Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(2); 174-180

ISSN: 0975-4873

Research Article

Protective Potential of *Azadirachta indica* Leaf Extract in Diabetic Rat Liver

Gupta N K[1,2*], Srivastva N[2], Bubber P[3], Garg S[4], Mohammad O[5]

[1]*Biotechnology Branch, UIET. Panjab University, Chandigarh-160014, India,*
[2]*Dept of Biotechnology, Banasthali University, Banasthali (Rajasthan),*
[3]*Dept. of Biochemistry, IGNOU, Delhi,*
[4]*Dept of Histopathology, Dental College and Hospital, Panjab University, Chandigarh.*
[5]*Dept of Experimental Medicine, PGIMER, Chandigarh.*

Received:11th Oct, 16; Revised: 14th Dec, 16; Accepted:1st Feb, 17; Available Online: 25th February, 2017

ABSTRACT
Diabetes mellitus is a debilitating metabolic disorder and emerged as a major complication nowadays. The role of plants and their derivatives is indispensable in the treatment of various diseases. *Azadirachta indica* is a medicinal plant and holds a potential to attenuate the pathological changes associated with diabetes. In the present study streptozotocin (STZ) induced diabetic rats received the *Azadirachta indica* leaf extracts (ALE) treatment for a period of seven consecutive days (600 mg/Kg body weight) and then evaluated for changes in the liver tissue. The diabetic rats exhibited significant ($p \leq 0.001$) increase in the lipid peroxidation levels, decrease in GSH levels and modulation in the activities of various antioxidant enzymes including catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase. However, following ALE treatment to diabetic rats reported decrease in the blood glucose levels, lipid peroxidation levels, improved GSH levels and restored the activities of various antioxidant enzymes towards control levels. Besides this, light microscopic and ultramicroscopic examination of liver tissue also demonstrated less fragmentation, inflammation and decreased tissue level damage after ALE treatment, and corroborated the results of biochemical parameters. Thus, the results of present study revealed the hepatoprotective potential of *Azadirachta indica* in diabetes.

Keywords: Hepatoprotection, diabetes mellitus, *Azadirachta indica*, lipid peroxidation, free radicals.

INTRODUCTION

Diabetes mellitus is one of the leading causes of death in the world after heart attack and cancer. Every year about one million deaths occur due to diabetes and its associated complications[1]. Recently the number of diabetic cases has increased tremendously in the developing countries viz. India, China and Bangladesh, mainly comprise young adults and children[1,2]. Hyperglycemia is a hallmark of diabetes and leads to multitargeted damage in vital tissues causing retinopathy, neuropathy, nephropathy, cardiomyopathy and hepatic changes in the diabetic patients. Persistent hyperglycemia develops oxidative stress (both in type 1 and type 2 diabetes) via increased production of reactive oxygen species (ROS)[3]. Moreover, in type1 diabetes these ROS cause severe diabetic complications but in case of type 2 diabetes besides insulin resistance also cause destruction of beta cells in pancreas. However, malfunctioning of mitochondria during diabetes elicits an aggravated response of oxygen free radicals that causes glycation of proteins, accumulation of sorbitol and develops structural and functional abnormalities in the tissue architecture[4,5]. Liver is being considered as the centre of metabolic activities carry out glycolysis, glycogenolysis, glyconeogenesis for glucose homeostasis, get severally affected during diabetes. The ROS reacts with the membrane lipids of hepatocytes, to damage cellular proteins, nucleic acids and develop microangiopathic modifications viz. hypentrophy, necrosis, inflammation, and fragmentation[4,6]. In long term, these structural abnormalities affect the physiology of the liver and cause organ dysfunction. According to a latest reports tight control of blood glucose along with maintenance of antioxidant levels in the body could be a best therapy to avoid the diabetes related complications[7,8]. In current scenario, the treatment of diabetes is being carried out with several synthetic drugs, which are efficacious, but associated with some adverse effects in their chronic use[9]. Besides this, few of the synthetic drugs are very expensive and less available to the patients. However, medicinal plants and their derivatives have been found to be efficacious as well as pose limited or no side effects[10]. Therefore, the research interest of basic scientists has been inclined towards phyto medicines and functional food. Thus, plants are emerging as a better option in the diabetic treatment. A*zadirachta indica* (Neem) a well known medicinal plant and belongs to the family Meliaceae. The various parts (the root, shoot, leaves, fruit and flower) of this plant are used in soap industry, tooth paste, detergents insect killers and antibacterial effects[11]. The multiple medicinal properties of neem include anti

