Short Communication

Evaluation of free radical scavenging properties and hypoglycemic activity of ethanolic extract of *Tridax procumbens* Linn. in Wistar rats

Abstract

Objective: The aim was to evaluate the free radical scavenging properties and hypoglycemic effect of ethanolic extract of whole plant of *Tridax procumbens* in Wistar rats. **Materials and Methods:** The plants parts were powdered and extracted with ethanol. The ethanolic extract was used for screening of hypoglycemic activity. The free radical scavenging properties and hypoglycemic effect of ethanolic extract of *T. procumbens* were studied using 2,2-diphenyl-1-picrylhydrazyl methods and oral glucose tolerance test. **Result:** The free radical scavenging properties of the ethanolic extract of *T. procumbens* was comparable with ascorbic acid. The plant extract was not affecting normal animal glucose levels, whereas its affects hyperglycemia induced by glucose load. The glucose lowering effect (hypoglycemic) of ethanolic extract of *T. procumbens* was dose dependent. **Conclusion:** The ethanolic extract of *T. procumbens* did not affect the normal animal glucose levels and preventing the hyperglycemia induced by glucose load in rodents.

Key words:
2,2-diphenyl-1-picrylhydrazyl, diabetes, hyperglycemia, Tridax procumbens

Introduction

Diabetes is a metabolic disorder affecting nearly 10% of population worldwide. Increased frequency of diabetes is noted in developing countries.[1] Most of the drugs were discovered form natural sources and many more are yet to discover. In ancient India, Ayurvedic system of medicine was used for treating wide variety of disorders/diseases, but the scientific evidence for treating diseases with herbs and fixed herbal formulations are poorly documented. *Tridax procumbens* is commonly used for the treatment of diabetes and some the *T. procumbens* is proven to have anti-diabetic activity. The anti-diabetic activity of leaves of *T. procumbens* was already reported and the hypoglycemic and anti-diabetic activity of flower and whole plant extract is remains unknown.[2]

Tridax procumbens Linn. (*Asteraceae*) is a species of flowering plants also known as *Tridax* daisy. This plant is native of tropical America and naturalized in tropical Africa, Asia, and Australia. *T. procumbens* mainly contains proteins, fibers, soluble carbohydrates, calcium oxide, luteolin, glucoluteolin, quercetin, isoquercetin and oleanolic acid. Oleanolic acid is triterpenoid and widely used for liver disorders and its anti-diabetic activity was studied in animals.[3,4] The leaves have been reported to have effective anti-arthritic, analgesic, antipyretic, and anti-inflammatory properties.[5] Free radicals and reactive oxygen species are attenuation the disease progression and most of the herbs reported to be have antioxidant property. Hence, this study was planned to evaluate the free radical scavenging properties and hypoglycemic effect of ethanolic extract of whole plant of *T. procumbens* in Wistar rats.

Access this article online	
Website: www.ddtjournal.org	Quick Response Code

Subramani Parasuraman, Rajendran Ramesh Petchi[1], Chockalingam Vijaya[1], Sokkalingam Arumugam Dhanaraj[2]

Pharmacology Unit, [2]Unit of Pharmaceutical Technology, Faculty of Pharmacy, AIMST University, Bedong, Kedah 08100, Malaysia, [1]Department of Pharmacology, Ultra College of Pharmacy, Madurai, Tamil Nadu, India

Address for correspondence:
Dr. S. Parasuraman,
Pharmacology Unit, Faculty of Pharmacy, Asian Institute of Medicine, Science and Technology University, Bedong-Semeling Road, Bedong, Kedah 08100, Malaysia.
E-mail: parasuphd@gmail.com

Materials and Methods

Materials

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

Animals

The experimental animals were purchased form Sainath Enterprises, Hyderabad, India and they were acclimatized to laboratory conditions for a period of 1 week. The animals were housed in large, spacious poly acrylic cages at an ambient room temperature with 12-h-light/12-h-dark cycle. Rats had free access to tap water and dry rat pellets obtained from Hindustan Lever Ltd., Bangalore, India. The study was approved by the Institute Animal Ethics Committee of Ultra College of Pharmacy, Madurai, Tamil Nadu, India. All the animal experiments were carried out according to Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines.

Extraction of *Tridax procumbens*

The whole plant powder was packed into a Soxhlet apparatus and extracted with 95% ethanol at 50°C (approximately) for 18 h. The ethanolic extract of *T. procumbens* was distilled and concentrated to dry mass. The extract was stored in desiccators at room temperature until analysis. The yield was found to be 6% w/v.

