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The Antidiabetic Efficacy of *Azadirachta indica* leaves and seeds in Alloxan induced diabetic Albino mice

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ABSTRACT:

Diabetes mellitus is a metabolic disorder in endocrine system. In order to management of diabetic complications different part of Azadirachta indica alcoholic extraction was used in Alloxan induced diabetic mice. It was observed that treatment of the diabetic mice with ethanolic extract of leaves and seeds of A.indica at a dose of 500 mg/kg body weight and 200 mg/kg body weight for 28 days. It was observed that blood glucose level total cholesterol, triacylglycerol, ALP, SGPT and SGOT were gradually decreased after treatment with leaves and seed extract (significant fall ($P<0.05$). The data suggests that seeds of A.indica could be of more useful in diabetes mellitus in controlling the blood sugar or may also be helpful in preventing or delaying the onset of the disease.

Key words: Azadirachta.indica, Diabetes mellitus, Alloxan, Antidiabetic

INTRODUCTIONS:

Diabetes is a chronic disorder disease. It may be caused by either produces little or cease insulin production, or becomes progressively resistant to its action [Ranjan C et al., 2002]. Such types of disorder becomes in metabolism of carbohydrates, proteins and fat due to absolute or relative deficiency of insulin secretion with/without varying degree of insulin resistance [Barar FSK 2000; and Devlin MT 1997]. Now it becomes an epidemic with a worldwide incidence of 5% in the general population. The number of adult diabetes person in the world will rise from 135 million in 1995 to 300 million in the year 2025 [Torban H. 2002]. The countries with the largest number of diabetic people in the year 2025 will be India, China and United States [Ramchandran A. 2002]. There are more than 30 million people with diabetes mellitus in India and the incidence is increasing [Shankar P *et al,.* 2001] but many patients in the community are undiagnosed. Main disabler and killer in the next coming 25 years will be due to diabetes [Edvin *et al.,* 2006]. Patients with diabetes experience significant morbidity, mortality from microvascular (Retinopathy, neuropathy, nephropathy)

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and macrovascular complications (heart attack, stroke and peripheral vascular disease). The complications are far less common and less severe in people who have well-controlled blood sugar levels [Andrew, 2000]. Acute complications include diabetic ketoacidosis, nonketotic hyperosmolar coma and diabetic coma. In case of chronic complication, chronic elevation of blood glucose level leads to damage to blood vessels. In diabetes, the resultant problems are grouped under "microvascular disease" (due to damage to small blood vessels) and "macrovascular disease" (due to damage to the arteries) [Andrew,2000]. Microvascular disease leads to retinopathy, neuropathy and nephropathy (nephropathy leads to anaemia) [Halder *et al.,* 2003, Merlin *et al.,* 2005]. Macrovascular disease leads to cardiovascular disease, mainly by accelerating atherosclerosis. These disorders include: (1) Coronary artery disease, leading to myocardial infarction (heart attack) or angina (2) Stroke (mainly ischemic type) (3) Peripheral vascular disease, which contributes to intermittent claudication (exertion-related foot pain) as well as diabetic foot [Andrew, 2000].

In the despite of great efforts that have been made in the understanding and management of diabetes and disease related complications are increasing unabated. Diabetes mellitus represents a heterogeneous group of disorders causing hyperglycemia, which is due to impaired carbohydrate "glucose" utilization resulting from a defective or deficient insulin secretary response. Along with hyperglycemia, there are also abnormalities in serum lipids [Reaven, 1988]. The disease causes morbidity and long-term complications and an important risk factor for cardiovascular diseases (G.Y Yeh *et al.,* 2003). Now a days, there are different groups of oral hypoglycemic drugs and insulin for clinical use, having characteristic profiles of side effects. Management of diabetes without any side effects is still a challenge to the modern medical system. This leads to increasing the demand for complementary and alternative medicine with antidiabetic activity to overcome the side effects and toxicity of synthetic drugs. There are many reports of herbal extracts being used in Ayurvedic literature as antidiabetic which are directly or indirectly used for the preparation of many modern drugs. However, these plants have not gained much importance as medicines and one of the factors is lack of specific standards being prescribed for herbal medicines and supportive animal/clinical trials. The problem becomes complicated when several active compounds are in involved in the medicinal action. Neem (Azadirachta indiaca) has also important medicinal properties and biological activities (Biswas. 2002; Kale, 2003). The efficacy of an herbal drug, to control hyperglycemia, is

depends on their active constituents. This work was designed to investigate the antidiabetic efficacy of Azadirachta indica leaf and seed extract in diabetic Swiss albino mice.

