Original Research Article Website : www.ijbasr.org ISSN : 2349-1965
International Journal of Basic & Applied Science Research
[2014; 1 (1): pp 28-34]

HEPATOPROTECTIVE ACTIVITY OF *AZADIRACHTA INDICA* LEAVES ON ALLOXAN INDUCED DIABETIC SWISS ALBINO MICE

Kumari R.[1]*, Prakash R.[1], Suman P. K.[2] and Padmadeo S. R.[1]
[1]Department of Biochemistry, Patna University, Patna, Bihar
[2]C. R. P. F., Jhargram, West Bengal

ABSTRACT :

Insulin-dependent and non-insulin dependent diabetes is a common and serious metabolic disorder throughout the world. These conditions lead to excess glucose in the blood due to production of insufficient amount of insulin or unable to use of insulin in the body cells. In the present study diabetes was induced by intra peritoneal administration of Alloxan (150mg/kg b.w) and ethanolic extract of *Azadirachta indica* leaves (500mg/kg bw) was orally administered for 28 days. Blood Glucose tests as well as SGPT, SGOT, ALP and Bilirubin levels were estimated. It was analysed that serum glucose levels as well as SGPT, SGOT, ALP and Bilirubin levels significantly decrease due to oral administration of ethanolic extract of *Azadirachta indica* leaves. This study suggests that *Azadirachta indica* possesses antidiabetic as well as hepatoprotective effects.

KEY WORDS : *Azadirachta indica, Ethanolic extract, Hypoglycemic activity and Alloxan*

INTRODUCTION:

Diabetes mellitus is a common prevalent disease, affecting the citizens of both developed and developing countries. According to International diabetes federation, 382 million people have diabetes in 2013; by 2035 this will rise to 592 million. Diabetes is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin (ADA, 2007). This disease complication is associated with a high risk of atherosclerosis (Wakabayashi, *et al.*, 2004), coronary heart disease (Feher, 2004) and peripheral vascular disease (Thomas, *et al.*, 2004). Different types of synthetic oral hypoglycemic agents such as biguanides and sulfonylureas are available along with insulin for the treatment of diabetes. There is an increasing demand by patients to use the natural products with antidiabetic activity to overcome the side effects and toxicity of synthetic drugs (Flower, 2007). There are many reports of herbal extract being used in Ayurvedic literature as antidiabetic which are directly or indirectly used for the

Corresponding Author :
Rajani Kumari
E-mail : rajanicool@gmail.com
Date of Acceptance : 01.03.2014
Date of Publication : 20.04.2014

preparation of many modern drugs (Patwardhan, *et al.,* 2004). The herbal drugs with anti-diabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional medicine systems. The plants have potential source of hypoglycemic drugs because many plants and plant derived compounds have been used in the treatment of diabetes. Many Indian plants have been investigated for their beneficial use in different types of diabetes and reported occur in numerous scientific journals. Hence, they play an important role as alternative medicine due to less side effects and low cost. The active principles present in medicinal plants have been reported to possess pancreatic beta cells re-generating, insulin releasing and fighting the problem of insulin resistance (Chattopadhyay, 1996; and Chattopadhyay, 1999).

Different parts of Neem (*Azadirachta indica*) is most useful for traditional medicinal have antiseptic, wound healing, skin disease curing and antiulcer activity (Kirtikar, *et al.,* 1933; Chopra, *et al.,* 1956). The hypoglycemic activities of its stem bark and seeds have been reported by various researchers (Biswas, *et al.*, 2002; Ebong, *et al.*, 2008). Azadirachtin, Nimbin, Nimbidin and Nimbidiol are effective alkaloids found in Neem (Chawla, *et al.*, 1994). They act not only as blood purifiers but also controls sugar level very effectively. It has been reported that an aqueous extract of tender leaves of neem tree reduced blood sugar in dogs (Murthy, *et al.,* 1978). Aqueous extract of Neem leaves significantly decreases blood sugar level and prevents adrenaline as well as glucose-induced hyperglycaemia (Shukla, *et al.,* 1973). Aqueous leaf extract also reduces hyperglycaemia in streptozotocin diabetes and the effect is possibly due to presence of a flavonoid, Quercetin (Nuraliev, *et al.,* 1992). A significant hypoglycaemic effect was also observed by feeding Neem oil to fasting rabbits. Recently, hypoglycaemic effect was observed with leaf extract and seed oil, in normal as well as Alloxan-induced diabetic rabbits (Khosla, *et al.,* 2000). The possible mechanisms underlying the hypoglycaemic activity of the aqueous leaf extract have also been discussed (Chattopadhyay, 1996).

