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Antidiabetic activity of *Girardinia diversifolia* on alloxan-induced diabetes mellitus in rats-A preliminary study

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ABSTRACT

Background: According to World Health Organization, about 80% of the world population relies on traditional systems of medicine for primary health care. The objective of the present study was to evaluate antihyperglycemic activity of aqueous and alcoholic extract of *Girardinia diversifolia* (AQGD and ALGD respectively).
Material and methods: In the present study, the leaves of *Girardinia diversifolia* were subjected to successive extraction by using petroleum ether, chloroform, acetone, alcohol and water. Animals were divided into seven groups (n=6). Group I served as normal control (normal saline); group II served as alloxan diabetic control (120 mg/kg, i.p.); group III served as positive control, glibenclamide (10 mg/kg, p.o.); group IV and VII served as test groups, two doses of alcoholic extracts of *Girardinia diversifolia* (200 & 400 mg/kg p.o.), and two doses of aqueous extract of *Girardinia diversifolia* (200 and 400 mg/kg p.o.) respectively.
Results: The preliminary phytochemical investigation revealed the presence of alkaloids, carbohydrates, glycosides, saponins, proteins, flavonoids, tannins, and phenolic compounds. The present study showed significant antihyperglycemic effect on repeated administration of aqueous and alcoholic extract of *Girardinia diversifolia* in Wistar rats.
Conclusion: Based on the present study, it can be concluded that the alcoholic extract is having good antidiabetic activity as compared to aqueous extract of *Girardinia diversifolia*. Further experiments are required to isolate and identify the antioxidant and antidiabetic components, and assess the mechanism of action.

INTRODUCTION

Diabetes is an important human illness afflicting many from a variety of walks of life in different countries. In India it is proving to be a key health problem, especially in the urban areas (Ablat et al., 2014). Diabetes mellitus is a chronic metabolic disorder, hyperglycemia occurs because of uncontrolled hepatic glucose output and reduced uptake of glucose spills over into the urine (glycosuria) and causes an osmotic diuresis (polyuria), which in turn results in dehydration, thirst and increased drinking (polydipsia) (Rang et al., 2003). The IDF (International Diabetes federation) has subsequently released estimates of the numbers of people with diabetes for 2003 and forecasts for 2025 of 194 million and 334 million, respectively (Wild et al., 2004).

It has been demonstrated an initial rise of glucose is followed by a decrease, probably due to depletion of islets from insulin, again followed by a sustained increase of blood glucose by single intraperitoneal injection of 120 mg/kg body weight of freshly prepared alloxan. Diabetes results from irreversible damage of insulin-producing ß-cells. In laboratory animals, diabetes can be induced with alloxan, a 2, 4, 5, 6-tetraoxopyrimidine. Alloxan is a potent generator of reactive oxygen species (ROS), which can mediate ß-cell toxicity. Glucose transporter 2 (GLUT2) and glucokinase (GK) are target molecules for alloxan (Szkudelski, 2001). According to World Health Organization estimate, about 80% of the world population relies on traditional systems of medicine for primary health care. Traditional systems of medicine like Unani, Ayurveda, Siddha etc. are of the ancient health care systems. The development of traditional system of medicines with perspectives of safety, efficacy and quality in developing and developed countries will help not only to preserve this traditional heritage but also to rationalize the use of natural products in the health care. The plant species mentioned in the ancient texts of Ayurveda and other Indian systems of medicines may be explored with the modern scientific approaches for better leads in the health care (Mukherjee et al, 2006). Girardinia diversifolia contain phytoconstituents like ß-sitosterol, accholine, histamine, 5-OH tryptamine. The leaves of Girardinia diversifolia is traditionally used for

fever and gastric trouble (Manandhar et al, 2002). The present study was an attempt to investigate antidiabetic activity of leaves of Girardinia diversifolia. We have investigated the effect of extracts of its leaves on fasting blood sugar levels and serum biochemical analysis.

