Available online on 10.01.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Antidiabetic and anticataract evaluation of different extracts of *Butea monosperma* Linn. in STZ induced diabetic animals

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ABSTRACT

Aim- The main aim of study is to evaluate the anti-diabetic and anti-cataract activity of different extracts of *Butea monosperma* in diabetic animals. **Material & Methods-** Different extracts were prepared by successive solvent extraction methods. All the extracts were examined for the presence of different phytoconstituents. Diabetes was induced by single injection of STZ (40mg/kg) in Wistar albino rats and diabetes was confirmed by measurement of blood glucose level with the help of glucometer which is based on glucose oxidation methods. For the anti-cataract activity cataract was induced by solution of glucose in goat lenses and different biochemical parameters i.e. MDA, Na+, K+ were measured. **Results-** Preliminary phytochemical screening methods showed the presence of alkaloids, flavonoids, terpenoids, glycosides etc. In anti-diabetic evaluation, butanolic extract showed a highly significant activity in diabetic animals as compared to other extracts. So butanolic extract was further selected for the anti-cataract activity in goat eye lenses. Butanolic extract also highly significantly affect cataract formation all related biochemical parameters and electrolyte level. **Conclusion-** Administrations of butanolic extract have significant antidiabetic effect in STZ induced diabetes. Butanolic extract exhibited potent anti-hyperglycemic, antioxidant and anti-cataract effects in STZ induced type 2 diabetic rats. This effect may be presence of flavonoids in butanolic extract.

Keywords: Cataract, STZ induced diabetes, *Butea monosperma*, Butanolic extract, Hyperglycemia

Article Info: Received 25 Sep 2018; Review Completed 29 Nov 2018; Accepted 26 Dec 2018; Available online 10 Jan 2019

Cite this article as:

Malvia S, Jain NK, Yadav S, Tomar V, Antidiabetic and anticataract evaluation of different extracts of *Butea monosperma* Linn. in STZ induced diabetic animals, Journal of Drug Delivery and Therapeutics. 2018; 8(6-A):53-57

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INTRODUCTION

Diabetes is defined as circumstances in which homeostasis of carbohydrate, protein and lipid metabolism is improperly keeping pace as a consequence of a relative or absolute deficiency of insulin emission, resistance to insulin action or both at one or more points in the multifaceted pathways of hormone action[1].

As diabetes progresses and β-cell function deteriorates, many complications occur due to hyperglycemia and other vascular changes. When oxidative injure to the lens and its proteins become extensive, the lens becomes adequately cloudy to obstruct vision, and the individual is said to have a cataract. Alternatively, events that cause loss of order and induce abrupt fluctuations in refractive index result in greater than before light dispersion and loss in transparency, commonly called cataract[2].

As per the literature review, it has been observed that flowers of *Butea monosperma* Linn. is listed among the various medicinal plants widely been used as a antibacterial, demulcent, bitter tonic, laxative, carminative, refrigerant, and febrifuge, diuretic, useful in chronic cystitis, gonorrhea and cadiotonic, acute-chronic inflammatory conditions and in treatment of diabetes mellitus[3].

In the absence of any scientific evidence for their anti-diabetic activity in diabetic animals and use of these medicinal plants in diabetic complication i.e. cataract formation, there is a need in scientifically establishing the anti-diabetic activity and anti-cataract activity in diabetic animals, so that we are able to come up with a more effective and potent bioactive extract with fewer side effects in comparison with existing synthetic drugs[4].

MATERIAL & METHODS

Collection and authentication:

The flowers of *Butea monosperma* was collected from outfield during the month of March. The flowers were washed thoroughly in tap water, dried in shade, finely powdered and used for successive extraction methods. The material was submitted to Department of Botany, Janata PG College, A.P.S. University, Rewa, M.P., and identified by Prof.

Dr. S.N Dwivedi, Professor and Head of Department, Janata PG College, A.P.S. University, Rewa, M.P. as Voucher Specimen Number-/J/BOT/H-312 .

Successive extraction methods:

Powdered drug 100gm was weighed and packed in soxhlet. The drug was continuously extracted with petroleum ether for about 72 hours. Defatted drug was subjected to extraction with chloroform in soxhlet apparatus, the extraction was completed in 17-18 hrs. The extract was dried & stored in dark place. Drug was subjected to extraction with methanol (90%), butanol and finally with water in soxhlet apparatus, the extraction was completed in 25 cycles. The extract was dried & stored in dark place. The % Yield of the Petroleum ether, chloroform, methanol, Butanol, & Aqueous extract of *Butea monosperma* were calculated by using the following formula[5].

$$\% \text{ Yield} = \frac{\text{Net weight of powder in gram after extraction}}{\text{Total weight of leaf powder in gram taken for extraction}} \times 100$$

Preliminary phytochemical studies:

Preliminary phytochemical screening was performed for the presence of fatty acids, terpenoids, alkaloids, flavonoids, steroids and other active phytochemicals[6].