The aim of the present study was to investigate the anti-hyperglycemic activity of ethanolic extract of *Azadirachta indica* leaves on Alloxan induced diabetic mice and its protective effect on liver.

MATERIALS AND METHODS:

Experimental animals:

8 weeks old male Swiss albino mice (25 to 30g) were carried out and acclimatized till 2 weeks at laboratory condition. Animals were housed in animal house at 25°C ± 2°C with 12 - 12 hrs dark- light cycle. Standard food and water provided *ad libitum* throughout the experimental period. Animals care and handling were taken according to standard protocol. This project was approved by the DRC, Department of Biochemistry, Patna University, Patna.

Plant material:

Fresh matured leaves of *Azadirachta indica* (Neem) were harvested from Science College Campus, Patna University, Patna and was identified by Dr. S. R. Padmadeo, Professor, Department of Botany, Patna University, Patna (Bihar). The leaves were washed with distilled water and dried completely under the mild sun. Crushed with electrical grinder in coarse powder and soaked in absolute ethanol (95%) for 48 hours. The supernatant was collected and the residue was further soaked in absolute ethanol (95%) for 24 hours. The supernatant was collected and filtered. The filtrate was subjected to Rota vapour extraction at a temperature below 60°C for 24 hours. The concentrated form of the extract was obtained and freeze-dried by lyophilisation. The dose was finally made to 500mg/kg body weight for oral administration after the LD_{50} estimation.

Induction of diabetes:

Alloxan (150mg/kg bw) was prepared in distilled water and administered intra-peritoneal to the mice three times at the interval of 72 hrs. Diabetes was confirmed by blood sugar test, with the help of glucometer (Lever Check Pvt. Ltd.) and its chemical method. Animals have more than 250 mg/dl blood sugar level were selected for further study and maintained up to 4 days in diabetic condition for well establishment.

Experimental protocol:

Mice were divided into three groups and each group had 6 mice as follows: Group I: Normal Control (NC), Group II: Alloxan induced diabetic Control (DC) and Group III: *A. indica t*reated (500mg/kg bw) Alloxan induced diabetic group. *A. indica* (500 mg/kg bw) was orally administered daily for 28 days. Whole blood was collected from retro orbital vein puncture into sodium fluoride treated tubes for estimation of glucose and blood was collected in plane tubes for other biochemical test. Serum was separated by centrifugation at 2500rpm at 4°C for 15 minutes.

Statistical Analysis :

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 = 6. P-Values < 0.05 were considered to be statistically significant.

RESULTS:

This investigation showed that 120 ± 1.8 mg/dl blood glucose level in normal control, in alloxan control group blood glucose (390 ± 23.7mg/dl, P<0.001) was observed and in Azadirachta indica treated group blood glucose concentration was observed 225 ± 12.3 mg/dl, P< 0.01 (Fig.1).On the other hand, the present data also indicated that there was a significant (P<0.001) elevation in SGPT, SGOT, ALP and Bilirubin level in diabetic control mice when compared with non diabetic control mice. The oral administration of A.indica extracts (500mg/kg/b.wt.) to diabetic mice significantly (P<0.01) improved the SGPT, SGOT, ALP and Bilirubin as compared to diabetic control group (Figures 2, 3, 4 and 5) that denote its hepatoprotective activities.

