

**ANTIDIABETIC ACTIVITY OF *MALLOTUS ROXBURGHIANUS* LEAVES
IN DIABETIC RATS INDUCED BY STREPTOZOCIN**

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Summary

Mallotus roxburghianus Muell. (Euphorbiaceae) is a native plants widely distributed in Mizoram (India). The decoction of leaves is used by the tribal people of Mizoram for the treatment of diabetes. We investigated the methanolic extract for its anti-diabetic activities in streptozocin induced type 2 diabetic models in rats. Two graded doses of the extract and Glibenclamide as standard are given to the diabetic rats for 12 days. There is a significant reduction in fasting blood glucose levels, serum triglycerides levels and serum total cholesterol levels in diabetic rats. In addition, other parameters like changes in body weights; glycogen content of liver and skeletal muscles; glutathione levels and Thiobarbituric Acid Reactive Substances in liver assessed in the extract treated diabetic rats are compared with diabetic control and normal animals. Significant results were observed in all the above parameters, thereby justifying the use of the plants in the indigenous system of medicines.

Key Words: *Mallotus roxburghianus*, Anti-diabetic activity, methanolic extract, streptozocin

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Introduction

Diabetes is a serious metabolic disorder with micro and macro vascular complications that results in significant morbidity and mortality. The increasing number of ageing population, consumption of calories rich diet, obesity and sedentary life style have led to increase the number of diabetes world wide. The current treatment, although provide a good glycemic control but do a little in preventing complications (1). Besides, these drugs are associated with side effects (2). There is an increased demand to use natural products with antidiabetic activity due to the side effects associated with the use of insulin and oral hypoglycemic agents (3, 4). The World Health Organization (WHO) (1980) has also recommended the evaluation of the effectiveness of plants in condition where we lack safe modern drugs (5).

Mallotus roxburghianus Muell. (Euphorbiaceae) is native plants of Mizoram (India) found particularly in the tropical evergreen forests and mixed bamboo forests. It is distributed in the region of Chitagong Hill tract of Bangladesh and Myanmar. The tribal people of Mizoram used the decoction of leaves for the treatment of Diabetes (6). The ethanol extract of *Mallotus roxburghianus* has been reported for its anti-oxidant activity (7). The current investigation is an attempt to study the antidiabetic activity of the methanol extract of *Mallotus roxburghianus* in streptozocin induced type 2 diabetic model in rats.

Materials and Methods

Animals

The study was conducted in male Wistar strain albino rats, weighing about 180 – 225 g. Before and during the experiment, animals were fed with normal laboratory diet and water *ad libitum*. The animals were acclimatized for a period of 3 days in the new environment before initiation of experiment. The study was approved by the Institutional Animal Ethics Committee.

Chemicals and instruments

Chemicals were procured as follows: Streptozocin, Thiobarbituric acid and Glutathione were obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. All other chemicals and solvents used were of analytical grades. Blood glucose levels were estimated by glucose oxidase peroxidase reactive strips (Accu-check, Roche Diagnostics, USA). Serum protein and lipid profiles (TG and TC) were estimated by enzymatic kit (Span Diagnostic Ltd., India). UV-VIS Spectrophotometer (Genesys 10 UV, USA).

Collection of plants materials:

The leaves of *Mallotus roxburghianus* were collected during the month of April 2005 from Saitual Village at Aizawl District of Mizoram, India. The plant was identified by the Botanical Survey of India, Eastern Zone, Shillong and the voucher specimen was deposited in the Herbarium of the Department of Pharmacy, RIPANS (Herbarium Number M-15).

Preliminary phytochemical screening

Preliminary phytochemical screening (8,9) revealed the presence of Alkaloids, Glycosides and Tannins in the leaves of *Mallotus roxburghianus*.

Preparations of methanol leaf extract

The air dried leaves were coarsely powdered. 100 g was taken and extracted by 500 ml of petroleum ether (b.p 60-80°) for 18 hours, followed by 500 ml of Methanol for 18 hours. The methanol extract was collected and filtered. The filtrate was concentrated by Rotary vacuum evaporator, the residue (yield 8.72 g) was stored in the refrigerator at 2-8°C for use in subsequent experiment.

Acute toxicity studies

Healthy adult Wistar strain of albino rats weighing between 190-225 g of either sex, starved overnight were taken and divided into four groups (n=6). They were orally fed with the methanol extract of *Mallotus roxburghianus* in increasing dose levels of 100, 500, 1000, 3000 mg/kg body weight (10). The mice were observed continuously for 2 h for behavioral, neurological and autonomic profiles and after a period of 24 and 72 h for any lethality or death (11).

