

EFFECT OF HIBISCUS SABDARIFFA CALYX EXTRACT ON STRESSED RABBIT PLASMA CHOLESTEROL STATUS

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ABSTRACT

Hibiscus sabdariffa aqueous extract has been shown to have antioxidant and lipid lowering effects in animal studies. This study is aimed at investigating the effects of the aqueous extract of *Hibiscus sabdariffa* calyx on the lipid profile of rabbits subjected to stress.

The rabbits were stressed by suspension for 2 minutes, twice daily, for 15 days. After 10 days of stress, there was significant increase in plasma LDL-cholesterol but a decrease in plasma HDL-cholesterol and total cholesterol when compared to the control. Administration of extract significantly ($P < 0.05$) reduced LDL-cholesterol and total cholesterol in stressed rabbits relative to control and stressed group and an increased HDL-cholesterol level ($P < 0.05$) relative to stressed group only. After 15 days, similar results were obtained. However, there was no significant difference in HDL-cholesterol levels between stressed and stressed plus extract-treated rabbits.

Conclusion: These data suggest that *Hibiscus sabdariffa* calyx extract may be protective against stressed-induced LDL-cholesterol increase.

INTRODUCTION

Stress is an adaptive response that prepares the organism for a life-threatening situation. Stress can occur when our perception of events does not meet our expectations and we are unable to manage our reaction¹. It induces strain upon both emotional and physical endurance which have been considered a basic factor on the aetiology of a number of diseases². Stress response usually brings about metabolic effects by the interaction of the hypothalamus, the anterior pituitary gland and adrenal glands situated just above the kidney

(hypothalamic-pituitary-adrenal axis) along with the sympathetic nervous system. The hypothalamic-pituitary-adrenal axis is responsible for releasing cortisol, most commonly known as the stress hormone. Stress also promotes the release of inflammatory cytokine³ and prolactin⁴, and suppresses the production of growth hormone and luteinizing hormone, which is an important intermediary hormone between the environment and internal state⁵. Apart from the level of circulating hormones such as cortisol and prolactin, there are indications that cholesterol can also be used as an index of stress^{6,7,8}.

KEYWORDS: *Hibiscus sabdariffa*, stress, cholesterol

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Cholesterol, which is the principal sterol synthesized in animals travels, through the blood attached to proteins to form lipoproteins which are classified based on the protein content in relation to fat. The biochemical feature which has attracted

the most sustained and widespread attention in relation to aetiology and prevention of these diseases is serum cholesterol or its fractions like low density lipoproteins (LDL) and high density lipoproteins (HDL) ⁹. It is well established that increased levels of blood cholesterol especially low density lipoprotein-cholesterol (LDL-C) is an important risk factor for cardiovascular complications since it favours lipid deposition in tissues including blood vessels. Evidences from lipid lowering trials have clearly established that reduction of total cholesterol or low density lipoprotein-cholesterol (LDL-C) is associated with decreased risk of atherosclerosis and coronary heart disease ^{10,11}. Furthermore, Epidemiological studies have also shown an inverse correlation between high density lipoprotein-cholesterol (HDL-C) level and the risk of cardiovascular disease ¹².

Hibiscus sabdariffa Linn (Family: Malvaceae) is an annual dicotyledonous herbaceous shrub popularly known as Roselle or Sorrel in English ^{13,14}. There are many published reports on the constituents of different plant parts of *H. sabdariffa*, which have been summarized by Ross ¹⁵. The dried calyces contain the flavonoids; gossypetin, hibiscetine and sabdaretine ¹⁶. Certain amounts of delphinidin-3-monoglucoside and cyanidin-3-monoglucoside and delphinidin are also present ¹⁷. There are indications that extracts from the red calyces of *Hibiscus sabdariffa* (popularly called Zobo in Nigeria) contain antioxidant principles ^{18,19,20}. There are indications that stress cause remarkable changes in blood cholesterol concentration. This study therefore aimed to ascertain the extent of these changes and to determine the effect of *Hibiscus sabdariffa* calyx extract on the

stress-induced changes in the blood cholesterol.

MATERIALS AND METHODS

Hibiscus sabdariffa calyx

Dried calyces of *Hibiscus sabdariffa* were purchased from a local market in Benin city.

Reagents

Cholesterol assay kits (Randox laboratories ltd, Antrim, United Kingdom), Xylene (NAFFCO, New York).

