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Original Research Article

Antidiabetic, Antihyperlipidemic and Antioxidant Activities of Buchanania lanzan Spreng Methanol Leaf Extract in Streptozotocin-Induced Types I and II Diabetic Rats

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Abstract

Purpose: *To evaluate the antidiabetic, antihyperlipidemic and antioxidant activities of Buchanania lanzan.*
Methods*: Wistar rats were divided into nine groups of six animals each, and 40 mg/kg of streptozotocin or streptozotocin + nicotinamide was administered intraperitonially to induce types I and II diabetes. Those with blood glucose levels > 190 ± 8 mg/dl were administered the methanol leaf extract of Buchanania lanzan (MEBL, 100 or 200 mg/kg, p.o.) or positive control for 21 days. Blood glucose, lipid profile, antioxidant enzymes and oxidative stress markers were evaluated.*
Results: *Following induction, blood glucose level rose to 327.7 ± 47.4 mg/dl, compared to the normal value of 910 ± 3.2 mg/dl. Administration of MEBL (100 or 200 mg/kg) significantly ($p < 0.05$) decreased blood glucose level, serum lipid profile, and significantly ($p < 0.05$) increased antioxidant activity as evidenced by increase in super oxide dismutase (SOD), catalase, glutathione (GSH), and decrease in the activity of lipid peroxidation (LPO).*
Conclusion: *MEBL exhibits antidiabetic, antihyperlipidemic and antioxidant activities in diabetic rat and needs to be further investigated for the treatment of both types I and II diabetes mellitus.*

Keywords: *Antidiabetic, Antihyperlipidemic, Antioxidant, Buchanania lanzan.*

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disease caused by an absolute or relative lack of insulin and or reduced insulin activity, which results in hyperglycemia and abnormalities in carbohydrate, protein and fat metabolism. Hyperlipidemic condition is metabolic complication of both clinical and experimental diabetes [1]. Low density lipoprotein in diabetic patients leads to abnormal metabolism and is associated with increase in very low density lipoprotein (VLDL) secretion and impaired VLDL catabolism. Ultimately, this leads to atherosclerotic plaque formation. Patients with diabetic mellitus are more likely to develop microvascular and macrovascular complications than the non diabetic population. Though different types of oral hypoglycaemic agents are available along with insulin for the treatment of diabetes mellitus, there is growing interest in herbal remedies due to the side effects associated with these therapeutic agents [2]. The

investigation of anti-diabetic agents of plant origin which are used in traditional medicine is thus of great importance.

Management of diabetes with synthetic drugs is costly and chances of side effects are high. Therefore, it is prudent to look for options in herbal medicines for diabetes as well. Some active principles present in medicinal plants have been reported to possess pancreatic beta cells regenerating, insulin releasing and fighting the problem of insulin resistance [3]. It is claimed, not only in Ayurveda, but also in several other traditional systems of medicine, that plants useful in diabetes management also possess strong antioxidant/free-radical scavenging properties [4]. Free radicals meet many of the criteria required for a role in the pathogenesis of diabetic syndrome [6].

Buchanania lanzan Spreng (locally called as Chironji), a member of family Anacardiaceae is a commercially useful tree species found in several areas of India. The plant has well-known traditional uses and its seeds are used as expectorant and tonic. The oil extracted from kernels is applied on skin diseases and also to remove spots and blemishes from the face. The root is used as expectorant, in biliousness and also for curing blood diseases. The juice of the leaves is digestive, expectorant, aphrodisiac and purgative. The rhizome of *B. lanzan* finds an important place in indigenous medicine as an expectorant, diuretic and carminative. It is also found to have anticancer, antihypertensive, larvicidal and anti-diabetic activities [7-10]. The present study was carried out to evaluate the antidiabetic, antihyperlipidemic and antioxidant activity of the methanol extract of *Buchanania lanzan* in streptozotocin-induced types I and II diabetic rats.

