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# Hypoglycemic potensial of *Aegle marmelos leaf extract in alloxan induced diabetes in albino wistar rats*

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ABSTRACT

The present study was undertaken to evaluate the Anti-diabetic activity of *Aegle Marmelos,* family Rutaceae. Ethyl acetate extract of plant leaf of *Aegle Marmelos Linn* were prepared successively. Preliminary photochemical studies revealed the presence of chemical constituents like of Saponins and Tannins in ethyl acetate extract. The experiment was started with the treatment of Alloxan monohydrate by intraperitoneal route in a dose of 150 mg/kg body weight to all rats except group-I animals for 3 days alternatively for induction of diabetes. After induction of diabetes Group-I animals served as untreated control. Group-II animals were treated with 1ml normal saline orally for 7 days. Group-III animals were treated with 0.5ml DMSO orally for 7 days. Similarly Group-IV and Group-V animals were treated with 250mg and 500mg of *Aegle Marmelos* extract for 7days respectively. And after 7 days animals were fasted for 48 hrs and anaesthetized with thiopentone sodium and sacrificed. Blood samples were collected by carotid artery, then immediately blood glucose was measured with glucometer and centrifuged at 4500 rpm for 15 minutes. The supernatant was separated and used for estimation of various biochemical parameters. Anti-diabetic activity produced in ethyl acetate extract of *Aegle Marmelos* decreases the levels of Blood Glucose, Cholesterol, Triglycerides, HDL, LDL, VLDL.

## INTRODUCTION

Plants have been utilised as a natural source of medicinal compounds since thousands of years.Human is using numerous plants and plant derived products to cures and relief from various physical and mental illness. These plants are used in traditional Chinese, Ayurveda, Siddha,Unani and Tibetan medicines. Ancient literature such as Rigveda, Yajurveda, Atharvaveda,Charak Samhita and Sushrut Samhita also describes the use of plants for the treatment of various health problems[28].

World Health Organization has listed over 21000 plant species used around the world for medicinal purposes. In India, about 2500 plant species belonging to more than 1000 genera are being used in indigenous system of medicine. In terms of both quaninty and value of the medicinal plants exported, India ranks second in the world. India is one of the 12 mega biodiversity centers of the world wit 16-agro-climatic zones and has about 45000 plant species of which 15000 species are of flowering plants having about 7000 species identified as medicinal plants. There are about 400 families in the world of the flowering plants, of which at least 315 are represented by India. Despite of our rich heritage and knowledge of the use of plant drugs little attention has been paid to harness the inexpensive remedies to modern requriments[29].

Diabetic mellitus is a metabolic disease as old as mankind[3], affects more than 100 million people worldwide and in next five years it affects about five times more people than it now [4]. It is a growing health problem in most countries [5]. The prevalence rate of diabetes is estimated to be 1 to 5% in India [4]. The increasing number of agency population, consumption of calories rich diet, obesity and sedentary life style have lead to a tremendous increase in number of diabetes worldwide [6].

## MATERIALS AND METHODS

The plant leaf was collected during the month December to January and was identified

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pharmacognostically by a botanist. Then it was dried in shade, powdered, weighed and stored in a clean, dry and air tight container. The powder was subjected for successive extraction with solvents n-Hexane and ethanol.

**Powder Extraction**

Powder was packed in a round bottom flask and extracted with n-Hexane at $70^0$C temperature by soxhlet apparatus to remove the chlorophil content from palnt. After that the powder is extracted with ethyl acetate at $80^0$C by Soxhlet extactor. After extraction the residue was dried on water bath at $100^0$C to get a solid mass.

**Pharmacological Activities**

*Dosage*

Based on the references of previous acute toxicity studies of the plant, 250mg/kg & 500mg/kg doses were selected as low and high dose[7].

*Experimental Animals*

Adult male or female Wister rats, weighing 200 to 250g were used in the study. The study protocol was reviewed and approved by the institutional animal ethical committee and conforms to the Indian national science academy guidelines for the use and care of experimental animals in research. Animals were obtained from the Sri Venkateswara Enterprises, Bangalore. Rats were housed in polyacrylic cages (38X23X10 cm) with not more than four animals per cage. They were housed in an air conditioned room and were kept in standard laboratory conditions under natural light and dark cycle (approximately 14 h light/ 10 h dark) and maintained humidity 60±5% and an ambient temperature of 25±2°C. All experiments were performed between 9:00am and 4:00pm. the animals were free access to standard diet and tap water *ad libitum* and allowed to acclimatize for one week before the experiments. Commercial pellet diet contained 22 % Protein, 4% Fat, 4% Fiber, 36% Carbohydrates and 10% Ash w/w, supplied by Sri Venkateswara Enterprises, Bangalore was used.

