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# Hypolipidemic properties of *bauhinia rufescens* in alloxan-induced diabetic rats

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ABSTRACT

The present investigation was aimed at evaluating the hypolipidemic properties of methanolic leaf extracts of *Bauhinia rufescens* (*MLEBR*) on alloxan-induced diabetic rats. Alloxan was injected intraperitoneally as a single dose of 150mg/kg for diabetes induction in the rats. Animals were orally treated for 4 consecutive weeks with *MLEBR* at doses of 200, 300 400mg/kg and Glibenclamide. The effects of the extracts and Glibenclamide on lipid profile and body weight (BW) were examined on the diabetic rats. Total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLD-C) and triglycerides (TG) levels at sacrifice (day 29) were estimated. Normal and Diabetic rats that were given normal saline only were used for comparison. Alloxan-induced diabetic rats showed moderate to significant increases in the levels of TC, LDL-C, VLDL-C and TG while body weight and HDL-C levels decreased compared to controls (non diabetic rats). Administration of the plant extracts to Alloxan-induced diabetic rats resulted in a significant decrease in TC, LDL-C, VLDL-C and TG and the dose 200 mg/kg of the *MLEBR* was the most effective; HDL-C level was markedly increased after four weeks post treatment compared to untreated diabetic rats. It can also be noticed that the *MLEBR,* especially the dose 200 mg/kg ($p<0.01$), produced more effects than glibenclamide. Rats treated with glibenclamide (5mg/kg) generally gave lower results compared to groups treated with the plant extracts. Results of the present study showed that methanolic leaf extracts of *Bauhinia rufescens* has beneficial effects on diabetic

hyperlipidemia as such could be advanced in preventing the development of atherosclerosis and possible related cardiovascular pathologies associated with diabetes.



**1. Introduction**

Diabetes mellitus is a broadly applied term used to denote a complex group of syndromes that have in common a disturbance in the oxidation and utilization of glucose, which is secondary to a malfunction of the beta cells of the pancreas, whose function is the production and release of insulin. Because insulin is involved in the metabolism of carbohydrates, proteins and fats, diabetes is not limited to a disturbance of glucose homeostasis alone (Saunders Comprehensive Veterinary Dictionary. 2007). Diabetes mellitus is a chronic disease that causes serious health complications including renal (kidney) failure, heart disease, stroke, and blindness. Diabetes mellitus clutches a group of chronic disorders characterized by derangement in carbohydrates, proteins and fat metabolisms caused by hyperglycemia or reduced insulin secretion or action or both. (American Diabetes Association, 2007).

The various forms of diabetes have been organized into categories developed by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus of the American Diabetes Association. Type 1 diabetes mellitus in this classification scheme includes patients with diabetes caused by an autoimmune process, dependent on insulin to prevent ketosis. This group was previously called type I, insulin-dependent diabetes mellitus, juvenile-onset diabetes, brittle diabetes, or ketosis-prone diabetes. Patients with type 2 diabetes mellitus are those previously designated as having type II, non-insulin-dependent diabetes mellitus, maturity-onset diabetes, adult-onset diabetes, ketosis-resistant diabetes, or stable diabetes. Those with gestational diabetes mellitus are women in whom glucose intolerance develops during pregnancy. Other types of diabetes are associated with a pancreatic disease, hormonal changes, adverse effects of drugs, or genetic or other anomalies.

In diabetes, the plasma cholesterol level is usually elevated, and this plays a role in the accelerated development of the atherosclerotic vascular disease that is a major long-term complication of diabetes in humans. The rise in plasma cholesterol level is due to an increase in the plasma concentration of very low density lipoprotein (VLDL) and low density lipoprotein (LDL), which may be due to increased hepatic production of VLDL or decreased removal of VLDL and LDL from the circulation.

In individuals with elevated plasma cholesterol levels, there is an increased incidence of atherosclerosis and its complications. The normal range for plasma cholesterol is said to be 120-200 mg/dL, but in men, there is a clear, tight, positive correlation between the death rate from ischemic heart disease and plasma cholesterol levels above 180 mg/dL. Furthermore, it is now clear that lowering plasma cholesterol by diet and drugs slows and may even reverse the progression of atherosclerotic lesions and the complications they cause.

