Original Article

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

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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 albumin-to-globulin ratio (A/G ratio), albumin, amylase, bilirubin, blood urea, blood urea nitrogen, calcium, direct bilirubin, globulin, glucose-6-phosphate, glycated hemoglobin (HbA1c), homocysteine, indirect bilirubin, inorganic phosphate, lipase, mean blood glucose, serum uric acid, and vitamin D3. **Results:** No significant changes were observed in A/G ratio among the treatment groups when compared with the diseased and control treatment groups. Low- and medium-dose-treated animals showed a significant change in albumin, bilirubin, calcium, direct bilirubin, indirect bilirubin, globulin, glucose-6-phosphate, homocysteine, inorganic phosphate, lipase, and vitamin D3 levels when compared with standard treatment group as well as diseased group. Low-dose treatment group animals showed a significant increase in amylase and mean blood glucose levels than the diseased treatment groups, whereas low-dose treatment group animals showed a significant decrease in HbA1c levels than the diseased treatment groups. **Conclusion:** Through the biochemical changes, it is evident that the low and medium dose of methanolic leaf extract of *A. marmelos* can be used in the treatment of diabetes and its complications.

Keywords: *Aegle marmelos, alloxan, bael, diabetes, Rutaceae*

Introduction

Natural products have a very special place in drug research and development. Plants as a source of therapeutically useful drugs have been proved to 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 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] Analgesic, antioxidant, antibacterial, antifungal, anticancer, antidiarrheal, immunomodulant, antihyperlipidemic, antiulcer, diuretic, antifilarial, and hepatoprotective activities have been reported in various plant extracts of the plant.[3-28] Most of the phytocompounds are found to be accumulated in the leaves of the plants. Therefore, the present research work aimed at the evaluation of biochemical changes in diabetic rats treated with methanolic leaf extracts from *A. marmelos.*

Materials and Methods

Collection of plant material

The leaves of *A. marmelos* (L.) were collected from Dolas Nagar, Tadepalli 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

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extracts were 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.

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. 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 ± 2°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 the experiment.

All experimental procedures involving animals were conducted in accordance with the guidelines of the 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 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 a vehicle solution of 0.5% dimethyl sulfoxide [DMSO] and a dose of l mL/kg; body weight 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 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.

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 were selected for further biochemical evaluation. The blood glucose levels were estimated using one-touch glucometer. The biochemical effects were evaluated by the determination of albumin-to-globulin ratio (A/G ratio), albumin, amylase, bilirubin, blood urea, blood urea nitrogen, calcium, direct bilirubin, globulin, glucose-6-phosphate, HB1Ac, homocysteine, indirect bilirubin, inorganic phosphate, lipase, mean blood glucose, serum uric acid, and vitamin D3 [Figure 1].

Statistical analysis

Results of the study were presented as mean ± 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 a value of $P < 0.05$ was considered as significant.

Results and Discussion

Drugs from plants are researched in laboratory animals both for therapeutic efficacy and safety. This is significant on the grounds that any hepatic and renal harm will modify design and capacity of these fundamental organs and effects in general digestion. The liver is the main organ in the metabolism of plant drugs and different substances. Liver cell obliteration shows its belongings generally as significant in the liver cell film penetrability, which brings about the spilling out of tissue content into the circulatory system. In a few organs, cell film harm is trailed by arrival of various cytoplasmic chemicals to the blood, a marvel that gives the premise to clinical analysis. Abnormal biochemical changes in bilirubin, direct bilirubin, indirect bilirubin, *D3,* amylase, blood urea, blood urea nitrogen, calcium, globulin, glucose-6-phosphate, homocysteine, inorganic phosphate, lipase, serum uric acid, and vitamin levels are of clinical and toxicological significance, being characteristic of tissue harm by poisons or infection condition.[30]

Effects of methanolic leaf extracts on serum albumin-to-globulin ratio of the treated animals

Higher A/G ratio is an indication of disease in the liver, kidney, or intestines. It is also linked to diabetes, low thyroid activity, and leukemia. Similar to earlier studies, when compared to normal control rats, alloxan-induced diabetic rats had significantly higher serum A/G ratio concentrations.[30] No significant changes were observed in serum A/G ratio among the treatment groups when compared with the diseased (1.81 ± 0.81) and control treatment groups (1.74 ± 0.10). The statistical significance between the groups was found to be $P > 0.05$. The effects of methanolic leaf extracts on serum A/G ratio of the treated rats are shown in Table 1.

