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Research Article

Hypoglycaemic Activity and Nephro-Protectective Effect of *Bauhinia rufescens* in Alloxan-Induced Diabetic Rats

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ABSTRACT

The study aims to investigate the hypoglycaemic effects of methanolic leaf extracts of *Bauhinia rufescens* (MLEBR) on alloxan-induced diabetic rats. Alloxan was injected intraperitoneally at a single dose of 150mg/kg body weight (b.w.) for diabetes induction in the rats. The oral administration of 200, 300, and 400mg/kg b.w. of extract (once a day, for four weeks) significantly lowered ($P<0.01$) blood glucose levels in all treated diabetic rats. Furthermore, extract significantly ($P<0.01$) attenuated the elevated serum concentrations of urea and creatinine levels when compared to the untreated diabetic rats. The results show that chronic oral administration of MLEBR at doses 200, 300 and 400mg/kg b.w. may be a safe alternative antihyperglycaemic and nephro-protective agent due to its beneficial effect of improving blood glucose level and kidney health.

Keywords: Alloxan, *Bauhinia rufescens*, Diabetic rats, and Hypoglycaemic effects.

INTRODUCTION

Diabetes is a chronic disorder in metabolism of carbohydrates, proteins and fats. It is characterized by defects in insulin secretion, insulin action or both (Barar, 2000). Diabetes is a condition in which a person has a high blood sugar (glucose) level, either because the body does not produce enough insulin, or because body cells do not properly respond to the insulin that is produced. It has now become an epidemic with a worldwide incidence of 5% in the human population. The number of adults with diabetes in the world will rise from 135 million in 1995 to 300 million in the 2025 (Torben, 2002).

Decreased physical activity, increasing obesity, stress and changes in food consumption have been implicated in the increasing prevalence of diabetic cases in the past two decades (Edwin, 2006). As of 2000, at least 171 million (2.8%) people worldwide suffer from diabetes (Wild *et al.*, 2004).

Diabetes is characterised by polyuria, polydipsia, weight loss inspite of polyphagia, hyperglycaemia, glycosuria, ketosis, acidosis and coma (Ganong, 2003).

The vast majority of cases of diabetes fall into two broad etiopathogenetic categories. In one category, type 1 diabetes, the cause is an absolute deficiency of insulin secretion. In the other, much more prevalent category, type 2 diabetes, the cause is combination of resistance to insulin action and an inadequate compensatory insulin-secretory response (American Diabetes Association, 2005).

Oral hypoglycaemic agents, especially the sulfonylureas, thiazolidinediones, and biguanides have many setbacks, ranging from development of resistance and adverse effects to lack of responsiveness in large segment of patients population. Sulfonylureas lose effectiveness for 44% of patients within six years. Also, these treatments are associated with side effects or even toxic effects e.g., thiazolidinediones may cause liver toxicity; sulfonylureas might worsen heart disease, lower the glucose level below the normal range and increase the body weight gain; bloating, flatulence, diarrhoea and abdominal discomfort and pains are major complaints with glucosidase inhibitors (Dey *et al.*, 2002; Michael *et al.*, 2005, and DeFronzo, 1999). Moreover, none of these glucose-lowering agents adequately controls the

hyperlipidemia that frequently met with the disease (Derek, 2001).

The limitation of currently available oral anti-diabetic agents either in terms of efficacy and/or safety coupled with the emergence of the disease into global epidemy have encouraged a concerted effort to discover drugs that can manage type II diabetes more efficiently (Ranjan *et al.*, 2002).

Plants are well known in traditional herbal medicine for their hypoglycaemic activities, and available literature indicate that there are more than 800 plants species showing hypoglycaemic activity (Rajagopal, 2008). Among the traditional plants used for diabetes, only a small number of them have received scientific and medical evaluation and these include; *Acacia arabica, Momordica charantia, Syzygium cumini, Allium cepa, Ficus bengalensis, Pterocarpus marsupium, and vernonia amygdalina* amongst others.

Therefore, plant materials are continuously being scrutinized and explored for their effect as hypoglycaemic agents. Plants used in traditional medicine to treat diabetes mellitus represent a valuable alternative for the control of this disease.

