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ANTI-HYPERGLYCEMIC AND ANTIOXIDANT ACTION OF *AZADIRACHTA INDICA* LEAF IN ALLOXAN INDUCED TYPE-I DIABETES MELLITUS IN EXPERIMENTAL DIABETIC SWISS ALBINO MICE: A HISTOPATHOLOGICAL INVESTIGATION

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ABSTRACT: Objective: To attenuates hyperglycemia-mediated oxidative stress and protects liver marker enzymes in Swiss albino mice. **Material and methods:** Hyperglycemia was induced in mice by single intraperitoneal injection of alloxan (150 mg/kg body weight). Three days after alloxan induction, the hyperglycemic mice were treated with *Azadirachta indica* orally at the dose of 200 and 400 mg/kg body weight daily for 28 days. Serum biochemical parameters such as serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), billirubin, glucose were estimated. Antioxidant properties were assessed by estimating lipid peroxidation, SOD and catalase (CAT). **Results and discussion:** *Azadirachta indica* at the dose of 200 and 400 mg/kg orally significantly ($P < 0.001$) and dose-dependently reduced and normalized blood glucose levels as compared with that of alloxan control group. Serum biochemical parameters were significantly ($P < 0.001$) restored toward normal levels in *Azadirachta indica* treated mice as compared with alloxan control. *Azadirachta indica* treatment also significantly ($P < 0.001$) decreased lipid peroxidation and recovered SOD level and CAT activity toward normal as compared with alloxan control. In Histopathological study also did show adverse alternation in the morphological architecture of the liver tissue. **Conclusion:** The present study infers that *Azadirachta indica* leaf demonstrated remarkable anti-hyperglycemic activity in alloxan-induced diabetic mice. The potential anti-hyperglycemic action is plausibly due to its underlying antioxidant role.

INTRODUCTION: Diabetes mellitus is a metabolic disorder which is the disturbance of lipid, carbohydrate and protein metabolism, its effect on a large number of people throughout the world as well as in India¹.

It is resulting from insulin deficiency or its resistance and high blood glucose level which appear to be the primary defects associated with diabetes². It is considered to be one of the lethal diseases around the world especially Asia and Africa, these are the most common regions where the disease is going on 2 - 3 folds in near future³.

Globally, Diabetes mellitus presents is increasingly important public health issues. The prevalence of DM in all age groups was estimated to be 2.8 % (170 million) in 2000 and the no of people will raise to 4.4 % (366 million) in 2030⁴.

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There is a growing interest in herbal remedies because of its effectiveness, minimal side and low costs. *A. indica* tree is being used in the traditional systems of medicine for the treatment of a variety of human ailments⁵. *A. indica* has been used to treat diabetes; there are several reports which suggest the hypoglycemic potential of *A. indica*⁶.

MATERIALS AND METHODS:

Collection and Preparation of *Azadirachta indica*

Ethanolic Extract: In the present study fresh leaves of *Azadirachta indica* were collected from the local garden and confirmed by Department of Botany in SHIATS, Allahabad (Uttar Pradesh). The collected fresh leaves of *Azadirachta indica* were thoroughly cleaned with distilled water, dried well and powdered used for extraction. It was soaked in 70% ethanol (72 h).

The resulted extract was filtered and concentrated by Soxhlet apparatus for 12 hr at 30 °C. Ethanol was evaporated using a rotary evaporator under reduced pressure and low temperature. Distilled water was used to reconstitute the solid extract to obtain a desired concentration for the studies⁷.

Preliminary Phytochemical Screening: Phytochemical analysis of *Azadirachta indica* leaves extract was tested for the presence of bioactive compounds by using following standard methods⁸.

Test for Proteins: Millon's Test: Crude extract when mixed with 2 ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Test for Phenols and Tannins: Crude extract was mixed with 2 ml of 2% solution of FeCl₃. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for Flavonoids: Shinoda Test: Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicated the presence of flavonoids.

Test for Saponins: Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for Alkaloids: Crude extract was mixed with 2 ml of 1% HCl and heated gently. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Experimental Animals: Healthy male Swiss albino mice (30 - 35 g) were selected for this experiment. The animals were maintained in well-ventilated room at room temperature with natural day-night 12:12 (light: dark cycle) cycle in polypropylene cages lined with standard environmental conditions (22 ± 2) °C, relative humidity (55 ± 10) %. The mouse was fed on a standard pellet diet *ad libitum* and free access to water. The experiments were approved by the Institutional Animal Ethics committee (MCS/RC/2012/244) and were carried out in accordance with the current guidelines for the care of laboratory animals.

Acute Toxicity Studies: The acute toxicity test of the extract was performed according to the OECD guidelines no. 423. The mice were divided into four groups, 3 mice in each group. The four groups of mice were orally administered extract with various doses of the extract ranging from 50 to 2000 mg/kg body weight. The animals were monitored for one week for mortality and behavioral, neurological, and autonomic responses.

No death occurred and also no abnormalities were detected in behavioral, neurological and autonomic responses till the end of one week. The extract was found to be safe up to the dose of 2000 mg/kg body weight. In the present study the extract dose of 200 and 400 mg/kg body weight was chosen during the study of alloxan-induced diabetic animal and the extract dose of 200 and 400 mg/kg body weight during the study of alloxan-induced diabetic Swiss albino mice.

