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Research Article

The Hypolipidaemic Effect of the Ethanolic Extract of *Hibiscus sabdariffa* L. Calyces on Induced Hyperlipidaemia in Albino Rats

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Abstract

Objectives: This study was conducted to evaluate the effect of the ethanolic extract of the calyces of roselle *(Hibiscus sabdariffa* L.*)* on serum levels of lipid profile (cholesterol, triglycerides, low density lipoprotein (LDL), and high density lipoprotein (HDL)) in hyperlipidaemic albino rats.

Methodology: The ethanolic extract of *Hibiscus sabdariffa* calyces was prepared by soaking the dried calyces of the plant in 80% ethanol for three days and then filtered and the filtrate was dried. 32 rats were divided randomly into four groups, of 8 rats each. All groups (A_n, A_p, B and C) were fed a standard normal diet for one week, then groups A_p, B and C were fed a hyperlipidaemic formula (2g/10ml), orally for one week (9.6g of cholesterol powder dissolved in 50 ml of ghee (milk lipids)). Groups B and C were administered 1000 mg/kg and 500 mg/kg of *Hibiscus sabdariffa* extract, respectively for four weeks. Groups A_n and A_p were kept as negative and positive controls, respectively. Blood samples were collected from all rats on days 0, 7, 14, 21, 28 and 35 for determination of lipids profile.

Results: The findings of this study revealed a significant ($P \leq 0.05$) hypolipidaemic effect produced by dose 500 mg/kg at day 35 which reduced serum cholesterol, triglycerides, LDL and increased serum HDL significantly ($P \leq 0.05$).

Conclusion: It was concluded that *Hibiscus sabdariffa* L. has a potent lipid lowering activity and this confirms its traditional use as a lipid lowering therapeutic agent.

Keywords: *Albino Rats; Lipid profile; Hibiscus sabdariffa; Hyperlipidaemia; Hypercholesterolaemia*

Introduction

Lipid is the scientific term for fats in the blood. At proper levels, lipids perform important functions in the body, but can cause health problems if it is present in excess. The term hyperlipidaemia means high lipid levels in blood. Hyperlipidemia results from genetic predisposition interacting with an individual's diet [1]. It is a metabolic disorder, specifically characterized by alterations in serum lipid and lipoprotein profile due to increase concentration of total cholesterol (TC), low Density lipoprotein cholesterol (LDL-C) very low Density lipoprotein cholesterol (VLDL-C) and triglyceride (TG), and a concomitant decrease in the concentration of High Density lipoprotein cholesterol (HDL-C) in blood circulation [2]. Hypercholesterolemia is the most prevalent indicator for susceptibility to cardiovascular diseases. The common causes of hypercholesterolemia are hypothyroidism, cholestatic liver disease, drugs such as diuretics, corticosteroids, androgens. The Common causes of hypertriglyceridemia is Diabetes Mellitus (typeII), hepatocellular disease, chronic renal diseases, abdominal obesity, excess alcohol and drugs (corticosteroids, retinoids) [3]. Hypercholesterolemia, therefore, is an

important risk factor for cardiovascular diseases. Therefore, reduction of plasma cholesterol and endothelial protection became important steps for the control of atherosclerotic disease and its complications such as acute myocardial infarction and systemic hypertension [4]. Atorvastatin, Gemfibrozil, Cholestyramine and Ezetimibe are the most common hypolipidemic drugs, Therefore, used in the treatment of hyperlipidema, which are promotes a reduction of lipids level in the blood or acts to prevention coronary heart disease, while these drugs have various side effects. The most common are headache, nausea, muscle pain, liver damage, gastrointestinal problem and hyperglycemia [1]. Herbal medicine sometimes referred to herbalism or botanical medicine, is the use of herbs for therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herbal plants produce and contain a variety of chemical substances that act upon the body [5]. *Hibiscus sabdariffa* L. has been reported to possess biological activities like antihypertensive, antimutagenic, chemopreventive, antioxidant, anticonvulsant, anxiogenic, CNS-depressant, serotoninergic activities, reducing oxidative liver damage, anti-inflammatory activities and hypoglycemic activity, but there is hardly any scientific reports on the hypolipidemic effect of the calyces of *Hibiscus sabdariffa* L. *Hibiscus sabdariffa*. Traditionally it has been used for many years ago against high blood pressure. Thus, this study is conducted to investigate the hypolipidemic effect of *Hibiscus sabdariffa* L. calyces in hyperlipidemic rats, to verify its traditional use as hypolipidemic agent, scientifically depending on laboratory findings.

