Original Article

Antidiabetic and antihyperlipidemic effects of an ethanolic extract of the whole plant of *Tridax procumbens (Linn.)* in streptozotocin-induced diabetic rats

Abstract

Objective: To study the antidiabetic and antihyperlipidemic effects of an ethanolic extract of the whole plant of *Tridax procumbens* (Asteraceae) in streptozotocin-induced diabetic rats.

Materials and Methods: The whole plant of *T. procumbens* was collected in different regions of Madurai districts, Tamil Nadu. The air dried whole plant of *T. procumbens* was extracted with ethanol (95%) in a Soxhlet apparatus for 72 h. Diabetes was induced in male Wistar rats by streptozotocin (50 mg/jk, i.p.) and nicotinamide (120 mg/kg, i.p) injection. The dry mass of the extract was used for preliminary phytochemical and pharmacological analysis. Diabetic rats were treated with glibenclamide (0.25 mg/kg, p.o.) or *T. procumbens* extract (250 and 500 mg/k, p.o.) for 21 consecutive days. The blood samples were collected at regular intervals to access hypoglycemic effect of an ethanolic extract of the whole plant of *T. procumbens*. At the end of the experiment, serum lipid profile and liver enzymes levels were analyzed for all the experimental animals and compared with diabetic control.

Results: The preliminary phytochemical analysis of an ethanolic extract of the whole plant of *T. procumbens* indicated the presence of alkaloids, tannins, flavonoids, saponins, and phenolic compounds. The ethanolic extract of the whole plant of *T. procumbens* at 250 and 500 mg/kg has significant antidiabetic and antihyperlipidemic activities. The diabetic control animals exhibited a significant decrease in body weight compared with control animals. *T. procumbens* inhibited streptozotocin-induced weight loss and significantly alter the lipid levels.

Conclusion: The ethanolic extract of the whole plant of *T. procumbens* showed significant antidiabetic and antihyperlipidemic activities against streptozotocin-induced diabetes in rats.

Key words:
Antidiabetic, antihyperlipidemic, diabetic mellitus, hyperlipidemia, T. procumbens

Introduction

The dynamics of the diabetes and hyperlipidemia are changing rapidly in low- to middle-income countries. In 2030, diabetes may affect 472 million (approx.) of world populations. The number of adult with impaired glucose tolerance may rise from 344 million (2010) to 472 million by 2030.[1] International diabetes federation (IDF) estimated that 80% of world diabetic population live in low- and middle-income countries in 2030. As per IDF 2011 report, China, India, and United States of America have 90.0, 61.3, and 23.7 million peoples were living with diabetes that may be increase up to 129.7, 101.2, and 29.3 million people, respectively, in 2030.[2] The treatment of any diseases with allopathic drugs causes moderate to severe adverse events, which could cause death. Hence, the alternative systems of medicine are being explored to treat diseases.[3] Diabetes mellitus is a multifactorial disease characterized by hyperglycemia and lipoprotein abnormalities.

India is one of the eight important vavilovian centers of origin and crop plant diversity. It is immensely rich in the medicinal

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and aromatic plants in diverse ecosystems. A large majority of Indian population is treated by traditional systems of medicine such as Ayurveda, Unani, and Siddha. Ayurveda, the Indian indigenous system of medicine, has been an integral part of Indian culture. Although Indian's traditional medicinal system are deeply rooted in the Indian psyche, today, we found that these system of medicine do not hold a "primary" status in the therapeutics. At the same, there is a global resurgence in the use of herbal medicines along with a growing scientific interest in them as a source of new drugs.[4]

Tridax procumbens Linn. (Asteraceae) is one of the common herb present throughout India, and this plant extract was commonly used for treating the cut wounds (anti-inflammatory) by rural Indians. Studies showed that *T. procumbens* has anti-inflammatory, hepatoprotective, wound healing, antimicrobial, antiseptic, hypotensive, and immunomodulatory properties.[5,6] The previous studies on *T. procumbens* extract showed presence of hypoglycemic effect and antidiabetic effect against alloxan-induced diabetes in rats.[7] Alloxan is used to induce non-insulin-dependent diabetes, and the effect of *T. procumbens* extract on insulin-dependent diabetes is unknown. Hence, the present study is undertaken to investigate the antidiabetic and antihyperlipidemic potential of an ethanolic extract of the whole plant of *T. procumbens* on streptozotocin (STZ)-induced insulin-dependent diabetes mellitus in rats.

