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Antihyperglycaemic activity of ethanolic extract of *cissus quadrangularis* (l.) Leaves in alloxan induced diabetic rats

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ABSTRACT

The present study was performed to explore the antihyperglyceamic activity of ethanolic extract of leaves of *Cissus quadrangularis* against alloxan induced diabetic rats. Ethanolic extract of *C. quadrangularis* and glyburide were administered orally in alloxan induced diabetic rats. In the acute study, the serum glucose level was estimated at 0, 2, 4, 6 and 24 h after drug administration. The subacute study involved repeated administration of the drugs for 28 days, a serum glucose level estimated at 7, 14, 21 and 28 days. In the OGTT, D-glucose (2.5 g/kg) was administered in diabetic rats half an hour after pre-treatment with EtCQ and glyburide. Serum glucose levels were estimated 30 min prior to glucose administration and at 0, 30, 60 and 120 min after glucose loading. In EtCQ (400 mg/kg), the onset was 4 h, the peak effect was 6 h but the effect waned at 24 h. In subacute study, repeated administration (once a day for 28 days) of the glyburide and EtCQ caused a significant reduction in the serum glucose level as compared to the vehicle treated group. EtCQ (400 mg/kg) treatment prevented a decrease in the body weight of the diabetic rats. In the OGTT, EtCQ (200 & 400 mg/kg) increased the glucose threshold at 30 min after the administration of glucose. The EtCQ (400 mg/kg) showed significant antihyperglyceamic activity than EtCQ (100 and 200 mg/kg). It can be concluded that ethanolic extract of *C. quadrangularis* has antihyperglyceamic activity.

INTRODUCTION

Diabetes mellitus (DM) is a global epidemic affecting >150 million people, a number that is expected to double by 2025 (Grover *et al.,* 2002). DM is considered as heterogeneous group of diseases characterized by major causes affecting to cardiovascular, renal, neurological and ophthalmic systems (Chakkarwar and Manjrekar, 2005). Currently available synthetic oral anti-hyperglycaemic agents may be associated with an increased risk of unwanted effects on prolonged use (Edwin *et al*., 2006). So there is clear need to investigate a newer herbal medicines which have less side effects, easy availability and economic (Shah *et al*., 2006a).

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C. quadrangularis L. (Vitaceae) is one of the most common plant in India. In Hindi, it is popularly known as harjora; other common names include bone setter (English), kandvel (Marathi), Asthishrinkla (Sanskrit), hathjod (Urdu), habhanga (Bengali), Mangaravalli (Kannad), parantai (Tamil), haddjor (Punjabi), Cannalamparanta (Malyalam) (Ayurvedic Pharmacopoeia of India, 2001).

C. quadrangularis is available throughout the year. The plant has been reported to possess wound healing (Mohanty *et al*., 2010), antiostoporotic (Shirwaikar *et al*., 2003), antioxidant (Chidambara *et al*., 2003), antipseudomonal and antibacterial (Kashikar and George, 2006), ulcer protective (Jainu and Devi, 2006a), antiplasmodial (Bah *et al*., 2007), anti-inflammatory (Jainu *et al*., 2006b; Jainu *et al*., 2006c; Panthong *et al*., 2007) and analgesic activity (Priyanka *et al*., 2010).

Use of plants as a sourse of medicine has been inherited and is an important component of the health care system in India.

Number of Indian medicinal plants has been claimed for their antidiabetic activity in the traditional system of medicine, but all of them have not been reported scientifically.

Many indigenous drugs have been claimed to have antidiabetic effect in Ayurvedic system of medicine but they were not properly investigated (Rangari, 2004). The objective of the present investigation was to study the effect of ethanolic extract of *C. quadrangularis* on serum glucose levels and on the oral glucose tolerance test (OGTT) in alloxan induced diabetic rats.

MATERIALS AND METHODS

Drugs and chemicals

Fresh *C. quadrangularis* leaves were collected from local area of Jalgoan district, Maharashtra, India in the months of July-October. This plant was identified, authenticated and voucher specimens No. 9160 have been kept in Department of Botany, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur, Maharashtra, India. Glyburide (Ranbaxy Pharma. Ltd. India), alloxan monohydrate (Spectrochem, India), glucose estimation kit (Accurex Biomedical Pvt. Ltd., India) and D-glucose (S.D. Fine-Chem. Ltd, India) were purchased from respective companies.