One of such plants is *Bauhinia rufescens*, a branched shrub or small tree that grows up to 7.62m high, with greenish-yellow to white and pale pink flowers. It is found in the entire Sahel and adjacent Sudan zone, from Senegal and Mauritania across Northern Ghana and Nigeria. Several *Bauhinia* species are utilized as folk medicines worldwide, including Africa, Asia, South America and Central America.

An extract of the root is used as an astringent or antipyretic in local medicine. Leaves and fruit are applied for the treatment of diarrhoea, dysentery and ophthalmic diseases. The bark of the roots and trunk is used to cure chest complaints, syphilis and other venereal diseases, leprosy, diarrhoea and dysentery and to reduce fever (Ayensu, 1978).

Aqueous extract from *B. foficata* L. and *B. monandra* Kurz leaves were shown to be hypoglycaemic in normoglycaemic mice by Monezes *et al.* (2005).

Aliyu *et al.* (2009) reported promising antioxidant activity from methanolic extracts of leaves of *B. rufescens*, indicating that it may be helpful in the treatment of the diseases caused by free radicals.

The aim of this study was to evaluate the anti-diabetic and nephro-protective potential of the leaf extracts of *Bauhinia rufescens.*

MATERIALS AND METHODS

Plant materials

The leaves of *Bauhinia rufescens* were collected around Samaru, Zaria, during the month of October, 2010. They were taxonomically authenticated by Mal. M. Musa at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria. A voucher specimen number 427 matched with the collected plant. The leaves of the plant (*Bauhinia rufescens*) were air dried and later ground to powder using pestle and mortar.

Experimental animals

Forty three male Wister 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 laboratory cages in this same animal house and were allowed to acclimatize to the laboratory conditions 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* during the stabilization period. This research was carried out in accordance with the rules governing the use of laboratory animals as accepted internationally.

Chemicals

Alloxan monohydrate used was obtained from Sigma Chemical Company, St. Louis Mo, USA. A digital glucometer (Accu-Chek Advantage, Roche Diagnostic, Germany) was used to determine the blood glucose levels of the animals. All other chemicals and drugs used were obtained commercially and were of analytical grade.

Plant extract

Four hundred grams (400g) of the powdered leaves of *Bauhinia rufescens* was macerated in 2.5L of methanol with regular shaking for 72 hours. The mixture was then filtered and the filtrate evaporated to dryness in a water-bath and the residue kept until needed (Njike, 2005).

Phytochemical screening

The methanolic leaf extract of *Bauhinia rufescens* (MLEBR) was subjected to preliminary phytochemical analysis to test for the presence or absence of secondary metabolites using methods of analysis described by Brain and Turner (1975).

Acute toxicity

The median lethal dose LD_{50} of the methanolic leaf extracts of *Bauhinia rufescens* (MLEBR) was determined using method described by Lorke (1983). In the first phase, nine rats were divided into three groups of 3 rats each. The first group received the extracts at a dose of 1000 mg/kg b.w., the second group received a dose of 100 mg/kg b.w. of the plant extract, while the Group 3 received 10 mg/kg b.w. of the same extract. The animals were observed for general signs and symptoms of toxicity including mortality over a period of 24 h. In the second phase, based on the results of the first phase four (4) rats were divided into four groups of one rat each. The extract was administered at the dose of 1200, 1600, 2900, and

5000 mg/kg b.w. to group 1, 2, 3 and 4 respectively. The rats were also observed for 24 h for signs of toxicity including death. The LD_{50} was then calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose, i.e. the geometric mean of the consecutive doses of which 0 and 100% survival rates were recorded (Lorke, 1983).

Induction of experimental diabetics

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.

Treatment of rats with plant extract and glibenclamide

In the diabetic study, 30 rats were used and divided into 6 groups of 5 rats each.

Oral administration of the plant extracts to the diabetic groups started the same day except for normal and diabetic control groups for a period of 4 weeks. During this period, animals in all groups were fed with standard diet and water. Blood sample was collected from the tail vein for blood glucose level test. On the last day, blood samples were collected from overnight fasted rats by cervical dislocation under mild ether anaesthesia for biochemical estimations.

Commercially available Glibenclamide tablets were dissolved in distilled water and administered orally to the rats in group F which served as positive control which was also for a 4 week duration.