Induction of Diabetes in Experimental Animals:

Diabetes mellitus was induced in overnight fasted mice by a single intraperitoneal injection of alloxan monohydrates at the rate of 150 mg/kg body weight⁹. After injection of alloxan, the animals were allowed free access to 5% glucose solution to overcome the drug induced hypoglycaemia. The blood glucose level was estimated within 72 hr, after alloxan induction. Diabetes was confirmed by blood samples collected from the tip of the tail

using a blood glucometer (Accu Sure, Taiwan). The level of blood glucose more than 200 mg/dl were selected for this experiment¹⁰.

Experimental Design: Animals were dividing into four groups, consisting of six animals each, as follows:

Group I: Normal control (CN)

Group II: Diabetic (DM)

Group III: diabetic mice were administered 200 mg/ kg body weight *A. indica* ethanolic extract per day orally for 28 days (DM + M₂₀₀)

Group IV: diabetic mice were administered 400 mg/ kg body weight *A. indica* ethanolic extract per day orally for 28 days (DM + M₄₀₀)

One week after the induction of diabetes in Swiss albino mice, the fasting blood glucose levels of fasted mice were measured. Mice with blood glucose more than 200 mg/dl were included in the study.

Collection of Blood Sample and Estimation of Serum Biochemical Investigations: After the last dose, animals were sacrificed and Blood samples were collected by orbital sinus puncture method¹¹. Meanwhile, serum levels of SGOT, SGPT, ALP and total bilirubin were estimated according to the protocol of the manual of diagnostic kits (Creast coral, Goa, India).

Superoxide dismutase (SOD) in serum was determined using photo-oxidation method¹². catalase measurement was carried out based on the ability of catalase to oxidize hydrogen peroxide¹³. Lipid peroxidation was determined by¹⁴.

Histological Study:

Transmission Electron Microscopy: Processing for electron microscopy and analysis were done according to the method¹⁵.

Light Microscopy Studies: At the end of the experimental, the liver tissues from each animal was removed after sacrificing the animal by cervical dislocation and a portion of the liver was cut into 2 to 3 pieces of approximately 6 mm³ sizes and fixed for 48 hr in 10% formalin saline were dehydrated by passing successfully in different mixture of ethyl alcohol-water, cleaned in xylene and embedded in paraffin wax. The thin sections of liver were made into permanent slides and examined¹⁶ under high resolution microscope with photographic facility and photo micrographs were taken.

Statistical Analysis: Data from the experiments were presented as mean \pm Standard deviation. Statistical analysis was done by using the GraphPad Prism Program (GraphPad Software, Inc., San Diego, USA). Paired student T-test was done to see any difference between the paired groups. The level of significance was set at $p < 0.001$.

RESULTS:

Body and Organs Weight: In the present study, alloxan induced diabetic mice showed significant ($P < 0.001$) reduction in body weight and liver tissue. Administration of ethanol extract of *A. indica* leaf (200 and 400 mg/kg) significantly increased the body weight and tissue weight within 21 days (Table 1).

Table 2 shows the photochemical constitute of *A. indica* extract. The preliminary studies indicated the presence of protein, alkaloids, reducing sugar, total phenol, tannis and saponins.

TABLE 1: EFFECT OF *A. INDICA* ON BODY WEIGHT AND LIVER WEIGHT IN ALLOXAN INDUCED DIABETIC MICE

Treatment	Body weight (gm)		Liver weight (gm)
	Initial	Final	
Normal	31.33 \pm 0.82	35 \pm 0.81	3.07 \pm 0.13
Diabetic	26 \pm 1.68#	18 \pm 0.23#	1.89 \pm 0.14#
Diabetic+M200	28.16 \pm 0.41*	31.83 \pm 0.981*	2.03 \pm 0.43*
Diabetic+M400	29.45 \pm 0.52*	33.54 \pm 1.41*	2.70 \pm 0.26*

The value represented as means \pm S.D for six mice per group. # $p < 0.001$ as compare to normal group and * $p < 0.001$ as compare to diabetic group. (Normal control), (Diabetic control), DM + M₂₀₀ (Diabetic+ *A. Indica* leaf extract at the dose of 200 mg /kg b.w). DM + M₄₀₀ (Diabetic+ *A. Indica* leaf extract at the dose of 400 mg /kg b.w)

TABLE 2: PHYTOCHEMICAL ANALYSIS OF METHANOL, ETHANOL EXTRACTS OF A. INDICA LEAVES

Solvent used for extraction	Protein	Flavonoids	Alkaloids	Reducing sugar	Phenols/Tannins	Saponins
Methanol	-	+	-	+	-	-
Ethanol	+	+	+	+	+	+
Acetone	-	-	-	+	-	+

Serum Glucose Level: After induction of diabetes by alloxan, the blood glucose level of diabetic mice was increased, the mean level of glucose in the control group of mice was evaluated to be 76.71 ± 2.60 mg/dl (range 50 - 98) whereas it was 220.00 ± 8.76 mg/dl (range values 186.6 - 256.8, $p=0.0001$, $t=26.37$) in alloxanized group. After the treatment of mice with the leaf extract of *Azadirachta indica* (200 mg/kg body weight) the glucose level decreased down to 186 ± 7.87 mg/dl ($p=0.0027$, $t=5.51$) having a range of 145 - 223 mg/dl and more potent effect at the dose of 400 mg/kg body weight of extract the level of glucose also significantly decreased to 119.4 ± 6.82 mg/dl ($p=0.0001$, $t=10.47$) having range of 85.87 - 146 mg/dl. These variations in glucose concentrations are evident from **Fig. 1**. The significant increase in glucose concentration in the diabetic animals than that of the control mice is evident on alloxanization. However, the oral administration of aqueous extract of *Azadirachta indica* significantly reduced the glucose level in serum when compared with alloxan induced diabetic mice.

