

## Comparative Study of Aegle Marmelos Correa and Eugenia Jambolana on Blood Glucose Level in Alloxan- Induced Diabetic Albino Rats



### Medical Science

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**Seema Baishnab**

Assoc Professor, Department of pharmacology, MAMC, Agroha, Hisar, Haryana

**Farhana R**

Assoc Professor, Department of pharmacology, Sree balaji medical college hospital, Chennai

### ABSTRACT

*The aim of the present study is to compare the action of extract of Aegle marmelos correa and of Eugenia Jambolana (Jamun) on blood glucose level in normal and alloxan-induced diabetic albino rats. The extracts (100mg/kg/d) was administered orally for four weeks to alloxan-induced diabetic rats. Blood glucose was estimated every week for four consecutive weeks. The test drugs that is Aegle marmelos correa and Eugenia Jambolana significantly ( $p < 0.01$ ) reduced the rise in blood glucose induced by alloxan individually but when compared with each other, they do not show significant ( $p > 0.01$ ) reduction of blood glucose.*

### Introduction

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia<sup>1</sup>. A changing lifestyle in developing countries like India has enormously increased the statistical figures of diabetes mellitus. A survey depicts that 4% of adults in India suffered from DM in year 2000 and it is expected to increase by 6% by the year 2025.<sup>2</sup>

Aegle marmelos correa is a tree belonging to the family Rutaceae, occurring throughout India, Srilanka, Pakistan, Bangladesh, Myanmar, Thailand and most Southeastern Asian countries. Aegle marmelos correa commonly known as Bael fruit is mildly astringent and used to cure dysentery, diarrhea,<sup>3</sup> hepatitis, tuberculosis, dyspepsia and good for heart and brain.<sup>4</sup> Phytochemical analysis of bael fruit has revealed the presence of alkaloids, coumarin, marmin, imperatorin, aegelin, tannins.<sup>5</sup> The powdered bael fruit is used by the local people of Assam in the treatment of diabetes.

Eugenia Jambolana (syn. Syzygiumcumini, jambul, black plum) belongs to the family Myryaceae.<sup>6</sup> It is a large evergreen tree, which grows up to 30 m height. It is widely distributed throughout India. It is commonly known as jamun, or Indian blackberry. It has valued in Ayurvedic and Unani system of medication for possessing variety of therapeutic properties.<sup>7</sup> EJ has been indicated in Ayurveda for use in diabetes.<sup>8</sup> EJ seeds have been reported to have anti-inflammatory<sup>9</sup>, neuropsychopharmacological<sup>10</sup>, anti-bacterial<sup>11</sup>, anti-HIV,<sup>12</sup> and anti-diarrhoeal effects.<sup>13</sup> EJ seed kernel decreased the oxidative stress in diabetic rats, which in turn may be due to its hypoglycemic property.<sup>14</sup>

Keeping this in view, the present study was aimed for comparing the effect of Aegle marmelos correa and Eugenia Jambolana on blood glucose level in alloxan-induced diabetic albino rats.

### Materials and Methods

#### Plant material and extraction

The fruit pulp of Aegle marmelos were collected from the local market in Dibrugarh in the months of June to August. The plant material was authenticated by Dr S.S.Dhawan, Professor of food and nutrition department, HAU, Hisar, Haryana. Eugenia Jambolana fruit was obtained from the local market in the months of June to August and identified by the Department of Botany, Assam University, Dibrugarh, Assam. The fruit pulp of Aegle marmelos were manually separated from the outer seed shell, air dried, powdered (1600 g) and aqueous extracts were prepared

using sufficient water by percolation method followed by steam evaporation. A final yield of 165 g of the extract was obtained.<sup>15</sup> The required amount of seed and fruit pulp of EJ were collected and dried in room temperature. About 500gm of both the seed and pulp were ground separately into powdered form and packed separately into the percolator along with 90% ethyl alcohol for 48hrs. Materials were collected from the percolater and alcohol was allowed to evaporate.

#### Animals

Healthy adult Wistar albino rats (*Rattus norvegicus*) weighing 200–250 grams each were used for the individual study. All the animals were taken care of under ethical consideration as per the guidelines of the CPCSEA with due approval from the Institutional Animal Ethical Committee (Registration no: 634/02/a/CPCSEA; dated 19/5/2002).

#### Chemicals used

Crude powder of glibenclamide was obtained from Sun Pharma Ltd., Mumbai while alloxan monohydrate was purchased from Sigma Aldrich India, Bangalore. The glucose kit for blood glucose estimation was obtained from Sigma Diagnostic (India) Pvt. Ltd., Baroda.

#### Experimental design for antidiabetic study

For Aegle marmelos - A total of thirty animals were equally divided into five groups with six animals in each group:

Group-A : Normal Control. Received normal saline, 2 ml/kg/d.

Group- B: Normal Test. Received normal saline, 2 ml/kg/d.

Group-C: Diabetic Control. Received normal saline, 2 ml/kg/d.

Group-D: Diabetic Test. Received aqueous extract of Aegle marmelos (AEAM), 100 mg/kg/d.

Group-E: Diabetic Standard. Received glibenclamide, 2.5 mg/kg/d.