Animals

Fifteen male English rabbits (*Oryctolagus cuniculus*) used for this study were purchased from a local breeder in Aduwawa market, Benin City, Edo state, Nigeria. The weights of the rabbits ranged from 800-1400g and they were housed in well-ventilated wooden cages with free access to food and water. They were maintained on growers mash (Bendel Feed and Flour Mill, Ewu, Edo State) and water ad libitum. They were left to acclimatize to our laboratory conditions for 24 days before the experiment commenced. The animals were protected from parasitic infestation by proper veterinary management (12 hour light/dark cycle, 22-28°C, sweeping and moping the floor with disinfectant, and cleaning the feed and water troughs) throughout duration of the treatment.

Preparation and solute content determination of *Hibiscus sabdariffa* calyx extract

Twenty-five grams (25g) of dried calyx of *Hibiscus sabdariffa* was put in a pot containing 250ml of distilled water and allowed to boil for 15 minutes. After the period of boiling, the mixture was left to

cool. Thereafter, it was filtered through four layers of cheese cloth, and a clear, red decoction was obtained. One milliliter of the *Hibiscus sabdariffa* calyx extract was put in a pre-weighed watch-glass. The watch-glass containing the extract was put on a hot plate and allowed to evaporate to dryness. The solute content of the extract was determined by subtracting the weight of the watch-glass + extract of *H. sabdariffa* from the pre-weighed watch-glass.

Experimental Design

At the end of acclimatization, the rabbits were re-weighed. Fifteen (15) rabbits weighing 950-1600g were thus used for this study. They were randomly selected into three (3) experimental groups of 5 rabbits each as shown below. The experiment lasted for 15 days.

Group 1: Water treated control. Each rabbit was given distilled water, 2.5 ml H₂O/kg body weight.

Group 2: Stressed but extract free. Induction of stress was done by wrapping the rabbits across the trunk using baft and suspended on a retord stand for two minutes every morning and evening, five days per week for three weeks.

Group 3: Stressed and extract treated. The rabbits were stressed by suspension and the aqueous *Hibiscus sabdariffa* calyx extract was immediately administered after stress. Extract was also administered twice a day, five days a week for three weeks by gavage. The dosage of the extract was 2500 µg/kg body weight.

Sample collection and sample treatment. Blood was collected from the veins in the pinnae. Xylene was applied to the pinnae with the aid of cotton wool to make the veins more prominent. Once the veins were made visible, blood was collected using hypodermic syringes and needles.

The blood collected was quickly transferred into heparinised bottles and left standing on ice. The blood samples were collected after ten and fifteen days of treatment respectively. The blood collected in the heparinised bottles was centrifuged for 10 minutes at 1250 x g to obtain plasma. The plasma samples were left at 4°C until required for cholesterol assay.

Biochemical assay

Total cholesterol assay was determined spectrophotometrically by the enzymatic endpoint method²¹. The HDL-cholesterol estimation was determined by the precipitation method²². LDL-cholesterol was calculated from primary measurements using the empirical formula of Friedawald equation²³.

Statistical analysis

The data obtained were subjected to standard analysis of variance (ANOVA) procedure of SAS²⁴. The treatment means were compared using Duncan procedure of the same software. The significance level was set at P < 0.05.

RESULTS

The effect of 10 day stress on plasma cholesterol status in the rabbits and the role of *Hibiscus sabdariffa* calyx extract on the stress-induced change in cholesterol is presented in Table 1. Relative to the control (group 1), the stressed rabbits had statistically significant (P < 0.05) increase in LDL-cholesterol level. The table also shows that exposure of stressed rabbits to aqueous extract of *Hibiscus sabdariffa* caused significant reduction (P < 0.05) in stress mediated increase in LDL-cholesterol when compared to the control (group 1) and stress only group (group 2). The stressed only rabbit also had

TABLE 1: Changes in Total cholesterol, HDL-cholesterol, LDL-cholesterol and HDL-cholesterol/Total cholesterol ratios of control, stressed and stressed plus *H. sabdariffa* calyx extract-treated rabbits after ten days.

Group	Treatment	No of rabbits (No of samples)	No of surviving rabbits	Plasma cholesterol concentration (mg/dl) Mean \pm SD (n) ^a			
				TC	LDL-C	HDL-C	HDL-C/TC
1	Control	5	5/5	152.83 \pm 16.58(3)	52.67 \pm 6.71 (2)	53.80 \pm 16.53(3)	0.35
2	Stressed	5	5/5	123.20 \pm 2.54(3) ^b	72.01 \pm 10.77(4) ^b	26.01 \pm 8.37(4) ^b	0.21
3	Stressed + aq. extract	5	4/5	45.41 \pm 6.01(3) ^{b,d}	28.79 \pm 10.39(2) ^{b,d}	45.42 \pm 4.91(3) ^{c,d}	1.00

^a number of values used for mean cholesterol estimation

^b Value significantly different from group 1 value ($P < 0.05$) within the same column

^c Value not significantly different from group 1 value ($P > 0.05$) within the same column

^d Value significantly different from group 2 value ($P < 0.05$) within the same column

TABLE 2: Changes in Total cholesterol, HDL-cholesterol, LDL-cholesterol and HDL-cholesterol/Total cholesterol ratios of control, stressed and stressed plus *H. sabdariffa* calyx extract-treated rabbits after fifteen days.

