ANTIHYPERGLYCAEMIC ACTIVITY OF AQUEOUS EXTRACT OF *VINCA ROSEA* LINN IN ALLOXAN INDUCED DIABETIC RATS

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Summary

An in vivo study of anti diabetic effect of aqueous extract of the leaves of *Vinca rosea* was conducted on alloxan induced diabetic rats. 7 days of the orally feeding V. rosea extract (400mg/kg) to alloxan (120 mg/kg, i.p., single dose) induced diabetic rats produced significant decrease in blood glucose level as compared to pathogenic diabetic rats. Further the aqueous extract treatment can significantly alter the pattern of glucose tolerance in normal and diabetic rats. The inhibitory effect on biochemical parameter (blood glucose level) induced by the Aqueous extract of *V.rosea* at a dose of 400 mg/kg was almost comparable to that of standard drug, glibenclamide 10 mg/kg. the present study demonstrates that the aqueous extract of *V.rosea* exhibits promising anti diabetic activity and helps to maintain good glycemic control.

Key words: Alloxan, anti diabetic activity, Vinca, catharanthus rosea, hypoglycemic.

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Introduction

Diabetis mellitus is a heterogeneous metabolic disorder characterized by hyperglyceamia resulting from defective insulin secretion, resistance to insulin action or both[1]. Type 2 diabetes usually occur in obese individuals and is associated with hypertension and dyslipidemia. The capacity of nutrients to stimulate insulin release from the pancreatic β cells reflects their capacity to augment oxidative fluxes in the islet cells[2]. Also, oxidant stress associated with insulin resistance and non insulin dependant diabetes mellitus [3,4]contributes to poor insulin action[5,7]. Thus, the treatment aims to reduce insulin resistance and to stimulate insulin secretion. In Diabetes mellitus, oxidative stress seems mainly to be due to an increased production of free radicals and/ or a sharp reduction of antioxidant defenses[8,10][Jank et., al]found that increased oxidative stress involved in the pathogenesis and progression of diabetic tissue damage[11].

Further, there is evidence that diabetes induced changes in the activities of antioxidant enzymes in various tissues[12]. Herbal medicines have been used for the treatment of diabetic patients since long and they are currently accepted as an alternative therapy for diabetic treatment.[13-15].Oral hypoglycemic agents are useful in the treatment of diabetes mellitus but their use is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects and the world health organization expert committee on diabetes has listed as one of its recommendations that traditional methods of treatment for diabetes should be investigated.[16]

The plant *vinca rosea* (= catharanthus roseas, catharanthus, periwinkle) is a dicotyledonous plant blonging to the family apocynaceae.This plant is cultivated in south India ,USA , Europe, Australia and west India as an ornamental plant as well as for its medicinal properties. It was used as antidysentric, anti hemorrhagic ,diuretic and for wound healing in European countries .Traditionally in Brazil it was used to treat tooth ache and in Jamaica it was used to treat diabetes in the form of tea. Vinca is a erect, Pubescent herb with branched tap root. Leaves are simple, Petiolate ,Ovate,entire with acute apex and glossy appearance. The Phytochemical studies reveals the presence of about 20 dimeric indoledinydroindole alkaloids.Among them vincristine & Vinblastine are most significant .Other are ajmalicine ,Lochnerine,serpentine,tetrahydroalstonine[17].

Materials and Methods

Plant Material

The fresh leaves of *V.Rosea* were collected from Srivilliputtur,Tamilnadu,India. The plant was authenticated by Dr.Stephen ,dept of botany American college ,madurai. The dried and coarsely powdered drug (100 gm) was packed in a soxhlet apparatus and was subjected to extraction with water over 72hrs .The filtrate was evaporates under vaccum drier and brown mass residue obtained was stored at 4ºC for further use.The average yield of the aqueous extract was approximately37gm.For Experimental study ,The weighed amount of aqueous extract (400 mg/kg) was dissolved in 1% tween80 in normal saline.

Animals

The experimental protocol was approved by the Institutional Animal Ethics Committee(IAEC) of kalasalingam college of pharmacy, Srivilliputtur which is registered with CPCSEA, Government of India.(Reg No:509/01/C/CPCSEA).Albino rats of either sex,weighing between 150-200 gms were procured from the central animal House, Kalasalingam College of Pharmacy and acclimatized under standard laboratory conditions at c2ºC, 50± 15 % RH and Normal photoperiod 12:12 hr eight:dark cycle for 7 days were used Commercial pellet diet and water were provided ad libitum.

