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Assessment of Antidiabetic Potential of Ethanolic Leaves Extracts from *Mangifera indica*, *Cynodon dactylon* and *Aegle marmelos* in Alloxan-Induced Diabetic Mice: A Combined Biochemical and Histopathological Analysis

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Abstract

Diabetes mellitus is a prevalent global health issue, often associated with oxidative stress and insulin resistance. This study investigated the antidiabetic effects of ethanolic extracts from *Aegle marmelos*, *Cynodon dactylon* and *Mangifera indica* on alloxan-induced diabetic mice. Diabetes was induced in adult and aged mice using alloxan monohydrate (150 mg/kg body weight), followed by oral administration of plant extracts (200 mg/kg body weight) for 20 days. Treatment significantly reduced fasting blood glucose levels in both adult and aged diabetic mice. Additionally, there was a marked decrease in lipid peroxidation (MDA) levels and an increase in superoxide dismutase (SOD) activity, indicating enhanced antioxidant defense. Histopathological analysis revealed partial restoration of β-cell morphology, improved islet architecture, and reduced inflammatory infiltration. These findings suggest that the ethanolic extracts of *Mangifera indica*, *Cynodon dactylon* and *Aegle marmelos* possess potent antidiabetic effects by reducing oxidative stress, boosting antioxidant enzyme activity, and promoting pancreatic β-cell regeneration. This study underscores the therapeutic potential of these medicinal plants as alternatives or supplements to conventional antidiabetic treatments.

***Key words*:** Antidiabetic activity, Diabetes mellitus, Ethanolic plant extracts, Oxidative stress

Chronic hyperglycemia brought on by compromised insulin production, action, or both is a hallmark of diabetes mellitus, a complex metabolic disease [1]. Persistent hyperglycemia leads to oxidative stress, which is a condition in which the body's production of reactive oxygen species (ROS) is out of balance with its antioxidant defense system, resulting in diabetic problems and gradual cellular damage [2]. The pathophysiology of diabetes is significantly influenced by oxidative stress, which worsens lipid peroxidation, lowers the activity of important antioxidant enzymes like catalase and superoxide dismutase (SOD), and further impairs pancreatic β-cell function [3-4].

Current antidiabetic treatments, including insulin therapy and oral hypoglycemic agents, have limitations such as long-term toxicity, limited efficacy, and the inability to fully prevent disease progression [5]. Given these challenges, there has been an increasing focus on alternative approaches, particularly plant-based therapies rich in bioactive compounds with antioxidant and hypoglycemic properties. Traditional medicinal plants offer a promising complementary strategy for diabetes management, with their phytochemical constituents demonstrating beneficial effects on oxidative stress and glucose metabolism [6]. Traditional medicine has made considerable use of ethanolic extracts of *Mangifera indica* (EEMI), *Cynodon dactylon* (EECD), and *Aegle marmelos* (EEAM) because of their various pharmacological characteristics [37].

These plants are known for their high concentrations of flavonoids, polyphenols, alkaloids, and terpenoids, which exhibit potent antioxidant and antidiabetic effects [6-8]. Flavonoids and polyphenols, in particular, contribute to free radical scavenging and lipid peroxidation inhibition, thereby improving antioxidant defense mechanisms [17]. Among these bioactive compounds, mangiferin, a polyphenolic compound in *Mangifera indica* leaves, has been reported to neutralize ROS through electron or hydrogen donation, thereby reducing oxidative damage [6]. *Cynodon dactylon* contains β-sitosterol, a plant sterol with structural similarity to cholesterol, which has shown hypoglycemic effects by modulating lipid metabolism and oxidative stress [7]. Similarly, *Aegle marmelos* leaves are rich in alkaloids such as aegeline, which enhance glucose metabolism, improve insulin sensitivity, and mitigate oxidative damage [8], [12].

There is growing recognition of the importance of total antioxidant capacity (TAC) and oxidative stress markers in diabetes studies. Instead of evaluating individual antioxidants separately, TAC offers a comprehensive measure of all

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antioxidants in a biological system, capturing their cumulative effect. This metric provides important information on the physiological state of oxidative stress and the possible benefits of antioxidant treatments [9-10], [31]. A thorough understanding of the antioxidant capacity of plant-based therapeutics in the management of diabetes can be obtained by measuring important oxidative stress markers, such as lipid peroxidation (malondialdehyde, MDA) and antioxidant enzyme activity (SOD) [20].

Using a model of diabetic mice caused by alloxan, this work examines the antidiabetic and antioxidant properties of ethanolic extracts from the leaves of *Mangifera indica, Cynodon dactylon,* and *Aegle marmelos*. The study intends to clarify the therapeutic effectiveness of these extracts in reducing oxidative stress, controlling blood glucose levels, and reestablishing pancreatic function by assessing indicators including lipid peroxidation and SOD activity. Investigating plant-based substitutes with strong bioactive chemicals may provide encouraging insights for safer and more effective diabetes management techniques, especially in light of the growing prevalence of diabetes and the shortcomings of current therapies.