Phytochemical analysis

The ethanolic extract of *T. procumbens* was dissolved in minimum volume of ethanol and used for the phytochemical analysis. The Salkowski reaction, Dragendroff 's reaction, ferric chloride solution test, Keller — Kiliani test and Shinoda test were carried out to detect the presence of phytosterols, alkaloids, tannins, glycosides and flavonoids/phenolic compounds, respectively.[5]

Evaluation of free radical scavenging properties

The antioxidant activity of ethanolic extract of *T. procumbens* was assessed on the basis of the free radical scavenging effect of Griess–Ilosvay reaction. The ethanolic extract of *T. procumbens* and standard compound ascorbic acid were dissolved in dimethyl sulfoxide separately and used for *in vitro* antioxidant testing by 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods. The assay was carried out in a 96 well microtiter plate. To 200 µl of DPPH solution, 10 µl of each of the test or standard solutions were added separately in wells of the 96 well microtiter plates. The final concentration of the test, standard solution used was 7.81–1000.00 µg/ml. The plate was incubated at 37°C for 30 min and the absorbance of each was measured at 490 nm using enzyme-linked immunosorbent assay reader (Bio-Rad Laboratories Inc., USA) against the corresponding test and standard blanks and the remaining DPPH was calculated. Measurements were taken in triplicate.[6] DPPH scavenging effect was calculated using following equation:

2,2-diphenyl-1-picrylhydrazyl scavenging effect (%) = $([A_0\text{-}A/A_0] \times 100]$, where A_0 is the absorbance of control and A is the absorbance of the extract. The results were reported as IC_{50} values and ascorbic acid equivalents of ethanolic extract of *T. procumbens*.

Acute toxicity testing of ethanolic extract of *Tridax procumbens*

The acute toxicity testing was performed using fixed-dose procedure using Swiss albino mice. The ethanolic extract of *T. procumbens* was administered at doses of 250, 500, 1000 and 2000 mg/kg and monitored for 2 weeks form the date of administration. During observation period, no gross behavioral changes and mortality were observed.[7]

Effect of ethanolic extract of *Tridax procumbens* on normal animals

Overnight fasted male Wistar albino rats (180 ± 20 g) were divided into two groups with six animals in each group. Groups I and II were treated with ethanolic extract of *T. procumbens* at the dose levels of 250 and 500 mg/kg respectively. The investigational product was suspended in 0.5% carboxymethylcellulose sodium (CMC) and administered orally. The effect of ethanolic extract of *T. procumbens* on blood glucose levels monitored at 0, 1, 2 and 3 h after drug administration. The rat was restrained in rodent restrainer and few drops of blood samples were collected from the tail vein. The blood glucose levels were measured using glucometer (Ortho-Clinical Diagnostics, Johnson and Johnson Company, USA).

Evaluation of hypoglycemic activity of ethanolic extract of *Tridax procumbens*

Hypoglycemic effect of ethanolic extract of *T. procumbens* was conducted on 30 adult male Wistar albino rats (180 ± 20 g). Prior to experiment, blood sample was collected form all the animals to check random blood glucose levels. The animals which had a glucose level >130 g/dl were excluded from the study. Rats were divided into five groups, with six animals in each group. Group I serves as normal control. Groups II to VI served as animals served as glucose control, glibenclamide (0.25 mg/kg, p.o.) ethanolic extract of *T. procumbens* (250 mg/kg) and ethanolic extract of *T. procumbens* (500 mg/kg) respectively. Dose of *T. procumbens* was selected based on its acute toxicity profile.

The prestudy blood sample was collected prior to the experiment through tail vein and rats were fasted for overnight. Sixty minutes prior to vehicle/drug administration all the animals received glucose (2 g/kg, p.o.) except Group I animals. The standard and investigational

products were dissolved in 0.5% CMC and administered as an oral suspension and blood samples were collected at 1, 2, 3 and 5 h after drug administration through tail vein and immediately estimated blood glucose level using glucometer.

Statistical analysis

The mean and SEM values were calculated for each parameter. Significant differences between the groups were determined using two-way ANOVA followed by Bonferroni *post-hoc* test using SPSS software (version 16). A *P*-value less than 0.05 was considered significant.

Results

The preliminary phytochemical analysis of the ethanolic extract of *T. procumbens* showed the presence of alkaloids, tannins, flavonoids/phenolic compounds. Acute toxicity testing of ethanolic extract of *T. procumbens* did not show any mortality or gross behavioral changes in observation period. The ethanolic extract of *T. procumbens* showed a moderate antioxidant activity by DPPL method with IC_{50} value of 113.38 ± 7.20 μg/ml when compare to standard ascorbic acid (IC_{50} = 53.84 ± 1.44 μg/ml) [Figure 1]. The ethanolic extract of *T. procumbens* (250 and 500 mg/kg) did not altered normal glucose levels of rodents whereas it having glucose lowering effect in glucose loaded animals.