Hypoglycemic activity ,Screening in Normal rats

Normal fasted rats

Normal albino rats were first for the screening of antidiabetic activity of the aqueous extract of *V.Rosea*. Overnight fasted normal rats were randomly divided into 4 groups of 5 rats each. The group I served as control ,which received 5% Tween 80 orally, Group II received glibenclamide 10 mg/kg and 400mg/kg of aqueous extract of *V.Rosea* respectively. Blood samples were Collected from retro-orbital plexus, before and 1,2 and

4 hr after treatment fasting Blood glucose (FBG) was determined by using commercial kit .

Glucose Tolerance in normal rats[20]

For GTT, overnight fasted rats were divided into three groups of 5 rat each. Group I served as control ,which required 5 % tween 80 orally .Group II received glibenclamide 10 mg/kg orally. Group III received 400 mg /kg of aqueous extract of *V.Rosea* orally.

After 30 minutes 3 gm/kg of glucose was orally administered to each rat. Blood samples were collected from retro-orbital plexus just before drug administration and at 0.5,1.5 and 2.5 hrs(after glucose administration) for determination of glucose.

Experimental Induction of Diabetes

Diabetes was induced by single intraperitoneal injection (120 mg/kg bodyweight) of alloxan to overnight fasted rats.After injection rats had free access to food and water and were given 5 % glucose solution to drink overnight to counter hypoglycemic shock.Diabtes in rats were confirmed by moderate polydipsia and marked polyurea.After 3 days the fasting blood glucose levels were determined by ortho-toluidine method[21].The Rats showing fasting bloodglucose more than 180mg/dl were considered diabetic and selected for the experiment. 25 rats were randomly divided into 5 groups of 5 animals each and treated as follows:-

GroupI:- **Normal Control**, Group II: Diabetic Control alloxan administrated rats.Group I and II rats received (% tween 80 in normal saline orally once a day for 7 days **Group III** – Glibenclamide treated: Diabetic rats received Glibenclamide(10 mg/kg) orally for 7 Days.**Group IV** :-**V.R 400 Treated**:Diabetic rats received aqueous extract of V.Rosea(400 mg/kg) orally for 7 days.The Experiment was terminated at the end of 7 days and the animals were fasted overnight.

Biochemical Analysis

Blood Samples were collected from retro-Orbital plexus using micro –Capillary technique ,From all the groups of overnight fasted rats .Whole blood was collected for the estimation of blood glucose[22]

Statistical Analysis

Data's were expressed as mean ± SEM and subjected to statistical analysis and glibenclamide treated groups were compared with the normal group $P<0.05$ was considered as significant.

Results

Hypoglycemic activity screening in normal rats

The onset of Hypoglycemic activity of aqueous extract of *V.Rosea* at 200 mg/kg was evident between 1-2 hr , the peak was found to be at 4 hr .The rats receiving 400 mg/kg of aqueous extract of *V.Rosea* showed the onset of effect at 1 hr with a peak effect at 4 hr.The Hypoglycemic effect of aqueous extract of *V.Rosea* at 400 mg /kg was 72.46 and it was significant to that of glibenclamide 58.64.

Glucose Tolerance Test (GIT) in Normal Rats

The plasma glucose levels of the normal rats reached a peak at 30 min after the oral administration of glucose (3 gm/Kg) and gradually decreased to 132 mg/dl in 2½

Hours. The pretreatment with aqueous extract at *V.Rosea* (400 ,g/kg) and glibenclamide(10 mg/kg) elicited decreased plasma glucose level significantly(P,0.02) as compared to the normal control group. The maximum reduction for glibenclamide treated group was from 184(at 30 mts) to 97 mg/dl(at-2½ hrs) whereas for 400 mg of aqueous extract it was from 194 mg/dl (at 30 mts) to 106.88 mg/dl(at 2½hrs).

Antidiabetic activity screening in experimentally induced diabetic rats

By 7th day the blood Glucose was increased in untreated diabetic group from 204 ± 10.8 to 368 ± 10.2 mg/dl .When compared to 108 ± 6.25 mg/dl in the healthy control Supplementation of aqueous extract of *V.Rosea* for 7 days decrease the blood glucose significantly P<0.05 .In extract treated group the blood glucose decreased from an initial of 204 ± 7.28 to 169 ± 11.4 mg/dl at the end of 7 days In glibenclamide treated group there was a significant decrease in the glucose from an initial level of 205 ± 12.4 to 154 ± 11.2 mg/dl after 7 days .But in the untreated diabetic group the glucose was increased.

