Evaluation of Antidiabetic and Antioxidant Activity of *Aegle marmelos* L. Correa Fruit Extract in Diabetic Rats

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ABSTRACT

Background: Diabetes mellitus is regarded as a serious chronic disease that carries a high risk for considerable complications. The use of natural plant products for management of diabetes is increasing due to their minimal side-effects and economical aspects. *Aegle marmelos* L. Correa (*A. marmelos*), family Rutaceae is highly reputed medicinal plant commonly known as bael. *A. marmelos* fruit is widely used in folk medicine for the treatment of diabetes mellitus.

Aim of the work: This study was aimed to evaluate the antidiabetic and antioxidant activity of *A. marmelos* fruit ethanolic extract against alloxan-induced diabetes in male rats.

Material and Methods: Twenty five male albino rats with an average body weight 180-195g were divided into two main groups; first group: control (n=5) and second group: diabetic rats (n=20), which were divided equally to four subgroups as follows: diabetic untreated rats , diabetic rats treated with 125 mg/kg/day *A. marmelos* fruit extract; diabetic rats treated with 250 mg/kg/day *A. marmelos* fruit extract and diabetic rats treated with 500 mg/kg/day *A. marmelos* fruit extract. Diabetes was induced by a single intraperitonial injection of alloxan (120 mg/kg).

Results: Phytochemical screening of *A. marmelos* fruit extract revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, tannins, coumarins, sterols and triterpenoids. Results of the biological study reported that alloxan-induced diabetic group exhibited hyperglycemia, hyperlipidemia, elevation in malondialdehyde (MDA) level accompanied with weight loss and reduction in high density lipoprotein cholesterol (HDL-C) level, reduced glutathione (GSH) level and superoxide dismutase (SOD) enzyme activity when compared to control group. Treatment with *A. marmelos* fruit extract at the three dose levels reported improvement in the biological evaluation, lipid profile, glucose, insulin, MDA and GSH levels and SOD enzyme activity when compared to the diabetic group. The improvement was most pronounced in 500 mg/kg *A.marmelos* treated group.

Conclusion: It could be concluded that *A. marmelos* fruit extract had hypoglycemic activity; this effect may be attributed to its antioxidant activity and its high content of active constituents which was proved in this study. Therefore, it could be recommended that *A.marmelos* fruit may be useful as a healthy food and in the development of antidiabetic drugs.

Keywords: *Aegle marmelos* fruit extract – phytochemichal - Antidiabetic - Antioxidant - Diabetic rats.

INTRODUCTION

Diabetes mellitus (DM) is the most common metabolic disorder characterized by persistent hyperglycemia, which is due to carbohydrate, protein and lipid metabolism disturbance caused by relative or absolute deficient in insulin secretion and/or insulin action in the peripheral tissues [1]. DM has become the third greatest "killer"after cancer and cardio-/cerebro-vascular diseases [2]. It is estimated that 5% of death in the world is caused by diabetes, a number which will increase by 50% in the next 10 years [3]. There are growing evidences that the excess generation of reactive oxygen species (ROS) in diabetes, which cause oxidative stress, may wholly or in part contribute towards the development of complications in a variety of tissues [4,5].

Because DM control without side effects is a challenge, drugs derived from plants may play an important role in the treatment of DM [6]. Natural products isolated from medicinal plant sources have been used for the prevention and treatment of various diseases pathologies, including cancers, heart disease, diabetes mellitus and high blood pressure [7,8]. Up to 2014, More than 800 species have been investigated and their hypoglycemic effects were reported [9].

Aegle marmelos L. Correa (*A. marmelos*), a medicinal plant of family Rutaceae which is commonly known as Bael, Bengal-quince, golden apple or wood/stone apple tree. It is a medium sized deciduous tree, up to 12-15 m tall with short trunk, thick, soft, flaking bark and spreading, sometimes spiny branches [10]. This plant is native to Northern India, but widely found throughout the Indian Peninsula and in Ceylon, Burma, Bangladesh, Thailand and China. It is also grown in some Egyptian gardens, in Surinam and Trinidad [11,12]. *A. marmelos* fruit is globose with smooth, hard and aromatic shell that is gray green in color when

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raw and yellowish when ripe. Fruit pulp is pale orange, sweet, resinous and highly aromatic [13,14].

