AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Pharmacological evidence of *Corchorus trilocularis* (L.) leaves in alloxan induced diabetic rats

R. L. Chaudhari.[1*], M. A. Mahajan[2], R. Y. Chaudhari[2], J. O. Bhangale[3]

1. Department of Pharmacology, Faculty of Pharmaceutical Sciences, Jodhpur National University, Jodhpur, India

2. Department of Pharmaceutical Chemistry, Tapi Valley Education Society's, Hon'ble, Loksevak Madhukarrao Chaudhari College of Pharmacy, Faizpur, 425 503, Maharashtra, India.

3. Department of Pharmacology, Smt. N. M. Padalia Pharmacy College, Ahmedabad, 382210, Gujarat, India.

ABSTRACT

The present study was performed to validate floklore claims of leaves of *Corchorus trilocularis* using ethanol extract for its antihyperglyceamic activity in alloxan induced diabetic rats. Ethanolic extract of C. *trilocularis* (EtCT) 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 estimation 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 EtCT 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 EtCT (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 EtCT caused a significant reduction in the serum glucose level as compared to the vehicle treated group. EtCT (400 mg/kg) treatment prevented a decrease in the body weight of the diabetic rats. In the OGTT, EtCT (400 mg/kg) increased the glucose threshold at 60 min after the administration of glucose. The EtCT (400 mg/kg) showed significant antihyperglyceamic activity than EtCT (100 and 200 mg/kg).It can be concluded that ethanolic extract of C. *trilocularis* has antihyperglyceaamic activity.

Keywords: *Corchorus trilocularis*, Alloxan, OGTT, Wistar rats

*Corresponding Author Email: jitu2586@gmail.com

Received 25 October 2012, Accepted 04 November 2012

Please cite this article in press as Chaudhary RL *et al.*, Pharmacological evidence of *Corchorus trilocularis* (L.) leaves in alloxan induced diabetic rats American Journal of PharmTech Research 2012.

INTRODUCTION

Diabetes mellitus (DM) is considered as heterogeneous group of diseases characterized by chronic hyperglycaemia from whatever cause leading to complications involving cardiovascular, renal, neurological and ophthalmic systems[1]. Now a day, there is clear need to investigate a newer agent in the treatment of diabetes mellitus because existing synthetic oral antihyperglycaemic agents have an unwanted effect on prolonged use[2]. The patients are using herbal medicines which have less side effects, easy availability and economic[3]

Corchorus trilocularis L. (*Tiliaceae*) is one of the most commonly plants in India. In Hindi it is popularly known as Kadukosta, Kadvapat, Hardikaket; other common names include Kaaduchunch (Marathi), Jangali jiraa (Oriya), chanchu (Sanskrit). The *C. trilocularis* is available throughout the year. The methanolic extract of *C. trilocularis* contain triterpenoid triloculerol A and triloculerol A 3 glucoside, these constituent has been shown to possess β-glucuronisidase inhibitory and enzyme inhibition activity[4]. The plant has been reported to possess anti-inflammatory[5], demulcent[6]. Seeds are used in fever and for cleaning bowls. In traditional folklore medicine in India, *C. trilocularis* is also used for syphilis[7].

Throughout the world number of 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[8].

The objective of the present investigation was to study the effect of ethanolic extract of C. *trilocularis* 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. *trilocularis* leaves were collected from local area of Jalgoan district, Maharashtra, India in the months of July-October. This plant was identified and authenticated by Dr. T. Chakraborty, Scientist D, Botanical Survey of India, Pune. Voucher specimens No. (MAACORTI1) have been kept in Botanical Survey of India, Pune, and 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 5.8 g/100 g. The C. *trilocularis* 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. *trilocularis* has been carried out[9].

Acute oral toxicity of the extract

Adult Albino mice (25-30 g) were divided into five groups containing ten mice each. The mice were fasted for 6 h and access only water *ad libitum* before experimental study. Group I received only vehicle (distilled water). Group II: EtCT (1000 mg/kg), Group III: EtCT (2000 mg/kg), Group IV: EtCT (3000 mg/kg) and Group V: EtCT (4000 mg/kg). 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[(10)].

Induction of experimental diabetes

Wistar rats were made diabetic by a single intraperitoneal injection of aqueous alloxan monohydrate (120 mg /kg) solution[(11)]. 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[(12,13)].

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[14] and serum glucose levels were expressed in mg/dl.