Materials and Methods

Rats: Adult Wister Albino rats, both sexes were purchased from the Faculty of Pharmacy, University of Khartoum.

Study plant: Calyces of Roselle (*Hibiscus sabdariffa*) were purchased from Omdurman Great Market.

Cholesterol powder: Was purchased from Lab Line Company in the form of white crystalline powder each containing 25g.

Kits for Biochemical analysis: Were purchased from Biomed Trading Enterprises, Khartoum, production Germany. Commercial diagnostic kits for estimating blood lipid profile (TC, TG, and HDL-c).

Plant preparation

Dry mature Roselle calyces used in this study were obtained from Omdurman market. The dried calyces were milled using pestle and mortar to get a powder used for the extraction [6].

Ethanolic extraction of (*Hibiscus sabdariffa*) Roselle

Extraction was carried out according to methods described by [6] in Medicinal and Aromatic Plants Research Institute (MAPRI). 300g of plant sample was soaked in 2.5 liters of 80% ethanol for three days. Extraction was carried out till the color of the solvent in the last time returned colorless. Solvent was evaporated under reduced pressure using rotary evaporator apparatus. The concentrated extract was exposed to air till complete dryness and the yield percentage was calculated.

Phytochemical screening

General phytochemical screening for the ethanolic extract was carried out for extract using the methods described by [6-9] with few modifications.

Experimental animals

Thirty -two Wistar Albino Rats (W.A.R.) of both sexes, aging from (4 - 6) months and weighting (150 - 270g) were used. The animals were housed in groups and kept under controlled conditions of temperature (25 ± 1°C). Animal were allowed to feed and drink water freely.

Ethical consideration

All rats received humane care according to the guidelines outline by the committee for the purpose of control and supervision on experiments on animals [10].

Collection of blood samples

Blood samples were collected from all rats from the retro-orbital plexus of rats eyes using heparinized capillary tubes. The animals were anaesthetized using chloroform and blood was drown from the retro orbital plexus of the eyes of rats using capillary glass tubes according to the method of [11]. Blood was collected in plain containers and let to stand for 30 minutes. Serum was then separated from the blood cells using centrifuge at 3000 rpm for 10 minutes. The sera were collected in new plain containers and kept in refrigerator in 4°C for estimation.

Preparation of the Hyperlipidaemic Formula:

50 ml of natural Animal Ghee was measured using measuring cylinder and 9.6g of cholesterol powder (stabilizer 95%) was weighed using a sensitive balance. The cholesterol Powder was added gradually to the animal ghee with continuous stirring with glass rod until a homogeneous solution became ready. A Fatty dietary formula with concentration of 2g/10ml ghee was prepared [12].

Induction of hyperlipidaemia

In this study the protocol of inducing hyperlipidemia was obtained according to a previous study conducted by Mini (2008), with slight modification to induce hyperlipidemia within a short period of time (7 days), instead of the common duration (72 days).

Experimental design

All rats were kept for 10 days after being brought from Khartoum University as a period of adaptation. They were divided into four groups, each group contained eight rats (n = 8). Group A_n, was left untreated (negative control) but was fed a standard normal diet for five weeks, while groups A_p (positive control), B and C received a hyperlipidemic formula for one week. At day 7 groups B and C were treated with a high dose (1000 mg/ kg) and a low dose (500 mg/ kg) of an ethanol extract of Roselle, respectively, for four weeks. While group A_p was kept as a control for treatment. The extract was administered orally using an oro- gastric feeding tube. Blood samples were collected from all rats from the retro-orbital plexus of rats eyes using heparinized capillary tubes at days 0, 7, 14, 21, 28 and 35, for determination of lipid profile (TC, TGs, LDL and HDL) of the four groups.

