Original research Articles

Preliminary Investigation of the Subchronic Hypolipidemic Actions of *Hibiscus Sabdariffa* in Rats

ABSTRACT

Hibiscus sabdariffa is a popular medicinal plant that is claimed to have hypotensive and hypocholesterolemic actions in animals. The present preliminary study was conducted to assess the subchronic hypolipidemic actions of *H. sabdariffa* calyx in normal rats when administered as an aqueous extract. Twenty (20) Wistar albino rats were used for this study and were divided into 4 groups. Group 1 rats were normal control and received distilled water (10 ml/kg) while Groups 2-4 rats received 200, 400 and 600 mg/kg body weight of the extract respectively. All treatments were through the oral route for 21 days. On the 21st day, blood samples for Lipid panel analyses were collected. The results disclosed statistically significant ($p < 0.05$), dose-dependent decreases in Low density lipoprotein (LDL), very low density lipoprotein (VLDL) and triacylglycerol (TAG) levels in all doses of the extract-treated groups (Groups 2-4) when compared to that of the normal control rats. No statistically significant change ($p > 0.05$) was observed in the total cholesterol level of Groups 2, 3 & 4 given 200, 400 and 600 mg/kg of the extract, respectively compared to the normal control (Group 1). However, there was a significant increase ($p < 0.05$) in high density lipoprotein (HDL) levels of the extract-treated group when compared to that of the normal control rats. It was concluded that aqueous extract of *H. sabdariffa*, administered subchronically, exhibited beneficial hypolipidemic effects in healthy rats and warrants further laboratory and clinical investigations to define its potential benefits and risk for the treatment of lipid disorders.

Keywords: *Hibiscus sabdariffa* , Lipid profile, Normal rat

INTRODUCTION

Hibiscus sabdariffa belongs to the family Malvaceae. It is a very common shrub in the tropics with many uses in culinary and therapy **[1]**. It is usually consumed after a hot water extraction of the calyces and subsequent addition of other ingredients. The calyces are soaked in hot water and filtered after an hour. Thereafter, sweeteners may be added to the filtrate. In Nigeria, this

preparation is called "Zobo" drink and it is cherished by most Nigerians. The shrub is found in so many tropical countries such as Indonesia, Thailand, Egypt, Mexico and Nigeria **[2, 3].** Researchers have reported blood-pressure lowering effects of the extract **[1, 4].** It is also on record that the calyx extract is used in the treatment of arteriosclerosis **[5]** and other ailments including hyperlipidemia and obesity **[6, 7].** In general, Hibiscus species is also used in treating diabetes mellitus **[8].**

Despite all the anecdotal medicinal uses of *H.sabdariffa* in man, there are insufficient investigations on its hypolipidemic actions associated with its subchronic administration in healthy human subjects and in patients with lipid disorders. Our study therefore investigated the alterations in the serum lipid profile of healthy albino Wistar rats subchronically administered *H.sabdariffa* aqueous extract.

MATERIALS AND METHODS

Materials

Plant Materials

Fresh calyces of *Hibiscus sabdariffa* were purchased from Ogige market, Nsukka, Enugu State of Nigeria and were identified by a botanist of the herbarium (UNH: 232b) Botany Department., University of Nigeria, Nsukka. The calyces were air-dried separately at room temperature and then pulverized.

Animals

Adult male Wistar albino rats of 10 to 16 weeks and average weight of 160 ± 15 g were obtained from the animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka. The animals were acclimatised for duration of 7 days under standard environmental conditions with a 12 hour light/dark cycle maintained on a regular feed (Vital feed) and water *ad libitum*. The experimental protocol used in this study was approved by the ethics committee of the University of Nigeria, Nsukka and conforms with the guide to the care and use of animals in research and teaching of University of Nigeria, Enugu state, Nigeria. ECUN: 2009/173451.

Chemicals/reagents/samples

All chemicals used in this study were of the analytical grade and products of May and Baker, England; BDH, England or Merck, Darmstadt, Germany.

