

Hypoglycemic and Hypolipidemic Effect of Methanol Extract of *Hibiscus Sabdariffa* Seed in Alloxan Induced Diabetic Albino Rats

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ABSTRACT

This study investigated the hypoglycemic and hypolipidemic effect of the oral administration of methanol extract of *Hibiscus sabdariffa* seed in alloxan induced diabetic albino rats. Two different doses (200 and 400 mg/kg) of the methanol extract of *Hibiscus sabdariffa* seed were administered daily to two groups of diabetic rats for a period of two weeks. Metformin (250 mg/kg) was given as the reference standard drug. Phytochemical, acute toxicity, fasting blood glucose, body weight, cholesterol, triglycerides and lipoproteins levels were determined using standard methods. Acute toxicity of methanol extract of *Hibiscus sabdariffa* seed was estimated to be greater than 5000 mg/kg body weight. There was an initial decrease in the body weight of the experimental animals after induction of diabetes which increased significantly ($p < 0.05$) by the second week of treatment. The fasting blood glucose levels of the treated diabetic rats were significantly decreased at the second week of treatment with the group that received 400 mg/kg of extract recording the lowest fasting blood glucose level. The cholesterol, triglycerides and low density lipoproteins levels of the diabetic treated rats were significantly reduced while the high density lipoproteins level significantly increased. It can therefore be concluded that *Hibiscus sabdariffa* seed has the ability to lower blood sugar and maintain a stable lipid level.

Keywords: Hypoglycemic, hypolipidemic, Alloxan, Phytochemical, *Hibiscus sabdariffa*

INTRODUCTION

Diabetes mellitus is a metabolic disorder distinguished by a persistent elevation in the blood glucose level. This persistent rise may either be triggered by the inability of β -cells of the pancreas to secrete insulin or the failure of the liver/skeletal muscles cells to respond to insulin action (Ruud *et al.*, 2017). These are the Type 1 and 2 diabetes mellitus which are the most common. The breakdown of insulin producing cells by auto immune response is the leading cause of Type 1 diabetes mellitus while Type 2 diabetes mellitus are caused by insulin resistance and a progressive insulin secretory defect due to the failure of β -cells (Mhya *et al.*, 2019). Alloxan is a compound experimentally used to induce Type 1 diabetes and in so doing, selectively destroys the pancreatic insulin producing β -cells (Ankur and Ali, 2012). Lifestyle and physical inactivity are some of the leading cause of diabetes mellitus affecting millions of people with high frequency rate worldwide. There are also evidences that genetic and environmental factors could lead to hyperglycaemia, dyslipidaemia and inflammation, resulting in β -cell dysfunction,

thereby triggering the pathogenesis of diabetes (Fu *et al.*, 2013).

Medicinal plants have long been used by traditional practitioners in management and control of diabetes in Nigeria with reduced burdens of unwanted side effects associated with the use of synthetic anti-diabetic drugs in the market.

Hibiscus sabdariffa L. (Malvaceae) also known as roselle is a local delicacy plant in the Northern and Western Nigeria. It is used traditionally as a flavouring agent in food industries, in beverages and in herbal medicines. *Hibiscus sabdariffa* extracts of different parts have been reported to exhibit antibacterial, anti-oxidant, nephro- and hepato-protective effects (Da-Costa-Rocha *et al.*, 2014). In this study, the hypoglycemic and hypolipidemic effects of methanol extract of *Hibiscus sabdariffa* seed in alloxan induced diabetic albino rats was evaluated.

MATERIAL AND METHODS

Plant material

Hibiscus sabdariffa (Rossel) plant was collected from Minna and identified at the department of Biological Science, Federal University of Technology, Minna, Nigeria. The pods of the seed were obtained by the removal of the flowery calyces of the plant. The pods were then cracked to access the seed. The seed were air dried under shade at ambient room temperature and ground with an electric grinder.

Plant Extraction

The powdered seed (600g) was extracted with 70% methanol (v/v methanol – water mixture) at a ratio of 1:5 (w/v) under reflux and the extraction lasted for 2 h as described by Ogbadoyi *et al.* (2011). Afterwards, the methanol extract was filtered with clean white cotton cheese cloth and filter paper (Whatmann filter paper no.1), concentrated using a rotary evaporator (Heidolph Germany) and finally dried in water bath at 50°C.

