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The Hypolipidemic Effect of Aqueous Extract of *Hibiscus sabdariffa* on Paracetamol-induced Hepatotoxicity in Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author AOO designed the study. Author JFE performed statistical analysis. Author EBU wrote the first draft and final manuscript. Author OEO proof read the final work. All authors read and approved the final manuscript.

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ABSTRACT

Aim of the Study: This study was undertaken to ascertain if *Hibiscus sabdariffa* extract can affect the lipid profile (Total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), very low density lipoprotein (VLDL), and low density lipoprotein (LDL)) levels in a paracetamol- induced hepatotoxicity using albino Wistar rat as a model.
Materials and Methods: Thirty (30) rats used for this study were divided into three groups. Group A (n=10) served as control. Group B (n=10) was administered paracetamol only at a dose of 750 mg/kg body weight. Group C (n=10) was administered paracetamol (dose 750 mg/kg body weight) and aqueous extract of *H. sabdariffa* (dose 10 ml/kg body weight) of the animal for 3 weeks. All animals were allowed free access to clean drinking water and normal rat chow.
Results: Results of the study revealed that TC was significantly lower ($p<0.05$) in the paracetamol + *H. sabdariffa*-treated group as compared to paracetamol-treated group and control respectively.

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Similar trend was observed with TG, VLDL-c, LDL-c and HDL-c. However, the decrease in HDL-c was not statistically significant when compared to control.
Conclusion: The presence of bioactive constituents vis; anthocyanins, flavonoids, polyvenols and free radical scavenging properties in *H. sabdariffa* enabled a hypolipidemic effect on the animals by lowering the levels of serum TG, VLDL-c, LDL-c despite challenge on the liver. However, it was unable to produce significant effect on HDL concentration -very important cholesterol required in high level to maintain homeostasis inside the body. This may be due to the challenge on the liver as a result of the paracetamol abuse.

Keywords: Paracetamol; hepatotoxic; Hibiscus sabdariffa; lipid profile; albino wistar rats.

ABBREVIATIONS

TC : Total Cholesterol
TG : Triglycerides
HDL : High Density Lipoprotein
VLDL : Very Low Density Lipoprotein
LDL : Low Density Lipoprotein
VLDL-c : Very Low Density Lipoprotein Concentration
LDL-c : Low Density Lipoprotein Concentration
HDL-c : High Density Lipoprotein Concentration
ANOVA : Analysis of Variance
SEM : Standard Error of Mean

1. INTRODUCTION

Hibiscus sabdariffa L. also known as Roselle is an ideal crop for developing countries, and it is widely grown in Central and West Africa and South East Asia [1]. The calyxes and dried leaves of *H. sabdariffa L.* can be used for various purposes in different countries and some of the uses include: culinary use – in the preparation of herbal drinks/ tea, fermented drinks, wine, flavoring agents, chocolates, jam and cake. In Nigeria and Sudan, the dried calyxes are boiled with sugar to make a popular drink known as "Zobo" or "Kakade" [2].

Several studies, both *in vitro* [3,4] and *in vivo* [5,6] have shown that the extract of *H. sabdariffa L.* has a potent antioxidant effect. The antioxidant activity of the extract is due to its strong scavenging effect on reactive oxygen and free radicals [6,7].

The aqueous extract of the red and green *H. sabdariffa* has also been reported to cause significant decrease in the LDL-c levels, while no significant effect was observed in HDL-c and Triglyceride levels [8].

H. sabdariffa has been documented to possess cardioprotective properties [9], hypocholesterolomic, antioxidative, hepato-protective qualities in animals [10,11]. The aqueous extract of *H. sabdariffa* has shown antioxidant activity than ascorbate due to anthocyanins present in its petals [12].

