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Effect of Citrus maxima (Merr.) Fruit Juice in Alloxan-Induced Diabetic Wistar Rats

Oyedepo, T.A.

Faculty of Science and Technology,
Adeleke University,
P.M.B. 250, Ede,
Osun State,
Nigeria
Email:oyedepo004tope@yahoo.com

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ABSTRACT

The effect of Citrus maxima Merr. fruit juice extract in alloxan-induced diabetes Wistar rats was investigated in this study. Sixteen adult male albino wistar rats were randomly divided into four groups [A - D]. Group A and C were administered with alloxan [150mg/kg] intraperitoneally to induce diabetes. Groups A and B were later treated with the fruit juice [10ml/kg body weight] for 22 days, while groups C and D served as negative and positive controls respectively. The juice treated diabetic rats group showed remarkable improvement in body weight and demonstrated significant decrease in the blood glucose levels [$p<0.05$] when compared to the diabetic control group. Also, the blood cholesterol and triglycerides levels decreased significantly [$p<0.05$] in treated diabetic rats, except for the HDL- cholesterol level which however showed an increased trend. There was no noteworthy difference [$p>0.05$] in AST and ALT activity for the juice treated groups. Furthermore, packed cell volume [PCV] and red blood cell [RBC] count increased significantly [$p<0.05$] in diabetic rats treated group when compared to the untreated diabetic control group. The present study shows that the Citrus maxima Merr. Fruit juice extract has potent hypoglycemic and hypocholesterolemic activities against alloxan -induced diabetes rats and the consumption can be encouraged for people living with type-2-diabetes.

KEYWORDS: Antioxidant, Diabetes, blood glucose, Alloxan, Citrus maxima

INTRODUCTION

The plant *Citrus maxima* Merr. (Rutaceae) is commonly known as shaddock or pomelo. *Citrus maxima* are native to Southeastern United States and they are also widely cultivated in some regions of West Africa. Shaddock (*Citrus maxima*) is a hybrid of grape (*Citrus vinifera*) and orange (*Citrus sinensis*) with a smooth yellowish skin and may either be seeded or seedless. In some cases, through selection and breeding, shaddock has been crossed with tangerine (*Citrus reticulata)* to obtain a variant with juicy, thick skinned, easy to peel and termed tangelo [Anih, 2006]. Although the name C*itrus grandis* (L.) Osbeck is sometimes used for shaddock, *Citrus maxima* (J. Burman) Merrill is correct under the International Code of Botanical Nomenclature [Scora and Nicolson, 1986].

The pulp is stated to possess the following properties as reported in ancient and medieval literature: appetizer, antitoxic, cardiac stimulant, and stomach tonic [Gallesio, 1811]. The major flavanones of shaddock are neohesperidin and naringin, which are high in the seed in case of unripe citrus fruits [Chung *et al*., 2005] and its extract showed antioxidant activity through free radical-scavenging in *vitro* and to reduce reactive oxygen species in H_2O_2-treated HepG2 cells [Chung *et al*., 2005]. 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and ferric-reducing antioxidant power values determined for the essential oil were 26.1 ± 1.2% and 2.3 ± 0.3 mM, respectively, which are significantly higher than those of other fruit pulp extracts [Lim *et al*, 2006]. Hesperidin and naringin are present in fruit juice. Caffeic, p-coumaric, ferulic, and vanillic acid are also present in the fruit juice [Jang *et al*., 2010]. *Citrus maxima* essential oil is composed of *α*-Pinene, Sabenine, *β*-Pinine, Methyl heptenone, *β*-myrcene, Hexanal, Sabenine, DL-Limonene, t-Ocimene, Linalool, 1-Hexene, 4-methyl; 1-Hexene,3,3-dimethyl; Geranyl formate, Z-Citral, Geranyl formate, E-Citral, Geranyl acetate, *β*-Farnesene, and at 750 ppm and 1000 ppm it inhibited mycelia growth of *A. flavus* [Tian-Shung, Shiow-Chyn and Pei-Lin, 2006].