The ethanolic extracts of *Mangifera indica*, *Cynodon dactylon*, and *Aegle marmelos* have demonstrated significant potential as antidiabetic agents [22]. The presence of mangiferin in *Mangifera indica* contributes to its antioxidant and anti-inflammatory activity [8]. *Cynodon dactylon* enhances antioxidant defense through its hypoglycemic effects and free radical scavenging properties. Similarly, *Aegle marmelos* leaves have shown promise in improving insulin sensitivity and reducing oxidative stress [21]. By examining these characteristics, this research aims to offer important insights into the therapeutic role of these plant extracts in the management of diabetes, potentially presenting substitutes for traditional treatments [19], [30].

MATERIALS AND METHODS

Gathering of therapeutic plants

Under the direction of traditional medical practitioners who shared their ethnobotanical knowledge, the leaves of *Mangifera indica, Aegle marmelos,* and *Cynodon dactylon* were gathered from their respective native habitats [13]. A botanist confirmed the identity of the plant species. An electric mill was used to grind the gathered leaves into a fine powder after they had been shade-dried at room temperature. Until it was needed again, the powdered material was kept dry and in sealed plastic bags.

Plant material extraction

The Soxhlet method was used to extract ethanol from the powdered leaves of each plant [38]. In short, ethanol was used to extract 3 g of powdered material over the course of 6 hours. After filtering, the extract was lyophilized for storage and evaporated at a lower pressure. Before being used in experimental tests, the resultant granules were kept in airtight containers at -20°C.

Animals used in experiments

The study employed healthy male Swiss albino mice measuring 25–30 g, aged 4–5 months (adult), and 20–24 months (aged). The animals were kept under conventional circumstances at the Rajarambapu College of Pharmacy's animal home in Kasegaon, Walva, Sangli, with 12-hour light-dark cycles, a temperature of 25 ± 5°C, and a humidity of 40–60%. Mice were housed in sterile polypropylene cages (29 × 22 × 14 cm) with sterile rice husk bedding, changed every 24 hours. Groups of 5–6 mice were maintained in separate cages and provided ad libitum access to water. The control group was fed a standard diet (Nutrinix Std-1020, Nutrivet Life Sciences, Pune, Maharashtra, India).

Following the recommendations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India, the study procedure complied with the ethical standards established by the Institutional Animal Ethics Committee (IAEC).

Diabetes induction

Mice that had fasted for the whole night were given a single intraperitoneal injection of alloxan monohydrate (Sigma Aldrich) diluted in acetate buffer at a dose of 150 mg/kg body weight to cause diabetes. A glucometer was used to measure the fasting blood glucose levels after 72 hours. Mice classified as diabetic and involved in the study had blood glucose levels more than 200 mg/dL.

Design of experiments

The following groups of mice were created:

I. *Control group*: A regular diet and 0.5 mL of distilled water were given to healthy mice.
II. *Diabetic control group*: Mice with diabetes induced by alloxan that are not treated.
III. *Recovery I*: Ethanolic extract of *Mangifera indica* (EEMI) was administered to diabetic mice at a dose of 200 mg/kg body weight per day for 20 days.
IV. *Recovery II*: Ethanolic extract of *Aegle marmelos* (EEAM) was administered to diabetic mice at a dose of 200 mg/kg body weight per day for 20 days.
V. *Recovery III*: For 20 days, diabetic mice were given 200 mg/kg body weight of ethanolic extract of *Cynodon dactylon* (EECD) every day.

Chemicals

Because it mimics type 1 diabetes mellitus by selectively harming insulin-producing pancreatic β-cells, alloxan monohydrate was utilized as a diabetogenic drug [23].

Tissue and blood collection and analysis

Following a 20-day course of treatment, sterile blood samples were drawn from the tail vein. A sterile needle was used to puncture the tail vein, and silver nitrate was applied to the puncture site to stop the bleeding [25]. Following the process, the tips of the tails were swabbed with 70% ethanol to disinfect them. Using a glucometer, blood samples were utilized right away to determine blood glucose levels [33]. All animals were sacrificed by cervical dislocation after blood collection [34]. Under sterile settings, a midline abdominal incision was made to gently remove pancreatic tissue [39]. The tissue was weighed, blotted dry using filter paper, and prepared for biochemical and histological studies, which included lipid peroxidation, antioxidant enzyme activity, and the assessment of oxidative stress indicators.

In order to examine the plant extracts potential for therapeutic use, this experimental design made it easier to measure blood glucose levels, lipid peroxidation, superoxide dismutase (SOD) activity, and pancreatic histology.

Analysis of data

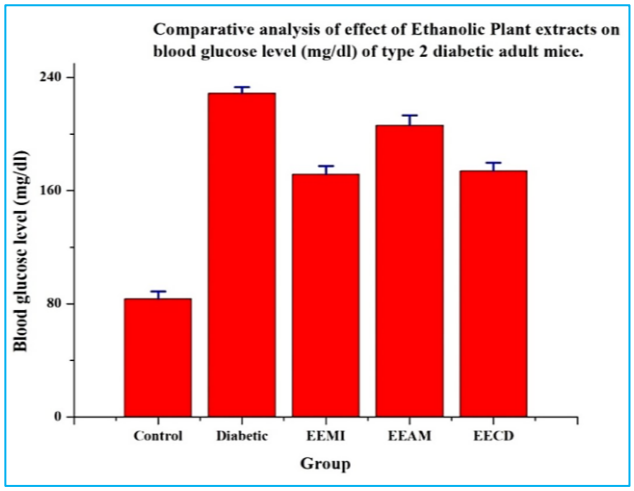
Microsoft Excel was initially used to organize and clean the gathered data. The mean ± standard deviation (SD) was used to express the results. For statistical comparisons, one-way analysis of variance (ANOVA) was employed. The groups

comprised diabetic mice treated with EEMI (recovery group I), diabetic mice treated with EEAM (recovery group II), diabetic mice treated with EECD (recovery group III), and untreated normal mice (control group I). Statistical significance was defined as a p-value of less than 0.05.

