British Journal of Pharmaceutical Research
4(3): 315-324, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Evaluation of Anti-diabetic Potentials of Methanol Extract of *Ficus hispida* Linn. Leaves Against Alloxan Induced Diabetic Rats

V. D. Ravichandra[1] and Padmaa M. Paarakh[2*]

[1]Department of Pharmacognosy, East West College of Pharmacy, Bangalore-91, India.
[2]Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore-68, India.

Authors' contributions

This work was carried out in collaboration between both authors VDR and PMP. Author PMP designed the study and given protocol to the author VDR. He preformed study and submitted his findings to the author PMP. Both the authors together wrote the manuscript and submitted to the journal. All authors read and approved the final manuscript.

Original Research Article

Received 7th June 2013
Accepted 19th September 2013
Published 25th November 2013

ABSTRACT

Objective: The present study was designed to evaluate the acute and chronic effects of the methanol extract of *Ficus hispida* against alloxan (150 mg/kg) induced diabetic rats.
Methods: In acute study, hypoglycemic potency of methanol extract of *Ficus hispida* was assessed by oral glucose tolerance test (OGTT) and in chronic study of 21 days, extract at different doses (ie 100, 200 and 400 mg/kg) was screened for itsanti-diabetic activity. Blood glucose level had been estimated at 0, 7th, 14th and 21st and addition to this serum concentrations of insulin, triglycerides, cholesterol, serum glutamate oxaloacetae transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and urea determined at 21st day of the study.
Results: In OGTT, standard glibenclamide and extract (200 and 400 mg/kg) have shown significant reduction in blood glucose level compared to control group. In chronic model, the methanol extract was effective in reducing the blood glucose levels ($P < 0.001$) at higher dose (200 mg/kg and 400 mg/kg) and effect was comparable to that of standard. The extract could also significantly ($P<0.001$) reduced level of SGOT, triglycerides and cholesterol in serum and significantly ($P<0.001$) increased the insulin level in blood which proves beneficial antidiabetic potentials of the extract in diabetic model. The change in concentrations of SGPT and urea were not significant ($P>0.05$).

**Corresponding author: Email: padmaparas@hotmail.com, rc.sunmoon2008@gmail.com;*

Conclusion: The methanol extract of *Ficus hispida* posses significant anti-diabetic activity in alloxan induced diabetis in rats model.

Keywords: Anti diabetic activity; Ficus hispida; alloxan; insulin; blood glucose.

1. INTRODUCTION

The Diabetes Mellitus is one of the common metabolic disorders with micro and macro vascular complications and it has become leading cause for mortality and morbidity in the world wide. The synthetic drugs used for its treatment have many serious complications. Hence always there is a scope for the development of alternative medicine for diabetes among which herbal medicine is first and foremost [1].Diabetes mellitus (Madhumeha in Sanskrit) has been known since ages and the sweetness of diabetic urine has been mentioned in Ayurveda by Sushruta. However the ayurvedic pharmacotherapy of diabetes is over 80 years old and includes use of several herbal medicines such as *Momordicacharantia*, *Aeglemarmelos* and Fenugreek seeds which have been scientifically proved [1,2].

The plant *Ficushispida* (*Moraceae)* commonly known as devil fig, hairy fig is grows in tropical and subtropical regions of India. The plant is used in traditional system, considered to be medicinally important and proved for many health benefits. A mixture of honey and the juice of these fruit is a good antihemorrhagic [3] but the barks and leaves are of particular interest from a medicinal point of view as antidiarrhoeal [4] cardioprotective[5] and hypoglycemic[6] activity among others. In Traditional Medicine, it is also used for the treatment of leukoderma, psoriasis, hemorrhoids, ulcers, jaundice, inflammations, fever and alopecia.The usefulness of this plant in diabetes is also described in Ayurveda and other folk cure books which have no scientific evidences [3].

Hence it was necessary to provide a clear background proof for the beneficial property of the plant in diabetes. In this attempt, study had been conducted to determine anti-diabetic potentials of methanol extract of *Ficushispida* leaves.

2. MATERIALS AND METHODS

2.1 Plant Material

The leaves of *Ficushispida* have been collected from Sri Venkateshwara University, Tirupati, India and dried under shade. The leaves were identified and authenticated by Dr. Madhava Chetty, Asst.Prof. Dept. of Botany and specimen herbarium (Reference No-SVU/OCT/2009/F18) were preserved at institute herbarium library. The leaves part were separated from other parts, washed, cleaned and dried for further use.