Diabetes mellitus describes a metabolic disorder of multiple etiologies. It is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Approximately 140 million people worldwide suffer from diabetes. The disease becomes a real problem of public health in developing countries, where its prevalence is increasing steadily and adequate treatment is often expensive or unavailable. Alternative strategies to the current modern pharmacotherapy of diabetes mellitus are urgently needed, because of the inability of existing modern therapies to control all the pathological aspects of the disorder, as well as the enormous cost and poor availability of the modern therapies for many rural populations in developing countries [Lyra *et al*., 2006]. Plants used in traditional medicine to treat diabetes mellitus represent a valuable alternative for the control of this disease.

The antihyperglycemic effect of various fruit extract in chemical-induced diabetes have been documented by several authors [Ahmed *et al*., 2001, Rajesh *et al*., 2010, Mohammed *et al*, 2010, Tao and Qin, 2007]. Therefore, the

Corresponding Author: Oyedepo, T.A.
Faculty of Science and Technology, Adeleke University, P.M.B. 250, Ede, Osun State, Nigeria
Email:oyedepo004tope@yahoo.com

purpose of this study was to investigate the effect of the fruit juice of Citrus maxima in alloxan-induced diabetes in rats. The effect of the juice extract on one of the major organs in the body [liver] under diabetic condition was also examined.

Materials and Methods

Experimental Animals

Sixteen (16) adult male albino Wistar rats weighing between 150 and 250g were used for this study. The rats were fed with standard feed [vital growers mesh] and water *ad libitum*. The feed nutrient composition includes; crude protein 14.5%, fat 7.0%, crude fiber 7.4%, calcium 0.8% and phosphorus 0.4%.

Preparation of the Fruit Juice
The Shaddock fruits were obtained locally and authenticated at Federal Research Institute of Nigeria [FRIN], Jericho, Ibadan, Nigeria. The fruits were thoroughly washed with potable water to remove dirt, soil and other foreign bodies in order to remove the microbial load. They were peeled; the epicarp and seeds were removed. They were also sliced; crushed and about 3 ½ liters of must/juice were obtained using Phillips Juice extractor. The Shaddock juice was prepared according to the method described by Anih [2006].

Induction of Diabetes in Experimental Animals

Group A and C rats were induced with diabetes by intraperitoneal injection of alloxan solution at a dose of 150mg/kg body weight. The alloxan monohydrate was obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Treatment with Citrus maxima Fruit Juice

The treatment was carried out two days after diabetes had been induced in group A and B rats. The treatment was done through oral administration of Citrus maxima juice at a dose of 10ml/kg body weight daily. This treatment lasted for 22 days.

Experimental Design

Sixteen healthy adult male albino Wistar rats weighing between 150 – 250 g were used for the study. They were randomly divided into 4 groups [A – D] with four rats in each group. They were grouped as follows:

Group A: Diabetic rats treated with Citrus maxima fruit juice and fed for 22 days.

Group B: Normal rats treated with Citrus maxima fruit juice and fed for 22 days.

Group C: Diabetic control rats untreated [negative control] and fed for 22 days.

Group D: Normal control rats untreated [positive control] and fed for 22 days.

Collection and Preparation of Blood Sample

At the end of the 22 days treatment with Citrus maxima fruit juice, blood samples were collected from the tail veins of the rats (both treated and untreated groups), for hematological analysis. After which blood was finally collected from the jugular vein into centrifuge tubes. The blood samples in each centrifuge tubes were allowed to clot and serum separated by centrifugation at 5,000rpm for 10minutes. The serum was used for biochemical analysis.

Analysis of Biochemical Parameters

Determination of Blood Glucose Level by Enzyme Method
Blood glucose level was determined using kit product of Fortress diagnostics [Trinider, 1969]

Determination of Serum Total Cholesterol Level by Enzyme Method

Serum total cholesterol level was determined using kit product of Randox diagnostics [Roeschlau, Bernt and Gruber, 1974].