Qualitative Phytochemical Screening

Qualitative phytochemical screening of the methanol extract of *Hibiscus sabdariffa* seed was carried out according to the methods described by Trease and Evans (1989) and Harborne (1973) to determine the presence of alkaloids, tannins, flavonoids, phenols, steroids, anthraquinones, terpenoids, saponins, glycosides and phlobatannins.

Acute Toxicity Study

Acute toxicity study was carried out in two phases as described by Lorke (1983). The first phase consist of three groups of animals which were selected randomly comprising three animals each, these groups were orally administered 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight of the methanol seeds extract respectively and were observed for 24 h. In the absence of death, the second phase was carried out which also consist of three animals each in three groups selected randomly, they were orally dosed with 1600 mg/kg, 2900 mg/kg and 5000 mg/kg respectively and observed for 24 hours.

Experimental Animals

Albino rats weighing between 110g and 145g were housed in plastic cages and allowed to acclimatize for two weeks at 26±2 °C room temperature and kept under twelve hours of dark/light cycle with free access to feed and water.

Alloxan Induction of Rats and Treatment Protocol

Alloxan was intraperitoneally injected at a single dose of 150mg/kg body weight in overnight fasted rats. Fasting blood glucose levels were measured after three days and the rats with blood glucose level above 150 mg/dl were considered diabetic.

Sixteen rats were randomly divided into five groups of four animals each described as follows:-

Group A: Non diabetic (normal control); Group B: Diabetic untreated; Group C: Diabetic treated with 200 mg/kg body weight of methanol extract; Group D: Diabetic treated with 400 mg/kg body weight of methanol extract. Group E: Diabetic treated with 250mg/kg body weight of Metformin. Treatment with extract followed for a period of 14 days. The fasting blood glucose was determined using an accu-check glucometer kit. The rats were fasted for 8 – 12 h after which the tails were punctured and blood dropped on the strip, which was inserted into the glucometer to obtain the blood glucose concentration in mg/dl for each rat in all the five groups at an interval of 3, 7 and 14 days. Weight changes of the rats were monitored weekly throughout the treatment period. On the 14th day of treatment, animals were fasted for 8-12 h after which blood samples was collected by cardiac puncture using chloroform as anesthesia.

Estimation of Lipid Profile

Serum total cholesterol and triglycerides were determined by method described by Yadav *et al.* (2008). High density cholesterol (HDL) cholesterol was determined by direct method (Sugiuchi *et al.*, 1995). Low density cholesterol

(LDL) was calculated using Friedewald's equation (Friedewald, 1972):-

$$\text{LDL} = \text{Total cholesterol} - \text{HDL} - (\text{Triglycerides}/5)$$

Statistical Analysis

The data obtained was analyzed by one-way analysis of variance (ANOVA) using SPSS software version 16.0. The results were expressed as mean \pm standard deviation (SD). Significant differences between the animal groups were compared using the Duncan Multiple Range Test. A probability level of less than 5 % ($p < 0.05$) was considered significant.

RESULTS

Qualitative Phytochemical Screening

The findings of the qualitative phytochemical screening of methanol extract of *Hibiscus sabdariffa* seed is shown in Table 1; Results revealed the presence of tannins, saponins, glycosides, steroids, phlobatannins, flavonoids, phenols and alkaloids while anthraquinones and terpenoids were absent.

Table 1: Qualitative phytochemical screening of methanol extract of *Hibiscus sabdariffa* seed

Phytochemicals	Result
Tannins	+
Saponins	+
Glycosides	+
Steroids	+
Phlobatannins	+
Flavonoids	+
Phenols	+
Alkaloids	+
Anthraquinones	-
Terpenoids	-

Note: + = detected; - = not detected

Acute Toxicity Test

There was no mortality recorded in the first and second phases of the oral administration of methanol extract of *Hibiscus sabdariffa* seed at doses of 10 mg/kg, 100 mg/kg, 1000 mg/kg, 1600 mg/kg, 2900 mg/kg and 5000 mg/kg body weight of rats respectively. Although, behavioural changes were observed at higher doses of 2900 mg/kg and 5000 mg/kg such as

isolation, increase in rate of breathing and scratching which lasted for about 3 hours, after which the animals resumed and maintained their normal activities.