Oral glucose tolerance test (OGTT)

The oral glucose tolerance test (12) was performed in overnight fasted (18 h) normal rats. Rats, divided into four groups (n=6) were administered vehicle, *Mallotus roxburghianus* methanol extract 200 and 400 mg/kg and glibenclamide 600 µg/kg (13) respectively. Glucose (2g/kg) was fed 30 min after the administration of extracts. Blood was withdrawn from the tail vein at 0, 30, 60, 90 and 120 min of extract administration, and fasting blood glucose levels were determined.

Induction of experimental diabetes

Non insulin dependant diabetes mellitus was induced in over night fasted male rats by a single intraperitoneal injection of streptozocin, with a dose of 65 mg/kg body weight (14) dissolved in cold citrate buffer (pH 4.5) (15). The elevated blood glucose level in the blood at 72 h and then on day 7 after injection confirmed hyperglycemia (16). Rats found with permanent high fasting blood glucose level > 300 mg/dl were included for the antidiabetic studies (1).

Experimental designs

In the experiment a total 30 rats (24 diabetic rats and 6 normal rats) were used. The rats were divided into five groups with six in each group. The treatment scheduled is as follows:

1. Group I - Normal untreated rats
2. Group II - Diabetic control received only 0.5 % CMC Solution.
3. Group III - Diabetic rats treated with Glibenclamide 600 µg/kg body weight.
4. Group IV - Diabetic rats treated with MR extract 200 mg/kg body weight
5. Group V - Diabetic rats treated with MR extract 400 mg/kg body weight.

The drugs solutions or vehicle were administered orally by gastric intubations tube once daily at 11: 00 am for 12 days (17). The fasting blood glucose levels were determined on day 0, day 1, day 5 and day 12. Changes in initial and final body weight were also measured.

On the 12th day, the animals were sacrificed by an over dose of ether. Different parameters like serum triglycerides and total cholesterol, liver and skeletal muscle glycogen content (18), reduced glutathione level in liver by the method of Ellman's reagent (19).

Fasting blood glucose level

Fasting blood glucose levels were estimated by glucose oxidase peroxidase reactive strips (Accu-check, Roche Diagnostic, USA) (16).

Serum triglycerides and Total cholesterol levels:

The content of triglycerides and total cholesterol levels in the serum were estimated by Diagnostic Reagent Kit obtained from Span Diagnostic Ltd., India (20).

Assay of glycogen content in liver and skeletal muscle

The glycogen content of liver and skeletal muscles was determined by using anthrone reagent (18). 100 mg tissue was taken and placed in a centrifuge tube containing 1 mL of 30% KOH. The tube was kept in a boiling water bath for 20 minutes, the digest was cooled, and 1.25 ml of 95% ethanol was added. The content is mixed with a stirring rod, and the rod was washed with a small quantity of 60% ethanol. The content of the tube were gently boil in a hot water bath, again cooled, and centrifuge for 15 min at 3000 rpm. The supernatant liquid was decanted and the residue was redissolved in 1 ml of distilled water and decanted as before. The sedimented glycogen in the above was dissolved exactly in 5 mL of water. The tube was submerged in cold water, 10 mL of the anthrone reagent was added, and the reactants were mixed by swirling the tubes. The cold tubes was covered with glass marbles and heated for 10 min in a boiling water bath. Then they were immediately cooled in cold water and read in a 620 nm against reagent blank. Result was expressed in mg/g of tissue.

Assay of reduced glutathione (GSH) in liver

The glutathione level in liver was determined by Ellman's reagent (19). Liver (200 mg) homogenized in 8.0 ml of 0.02 M EDTA in ice bath. Aliquots of 5.0 ml of the homogenates were mixed in 15.0 ml test tubes with 4.0 ml distilled water and 1.0 ml of 50 % trichloroacetic acid. The tubes were centrifuge for 5 min approximately 3000 x g. 2 ml of supernatant solution was mixed with 4.0 ml of 0.4 M Tris buffer, pH 8.9. 0.1 ml Ellman's reagent [5, 5'-dithiobis-(2-nitrobenzoic acid)] was added. Shaken and the absorbance was read at 412 nm against reagent blank with no homogenate (within 5 min of the addition of Ellman's reagent). Results were expressed in umol GSH/g tissue.

Statistical Analysis

All the data were statistically analyzed by using one-way ANOVA, followed by Dunnett test using GraphPad Instat software. The values were considered significant when $p < 0.05$.

