

A Screening on the Type I Anti-Diabetic Activity of the Methanolic Extract of *Aegle Marmelos* in Streptozotocin Induced Rat Model

ABSTRACT:

Aegle marmelos, generally acknowledged as Bael, is being ancient in Ayurveda for the therapy of a number of disorders. All the components on it tree along with stem, bark, root, leaves, fruit and seeds at all stages of maturity have medicinal virtues and have been aged in Ethno-medicine.

Aims: The present investigation study the screening on the Type I anti-diabetic activity of the methanolic extract of *Aegle marmelos* in STZ induced in rat's model.

Study design: In-vivo study in rat's model

Place and Duration of Study: Department of Pharmacology, Karnataka college of Pharmacy, Bangalore, India, between Jan 2021 to Dec 2021.

Methodology: Extracted *Aegle marmelos* was to be evaluated the toxicity as per the OECD guidelines and biochemical, hematological and gross pathological analysis has been assessed. Type I Diabetes has been induced in Wistar rats thru STZ 65mg/kg/b.w. I.P. During the experiment, Rat's BW and FBS level were monitored. At the end of study, animals among whole groups have been sacrificed and biochemical parameters; Lipid profile, C-Peptide, HbA1c, Serum insulin, pancreatic insulin, histology of pancreas had been performed. *Aegle marmelos* was also screened for pro-inflammatory cytokines viz., IL-1 β , IL-6 and TNF- α were observed by ELISA. Furthermore, Antioxidant Enzyme like SOD, CAT, LPO and GSH were performed.

Results: The observed extract *Aegle marmelos* was shown in safe in the toxicity data. And it has been shown significant impact *in vivo* to manage the diabetic markers like weight gain, blood glucose, lipid profile, C-Peptide, HbA1c, release of insulin secretion, and pancreatic insulin. Diabetic pancreas of rats confirmed fall of beta cell density and disruption of normal architecture. But treated group were found to restore the mass of beta cells. Mediator of inflammatory cytokines like increased in STZ group and was inhibited by test chemicals. Elevated oxidative enzymes also have been seen to control upon the treatment with *Aegle marmelos*.

Conclusion: All this findings and phytoconstituents present within the extract should be the possible chemical substances concerned in the prevention of diabetes.

Keywords: *Aegle marmelos*, STZ, Toxicity, Diabetes, Cytokines

Comment [p1]: Mention the names of the groups

1. INTRODUCTION:

Diabetes mellitus is the most common metabolic disease characterized by way of continual hyperglycemia, which is due in accordance with carbohydrate, protein and lipid metabolism

disturbance triggered through a relative or utmost deficiency in insulin secretion and/or insulin action within the peripheral tissues¹. DM has emerged as the 0.33 greatest "killer" after cancer and cardio, cerebrovascular diseases². It is estimated as 5% on demise in the world is caused by diabetes, a quantity which will increase with the aid of 50% within the next 10 years³. There is thriving proof as the excess generation of ROS into diabetes, as reason oxidative stress, may thoroughly and of part make a contribution towards the improvement of problems of a variety over tissues^{4, 5}. Because DM control without side effects is a challenge, pills derived from plants may additionally lead an essential function between the remedy over DM⁶. Natural merchandise isolated from medicinal plant sources have been ancient for the siege and therapy on a number of diseases pathologies, consisting of cancers, heart disease, diabetes mellitus or high blood pressure^{7, 8}. Up to 2014, More than 800 kinds have been investigated and theirs hypoglycemic results have been reported⁹.