In evaluating plasma cholesterol levels in relation to atherosclerosis, it is important to analyse the LDL and high density lipoprotein (HDL) levels as well. LDL deliver cholesterol to peripheral tissues, including atheromatous lesions, and the LDL plasma concentration correlates positively with myocardial infarctions and ischemic strokes. On the other hand, HDL pick up cholesterol from peripheral tissues and transport it to the liver, thus lowering plasma cholesterol.

It is interesting that women, who have a lower incidence of myocardial infarction than men, have higher HDL levels. In addition, HDL levels are increased in individuals who exercise and those who drink one or two alcoholic drinks per day, whereas they are decreased in individuals who smoke, are obese, or live sedentary lives. Moderate drinking decreases the incidence of myocardial infarction, and obesity and smoking are risk factors that increase it. Plasma cholesterol and the incidence of cardiovascular diseases are increased in familial hypercholesterolemia, due to various loss-of-function mutations in the LDL receptors (Ganong, 2003).

*Bauhinia rufescens* is a much-branched shrub or small tree reaching 5-8 m in height. Bark white to grey, smooth, fibrous and scaly in the old, slash pink. Twigs arranged in one plane like a fishbone, with ca 10 cm long thorn-like lignified lateral shoots. Leaves small, up to 2.5-4 cm long, glabrous, greyish green, bilobed almost to the base in cow-hoof shape, as in most species of the genus *Bauhinia* (Burkill, 1995).

Today, people around the globe are giving preference to alternative medicines such as ayurveda, naturopathy, homeopathy and herbal medicine due to low cost, easy availability and lesser side effects. Aliyu *et al*., (2009) reported that the methanolic leaves extracts of *Bauhinia rufescens* has promising antioxidant agents and may be helpful in the treatment of the diseases caused by free radicals. Several Bauhinia species are utilized as folk medicines worldwide, including Africa, Asia, South America and Central America (Ayensu, 1978).

## 2. Materials and methods

### 2.1. Collection of plant materials

The leaves of *Bauhinia rufescens* were collected in October 2010 from different localities within Samaru Zaria of Kaduna State Nigeria. The plant materials were authenticated by Malam M. Musa at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

The leaves of the plant were washed with distilled water and dried in shade, pulverized by mechanical grinder to get fine powder and stored in an airtight container.

### 2.2. Experimental animals

Thirty male Wistar strain albino rats weighing 170 – 240g were obtained from animal house of the Department of Pharmacology and Therapeutic, Ahmadu Bello University, Zaria. The animals were kept in well aerated polypropylene cages under standard laboratory condition and were allowed to acclimatize for a period of 2 weeks before the commencement of the experiment. The animals were maintained on standard animal feeds and drinking water *ad libitum* throughout the study. This research was carried out in accordance with the rules governing the use of laboratory animals as accepted internationally.

### 2.3. Preparation of the plant extracts

The powdered material (250g) obtained was macerated in 1.5L of methanol with regular shaking for 36 hours. The mixture was then filtered and the filtrate evaporated to dryness in a water-bath and the residue kept until needed (Njike, 2005). The extracts obtained were also subjected to phytochemical analysis.

### 2.4. Induction of experimental diabetes

Diabetes mellitus was induced in a batch of normoglycaemic albino rats starved for 12 hours by injecting, intraperitoneally, single dose of alloxan monohydrate (150 mg/kg body weight) reconstituted in sterile saline (Sharma, 2010). After 5 days, blood samples were obtained by tail snip method and blood sugar level of each animal determined using a glucometer (Accu Check Advantage, Roche, USA). All rats with blood glucose concentration of greater than 250 mg/dL were considered as hyperglycaemic (Sharma, 2010), and were selected for the study.

