Int J Pharm Bio Sci 2013 Oct; 4(4): (P) 533 - 541

Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ANTIDIABETIC ACTIVITY OF AQUEOUS EXTRACT OF *PARKINSONIA ACULEATA* IN ALLOXAN INDUCED DIABETIC RATS, WITH EMPHASIS ON DIABETIC COMPLICATIONS

GHALIB I HUNDEKARI*[1], SYEDA SHAHANA[1], M.A.SHUKOOR[1] AND NAGAPPA A.N[2].

[1]Dr Rafiq Zakaria Campus,Aurangabad. Maharashtra, India.
[2]Manipal College of Pharmaceutical Sciences, Manipal, India.

ABSTRACT

The present study focused on antidiabetic effect of Aqueous leaf extract of *Parkinsonia aculeata* in alloxan induced diabetic rats using Metformin as (positive control). The parameters measured were blood glucose, hepatic glycogen, triglycerides, SGOT, SGPT, Creatinine, LDL, CRP and urea, Emphasis was on evaluating effect of Aqueous leaf extract in Diabetic Neuropathy and memory dysfunction in rats using various behavioural models. Oral administration of Aqueous extract in dose of (500 mg/kg) for 18 days exhibited a significant reduction in Bld glucose, urea, triglycerides etc as compared to negative control. Also it was found to be very effective in preventing Diabetic neuropathy and memory dysfunction.The effects of Aqueous leaf extract on the metabolic and behavioural parameters was comparable to those observed in diabetic Metformin treated group. The results suggest that *P. aculeata* can be new clinical significant choice in diabetes mellitus and its complications.

KEY WORDS: ***Parkinsonia aculeata*, Alloxan, Diabetic Neuropathy, Metformin, Blood Glucose.**

GHALIB I HUNDEKARI
Dr Rafiq Zakaria Campus,Aurangabad. Maharashtra, India

Corresponding author

INTRODUCTION

Diabetes mellitus is a complex group of disorders that disturbs the metabolism of carbohydrates, fat and protein. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin. Several drugs such as biguanides and sulfonylureas are presently available to reduce hyperglycemia in diabetes mellitus. These drugs have side effects and thus searching for a new class of compounds is essential to overcome diabetic problems[1]. Management of diabetes without any side effect is still a challenge to the medical community. There is continuous search for alternative drugs. Therefore it is prudent to look for options in herbal medicines for diabetes as well. Although, herbal medicines have long been used effectively in treating diseases in Asian communities and through out the world, the mechanism of most of the herbals used has not been defined. Many traditional plants treatments for diabetes are also used. But most of the evidence for their beneficial effects is anecdotal[2] . Despite the introduction of hypoglycemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem. Many indigenous Indian medicinal plants have been found to be useful to successfully manage diabetes. One of the great advantages of medicinal plants is that these are readily available and have no side effects. The major chronic complications associated with diabetes include retinopathy, neuropathy, nephropathy and atherosclerotic coronary artery disease and peripheral atherosclerotic vascular disease. About 85% of all diabetics develop retinopathy, 25-50% develop kidney disease, and 60-70% have mild to severe forms of nerve damage. Diabetics are also 2-4 times more likely to develop cardiovascular disease and 2-4 times more likely to suffer a stroke[3]. Metabolic diseases such as diabetes and obesity have been associated with increased vulnerability to stress[4] and cognitive dysfunction[5]. Diabetes mellitus can lead to functional and structural deficits in both the peripheral and central nervous system. The pathogenesis of these deficits is multifactor and may involve microvascular dysfunction and oxidative stress[6]. Cognitive deficits are also reported to occur in animal models of diabetes which can be prevented, but not fully reversed by insulin treatment[26].

MATERIALS AND METHODS

Plant material

Leaves of *Parkinsonia aculeata* were collected from Sholapur district in Maharashtra and authenticated by Dept of Botany, Dr Rafiq Zakaria Campus, Maulana Azad College, Aurangabad**.** India.

Extraction

Leaves of *Parkinsonia aculeata* were sun dried, coarsely powdered and extracted by macerating leaves with water for 48 h and then boiling it.

Phytochemical screening

The extract of leaves was subjected to preliminary phytochemical screening to identify the presence of various phytoconstituents present. It showed the presence of alkaloids, glycosides, saponins, flavanoids etc.