This fruit is widely used in folk medicine for treatment of diabetes mellitus [15], as well it is used in treatment of chronic diarrhea, dysentery and peptic ulcers, as a laxative and to recuperate from respiratory affections [16]. *A. marmelos* fruit has been reported to possess antioxidant [17], radioprotective [18], gastroprotective [19], anti-ulcerative colitis [20], hepatoprotective [21], cardioprotective [22] and antidiabetic [23] activities.

A. marmelos fruit possess high nutritional value. The fruit is used to make juice, jam, sirup, jelly, toffee and other products. The pulp is reported to contain water, sugars, protein, fiber, fat, calcium, phosphorus, potassium, iron, minerals and vitamins (Vitamin A, B1, C and Riboflavin) [14,24], as well as bioactive compounds, like carotenoids, phenolics, alkaloids, pectins, tannins, coumarins, flavonoids and terpenoids [25,26].

There are few available reports on the pharmacological actions of *A. marmelos* fruit which grows in Egypt till date. Therefore, this study was aimed to evaluate the antidiabetic and antioxidant activity of *A. marmelos* fruit ethanolic extract against alloxan-induced diabetes in male rats.

MATERIAL AND METHODS

Plant material.

Fresh mature fruits of *Aegle marmelos* were collected during the month of August-September 2016 from El-Zohrya Botanical Garden, Giza, Egypt. Fruits were identified by Mrs. Threase Labib, consultant of plant taxonomy at Orman Botanical Garden and National Gene Bank.

Drugs and chemicals.

All chemicals used in this experiment were of analytical grade. Kits used for the quantitative determination of the different parameters were purchased from Biodiagnostic Co., Dokki, Giza, Egypt. Ethanol 95%, diethyl ether and formalin were obtained from Sigma-Aldrich Co. (St. Louis, MO USA). Alloxan monoyderate (Loba Chemie Co., Mumbai, India), Casein (85% protein), vitamins and minerals constituents, sucrose and glucose were obtained from El-Gomhoriya Pharm. and Chem. Ind. Co., Cairo, Egypt. Cellulose and D-L methionine were purchased from Morgan Co., Cairo, Egypt. Corn oil and corn starch were obtained from the local market.

Experimental animals.

Male Sprague Dawley rats, weighing about 180-195 g were purchased from the animal house of Helwan Station for Experimental Animals, Ministry of Health, Cairo, Egypt. All animals were allowed one week to acclimatize in animal housing conditions before being used for the study. The rats were housed in well aerated cages under hygienic conditions with free access to water and standard diet. The standard diet was formulated according to AIN-93 [27].

Preparation of alcoholic extract of *Aegle marmelos* fruit.

Fruits of *A. marmelos* were washed with running tap water. The pulp was removed from the peel, cut into slices and dried by hybrid solar convective drying system, belonging to the solar energy dept., National Research Center, Dokki, Egypt. The dried fruit pulp was ground into fine powder. The ethanolic extract of *A. marmelos* fruit was prepared by soaking 500 g of powdered fruit pulp in 1 liter of a solvent composed of 700 ml ethanol 95% and 300 ml distilled water at room temperature for 24 h with stirring . The infusion was filtered by a piece of double layer gauze and fresh solvent was then added to the plant material. The combined filtrates were evaporated to dryness under vacuum at 40° C using a rotary evaporator [28]. The extract was stored in the refrigerator for further use.

Phytochemical screening of *Aegle marmelos* fruit extract.

A.marmelos fruit extract was screened for the presence of the major chemical constituents including alkaloids, anthraquinones, carbohydrates, glycosides, flavonoids, saponins, tannins, coumarins as well as, unsaturated sterols and triterpenoids. Phytochemical screening was performed using standard procedures of analysis as described by **Evans** [29] and **Harborne** [30] .

Induction of diabetes mellitus.

Diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal (i.p.) injection of freshly prepared alloxan monohydrate at a dose level of 120 mg/kg b.wt. dissolved in normal saline (0.9% NaCl, pH 7) according to the method of **Ashok *et al.*** [31]. The rats were given 5% glucose solution in feeding bottles for the next 24 h to prevent hypoglycemia after alloxan injection. After 72 hours of the injection fasting blood samples were obtained by retro-orbital method to estimate fasting serum glucose. Rats had fasting serum glucose more than 180 mg/dL were considered diabetic and they were used for the experiment [32].