Antidiabetic activity

Experimental Animals

Wistar Albino rats of either sex (150 to 200 g) were purchased from the CPCSEA approved vendor New Delhi. They were maintained under standard laboratory conditions at 25 ± 2°C and normal 12-hour light-dark cycle were used for the experiment. Commercial pellet diet and water were provided *ad libitum* throughout the course of study. All the experiments on animals were done as per protocol approved by IAEC/CPCSEA guidelines.

Selection of Dose (Acute Toxicity Studies)

Acute oral toxicity test was carried out according to the OECD guideline No. 423. Wistar Albino Rats were kept for overnight fasting prior to drug administration. A total of three animals were used, which received a single oral dose in 2000 mg/kg, body weight of different extracts. The animals were observed for a period of 24 hr for the changes in behavior, hypersensitivity reactions etc. Mortality, if any, was determined over a period of 2 weeks. Hence in our studies we selected 1/10 and 1/5th dose i.e. 200 and 400 mg/kg dose.

Streptozotocin (STZ) induced diabetes in rats

After fasting 18 hours, the rats were injected intraperitoneal injection through tail vein with a single dose of 40 mg/kg Streptozocin (Sigma, St. Louis, Mo, USA), freshly dissolved in citrate buffer (pH 4.5). After injection, the rats had free access to food and water. The diabetes was confirmed by estimating the blood glucose level after 3 days by glucometer based on glucose oxidation method. Rats having blood glucose level more than 250 mg/dl were selected for further study[7].

Experimental Design

In order to assess the anti-diabetic activity of *Butea monosperma* the animals were divided in thirteen groups. Each group contains six animals.

Group 1: Normal control, 0.9% NaCl-treated animals

Group 2: Diabetic control, STZ -treated rats (40 mg/kg body weight)

Group 3: Diabetic animals treated with Pet. Ether extract of *Butea monosperma* (200 mg/kg body weight)

Group 4: Diabetic animals treated with Pet. Ether extract of *Butea monosperma* (400 mg/kg body weight)

Group 5: Diabetic animals treated with chloroform extract of *Butea monosperma* (200 mg/kg body weight)

Group 6: Diabetic animals treated with chloroform extract of *Butea monosperma* (400 mg/kg body weight)

Group 7: Diabetic animals treated with methanolic extract of *Butea monosperma* (200 mg/kg body weight)

Group 8: Diabetic animals treated with methanolic extract of *Butea monosperma* (400 mg/kg body weight)

Group 9: Diabetic animals treated with butanolic extract of *Butea monosperma* (200 mg/kg body weight)

Group 10: Diabetic animals treated with butanolic extract of *Butea monosperma* (400 mg/kg body weight)

Group 11: Diabetic animals treated with aqueous extract of *Butea monosperma* (200 mg/kg body weight)

Group 12: Diabetic animals treated with aqueous extract of *Butea monosperma* (400 mg/kg body weight)

Group 13: Diabetic animals treated with Standard drug, Glibenclamide-treated rats (5 mg/kg body weight)

Anti-cataract Activity of extract showing best activity in STZ induced diabetes

Collection of Eye Balls

Fresh goat eye balls of young and healthy goats were collected from the slaughter house, immediately after the slaughter. These eye balls were immediately transferred to the laboratory at 0-4°C. Sliced the Cornea from the front of the eye to gain access to the lens.

Lens culture

The lenses were incubated in artificial aqueous humor (NaCl 140mM, KCl 5mM, MgCl2 2mM, NaHCO3 0.5mM, Na2HPO4 0.5mM, CaCl2 0.4mM, Glucose 5.5mM) for 72 hours at room temperature at a pH of about 7.8 is maintained. In addition to this 32mg of penicillin and 250mg of streptomycin were added to prevent bacterial contamination. Glucose 55mM served as cataract inducer[8].

The grouping schedules are as follows.

Group I: Normal lens glucose 5.5mM (control)

Group II: Glucose 55mM (induced)

Group III: A. Glucose 55mM+ Treated with Butanolic extracts (100µg/ml)

B. Glucose 55mM+ Treated with Butanolic extracts (300µg/ml)

C. Glucose 55mM+ Treated with Butanolic extracts (500µg/ml)

Preparation of lens homogenate

After incubation, lenses were homogenized in 10 volumes of 0.1M potassium phosphate buffer, pH 7.0. The homogenate

was centrifuged at 10,000 rpm for 1 h and the supernatant was used for estimation of biochemical parameters[8].

