ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 15, Issue 12, 2022

Online - 2455-3891
Print - 0974-2441

Research Article

EVALUATION OF PROTECTIVE EFFECT OF *AEGLE MARMELOS* FRUITS EXTRACTS AGAINST STREPTOZOTOCIN-INDUCED TYPE 1 DIABETES MELLITUS IN RAT'S MODEL

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Received: 15 August 2022, Revised and Accepted: 25 August 2022

ABSTRACT

Objective: The present study aims to investigate the activity of methanolic extract of *Aegle marmelos* on Type I anti-diabetic in STZ induced in rat's model.

Methods: Extracted *A. marmelos* fruits were evaluated for anti-diabetic activity. Type I Diabetes has been induced in Wistar rats through STZ 65 mg/kg/b.w.I.P. During the experiment, the rat's body weight and fasting blood sugar levels were monitored. At the end of the study, animals in all groups have been sacrificed and biochemical parameters such as lipid profile, C-Peptide, HbA1c, serum insulin, pancreatic insulin, and histology of the pancreas have been observed. Furthermore, levels of antioxidant enzymes superoxide dismutase, catalase, and lipid peroxidation were measured.

Results: The observed extract *A. marmelos* was proven to be safe in the toxicity findings. It has been shown an *in vivo* significant effect to manage diabetic markers such as weight gain, blood glucose, lipid profile, C-Peptide, HbA1c, the release of insulin secretion, and pancreatic insulin. The diabetic pancreas of rats has been observed to fall over beta cell density and disruption of normal architecture, but treated groups have been determined to restore the mass over beta cells. Elevated oxidative enzymes also have been viewed to control the treatment with *A. marmelos*.

Conclusion: All its findings and phytoconstituents existing inside the extract must stay the viable chemical materials involved in the prevention of diabetes

Keywords: *Aegle marmelos,* Streptozotocin, Anti-diabetic activity, Glucose, Insulin.

 DOI: http://dx.doi.org/10.22159/ajpcr.2022v15i12.45840. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Diabetes mellitus (DM) is a very common metabolic disease characterized by high glucose levels in the blood due to dysfunction in the pancreas to produce sufficient amounts of insulin hormone [1]. Elevated levels of glucose in the blood are known as hyperglycemia leads to the urgent frequency of urination (polyuria), increasing thirst (polydipsia), and increasing hunger (polyphagia).

Criteria for the diagnosis of DM include one of the following:

1. Glucose level in the blood >7.0 mmol/L (126 mg/dL)
2. Clinical symptoms of diabetes plus random blood glucose levels>11.1 mmol/L or (200 mg/dL)
3. 2 h plasma glucose >11.1 mmol/L (200 mg/dL) during a 75 gm oral glucose tolerance test [2].

Treatment of almost all types of DM became available when insulin was discovered and produced in 1921. Meanwhile, DM type II can be treated either by changing lifestyle and diets in addition to the available medications. The two types of DM (I and II) are chronic diseases not curable but can be controlled. However, several experimental and clinical studies on pancreas transplants were conducted with minimal success, especially for type I. On the other hand, several surgical procedures such as gastric bypass have been proved to be effective in controlling DM type II. In some cases, gestational diabetes is very common among pregnant mothers but it can disappear naturally after delivering babies.

Untreated diabetes can eventually results in severe complications such as hypoglycemia, diabetic ketoacidosis, or non-ketotic hyperosmolar coma. Therefore, proper management of the lifestyle and diet with the aid of medications has a significant effect towards controlling DM pushing the blood glucose levels in the blood up to the normal borders. Taking insulin is unavoidable for patients with Type II [3].

METHODS

Plant information

Aegle marmelos is a medicinal plant of the family Rutaceae which is known as Bael, this plant is provincial to Northern India but extensively located throughout the Indian Peninsula and in Ceylon, Burma, Bangladesh, and Thailand.

It is extensively described in the Vedic literature for the treatment of various diseases. A fruit is broadly used in folks' remedies for the treatment of DM. Furthermore, it has been used in the treatment of chronic diarrhea, dysentery, and peptic ulcers, as a laxative and in conformity with getting better out of respiratory distress.

It also possesses antioxidant, radio-protective, gastro-protective, anti-ulcerative colitis, hepato-protective, cardio-protective, and anti-diabetic activities [4].

Collection of plant material

The fruits of *A. marmelos* were collected from Bangalore-Karnataka (India). Identification of plant materially was confirmed by the Department of Botany University of Rajasthan, Jaipur, and specimens was preserved in the Department with reference number of RUBL 211761.