Experimental design

After the period of adaptation, animals were divided into two main groups, as follows:

The first group: control group, rats (n=5) were received a single i.p. injection with 0.5 ml of normal saline (0.9% NaCl, pH 7) and given orally 1 ml of 0.5% Tween 80 daily. The **second group:** diabetic rats (n=20), which were divided equally to four subgroups as follows: **Subgroup 1:** diabetic untreated rats , animals were given orally 1 ml of 0.5% Tween 80 daily. **Subgroups 2, 3 and 4:** diabetic rats which were treated with alcoholic extract of *A. marmelos* fruit with a daily oral dose of 125 , 250 and 500 mg/kg b.wt., respectively according to the method of **Kamalakkannan and Prince** [23] **and Sundaram *et al.*** [33].

A.marmelos fruit extract doses were suspended in 1 ml of 0.5% Tween 80. During the experimental period food intake (FI) was recorded daily per each group and the animals were weighed initially, twice weekly, and at the end of the experiment. Body weight gain% (BWG %) and feed efficiency ratio (FER) were calculated according to the method of **Chapman *et al.*** [34].

Biochemical analysis

After 4 weeks of treatment, blood samples of the overnight fasted rats were collected from the abdominal aorta under ether anesthesia and divided into two portions. One portion was taken in EDTA tubes and used immediately for estimation of GSH and SOD. The other portion of blood was taken in clean dry centrifuge tubes and left to clot at room temperature for 15 minutes, then centrifuged at 3000 rpm for 15 minutes. The serum was carefully separated and transferred into labeled Epindorff's tubes and stored at -20 °C until used for biochemical analysis. It was used for estimation of glucose, insulin and lipid profile parameters and MDA.

Glucose was determined by enzymatic GOD / POD kits according to the method of **Kaplan** [35]. Insulin was estimated by enzyme linked immunosorbent assay ELISA method as described by **Kao *et al.*** [36]. Enzymatic colorimetric kits were used for determination of total cholesterol, triacylglycerols, high density lipoprotein cholesterol (HDL-C) as described by **Allain *et al.*** [37] **, Fossati and Prencipe** [38] **and Lopes-Virella *et al.*** [39] respectively, while low-density lipoprotein-cholesterol (LDL-C) and very low-density lipoprotein-cholesterol (VLDL-C) were calculated according to the method of **Friedwald *et al.*** [40]. Lipid peroxidation indicated by formation of malondialdehyde (MDA) was assessed using the method described by **Yoshioka *et al.*** [41]. Reduced glutathione (GSH) and superoxide dismutase (SOD) activities were detected according to the method of **Beutler *et al.*** [42] and **Minami a**nd **Yoshikawa** [43] respectively.

Statistical analysis

Results were expressed as a mean ± SE. Data were subjected to one-way analysis of variance (ANOVA), followed by an L.S.D. post hoc multiple comparisons to determine the statistical significance of the difference according to the method of **Snedecor and Cochron** [44]. The Statistical Package for Social Science (SPSS) version 23 was used for these calculations.

RESULTS

Phytochemical screening of *A. marmelos* fruit extract.

The preliminary phytochemical screening carried out on *A. marmelos* fruit extract revealed the presence of alkaloids, Carbohydrates, glycosides, flavonoids, tannins, coumarins sterols and triterpenoids. Anthraquinones and saponins were absent in the extract. (Table1).

Table (1): preliminary phytochemical screening of *A. marmelos* fruit extract.

Constituents	Results
Alkaloids	+
Anthraquinones	-
Carbohydrates	++
Glycosides	++
Flavonoids	++
Saponins	-
Tannins	+
Coumarins	++
Sterols	++
Triterpenoids	++

(+): present (++): High (-): absent

Table (2) showed the effect of *A.marmelos* fruit extract on biological evaluation in alloxan-diabetic rats. The results reported that diabetic group showed very highly significant differences ($p<0.001$) in BWG%, DFI and FER as compared with control group. All diabetic groups treated with *A.marmelos* fruit extract demonstrated very highly significant differences in all biological parameters ($p<0.001$) when compared with control group and untreated diabetic group.

Moreover, BWG% in diabetic group treated with 125 mg /kg *A.marmelos* fruit extract recorded very highly significant differences ($p<0.001$) when compared with both 250 and 500 mg/kg *A.marmelos* treated groups. Administration of *A.marmelos* fruit extract induced significant improvement in DFI and FER, especially at a dose level of 500 mg /kg, which recorded highly significant differences ($p<0.001$) as compared with groups treated at a dose level of 125mg/kg or 250 mg/kg.

