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ORIGINAL ARTICLE

Evaluation of Biochemical Changes in Diabetic Rats Treated with *Aegle marmelos* (L.) Methanolic Leaf Extract

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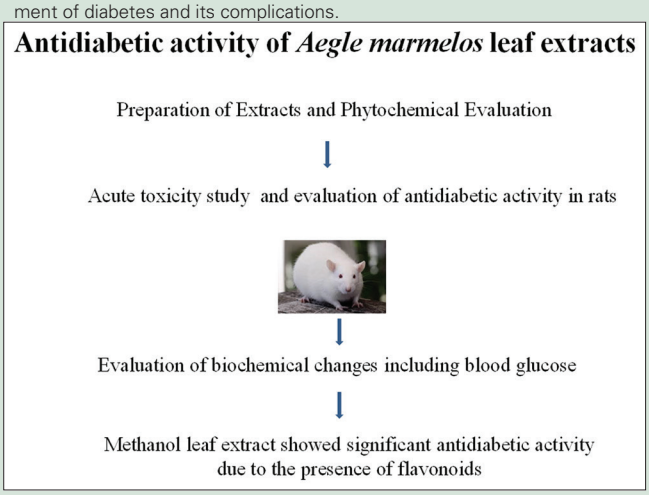
ABSTRACT

Background: *Aegle marmelos* (L.) Correa is a widely found plant in India as well as in South Asia. For more than several centuries, it is being widely used for its medicinal properties. **Objective:** The objective of this study was to evaluate the biochemical changes in alloxan-induced diabetic rats treated with methanolic leaf extracts of *A. marmelos*. **Materials and Methods:** Six treatment groups, namely control, diseased, standard (glimepiride), low dose (100 mg/kg), medium dose (250 mg/kg), and high dose (500 mg/kg) of methanolic leaf extracts, were used in the study. The biochemical effects were evaluated by the determination of bodyweight, blood glucose, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), total proteins, serum albumin, serum creatinine, and alkaline phosphatase. **Results:** A significant increase in the bodyweight of the animals was observed in the high-dose treated animals (350.0 ± 6.15) when compared to the diseased group animals (241.0 ± 7.23). A significant decrease in the blood glucose, SGOT, and SGPT levels was observed in the high-dose treated animals (142.3 ± 20.52, 71.6 ± 4.8, and 24.5 ± 2.42) when compared to the diseased group animals (292.8 ± 29.34, 146.3 ± 11.12, and 74.5 ± 2.88), respectively. Similarly, total proteins, serum albumin, serum creatinine, and alkaline phosphatase levels of the high-dose treated animals were also significantly decreased (6.1 ± 0.26, 4.2 ± 0.22, 0.4 ± 0.18, and 109.2 ± 14.58) when compared to the diseased group animals (9.7 ± 0.27, 5.4 ± 0.26, 1.0 ± 0.22, and 257.2 ± 8.22), respectively. **Conclusion:** Through the biochemical changes, it is evident that the high dose of methanolic leaf extract of *A. marmelos* can be used in the treatment of diabetes and its complications.

Key words: *Aegle marmelos*, alloxan, bael, diabetes, rutaceae

SUMMARY

- The presence of flavonoids in the methanolic leaf extracts of *Aegle marmelos* has yielded the antidiabetic activity and therefore can be used in the treatment of diabetes and its complications.



Abbreviations Used: SGPT: Serum glutamic pyruvic transaminase; SGOT: Serum glutamic oxaloacetic transaminase.

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INTRODUCTION

Natural products have a special place in pharmaceutical research. During their long evolution and selection, they have acquired qualities mostly in connection with biological functions of animal or plant organisms. In addition, natural products are noted for their highly complex molecular architectures, and they show amazing arrangements of functional groups, strained ring systems, and other attractive structural attributes. Traditional knowledge of medicinal plants has always guided the search for new cures. In spite of the advent of modern high-throughput drug discovery of valuable drugs, traditional plants are often economical, locally available, and consumable raw or as simple medicinal preparation. Many vegetable drugs are used in preparations prescribed by practitioners of indigenous medicine in different regions; the common people use others as household remedies.[1]

Aegle marmelos (L.) Correa is a widely found plant in India as well as in South Asia. For more than several centuries, it is being widely used for its medicinal properties. Several phytocompounds have been isolated and characterized by various plant parts, namely alkaloids, terpenoids, tannins, phenols, cardiac glycosides, steroids, flavonoids, and saponins. These phytocompounds are reported to possess therapeutic potential for various diseases and disorders.[2] Analgesic, antioxidant, anti-bacterial, antifungal, anticancer, antidiarrheal, immunomodulant, antihyperlipidemic, antiulcer, diuretic, antifilarial, and hepatoprotective activities have been reported in various plant extracts of the plants.[3-28] Leaves are considered to be the most common sites of accumulation of phytocompounds in plants. Therefore, the present research work was aimed at the evaluation of biochemical changes in diabetic rats treated with methanolic leaf extracts from *A. marmelos*.