*Experimental Protocol*

Rats were randomly divided into five groups, each consisting of six animals

Group 1: Normal group animals are untreated animals.

Group 2: Positive Control group animals treated with 1ml normal saline orally.

Group 3: Vehicle control group animals treated 0.5 ml of DMSO.

Group 4: Test group-I animals treated with 250 mg/kg *Aegle Marmelos* extract orally.

Group 5: Test group-II animals treated with 500 mg/kg *Aegle Marmelos* extract orally.

Note: All test agents were dissolved in DMSO and given orally for a period of 7 days for treatment.

*Chemical Substances*

Alloxan monohydrate was procured from the Avra Synthesis, Hyderabad. And all the other chemicals are procured from Merck laboratories, nice chemicals, Sd fine chemicals from local market.

*Drug Solutions*

Ethyl acetate extract of *Aegle Marmelos* is very sparingly soluble in aqueous solutions, to solubilise the extract DMSO was used as a vehicle and desired concentration of drug solutions were prepared. Alloxan was freshly prepared by dissolving in normal saline.

*Experimental Procedure*

In the present study albino wistar rats weighing about 200-250 gms were selected and divided into 6 groups containing 6 animals in each group. The experiment was started with the treatment of Alloxan monohydrate by intraperitoneal route in a dose of 150 mg/kg body weight to all rats except group-I animals for 3 days alternatively for induction of diabetes[64,70]. After induction of diabetes Group-I animals served as untreated control. Group-II animals were treated with 1ml normal saline orally for 7 days. Similarly Group-III and Group-IV animals were treated with 250mg and 500mg of *Aegle Marmelos* extract for 7days respectively. And after 7 days animals were fasted for 48 hrs and anaesthetized with thiopentone sodium and sacrificed. Blood samples were collected by carotid artery, then immediately blood glucose was measured with glucometer and centrifuged at 4500 rpm for 15 minutes. The supernatant was separated and used for estimation of various biochemical parameters by autoanalyser.

**Biochemical Estimations**

*Estimation of Serum Glucose[8]*

To 1500 µl of the reagent, 1500 µl of purified water, 20 µl of standard glucose (100 mg/dl) was added and incubated for 5 min at 37° C. This incubated mixture was aspirated and concentration of standard was calibrated to show a value of 100 mg/dl. The serum glucose was estimated by adding 20 µl of the serum sample to 1500 µl of the reagent, 1500 µl of purified water mixed well and incubated at 37° C for 5 min. This incubated mixture was aspirated and absorbance recorded against a reagent blank at 505 nm using Clinical Chemistry Analyzer.

*Triglycerides[9]*

To 1000 µl of the reagent, 10 µl of standard triglyceride (200 mg/dl) was added and incubated for 10 min at 37°C. This incubated mixture was aspirated and concentration of standard was calibrated to show a value of 200 mg/dl. The serum triglyceride was estimated by adding 10 µl of the serum sample to 1000 µl of the reagent, mixed well and incubated at 37° C for 10 min. This incubated mixture was aspirated and absorbance recorded against a reagent blank at 505 nm using Clinical Chemistry Analyzer.

*Cholesterol[10]*

To 1000 µl of the reagent, 10 µl of standard cholesterol (200 mg/dl) was added and incubated for 10 min at 37°

C. This incubated mixture was aspirated and concentration of standard was calibrated to show a value of 200 mg/dl. The serum cholesterol was estimated by adding 10 µl of the serum sample to 1000 µl of the reagent, mixed well and incubated at 37° C for 10 min. This incubated mixture was aspirated and absorbance recorded against a reagent blank at 505 nm using Clinical Chemistry Analyzer.

### Statistical Analysis

The data were represented as Mean ± S.D., and statistical significance between treated and diabetic control groups was analyzed using one-way ANOVA, followed by Tukey`s multiple comparison test. P < 0.0001 was considering statistically significant.