Results

Acute toxicity studies revealed that the methanol extract of *Mallotus roxburghianus* is a non-toxic in nature. There was no lethality or any toxic reactions found at any doses selected until the end of the study period. In Oral Glucose Tolerance Test, the two doses (200 mg/kg and 400 mg/kg) methanol extract of *Mallotus roxburghianus* and Glibenclamide (600 µg/kg) showed a significant reduction ($p < 0.05$; $p < 0.01$) of blood glucose levels from 30 min onwards (Table 1) but with no significant difference in results is observed by the two doses of extract. Induction of diabetes was confirmed by the presence of high blood glucose level (>300 mg/dl) on experimental rats. The

effect of the two doses of methanol extract of *Mallotus roxburghianus* and Glibenclamide on the blood glucose levels at day 0, day 1, day 5 and day 12 are presented in Table 2. A significant reduction in fasting blood glucose levels was seen on diabetic rats treated with two different doses of extract and glibenclamide when compared with diabetic control (untreated diabetic rats). However, the reduction of fasting blood glucose level was significant ($p < 0.05$) only on day 5 and day 12. The two doses of extract reduced the blood glucose level, but, there was no significant difference between the two selected doses. The effect on serum total cholesterol, total triglycerides levels are presented on (Table 3). The lowering effect on serum total cholesterol level was found significant ($p < 0.05$), but there was no difference in the results given by the two different doses of the extract. The glycogen content of liver and skeletal muscles are also restored by the extract and glibenclamide, significant ($p < 0.01$) was observed in both of the extract treated and glibenclamide treated group (Table 4), but no significant different in effect was found on the two selected doses of extract. There was also a significant increase ($p < 0.01$) of Reduced Glutathione in the liver of diabetic rats treated with the two extract doses and glibenclamide (Table 5) by comparing with diabetic control group, but there was not any significant different results given by the two different doses of extract.

Table 1. Effect of methanol extracts of *Mallotus roxburghianus* on oral glucose tolerance test.

		Blood glucose concentration (mg/dl)				
Group	Treatment	0 min	30 min	60 min	90 min	120 min
I	Control (vehicle)	93.66±1.520	118.83±1.641	113.66±3.303	106.5±2.71	98.83±3.390**
II	MR 200 mg/kg	93.16± 1.249	107.66 ± 4.028*	101.33±3.051**	98±3.502	94±2.556**
III	MR 400 mg/kg	93.16±2.136	106.83±1.701*	102.16±1.797*	96.16±3.351	90.16±2.301**
IV	Glibenclamide (600 µg/kg)	88.66 ± 2.431	96.16±3.429**	84±3.130**	75.5±3.019**	62.33±3.721**

* p<0.05, ** p< 0.01 significant vs. control

Table 2. Effect of methanol extract of *Mallotus roxburghianus* on fasting blood glucose levels.

		Mean Blood Glucose Concentration ± SEM (mg/dl)			
Group	Treatment	0 Day	1st Day	5th Day	12th Day
I	Normal	98.33±1.453*	97.66±1.202*	100.66±1.358**	102.83±1.302**
II	Diabetic Control	327.5±12.173	335±10.567	331.66±10.138	331.83±10.442**
III	Glibenclamide (600 µg/kg)	319.5 ± 7.654	313.83±10.355	269.16 ± 10.864**	198.33 ± 16.615**
IV	MR 200 mg/kg	325.66±11.612	320 ± 12.517	294 ± 11.690*	238.83 ± 13.70**
V	MR 400 mg/kg	321.83 ± 13.24	320.33 ± 10.960	88.66 ± 10.105*	241.33±10.620**

* P < 0.05. ** P< 0.01 Significant vs. control group

Table 3. Effect of methanol extracts of *Mallotus roxburghianus* on body weight .

Group	Treatment	Day 0	Day 12
I	Normal	214.7±8.919	220.21 ± 9.180**
II	Diabetic Control	189.8± 4.996	140.21 ± 4.279**
III	Glibenclamide (600 µg /kg)	194.7 ± 2.851	191.95 ± 3.623**
IV	MR 200 mg/kg	193.21 ± 3.499	174.61 ± 6.031**
V	MR 400 mg/kg	182.05 ± 3.962	168.23 ± 2.602**

p<0.01 Significant vs. control

Table 4. Effect of methanol extract of *Mallotus roxburghianus* on serum lipid profile.

Group	Treatment	Triglycerides (mg/dl)	Total Cholesterol (mg/dl)
I	Normal	69.29 ± 3.056	57.90 ± 3.144*
II	Diabetic control	74.98 ± 9.411	98.36 ± 2.957*
III	Glibenclamide (600 µg /kg)	39.35 ± 3.268 *	44.13± 6.056*
IV	MR 200 mg/kg	54.24 ± 2.674	52.86±3.114*
V	MR 400 mg/kg	55.98 ± 6.678	50.67 ± 3.095*

* P< 0.05 Represent statistical significance vs. Control

Table 5. Effect of methanol extracts of *Mallotus roxburghianus* on glycogen content of Liver and skeletal muscle.