Extraction of *A. marmelos* fruits using Soxhlet extractor and sample preparation [5-9]

Extract preparation

The fruits of *A. marmelos* were cut into tiny parts and dried in the laboratory at temperature of 25±2°C for 1 week. The dried fruits were

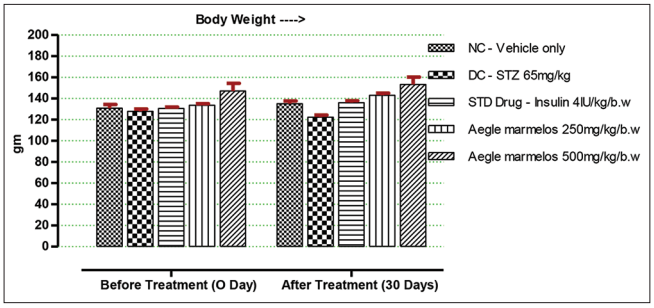

Fig. 1: Body weight. Mean±S.E.M of the animals' weight (n=6)

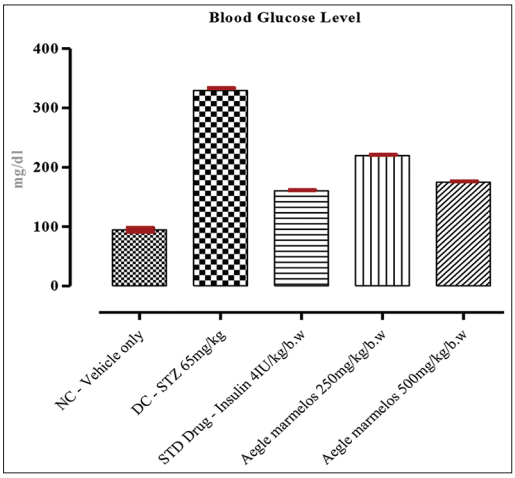

Fig. 2: Blood glucose level. Values are presented as Mean±S.E.M (n=6)

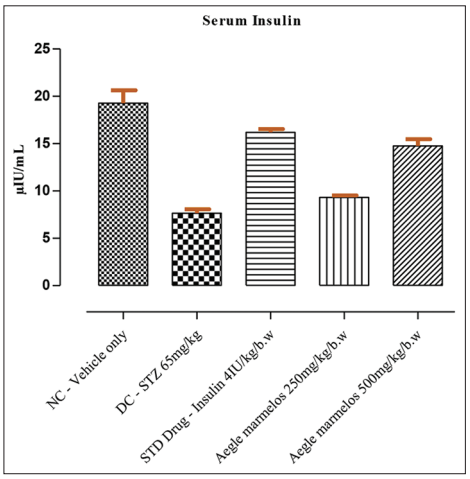

Fig. 3: Serum insulin level. Values are exhibited as Mean±S.E.M (n=6)

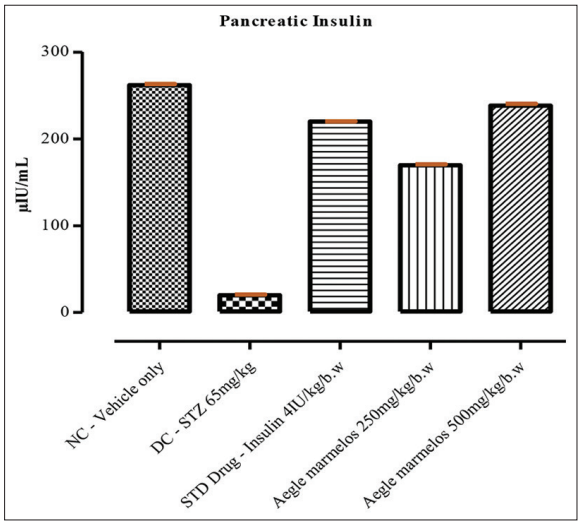

Fig. 4: Pancreatic insulin level. Values are exhibited as Mean±S.E.M (n=6)

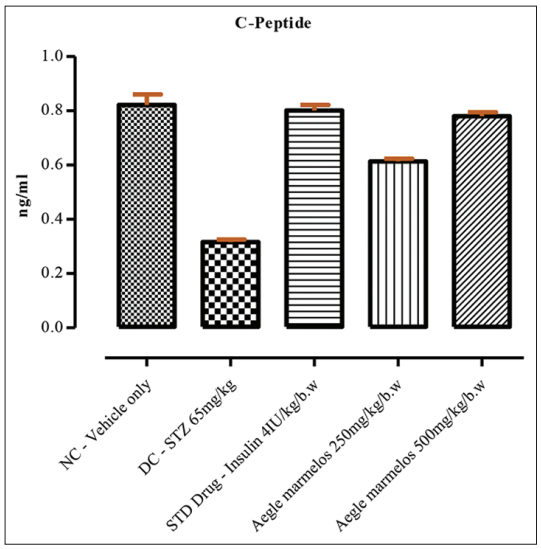

Fig. 5: C-peptide level. Values are exhibited as Mean ± S.E.M (n=6)

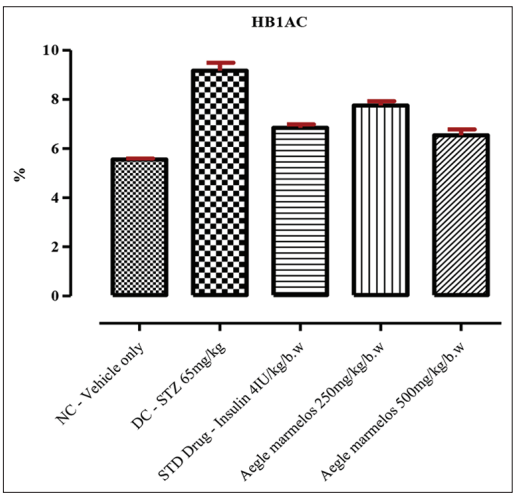

Fig. 6: Hb1AC level. Values are exhibited as Mean ± S.E.M (n=6)

grounded into fine powder and sieved (Coarse 10/40). The fine powder was utilized to prepare the extract in methanol.