Instruments/Equipment

Rotary evaporator (Model Modulyo 4K, Edward, England), Chemical Balance (Gallenkamp, England), Conical Flasks (Pyrex, England), (Gallenkamp, England), Centrifuge (PIC, England), Digital Photo Colorimeter (EI 312 Model, Japan), Adjustable Micropipette (Perfect, USA.), Refrigerator (Kelvinator, Germany),

Methods

Extraction of the Leaves of *Hibiscus sabdariffa*

The calyces of *Hibiscus sabdariffa* were air-dried separately at room temperature and then pulverized. The ground samples were extracted with aqueous solvent, using the cold maceration technique. About 1 kg of the ground calyces was soaked in 5 litres of distilled water with intermittent shaking every 2 h for 48 h. The samples were filtered using Whatman No 1 filter paper. The filtrates were concentrated to solid matter using rotary evaporator, which then become the stock sample for the aqueous calyces extract. These extracts were stored in the refrigerator compartment till the time of use.

Experimental design

Twenty (20) male albino Wistar rats were acclimatized at the same conditions of temperature and pressure, and the same animal feeds were used. The rats were assigned into four (4) groups of five (5) rats each as shown below:

Group 1 Normal Control

Group 2 200 mg/kg of aqueous calyces extract *Hibiscus sabdariffa*

Group 3 400 mg/kg of aqueous calyces extract *Hibiscus sabdariffa*

Group 4 600 mg/kg of aqueous calyces extract *Hibiscus sabdariffa*.

Administration of the extract was through the oral route using stomach tube once daily for 21 days. On day 21, serum samples were collected for lipid profile assay.

Blood sample collection

Blood samples were collected from the retrobulbar plexus of the median canthus of the eye of the rats**.** A microcapillary tube was carefully inserted into the medial canthus of the eye to

puncture the retrobulbar plexus and thus enable outflow of about 2 ml of blood into a clean glass tube. The blood was kept at room temperature for 30 minutes to clot. Afterwards, the test tubes containing the clotted blood samples were centrifuged at 3000 revolution per minute using a clinical table centrifuge. The clear serum supernatant was then carefully aspirated with syringe and needle and stored at $4^{o}C$ in a clean sample bottle for the serum biochemistry assays.

Determination of Lipid Profile

Determination of Serum Cholesterol

The serum cholesterol was determined by cholesterol oxidase-perioxidase method **[9].** High density lipoproteins (HDL)–cholesterol concentration was determined by the method of **[10]** using QCA commercial kit. Estimation of triacylglycerol concentration was done by method of **[11]** using QCA commercial kit while Very low density lipoprotein (VLDL) and Low density lipoprotein (LDL) -cholesterol concentration, were calculated using the Friedwald formula **[12].**

Statistical Analysis

Data obtained were analyzed using One-way Analysis of Variance (ANOVA). Variant means were separated using Duncan's multiple range Post hoc Test. P values ≤ 0.05 were considered significant. Results were presented as Mean ± Standard Error of the Mean (Mean ±SEM).

RESULTS

The results show that the serum total cholesterol levels of all the rats in all the groups (Groups 1-4) did not differ significantly ($p > 0.05$) among one another. There was no significant change between the total cholesterol level of the extract-treated and the distilled water-treated groups

(Figure 1). There was dose-dependent and statistically significant ($p < 0.05$) reductions in the serum triacylglycerol levels of Groups 2-4 rats when compared with that of the normal control rats. The serum triacylglycerol levels of Groups 2 and 3 rats were comparable ($p > 0.05$) to each other but were significantly ($p < 0.05$) higher than that of the Group 4 rats (Figure 2). There was significant ($p < 0.05$) increase in the serum HDL levels of Group 4 rats compared with that of the normal group (Group 1). The serum HDL levels of the rats in Groups 2 and 3 were statistically comparable ($p > 0.05$) but were significantly ($p < 0.05$) higher than that of the Group 1 rats (Figure 3). There was significant reduction in the VLDL levels of all the extract-treated rats (Groups 2-4) when compared with the normal control rats administered with distilled water. The VLDL levels of Groups 2 and 3 rats were comparable ($p > 0.05$) (Figure 4). There was significant ($p < 0.05$) reduction in the serum LDL levels of the rats in Groups 2-4 (Extract-treated groups) when compared to that of the Group 1 rats (Normal control) in a dose-dependent manner. There was also significant ($p < 0.05$) reduction in the LDL levels of the Group 3 rats when compared with that of the group 2 (Figure 5).

