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RESEARCH ARTICLE

Evaluation of the Antidiabetic Effects of Methanolic Extracts of Neem (*Azadirachta indica*) Seeds on the Streptozotocin-induced Wistar Rats

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A B S T R A C T

Plants are among the most prominent candidates in herbal medicine. Vast research is being performed for effective plants against diabetes. This experiment aimed to evaluate the effects of administering the methanolic extract of Neem (*Azadirachta indica*: MEN) at different concentrations, 150, 250 and 350 mg/kg body weight orally on the serum and body weight parameters of diabetic rats, and to compare these results with those of the standard medicated and non-diabetic control groups. The MEN and controls underwent a 28-day therapy, during which the impact on liver function parameters and serum glucose levels was observed. The estimated parameters included alanine aminotransferase, aspartate transferase, serum cholesterol, glucose, uric acid, urea, triglycerides, and creatinine. The results showed that the effects of the MEN were in a dose-dependent manner. There was a significant ($p<0.05$) increase in weight gain and a decrease in glucose levels in the treated groups. The lipid profile and liver and kidney function-related serum parameters remained normal in the groups treated with MEN at a dose rate of 350 mg/kg. The 350 mg/kg concentration results were comparable ($p>0.05$) to the standard medicated group. The findings of the study revealed that the administration of methanolic extract of Neem at a dose rate of 350 mg/kg can effectively control hepatic damage and serum parameters. Considering the findings from this research, it can be suggested that the methanolic extract of Neem seeds warrants further investigation as a potential antidiabetic agent. One important step in this process would be to identify the specific components of the extract that contribute to its effectiveness. Further research should be conducted to determine the optimal therapeutic dose of the methanolic extract of Neem seeds.

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INTRODUCTION

Diabetes is among the most threatening diseases affecting a great variety of species, including humans, canines, felines, and multiple other species globally (Huneif *et al.*, 2022). It may be a genetic abnormality or may be developed because of metabolic, nutritional, and other reasons (Choudhury and Rajeswari, 2021). Diabetes is present globally and the World Health Organization has reported an increase in mortalities because of diabetes in humans globally (Choudhury and Rajeswari, 2021). Veterinary diabetes is an under-reported problem because most pets remain subclinical or not cared for too much (Bakker and de la Garza, 2022). However, in Europe, it has been reported that the tendency of diabetes development in dogs and cats may be higher than in other animals (Burggraaf *et al.*, 2021). If untreated, diabetes can lead to severe complications that impede the functioning of the liver and other vital organs, especially kidneys and the cardiovascular system. The increased blood glucose, observed in diabetes, leads to altered blood pressure, changed renal functions, bradycardia and cardiac arrest (Oršolić *et al.*, 2021; Yu *et al.*, 2021). Hyperglycemia (increased blood glucose) may lead to increased risks of infectious diseases because of the facilitation of nutrition of bacteria inside the blood (Tang *et al.*, 2023).

Scientists have tried to develop effective control to manage diabetes so that a permanent solution may be developed to control diabetes (Choudhury *et al.*, 2020). Efforts have been made to improve gene therapy, so that we may control the production deficiencies of insulin as observed in diabetes type 1 (Akil *et al.*, 2021). Multiple other therapies, including insulin therapy, have been designed for the control and management of diabetes (Khursheed *et al.*, 2019). The treatment protocols for the treatment of diabetes in humans and pets are applied similarly i.e., the insulin therapy and nutritional strategies for the management of hyperglycemia and hyperglycosuria (Moshref *et al.*, 2019). The management of diabetes needs substances that can control glucose levels in the blood, urine and cholesterol levels of the body (Dwivedi and Pandey, 2020). Pharmaceuticals or nutraceuticals with hepatoprotective, hypoglycemic, and cholesterol-managing effects are considered the most suitable for the control of diabetes (Modi *et al.*, 2023).

Plants have always been a great candidate for providing nutraceuticals with their role in managing disease, improving body conditions, and showing multiple other medicinal effects (Morshdy *et al*., 2021; Moryani *et al*., 2021; Faheem *et al.*, 2022; Shazmeen *et al.*, 2022). Multiple plants have been reported to contain substances that have hepatoprotective and hyperglycemic effects which help to reduce diabetes (Murtaza *et al*., 2021; Mushtaq *et al*., 2021; Sok *et al.*, 2021; Mobasheri *et al.*, 2023). Multiple plants and plant products have been reported to contain antidiabetic effects in various studies (Bhatia *et al.*, 2019; Muhammad *et al.*, 2021; Faiza *et al*., 2022).