**Author for Correspondence: uietnaveengupta@gmail.com*

oxidative, anti viral, antibacterial, anti ulcer, anti malarial, etc[12]. Besides this, Subapriya et al. have reported antioxidative effect of A*zadirachta indica* in buccal poch cancer[13] while Raghvendra et al. have reported anti Alzimer and anti oxidative effect by conducting behavior analysis on rats[14]. The hypoglycemic activity has been reported by Doli et al., using single dose of A*zadirachta indica* (250mg/kg body weight) and observed decrease in blood glucose levels, serum cholesterol, triglyceride, urea, ceatinine and lipids levels[15]. Further multiple doses of A*zadirachta indica* have also reduced blood glucose levels significantly in comparison to diabetic rats. Chattopadhay et al. have also reported antidiabetic and antilipidemic activity of this plant. However, the microangiopathic modifications especially in liver, has been studied only a little and need further elucidation[16]. Keeping these views under consideration existing study was designed to investigate the hepatoprotective effects of A*zadirachta indica* in streptozotocin (STZ) induced diabetic rats.

MATERIAL AND METHODS

Chemicals

All chemicals used in the study were of analytical grade and purchased from standard companies including Hi media (Streptozotocin), Sigma Aldrich (glutathione reductase enzyme), SRL, (Sodium phosphate hydrogen monobasic, sodium phosphate hydrogen dibasic, potassium phosphate monobasic, Tris hydrochloride, EDTA) etc.

Azadirachta indica leaf Extract (ALE) Preparation

The leaves of *Azadirachta indica* were collected from herbal garden of Panjab University Chandigarh India. The leaves were washed and grounded in one litter of double distilled water then filtered and lypholysed to obtain powder extract. Every day this powder extract was dissolved in appropriate volume of distill water to prepare fresh doses of ALE. The volume of dose was kept fixed 0.5 ml (600mg/kg body weight) and administered orally once a day.

Animals

Healthy male *Sprague-Dawley* rats (150±10 gm) were procured from central animal house facility of Panjab University Chandigarh (India). Animals were fed on standard pellet diet (Hindustan liver ltd.) with free access of water. All procedures and protocols related to animals studies were carried in accordance with guidelines issued by the committee for the purpose of control and supervision of experimentation on animals (CPCSEA) of the Panjab University, Chandigarh.

Experimental design

All animals used in the current study were acclimatized to laboratory conditions for one week and then randomly divided into 3 groups, containing 6-7 animals in each group. The groups were designated as Group 1 Control, Group 2 Diabetic (D), and Group 3 Diabetic treated with ALE (D+ALE).

A single dose of STZ (60 mg/kg body weight) was induced intraperitoneally to group 2 and group 3 animals, and diabetes was confirmed on the 3rd day of STZ induction (fasting blood glucose >250 mg/dl). Post one week of STZ induction and stabilization of blood glucose levels, the ALE treatments (600mg/kg body weight) were started with group 3 animals (D+ALE) and continued for 7 consecutive days. The concentration of ALE used in present study was selected on the basis of blood glucose lowering response curve as mentioned in our previous report[17]. However, parallel to this, group 1 and group 2 animals received 0.5 ml of distilled water instead of ALE treatment. After 14 days, all animals were sacrificed followed by overnight fast. Liver tissues were carefully excised and washed in cold normal saline solution. An appropriate weight of tissue was taken and homogenized in 100 mm Tris buffer pH 7.4 to prepare tissue homogenate (10%, w/v). Finally, homogenate was centrifuged at 12000 rpm for 30 min to prepare post mitochondrial supernatant (PMS).

Prooxidants profile

The prooxidants status of liver tissue was analyzed by measuring the levels of Glutathione (GSH), lipid peroxidation (LPO) and activities of various antioxidant enzymes. The levels of GSH were measured by the method of Ellman[18] and levels of LPO were estimated by the method of Wills[19]. The Catalase (CAT) activity was measured by the method of Luck[20] and Superoxide dismutase (SOD) activity was determined by method of Kono et.al.[21]. Activity of glutathione reductase (GRx) was assayed by the method of Carlberg and Mannervik[22] while the activity of glutathione peroxidase (GPx) was measured by the method of Flohe and Gunzler[23]. The activity of glutathione S transferase (GST) was measured by the method of Warholm et al[24] and the concentration of protein in tissue homogenate was estimated by the method of Lowry et al.[25].

Histopathological analysis

Small sections of liver tissue were cut for light microscopic examination (LM) and transmission electron microscopic (TEM) examination. For LM the tissue was fixed in formalin solution, then washed and dehydrated in ascending grades of alcohol. Embedding was carried out in paraffin wax; thin sections (5µ) were cut using microtome and then stained with Hematoxyline and Eosin (H&E) stain.

For TEM examination ~1 mm size tissue pieces were fixed in Karnosky fixative (Glutralaldehyde solution) and then dehydrated in acetone. The tissue was cleaned in toluene and then embedded with liquid araldite. Finally, the thin sections (60-80 nm) were cut and viewed under TEM at AIIMS New Delhi.