RESULTS AND DISCUSSION

a. *A comparison of the impact of ethanolic plant extracts on the blood glucose levels (mg/dl) of adult and elderly mice with type 2 diabetes*

Graph No. 1 presents the comparative analysis of blood glucose levels across different treatment groups in adult diabetic mice. The control group maintained a normal blood glucose level of 80.00 ± 2.5298 mg/dL. In contrast, the diabetic group exhibited a significant hyperglycemic state with a blood glucose level of 228.67 ± 8.0664 mg/dL, which was much greater than that of the control group (P<0.01).



Graph No. 1 shows a comparison of the impact of ethanolic plant extracts on the blood glucose level (mg/dl) of adult mice with type 2 diabetes

Blood glucose levels were considerably lowered by treatment with ethanolic extracts of *Mangifera indica* (EEMI), *Aegle marmelos* (EEAM), and *Cynodon dactylon* (EECD) in comparison to the diabetic group (P<0.01). Blood glucose levels were lowered to 167.00 ± 3.5777 mg/dL by EEAM, which was the most effective of the treated groups. EEMI (171.33 ± 6.6833 mg/dL) and EECD (173.17 ± 4.9565 mg/dL) were next in line. These results demonstrate the plant extracts' antidiabetic potential in reducing adult mice's hyperglycemia.

Blood glucose levels in aged mice are shown in Graph No. 2. The diabetic group had a significantly higher blood glucose level of 258.17 ± 4.5789 mg/dL than the adult diabetic group (P<0.01), with the control group having a blood glucose level of 83.50 ± 5.1672 mg/dL.

EEMI treatment decreased the blood glucose level to 171.67 ± 5.6451 mg/dL., demonstrating substantial improvement (P<0.01). Similarly, EECD treatment lowered the glucose level to 174.00 ± 5.7800 mg/dL, comparable to the EEMI group. However, the EEAM group displayed a moderate reduction to 206.17 ± 6.9690 mg/dL, suggesting relatively lower efficacy in aged mice compared to its effect in adult mice.

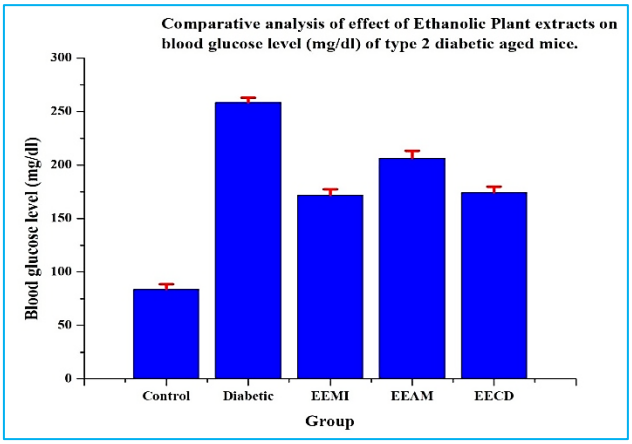
b. *Comparative analysis of effect of Ethanolic Plant extracts on Superoxide Dismutase (Unit/mg protein/hr.) of type 2 diabetic adult and aged mice*

The evaluation of ethanolic plant extracts' effects in comparison to activity of Superoxide Dismutase (SOD) in mice with type 2 diabetes both adult and aged groups, demonstrated significant differences between the control, diabetic, and treatment groups.

Graph No. 3. Comparative analysis of effect of Ethanolic Plant extracts on Superoxide Dismutase (Unit/mg protein/hr.) of type 2 diabetic adult mice.

SOD activity was considerably lower in the diabetes group (27.365 ± 0.4168 U/mg protein/hr.) than in the control group (55.440 ± 0.6748 U/mg protein/hr.).indicating oxidative stress due to diabetes. Treatment with ethanolic extracts of *Mangifera indica* (EEMI), *Aegle marmelos* (EEAM), and *Cynodon dactylon* (EECD) showed varying degrees of improvement in SOD activity:

1. EEMI: SOD activity increased to 64.583 ± 0.4755 U/mg protein/hr., exceeding the control group and indicating a strong antioxidative effect.
2. EEAM: SOD activity was restored to 52.634 ± 0.4589 U/mg protein/hr., approaching control levels.
3. EECD: SOD activity was 57.083 ± 0.4108 U/mg protein/hr., slightly exceeding control levels.