Azadirachta indica (Neem) is a well-known plant belonging to the family Meliaceae (Latif *et al.*, 2020). Neem is famous for its biological and medicinal effects (Ahmad *et al.*, 2019). Various preparations of neem have been reported to contain multiple compounds that are useful in controlling and managing diseases (Pradhan *et al.*, 2022). The proportion of effective compounds varies because of the solvent and methods used for extraction from a single plant (Abubakar and Haque, 2020), so various preparations of plants are investigated to achieve effective preparation for the control of diabetes (Dey *et al.*, 2020). Previously, multiple preparations containing neem have been reported to have antidiabetic effects (Ezeigwe *et al.*, 2020a; Mohammed *et al.*, 2023; Nguyen *et al.*, 2023).

In this research, we have conducted trials to estimate the antidiabetic potential of methanolic extract of Neem seeds in the rat model to discover the effects of methanolic extract of Neem (MEN) on the control of diabetes. The study contained serum parameters of aspartate transferase, alanine aminotransferase, creatinine, urea, serum cholesterol and serum glucose. The hypoglycemic and hepatoprotective effects were analyzed.

MATERIALS AND METHODS

Animals: In this research work, the male rats of the Wistar breed weighing 150 to 200g were used. Housing of the rats was done in groups, each having 10 rats per cage with an average space provided to each rat being 850cm^2 with a height of 25 centimeters. Rats were fed Laboratory feed *ad libitum* containing proteins at 27%, fats at 10%, carbohydrates at 33% and vitamins at 4% approximately. The room temperature was adjusted between 20-25°C and a relative humidity of 50-60% with 12 hours of light provided to them. All the animal ethics guidelines were followed during the experiment (Prager *et al*., 2011).

Extract preparation: The seeds of neem were collected from the verified source and identified by the botanist. Seeds were dried in the sunlight to remove any moisture and ground into powder. Extraction was performed in the Soxhlet apparatus by placing the seeds in the extraction chamber using methanol as a solvent. After extraction, filtration was done, and the extract was dried to achieve a yield of 12%. The dried extracts were placed at -4°C for the research (Ishaq *et al*., 2022).

Induction of diabetes in the rats: For the induction of diabetes in the rats, we injected an aqueous solution of streptozotocin at 60 mg/kg body weight (intraperitoneally). Fasting glucose levels were measured before the administration of the injection. The fasting glucose levels of rats were observed at 3, 7, and 10-day intervals to determine the fasting glucose levels of blood. On the 10th day, the rats exhibiting a glucose level of more than 250 mg/dL were selected for the induction of treatments (Gajdosik *et al*., 1999).

Experimental Design: The random division of the 150 rats was done into 5 groups, labeled with letters A to E, each having 3 replicates with 10 rats in each replicate. Group A contained diabetic rats with no medication, served as a negative control. Groups B, C, and D had diabetes and received a methanolic extract of Neem (MEN) at 150, 250, and 350 mg/kg. Group E contained diabetic rats and received an oral dose at a rate of 10 mg/kg body weight of Glibenclamide as a standard control, while group F was non-diabetic, non-medicated served as control. All the treatments started 10 days' post streptozotocin (considered as day 0 of treatment). The experiment continued for 4 weeks, and the observations were recorded. The blood samples were collected humanely from the direct cardiac injection.

Parameters

Weight gain: Weekly weights were observed in the rats during the experiment and the weight gains and percentage of body weights were measured at the end of the experiment by the following formula (Oyedemi *et al*., 2011).

$$\% \text{ Weight Gain} = ((\text{Initial weight-final weight})/\text{Final weight}) \times 100$$

Glucose levels: The glucose levels were estimated on a weekly basis from the day of the start of therapy to the 28th day of the experiment using a Glucometer (OnCall® *Ez II;* S. no. 303S0014E09) (Muzaffar *et al*., 2019).

Lipid profile of the rats: Serum collected from the rats was used for the estimation of the lipid profile of the rats on the 28th day of the experiment. Cholesterol, low-density lipids (LDL), very-low-density lipids (VLDL), high-density lipids (HDL), cholesterol, and glycoproteins were estimated using the spectrophotometric kits (Gobinath *et al.,* 2022).