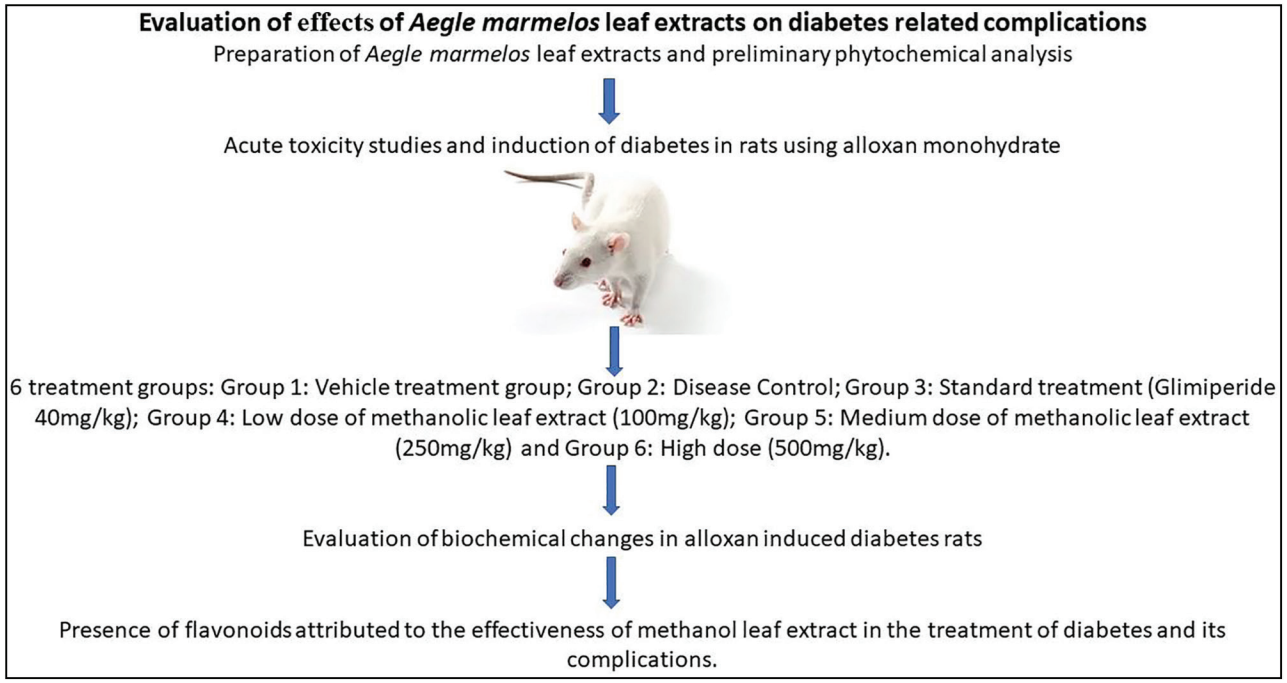

Figure 1: Graphical summary of evaluation of effects of plant extracts on diabetic rats

Effects of methanolic leaf extracts on bilirubin, direct bilirubin, and indirect bilirubin levels of the animals

In the systemic circulation, bilirubin is the end result of hemoglobin breakdown. Stocker *et al.* discovered in 1987 that bilirubin served as a physiological antioxidant. Recent research has discovered that a slight increase in bilirubin levels within the physiological range protects against metabolic disorders. The insulin resistance status may have a role in the connection between certain metabolic disorders and bilirubin.