Method of extraction

Each 100 g powder was used for methanol extraction with 1 l of solvent in reflux condenser for three cycles of 7 h. When the volume was the methanol volume reduced by 50%, Whatman filter papers No. 1 was used to filter the extract and dried to record a constant dry-weight.

Experimental animals

Males of *Wistar rats* age 8–10 weeks with a constant weight between 150 and 200 g have been used in this study. Before starting the experiments, the animals were maintained in the laboratory for adjustment and adaptation for 7 days. All rats were maintained in the

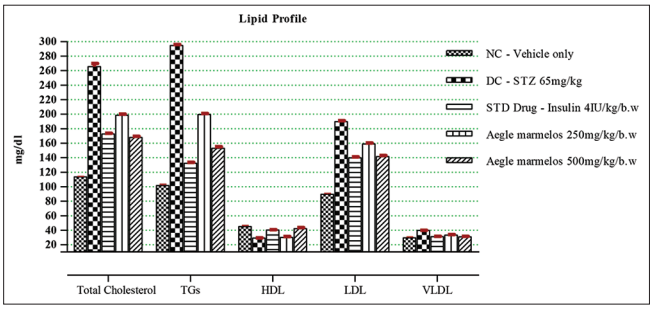

Fig. 7: Serum lipid profile level. Values are presented as Mean±S.E.M (n=6)

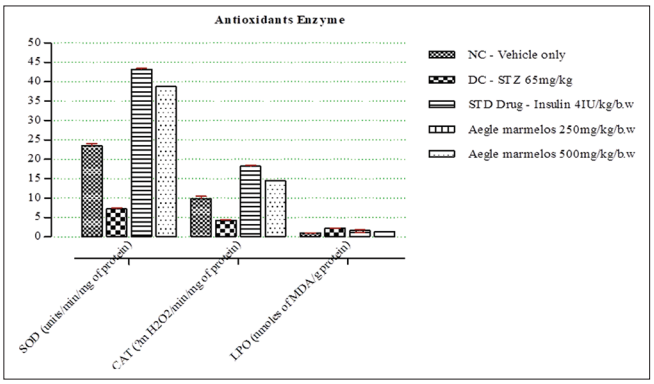

Fig. 8: Anti-oxidants enzyme levels. Values are expressed as Mean±S.E.M (n=6)

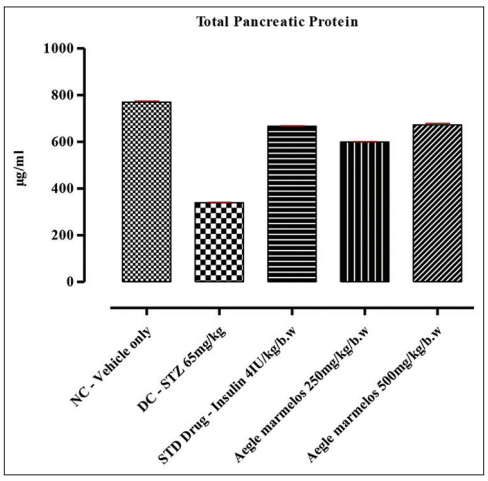

Fig. 9: Total pancreatic protein level. Values are expressed as Mean±S.E.M (n=6)

laboratory cages with proper ventilation at a photoperiod of 12:12 h and room temperature (25±2°C). All animals have a clear accessible path to a chewable diet and water *ad libitum.* All the experiments were carried out following the standardized procedures and experimental protocol previously approved by the ethics committee of the Institutional Animal Ethical Committee (IAEC) of Karnataka College of Pharmacy, Bengaluru (Reg. Number: IAEC/09/21-22/01/18/12/21).

Model for type I DM

STZ-induced DM

Diabetes induced through I.P., a dose of STZ 65 mg/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. To avoid hypoglycemia, STZ-Rats were fed 5% w/v glucose solution for 24 h. After 72 h, rats were recorded with fasting blood sugar (FBS) >180 mg/dL and chosen for the analysis. Experimental animals were given straight access to the drinking water and food and held in polyethylene cages at room temperature. Rat's body weight and FBS levels of rats were taken with a one-touch glucometer before and after the end of the test, that is, 0 and 30 days [10].