MATERIALS AND METHODS:

Plant: - Fresh matured leaves and seeds of A. indica were collected from botanical garden and were taxonomically identified by Botany Department of Patna University, Bihar, India.

Preparation of Plant Extracts:

One kilogram air-dried fresh mature leaves and seeds of neem (Azadirachta indica) plants were taken, ethanolic extract was prepared at room temperature by dipping it into 95% ethanol for 48 hrs and percolate. The extract of leaves and seeds was concentrated under reduced pressure (bath temperature 450-50°C) and finally dried in a vacuum desiccator. LD50 was calculated and administered dose 500 mg/kg b.w. and 200 mg/kg b.w were prepared with suitable diluting distilled water.

ANIMALS:

The experiments were carried out with 25 to 30 gms male Swiss albino mice. Animals were housed in cage at a temperature of 25°C + 2°C with 12-12 hrs dark light cycle. Food and water were provided ad libitum (prepared mixed formulated feed by the laboratory itself).This project was approved by the DRC, Department of Biochemistry, Patna University, Patna.

INDUCTION OF DIABETES:

Alloxan (150mg/kg body wt.) [Szkudelski, 2001] was prepared in distilled water and administered into experimental Swiss albino mice by intra peritoneal (IP) injection of three times at the interval of 72 hrs. After 48 hrs of last shots of alloxan injection, diabetes was confirmed by testing blood sugar level, with the help of glucometer (Lever check Pvt. Ltd.) and chemical method. Animals have more than 200 mg/dl blood sugar level was selected for the further study and maintained till 4 days in diabetic condition for well establishment.

Experimental design: - Swiss albino mice were divided into four groups:-

Group 1- Healthy normal animals.

Group II- Untreated diabetes induced animals.

Group III- Diabetes animals treated with ethanolic extract of A.indica leaves

Group-IV- Diabetes animal treated with ethanolic extract of A.indica seeds

500 mg/kg body weight of ethanolic extract of *A.indica* leaves and 200 mg/kg b.w of *A.indica* seeds was orally administered per day for successive 28 days. Whole blood was collected from retro orbital vein puncture (Madway *et al.,* 1969) into sodium fluoride treated tubes and for other biochemical test; blood was collected in plane tubes. Plasma / Serum were separated by centrifugation.

Assay :

Serum glucose, Total cholesterol, Triacylglycerol, ALP, SGPT and SGOT were measured by standard kits.

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Statistical Data :

Comparison between control and drug treated groups were analysed by Graph Pad Prizm 5.04 software with one way ANOVA. The results were expressed as mean ± Standard Error of Mean (S.E.M), n = 10. P-Values < 0.001 were considered to be statistically significant.

Result and discussion:

Table 1 presents data of various biochemical parameters of peripheral blood serum of different groups of Swiss albino mice. Group I healthy Normal animals, Group II Untreated diabetes induced animals, Group III Diabetes animals treated with ethanolic extract of *A.indica* leaves, Group-IV- Diabetes animal treated with ethanolic extract of *A.indica* seeds. Experimental results showed a significant increase in blood glucose level in the alloxan treated diabetic group by 226 %, (Table -1), compared to that of normal control. While the oral administration of ethanolic extract of A.indica leaves and seeds significantly decreased by 41.83 %, and 48.7%, respectively as compared with the diabetic control mice. This finding run parallel with that obtained by Sonia Bajaj *et al.,* (1999), Halim (2003), Akbar Waheed *et al.,* (2006), Hamdy *et al.,* (2008) and Atangwho *et al.,* (2009). The exact mechanism in reducing blood glucose level is not well understood.

Table 1:Effect of ethanolic extract of leaves and seeds of A.indica on the blood glucose and some other biochemical assay.