Animals

Adult Swiss albino mice and Wistar rats, weighing between 25-30 g and 150-180 g respectively were used and acclimatized to laboratory conditions for one week.

All animals were housed in well ventilated polypropylene cages at 12:12 h light/dark schedule with 25±2ºC and 55-65% relative humidity. The rats had fed with commercial pellet rats chow and water *ad libitum* as a standard diet. Experimental protocol was approved by institutional animal ethics committee in accordance with CPCSEA.

Preparation of leaf extract

The leaves were collected and dried in shade and ground. Coarsely powdered leaves were used for the study. Coarsely powdered plant material (1000 g) was subjected to hot continuous extraction with ethanol (60 – 80°C) in a soxhlet extractor at a temperature of 45-50°C to 40 cycles per batch for 2 batches.

The extraction was continued until the solvent in the thimble becomes clear indicating the completion of the extraction. After each extraction the solvent was distilled off and concentrated extract was transferred to previously weighed petri dish and evaporated to dryness at room temperature to obtain dried extracts. After completion of drying the petri dish was weighed again. The yield of extract was calculated by subtracting original weight of empty petri dish. The yield was 3.5 g/100 g. The *C. quadrangularis* extract was dissolved in distilled water to prepare the drug solution of concentration of 100 mg/ml and used for pharmacological studies.

Preliminary phytochemical studies

Preliminary qualitative phytochemical screening for the identification of the phytoconstituents of the ethanolic extract of *C. quadrangularis* has been carried out (Harborne, 1998).

Acute oral toxicity of the extract

Adult Albino mice were divided into five groups containing ten mice each. The mice were fasted for 6 h and access only water *ad libitum* before experimrntal study. Gr. I received only vehicle (distilled water). Gr. II, III, IV and V animals received with different doses of EtCQ i.e. 1000, 2000, 3000 and 4000 mg/kg respectively. All the doses and vehicle were administered orally. The mice were observed continuously for 2 h for behavioral, neurological and autonomic profiles for any lethality or death for the next 48 h (Ravichandran *et al*., 2007).

Induction of experimental diabetes

Wistar rats were made diabetic by a single intraperitoneal injection of aqueous alloxan monohydrate (120 mg /kg) solution (Kameswararao *et al*., 1999). After 48 h, blood samples were collected and serum glucose levels were determined to confirm the development of diabetes. Only those animals which showed hyperglycaemia (blood glucose levels > 200 mg/dl) were used in the study (Ewart *et al*., 1975; Cetto *et al*., 2000).

Collection of blood and determination of serum glucose

Blood samples from the experimental rats were collected by retro orbital plexus technique using heparinised capillary glass tubes. The collected blood samples were analyzed for glucose levels by the glucose oxidase peroxidase (GOD/POD) method (Abdel Barry *et al*., 1997) and serum glucose levels were expressed in mg/dl.

Effect of EtCQ on serum glucose in alloxan-induced diabetic rats

Diabetic wistar rats of either sex were fasted overnight and divided into five groups (n =10) viz; Gr. I: vehicle (distilled water, 10 ml/kg), Gr. II: glyburide (10 mg/kg) (Shah *et al*., 2006a), Gr. III: EtCQ (100 mg/kg), Gr. IV: EtCQ (200 mg/kg) and Gr. V: EtCQ (400 mg/kg). EtCQ and glyburide were administered orally.

The acute study involved estimation of serum glucose levels at 0, 2, 4, 6 and 24 hour after EtCQ and glyburide administration. The animals had free access to feed and water after 6 h. The subacute study involved repeated administration of EtCQ and glyburide for 28 days (once a day) at a prefixed time and serum glucose levels were estimated in samples withdrawn after 2 h on day 7, 14, 21 and 28.

At the end of 28 days, EtCQ and glyburide administration was stopped and a rest period of 7 days was given to the animals to study effect of EtCQ and glyburide treatment on serum glucose levels after 7 days (Dunn and Mcletchie, 1943). The animals had free access to feed and water during this period. During the study period of 35 days the rats were weighed daily and their body weights were recorded.