Materials and Methods

Plant collection

Taxonomically identified *T. procumbens Linn.* (Asteraceae) plant was collected from different regions of Madurai District, Tamil Nadu, India, in July 2011. Plant was identified and authenticated by Botanist of Agricultural College and Research Institute, Madurai, Tamil Nadu. The whole plant was dried under the shade for a few days and grinded in electrical grinder to coarse powder.

Extraction of leaves

The whole plant powder of *T. procumbens* was packed in the Soxhlet apparatus and extracted with 95% ethanol. The extraction was carried out for 72 h at about 50^{0}C. Later on, the extract was filtered, and the filtrate was concentrated to a dry mass by simple distillation. The yield was found to be 6% w/v. The extract was stored in desiccators at room temperature until analysis.

Animals

The male Wistar albino rats, aged 4 months (body weight: 180 ± 10 g), were obtained from Sainath enterprises, Hyderabad, India. The animals were housed in large, spacious poly acrylic cages at an ambient room temperature with 12-h-light/12-h-dark cycle. Rats have free access to water and rat pellets (Hindustan Lever Ltd., Bangalore, India). The study was approved by the Institute Animal Ethics Committee of Ultra College of Pharmacy, Madurai, India. All the animal experiments were carried out according to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

Chemicals and reagents

STZ was procured from Avra Synthesis Pvt. Ltd., Hyd. Glibenclamide was received as a gift drug from Aurobindo Pharma Ltd., Hyderabad. Biochemical assay kits for glucose, serum glutamic pyruvate transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), total cholesterol, total protein, triglyceride, and glucose-6-phosphate dehydrogenase kits were procured from Coral diagnostics Ltd., Mumbai. All other chemicals used were of analytical grade obtained from SD-Fine, India.

Phytochemical screening

The extract was subjected for phytochemical screening to screen the presence of various constituents such as sterols, alkaloids, tannins, glycosides, flavonoids and saponins/ phenolic compounds by Libermann-Burchard reaction, Dragendroff 's reaction, ferric chloride solution test, Keller-Kiliani test, Shinoda test and lead acetate test respectively.[8,9]

Anti-diabetic screening of ethanolic extract of whole plant of *T. procumbens*

Adult, healthy male Wistar rats weighing between 180 ± 10 g were used for the experiment. The rats were divided into five different groups of six animals as follows.

Group I - Normal control rats
Group II - Diabetic control
Group III - Diabetic rats treated with glibenclamide (0.25 mg/kg)
Group IV - Diabetic rats treated with ethanolic extract of the whole plant of *T. procumbens* (250 mg/kg)
Group V - Diabetic rats treated with ethanolic extract of the whole plant of *T. procumbens* (500 mg/kg).

Diabetes was induced in overnight-fasted rats by a single intra-peritoneal injection of freshly prepared STZ 50 mg/kg b.w. followed by 120 mg/kg of nicotimanide in 0.1 M citrate buffer (pH 4.5) in a volume of 0.5 ml/kg b.w.[10,11] Diabetes was confirmed in the STZ-treated rats by measuring fasting blood glucose levels after 48 hours of STZ injection using One-Touch Horison glucometer, with gluco-strips (Ortho-Clinical Diagnostics, Johnson and Johnson Company, USA). After 24 h of STZ + nicotinamide injection, animals were given 5% w/v of glucose solution (2 ml/kg b.w.) to prevent initial drug-induced hypoglycemic mortality. Rats with fasting blood glucose of more than 200 mg/dl were considered as diabetics.[12]

The diabetic rats were divided randomly into group- II to group- V. The ethanolic extract of the whole plant of *T. procumbens Linn. (Asteraceae)* doses were selected form previously published reports (i.e., 250 and 500 mg/kg).[13] The standard (glibenclamide) and investigational drugs were suspended in 0.5% w/w carboxymethyl cellulose (CMC) and administered once daily through oral gavage for 21 consecutive days. The blood sample (few drops) were collected on 1st, 7th, 14th, and 21st day from the tail vein of rats by pricking with sharp tip needle and immediately used for the estimation of blood glucose with glucometer.[14] Weekly body weight variations were monitored for all the experimental animals. At the end of the experiment blood sample was withdrawn

from all the experimental animals through retro-orbital plexus puncture, and serum was separated.[14] The serum was used for estimation of the biochemical parameters.