Aegle marmelos is a medicinal plant of family Rutaceae which is typically acknowledged as like Bael, Bengal-quince, golden apple or wood/stone apple tree. It is a medium-sized deciduous tree, up to 12-15 m tall with a short trunk, thick, soft, flaking bark and spreading, occasionally spiny branches¹⁰. This plant is provincial to Northern India but extensively located throughout the Indian Peninsula and in Ceylon, Burma, Bangladesh, Thailand and China. It is also grown in partial Egyptian gardens, within Surinam and Trinidad^{11, 12}. *A. marmelos* crop plants are globose with a smooth, hard and aromatic shell that is grey-green when raw and yellowish when ripe. The fruit pulp is faded orange, sweet, resinous and noticeably aromatic^{13, 14}. This fruit is broadly used into folks remedy for the treatment of diabetes mellitus¹⁵, so properly it is used in the treatment over chronic diarrhea, dysentery and peptic ulcers, as a laxative and in conformity with get better out of respiratory affections¹⁶. *A. marmelos* crop plants has been acknowledged in imitation of possess antioxidant¹⁷, radioprotective¹⁸, gastroprotective¹⁹, anti-ulcerative colitis²⁰, hepatoprotective²¹, cardioprotective²² and antidiabetic²³ activities. *A. marmelos* fruit possesses excessive nutritional value. The crop is aged to redact juice, jam, syrup, jelly, toffee and other products. The pulp is observed to contain water, sugars, protein, fiber, fat, calcium, phosphorus, potassium, iron, minerals yet nutritional vitamins (Vitamin A, B1, C then Riboflavin)^{14, 24} as like well as bioactive compounds, kind of carotenoids, phenolics, alkaloids, pectens, tannins, coumarins, flavonoids and terpenoids^{25, 26}. Therefore, this study was aimed to evaluate the anti-diabetic of *A. marmelos* fruits of methanolic extract against STZ induced diabetes in rats.

2. MATERIALS AND MEHTODS:

2.1 Collection of Plant Material:

The fruits of *Aegle marmelos* were brought from Bangalore, Karnataka, India. The plant specimen has been identified and authenticated by department of botany, University of Rajasthan, Jaipur and specimens were kept for the reference. And reference number was RUBL 211761.

2.2 Extraction of Fruits of *Aegle marmelos*²⁷⁻³¹

Preparation of Extract: The fruits of *Aegle marmelos* were chopped into small pieces and dried under shade at room temperature for seven days. The dried fruits were powdered and passed through the sieve (Coarse 10/40). The powder was used for the preparation of methanolic extract.

Method of extraction: Each 100gm powder was subjected to extraction with 1000ml methanol in a reflux condenser for 3 cycles of 7hrs each till the volume reduced to half. Extract was filtered through Whatman filter paper No.1 and evaporated to dryness to get constant weight.

2.3 Experimental Animals:

Female *Albino* mice weighing between 25-35gm for toxicity studies and *Wistar* male rats (8-10 weeks old) weighing 150-200gm were used for the main experiment. All the experiments conducted on the animals were in accordance with the standards set for the use of the laboratory animal use and the experimental protocols were duly approved by

the IAEC of Karnataka College of pharmacy, Bangalore (Reg. Number: 1564/PO/Re/S/11/CPCSEA).

2.4 Experimental design:

2.4.1 Acute oral toxicity study:

The acute oral toxicity study was performed according to the OECD guidelines No. 425. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. Parameters were analyzed: Body weight, Blood Glucose Level, Lipid Profile, Renal Function Test (RFT), Liver Function Test (LFT), Hematological parameters Blood samples were performed using an Automatic Hematology Analyzer, and Vital Tissue Histology (i.e. Kidney, Liver, Spleen, Heart, and Lung). A dose of 1/10th and 1/20th were considered to be high dose and low dose prepared by dissolving in milliQ water. The doses were prepared as per the OECD guideline No. 425.

2.4.2 Model for Type I Diabetes Mellitus:

2.4.2.1 STZ induced Diabetes Mellitus³²:

Wistar male rats (150-200g) were considered for this analysis and diabetes induced through I.P., dose of STZ 65mg/kg/b.w. STZ was made freshly before administration and dissolved in the buffer of 0.1 M cold sodium citrate and pH 4.5. In order to avoid hypoglycaemia, STZ-Rats were fed 5% w/v glucose solution for 24 hours. After 72h, rats were recorded FBS >180 mg/dL and chosen for the analysis. All the animals were given free access to have the tap water and pellet diet and held in polyethylene cages at room temperature. Rat's body weight, FBS levels of rats were taken with one-touch glucometer prior to and after the end of the test, i.e. 0 and 30 days.

Table 1. Groupings were done by following manner, Where N = 6 animals in each group;

01.	STZ induced Diabetes Mellitus in Rat's Model	Group I: Normal Control Group – Vehicle Only.	6 rats
		Group II: Disease Control, Received STZ 65mg/kg/b.w I.P	6 rats
		Group III: Standard drug, Received Insulin 4U/kg/b.w. i.p + STZ 65mg/kg/b.w I.P	6 rats
		Group III: Test drug (Low dose), Received <i>Aegle marmelos</i> X mg/kg/b.w P.O + STZ 65mg/kg/b.w I.P	6 rats
		Group IV: Test drug (High dose), Received <i>Aegle marmelos</i> Y mg/kg/b.w P.O + STZ 65mg/kg/b.w I.P	6 rats

Comment [p2]: Why was the number of each mouse in the even group selected ???