Bilirubin may play a role in the insulin signaling pathway's signal transduction, improving insulin sensitivity by lowering oxidative stress and inflammatory reactions. The physiological properties of bilirubin may explain its ability to protect against the progression of diabetes. These properties make bilirubin a potential clinical biomarker or therapeutic target for a variety of disease states. Similar to earlier studies, an extremely significant increase in the bilirubin, direct bilirubin, and indirect bilirubin levels was observed in the standard, low-, and medium-dose-treated animals (142.3 ± 20.52) when compared to the normal group animals.[31] The statistical significance between the groups was found to be $P < 0.05$. The effects of methanolic leaf extracts on bilirubin, direct bilirubin, and indirect bilirubin levels of the treated rats are shown in Table 2.

Effects of methanolic leaf extracts on albumin levels of the animals

Blood plasma proteins are the first to get modified as they are directly exposed to higher glucose concentrations and a number of them have been identified. Human serum albumin is one of the most abundant plasma proteins and is heavily glycated in diabetes. Albumin constitutes more than 50% of plasma proteins, and any variation in levels of albumin may change the stoichiometry of glycation of other plasma proteins glycation. In diabetic patients, albuminuria is an important predictive marker for progressive diabetic renal impairment, cardiovascular disease, and diabetic retinopathy. Untreated diabetic animals developed albuminuria, which could be caused by decreased tubular reabsorption or albumin leakage due to a damaged glomerular membrane. Similar to the earlier studies, an extremely significant increase in albumin levels was observed in the standard, low-, and medium-dose-treated animals when compared to the normal group animals.[32] The statistical significance between the groups was found to be $P < 0.05$. The effects of methanolic leaf extracts on the albumin levels of the treated rats are shown in Table 3.

Effects of methanolic leaf extracts on amylase, blood urea, blood urea nitrogen, calcium, globulin, glucose-6-phosphate, homocysteine, inorganic phosphate, lipase, serum uric acid, and vitamin D3 levels of the animals

Insulin inhibits HMG-CoA reductase, which creates cholesterol, and activates lipoprotein lipase, which hydrolyzes triglycerides, in normal conditions. Hypercholesterolemia and hypertriglyceridemia arise as a result of insulin insufficiency in diabetes. As a result, treatment with a polyherbal formulation may be responsible for lowering lipid levels via insulin release or insulin sensitization.[33]

Phosphorus is important for energy storage, transmission, and liberation in the body, as well as intermediary metabolism of carbs, fats, and proteins. Pi is an inorganic phosphate that is found in DNA and RNA and is involved in both glycolysis and oxidative phosphorylation. Pi is a substrate for glyceraldehyde-3-phosphate dehydrogenase, which accelerates glycolysis. Mitochondria are our bodies' power factories, and their major job is to produce adenosine

triphosphate (ATP), which provides 90%–95% of all cellular energy through oxidative phosphorylation. As has been well documented, the concentration of plasma Pi, and hence red cell 2,3-diphosphoglycerate and ATP levels, and oxygen transport to tissue are intimately linked with diabetes and other diseases (hyperalimentation and uremia).

Similar to earlier studies, an extremely significant increase in the amylase, blood urea, blood urea nitrogen, calcium, globulin, glucose-6-phosphate, homocysteine, inorganic phosphate, lipase, serum uric acid, and vitamin D3 levels was observed in the standard, low-, and medium-dose-treated animals when compared to the normal group animals.[34] The statistical significance between the groups was found to be $P < 0.05$. The effects of methanolic leaf extracts on amylase, blood urea, blood urea nitrogen, calcium, globulin, glucose-6-phosphate, homocysteine, inorganic phosphate, lipase, serum uric acid, and vitamin D3 levels of the treated rats are shown in Table 4.