Biochemical analysis:

The collected blood was allowed to clot for 30 min and centrifuged at 3000 RPM for 20 min. The serum was used for estimation of biochemical parameters such as liver biomarkers (SGOT and SGPT) and cholesterols levels (total cholesterol, triglycerides, and HDL-cholesterol). The total cholesterol, triglyceride, HDL-cholesterol, SGOT and SGPT levels in significance serum were analyzed using an auto-analyzer (Robonik prietest, India) using enzymatic kits of Coral diagnostics Ltd., Mumbai. LDL levels were calculated mathematically.[15]

Statistical analysis

All the data was expressed as mean ± SEM. Statistical significance between the groups were tested using one-way ANOVA followed by Dennett's post-hoc test. A *P* less than 0.5 was considered significant.

Result

The preliminary phytochemical analysis of an ethanolic extract of the whole plant of *T. procumbens* showed the presence of alkaloids, tannins, flavonoids, saponins, and phenolic compounds.

Throughout the study, diabetic control animals showed a significant increases of blood glucose levels ($P<0.001$). The standard and investigational drugs treated animals showed a significant reduction in glucose levels when compare to diabetic control. At the end of the study, *T. procumbens* (500 mg/ kg) and glibenclamide (0.25 mg/kg) treated animals reversed the hyperglycemia induced by STZ to normal levels. Glibenclamide and ethanolic extract of the whole plant of *T. procumbens* (250 and 500 mg/kg) showed significant antidiabetic activity after two weeks of administration. Ethanolic extract of the whole plant of *T. procumbens* (500 mg/kg) treated animals showed significant antidiabetic potential from first week of the drug administration onwards [Table 1]. The whole plant ethanolic extract of *T. procumbens* and glibenclamide did not affected the body weight of the animals, but significant reduction in body weight was observed in diabetic control animals [Figure 1; $P < 0.001$].

As shown in Table 2, elevated total cholesterol, triglyceride, LDL cholesterol levels, and reduced HDL cholesterol level

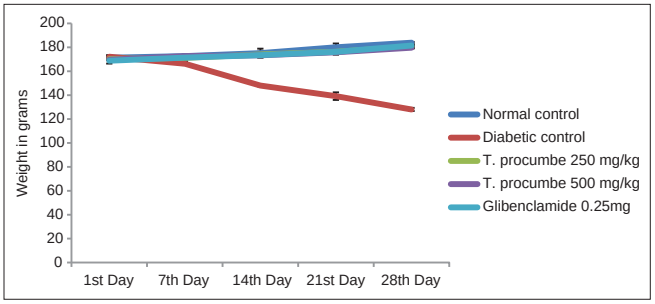

Figure 1: Effect of ethanolic extract of the whole plant of *T. procumbens* on body weight

Table 1: Effect of an ethanolic extract of the whole plant of *T. procumbens* on blood glucose levels in streptozotocin-induced diabetes

Treatment	Blood glucose (mg/dl)			
	0th day	7th day	14th day	21st day
Normal control	80.6±1.36	76.18±1.1	79.1±1.49	81.2±1.22
Diabetic control	260.1±3.57	301.17±2.18	341.2±0.52	365.6±1.48
T. procumbens 250 mg/kg	255.57±2.77	233.6±3.38***	195.93±2.94***	151±1.97***
T. procumbens 500 mg/kg	254.63±3.76	200.2±2.04**	180.33±1.78***	120.2±2.11***
Glibenclamide 0.25 mg	257.93±2.77	190.73±3.46***	160.73±2.54***	110.4±2.26***