Recently, our laboratory studies have shown that despite the free radical scavenging property and presence of natural antioxidants in *H. sabdariffa,* these effects could not ameliorate the challenge on the liver due to paracetamol abuse in rats [13]. Based on this finding, it became expedient to investigate further to know if the aqueous extract of *H. sabdariffa* could positively affect lipid profile in a paracetamol-induced hepatotoxicity, hence this study.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh leaves of *H. sabdariffa* were purchased from Watt market in Calabar South, Cross River State, Nigeria. The leaves were authenticated by the Chief Botanist, Department of Botany, University of Calabar, Cross River State, Nigeria. 20 grams of the *H. sabdariffa* was weighed and grounded in an electric mill to obtain particles less than 2 mm. It was used to make an infusion by adding 1 liter of clean water and allowed to stand for 48 hours. The solution was filtered using Whatman's No. 1 filter paper. The filtrate was stored in clean plastic containers and refrigerated. The extract was brought out of the refrigerator 2 hours to oral administration. The extraction was carried out according to the method of [14] with little modification.

2.2 Experimental Animals

Thirty (30) albino Wistar rats of both sexes weighing between 100-200 g from the start of the experiment were used for this study to determine the following: Total cholesterol concentration, LDL-c, HDL-c, TG, VLDL-c concentrations. They

were maintained in the animal facility of the Department of Physiology, University of Calabar, Nigeria, at a temperature of 30 ± 2°C and 12 h light/dark cycles. The rats were kept singly in improvised plastic metabolic cages with wire net covers. The rats were randomly assigned into three groups (A = control, B = paracetamol-treated group and C = paracetamol + aqueous leaf extract of *H. sabdariffa*-treated group). Each group consisted of ten rats. They were allowed free access to normal rat chow and clean drinking water. The paracetamol-treated group received intraperitoneal inducement of paracetamol (750 mg/kg body weight [15]. The paracetamol + extract of *H. sabdariffa* treated group received intraperitoneal inducement of paracetamol (750 mg/kg body weight) [15] and oral administration of aqueous leaf extract of *H. sabdariffa* (10 ml/kg body weight of rats). The animals in the control group were fed with only normal rodent chow and clean drinking water. Drug administration was done once daily for 3 weeks after which the blood samples were collected for analyses.

2.3 Preparation of Paracetamol Sample

The stock concentration of paracetamol was prepared by dissolving 750 mg of standard drug in 5 ml of distilled water bringing the stock concentration to 75 mg m/L. The dose used was 750 mg/kg body weight [15].

2.3.1 Preparation of animals for collection of blood samples

This was done by the method of [16] and used by [17].

2.3.2 Biochemical assays

Total cholesterol, LDL- cholesterol, HDL-cholesterol, Triglycerides was done using the laboratory procedure manual. Method: Hitachi 704 Analyzer serviced by Roche Diagnostics [18].

VLDL- cholesterol concentration

The concentration of very low density lipoprotein cholesterol was determined using lipoprotein electrophoresis and ultracentrifugation [19].

2.3.3 Determination of Plasma LDL-c for equation

LDL-c = (Total cholesterol – Triglyceride – HDL-c)/5

2.4 Statistical Analysis

All results are presented as mean ± standard error of mean (SEM). The data were analyzed using a one-way analysis of variance (ANOVA) and $p<0.05$ was considered statistically significant [20].

2.4.1 Acknowledgement

All biochemical assays were carried out by Medichecks Diagnostic and Research Laboratory, Calabar, Cross River State, Nigeria.

3. RESULTS

Based on laboratory findings, the results of this research are expressed below.

3.1 Effect of Paracetamol and *H. sabdariffa* on TC Concentration

TC concentration in the control, paracetamol-treated and paracetamol + *H. sabdariffa*-treated groups are illustrated in Table 1. There was a significant decrease ($p<0.05$) in TC concentration in the paracetamol + *H. sabdariffa*-treated group as compared to control and paracetamol-treated groups respectively.