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MATERIALS AND METHODS

Collection of plant material

The leaves of *A. marmelos* (L.) were collected from Dolas Nagar, Tadepalle Mandal, Guntur District, Andhra Pradesh, India. Authentication was performed by Dr. P. Satya Narayana Raju, Plant Taxonomist, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India. The reference specimen is preserved in the Department of Botany, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur.

Preparation of plant extracts

The collected leaves were washed thoroughly with water and shade dried. Methanolic leaf extracts were obtained by extracting powder with 85% ethanol by the Soxhlet extraction method for 72 h. After completion of the extraction, the excess solvent was removed by rotary evaporation. The methanolic leaf extract was used for further evaluation of biochemical changes in alloxan-induced diabetes.

Preliminary phytochemical analysis

The methanolic leaf extract from *A. marmelos* (L.) was subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents; it revealed the presence of glycosides, saponins, tannins, and flavonoids.

Animals

Normal healthy male Wistar albino rats, 9–12 weeks old with an average weight of 200–250 g were procured from the Mahaveer Enterprises (CPCSEA Regd No: 146/99/CPCSEA), Bagh Amberpet, Hyderabad, India. They were housed in polypropylene cages and fed with a standard chow diet and water *ad libitum*.

The animals were acclimatized to the conditions by maintaining them at a temperature 25°C ± 2°C and relative humidity 55 ± 10 at 12 h and each at dark and light cycle for about 7 days before dosing and during the commencement of the experiment.

All experimental procedures involving animals were conducted in accordance with the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) with prior approval from the Institutional Animal Ethics Committee (IAEC Approval No. ANUCPS/IAEC/AM/P/26/2019) of College of Pharmaceutical Sciences, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India.

Treatment groups

The biochemical changes were evaluated using the alloxan-induced diabetes model.[29] A total of 36 rats were used. The rats were divided into six groups of six rats each: Group 1: vehicle treatment group, Group 2: disease control, Group 3: standard treatment (glimepiride 40 mg/kg), Group 4: low dose of methanolic leaf extract (100 mg/kg), Group 5: medium dose of methanolic leaf extract (250 mg/kg), and Group 6: high dose (500 mg/kg). Plant leaf extracts were suspended in vehicle solution of 0.5% dimethyl sulfoxide and a dose of l ml/kg, bodyweight was administered orally using an intragastric tube for 15–45 days to the respective groups.

Chemicals

Alloxan monohydrate was procured from Sigma Aldrich, Bangalore, India. All the other chemicals and solvents used in the study were of analytical grade and obtained from local suppliers.

Acute toxicity studies

The acute toxicity studies were carried out in accordance with OECD Test Guideline 423: Acute oral toxicity-acute toxic class method. The methanolic leaf extract of *A. marmelos* (L.) was found to be safe up to 2000 mg/kg bodyweight after oral administration of the test compound. 100 mg/kg, 250 mg/kg, and 500 mg/kg were used for further animal pharmacological study.

Parameters evaluated: diabetes was induced by the administration of alloxan monohydrate (150 mg/kg bodyweight) with normal saline as a vehicle. After 72 h, rats with blood glucose levels more than 150 mg/dl were selected for further biochemical evaluation. The blood glucose levels were estimated using the one-touch glucometer. The biochemical effects were evaluated by the determination of bodyweight, blood glucose, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), total proteins, serum albumin, serum creatinine, and alkaline phosphatase.

Statistical analysis

The results of the study were presented as mean ± standard error of the mean. The statistical significance of the groups was determined using the one-way analysis of variance followed by Dunnett's test using GraphPad PRISM software. $P < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Effects of methanolic leaf extracts on bodyweight of the treated animals

A significant increase in the bodyweight of the animals was observed in the high-dose treated animals (350.0 ± 6.15) when compared to the diseased group animals (241.0 ± 7.23). The statistical significance between the groups was found to be $P < 0.05$. The effects of methanolic leaf extracts on bodyweight of the treated rats are shown in Figure 1.

Effects of methanolic leaf extracts on blood glucose of the animals

A significant decrease in the blood glucose levels was observed in the high-dose treated animals (142.3 ± 20.52) when compared to the diseased group animals (292.8 ± 29.34). The statistical significance between the groups was found to be $P < 0.05$. The effects of methanolic leaf extracts on blood glucose levels of the treated rats are shown in Figure 2.