Table 1: Lipid profile comparison of methanolic extract treated diabetic Wistar rats compared to control groups.

Groups Parameters	VLDL	LDL	HDL	Cholesterol	Triglycerides
A	43.33 ± 2.08^{a}	105.66 ± 4.04^{a}	18.00±2.00^{a}	183.66±18.03^{a}	170.66±4.04^{a}
B	37.00±2.00^{b}	97.33±2.51^{a}	20.00±2.00^{a}	132.66±2.08^{b}	156.66±12.58^{a}
C	30.66±2.08^{c}	78.33±13.31^{b}	24.33±2.08^{a}	114.0±9.64^{b}	136.66±7.63^{b}
D	22.66±2.08^{d}	45.66±2.08^{c}	32.66±2.51^{b}	87.66±6.42^{c}	100.0±5.00^{c}
E	19.00±1.00^{de}	43.00±2.64^{c}	34.33±1.52^{bc}	86.00±3.60^{c}	101.66±2.88^{c}
F	16.33±1.52^{e}	37.33±2.51^{c}	36.66±0.57^{c}	74.00±6.55^{c}	97.33±2.51^{c}

Groups with similar superscripts within a column are statistically comparable (p>0.05): A: Control group of Diabetic rats; B: Diabetic rats receiving the methanolic extract of Neem @ 150 mg/kg; C: Diabetic rats receiving the methanolic extract of Neem @ 250 mg/kg; D: Diabetic rats receiving the methanolic extract of Neem @ 350 mg/kg; E: Diabetic rats and receiving an oral dose @10 mg/kg body weight of Glibenclamide as a standard control; F: Non-medicated nondiabetic Wistar rats.

Liver and renal function assays: Liver function enzymes, Alanine aminotransferase (ALT), Aspartate transferase (AST), Alkaline phosphatases (ALKP), bilirubin, globulins, albumins, albumin to globulin (A/G) ratio, urea, uric acid, creatinine, were estimated using spectrophotometric methods (Gobinath *et al*., 2022).

Statistical analysis: All the data were recorded in Microsoft Excel® and analyzed for statistical analysis in Minitab® software. One-way Analysis of Variance (ANOVA) was used for statistical variance and mean comparisons were done by the Tuckey Post-Hock tests. All the means were significant statistically at a confidence interval of 95% ($p \leq 0.05$).

RESULTS

Effects of weight gain: The weights of all the groups were recorded every week and statistical comparisons were made. The results revealed that the effects of MEN were in a dose-dependent manner. Wistar rats receiving MEN at 350 mg/kg had comparable ($p>0.05$) values for rats receiving the standard medication (Table 1).

Effects on glucose levels: The effect of the treatments was compared on the glucose levels of the Wistar rats weekly and the results were collected. The results of all the groups were compared. The data suggested that the effects of the MEN were in a dose-dependent manner and the MEN @ 350 mg/kg had a glucose-lowering efficiency comparable ($p>0.05$) to standard medicated control (Fig. 2).

Effects on lipid profile: Cholesterol, LDL, HDL, VLDL, and Glycoproteins were observed, and statistical comparisons were performed. The results of all the groups were compared. The data suggested that the effects of the MEN were in a dose-dependent manner and the MEN @ 350 mg/kg had a lipid-reducing efficiency comparable ($p>0.05$) to standard medicated control (Table 1).

Effects on liver and renal function parameters: Liver function parameters ALKP, AST, ALT, Bilirubin, Creatinine, Urea, Albumins, Globulins, TSP, and A/G ratios were determined to evaluate the effects of MEN as hepatoprotective in Wistar rats. Statistically comparable ($p>0.05$) results were observed for the standard medicated control and the MEN @ 350 mg/kg concentration (Fig. 3).