TABLE --1
EFFECT OF *VINCA ROSEA* [LINN] LEAVES EXTRACT ON BLOOD GLUCOSE LEVELES IN NORMAL RAT

GROUPS	FASTING	BLOOD GLUCOSE LEVEL mg/dl(Mean ± SEM		
		1	2	4
Control	91.26 ±4.85	94.62 ±6.24	92.84 ±6.53	93.38 ±6.45
Glibenclamide	96.67 ±8.21	75.64 ±5.25*	63.26 ±5.18*	58.64 ±4.21*
Test Extract 200 mg/kg	98.45 ±7.09	94.18 ±7.13**	89.72 ±6.70**	86.32 ±5.08
Test Extract 400 mg/kg	93.87 ±6.26	85.28 ±5.31*	76.65 ±6.87*	72.46 ±6.08

Data are expressed as the mean ± SEM(n=6) in each group when compared to control and treated group by Student's "t" test;*P< 0.02 as compared to control group.**Non Significant

TABLE 2
EFFECT OF *VINCA ROSEA* [LINN] LEAVES EXTRACT ON ORAL GLUCOSE TOLERANCW IN NORMAL RAT

GROUPS	FASTING	BLOOD GLUCOSE LEVEL Mg/dl(Mean ±SEM)		
		AFTER TREATMENT		
		30 mts	90 mts	150 mts
Control with Glucose	96.82±8.62	216.84±11.32	186.88±9.62	132.58±13.24
Standard with Glucose	88.59±12.81	184.23±12.94*	146.83±18.64*	97.62±13.43*
Test Extract with Glucose	102.87±3.36	194.82±12.83*	134.85±10.20*	106.88±8.34*

Data are expressed as the mean ±SEM(n=6) in each group when compared to control and treated group by student's "t" test; *P <0.02 as compared to control group.

TABLE 3
EFFECT OF *VINCA ROSEA* [LINN] LEAVES EXTRACT ON BLOOD GLUCOSE LEVELES IN ALLOXAN INDUCED DIABETIC RAT

GROUPS	FASTING	BLOOD GLUCOSE LEVEL Mg/dl(Mean ±SEM)		
		[DAYS] AFTER TREATMENT		
		1	3	7
Control	102.43± 6.24	107.81±3.45	103.61±8.13	108.25±6.25
Diabetic	206.02±10.89	207.63±7.52	207.92±9.84	209.48±8.47
Diabetic with glibenclamide 10 mg/kg	205.23±12.45	191.80±11.23*	183.84±9.65*	174.58±11.24*
Diabetic with test Extract 400 mg/kg	204.37±8.34	194.45±7.28*	173.51±12.34*	169.46±11.42*

Data are expressed as the mean ±SEM(n=6) in each group
When compared to control and treated group by student's "t" test
*P <0.05 as compared to control group.

