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Original Research Article

Study of antidiabetic activity of *Aegle marmelos* in streptozotocin-induced hyperglycemic Wistar rats in a tertiary care hospital

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ABSTRACT

Background: Diabetes has become a major metabolic disorder disease that is increasingly becoming a killer disease worldwide. The aim of this study was to evaluate (bael tree) *Aegle marmelos* whole plant extract has the potential efficiency to control hyperglycemia, that affects kidney and lipid profile, in streptozotocin induced rats. This study aimed to evaluate the Antidiabetic activity of, *Aegle marmelos* in streptozotocin-induced hyperglycemic Wistar rats.
Methods: Wistar rats of either sex were used grouped into six groups of six rats each. The first group was used as control, the second group was induced diabetes by inducting streptozotocin 70 mg/kg i.p., The 3rd,4th, 5th and 6th group was induced diabetes and treated with Metformin 600 mg/kg, *A. marmelos* methanol whole plant extract in the increasing dosage of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight from the 7th to 28th day respectively. The blood samples were collected at day 0,7 and 28th day for estimation of fasting blood sugar, serum cholesterol, triglycerides and creatinine. Results was presented as the mean standard deviation (SD). A one-way analysis variance was performed using SPSS-17 and Graphpad Prism 5 statistical software.
Results: After administration of *Aegle marmelos* extract in increasing level, fasting glucose level was significantly ($p < 0.05$) reduced. Also, there is significant reduction in serum cholesterol level, triglyceride level and creatinine level. The effect was more after 28 days of treatment.
Conclusions: The study results indicate the active compounds in the *Aegle marmelos* whole plant methanol extract has antidiabetic and antilipidemic activity. It can also protect kidney to some extent.

Keywords: *Aegle marmelos*, Anti-hyperglycemic activity, Hyperglycemic rats

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia related with diabetes mellitus is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels.[1] The global diabetes prevalence in 20-79-year-olds in 2021 will be estimated to be 10.5% (536.6 million people), rising to 12.2% (783.2 million) in 2045.[2]

The treatment approach for type 2 diabetes includes several conventional therapeutics, namely, sulfonylureas and repaglinide enhance insulin secretion, troglitazone increases insulin action in fat and muscle, metformin promotes insulin mechanism in liver tissue, and miglitol

and acarbose enact delayed carbohydrate absorption from food intake, respectively. The drugs used for the treatment of type 2 diabetes poses limitations in the sense that they have significant side effects.[3]

For this possible reason now a days the common people are shifting from allopathic system to Ayurveda for management of diabetes. The herbal drugs possessing multiple beneficial properties and enriched in many phytoconstituents have been used since ancient times for treating various diseases as medicines. As they are accomplished with natural occurrence, more efficacy and lesser side effects, the traditional herbal medicines are generally considered to be safe.

There is a long history in India for using medicinal plants to manage diabetes. Around 800 plants have been reported by World ethnobotanical information, to be used for controlling diabetes mellitus, of which the experimental proofs are available for only 410 plants for being anti-diabetic and for only 109 plants, the anti-diabetic mechanism is known.[4]

The use of herbs for treating diabetes is considered more advantageous as they cause much lesser side-effects. These herbal medicines reduce the blood glucose level and protect β-cells from harmful effects of diabetic condition by acting through different mechanism.[5]

Aegle marmelos is also known as Bael or Billipatra in India and had been in use since prehistoric or Vedic times for treating various medical ailments. The beneficial effects of the plant have been described by Charak in an ancient medical-treatise called Charak Samhita where they called it as "Rasayana" for its beneficial effects in curing diseases. The antihyperglycemic effects of the leaf extract of *Aegle marmelos* are well known.[6]

Therefore, this study was planned to see antidiabetic activity of, *Aegle marmelos* in streptozotocin-induced diabetic rats. Also, to estimate fasting blood sugar level of hyperglycemic rats treated with *Aegle marmelos* in comparison with standard drug and to estimate serum cholesterol, triglycerides and creatinine level of hyperglycemic rats treated with *Aegle marmelos* in comparison with standard drug.

METHODS

The experiment was initiated after obtaining approval from institutional animal ethics committee. (Protocol no: 003/Pharmac/IAEC/2023) in November 2023. The duration of experiment was 4 weeks from Jan 2024 to Feb 2024.