Group	Treatment	Liver glycogen Mean Concentration (mg/g)± SEM	Skeletal muscle Mean Concentration (mg/g)± SEM
I	Normal	17.94±0.238**	11.73±0.369**
II	Diabetic Control	7.13 ± 0.364 **	3.01±0.281**
III	Glibenclamide (600 µg /kg)	16.88±0.220**	12.37±0.600**
IV	MR 200 mg/kg	13.12±0.452**	6.21±0.352**
V	MR 400 mg/kg	14.09 ± 0.289**	7.66 ± 0.386**

** p<0.01 Significant vs. control

Table 6. Effect of methanol extracts of *Mallotus roxburghianus* on Reduced Glutathione content of Liver.

Group	Treatment	GSH level ($\mu\text{mol/g}$) \pm SEM
I	Normal	106.95 \pm 2.350**
II	Diabetic Control	79 \pm 4.642**
III	Glibenclamide (600 μg /kg)	100.05 \pm 3.048**
III	MR 200 mg/kg	94.95 \pm 2.033**
IV	MR 400 mg/kg	96.8 \pm 1.876**

* $p < 0.01$ Significant vs. control

Discussion

The objectives of the present studies were to explore the antidiabetic potential of the methanol extract of *Mallotus roxburghianus* on streptozocin induced diabetic rats. The induction of diabetes by single intraperitoneal injection of Streptozocin (65 mg/kg) was already shown (1, 21).

The acute toxicity studies did not produce any muscular weakness, neither gross behavioral disturbances nor death in any of the rats, thereby suggesting its non toxic in nature. The oral glucose tolerance test had revealed that the extract has significant capability to reduce blood glucose levels (Table-1).

The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves over production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues (22). Administration of two doses 200 mg/kg and 400 mg/kg of the methanol extract for a period of 12 days produced a significant decrease in fasting blood glucose levels in diabetic rats when compared with the diabetic control (Table 2).

Induction of diabetes with streptozocin is associated with the characteristic loss of body weight which is due to increased muscle wasting in diabetes (23). Diabetic rats treated with the methanol extract shows an increase in body weight as compared to the diabetic control (Table 3). The increase in body weight of extract treated diabetic rats may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis.

The result in our studies had shown that there is an increased serum triglycerides and total cholesterol level in diabetic rats when compared to normal animals (Table 4). The increase in the serum triglycerides and total cholesterol in diabetic rats are in agreement with previous report (24, 16). The level of serum lipids is usually raised in diabetes and such elevation represents a risk factor for coronary heart disease (25). The abnormal high concentration of serum lipids in diabetes is mainly due to an increase in the mobilization of free fatty acids from the peripheral depots, since

insulin inhibits the hormone sensitive lipase. Under normal circumstances insulin activates enzymes lipoprotein lipase and hydrolyses triglycerides and insulin deficiency results in failure to activate the enzymes thereby causing hypertriglyceridemia (17). The results in our studies (Table 4) shown that the extract lower the serum cholesterol and triglycerides levels in diabetic rats.

Glycogen is the primary intracellular storable form of glucose and its levels in various tissue especially skeletal muscles are a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Since Streptozocin causes selective destruction of β -cells of Islet of langerhans resulting in marked decrease in insulin levels, it is rational that glycogen levels in liver and skeletal muscles are decreased as they depend on insulin for influx of glucose (26, 27, 1). The result in our studies shown that there is an increase in the glycogen content of liver and skeletal muscle of diabetic rats treated with extract and glibenclamide when compared to diabetic control rats. The same results was already reported in earlier studies (27,28). The decrease of glycogen content in this study is probably due to lack of insulin in the diabetic state which results in the inactivation of glycogen synthase systems. The significant increase in the glycogen content of extract treated rats may be due to the reactivation of glycogen synthase system.

It has been shown that the antioxidant status of tissues is an important factor in the development of diabetic complications (29). Reduced Glutathione plays an important role mainly in the detoxication and metabolism as a cofactor or a substrate for some enzymes and in this way it is an antioxidant agent protecting tissues from oxidative stress and thus measured as a common marker of free radical damage. In our studies, the Reduced Glutathione levels of the extract treated diabetic rats are significantly increased by comparing with diabetic control rats. The same results were obtained in earlier studies (30,20).

From the present studies, the results have shown that the methanol extract of *Mallotus roxburghianus* leaves have the antidiabetic properties on streptozocin induced diabetic models of experimental animals. However, the activity may not be the dose dependent as the two different doses of extract (200 mg/kg and 400 mg/kg) do not show any significant variation in the results. It may be concluded that the leaves of *Mallotus roxburghianus* may be promising for the development of potent phytomedicine for diabetes. Further comprehensive investigations are progress in our laboratory to elucidate the exact mechanism of its antidiabetic activities.

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