Table 1. Comparison of TC levels in rats between the control, paracetamol-treated and paracetamol + *H. sabdariffa*-treated groups

Group	TC (mmol/L)
Control	1.18 ± 0.04
paracetamol-treated	0.93± 0.04$^{###}$
paracetamol + *H. sabdariffa*-treated	0.45 ± 0.01 ***

*Values are mean ± SEM, n = 10, $^{###}$ p<0.05 vs. control***p<0.05 vs. control and paracetamol-treated groups*

3.2 Effect of Paracetamol and *H. sabdariffa* on HDL-c Concentration

The HDL-c concentration in the control, paracetamol-treated and paracetamol + *H. sabdariffa*-treated groups are illustrated in Table 2. There was a significant decrease ($p<0.05$) in HDL-c in the paracetamol + *H. sabdariffa*-treated group as compared to control.

3.3 Effect of Paracetamol and *H. sabdariffa* on TG Concentration

TG concentration in the control, paracetamol-treated and paracetamol + *H. sabdariffa*-treated

groups are illustrated in Table 3. There was a significant decrease (*p<0.05*) in TG concentration in the paracetamol + *H. sabdariffa*-treated group as compared to control and paracetamol-treated groups respectively.

Table 2. Comparison HDL-c concentration in rats between the control, paracetamol-treated and paracetamol + *H. sabdariffa*-treated groups

Group	HDL-c (mmol/L)
Control	0.30 ± 0.01
paracetamol-treated	0.27 ± 0.01
paracetamol +*H. sabdariffa*-treated	0.23 ± 0.00***

*Values are mean ± SEM, n = 10, *** (p<0.05) vs. control*

3.4 Effect of Paracetamol and *H. sabdariffa* on VLD-c Concentration

The VLDL-c concentration in the control, paracetamol-treated and paracetamol + *H. sabdariffa*-treated groups are illustrated in Table 4. There was a significant decrease (*p<0.05*) in VLDL-c concentration in the paracetamol + *H. sabdariffa*-treated group as compared to control and paracetamol-treated groups respectively.

Table 3. Comparison of TG concentration in rats between the control, paracetamol-treated and paracetamol + *H. sabdariffa*-treated groups

Group	TG (mmol/L)
Control	0.72 ± 0.01
paracetamol-treated	0.61 ± 0.01###
paracetamol +*H. sabdariffa*-treated	0.50 ± 0.01 ***

*Values are mean ± SEM, n = 10, ### p<0.05 vs. control, ***p<0.05 vs. control and paracetamol-treated groups*

Table 4. Comparison of VLDL-c concentration in rats between the control, paracetamol-treated and paracetamol + *H. sabdariffa*-treated groups

Group	VLDL-c (mmol/L)
Control	0.33 ± 0.00
paracetamol-treated	0.23 ± 0.01###
paracetamol +*H. sabdariffa*-treated	0.13 ± 0.00 ***

*Values are mean ± SEM, n = 10, ### p<0.05 vs. control, ***p<0.05 vs. control and paracetamol-treated groups*

3.5 Effect of Paracetamol and *H. sabdariffa* on LDL-c

The LDL-c concentration in the control, paracetamol-treated and paracetamol + *H. sabdariffa*-treated groups are illustrated in Table 5. There was a significant decrease (*p<0.05*) in LDL-c concentration in the paracetamol + *H. sabdariffa*-treated group as compared to control and paracetamol-treated groups respectively.

Table 5. Comparison of LDL-c concentration in rats between the control, paracetamol-treated and paracetamol + *H. sabdariffa*-treated groups

Group	LDL-c (mmol/L)
Control	0.55 ± 0.03
paracetamol-treated	0.53 ± 0.03
paracetamol +*H. sabdariffa*-treated	0.46 ± 0.00 ***

*Values are mean ± SEM, n = 10, ***p<0.05 vs. control and paracetamol-treated groups*

4. DISCUSSION

Lipid profile/ lipid panel is a panel of blood tests [21,22] that serves as an initial screening tool for abnormalities in lipids [23] such as cholesterol [24] and triglycerides [25]. The results of this test can identify certain genetic diseases and can determine approximate risks for cardiovascular disease [26] certain forms of pancreatitis [27,28] and other diseases.

