Research Paper

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Effect of Bitters on the Body Weight, Lipid Profile, Catalase and Lipid Peroxidation in Experimental Animals

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This study was designed to investigate the potential effect of bitters on body weight, lipid profile (total cholesterol, triglyceride, high density lipoprotein (HDL)-cholesterol and low density lipoprotein (LDL)-cholesterol), catalase and lipid peroxidation in the plasma and heart tissue of Albino rats. Three brands of bitters (Yoyo, Swedish and Living bitters) were administered daily for thirty-two days through oral rout to age matched twenty eight Albino rats of both sexes. The rats were grouped into four with seven rats per group. The control group was administered normal saline, the treatment groups were administered with Yoyo, Swedish and Living bitters respectively (15 mL kg^{-1}). The animals were sacrificed after thirty-two days. There was a significant ($p<0.05$) decrease in body weight, a reduction in cardiac total cholesterol, lipid peroxidation, triglyceride and LDL-cholesterol levels and an increase in catalase activity. There was also a significant reduction in plasma total cholesterol and triglyceride levels but significant increase in plasma catalase activity. Hence, bitters could be recommended to be taken as digestive, as it may help to reduced the body weight, cardiac total cholesterol, triglyceride, LDL-cholesterol levels, lipid peroxidation level and increase catalase activity.

Key words: Bitters, plasma, cholesterol, triglyceride, catalase

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INTRODUCTION

Bitters are prescription and natural remedies that are commonly used in developing countries as a cure for indigestion and other stomach ailments and for treatment of various diseases. Its use has been on the increase recently without investigations/examinations of the possible toxic effect or metabolic alteration. Studies or reports over the last two and a half decades have constantly demonstrate increasing use of herbal remedies in both developing countries and in developed countries where modern medicines are predominantly used medicine as the most common form of alternative medicine and is used by about 60-80% of the world (Bussmann *et al*., 2010). Bitters include but are not limited to: Gentian root (*Gentiana* spp.) (Jaeschke *et al*., 2010), Aloe (*Aloe vera* syn. *A. arbadensis*) (Reynolds and Dweck, 1999), Wormwood (*Artemisia absinthium*) (Kharoubi *et al*., 2008) from which absinthe was made, Dandelion root (*Taraxacum officinale*) (Choi *et al*., 2010), Angelica root (*Angelica archangelica*) (Sarker and Nahar, 2004), Senna leaves (*Cassia senna*) (El-Kamali and El-Amir, 2010), Zedoary root (*Curcuma zedoaria*) (Krishnamoorthy *et al*., 2009), Myrhh (*Commiphora molmol*) (Tariq *et al*., 1985), Cinchona bark (*Cinchona* spp.) (Schulz and Albroscheit, 1988), Turmeric (*Curcuma longa* syn. *C. domestica*) (Kim and Kim, 2010), Shitetta (*Swertia chirata* syn. *Ophelia chirata*) (Verma *et al*., 2008) and Saffron (*Crocus sativa*) (Motamedi *et al*., 2010). Bitters generally have been reported to prevent kidney and bladder infections, help to regulate blood pressure and dilate arteries, facilitate digestion, prevent disorder like ulcers, gastritis, insomnia, stress and depression and prevent overweight and excess body fat (Ogbonnia *et al*., 2010). The medicinal use of the extract from these plants are well documented but there exist little or no information as regard the effect on lipid profile (total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol), catalase and lipid peroxidation (thiobarbituric acid reactive substances (TBARS)) of the heart by the extract these plants when used in combination (Krishnamoorthy *et al*., 2009). The herbs are used in combination creates the desired synergistic effect (Krishnamoorthy *et al*., 2009). The aim of this study was to investigate the potential effect of bitters on the lipid profile (total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol), catalase and lipid peroxidation (TBARS) in the plasma and heart tissue of Albino rats.