Group	Treatment	No of rabbits (No of samples)	No of surviving rabbits	Plasma cholesterol concentration (mg/dl) Mean \pm SD (n) ^a			
				TC	LDL-C	HDL-C	HDL-C/TC
1	Control	5	5/5	74.57 \pm 9.46(5)	34.56 \pm 5.63(3)	46.80 \pm 3.44(3)	0.61
2	Stressed	5	4/5	61.18 \pm 7.57(4) ^b	54.18 \pm 15.00(2) ^b	20.56 \pm 4.45(3) ^b	0.33
3	Stressed + aq. extract	5	4/5	48.30 \pm 14.14(3) ^{b,d}	16.31 \pm 3.37(3) ^{c,e}	20.29 \pm 6.79(3) ^{b,d}	0.44

^a number of values used for mean cholesterol estimation

^b Value significantly different from group 1 value ($P < 0.05$) within the same column

^c Value not significantly different from group 1 value ($P > 0.05$) within the same column

^d Value not significantly different from group 2 value ($P > 0.05$) within the same column

^e Value significantly different from group 2 value ($P < 0.05$) within the same column

significantly decreased ($P < 0.05$) HDL-cholesterol level relative to the control group. As is also evident from the table, exposure of stressed rabbits to *H. sabdariffa* calyx extract led to significant ($P < 0.05$) increase in HDL-cholesterol when compared to the stress only group. Relative to the control group, stress caused significant decrease ($P < 0.05$) in the total cholesterol level. Administration of the extract caused a further significant decrease ($P < 0.05$) in plasma total cholesterol when compared to the control and stressed group.

The effect of 15 day stress on the rabbit plasma total cholesterol, HDL-cholesterol and LDL-cholesterol status is presented in Table 2. Also shown is the effect of aqueous extract of *Hibiscus sabdariffa* calyces on stress mediated changes in the various clans of cholesterol. Stress caused a significant decrease in rabbit plasma total cholesterol, a 17.96% decrease. Stressed rabbits treated with the extract had plasma total cholesterol that is statistically ($P < 0.05$) lower than that of the control (group 1). Stress significantly ($P < 0.05$) increased plasma LDL-cholesterol. It is also evident from the data that exposure of stressed rabbits to aqueous extract of *H. sabdariffa* calyx significantly reduced the plasma LDL-cholesterol when compared to the value of stress only rabbits. Stress rabbits had significant decreased ($P < 0.05$) HDL-cholesterol level relative to the control group. As is also evident from the table that exposure of stressed rabbits to *Hibiscus sabdariffa* calyx extract led to significantly lower HDL-cholesterol relative to the control.

From this study, it was also observed that the HDL-cholesterol/total cholesterol ratio of stressed only rabbits decreased

relative to the control. Treatment of the stressed rabbits with *Hibiscus sabdariffa* extract resulted in a HDL-cholesterol/total cholesterol ratio higher than the stressed only rabbits (Table 1 and 2).

DISCUSSION

The scientific basis for the statement that plants and their active constituents play an important role in the prevention of chronic and degenerative diseases is continuously advancing. The efficiency of *Hibiscus sabdariffa* as a functional plant has been revealed lately, especially for its anti-oxidant bioactivity²⁵. In this study, the benefits of *Hibiscus sabdariffa* were further studied using stressed male English rabbits, and cholesterol concentration levels were used as an index of stress in the rabbits. The levels were also used as a measure of the ability of *Hibiscus sabdariffa* calyx extract to ameliorate the stress. The results presented in Table 1 show that stress caused significant increase in LDL-cholesterol but a decrease in HDL-cholesterol. However, the situation was reversed when the calyx extract was administered to stressed rabbits.

