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Antidiabetic Activity of Aqueous Extract of *Eucalyptus citriodora* Hook. in Alloxan Induced Diabetic Rats

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ABSTRACT

The present study was undertaken to study the antidiabetic activity of the aqueous extract of *Eucalyptus citriodora* Hook. leaf in alloxan-induced diabetic rats. The activity of the extract was studied on glucose loaded and alloxan-induced diabetic rats. In both the tests, the extract has shown significant and considerable antidiabetic effect in a dose dependent manner. On oral administration of the extract at a dose of 500 mg/kg of body weight, the reduction of blood glucose level was 22.9% after 4th hr and on continuous administration the reduction in blood glucose level after 21 days was 49.9 and 56.8% with dose of 250 and 500 mg/kg of body weight respectively. Aqueous extract of leaves of *E. citriodora* exhibited significant antidiabetic activity which was comparable with the standard drug Glibenclamide.

KEYWORDS: *Eucalyptus citriodora*, antidiabetic, flavonoids, glibenclamide, tannins.

INTRODUCTION

Eucalyptus citriodora Hook. (Myrtaceae) is also known as lemon scented gum or citron scented gum and found in different states as Assam, Madhya Pradesh, Bihar, Kerala, Maharashtra and Uttar Pradesh (1–2). Incorporation of *Eucalyptus globulus* in diet (62.5 g/kg) and drinking water (2.5 g/kg) or administration of aqueous extract (0.25 – 0.5 g/kg) reduced the hyperglycemia and associated weight loss of streptozotocin treated mice (3). The pronounced anti HIV, antitumor, antigranulation, antimalarial, antidiabetic, anti-inflammatory and hepatoprotective activities of nonvolatile constituents of some species of eucalyptus has been reported. Some of the nonvolatile constituents of *Eucalyptus citriodora* are triterpenes, tannins, flavonoids, anthocyanins, phenolic compounds etc. (4). Eucalyptus leaf in folk medicine is used internally for the treatment of diabetes, asthma, fever, whooping cough, liver and gallbladder complaints, ulcer, neuralgia, stomatitis, pain, gonorrhea, rheumatism and as a gastrointestinal remedy (5). Present study was carried out to evaluate the antidiabetic activity of the aqueous extract of leaves of *E. citriodora.*

MATERIALS AND METHODS

Plant material collection and preparation of extract

The leaves of *E. citriodora* were collected from Ranchi, India and authenticated through Birsa Agricultural University, Kanke, Ranchi and a voucher specimen was preserved in the Department of Pharmaceutical sciences, Birla Institute of Technology, Mesra, Ranchi. The leaves were dried in oven at 45°C and then coarsely powdered.

The powdered material was extracted with water by maceration. The extract was dried by rotary vacuum evaporator. The yield of the aqueous extract of *E. citriodora* (ECAE) was 1.6 % w/w.

Animals

Albino rats of either sex weighing between 140 – 180 g were used for the experiment. Approval was taken from the Institutional Animal Ethical Committee (IAEC) of Birla Institute of Technology, Mesra. Animals were allowed free access to standard pellet diet and water *ad libitum*.

Effect of E. citriodora extract on oral glucose tolerance in rats

Fasted rats were divided into three groups of six animals each. Group I served as glucose control. Group II & III were treated with plant extract (250 mg/kg of body weight) and glibenclamide (600 µg/kg of body weight) orally respectively. After 30 min of extract and standard drug administration, the rats of all the groups were treated with 2 g/kg of glucose. Blood glucose levels were determined by collecting the blood from retro orbital plexus at 30 and 90 min after glucose administration by O-toluidine method (6–7).

Effect of E. citriodora extract on alloxan-induced diabetic rats

Diabetes was induced in rats by a single i.p. injection of 150 mg/kg of body weight of alloxan monohydrate in sterile saline (8). After 72 hr of alloxan injection, the diabetic rats (glucose level > 250 mg/dl) were separated (9) and divided into four groups of six animals each as below:

Group I: Diabetic control rats received distilled water

Group II: Diabetic rats treated with ECAE 250 mg/kg of body weight in distilled water.

Group III: Diabetic rats treated with ECAE 500 mg/kg of body weight in distilled water.

Group IV: Diabetic rats treated with Glibenclamide 600 µg/kg of body weight in aqueous solution.

Blood samples were collected from retro orbital plexus at zero time (before extract and glibenclamide administration) and after 2, 4, 6 hr of treatment. Blood glucose levels were determined by O-toluidine method. In multidose study (sub acute study) the animals were treated with the same dose for three weeks. Blood glucose levels were determined by O-toluidine method on 7th, 14th and 21st day of treatment.