Effects of methanolic leaf extracts on glycated hemoglobin levels of the animals

The diabetic untreated rats had considerably higher glycosylated hemoglobin (HbA1c) levels than the normal control rats. This could be due to the action of alloxan, which caused hyperglycemia by causing insulin secretion to decrease as a result of pancreatic beta-cell death. Increased blood glucose causes nonenzymatic adduction of glucose to the free amino groups at the *N*-terminal of hemoglobin's beta chain, resulting in glycosylated hemoglobin.[35] Similarly, Hb1Ac levels of the low-dose-treated animals were also significantly decreased (5.85 ± 0.18) when compared to the diseased group animals (6.18 ± 0.30). High HbA1c levels mean high risk of diabetes. The statistical significance between the groups was found to be $P< 0.0001$, which was considered extremely significant. Variation among column means is significantly greater than expected by chance. The effects of methanolic leaf extracts on HbA1c levels of the treated rats are shown in Table 5.

Effects of methanolic leaf extracts on mean blood glucose levels of the animals

Elevated blood glucose induces insulin release from pancreatic cells, which increases peripheral glucose consumption and regulates glucose homeostasis via many processes. In diabetics, this is disrupted, resulting in glucose intolerance.[36] Similar to earlier studies, mean blood glucose levels of the low-dose-treated animals were also decreased (104.83 ± 1.24) when

Table 1: Effects of *Aegle marmelos* (L.) methanolic leaf extracts on albumin-to-globulin ratio in diabetic rats

S. no.	Parameter(s)	Normal	Diseased	Glimepiride	Low dose	Medium dose	High dose
1	A/G ratio	1.74 ± 0.10	1.81 ± 0.81	1.86 ± 0.03	1.78 ± 0.03	1.92 ± 0.11	1.96 ± 0.08

Table 2: Effects of methanolic leaf extracts on bilirubin, direct bilirubin, and indirect bilirubin levels in diabetic rats

S. no.	Parameter(s)	Normal	Diseased	Glimepiride	Low dose	Medium dose	High dose
1	Bilirubin	0.08 ± 0.01	0.22 ± 0.01**	0.16 ± 0.00**	0.20 ± 0.00**	0.18 ± 0.00**	0.09 ± 0.01
2	Direct bilirubin	0.03 ± 0.001	0.063 ± 0.001**	0.044 ± 0.001**	0.053 ± 0.000**	0.04 ± 0.000**	0.034 ± 0.001
3	Indirect bilirubin	0.034 ± 0.001	0.063 ± 0.001**	0.044 ± 0.001**	0.053 ± 0.000**	0.040 ± 0.000**	0.034 ± 0.001

**$P < 0.05$ compared with control group

Table 3: Effects of methanolic leaf extracts on albumin levels in diabetic rats

S. no.	Parameter(s)	Normal	Diseased	Glimepiride	Low dose	Medium dose	High dose
1	Albumin	3.43 ± 0.12	5.36 ± 0.13**	4.31 ± 0.18**	5.45 ± 0.11**	4.45 ± 0.09**	3.73 ± 0.10

**$P < 0.05$ compared with control group

Table 4: Effects of methanolic leaf extracts on amylase, blood urea, blood urea nitrogen, calcium, globulin, glucose-6-phosphate, homocysteine, inorganic phosphate, lipase, serum uric acid, and vitamin D3 levels in diabetic rats