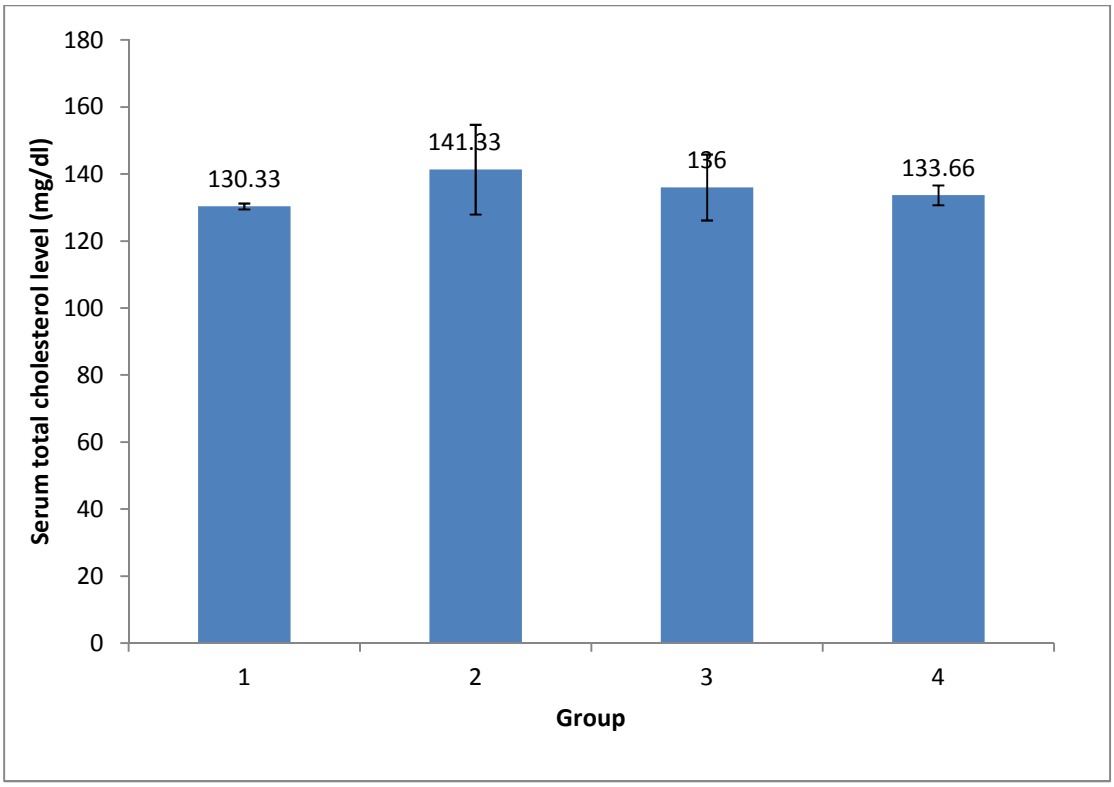

Figure 1: The serum total cholesterol levels of rats administered with aqueous extract of *H.sabdariffa.*