Comment [p3]: Why are the names of the two groups similar?

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At last, Animals were finally anaesthetized with high dose of Phenobarbital. Blood was collected by Cardiac puncture and tissues were collected and then examined. The parameters;

- Blood Glucose Level (Using Digital Glucometer, One touch select, LifeScan Scotland Ltd, UK), Serum Insulin, Pancreatic Insulin (Sandwich ELISA Assay), C-peptide, Hb1Ac (Span Diagnostic), and Lipid Profile (DELTA LABS Kit, Bangalore, India)
- Measurement of Pro-Inflammatory Cytokines, Markers of disease severity; IL-6, IL-1beta, and TNF-alpha by Sandwich ELISA Assay (Commercial Available kit, Mercodia, Sweden)³³⁻³⁷.
- Antioxidant Enzyme Studies: Lipid Peroxidation (LPO)^{38, 39}, Reduced Glutathione (GSH)^{40, 41}, Superoxide dismutase (SOD)⁴², and Catalase (CAT)⁴³.
- Histopathology Study: Pancreas⁴⁴

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Histology of Pancreas tissue – H&E Staining:

The animals were euthanized using high dose of Pentobarbital and then sacrificed and the pancreas of each animal was isolated and was cut into small pieces, preserved and fixed in

10% formalin for two days., dehydrated with alcohol, embedded in paraffin, cut into 4-5 m thick sections, and stained with Haematoxylin-Eosin dye for photomicroscopic observation. The microscopic features of the organs of rats were compared with the control group.

3.0 STATISTICAL ANALYSIS:

The results are expressed as Mean \pm SEM from N=6 rats in each group. Data were analysed using statistical software Microsoft Excel worksheet. The significance of difference among the groups was assessed using Student t-test compared between Normal control (Untreated) vs. all groups $p < 0.05$ were considered significant.

4.0 RESULTS:

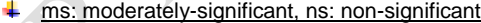
The yield of methanolic extract of fruits of *Aegle marmelos* was calculated and the % Yield was 27.5.

Mortality was not seen in the acute toxicity up to a dose of 5000mg/kg.

Dose: Selection of dose was done on the basis of acute toxicity OCED guideline 425. 5000 mg/kg body weight was tolerated dose and no signs of toxicity have been found, after performing the acute oral toxicity studies. 1/20th and 1/10th of the same dose was selected; 250mg/kg and 500mg/kg respectively and the further study were carried out.

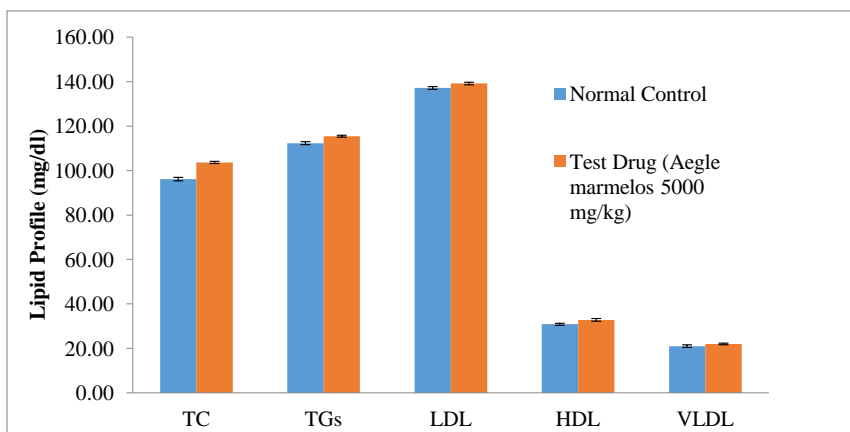
4.1 Toxicity Reports of Acute toxicity on 5000 mg/kg/B.W. of Dose of *Aegle marmelos*

Table 2. Body weight and Blood Glucose Level

PARAMETERS	RESULTS			
	Normal Control	\pm S.E.M.	Test Drug (<i>Aegle marmelos</i> 5000 mg/kg)	\pm S.E.M.
Body weight in gm.	26.17	0.088	26.83	0.042
Normal Control Vs. Test Drug	t-Test: Paired Two Sample for Means $P(T \leq t)$ one-tail - 0.0013 ^{ms}			
Blood Glucose Level (mg/dl)	82.67	0.667	82.67	0.494
Normal Control Vs. Test Drug (<i>Aegle marmelos</i> 5000 mg/kg)	t-Test: Paired Two Sample for Means $P(T \leq t)$ one-tail - 0.5 ^{ns}			
				

Values are expressed as Mean \pm S.E.M; (n =6/group).