Chemicals

All chemicals and drugs was obtained commercially and was of analytical grade. Streptozotocin was used to induce hyperglycemia (Sigma). Metformin (600 mg/kg) was used as standard (Merck Serono).[7] Commercial kits for the estimation of serum glucose, cholesterol, triglyceride and creatinine was procured.

Plant material

Whole plant of *Aegle marmelos* was collected locally. The plant was identified by the Department of Botany at MGM Agriculture University, Auranagabad.

Preparation of extract

For whole plant extract, 100 gm of shade dried leaves, stem and bark was grinded in an electrical grinder and dissolved in 70% methanol solution. The mixture was left for 24 hrs with a magnetic stirrer at room temperature. The next day the mixture was strained out in a fine sieve and the crude extract was air evaporated for 3 days. The concentrated extract of plant was then orally administered to the rats in the different treatment groups (100 mg/kg, 200 mg/kg and 400 mg/kg body weight) using a syringe.[8,9]

Experimental animals

Wistar rats of either sex weighing 150-300 gm were used. The animals were fed with standard laboratory chow and has free access to water under well ventilated conditions of 12 hrs day and 12 hrs dark cycles. The animals were acclimatized to laboratory conditions prior to the experiment.

Induction of diabetes

The rats were made to fast 12 hrs before the induction of diabetes. Thereafter they were injected with streptozotocin (70 mg/kg, i.p.).[8] seven days after injection the rats with fasting blood glucose higher than 200 mg/dl were considered hyperglycemic and used for the experiment. Feeding was stopped 12 hrs before blood sampling.[10]

Experimental design

The experimental period was of 4 weeks. The first 7 days was for the induction of diabetes in rats and the following 3 weeks was the investigational period with crude aqueous extracts of bael whole plant which was administered separately. There was Six groups of six rats each.[10]

Blood sampling and biochemical analysis

Before and after administration of the methanol whole plant extracts and metformin, rats were anaesthetized using ketamine. Venous retro orbital blood samples were collected in the fasting state at specific intervals (day 0, 1, 7, 14, 28) using a glass capillary and collected in polystyrene tubes without the anticoagulant.[11] Serum was separated by centrifugation at 3000 rpm for 10 mins after which it was tested for sugar level by using glucose reagent kit (GOD-POD method). At the end of the experimental period (day 28) the blood samples collected was also tested

for serum cholesterol, triglycerides and creatinine. Samples were stored at -20ºC until assayed. Body weight was also being measured.

Table 1: Study group with drug and its dose.

Groups	Drugs	Drug dose
Group I	Distill water	1 ml distill water /oral route
Group II	Streptozotocin	70 mg/kg, i.p.
Group III	Streptozotocin + metformin	70 mg/kg, i.p. + 600 mg/kg bw oral
Group IV	Streptozotocin + *Aegle marmelos* plant extract	70 mg/kg, i.p. + 100 mg/kg bw oral
Group V	Streptozotocin + *Aegle marmelos* plant extract	70 mg/kg, i.p. + 200 mg/kg bw
Group VI	Streptozotocin + *Aegle marmelos* plant extract	70 mg/kg, i.p. + 400 mg/kg bw

Statistical analysis

Results were presented as the mean standard deviation (SD). A one-way analysis variance was performed using SPSS-17 and Graphpad Prism 5 statistical software. ANOVA test was used for inter group comparison. The values were considered significantly different when the p value was lower than 0.05.

RESULTS

We observed that fasting blood sugar level in Control group was 97.6±5.8 mg% at day 0, 96.2±3.6 at day 7 and 99.4±4.6 at day 28. The second group was treated with streptozocin 70 mg i.p. for 7 days. The fasting blood sugar at day 0, day 7 and day 28 was 100.4±4.4, 220.6±8.4 and 216.4±7.2 mg% respectively. The statistical significance between the groups was found to be P<0.05 (Table 2).

Table 2: Fasting blood sugar level in control group and streptozocin induced hyperglycemic group at day 0, day 7 and day 28.