Effect of EtCQ on oral glucose tolerance test (OGTT) in normal and diabetic rats

The diabetic animals were fasted overnight before commencing the experiment. Nondiabetic and diabetic rats were divided into five groups (n = 10) viz; Gr. I: vehicle (distilled water, 10 ml/kg), Gr. II: glyburide (10 mg/kg), Gr. III: EtCQ (100 mg/kg), Gr. IV: EtCQ (200 mg/kg) and Gr. V: EtCQ (400 mg/kg). The rats of all the groups were loaded with D-glucose (2.5 g/kg, p.o.) solution after half an hour of drug administration (Latha and Pari, 2003; Badole *et al*., 2006a; Badole *et al*., 2006b). Blood samples were withdrawn by the retro orbital plexus technique before drug administration and at 30, 60, and 120 minutes after glucose loading. The serum glucose was estimated immediately thereafter.

Statistical analysis

Data was expressed as mean ± SEM and statistical analysis was carried out by two-way ANOVA with *post hoc* Dunnett's test performed using GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego California USA. The significance level was considered at $2\alpha=0.05$.

RESULTS AND DISCUSSION

Throughout the world diabetes is the world's fastest growing metabolic disorder and C. quadrangularis is one of the traditional medicine used for the treatment of diabetes mellitus (Bailey and Day, 1989). Aqueous extracts of leaves of *C. quadrangularis* showed more significant reduction in blood glucose level in alloxan induced diabetic rats as compared to control and glibenclamide treated rats.

Glyburide is a potent, second-generation, oral sulfonylurea antidiabetic agent used as an adjunct to diet to lower blood glucose levels in patients with diabetes mellitus. The hypoglycaemic action of glyburide is due to stimulation of pancreatic islet cells, which results in an increase in insulin secretion. The effects of sulfonylurea are initiated by binding to and blocking on ATP sensitive K^+ channel, which have been cloned. The drugs thus resemble physiological secretagogues (e.g. glucose, leucine) which also lower the conductance of this channel. Reduced K^+ conductance causes membrane depolarization and influx of Ca^{+2} through voltage sensitive Ca^{+2} channel. Prolonged administration of glyburide also produces extrapancreatic effects that contribute to its hypoglycaemic activity (Shah *et al*., 2006b).

The EtCQ was found to be safe at all the doses used and there was no mortality found up to the dose of 5000 mg/kg as administered orally. Therefore, we have taken 500 mg/kg as the therapeutic dose and made variations by taking 100 mg/kg as lower dose and 400 mg/kg as higher dose.

A single administration of EtCQ (400 mg/kg) as well as glyburide (10 mg/kg) significantly reduced serum glucose levels at 4 and 6 h after administration. The reduction in serum glucose from basal value (before) at 6 h after glyburide and EtCQ (200 and 400 mg/kg) were 144.69, 100.5, and 165.18 mg/dl respectively. The onset of the antihyperglycaemic effect of glyburide was at 2 h and EtCQ (400 mg/kg) was at 4 h; the peak effect was 6 h but the effect waned at 24 h. EtCQ (400 mg/kg) resulted in lowered serum glucose at 24 h (Table 1).

In the subacute study, repeated administration (once a day for 28 days) of EtCQ and glyburide caused significant reduction in the serum glucose level as compared to vehicle treated group. On the 35th day, the reductions in serum glucose level of glyburide and EtCQ (200 and 400 mg/kg) were 269.77, 158.14 and 239.53 respectively (Table 2). The body weight of vehicle treated diabetic rats decreased during the study period. Glyburide and EtCQ (400 mg/kg) prevented the decreased in body weight of diabetic rats (Table 3). Subacute treatment for 35 days with the EtCQ in the treated doses brought about improvement in body weights indicating its beneficial effect in preventing loss of body weight in diabetic rats (Xie *et al*., 2003). The ability of EtCQ to prevent body weight loss seems to be due to its ability to reduced hyperglycaemia.