Table (2). Effect of *A. marmelos* fruit extract on biological evaluation in diabetic rats.

Experimental groups	Control	DM	DM + *A.marmelos* (125mg/kg)	DM + *A.marmelos* (250 mg/kg)	DM + *A.marmelos* (500 mg/kg)
%BWG	44.07 ± 0.16	a *** 16.43 ± 0.26	a *** b *** d *** 30.41 ± 0.18	a *** b***c*** 32.57±0.19	a***b*** 32.80±0.24
FI (g/rat/day)	19.16 ± 0.71	a *** 26.50 ± 0.69	a *** b *** d *** 23.35 ± 0.01	a***b *** c* **d*** 23.14 ± 0.08	a***b*** 22.54±0.10
FER	0.154 ± 0.008	a *** 0.042 ± 0.005	a *** b ***d*** 0.087 ± 0.003	a*** b *** c***d*** 0.094 ± 0.007	a***b*** 0.096±0.006

BWG%: Body weight gain percent. **DFI**: Daily feed intake. **FER**: Feed efficiency ratio.

- Each value represents mean of 5 rats ± SE.

[a]: Significant difference between control and diabetic groups.

[b]: Significant difference between diabetic and diabetic treated groups.

[c]: Significant difference between diabetic treated with 125 mg/kg and diabetic treated with 250 mg/kg of *A. marmelos* fruit extract

[d]: Significant difference between diabetic treated with 125 mg/kg or 250 mg/kg and diabetic treated with 500 mg/kg of *A. marmelos* fruit extract.

(* $P< 0.05$, ** $p<0.01$ and ***$p<0.001$)

Table (3) showed the effect of *A.marmelos* fruit extract on serum glucose concentration and insulin level in alloxan-diabetic rats. In diabetic rats, there was very highly significant elevation ($p<0.001$) in glucose concentration accompanied with very highly significant reduction ($p<0.001$) in insulin level as compared with control group. Oral administration of *A. marmelos* fruit extract at dose level of 125, 250 and 500 mg/kg remarkably ameliorated the elevation in glucose concentration and the reduction in insulin level , there was very highly significant improvement ($p<0.001$) in glucose concentration and insulin level as compared with untreated diabetic group. The results also demonstrated that serum glucose concentration in diabetic group treated with 125 mg/kg *A.marmelos* fruit extract recorded highly significant differences ($p<0.01$) when compared with 250 mg/kg *A.marmelos* treated group. Treatment with 500 mg/kg *A.marmelos* fruit extract showed non significant differences when compared with 250 mg/kg *A.marmelos* treated group while demonstrated significant differences with respect to 125 mg/kg *A.marmelos* treated group in both glucose concentration ($p<0.001$) and insulin level ($p<0.01$).

Table (3): Effect of *A. marmelos* fruit extract on glucose concentration and insulin level in diabetic rats

Experimental groups	Control	DM	DM + *A.marmelos* (125mg/kg)	DM + *A.marmelos* (250 mg/kg)	DM + *A.marmelos* (500 mg/kg)
Glucose (mg/dl)	68.38 ± 4.11	a *** 195.48 ± 4.07	a *** b *** d *** 97.48 ± 1.72	a ** b***c** 82.72± 3.85	a*b*** 78.82±2.64
Insulin (µIU/ml)	17.86 ± 1.10	a *** 6.58 ± 0.51	a *** b *** d ** 12.46 ± 0.37	a**b *** 14.34 ± 0.61	a*b*** 15.64±0.45

- Each value represents mean of 5 rats ± SE.

[a]: Significant difference between control and diabetic groups.

[b]: Significant difference between diabetic and diabetic treated groups.

[c]: Significant difference between diabetic treated with 125 mg/kg and diabetic treated with 250 mg/kg of *A.marmelos* fruit extract.

[d]: Significant difference between diabetic treated with 125 mg/kg or 250 mg/kg and diabetic treated with 500 mg/kg of *A. marmelos* fruit extract.

(*$P<0.05$,**$p<0.01$and***$p<0.001$).

Table (4) showed the effect of *A.marmelos* fruit extract on serum lipid profile levels in alloxan-diabetic rats. Results indicated very highly significant elevation ($p<0.001$) in TC, TG, LDL-C and VLDL-C levels concurrent with very highly significant reduction ($p<0.001$) in HDL-C level in diabetic group as compared with control group. All treated diabetic groups showed significant improvement in lipid profile levels.