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

Diabetic wistar rats of either sex were fasted overnight and divided into five groups (n =6) viz; Group I: vehicle (distilled water, 10 ml/kg), Group II: glyburide (10 mg/kg), Group III: EtCT (100 mg/kg), Group IV: EtCT (200 mg/kg) and Group V: EtCT (400 mg/kg). EtCT and glyburide were administered orally.

The acute study involved estimation of serum glucose levels at 0, 2, 4, 6 and 24 h after EtCT and glyburide administration. The animals had free access to feed and water after 6 h.

The subacute study involved repeated administration of EtCT and glyburide for 28 days (once a day) at a prefixed time (10:00-11:00 am) 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, EtCT and glyburide administration was stopped and a rest period of 7 days was given to the animals to study effect of EtCT and glyburide treatment on serum glucose levels after 7 days[15]. 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 EtCT 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 = 6) viz; Group I: vehicle (distilled water, 10 ml/kg), Group II: glyburide (10 mg/kg), Group III: EtCT (100 mg/kg), Group IV: EtCT (200 mg/kg) and Group V: EtCT (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[16-18]. Blood samples were withdrawn by the retro orbital plexus technique before drug administration and at 30, 60, and 120 min 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

Plants have been used for the treatment of diabetes in developing countries where the cost of the conventional medicines represents a burden to the population. Throughout the world, number of medicinal plant has been claimed for the treatment of diabetes[19]. *C. quadrangularis* is used as a medicine for the treatment of diabetes mellitus. 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[20].

The EtCT was found to be safe at all the doses used and there was no mortality found up to the dose of 5000 mg/kg of EtCT when administered orally. Therefore, we have selected 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 EtCT (100, 200 and 400 mg/kg) as well as glyburide (10 mg/kg) significantly reduced serum glucose levels at 2, 4 and 6 h.

The reduction in serum glucose from basal value (before) at 6 h after glyburide and EtCT (100, 200 and 400 mg/kg) were 216.39, 46.01, 96.09 and 165.34 respectively. The onset of the antihyperglycaemic effect of glyburide was at 2 h and EtCT (400 mg/kg) was at 4 h; the peak effect was 6 h but the effect waned at 24 h. EtCT (400 mg/kg) resulted in lowered serum glucose at 24 h. The significant reduction in serum glucose from basal value (before) at 24 h was 102.35 mg/dl (Table 1)

In the sub acute study, repeated administration (once a day for 28 days) of EtCT 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 EtCT (100, 200 and 400 mg/dl) were 275.99, 100.17, 142.36 and 225.09 respectively. (Table 2) The body weight of vehicle treated diabetic rats decreased during the study period. Glyburide and EtCT (400 mg/kg) prevented the decreased in body weight of diabetic rats (Table 3).

Table 1: Effect of EtCT 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	440.16±15.86	446.21±9.93	450.64±18.12	452.68±20.7	461.74±16.70
Glyburide (10 mg/kg)	446.47±6.27	356.67±17.9*	320.37±23.77***	230.08±21.17***	380.22±24.16
EtCT (100 mg/kg)	480.32±14.91	471.02±11.57	448.39±20.4	434.31±20.71	461.34±27.1
EtCT (200 mg/kg)	475.09±17.69	434.13±21.84	422.87±17.21	379.64±20	449.37±19
EtCT (400 mg/kg)	484.53±15.67	403.58±19.45	350.13±19.72**	319.19±31.49***	382.18±25.13

n = 6, 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 (distilled water, 10 ml/kg). The significance level was considered at 2a=0.05

Table 2: effect of EtCT 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	day 7 rest period
Vehicle	440.16±15.86	470.27±17.94	471.13±15.6	489.09±17	496.21±11.77	520.08±17.55
Glyburide (10 mg/kg)	446.47±6.27	344.42±18.2***	296.11±29.34***	244.65±29.9***	189.11±21.7***1	170.48±22.04***
EtCT (100 mg/kg)	480.32±14.91	452.4±21.53	438.17±27.87	453.49±21.87	396.08±22.36**	380.15±30.27***
EtCT (200 mg/kg)	475.09±17.69	425.18±17.24	396.81±20.44	356.41±20.74***	344.75±22.92***	332.73±30.45***
EtCT (400 mg/kg)	484.53±15.67	400.06±21.75	371.03±18.42**	335.32±20.1***	319.52±28.97***	259.44±25.7***

n = 6, 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 (distilled water, 10 ml/kg). The significance level was considered at 2a=0.05