Antioxidant activity

Antioxidant activity of the aqueous extract was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical (10–12). Various concentrations of the extract were added to 0.004% methanolic solution of DPPH. After 30 min the absorbance at 517 nm was determined, and the percent inhibition activity was calculated using the following formula.

$$\% \text{ inhibition} = [(Ac - At) / Ac] \times 100$$

Where, Ac = absorbance of control sample and At = the absorbance of test sample.

The IC_{50} was determined as the concentration in µg required to scavenge 50% DPPH free radical.

"Table 1: Effect of aqueous extract of *E. citriodora* leaf on oral glucose tolerance in rats"

Group	Treatment (p.o.)	Blood Glucose Level (mg/dl)		
		Fasting	30 min	90 min
I	Glucose (2 g/kg)	83.7 ± 10.2	168.5 ± 9.4	132.7 v 8.6
II	Extract (250 mg/kg) + Glucose (2 g/kg)	87.4 ± 5.8	153.6 ± 6.4*	123.9 ± 7.3*
III	Glibenclamide (600 µg/kg) + Glucose (2 g/kg)	78.8 ± 3.9	127.8 ± 6.2*	103.9 ± 6.8*

Values are Mean ± S.D. (n = 6);

*p < 0.01 vs. group I.

"Table 2: Effect of aqueous extract of *E. citriodora* leaf on alloxan-induced diabetic rats (single dose short term study)"

Group	Treatment (p.o.)	Blood Glucose Level (mg/dl)			
		0 hr	2 hr	4 hr	6 hr
I	Diabetic Control (Distilled water)	374.6±12.4	381.6±9.8	372.6±13.8	368.8±16.2
II	ECAE (250 mg/kg)	388.2±16.4	340.2±15.7*	312.6±13.2*	335.5±17.3*
III	ECAE (500 mg/kg)	379.4±12.8	324.3±9.8*	292.6±10.8*	312.5±13.3*
IV	Glibenclamide (600 µg/kg)	384.4±14.2	323.6±11.9*	284.9±15.8*	275.1±16.2**

Values are Mean ± S.D. (n = 6);

*p < 0.05,

**p < 0.01 vs. diabetic control

"Table 3: Effect of aqueous extract of *E. citriodora* leaf on alloxan-induced diabetic rats (multidose long term study)"

Group	Treatment (p.o.)	Blood Glucose Level (mg/dl)			
		0 day	7th day	14th day	21st day
I	Diabetic Control (Distilled water)	374.6±12.4	362.6±10.8	342.9±12.2	322.5±16.7
II	ECAE (250 mg/kg)	388.2±16.4	319.8±14.2*	274.8±16.9**	194.5±9.9**
III	ECAE (500 mg/kg)	379.4±12.8	297.1±11.7*	229.3±15.1**	163.8±16.2**
IV	Glibenclamide (600 µg/kg)	384.4±14.2	290.1±12.7*	208.8±10.8**	128.2±13.6**

Values are Mean ± S.D. (n = 6);
*p < 0.05,
**p < 0.01 vs. diabetic control

Statistical Analysis

The results were expressed as Mean ± S.D. of six animals and the data were statistically analyzed by student's t-test.

RESULTS

In glucose tolerance test, the extract has reduced the increased blood glucose level significantly after administration of glucose. The maximum glucose tolerance of the extract was observed at 30th min (Table 1).

Both in single dose short term study and multidose long term study the extract exhibited significant reduction of blood glucose level in a dose dependent manner. In single dose treatment the reduction of blood glucose level was 19.5 and 22.9 % at a dose of 250 and 500 mg/kg of body weight respectively (Table 2). In subacute study the reduction of blood glucose after 21 days was 49.9 and 56.8 % with 250 and 500 mg/kg of body weight respectively. These values are comparable with the standard drug Glibenclamide, where the reduction of blood glucose level was 66.6 % (Table 3). In antioxidant study the IC50 of the extract was found to be 352 µg/ml.

