

Effects of *Aegle marmelos* (L.) Methanolic Leaf Extracts on Cardiovascular Parameters in Diabetic Rats

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Abstract: *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. The objective of the study was to evaluate the cardiovascular changes in alloxan induced diabetic rats treated with methanolic leaf extracts of *Aegle marmelos*. 5 treatment groups (namely control, diseased, low dose (100mg/kg), medium dose (250mg/kg) and high dose (500mg/kg) of methanolic leaf extracts were used in the study. The cardiovascular effects were evaluated by the determination of Very-Low-Density Lipoprotein (VLDL), Serum Sialic Acid, Glutathione Peroxidase, Serum Catalase, Ascorbic acid, Sodium, Potassium, and Chloride levels. High dose treatment group showed significant decrease in Very-Low-Density Lipoprotein (VLDL), Serum Sialic Acid, Glutathione Peroxidase, Serum Catalase, Ascorbic acid, Sodium, Potassium, and Chloride levels when compared with the diseased treatment groups. Though Low and medium dose treated animals showed insignificant decrease in these cardiovascular parameters when compared with high dose treatment group as well as diseased group. The effects of high dose treatment on cardiovascular parameters were very significant as that of control group. Through the cardiovascular parameters it is evident that the high dose of methanolic leaf extract of *Aegle marmelos* can be used the treatment of diabetes and its cardiovascular complications.

Keywords: *Aegle marmelos*, Rutaceae, Bael, Alloxan, Diabetes, Cardiovascular Parameters

1. Introduction

Natural products have a very special place in drug research and development. Plants as a source of therapeutically useful drugs have been proved the evidence of high economic importance. Search for new drugs from various plant sources occurs throughout the globe. In India though there are certain limitations or challenges in the resources, standardization of medicinal plants has gained significance in the recent times [1].

Aegle marmelos (L.) Correa is a widely found medicinal plant in India and South Asia. It is being commonly used for its therapeutic properties [2]. Antinociceptive activity [3], hepatoprotective activity [4, 5], antioxidant activity [4, 10, 17-20], antimicrobial activity [6], antibiofilm activity [6], cytotoxic activity [6, 10], antifeedant activity [6], larvicidal

activity [6], antiproliferative activity [7, 13, 19], cholinergic agonist activity [8], serotonergic agonist activity [8], adrenergic agonist activity [8], antifungal activity [9, 11], transcriptome gene activity [12], anticancer activity [13], antidiarrhoeal activity [14], protective effects against fructose induced hepatic insulin resistance [15], immunomodulatory activity [16], protective effects against chronic fatigue syndrome [20], antilipidemic activity [21, 28], antihypercholesterolemic activity [21, 28], anti ulcer activity [22], detoxifying activity [23], diuretic activity [24], antibacterial activity [25], anti filarial activity [26], antidiabetic activity [27] has been reported in various plant extracts of the plant. Most of the phytochemicals are found to be accumulated in the leaves of the plants. Therefore, the present research work was aimed at the evaluation of cardiovascular changes in diabetic rats treated with methanolic leaf extracts from *Aegle marmelos*.

2. Materials and Methods

2.1. Collection of Plant Material

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

2.2. Preparation of Plant Extracts

The collected leaves were washed thoroughly with water and shade dried. Methanolic leaf extracts was obtained by extracting powder with 85% ethanol by 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.

2.3. Preliminary Phytochemical Analysis

The methanolic leaf extract from *Aegle 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.

2.4. Animals

Normal healthy male wistar albino rats, 9-12 weeks old with an average weight of 200-250gm were procured from the Mahaveer Enterprises (CPCSEA Regd No: 146/99/CPCSEA), Bagh Amberpet, Hyderabad. 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 $25\pm 2^{\circ}\text{C}$ and relative humidity 55 ± 10 at 12 h each at dark and light cycle for about 7 days prior to dosing and during the commencement of 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 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.

2.5. Treatment Groups

The cardiovascular changes were evaluated using alloxan induced diabetes model. [29, 30] A total of 30 rats were used. The rats were divided into 5 groups of 6 rats each. Group 1: Vehicle treatment group; Group 2: Disease Control; Group 3: Low dose of methanolic leaf extract (100mg/kg); Group 4: Medium dose of methanolic leaf extract (250mg/kg) and Group 5: High dose (500mg/kg). Plant leaf extracts were

suspended in vehicle solution of 0.5% dimethyl sulfoxide [DMSO] and a dose of 1ml/kg, body weight was administered orally using an intragastric tube for 15-45 days to the respective groups.

2.6. Chemicals

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

2.7. 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 *Aegle marmelos* (L.) was found to be safe up to 2000 mg/kg body weight after oral administration of the test compound. 100 mg/kg, 250 mg/kg and 500 mg/kg were used for further animal pharmacological study.