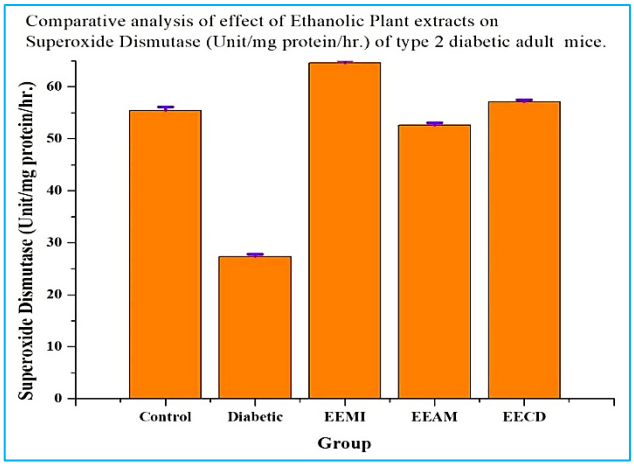


Graph No. 2 shows a comparison of the impact of ethanolic plant extracts on the blood glucose level (mg/dl) of elderly mice with type 2 diabetes

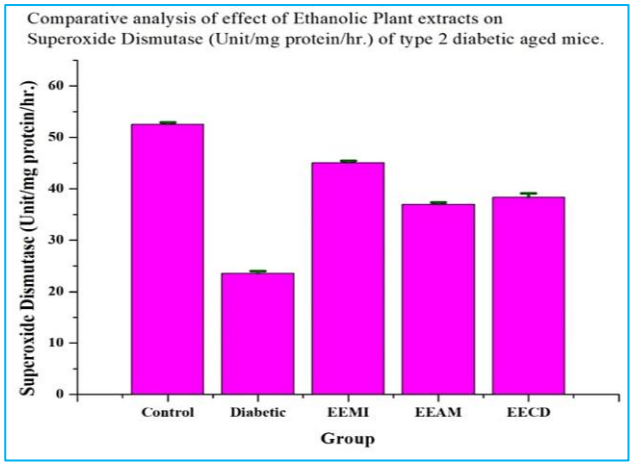
According to statistical research, the variations between the diabetic group and every group that received treatment were highly significant (P<0.01). The findings imply that the SOD activity was increased by extracts, with EEMI showing the most pronounced effect in adult mice. Graph No. 4. Comparative analysis of effect of Ethanolic Plant extracts on Superoxide Dismutase (Unit/mg protein/hr.) of type 2 diabetic aged mice.

In the aged mice, the diabetic group exhibited significantly lower SOD activity (23.569 ± 0.4080 U/mg protein/hr.) contrasting with the control group (52.577 ± 0.3752 U/mg protein/hr.), indicating exacerbated oxidative stress due to aging and diabetes. Treatment with the plant extracts improved SOD activity as follows:

1. EEMI: SOD activity increased to 45.035 ± 0.4096 U/mg protein/hr., partially restoring antioxidant defenses.
2. EEAM: SOD activity was 36.978 ± 0.3818 U/mg protein/hr., reflecting moderate recovery.
3. EECD: SOD activity was 38.383 ± 0.7360 U/mg protein/hr., showing a slight improvement over the diabetic group. When compared to the diabetic group, SOD activity improved statistically significantly in all treatment groups (P<0.01). However, compared to adult mice, the degree of restoration was less pronounced in old mice, indicating age-related restrictions in antioxidant response.



Graph No. 3. Comparative analysis of effect of Ethanolic Plant extracts on Superoxide Dismutase (Unit/mg protein/hr.) of type 2 diabetic adult mice



Graph No. 4. Comparative analysis of effect of Ethanolic Plant extracts on Superoxide Dismutase (Unit/mg protein/hr.) of type 2 diabetic aged mice

c. *Lipid peroxidation in type 2 adult and elderly diabetic mice: The impact of ethanolic plant extracts*

Malondialdehyde (MDA) levels, which indicate lipid peroxidation, are a sign of oxidative stress. Lipid peroxidation in adult and old type 2 diabetic mice was compared, and the control, diabetic, and treated groups showed notable changes.
Graph No. 5. Comparative analysis of effect of Ethanolic Plant extracts on Lipid peroxidation (n mole MDA/mg wet tissue) of type 2 diabetic adult mice.

In adult mice, lipid peroxidation levels were significantly elevated in the diabetic group (55.3748 ± 0.2384 n mole MDA/mg wet tissue) in contrast to the control group. (35.6739 ± 0.2655 n mole MDA/mg wet tissue), indicating increased oxidative stress due to diabetes. Treatment with ethanolic plant extracts resulted in reduced MDA levels as follows:

1. EEMI (Ethanolic Extract of *Mangifera indica*): Lipid peroxidation decreased significantly to 40.3490 ± 0.1682 n mole MDA/mg wet tissue.
2. EEAM (Ethanolic Extract of *Aegle marmelos*): MDA levels were reduced to 46.1822 ± 0.5981 n mole MDA/mg wet tissue.
3. EECD (Ethanolic Extract of *Cynodon dactylon*): Lipid peroxidation was lowered to 47.7897 ± 0.8679 n mole MDA/mg wet tissue.