### 2.5. Treatment of rats with plant extract and glibenclamide

In this experiment, a total of 30 rats were divided into 6 groups of 5 rats each for the antilipidemic study. The groupings were as follows:

Group A: served as normal control and did not receive any treatment
Group B: Diabetic rats that received normal saline (5 ml/kg b.w.).
Group C: Diabetic rats were treated with MLEBR of graded dose *200*mg/kg b.w.
Group D: Diabetic rats received MLEBR of graded dose *300*mg/kg b.w.
Group E: Diabetic rats were treated with MLEBR of graded dose 4*00*mg/kg b.w.
Group F: Diabetic rats + glibenclamide 5mg/kg b.w. and served as positive control (Sharma, 2010).

### 2.6. Biochemical estimations and body weight measurements

On the last day, blood samples were collected from overnight fasted animals by cervical dislocation under mild anaesthesia for biochemical estimations.

About 4ml of blood collected from each rat was put into centrifuge tubes and centrifuged in a Denley BS400 centrifuge (England) at 5000 r.p.m. for 5-minutes. The supernatant (serum) collected was used for the biochemical estimations. The serum levels of triglyceride (TGL), total cholesterol (TC) and high-density lipoprotein-cholesterol

(HDLC) were determined spectrophotometrically, using enzymatic colorimetric assay kits (Randox, Northern Ireland) while low-density lipoprotein cholesterol (LDLC), very low-density lipoprotein cholesterol (VLDLC) and atherogenic index (TC/HDLC ratio) were obtained by calculations. The weekly body weights of the animals were measured using a top loader weighing balance.

**2.7. Assay for triglycerides**

The serum levels of TGL were determined by the method as described by Tietz *et al.* (1990). 1000µl of the reagent was added to 10µl each of the sample and standard. These were incubated for 10 minutes at 20-25 $^{0}$ C and the absorbance of the sample ($A_{sample}$) and standard ($A_{standard}$) measured against the reagent blank within 30 minutes.

$$\text{TGL concentration} = \frac{\text{A sample}}{\text{A standard}} \text{X} 2.29 \text{mmol/L}$$

**2.8. Assay for total cholesterol**

The serum levels of TC were determined after enzymatic hydrolysis and oxidation of the sample (Richmond, 1973; Roeschlau *et al., 1974).* 1000µl of the reagent was added to 10µl each of the sample and standard. These were incubated for 10 minutes at 20-25 ° C and the absorbance of the sample ($A_{sample}$) and standard ($A_{standard}$) were measured against the reagent blank within 30 min.

$$\text{TC Concentration} = \frac{\text{A sample}}{\text{A standard}} \text{X} 5.17 \text{mmol/L}$$

**2.9. High density lipoprotein cholesterol (HDLC) assay**

Low-density lipoproteins (LDL and VLDL) and chylomicron fractions in the sample were precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 minutes at room temperature, centrifuged for 10 minutes at 4000rpm. The supernatant represents the HDLC fraction. The cholesterol concentration in the HDL, which remained in the supernatant, was determined, and taken as high density lipoprotein cholesterol concentration.

**2.10. Low density lipoprotein – cholesterol**

The concentrations of LDL cholesterol were derived through calculations in mmol/L using Friedewald's equation as stated below (Friedewald *et al*.,1972).

$$\text{LDLC} = \text{TC} - (\text{HDLC} + \tfrac{\text{TGL}}{2.2})$$

**2.11. Very low density lipoprotein – cholesterol**

The concentration of VLDL cholesterol were derived by calculations in mmol/L using Friedewald's equation as stated below (Friedewald *et al*., 1972).

$$\text{VLDL} = \frac{\text{TGL}}{2.2}$$

**2.12. Statistical analysis**

Results were expressed as mean ± standard error of mean. The data was statistically analysed using analysis of variance (ANOVA) with multiple comparisons versus control groups. The values of $p<0.05$ was considered as significant.