Drugs and chemicals

Parkinsonia aculeata extract, Alloxan and standard drug Metformin. All other chemicals were of analytical grade.

Animals

Albino Wistar rats weighing 150-200 gms of both sexes were used. Animals were housed in standard environmental conditions of Temperature and Humidity, with standard Pellet diet and Water *ad libitum*. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of Dr Rafiq Zakaria Campus,Y.B. Chavan College of Pharmacy. Aurangabad. (Approval number-CPCSEA/IEAC/KNCP/2011-2012/58)

Induction of Experimental diabetes

Overnight fasted experimental rats from groups 2, 3, 4, were injected with Alloxan (Sigma, USA) at a dose of 150 mg/kg body weight. The chemical was injected intraperitoneally (i.p) within 10 min after dissolving in distilled water. The rats in group I were injected with distilled

water as a vehicle control. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induce hypoglycemia. Fasting blood glucose (FBG) was estimated at the time of induction of diabetes and postprandial glucose (PPG) was checked regularly until stable hyperglycemia was achieved. After a week time for the development of diabetes, the rats with moderate diabetes having hyperglycemia (blood glucose levels of 250 mg/dl) were included in the study as stable hyperglycemic animals. Once the stable hyperglycemia was achieved, the rats belonging to group 3 were treated with an oral dose of metformin (120 mg/kg bw)[27], and Group 4 with Aqueous extract (500mg/kg), once every day for 17 consecutive days while groups 1 and 2 rats received only Distill water (as vehicle control). The animals were divided into 4 groups and each group consisted of 6 rats.

Grp1: Normal control (vehicle only)
Grp 2: Diabetic control (vehicle only)
Grp 3: Diabetic rats treated with Metformin (120 mg/kg) (standard)
Grp 4: Diabetic rats treated with aqueous *Parkinsonia aculeata* extract (500mg/kg b.w)

Biochemical Analysis

Blood glucose concentration was determined in serum by commercially available glucose kit (Accu-check). The glucose levels were expressed as mg/dl. On day 17th of treatment with *P.aculeata* extract the animals were sacrificed under ether anesthesia, blood sample was collected and Liver was isolated and preserved in 10% solution of formaldehyde. Liver glycogen was estimated as per method described by J. Van Der Vies, 1954. The Biochemical Parameters Evaluated were Bld Glucose, LDL, Triglycerides, Urea, Creatinine, CRP, Liver Glycogen, SGOT and SGPT. Behavioural Models of Diabetic Neuropathy and Learning and Memory Dysfunction were Rota Rod, Tail Flick, Immersion in Hot and Cold Water, Hot Plate, Elevated Plus Maze, Open Field Test and Object Recognition Test.

STATISTICAL ANALYSIS

All observations are given mean ± SEM and data were analysed using One way ANOVA followed by Dunnet's test.

RESULTS

Blood Glucose

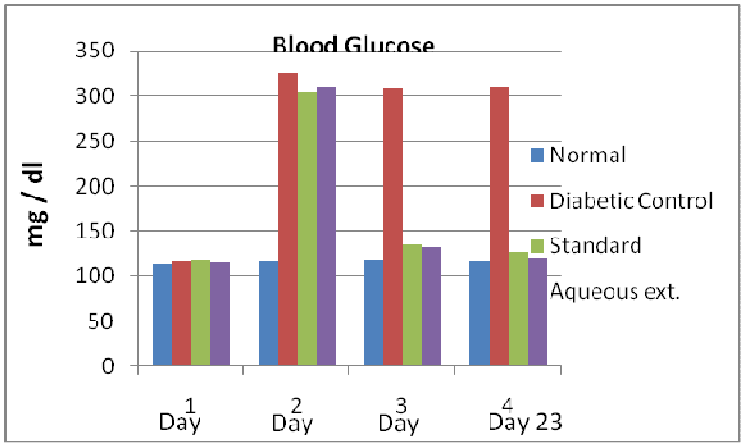

Figure 1
Effect of Aqueous PA extract on blood glucose.