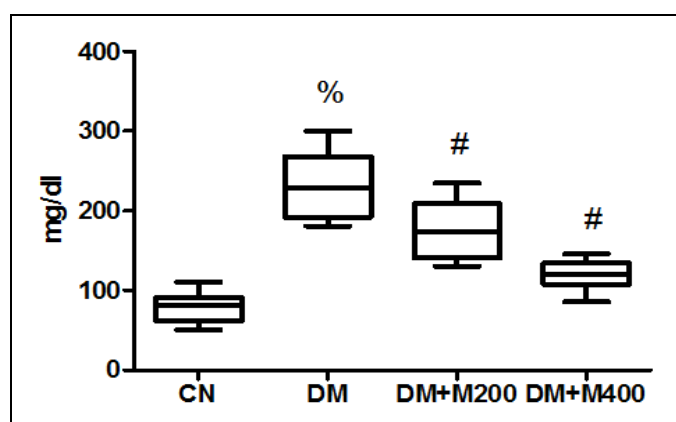


FIG. 1: EFFECT OF ETHANOLIC EXTRACT OF AZADIRACHTA INDICA (200 AND 400 mg/kg b.w) ON THE SERUM GLUCOSE LEVELS IN ALLOXAN INDUCED MICE

Values are the means \pm S.D for six animals in each group. Values are significant at $p<0.001$, statistical significance was compared within groups as follows. % Diabetic mice were compared with normal mice. # DM + M₂₀₀ and DM + M₄₀₀ treated diabetic mice were compared with diabetic mice

Activity of Liver Marker Enzymes: Serum Glutamic Pyruvate Transaminase (SGPT): In

Control group of mice SGPT activity was found to be 19.53 ± 1.07 IU/ml having the range of 14.56 to 24.34 IU/ml. In diabetics, its activity got raised to 29.84 ± 1.23 IU/ml ($p= 0.0003$, $t=9.05$) with variations range from 25.56 to 34.00. However, extract (200 and 400 mg/kg b.w) treatment of this group for four weeks resulted in decrease of SGPT activity to 26.89 ± 1.25 and 22.58 ± 5.31 ($p=0.1171$, $t=1.89$ and $p=0.0360$, $t=2.84$) having values ranging from 20 to 32 IU/ml and 15.56 to 31 IU/ml. These variations are depicted by the box-plot in **Fig. 2**.

Serum Glutamate Oxaloacetate Transaminase (SGOT): In Control group of mice SGPT activity was found to be 22.06 ± 1.57 IU/ml having the range of 17.12 to 27.67 IU/ml. In diabetics, its activity got raised to 39.74 ± 1.06 IU/ml ($p= 0.0001$, $t=19.84$) with variations range from 35.56 to 43.90. However, extract (200 and 400 mg/kg b.w) treatment of this group for four weeks resulted in decrease of SGOT activity to 34.26 ± 1.51 and 29.04 ± 1.34 ($p=0.0180$, $t=3.46$ and $p=0.0005$, $t=7.90$) having values ranging from 29.45 to 39.98 IU/ml and 22.45 to 34 IU/ml. These variations are depicted by the box-plot in **Fig. 3**.

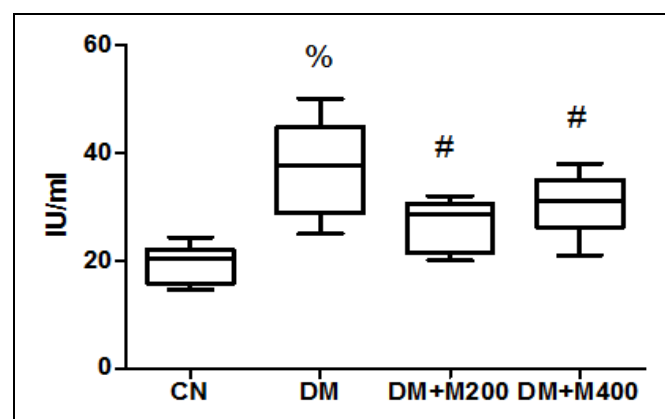


FIG. 2: EFFECT OF ETHANOLIC EXTRACT OF AZADIRACHTA INDICA (200 AND 400 mg/kg b.w) ON THE SERUM GLUTAMIC PYRUVATE TRANSAMINASE LEVELS IN ALLOXAN INDUCED MICE

Values are the means \pm S.D for six animals in each group. Values are significant at $p<0.001$, statistical significance was compared within groups as follows. % Diabetic mice were compared with normal mice. # DM + M₂₀₀ and DM + M₄₀₀ treated diabetic mice were compared with diabetic mice