Statistical analysis

Results were calculated carefully and represented as ± standard error mean (SEM) of six animals. Variation among the groups was analyzed by one way applying analysis of variance (ANOVA) followed by LSD post hoc test using SPSS software, the value $p<0.05$, was considered statistically significant.

RESULTS

Blood Glucose and body weight

Streptozotocin is a potent diabetogen and animals treated with STZ showed elevation in the blood glucose levels and reduction in their body weights. The levels of blood

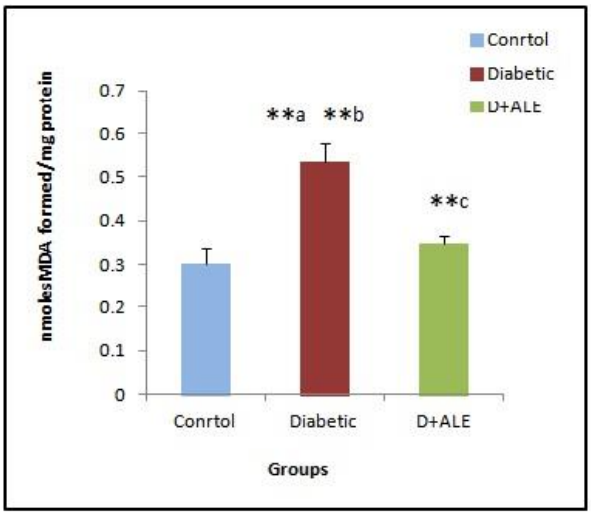

Figure (A): LPO levels

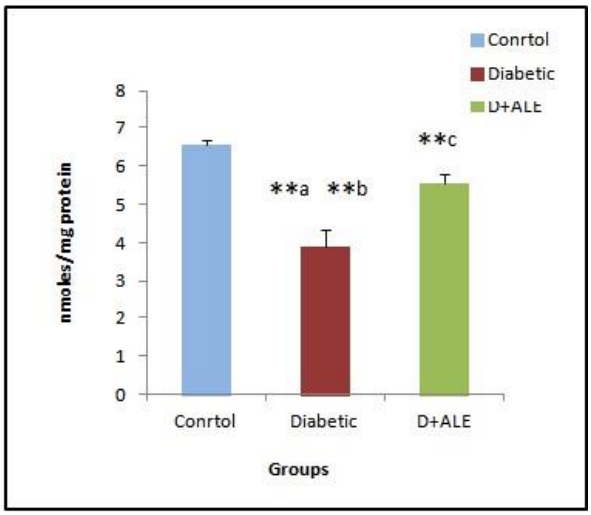

Figure (B): GSH levels

Figure 1: (A) Evaluation of LPO levels in the liver tissue of three groups of animals and levels expressed as nmoles MDA formed/min/mg protein. Figure 1: (B) Evaluation of GSH levels in the liver tissue of three groups of animals and expressed as nmoles /mg protein. ALE: *Azadirachta indica* leaf extract, D: Diabetic. All the values are expressed as mean ± SEM, n=6. Statistically Significant: * Significant at 0.05 levels. ** Significant at 0.001 levels. [a] compared with control group, [b] compared with D+ALE group, [c] compared with diabetic group.

glucose in diabetic animals were found to be elevated on 3rd day and increased up to ~ 4 folds till 7th day, after that remained constant till 14th day of STZ induction. These levels of blood glucose and body weights have already been mentioned in our previous study[17] (carried out on heart tissue) where increase in the blood glucose level was found to be significant ($p \leq 0.001$) in comparison to control animals. However, ALE treatment to D+ALE animals significantly reduced ($p \leq 0.001$) blood glucose levels in comparison to untreated diabetic rats. Besides this, change in body weight was also significant. Both the groups' i.e. diabetic and D+ALE animals reduced their body weights during the course of diabetes in comparison to control animals till 7th day of STZ induction. However, the initiation of ALE treatment to D+ALE animals prevented the further reduction in their body weights and improved the overall condition of the animals[17].

Prooxidant Analysis

Analysis of LPO and GSH levels

Hyperglycemia influenced the prooxidants system and during diabetes a disturbed pattern was observed in the various biochemical parameters. The Figure 1 (A) and 1 (B) represent the levels of LPO and GSH respectively. An elevated the levels of LPO were observed in the group 2 animals (diabetic animals) (Figure A). These levels were found to be significant ($p \leq 0.001$) in comparison to controls (group 1animals). However, ALE treatment to D+ALE animals (group 3 animals) exhibited significant decrease ($p \leq 0.001$) in the LPO levels. Similarly, the concentration of GSH was also measured in the liver tissues of all the animals and found to be decreased in the diabetic rats in comparison to control rats ($p \leq 0.001$). Figure 1 (B) clearly denotes the change in the levels of GSH during diabetes and ALE treatment. The ALE treated (D+ALE) rats exhibited significantly increase in the levels of GSH ($p \leq 0.001$) in comparison to untreated diabetic rats following ALE treatment.