## RESULTS AND DISCUSSION

### Biochemical Parameters

*Blood Glucose*

With normal group Blood glucose levels were found to be **114.6 ± 5.25** with Control (Saline) Blood glucose levels were found to be **321.9 ± 4.88** and with vehicle control (DMSO) Blood glucose was reduced to **311.0 ± 1.24.** Hence all the values of treatment groups were compared with control group. When EAEAM was administered orally at the dose of 250mg/kg Blood glucose was significantly reduced to **130.81 ± 2.89 (*p<0.0001*)**. When EAEAM was administered orally at the dose of 500mg/kg Blood glucose was significantly reduced to **184.18 ± 3.56 (*p<0.0001*)**. There was a significant decrease in Blood glucose with EAEAM 250mg/kg.

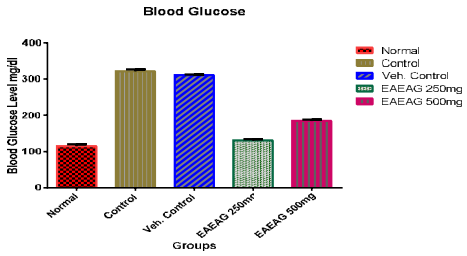


**Figure 1. Effect of extract on blood glucose**

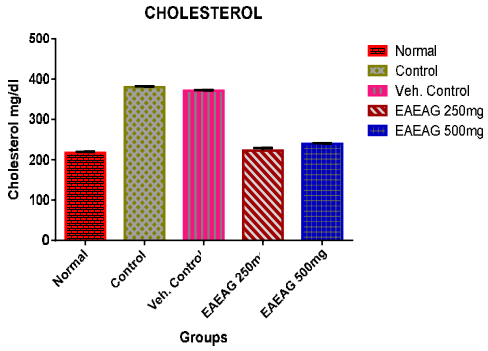


**Figure 2. Effect of extract on blood cholesterol**

*Cholesterol*

With normal group Cholesterol levels were found to be **217.91± 1.77,** with Control (Saline) Blood Cholesterol levels were found to be **380.08 ± 2.71** and with vehicle control (DMSO) Blood Cholesterol was reduced to **371.5 ± 1.63.** Hence all the values of treatment groups were compared with control group. When EAEAM was administered orally at the dose of 250mg/kg Cholesterol was significantly reduced to **223 ± 6.83 (*p<0.0001*)**. When EAEAM was administered orally at the dose of 500mg/kg Cholesterol was significantly reduced to **239.75 ± 1.33 (*p<0.0001*)**. There was a significant decrease in Cholesterol with EAEAM 250mg/kg and 500mg/kg.

*Triglycerides*

With normal group triglyceride levels were found to be **146.43± 1.39** with Control (Saline) triglyceride levels were found to be **253.08 ± 1.93** and with vehicle control (DMSO) triglyceride was reduced to **242.31 ± 1.25.** Hence all the values of treatment groups were compared with control group. When EAEAM was administered orally at the dose of 250mg/kg triglyceride levels was significantly reduced to **169.46 ± 5.03 (*p<0.0001*)**. When EAEAM was administered orally at the dose of 500mg/kg triglyceride levels was significantly reduced to **198.75 ± 1.46 (*p<0.0001*)**. There was a significant decrease in triglyceride with EAEAM 250mg/kg.

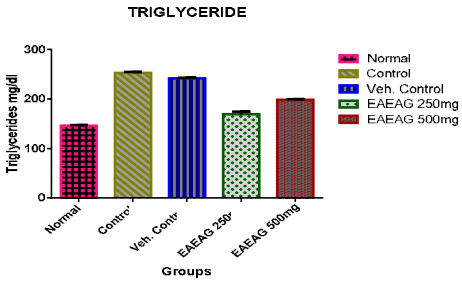


**Figure 3. Effect of extract on triglyceride**

*High Density Lipoprotein*

With normal group HDL levels were found to be **36.60 ± 0.35** with Control (Saline) HDL levels was found to be **63.26 ± 0.48** and with vehicle control (DMSO) HDL was reduced to **60.58 ± 0.3.** Hence all the values of treatment groups were compared with control group. When EAEAM was administered orally at the dose of 250mg/kg HDL levels was significantly reduced to **42.36 ± 1.26 (*p<0.0001*)**. When EAEAM was administered orally at the dose of 500mg/kg HDL levels was significantly reduced to **49.68 ± 0.36 (*p<0.0001*)**. There was a significant decrease in HDL with EAEAM 250mg/kg.