The groupings were done as follow:

Group A: Served as normal control and did not receive any treatment.

Group B: Diabetic rats receiving normal saline only (5 ml/kg b.w.).

Group C: Diabetic rats treated with MLEBR of graded dose *200*mg/kg b.w.)

Group D: Diabetic rats treated with MLEBR of graded dose *300*mg/kg b.w.)

Group E: Diabetic rats treated with MLEBR of graded dose *400*mg/kg b.w.).

Group F: Diabetic rats + glibenclamide 5mg/kg, p.o. and served as positive control (Sharma, 2010).

Biochemical estimations

The blood samples collected from the sacrificed rats were centrifuged in a Denley BS400 centrifuge (England) at 5000 rpm for 5-minutes. The supernatant (serum) collected was assayed for the serum urea and creatinine.

Determination of serum creatinine (using Jaffe's reaction)

To 0.5ml of test sample was added 1.5ml of distilled H_2O + 0.5ml of 10% sodium tungstate + 0.5ml of 2/3 normal H_2SO_4. Mixed well and allowed to stand for 15 minutes for the colour to develop. The optical density of the developed colour was read at 520nm.

Determination of serum urea (using diacetyl monoxime method)

Blood serum was deproteinized with Trichloacetic acid and the resulting filtrate heated for 20min with Diacetylmonoxime reagent in the presence of Perchloric acid to develop a stable yellowish colour. The coloured solution was measured at 520nm.

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 (Duncan *et al.*, 1977).

RESULTS

Phytochemical screening

Phytochemical screening of methanolic leaf extract of *B. rufescens*showed the presence of tannins, saponins, flavonoids, alkaloids, and terpenoids/steroids.

Acute toxicity study

There were no deaths recorded in the first and second phase of acute toxicity test after the extract administration.

Hypoglycaemic activity of methanolic leaf extract of *Bauhinia rufescens* and Glibenclamide at 0, 1, 2, 3 and 4th week of treatment in alloxan induced diabetic rats

The methanolic leaf extract of *Bauhinia rufescens* at different dosing of 200, 300, and 400mg/kg b.w. and Glibenclamide 5mg/kg b.w. significantly ($p<0.01$) reduced the blood glucose levels of the diabetic rats when compared with untreated diabetic control at 1st, 2nd, 3rd and 4th weeks post treatment. The extract at 300 and 400mg/kg dosing proved to have a better lowering effect on the blood glucose level than Glibenclamide treated group.

There was an astronomical increase in the plasma glucose level of the alloxan-induced untreated diabetic rats when compared with the control. Glibenclamide a standard drug caused reduction in blood glucose level in 1st week post treatment, but this blood glucose value was found statistically

insignificant (P>0.05). Variant doses of the MLEBR and the standard drug Glibenclamide produced significant (p<0.01) hypoglycaemic effects in the diabetic rats at 2nd, 3rd, and 4th week post treatment when compared with untreated diabetic control. The administration of MLEBR at doses equivalent to 300 and 400mg/kg b.w. four week post treatment reduced the plasma glucose level of the rats to mean values close to that of the normal control.

The 300mg/kg b.w. dose reduced the blood glucose level of diabetic rats from an initial mean value of 413.8±49.13mg/dL at week 0 to a mean value of 100.0±16.83mg/dL at 4th week, while the 400mg/kg b.w. dose reduced the blood glucose level of diabetic rats from the initial value of 435.2±71.53 to 101.8±7.88. The fourth week values of 100.0±16.83 and 101±7.88 of doses 300 and 400mg/kg b.w. respectively compare with the normal control value of 91.0±2.02, and were more remarkable than that of the standard drug Glibenclamide that reduced the initial plasma glucose of 431.2±61.63 at week 0 to 181.0±44.79 at the 4th week. These findings are summarized in Table 1.

Effects of methanolic leaf extract of *Bauhinia rufescens* (MLEBR) on serum urea and creatinine in alloxan-induced diabetic rats

Serum urea and creatinine levels decreased significantly (P<0.05) in all MLEBR or glibenclamide treated groups when compared to untreated diabetic control group as shown in Figure 1 and 2.