Biochemical Parameters

Estimation of total protein content

To 0.1 ml of lens homogenate, 4.0ml of alkaline copper solution was added and allowed to stand for 10min. Then, 0.4 ml of phenol reagent was added very rapidly and mixed quickly and incubated in room temperature for 30 mins for colour development. Reading was taken against blank prepared with distilled water at 610 nm in UV-visible spectrophotometer. The protein content was calculated from standard curve prepared with bovin serum albumin and expressed as µg/mg lens tissue[8].

Estimation of malondialdehyde (MDA)

Lenses were homogenized in10% (w/v) 0.1 M Tris–HCl buffers (pH 7.5). One milliliter of the homogenate was combined with 2 ml of TCA–TBA–HCl reagent 15% trichloroacetic acid (TCA) and 0.375% thiobarbituric acid (TBA) in 0.25 N HCl and boiled for 15 min. Precipitate was removed after cooling by centrifugation at 1000g for 10 min and absorbance of the sample was read at 535 nm against a blank without tissue homogenate. The values are expressed as nmoles of MDA/ min/ mg lens protein[8].

Estimation of Different electrolytes

Sodium and potassium levels were estimated by flame photometry[8].

Statistical Analysis

The values are expressed in mean ± SEM. The results were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's "t" test to determine the statistical significance. $p< 0.05$ was chosen as the level of significance. Statistical analysis was performed using Graph Pad Prism Software 5.0 version.

RESULTS

Streptozotocin induced antidiabetic activity of *Butea monosperma*

Effect on Blood glucose level

The induction of diabetes with streptozotocin increases the blood glucose level significantly ($p<0.001$) in group II rats as compared to normal rats. In 21 day study glibenclamide the standard drug restored the blood glucose highly significantly with the $p<0.001$ in 14 days whereas butanolic extract (200 & 400 mg/kg) reduced the glucose level moderately and highly significant with $p<0.01$ & $p<0.001$. Petroleum ether, chloroform, methanolic and aqueous extracts had moderately significant effects ($p<0.01$) on 14th and 21st days.

Table 1: Effect of different extracts on glucose level in streptozotocin induced diabetic rats

Group No	Group	Blood Sugar level				
		Long Term Study (Days)				
		Before inducing Diabetes	3	7	14	21
I	Normal control	81.5 ± 2.44	82.2 ± 2.48	82.5 ± 3.77	83.5 ± 3.77	82.11 ± 3.44
II	Diabetic control	82.4 ±2.83	242.1 ± 4.55	273.8 ± 3.77***	262.3 ±3.51 ***	292.1 ± 3.24***
III	Pet. Ether extract (200 mg/kg)	78.1 ± 3.33	240.6 ± 2.55	239.9 ± 4.33**	234.8± 4.44**	227.6 ± 4.22**
IV	Pet. Ether extract (400 mg/kg)	82.72 ± 3.58	244.4 ± 3.74	224.3 ± 3.49**	218.8± 3.61**	206.4 ± 4.41**
V	Chloroform extract (200 mg/kg)	81.4 ± 3.46	242.6 ± 3.58	222.9 ± 3.51	214.8± 4.33**	212.6 ± 3.65**
VI	Chloroform extract (400 mg/kg)	80.4 ± 3.45	243.6 ± 3.68	220.2 ± 3.41	214.8± 2.99**	215.6 ± 3.88**
VII	Methanolic extract (200 mg/kg)	79.4 ± 3.44	242.7 ± 3.69	226.3 ± 4.41	218.3 ±3.49**	215.1 ± 3.55**
VIII	Methanolic extract (400 mg/kg)	82.3 ± 2.44	243.6 ± 3.42	221.9 ± 3.44	215.8± 4.55**	217.6 ± 3.78**
IX	Butanolic extract (200 mg/kg)	84.27 ± 2.49	245.1 ± 3.65	215.2 ± 3.66***	201.8±3.38***	193.2± 4.55***
X	Butanolic extract (400 mg/kg)	87.78 ± 3.49	243.6 ± 3.69	205.2 ± 4.71***	190.6±4.62***	170.3± 4.55***
XI	Aqueous extract (200 mg/kg)	82.4 ±3.92	241.7 ± 3.44	271.8 ± 3.44	265.3 ±3.11*	262.1 ± 3.33*
XII	Aqueous extract (400 mg/kg)	83.4 ±2.83	241.7 ±3.44	268.2± 4.31	262.3 ±2.66*	259.1 ± 3.44*
XIII	Glibernclamide (5 mg/kg)	83.25 ± 2.44	244.8 ± 2.61	198.2 ± 3.66**	168.3±4.55***	159.7± 3.24***