2.2 Preparation of Extract

The shade dried leaves were pulverised into powder and sieved through No. 22 mesh. About 350 g (appx.) of coarse leave powder was defatted with petroleum ether and marc left over was extracted with methanol in soxhlet's apparatus[7] and solvent was evaporated using Rota-Evaporator, dried and used for further experiments [FHME].

2.3 Preliminary Phytochemical Investigation

The preliminary phytochemical investigation for the methanol extract of *Ficushispida* was conducted as per procedure prescribed by Khandelwal[8].

2.4 Drugs and Chemicals

Glibenclamide was procured from Aventis Pharma Ltd., India.Alloxan was obtained from Sigma Laboratory, India**.** The glucometer (BIOCON) was used for the estimation of blood glucose level**.** The reagents kits for the estimation of insulin, urea, SGPT, SGOT, Triglycerides and cholesterol are purchased from SPAN Diagnostics Pvt Ltd., Bangalore. All other reagents and chemicals were obtained commercially and were of analytical grade.

2.5 Animals

Wistar male rats (180-220 g) were procured from Yash farm, Pune. The animals were housed under standard conditions of temperature (22 ± 10ºC), relative humidity (55 ± 10%), 12hr light/dark cycles and fed with standard pellet diet (Amrut, Pranav Agro Industries Ltd., Sangli, India) and water ad libitum. After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under above said environmental conditions. The experimental protocol has been approved by the Institutional Animals Ethics Committee (Ref.no.IJAHSM/IAEC/2011/012) with the permission from Committee for thePurpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

2.6 Acute Oral Toxicity Studies

The acute oral toxicity study was done according to the OECD guidelines 423 (Acute Toxic Method). A starting dose used was 2000 mg/kg body weight p.o. of extract (FHME) was administered to 6 male rats, observed for 14 days. The experiments were repeated again with the same dose level, 2000 mg/kg body weight p.o. of extracts for 3 days more, and observed for 14 days [9].

2.7 Evaluation of Anti-diabetic Activity

2.7.1 Induction of Diabetes in experimental animals

In both acute and chronic models, rats were made diabetic by a single intra peritoneal injection of alloxan monohydrate (150 mg/kg). Alloxan was first weighed individually for each animal according to the body weight and then solubilized with 0.2 ml saline (154 mMNaCl) just prior to injection. Two days after alloxan injection, rats with plasma glucose levels of >140 mg/dl were included in the study [10].

2.7.2 Oral glucose tolerance test [OGTT]

The animals were divided into six groups consisting of six animals in each and all the animals except normal (Group I) were induced diabetes by administering single dose of alloxan as explained above. At third day, the suspensions of standard drug glibenclamide (5 mg/kg) and extract were prepared using Tween 20 as suspending agent and administered to respective animals with help of oral feeding tubes according to below protocol[11].

Group I served as Normal control treated with normal saline (1 ml p.o) alone, Group II served as Diabetic control treated with alloxan and vehicle, Group III - was Standard group treatedalloxan and Glibenclamide(5 mg/kg), Group IV, V and VI were test groups treated with alloxan and 100 mg/kg, 200 mg/kg and 400 mg/kg of methanol extract of *Ficushispida* (FHME) respectively.

One hour after administration of extracts, all rats were fed with oral glucose solution (2 g/kg) and blood samples from each rat were collected at different intervals of 0 mins, 30 mins, 60 mins, 90 mins and 120 mins and estimated for blood glucose.

2.7.3 Evaluation of anti-diabetic activity by chronic study model

The animals were divided into six groups consisting of six animals and all the animals except normal (Group I) were induced diabetes by administering single dose alloxan before two days of the study as explained above. From the third day of the study, the suspensions of standard drug glibenclamide and extracts were prepared using Tween 20 as suspending agent and administered for 21 days to respective animals with help of oral feeding tubes according to below protocol.