DISCUSSION AND CONCLUSION

The result of this study showed that oral administration of methanolic leaf extract of *Bauhinia rufescens* (MLEBR) had a beneficial effect on the diabetic state by reducing hyperglycaemia. The MLEBR at the various graded doses of 200, 300 and 400mg/kg body weight caused a statistically significant (P<0.01) reduction in blood glucose level in alloxan-induced diabetic rats. From the results of clinical studies (Knekt *et al.*, 2002), it is evident without any doubt that the reduction of hyperglycaemia is the most important factor in the prevention of chronic microvascular complications of diabetes mellitus (retinopathy, nephropathy, neuropathy and diabetic foot) as well as in the prevention of accelerated atherosclerosis-related condition (myocardial infarction, stroke, *etc.*).

The exact mechanism involved in the hypoglycaemic action is not clear, the extract may stimulate insulin secretion by the pancreas or/and enhance insulin sensitivity in various organs especially the muscles by promoting glucose uptake and metabolism inhibiting hepatic gluconeogenesis.

Phytochemical screening of methanolic leaf extract of *Bauhinia rufescens* revealed the presence of flavonoid, tannins, saponins, cardiac glycosides, steroids and triterpenes The mechanism by which the extract exert the hypoglycaemic effect may appear to be related to presence of flavonoid among other secondary metabolites or bioactive chemical constituents found in the plant extract which may be an active constituents in a group or as an individual responsible for the hypoglycaemic activity of the plant extract (Marles and Farnsort,1995). Flavonoids have been shown to exert their antioxidant activity by various mechanism by scavenging or quenching free radicals or by inhibiting enzymatic systems responsible for free radical generation (Blaha *et al*., 2004; Dias *et al.*,2005; Lukacinova *et al.*,2008). Apart from being antioxidants, flavonoids have been reported to inhibit sodium-dependent vitamin C transporter 1 (SVCT 1) and glucose transporter Isoform 2 (Glut 2), the intestinal transporters for vitamin C and glucose, leading to a decrease in the intestinal absorption of glucose, hence decrease in the blood glucose concentration (Song *et al.*, 2002). Several researchers have also demonstrated that flavonoids act as reducer of hyperglycemia by causing inhibition of renal glucose reabsorption through inhibition of the sodium-glucose symporters located in the proximal renal convulated tubule (Hungo *et al.*, 1998; Maghrani *et al.*, 2005; Lukacinova *et al.*, 2008). Like the plant extract, Glibenclamide also produced a significant reduction in the blood glucose level of diabetic rats. Glibenclamide exert its action mainly by increasing the secretion of insulin. They only work in diabetics with some remaining B cells. They bind to the ATP-inhibited K^+ channels in the B cell membranes and inhibit channel activity, depolarizing the B cell membrane and increasing Ca^{2+} influx and hence insulin release (Ganong, 2005). The Comparable effect of the plant extract with Glibenclamide in this study may suggest similar mechanism of action. This findings appears to be in consonance with the earlier suggestion of Jackson and Bressler (1981) that sulfonylureas such as Glibenclamide have extra-pancreatic hypoglycaemic mechanism of action secondary to their causing insulin secretion and the attendant glucose uptake into and utilization by the tissues

A significant increase in serum urea and creatinine levels (Figure 1 and 2 respectively) were observed in diabetic control group. Treatments with MLEBR and standard drug Glibenclamide reduced significantly (P<0.01) the levels of creatinine and urea. These results suggest that the methanolic leaf extract of *B. rufescens* may be able to prevent altered protein metabolism and/or impaired renal function in diabetes mellitus.

From this study, it was observed that the methanolic leaf extract of *Bauhinia rufescens* has

significant hypoglycaemic activity in alloxan-induced diabetic rats, as well as improving kidney health.