Figure 1. Lipid Profile (mg/dl)



Values are expressed as Mean \pm S.E.M; (n =6/group).

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Lipid Profile	TC	TGs	LDL	HDL	VLDL
Normal Control Vs. Test Drug (<i>Aegle marmelos</i> 5000 mg/kg)	0.00086 ^{ss}	0.0088 ^{ms}	0.071 ^{ns}	0.016 ^{ws}	0.055 ^{ns}
t-Test: Paired Two Sample for Means <i>P</i> (T<=t) one-tail					
* <u>ss: strongly-significant, ms: mildly-significant, ws: weakly-significant, ns: non-significant</u>					

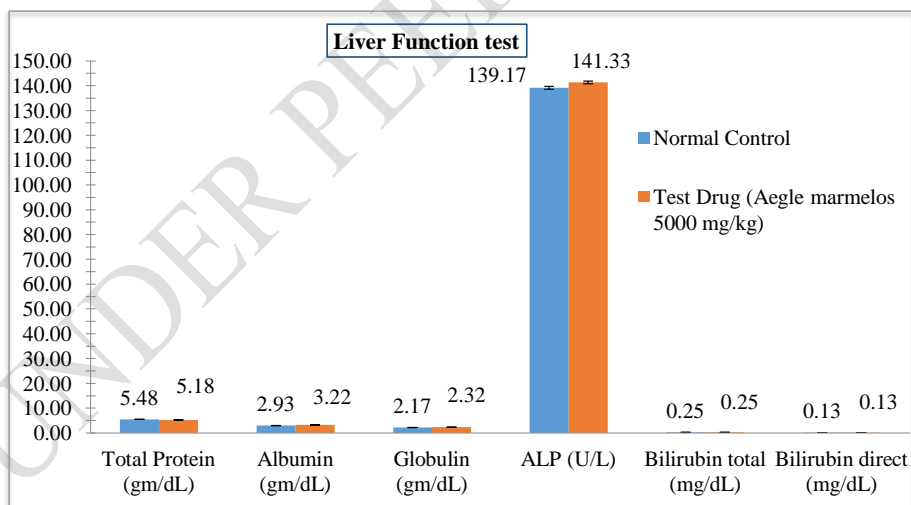
Table 3. Interpretation between the groups

Table 4. Serum Electrolytes

Serum Electrolytes	Normal Control \pm S.E.M.	Test Drug (<i>Aegle marmelos</i> 5000 mg/kg) \pm S.E.M.	P(T<=t) one-tail
<i>t</i> -Test: Paired Two Sample for Means (Normal Control Vs. Test Drug)			
Sodium (m mol/L)	136.00 \pm 0.632	140.00 \pm 0.365	0.0013 ^{ms}
Potassium (m mol/L)	3.72 \pm 0.060	3.95 \pm 0.043	0.016 ^{ws}
Chloride (m mol/L)	107.50 \pm 0.764	113.50 \pm 0.764	0.0052 ^{ns}
Urea (mg/dl)	24.83 \pm 0.401	27.33 \pm 0.882	0.032 ^{ws}
Creatinine (mg/dl)	0.20 \pm 0.052	0.32 \pm 0.060	0.067 ^{ns}
Uric acid (mg/dl)	2.60 \pm 0.052	2.90 \pm 0.058	0.0071 ^{ms*}
🚩 <u>ms: mildly-significant, ws: weakly-significant, ns: non-significant, ms*: moderately significant</u>			

Values are expressed as Mean \pm S.E.M; (n =6/group).

Figure 2. Liver Function Profile



Values are expressed as Mean \pm S.E.M; (n =6/group).