DISCUSSION

Diabetes is a metabolic disorder that may cause several issues in humans and pets (Khursheed *et al.*, 2019). Diabetes causes several complications, including hyperglycemia, hyperglycosuria, renal impairment, disturbance in liver functions, immune system imbalance, and oxidative stress in the affected patients (Martín-Carro *et al.*, 2023). Multiple therapies are suggested to control diabetes by maintaining the blood glucose levels in a normal position, hepato-protective action management, and maintenance of renal functions (Singh *et al.*, 2021). Plant extracts have always been a substantial source of medicinal substances and remained under consideration by researchers because of their activities (Najmi *et al.*, 2022). Neem (*A. indica*) is a plant with great medicinal properties, including antioxidant, hepatoprotective, and immunomodulatory activities (Kharwar *et al.*, 2020). Neem has been used for the treatment of diabetes in research in multiple forms by researchers (Jalil *et al.*, 2013; Ezeigwe *et al.*, 2020b; Pingali *et al.*, 2020; Patil *et al.*, 2022; Abdullah *et al.*, 2023; Nguyen *et al.*, 2023).

In this experiment, we used a methanolic extract of Neem (*A. indica*; MEN) to treat diabetes in streptozotocin-induced diabetic rats. The experiment contained 3 different levels of MEN (150, 250, and 350 mg/kg body weight) and compared to diabetic non-medicated, diabetic standard medicated, and nondiabetic non-medicated trials. The results were analyzed by Analysis of Variance and Tuckey tests as post hoc mean comparison tests. Multiple other researchers have conducted similar studies using herbal extracts as antidiabetic agents using a rat model for the experiment (Airaodion *et al.*, 2019; Yazid *et al.* 2020; Samadi-Noshahr *et al.,* 2021). This was a pioneer study featuring the methanolic extract of Neem for the control of diabetes in rats.

Neem is a popular plant to be searched for medicinal properties (Pingali *et al.*, 2020; Patil *et al.*, 2022). Neem and its extracts have been used to treat multiple metabolic and infectious disorder (Patil *et al.*, 2022). Because of its medicinal properties, multiple researchers have conducted trials on various preparations developed from various parts to check its effects on diabetes (Jalil *et al.*, 2013; Ezeigwe *et al.*, 2020b; Pingali *et al.*, 2020; Abdullah *et al.*, 2023; Nguyen *et al.*, 2023). Jalil *et al.* (2013) tried to investigate the effective compounds and compared the efficacy of various compounds of Neem and explained that the compounds of Neem have potential to control diabetes. They could not state the final selection for control of diabetes. Ezeigwe *et al.*, (2020a, b) also used the aqueous and methanolic extracts obtained from the leaves of Neem and stated that these extracts had efficacy but not to the parallel chemicals, and they stated that extracts with other parts and solvents may reach the therapeutic levels. Pingali *et al.* (2020) had almost the same effects using aqueous extracts of leaves and twigs