Group I served as Normal control treated with normal saline (1 ml p.o) alone, Group II served as Diabetic control treated with alloxan and vehicle, Group III was Standard control and treated with alloxan and standard drug Glibenclamide(5 mg/kg), Group IV, V and VI were test groups treated with alloxan and 100 mg/kg, 200 mg/kg and 400 mg/kg of methanol extract of *Ficushispida*(FHME) respectively[12]. Blood samples from each rat were collected on day 1^{st}, 7^{th}, 14^{th} and 21^{st} and estimated for blood glucose. On last day of study, blood samples were also estimated for SGPT, SGOT, cholesterol, Triglycerides, urea and insulin

.

2.7.4 Collection of blood sample and estimation of parameters

Blood samples were collected from retro-orbital plexus under mild ether anesthesia from rats. The blood glucose estimation done by GOD-POD kit using UV spectrophotometer (Shimadzu 1700). On the 21^{st} day, serum was separated from blood samples and analyzed for serum cholesterol and serum triglycerides by enzymatic DHBS colorimetric method. Serum SGOT, serum SGPT, serum urea and serum insulin were estimated using standard kits.

2.8 Statistical Analysis

All the values of blood sugar level and biochemical estimations were expressed as mean ± Standard Error of Mean (S.E.M.) and analyzed for ANOVA and Post Hoc Dunnet's t-test using Graph pad Prism 5 software. Differences between groups were considered significant at $P<0.05$ levels.

3. RESULTS

3.1 Preliminary Phytochemical Study

The percentage yield of the FHME was found to be 7.37% w/w. The preliminary phytochemical investigation for the methanol extract of *Ficushispida* revealed the presence of flavonoids, tannins, poly phenols, steroids and carbohydrates in the leaves [12].

3.2 Acute Toxicity Studies

The single dose of 2000 mg kg^{-1} b.w. of FHME was safe and caused neither mortality nor any signs of clinical abnormality in the tested animals during the observation period of 14 days after administration of highest dose. There was no considerable change in body weight before and after treatment of the experiment and no signs of toxicity were observed. When the experiments were repeated again with the same dose level, 2000 mg/kg body weight p.o. of extracts for 3 days more, no changes were observed for 14 days. As per the results obtained in acute oral toxicity study, doses were selected as 100, 200 and 400 mg/kg.

3.3 Evaluation of Anti-diabetic Activity

3.3.1 Oral glucose tolerance test

In oral glucose tolerance test, animals of diabetic control group have shown significant elevation in blood glucose level through entire study when compared to normal animals. But treatment with standard drug, glibenclamide and methanol extract (100 mg/kg, 200 mg/kg and 400 mg/kg) of *Ficushispida* could able to reduce significantly (P<0.001) blood glucose level in treatment groups after 60 mins and 120 mins. The results of OGTT have shown in [Fig. 1].

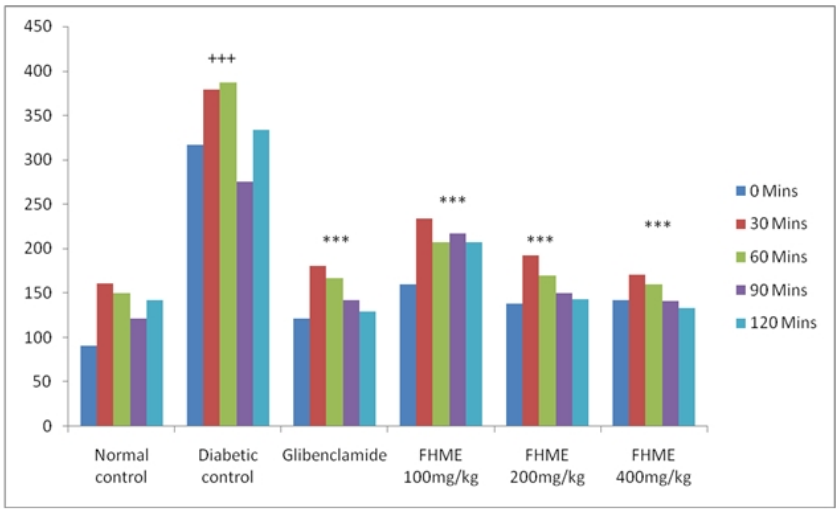

Fig. 1. Effect of methanolic extract of *Ficushispida* on oral glucose administration in rats in OGTT.