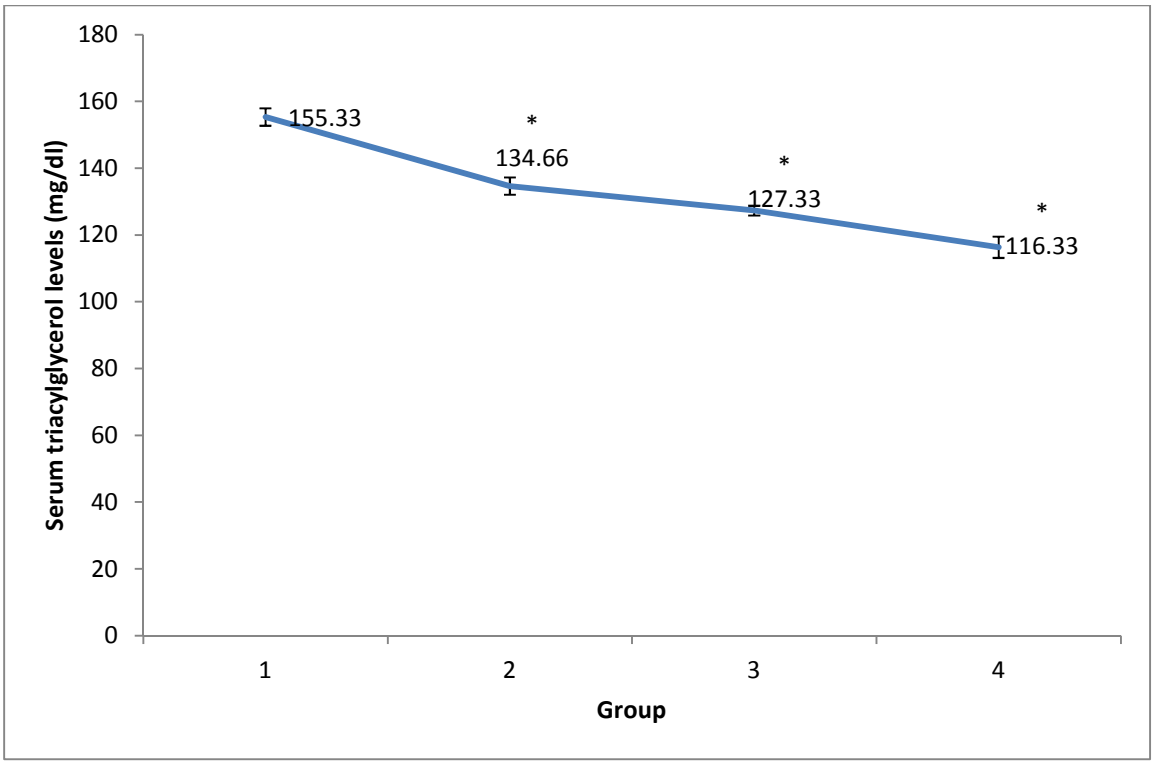

Figure 2: Serum triacylglycerol levels of albino Wistar rats dosed with aqueous extract of *H. sabdariffa*