Values are expressed as mean ± SEM ($n=6$). ***$P<0.001$ compared with diabetic control (One-way ANOVA followed by Dennett's *post-hoc* test)

Table 2: Effect of an ethanolic extract of the whole plant of *T. procumbens* on lipid profile in streptozotocin-induced diabetes

Treatment	Total cholesterols (mg/dl)	Triglyceride (mg/dl)	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)
Normal control	61.16±1.07	70.25±0.81	28.51±0.44	28.391±0.10
Diabetic control	110.04±0.78***	132.12±0.08***	16.14±0.16***	80.64±0.18***
T. procumbens 250 mg/kg	65.31±0.83	82.05±0.70	25.36±0.03	39.41±1.00
T. procumbens 500 mg/kg	68.90±0.52	81.11±0.46	25.36±0.13	36.52±0.36
Glibenclamide 0.25mg/kg	68.60±0.10	85.12±1.1 2	26.35±0.33	35.33±0.91

Values are expressed as mean ± SEM ($n=6$). ***$P < 0.001$ compared with diabetic control (One-way ANOVA followed by Dennett's *post-hoc* test).

were observed in the diabetic control compared with that of normal controls. Administration of the ethanolic extract of the whole plant of *T. procumbens* to STZ-diabetic rats protected the alterations of lipid levels. The liver biomarkers such as SGPT, SGOT found to be normal in ethanolic extract of the whole plant of *T. procumbens*- and glibenclamide-treated rats, but the diabetic control showed elevated SGPT, SGOT levels at the end of the experiment ($P < 0.001$).

Discussion

The ethanolic extract of the whole plant of *T. procumbens* at 250 and 500 mg/kg showed significant antidiabetic activity against STZ-induced diabetes mellitus in rats, and the effect was comparable with that of the standard drug glibenclamide.

Loss in body weight was observed in STZ-induced diabetes mellitus in rats and was controlled by treatment with ethanolic extract of the whole plant of *T. procumbens*. Administration of an ethanolic extract of the whole plant of *T. procumbens* to diabetic rats resulted in an increase in body weight compared to diabetic rats. The present study findings suggested that *T. procumbens* treatment has positive effect on maintaining body weights in diabetic rats. The protective effect of plant fraction on body weight of diabetic rats may be due to its ability to reduce hyperglycemia. A gradual increase in body weights of glibenclamide treated groups was similar to that of normal control rats. STZ-induced diabetes mellitus was characterized by severe loss of body weight due to increased muscle wasting in diabetes.[16] STZ is a glucosamine-nitrosourea derived from *Streptomyces achromogenes* (gram-positive bacterium), and it is used for the treatment of pancreatic beta cell carcinoma. STZ inducing diabetes, hyperinsulinemia, or hyperglycemia by damaging the pancreatic beta cells.[17]

Alcoholic and petroleum ether extracts of leaves of *T. procumbens* (200 mg/kg) showed significant antidiabetic activity in alloxan monohydrate-induced diabetes mellitus in rats.[5] Methanolic extract of the whole plant of *T. procumbens* showed significant antidiabetic activity in alloxan-induced diabetes mellitus in rats, and the antidiabetic activity might be due to individual or synergistic activity of flavonoids and other active phytoconstituents of the plant.[6] In previous studies, alcoholic and methanolic extract of *T. procumbens* showed significant antidiabetic activity against alloxan-induced diabetes in rodents, and the present study result findings also suggest the same. The antidiabetic effect of *T. procumbens* might be presence of flavonoids.

Plant which have flavonoids, terpenoids, alkaloids, and glycosides have antioxidant activity and claimed to possess antidiabetic effect. Flavonoids present in the plant regenerate the damaged beta cells of pancreases, and the polyphenolic compounds and saponin present in the plants inhibit glucose transport by inhibiting sodium glucose co-transporter-1 (S-GLUT-1) in intestine.[18,19] Antidiabetic effect of an ethanolic extract of the whole plant of *T. procumbens* might be due to presence of flavonoids, polyphenolic compounds, and saponin in the extract.

On bases of the present and pervious study reports, *T. procumbens* has significant antidiabetic activity against both alloxan- and STZ-induced diabetes mellitus in rodents.[5] The action may be due to presence of flavonoids, and further investigation required to rollout the bioactive compound present in the plant.