**3. Results**

A significant decrease ($P<0.05$) in the plasma total cholesterol (CHOL) level was observed in all the diabetic animals treated with extract or Glibenclamide when compared with untreated diabetic control group, while a very significant increase ($P<0.01$) in CHOL was observed in the untreated diabetic rats (Table 1). There was also a significant decrease ($P < 0.05$) in TRIGS, VLDL, and LDL levels while a non-significant increase in HDL were observed

in all diabetic animals treated with extract or glibenclamide. The untreated diabetic rats showed a significant increase in TRIGS, VLDL, and LDL levels and a significant decrease in HDL levels (Table 1 and 2). There was no significant change (P>0.05) observed in potassium sodium level and packed cell volume (PCV) in all the six experimental groups (Table 2). The atherogenic index value, HDL:TC (ratio of HDL to total cholesterol) were significantly (P<0.05) reduced in all extract treated diabetic groups or the glibenclamide when compared to the untreated diabetic control group. The atherogenic index value was significantly increased (P<0.05) in untreated diabetic rats when compared to normal control rats (Table 2). The normal control, untreated diabetic control and treated diabetic groups showed increase in the mean weekly body weights. These increase are not statistically significant (p>0.05). The untreated diabetic rats showed less drastic increase when compared with normal control or treated groups. The treated diabetic groups showed progressive and steady increase in their mean weekly body weights (Table 3).

**Table 1**

Effects of methanolic leaf extract of *Bauhinia rufescens* on serum total cholesterol, triglycerides, very low density lipoprotein (VLDL), and high density lipoprotein cholesterol (HDL) in alloxan induced diabetic rats.

| Group | CHOL (mg/dL) | TRIGS (mg/dL) | VLDL (mg/dL) | HDL (mg/dL) |
|---|---|---|---|---|
| A | 112.0±9.0 | 138.2±14.2 | 63.2±6.4 | 45.0±4.9 |
| B | 152.2±1.8$^{\#\#}$ | 179.0±10.2$^{\#}$ | 81.6±4.6$^{\#}$ | 41.8±4.2$^{ns}$ |
| C | 114.4±5.1** | 116.8±6.6*** | 52.8±2.9*** | 54.0±5.7$^{ns}$ |
| D | 124.8±16.7* | 136.4±10.2** | 61.6±4.6** | 47.8±5.9$^{ns}$ |
| E | 122.0±9.6* | 141.8±11.2* | 64.0±5.0* | 46.2±2.9$^{ns}$ |
| F | 99.6±1.3*** | 122.2±8.2*** | 55.6±3.9*** | 43.0±3.0$^{ns}$ |

Values are expressed as mean ± S.E.M., n =5, *P<0.05, **P<0.01 when compared with diabetic control, $^{\#}$P<0.05, $^{\#\#}$P<0.01 when compared with normal control.

Group A: normal control; Group B: diabetic control; Group C: diabetic rats treated with 200mg/kg of extract; Group D: diabetic rats treated with 300mg/kg b.w. of extract; Group E: diabetic rats treated with 400mg/kg b.w. of extract; Group F: diabetic rats treated with glibenclamide 5mg/kg b.w. ns: not significant.

**Table 2**

Effects of methanolic leaf extract of *Bauhinia rufescens* on Low Density Lipoprotein (LDL), Total Cholesterol High Density Liprotein Cholesterol ratio, atherogenic Index, and Packed Cell Volume in alloxan induced diabetic rats.

| Group | LDL (mg/dL) | TC:HDL | AAI | PCV (%) |
|---|---|---|---|---|
| A | 36.8±5.2 | 2.56±0.20 | 68.8±10.2 | 37±2.3 |
| B | 75.6±5.9$^{\#}$ | 3.76±0.34$^{\#}$ | 38.8±6.6 | 32±2.0$^{ns}$ |
| C | 37.8±8.2* | 2.24±0.33* | 107.6±31.0$^{*}$ | 29±1.$^{ns}$ |
| D | 50.2±21.8$^{ns}$ | 2.88±0.66$^{ns}$ | 86.0±29.9$^{*}$ | 34±1.6$^{ns}$ |
| E | 48.0±12.9$^{ns}$ | 2.78±0.46$^{ns}$ | 76.4±25.2$^{*}$ | 33±1.7$^{ns}$ |
| F | 33.2±5.5* | 2.40±0.25* | 78.4±9.1$^{*}$ | 31±1.6$^{ns}$ |

Values are expressed as mean ± S.E.M., n =5, *P≤0.05 when compared with diabetic control, $^{\#}$P<0.05 when compared with normal control.