The above drugs were administered orally, once daily, for four weeks.

For E Jambolana - The animals were selected randomly and divided in five groups consisting of 6 animals in each group. All the animals were treated for 4 weeks.

**Group 1:** Normal control (3% gum acacia suspension) 5ml/

kg b.w/day p.o.

**Group 2:** Diabetic control (3% gum acacia suspension) 5ml/kg b.w/day p.o. **Group 3:** Seed of *E. jambolana* 100 mg/kg b.w./day p.o.

**Group 4:** Pulp of *E. jambolana* 100 mg/kg b.w/day p.o.

**Group 5:** Glibenclamide 25 mg/kg b.w/day p.o

After every 1 week fasting blood samples were taken from the orbital sinus of the

treated animals and blood glucose level were measured by glucose oxidase method.

#### Induction of Diabetes

A single dose (150mg/kg b.w. ip) of alloxan monohydrate dissolved in normal saline were used for induction of Type II diabetes in rats after overnight fasting. After 1 hr of alloxan administration, the animals were fed standard pellets and water *ad libitum*. The animals were stabilized for 72hours and animals showing blood glucose level (estimated by glucose oxidase method)<sup>16</sup> more than 200mg/dl were selected for the study.

#### Statistical analysis

The data was statistically analysed for individual effect by using One-way ANOVA<sup>17</sup> followed by Dunnett's multiple comparison test.<sup>18</sup> Values of  $p < 0.01$  were considered significant. For comparative study Mann Whitney U Test (non parametric test)<sup>19</sup> done. Values of  $p < 0.01$  were considered significant.

#### Results

##### Effect on blood glucose level

##### For Aegle Marmelos group –

Normal Rats: No significant ( $p > 0.01$ ) difference of blood glucose level was found in Normal Control Group and Normal test Group after four weeks of drug administration .

Diabetic Rats: On repeated administration of the extract and glibenclamide for four weeks, a significant ( $p < 0.01$ ) decrease in blood glucose was found in Diabetic Test Group and Diabetic Standard Group respectively as compared to Diabetic Control Group which showed a significant  $p < 0.01$  rise in blood glucose as compared to Normal Control Group. However, both the drugs failed to restore the blood glucose level to that of the Normal Control Group.

##### For E Jambolana group –

Analysis of data shows a decrease in the blood glucose level on treatment with the ethanolic extract of seed and pulp of *E. jambolana* (100mg/kg bd wt). Both the extract exhibited a statistically significant decrease ( $p < 0.01$ ) in the blood glucose level when comparison was done with diabetic control group .Values are expressed as Mean  $\pm$  SEM; n=6 rats in each group. One-way ANOVA followed by Dunnett's multiple comparison tests was done.  $p < 0.01$  when compared to Normal Control Group.  $p < 0.01$  when compared to Diabetic Control Group.

For compared groups between Aegle Marmelos fruit and E Jambolana seeds –

Analysis of data shows no significant reduction of blood glucose level when compared with each other( $p \geq 0.05$ ).

Comparison done by Mann Whitney U Test (non parametric test).

#### Discussion

From the study, it was seen that A Mermelos and E. Jambolana significantly ( $p < 0.05$ ) lowered the blood glucose level in diabetic rats. However, the anti hyperglycemic action of A Mermelos may be attributed to the insulin-like effects of the constituents of the fruit pulp of Aegle Marmelos. The study also reports the antidiabetic activity of ethanolic extract of seed and pulp of *E. jambolana*, which is a well known fruit in Ayurveda . But when both extracts compared with each other, there is no significant difference in lowering the blood glucose level. Raised blood glucose level is the principal stimulus for insulin secretion.<sup>20</sup>

Alloxan, a  $\beta$ -cytotoxic agent, rapidly and selectively accumulates in pancreatic  $\beta$ -cells and causes  $\beta$ -cell death and apoptosis by generation of reactive oxygen species (ROS), superoxide radicals and hydrogen peroxide.<sup>21,22</sup>  $\beta$  cell death causes hyperglycemia due to insulin deficiency which further aggravates the oxidative stress induced by alloxan.<sup>23</sup>

The antidiabetic activity of the fruit pulp of Aegle Marmelos might be attributed to the presence in it of Coumarins which potentiate the insulin secretion from existing beta cells of the islets of langerhans.<sup>24</sup> Flavonoids are also known to regenerate the damage beta cells in the alloxan diabetic rats.<sup>25</sup> The flavonoids may be responsible for hypoglycaemic action if E Jambolana. The antioxidant activity might be due to the presence of phenolic compounds such as flavonoids.<sup>26</sup> Furthermore, previous studies have reported the protective action of flavonoids against oxidative stress induced cellular damage<sup>27</sup> and also the ability of flavonoids to regenerate  $\beta$ -cells.<sup>28</sup>

#### Conclusion

Thus, there is antidiabetic effect of Aegle Marmelos and E.Jambolana when studied individually but when compared with each other for antidiabetic effects, the result is insignificant. However, further studies to isolate the active principle of Aegle Marmelos and E.Jambolana responsible for hypoglycemia, together with studies on serum insulin assay to confirm its insulin releasing action have to be undertaken.

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