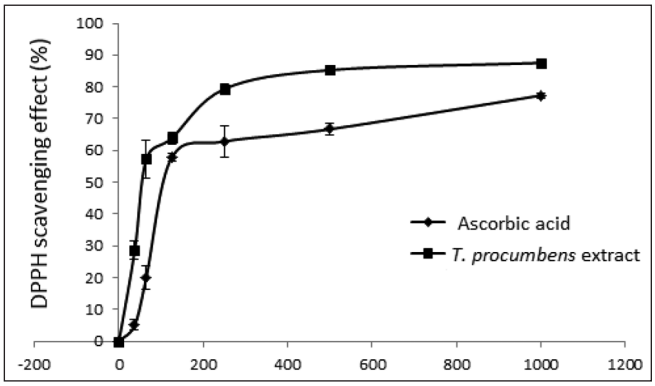

Figure 1: 2,2-diphenyl-1-picrylhydrazyl scavenging effect of ethanolic extract of whole plant of *Tridax procumbens* (*T. procumbens*) and ascorbic acid (μg/ml). Ethanolic extract of *T. procumbens* showed a moderate antioxidant activity by DPPL method with IC_{50} value of 113.38 ± 7.20 μg/ml when compare to standard ascorbic acid (IC_{50} = 53.84 ± 1.44 μg/ml)

Glucose loaded animals showed significant increase in glucose levels form 0.5 h onwards. Ethanolic extract of *T. procumbens* (500 mg/kg) significantly reduced the blood glucose levels after 60 min of drug administration in glucose load animals when compare to glucose control animals whereas ethanolic extract *T. procumbens* (250 mg/kg) showed significant reduction in glucose levels after 2 h of drug administration [Table 1]. The hypoglycemic potential of ethanolic extract of *T. procumbens* (500 mg/kg) is comparable with standard drug glibenclaimde.

Discussion

Free radicals are formed in the cells as a consequence of both oxidative biochemical reaction and have been implicated in the pathogenesis of wide variety of clinical disorders, resulting from deficient natural antioxidant defenses.[8] Ethanolic extract of *T. procumbens* possesses a series of substituted phenolic compounds and variety of flavones, which have known antioxidant property.[5]

Ethanolic extract of *T. procumbens* did have any glucose lowering effect on normal rat where as its reducing the glucose levels in glucose loaded rats (hyperglycemic rats). The results of the present study suggesting that, the ethanolic extract of *T. procumbens* did not have any glucose lowering effect on normal rats. The biological acidity of *T. procumbens* leaves and plants parts were reported and this effect may be due to flavonoid present in the plant.[5]

Conclusion

This study concludes that the whole plant ethanolic extract of *T. procumbens* has moderate antioxidant activity and hypoglycemic effect in glucose loaded animals. Whole plant ethanolic extract of *T. procumbens* did not have any glucose lowering effect in normal animals.

Table 1: Hypoglycemic effect of ethanolic extract of whole plant of *T. procumbens* in glucose loaded rats

Groups	Blood glucose levels (mg/dl)					
	0 min	0.5 h	1 h	2 h	3 h	5 h
Normal control	86.50±3.03	85.00±2.96	86.33±1.89	86.67±3.60	83.00±3.89	82.00±2.78
Glucose control	88.33±2.60	137.00±6.06***	174.67±5.72***	183.00±9.32***	158.33±3.84***	147.00±14.82***
Glibenclamide (0.25 mg/kg	87.83±2.95	126.17±5.58	177.00±7.04	146.50±4.62$^{\$\$}$	115.33±4.43$^{\$\$\$}$	101±4.75$^{\$\$\$}$
T. procumbens (250 mg/kg)	87.33±3.57	129.00±7.43	170.33±4.11	159.00±4.89	111.33±4.92$^{\$\$\$}$	98.67±3.12$^{\$\$\$}$
T. procumbens (500 mg/kg)	83.67±3.63	121.33±4.25	169.67±3.24	131.67±4.80$^{\$\$\$}$	109.67±4.60$^{\$\$\$}$	92.67±4.05$^{\$\$\$}$

All the values mean ± SEM (n = 6); ***P < 0.001 compared with normal control; $^{\$\$}P$ < 0.01; $^{\$\$\$}P$ < 0.001 compared with glucose control; Two-way ANOVA followed by Bonferroni *post-hoc* test; *T. procumbens* – *Tridax procumbens*; SEM – Standard error of mean

References

1. 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.
2. Bhagwat DA, Killedar SG, Adnaik RS. Anti-diabetic activity of leaf extract of *Tridax procumbens*. Int J Green Pharm 2008;2:126-8.
3. Mundada S, Shivhare R. Pharmacology of *Tridax procumbens* a weed: review. Int J PharmTech Res 2010;2:1391-4.
4. Liu J. Pharmacology of oleanolic acid and ursolic acid. J Ethnopharmacol 1995;49:57-68.
5. Petchi RR, Vijaya C, Parasuraman S. Anti-arthritic activity of ethanolic extract of *Tridax procumbens* (Linn.) in Sprague Dawley rats. Pharmacognosy Res 2013;5:113-7.
6. 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.
7. Parasuraman S. Toxicological screening. J Pharmacol Pharmacother 2011; 2:74-9.
8. Halliwell B, Gutteridge JM, editors. Free Radicals in Biology and Medicine. 2nd ed. London: Clarendon Press; 1989.

How to cite this article: Parasuraman S, Petchi RR, Vijaya C, Dhanaraj SA. Evaluation of free radical scavenging properties and hypoglycemic activity of ethanolic extract of *Tridax procumbens* Linn. in Wistar rats. Drug Dev Ther 2014;5:164-7.

Source of Support: Nil. **Conflict of Interest:** None declared