Determination of Serum High Density Lipoprotein (HDL – Cholesterol) Level

Serum HDL-Cholesterol was determined using kit product of Fortress diagnostics [Friedewald and Fredrickson, 1972].

Determination of Serum Triglycerides Level

Serum triglycerides level was determined using kit product of Stambino diagnostics [Wahlefeld, 1974].

Determination of Serum Alanine Aminotransferases (ALT) Activity

Serum ALT activity was determined using Fortress diagnostics kit product [Henry, 1972].

Determination of Serum Aspartate Aminotransferases (AST) Activity

Serum AST activity was determined using Fortress diagnostics kit product [Henry, 1972].

Determination of Serum Alkaline Phosphate (ALP) Activity

Serum ALP activity was determined using kit product of Quimica Clinica Aplicada [Klein, Read and Babson, 1960].

Analysis of Hematological Parameters

Determination of Packed Cell Volume (PCV)
The packed cell volume (PCV) was determined using Micro-hematocrit method [McInroy, 1954].

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Determination of Red Blood Cell Count (RBC)

995µl of Hayem's solution ($HgCl_2$, NaCl and Na_2SO_4. H_2O) was dispensed into test tubes, followed by the addition of 5µl of blood. The cover slip was placed over the counting chamber and the diluted blood was mixed and introduced into the counting chamber at an angle of 45°. The chamber was focus on microscope for counting under X40 objective lens, to count the red blood cells.

Statistical Analysis

The results were expressed as mean ± standard error of mean (s.e.m.) for four rats in each group and all grouped data were statistically analyzed using students't-test. $P < 0.05$ was considered significant while $P > 0.05$ was considered insignificant

Results

Weight Changes of Rats

Table 1 shows the body weight of the rats on day 1 and 22 (final day) of the experiment, as well as the percentage (%) weight gain / loss of the rats during the period of the experiment. Group A, B and D rats showed significant weight gained while group C rats showed significant weight loss.

Table 1: Weight Changes of Rats

Group	Initial Weight	Final Weight(g)	% Weight Gain/Loss
A [diabetic + extract]	169.70 ± 10.20	205.80 ± 2.10	21.27
B [normal + extract]	168.50 ± 0.35	218.45 ± 2.55	29.64
C [diabetic control]	172.65 ± 7.34	152.42 ± 2.05	- 11.71
D [normal control]	198.10 ± 11.20	252.05 ± 1.24	27.23

Values are expressed as means ± s.e.m. for n =4

Blood Glucose Level

Table 2 shows the blood glucose level [mmol/L] in the diabetic and normal rat groups. Induction of diabetes in the experimental rats group was confirmed by the significant increase [$p < 0.05$] in the blood glucose level of the diabetic control rats group[group C] when compared with the normal control rats group[group D]. Following the oral treatment with Citrus maxima fruit juice, the blood glucose level of the treated diabetic group [group A] decreased significantly [$p < 0.05$] when compared with the diabetic control rats group [group C].

Table 2: Effect of Citrus maxima Fruit Juice on Serum Glucose Level in Normal and Diabetic Rats

Group	Blood Glucose Level [mmol/L]
A [diabetic + extract]	5.08 ± 0.89[a],[b]
B [normal + extract]	4.52 ± 0.77[b]
C [diabetic control]	12.00 ±1.20c
D [normal control]	5.01 ± 0.22

Values are expressed as means ± s.e.m for 3 determinations, n=4
a = Statistically significant decrease [$p < 0.05$] when compared with diabetic control.
b = Statistically insignificant difference [$p > 0.05$] when compared with normal control.
c = Statistically significant increase [$p < 0.05$] when compared with normal control

Serum Lipid Profile

Table 3 shows the blood lipid profile [mmol/L] in the diabetic and normal rats groups. In the diabetic control rats groups, the cholesterol and triglycerides levels increased significantly [$p < 0.05$] when compared with the normal control rats groups. In diabetic rat group treated with Citrus maxima juice, the blood cholesterol and triglycerides levels decreased significantly [$p < 0.05$] when compared with the untreated diabetic control group, except for high density lipoprotein [HDL] -cholesterol which increased. The normal rats group treated with the fruit juice shows no significant difference [$p > 0.05$] in blood lipid profile when compared with the normal control group.