The increase in LDL-cholesterol and decrease in HDL-cholesterol in the stressed rabbits corroborates earlier reports^{26,27}. Similar results were also obtained in rabbits under the effect of starvation stress²⁸. Our result agree with findings of Nayanatara et al.,²⁹ with adult albino rats exposed to chronic unpredictable stressors for 10 days that showed significantly increased LDL-cholesterol when compared to non-stressed rats. Several potential mechanisms of stress responses may explain the elevations in LDL-cholesterol. One possibility is that individual differences in stress-induced lipolysis are

responsible³⁰. Mammals have evolved so that in time of stress, extra energy is supplied to the blood in the form of metabolic fuels- namely, fatty acids and glucose. Catecholamine stimulate lipolysis in adipose tissue, through activation of hormone-sensitive lipase, leading to the breakdown of triacylglycerol into fatty acids and glycerol. This effect is sensitized by cortisol³⁰. Increased levels of fatty acid and cortisol lead to insulin insensitivity in tissues and promote triacylglycerol synthesis and apolipoprotein B secretion by the liver. These combined effects result in increased hepatic production and secretion of very low density lipoprotein, which is ultimately converted to LDL, the principal carrier of cholesterol in the blood. Another potential mechanism is stress-induced down regulation of the hepatic LDL receptor. LDL is normally cleared from the blood through binding to this receptor. LDL receptor expression is stimulated by insulin and inhibited by cortisol³¹. Stress-induced insulin resistance, together with increased production of cortisol, could delay LDL clearance by inhibiting expression of its receptor. During blood circulation, HDL-cholesterol mediates the transport of excess cholesterol from the peripheral cells to the liver for its catabolism by a pathway termed as “reverse cholesterol transport” hence increased HDL-cholesterol levels, caused by the *Hibiscus sabdariffa* calyx extract, may prove beneficial in lipid disorders and might also serve as a cardioprotective factor to prevent the gradual initiation of atherosclerotic process³². Continued treatment with *Hibiscus sabdariffa* calyx extract led to a decrease in HDL-cholesterol levels which agree with the findings of Ghislain et al.³³, thus suggesting that *Hibiscus sabdariffa* calyx

extract may only exhibit increasing effect on HDL-cholesterol in hyperlipidemic conditions³⁴.

Cholesterol serves as a precursor for the biosynthesis of cortisol, which is rapidly synthesized and secreted in response to stress. Elevations in levels of cortisol causes the body to increase energy production and utilization, and thus transiently shut-off biosynthetic pathways of cholesterol and fatty acids. These combined effects in response to stress by suspension may possibly be reasons for decreased total cholesterol concentration. However, the decrease in plasma total cholesterol of the stressed only rabbits is uncertain as stress leads to increased plasma total cholesterol. However, the treatment with *Hibiscus sabdariffa* led to significant decrease in total cholesterol when compared to the control and stress only rabbits. Results on the effect of the *Hibiscus sabdariffa* calyx extract on stress obtained from this study also agrees with earlier reports^{25,35}. The anti-hypercholesterolemic action of the aqueous extract of *Hibiscus sabdariffa* may be due to increased inhibition of intestinal absorption of cholesterol, interference with lipoprotein production, increased expression of hepatic LDL-receptors and their protein leading to an increased removal of LDL-cholesterol from the blood and its increased degradation and cholesterol metabolism in the body. All these either singly or in combination may have led to decreased plasma LDL-cholesterol which may have also reduced plasma total cholesterol level during the treatment with *Hibiscus sabdariffa* calyx aqueous extract^{36,37}.

The lipoprotein fractions are more predictive of developing coronary artery disease instead of total cholesterol. LDL-

cholesterol is well recognized as a risk factor and HDL-cholesterol as a protective factor against atherosclerosis³⁸. It has been shown that HDL-cholesterol/total cholesterol ratio is one of the most powerful predictors of risk of developing coronary artery diseases²⁷. An additional justification for using this ratio might be that it includes the amount of cholesterol in the triacylglycerol-rich VLDL fraction, which also positively correlates with coronary artery disease risk³⁹. Our findings on the effect of stress on the HDL-cholesterol/total cholesterol ration agrees with the results obtained by forced immobilization stress on rabbits²⁷. Reduction in hyperlipidemia occurs simultaneously with an increase in the HDL-cholesterol/total cholesterol ratio which is associated with a reduced incidence of atherosclerosis⁴⁰.

CONCLUSION

The hypocholesterolemic effect of aqueous extract of *Hibiscus sabdariffa* has been shown by the dual effect it exhibited in lowering plasma total and LDL-cholesterol in the rabbits. Evidently, stress caused increased plasma level of the “bad cholesterol” and a decrease in “good cholesterol”. *Hibiscus sabdariffa* calyx extract however caused a reverse of the changes brought about by the stress by decreasing “bad cholesterol” and increasing “good cholesterol”. Thus stress potentiates increased risk of cardiovascular diseases while *Hibiscus sabdariffa* calyx extract may prove beneficial in lipid disorder, and serve as a cardio-protective factor to prevent cardiovascular disease. It can therefore be concluded that *Hibiscus sabdariffa* is a good anti-stress agent.

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