MATERIALS AND METHODS

Food supplements: Yoyo bitters were obtained from Ablatt Pharmaceuticals Limited, Nigeria, Swedish Bitters was purchased from Swedish Bitters Herb Company, USA and Living bitters from African Angel, USA.

Reagents and chemicals: Bovine serum albumin, sodium hydroxide, copper sulphate, sodium potassium tartarate, potassium iodide, sodium dihydrogen phosphate, disodium hydrogen phosphate and sodium chloride were products of Aldric Chemicals, UK. All other chemicals are of analytical grade.

Animal: Age matched 28 Albino rats of both sexes which were purchased from the Physiology Department of the University of Ibadan, Ibadan, Oyo State, Nigeria. The rats were randomly grouped into four (YB-animal fed with Yoyo Bitters, SB-Swedish Bitters, LB-Living Bitters and CT as control) and kept in wooden cages, which were well ventilated. The animal were allowed to acclimatize to their new environment for two weeks and fed with normal rat chow (Guinea Feed Ltd, Nigeria) and water *ad libitum*. After two weeks of acclimatization, the four groups of animals were administered, Yoyo Bitters (YB), Swedish Bitters (SB), Living Bitters (LB) (15 mL kg^{-1} b.wt.) and normal saline (CT) through oral rout once daily, in addition to the normal rat chow and water for 32 days (about 5 weeks).

Body weight: At least twice every week the body weight of each animal was measured.

Sample collection: After 32 days, the rats were sacrificed by cervical decapitation under the influence of diethyl ether anaesthesia. Blood samples were collected from each animal by cardiac puncture with sterile needle and transferred to heparinised tubes, centrifuged at 5,000 rpm for 10 min and the supernatant and plasma, was separated and stored at a temperature of -4°C until it is required for assaying. The heart was dissected out, washed in ice-cold saline, dried and weighed. The heart was then homogenized in normal saline solution in the ratio 1:4 (1 mg of heart tissue to 4 mL of normal saline solution) and the homogenate was centrifuge at 4000 rpm for 5 min while the supernatant was separated and stored at a temperature of -4°C until it is required for assaying.

Biochemical analysis: Triglyceride level was determined by the method of Mochin and Leyva (1984), total cholesterol and HDL-cholesterol levels were determined by the method of De Hoff *et al*. (1978) and LDL-cholesterol level was calculated by the method of Nauck *et al*. (2000). Catalase activity was determined by the method of Johansson and Borg (1988) and TBARS were determined by the method of Buege and Aust (1978).

Statistical analysis: All the results were expressed as Mean±Standard Error of Mean (SEM) for animal in each group. All the grouped data were statistically evaluated using SPSS 15.0 software. Hypothesis testing methods included One-way Analysis of Variance (ANOVA) and subsequent comparisons among groups were made using Duncan's Multiple Range Test (DMRT). Statistical significance was set at $p<0.05$.

RESULTS

Effect of bitters on body weight: There was a significant decrease in body weight of the animal fed with Yoyo bitters whereas a significant reduction was observed in animal fed with Swedish and Living bitters from 15 days as shown in Fig. 1.

Effect of bitters on lipid profile: The lipid profile result (Table 1, 2) shows a significant reduction in the total cholesterol level of the plasma and heart tissue, respectively in all the animal fed with Yoyo bitters (162.78±2.19, 653.97±56.07), Swedish bitters (161.52±2.52, 703.50±35.98) and Living bitter (164.05±2.52, 668.54±29.80) when compared with the control (181.71±5.55, 1123.78±212.66) at $p<0.05$; a significant reduction in the triglyceride level of the heart tissue in animal fed with Yoyo bitters (1519.77±111.59), Swedish bitters (1999.34±43.51) and Living bitters (1964.09±116.67) when compared with the control (3219.67±583.12) at $p<0.05$ whereas no significant difference was observed in the plasma triglyceride level in the animal fed with Yoyo bitters (89.64±9.78), Swedish bitters (88.55±5.18) and Living bitter (89.66±3.79) when compared with the control (88.17±0.76) at $p<0.05$; a significant elevation in LDL-cholesterol level of the plasma and heart tissue, respectively in the animal fed with Yoyo bitters (39.32±2.22, 181.44±24.91), Swedish bitters (39.18±2.29, 135.72±25.57) and Living bitter (33.71±6.19, 114.10±25.35) when compared with the control (64.76±10.77, 282.99±54.82) at $p<0.05$; but no significant difference in HDL-cholesterol level of the plasma and heart tissue respectively in the animal fed with Yoyo bitters (105.53±2.44, 168.37±34.91), Swedish bitters (104.63±1.77, 167.91±35.09) and Living bitter (112.40±5.98, 162.26±37.46) when compared with the control (99.31±10.11, 196.86±23.74) at $p<0.05$.