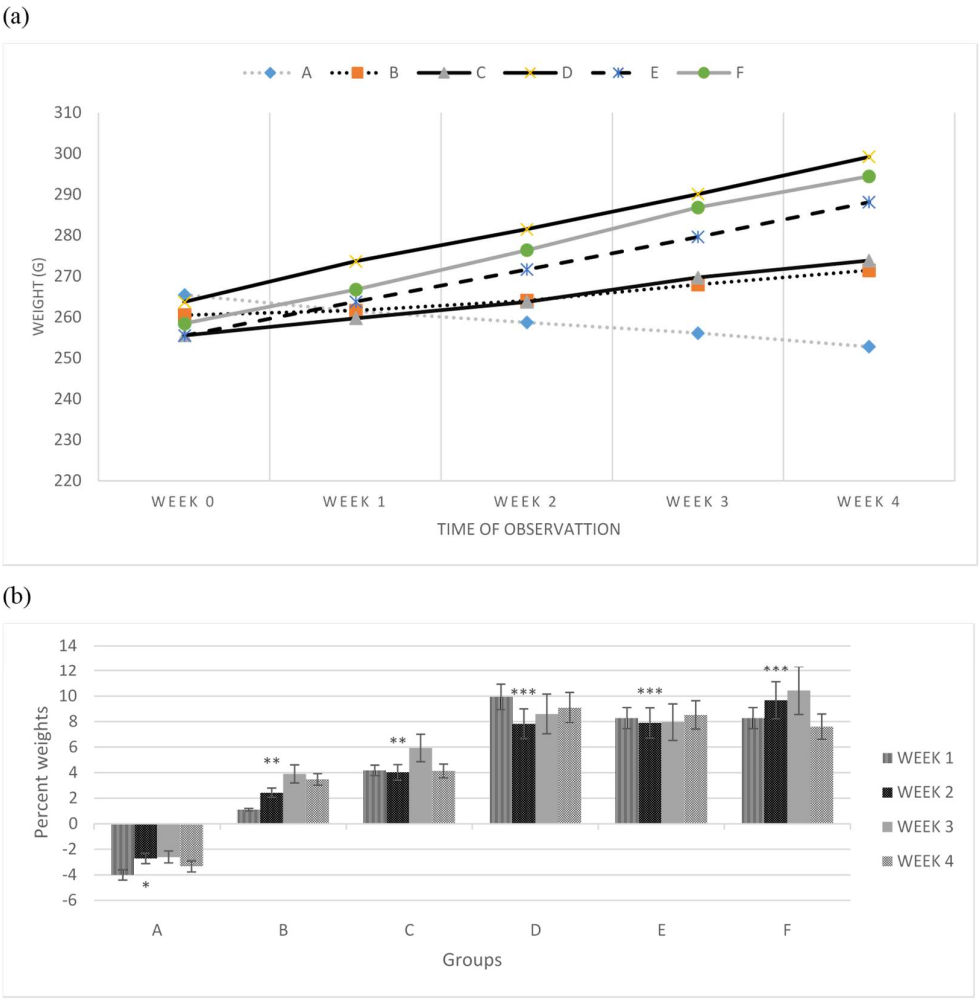

Fig. 1: (a) Week-wise weights of the Neem methanolic extracted treated Wistar rats compared to controlled groups; (b) Percent weekly weight gain of the Neem methanolic extracted treated Wistar rats compared to controlled groups: Groups with similar "*" signs are statistically comparable ($p>0.05$): A: Control group of Diabetic rats; B: Diabetic rats receiving the methanolic extract of Neem @ 150mg/kg; C: Diabetic rats receiving the methanolic extract of Neem @ 250mg/kg; D: Diabetic rats receiving the methanolic extract of Neem @ 350mg/kg; E: Diabetic rats and receiving an oral dose @10mg/kg body weight of Glibenclamide as a standard control; F: Non-medicated nondiabetic Wistar rats.

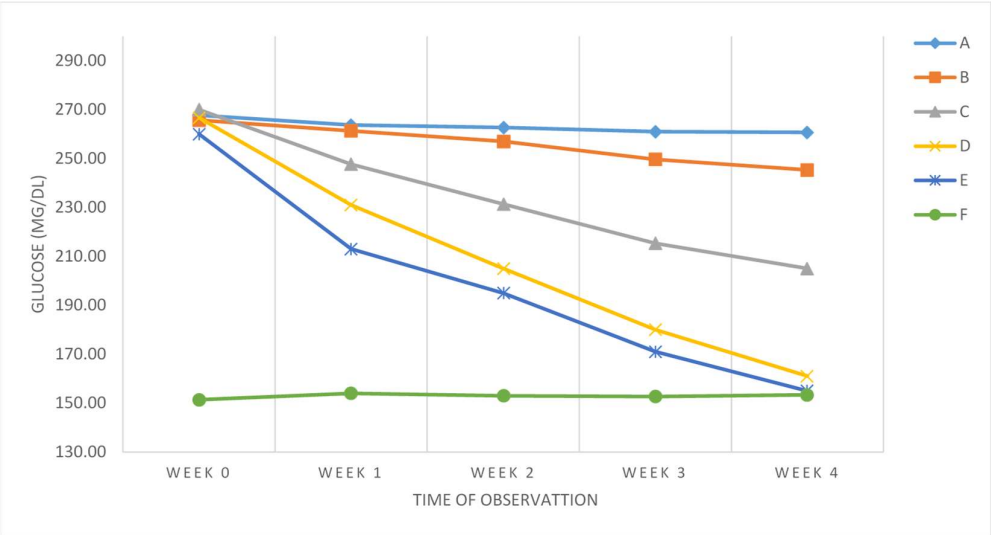

Fig. 2: The methanolic extract of Neem lowers the fasting glucose levels (mg/dL) every week: A: Control group of Diabetic rats; B: Diabetic rats receiving the methanolic extract of Neem @ 150mg/kg; C: Diabetic rats receiving the methanolic extract of Neem @ 250mg/kg; D: Diabetic rats receiving the methanolic extract of Neem @ 350mg/kg; E: Diabetic rats and receiving an oral dose @10mg/kg body weight of Glibenclamide as a standard control; F: Non-medicated nondiabetic Wistar rats.