Diabetic group treated with 125 mg/kg *A.marmelos* fruit extract showed very highly significant differences ($p<0.001$) in lipid profile levels as compared with control group. TC, TG and LDL-C values recorded very highly significant differences ($p<0.001$) and HDL-C and VLDL-C values recorded highly significant differences ($p<0.01$) in 250 mg/kg *A.marmelos* treated group comparing with control group. While, TC and LDL-C values recorded very highly significant differences ($p<0.001$) and TG, HDL-C, and VLDL-C values recorded significant differences ($p< 0.05$) in 500 mg/kg *A.marmelos* treated group compared with control group.

Oral administration of *A.marmelos* fruit extract at dose level of 125, 250 and 500 mg/kg remarkably ameliorated the elevation in TC, TG, LDL-C ,VLDL-C and the reduction in HDL-C levels, there was very highly significant improvement ($p<0.001$) in lipid profile levels as compared with untreated diabetic group. Results also revealed that LDL-C level recorded very highly significant difference ($p< 0.001$), but HDL-C level recorded highly significant difference ($p<0.01$) and TC level recorded significant difference ($p<0.05$) in 250 mg/kg *A.marmelos* treated group when compared with 125 mg/kg *A.marmelos* treated group.Treatment with 500 mg/kg *A.marmelos* fruit extract showed non-significant differences when compared with 250 mg/kg *A.marmelos* treated group. While treatment with 500 mg/kg *A.marmelos* fruit extract demonstrated very highly significant differences ($p<0.001$) in TC, HDL-C and LDL-C levels and highly significant differences ($p<0.01$) in TG and VLDL-C levels with respect to 125mg/kg *A.marmelos* treated group.

Table (4): Effect of *A. marmelos* fruit extract on lipid profile levels in diabetic rats.

Experimental groups	Control	DM	DM + *A.marmelos* (125mg/kg)	DM + *A.marmelos* (250 mg/kg)	DM + *A.marmelos* (500 mg/kg)
TC (mg/dl)	99.40 ± 0.87	a *** 183.10 ± 3.28	a *** b *** d *** 131.28 ± 3.24	a *** b***c* 124.38± 1.85	a***b*** 118.82±0.67
TG (mg/dl)	70.54 ± 1.08	a *** 171.08 ± 3.60	a *** b *** d ** 89.40 ± 3.40	a***b *** 84.12 ± 2.05	a*b*** 79.32±0.74
HDL-C (mg/dl)	47.84± 1.17	a*** 18.26±0.67	a***b***d*** 35.62± 1.43	a**b***c** 41.94±1.58	a*b*** 43.74±1.09
LDL-C (mg/dl)	37.44±1.74	a*** 126.28±2.17	a***b***d*** 77.50±2.80	a***b***c*** 64.40±2.35	a***b*** 60.38±2.44
VLDL-C (mg/dl)	14.08±0.21	a*** 34.20±0.73	a***b***d** 17.88±0.68	a**b*** 16.78±0.43	a*b*** 15.84±0.15

TC: Total Cholesterol, **TG**: Triglycerides, **HDL-C**: High Density Lipoprotein Cholesterol, **LDL-C**: Low Density Lipoprotein Cholesterol and **VLDL-C**: Very Low Density Lipoprotein Cholesterol.

- Each value represents mean of 5 rats ± SE.

a: Significant difference between control and diabetic groups.

b: Significant difference between diabetic and diabetic treated groups.

c: Significant difference between diabetic treated with 125 mg/kg and diabetic treated with 250 mg/kg of *A.marmelos* fruit extract

d: Significant difference between diabetic treated with 125 mg/kg or 250 mg/kg and diabetic treated with 500 mg/kg of *A. marmelos* fruit extract.

(* $P< 0.05$, ** $p<0.01$ and ***$p<0.001$).

Table (5) showed the effect of *A.marmelos* fruit extract on MDA and GSH levels and SOD enzyme activities in alloxan-diabetic rats. In diabetic group, there was very highly significant elevation ($p<0.001$) in MDA levels accompanied with very highly significant reduction ($p<0.001$) in GSH and SOD enzyme activities as compared with control group. Diabetic groups treated with

A.marmelos fruit extract at dose level of 125, 250 and 500 mg/kg showed improvement in MDA and GSH levels and SOD enzyme activities, but there values showed significant differences as compared with control group.