Biochemical analysis

Blood samples were analyzed for presence of (TC, TG, HDL-c and LDL-c), according to Enzymatic method (Peroxidase method), using Spectrophotometer (Mindray BA-88A P.R.C).

Determination of Plasma LDL-c for equation

$$\text{LDL-c} = \frac{\text{Total cholesterol} - \text{Triglyceride} - \text{HDL-c}}{5}$$

Statistical analysis

The one-way analysis of variance (ANOVA) and mean separation were conducted to test significant difference level of groups ($p < 0.05$), (Gomez and Gomex, 1984). T-test was carried out according to Statistical package for Social Science (SPSS) program [13].

Results

The findings of this research were expressed below based on observation and laboratory findings.

Observation

During hyperlipidemia formula feeding, abnormal behaviors and manifestation that were observed in rats as shown in table 1.

Before Treatment	After treatment
Manifestation of abscess	Recovery of abscess
Hair loss	Hair growth
Weight gain	Weight loss
Yellowish stool	Stool color return again (Dark)
Laziness	Alertness

Table 1: *Result of observations.*

Phytochemical screening of *Hibiscus sabdariffa* calyces

Phytochemical screening of *Hibiscus sabdariffa* was carried out in the Medicinal and Aromatic Plants Research Institute and it was found to possess sterols, Triterpene, Flavonoids, Saponins, Cumarins and Tannins as shown in table 2.

Test	Observation	Results
Alkaloids	No change in color	-
Sterols	Pale green	+
Triterpenes	Pale purple	+
Flavonoids	yellow color	+
Saponins	Foam	++
Coumarins	UV fluorescence	++
Tannins	Black green colour	++
Anthraquinones	No change in color	-

Table 2: *Phytochemical screening.*

Key: *-: Negative; +: Trace; ++: Moderate.*

Effect of *Hibiscus sabdariffa* ethanolic extract on the level of total cholesterol (TC)

According to the result in tables 3a and 3b serum cholesterol level after fatty diet was elevated in group A_p (+ve control), B and C compared to group A_n (-ve control). The serum cholesterol level of the rats of group B and C decreased gradually from day 7 up to day 28 compared to group A_p (+ve control). Groups B and C showed a significant reduction ($P < 0.05$) in serum cholesterol at days (21 and 28) and days (14, 21 and 28) respectively.

	Cholesterol (mg/dl)	
Group	**Before fatty-diet**	**After fatty-diet**
An (Negative Control)	147.0 ± 18.7	138.0 ± 17.0
Ap (Positive Control)	123.2 ± 21.8	224.8 ± 33.9
B (1000 mg/kg)	102.4 ± 11.9	297.3 ± 41.9
C (500 mg/kg)	110.3 ± 15.9	308.3 ± 26.0

Table 3a: *Effect of fat-diet on cholesterol level.*

Data are expressed in mean ± Standard error of mean.

	Cholesterol (mg/dl)				
Group	**Day (0)**	**Day (7)**	**Day (14)**	**Day (21)**	**Day 28**
An (Negative Control)	138.0 ± 17.0	128.0 ± 11.7	122.9 ± 13.9	130.0 ± 14.9	120.3 ± 11.9
Ap (Positive Control)	224.8 ± 33.9	251.8 ± 3.7	222.6 ± 4.1	222.8 ± 6.9	216.8 ± 4.9
B (1000 mg/kg)	297.3 ± 41.9	201.1 ± 25.4	169.0 ± 20.5	138.7 ± 15.7*	138.6 ± 15.0*
C (500 mg/kg)	308.3 ± 26.0	225.6 ± 21.6	161.4 ± 18.7*	134.9 ± 14.8*	135.7 ± 9.3*

Table 3b: *Effect of Hibiscus sabdariffa extract on cholesterol level of hyperlipidemic rats.*

Data are expressed in mean ± Standard error of mean.