EXPERIMENTAL

Chemicals

Streptozotocin was purchased from Himedia Research Laboratories, Mumbai, India. Nicotinamide was purchased from Sisco Research Laboratories, Mumbai, India. Assay kits for serum triglycerides (TGL), total cholesterol (TC) and high density lipoproteins (HDL) were obtained from Coral Diagnostics Ltd, Mumbai, India. All other chemicals used were of analytical grade.

Plant material and preparation of extract

The leaves of *Buchanania lanzan* were obtained from the local market and authenticated by V. Krishna Rao of Kovel Foundation, Visakhapatnam, India. Voucher number of the specimen is KF VS 7274 and the specimen is kept in the herbarium of Kovel Foundation, Visakhapatnam.

The leaves were air-dried and then powdered. Lipids were removed via petroleum ether extraction by maceration method. It was then filtered and the filtrate discarded. The residue was successively extracted with methanol (99%) using a Soxhlet apparatus. The solvent was completely evaporated under reduced pressure and the dry extract dissolved in normal saline prior to further study.

Experimental animals

Male albino Wistar rats (aged, 4 months; body weight, 180 ± 10 g), used in the study, were procured from Sainath Enterprises, Hyderabad, India. The animals were housed in polyacrylic cages with not more than six animals per cage, at an ambient temperature of 18 ± 2 ^{0}C and 12 h light/12 h-dark cycle. The rats had free access to standard chow diet and water *ad libitum*. The care and handling of the animals were carried out as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi and OECD TG 452. [19]. The research protocols were approved by the Institutional Animal Ethical Committee (IAEC) as per approval ref no. 1028/SPIPS/Wgl/IAEC/2011.

Acute toxicity study

Acute toxicity assay was performed in rats according to OECD 423 guidelines [20] and no lethality was found in any of the groups after treatment up to 2000 mg/kg.

Experimental design

Nine groups of six animals each were used for study. Group I animals served as normal control rats and received normal saline. Group II served as type I diabetic control and received streptozotocin (40 mg/kg, ip). Group III was type-II diabetic control and received streptozotocin and nicotinamide (230 mg/kg, ip) Group IV animals were type I diabetic rats treated with 100 mg/kg of the extract of *Buchanania lanzan* (MEBL) while Group V animals were type-II diabetic rats treated with 100 mg/kg of MEBL Group VI served as type I diabetic treated with 200 mg/kg of MEBL, Group VII were type II diabetic rats treated with 200 mg/kg of MEBL; Group VIII animals were type I diabetic rats treated with the standard drug, insulin (4 IU/kg,

sc) [2] while Group IX type II diabetic rats treated with the standard drug, glibenclamide (0.5 mg/kg, orally) [2].

Induction of type I diabetes

A single dose of streptozotocin (40 mg/kg, ip) was administered to Groups II, IV, VI, and VIII rats. While normal control rats were injected with saline alone. Streptozotocin was dissolved in freshly prepared 0.1M citrate buffer (pH 4.5).[11] Streptozotocin-injected animals were given 5 % glucose for 24 h to prevent initial streptozotocin-induced hypoglycemic mortality [11].

Induction of type II diabetes (T2DM)

Experimental T2DM was induced in groups III, V, VII and IX rats by administering nicotinamide (NA, 230 mg/kg, ip, dissolved in normal saline) 15 min prior to administration of Streptozocin (STZ, 40 mg/kg, ip, freely dissolved in 0.1M citrate buffer pH 4.5).

Blood glucose was evaluated 3 days later and the animals with glucose level > 190 ± 8 mg/dl were selected for the study. The reference drugs and extract were administered once daily for 21 days. Blood glucose level was monitored on days 7, 14 and 21.

Assessment of biochemical parameters

After 21 days of the experiment, blood samples were collected by retro orbital sinus puncture, under mild ether anaesthesia from all the animals. Then the blood samples were centrifuged at 3000 rpm for 15 min and serum was collected. The collected serum was used for the evaluation of biochemical parameters.