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

Treatment	Mean body weight (g)±SEM					
	0	7	14	21	28	day 7 rest period
Vehicle	29±0.48	27±0.52	27±0.26	26±0.45	22±1.06	18±0.82
Glyburide (10 mg/kg)	30±0.58	29±0.82	31±0.89**	30±0.68**	29±0.82***	30±1.37***
EtCT (100 mg/kg)	29±0.26	28±0.68	27±0.52	27±1.15	27±1.15***	25±1.06***
EtCT (200 mg/kg)	30±0.52	30±0.37	29±0.86	28±1.24	28±1.41***	26±0.77***
EtCT (400 mg/kg)	25±0.37	30±0.73	29±0.52	30±0.89**	30±0.73***	31±1.13***

n = 6, 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 (distilled water, 10 ml/kg). The significance level was considered at 2a=0.05

Subacute treatment for 35 days with the EtCT in the treated doses brought about improvement in body weights indicating its beneficial effect in preventing loss of body weight in diabetic rats[21]. The ability of EtCT 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 and alloxan treated diabetic rats. Glyburide (10 mg/kg) and EtCT (100, 200 and 400 mg/kg) produced a significant increase in the glucose threshold within 60 min, which was then reversed at 120 min after glucose loading nondiabetic (Table 4) as well as alloxan induced diabetic rats (Table 5).

Table 4: Effect of alcoholic extract of EtCT 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	117.63±7.17	326.22±8.87	196.38±9.22	138.38±7.20	151.10±9.69
Glyburide (10 mg/kg)	402.90±17.44	497.19±11.51	440.54±15.72***	394.38±5.99*	505.43±9.27
EtCT (100 mg/kg)	449.93±19.61	523.27±9.64	331.58±16.53	322.54±4.60	442.12±19.02
EtCT (200 mg/kg)	476.60±17.73	538.42±15.52	475.14±9.37*	390.69±13.87**	501.14±9.81
EtCT (400 mg/kg)	465.13±17.88	530.49±20.28	361.02±4.92**	321.71±10.31***	452.50±11.28

n = 6, 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 (distilled water, 10 ml/kg). The significance level was considered at 2a=0.05

Table 5:Effect of EtCT 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	402.90±17.44	497.19±11.5	440.54±15.72	394.38±5.99	505.43±9.27
Glyburide (10 mg/kg)	449.93±19.61	523.27±9.64	331.58±16.53***	322.54±4.60**	442.12±19.0
EtCT (100 mg/kg)	476.60±17.73	538.42±15.5	475.14±9.37	390.69±13.87	501.14±9.81
EtCT (200 mg/kg)	465.13±17.88	530.49±20.2	361.02±4.92*	321.71±10.31**	452.50±11.2
EtCT (400 mg/kg)	482.59±14.50	541.68±16.0	334.77±13.14**	290.13±8.36***	471.88±10.7

n = 6, 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 (distilled water, 10 ml/kg). The significance level was considered at 2a=0.05

EtCT significantly enhanced glucose utilization in OGTT in both nondiabetic and diabetic rats. From the data obtained OGTT, it is clear that administration of EtCT 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 EtCT may results

from improved pancreatic cytoarchitecture. In this context, other medicinal plants, such as *Cassia auriculata*[16], *Pleurotus pulmonarius*[18] 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[22]. Apart from flavonoids, alkaloids, tannins and phenolics are the other bioactive principles reported to possess antihyperglycaemic activity[23] Flavonoids regenerate the damaged ß cells in the alloxan diabetic rats[24].

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

Table 6: Phytochemical screening of the ethanolic extract of C. *trilocularis*

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

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, 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[25].

CONCLUSION

Antihyperglycaemic activity of ethanolic extract of *C. trilocularis* may probably be due to the presence of several bioactive antidiabetic principals.

REFERENCES

1. Chakkarwar PN, Majrekar NA. Insulin glargine: A long acting insulin analog. J Postgrad Med 2005; 51(1): 68-71.
2. Edwin E, Sheeja E, Chaturvedi M, Sharma S, Gupta VB. A comparative study on antihyperglycemic activity of fruits and barks of *Ficus bengalensis (L.*). Adv Pharmacol Toxicol 2006; 7(3): 69-71.
3. Shah SN., Bodhankar SL., Bhonde R., Mohan V. Hypoglycaemic activity of the

combination of active ingredients isolated from *Trigonella foenumgraecum* in alloxan induced diabetic mice. Pharmacologyonline. 2006a; 1: 65-82.

