

Antidiabetic Activity of Methanolic Extracts of Leaves of *Butea Monosperma* Roxb

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder that imposes a huge health and economic burden on societies. Because the currently available medications have many drawbacks, it is important to search for alternative therapies. Medicinal plants used in traditional medicines are ideal candidates. *Butea monosperma* Roxb. belongs to the family Fabaceae, have pharmacological actions like Antioxidant, Antimicrobial, anti-inflammatory, anti-ulcer activity. The main objective is to investigate antidiabetic activity of methanolic leaf extract of *Butea monosperma* Wild., on alloxan induced diabetic rats. Thirty wistar albino rats were randomly divided into five groups. Either sex of Wistar strains of albino rats were divided in to 5 groups were Group 1 served as normal control, Group 2 served as alloxan control, Group 3 were administered with standard drug (Glibenclamide), Group 4 and Group 5 were administered to different doses of methanolic extract of *Butea monosperma* Roxb., (i.e. 200 and 300 mg/kg/Kg body weight). The anti-diabetic activity was determined by glucometer in both normal and alloxan-induced diabetic rats. The methanolic extract of *Butea monosperma* Roxb showed significant reduction in blood glucose levels in albino rats due to presence of phytochemicals such as alkaloids. The Results are altered levels of the FBGL and OGTT in alloxan induced rats were brought back to normal on treatment with methanolic extract of *Butea monosperma* Roxb., Thus the positive results suggest that *Butea monosperma* extract should be further studied to determine bioactive chemical compounds as well as to understand possible mechanism of action and evaluate their toxicity looking towards pharmaceutical actions. The Conclusion was concluded from result that the methanolic extract of *Butea monosperma* Roxb, showed significant antidiabetic activity in a dose dependent manner.

Keywords: Antidiabetic activity, *Butea monosperma* Roxb. Glibenclamide.

INTRODUCTION

In current scenario, herbs are the potent sources of medicines used in treatment of various diseases and disorders. The Pharmacognostical evaluation of indian medicinal plants viz., *Butea monosperma* Roxb studied which include morphological and physicochemical studies^[1]. Morphological studies of species plant part were studied which will be beneficial for validation and assessment of quality control parameters of these plants to find out presence of adulterants if any in order to establish quality, safety and efficacy^[2].

Diabetes mellitus (DM):

Diabetes is one of most common non-communicable diseases. It is a heterogeneous group of metabolic disorders characterized physiologically by dysfunction of pancreatic beta cells and deficiency in insulin secretion or insulin activity and clinically by hyperglycemia or impaired glucose tolerance and other manifestable disorders^[3]. It is an endocrinological syndrome abnormally having high levels of sugar in blood. Type 1 diabetes most commonly occurs in children and is a result of body's immune system attacking and destroying beta cells. The trigger for this autoimmune attack is not clear, but result is end of insulin production. Multiple risk factors for development of Type 2 diabetes mellitus^[4]:

- Family history (parents with diabetes).
- Obesity (i.e., ≥ 20 % over ideal body weight or body mass index ≥ 25 kg/m²).
- Habitual physical inactivity.
- Impaired glucose tolerance.
- Hypertension ($\geq 140/90$ mm Hg in adults).
- High density lipoprotein (HDL) cholesterol ≤ 35 mg/dl and/or triglyceride level ≥ 250 mg/dl ^[5].

In the present work an attempt was made for pharmacological evaluation of antidiabetic activity of methanolic extracts of leaves of *Butea monosperma* Roxb on alloxan induced diabetes in albino rats.

MATERIALS AND METHODS

Chemicals and Equipments

The chemicals, solvents and drugs used to conduct this experiment were analytical grade. Alloxan (Thermo Fisher Scientific, Waltham, MA, USA), Glibenclamide active powder (Thermo Fisher Scientific), methanol (Carlo Erba Reagents, Val-deReuil, France), n-butanol (Carlo Erba Reagents), dimethyl sulfoxide (UNI-CHEM chemical reagents, UK), chloroform (Labort Fine Chem Pvt. Ltd, Surat, Gujarat, India), n-hexane (Blulux Laboratories Ltd, Faridabad, Haryana, India), glucometer, 3,5-dinitrosalicylic acid (DNSA) (Sigma-Aldrich Co., St Louis, MO, USA), and Accu-Check® Active glucometer test strips (Hoffman-La Roche Ltd., Basel, Switzerland) were used to carry out the experiment.