Effect of bitters on catalase and lipid peroxidation: The catalase activity and lipid peroxidation results (Table 3, 4) also showed a significance increase in catalase activity of the plasma and heart tissue respectively in animal fed with Yoyo bitters (8.50±0.03, 8.48±0.11), Swedish bitters

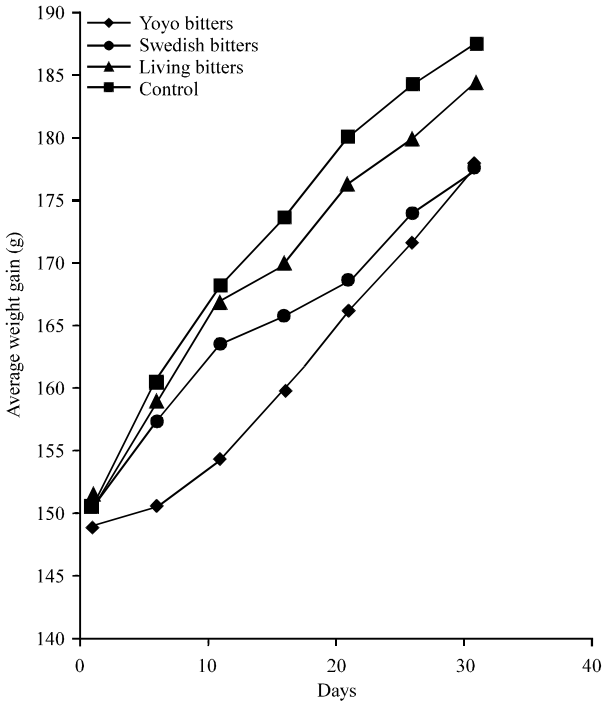


Fig. 1: Effect of Yoyo, Swedish and Living bitters on body weight of experimental animal when compared with control

Table 1: Effect of bitters on plasma lipid profile

Bitters	Total cholesterol	Triglyceride	LDL-cholesterol	HDL-cholesterol
Yoyo bitters	162.78±2.19^a	89.64±9.78^a	39.32±2.22^a	105.53±2.44^a
Swedish bitters	161.52±2.52^a	88.55±5.18^a	39.18±2.29^a	104.63±1.77^a
Living bitters	164.05±2.52^a	89.66±3.79^a	33.71±6.19^a	112.40±5.98^a
Control	181.71±5.55^b	88.17±0.76^a	64.76±10.77^b	99.31±10.11^a

All value are expressed as Mean±SEM. Values with different superscript indicate significant difference at $p<0.05$

Table 2: Effect of bitters on cardiac lipid profile

Bitters	Total cholesterol	Triglyceride	LDL-cholesterol	HDL-cholesterol
Yoyo bitters	653.97±56.07^a	1519.77±111.59^a	181.44±24.91^a	168.37±34.91^a
Swedish bitters	703.50±35.98^a	1999.34±43.51^a	135.72±25.57^a	167.91±35.09^a
Living bitters	668.54±29.80^a	1964.09±116.67^a	114.10±25.35^a	162.26±37.46^a
Control	1123.78±212.66^b	3219.67±583.12^b	282.99±54.82^b	196.86±23.74^a