FIG-1 EFFECT OF *VINCA ROSEA* [LINN] LEAVES EXTRACT ON BLOOD GLUCOSE LEVELES IN NORMAL RAT

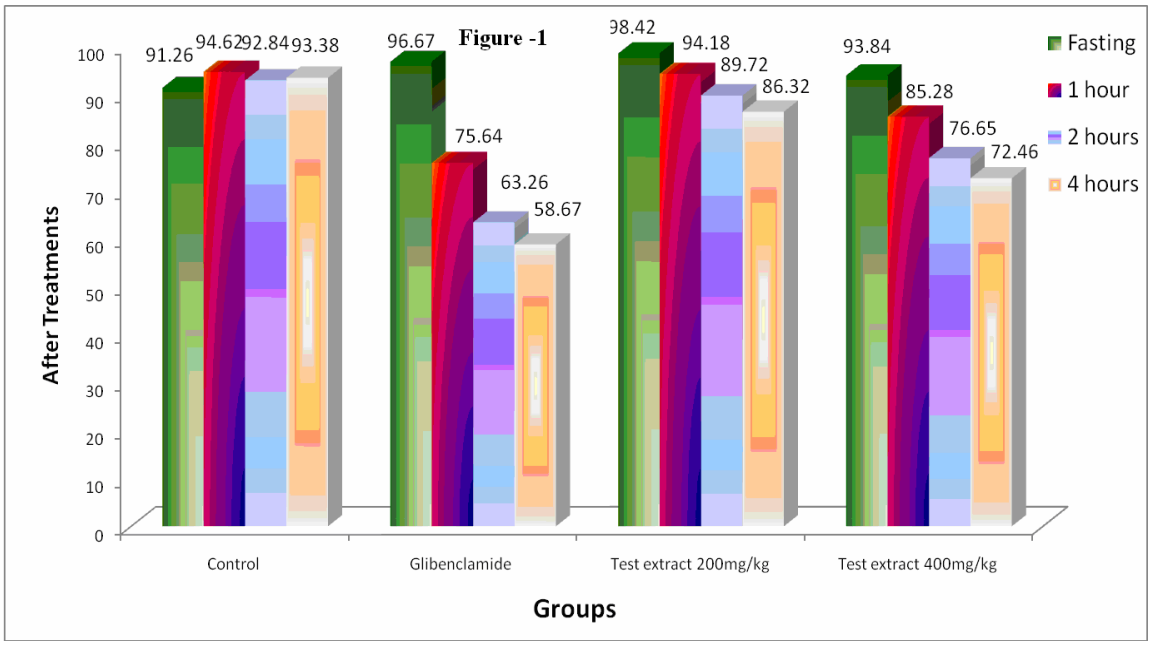


FIG-2 EFFECT OF *VINCA ROSEA* [LINN] LEAVES EXTRACT ON ORAL GLUCOSE TOLERANCW IN NORMAL RAT

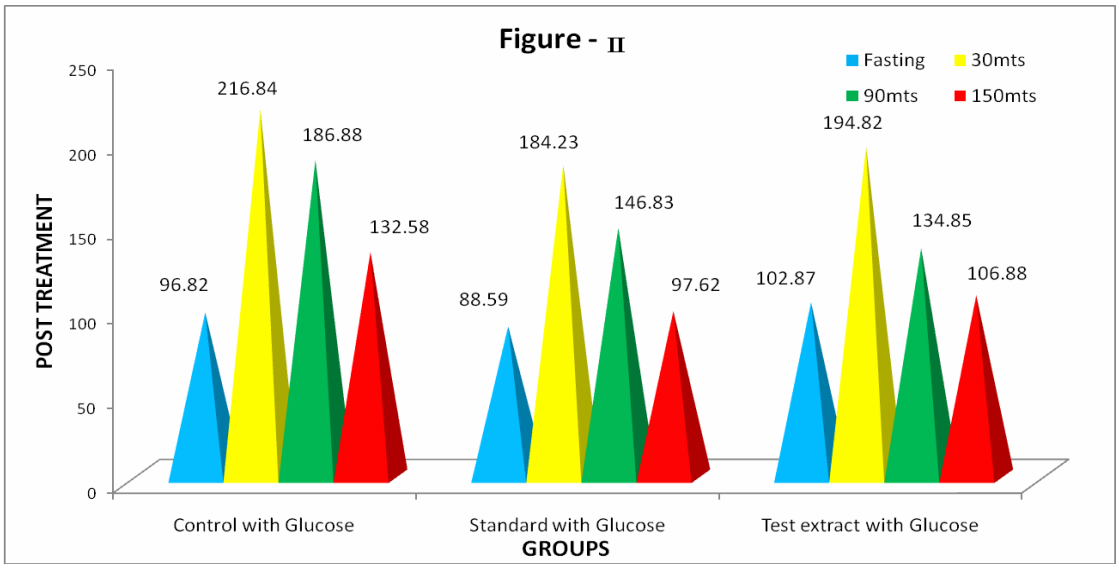
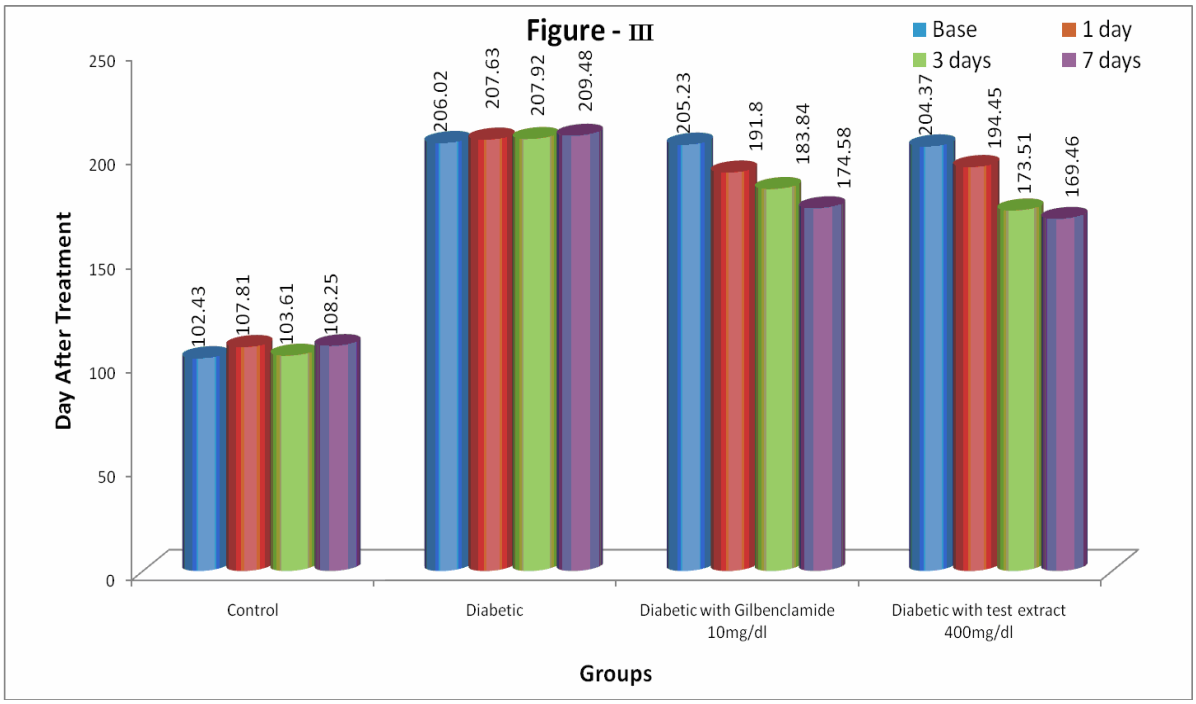