Where- *p<0.05, **p<0.01, ***p<0.001 compared with diabetic control vs treated groups

Anti-cataract Activity in Goat Eye Lenses

Glucose (55mM) treated lenses showed significantly higher Na+ and lower K+ activity ($P<0.001$) compared with normal lenses. Butanolic extract treated lenses showed significantly increased level of K^+ ($P<0.001$) with increasing concentration and the maximum activity was registered at concentration of 500 µg/ml respectively.

Butanolic extract treated lenses showed significantly decreased level of Na^+ ($P<0.001$) with increasing concentration and the maximum activity was registered at concentration of 500 µg/ml respectively.

Table 2: Effect of butanolic extract on Na^+ and K^+ activity in lens homogenate

Description	Na^+(meq/gm)	K^+(meq/gm)
Normal Goat lens	152±4.14	15.1±1.4
Goat lens + Glucose55mM	221.4±3.35***	6.6±0.3***
Goat lens + Glucose55mM + Butanolic extract in conc. of		
a) 100 µg/ml	167.6±4.73**	8.4±0.44*
b) 300 µg/ml	159.7±3.41**	11.5±0.4**
c) 500 µg/ml	153.4±13.56***	14.4±2.6***

Where- *p<0.05, **p<0.01, ***p<0.001 compared with diabetic control vs treated groups

Effect of Butanolic Extract on total protein and MDA level

Group II (disease control) showed significant decrease (P<0.001) in protein content as compared to group I. On the treatment of butanolic extract, the total protein content was highly significantly increased in treated groups.

There is a significant increase in MDA levels in groups treated with glucose. Butanolic extract treated lens had showed significant decrease in lipid peroxidation levels when compared to glucose 55 mM treated lens. The level of MDA was expressed in nmoles of MDA formed/mg protein.

Table 3: Effect of butanolic extract on total protein and MDA level in lens homogenate

Description	Total Protein (mg)	MDA (nmoles/mg)
Normal Goat lens	182.5±3.43	33.9±2.43
Goat lens + Glucose55mM	153.2±3.12**	60.5±3.22
Goat lens + Glucose55mM + Butanolic extract in conc. of		
a) 100 µg/ml	167.5±3.32*	50.2±3.95*
b) 300 µg/ml	180.4±4.27**	44.1±2.74**
c) 500 µg/ml	186.5±2.35***	36.4±4.34***

Where- *p<0.05, **p<0.01, ***p<0.001 compared with diabetic control vs treated groups

DISCUSSION

The research was envisaged for antidiabetic and anticataract activity of different extracts of flowers of *Butea monosperma* by STZ induced diabetes.

The islet β-cells are susceptible to damage caused by oxygen free radicals [9,10] since the antioxidant defense system is weak under diabetic condition. The levels of antioxidant protection structure are altered in streptozotocin-induced diabetic rats, which are in good association with the present observation. Non protein thiols like glutathione are one of the significant primary defenses that frustrate the oxidative stress. Decreased levels of serum glutathione in streptozotocin diabetic rats, which is in dependable with earlier reports[10] ·The butanolic extract of *Butea monosperma* produced a marked decrease in blood glucose levels at 200 mg/kg and 400 mg/kg body weight in streptozotocin-diabetic rats after 21 days treatment. The antidiabetic effect *Butea monosperma* may be due to increased release of insulin from the existing β-cells of pancreas.

Butanolic extract showed the presence of flavonoids and phenolic compounds. From the previous reported literature, triterpenoids, flavonoids and phenolic compounds are responsible for anti-diabetic effect. So almost certainly, antidiabetic effect of plants may be due to presence of flavonoids.

Lens being rich in structural proteins has long been hypothetical that eventual event in cataractogenesis is essentially a disturbance in the state of lens proteins. Most of the changes observed in protein structure are due to ageing of the proteins [11,12] which may lead to the defeat of transparency[13]. The lens yellows with age and this modify has been attributed to insoluble fraction in both ordinary[14] and cataractous lenses[15].

In our study we found that, on treatment of both butanolic extract had significantly increased protein content in lens. The probable mechanism of anti-cataract activity may be via alteration of protein content in eye.