All value are expressed as Mean±SEM. Values with different superscript indicate significant difference at $p<0.05$

Table 3: Effect of bitters on plasma catalase activity and lipid peroxidation

Bitters	Catalase	TBARS
Yoyo bitters	8.50±0.03^b	12019.23±3582.25^a
Swedish bitters	8.34±0.06^b	15000.00±1453.17^a
Living bitters	8.60±0.05^b	8226.49±1830.29^a
Control	8.07±0.03^a	25213.68±2230.83^b

All value are expressed as Mean±SEM. Values with different superscript indicate significant difference at $p<0.05$

Table 4: Effect of bitters on cardiac catalase activity and lipid peroxidation

Bitters	Catalase	TBARS×10^{-6}
Yoyo Bitters	8.48±0.11^{b}	4.33±0.80^{a}
Swedish Bitters	8.63±0.06^{b}	4.74±0.83^{a}
Living Bitters	8.51±0.10^{b}	4.17±0.64^{a}
Control	8.07±0.13^{a}	3.63±0.94^{a}

All value are expressed as Mean±SEM. Values with different superscript indicate significant difference at $p<0.05$

(8.34±0.06, 8.63±0.06) and Living bitter (8.60±0.05, 8.51±0.10) when compared with the control (8.07±0.03, 8.07±0.13) at $p<0.05$ but there was a decrease in TBARS level of the plasma and heart tissue, respectively in the animal fed with Yoyo bitters (12019.23±3582.25, 4.33±0.80), Swedish bitters (15000.00±1453.17, 4.74±0.83) and Living bitter (8226.49±1830.29, 4.17±0.64) when compared with the control (25213.68±2230.83, 3.63±0.94) at $p<0.05$.

DISCUSSION

The present study was designed to investigate the effect of bitters on the body weight, lipid profile (total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol), catalase and lipid peroxidation (TBARS) in the plasma and heart tissue of Albino rats.

The study showed that all experimental animals exerted a significant decrease in body weight in the experimental animal fed with bitters (Yoyo, Swedish and Living bitters) when compared with control. Intake of bitters was also shown to lower total cholesterol and LDL-cholesterol levels in the plasma and heart tissue; and triglyceride in the heart tissue. Lowering total cholesterol, triglyceride and LDL-cholesterol levels of the plasma and heart tissue reduces the risk of hypercholesterolemia and hyperlipidemia that may precipitate into coronary atherosclerosis and other related cardiovascular diseases (Witztum and Steinberg, 2001). This could be as a result of the fact that bitter helps in oxidation of total cholesterol and triglyceride plus reduction in LDL-cholesterol level. The study also showed an increase in catalase activity and a decrease in the level of lipid peroxidation products (TBARS) in both the plasma and the heart tissue. Oxidative stress is one of the causative factors that link hypercholesterolemia with atherogenesis. Malondialdehyde (MDA), the product of lipid peroxidation, is an index of the level of oxygen free radicals. The increased catalase activity help to scavenge oxygen free radicals (Jaeschke *et al*., 2010).

CONCLUSION

In conclusion, the present results show that moderate consumption of bitters may lower body weight, total cholesterol, LDL-cholesterol and lipid peroxidation and increase catalase activity.

REFERENCES

Buege, J.A. and S.D. Aust, 1978. Microsomal lipid peroxidation. Meth. Enzymol., 52: 302-310.

Bussmann, R.W., A. Glenn, K. Meyer, A. Kuhlman and A. Townesmith, 2010. Herbal mixtures in traditional medicine in Northern Peru. J. Ethnobiol. Ethnomed, Vol. 6 10.1186/1746-4269-6-10

Choi, U.K., O.H. Lee, J.H. Yim, C.W. Cho, Y.K. Rhee, S.I. Lim and Y.C. Kim, 2010. Hypolipidemic and antioxidant effects of dandelion (*Taraxacum officinale*) Root and Leaf on Cholesterol-Fed Rabbits. Int. J. Mol. Sci., 11: 67-78.