Table 5. Interpretation between the groups

Liver Function Profile	TP	Alb	Glb	ALP	BT	BD

Normal Control Vs. Test Drug (<i>Aegle marmelos</i> 5000 mg/kg)	0.0046 ^{ms}	0.017 ^{ws}	0.015 ^{ws}	0.046 ^{ws}	0.5 ^{ns}	0.5 ^{ns}
	t-Test: Paired Two Sample for Means <i>P</i> (<i>T</i> ≤ <i>t</i>) one-tail					
✚ ms: mildly-significant, ws: weakly-significant, ns: non-significant						

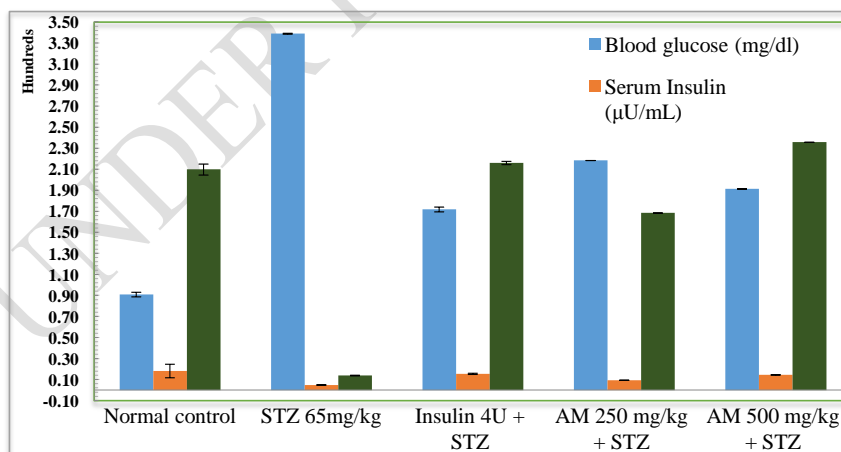
Table 6. Haematological Parameters

Haematological Test	Normal Control ± S.E.M.	Test Drug (<i>Aegle marmelos</i> 5000 mg/kg) ± S.E.M.	<i>P</i> (<i>T</i> ≤ <i>t</i>) one-tail
<u>t-Test: Paired Two Sample for Means (Normal Control Vs. Test Drug)</u>			
Hb gm/dL	13.98 ± 0.060	14.42 ± 0.087	0.004 ^{ms}
WBC (c/cmm)	7905.17 ± 25.060	8633.33 ± 42.164	1.48 ⁻⁰⁷ (<0.001) ^{ss}
RBC (m/cmm)	8.23 ± 0.088	8.58 ± 0.048	0.015 ^{ws}
Neutrophil (%)	56.83 ± 0.601	62.33 ± 0.667	0.001 ^{ms*}
Lymphocyte (%)	33.00 ± 0.365	33.50 ± 0.764	0.28 ^{ns}
Platelet (lakh/cmm)	3.23 ± 0.009	3.26 ± 0.006	0.02 ^{ns}
NLR	1.72 ± 0.029	1.87 ± 0.060	0.042 ^{ws}
PLR	97.83 ± 1.069	97.48 ± 2.306	0.44 ^{ns}
✚ ms: mildly-significant, ss: strongly-significant, ws: weakly-significant, ns: non-significant, ms*: moderately significant			

Values are expressed as Mean ± S.E.M; (n =6/group).

4.2 EFFECT OF AM ON TYPE I DIABETES MELLITUS RAT

Figure 3. Effect of Blood Glucose, Serum Insulin, and Pancreatic Insulin with the treatment of *Aegle marmelos* (AM) in Diabetic Rats



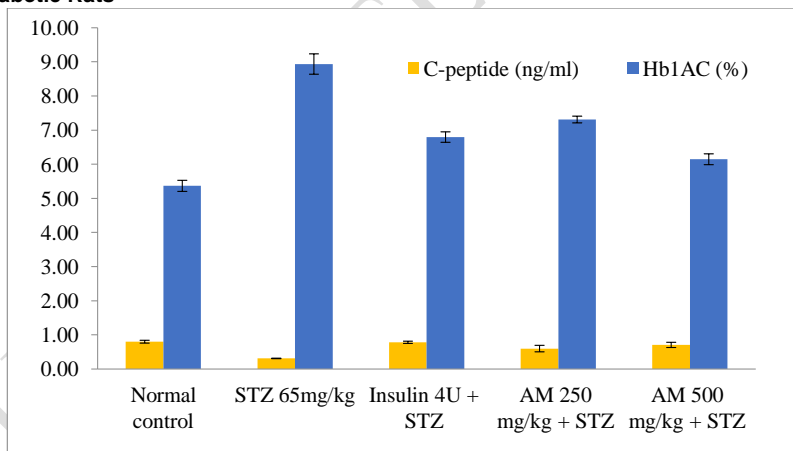
Values are expressed as Mean ± S.E.M; (n =6/group).