Analysis of antioxidant enzymes

The Figure 2A-2E demonstrates the activities of various antioxidant enzymes, which showed modulation in their activities during diabetes and ALE treatment. The activity of catalase was found increased ($p \leq 0.001$) while the activities of SOD ($p \leq 0.001$), GPx ($p \leq 0.001$), GRx ($p \leq 0.001$) and GST ($p \leq 0.001$) were found to be decreased in the diabetic animals in comparison to control animals. The oral administration of ALE to D+ALE animals significantly ($p \leq 0.001$) altered the activities of these enzymes and brought them near to the control levels. As seen in Figure 2(A) the enzyme activity of catalase following ALE treatment was found to be reversed and restored towards control levels. However, the activity of SOD was improved 2(A) but found to be statistically insignificant in comparison to untreated diabetic animals. Similarly, the ALE treatment to D+ALE rats further restored the activities of GPx, GRx and GST in comparison to untreated diabetic rats. The level of significant restoration, was different in different enzymes and found to be ($p \leq 0.01$), ($p \leq 0.001$) and ($p \leq 0.01$) for GPx, GRx and GST respectively.

Histological analysis

The liver sections were viewed under light microscope and Transmission Electron microscope to analyze the histological alterations (Figure 3 A- F) during diabetes. The light microscopic analysis was carried out by comparing the control sections (Figure 3 A) with diabetic (Figure 3B) and D+ALE sections (Figure 3C). The control sections showed normal tissue architecture, round oval hepatocytes, compactly arranged sinusoids, normal central vein and absence of inflammation etc. whereas diabetic sections (Figure 3B) revealed ruptured sinusoids,