Whole blood was used to determine glucose concentration with the aid of a glucometer [11] with reagent strips (Aspen Diagnostics (P) Ltd, Delhi, India). Serum total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides (TG) were measured using a standard kit (Coral Laboratories) [12]. The amount of lipid peroxidation products (LPO) present in serum was determined by thiobarbituric acid reactive substances (TBARS) method, which measures malondialdehyde (MDA) reactive products using UV-visible spectroscopy (SL-150, Elico Ltd, India) at 532 nm. LPO was expressed in nmol/mg protein [13]. Superoxide dismutase (SOD) in serum was determined using photo-oxidation method [14]. Change in absorbance was recorded spectrophotometrically at 460 nm for 4 min and SOD was expressed in nmol/mg protein. Glutathione was determined spectrophotometrically at a wavelength of 319 nm based on the formation of a coloured complex between glutathione and 5,5'-dithiobis-(2-nitrobenzoic acid (DTNB) [15]. Glutathione was expressed in µmol/mg pr. Catalase measurement was carried out based on the ability of catalase to oxidize hydrogen peroxide [16]. The change in absorbance of hydrogen peroxide solution was measured at 240 nm for 3 min at 1 min interval. On decomposition of hydrogen peroxide by catalase, the absorbance decreases with time. Catalase was expressed in nmol/mg protein.

Statistical analysis

Statistical analysis was done by using one way and two way ANOVA followed by Bonferroni's multiple comparison using Graph Pad Prism software, version 5.0 test Spreng (Graph Pad Software, Inc, USA). All results are expressed as mean ± SEM. A probability of $p < 0.05$ was considered as significant.

RESULTS

Effect of MEBL on blood glucose level

Diabetes induction using STZ showed significant hyperglycaemia in both types I and II diabetic groups. Oral administration of the MEBL (100 and 200 mg/kg) and the standard drugs (insulin and glibenclamide) for 21 days significantly ($p < 0.05$) lowered blood glucose levels (Table1). The effect was dose-dependently.

Effect of MEBL on serum lipid profile of type I and type II diabetic rats

The levels of LDL, VLDL, cholesterol and triglycerides in types I and II diabetic rats significantly ($p < 0.05$) increased following treating with streptozotocin while HDL levels decreased significantly ($p < 0.05$), when compared with normal control (Table 2). Treatment with standard drugs (insulin and glibenclamide) and MEBL produced significant ($p < 0.05$) decrease in LDL, VLDL, cholesterol and triglycerides, but a significant ($p < 0.05$) increase in HDL levels, compared to diabetic control groups.

Effect of MEBL on antioxidant activity in diabetic rats

The results for the anti-oxidant activity of the extract are shown in Table 4. Both the extract and reference drugs significantly ($p < 0.05$) reduced altered LPO damage. The lowest activities of CAT, SOD and GSH was seen in the

Table I: Effect of MEBL on blood glucose level (mean ± SEM, n = 6) of diabetic rats

Group	Blood glucose level (mg/dL)				
	Pre-STZ	Day 0	Day 7	Day 14	Day 21
I (Normal control)	101.66±4.70	109.66±4.84	117.00±0.57	111.33±1.20*	115.66±5.23*
II (Diabetic control, Type, I)	91.00±3.21	327.66±47.41*	353.66±46.00*	381.66±40.44*	405.33±27.51*
III (Diabetic control, Type II)	105.00±8.73	511.33±120.57*	429.00±91.000*	254.66±60.93*	109.66±40.17*
IV (Extract 100mg, Type I)	79.66±7.17	213.66±5.81	243.66±6.88	270.33±7.21	357.33±3.18
V (Extract 100mg, Type II)	130.66±19.83	335.00±14.10	259.66±31.15	149.33±10.68	119.00±4.72*
VI (Extract 200mg, Type I)	95.66±10.08	425.33±79.68*	337.66±64.15	303.33±73.60	154.66±22.21*
VII (Extract 200mg, Type II)	86.33±13.59	272.00±59.80	203.33±26.03	169.33±21.85	129.66±20.34*
VIII (STZ+INS	103.66±9.93	392.66±176.68	293.33±104.83	208.33±43.09	111.33±8.11*
IX (STZ+NIC+GLI)	90.33±7.21	392.33±61.86*	352.33±53.45	176.33±28.98	137.66±23.82*