2.8. Parameters Evaluated

Diabetes was induced by the administration of alloxan monohydrate (150 mg/kg b.w.) with normal saline as vehicle. After 72 h, rats with blood glucose levels more than 150 mg/dl was selected for further biochemical evaluation. The blood glucose levels were estimated using one touch glucometer. The cardiovascular effects were evaluated by the determination of Very-Low-Density Lipoprotein (VLDL), Serum Sialic Acid, Glutathione Peroxidase, Serum Catalase, Ascorbic acid, Sodium, Potassium, and Chloride levels [31].

2.9. Statistical Analysis

Results of the study were presented as mean \pm standard error of the mean. The statistical significance of the groups was determined using one-way analysis of variance followed by Dunnett's test using Graph Pad PRISM Software and $P < 0.05$ was considered as significant.

3. Results and Discussion

3.1. Effects of Methanolic Leaf Extracts on Very-Low-Density Lipoprotein (VLDL) of the Treated Animals

Higher Very-Low-Density Lipoprotein (VLDL) is an indication of disease in the heart, blood vessels, liver, kidney or intestines. It's also linked to diabetes, low thyroid activity and leukemia. There were significant changes in Very-Low-Density Lipoprotein (VLDL) among the treatment groups when compared with the diseased [32]. There were no significant changes in VLDL levels between treatment and control treatment groups. The statistical significance between the groups was found to be $P < 0.05$. The effects of methanolic leaf extracts on Very-Low-Density Lipoprotein (VLDL) of the treated rats are shown in Table 1.

Table 1. Effects of *Aegle marmelos* (L.) methanolic leaf extracts on cardiovascular parameters in diabetic rats.

S. No	Parameter (s)	Normal	Diseased	Low Dose	Medium Dose	High Dose
1	VLDL (mg/dl)	26.36±1.26	36.08±1.87**	31±1.73	28.11±1.63	26.65±0.55**
2	Serum Sialic Acid (mg/%)	67.33±1.36	84.93±1.66**	75.66±1.16	72.06±1.48	68.91±0.88**
3	Glutathione Peroxidase (mg/L)	91.81±1.24	153.61±1.38**	126.91±1.01	122.91±2.01	91.76±0.80**
4	Serum Catalase (MU/L)	111.71±0.01	126.58±1.48**	124.61±1.06	121.62±2.00	112.23±1.34**
5	Ascorbic Acid (mg/dl)	0.5±0.14	3.65±0.26**	2.57±0.77	1.96±0.37	0.53±0.05**
6	Sodium (mg/dl)	143.48±0.31	166.26±0.82**	163.15±1.73	157.88±1.44	143.56±1.10**
7	Potassium (mg/dl)	5.5±0.23	6.38±0.24**	5.66±0.16	5.55±0.38**	5.36±0.21**
8	Chloride (mEq/L)	104.23±2.88	144.68±2.69**	138.45±1.78	127.26±1.47	104.35±1.39**

The cardiovascular parameters of diseased groups were compared with control group and treatment groups (p<0.05).

3.2. Effects of Methanolic Leaf Extracts on Serum Sialic Acid and Serum Catalase Levels of the Animals

The physiological properties of serum sialic acid and serum catalase may explain its ability to protect against the progression of diabetes. These properties make serum sialic acid and serum catalase a potential clinical biomarker or therapeutic target for a variety of disease states. Extremely significant decrease in the serum sialic acid and serum catalase levels was observed in the high dose treated animals when compared to the diseased group animals [33]. The statistical significance between the groups was found to be P< 0.05. The effects of methanolic leaf extracts on serum sialic acid and serum catalase levels of the treated rats are shown in Table 1.

3.3. Effects of Methanolic Leaf Extracts on Glutathione and Ascorbic Acid Levels of the Animals

The physiological properties of glutathione and ascorbic acid levels may explain its ability to protect against the progression of diabetes [34]. These properties make glutathione and ascorbic acid a potential clinical biomarker or therapeutic target for a variety of disease states. Extremely significant decrease in the glutathione and ascorbic acid levels was observed in the high dose treated animals when compared to the diseased group animals. The statistical significance between the groups was found to be P< 0.05. The effects of methanolic leaf extracts on glutathione and ascorbic acid levels of the treated rats are shown in Table 1.

3.4. Effects of Methanolic Leaf Extracts on Sodium, Potassium and Chloride Levels of the Animals

Extremely significant decrease in the sodium, potassium and chloride levels was observed in the high dose treated animals when compared to the diseased group animals [35]. The statistical significance between the groups was found to be P< 0.05. The effects of methanolic leaf extracts on sodium, potassium and chloride levels of the treated rats are shown in Table 1.

4. Conclusion

Through the cardiovascular parameters it is evident that the high dose of methanolic leaf extracts from *Aegle*

marmelos can be used the treatment of diabetes and its cardiovascular 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

The authors declare that they have no competing interests.

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