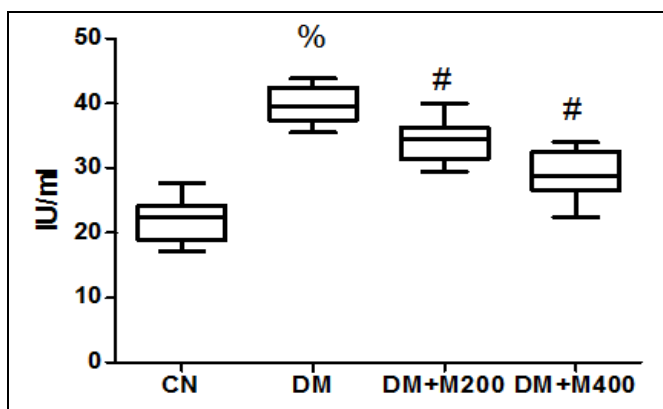


FIG. 3: EFFECT OF ETHANOLIC EXTRACT OF AZADIRACHTA INDICA (200 AND 400 mg/kg b.w) ON THE SERUM GLUTAMATE OXALOACETATE TRANSAMINASE LEVELS IN ALLOXAN INDUCED MICE

Values are the means \pm S.D for six animals in each group. Values are significant at $p < 0.001$, statistical significance was compared within groups as follows. % Diabetic mice were compared with normal mice. # DM + M₂₀₀ and DM + M₄₀₀ treated diabetic mice were compared with diabetic mice

Serum Alkaline Phosphates: The estimation of serum alkaline phosphates level in control group revealed its level to be 5.26 ± 1.12 KA with individual level variations range from 3.89 to 6.84 KA. In alloxan treated diabetic group, the mean value was 11.70 ± 1.23 KA with individual level variations range from 9.84 to 13.45 KA whereas in extract treated at dose of 200 mg/kg b.w, the mean value was 9.20 ± 1.29 KA of the values ranging from 7 to 11.23 KA and at dose of 400 mg/kg b.w, its mean value was 5.67 ± 1.07 KA with ranging from 4 to 7.09. This showed a significant increase ($p = 0.0002$, $t = 10.04$) when compared with control group, however, the decline in extract treated level was significant ($p = 0.0077$, $t = 4.29$ and $p = 0.0006$, $t = 7.75$) as compared to alloxan treated group (Fig. 4).

Serum Bilirubin Levels: Bilirubin level of control mice was observed to be 0.48 ± 0.093 mg/dl (values ranging between 0.37 to 0.65) which got increased to 0.98 ± 0.272 mg/dl in alloxan induced diabetic mice. Bilirubin contents ranged from 0.44 to 1.20 ($p = 0.0024$, $t = 5.65$) in diabetic mice. However, after the treatment of diabetic mice with the leaf extract of *Azadirachta indica* at the dose of 200 and 400 mg/kg b.w, the bilirubin level decreased down to the mean value of 0.86 ± 0.253 and 0.69 ± 0.203 ($p = 0.0159$, $t = 3.57$ and $p = 0.0133$, $t = 3.74$) having values ranging from 0.40 to 1.10 mg/dl, 0.44 to 1.00 mg/dl. These variations along

with statistical significance are depicted by box-plot as shown in Fig. 5.

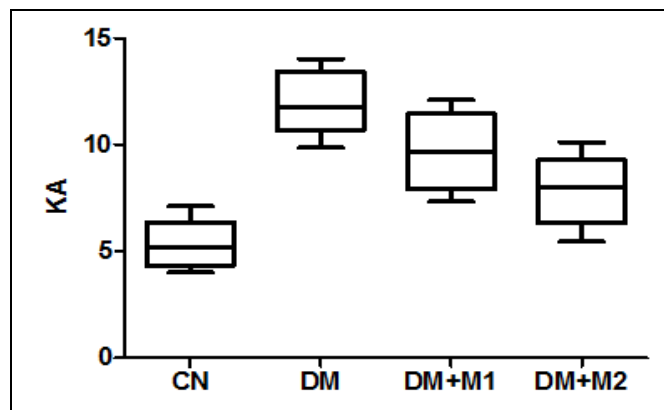


FIG. 4: EFFECT OF ETHANOLIC EXTRACT OF AZADIRACHTA INDICA (200 AND 400 mg/kg b.w) ON THE SERUM ALKALINE PHOSPHATES LEVELS IN ALLOXAN INDUCED MICE

Values are the means \pm S.D for six animals in each group. Values are significant at $p < 0.001$, statistical significance was compared within groups as follows. % Diabetic mice were compared with normal mice. # DM + M₂₀₀ and DM + M₄₀₀ treated diabetic mice were compared with diabetic mice

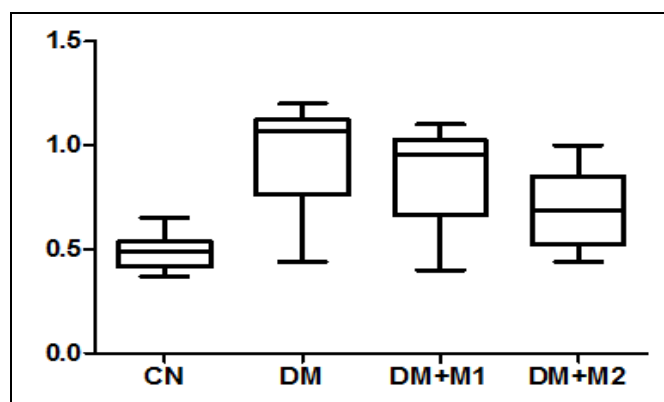


FIG. 5: EFFECT OF ETHANOLIC EXTRACT OF AZADIRACHTA INDICA (200 AND 400 mg/kg b.w) ON THE SERUM BILIRUBIN LEVELS IN ALLOXAN INDUCED MICE