H. sabdariffa has been reported to possess lipid lowering activity that could prevent diseases like hyper-lipidemia and cardiovascular diseases [29,30,9]. In 2003, [31] had reported the anti-cholesterol action of *H. sabdariffa* in reducing serum concentration of TGs, TC and LDL-C. These reports corroborate our result findings.

The main constituents of *H. sabdariffa L.* relevant for its pharmacological study are organic acid, anthocyanins and flavonoids [10,32]. These properties highlight the antioxidant activity of *H. sabdariffa* extract.

In the present study, *H. sabdariffa* revealed hypolipidemic activity in paracetamol-induced hepatotoxicity. 750 mg/kg-1 body weight of paracetamol and 10 ml/kg body weight of *H. sabdariffa* extract administered on rats caused significant reduction (P< 0.05) in serum cholesterol level (Table 1), serum TG (Table 3), serum VLDL-C (Table 4), and serum LDL-C

(Table 5) respectively as compared to paracetamol-treated group and control respectively. There was however, no significant decrease in the serum HDL-C (Table 2) when compared to paracetamol-treated group and control respectively.

Olatunji et al. [8] had demonstrated in 2005 that aqueous extract of red and green *H. sabdariffa* caused significant decrease in LDL-c level, while no significant effect was observed in HDL-c and TGs level. In 2007, [33] also reported the ability of *H. sabdariffa* extract to decrease LDL-c, TGs level, TC, lipid peroxidation *in vivo* and VLDL-c. These reports corroborate with our findings.

Egbal and Howeida [34] have reported hypolipidemic effect of ethanolic extract of *H. sabdariffa* L. calyces on induced hyperlipidemia in albino rats. In 2006, [35] also reported significant reduction in serum TG and LDL level after feeding rats with 1000 mg/kg and 500 mg/kg of dried calyces extract of Roselle. Harrison et al. [36] had earlier demonstrated that administration of 5% and 10% extract of *H. Sabdariffa L*. to cholesterol rich basal diet resulted in significant reduction of serum lipids level. The result of this study is in consonance with the above reports.

However, [37] had reported increased serum levels of HDL-c with *H. sabdariffa* extract; this is contrary to the findings of the present study. The HDL-c showed no significant change in the paracetamol + *H. sabdariffa*-treated group when compared to control. This may be specie specific, and also may depend on the dosage of substance abused. A high level of HDL-c is good because it helps remove other forms of cholesterol from bloodstream by scrubbing and keeping clean the endothelium of blood vessels. Conversely, a low HDL concentration as seen in this study predisposes the heart to attack and other forms of diseases.

Recent studies have shown that *H. sabdariffa* may not be able to ameliorate the challenge on liver due to paracetamol abuse in rats despite its free radical scavenging property and presence of natural antioxidants [13]. It is however not surprising that the aqueous extract of *H. sabdariffa* could not also exert its antioxidant property on the liver to boast the level of HDL concentration - very important cholesterol required in high level to maintain homeostasis inside the body. This implies that when paracetamol is abused by users, the effect can be detrimental to cells, tissues, and organs of the body. This should be seriously warned against.

5. CONCLUSION

The effect of *H. sabdariffa* on lipid profile level in a paracetamol-induced hepatotoxicity was investigated. Results from the findings showed that the aqueous extract of *H. sabdariffa* reduced levels of serum TG, VLDL-c, LDL-c despite challenge on the liver of cholesterol in the animal model. However, it was unable to produce significant effect on HDL concentration -very important cholesterol required in high level to maintain homeostasis inside the body this may be due to the challenge on the liver as a result of the paracetamol abuse. Conclusively, it may be adduced from the recent that the presence of bioactive constituents viz; anthocyanins, flavonoids, polyvenols and free radical scavenging properties in *H. sabdariffa* enabled a hypolipidemic effect on the animals despite challenge on the liver. However, it is recommended that future studies be undertaken to ascertain the mechanism of action of this extract as the scope of this study was limited to lipid profile level estimation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Permission was sought for, and obtained from the Faculty Animal Research Ethics Committee of the Faculty of Basic Medical Sciences for the study. The ethical approval number: FAREC-FBMS/20/2018

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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