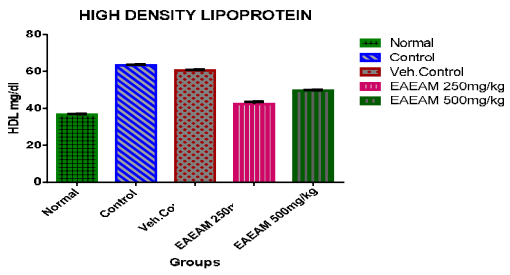


**Figure 4. Effect of extract on HDL**

*Low Density Lipoprotein*

With normal group LDL levels were found to be **181.31 ± 1.80** with Control (Saline) LDL levels was found to be **316.81 ± 3.03** and with vehicle control (DMSO) LDL was reduced to **313.71 ± 6.77.** Hence all the values of treatment groups were compared with control group. When EAEAM was administered orally at the dose of 250mg/kg LDL levels was significantly reduced to **180.65 ± 6.49 (*p<0.0001*)**. When EAEAM was administered orally at the dose of 500mg/kg LDL levels was significantly reduced to **190.06 ± 1.31 (*p<0.0001*)**. There was a significant decrease in LDL with EAEAM 250mg/kg.

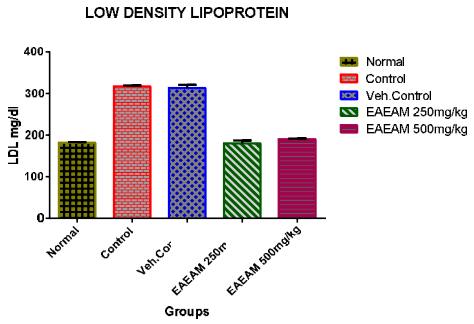


**Figure 5. Effect of extract on LDL**

*Very Low Density Lipoprotein*

With normal group VLDL levels were found to be **43.58 ± 0.35** with Control (Saline) VLDL levels were found to be **76.01 ± 0.54** and with vehicle control (DMSO) VLDL was reduced to **74.3 ± 0.32.** Hence all the values of treatment groups were compared with control group. When EAEAM was administered orally at the dose of 250mg/kg VLDL levels was significantly reduced to **44.6 ± 1.36 (*p<0.0001*)**. When EAEAM was administered orally at the dose of 500mg/kg VLDL levels was significantly reduced to **47.95 ± 0.26 (*p<0.0001*)**. There was a significant decrease in VLDL with EAEAM 250mg/kg.

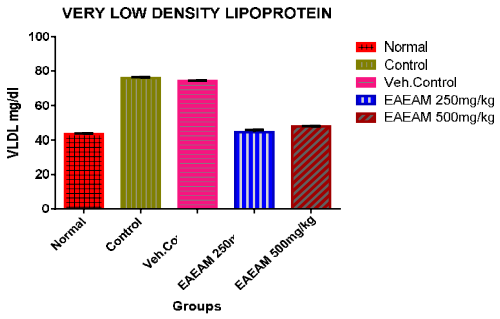


**Figure 6. Effect of extract on VLDL**

## DISCUSSION

Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted[74]. Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from Streptozotocin. Alloxan has a destructive effect on the beta cells of the pancreas[11]. Alloxan, a beta cytotoxin, destroys betacells of islets of langerhans of pancreas resulting in a decrease endogenous insuline secretion and paves the ways for the decreased utilization of glucose by the tissue. It results in elevation of blood glucose level. Expresion of elivated pasting blood glucose level conformed induction of diabetes in alloxan induced experimental rats, thereby inducing hyperglycaemia. Experimental studies reveal that ethyl acetate extract from *Aegle Marmelos* (250mg/kg&500mg/kg) orally administered for 7days produce a significant decrease in the blood glucose level in the model of alloxan induced diabetes in rats. It also proves the traditional claim with regard to *Aegle Marmelosc* for its anti-diabetic activity.

## CONCLUSION

In the present investigation it was observed that Diabetes produced by the 150mg of Alloxan is prevented by treatment of groups ethyl acetate extract of *Aegle Marmelos* decreases the levels of Blood Glucose, Cholesterol, Triglycerides, HDL, LDL, VLDL. This protective effect of ethyl acetate extract may be due to the presence of Alkaloids, Triterpenoids and Tannins. Further studies are needed to elucidate the particular Alkaloids, Triterpenoids and Tannins which is responsible for Anti-diabetic activity.

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