MATERIAL AND METHODS

Plant material and extract preparation

The leaves of *Girardinia diversifolia,* belonging to family *Urticaceae,* were collected from Chickmangalore, Karnataka, India. The Plant material was authenticated by Dr. K Lakshman, Professor and Head of department of pharmacognosy, PES College of Pharmacy and voucher specimen was submitted to the same department. The plant material was dried, powdered then passed through sieve no. 44 to get uniform powder. Sieve powder was stored in air tight, high density polyethylene container before extraction. The powder was subjected to hot continous soxhlet extraction with methanol (50-55 °C), after defatting with petroleum ether. Solvent with residue extraction was distilled off and excess solvent was removed by using rotary flash evaporator.

Animals

Healthy Wistar albino rats (150-200 g) were used for acute oral toxicity studies and anti-diabetic models. They were purchased from Bioneeds, Nelamangala, Tumkur, KA, India. They were maintained in the animal house of PES College of Pharmacy, Bangalore for experimental purpose. The rats were housed in polypropylene cages and maintained under standard condition (12/12h light/dark cycle; 25 ± 3 °C; 35-60% humidity). The animal had free access to standard lab chow and tap water. The study was conducted after obtaining Institutional animal ethical committee clearance. (PESCP/IAEC/2007-8/1-8)

Acute toxicity

The acute toxicity of alcoholic and aqueous extracts of *G. diversifolia* were determined by using female albino Wistar rats (150-200 g) those maintained under standard husbandry conditions. The animals were fasted 12 hour prior to the experiment, up and down procedure was adopted for toxicity studies. Animals were administered with single dose of extract of *G. diversifolia* and observed for its mortality during 2 days and 7 days study period (short term) toxicity. Based on short-term profile the dose of the drugs for experimental study had been determined.

Experimental procedure

In the present study, the leaves of *G. diversifolia* were subjected to successive extraction by using petroleum ether, chloroform, acetone, alcohol and water. The preliminary phytochemical investigation revealed the presence of alkaloids, carbohydrates, glycosides, saponins, proteins, flavonoids, tannins, and phenolic compounds. Animals were divided into seven groups (n=6). Group I served as normal control (normal saline); group II served as alloxan diabetic control, received alloxan (120 mg/kg, i.p.); group III served as positive control, received glibenclamide (10 mg/kg, p.o.); group IV & VII served as test groups, received two doses of alcoholic extracts of *G. diversifolia* (ALGD; 200 & 400 mg/kg p.o.), and two doses of aqueous extract of *G. diversifolia* (AQGD; 200 and 400 mg/kg p.o.) respectively (Jamal et al., 1997).

Induction of diabetes

Alloxan was dissolved in normal saline immediately before use. Diabetes was induced in 16 hour fasted rats by single intraperitoneal injection of 120 mg/kg body weight of freshly prepared alloxan. The rats after alloxan injection were given 5% w/v glucose solution in feeding bottles for next 24 hours in their cages to prevent hypoglycemia. After 72 hours rats with fasting blood glucose levels greater than 200 mg/dl and lesser then 400 mg/dl were selected and used for further observation.
Animals were grouped randomly into seven groups of six each and were fasted over night. Drug treatment was made as mentioned above. The blood samples were withdrawn from retro orbital plexus at 0 hour i.e., just prior to oral administration of all the drugs. The treatment was continued for next 7 days. The blood samples were also withdrawn on 4th and 7th day after 1 hour administration of drug for sub acute study. Blood glucose level was estimated (Kesari et al, 2006; Ghosh et al., 2001; Sharma et al., 2006).

Oral glucose tolerance test (OGTT) in alloxan-induced diabetic rats

On the 8th day OGTT is carried out, after 60 min of drug administration, the rats of all the groups were orally treated with 2 g/kg of glucose. The blood samples were collected from retro orbital plexus at 0 hour i.e. just prior to the administration of glucose load and at 1st hour, 2nd hour, 3rd hour and 6th hour after the administration of the glucose load. Blood glucose level was estimated at various time intervals. Serum glucose was estimated by the GOD/POD.

Statistical analysis

All values were expressed as mean ± SEM. Statistical analysis was made by using one-way ANOVA followed by Dunnett's test. *P*-values less than 0.05 were considered as statistically significant.