In the oral glucose tolerance test, administration of glucose load (2.5 g/kg) increased serum glucose levels significantly after 30 min in non diabetic (Table 4) and alloxan treated diabetic rats (Table 5). Glyburide (10 mg/kg) and EtCQ (400 mg/kg) produced a significant increase in the glucose threshold within 30 min.

EtCQ significantly enhanced glucose utilization in OGTT in both nondiabetic and diabetic rats. From the data obtained OGTT, it is clear that administration of EtCQ effectively prevented the increase in serum glucose level without causing a hypoglycaemic state. The effect may be due to restoration of the delayed insulin response. The results of both acute and subacute study hypothesized that the late onset of action and prolonged duration of action of EtCQ may results from improved pancreatic cytoarchitecture. These results confirmed the use of *C. quadrangularis* in folklore practice as an antidiabetic (Krishnamurthy, 2003). In this context, other medicinal plants, such as *Cassia auriculata* (Latha and pari, 2003), *Pleurotus pulmonarius* (Badole *et al*., 2006a) have been reported to possess similar effects.

Flavonoids are potent antioxidant and known to modulate the activities of various enzymes due to their interaction with various biomolecules (Catopano, 1997). Apart from flavonoids, alkaloids, tannins and phenolics are the other bioactive principles reported to possess antihyperglyceamic activity (Kameswararao *et al*., 1997). Flavonoids regenerate the damaged ß cells in the alloxan diabetic rats (Chakravarthy *et al*., 1980).

Preliminary phytochemical analysis indicated that, the leaves extracts of *C. quadrangularis* contain alkaloids, flavonoids, tannins, sterols, carbohydrates and glycosides (Table 6).

The traditional medicinal plants with various active principles and properties have been used since ancient times by physicians and laymen to treat a great variety of human diseases such as diabetes, coronary heart disease and cancer.

The beneficial multiple activities like manipulating carbohydrate metabolism by various mechanisms, preventing and restoring integrity and function of β-cells, insulin releasing activity, improving glucose uptake and utilization and the antioxidant properties present in medicinal plants offer exciting opportunity to develop them into novel therapeutics (Tiwari and Rao, 2002).

Antihyperglycaemic activity of ethanolic extract of *C. quadrangularis* may probably be due to the presence of several bioactive components.

Table. 1: Effect of EtCQ on serum glucose level in alloxan-induced diabetic rats (Acute study).

Treatment		Mean fasting glucose level (mg/dl)±SEM				
		0 h	2 h	4 h	6 h	24 h
Vehicle		402.70±42.95	446.90±7.15	449.73±7.44	455.61±7.48	463.32±6.70
Glyburide (10 mg/kg)		445.12±7.06	383.82±13.70*	335.93±12.46***	300.43±2.85***	348.78±20.15***
EGA	(100 mg/kg)	481.99±14.16	460.73±12.95	441.40±13.49	436.53±16.41	462.28±21.12
	(200 mg/kg)	477.56±17.87	442.96±10.33	422.23±7.56	377.06±13.75**	422.51±2.71
	(400 mg/kg)	481.63±9.40	396.72±13.98	344.04±11.49***	316.45±14.91***	404.31±16.33

n = 10, Data was analyzed by two-way ANOVA with *post hoc* Dunnett's test using Graphpad Instat software, *P<0.05, **P<0.01, ***P<0.001 as compared with vehicle-treated group. The significance level was considered at 2a=0.05

Table. 2: Effect of EtCQ on serum glucose level in alloxan-induced diabetic rats (Subacute study).

Treatment		Mean fasting glucose level (mg/dl)±SEM					
		0 day	7 day	14 day	21 day	28 day	After day 7 rest period
Vehicle		402.70±42.95	467.88±9.79	483.51±7.16	507.86±8.91	522.03±6.83	540.50±8.86
Glyburide (10 mg/kg)		445.12± 7.06	337.07±16.47***	279.86±21.73***	248.43±19.90***	205.06±17.49***	175.35±21.26***
EGA	(100 mg/kg)	481.99±4.16	465.30±20.88	435.64±19.92	439.98±19.16*	449.16±25.84*	388.34±27.49
	(200 mg/kg)	477.56±17.87	394.44±6.49**	368.72±5.86***	357.23±11.53***	337.59±14.44***	319.42±22.48***
	(400 mg/kg)	481.63±9.40	361.56±10.98***	324.23±5.90***	300.08±8.38***	278.45±9.16***	242.10±16.68***

n = 10, Data was analyzed by two-way ANOVA with *post hoc* Dunnett's test using Graphpad Instat software, *P<0.05, **P<0.01, ***P<0.001 as compared with vehicle-treated group. The significance level was considered at 2a=0.05

Table. 3: Effect of EtCQ on body weight in alloxan-induced diabetic rats.