Values are mean ± S.E.M, n=6; symbols represent statistical significance.
^{ns}p>0.05, + p<0.05, $^{++}$ p<0.01, $^{+++}$p<0.001 Normal control vs Diabetic control;
*^{ns}p>0.05, * p<0.05,** p<0.01,***p<0.001 Diabetic control vs therapeutic groups*

3.3.2 Determination of chronic anti-diabetic activity

In chronic study, there was significant increase in blood glucose level (P<0.001) in diabetic control animals compared to normal animals while extract treated animals at 200 mg/kg and 400 mg/kg have shown significant (P<0.001) reduction in blood glucose levels at 14th and 21st day when compared to diabetic control animals [Table No .1].

Table 1. Effect of prolonged treatment of *Ficushispida* extract on blood glucose in alloxan induced diabetic animals

	Concentration of Blood glucose (mg/dl)			
Treatment	**Day 1**	**Day 7**	**Day 14**	**Day 21**
Normal Control	119.1± 1.533	120.7± 0.941	121.0± 1.585	122.3± 1.394
Diabetic control	334.6^{+++}± 3.773	345.8^{+++}± 4.094	362.4^{+++}± 3.198	416.8^{+++}± 4.561
Glibenclamide	370.5± 1.218	282.7± 4.229***	248.0± 2.166***	170.2± 3.932***
FHME 100mg/kg	399.1± 1.312	360.3± 3.464	348.8± 1.885	297.8± 2.560*
FHME 200mg/kg	402.1± 1.831	321.0± 2.991*	279.9± 3.300***	186.8± 2.927***
FHME 400mg/kg	392.11± 5.140	316.11± 0.835***	271.1± 0.956***	178.4 ± 1.274***

Values are mean ± S.E.M, n=6; symbols represent statistical significance.
^{ns}p>0.05, + p<0.05, $^{++}$ p<0.01, $^{+++}$p<0.001 Normal control vs Diabetic control;
*^{ns}p>0.05, * p<0.05,** p<0.01,***p<0.001 Diabetic control vs therapeutic groups*

The concentration of insulin was found to be declined significantly (P<0.001) in diabetic control animals in comparison to normal animals due to the administration of alloxan. In animals treated with glibenclamide and FHME (100 mg/kg and 200 mg/kg), there was significant (P<0.001) increasing in blood insulin concentration compared to diabetic control animals and the results were comparable to normal animals. The concentration of liver enzymes in the blood was found to be elevated in diabetic animals compared to normal animals. The increase in SGOT concentration was more significant (P<0.001) whereas increase in SGPT level was less significant (P<0.01). In animals treated with glibenclamide there was significant decrease in both SGPT and SGOT levels. The FHME at 400 mg/kg shown significant reduction in SGPT (P<0.01) and SGOT (P<0.001) level but no significant reduction observed in groups treated with FHME 100mg/kg and FHME 200mg/kg.

The concentration of serum cholesterol and triglycerides in the blood were significantly (P<0.01)increased in diabetic animals when compared to normal animals and there was reduction in the serum cholesterol and triglycerides concentration found in glibenclamide and FHME (400 mg/kg) treated animals when compared to diabetic control animals but the effect was less significant (p<0.05).

It is found that there is no significant change in blood urea (p>0.05) concentration in animals of diabetic control and other treatment groups in comparison to normal animal [Table No. 2].

Table 2. Effect of prolonged treatment of *Ficushispida* extract Insulin, lipid profile, SGPT, SGOT and urea in alloxan induced diabetic animals

Treatment	Insulin (µU/ml)	Cholesterol mg/dl	Triglycerides mg/dl	SGPT U/L	SGOT U/L	Urea Mg/dl
Normal Control	134.3± 0.945	81.32± 1.447	103.6± 1.031	72.35± 0.722	136.4± 0.568	78.18± 1.276
Diabetic control	59.67^{+++}± 0.966	104.8^{++}± 1.62	125.4^{++}± 1.324	96.98^{++}± 0.686	192.7^{+++}± 3.170	93.27± 1.060
Glibenclamide	120.4± 0.858^{***}	84.65± 0.888^{**}	104.1± 0.990^{**}	81.27± 0.681^{**}	148.9± 0.876^{***}	79.82± 0.881
FHME 100mg/kg	83.33± 0.673	95.37± 0.638	121.1± 1.007	93.20± 0.312	186.7± 0.574	85.38± 0.405
FHME 200mg/kg	104.9± 0.469^{***}	85.57± 1.387^{*}	112.3± 1.037	85.65± 1.055	165.1± 0.842	80.63± 0.910
FHME 400mg/kg	115.1± 0.822^{***}	85.36± 0.219^{*}	106.8±0.205*	83.65± 0.195^{**}	153.7± 0765^{***}	80.4± 0.272