Plant material collection

The leaves of *Butea monosperma* Roxb was collected from vangapally and was identified and authenticated by Dr. Srinivas Reddy, a botanist, SLNS Degree and PG College, Yadadri-Bhongir. Plant material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process ^[6].



Fig. 1- Leaves of Plant of *Butea monosperma* Roxb

Preparation of methanolic extract:

Fresh leaves of *Butea monosperma* Roxb were collected and washed under tap water. Leaf extract used was prepared by taking 20 gms of finely cut leaves into 250 ml beaker containing 200 ml of methanol. The contents were mixed well and then mixture was boiled upto 50-60°C for 4-5 hrs. Further extract was filtered with whatmann filter paper. The filtrate was boiled until concentrated residue is formed. Concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities ^[7].

Phytochemical screening

Preliminary Phytochemical Screening Preliminary phytochemical screening of the 80% methanolic stem leaf extract of *Butea monosperma* Roxb revealed the presence of tannins, saponins, polyphenols, flavonoids, terpenoids, and steroids while alkaloids were not detected^[8].

Experimental animals

Animal experimentation part was performed strictly adhering to Indian regulations and approved by Institutional Animal Ethical Committee. Healthy adult wistar albino rats weighing 200-250 gms of either sex were selected for study. Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory Animal Diet) and water ad libitum. They were fasted overnight before day of experiment, after 72 hours of fasting from day of alloxan introduction^[9]. Animals were housed within departmental animal house and room temperature was maintained at 27° C. Animal studies had approval of IAEC.

Selection of dose for animal study

The dose considered for experiment on rats was obtained from conversion of human dose of *Butea monosperma* wild (3-5 g/kg). Conversion factor of human dose (per 200 g body weight) is 0.018 for rats and 0.002 for mice (Ghosh 1984). Hence calculated dose for rats (considering human dose 3 and 5 g/kg) is 200 and 300 mg/kg. Acute toxicity was done at dose of 2000 mg/kg body weight^[10].

Preparation of extracts: Aqueous and alcoholic extracts of *Butea monosperma* Roxb suspended in water in presence of 3% v/v Tween-80 solution. All drugs were administered orally for experimental purpose. Each time preparations of extracts were prepared when required. Drugs were administered at a constant volume of 10 ml/kg for each animal^[11].

Acute oral toxicity:

Acute oral toxicity of methanolic extract of *Butea monosperma* Roxb was determined by using albino wistar rats (200-250 g) which were maintained under standard conditions. Animals were fasted 12 hour prior to experiment, up and down procedure OECD guideline no. 425 were adopted for toxicity studies. Animals were administered with single dose of individual extract upto 2000 mg/kg and observed for its mortality during 2 days and 7 days study period (short term) toxicity and observed upto 7 days for their mortality, behavioral and neurological profiles^[12].

Procedure:

Animals were grouped and divided randomly into four groups of four and each was fasted to overnight. The blood samples were withdrawn by tail vein at 0 hour i.e. before I.P administration of extracts/standard/vehicle. Then blood was collected at an interval of 1, 2, 4, and 8 hour after the administration, and according to procedure blood glucose levels were measured by glucometer (ONE TOUCH glucometer). Administration of drug was continued upto fifteen days^[13].

Oral glucose tolerance test (OGTT) in normal rats:

On next day (16th day) after assessment of hypoglycemic activity OGTT was carried out in same normal animals.

Procedure: All animals in each group were administered 2 g/kg of glucose one hour after extract/ glibenclamide/ vehicle administration. Blood samples were collected by tail vein at 0 hour, 0.5 hour, 1 hour, 1.5 hour and 2 hour after administration of glucose load. Blood glucose levels were measured by glucometer^[14].

Assessment of anti-diabetic activity in alloxan induced diabetic rats:

Albino wistar rats of either sex weighing 200-250 g were selected for the study. All animals were allowed free access to water and pellet diet and maintained at room temperature in rat cages. Alloxan was dissolved in normal saline immediately before use. Diabetes was induced in 16 hour fasted rats by single intraperitoneal injection of 120 mg/kg body weight of freshly prepared alloxan in normal saline.

The rats after alloxanization were given 5 % w/v glucose solution in feeding bottles for next 24 hours in their cages to prevent hypoglycemia. After 72 hrs rats with fasting blood glucose levels greater than 200 mg/dl were selected and used for further studies. All animals were observed for seven days for consistent hyperglycemia (fasting blood glucose level greater than 200 mg/dl and lesser than 400 mg/dl) and such animals were selected and divided into six groups of four each and used for study of following experimental models^[15].