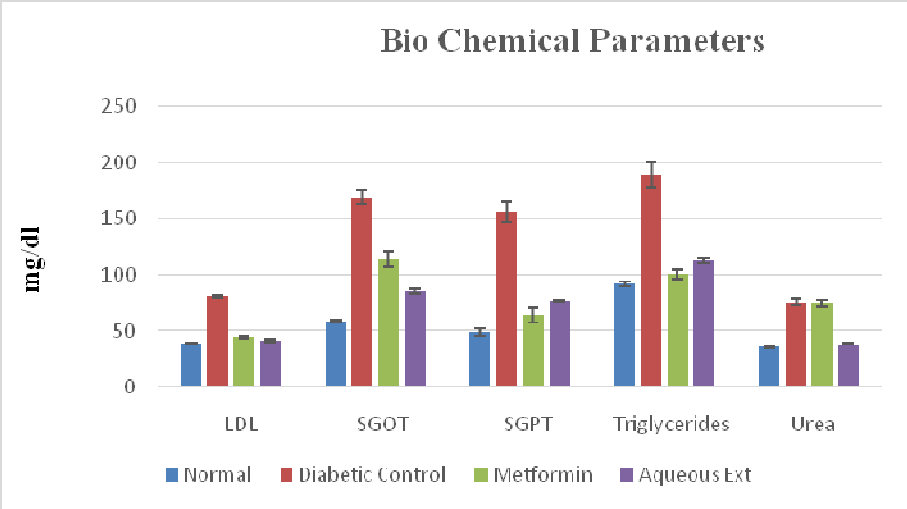

Figure 2
Effect of Aq PA extract on various biochemical parameters

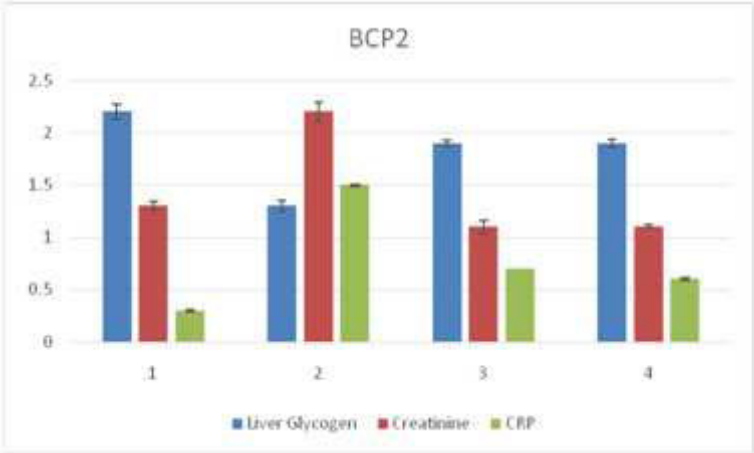

Figure 3
Effect of Aq PA extract on other biochemical parameters

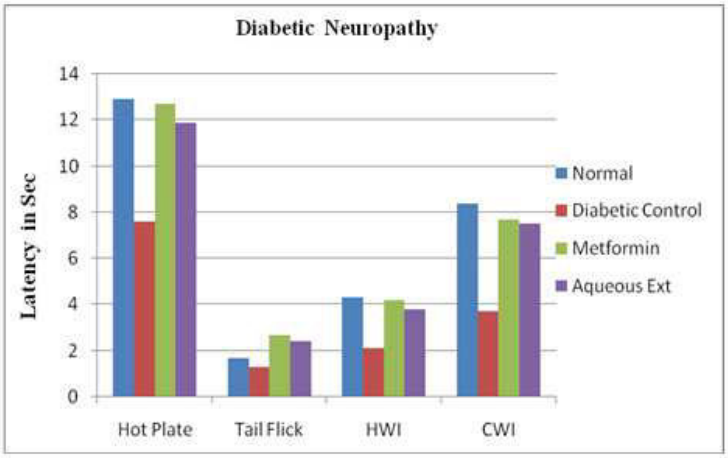

Figure 4
Diabetic Neuropathy

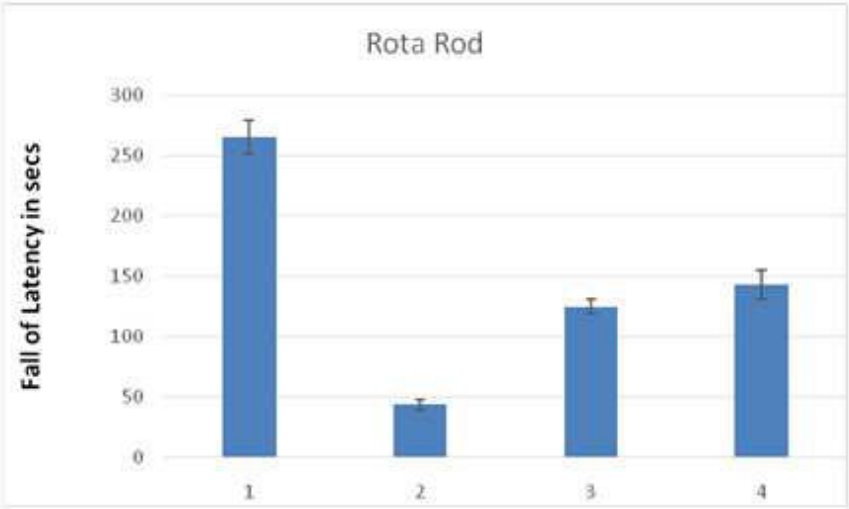

Figure 5
Rotarod

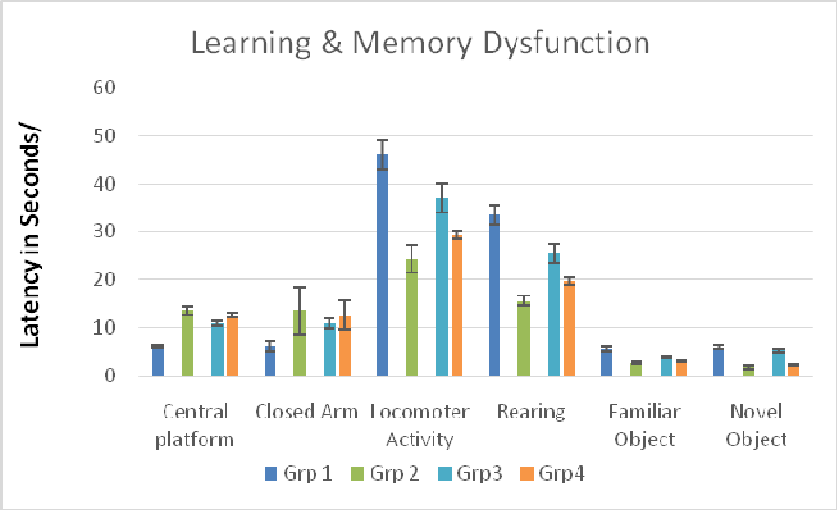

Figure 6
Learning and Memory Dysfunction

DISCUSSION

Present study was conceived with a view to provide scientific and pharmacological evidences for hypoglycemic potential of *Parkinsonia aculeata* with stress on diabetic complications. Before the advent of insulin and oral hypoglycemic agents, the major form of treatment for diabetes was plant extracts or different folk plant preparations prescribed by traditional practitioners. Multi-drug therapy used in the management of these ailments may lead to hazardous drug interactions and side effects in the long run. Three weeks treatment with Aqueous extract and Metformin lowered elevated blood glucose level, which was high in diabetic control animals. Maximum reduction in the blood glucose level was noted with Aqueous extract (Table 1).

Table 1
Effect of Aqueous PA extract on blood glucose

Animal: Rats ***Alloxan: 150mg/kg, i.p.***

Group	Treatment	Fasting blood glucose level (mg/dl)			
		Initial	1st week	2nd week	3rd week
1	Normal	113.5±2.1	116.8±1	117.5±0.9	115.6±1.7
2	Diabetic control	115.8±1.6**	325±15.5**	309±8.4**	309.6±7.7**
3	Diabetic + Metformin (120mg/kg)	117.8±2**	305±6.5**	135.5±2.1*	127.1±2.4*
4	Diabetic +Aqueous 500mg/kg	114.8±1.4**	309.5±5.5**	132.6±1.8*	119.3±1.6*

Results are expressed as Mean ± SEM (n=6).
The data was analysed using One-way Analysis of Variance (ANOVA) followed by Dunnett's - test.
****P < 0.5 vs Normal **P < 0.01 vs Normal***

Thus Aqueous extract proved significant hypoglycemic activity in diabetic rats, which was comparable to the standard drug used i.e. Metformin. The results showed that Aqueous extract and Metformin significantly decreased serum enzyme levels of SGOT, SGPT, Triglycerides, Urea, LDL and Creatinine (Table 2 & 3).