Group	Blood Glucose (mg/dl)	Cholesterol (mg/dl)	Triacylglycerol (mg/dl)	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)
Normal Control	120.2±1.8	71.8±3.1	96.1±4.7	18.8 ± 1.3	35.1±1.7	32.3 ± 2.5
Diabetic Control	392.8±23.7	150.2±8.2	214.5± 8.2	42 ±2.1	56.9±2.2	71.9 ± 1.9
Diabetic+ *A. indica* leaves	228.5±12.3	106.4±2.5	170.2±9.8	24.2 ±1.7	32.4±3.1	37.5 ±3.0
Diabetic + *A. indica* seed	201.5 ± 10	111.4±3	151.6±5.0	23.1±1.5	28.5 ± 3.0	40.2±3.0

Mean + SEM, n=10 , P< 0.01 in student's test

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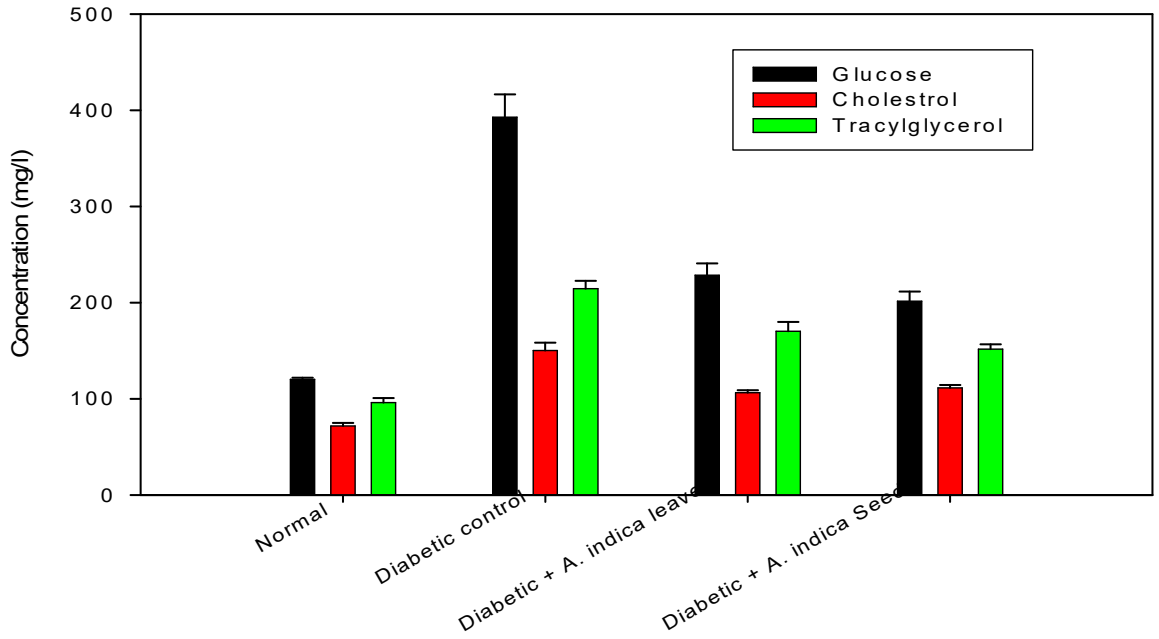

Fig 1 Different group of Swice albino mice

Fig.1. Effect of aqueous extract of leaves and seeds of *A.indica* on the blood glucose, cholesterol and Triacylglycerol assay.