RESULTS

Fasting blood glucose (FBG) levels in normal rats were range of 90-100 mg/dl. Treatment with alloxan (120 mg/kg, i.p.) had increased the BGL to a range of 250-350 mg/dl after 7 days. Changes in the fasting blood glucose levels in different groups were tabulated in table 1 and represented in graph 1. This data shown that the blood glucose levels of normal control animals have maintained throughout the study period.

The group II which is the diabetic control group has shown significant increase in fasting glucose levels during this 7 day study period. The group III glibenclamide (10 mg/kg) treated group showed significant decrease in

Table. 1 Effect of various extracts of *G. diversifolia* on blood glucose level in alloxan (120 mg/kg i.p.)-induced diabetic rats.

S. no.	Treatment	Blood glucose level (mg/dl)		
		Day 0	Day 4	Day 7
I	Normal control	97.28 ± 2.11	95.58 ± 1.21	96.62 ± 1.56
II	Alloxan diabetic control (120 mg/kg)	262.92 ± 0.85**	282.71 ± 0.55**	280.48 ± 0.56**
III	Glibenclamide (10 mg/kg)	265.54 ± 0.88	149.85 ± 0.75**	105.25 ± 0.38**
IV	ALGD (200 mg/kg)	258.44 ± 0.74	251.35 ± 0.79	238.26 ± 0.87**
V	ALGD (400 mg/kg)	266.66 ± 0.92	241.48 ± 0.57**	196.48 ± 0.57**
VI	AQGD (200 mg/kg)	264.09 ± 1.06	258.90 ± 0.43	240.23 ± 0.56
VII	AQGD (400 mg/kg)	257.01 ± 0.83	251.94 ± 0.67	222.25 ± 1.14

ALGD: alcoholic extract of *Girardinia diversifolia* AQGD: aqueous extract of *Girardinia diversifolia.*
Values are expressed as mean ± SEM, n=6.
Data were analyzed by using one-way ANOVA followed by Dunnett's test.
***P* <0.01 vs. vehicle control.

fasting blood glucose level during 4th, and 7th day of study period. ALGD extracts of 200 and 400 mg/kg showed significant decrease in fasting blood glucose on 4th and 7th day of study period. The other extracts AQGD, at both dose levels did not show significant decrease in blood glucose level throughout the study period. These results suggest that the ALGD 200 and 400 mg/kg possess anti-diabetic activity (Table 1).

Oral glucose tolerance test (OGTT) in alloxan-induced diabetic rats on 8th day

Repeated administration of ALGD (200 & 400 mg/kg) and glibenclamide (10 mg/kg) had significantly reduced the increase in BGL at 1st, 2nd, 3rd and 6th hour after glucose loading (2 g/kg) in alloxan-induced diabetes in rats, while AQGD (200 and 400 mg/kg) couldn't prevent the increase in glucose level after glucose loading in alloxan induced diabetic rats (Table 2).

Table 2. Effect of various extracts of *G. diversifolia* on glucose tolerance test in alloxan (120 mg/kg i.p.)-induced diabetic rats.

Group	Treatment	Blood glucose level (mg/dl) / Percentage change in Blood glucose level (%)								
		0 hour	1st hour		2nd hour		3rd hour		6th hour	
		BGL	BGL	% change	BGL	% change	BGL	% change	BGL	% change
I	Normal control	96.60 ± 0.56	137.12 ± 0.71	41.94 ± 2.29	112.79 ± 0.86	16.75 ± 1.38	97.34 ± 0.73	0.76 ± 0.47	96.89 ± 0.73	0.30 ± 0.43
II	Alloxan diabetic control (120 mg/kg)	282.10 ± 0.91**	416.95 ± 0.89**	47.4 ± 3.06**	357.32 ± 3.11**	26.66 ± 0.60**	293.08 ± 1.24**	3.89 ± 0.86**	282.27 ± 0.99**	0.06 ± 0.47**
III	Glibenclamide (10 mg/kg)	104.38 ± 1.52	132.85 ± 0.98	27.27 ± 1.90**	115.71 ± 1.27	10.83 ± 0.70**	105.68 ± 1.26	1.24 ± 0.51***	105.85 ± 1.06	1.40 ± 0.44
IV	ALGD (200 mg/kg)	262.81 ± 1.03	366.24 ± 1.58	39.35 ± 1.52*	310.50 ± 1.39	18.17 ± 1.45**	279.64 ± 1.608	6.40 ± 0.42**	276.97 ± 1.49	5.38 ± 0.42
V	ALGD (400 mg/kg)	215.24 ± 1.61	280.77 ± 1.37	30.44 ± 1.30**	241.63 ± 0.96	12.26 ± 1.61**	222.01 ± 1.19	3.14 ± 0.24**	219.07 ± 0.94	1.77 ± 0.19
VI	AQGD (200 mg/kg)	278.87 ± 0.47	411.75 ± 1.53	47.64 ± 1.93	338.95 ± 0.66	21.54 ± 3.05	291.92 ± 5.11	4.67 ± 0.74	282.90 ± 1.51	1.44 ± 0.64
VII	AQGD (400 mg/kg)	282.95 ± 0.85	415.62 ± 1.20	46.88 ± 3.76	344.00 ± 1.26	21.57 ± 1.08	293.21 ± 1.04	3.66 ± 0.71	284.74 ± 0.48	0.63 ± 0.35