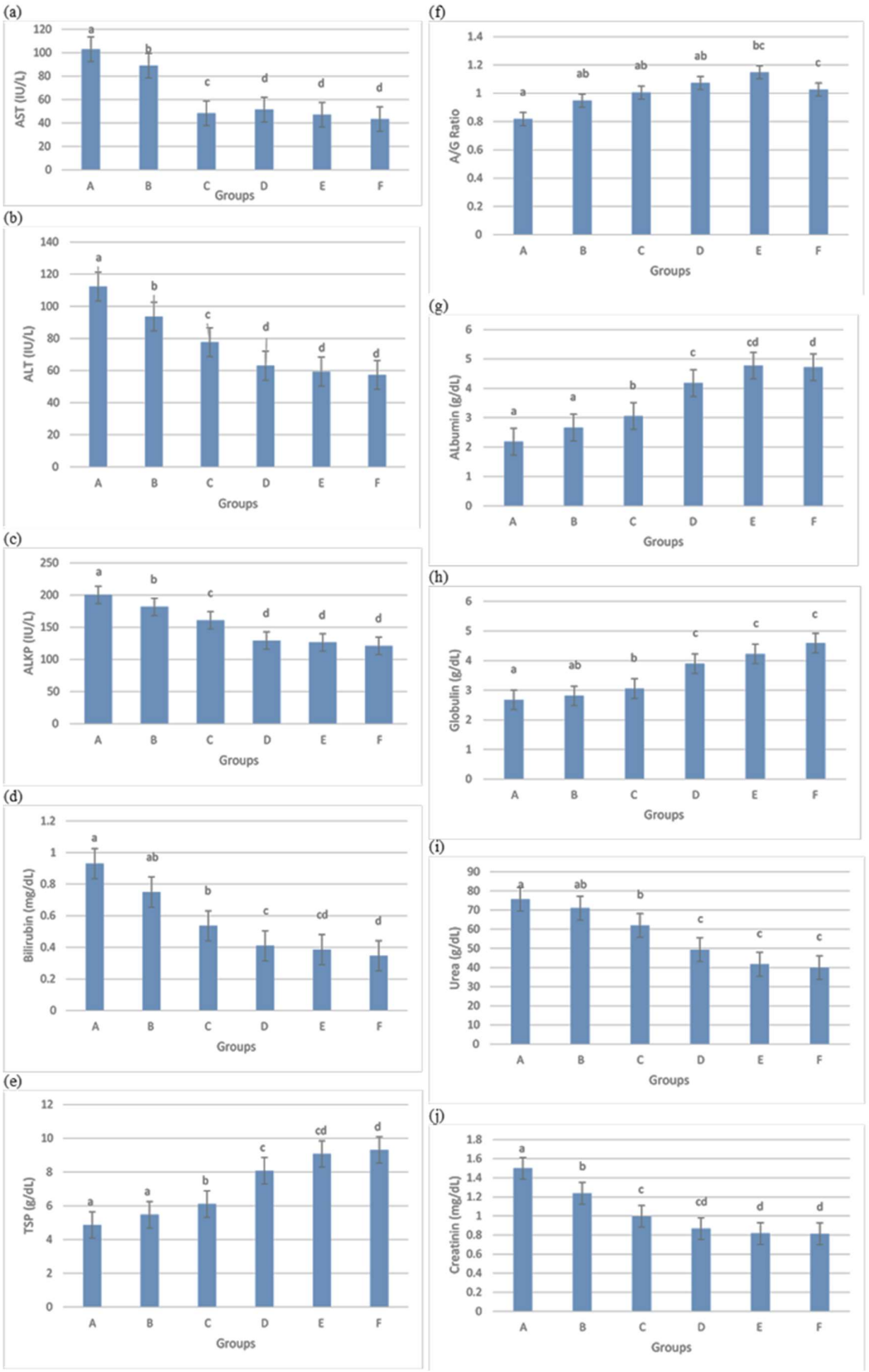

Fig. 3: Liver and serum parameters (a) ALT, (b) AST, (c) ALKP, (d) Bilirubin, (e) TSP, (f) A/G ratio(g) Albumin, (h) Globulin, (i) Urea, and (j) Creatinine of methanolic extract treated diabetic Wistar rats compared to the control groups: Groups with similar superscripts are statistically comparable ($p>0.05$): A: Control group of Diabetic rats; B: Diabetic rats receiving the methanolic extract of Neem @ 150mg/kg; C: Diabetic rats receiving the methanolic extract of Neem @ 250 mg/kg; D: Diabetic rats receiving the methanolic extract of Neem at 350 mg/kg; E: Diabetic rats and receiving an oral dose at 10 mg/kg body weight of Glibenclamide as a standard control; F: Non-medicated nondiabetic Wistar rats; ALT: alanine aminotransferase; AST: Aspartate transferase; ALKP; Alkaline phosphatase; TSP: total serum proteins; A/G ratio: Albumins to Globulins Ratio.

of Neem and stated that it could reduce the inflammation and glucose levels in comparison to placebo induces diabetic patients.

Body weight loss is among the most prominent signs of diabetes (Awuchi *et al.*, 2020). In type 2 diabetes, a decrease in weight is observed. The decrease in body weight is because of the faulty metabolism of glucose and its unavailability for use by the body (Shakib *et al.*, 2019). This problem causes the body to starve and a decline in weight gain is observed. In this experiment, similar results were observed when the diabetic rats had a sharp decline in body weight. MEN helped the rats to maintain their weight gain (Fig. 1). The treated groups showed a positive weight gain and had similar results to the standard medicated control group (Fig. 1).

Hyperglycemia is the major indicator of diabetes and is the principal factor in diabetes-related pathologies, so its management has prime importance in diabetes management (Ansaripour and Abbasi, 2022). MEN at 350 mg/kg was effective in controlling glucose. In this study, the effect on glucose levels was estimated, and found that MEN was effective in controlling the blood glucose levels, and comparable ($p>0.05$) results of the standard medicated control were achieved (Fig. 2). The results of our study fit in line with the results of the study of Yazid *et al.* (2020), who found that the *Vernonia amygdalina* plant was effective in reducing glucose levels in the rats. Airaodion *et al.* (2019) and Samadi-Noshahr *et al.* (2021) also suggested similar findings while working on *Carica papaya* and fennel seeds, respectively. the mechanism of action may include the involvement of phenolic compounds, which trigger multiple pathways leading to increased utilization of the sugar from the blood into tissues (El-Hadary and Ramadan, 2019).