**p < 0.05, Type 1 and Type II diabetic controls were compared to normal control. Test and standard groups were compared to respective diabetic control groups.*

Table 2: Effect of MEBL on serum lipid profile of type I and type II diabetic rats

Group	Serum lipid level			
	HDL (mg/dl)		*LDL (mg/dl)*	
	Day 0	*Day 21*	*Day 0*	*Day 21*
I (Normal control)	21.66±1.45	21.33±1.76[b]	53.46±1.75	51.33±1.34[b]
II (Diabetic control, Type I)	20.33±1.20	8.66±1.20	55.73±3.16	59.66±9.35
III (Diabetic control, Type II)	18.33±2.90	10.33±2.33	61.66±5.50	85.20±13.11
IV (Extract 100mg, Type I)	21.66±1.20	26.00±1.15[c]	54.20±0.41	33.8 ±7.05[a]
V (Extract 100mg, Type II)	18.00±2.30	26.00±1.15[d]	62.00±3.52	42.06±2.22[c]
VI (Extract 200mg, Type I)	20.33±2.02	21.00±1.15[b]	54.00±4.10	42.46±2.71
VII (Extract 200mg, Type II)	19.66±1.66	20.00±0.57[a]	54.46±5.42	44.60±4.42[c]
VIII (STZ+INS	22.66±2.02	25.33±1.76[c]	51.20±3.06	34.00±0.91[a]
IX (STZ+NIC+GLI)	19.00±2.08	24.66±1.76[c]	59.33±7.51	40.73±3.65[c]

Note: *HDL = high density lipoproyein; LDL = low density lipoprotein*

Table 3: Effect of MEBL on additional serum lipid parameters of types I and II diabetic rats

Group	VLDL (mg/dl)		TGL (mg/dl)		CHOL (mg/dl)	
	Day 0	*Day 21*	*Day 0*	*Day 21*	*Day 0*	*Day 21*
I (Normal control)	13.53±0.37	14.66±0.29[d]	67.66±1.85	73.33±1.45[d]	88.66±0.88	87.33±1.45[d]
II (Diabetic control, Type I)	14.60±0.92	6.33±3.03	73.00±4.61	231.66±15.16	90.66±2.90	114.66±7.05
III (Diabetic control, Type II)	16.33±0.76	42.46±2.17	81.66±3.84	212.33±10.86	96.33±3.28	138.00±10.39
IV (Extract 100mg, Type I)	16.46±1.34	16.60±0.34[c]	82.33±6.74	83.00±1.73[c]	92.33±1.76	76.66±5.60[c]
V (Extract 100mg, Type II)	15.33±0.92	18.60±0.34[d]	76.66±4.63	93.00±1.73[d]	95.33±2.18	86.66±1.20[d]
VI (Extract 200mg, Type I)	14.66±1.37	23.20±1.00[c]	73.33±6.88	116.00±5.03[c]	89.00±3.78	86.66±2.02[b]
VII (Extract 200mg, Type II)	19.20±0.41	27.40±1.32[d]	96.00±2.08	137.00±6.65[d]	93.33±6.22	92.00±6.02[d]
VIII (STZ+INS	15.13±0.69	18.00±0.91[c]	75.66±3.48	90.00±4.58[c]	89.00±1.73	77.33±1.76[c]
IX (STZ+NIC+GLI)	17.00±0.83	18.60±1.70[d]	85.00±4.16	93.00±8.54[d]	95.33±8.81	85.00±3.21[d]