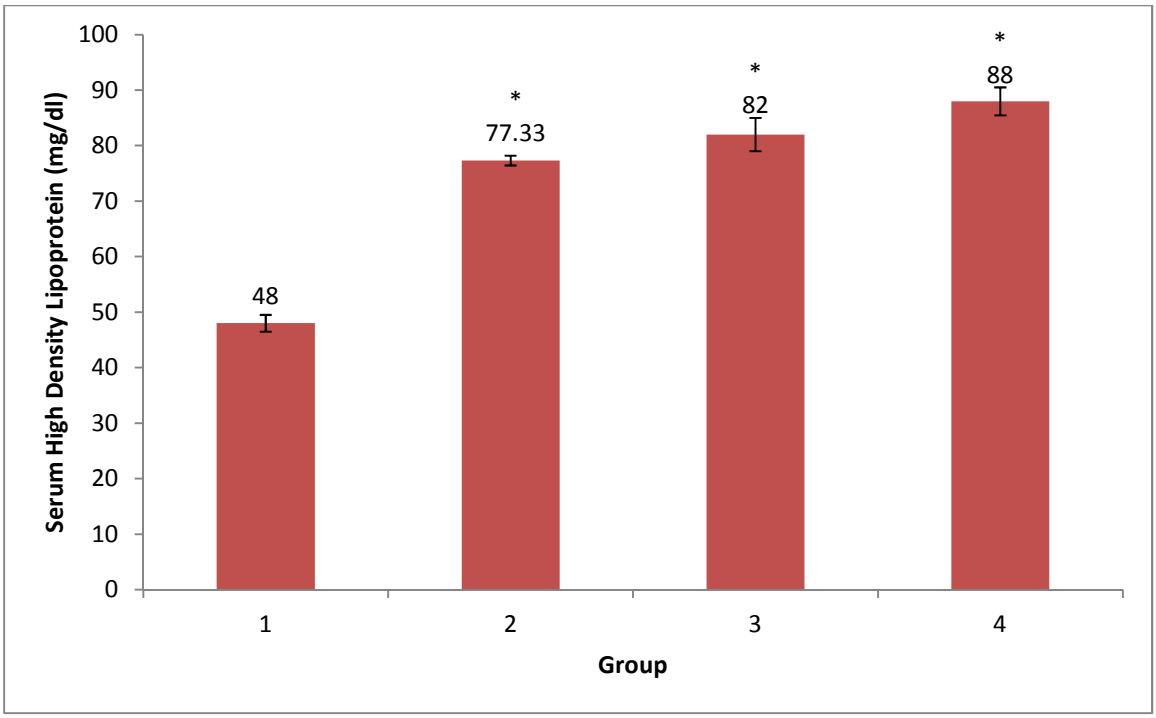

Figure 3: High density lipoprotein levels of rats administered with graded doses of aqueous extract of *H.sabdariffa*

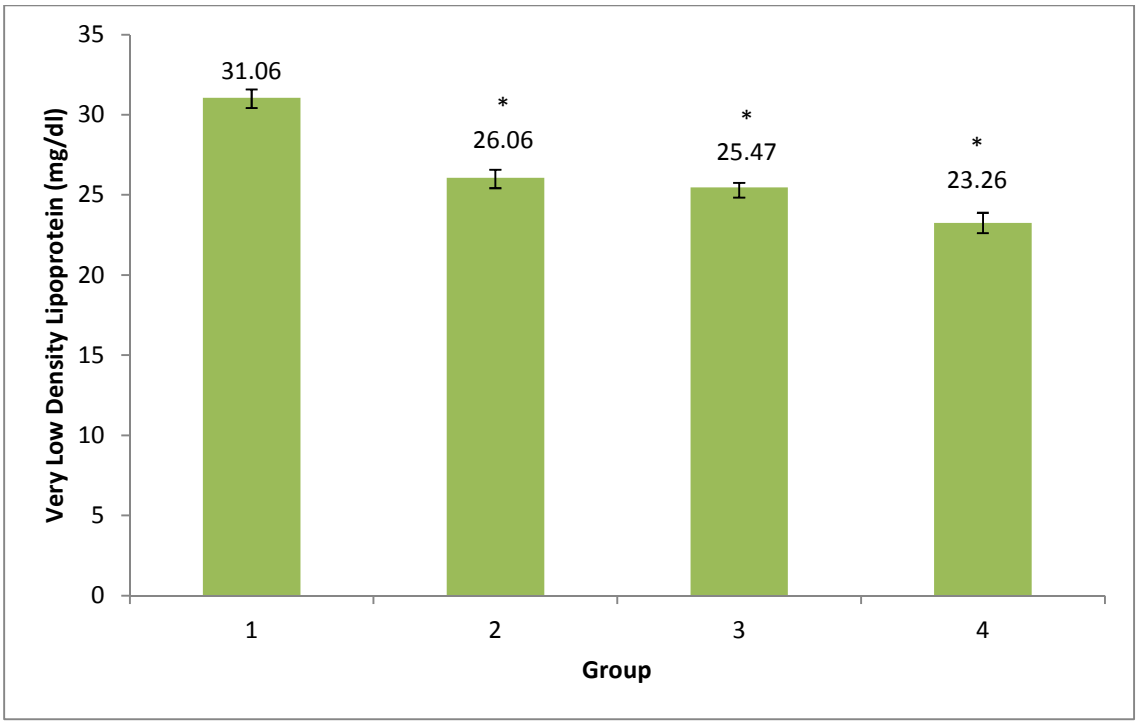

Figure 4: Effect of administration of *H.sabdariffa* aqueous extract on serum very low density lipoprotein levels of albino Wistar rats