Groupings were done in the following manner, where n = 6 animals in each group;

Table 1: Statistics of body weight -> comparison between the groups: Bonferroni post-tests

Row factor	Difference	t	p-value	Summary
NC - Vehicle only versus DC - STZ 65 mg/kg				
Before treatment (0 Day)	−3.000	0.5603	>0.05	ns
After treatment (30 Days)	−12.67	2.366	<0.05	*
NC - Vehicle only versus STD Drug - Insulin 4 IU/kg/b.w				
Before treatment (0 Day)	−0.1600	0.02988	>0.05	ns
After treatment (30 Days)	1.000	0.1868	>0.05	ns
NC - Vehicle only versus. *Aegle marmelos* 250 mg/kg/b.w				
Before treatment (0 Day)	2.840	0.5304	>0.05	ns
After treatment (30 Days)	8.000	1.494	>0.05	ns
NC - vehicle only versus *Aegle marmelos* 500 mg/kg/b.w				
Before treatment (0 Day)	16.34	3.052	<0.01	**
After treatment (30 Days)	18.17	3.393	<0.01	**
DC - STZ 65 mg/kg versus STD drug - insulin 4 IU/kg/b.w				
Before treatment (0 Day)	2.840	0.5304	>0.05	ns
After treatment (30 Days)	13.67	2.553	<0.05	*
DC - STZ 65 mg/kg versus *Aegle marmelos* 250 mg/kg/b.w				
Before treatment (0 Day)	5.840	1.091	>0.05	ns
After treatment (30 Days)	20.67	3.860	<0.001	***
DC - STZ 65 mg/kg versus *Aegle marmelos* 500 mg/kg/b.w				
Before treatment (0 Day)	19.34	3.612	<0.01	**
After treatment (30 Days)	30.84	5.760	<0.001	***
STD drug - insulin 4 IU/kg/b.w versus. *Aegle marmelos* 250 mg/kg/b.w				
Before treatment (0 Day)	3.000	0.5603	>0.05	ns
After treatment (30 Days)	7.000	1.307	>0.05	ns
STD drug - insulin 4 IU/kg/b.w versus. *Aegle marmelos* 500 mg/kg/b.w				
Before treatment (0 Day)	16.50	3.081	<0.01	**
After treatment (30 Days)	17.17	3.207	<0.01	**
Aegle marmelos 250 mg/kg/b.w versus. *Aegle marmelos* 500 mg/kg/b.w				
Before treatment (0 Day)	13.50	2.521	<0.05	*
After treatment (30 Days)	10.17	1.899	>0.05	ns

Table 2: Statistics of blood glucose level -> Comparison between the groups: Tukey's multiple comparison tests

Tukey's multiple comparison test	Mean Diff.	Significant? p<0.05?	Summary
NC - Vehicle only versus. DC - STZ 65 mg/kg	−234.9	Yes	***
NC - Vehicle only versus. STD Drug - Insulin 4IU/kg/b.w	−65.85	Yes	***
NC - Vehicle only versus. *Aegle marmelos* 250 mg/kg/b.w	−125.2	Yes	***
NC - Vehicle only versus. *Aegle marmelos* 500 mg/kg/b.w	−80.52	Yes	***
DC - STZ 65 mg/kg versus. STD Drug - Insulin 4IU/kg/b.w	169.0	Yes	***
DC - STZ 65 mg/kg versus. *Aegle marmelos* 250 mg/kg/b.w	109.7	Yes	***
DC - STZ 65 mg/kg versus. *Aegle marmelos* 500 mg/kg/b.w	154.3	Yes	***
STD Drug - Insulin 4IU/kg/b.w versus. *Aegle marmelos* 250 mg/kg/b.w	−59.33	Yes	***
STD Drug - Insulin 4IU/kg/b.w versus. *Aegle marmelos* 500 mg/kg/b.w	−14.67	Yes	**
Aegle marmelos 250 mg/kg/b.w versus. *Aegle marmelos* 500 mg/kg/b.w	44.67	Yes	***

Table 3: Statistics of serum insulin level -> comparison between the groups: Tukey's multiple comparison tests

Tukey's multiple comparison test	Mean Diff.	Significant? p<0.05?	Summary
NC - Vehicle only versus DC - STZ 65 mg/kg	11.61	Yes	***
NC - Vehicle only versus STD Drug - Insulin 4IU/kg/b.w	3.072	Yes	*
NC - Vehicle only versus *Aegle marmelos* 250 mg/kg/b.w	9.952	Yes	***
NC - Vehicle only versus *Aegle marmelos* 500 mg/kg/b.w	4.502	Yes	**
DC - STZ 65 mg/kg versus STD Drug - Insulin 4IU/kg/b.w	−8.540	Yes	***
DC - STZ 65 mg/kg versus *Aegle marmelos* 250 mg/kg/b.w	−1.660	No	ns
DC - STZ 65 mg/kg versus *Aegle marmelos* 500 mg/kg/b.w	−7.110	Yes	***
STD Drug - Insulin 4IU/kg/b.w versus *Aegle marmelos* 250 mg/kg/b.w	6.880	Yes	***
STD Drug - Insulin 4IU/kg/b.w versus *Aegle marmelos* 500 mg/kg/b.w	1.430	No	ns
Aegle marmelos 250 mg/kg/b.w versus *Aegle marmelos* 500 mg/kg/b.w	−5.450	Yes	***

Table 4: Statistics of pancreatic insulin level -> comparison between the groups: Tukey's multiple comparison tests