Antioxidant enzymes are able to catalytically take away free radicals and other reactive kind. A wide array of enzymatic antioxidant military protection exists, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX)[16]. Appearance of some of these enzymes is controlled by redox-sensitive transcription factors, allowing the antioxidant system to respond to fluctuations in production of oxidizing species caused by photo oxidative processes, especially during sustained exposure[17].

In our study we found that, on administration of butanolic extract had significantly decreased the MDA level. MDA is a powerful oxidant radical and can smash up the lens. Since flavonoids act as an antioxidant derivative, so by inhibiting the formation of these radicals they prevent further spoil by oxidation.

Na+ K+ ATPase is significant in maintaining the ionic equilibrium in the lens, and its impairment causes accumulation of Na^+ and loss of K^+ with hydration and swelling of the lens fibers leading to cataractogenesis (Wilbur, 1949). In the present study, cataract lens treated with butanolic extract eminent the activity of total proteins and K^+ ions to the level of normal level while reduced concentrations of Na^+ ions. This clearly evidenced that butanolic extract prevent the alteration of Na^+ and K^+ imbalance, which may be due to a direct effect on lens membrane Na+ K+ - ATPase or indirect through their free radical scavenging activity.

CONCLUSION

Administrations of butanolic extract have significant antidiabetic effect in STZ induced diabetes. Butanolic extract exhibited potent anti-hyperglycemic, antioxidant and anti-cataract effects in STZ induced type 2 diabetic rats. Thus, our results strongly sustain the notion that behavior of butanolic extract to diabetics would help in achieving good glycemic and metabolic manage due to its antidiabetic and anti-cataract effect.

REFERENCES

1. Sangal A, Role of cinnamon as beneficial antidiabetic food adjunct: a review, Advances in Applied Science Research, 2011; 2(4):440-450.
2. Bunce GE, Nutrition and eye disease of the elderly, *Journal of Nutritional Biochemistry, 1994;* 5(1):66-77.
3. Khandelwal KR, *Practical Pharmacognosy*. Pune, Nirali Prakashan, 2006.
4. Kirtikar KR and Basu BD, *Indian Medicinal Plants*. India, International Book Distributors Book Sellers & Publisher,2005.
5. Kokate CK, *Practical Pharmacognosy*. Delhi, Vallabh Prakashan, 1996.
6. Khan MSY, Bano S, Javad K, Asad MM, A comprehensive review on the chemistry and pharmacology of Corchorus species- A source of cardiac glycosides, triterpenoids, ionones, flavonoids, coumarins, steroids and some other compounds" Journal of Scientific & industrial research, 2006; 66:283-298.
7. Choudhary NK, Jha AK, Sharma S, Goyal S, Dwivedi J, Antidiabetic Potential of Chloroform Extract of Flowers of *Calotropis gigentea*: An In-vitro and In-Vivo study. International Journal of Green Pharmacy, 2011; 5(4):296-301.
8. Gehlot V. Dave K. Choudhary NK, Goyal S, Berberine from roots of *Berberis aristata* prevents cataract formation in isolated goat eye lens: An In-vitro study. International Journal of Pharmaceutical and Biological Archives. 2012; 3(5):1265-1270.
9. Prince PSM, Menon VP, Effect of *Syzigium cumini* in plasma antioxidants on alloxan-induced diabetes in rats, Journal of Clinical Biochemistry and Nutrition, 1998; 25(2):81–86.
10. Cai L, Wang J, Li Y, Sun X, Wang L, Zhou Z, Kang Y J, Inhibition of superoxide generation and associated nitrosative damage is involved in metallothionein prevention of diabetic cardiomyopathy. Diabetes, 2005; 54:1829-1837.
11. Waley SG, In The eye. (ed:Dayson, H.), Academic press, London,1969; 1:299- 379.
12. Bloemendal, H, Lens proteins. *CRC Cri. Rev. Biochem, 1982*:1-38.
13. Spector A, Roy D and Stauffer J, Isolation and characterization of an age dependent polypeptide from human lens with non-tryptophan fluorescence. *Exp. Eye Res, 1975;* 21:9-24.
14. Pirie A, Color and solubility of proteins of human cataracts. *Invest. Ophthalmol, 1968;* 7:634-650.
15. Halliwell B & Gutteridge JMC, *Free Radicals in Biology and Medicine*. Oxford University Press Inc, New York, 1999.
16. Rahman I . Regulation of nuclear factor-kB, actiivator protein-1, and glutathione levels by tumor necrosis factor-alpha, and dexamethasone in alveolar epithelial cells. *Biochemical Pharmacology, 2000;* 60(8):1041-1049.
17. Wilbur KM, Estimation of lipid peroxide. Arch Biochem Biophysics, 1949; 24:305-15.