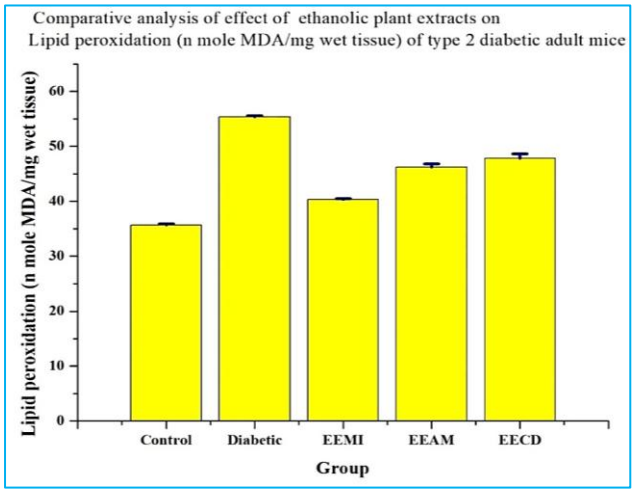
Statistical analysis showed that the differences between the diabetic group and each the group that was treated were highly significant ($P<0.01$). Among the treatments, EEMI demonstrated the most pronounced effect, bringing MDA levels closer to control values.

Graph No. 6. Comparative analysis of effect of ethanolic plant extracts on lipid peroxidation (n mole MDA/mg wet tissue) of type 2 diabetic aged mice.

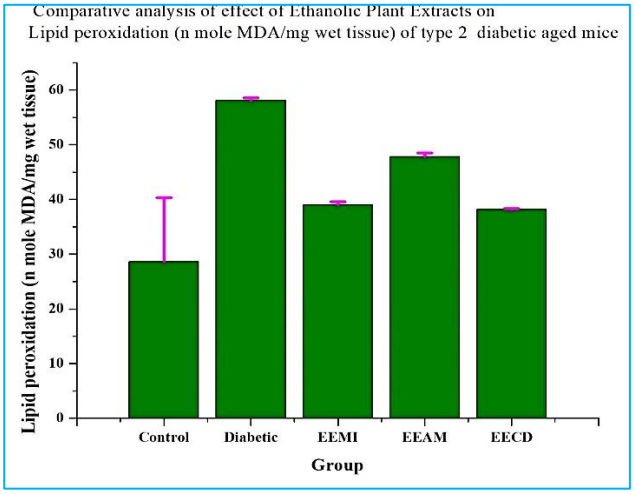
In aged mice, the diabetic group exhibited even higher lipid peroxidation (58.1240 ± 0.4150 n mole MDA/mg wet tissue) compared to the control group (28.6547 ± 11.7120 n mole MDA/mg wet tissue), reflecting compounded oxidative stress due to aging and diabetes. Treatment with the ethanolic plant extracts yielded the following results:

1. EEMI: Lipid peroxidation was reduced to 39.0040 ± 0.5960 n mole MDA/mg wet tissue, indicating significant improvement.
2. EEAM: MDA levels decreased to 47.8447 ± 0.6610 n mole MDA/mg wet tissue, showing moderate recovery.
3. EECD: Lipid peroxidation decreased to 38.1940 ± 0.1860 n mole MDA/mg wet tissue, approaching the effect of EEMI.

MDA levels were significantly lower in all treatment groups than in the diabetes group ($P<0.01$). Similar to adult mice, EEMI exhibited the greatest antioxidative effect in aged mice, though the improvement was less pronounced compared to adult counterparts, suggesting age-related limitations in oxidative stress mitigation.



Graph No. 5. Comparative analysis of effect of Ethanolic Plant extracts on Lipid peroxidation (n mole MDA/mg wet tissue) of type 2 diabetic adult mice



Graph No. 6. Comparative analysis of effect of Ethanolic Plant extracts on Lipid peroxidation (n mole MDA/mg wet tissue) of type 2 diabetic aged mice

d.i. *Effect of ethanolic plant extracts on histological structure in type 2 adult mice*