Tukey's multiple comparison test	Mean Diff.	Significant? p<0.05?	Summary
NC - Vehicle only versus DC - STZ 65 mg/kg	241.8	Yes	***
NC - Vehicle only versus STD Drug - Insulin 4IU/kg/b.w	41.91	Yes	***
NC - Vehicle only versus *Aegle marmelos* 250 mg/kg/b.w	92.25	Yes	***
NC - Vehicle only versus *Aegle marmelos* 500 mg/kg/b.w	23.39	Yes	***
DC - STZ 65 mg/kg versus STD Drug - Insulin 4IU/kg/b.w	−199.9	Yes	***
DC - STZ 65 mg/kg versus *Aegle marmelos* 250 mg/kg/b.w	−149.6	Yes	***
DC - STZ 65 mg/kg versus *Aegle marmelos* 500 mg/kg/b.w	−218.4	Yes	***
STD Drug - Insulin 4IU/kg/b.w versus *Aegle marmelos* 250 mg/kg/b.w	50.34	Yes	***
STD Drug - Insulin 4IU/kg/b.w versus *Aegle marmelos* 500 mg/kg/b.w	−18.52	Yes	***
Aegle marmelos 250 mg/kg/b.w versus *Aegle marmelos* 500 mg/kg/b.w	−68.86	Yes	***

Table 5: Statistics of C-peptide level -> comparison between the groups: Tukey's multiple comparison tests

Tukey's Multiple Comparison Test	Mean Diff.	Significant? 0.05?	Summary
NC - Vehicle only versus. DC - STZ 65 mg/kg	0.5050	Yes	***
NC - Vehicle only versus. STD Drug - Insulin 4IU/kg/b.w	0.02000	No	ns
NC - Vehicle only versus. *Aegle marmelos* 250 mg/kg/b.w	0.2083	Yes	***
NC - Vehicle only versus. *Aegle marmelos* 500 mg/kg/b.w	0.04167	No	ns
DC - STZ 65 mg/kg versus. STD Drug - Insulin 4IU/kg/b.w	−0.4850	Yes	***
DC - STZ 65 mg/kg versus. *Aegle marmelos* 250 mg/kg/b.w	−0.2967	Yes	***
DC - STZ 65 mg/kg versus. *Aegle marmelos* 500 mg/kg/b.w	−0.4633	Yes	***
STD Drug - Insulin 4IU/kg/b.w versus. *Aegle marmelos* 250 mg/kg/b.w	0.1883	Yes	***
STD Drug - Insulin 4IU/kg/b.w versus. *Aegle marmelos* 500 mg/kg/b.w	0.02167	No	ns
Aegle marmelos 250 mg/kg/b.w versus. *Aegle marmelos* 500 mg/kg/b.w	−0.1667	Yes	***

Table 6: Statistics of Hb1AC level -> comparison between the groups: Tukey's multiple comparison tests

Tukey's multiple comparison test	Mean Diff.	Significant? p<0.05?	Summary
NC - Vehicle only versus. DC - STZ 65 mg/kg	−3.612	Yes	***
NC - Vehicle only versus. STD Drug - Insulin 4IU/kg/b.w	−1.287	Yes	**
NC - Vehicle only versus. *Aegle marmelos* 250 mg/kg/b.w	−2.200	Yes	***
NC - Vehicle only versus. *Aegle marmelos* 500 mg/kg/b.w	−0.9833	Yes	*
DC - STZ 65 mg/kg versus. STD Drug - Insulin 4IU/kg/b.w	2.325	Yes	***
DC - STZ 65 mg/kg versus. *Aegle marmelos* 250 mg/kg/b.w	1.412	Yes	***
DC - STZ 65 mg/kg versus. *Aegle marmelos* 500 mg/kg/b.w	2.628	Yes	***
STD Drug - Insulin 4IU/kg/b.w versus. *Aegle marmelos* 250 mg/kg/b.w	−0.9133	Yes	*
STD Drug - Insulin 4IU/kg/b.w versus. *Aegle marmelos* 500 mg/kg/b.w	0.3033	No	ns
Aegle marmelos 250 mg/kg/b.w versus. *Aegle marmelos* 500 mg/kg/b.w	1.217	Yes	**

Table 7: Statistics of serum lipid profile level -> comparison between the groups: Bonferroni post-tests