Effect of Methanol Extract of *Hibiscus Sabdariffa* Seed on Body Weight of Alloxan Induced Diabetic Albino Rats

The changes in the body weight are presented in Figure 1. Diabetes induced by alloxan resulted in a significant ($p < 0.05$) reduction in the body weight of rats in groups B - E by the 3rd day and progressively continued in the untreated group (Group B). While the treated groups (Groups C - E) showed a significant ($p < 0.05$) increase in body weight by the 2nd week of treatment.

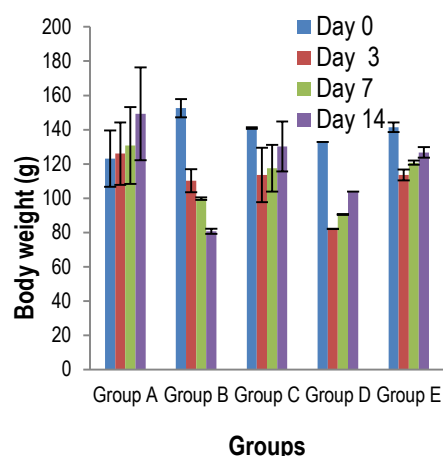


Figure 1: Effect of methanol extract of *Hibiscus sabdariffa* (MEHs) seed on body weight of alloxan induced diabetic albino rats. Group A: Normal control; Group B: Diabetic untreated; Group C; diabetic treated with 200 mg/kg MEHs; Group D: diabetic treated with 400 mg/kg MEHs and Group E: diabetic treated with 250 mg/kg MEHs.

Effect of methanol extract of *Hibiscus sabdariffa* seed on fasting blood glucose levels of alloxan induced diabetic albino rats

Administration of methanol extract showed a significant reduction ($p < 0.05$) of the fasting blood glucose level in all treated diabetic groups except for 200 mg/kg treated rats (group C) which showed a decrease at week 1 and then an increase at week 2 (Table 2).

Table 2: Effect of methanol extract of *Hibiscus sabdariffa* seed on fasting blood glucose levels of alloxan induced diabetic albino rats.

Treatment	After induction (mg/dl)	Treatment wk1 (mg/dl)	Treatment wk2 (mg/dl)
Group A	63.75±8.88 ^a	66.50±6.61 ^a	71.50±1.29 ^c
Group B	232.00±1.82 ^c	221.75±3.59 ^d	240.50±6.45 ^e
Group C	201.75±69.51 ^c	76.50±6.61 ^b	86.00±6.38 ^d
Group D	214.50±1.29 ^c	72.12±0.85 ^{ab}	50.00±0.71 ^a
Group E	150.50±11.82 ^b	96.50±3.69 ^c	61.50±1.29 ^b

Key: Mean ± SD; Values with different superscripts down the column differ significantly ($p < 0.05$). wk = week. Group A: Normal control; Group B: Diabetic untreated; Group C; diabetic treated with 200 mg/kg MEHs; Group D: diabetic treated with 400 mg/kg MEHs and Group E: diabetic treated with 250 mg/kg MEHs.

Table 3: Effect of methanol extract of *Hibiscus sabdariffa* seed on lipid profile of alloxan induced diabetic albino rats.

Treatment	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Group A	48.20±1.55 ^a	52.79±0.76 ^a	32.80±1.55 ^b	4.85±0.14 ^a
Group B	131.76±0.62 ^d	96.55±0.07 ^b	25.84±0.11 ^a	86.66±0.59 ^d
Group C	53.49±0.22 ^c	38.95±17.36 ^a	36.62±5.11 ^{bc}	8.92±1.64 ^b
Group D	53.38±0.07 ^c	51.38±0.38 ^a	31.61±0.12 ^b	11.49±0.11 ^c
Group E	50.25±0.07 ^b	54.94±0.07 ^a	30.35±0.07 ^b	8.91±0.12 ^b

Data are mean ± SD; Values with different superscripts down the column differ significantly ($p < 0.05$). . Group A: Normal control; Group B: Diabetic untreated; Group C; diabetic treated with 200 mg/kg MEHs; Group D: diabetic treated with 400 mg/kg MEHs and Group E: diabetic treated with 250 mg/kg MEHs; HDL: High density lipoproteins; LDL: Low density lipoproteins.