Oral administration of *A.marmelos* fruit extract at dose level of 125, 250 and 500 mg/kg remarkably ameliorated the elevation of MDA level and the reduction in GSH levels and SOD enzyme activities. Diabetic group treated with 500 mg/kg *A.marmelos* fruit extract showed very highly significant improvement ($p<0.001$) in MDA and GSH levels and SOD enzyme activities as compared with untreated diabetic group. MDA and GSH values recorded very highly significant differences ($p<0.001$) and SOD values recorded highly significant differences ($p<0.01$) in 250 mg/kg *A.marmelos* treated group comparing with untreated diabetic group.While MDA values recorded very highly significant differences ($p<0.001$) and GSH values recorded highly significant differences($p<0.01$) in 125 mg/kg *A.marmelos* treated group comparing with untreated diabetic group.

Data also demonstrated that GSH values in 250 mg/kg *A.marmelos* treated group recorded highly significant differences ($p<0.01$) when compared with 125 mg/kg *A.marmelos* treated group. Treatment with 500 mg/kg *A.marmelos* fruit extract showed non-significant differences when compared with 250 mg/kg *A.marmelos* treated group. while, demonstrated significant differences when compared with 125 mg/kg *A.marmelos* treated group in GSH levels ($p<0.001$) and SOD ($p<0.05$)enzyme activities .

Table (5): Effect of *A. marmelos* fruit extract on malondialdehyd (MDA), reduced glutathione (GSH) levels and sueroxide dismutase (SOD) activities in diabetic rats

Experimental groups	Control	DM	DM + *A.marmelos* (125mg/kg)	DM + *A.marmelos* (250 mg/kg)	DM + *A.marmelos* (500 mg/kg)
MDA (µmol/dl)	85.80 ± 3.41	a *** 133.00 ± 1.44	a *** b *** 108.50 ± 2.53	a ** b*** 102.34± 4.32	a*b*** 100.46±5.80
GSH (mg/dl)	47.56 ± 1.54	a *** 26.90 ± 1.13	a *** b ** d *** 32.26 ± 1.06	a***b ***c** 37.36 ± 0.54	a***b*** 38.98±0.72
SOD (U/ml)	59.96± 1.29	a*** 40.26±1.57	a***d* 44.48± 1.87	a***b** 47.86±1.54	a***b*** 49.80±0.97

MDA: Malondialdehyde, **SOD**: Superoxide dismutase, **GSH**: Reduced glutathione.

- Each value represents mean of 5 rats ± SE.

a: Significant difference between control and diabetic groups.

b: Significant difference between diabetic and diabetic treated groups.

c: Significant difference between diabetic treated with 125 mg/kg and diabetic treated with 250 mg/kg of *A.marmelos* fruit extract.

d: Significant difference between diabetic treated with 125 mg/kg or 250 mg/kg and diabetic treated with 500 mg/kg of *A. marmelos* fruit extract.

(* P< 0.05, ** p<0.01 and ***p<0.001).

DISCUSSION

Diabetes mellitus is a chronic metabolic disorder which affects almost all systems of the body and the management includes various treatment modalities [45]. Apart from the currently available drug regimens for management of diabetes, a wide range of drugs extracted from plant species were examined for their possession of antidiabetic properties [46]. These drugs found to be relatively less toxic with minimal adverse effects in comparison with common allopathic drugs. Alloxan a beta-cytotoxin induces chemical diabetes (alloxan diabetes) in a wide variety of animal species by damaging the insulin secreting cells of the pancreas. This damages a large number of beta-cells, which paves the ways for the decreased utilization of glucose by the tissue [47]. On these backgrounds, the present study was designed to evaluate the hypoglycemic and antioxidant effects of *A. marmelos* fruit extract on biological, and biochemical parameters of alloxan induced diabetic rats.

A.marmelos fruit is an important medicinal plant and its fruits have been used in the treatment of various diseases. Phytochemical screening of *A.marmelos* fruit extract revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, tannins, coumarins,

sterols and triterpenoids. In accord with the present results **Manjula and Kumar** [48] reported that, phytochemical screening of *A. marmelos* ethanolic fruit extract revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, tannins, coumarins and triterpenoids. Moreover, the antioxidant activity of ethanolic extract of *A. marmelos* fruit was evaluated by various assays indicated that, fruit possesses considerable antioxidant activities. Higher amounts of flavonoids, and phenolic compounds were observed [49].