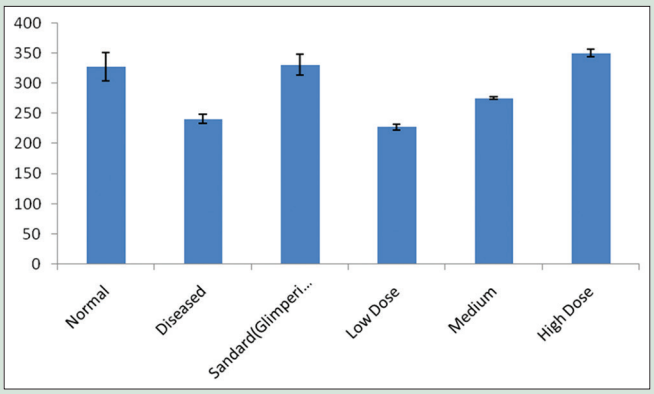

Figure 1: Effects of methanolic leaf extracts on bodyweight of the treated animals

Effects of methanolic leaf extracts on serum glutamic oxaloacetic transaminase of the animals

A significant decrease in the SGOT levels was observed in the high-dose treated animals (71.6 ± 4.8) when compared to the diseased group animals (146.3 ± 11.12). The statistical significance between the groups was found to be $P < 0.05$. The effects of methanolic leaf extracts on SGOT levels of the treated rats are shown in Figure 3.

Effects of methanolic leaf extracts on serum glutamic pyruvic transaminase of the animals

A significant decrease in the SGPT levels was observed in the high-dose treated animals (24.5 ± 2.42) when compared to the diseased group animals (74.5 ± 2.88). The statistical significance between the groups was found to be $P < 0.05$. The effects of methanolic leaf extract on SGPT levels of the treated rats are shown in Figure 4.

Effects of methanolic leaf extracts on total proteins, serum albumin, and serum creatinine levels of the animals

Similarly, total proteins, serum albumin, and serum creatinine levels of the high-dose treated animals were also significantly decreased (6.1 ± 0.26, 4.2 ± 0.22, and 0.4 ± 0.18) when compared to the diseased group animals (9.7 ± 0.27, 5.4 ± 0.26, and 1.0 ± 0.22), respectively. The statistical significance between the groups was found to be $P < 0.05$. The effects of methanolic leaf extracts on total proteins, serum albumin, and serum creatinine levels of the treated rats are shown in Figures 5-7.

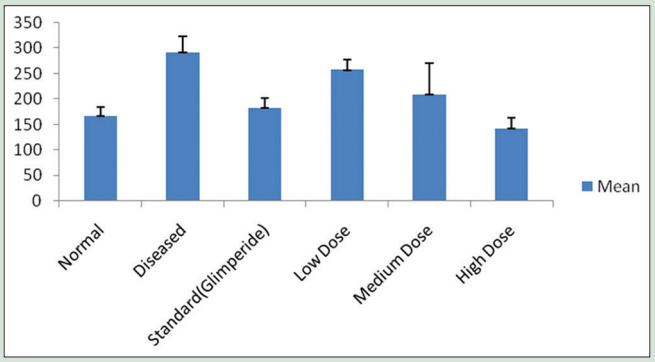

Figure 2: The effects of methanolic leaf extracts on blood glucose levels of the treated rats

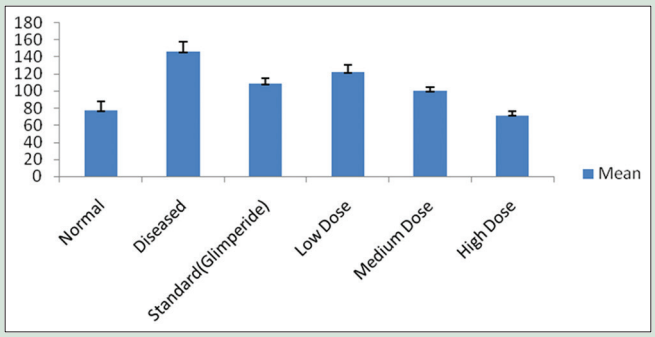

Figure 3: The effects of methanolic leaf extracts on serum glutamic oxaloacetic transaminase levels of the treated rats

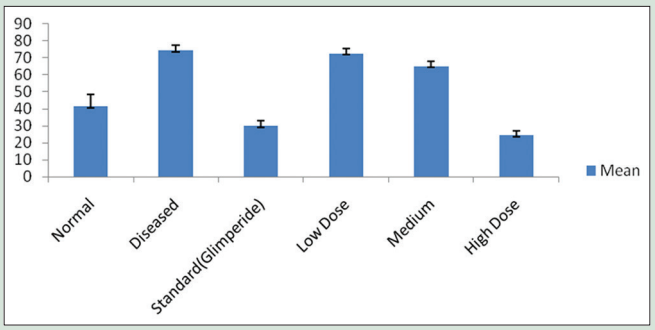

Figure 4: The effects of methanolic leaf extracts on serum glutamic pyruvic transaminase levels of the treated rats