Effect of the administration of methanol extract of *Hibiscus sabdariffa* seed on lipid profile of alloxan induced diabetic albino rats

The cholesterol, triglycerides, low density lipoproteins (LDL) concentrations of the untreated diabetic groups increased significantly ($p < 0.05$) when compared with those of normal control group as presented in Table 3. However, rats treated with methanol extract had significantly reduced concentrations of cholesterol, triglycerides, low density lipoproteins (LDL) and increased concentration of high density lipoproteins (HDL).

alkaloids) revealed from the screening of methanolic extract of *Hibiscus sabdariffa* seeds could be responsible for the significant reduction in the fasting blood glucose level and lipid profile in all diabetic rats treated with the plant seed extract.

The absence of death from acute toxicity study of the methanolic extract of *Hibiscus sabdariffa* seeds at a dose up to 5000 mg/kg is in agreement with findings of Gaya et al. (2009), who reported similar result. This may imply that the extract has low toxicity level and a wide margin of safety.

DISCUSSION

Medicinal plants are traditionally used in treatment of different ailments owing to their efficacy. This may be due to the presence of some essential phytochemicals. The phytochemical (tannins, saponins, glycosides, phlobatannins, flavonoids, phenols and

Alloxan induced diabetes is associated with a characteristic reduction in body weight of animals which was observed in the untreated diabetic rats (group B). While the diabetic treated rats (groups C, D and E) showed increased body weight after 2 weeks of treatment. This may be due to the effect of the

extract in controlling muscle wasting and tissue protein loss (Ewenighi *et al.*, 2015).

Blood glucose level at the 3rd day of administering alloxan was increased significantly in all the diabetes induced groups B to E compared with the normal control group A. This increase in the blood glucose levels maybe as a result of the insensitivity of the cells to insulin or the destruction of the insulin producing beta cells of the pancreatic islets (Ankur and Ali, 2012). It was observed a week after diabetes induction that the blood glucose levels in groups B and C rats reduced but significantly increased by the 2nd week of treatment. This fluctuations observed in the blood glucose levels in groups B and C may be due to an attempt by the β -cells of the pancreas to regenerate itself after exposure to alloxan. The significant decreased blood glucose level in the treated groups of rats compared with the untreated rats could be due to the presence of one or more phytochemicals contained in the methanolic seeds extract of *Hibiscus sabdariffa* capable of either enhancing insulin secretion or regenerating insulin-producing pancreatic beta-islets (Farzaei *et al.*, 2015). Flavonoids and phenols have been reported to possess antioxidant activities which could be responsible for the antidiabetic properties of the extract via insulin modulation (Mashi *et al.*, 2019). Saponins are effective in lowering blood glucose levels either by restoring insulin response, the induction of the release of insulin from the pancreas.

Dyslipidemia is a condition characterized by high or low levels of lipids in the blood leading to cardiovascular diseases. It is associated with diabetes wherein insulin resistance affects the regulation of lipids and lipoproteins metabolism. In this study, the diabetic untreated rats had significantly elevated cholesterol, triglycerides, LDL and low HDL compared to the normal control rats. The methanol seed extract of *Hibiscus sabdariffa* administered to the diabetic treated rats remarkably reduced the cholesterol, triglyceride and LDL concentrations while HDL concentrations were significantly increased. Saponins, have been reported to possess

ability to reduce lipid profile by effectively reducing absorption of fats after digestion by pancreatic lipases thereby inhibiting adipogenesis (Marrelli *et al.*, 2016).

CONCLUSION

The methanol extract of *Hibiscus sabdariffa* seed was found to be of low toxicity and it reduced the fasting blood glucose level progressively at the dose of 400 mg/kg. The methanol extract of *Hibiscus sabdariffa* seed also helped in maintaining a stable lipid and lipoprotein profile. Thus, *Hibiscus sabdariffa* seed may be a potent source of anti-diabetic agents and a beneficial remedy for the management of hyperlipidemia.

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