The lipid profile is very important in diabetes because the elevated levels of cholesterols, glycoproteins and LDLs are increased in diabetes and their estimation can help to estimate the risks of diabetes (Sheikh and Gallehdari, 2023). HDLs are reduced in diabetes, leading to increased cardiovascular disease risks. The lipid profile is an economical and early tool for the detection of hepatic injuries, including diabetes (Shao *et al.*, 2022). MEN at 350 mg/kg showed positive effects on the reduction of lipid profile. The lipid profile of the diabetes-affected rats was also maintained in normal ranges by the MEN @ 350 mg/kg concentration. Cholesterol, LDL, VLDL, HDL, and glycoproteins were maintained in normal ranges (Table 1). Shao *et al.* (2022) conducted a trial and found that the herbal extract of *Acacia pennata* was highly effective in controlling the lipid profile of the rats. Multiple other authors have also claimed similar results in their studies (Gobinath *et al.*, 2022).

Liver and renal function profiles were also estimated, and the protective effects were estimated. ALT, ALKP, bilirubin, and AST are determinants for proper liver functions while Creatinine, and Urea, are the determinants for the function of kidneys. While Albumins, Globulins, A/G ration, and TP serve for both the functioning of kidneys and the liver. All these parameters were effectively controlled by the MEN @ 350 mg/kg body weight dose rate (Fig. 3). These effects were predictable because many other researchers have claimed similar results using herbal formulations (Mobasheri *et al.*, 2023). The hepatoprotective and renal function maintenance can be attributed to the active compounds that have been found effective in controlling hepatic and renal injuries.

Conclusions: This research concludes that the Methanolic extract of Neem can control diabetes because of its positive effects on weight gain, glucose levels, lipid profile, and hepatoprotective agents. The MEN @ 350 mg/kg can manage the pathological conditions produced by diabetes.

Authors contribution: Methodology and conceptualization by AMA and AEA; data analysis and write-up of the paper by AMA, MAB and AAA.

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