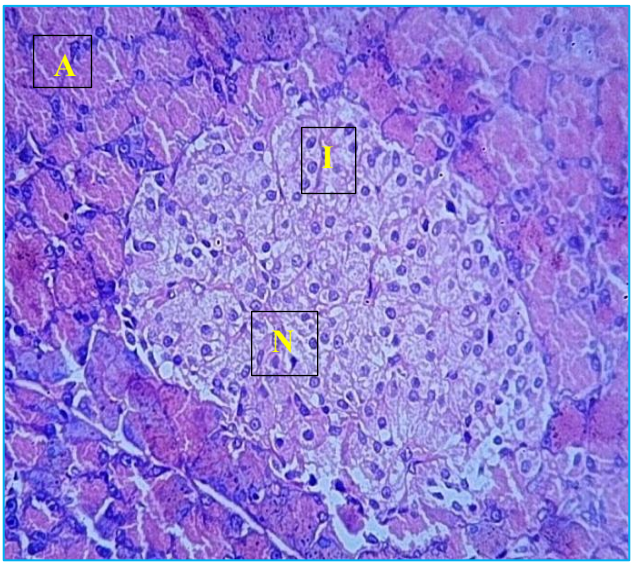


Fig 1 T. S. of adult mice from the control group

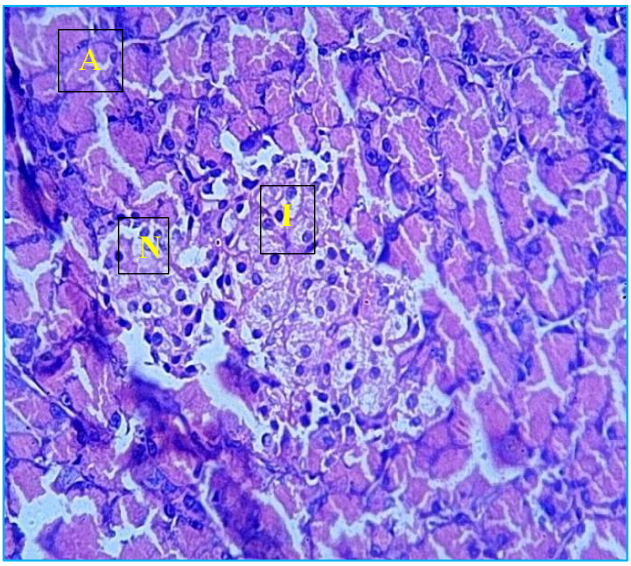


Fig 2 T. S. of adult mice from the diabetic group

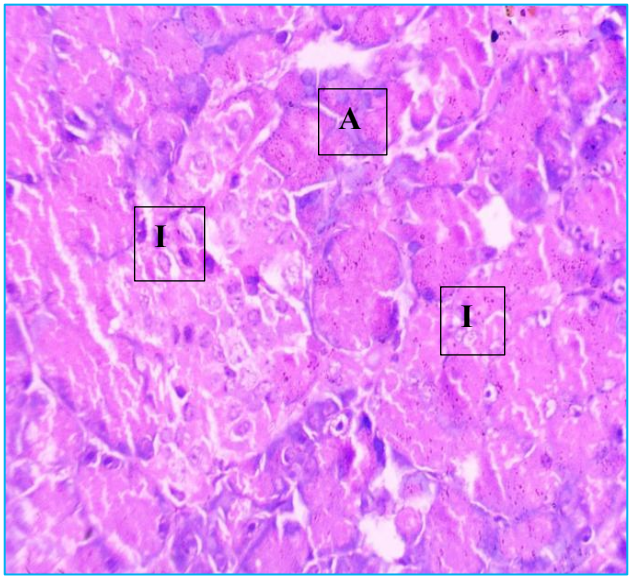


Fig 3 T. S. of adult mice from the EEMI group

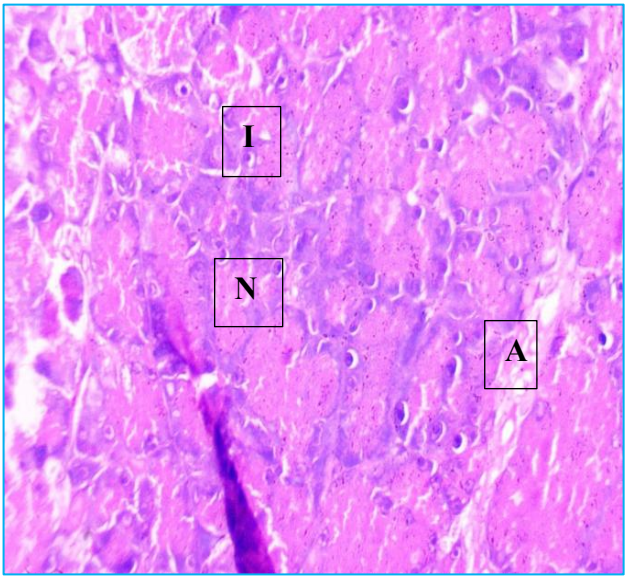
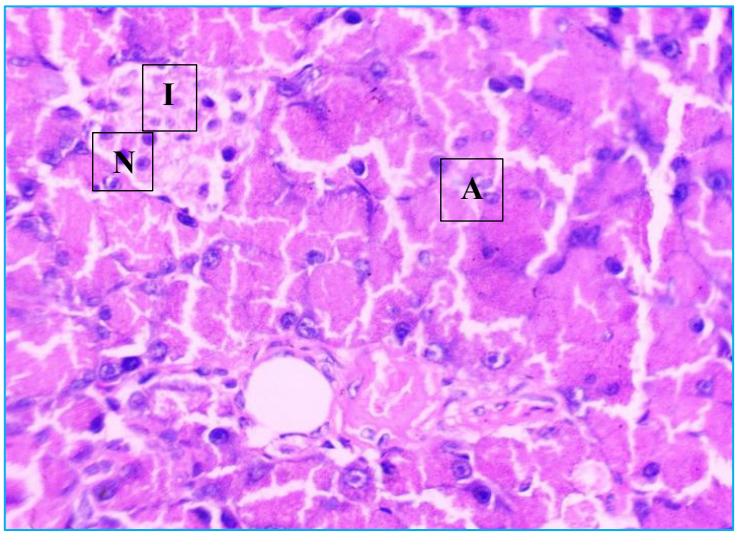


Fig 4 T. S. of adult mice from the EEAM group



A: Acinar cells
N: Nucleus
I: Islets of langerhans

Fig 5 T. S. of adult mice from the EECD group

Fig 1-5 T. S. of adult pancreas of control, diabetic and all recovery group mice exhibiting a normal, necrotic and regenerating structure of pancreatic acini (X400)

d.ii. *Effect of Ethanolic plant extracts on histological structure in Type 2 aged mice*