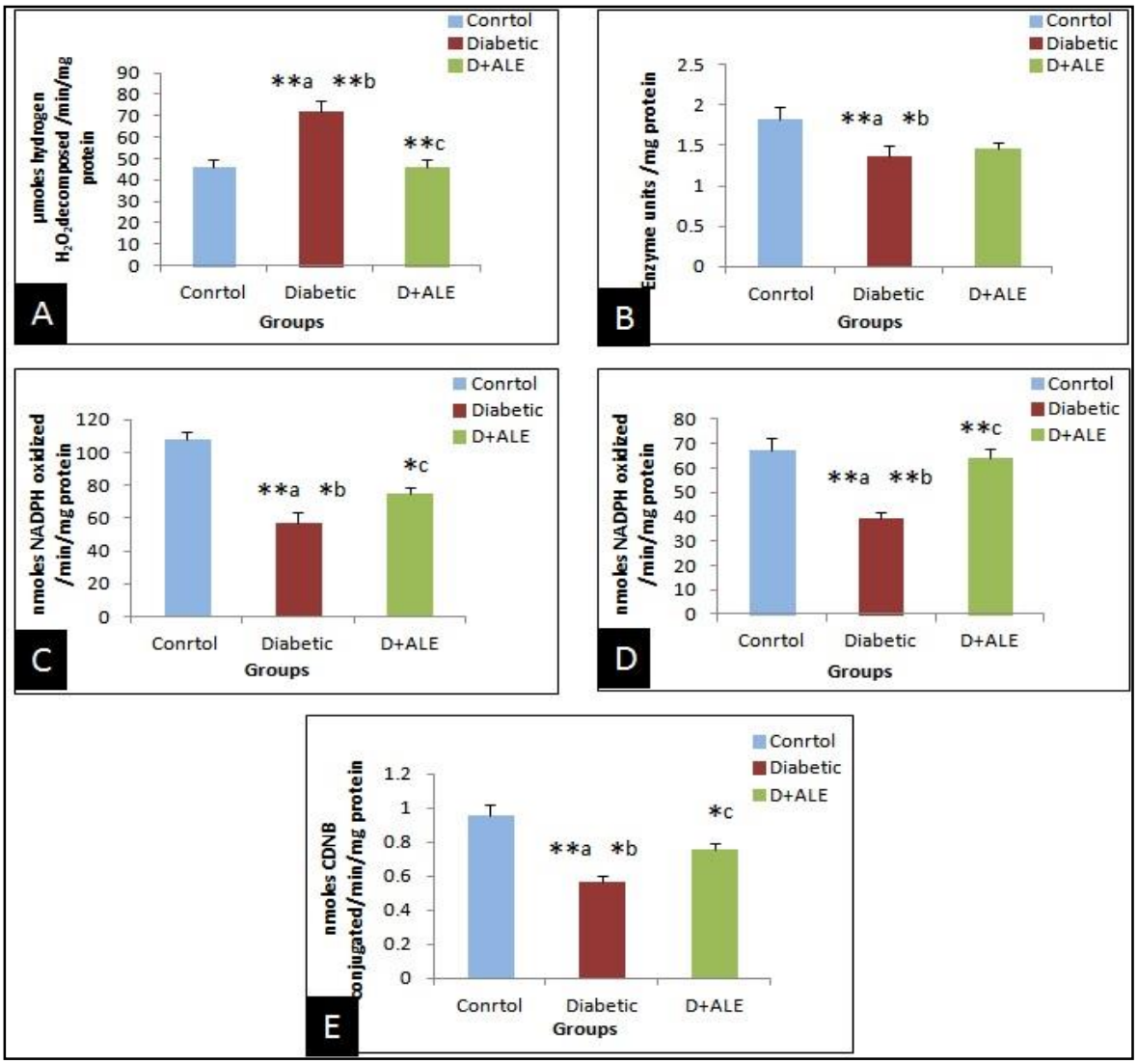

Figure 2: Evaluation of antioxidant enzymes in the liver tissue of three groups of animals. (2A) catalase activity expressed as µmoles hydrogen peroxide (H_2O_2) decomposed /min/mg protein. (2B) SOD activity expressed as enzyme units /mg protein where one unit of enzyme is the amount of enzyme inhibiting the rate of reaction (NBT reduction) by 50%. (2C) Glutathione peroxidase activity expressed as nmoles NADPH oxidized/min/mg protein (2D) Glutathione reductase activity expressed as nmoles NADPH oxidized/min/mg protein. (2E) Glutathione S transferase activity expressed as nmoles CDNB conjugated/min/mg protein. ALE: *Azadirachta indica* leaf extract, D: Diabetic. All the values are expressed as mean ± SEM, n=6. Statistically Significant: * Significant at 0.05 levels. ** Significant at 0.001 levels. [a] compared with control group, [b] compared with D+ALE group, [c] compared with diabetic group.

hepatocytes with necrosis, and inflammation. On the other hand the section prepared from D+ALE treated rats liver (Figures 3C) showed mild structural changes and restored the normal tissue architecture in comparison to the section of untreated diabetic rats. However, TEM analysis further revealed a close view of tissue architecture at ultra structural level changes. The diabetic section again underlined the above mentioned changes along with decreased number of glycogen granules and fragmented endoplasmic reticulum (Figures 3E) whereas the D+ALE section (Figures 3F) showed comparatively less fragmentation and more pronounced endoplasmic reticulum. While all other changes in D+ALE section were further found to be similar to the control sections. Over all the TEM analysis also confirmed these changes.

DISCUSSION

Diabetes mellitus is a chronic metabolic disorder having a complex pathophysiology. The animal models could represent these pathophysiological changes in a better way therefore; animal models are preferred in the diabetic research[26]. In the present study, the animal model of diabetes was prepared by inducing streptozotocin to the SD rats at a dose of 60 mg/kg body weight. STZ is a well known diabetogen and all doses above 50mg/kg body weight can produce symptoms similar to insulin dependent diabetes[27]. However, the mode of action of this drug is very specific and involves alkylation and fragmentation of DNA present in beta cells[27]. The destruction of beta cells and deficiency of insulin leads to the development of