Table 7. Interpretation between the groups

Comparisons Between The Group	BGL	S. Insulin	P. Insulin
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NC Vs. DC	2.9 ⁻¹² (<0.001) ^{ss}	3.45 ⁻¹¹ (<0.001) ^{ss}	9.2 ⁻¹⁶ (<0.001) ^{ss}
DC Vs. STD	1.005 ⁻⁰⁹ (<0.001) ^{ss}	4.18 ⁻¹¹ (<0.001) ^{ss}	1.9 ⁻¹⁷ (<0.001) ^{ss}
STD Vs. AM 250mg/kg	1.4 ⁻¹¹ (<0.001) ^{ss}	3.8 ⁻⁰⁹ (<0.001) ^{ss}	2.02 ⁻¹¹ (<0.001) ^{ss}
STD Vs. AM 500mg/kg	0.0043 ^{ms}	0.014 ^{ws}	2.01 ⁻⁰⁷ (<0.001) ^{ss}
t-Test: Two-Sample Assuming Equal Variances P(T<=t) one-tail			
* ss: strongly -significant, ms: moderately-significant, ws: weakly-significant			

Figure 4. Effect of C-Peptide and Hb1AC with the treatment of *Aegle marmelos* (AM) in Diabetic Rats



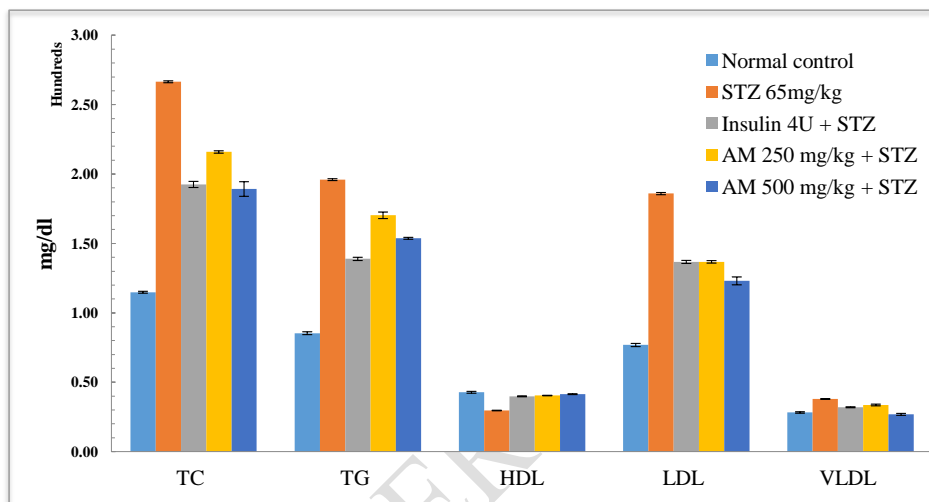
Comparisons Between The Group	C-Peptide	Hb1AC
NC Vs. DC	1.7 ⁻⁰⁷ (<0.001) ^{ss}	4.99 ⁻⁰⁷ (<0.001) ^{ss}
DC Vs. STD	3.1 ⁻⁰⁸ (<0.001) ^{ss}	3.9 ⁻⁰⁵ (<0.001) ^{ss}
STD Vs. AM 250mg/kg	0.048 ^{ws}	0.008 ^{ms}
STD Vs. AM 500mg/kg	0.18 ^{ns}	0.007 ^{ms}

t-Test: Two-Sample Assuming Equal Variances $P(T \leq t)$ one-tail
+ ss: strongly –significant, ms: mildly-significant, ns: non-significant, ws: weakly-significant

Values are expressed as Mean \pm S.E.M; (n =6/group).