Table 1: Group classification for assessment of ant diabetic activity

Group	Treatment	Dose (mg/kg)
Group 1	Normal control received distilled water	10ml/kg
Group 2	Diabetic control received distilled water	10ml/kg
Group 3	Standard group received Glibenclamide	10ml/kg
Group 4	Methanolic extract of <i>B.monosperma</i>	200
Group 5	Methanolic extract of <i>B.monosperma</i>	300

Effect of methanolic extract of *Butea monosperma* Roxb on blood glucose levels in alloxan induced diabetic rats:

All animals of above groups were administered as per treatment protocol mentioned above. Blood samples were collected by retro orbital puncture at 0, 1, 2, 4 and 8 hour. Treatment was continued for next 22 days. Again blood samples were also collected on 4th, 7th, 14th and 21st day after 1 hour administration for sub acute study. Blood glucose level was estimated at various time intervals by subjecting the collected blood to cold centrifugation for serum separation. Serum obtained was used for estimating glucose level using GOD/POD (span) kit ^[16].

Oral glucose tolerance test (OGTT) in alloxan induced diabetic rats:

On 8th, 15th and 22nd day OGTT was carried out on same alloxan induced diabetic animals used for assessment of anti-diabetic activity studies.

Procedure: All animals in each group were administered 2 g/kg of glucose one hour after extract/ glibenclamide/ vehicle administration. The blood samples were collected by retro orbital puncture at 0 hour, 0.5 hour, 1 hour, 1.5 hour and 2 hour after administration of glucose load. Serum was treated with solutions of GOD/POD kit and according to procedure blood glucose levels were measured under by biochemical analyzer ^[17].

Statistical analysis

The values were expressed as mean ± SEM data was analyzed using one-way ANOVA followed by T-test. Two sets of comparison had made. i.e.

1. Normal control Vs All treated groups.
2. Diabetic control Vs All treated groups.

Differences between groups were considered significant at $P < 0.001$ and $P < 0.05$ levels¹⁸.

RESULTS

Acute toxicity testing

Acute toxicity studies revealed that the alcoholic extracts of *Butea monosperma* Roxb were safe up to 2000 mg/kg of body weight and approximate LD 50 is more than 2000 mg/kg. No lethality or any toxic reactions was observed up to end of study period.

Anti-diabetic activity in alloxan induced diabetic rats

Fasting blood glucose levels (FBGL) in normal rats were in range of 90-100 mg/dl. Treatment with alloxan (120 mg/kg, I.P.) had increased the FBGL to range of 252-266 mg/dl after 72 hours. These values on subsequent days got stabilized by day seven on an average of 255 mg/dl. Changes in fasting blood glucose levels in different groups are tabulated in Table 3. This data shown that blood glucose level of normal control animals has maintained throughout study period. The group 1 which is diabetic control group has shown significant increase in fasting blood glucose levels during this 21st day study period. The group 2 glibenclamide (10 mg/kg) treated group has shown ($p < 0.005$) significant decrease in fasting blood glucose level during 7th, 14th and 21st day of study period.

Effect of methanolic extract of *Butea monosperma* on antidiabetic activity in alloxan induced diabetic rats

The animals treated with 200 and 300 mg/kg of methanolic extract of *Butea monosperma* Roxb shown significant decrease ($P < 0.005$) in FBGL on 7th, 14th and 21st day of treatment when compare to other groups of animals. These extract have reduced more (%) in FBGL when compared to control group. The detailed results are summarized in Table No: 2.

Table 2: Effect of methanolic extract of *Butea monosperma* on fasting blood glucose level (FBGL) in alloxan induced diabetic rats.

Treatment	Dose (mg/kg)	Blood glucose level (mg/dl)			
		0 day	7 th day	14 th day	21 st day
Normal control	-	100±3	98±2	95±1	91±1
Diabetic control	10	240±1	267±2	274±3	285±2
Glibenclamide	10	99±2	95±3	78±1	71±1
Methanolic extract of <i>Butea monosperma</i>	200	88±1	78±2	70±1	62±2
Methanolic extract of <i>Butea monosperma</i>	300	95±1	83±1	78±2	60±3

Values are expressed as mean \pm S.E.M. n=3. Significant values were compared with $P < 0.005$. Normal control Vs all groups. Paranthesis indicates percentage reduction in BGL.