Table 2
Effect of Aq PA extract on various biochemical parameters

Group	LDL mg/dl	SGOT mg/dl	SGPT mg/dl	Triglycerides mg/dl	Urea mg/dl
1	39	58.3	49	92.1	36.5
2	80.7**	169**	155.5**	188.8**	76**
3	44.5*	114.1**	64*	100.3*	74.8**
4	41.5*	85.3**	76.8**	112.8*	38.8*

Results are expressed as Mean ± SEM (n=6).
The data was analysed using One-way Analysis of Variance (ANOVA) followed by Dunnett's- test.
****P < 0.5 vs Normal **P < 0.01 vs Normal***

Table3
Effect of Aq PA extract on other biochemical parameters

Grp	Liver Glycogen mg/2gm liver wt	Creatinine mg/dl	CRP mg/L
1	2.2	1.05	0.3
2	1.3**	2.24**	1.5**
3	1.9**	1.1*	0.71**
4	1.9*	1.1*	0.63**

Results are expressed as Mean ± SEM (n=6).
The data was analysed using One-way Analysis of Variance (ANOVA) followed by Dunnett's- test.
****P < 0.5 vs Normal **P < 0.01 vs Normal***

Plasma C-reactive protein (CRP) concentrations are increased in obese and / or hyperinsulinemic individuals. CRP concentrations are related to degree of insulin resistance / hyperinsulinemia. Pakinsonia extract was effective in reducing C reactive proteins also. Aqueous extract was more effective in reducing CRP level (Table 3). Liver Glycogen was also elevated by PA extract almost comparable to normal (Table 3). Pathophysiology of diabetes remains is complicated but free radicals play a major role in diabetic complications. Alloxan-induced diabetic model exhibits a profound hyperalgesia and motor incoordination which was evident within 2 weeks of alloxan injection and lasted for at least 4 weeks. Diabetic rats with autonomic neuropathy presented lower scores in cognitive tests of memory than non-diabetic subjects. Neuropathic pain is the most common symptom associated with diabetic neuropathy. Approximately 10% of patients with diabetes experience persistent pain from neuropathy. Diabetic rats display exaggerated hyperalgesic behavior in response to noxious stimuli that may serve as model of painful diabetic neuropathy. Alloxan injected rats had nociceptive threshold significantly lower than

non-diabetic rats as observed by tail withdrawl and paw licking time in hot plate, tail flick, hot water tail immersion, cold water tail immersion tests indicating that diabetic animals exhibit thermal hyperalgesia (Table 4).

Table 4
Diabetic Neuropathy

Group	Treatment	Hot Plate (in sec)	Tail Flick (in sec)	Hot Water Immersion (in sec)	Cold Water Immersion (in sec)	Rota Rod (in sec)
1	Normal	12.9±0.48	1.7±0.1	4.3±0.1	8.4±0.2	265±14
2	Diabetic control	7.6±0.3**	1.3±0.03**	2.1±0.11**	3.7±0.2**	44±4.4**
3	Diabetic+Metformin (120mg/kg)	12.7±0.39*	2.7±0.08**	4.2±0.13*	7.7±0.28**	125±6.5**
4	Diabetic +Aqueous 500mg/kg	11.9±0.45*	2.4±0.1**	3.8±0.11*	7.5±0.17*	143±12**

Results are expressed as Mean ± SEM (n=6).
The data was analysed using One-way Analysis of Variance (ANOVA) followed by Dunnett's- test.
****P < 0.5 vs Normal **P < 0.01 vs Normal***