ALGD: alcoholic extract of *Girardinia diversifolia* AQGD: aqueous extract of *Girardinia diversifolia.*
Values are expressed as mean ± SEM, n=6.
Data were analyzed by using one-way ANOVA followed by Dunnett's test.
**P* <0.05, ***P* <0.01 & ****P* <0.01 vs. vehicle control.

DISCUSSION

Diabetes mellitus of long duration is associated with several complications such as atherosclerosis, myocardial infarction, neuropathy nephropathy, etc. These complications have long been assumed to be related to chronically elevated glucose levels and subsequent oxidative stress. Mechanism that contributes to increased oxidative stress in diabetes includes non- enzymatic

glycosylation, auto-oxidation glycoslyation and metabolic stress (Verma et al., 2010).

The present study was designed to investigate the hypoglycemic and anti-diabetic activity of leaves of *G. diversifolia* in rats. In the present study, the leaves of *G. diversifolia* were subjected to successive extraction by using petroleum ether, chloroform, acetone, alcohol and water. The preliminary phytochemical investigation revealed the presence of alkaloids, carbohydrates, glycosides, saponins, proteins, flavonoids, tannins, and phenolic compounds. Administration of alloxan (120 mg/kg, i.p.) produced significant increase in BGL after 7 days. It has been postulated that glucose transporter and glucokinase are the target molecule for alloxan, leading to decreased insulin levels and uncontrolled BGL (Maroo et al., 2002; Kumar et al., 2007). It has also been established that there is a steady decrease in beta-cell function and mass that may occur in persons at high risk of developing type II diabetes. Beta-cell stabilization or regeneration must take place to inhibit the loss of beta-cell function and mass (Henry, 2006). The regeneration of ß-cells in diabetes has been studied in numerous animal models of alloxan-induced diabetes (Chakravarthy et al., 1982; Ghosh and Suryawanshi, 2001). Development of type II diabetes is chiefly due to loss of pancreatic ß-cell function, which results in augmented impairment of patient's ability to produce insulin in response to augmented blood glucose.

Repeated dose administration of AQGD didn't show any hypoglycemic activity, indicating the extract is ineffective to produce hypoglycemia after repeated administration. However, repeated dose administration of ALGD for 7 days produced significant decrease in BGL on 4th and 7th day and showed improved glucose tolerance on 8th day as compared with alloxan diabetic control rats. These results suggested that ALGD on repeated administration produced hypoglycemic activity. However, the hypoglycemic activity was less than that of glibenclamide, a standard hypoglycemic agent. The effect of glibenclamide on glucose tolerance has been attributed to the improved activity of beta-cells of the pancreas, resulting in the better secretion of insulin. Therefore, the mechanism after this antihyperglycemic activity of plant extracts involves an insulin-like effect, probably, through peripheral glucose consumption or enhancing the sensitivity of beta-cells to glucose, resulting in increased insulin release (Muniappan et al., 2004). The antidiabetic activity of *G. diversifolia* may be due to the presence of flavonoids. It has been reported that flavanoids represent the active biological principles of most medicinal plants with hypoglycemic and antidiabetic properties (Wollenweber et al., 1988). However, the extract should further be subjected to bioactivity-guided drug discovery to isolate the lead compound responsible for antidiabetic activity and possible mechanisms(s) of action.