Values are the means \pm S.D for six animals in each group. Values are significant at $p < 0.001$, statistical significance was compared within groups as follows. % Diabetic mice were compared with normal mice. # DM + M₂₀₀ and DM + M₄₀₀ treated diabetic mice were compared with diabetic mice

Effect of Antioxidant Activity:

Serum Lipid Peroxidation Activity: The estimation of serum lipid peroxidation level in control group revealed its level to be 1.18 ± 0.12 nmole/ml with individual level variations range from 1.01 to 1.35 mg/dl. In alloxan treated diabetic group, the mean value was 2.10 ± 0.25 nmole/ml with individual level variations range from 1.67 - 2.38 nmole/ml whereas in extract treated at dose of 200 mg/kg

b.w, the mean value was 1.69 ± 0.41 nmole/ml of the values ranging from 1.21 to 2.34 nmole/ml and at dose of 400 mg/kg b.w, its mean value was 1.29 ± 0.13 nmole/ml with ranging from 1.10 to 1.49. This showed a significant increase ($p=0.0002$, $t=9.72$) when compared with control group, however, the decline in extract treated level was significant ($p=0.0461$, $t=2.63$ and $p=0.0006$, $t=7.75$) as compared to alloxan treated group (Fig. 6).

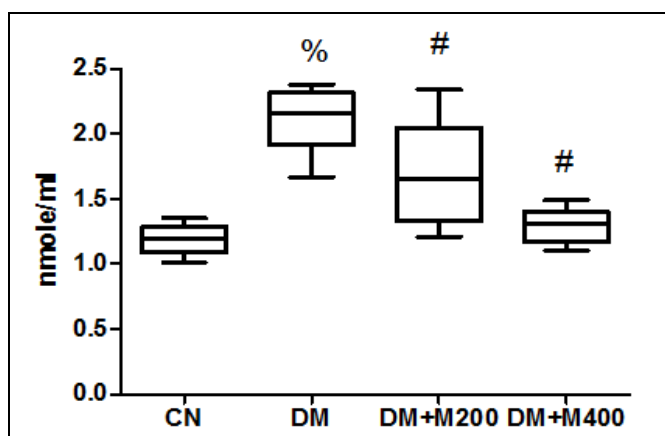


FIG. 6: EFFECT OF ETHANOLIC EXTRACT OF *AZADIRACHTA INDICA* (200 AND 400 mg/ kg b.w) ON THE SERUM LIPID PEROXIDATION ACTIVITY IN ALLOXAN INDUCED MICE

Values are the means \pm S.D for six animals in each group. Values are significant at $p<0.001$, statistical significance was compared within groups as follows. % Diabetic mice were compared with normal mice. # DM + M₂₀₀ and DM + M₄₀₀ treated diabetic mice were compared with diabetic mice

Superoxide Dismutase (SOD): SOD level of control mice was observed to be 6.15 ± 1.07 nmol/mg protein (values ranging between 4.40 - 7.78) which got decreased to 3.96 ± 0.58 nmol/mg protein in alloxan induced diabetic mice. SOD contents ranged from 3.23 - 5.00 ($p=0.0016$, $t=6.16$) in diabetic mice. However, after the treatment of diabetic mice with the leaf extract of *Azadirachta indica* at the dose of 200 and 400 mg/kg b.w, the SOD level increased to the mean value of 5.21 ± 0.79 and 5.60 ± 1.09 ($p=0.0098$, $t=4.055$ and $p=0.0451$, $t=2.657$) having values ranging from 4.23 - 7.10 nmol/mg protein, 4.20 - 6.34 nmol/mg protein. These variations along with statistical significance are depicted by box-plot as shown in Fig. 7.

Catalase: Catalase level of control mice was observed to be 72.00 ± 4.40 nmol/mg protein (values ranging between 60 to 85) which got decreased to 48.83 ± 2.35 nmol/mg protein in

alloxan induced diabetic mice. Catalase contents ranged from 35 - 59 ($p=0.0068$, $t=4.44$) in diabetic mice. However, after the treatment of diabetic mice with the leaf extract of *Azadirachta indica* at the dose of 200 and 400 mg/kg b.w, the serum catalase level increased to the mean value of 53.41 ± 7.87 and 65.00 ± 3.66 ($p=0.1612$, $t=1.643$ and $p=0.0421$, $t=2.713$) having values ranging from 45 to 65 nmol/mg protein, 55 to 80 nmol/mg protein. These variations along with statistical significance are depicted by box-plot as shown in Fig. 8.