Row factor	Difference	t	p-value	Summary
NC - Vehicle only versus. DC - STZ 65 mg/kg				
Total cholesterol	152.1	70.41	<0.001	***
TGs	193.1	89.37	<0.001	***
HDL	−16.09	7.449	<0.001	***
LDL	99.91	46.25	<0.001	***
VLDL	10.10	4.676	<0.001	***
NC - Vehicle only versus. STD Drug - Insulin 4IU/kg/b.w				
Total cholesterol	59.40	27.50	<0.001	***
TGs	30.91	14.31	<0.001	***
HDL	−5.080	2.352	>0.05	ns
LDL	50.25	23.26	<0.001	***
VLDL	1.530	0.7083	>0.05	ns
NC - Vehicle only versus. *Aegle marmelos* 250 mg/kg/b.w				
Total cholesterol	85.26	39.47	<0.001	***
TGs	98.11	45.42	<0.001	***
HDL	−15.31	7.088	<0.001	***
LDL	69.18	32.03	<0.001	***
VLDL	3.740	1.731	>0.05	ns
NC - Vehicle only versus. *Aegle marmelos* 500 mg/kg/b.w				
Total cholesterol	54.52	25.24	<0.001	***
TGs	51.58	23.88	<0.001	***
HDL	−3.000	1.389	>0.05	ns
LDL	51.80	23.98	<0.001	***
VLDL	1.270	0.5879	>0.05	ns
DC - STZ 65 mg/kg versus. STD Drug - Insulin 4IU/kg/b.w				
Total cholesterol	−92.69	42.91	<0.001	***
TGs	−162.1	75.06	<0.001	***
HDL	11.01	5.097	<0.001	***
LDL	−49.66	22.99	<0.001	***
VLDL	−8.570	3.967	<0.001	***
DC - STZ 65 mg/kg versus. *Aegle marmelos* 250 mg/kg/b.w				
Total cholesterol	−66.83	30.94	<0.001	***
TGs	−94.94	43.95	<0.001	***
HDL	0.7800	0.3611	>0.05	ns
LDL	−30.73	14.23	<0.001	***
VLDL	−6.360	2.944	<0.05	*
DC - STZ 65 mg/kg versus. *Aegle marmelos* 500 mg/kg/b.w				
Total cholesterol	−97.57	45.17	<0.001	***
TGs	−141.5	65.49	<0.001	***
HDL	13.09	6.060	<0.001	***
LDL	−48.11	22.27	<0.001	***
VLDL	−8.830	4.088	<0.001	***
STD Drug - Insulin 4IU/kg/b.w versus. *Aegle marmelos* 250 mg/kg/b.w				
Total cholesterol	25.86	11.97	<0.001	***
TGs	67.20	31.11	<0.001	***
HDL	−10.23	4.736	<0.001	***
LDL	18.93	8.763	<0.001	***
VLDL	2.210	1.023	>0.05	ns
STD Drug - Insulin 4IU/kg/b.w versus *Aegle marmelos* 500 mg/kg/b.w				
Total cholesterol	−4.880	2.259	>0.05	ns
TGs	20.67	9.569	<0.001	***
HDL	2.080	0.9629	>0.05	ns
LDL	1.550	0.7176	>0.05	ns
VLDL	−0.2600	0.1204	>0.05	ns
Aegle marmelos 250 mg/kg/b.w versus *Aegle marmelos* 500 mg/kg/b.w				
Total cholesterol	−30.74	14.23	<0.001	***
TGs	−46.53	21.54	<0.001	***
HDL	12.31	5.699	<0.001	***
LDL	−17.38	8.046	<0.001	***
VLDL	−2.470	1.143	>0.05	ns

HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, TG: Triglycerides

01.	STZ-induced diabetes mellitus in rat's model	Group I: Normal control group – vehicle only	6 rats
		Group II: Disease control, received stz 65 mg/kg/b.w i.p single dose	6 rats
		Group III: Standard drug, received insulin 4IU/kg/b.w.i.p+STZ 65 mg/kg/b.w I.P	6 rats
		Group IV: Test drug (low dose), received *Aegle marmelos* 250 mg/kg/b.w P.O+STZ 65 mg/kg/b.w I.P	6 rats
		Group V: Test drug (High dose), received *Aegle marmelos* 500 mg/kg/b.w P.O+STZ 65 mg/kg/b.w I.P	6 rats

Rat's body weight and FBS levels of rats were taken with a one-touch glucometer before and after the end of the test, that is, 0 and 30 days. At last, animals were finally anesthetized with a heavy phenobarbital dosage. Samples of blood were obtained through cardiac puncture and samples were centrifuged at 2500 rpm for 15 min and examined. The parameters;

Observed parameter

Body weight (Pre- and post-treatment), Blood Glucose Level (Pre and Post-treatment), (Using Digital Glucometer, one-touch selects, Life Scan Scotland Ltd, UK), Serum Insulin, Pancreatic Insulin (Sandwich ELISA Assay), C-peptide, Hb1AC (Span Diagnostic), and Lipid Profile (DELTA LABS Kit, Bengaluru, India). Assessment of Lipid Profile (triglyceride, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very low density lipoprotein (VLDL)) were recorded and instructions were provided (DELTA LABS kit) [11-15]. Determination of pancreatic total protein [16,17]. Determination of the total protein in the pancreas tissues was conducted following the standard method of Lowry *et al*. with modification as stated bybyHartree). Antioxidant Enzyme Studies: [18-21] Lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), and histopathology study [22]. The pancreas was removed and cut into two parts; the first part washomogenized to be utilized for measurement the pancreatic insulin, pancreatic protein, and anti-oxidant enzyme. However, the second part was preserved in formalin (10%) for histopathological preparation and study.