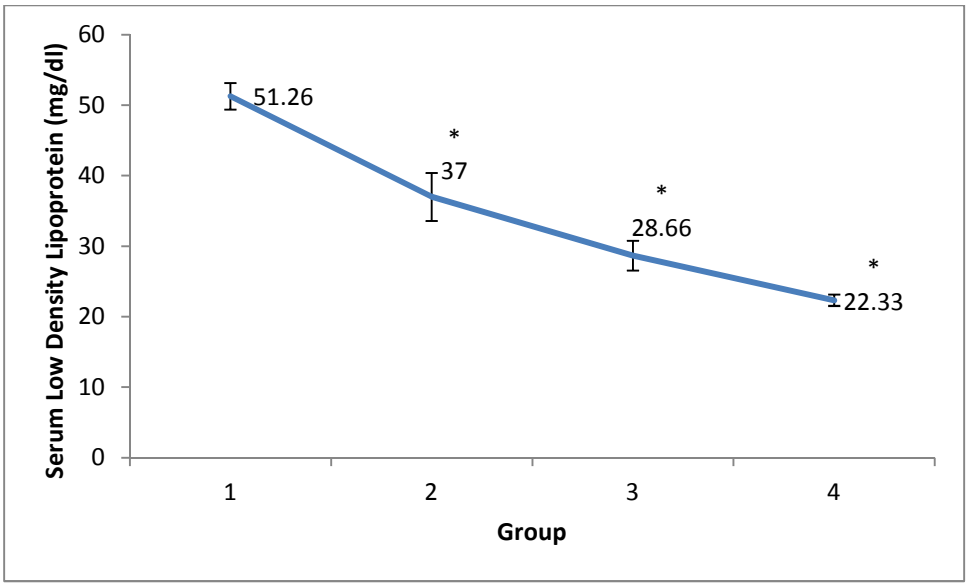

Figure 5: Effects of graded doses of *H.sabdariffa* aqueous extract administration on the serum low density lipoprotein of albino Wistar rats

DISCUSSION

Assessment of the lipid panel following 21 days administration of *H.sabdariffa* aqueous extract at varying doses revealed significant decreases in the serum levels of TAG, LDL, VLDL and significant increases in the serum levels of HDL without any significant changes in the serum total cholesterol levels of the extract-treated rats compared to the normal control rats.

Reductions in the serum levels of TAG, LDL and VLDL by aqueous extract of *H.sabdariffa* may have been mediated by the antioxidant properties of the extract. Calyces of *H. sabdariffa* are one of the best sources of vitamin C and anthocyanins **[8, 13, 14].** The dried calyces contain flavonoids **[15]** which act as antioxidants. Some researchers have also associated the lipid-lowering effects of *H.sabdariffa* with the presence of water soluble fibers contained in it **[16, 17].**

The result of this present study is not consistent with the findings of **[18]** and **[19].** However, the finding of non-significant changes in the total cholesterol levels of the extract-treated rats compared to that of the normal control rats did not agree with the findings of **[20]** who reported reductions in the serum levels total cholesterol following treatment with *H.sabdariffa* extract. In the same vein, many researchers have reported decrease in the serum total cholesterol levels upon administration of *H.sabdariffa* **[16, 17].** The finding of significant increase in the serum HDL levels of the extract-treated rats when compared with that of the normal control rats is in agreement with the submissions of **[18, 19, 20]**.

CONCLUSION

We conclude that aqueous extract of *H. sabdariffa* aqueous, administered subchronically, exhibited beneficial hypolipidemic effects in healthy rats and warrants further laboratory and clinical investigations to define its potential benefits and risks for the treatment of lipid disorders.

CONSENT

Not applicable

ETHICAL APPROVAL

All authors hereby declare that “principles of laboratory animal care” (NIH publication No 85-23, revised 1985) were followed, as well as specific national laws. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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