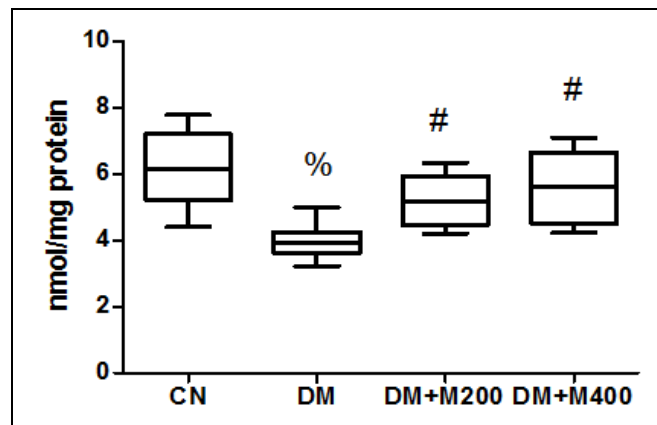


FIG. 7: EFFECT OF ETHANOLIC EXTRACT OF *AZADIRACHTA INDICA* (200 AND 400 mg/ kg b.w) ON THE SUPEROXIDE DISMUTASE (SOD) LEVELS IN ALLOXAN INDUCED MICE

Values are the means \pm S.D for six animals in each group. Values are significant at $p<0.001$, statistical significance was compared within groups as follows. % Diabetic mice were compared with normal mice. # DM + M₂₀₀ and DM + M₄₀₀ treated diabetic mice were compared with diabetic mice

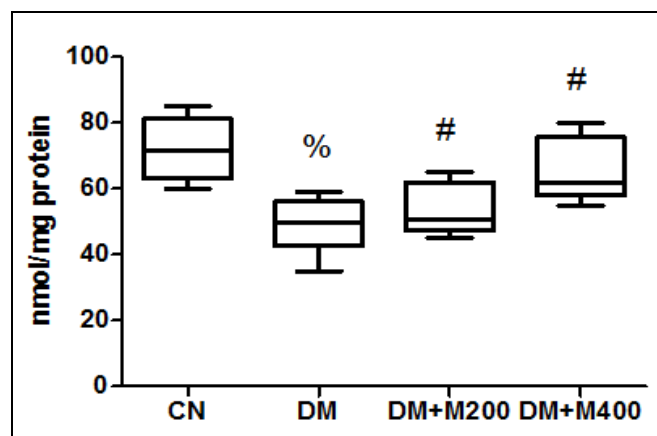


FIG. 8: EFFECT OF ETHANOLIC EXTRACT OF *AZADIRACHTA INDICA* (200 AND 400 mg/ kg b.w) ON THE SERUM CATALASE LEVELS IN ALLOXAN INDUCED MICE

Values are the means \pm S.D for six animals in each group. Values are significant at $p<0.001$, statistical significance was compared within groups as follows. % Diabetic mice were compared with normal mice. # DM + M₂₀₀ and DM + M₄₀₀ treated diabetic mice were compared with diabetic mice

Histopathological Study:**Morphological Findings of Light Microscopy:**

Microscopically examined liver section showed normal central vein with prominent small-sized nuclei, with the hepatocytes well arranged in sinusoids. (Fig. 9) However, in the diabetic group showed abnormal central vein with a relatively large sized nuclei also the hepatocytes are not well arranged in sinusoids in the diabetic mice (Fig.10). In the treated group showed architecture of central vein and hepatocytes toward normal and there was no evidence of inflammation (Fig. 11).

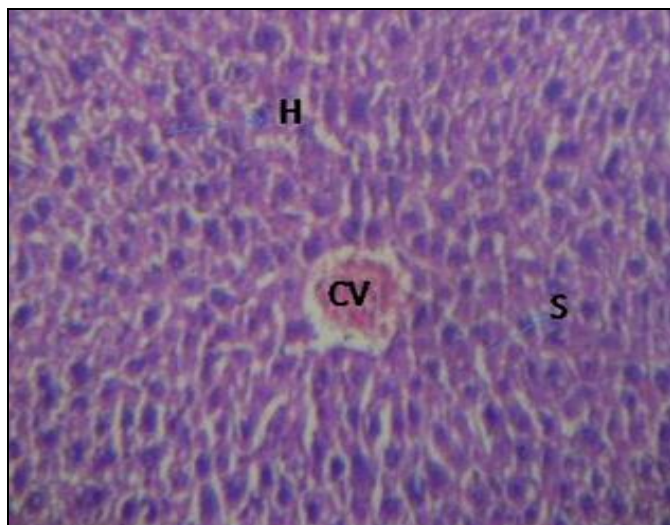


FIG. 9: IN CONTROL MICE LIVER SECTION SHOWED NORMAL STRUCTURE OF CENTRAL VEIN (CV), HEPATOCYTES (H) WELL PRESERVED AND ESSENTIALLY NORMAL, CYTOPLASM NOT VACUOLATED, SINUSOID(S) WELL DEMARCATED (H AND E x 200)

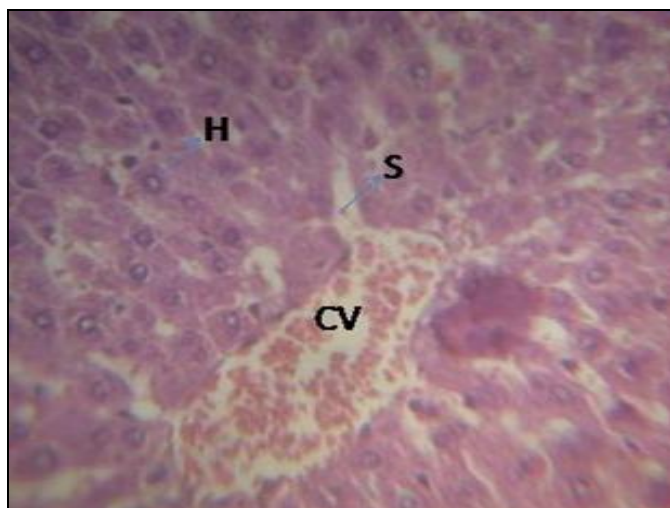


FIG. 10: IN DIABETIC MICE LIVER SECTION SHOWED ABNORMAL STRUCTURE OF CENTRAL VEIN (CV), SINUSOID (S) AND HEPATOCYTE(H) ARE NOT ARRANGED IN SINUSOID. CYTOPLASMA IS VACUOLATED (H AND E x 200)