** = Significant difference (P < 0.05).*

Effect of *Hibiscus sabdariffa* ethanolic extract on the level of triglycerides (TGs)

As demonstrated in tables 4a and 4b serum Triglycerides level increased after fatty diet become in group A_p (+ve control), B and C compared to group A_n (-ve control). Then serum Triglycerides level of the rats of groups B and C decreased gradually from day 7 up to day 28 as compared to group A_p (+ve control). Groups B and C showed a significant reduction ($P < 0.05$) in serum triglycerides at days (21 and 28) and days (14, 21 and 28) respectively.

	Triacyl glycerides (mg/dl)	
Group	**Before fatty-diet**	**After fatty-diet**
An (Negative Control)	107.0 ± 8.8	100.4 ± 10.9
Ap (Positive Control)	67.6 ± 4.8	162.2 ± 18.1
B (1000 mg/kg)	55.3 ± 11.7	197.3 ± 11.3
C (500 mg/kg)	75.9 ± 6.4	215.4 ± 18.8

Table 4a: *Effect of fat-diet on the level of triacyl glycerides.*

Data are expressed in mean ± Standard error of mean.

Effect of *Hibiscus sabdariffa* extract on serum LDL level of hyperlipidemic rats

The findings of this study in tables 5a and 5b, revealed an elevated serum LDL level (after fatty diet) in groups A_p, B and C compared to group A_n. The serum LDL level of the rats of groups B and C decreased gradually from day 7 up to day 28 as compared to group A_p (+ve control). Groups B and C showed a significant reduction ($P < 0.05$) in serum triglycerides at days (21 and 28) and days (14, 21 and 28) respectively.

	Low density lipoprotein (mg/dl)	
Group	**Before fatty-diet**	**After fatty-diet**
An (Negative Control)	89.5 ± 11.5	74.5 ± 12.9
Ap (Positive Control)	74.2 ± 8.8	118.0 ± 25.8
B (1000 mg/kg)	98.6 ± 9.6	140.4 ± 22.6
C (500 mg/kg)	86.6 ± 5.1	137.7 ± 29.9

Table 5a: *Effect of fat-diet on the level of low density lipoprotein.*

Data are expressed in mean ± Standard error of mean.

	Low density lipoprotein (mg/dl)				
Group	**Day (0)**	**Day (7)**	**Day (14)**	**Day (21)**	**Day 28**
An (Negative Control)	74.5 ± 12.9	67.1 ± 7.4	50.6 ± 6.8	54.5 ± 5.8	46.6 ± 5.5
Ap (Positive Control)	118.0 ± 25.8	95.4 ± 11.8	76.6 ± 7.4	73.0 ± 12.7	73.2 ± 10.7
B (1000 mg/kg)	140.4 ± 22.6	100.4 ± 13.4	80.7 ± 11.9	63.4 ± 9.9*	52.6 ± 7.5*
C (500 mg/kg)	137.7 ± 29.9	104.9 ± 15.8	77.4 ± 12.9*	55.6 ± 4.9*	49.9 ± 8.3*

Table 5b: *Effect of Hibiscus sabdariffa extract on serum LDL level of hyperlipidemic rats.*

Data are expressed in mean ± Standard error of mean.

** = Significant difference (P < 0.05).*

Effect of *Hibiscus sabdariffa* extract on serum HDL level of hyperlipidemic rats

The findings of this study (as demonstrated in tables 6a and 6b), revealed that the level of serum HDL (after administration of fatty diet) decreased in groups A_p (+ve control), B and C compared to group A_n (-ve control). The serum HDL level of the rats of group B and C increased gradually from day 7 up to day 28 as compared to group A_p (+ve control). Groups B and C showed a significant increase ($P < 0.05$) in serum level of HDL at days (21 and 28) and days (14, 21 and 28) respectively.