In the present study, Alloxan induced diabetic rats showed very highly significant decrease in BWG%, FER and highly significant increase in DFI as compared with control rats. All diabetic rats treated with *A.marmelos* fruit extract showed significant improvement in all biological parameters where there was highly significant increase in BWG%, FER and highly significant decrease in DFI as compared with untreated diabetic rats. The body weight is a sensitive indicator that reflects the state of health of experimental animals and the decrease in body weight correlates with defects in body metabolism [50]. The expected reduction of body weight after alloxan injection was in agreement with **Hassan and Emam** [51] and **Ojo *et al.*** [52] who attributed this reduction to the amelioration of hyperglycemia. The increase in the blood glucose resulting from the defective cellular uptake of glucose, forces the cells to utilize amino acids and fatty acids as a source of energy which eventually leads to the reduction of fats and tissue proteins which normally represents about 30 to 40% of total body weight. Thus, the excessive breakdown of tissue proteins due to diminished insulin response as well as the unavailability of carbohydrate for energy metabolism in diabetes mellitus results in decreased body weight. Interestingly, *A.marmelos* fruit extract administration to diabetic rats at the three treated doses caused a significant increase in body weight, an effect that was resembled in the experimental research of **Kamalakkannan and Prince** [23] as well as **Kamalakkannan and Stanely Mainzen Prince** [53].

Regarding to the food intake, alloxan injection to normal rats in this study showed marked hyperphagia which may be attributed to the hyperglycemia. **Guyton and Hall** [54] explained this hyperphagia on physiological basis that the decrease in blood glucose concentration causes hunger, which has led to the so called glucostatic theory of hunger and feeding regulation. The satiety center are sensitive to arterio-venous gradient of blood glucose level, so high arterio-venous blood glucose gradient stimulates the satiety center and inhibits the feeding center inducing anorexia. In diabetes, although the blood glucose level is high, polyphagia is increased because the arterio-venous gradient is low as the cells cannot use the glucose due to absence of insulin. The effect of *A.marmelos* fruit extract in preventing body weight loss and improving food intake seems to be due to its ability to reduce hyperglycemia.

The present results showed very highly significant increase in serum glucose concentrations accompanied with very highly significant decrease in serum insulin levels of diabetic rats when compared to the control rats. **Etuk and Muhammed** [55] and **Adeyi *et al.*** [56] attributed this increase in glucose levels to the reactive oxygen species induced by alloxan; this, in conjunction with a simultaneous massive increase in cytosolic calcium concentrations led to rapid destruction of pancreatic islet cells and a concomitant reduction in synthesis/release of insulin.

Interestingly, treatment of diabetic rats with *A.marmelos* fruit extract here resulted in significant improvement in glucose concentrations and insulin levels, where there was very highly significant decrease in serum glucose concentrations accompanied with very highly significant increase in insulin levels when compared to untreated diabetic rats. The most pronounced hypoglycemic and hyperinsulinemic effect was obtained with dose of 500 mg/kg. The antihyperglycemic effect of *A.marmelos* fruit extract was in agreement with that reported by **Kamalakkannan and Prince** [17] . The previous authors reported that oral administration of aqueous extract of *A.marmelos* fruit had hypoglycemic effect against STZ-induced diabetes in rats. **Kamalakkannan and Stanely Mainzen Prince** [57] and **Kamalakkannan and Prince** [23] also agree with our results as oral administration of aqueous extract of *A.marmelos* fruit prevented significantly the STZ-induced hyperglycaemia and hypoinsulinemia. The antihyperglycemic effect of *A.marmelos* fruit extract may result from improvement in the cellular glucose entry and metabolism through potentiation of insulin from existing β-cells of the islets of Langerhans, insulin sensitivity, β-cells function and regeneration. This effect may be due to the presence of several bioactive

antidiabetic principles as shown by phytochemical analysis results. It has been reported that many bioactive principles possess antihyperglycemic activity as flavonoids, sterols, triterpenoids, alkaloids [58] and cumarins [59]. Flavonoids are known to regenerate the damaged β-cells in the alloxan induced diabetic rats [60]. Diabetes management can be achieved by delaying enzyme *α*-amylase activity [61]. *A.marmelos* fruit extract has been shown to inhibit pancreatic *α*-amylase and intestinal *α*-glucosidase activity **[62]**. **Das *et al.*** [63] showed that *α*-amylase inhibition property can be achieved by flavonoids.