Liver Enzyme Activity

Table 4 shows liver enzyme activities of normal and diabetic rats groups. In diabetic control rats group, AST and ALT activity increased significantly [$p< 0.05$] when compared with the normal control except for ALP activities which shows no significant difference [$p > 0.05$].The diabetic rats group and the normal rats group treated with the fruit juice show no significant difference in ALT and AST activities when compared with the normal control group untreated except for ALP activities which decreased significantly [$p< 0.05$] when compared with the normal control group.

Table 3: Effect of the Fruit Juice of Citrus maxima on Serum Lipid Profile in Normal and Diabetic Rats

Group	Cholesterol [mmol/L]	HDL[mmol/L]	Triglycerides [mmol/L]
A [diabetic + extract]	4.45 ± 0.11[a,c]	4.56 ±0.20 [a,b]	0.57 ±0.13 [a,b]
B [normal + extract]	3.18 ±0.79[c]	6.40 ± 1.15[b]	0.24 ± 0.19[b]
C [diabetic control]	8.68 ± 1.18[d]	1.13 ±0.25[d]	1.37 ± 0.40[d]
D [normal control]	4.77± 0.22	4.88 ±1.39	0.36 ± 0.12

Values are expressed as means ± s.e.m. for 3 determinations, n=4
a = Statistically significant decrease [p< 0.05] when compared with diabetic control except HDL-cholesterol which increased.
b= Statistically insignificant difference [p > 0.05] when compared with normal control.
c= Statistically significant decrease [p< 0.05] when compared with normal control.
d = Statistically significant increase [p< 0.05] when compared with normal control except HDL- cholesterol which decreased.

Hematological Parameters

Table 5 shows the hematological parameters; packed cell volume [PCV] and red blood cell count [RBC] of the normal and diabetic rats group. In diabetic control rat group, PCV and RBC decreased significantly when compared with the normal control rat group. The diabetic rat group treated with the fruit juice show insignificant difference [p >0.05] in the PCV and RBC level when compared with the normal control rats group.

Discussion

Diabetic control rats group showed significant weight loss as indicated by the percentage weight loss in table 1. The observed weight loss was consistent with the low level of feed intake as the rats showed poor appetite during the experiment. In addition, the weight loss may be due to fluid depletion and accelerated breakdown of fats and adipose muscles. Diabetic and normal rat groups [Group A and B] treated with the extracts showed significant weight gain than the normal control group which was untreated [Table1]. These may be due to the presence of several phytochemicals such as alkanoids and flavonoids in the fruit juice. These phytochemicals are believed to have facilitated glucose utilization by peripheral tissues [Egesie et al., 2006].

Alloxan monohydrate, a pyrimidine derivative is a very selective toxin of pancreatic ?-cells, through its inhibition of glucokinase, thus making it a good model for screening plant with antidiabetic properties [Egesie et al., 2006]. Induction of diabetes with alloxan was confirmed by the significant increase [p< 0.05] in the blood glucose level of the diabetic control group when compared with the normal control [Table 2]. Treatment with the Citrus maxima fruit juice significantly decreased [p< 0.05] the blood glucose level of the diabetic rats group treated with the fruit extracts when compared with diabetic control. The normal rats group treated with the fruit juice shows no significant difference [p >0.05] in blood glucose level when compared with normal control group. This result agrees with the work of Lenzen [2008].