Table 1: Hypoglycaemic activity of methanolic leaf extract of *Bauhinia rufescens* at 0, 1, 2, 3, and 4th week of treatment in alloxan induced diabetic rats

GROUP	Weekly blood glucose level of the animal (mg/dL)				
	Week0	Week1	Week2	Week3	Week4
A	87.0±2.84	89.4±2.54	86.2±2.31	92.0±1.70	91.0±2.02
B	437.2±26.17	489.5±54.52	445.0±25.04	470.6±31.42	460.2±25.05
C	435.4±65.42	186.2±31.87**	253.8±32.37**	200.4±36.34**	190.2±28.70***
D	413.8±49.63	236.0±67.93**	210.2±65.66**	132.8±20.28**	100.0±16.83***
E	435.0±71.53	238.0±53.99**	229.2±47.40**	155.6±32.95**	101.8±7.88***
F	431.2±61.63	355.6±63.33^{ns}	253.0±67.60**	277.4±75.46**	181.0±44.79***

Values are expressed as mean ± S.E.M., n =5, *P<0.05, **P<0.01, ***P<0.001 when compared with diabetic control, #P<0.05, ##P<0.01 when compared with normal control.
Group A: distilled water; Group B: diabetic rats untreated; Group C: diabetic rats treated with 200mg/kg of MLEBR; Group D: diabetic rats treated with 300mg/kg b.w. of MLEBR; Group E: diabetic rats treated with 400mg/kg b.w. of MLEBR; Group F: diabetic rats treated with Glibenclamide 5mg/kg b.w., ns: not significant.