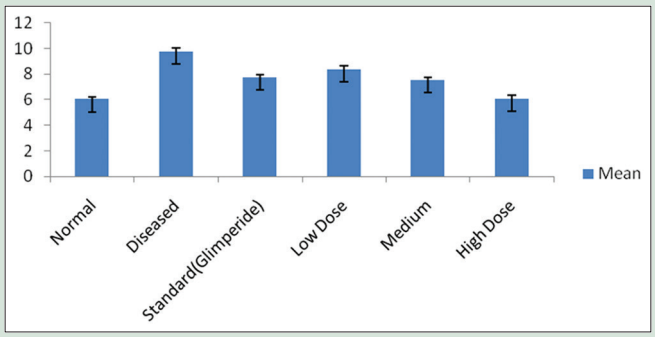

Figure 5: The effects of methanolic leaf extracts on total proteins of the treated rats

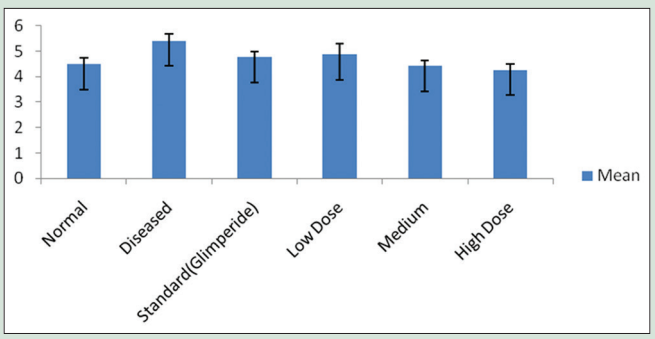

Figure 6: The effects of methanolic leaf extract on total proteins levels of the treated rats

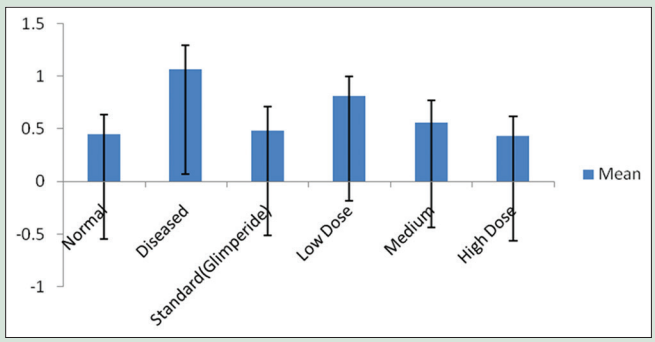

Figure 7: The effects of methanolic leaf extract on serum creatinine levels of the treated rats

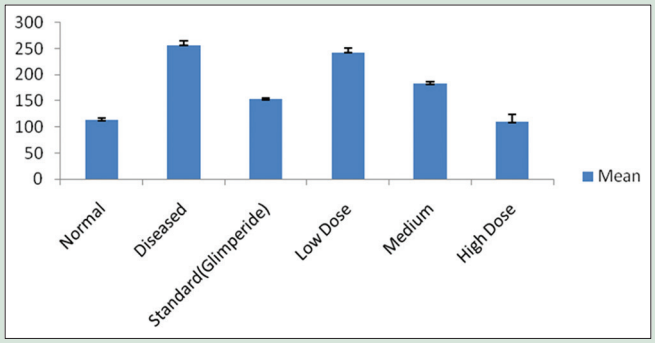

Figure 8: The effect of methanolic leaf extracts on serum creatinine levels of the treated rats

Effects of methanolic leaf extracts on total proteins, serum albumin, and serum creatinine levels of the animals

Alkaline phosphatase levels of the high-dose treated animals were also decreased (109.2 ± 14.58) when compared to the diseased group animals (257.2 ± 8.22). The statistical significance between the groups was found to be $P < 1.0000$. The effects of methanolic leaf extracts on alkaline phosphatase levels of the treated rats are shown in Figure 8.

Earlier studies carried out on various fruit and bark extracts from the *A. marmelos* (L.) also showed significant pharmacological evidence for its therapeutic benefits in various animal models for diabetes and its related complications such as diabetic cataract and diabetic retinopathy.[30-32]

CONCLUSION

Through the biochemical changes, it is evident that the high dose of methanolic leaf extracts from *A. marmelos* can be used in the treatment of diabetes and its complications. The antidiabetic activity could be attributed to the presence of flavonoids in the extracts. However, there is a need for further cellular and molecular pharmacological studies to elucidate the exact mechanisms for its antidiabetic potential.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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