	High density lipoprotein (mg/dl)	
Group	**Before fatty-diet**	**After fatty-diet**
An (Negative Control)	109.1 ± 12.3	56.9 ± 11.9
Ap (Positive Control)	75.2 ± 10.7	49.8 ± 15.5
B (1000 mg/kg)	57.1 ± 9.6	23.1 ± 6.1
C (500 mg/kg)	61.3 ± 10.6	21.6 ± 6. 6

Table 6a: *Effect of fat-diet on the level of high density lipoprotein.*

Data are expressed in mean ± Standard error of mean.

	High density lipoprotein (mg/dl)				
Group	**Day (0)**	**Day (7)**	**Day (14)**	**Day (21)**	**Day 28**
An (negative control)	56.9 ± 11.9	66.4 ± 18.2	66.1 ± 15.3	51.1 ± 10.1	58.6 ± 8.4
Ap (positive control)	49.8 ± 15.5	55.2 ± 6.2	91.8 ± 14.1	68.8 ± 5.6	68.4 ± 7.9
B (1000mg/kg)	23.1 ± 6.1	37.9 ± 5.3	48.9 ± 6.6	68.0 ± 7.3*	72.3 ± 8.8*
C (500mg/kg)	21.6 ± 6. 6	26.7 ± 5.8	40.4 ± 6.9*	55.0 ± 9.3*	60.4 ± 8.3*

Table 6b: *Effect of Hibiscus sabdariffa extract on serum high density lipoprotein level of hyperlipidemic rats.*

Data are expressed in mean ± Standard error of mean.

** = Significant difference (P < 0.05).*

Discussion

Herbal drugs are prescribed widely because of their effectiveness, less side effect and relatively low cost. In present study, HSEE revealed hypolipidemic activity. *Hibiscus Sabdariffa* when used either in high (1000 mg/kg) or low (500 mg/kg) dose orally caused a

significant reduction ($p < 0.05$) in serum Cholesterol level (Table 3b), serum TGs level (Table 4b), serum LDL level (Table 5b), respectively while HDL level was significantly increased ($p < 0.05$) by the two doses (Table 6b).

Ochani P [2] reported that the calyces and leaves of *Hibiscus sabdariffa* reduce total cholesterol and increase HDL level. This totally agreed with the finding of our current research. In addition [14] reported the anticholesterol action of *Hibiscus Sabdariffa* in reducing the serum concentration of TGs, TC and LDL-C. His study showed a significant increase ($p < 0.05$) in HDL-C, since HDL-C is a protective factor coronary heart diseases.

Concerning serum Triglycerides and LDL level, the present finding agrees with study of [15] who demonstrated that serum TGs and LDL level decrease significantly after feeding rats with 1000 mg/kg and 500 mg/kg of dried calyces extract of Roselle. Harrison D., *et al.* [16] reported that administration of 5% and 10% ethanolic extract from flowers of *Hibiscus Sabdariffa* L. to cholesterol rich basal diet showed better results in the reduction of serum lipids level.

On the other hand, the present findings agrees with study of [7] who reported the flowers of *Hibiscus sabdariffa* contain anthocyanins, flavonoids and polyphenols. And it have highlighted the role of polyphenolic acid, flavonoids and anthocyanins that may act as antioxidants or have other mechanisms contributing to the cardio protective actions. Reduction of cholesterol, TGs and LDL and increase of HDL in serum lipids can be attributed to the presence of sterols, Triterpenes, flavonoids, Coumarins, Saponins and Tannins.

Conclusion

Based on the finding of this research, it can be concluded that *Hibiscus sabdariffa* is a potent lipids lowering agent. Accordingly, it can be predicted that it probably possesses a cardioprotective and antiatheriosclerotic potential.

Further studies are recommended to investigate the effect of *Hibiscus sabdariffa* on cardiovascular system as well as on liver enzymes.

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