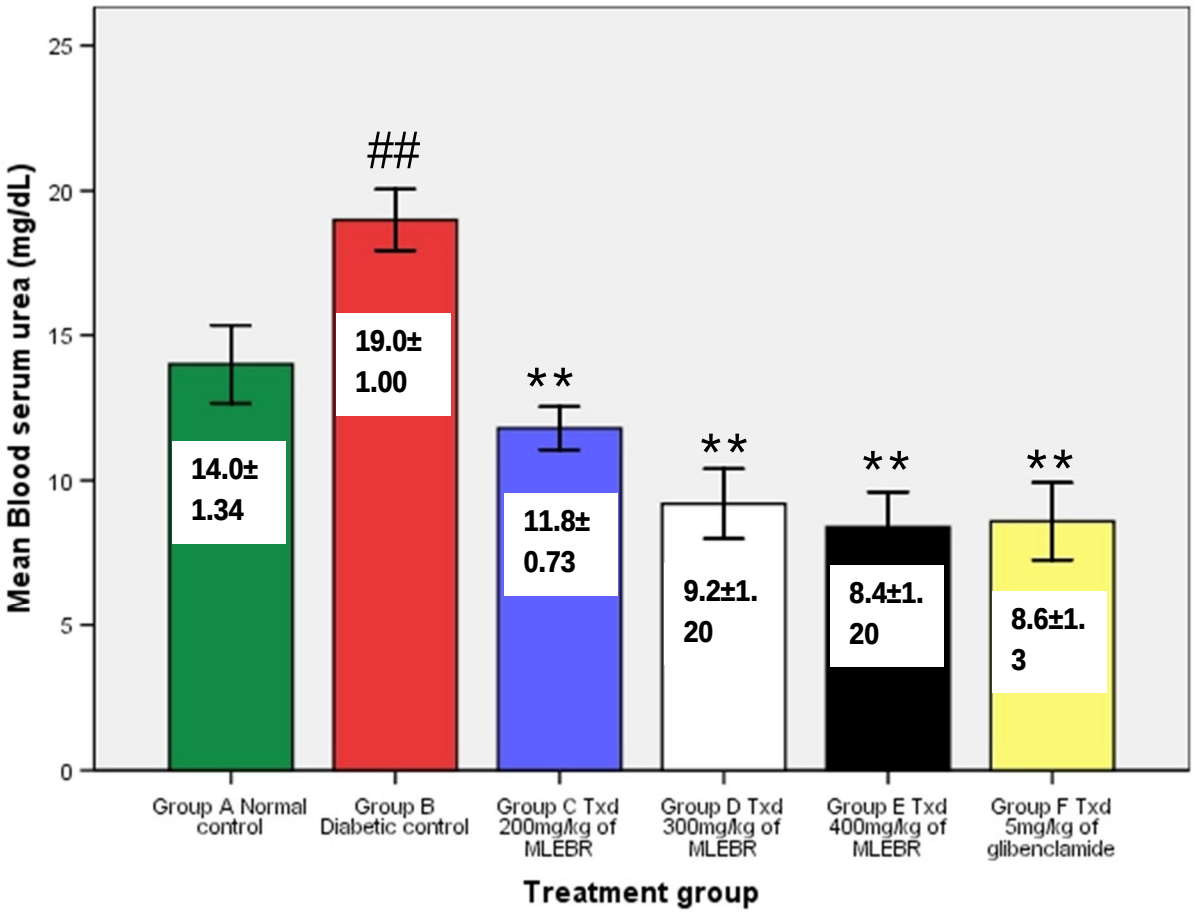

Fig. 1: Effects of methanolic leaf extract of *Bauhinia rufescens* on the serum urea in alloxan induced diabetic rats, **P<0.01 vs diabetic control and ##P<0.01 vs normal control.
Note: Vertical black bars represent SEM

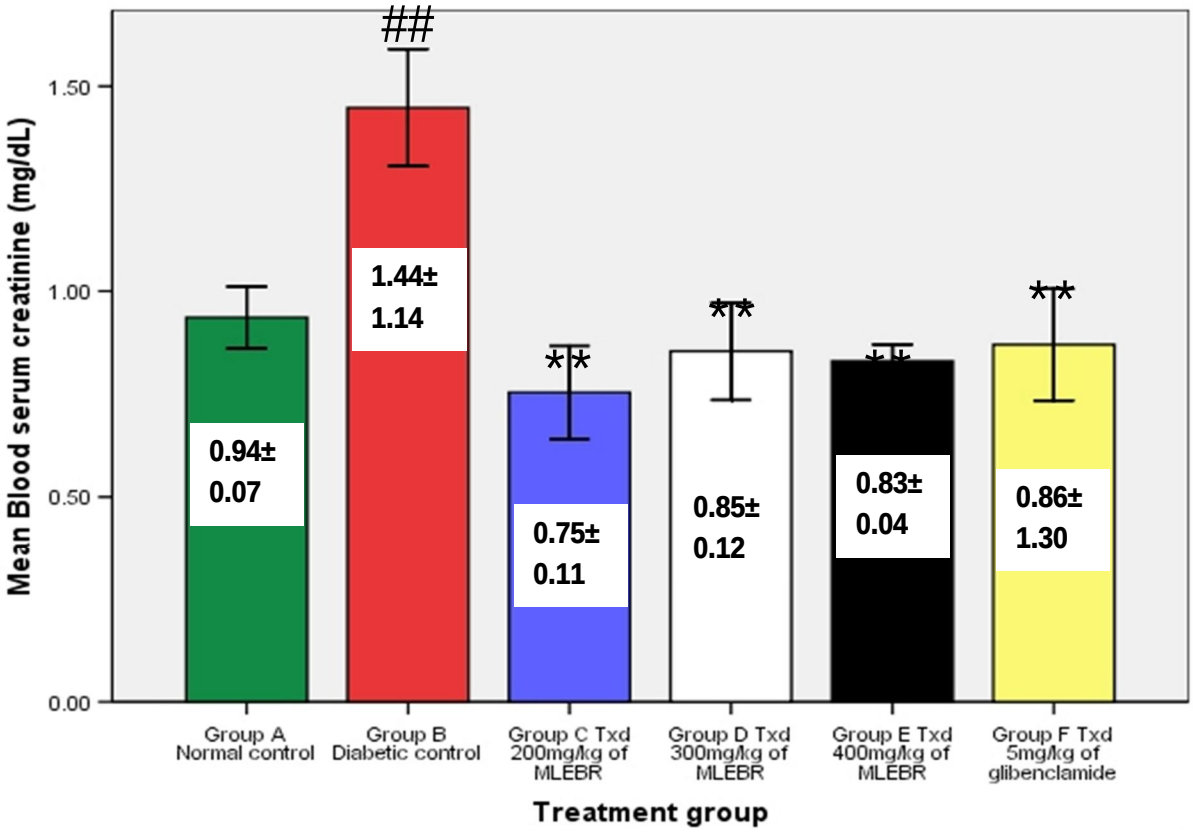

Fig. 4.10: Effects of methanolic leaf extract of *Bauhinia rufescens* on the serum creatinine in alloxan induced diabetic rats, **P<0.01 vs diabetic control and ##P<0.01 vs normal control.
Note: Vertical black bars represent SEM

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