Experimental animals were killed with high dose of Pentobarbital. Then, the pancreas of each rat was dissected and chopped into minute portions followed by preservation and fixation in 10% formalin for 48 h. The tissue was dehydrated using alcohol and then embedded in paraffin. The microtome was used to prepare the slides (4–5 mm thick). Hematoxylin-Eosin dye was used for staining and the mounted slides were examined under a light microscope. The histological profile of the pancreas from each animal group was compared to those of the control group.

Biochemical markers were determined following the standard protocols provided by the manufacturer.

Statistical analysis

Data of the presented study were analyzed descriptively for the mean and standard error (mean±SEM) from the number of experimental animals in each group (n-6). The significant differences in the mean were determined using a one-way analysis of variance at the significance level of 0.05. Tukey's multiple comparison test was used to detect the pair-wise significance differences between the groups. All statistical analyses were conducted using the GraphPad Prism software package (version 5).

RESULTS

The Control group had an equal volume of "vehicle" only, Disease control group received "STZ" 65 mg/kg/b.w I.P, Standard group received "Insulin" at the dose of 4 IU/kg/b.wi.p and Test drug, "*A. marmelos*"

Table 8: Statistics of anti-oxidants enzyme level -> comparison between the groups: Bonferroni post-tests

Row factor	Difference	t	p-value	Summary
NC - Vehicle only versus. DC - STZ 65 mg/kg				
SOD (units/min/mg of protein)	−16.22	12.31	<0.001	***
CAT (µm H2O2/min/mg of protein)	−5.630	4.274	<0.001	***
LPO (nmoles of MDA/g protein)	1.180	0.8958	>0.05	ns
NC - Vehicle only versus. STD Drug - Insulin 4IU/kg/b.w				
SOD (units/min/mg of protein)	19.74	14.99	<0.001	***
CAT (µm H2O2/min/mg of protein)	8.340	6.332	<0.001	***
LPO (nmoles of MDA/g protein)	0.1400	0.1063	>0.05	ns
NC - Vehicle only versus. *Aegle marmelos* 250 mg/kg/b.w				
SOD (units/min/mg of protein)	2.880	2.186	>0.05	ns
CAT (µm H2O2/min/mg of protein)	7.380	5.603	<0.001	***
LPO (nmoles of MDA/g protein)	0.6900	0.5238	>0.05	ns
NC - Vehicle only versus. *Aegle marmelos* 500 mg/kg/b.w				
SOD (units/min/mg of protein)	15.31	11.62	<0.001	***
CAT (µm H2O2/min/mg of protein)	4.600	3.492	<0.01	**
LPO (nmoles of MDA/g protein)	0.3300	0.2505	>0.05	ns
DC - STZ 65 mg/kg versus. STD Drug - Insulin 4IU/kg/b.w				
SOD (units/min/mg of protein)	35.96	27.30	<0.001	***
CAT (µm H2O2/min/mg of protein)	13.97	10.61	<0.001	***
LPO (nmoles of MDA/g protein)	−1.040	0.7896	>0.05	ns
DC - STZ 65 mg/kg versus. *Aegle marmelos* 250 mg/kg/b.w				
SOD (units/min/mg of protein)	19.10	14.50	<0.001	***
CAT (µm H2O2/min/mg of protein)	13.01	9.877	<0.001	***
LPO (nmoles of MDA/g protein)	−0.4900	0.3720	>0.05	ns
DC - STZ 65 mg/kg versus. *Aegle marmelos* 500 mg/kg/b.w				
SOD (units/min/mg of protein)	31.53	23.94	<0.001	***
CAT (µm H2O2/min/mg of protein)	10.23	7.767	<0.001	***
LPO (nmoles of MDA/g protein)	−0.8500	0.6453	>0.05	ns
STD Drug - Insulin 4IU/kg/b.w versus. *Aegle marmelos* 250 mg/kg/b.w				
SOD (units/min/mg of protein)	−16.86	12.80	<0.001	***
CAT (µm H2O2/min/mg of protein)	−0.9600	0.7288	>0.05	ns
LPO (nmoles of MDA/g protein)	0.5500	0.4176	>0.05	ns
STD Drug - Insulin 4IU/kg/b.w versus. *Aegle marmelos* 500 mg/kg/b.w				
SOD (units/min/mg of protein)	−4.430	3.363	<0.01	**
CAT (µm H2O2/min/mg of protein)	−3.740	2.839	<0.05	*
LPO (nmoles of MDA/g protein)	0.1900	0.1442	>0.05	ns
Aegle marmelos 250 mg/kg/b.w versus. *Aegle marmelos* 500 mg/kg/b.w				
SOD (units/min/mg of protein)	12.43	9.437	<0.001	***
CAT (µm H2O2/min/mg of protein)	−2.780	2.111	>0.05	ns
LPO (nmoles of MDA/g protein)	−0.3600	0.2733	>0.05	ns

LPO: Lipid peroxidation, CAT: Catalase, SOD: Superoxide dismutase

Table 9: Statistics of total pancreatic protein level -> comparison between the groups: Tukey's multiple comparison tests