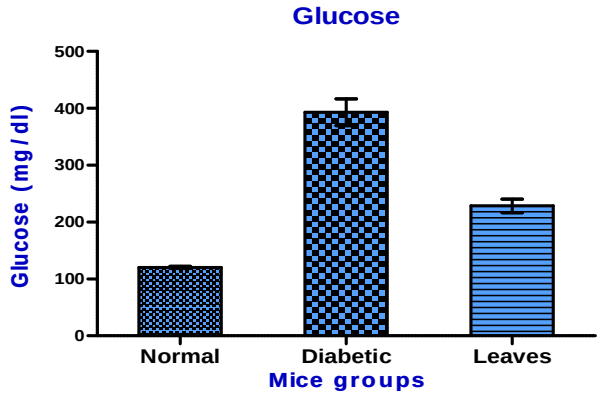

Fig1. Blood glucose concentration in different groups of mice (data shown mean values of six replicate)

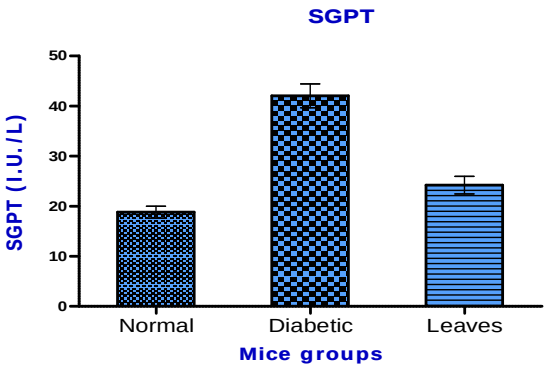

Fig 2. SGPT concentration in different groups of mice (data shown mean values of six replicate)

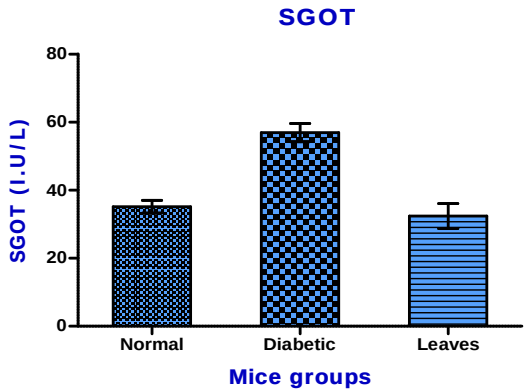

Fig 3. SGOT concentration in different group of mice (data shown mean values of six replicate)

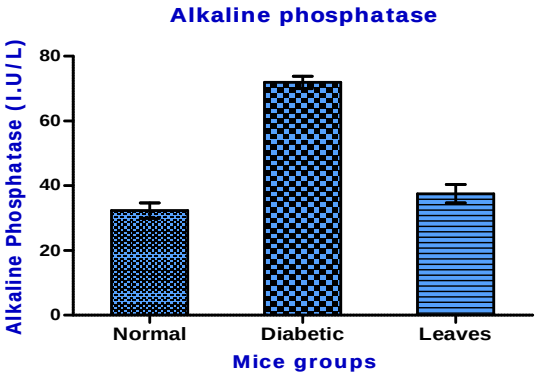

Fig4. ALP concentration in different groups of mice (data shown mean values of six replicate

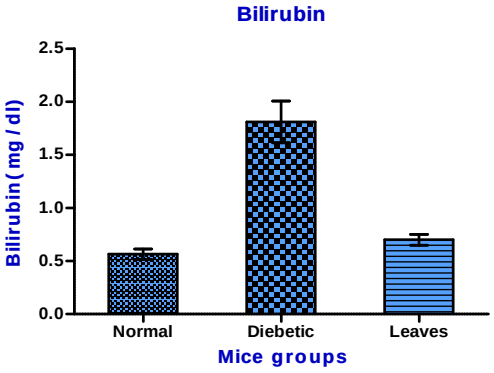

Fig 5. Bilirubin concentration in different groups of mice (data shown mean values of six replicate)

DISCUSSION:

Diabetes is a serious metabolic disorder with micro and macrovascular complications that results in significant morbidity and mortality (Wakabayashi, *et al.*, 2004; Feher, 2004; and Thomas, *et al.*, 2004). Different animals like mice and rat were hyperglycemic by intraperitonial induction of Alloxan through shrinking the pancreatic β-cell and insulin secretion was decreased (Szkudelski, 2001; (Hussein, *et al.*, and 2006; Badole, *et al.*, 2006). After treatment with *A. indica* leaves (500mg/kg/bw) to Alloxan induced diabetic mice, blood glucose reduced upto 42%. Diabetes mellitus is one of the commonest causes of liver failure and hepatomegaly reported by (Chatila, *et al.*, 1996). SGPT, SGOT, ALP and Bilirubin are important assay in the diagnosis of liver damage (Agarwal, *et al.*, 2012). It was analysed that increased concentration of SGPT, SGOT, ALP and Bilirubin may be due to the leakage of these enzymes (Navarro, *et al.*, 1993) that indicate the hepatotoxic effect (Defronzo, *et al.*, 1999; and Tanaka, *et al.*, 1988). These activity are in the absence of insulin because it increases the availability of amino acid in diabetes which is responsible for ketogenesis and gluconeogenesis (Chalasani, *et al.*, 2004). Alloxan induced diabetes increases ALP activity 2 times (Roy, 1998 and Vuksan, 2000) and Bile acids also induced ALP synthesis allowing the leakage into serum (Kaplan, 1986; Kaplan, 1993).

After treatment of *A. indica* for 28 days to alloxan induced mice showed antidiabetic effect as the serum glucose levels reached their normal levels and also controlled SGPT, SGOT and Bilirubin levels denote the antitoxic effects. *A. indica* have hepatoprotective against drug induced injury (Kale, *et al.*, 2003; and Chattopadhyay, *et al.*, 2003, 2005) although in this study the hepatotoxity was caused by diabetes.

ACKNOWLEDGEMENT:

This study was supported by the Department of Biochemistry, Patna University, Patna.

REFERENCES:

1. Agarwal V., Sharma A. K., Upadhyay A., Singh G., and Gupta R., Hypoglycemic effects of Citrullus colocynthis roots. *Acta Pol Pharm* 2012; 69: 75 - 79.
2. Badole S., Patel N., Bodhankar S., Jain B., and Bhardwaj S., Anti-hyperglycemic activity of aqueous extract of leaves of Cocculus hirsutus (L.) Diels in alloxan-induced diabetic mice. *Ind J Pharmacol.*, 2006; 38: 49 - 53.
3. Biswas K., Chattepadhya I., Banergee R. K. and Bandyopadhyayi U., Biological activities and medicinal properties of neem (*Azadirachta indica*). *Current Science*, 2002; 82 (2): 1336 - 1345.
4. Chatila R. and West A. B. Hepatomegaly and abnormal liver tests due to glycogenosis in adults

with diabetes. *Medicine (Baltimore)* 1996; 75: 327 - 333.