Mean±SD	Day 0	Day 7	Day 28
Control	97.6±5.8	96.2±3.6	99.4±4.6
Hyperglycemic	100.4±4.4	220.6±8.4*	216.4±7.2*

P value * <0.05

Table 3 depicts comparison of fasting blood sugar level between the groups. In metformin treated group III the fasting blood sugar level was significantly reduced to 200±9.6 and 164±8.2 mg% at day 14 and day 28 from 211.4±12.3 at day 7. In *A. marmelos* 100mg/kg treated group IV the fasting blood sugar level was significantly reduced to 204±7.4 and 198±5.6 mg% at day 14 and day 28 from 224.6±10.4 at day 7. In *A. marmelos* 200mg/kg treated group V the fasting blood sugar level was significantly reduced to 206±8.2 and 186±4.8 mg% at day 14 and day 28 from 216.4±6.3 at day 7. In *A. marmelos* 400mg/kg treated group VI the fasting blood sugar level was significantly reduced to 201±6.2 and 170±5.4 mg% at day 14 and day 28 from 224.6±12.8 at day 7. The statistical significance between the groups was found to be P<0.05.

Table 4 depicts comparison of serum cholesterol level between the groups. In metformin treated group III the serum cholesterol level was little bit increased after treatment with streptozocin and reduction was seen after 28 days i.e. 34.6±2.2. All the three doses of *A. marmelos* caused reduction of serum cholesterol after 28 days. The results were statistically significant for 200 and 400 mg/kg.

Table 3: Comparison of fasting blood sugar level between the groups (control, metformin, and different doses of *A. marmelos* plant extract).

	Group III- Metformin	Group IV- *A. marmelos* 100 mg/kg	Group V- *A. marmelos* 200 mg/kg	Group VI- *A. marmelos* 400 mg/kg
Day 0	93.6±6.7	96.6±5.6	95.6±7.6	98.6±5.2
Day 7	211.4±12.3	224.6±10.4	216.4±6.3	224.4±12.8
Day 14	200±9.6*	204±7.4*	206±8.2*	201±6.2*
Day 28	164±8.2**	198±5.6*	186±4.8*	170±5.4**

P value * <0.05, ** <0.01

Table 5 depicts comparison of serum triglyceride level between the groups. In metformin treated group III the triglyceride level was 108±5.4mg% at day 0 which was increased after induction of hyperglycemia i.e. 122±4.6 mg% at day 7. This value was significantly reduced to 110±3.2 mg% at day 28. All the three doses of *A. marmelos* caused significant reduction in serum triglyceride level after 28 days. The values were 120±4.8, 108±5.3 and 114±6.1 mg% respectively.

Table 6 depicts comparison of serum creatinine level between the groups. In metformin treated group III the creatinine level was increased initially to 1.3±0.1 from 0.8±0.2 at day 0. Then there was a reduction observed at day 28 i.e. 1.2±0.1 mg% which was not significant. In A.

marmelos 100mg/kg treated group IV the creatinine level was reduced to 1.3±0.1 from 1.4±0.1 at day 28. In *A. marmelos* 200mg/kg treated group V the creatinine level was significantly reduced to 1.1±0.1 at day 28 from 1.3±0.1 at day 7. In *A. marmelos* 400mg/kg treated group VI the creatinine level was 1.4±0.1 at day 7 and 1.1±0.1 at day 28. The reduction was found to be statistically significant.

Table 4: Comparison of serum cholesterol level between the groups (control, metformin, and different doses of *A. marmelos* plant extract).

	Group III-Metformin	Group IV- *A. marmelos* 100 mg/kg	Group V- *A. marmelos* 200 mg/kg	Group VI- *A. marmelos* 400 mg/kg
Day 0	34.8±1.5	36.80±2.4	30.6±3.4	32.1±4.1
Day 7	36.2±1.8	40.2±3.2	38.2±2.6	44.4±3.3
Day 28	34.6±2.2	38.2±3.4	32.4±3.2*	35.6±2.8*

P value * <0.05

Table 5: Comparison of Serum triglyceride level between the groups (control, metformin, and different doses of *A. marmelos* plant extract).

