Figure 3. The result of the proximate analysis of the extract shows that its moisture content is the highest which is as a result of its liquid form.

Haematological Analysis

The blood samples of the treated rats were analyzed and compared to the control and results were obtained as shown in Figure 4A and B.

Liver Function Assay

The livers of the rats in the test and control groups were analysed to ascertain the condition they were in and results obtained are shown in Figure 5.

Blood Glucose Levels

The level of glucose in the blood of the rats in the two groups was analysed and the results obtained is shown in Figure 6.

Lipid Profile

The lipid profile of the rats (test and control) was measured and the results are shown in Figure 7.

Antioxidant Enzyme Assay

The blood samples of the rats in the test and control groups were analyzed for the following antioxidant parameters; SOD, CAT, GSH and MDA. The results showed that the levels of SOD CAT and GSH were lower compared to the control whereas the level of MDA in the test group was higher compared to the control. This is shown in Figures 8A and B.

DISCUSSION

The phytochemical analysis of Swedish bitters confirmed the presence of phenol and tannins in relatively large concentrations of 418.61 and 817.27 μg GAE / mg, respectively. The antioxidant analysis of Swedish bitters revealed that it has a higher total reducing antioxidant power compared to the standard garlic acid used, with the highest level occurring at 25 %. Also, the DPPH scavenging activity of the extract increased with increasing concentrations. This places the tonic in a high class of antioxidants, with its potency much higher than most reported herbal tonics with similar pharmacodynamics¹³. The result for the lipid profile determination carried out on the blood samples of the rats in the test and control rats showed that there was a marked reduction in the levels of LDL and total cholesterol in the test group compared to the control. However, the reduction was not so significant for HDL levels. The reduction in the level of cholesterol in the test group suggests that Swedish bitters might help prevent Hypercholesterolemia (high cholesterol levels)¹⁴, even though there was an increase in the level of triglycerides in the test group compared to the control. The results of the liver function assay conducted showed that; there was an increase in the level of Total protein in the test

group relative to the control. Also, there was a reduction in the levels of AST, ALT, Creatinine, Albumin and ALP in the test group relative to the control. These results seem to suggest that the administration of Swedish bitters to the rats did not affect the optimal functioning of their liver. The antioxidant enzyme assays which were carried out on the sera of the rats showed that there was a marked decrease in the levels of SOD and CAT in the test group compared to the control. There was also a slight decrease in the level of GSH in the test group compared to the control. In addition, the level of MDA in the test group increased when compared with the control. These results suggest that the activities of the antioxidant were negatively affected by the tonic which led to an increase in the rate of lipid peroxidation reflected by the higher MDA level. There was a slight reduction in the glucose level of the treated rats relative to the control. The explanation for this slight reduction, instead of a marked one, could probably be due to the short duration of the study. Also this herbal tonic could be said to possess detoxification potentials as the results of this study detected the presence of phytochemicals and antioxidants in the tonic, although the tonic might have some defence-lowering effects on the body as shown by a decrease in the levels of antioxidant enzyme and an increase in lipid peroxidation in the treated rats.

CONCLUSION

From the study, it can be concluded that short-term administration of the richly antioxidant Swedish bitters had no marked end-organ damage, but rather helped reduce the level of cholesterol. There is also the possibility that in long-term administration, Swedish bitters can help regulate blood glucose level and the increased triglyceride levels and mildly distorted antioxidant status of the rats observed in this study. Further chronic and sub-chronic research studies are on-going in our laboratories.

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