5. Chattopadhyay R. R., and Banyopadhyay M., Effect of *Azadirachta indica* leaf extract on serum lipid profile changes in normal and sreptozotocin induced diabetic rats. *African Journal of Biomedical Research*, 2005; 8(2): 101 - 104.
6. Chattopadhyay R. R., A comparative evaluation of some blood sugar lowering agents of plant origin. *Journal of Ethnopharmacology*, 1999; 67: 367 - 372.
7. Chattopadhyay R. R., Possible mechanism of antibyperglycemic effect of *Azadirachta indica* leaf extract, Part: IV. *General Pharmacology (USA)*, 1996; 27 (3): 431- 434.
8. Chattopadhyay R. R., Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract, Part II. *Journal of Ethnopharmacology,* 2003; 89: 217 - 219.
9. Chawla A. S., Kumar M. and Bansal I., Chemical constituents and biological activity of neem-a review. *Indians Drugs*, 1994; 32: 57 - 60.
10. Chopra R. N. Nayar S. L. and Chopra I. C., Glossary of Indian Medicinal Plants. *CSIR, New Delhi*, 1956; 31 - 43.
11. De Fronzo R. A., Pharmacologic therapy for type 2 diabetes mellitus. *Ann Intern Med.*, 1999; 131: 281 - 303.
12. Ebong P. E., Atangwho I. J., Eyong E. U. and Egbung G. E., The Antidiabetic Efficacy of Combined Extracts from Two Continental Plants: *Azadirachta indica* (Neem) and *Vernonia amygdalina* (African Bitter Leaf). *American Journal of Biochemistry and Biotechnology*, 2008; 4 (3): 239-244.
13. Feher Md., Diabetes: preventing coronary heart disease in a high risk group. *Heart*, 2004; 90: 18 - 21.
14. Fowler M. J., Diabetes Treatment, Part 2: Oral agents for glycemic management. *Clin Diabetes*, 2007; 25: 131 - 134.
15. Hussein H. M., EI-Sayed E. M., and Said A. A., Anti-hyperglycemic, Antihyperlipidemic and Antioxidant Effects of *Zizyphus spina* christi and *Zizyphus jujuba* in Alloxan Diabetic Rats. *International Journal of Pharmacology*, 2006; 2: 563 - 570.
16. Kale B. P., Kothekar M. A., Tayode H. P., Jaju J. B., and Mateenuddin M., Effect of aqueous extracts of *Azadirachta indica* leaves on hepatotoxicity induced by antitubercular drug in rats. *Ind. J. Pharm*, 2003; 35: 177 - 180.
17. Khosla P., Bhanwra S., Singh, J., Seth S. and Srivastava R. K. A study of hypoglycemic effects of *Azadirachta indica* (Neem) in normal and alloxan diabetic rabbits. *Indian J Physiol Pharmacol.*, 2000; 44(1): 69 - 74.
18. Kirtikar K. R. and Basu B. D., Indian Medicinal Plants. *Basu, Allahabad*, 1933; 3 - 14.

19. Murthy, *et al.*, A preliminary study on hypoglycemic and antihyperglycemic effects of *Azardirachta indica*. *Indian. J. Pharmacol.,* 1978; 10: 247 - 50.

20. Nuraliev I. N. and Avezov G. A., The efficacy of quercetin in alloxan diabetes. *Eks. Clin Pharmacol,* 1992; 55: 42 - 44.

21. Patwardhan B., Vaidya A. D. B., and Chorghade M., Ayurveda and natural products drug discovery. *Curr Sci;* 2004; 86; 789 - 799.

22. 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: 692 - 699.

23. Shukla R., Singh S. and Bhandari C. R., Preliminary clinical trials on antidiabetic actions of *A. Indica. Medicine and surgery;* 1973; 134: 11 - 20.

24. Szkudelski, T., The mechanism of alloxan and streptozotocin action in β – cells of the rat pancreas. *Physiol. Res.*, 2001; 50: 536 - 546.

25. Tanaka, K.; Nanbara, S.; Tanaka, T.; Koide, H. and Hayshi, T., Amino tranferase activity in the liver of diabetic mice. *Diabetic Res. Clin. Pract.* 1988; 5 (1): 71 - 75.

26. Thomas G. N., Lin J. W., Lam W. W., Tomlinson B., Yeung V., Chan J. C., Liv R., and Wong K. S., Increasing severity of cardiovascular risk factors with increasing middle cerebral artery stenotic involvement in type-II diabetic Chinese patients with a symptomatic cerebro vascular disease. *Diabetes care;* 2004; 27: 1121 - 1126.

27. Vuksan V., Stavro M. P., Sievenpiper J., Beljanzdrakovic U., Leiter L. A., Josse R. G. and Xu Z., Similar postprandial Glycemic reductions with Escalation of dose and Administration. *Time of diabetis Care,* 2000; 23: 1221 - 1226.

28. Wakabayashi I. and Masuda H., Age-dependent relation of serum sialic acid concentration to aortic pulse wave velocity in type II diabetes. *Diabet Metabol,* 2004; 30: 441 - 449.

***