FIG. 11: IN AZADIRACHTA INDICA MICE LIVER SECTION SHOWED TOWARDS NORMAL STRUCTURE OF CENTRAL VEIN (CV), SINUSOID (S) AND ALSO ARRANGED HEPATOCYTE(H) IN SINUSOID. CYTOPLASM NOT VACUOLATED (H AND E x 200)

Ultra Structural Findings of Transmission Electron Microscopy:

Ultra structural Findings of liver tissues showed the significant differences between control and diabetic, in control mitochondria (m) and nucleus (N) are arranged well but in diabetic condition nucleus has ruptured and size of the rough endoplasmic reticulum (rER) changed. After treatment of extract on diabetic animal's nucleus is going on towards normal, mitochondrial has well in shaped as well as rough endoplasmic reticulum (Fig. 12 - 14).

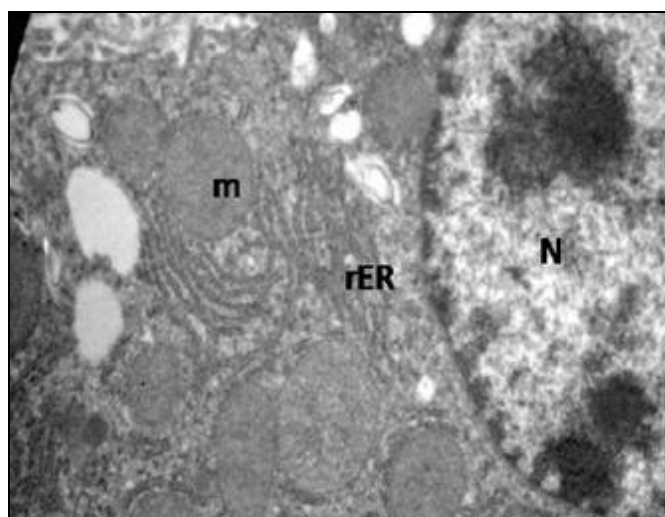


FIG. 12: ELECTRON MICROGRAPHS OF CONTROL GROUP SHOWING NORMAL - APPEARING OF HEPATOCYTES, NUCLEUS (N) MITOCHONDRIA (M) AND ROUGH ENDOPLASMIC RETICULUM (RER) WITH THEIR PRESERVED STRUCTURES. (14000X)

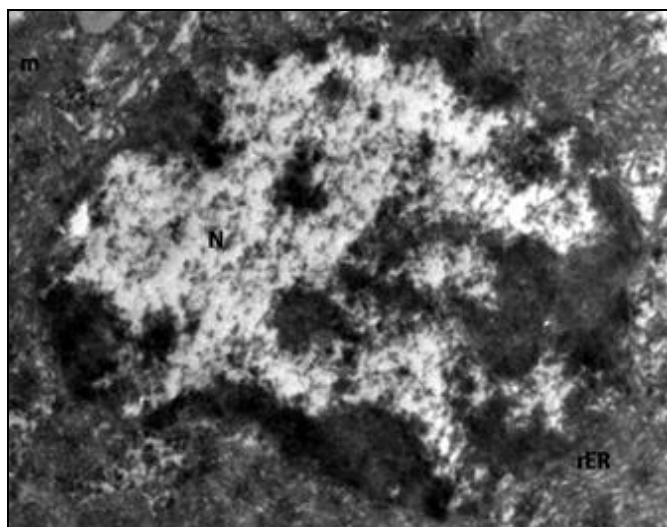


FIG. 13: ELECTRON MICROGRAPHS OF DIABETIC GROUP SHOWING DEGENERATION IN NUCLEUS (N), MITOCHONDRIA (M) AND ROUGH ENDOPLASMIC RETICULUM (RER) AND ABNORMAL HEPATOCYTES. (14000X)

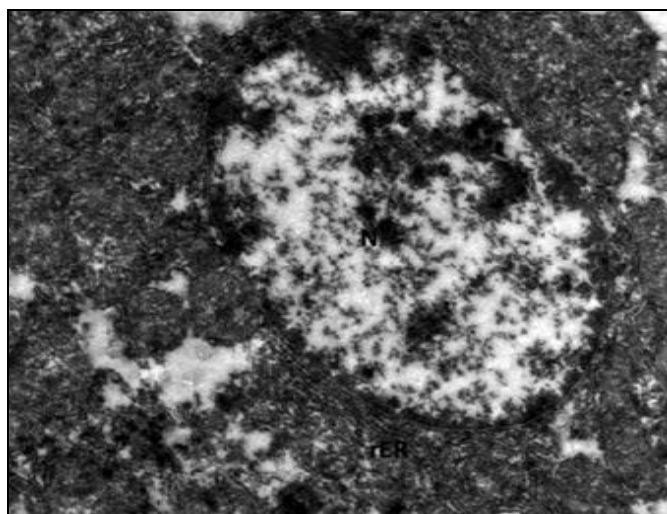


FIG. 14: ELECTRON MICROGRAPHS OF EXTRACT TREATED GROUP SHOWING WELL STRUCTURE OF NUCLEUS (N), MITOCHONDRIA (M) AND ROUGH ENDOPLASMIC RETICULUM (RER). (14000X)