Oral glucose tolerance test (OGTT) on 22nd day-

MESS (200 and 300 mg/kg) significantly ($P < 0.005$) suppress the rise in FBGL after glucose load (2 g/kg) in rats, at first half-an-hour and up to 2 hr time period as compare with other groups extract glibenclamide on 15th day. While MESS produced significant reduction in FBGL. Glibenclamide (10 mg/kg) showed ($P < 0.005$) significant suppression in FBGL rise at 1st, 8th, 15th & 22nd day normalized FBGL within 2 hrs. The detailed results are summarized in Table 3.

Table 3: Effect of extract of methanolic extract of *Butea monosperma* on 22nd day in normal rats.

Treatment	Dose (mg/kg)	Blood glucose level (mg/dl)			
		1 st day	8 th day	15 th day	22 nd day
Normal control	-	105 \pm 1	150 \pm 3	121 \pm 1	133 \pm 1
Diabetic control	10	231 \pm 2	375 \pm 4	351 \pm 2	221 \pm 1
Std. (Glibenclamide)	10	100 \pm 1	120 \pm 3	116 \pm 1	110 \pm 2
Methanolic extract of <i>Butea monosperma</i>	200	274 \pm 2	251 \pm 5	187 \pm 1	139 \pm 3
Methanolic extract of <i>Butea monosperma</i>	300	187 \pm 2	170 \pm 3	147 \pm 2	117 \pm 1

Values are expressed as mean \pm S.E.M. n=3. Significant values were compared with $P < 0.005$. Normal control Vs all groups. Paranthesis indicates % reduction in BGL.

DISCUSSION

The present study was aimed at evaluating antidiabetic activity of methanolic extract of *Butea monosperma* Roxb at a dose of 200 and 300 mg/kg showed significant effect on glucose tolerance and extract also showed reduction in fasting blood glucose levels in normal and alloxan induced diabetic rats. These findings indicate that the extracts might be producing hypoglycemic effect by a mechanism independent from insulin secretion e.g. by the inhibition of endogenous glucose production or by the inhibition of intestinal glucose absorption. Alloxan monohydrate is one of chemical agent used to induce diabetes mellitus in animals. It induces diabetes by dose dependent destruction of β -cells of islets of langerhans. It was observed that single intravenous dose of alloxan exhibited significant hyperglycemia. Excessive hepatic glycogenolysis and gluconeogenesis associated with decreased utilization of glucose by tissues is fundamental mechanism underlying hyperglycemia in diabetic state. As hyperglycemia induced by alloxan falls under category of mild diabetes and may reverse after a few weeks, hypoglycemic effect of plant in hyperglycemic rats was studied during 22 days treatment. Difference observed between initial and final fasting blood glucose levels of extract treated hyperglycemic rats revealed antihyperglycemic effect of *Butea monosperma* Roxb wild throughout period of study. Effect of methanolic extract of *Butea monosperma* Roxb wild was compared with that of reference standard, glibenclamide and it has shown a significant results.

CONCLUSION

The data of blood glucose level of rats treated with alloxan (150 mg/kg body weight) produced diabetes within 72 hrs. After 72 hrs of Alloxan administered blood glucose levels of rats were observed. It was observed that significant lowering of sugar in alcoholic extract. Administration of MESP at a dose of 200 and 300 mg/kg showed significant anti-hyperglycaemic effect at 21st day which was evident from the 1st day on wards as compared to standard alcoholic extract of three extract combination has showed better efficacy than individual extract in all treated extract. Anti-hyperglycaemic effect of the extract on the fasting blood sugar levels on diabetic rats. Decreasing blood glucose levels are comparable with that of 10 mg/kg of glibenclamide, glibenclamide (10 mg/kg body weight) shows significant effect on compare to initial and more significant effect on 7th day compare to initial. Methanolic extracts of *Butea monosperma* Roxb (200 mg/kg body weight) shows significant ($P^* < 0.01$), effect. Results of anti-diabetic activity of extract established a scientific basis for utility of these plants in treatment of diabetes. The methanolic extract had shown significant reduction in blood glucose levels in alloxan induced diabetic rats and produced maximum anti-diabetic activity and are higher than hypoglycemic activity of glibenclamide in diabetic rats. In conclusion, these extract showed significant anti-diabetic effect in diabetic rats after oral administration.

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