Values are mean ± S.E.M, n=6; symbols represent statistical significance.
$^{ns}p>0.05$, + $p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ Normal control vs Diabetic control;
*$^{ns}p>0.05$, * $p<0.05$,** $p<0.01$,***$p<0.001$ Diabetic control vs therapeutic groups*

4. DISCUSSION

Diabetes mellitus is a debilitating and often life threatening disorder with increasing incidence throughout the world [13]. It is estimated that 1 in 5 may be diabetic by 2025 year[14]. Alloxan, a cyclic urea derivative, which selectively destroys insulin-producing pancreatic cells by free radical mediated damage and when administered to rodents cause an insulin-dependent diabetes mellitus. Hence it was reported as a potent diabetogenic agent [15]and has been widely used for the induction of experimental diabetes in animals.

The glucose tolerance test is a medical test in which glucose is given and blood samples taken afterward to determine how quickly it is cleared from the blood [16,17]. This test usually performs to diagnose diabetes mellitus and hence here this test had been conducted to determine acute effect of methanol extract of *Ficus hispida* in normal and diabetic rats.

In oral glucose tolerance test, induction of diabetes in positive control rats resulted in increased concentration of blood glucose due to inability of the system to utilize glucose in the absence of insulin. But in extract and glibenclamide treated animals, blood glucose level was significantly reduced than control group which clearly shows the ability of the extract to increase the utilization of the glucose by cells and tissues.

In chronic study, diabetes was induced in all animals except normal group by administering alloxan before three days of study. Hence there was significant decrease in insulin secretion in diabetic animals due to the destruction pancreatic cells which resulted in decreased utilization of glucose and hence the blood glucose level was elevated. But in groups treated with standard drug glibenclamide, FHME (200 mg/kg and 400 mg/kg), significant increase in insulin release and subsequent decrease in blood glucose concentration was found.

The diabetes mellitus is a chronic metabolic disorder and it is also associated with several secondary complications such as hyperlipidemia, atherosclerosis, hypertension, diabetic nephropathy, diabetic neuropathy and diabetic keto acidosis. Hyperlipidemia is one of such common complication of diabetes which is characterized by increase in serum total

cholesterol (TC), triglycerides (TG), LDL and VLDL. The azotemia is condition which is due to the accumulation of nitrogenous waste products like urea and creatinine in blood and usually found during diabetic nephropathy [18,19].

In our present study administration of alloxan in control animals caused elevation of serum cholesterol, triglycerides, and urea as a consequence of secondary complications of diabetes. In animals of treatment groups with FHME (200 mg/kg and 400 mg/kg) have shown significant reduction in above serum parameters.

Liver injury is also a common consequence of diabetes mellitus due to the deposition of glycogen in liver which may leads to cirrhosis, fibrosis and liver failure. Hence concentration of liver enzymes SGPT and SGOT is elevated in diabetes mellitus[20].

In our study, diabetic control animals have shown higher concentrations of SGPT and SGOT where as extract and glibenclamide treated animals shown significant reduction when compared to control group.

Although the exact mechanism of action of alloxan is not fully understood, evidences indicate that the alloxan causes pancreatic β cell damage followed by insulin deficiency and diabetes mellitus [21,22,23]. In the present study, the extract was successful to maintain the normal glucose level in the diabetic animals and increased insulin secretion.

5. CONCLUSION

The methanol extract of *Ficus hispida* posses significant anti-diabetic activity in alloxan induced diabetic animal model. But further study is required to evaluate the anti-diabetic activity of the isolated compounds from the plant extract.

ACKNOWLEDGEMENTS

The authors are thankful to The principal and management of Oxford College of Pharmacy and East West College of Pharmacy for providing facilities to conduct this research work and Ramesh C, Senior Lecturer, Department of Pharmacology for his enormous support during the research.