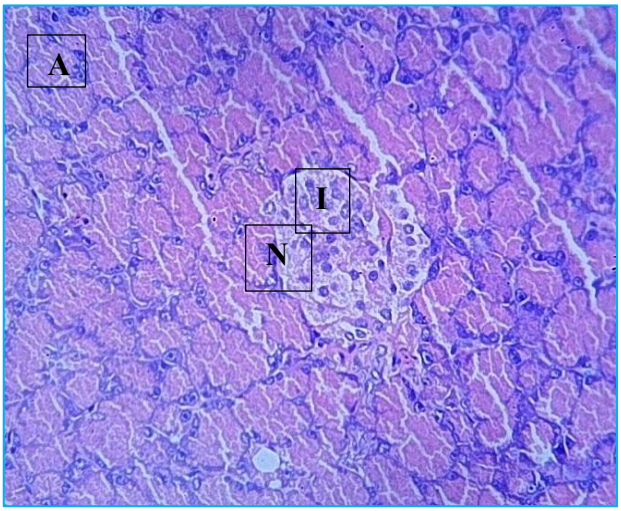


Fig 1 T. S. of aged mice from the control group

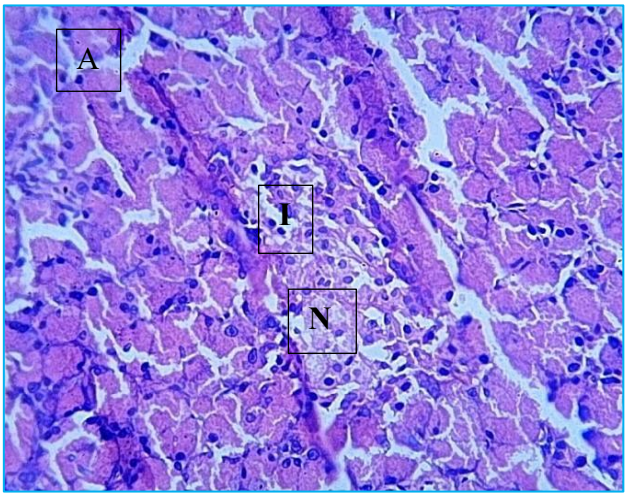


Fig 2 T. S. of aged mice from the diabetic group

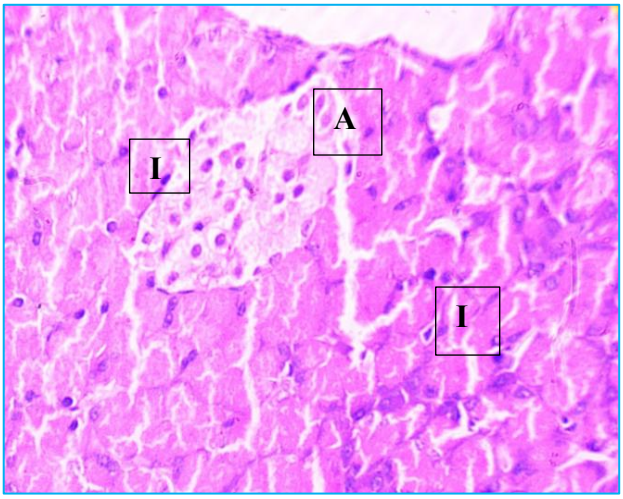


Fig 3 T. S. of aged mice from the EEMI group

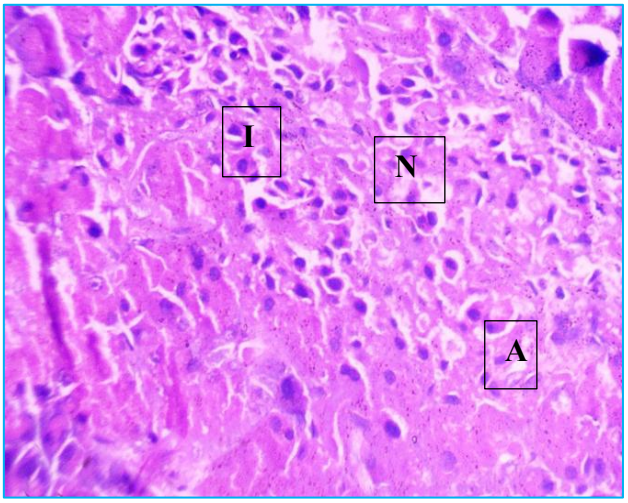
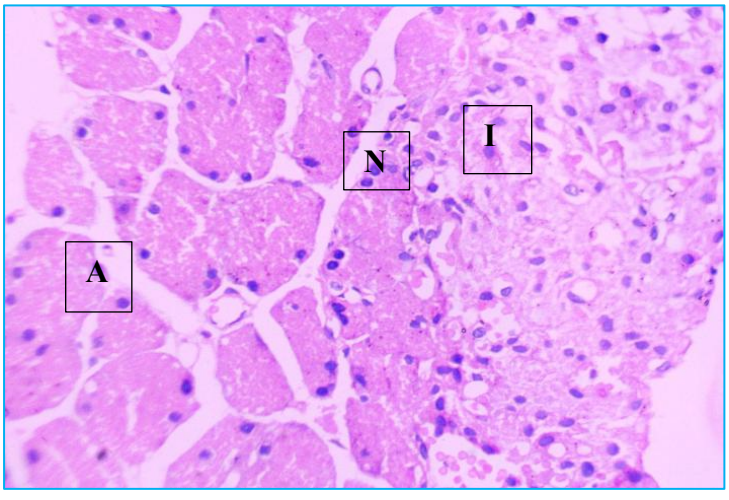