Group A: normal control; Group B: diabetic control; Group C: diabetic rats treated with 200mg/kg of extract; Group D: diabetic rats treated with 300mg/kg b.w. of extract; Group E: diabetic rats treated with 400mg/kg b.w. of extract; Group F: diabetic rats treated with glibenclamide 5mg/kg b.w. ns: not significant.

## 4. Discussion

Hyperglycaemia and dyslipidemia as well as oxidative stress generally coexist in diabetes subjects. Dyslipidemia which includes not only quantitative but also qualitative abnormalities of lipoprotein, plays a significant role in the

proatherogenesis of vascular complications in diabetes (Sobngwi *et al*., 2001; Beckman *et al.,* 2002; Rotimi *et al*., 2011). High cholesterol levels and hyperlipidemia are associated consequences of diabetes (Mironova *et al*., 2000; Odetola *et al.,* 2006). The present study showed an increase in the concentration of total cholesterol, triglyceride, very low density lipoprotein cholesterol (VLDL), low-density lipoprotein cholesterol (LDL-C) and decrease in HDL-C in alloxan-induced diabetic untreated rats (Table 1 and 2).

**Table 3**
Effects of methanolic leaf extract of *Bauhinia rufescens* on body weight in alloxan induced diabetic rats.

| Group | Body weight of the animal (g) | | | | |
|---|---|---|---|---|---|
| | Week0 | Week1 | Week2 | Week3 | Week4 |
| A | 199.2±5.05 | 205.8±5.45 | 209.6±5.39 | 214.0±4.15 | 220.2±5.25 |
| B | 190.8±7.91 | 188.4±9.62 | 189.0±8.02 | 194.4±7.35 | 195.4±8.54 |
| C | 187.2±5.52 | 189.6±6.85 | 194.6±7.10 | 194.8±7.10 | 200.0±9.32 |
| D | 198.4±12.60 | 197.2±14.18 | 202.0±16.43 | 206.8±16.65 | 212.0±17.06 |
| E | 182.6±4.82 | 182.4±2.54 | 191.6±3.35 | 196.6±6.03 | 200.8±6.59 |
| F | 206.6±9.40 | 210.6±11.09 | 212.6±12.58 | 217.6±13.89 | 221.0±17.44 |

Values are expressed as mean ± S.E.M., n =5.
Group A: non diabetic rats treated with distilled water; Group B: diabetic rats untreated; Group C: diabetic rats treated with 200mg/kg of extract; Group D: diabetic rats treated with 300mg/kg b.w. of extract; Group E: diabetic rats treated with 400mg/kg b.w. of extract; Group F: diabetic rats treated with glibenclamide 5mg/kg b.w.