Conclusion

The ethanolic extract of the whole plant of *T. procumbens* exerts an antidiabetic activity against STZ-induced diabetes mellitus in rats. It is also found to be effective in managing the complications associated with diabetes mellitus, such as hyperlipidaemia, and prevents the defects in lipid metabolism.

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References

1. Hu FB. Globalization of Diabetes the role of diet, lifestyle, and genes. Diabetes Care 2011;34:1249-57.
2. IDF diabetes atlas, fifth edition. Available form: http://www.idf.org/diabetesatlas/5e/the-global-burden [Last accessed on 2013 Apr 20].
3. Parasuraman S, Kumar E, Kumar A, Emerson S. Free radical scavenging property and diuretic effect of triglize, a polyherbal formulation in experimental models. J Pharmacol Pharmacother 2010;1:38-41.
4. Catherine T. The Role of Ayurvedic Therapies for HIV/AIDS Care: A comprehensive review of the literature. J Glob Health Perspect Available from: http://jglobalhealth.org/article/the-role-of-ayurvedic-therapies-for-hivaids-care- a-comprehensive-review-of-the -literature-2/. [Last accessed on 2013 Jan 05].
5. Bhagwat DA, Killedar SG, Adnaik RS. Anti-diabetic activity of leaf extract of *Tridax procumbens*. Int J Green Pharm 2008;2:126-8.
6. Pareek H, Sharma S, Khajja BS, Jain K, Jain GC. Evaluation of hypoglycemic and anti-hyperglycemic potential of *Tridax procumbens* (Linn.). BMC Complement Altern Med 2009;9:48.
7. Vogel HG, editor. Drug Discovery and Evaluation: Pharmacological Assay. 2nd ed. Berlin: Springer; 2002.
8. Agbafor KN, Nwachukwu N. Phytochemical analysis and antioxidant property of leaf extracts of vitex doniana and mucuna pruriens. Biochem Res Int 2011;2011:4.
9. Kokate CK, Kokate CK. Practical Pharmacognosy. 4th ed. 1994: Vallabh Publications/Prakashan.
10. Rabbani SI, Devi K, Khanam S. Protective role of glibenclamide against nicotinamide-streptozotocin induced nuclear damage in diabetic Wistar rats. J Pharmacol Pharmacother 20101;1:18-23.
11. Alenzi FQ. Effect of nicotinamide on experimental induced diabetes. Iran J Allergy Asthma Immunol 2009;8:11-8.
12. Liu Z, Li J, Zeng Z, Liu M, Wang M. The antidiabetic effects of cysteinyl metformin, a newly synthesized agent, in alloxan- and streptozocin-induced diabetic rats. Chem Biol Interact 2008;173:68-75.
13. Petchi RR, Vijaya C, Parasuraman S. Anti-arthritic activity of ethanolic extract of *Tridax procumbens* (Linn.) in Sprague Dawley rats. Phcog Res 2013;5:113-7.
14. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother 2010;1:87-93.
15. Parasuraman S, Babuji SSH, Thing GS, Kumari KS, Yoganishalini A, Lian CW, Kumutha M, Kassim T, Dhanaraj SA. Antihyperlipidemic effect of Angiosifa, a polyherbal formulation, in Sprague–Dawley rats. Phcog J [In Press]. [DOI: 10.1016/j.phcgj.2013.08.001].
16. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic

complications: A new perspective on an old paradigm. Diabetes 1999;48:1-9.

17. Graham ML, Janecek JL, Kittredge JA, Hering BJ, Schuurman HJ. The streptozotocin-induced diabetic nude mouse model: Differences between animals from different sources. Comp Med 2011;61:356-60.
18. Hakkim FL, Girija S, Kumar RS, Jalaluddeen MD. Effect of aqueous and ethanol extracts of Cassia auriculata L. flowers on diabetes using alloxan induced diabetic rats. Int J Diabetes Metab 2007;15:100-6.
19. Tiwari AK, Rao JM. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. Curr Sci 2002;83:30-8.

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