Table 4: *Effect of MEBL on antioxidant enzyme levels*

Group	Antioxidant enzyme			
	Lipid Peroxidation (η mol/ml serum)	***Catalase (unit/mg protein)***	***Glutathione (mg%, 10^{-3})***	***SOD (unit/mg protein)***
I (Normal control)	0.61±0.05[a]	155.1±6.5[a]	0.0080±0.0009[a]	0.291±0.025[b]
II (Diabetic control, Type, I)	7.212±1.3	17.68±3.0	0.0024±0.0003	0.078±0.01
III (Diabetic control, Type II)	5.242±1.0	30.31±5.4	0.0025±0.0004	0.069±0.022
IV (Extract 100mg, Type I)	0.66±0.00[a]	120.7±7.9[b]	0.0063±0.0001[a]	0.286±0.009[b]
V (Extract 100mg, Type II)	0.80±0.11[a]	134.3±8.5[b]	0.0065±0.0004[c]	0.289±0.011[b]
VI (Extract 200mg, Type I)	1.78±0.42[a]	104.7±19.7[b]	0.0043±0.0001	0.159±0.030
VII (Extract 200mg, Type II)	0.88±0.10[b]	99.78±16.4[c]	0.0052±0.0011	0.117±0.025
VIII (STZ+INS	0.70±0.00[a]	111.3±20.0[b]	0.0055±0.0003[b]	0.233±0.043[c]
IX (STZ+NIC+GLI)	0.88±0.10[a]	129.7±19.8[b]	0.0059±0.0011	0.246±0.046[b]

Data are expressed as mean ± SEM (n = 6); [a] $p < 0.001$, [b] $p < 0.01$, [c] $p < 0.05$ compared with treated and normal diabetic and control groups

diabetic rats both types I and II). However, following treatment with the extract (both doses) normalized ($p < 0.05$) the altered antioxidant enzymes levels, unlike the untreated diabetic rats.

DISCUSSION

The present study was undertaken to evaluate the antidiabetic, antihyperlipidemic and antioxidant activity of methanolic extract of *Buchanania lanzan* in Streptozotocin induced types I and II diabetic rats. Streptozotocin was given to rats for induction of diabetes and it is known that streptozotocin is toxic to cells by causing damage to the DNA, though other mechanisms may also contribute. DNA damage induces activation of poly-ADP-ribosylation, which is likely more important for diabetes induction than DNA damage itself. Nicotinamide (NA) is given before giving streptozotocin to induce type II diabetes and nicotinamide is proved to be effective in protecting pancreatic islets by inhibition of PARP-1 activity. NA inhibits this enzyme, preventing depletion of NAD(+) and ATP in cells exposed to STZ. Moreover, NA serves as a precursor of NAD(+) and thereby additionally increases intracellular NAD(+) levels. [21]

Administration of extract for 21 days resulted in significant diminution of fasting blood glucose level in comparison with diabetic rats. The presence of abundant flavonoids may be responsible for its activity. It was observed that there was increase in serum levels of glucose, triglycerides, total cholesterol, VLDL and LDL-C with a concomitant decrease in serum HDL-C in STZ-induced types I and II diabetes. Its effect on the triglycerides is likely to increase the risk of developing coronary heart disease [17]. It is noteworthy, however, that the extract significantly restored the serum lipids of both types I and II diabetic rats to their pre-diabetic levels. Oxidative stress is produced under diabetic conditions and it is likely to be involved in the progression of pancreatic β-cell dysfunction. Also, because of the relatively low expression of antioxidant enzymes, such as catalase and superoxide dismutase, pancreatic β- cells may be vulnerable to reactive oxygen species ROS attack when the system is under oxidative stress. It was observed that there was a decrease in the activity of antioxidant enzymes - superoxide dismutase (SOD), catalase (CAT), glutathione peroxidise (GPx) - in the serum of the diabetic rats. However, the extract restored the activity of these enzymes. [20].

It has been shown that dietary supplementation with natural antioxidants such as vitamins C and E, melatonin and flavonoids attenuated the oxidative stress and diabetic state induced by STZ. Ancient literature claim that the leaves of *Buchanania lanzan* may contain glycosides, carbohydrates, sterols and flavonoids which may have anti-diabetic activity [21]. Therefore, the antidiabetic, antihyperlipidemic and antioxidant activities of this plant may due to its active constituents, including glycosides, carbohydrates, sterols and flavonoids.