Diabetic-induced hyperlipidemia is attributable to excess mobilization of fat from the adipose tissue due to underutilization of glucose (Krishnakumar *et al.,* 2000; Nimenibo-uadia, 2003). The lack of insulin and elevations of the counter-regulatory hormones lead to activation of enzymes (hormone-sensitive lipase) that stimulate lipolysis and enhanced release of free fatty acids from adipose tissue (Subbiah *et al.,* 2006; Rotimi *et al*., 2011). The fatty acids from adipose tissues are mobilized for energy purpose and excess fatty acids are accumulated in liver, which are converted to triglyceride (Suryawanshi *et al.,* 2006). The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of unlimited actions of lipolytic hormones on the fat depots (Claudia *et al.*, 2006). Lowering of serum lipids levels through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease in diabetes (Claudia *et al.*, 2006). In this study, administration of all doses of methanolic leaf extract of *Bauhinia rufescens* significantly reduced serum levels of total cholesterol, triglyceride, low-density lipoprotein, atherogenic index and increased serum levels of high-density lipoprotein cholesterol in the alloxan-induced diabetic treated rats. Many dietary factors have been reported to contribute to the ability of herbs to improve dyslipidemia (Nimenibo-uadia, 2003; Rotimi *et al.*, 2011). Preliminary phytochemical screening of the extract revealed the presence of saponin among other polyphenolic compounds. This may be responsible for the lipid-lowering effect of *Bauhinia rufescens* on plasma lipid. Saponins are known antinutritional factors, which lower cholesterol by binding with cholesterol in the intestinal lumen, preventing its absorption, and/or by binding with bile acids, causing a reduction in the enterohepatic circulation of bile acids and increase its faecal excretion (James *et al.,*2010 ; Nimenibo-uadia, 2003 ; Rotimi et al., 2011). Increased bile acid excretion is offset by enhanced bile acid synthesis from cholesterol in the liver and consequent lowering of the plasma cholesterol (Rotimi *et al.,* 2011). Hence, saponins have been reported to have hypocholesterolic effect (James *et al.,* 2010). Kumarappen *et al*., (2007) reported that administration of polyphenolic to alloxan-induced diabetic rats reduced hyperlipidemia, and attributed this to a reduction in the activity of hepatic HMG-CoA reductase, which is the first committed enzymatic step of cholesterol synthesis. This lowers elevated LDL cholesterol levels, resulting in a substantial reduction in coronary events and deaths from coronary heart disease (CHD) that occurs in diabetics (Richard and Pamela, 2009). Thus, the observed hypolipidemic effect of *Bauhinia rufescens* can therefore be linked to the synergistic actions of phytochemicals. It is reported that the derangement of glucose, fat and protein metabolism during diabetes, results into the development of hyperlipidemia (Austin and Hokanson. 1994; Brown and Goldstein, 1983). In this study, all doses of the plant extract produced a significant beneficial effect on serum lipid profile in alloxan-induced diabetic rats, the beneficial effects on the lipid profile may be secondary to glycaemia control .The significantly lowered cholesterol may have contributed to the observed higher levels of high-density lipoprotein cholesterol in the animals. About 30% of blood cholesterol is carried in the form of HDL-C. HDL-C function to remove cholesterol atheroma within arteries and transport it back to the liver for its excretion or reutilization, thus

high level of HDL-C protect against cardiovascular disease (Kwiterovich, 2000; James *et al.,*2010). Therefore, the observed increase in the serum HDL-C level on administration of various doses of the extract in alloxan-induced diabetic rats indicates that the extract have HDL-C boosting effect. Moreso, the stabilization of serum triglyceride and cholesterol levels in rats by the plant extract may be attributed to glucose utilization and hence depressed mobilization of fat (Momo *et al.,* 2006; Iweala and Oludare, 2011). This result implies that the plant extract may be helpful in reducing the complications of hyperlipidemia and hypercholesterolemia which coexist quite often in diabetics (Sharma *et al.,* 2003). The study also revealed that administration of the extract at various doses significantly lowered the serum LDL-C in alloxan-induced diabetic rats. Studies have shown that chronic insulin deficiency as observed in alloxan-induced diabetes in experimental animals is associated with diminished levels of LDL-C receptors. This results to an increase in LDL particles and consequently increases serum level of LDL-C (Suryawanshi *et al.,* 2006).

The diabetic treated rats in 200, 300 and 400mg/kg b.w. extract and 5mg/kg b.w. Glibenclamide standard drug treatment groups showed steady increase in their mean weekly body weight, while the diabetic untreated rats have almost steady mean weekly weight gain (Table 3). This could be due to wasting associated with diabetic patients as a result of increased utilization of fats from the adipose tissue for generation of energy in the body.

**5. Conclusion**

The findings revealed that methanolic leaf extracts of *Bauhinia rufescens* has antilipidemic property on alloxan-induced diabetic rats , therefore, be considered as a potential strong candidate for future application as a functional supplement for the treatment and prevention of atherosclerosis.

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