DISCUSSION: There are various pathways and mechanisms involved in the demonstration of diabetes, which cannot be cured by a single drug. The preparations of medicinal have contained a variety of herbal and non-herbal constituents which act on targets by various modes and mechanisms. In the present investigation alloxan was used to prepare diabetic model and serum glucose level was found to be significantly higher than in normal albino mice. Alloxan causes a massive reduction in insulin release by the destruction of β -cells of the islets of Langerhans and thereby induces hyperglycaemia¹⁷. On the other hand, treatment

with ethanolic extract of *Azadirachta indica* showed significant anti-hyperglycemic activity. The reduction in glucose levels was observed in groups receiving 200 and 400 mg/kg of the extract for four weeks.

These reductions due to reported mechanisms of hypoglycaemic effect of *Azadirachta indica* include inhibition of adrenalin-induced glycogenolysis and an insulin tropic effect¹⁸. Similar reported, there are possible mechanisms of hyperglycemia action of *azadirachta indica* which was observed during the present work as well as in the previous studies, the first mechanisms may be attributed to the blocking of *Azadirachta indica* action on epinephrine, this suggestion is accordance with¹⁹ because epinephrine has been reported to induced hyperglycemia due to the dual action on carbohydrate metabolism and its causes increased liver glycogenolysis and reduction in peripheral utilization of glucose and another postulated that mechanism may be due to blocking impact of *azadirachta indica* on the inhibitory effect of serotonin on insulin secretion²⁰. On these outcomes, we have selected the glucose-induced hyperglycaemic model to screen the anti-hyperglycaemic activity of the plant extracts.

In this study has revealed that changes in body weight and organs weight in control and treated animals for the entire period of the study. As decrease in body weight is considered as a marker for the development of diabetes, these changes has occurred due to continuous excretion of glucose and decrease in peripheral uptake of glucose and glycogen synthesis²¹. This increase in body weight of diabetic mice as a result of ethanolic extract treatment may be ascribed to the increase in insulin release.

In this study demonstrated that Swiss albino mice with type-1 diabetes mellitus induced by i.p alloxan presented biochemical changes in liver marker enzymes, the level of SGOT, SGPT, bilirubin and ALP level were significantly increased in alloxan induced diabetic mice in comparison with normal mice²². The increase in the activities of these serum enzymes indicated that diabetes might induce hepatic dysfunction and reflect active liver damage. Therefore, the increase in AST, ALT, and ALP activity may be mainly due to the leakage of

these enzymes from the liver cytosol into the blood serum, which gives an indication of the hepatotoxic effect of alloxan²³. Furthermore, after treatment with ethanolic extract of *Azadirachta indica* is in parity with findings with other plants reported by other workers²⁴⁻²⁵.

In the histological section of liver observed that the portal areas, sinusoids and hepatocytes, nuclei and intra cytoplasmic organelles were degenerated. Further, ultra structural changes in hepatocytes, particularly the mitochondria, the rough endoplasmic reticulum (rER) and cell nuclei were also observed. Our results similar to earlier reported²⁶ who reported that morphological changes in the liver of STZ treated animals. An increased number of intra cytoplasmic acidophilus granules and bile duct hyperplasia. However, treatment of alcoholic extract for 28 days, ultra structure of liver towards normal.

We observed a decrease in antioxidant enzymes (SOD and CAT) with elevation of lipid peroxidation in alloxan induced diabetic mice. The cellular tissue damage has occurred due to chronic hyperglycemia and its effect on the structures and functions of an organ during hyperglycemia. These changes may be due to the glucose oxidation and formation of free radical generation and nitric oxide donor property of alloxan²⁷. Administration of *Azadirachta indica* significantly increased SOD and CAT level in diabetic mice, this increased activity due to its free radical scavenging potential. Lipid peroxides are the secondary products of oxidative stress and result of the toxic effect of reactive oxygen species produced during lipid peroxidation in diabetes²⁸.

During this study, elevated levels of lipid peroxidation were noticed in alloxan treated mice. There are several reports in the literature that demonstrated the elevated levels of lipid peroxides in the alloxan induced diabetes²⁹. Administration of extract on diabetic mice, the level of lipid peroxidation decreased.

Our current investigation suggested *Azadirachta indica* possesses antidiabetic and antioxidant activity, one or more antidiabetic compounds in the plant extract improve the physiology of mice affected by type 1 diabetes and bioactive constituents responsible for improving the

physiology of type 1 diabetic mice need to be isolated and characterized to contribute to better treatment of diabetes mellitus.

CONCLUSION: The present study infers that *Azadirachta indica* leaf demonstrated remarkable anti-hyperglycemic activity in alloxan-induced diabetic mice. The potential anti-hyperglycemic action is plausibly due to its underlying antioxidant role.

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