S. no.	Parameter(s)	Normal	Diseased	Glimepiride	Low dose	Medium dose	High dose
1.	Amylase	1492.5 ± 90.38	434.16 ± 68.05**	1634.83 ± 89.81	2302.33 ± 132.24**	1780.5 ± 61.33	1503.5 ± 41.94
2.	Blood urea	18.83 ± 1.42	29.5 ± 0.99**	20.00 ± 1.29	27.66 ± 1.08**	23.66 ± 0.88*	17.83 ± 1.42
3.	BUN	14.5 ± 1.38	25.83 ± 1.16**	15.5 ± 0.99	24.66 ± 1.20**	23.5 ± 1.17**	14.66 ± 1.11
4.	Calcium	10.3 ± 0.23	12.18 ± 0.16**	11.05 ± 0.07**	12.3 ± 0.10**	11.5 ± 0.11**	10.36 ± 0.16
5.	Globulin	1.76 ± 0.08	3.11 ± 0.11**	2.4 ± 0.11**	2.93 ± 0.08**	2.5 ± 0.05**	1.96 ± 0.08
6.	Glucose 6- phosphate	4.96 ± 0.08	14.36 ± 0.14**	13 ± 0.16**	13.98 ± 0.21**	13.43 ± 0.14**	4.61 ± 0.19
7.	Homocysteine	7.65 ± 0.12	16.1 ± 0.10**	13.13 ± 0.17**	15.03 ± 0.08**	13.78 ± 0.18**	8.05 ± 0.20
8.	Inorganic phosphate	5.85 ± 0.12	11 ± 0.11**	9.86 ± 0.13**	11.33 ± 0.12**	9.9 ± 0.14**	8.16 ± 0.32**
9.	Lipase	7.65 ± 0.12	16.1 ± 0.10**	13.13 ± 0.17**	15.03 ± 0.08**	13.78 ± 0.18**	8.05 ± 0.20
10.	Serum uric acid	3.96 ± 0.24	6.7 ± 0.11**	3.75 ± 0.14	6.43 ± 0.08**	5.51 ± 0.11**	3.46 ± 0.23
11.	Vitamin D3	26.91 ± 0.81	94.63 ± 1.01**	74.73 ± 1.20**	88.05 ± 0.66**	74.55 ± 1.23**	27.18 ± 1.18

**$P < 0.05$ compared with control group

Table 5: Effects of methanolic leaf extracts on *HbA1c* levels in diabetic rats

S. no.	Parameter(s)	Normal	Diseased	Glimepiride	Low dose	Medium dose	High dose
1	HbA1c	4.95 ± 0.28	6.18 ± 0.30**	5.15 ± 0.30	5.85 ± 0.18**	5.28 ± 0.27	4.65 ± 0.43

HbA1c = glycated hemoglobin

**$P < 0.05$ compared with control group

Table 6: Effects of methanolic leaf extracts on mean blood glucose levels in diabetic rats

S. no.	Parameter(s)	Normal	Diseased	Glimepiride	Low dose	Medium dose	High dose
1	Mean blood glucose	87.66 ± 2.24	116.33 ± 3.22**	83.83 ± 1.13	104.83 ± 1.24**	91.83 ± 1.64	81.83 ± 1.01