Fig 4 T. S. of aged mice from the EEAM group



A: Acinar cells
N: Nucleus
I: Islets of langerhans

Fig 5 T. S. of aged mice from the EECD group

Fig 1-5 T. S. of aged pancreas of control, diabetic and all recovery group mice exhibiting a normal, necrotic and regenerating structure of pancreatic acini (X400). Captions: A-Acinar cells, N-Nucleus, I- Islets of Langerhans

Histological analysis of adult and aged mice groups

Histopathological analysis of the pancreatic tissues in adult and aged mice revealed distinct differences in structural integrity and regenerative capacity between the two groups. In the adult mice, the islets of Langerhans demonstrated a higher degree of restoration, with noticeable regeneration of β-cells and reduced necrotic changes following treatment. The exocrine tissue exhibited minimal inflammation, indicating a better response to therapeutic interventions. In contrast, the aged mice displayed less pronounced recovery, with smaller

and irregularly shaped islets and limited β-cell regeneration. The exocrine tissue in aged mice showed moderate inflammatory infiltration and structural disruptions, suggesting reduced resilience and regenerative potential compared to the adult group. These findings highlight age-related differences in the capacity of pancreatic tissue to recover from diabetes-induced damage.

In both adult and elderly diabetic mice, the results of this study unequivocally show the antidiabetic efficacy of ethanolic extracts from *Mangifera indica, Cynodon dactylon,* and *Aegle marmelos.* The hypoglycemic benefits of these plant extracts were demonstrated by the significantly lower blood glucose levels in the groups treated with the extracts as compared to the diabetic control group that was not treated. The most promising outcomes in lowering blood glucose levels were demonstrated by the ethanolic extract of *Mangifera indica* (EEMI), which was followed by *Cynodon dactylon* (EECD) and *Aegle marmelos* (EEAM). These findings are in line with earlier research that documented the antidiabetic qualities of these plants because of their bioactive components, which include terpenoids, alkaloids, and flavonoids. These chemicals are well-known for their capacity to lower oxidative stress and regulate glucose metabolism [6-7].

The antioxidant defense system was noticeably strengthened in addition to the improvement in blood glucose levels [9]. The idea that plant extracts may reduce oxidative stress linked to diabetes is supported by the fact that Superoxide Dismutase (SOD) activity was noticeably higher in the treatment groups than in the diabetic control. This is consistent with other research that suggests a mechanism for lowering oxidative damage in diabetic circumstances is increased antioxidant enzyme activity [8-9]. *Mangifera indica* was particularly effective in restoring SOD activity, which could explain its superior efficacy in reducing blood glucose levels.

Furthermore, Malondialdehyde (MDA) values, a measure of lipid peroxidation, were considerably lower in the treated groups. This suggests that the plant extracts help protect cellular membranes from oxidative damage, which is commonly elevated in diabetic conditions due to increased free radical generation [14]. The reduction in MDA levels aligns with the known antioxidant properties of these plants, which may play a role in mitigating diabetic side effects, including retinopathy, neuropathy, and nephropathy [10-11].

Histopathological analysis of pancreatic tissues revealed promising changes in the structure of β-cells in the pancreas. In the diabetic control group, β-cell degeneration was evident, with reduced islet size and an increased presence of inflammatory cells. In contrast, the treated groups exhibited partial restoration of β-cell architecture, with more intact islets and fewer inflammatory infiltrates. These histological improvements imply that the plant extracts could be protective for pancreatic β-cells, which is crucial for restoring insulin secretion in diabetic individuals.

CONCLUSION

In conclusion, the ethanolic extracts of *Mangifera indica*, *Cynodon dactylon*, and *Aegle marmelos* exhibited significant antidiabetic effects in both adult and aged diabetic mice. The improvements in blood glucose levels, antioxidant enzyme activities, lipid peroxidation, and pancreatic β-cell structure strongly suggest the therapeutic potential of these plants in managing diabetes. Among the three extracts, *Mangifera indica* showed the most pronounced antidiabetic effect, likely due to its higher antioxidant content. The histopathological findings also support the protective effects of the plant extracts on pancreatic βeta cells, highlighting their potential in restoring insulin secretion and preventing diabetic complications. These results provide a basis for further clinical studies to explore the efficacy of these plant extracts in human diabetic patients, offering a promising alternative to conventional antidiabetic therapies. Additionally, the use of plant-based remedies could help alleviate the adverse side effects commonly associated with synthetic drugs, presenting a more sustainable and accessible option for diabetes management. Future research focusing on the separation and description of these plants' bioactive substances could further enhance our understanding of their mechanisms of action and their potential applications in diabetic treatment.

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Nil.

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Authors' contribution

Conceptualization and designing of research work (TAG, RDB); Execution of field/lab experiments and data collection (TAG); Analysis of data and interpretation (TAG); Preparation of manuscript (TAG).

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