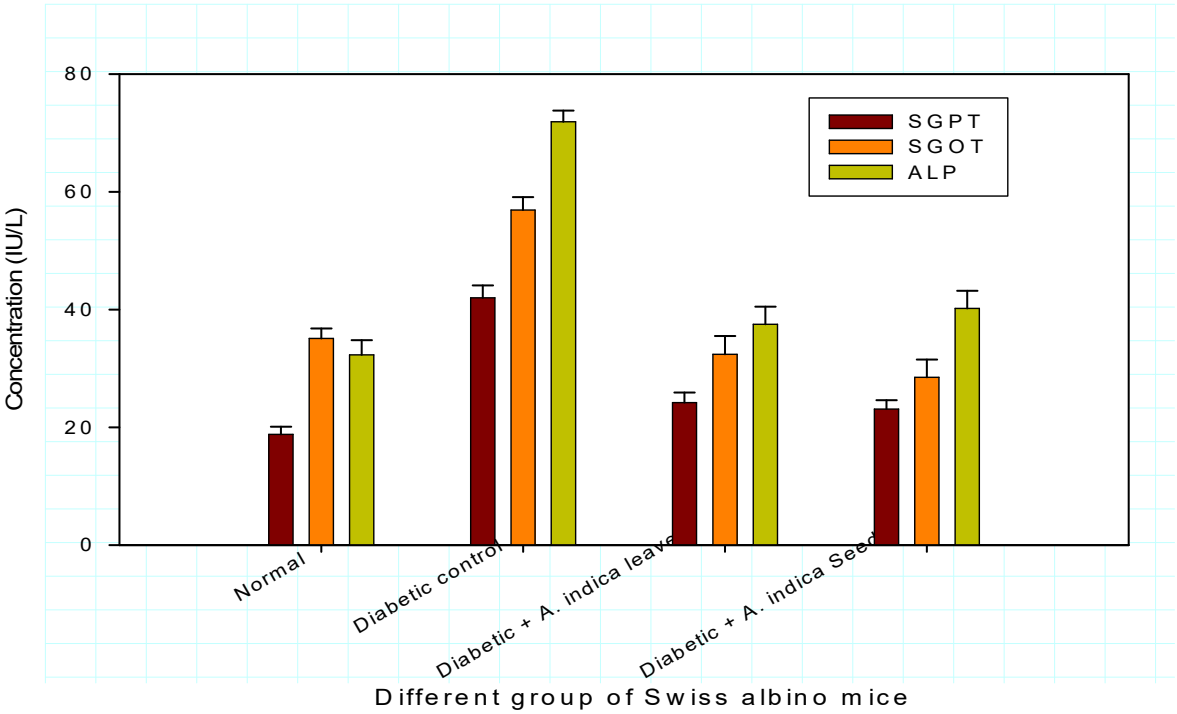

Fig. 2 Effect of ethanolic extract of leaves and seeds of A.indica on the blood

Moreover, our study was run to evaluate the effect of ethanolic extract of A.indica on lipid profile and liver function in the alloxan diabetic mice. In Alloxan-induced diabetes mellitus, the rise in blood glucose is accompanied by an increase in total cholesterol, triacylglycerol, ALP, SGPT and SGOT level. The results are agree with earlier data (Sharma *et al.,* 1996; Wasan *et al.,* 1998; Roy *et al.,* 1998; Defronzo 1999; Vuksan *et al.,* 2000; Pushparaj *et al.,* 2000). The present study revealed that the treatment of diabetic mice with *A.indica* ethanolic extract of leaves and seeds for a 28 days period caused a significant reduction in total cholesterol and triacylglycerol is (29.16%), and (20.64%) & 25.6% and 24.6% respectively in fig.1. Similarly upon treatment with the extracts of *A. indica* leaves and seeds, the activities of SGPT, SGOT and ALP are reduced significantly by SGPT (42.44%), SGOT (43.06%) and ALP (47.84%) & SGPT (45%), SGOT (49.9%) and ALP (44.0%) respectively was shown in fig.2. The mechanism of reducing blood glucose might be due to increased uptake of glucose peripherally, increased sensitivity of insulin receptor or blocking the action of epinephrine on glycogenolysis (Chattopadhyay 1996, 2005).

CONCLUSION:

By the present investigation ethanolic extract of leaves and seeds of A.indica has properties to reduced blood glucose level as well as lipid profile & liver function test (SGPT and SGOT). Although the ethanolic extract of leaves and seeds of A.indica chemical compounds for the hyperglycaemic effects still remain speculative, experimental evidence obtained in the present laboratory animal study indicates that *A.indica* leaves and seeds extract possesses antidiabetic property. The data suggests that seeds of *A.indica* could be of more useful in preventing in controlling the blood sugar. Future research to refine the extraction procedure of *A.indica* leaves and seeds can lead to improve and more experiments is needed for knowing mechanism of action of these plant extract. This plant leaves and seed may be help full in the controlling of diabetes and its complications.

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REFERENCES:

1. Barar FSK. Essentials of Pharmacotherapeutics. 3rd ed. S.Chand and Company Ltd: New Delhi; 2000.
2. Devlin MT. Text book of Bio Chemistry. 4th edn. Wileyliss Inc: NewYork; 1997.