**$P < 0.05$ compared with control group

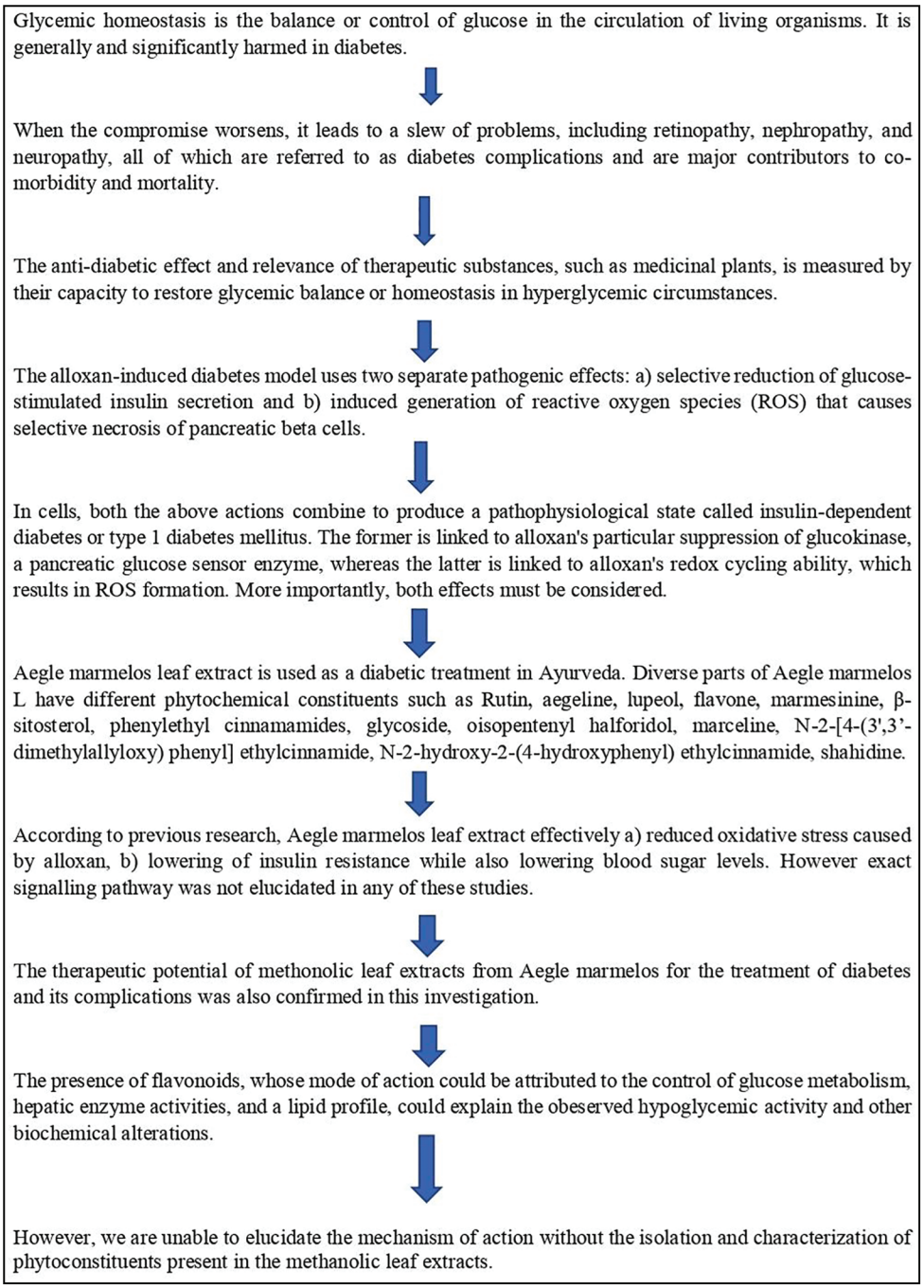

Figure 2: Schematic illustration of the antidiabetic activity of *Aegle marmelos* methanol leaf extract

compared to the normal group animals (87.66 ± 2.24). The statistical significance between the groups was found to be $P < 0.0001$, which was considered extremely significant. The effects of methanolic leaf extracts on mean blood glucose levels of the treated rats are shown in Table 6.

Earlier studies carried out on various leaf, 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.[37-39] Though we were are unable to elucidate the exact mechanism of action without the isolation and characterization of phytoconstituents present in the methanolic leaf extracts, based on the earlier studies the observed antidiabetic activity could be due to the presence of the following compounds such as (a) Rutin: (i) through inhibition of tissue gluconeogenesis, (ii) decrease in carbohydrate absorption from the small intestine, (iii) increase of tissue glucose uptake, (iv) stimulation of insulin secretion from beta cells, (v) prevention of Langerhans islet against degeneration, and (vi) decrease the formation of sorbitol, reactive oxygen species, advanced glycation end-product precursors, and inflammatory cytokines,[40] (b) Aegeline—through activation of Akt and Rab proteins,[41] and (c) Lupeol—through inhibition of α-amylase and α-glucosidase.[42] The findings of the earlier and this study in presented in Figure 2.

Conclusion

Through the biochemical changes, it is evident that the low dose of methanolic leaf extracts from *A. marmelos* can be used 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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Conflicts of interest

There are no conflicts of interest.

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