CONCLUSION

Administration of the methanol extract of *Buchanania lanzan* produced antidiabetic, Antihyperlipidemic and antioxidant activities. However, further phytochemical and

pharmacological investigations are required to elucidate the detailed mechanisms of action of the extract as well as identify the active constituent(s) responsible for these actions.

REFERENCES

1. *Gandhi HR. Diabetes and coronary artery disease. Importance of risk factors. Cardiol Today. 2001; 1: 31-34.*
2. *Kamesawara BR, Giri R, Kesavulu MM and Apparao CH. Effect of oral administration of bark extracts of Pterocarpus santalinus L. on blood glucose level in experimental animals. J. Ethnopharmacol. 2001 ; 74(1): 69–74.*
3. *Welihinda J, Arvindson G, Gylfe E, Hellman B, Karlsson E. The insulin-releasing activity of the tropical plant Momordica charantia. Acta Biol Med Ger. 1982; 41(12): 1229-1240.*
4. *McCune LM, Jhons T. Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous peoples of the North American boreal forest. J. Ethnopharmacol. 2002; 82(2-3): 197-205.*
5. *Giron MD, Salto R, Gonzalez Y, Giron N, Nieto JL, Periago JL. Modulation of hepatic and intestinal glutathione-Stransferases and other antioxidant enzymes by dietary lipids in streptozotocin diabetic rats. Chemosphere. 1999; 38: 3003–3013.*
6. *Gul M, Kutay FZ, Temocin S, Hanninen O. Cellular and clinical implications of glutathione. Ind J Exp Biol. 2000; 38: 625–634.*
7. *Achuthan CR, Jose P. Hypolipidemic effect of Alpinia galangal (Rasna) and Buchanania lanzan (Kachoori). Ind J Clin Biochem. 1997; 12(1): 55–58.*
8. *Xue Y, Chen H. Study on the anticarcinogenic effect of three compounds of Buchanania lanzan L. Xei Shen Yan Jiu,. 2002; 31(4): 247–248.*
9. *Othman R, Ibrahim H, Mohd MA, Awang K, Gilani AV, Musthafa MR. Vaso relaxant effect of Buchanania lanzan on smooth muscles of rat aorta. Planta Med. 2002; 68(7): 655–657.*
10. *Choochote W, Kanjanapothi D, Panthanga A, Taesotikul T, Jitpakdi A, Chaithong U et al. Larvicidal and repellent effects of Kaempferia galanga. Southeast Asian J Trap Med Public Health. 1999; 30(3): 470–476.*
11. *Animal models of diabetic complications consortium protocols. Low-Dose Streptozotocin Induction Protocol, Animal Models of Diabetic Complications Consortium, RAM, Ed. 2003: pp 1-17*
12. *William TF, Robert IL, Donald SF. Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge.* Clin Chem. *1972; 18(6): 499-502.*
13. *Okhawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Ann Biochem. 1979; 95: 351–358.*
14. *Misra HP, Fridovich I. Superoxide dismutase, a photochemical augmentation assay. Arch Biochem Biophys. 1977 May; 181(1): 308-312.*
15. *Beutler E, Durgun O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963; 51: 882-888.*
16. *Beer RF Jr, Seizer IW. A spectrophotometric method for measuring breakdown of hydrogen peroxide by catalase. J Biol Chem. 1952 March; 195(1): 130–140.*
17. *Lenzen S, Drinkgern J, Tiedge M. Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. Free Radical Biol. Med. 1996; 20(3): 463-466.*
18. *Shalini KM, Swarupananda M, Jaiprakash B. Preliminary Phytochemical Investigation on leaves of Buchanania lanzan (Chironji). Int J Pharm Sci Rev Res. 2010 July- August; 3 (2): 55-59.*
19. *http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECDtg425.pdf*
20. *http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECD_GL423.pdf*
21. *Szkudelski T, Streptozotocin-nicotinamide-induced diabetes in the rat: Characteristics of the experimental model, Exp Biol Med 2012; 237(5): 481-490*