Hypoglycemia is accompanied with dyslipidemia and represents a risk factors for coronary heart disease [64]. In the present results, alloxan diabetic rats showed very highly significant elevation in serum TC, TG , LDL-C and VLDL-C levels accompanied with very highly significant reduction in HDL-C level as compared with control group. The dyslipidemia observed in the untreated diabetic rats could indicate an increase in the mobilization of free fatty acids from the peripheral fat depots. This could result from the uninhibited actions of lipolytic enzyme lipase caused by insulin deficiency characteristic of the diabetic state [65]. Under normal conditions, insulin activates the enzyme lipoprotein lipase, which hydrolysis triglycerides [66,67]. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglycemia and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities [64].

Treatment of diabetic rats with *A.marmelos* fruit extract showed remarkably ameliorated effects in all lipid profile parameters.The group treated with 500 mg/kg *A.marmelos* fruit extract exhipited the greatest improvement in lipid profile among the treatment groups.The reduction in serum lipids may be due to decreased fat mobilization and synthesis. These results are in agreement with previous studies suggested that aqueous extract of *A. marmelos* fruits can be used as an antihyperlipidaemic agent as found in the streptozotocin- induced diabetic wistar rats [68,53].The lipid lowerimg effect of the extract might be due to the action of flavonoids and other phenolic compounds, triterpenoids, alkaloids, steroids and glycosides. Normalized rate of lipogenesis is due to the insulin-like activity of triterpenoids [69] or activating normoglycemia by the insulinotropic effect of flavonoids [70].

It is well known that hyperglycemia is the most important event to development of oxidative stress, but hyperlipidemia is also implicated in excessive ROS production and reduced antioxidant defense system, leading to oxidation of macromolecules such as lipids and DNA damage, contributing to apoptosis and necrosis [71]. Overproduction of reactive oxygen species such as hydrogen peroxide and molecular oxygen modulates biological function of all biomolecule, being lipids target to oxidation to generate MDA, a marker of lipid damage [72,73]. Consistent with this finding, the present study showed that alloxan produced very highly significant increase in MDA levels accompanied with very highly significant decrease in GSH levels and SOD activities when compared with control group. Similar results were reported by **Khashana and Al-Turfib** [74]. The elevated levels of oxidative stress in diabetic rats were due to autoxidation of glucose, protein glycation, lipid peroxidation, and low activities of antioxidant enzymes [75]. The depletion of GSH level in diabetic rats might be due to its utilization to alleviate the oxidative stress in diabetes [76].

Treatment of diabetic rats with *A.marmelos* fruit extract showed remarkably ameliorated effects, there was very highly significant improvement in MDA and GSH levels and SOD activities when compared to untreated diabetic group. The most effective dose of the extract was found to be 500 mg/kg, it exhibits remarkable oxidative stress control in diabetic group. Consistent with our findings, **Malik *et al.*** [77] reported that treatment of alloxan-diabetic mice with *A.marmelos* fruit extract significantly decreased the levels of MDA and increased the levels of GSH and SOD. **Kamalakkannan and Prince** [17] **and Kamalakkannan and Stanely Mainzen Prince** [78] reported that treatment of STZ-diabetic rats with *A.marmelos* fruit extract significantly decreased the levels of thiobarbituric acid reactive substances and hydroperoxides and increased the levels of GSH and SOD in both plasma and tissues. **Jagetia et al.** [18] indicated that treatment of the *A.marmelos* fruit extract before irradiation caused a significant decrease in the lipid peroxidation accompanied by a significant elevation in the GSH concentration in mice tissues. The antioxaditive effect of *A.marmelos* fruit extract was explained by

Manjula and Kumar [48] who found that *A.marmelos* fruit extract has a potent *in vitro* antioxidant activity which was correlated with its content of bioactive compounds. The ameliorated effect of *A.marmelos* fruit extract on lipid peroxidation may be attributed to the antioxidative phytochemicals present in it especially flavonoids. Flavonoids are the most promising agents for treatment of oxidative stress-related disease [79].

CONCLUSION

From the present results, it could be concluded that, ethanolic extract of *A.marmelos* fruit possesses significant hypoglycemic, hypolipidemic and antioxidant effects. The presence of several bioactive principles in this medicinal plant extract might be responsible for these effects. Therefore, the extract should be further investigated to isolate the active compounds responsible for its antidiabetic activity and develop new drug for treatment of diabetes.

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