Tukey's multiple comparison test	Mean Diff.	Significant? p<0.05?	Summary
NC - Vehicle only versus. DC - STZ 65 mg/kg	430.9	Yes	***
NC - Vehicle only versus. STD Drug - Insulin 4 IU/kg/b.w	102.7	Yes	***
NC - Vehicle only versus. *Aegle marmelos* 250 mg/kg/b.w	170.8	Yes	***
NC - Vehicle only versus. *Aegle marmelos* 500 mg/kg/b.w	96.96	Yes	***
DC - STZ 65 mg/kg versus. STD Drug - Insulin 4IU/kg/b.w	−328.3	Yes	***
DC - STZ 65 mg/kg versus. *Aegle marmelos* 250 mg/kg/b.w	−260.1	Yes	***
DC - STZ 65 mg/kg versus. *Aegle marmelos* 500 mg/kg/b.w	−334.0	Yes	***
STD Drug - Insulin 4IU/kg/b.w versus. *Aegle marmelos* 250 mg/kg/b.w	68.16	Yes	***
STD Drug - Insulin 4IU/kg/b.w versus. *Aegle marmelos* 500 mg/kg/b.w	−5.697	No	ns
Aegle marmelos 250 mg/kg/b.w versus. *Aegle marmelos* 500 mg/kg/b.w	−73.86	Yes	***

extract had low dose 250 mg/kg and high dose 500 mg/kg p.o., respectively.

The following parameters were observed post-treatment;

Body weight before (0 days) and after (30 days) of treatment.

Blood glucose level post-treatment

Serum insulin level post-treatment

Pancreatic insulin level post-treatment

C-peptide level post-treatment

Hb1AC level post-treatment

Serum lipid profile level post-treatment

Anti-oxidants enzyme level post-treatment

Total pancreatic protein level post-treatment

DISCUSSION

This study presents data on the anti-diabetic profiles of fruits of *A. marmelos,* which were shown to be comparable to or sometimes much higher than those, suggesting its potential as an alternative source for anti-diabetic activities. There were some reported data was there like Gupta *et al*., 2011 the influence of *A. marmelos* fruit extract in Streptozotocin-induced diabetes. In that study, the histopathological profiles were examined for anti-diabetic activities. The present study aimed to understand the influence of the *A. marmelos* fruit extract on the patterns of histological changes in the pancreas in Streptozotocin-induced diabetic rats. The oral intake of*A. marmelos* fruit extract at doses of 125 and 250 mg/kg 2 times a day to rats with diabetes for 1 month resulted in a remarkable elevation in the body mass, weight of the pancreas and insulin levels associated with an obvious decrease in fasting blood glucose levels. In the treated group of animals with the fruit, extract was characterized by significant improvement in the beta-cells of the pancreas indicating possible restoration of the damaged cells by Streptozotocin. This study indicated the potential of *A. marmelos* fruit extract to improve pancreas function. The findings of this study exhibit better results compared to animals treated with glibenclamide (300 μg/kg) [23-25].

This study was compared with the presented data on the treatment of diabetic markers, which were shown to be comparable efficacy then the standard one as insulin, *A. marmelos* has shown a marked decrease in the serum glucose level, total cholesterol, triglycerides, LDL, VLDL, and glycosylated hemoglobin, was also found to be a limited range. The HDL cholesterol, serum insulin, and pancreatic insulin increased with the test drug, increase in islet area was quite considerable. Histology of the pancreas of diabetic animals showed regeneration of mass, and restoration of normal tissue architecture was also observed upon the treatment of the test drugs. Similarly, the total pancreatic protein was assessed and analysis showed *A. marmelos* inhibited moderately in STZ-stimulated rats. Free-radical concentrations were screened in terms of SOD, CAT, MDA, and data revealed that there were significantly changes in the treated groups as compared with STZ rats. The data observed in the test drug is quite considerable and better in some markers those in animals treated with Insulin. The data suggest that it has the potential alternative and sustainable source for Ayurveda drugs.

It is evident that the chemical constituents in the plant fruit extract have the possible potential in diabetes prevention.

CONCLUSION

It was concluded that anti-diabetic activity of *A. marmelos* dried fruits extract in STZ-induced Type I DM in rats model. The crude extract from the *A. marmelos* plant produced significant anti-diabetic activity. *A. marmelos* has the production of serum insulin and pancreatic insulin while comparing to *Standard - Insulin*. The protection of the pancreas with this treatment is ideal for diabetes patients. All other diabetic markers such as blood glucose, lipid profile showed a dose-dependent manner with respect to their control group. The antioxidants enzymes (SOD, CAT, and LPO), C-Peptide, and HbA_{1c} also improved significantly. Histology of the pancreas of diabetic animals showed regeneration of pancreatic beta cells or mass, and restoration of normal tissue architecture was also observed upon the treatment of the test drugs.

ACKNOWLEDGMENT

The authors are grateful to the Department of Pharmacology, Karnataka College of Pharmacy, Bengaluru, India, for their support.

AUTHORS CONTRIBUTIONS

All the authors contributed equally in the final draft of the manuscript preparation, editing, and revising.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest

AUTHORS FUNDING

The authors did not receive any funding for this research work.

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