In Rota rod test motor incoordination was observed in diabetic animals (Table 4) with Diabetic control Grp having lower fall of time than normal, metformin and extract treated rats. Treatment with *Pakinsonia aculeata* extracts restored blood glucose, along with increasing pain threshold and preventing motor in coordination in diabetic rats. Hyperglycemia effects learning and memory also (Tab 5). Antioxidant effect of *Pakinsonia aculeata* helps reduce the oxidative stress caused by free radicals formed as a result of various metabolic processes in the body and also prevents atherosclerosis, or plaque formation in the blood vessels. Open field test observations indicated that the impaired performance of diabetic rats is related to cognitive dysfunction rather that thygmotaxic behavior, since the number of locomotor counts and rearing of diabetic rats were decreased to non-diabetic rats. The EPM test is a simple method for the evaluation of learning and memory process. Since the animals are able to remember the configuration of the open and enclosed arms, they escape from the unsafe open arm more rapidly on the second trial. It is possible to evaluate the fear motivated learning, which underlies the transfer latency procedure in this test. Shortened transfer latency on third day's trial in rats is used as a parameter for retention or consolidation of memory. Transfer latency of *Pakinsonia aculeata* treated rats was less comparable to normal rats showing that they are effective in memory dysfunction also (Table 5).

Table 5
Learning and Memory Dysfunction

	Transfer latency		Locomotor Activity		Exploration	
Group	(EPM) (in Sec)		(Open Field) (in Sec)		(Object Recogn)(in Sec)	
	Central platform	Closed Arm	Locomoter Activity	Rearing	Familiar Object	Novel Object
1	5.98	5.98	46.1	33.5	5.5	5.9
2	13.5**	13.5**	24.3**	15.5**	2.5**	1.7**
3	10.9**	10.9*	37*	25.5*	3.8**	5.2*
4	12.5**	12.5**	29.3**	19.6**	3**	2.1**

Results are expressed as Mean ± SEM (n=6).
The data was analysed using One-way Analysis of Variance (ANOVA) followed by Dunnett's- test.
****P < 0.5 vs Normal **P < 0.01 vs Normal.***

In an object recognition test exploration of the animal to the novel object and familiar object is similar indicating memory impairment in diabetic rats. The current study explored the findings demonstrating that diabetes reduced learning and memory performance whereas Aqueous extract was very effective in showing positive effect in object recognition test shown by increased exploration of novel object than familiar object. Oxidative damage to the synapses in the rats cerebral cortex and hippocampus is reported to contribute to the deficit of cognitive functions. Therefore, anti-oxidants might be of use in the prevention of

the neurodegeneration and cognitive dysfunctions associated with diabetes. *Pakinsonia aculeata* was effective in Diabetic neuropathy and memory Dysfunction maybe due to its antioxidant activity. Therefore, the reversal of hyperalgesia was also seen.

CONCLUSION

Extract of *Pakinsonia aculeata* were found to be very effective in Diabetes and Diabetic complications with slight difference in various parameters being evaluated compared to standard drug Metformin. However the mechanism at molecular level is yet to be understood particularly in diabetic complications and need further investigation.