De Hoff, J.L., L.M. Davidson and D. Kritchevsky, 1978. An enzymatic assay for determining free and total cholesterol in tissue. Clin. Chem., 24: 433-435.

El-Kamali, H.H. and M.Y. El-Amir, 2010. Antibacterial activity and phytochemical screening of ethanolic extracts obtained from selected Sudanese medicinal plants. Curr. Res. J. Biol. Sci., 2: 143-146.

Jaeschke, H., C.D. Williams, M.R. McGill and A. Farhood, 2010. Herbal extracts as hepatoprotectant against acetaminophen hepatotoxicity. World J. Gastroentrol., 16: 2448-2450.

Johansson, L.H. and L.A. Borg, 1988. A spectrophotometric method for determination of catalase activity in small tissue samples. Anal. Biochem., 174: 331-336.

Kharoubi, D., M. Slimani, D. Krouf, L. Seddik and A. Aoeus, 2008. Role of wormwood (*Artemisia absinthium*) extract on oxidative stress in ameliorating lead induced hematotoxicity. Afr. J. Tradit. Complement. Altern. Med., 5: 263-270.

Kim, M. and Y. Kim, 2010. Hypocholesterolemic effects of curcumin via up-regulation of cholesterol 7a-hydroxylase in rats fed a high fat diet. Nutr. Res. Pract., 4: 191-195.

Krishnamoorthy, J.R., M.S. Ranjith and S. Gokulshankar, 2009. Effect of the extract combinations of *Curcuma zedoaria* and Aloe vera in retarding melanin synthesis in murine melanoma cells. Egyptian Dermatol. Online J., Vol. 5

Mochin, M.C. and J.A. Leyva, 1984. A new spectrophotometric method for determining triglyceride in serum. Clin. Chim. Acta, 142: 281-285.

Motamedi, H., E. Darabpour, M. Gholipour and S.M. Seyyed Nejad, 2010. *In vitro* assay for the anti-brucella activity of medicinal plants against tetracycline-resistant *Brucella melitensis*. J. Zhejiang Univ. Sci. B, 11: 506-511.

Nauck, M., M.S. Graziani, D. Bruton, C. Cobbaert and T.G. Cole *et al*., 2000. Analytical and clinical performance of a detergent-based homogeneous LDL-cholesterol assay: A multicenter evaluation. Clin. Chem., 46: 506-514.

Ogbonnia, S.O., G.O. Mbaka, N.H. Igbokwe, E.N. Anyika, P. Alli and N. Nwakakwa, 2010. Antimicrobial evaluation, acute and subchronic toxicity studies of Leone bitters, a Nigerian polyherbal formulation, in rodents. Agric. Biol. J. N. Am., 1: 366-376.

Reynolds, T. and A.C. Dweck, 1999. Aloe vera leaf gel: A review update. J. Ethnopharmacol, 68: 3-37.

Sarker, S.D. and L. Nahar, 2004. Natural Medicine: The genus *Angelica*. Curr. Med. Chem., 11: 1479-1500.

Schulz, H. and G. Albroscheit, 1988. High-performance liquid chromatographic characterization of some medical plant extracts used in cosmetic formulas. J. Chromatogr. A, 442: 353-361.

Tariq, M., A.M. Ageel, A.M. Al-Yahya, J.S. Mossa, M.S. Al-Said and N.S. Parmar, 1985. Anti-inflammatory activity of *Commiphora molmol*. Agents Action, 17: 381-382.

Verma, H., P.R. Patil, R.M. Kolhapure and V. Gopalkrishna, 2008. Antiviral activity of the Indian medicinal plant extract *Swertia chirata* against herpes simplex viruses: A study by *in vitro* and molecular approach. Indian J. Med. Microbiol., 26: 322-326.

Witztum, J.L. and D. Steinberg, 2001. The oxidative modification hypothesis of atherosclerosis: Does it hold for humans? Trends Cardiovasc. Med., 11: 93-102.