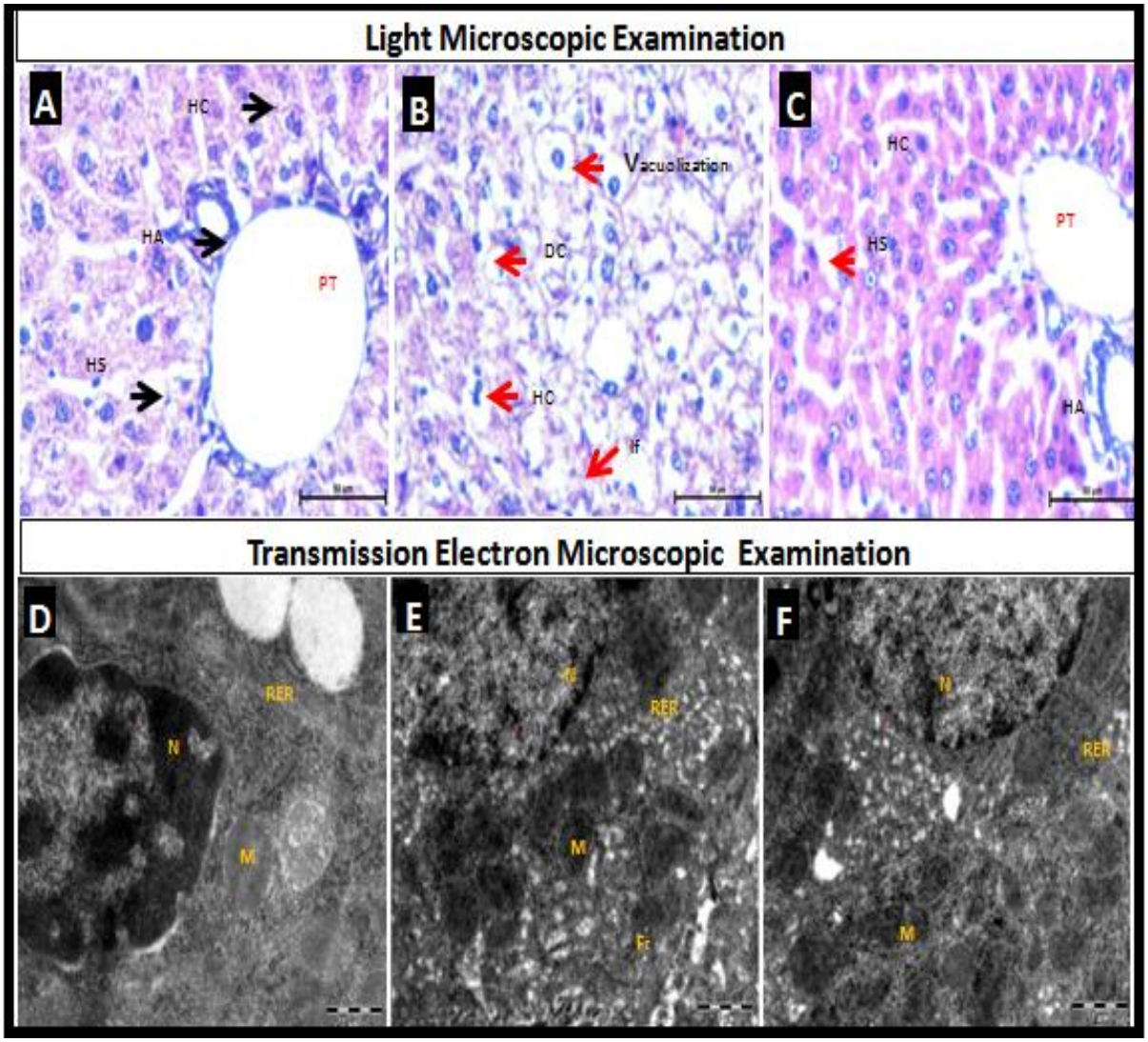

Figure 3: Histological analysis of liver. Light microscopic analysis at 40X, 3(A-C). Transmission Electron Microscopic analysis at 3200X, 3(D-F). ALE: *Azadirachta indica* leaf extract, D Diabetic. PT: Portal tract, HC: Hepatocytes, HA; Hepatic artery, DC: Degenerative changes, Fr: Fragmentation M: Mitochondria, N: Nucleus, RER: Rough endoplasmic reticulum, LM Section: (3A) Control liver Shows normal structure of hepatocytes, compact arrangement, sinusoids, and hepatic artery (Black arrow). Section (3B) Diabetic liver shows vacuolization, degenerative changes (thinning of sinusoidal boundaries) hepatocytes and inflammation (Red arrow). Section (3C) D+ALE liver shows decrease in diabetic changes, in hepatocytes and improvement in hepatic tissue architecture. TEM sections: (3D) Control liver shows compact arrangement of tissue and normal distribution of mitochondria, intact nuclear membrane and rough endoplasmic reticulum (RER). Section (3E) Diabetic liver (STZ) shows deformed mitochondria, fragmentation and decrease in RER. Section (3F) D+ALE treated liver shows less number of deformed mitochondria, well arranged RER, and over all less disturbed tissue architecture.

hyperglycemia; similar results were observed in our study after STZ induction to the rats[17]. The observation of elevated blood glucose levels in the diabetic rats might be due to the metabolism of stored glycogen and fats[28]. According to a recent report, the maintenance of hyperglycemic status requires the regular breakdown of structural proteins of the body, which affect over all body weight later on, leading to reduced body weight in the diabetic animals compared to control animals[29]. Although, the increase in body weight in D+ALE rats was not found to be significant, but indicated that ALE treatment could prevent the weight loss and confirmed the findings of Chattopadhay et al. and Khosla et al[30,31]. However, the exact mechanism involved in the reduction of blood glucose levels is largely unknown. Additionally, ALE treatment decreased the rate of weight loss in D+ALE treated rats in comparison to diabetic rats, which could be a response to decreased hyperglycemia. It is also possible that controlled blood glucose levels reduced the breakdown of structural protein causing the decrease in weight loss in D+ALE rats.