3. Ranjan C, Ramanujam R. Diabetes and insulin resistance associated disorders: Disease and the therapy. Curr Sci. 2002;83:1533-38.
4. Torben H. Genetics of Type 2 diabetes. *Curr Sci* 2002;83:1477-82.
5. Ramachandran A, Snehalatha C, Vijay V. Burden of type 2 diabetes and its complications – The Indian scenario. Curr Sci 2002;83:1471-76.
6. Shankar P, Sundarka MK. Management of Type 2 Diabetes: Evidence Based Approach. J Indian Acad Clin Med 2001;2:244- 50.
7. Edwin E, Sheeja E, Gupta VB, Jain DC. Fight Diabetes the herbal way. Express Pharma review 2006;1:41-2.
8. Andrew JK. Diabetes. Churchill living stone: New York; 2000.
9. Halder N, Joshi S, Gupta SK. Lens aldose reductase inhibiting potential of some indigenous plants. J Ethnopharmacol 2003;86:113–6.
10. Merlin T, Con T, Richard M, George J. Anaemia in Diabetes: An Emerging Complication of Microvascular Disease. Curr Diab Rev 2005;1:107-26.
11. Alam K, Mahpara S. Role of Diet, Nutrients, Spices and Natural Products in Diabetes Mellitus. Pakistan J Nutr 2003;2:1-12.
12. Lokesh D, Amit SD. Diabetes mellitus- its possible pharmacological evaluation techniquesand naturotherapy. Int J Green Pharm 2006; 1:15-28.
13. Akbar Waheed, Ga Miana and Si Ahmad. Clinical investigation of hypoglycaemic effect of seeds of Azadirachta indica in Type -2 (NIDDM) diabetes mellitus. *Pak. J. Pharm. Sci.* Vol. 2006; 19(4), 322-325.
14. Biswas K.; Chattepadhya, I.; Banergee, R. K.; BandyopadhyayiU. Biological activities and medicinal properties of neem (Azadirachta indica). Current Science, 2002; 82(II), 1336 –1345.
15. Chattopadhyay, R.R., Possible mechanism of antibyperglycemic effect of Azadirachta indica leaf extract, Part: IV. General Pharmacology (USA) 1996; 27 (3), 431–434.
16. DeFronzo RA. Pharmacologic therapy for type 2 diabetes mellitus. Ann Intern Med 1999; 131:281-303.
17. Halim, E. M. Lowering of blood sugar by water extract of Azadirachta indica and Abroma augusta in diabetes rats. Indian. J. Exp. Biol., 2003; 41(6):636-640.
18. Hamdy M. A. Hassanein and Hanan A. Okail. Toxicity determination and hypoglycaemic effect of neem biopesticide on the grass carp “Ctenopharyngodon idella”. Egypt. Acad. J. biolog. Sci., 2008; 1 (2) 37 - 49

19. Item Justin Atangwho, Patrick E Ebong, Godwin E. Egbung and Ime F. Ani. Effects of Co-administration of Extracts of Vernonia Amygdalina and Azadirachta indica on Serum Electrolyte Profile of Diabetic and non-diabetic Rats. Australian Journal of Basic and Applied Sciences , 2009; 3(3): 2974-2978.
20. Kale, B.P., M.A. Kothekar, H.P.Tayode, J.B.Jaju and M. Mateenuddin, Effect of aqueous extracts of Azadirachta indica leaves on hepatotoxicity induced by antitubercular drug in rats. Ind.J.Pharm, 2003; 35: 177-180.
21. Madway *et al.,* W. Madway, I.E. Prier and J.S. Wilkinson, A Text Book of Veterinary Clinical Pathology, The Williams and Wilkins Co., Baltimore (1969).
22. Pushparaj, P., c.h. Tan and B.K.H. Tan. Effect of Averrhoe bilimli leaf extract on Blood gulcose and lipids in streptozotocin diabetic rats. J.Ethnopharmacol. 2000; 72:69-76.
23. Reaven, G.M. Reaven, Role of insulin resistance in human disease, Diabetes 37 (1988), 1597–1607.
24. Roy, O.; Perrault,M. and Mareffe, A. Insulin stimulation of glucose uptake in skeletal muscle and adipose tissue in vivo is no dependent. Am. J. Physiol. 1998; 274: E 692: E 699.
25. R. R. Chattopadhyay and M. Banyopadhyay (2005). Effect of Azadirachta indica leaf extract on serum lipid profile changes in normal and sreptozotocin induced diabetic rats. African Journal of Biomedical Research, Vol.8 No.2, 101-104.
26. Szkudelski, T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol. Res., 2001; 50: 536-546.
27. Sonia Bajaj, *et al.* Investigations into the anti-diabetic activity of Azadirachta indica. Indian J. of Pharmacology; 1999; 31: 138-141.

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