CONSENT

Not Applicable

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Institutional Animal Ethics committee (Ref. no. IJAHSM/IAEC/2011/012)and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Satoskar RS, Bhandarkar SD, Ainapure SS. Pharmacology and Pharmacotheraputics. Popular Prakashan: Mumbai. 1999;16,874.
2. Jaspreet V, Sivakami S, Shahini S. Antihyperglycemic effects of three extracts from Momordicacharantia. J Ethnopharmacolo. 2003;88:107-11.
3. Sergio R, Peraza Sanchez. Constituents of leaves and twigs of Ficus hispida. Planta Medica. 2002;68:186-8.
4. Subhash C, Mandal CK, Ashok Kumar. Studies on anti-diarrhoeal activity of *Ficus hispida* leaf extract in rats. Fitoterapia. 2002;73:663–7.
5. Shanmugarajan TS, Arunsunda M. Cardio protective effect of *Ficus hispida* Linn on cyclophosphamide provoked oxidative myocardial injury in a rat model. Int J Pharmacol. 2008;1:1-10.
6. Ghosh R, Sharatchandra KH, Rita S, Thokchom IS. Hypoglycemic activity of *Ficus hispida* (bark) in normal and diabetic albino rats. Indian J Pharmacol. 2004;36:222-5.
7. Kokate CK. Practical Pharmacognosy. Vallabh Prakashan: New Delhi. 1994;4:110-1.
8. Khandelwal KR, Practical Pharmacognosy-Techniques and Experiments. Pune; Nirali Prakashan; 2000.
9. OECD. Acute Oral Toxicity-Acute Oral Toxic Class Method. Guideline 423, adopted 23.03.1996. In: Eleventh Addendum to the OECD Guidelines for the Testing of Chemicals. Organisation for Economic Co-operation and Development, Paris; 2000.
10. Ajabnor MA, Tilmisany AK. Effects of *Trigone llafeonum graceum* on blood glucose levels in normal and Alloxan-diabetic mice. J. Ethnopharmacol. 1998;22:15-49.
11. Vogel G, Vogel H. Drug Discovery and Evaluation, Pharmacological assays. Methods to induce experimental *diabetes mellitus*. New York; 2002. Springer Links 2nd Edition, ISBN 3-540-42396-6;948-953.
12. Ravichandra VD, Padmaa MP. Pharmacognostic and phytochemical investigation on leaves of *Ficus hispida*. IJPPS. 2011;3(2):131-4.
13. Koteeswara Rao N, Srinivas N. Anti-diabetic and Renoprotective effects of the chloroform extracts of *Terminalia chebula* Retz., seeds in streptozotocin induced diabetic rats. Comp and Alt. Med. 2006;6-17.
14. Subramonium A, Babu V. Standardised Phytomedicines for Diabetes. In: Role of Biotechnology in Medicinal and Aromatic Plants .Irfan Ali Khan and Atiya Khanum Eds. India. 2005;180-205.
15. Dunn JS, Sheehan HL, McLetchie NG. Necrosis of langerhans produced experimentally. Lancet. 1943;1:484-7.
16. Pamela CC, Richard AH, Denise RF. Type 1 Diabetes Mellitus: Lippincott's WIliams and Wlikins, India, 1st edition. 1994;336-7.
17. Satyanarayana U, Chakrapani. Insulin, Glucose Homeostasis and Diabetes Mellitus. Fundamentals of Biochemistry. Kolkata; Third edition. 2008;678-80.
18. Harsh M. The Endocrine System: Textbook ofPathology. New Delhi, Jaypee Brothers Medical Publishers (P) Ltd: 4th ed; 2002;849-51.
19. Vinay K, Abul KA, Nelson F. Diabetes mellitus: Pathologic basis of Disease. Robbins and Cotran. 7th Ed, India. 2004;1189-226.
20. Harsh M. The Glycogen Storage Disorders: Textbook ofPathology. 5th ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd. 2005;268-71.
21. Cohen G, Heikkila KE. Generation of hydrogen peroxide, superoxide radical by 6-hydroxy dopamine, dialuric acid and related agents. J Biol and Chem. 1974;49:2447-52.

22. Okamoto H. Molecular basis of experimental diabetes: degeneration, oncogenesis and regeneration of pancreatic β-cells. Bio Essays. 1995;2:15-21.
23. Takasu N, Asawa T, Komiya I, Nagaswa Y, Yamada T. Alloxan-induced DNA strand breaks in pancreatic islets: Evidence for H_2O_2 as an intermediate. J Biol and Chem. 1991;266:2112-14.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=357&id=14&aid=2596