Hyperglycemia often increases the influx of glucose in the cells, thereby increasing the metabolic load on the mitochondria, which further undergoes malfunctioning and produces free radicals. Normally small amount of free radicals are routinely produced and get scavenged by intra

cellular antioxidant systems, but during diabetes, the production of free radicals gets increased many fold and the scavenging system gets disturbed. The over produced free radicals react with cellular components and cause structural and functional level destruction. The attack on cellular lipids present in the cell membrane may cause lipid peroxidation[6], which might have damaged the hepatocytes in the present study. GSH is an important component of the proxidant system and protects the cells from the effects of free radicals. Moreover, free radicals further evoke the antioxidant enzymes, which also utilize cellular GSH to protect their activities, causing the observed significant modulation in the activities of various antioxidant enzymes. However, ALE is considered to be a rich source of a large number of compounds viz. Myricetin-3-O-Quercetin-3-O-B-D-glucoside, rutinoside, Quercetin-3-O-rutinoside, Kaempferol-Kaempferol-3-O-B-D-glucoside, 3-O rutinoside, and Quercetin-3-O-L-rhamnoside[16]. Quercetin-3-O-L-rhamnoside, known for its antioxidative and hypoglycemic property, scavenged the free radicals and decreased oxidative stress. Therefore, ALE treatment to diabetic rats seemed to improve the GSH content, decreased the lipid peroxidation, and restored the activities of antioxidant enzymes.

According to the previous reports, persistent hyperglycemia is destructive for the cellular components and develops morphological and physiological alterations in the tissues[6,32,33]. Initially, the changes appear at the tissue level as microangiopathic changes; however, with passage of time, these changes get accumulated and become a major reason of organ dysfunction. In the present study, the hyperglycemia in diabetic animals displayed the changes in prooxidant status of the tissue, which might have further developed histopathologic changes, such as necrosis, inflammation, and damaged sinusoids in the liver. Additionally, the studies of Zou et al. have also reported that enhanced LPO levels and depleted GSH levels could also cause structural and architectural damage to the tissues[6]. Moreover, Chen et al. also suggested that augmented oxidative stress and decreased antioxidant levels may cause hepatic injury[34]. Thus, decreased GSH levels, increased LPO levels and modulated oxidative stress parameters during diabetes in the present study, might have caused histopathologic damage in the liver tissue. However, ALE treatment decreased the inflammation, necrosis and improved the overall histological architectural of the tissue. Thus, decrease in blood glucose levels, improvement in the biochemical and prooxidant parameters upon ALE treatment corroborated the anti oxidative properties of ALE. Therefore, present study clearly demonstrates that ALE possesses excellent antioxidative, antidiabetic, and hepatoprotective properties.

CONCLUSION

The *Azadirachta indica* leaf extract has a potential to attenuate the diabetic and oxidative stress related pathological alterations developed in the liver tissue. The *Azadirachta indica* leaf extract demonstrated the protective effect by restoring the antioxidant biomarkers and improving the ultra structure of the tissue.

ACKNOWLEDGEMENT

The authors are grateful to the department of UIET (Panjab University, Chandigarh) and Banasthali University for providing infrastructure and facilities to carry out this research.

REFERENCES

1. Guariguata L, Nolan T, Beagley J, Linnenkamp U, Jacqmain O. IDF Diabetes Atlas. International Diabetes Federation; 2013.
2. Ziegler AG, Pflueger M, Winkler C, Achenbach P, Akolkar B, Krischer JP, Bonifacio E. Accelerated progression from islet autoimmunity to diabetes is causing the escalating incidence of type 1 diabetes in young children. Journal of Autoimmunity 2011; 37(1):3-7.
3. Rochette L, Zeller M, Cottin Y, Vergely C. Diabetes, oxidative stress and therapeutic strategies. Biochimica et Biophysica Acta (BBA)-General Subjects 2014; 1840(9):2709-29.
4. Naudi A, Jove M, Ayala V, Cassanye A, Serrano J, Gonzalo H, Boada J, Prat J, Portero-Otin M, Pamplona R. Cellular dysfunction in diabetes as maladaptive response to mitochondrial oxidative stress. Experimental Diabetes Research 2012; 2012:1-14.
5. Forbes JM, Cooper ME. Mechanisms of diabetic complications. Physiological Reviews 2013; 93(1):137-88.
6. Zou C, Qiu Q, Chen H, Dou L, Liang J. Hepatoprotective effects of selenium during diabetes in rats. Human & Experimental Toxicology 2016; 35(2):114-23.
7. Nabavi SF, Habtemariam S, Daglia M, Shafighi N, Barber AJ, Nabavi SM. Anthocyanins as a potential therapy for diabetic retinopathy. Current Medicinal Chemistry 2015; 22(1):51-8.
8. Nishikawa T, Araki E. Mechanism-based antioxidant therapies promise to prevent diabetic complications. Journal of Diabetes Investigation 2013; 4(2):105-7.
9. Stein SA, Lamos EM, Davis SN. A review of the efficacy and safety of oral antidiabetic drugs. Expert Opinion on Drug Safety 2013; 12(2):153-75.
10. Jia W, Gao W, Tang L. Antidiabetic herbal drugs officially approved in China. Phytotherapy Research 2003; 17(10):1127-34.
11. Raj A, Toppo P. Beneficial and Harmful Effects of Azadirachta Indica: A Review. Environmental Science 2015; 4(3).
12. Pathak GP, Sharma V, Singh G, Kour A. Neem (*Azadirachta Indica*): Traditional Medicine for House-Hold Remedy Against Various Human and Animal Ailments: Review. World Journal of Pharmacy and Pharmaceutical Sciences 2013; 2(5):3854-3860.
13. Subapriya R, Kumaraguruparan R, Nagini S. Expression of PCNA, cytokeratin, Bcl-2 and p53 during chemoprevention of hamster buccal pouch carcinogenesis by ethanolic neem (Azadirachta indica)