CONCLUSION

In conclusion, *G. diversifolia* exhibited significant antihyperglycemic activities in alloxan-induced diabetic rats. The decreasing effect in blood glucose levels of the *G. diversifolia* extracts justified the use of *G. diversifolia* as a anti-diabetic agent. Based on the present study it can be concluded that alcoholic extract of *Girardinia diversifolia* is having good anti-diabetic activity as compared to aqueous extract. Further, experiments are required to isolate and identify the antidiabetic components, and assess the mechanism of activity.

CONFLICT OF INTEREST

None declared.

REFERENCES

Ablat A, Mohamad J, Awang K, Shilpi JA, Arya A. Evaluation of antidiabetic and antioxidant properties of Brucea javanica seed. *Scient World J*. 2014, 786130, 2014.

Chakravarthy BK, Gupta S, Gode KD. Functional beta cell regeneration in the islets of pancreas in alloxan-induced diabetic rats by (-)-epicatechin. *Life Sci*. 31, 2693–2697, 1982.

Ghosh S, Suryawanshi SA. Effect of *Vinca rosea* extracts in treatment of alloxan diabetes in male albino rats. *Indian J Exp Biol*. 39,748-759, 2001.

Henry RR. Resurrecting the Beta Cell in Type 2 Diabetes: Clinical Impact of Therapies Directed at Beta-cell Preservation 2006.

Jamal AA, Issa AA, Mohammad HH. Hypoglycaemic and antihyperglycaemic effects of *Trigonella foenum-graecum* leaf in normal and alloxan-induced diabetic rats. *J Ethnopharmacol*. 58, 149–155, 1997.

Kesari AN, Gupta RK, Singh SK, Diwakar S, Watal G. Hypoglycemic and antihyperglycemic activity of *Aegal marmelos* seed extracts in normal and diabetic rats. *J of Ethenopharmacol*. 107, 374-379, 2006.

Kumar BRP, Praveen TK, Nanjan MJ, Karvekar MD, Suresh B. Serum glucose and triglyceride lowering activity of some novel glitazones against dexamethasone-induced hyperlipidemia and insulin resistance. *Indian J Pharmacol*. 39, 299-302, 2007.

Manandhar NP. Plants and people of Nepal timber press. Oregon. ISB 088192-527-6, 2002.

Maroo J, Vasu VT, Aalinkeel R, Gupta S. Glucose lowering effect of aqueous extract of *Enicostemma littorale* Blume in diabetes: a possible mechanism of action. *J Ethnopharmacol*. 81, 317-320, 2002.

Mukherjee P, Wahile A. Integrated approaches towards drug development from ayurveda and other Indian system of medicines. *J Ethnopharmacol*. 105, 25-35, 2006.

Muniappan L, Leelavinothan P, Sandhya S, Ramesh B. Insulin-secretagogue activity and cytoprotective role of the traditional antidiabetic plant *Scoparia dulcis* (Sweet Broomweed) *Life Sci*.75, 2003–2014, 2004.

Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. New Delhi: Churchill Livingstone, 2003.

Sharma SB, Nasir A, Prabhu KM, Murthy PS. Antihyperglycemic effect of the fruit pulp of *Eugenia jambolana* in experimental diabetes mellitus. *J Ethnopharmacol*. 104, 367-73, 2006.

Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol Res*. 50, 537-546, 2001.

Verma L, Khatri A, Kaushik B, Patil UK, Pawar RS. Antidiabetic activity of Cassia occidentalis (Linn) in normal and alloxan-induced diabetic rats. *Indian J Pharmacol*. 42, 224-228, 2010.

Wild S, Roglic G, Green A, Sicree R, King H. Global Prevalence of Diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care*. 27, 1047-53, 2004.

Wollenweber LE, Cody V, Middleton EJ, Harborne JB, Beretz A. Plant flavanoids in biology and medicineII: Biochemical, cellular and medicinal properties. *Prog Clin Biol Res*. 280, 1-461, 1988.