Treatment		Mean body weight (g)±SEM					
		0	7	14	21	28	After day 7 rest period
Vehicle		180.67±1.43	175.33±1.28	172.50±1.02	167.00±1.63	155.83±2.24	144.33±2.38
Glyburide (10 mg/kg)		178.00± 0.93	182.17±0.91	188.00±0.82	194.67±1.61**	200.17±2.10***	206.83±1.92***
EGA	(100 mg/kg)	179.50± 0.85	179.67±1.09	180.83±0.87	180.83±0.70	182.17±1.22**	183.33±1.02***
	(200 mg/kg)	178.67±0.49	183.00±1.03	185.33±1.69	188.83±1.33*	193.33±1.15***	166.67±29.54*
	(400 mg/kg)	179.00± 1.34	185.67±1.28	195.83±1.35*	203.33±0.49***	206.33±1.71***	212.83±1.64***

n = 10, Data was analyzed by two-way ANOVA with *post hoc* Dunnett's test using Graphpad Instat software, *P<0.05, **P<0.01, ***P<0.001 as compared with vehicle-treated group. The significance level was considered at 2a=0.05

Table. 4: Effect of EtCQ on oral glucose tolerance test (OGTT) in nondiabetic rats.

Treatment		Mean Fasting glucose level (mg/dl)±SEM				
		Before glucose	0 min	30 min	60 min	120 min
Vehicle		140.08±5.08	338.72±11.82	260.46±13.78	211.82±11.09	212.99±12.00
Glyburide (10 mg/kg)		123.86±4.53	324.22±8.86	198.24±8.81***	153.59±7.90***	170.80±8.87**
EGA	(100 mg/kg)	112.65±3.44	299.83±10.43	234.64±8.46	199.43±5.70	144.66±8.28***
	(200 mg/kg)	111.08±3.72	329.12±5.86	253.87±13.54	209.17±11.58	170.22±8.94**
	(400 mg/kg)	116.27±1.96	328.18±8.80	207.38±11.72***	148.09±5.76***	161.92±9.75***

n = 10, Data was analyzed by two-way ANOVA with *post hoc* Dunnett's test using Graphpad Instat software, **P<0.01, ***P<0.001 as compared with vehicle-treated group. The significance level was considered at 2a=0.05

Table. 5: Effect of EtCQ on oral glucose tolerance test (OGTT) in diabetic rats.

Treatment		Mean Fasting glucose level (mg/dl)±SEM				
		Before Glucose	0 min	30 min	60 min	120 min
Vehicle		409.18±18.62	494.75±15.89	506.39±11.42	462.51±14.23	508.45±4.80
Glyburide (10 mg/kg)		451.68±12.58	533.47±7.04	357.22±5.40***	307.09±5.62***	421.75±8.23***
EGA	(100 mg/kg)	478.27±16.52	528.82±5.26	489.32±5.77	440.76±7.89	461.28±13.52
	(200 mg/kg)	466.47±14.57	522.57±16.51	379.12±6.92***	306.79±6.85***	370.10±27.96***
	(400 mg/kg)	455.16±37.88	537.99±16.42	340.22±10.82***	292.43±8.81***	445.03±12.02*

n = 10, Data was analyzed by two-way ANOVA with *post hoc* Dunnett's test using Graphpad Instat software, *P<0.05, ***P<0.001 as compared with vehicle-treated group. The significance level was considered at 2a=0.05

Table. 6: Phytochemical screening of EtCQ.

Sr. No.	TEST	Inference
1	Alkaloids	+ve
2	Flavonoids	+ve
3	Saponins	-ve
4	Tannins	+ve
5	Sterols	+ve
6	Carbohydrates	+ve
7	Glycosides	+ve

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