leaf extract. Clinical Biochemistry 2006; 39(11):1080-7.
14. Raghavendra M, Maiti R, Kumar S, Acharya SB. Role of aqueous extract of Azadirachta indica leaves in an experimental model of Alzheimer's disease in rats. International Journal of Applied and Basic Medical Research 2013; 3(1):37.
15. Dholi SK, Raparla R, Mankala SK, Nagappan K. Invivo Antidiabetic evaluation of Neem leaf extract in alloxan induced rats. Journal of Applied Pharmaceutical Science 2011; 1(4):1.
16. Chattopadhyay RR, Bandyopadhyay M. Effect of Azadirachta indica leaf extract on serum lipid profile changes in normal and streptozotocin induced diabetic rats. African Journal of Biomedical Research 2006; 8(2):101-4.
17. Gupta NK, Srivastva N, Bubber P, Puri S. The Antioxidant prtential of *Azadirachta indica* ameliorates cardioprotection following diabetic mellitus induced microangiopathy. Pharmacognosy Magazine 2016; (in press).
18. Ellman GL. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics. 1959; 82(1):70-72.
19. Wills ED. Mechanisms of lipid peroxide formation in animal tissues. Biochemical Journal 1966; 99(3):667.
20. Luck H, Catalase in: Methods of analysis H.U. Bergmeyer (Ed), Academic press, New Yark USA, 1971, 885-893.
21. Kono Y. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Archives of Biochemistry and Biophysics 1978; 186(1):189-95.
22. Carlberg IN, Mannervik BE. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. Journal of Biological Chemistry 1975; 250(14):5475-80.
23. Flohé L, Günzler WA. Assays of glutathione peroxidase. Methods in Enzymology 1984; 105:114-20.
24. Warholm M, Guthenberg C, Mannervik B. Molecular and catalytic properties of glutathione transferase. mu. from human liver: an enzyme efficiently conjugating epoxides. Biochemistry 1983; 22(15):3610-3617.
25. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 1951; 193(1):265-75.
26. Islam MS, Koya D, Portha B. Animal Models of Diabetes and Its Associated Complications. Journal of Diabetes Research 2013.
27. Lenzen S. The mechanisms of alloxan-and streptozotocin-induced diabetes. Diabetologia 2008; 51(2):216-26.
28. Ugochukwu NH, Babady NE. Antihyperglycemic effect of aqueous and ethanolic extracts of Gongronema latifolium leaves on glucose and glycogen metabolism in livers of normal and streptozotocin-induced diabetic rats. Life Sciences 2003; 73(15):1925-38.
29. Irfan HM, Asmawi MZ, Khan NA, Sadikun A. Effect of Ethanolic Extract of Moringa oleifera Lam. Leaves on Body Weight and Hyperglycemia of Diabetic Rats. Pakistan Journal of Nutrition. 2016; 15(2):112.
30. Chattopadhyay RR. Possible mechanism of antihyperglycemic effect of Azadirachta indica leaf extract. Part IV. General Pharmacology: The Vascular System 1996; 27(3):431-4.
31. Khosla P, Bhanwra S, Singh J, Seth S, Srivastava RK. A study of hypoglycaemic effects of Azadirachta indica (Neem) in normal and alloxan diabetic rabbits. Indian Journal of Physiology and Pharmacology 2000; 44(1):69-74.
32. Lucchesi, AN, Cassettari, LL Spadella, CT, 2015. Alloxan-Induced Diabetes Causes Morphological and Ultrastructural Changes in Rat Liver that Resemble the Natural History of Chronic Fatty Liver Disease in Humans. Journal of diabetes research 2015; 2015:1-11.
33. Muthulakshmi S, Saravanan R. Protective effects of Azelaic acid against high-fat diet-induced oxidative stress in liver, kidney and heart of C57BL/6J mice. Molecular and Cellular Biochemistry 2013; 377(1-2):23-33.
34. Chen Y, Dong H, Thompson DC, Shertzer HG, Nebert DW, Vasiliou V. Glutathione defense mechanism in liver injury: insights from animal models. Food and Chemical Toxicology 2013; 60:38-44.