Table 8. Interpretation between the groups

Figure 5. Effect of Lipid Profile with the treatment of *Aegle marmelos* (AM) in Diabetic Rats

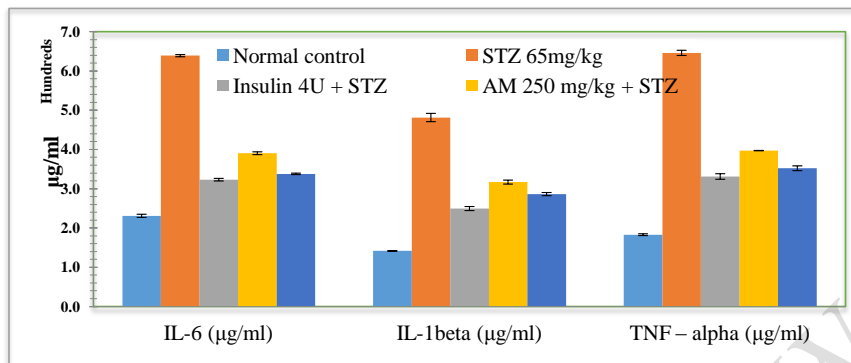


Values are expressed as Mean \pm S.E.M; (n =6/group).

Table 9. Interpretation between the groups

Comparisons Between The Group	TC	TG	HDL	LDL	VLDL
NC Vs. DC	1.2 ⁻¹⁸ (<0.001) ^{ss}	3.2 ⁻¹⁶ (<0.001) ^{ss}	1.9 ⁻⁰⁹ (<0.001) ^{ss}	1.5 ⁻¹⁵ (<0.001) ^{ss}	9.2 ⁻⁰⁹ (<0.001) ^{ss}
DC Vs. STD	1.02 ⁻¹¹ (<0.001) ^{ss}	6.6 ⁻¹³ (<0.001) ^{ss}	7.5 ⁻¹¹ (<0.001) ^{ss}	3.1 ⁻¹² (<0.001) ^{ss}	7.4 ⁻⁰⁸ (<0.001) ^{ss}
STD Vs. AM 250mg/kg	8.6 ⁻⁰⁷ (<0.001) ^{ss}	1.6 ⁻⁰⁷ (<0.001) ^{ss}	0.07 ^{ns}	0.47 ^{ns}	0.02 ^{ws}
STD Vs. AM 500mg/kg	0.28 ^{ns}	3.7 ⁻⁰⁷ (<0.001) ^{ss}	0.002 ^{ms}	0.0005 ^{ms*}	3.4 ⁻⁰⁵ (<0.001) ^{ss}
t-Test: Two-Sample Assuming Equal Variances $P(T \leq t)$ one-tail					
+ ss: strongly-significant, ns: non-significant, ms: mildly-significant, ws: weakly-significant, ms*: moderately-significant					

Figure 6. Effect of Pro-Inflammatory Cytokines with the treatment of *Aegle marmelos* (AM) in Diabetic Rats



Values are expressed as Mean ± S.E.M; (n =6/group).

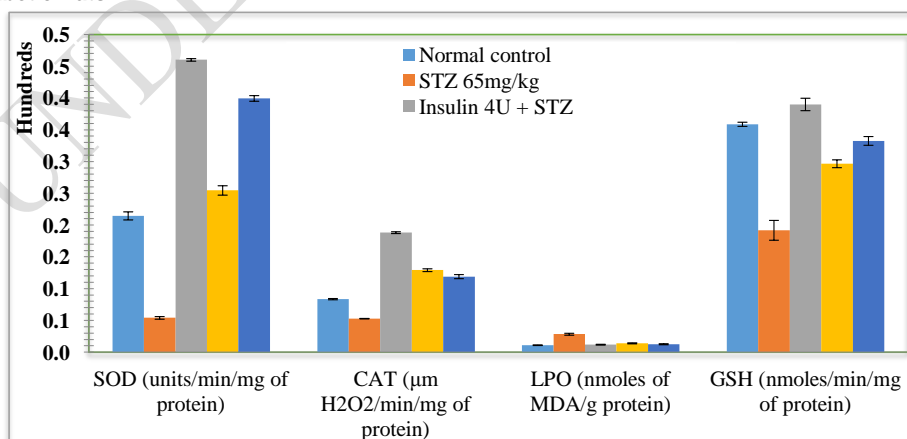
Table 10. Interpretation between the groups