REFERENCES

1. Ayesha Noor, S Gunashekaran, A Soosai, M.A.Vijaylaxmi. Antidiabetic cativity of aloe Vera and Histology of organs in STZ induced diabetic rats, Current Science Vol 94, No 8 2008.
2. Bailay CJ, Day C. Traditional Plant treatments for diabetes Mellitus: Pharmaceutical Foods, British Journal of Nutrition (1998), 80, 5–6.
3. American Diabetes Association (1997).
4. Débora Cristina Damasceno, Gustavo Tadeu Volpato, Iracema de Mattos Paranhos Calderon, Marilza Vieira Cunha Rudge. Oxidative stress and diabetes in pregnant rats. Animal Reproduction Science, Volume 72, Issues 3–4, 15 August 2002, Pages 235–244.
5. Nazer J, Ramírez R. Congenital malformations in the offspring of diabetic mothers. Rev Med Chil. 2000 Sep; 128(9): 1045-52.
6. Chandrashekhar S. Patil, Vijay Pal Singh, Shrinivas K. Kulkarni. Modulatory effect of sildenafil in diabetes and electroconvulsive shock-induced cognitive dysfunction in rats. Pharmacological reports 2006, 58.373-380.
7. Anurag Kuhad, Kanwaljit Chopra . Effect of sesamol on diabetes-associated cognitive. Experimental Brain Research , March 2008, Volume 185, Issue 3, pp 411-420.
8. Amol Kumar K Hule, Abhishek S Shah, Manoj N Gambhire, Archana R Juvekar, An evaluation of the antidiabetic effects of *Elaeocarpus ganitrus* in experimental animals, Indian journal of Pharmacology, Vol.43, No. 1 Jan – feb, 2011,pp. 56-59.
9. Development and Updation of the Diabetes Atlas of India**,** S. Sandeep, Mr. A. Ganesan, V. Mohan Madras Diabetes Research Foundation, Chennai.
10. Wadkar A, Magdum C.S, Patil S.S and Naikwade N.S. Anti-Diabetic Potential And Indian Medicinal Plants, Journal of Herbal Medicine and Toxicology 2 (1) 45-50 (2008).
11. Peggy Vaughn. Stress hormone impacts memory, learning in diabetic rodents February 17, 2008 301-496-1752.
12. Shari S. Bassuk, Nader Rifai, and Paul M. High-Sensitivity C-Reactive Protein: Clinical Importance Curr Probl Cardiol 2004; 29: 439-493.
13. Arshag D Mooradian.Dyslipidemia in type 2 diabetes mellitus, Nature Clinical Practice Endocrinology & Metabolism (2009) 5, 150-159.
14. G Subbulakshmi, Mridula Naik. Indigenous foods in the treatment of diabetes mellitus Department of Post Graduate Studies and Research in Home Science, SNDT Women's University, Juhu, Mumbai - 400 049. Review
15. Ahren, B., Sundkvist, G. (1995) long term effects of alloxan in mice. Int. J. Pancreatol. 17(2), 197-201.
16. Kelli A. S, Hayes J. M, Wiggin T. D, Backus C, Sang S. O, Lentz S. I, Brosius F and Feldmana E. L. (2007) Mouse models of diabetic neuropathy, Neurobiology of Disease*,* 28, 276–285.
17. Kirtikar R and Basu B.D. Cucurbitaceae. Indian Mediccinal Plants. 2nd edition. Allahabad: Lalit Mohan Basu Publication; 1935, 1137.

18. Muranyi M, Fujioka M, He Q P. *(*2003) Diabetes activates cell death pathway after transient focal cerebral ischemia. J. Diabetes, 52 (2), 481- 6.
19. Sabu M.C and Kuttan R. (2002) Antidiabetic activity of medicinal plants and its relationship with their antioxidant property. J Ethnopharmacol. 81(2), 155-60.
20. Somani R.S and Shaikh A.S. (2010) Animal models and biomarkers of neuropathy in diabetic rodents. Indian J Pharmacol. 42,129-34.
21. Szkudelski T. (2001) The mechanism of alloxan and Streptozotocin action in β-cells of the pancreas. Physiol. Res. 50,536-546.
22. Tiwari A.K and Rao J.M. Diabetes mellitus and multiple therapeutics approaches of phytochemical present status and future prospects. Curr. Sci. 83(1), 30-38.
23. Viswanathan V.(1999) Type 2 diabetes and diabetic nephropathy in India—magnitude of the problem**.** ephrol. Dial. Transplant*,* 14 (12),2805-2807.
24. World Health Organization. (1999) Defination, diagnosis & classification of diabetes mellitus and its complications-repoirt of a WHO consulation. Geneva, 9 & 25-34.
25. Vogel G.H., (2001) Drug discovery and evaluation-Pharmacological assays. second ed., Berlin: Spinger-Verlag, 1095-1125.
26. Anurag Kuhad, Kanwaljit Chopra. Effect of sesamol on diabetes-associated cognitive experimental Brain Research, March 2008, Volume 185, Issue 3, pp 411-420.
27. Amol Kumar K Hule, Abhishek S Shah, Manoj N Gambhire, Archana R Juvekar, An evaluation of the antidiabetic effects of *Elaeocarpus ganitrus* in experimental animals, Indian journal of Pharmacology, Vol.43, No. 1 Jan – feb, 2011,pp. 56-59.
28. V Varun Raj, L Srikanth, L Venkateshwarlu. In-Vivo Animal Models For Screening of Anti-Diabetic Activity International Journal of Pharma and Bio Sciences. Volume 1, Issue 4, 2010. 386-393.
29. Nagendra Kumar, Kshitish Kumar Kshitiz, Surajeet Kumar Patra. Evaluation of Role of Oral Anti-Diabetic Drugs on Lipid Profile In Type 2 Diabetes Mellitus Patients. International Journal of Pharma and Bio Sciences. Volume 1, Issue 4, 2010. 721-733.