FIG-3 EFFECT OF *VINCA ROSEA* [LINN] LEAVES EXTRACT ON BLOOD GLUCOSE LEVELS IN ALLOXAN INDUCED DIABETIC RAT



Discussion

In the present Experiments, the aqueous extract like glibenclamide, showed hypoglycemic effect at 400 mg/kg in both normal and Glucose loaded normal fasted rats..It is to be studied that the extract brought about these changes by acting through pancreatic mechanism similar to that of glibenclamide[23] or by inhibition of glucose absorption through gastrointestinal tract other herbs(P.marsupium and M.Charantia) [24,25]. The hypoglycemic effect of aqueous extract of *V.Rosea* (in Normal Rats) at 400 mg/kg was 72.46 and it was comparable to that of glibenclamide 58.64.

The glucose tolerance test in normal rats showed that the pretreatment with aqueous extract of *V.Rosea* (400 mg/kg) and glibenclamide (10 mg/kg) elicited decreased plasma glucose level significantly($P<0.02$) as compared to the normal control group.

Alloxan causes diabetes through its ability to destroy the insulin producing beta cells of the pancreas [26,27].In vitro Studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to the induction of cell necrosis [28,29]. The Cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration ,leading to a rapid destruction of beta cells [30].

The 7 days treatment of alloxan diabetic rats with 400 mg/kg glibenclamide. The aqueous of *V.Rosea* was comparable to that of glibenclamide. The aqueous extract decrease the blood glucose significantly ($P<0.05$) compared to control .At the end of 7 days in extract treated group blood glucose decreased to 169 ± 11.4 mg/dl and for glibenclamide treated group it was about 154.58±11.2 mg/dl .But in untreated diabetic group the blood glucose was increased.

In conclusion, it can be stated that the aqueous extract of *V.Rosea* has a significant antihyperglycemic activity in normal as well as in alloxan induced diabetic rats. The further investigation in the isolation of active principle responsible for this activity may give a potent Drug molecule from herbal source in the treatment of Diabetes.

References

1. Gavin JR III, Albert KGMM Davdison MB et..al, Reprot of the expert committee on the diagnosis and classification of diabetes mellitus, *Diabetes care*, 20 (1997), 1183.
2. Malaisse WJ, Insulin release: The fuel concept, *Diabmetab*, 9 (1983), 313.
3. Gopaul Nk, Anggard EE, Mallet AI, Plasma 8 epi prostaglandin F 2α levels are elevated individuals with non-insulin dependent diabetes mellitus, *FEBS Lett*, 368(1995) 225.
4. Nourooz ZJ, Tajaddini SJ, McCarthy S, Elevated levels of authentic plasma hydroperoxides in NIDDM, *Diabetes*, 44 (1995) 1054.
5. Paolisso G, D'Amore A, Volpe C. Evidence for a relationship between oxidative streess and insulin action in non insulin dependent (Type II) diabetic patients,*Metabolism*,43(1994)1426.