Comparisons Between The Group	IL-6	IL-1Beta	TNF-Alpha
NC Vs. DC	$8.1^{-16} (<0.001)^{ss}$	$1.02^{-11} (<0.001)^{ss}$	$8.3^{-15} (<0.001)^{ss}$
DC Vs. STD	$3.1^{-15} (<0.001)^{ss}$	$1.2^{-09} (<0.001)^{ss}$	$9.1^{-12} (<0.001)^{ss}$
STD Vs. AM 250mg/kg	$3.6^{-08} (<0.001)^{ss}$	$1.5^{-06} (<0.001)^{ss}$	$1.5^{-06} (<0.001)^{ss}$
STD Vs. AM 500mg/kg	0.0015^{ms*}	0.0001^{ss}	0.023^{ws}

t-Test: Two-Sample Assuming Equal Variances
 $P(T \leq t)$ one-tail

ss: strongly-significant, ws: weakly-significant, ms*: moderately-significant

Figure 7. Effect of Antioxidant Enzyme with the treatment of *Aegle marmelos* (AM) in Diabetic Rats



Values are expressed as Mean ± S.E.M; (n =6/group).

Table 11. Interpretation between the groups

Comparisons Between The Group	SOD	CAT	LPO	GSH
NC Vs. DC	1.8 ⁻¹⁰ (<0.001) ^{ss}	3.5 ⁻¹¹ (<0.001) ^{ss}	3.7 ⁻⁰⁷ (<0.001) ^{ss}	5.2 ⁻⁰⁷ (<0.001) ^{ss}
DC Vs. STD	3.5 ⁻¹⁸ (<0.001) ^{ss}	3.1 ⁻¹⁶ (<0.001) ^{ss}	7.3 ⁻⁰⁷ (<0.001) ^{ss}	4.1 ⁻⁰⁷ (<0.001) ^{ss}
STD Vs. AM 250mg/kg	5.6 ⁻¹¹ (<0.001) ^{ss}	2.8 ⁻¹⁰ (<0.001) ^{ss}	0.032 ^{ws}	5.5 ⁻⁰⁶ (<0.001) ^{ss}
STD Vs. AM 500mg/kg	9.5 ⁻⁰⁸ (<0.001) ^{ss}	1.2 ⁻⁰⁹ (<0.001) ^{ss}	0.27 ^{ns}	0.0003 ^{ss}
t-Test: Two-Sample Assuming Equal Variances $P(T \leq t)$ one-tail				
✚ ss: strongly-significant, ws: weakly-significant, ns: non-significant				

DISCUSSION:

Medicinal herbs are an essential part of our natural prosperity and had been a promising future; there are approximately half million natural plants around the world, and yet almost about to them their therapeutics activities had not been investigated so far, and their activities ought to keep ultimate among the treatment of present or may be in future studies. Different parts of *Aegle marmelos* plant are being used for various therapeutic purposes such as asthma, allergy, diabetes, healing wounds, and swollen joints etc⁴⁵. In screening of the toxic concerning of an herbal extract found to be safe and no impact on the test in rats after 14 days of observation. This study presents data on the treatment of diabetic markers, which were shown to be comparable efficacy then the standard one as Insulin, *Aegle marmelos* has shown marked decrease in the serum glucose level, Total cholesterol, triglycerides, LDL, VLDL, glycosylated hemoglobin, were also found to be a limited range. The HDL cholesterol, serum insulin and pancreatic insulin increased with test drug, increase in islet area was quite considerable. Similarly, mediator of inflammation was assessed and analysis showed *Aegle marmelos* inhibited moderately in STZ stimulated rats. Free radical concentrations were screened in terms of SOD, CAT, MDA, & GSH. And data revealed that there were significant changes in the treated groups as compared with STZ rats. The data suggesting, it has the potential alternative and sustainable source for Ayurveda drugs.

CONCLUSION:

In drawing the conclusion of the research carried out, the analysis is mainly focused on the toxicity and diabetic markers. *Aegle marmelos* has significant anti-diabetic activity executed of the present investigation should remain outcome of lower blood glucose levels, enhanced body mass, improvised lipid profile, and notable occurrence of beta cell mass in histopathology studies. The treated diabetic group confirmed notably lowered within the HbA1c levels. Similarly the increase in serum insulin and pancreatic insulin, controlled pro-inflammatory cytokines, anti-oxidant enzyme may additionally facilitate in conformity with prevent diabetic complication.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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