4. Ahmed A, Asim M, Zahid M, Ali A, Ahmad VU. New triterpenoids from *Corchorus trilocularis.* Chem Pharm Bull 2003; 51 (7): 851-853.
5. Ahirrao RA, Borse LB, Pawar SP, Rane BR, Desai SG, Alagawadi KR. Evaluation of anti-inflammatory activity of Corchorus trilocularis Linn. Seed oil. Adv Pharmac Toxic, 2009; 10 (1): 117-200.
6. Senthilkumar M, Gurumoorthi P, Janardhanan K. Some medicinal plants used by Irular, the tribal people of mrudhamalai hills, Coimbtore, Tamilnadu. Natural Product Radiance 2006; 5(5): 382-388.
7. Ishtiaq Ch M, Khan MA. An ethanomedicinal inventory of plants used for family planning and sex diseases in Samahni valley, Pakistan. Ind J of Traditional knowledge 2008; 7 (2): 277-283.
8. Rangari VD. Pharmacognosy and Phytochemistry. 1st edn, Career Publications, Nashik; 2004.
9. Harborne JB. *Phytochemical methods*, 3rd edn, Chapman and hall, London; 1998.
10. Ravichandran V, Suresh B, Sathishkumar MN, Elango K, Srinivasan R. Antifertility activity of hydroalcoholic extract of *Ailanthus excels* (Roxb): An ehanomedicines used by tribals of Nilfiris region in Tamilnadu. J Ethanopharmacol 2007; 112: 189-191.
11. Kameswararao BK, Kesavulu MM, Giri R, Apparao C. Antidiabetic and Hypolipidemic effects of *Momordica cymbalaria* Hook. Fruit powder in alloxan induced diabetic rats. J Ethnopharmacol 1999; 67: 103-109.
12. Ewart RBL, Kornfeld S, Kipnis DM. Effect of lectins on hormone release from isolated rat islets of langerhans. Diabetes 1975; 24: 705-714.
13. Cetto AA, Weidenfeld H, Revilla MC, Sergio IA. Hypoglycemic effect of *Equisetum mriochaetum* aerial parts on STZ diabetic rats. J Ethnopharmacol 2000; 72: 129-133.
14. Abdel-Barry JA, Abdel-Hassan IA, Al-Hakiem MH. Hypoglycaemic and antihyperglycaemic effects of *Trigonella foenum-graecum* leaf in normal and alloxan induced diabetic rats. J Ethnopharmacol 1997; 58: 149-155.
15. Dunn JS, McLetchie NGB. Experimental alloxan diabetic in rats. Lancet 1943; 2: 384-387.
16. Latha M, Pari L. Antihyperglycaemic effect of *Cassia auriculata* in experimental

diabetes and its effect on key metabolic enzymes involved in carbohydrate metabolism. Clin Exp Pharmacol Physiol 2003; 30: 38-43.

17. Badole SL, Bodhankar SL, Thakurdesai PA. Study of interaction of aqueous extract of *Pleurotus pulmonarius* (Fr.) Quel- Champ with rosiglitazone in alloxan induced diabetic mice. Pharmacologyonline 2006b; 3: 64-72.
18. Badole SL, Shah SN, Patel NN, Thakurdesai PA, Bodhankar SL. Hypoglycemic activity of aqueous extract of *Pleurotus pulmonarius* (Fr.) Quel- Champ in alloxan induced diabetic mice. Pharma Biol 2006a; 44(6): 421-425.
19. Bailey CJ, Day C. Traditional plant medicines as treatments for diabetes. Diab care 1989; 12(8): 533 - 564.
20. Shah SN, Bodhankar SL, Badole SL, Kamble HV, Mohan V. Effect of trigonelline: an active compound from *Trigonella foenumgraecum* Linn. in alloxan induced diabetes in mice. J Cell Tissue Res 2006b; 6(1): 585-590.
21. Xie TT, Wang A, Mehendale S, Wu J, Aung HH, Dey L, Qiu S, Yuan CS. Antidiabetic effect of *Gymnema yannaense* extract. Pharmacol Res 2003; 47: 323-329.
22. Catopano AL. Antioxidant effect of flavonoids. Angiol 1997; 48: 39-46.
23. Kameswararao B, Giri R, Kesavulu MM, Apparao C. Herbal medicines, In the treatment of diabetes mellitus. Manphar Vaidya Patrika 1997; 1: 33-35.
24. Chakravarthy BK, Gupta S, Gambir SS, Gode KD. Pancreatic β cell generation- a novel antidiabetic mechanism of *Pterocarpus marsupium* Rox. Ind J Pharmacol 1980; 12: 123-127.
25. Tiwari AK, Rao JM. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: present status and future prospects. Curr Sci 2002; 83(1): 30-38.