6. Faure P , Rossini E, Lafond JL, Vitamin E improves the free radical defense system potential and insulin sensitivity of rats fed high fructose diet, *J Nutr*., 127(1997)103.
7. Reedich M. Kozlovsky N, Potashnix R, oxidant stress reduces insulin responsiveness in 373-L adipocytes, *AM J Physiol*, 272 (1997) E935.
8. Cross CE, Halliwell B, Oxygen radicals and human disease, *Ann Intern Med*, 107 (1987) 526.
9. Giugliano D, Ceriello A, Oxidative stress and diabetic vascular complications, *Diabetic care*, 19 (1996) 257.
10. Low PA, Nickander KK, The roles of oxidative stress and antioxidant treatment in *experimental diabetic neuropathy Diabetes*, 46, (1997) S 38.
11. Jang YY, Song JH, Shin YK, Protective effect of boldine on oxidative mitochondrial damage in streptozoto in-induced diabetic rats, *pharmacological Res,* 42 (2000) 361.
12. Oberley LW, Free radicals and diabetes, *Free Radical Biol Med*, 5, (1988) 113.
13. Battua GR, Mamidipalli 8N, Parimia R, Hypoglycemic and Antihyperglycemic effect of alcoholic extract of Benincasa hispida in Normal and in alloxan induced Diabetic rats, *Pharmacog Mag*, 3(2007).
14. Modak M, Dixit P, Londhe J, Indian Herbs and Herbal drugs used for the treatment of Diabetes, *J Clin Biochem Nutr*, 40(2007)163.
15. BaddeS, Patel N, Bodhankar S, Antihyperglycemic activity of aqueous extract of leaves of cocculus hirsutus (L) Diets in alloxan induced Diabetic mice, *Indian J.Pharmacol*, 38(2006) 49.
16. Naggar EM, Antidiabetic effect of Cleome droserifolia aerial parts: Lipid peroxidation-induced oxidative stress in diabetic rats, *Acta Vet, Brno*, 74(2005)347.
17. Wealth of India – Raw materials vol – II, V revised Edition, 6, 7.
18. Skim F, Kaaya A, Jaouhari JT, Hypoglycemic activity6 of Globularia alypum leaves in rats, *Fitoterapia*, 70(1999) 382.
19. Sabu MC, Kuttan R, Antidiabetic activity of Medicinal plants and its relationship with their antioxidant property, *J Ethnopharmacol*, 81(2002)155.
20. Williamson EM, Okpako DT, Diabetes mellitus in pharmacological methods in *phytotherapy research, Wiley and sons publication, England*, 1996, 158.
21. Scientific and Research Publication from Indian Drug Manufacturers Association. *Indian Drugs*, Vol.,44 No.,8 Aug2007.
22. Hyavarina A, Nikkita E, Specific determination of blood glucose with ortho-toluidine, *clin chim Acta*, 7(1962)140.
23. Best CH, Tayler NB, *Physiological basis of Medical practice 6th edn*, Williams and Williams company, Baltimore, 1959, 68.
24. Mukhtar HM, Ansari SH, Ali M, Effect of aqueous extract of pterocarpus marsupium wood on alloxan induced diabetic rats, *pharmazie*, 60(2005)478.
25. Meir P, Yaniv Z, An in vitro study of Momordica charantia on glucose uptake and glucose metabolism in rats , *plants medica*, 1(1985)12.
26. Lenzen S, Panten U. Alloxan: History and Mechanism of action, *Diabetologia*, 1988, 31, 337-42.
27. Oberley LW, Free radicals and diabetes, *Free Rad Biol Med*, 1988, 5, 113-24.

28. Jorns A, Munday R, Tiedge M, comparative toxicity of alloxan, N-alkyl alloxans, and ninhydrin to isolated pancreatic islets in vitro, *J Endocrinol*, 1997, 155, 283-93.
29. Ledoux SP, Woodley SE, Palton NJ, Mechanism of nitrosourea induced Beta cells damage alterations in DNA, *Diabetes*, 1986, 35, 866-72.
30. Szicudelsice T, The mechanism of alloxan and streptozotocin action in beta cells of the rat pancreas, *physiol Res*, 200, 50, 537-46.