Table 4: Effect of Fruit Juice of Citrus maxima on Liver Enzymes Activities in Normal and Diabetic Rats

Group	AST [Units/L]	ALT[Units/L]	ALP[Units/L]
A [diabetic + extract]	12.50 ± 1.11[a,b]	6.38 ±1.20 [a,b]	25.83 ±1.18 [a,b]
B [normal + extract]	11.72 ±0.48[b]	6.40 ± 1.15[b]	25.38 ± 0.99[b]
C [diabetic control]	15.50 ± 0.78[c]	8.55 ±1.25[c]	31.67 ± 1.40[c]
D [normal control]	12.48 ± 1.22	5.18 ±1.39	30.86 ± 1.17

Values are expressed as mean ± s.e.m for 3 determinations, n=4
a = statistically significant decrease [p< 0.05] when compared with diabetic control
b = statistically insignificant difference [p >0.05] when compared with normal control except ALP which decreased.
c= statistically significant increase [p< 0.05] when compared with normal control except ALP which shows no significant difference.

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Blood cholesterol and triglycerides level increased significantly [p< 0.05] in diabetic control group except for HDL-Cholesterol level which decreased when compared with normal control group [table 3]. The increase in blood cholesterol and triglycerides is due to the action of hormone sensitive lipase, which promotes lipolysis and subsequently increases the level of plasma free fatty acids and triglycerides. These free fatty- acids are catabolized to acetyl coA which is further channeled to cholesterol synthesis, thus increasing blood cholesterol level. Treatment with the fruit juice significantly decreased [p< 0.05] blood cholesterol and triglyceride levels in the diabetic rats group treated, except for HDL -cholesterol which increased close to normal. In the normal rats group treated with the fruit juice, blood cholesterol level decreased significantly [p< 0.05] when compared with the normal control.

The fruit juice extract shows no significant toxic effect on vital body organs like the liver as determined by the liver enzymes biochemical indices [Table 4]. Diabetic control group showed significant increase [p< 0.05] in AST and ALT activity when compared with normal control groups except for ALP activity which was insignificantly different when compared with normal control group.

Treatment with the fruit juice showed no significant difference [p >0.05] in AST and ALT activity in both diabetic and normal rats groups treated with fruit juice, except for ALP activity which significantly decreased when compared with normal control group.

Table 5: Effect of Citrus maxima Fruit Juice on PCV and RBC

Group	PCV%	RBC [mm3]
A [diabetic + extract]	54.80 ± 1.65^{a,b}	6023.38 ±71.60 X $10^{3a,b}$
B [normal + extract]	59.10 ±3.48^{b}	6531.40 ± 41.15 X $10^{3\ c}$
C [diabetic control]	48.82 ± 3.78^{d}	5257.75 ±31.25 X $10^{3\ d}$
D [normal control]	55.19 ± 3.22	6105.18 ±51.69 X 10^{3}

Values are expressed as mean ± s.e.m. for 3 determinations, n=4
a = statistically significant increase [p< 0.05] when compared with diabetic control.
b = statistically insignificant difference [p >0.05] when compared with normal control.
c = statistically significant increase [p >0.05] when compared with normal control.
d = statistically significant decrease when compared with normal control.

PCV and RBC levels decreased significantly [p< 0.05] in diabetic control group when compared with normal control group [Table5]. However, treatment with the fruit juice significantly increased the PCV and RBC levels in diabetic rats group treated, while the normal rats group which was also treated with the fruit juice, show no significant difference in PCV level except RBC count which increased significantly [p< 0.05] when compared with the normal control group.

Conclusion

This study have shown that the fruit juice of Citrus maxima possess both hypoglycemic and hypolipidemia properties. This work is unique when compared to a similar work carried out by Sriparna KunduSen et al., 2011 [Sriparna et al.] because Citrus maxima fruit juice have better taste than the Citrus limetta Fruit Peel and diabetic patients will find it easy to drink the fruit juice. Those living with type-2-diabetes can thus be encouraged to be drinking the juice of Citrus maxima fruits in addition to other treatments so as to put a check on hyperglycemia which is common with the disease. The juice is quite safe for consumption as the study indicated no toxicity to the vital organs of the body.

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