DISCUSSION

Administration of alloxan causes destruction of β-cells of the pancreas (13) and increases the blood glucose level. Some Flavonoids and saponins isolated from medicinal plants significantly decrease the elevated blood glucose levels (14–15). Literature survey suggests the presence of triterpenes, tannins, flavonoids, anthocyanins, phenolic compounds etc. in the nonvolatile fraction of *E. citriodora*. Flavonoid glycosides stimulate the secretion of insulin in β-cells of the pancreas (16). Therefore, the significant antidiabetic activity of the aqueous extract of leaves of *E. citriodora* may be due to the presence of tannins and flavonoids. Further, free radical formation is associated with a number of diseases including diabetes (17–18). Hence, the antidiabetic activity may be due to its antioxidant property.

In conclusions, this study shows significant antidiabetic activity of *E. citriodora* leaf. However, it will be interesting to isolate the compounds responsible for antidiabetic activity and to elucidate their mechanism of action.

REFERENCES

1. Atal C.K. and Kapur B.M., Cultivation and Utilization of Aromatic Plants, (Publication and Information Directorate, CSIR, Hillside Road, New Delhi, 1982) 431.
2. Anonymous. The Wealth of India (Raw Materials), Vol. III (D-E), (CSIR, New Delhi, 1952) 203.
3. Gray A.M. and Flatt P.R. Antihyperglycmic actions of *Eucalyptus globulus* (Eucalyptus) are associated with pancreatic and extra-pancreatic effects in mice. *J Nutr.* **128**(12): 2319–2323 (1998).
4. Singh A.K., Khare M. and Kumar S. Non-volatile constituents of Eucalyptus, A review on chemistry and biological activity. *J Med Aro Plant Sc.* **21**: 375 (1999).
5. Anonymous. *PDR FOR HERBAL MEDICINE*, 2nd Edn., (Medical Economy Company, Montvale, New Jersey, 2000) 283.
6. Rajgopal G. and Toora B.D. *Practical Biochemistry- For Medical, Dental and Allied Courses*, (Ahuja Book Company Pvt. Ltd., 2002) 74.
7. Sasaki T., Matzy S. and Sonal A. Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for blood glucose estimation. *Rinsho Kagaku* **1**: 346–353 (1972).
8. Williamson E.M., Okpako D.T. and Evans F.J. *Selection, Preparation and Pharmacological Evaluation of Plant Material*, Vol. I, (John Wiley & Sons, England, 1996) 155.
9. Raphael K.R., Sabu M.C. and Kuttan R. Hypoglycemic effect of methanolic extract of *Phyllanthus amarus* Schum and Thonn on alloxan induced diabetes mellitus in rats and its reaction with antioxidant potential. *Ind J Expt Biol.* **40**: 905–909 (2002).
10. Bang Y.H., Hang S.K., Jeong H.L., Young S.H., Jai S.R., Kyong S.L. and Jung J.L. Antioxidant benzoylate flavan-3-ol glycoside from *Clastrus orbiculatus*. *J Nat Prod.* **64**: 82–84 (2001).
11. Blois M.S. Antioxidant determination by the use of a stable free radical. *Nature* **181**: 1199–1200 (1958).
12. Dasgupta N. and De B. Antioxidant activity of some leafy vegetables of India: A comparative study. *Food Chemistry* **101**: 471–474 (2007).
13. Vekatesh S., Reddy G.D., Madhava B., Ramesh M. and Rao A.A.V.N., Antihyperglycemic activity of *Caralluma attenuate*. *Fitoterapia* **74**: 274–279 (2003).
14. Abdel-Hassan I.A., Abdel-Barry J.A. and Mohammeda S.T. The hypoglycemic and antihyperglycaemic effect of Citrullus colocynthis fruit aqueous extract in normal and alloxan diabetic rabbits. *J Ethnopharmacol.* **71**: 325–330 (2000).
15. Nakashima M., Kimura I., imura M. andMatsura H. Isolation of pseudoprototimosaponin A III from *Anemarrhena asphodeloides* and its hypo-

glycemic activity in streptozotocin-induced diabetic mice. *J Nat Prod*. **56**: 345–350 (1993).

16. Hii C.S.T. and Howell S.L. Effects of flavonoids on insulin secretion and $^{15}Ca^{2+}$ handling in rat islet of langerhans. *J Endocrinolo*. **107**: 1–8 (1985).
17. Vijayakumar M., Govindarajan R., Rao G.M.M., Rao Ch.V., Shirwaikar A., Mehrotra S. and Pushpangadan P. Action of *Hygrophila auriculata* against streptozotocin-induced oxidative stress. *J Ethnopharmacol*. **104**: 356–361 (2006).
18. Grankvist K., Marklund S. and Taljedal I.B. Superoxide dismutase on prophylactic against alloxan diabetes. *Nature* **294**: 158–161 (1981).