	Group III-Metformin	Group IV- *A. marmelos* 100 mg/kg	Group V- *A. marmelos* 200 mg/kg	Group VI- *A. marmelos* 400 mg/kg
Day 0	108±5.4	114±6.6	118±6.2	120±6.6
Day 7	122±4.6	132±8.2	134±4.1	142±7.2
Day 28	110±3.2**	120±4.8*	108±5.3**	114±6.1**

P value * <0.05, ** <0.01

Table 6: Comparison of creatinine level between the groups (control, metformin, and different doses of *A. marmelos* plant extract).

	Group III-Metformin	Group IV- *A. marmelos* 100 mg/kg	Group V- *A. marmelos* 200 mg/kg	Group VI- *A. marmelos* 400 mg/kg
Day 0	0.8±0.2	0.9±0.3	0.7±0.1	0.8±0.1
Day 7	1.3±0.1	1.3±0.1	1.3±0.1	1.4±0.1
Day 28	1.2±0.1	1.2±0.1	1.1±0.1*	1.1±0.1*

P value * <0.05

DISCUSSION

The latest methods for treatment of diabetes have various shortcomings such as side effects or failure in management. The use of herbs for treating diabetes is considered more advantageous as they cause much lesser side-effects. These herbal medicines reduce the blood glucose level and protect β-cells from harmful effects of diabetic condition by acting through different mechanism.[5] *Aegle marmelos* whole plant methanolic extract was used in this study which is evaluated for its antihyperglycemic effects.[6]

We observed that fasting blood sugar level in Control group was 97.6±5.8 mg% at day 0 and 99.4±4.6 at day 28. The second group was treated with streptozocin 70 mg i.p. for 7 days. The fasting blood sugar at day 0, day 7 and day 28 was 100.4±4.4, 220.6±8.4 and 216.4±7.2 mg% respectively. The statistical significance between the groups was found to be p<0.05 (Table 1). We used streptozotocin was used to induce diabetes since it is reported not to cause cell damage of the pancreas unlike nitric oxide.[12]

Table 2 depicts comparison of fasting blood sugar level between the groups. There was significant reduction in fasting blood sugar level after 28 days in all the groups which is more with the use of high dose extract. Similar results were obtained by Marypatience Muyuka in their study.[13] After induction of streptozotocin in increasing dosage of 250 mg/kg, 350 mg/kg, 450 mg/kg there was increased blood sugar level. On administration of *Aegles marmelos* extract there was significant p<0.05 control within 14 days.

Another study conducted by Birudu et al showed 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).[7] The statistical significance between the groups was found to be p<0.05.

There was increased sugar levels in diabetic rats while those rats treated with plant extract showed lower glucose elevation thereby displaying improvement in glucose tolerance pattern, this means the plant utilized the blood glucose.[14] This hypoglycaemic effect action was either by refurbishing the islet function increasing insulin output or by facilitation of metabolites generated due to insulin action. Diabetes has become the primary cause of renal disease.[15]

In our study we observed that Streptozotocin induction elevates the level of cholesterol.[16] After treatment with whole plant extract of *Aegle marmellos* there was reduction in cholesterol level after 28 days which was significant for 200 and 400 mg/kg dose. This may be due to degradation of cholesterol by inhibition of endogenous synthesis of the cholesterol with the use of plant extract. Similar results were observed by Marypatience Muyuka.[13] During diabetes the levels of cholesterol are increased, insulin depletes level of lipoprotein lipase leading to deranged lipids.[17] Diabetes care 1991stated that reduction in lipids would be beneficial in long-term prognosis patients. This showed this plant extract could be useful in reducing hyperlipidaemia in diabetic patients.[18]

We also observed improvement in triglyceride level by whole plant extract of *Aegle marmellos* in our study. Narender et al also observed improvement in total cholesterol and triglyceride level in their research.[19]

There was little increase in serum creatinine level after induction of hyperglycaemia. When the rats were treated with the whole plant extract there was improvement in creatinine level. We can say that the plant extract may serve as reno protective.[20] Similar results were obtained by Marypatience Muyuka and Birudu et al.[7,13]

Limitation

The limitation of this study was short term duration. There is a need of long-term study to prove further benefits.

CONCLUSION

The study results indicate the active compounds in the *Aegle marmelos* whole plant extract may possess diverse biological action and therapeutic value based on its antidiabetic and antilipidemic and nephroprotective action. Through the biochemical changes, it is evident that the high dose of whole plant 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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