# Original article

# Effect of Exotic Fruit "Pepino" (Solanum muricatum Aiton.) on Blood Glucose Level

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Solanum muricatum is an exotic plant from Solanaceae family that is cultivated in the south of Turkey. However, fruit of the plant is known as pepino melon and introduced in the market for its antidiabetic and hepatoprotective effects; there is no sufficient scientific evidence for these activities. Therefore, this study was aimed to evaluate whether pepino fruits have lowering effect on blood glucose level or not. Juice of fruits was obtained just before ripening and hypoglycaemic effect of juice was investigated on normoglycaemic and glucose loaded rats at two different doses (250 and 500 mg/kg). Additionally acute antidiabetic activity was evaluated on streptozotocin induced diabetic rats. Tolbutamide was used as a reference drug at 100 mg/kg dose. Moreover, alpha glucosidase and alpha amylase inhibitory effects of fruit juice were also investigated. Antioxidant activity was examined by using DPPH radical scavenging assays. Furthermore, total phenolic content of fruit juice was determined to enlighten the chemical profile. Present study demonstrated that pepino juice was found to have a significant antidiabetic activity on diabetic animals at 500 mg/kg dose (27.1%). Pepino fruit should to be consumed by eating the whole fruit and it may be recommended to diabetic patients as a beneficial fruit with its low sugar content, high dietary fibers and its high antioxidant properties.

Key words: Antidiabetic, Pepino, Solanaceae, Solanum muricatum, Streptozotocin.

# Egzotik Meyve "Pepino"nun (*Solanum muricatum* Aiton.) Kan Glukoz Seviyesi Üzerindeki Etkisi

Solanum muricatum, Türkiye'nin güneyinde kültürü yapılan Solanaceae familyasından egzotik bir bitkidir. Bitkinin meyvesi pepino kavunu adıyla antidiyabetik ve hepatoprotektif etkili olduğu söylenerek pazarlansa da bu uygulamalar için yeterli bilimsel kanıt bulunmamaktadır. Bu sebeple, bu çalışmada pepino meyvelerinin kan glukoz seviyesini düşürücü etkili olup olmadığının araştırılması amaçlanmıştır. Meyveler olgunlaşmadan hemen önce meyve suyu elde edilmiş ve usarenin hipoglisemik etkisi iki farklı dozda (250 ve 500 mg/kg) normoglisemik ve glukoz yüklenmiş sıçanlarda incelenmiştir. Buna ilaveten, streptozotosin nedenli diyabetik sıçanlarda akut antidiyabetik etki değerlendirilmiştir. Referans ilaç olarak 100 mg/kg dozda tolbutamit kullanılmıştır. Ayrıca, usarenin alfa glukozidaz ve alfa amilaz inhibitörü etkileri araştırılmıştır. Antioksidan etkinin tayininde, DPPH radikal süpürücü etkinin yanısıra, usarenin kimyasal profilinin aydınlatılması amacıyla total fenol miktarı tayin ölçülmüştür. Bu çalışmada pepino meyve suyunun 500 mg/kg dozda diyabetik hayvanlarda anlamlı antidiyabetik etki gösterdiği bulunmuştur (% 27.1). Diyabet hastalarına, düşük şeker içeriği, yüksek miktarda bulundurduğu lif ve antioksidan etkisinden ötürü pepino meyvesini bütün olarak yemeleri önerilmelidir.

Anahtar kelimeler: Antidiyabetik, Pepino, Solanaceae, Solanum muricatum, Streptozotosin.

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#### INTRODUCTION

Pepino (Solanum muricatum Aiton) is an evergreen plant native to the temperate Andean region of Colombia, Peru and Chile. It is estimated for its edible fruit, a berry that is juicy, scented, mild sweet, and that can be highly variable in shape and color. It was an important crop during the times of the Inca Empire (1) and it has been grown for thousands of years. Nowadays, it has kindled an increasing interest in the exotic fruit markets and grown as a commercial crop in some Andean countries, New Zealand, and Australia. On the other hand, experiments related to pepino cultivation have recently been undertaken in many countries, such as Spain, Italy, France, Netherlands, the United States, Israel, Turkey, Korea and China (2).

Pepino gets its name from the Spanish word for "cucumber" due to some similarities of taste and texture between the two. This member of Solanacaeae family has a sweet taste and muskmelon-resembling flavor when ripe, so it is called pepino dulce, "sweet cucumber". In addition, it is a very versatile fruit and has a cucumber-like scent and flavor when it is not fully ripe. Thus, it is used as a component of different meals like soups, seafoods, sauces, meats, fruit salads and consumed as a fresh dessert. Fruits can also be frozen, jellied, dried, canned or bottled (2, 3).

Pepino has a high content of water (> 92%), and contains significant amounts of potassium (> 1000 mg/kg) and vitamin C (> 200 mg/kg) but low in calories (250 kcal/kg) Additionally, several (4-6).biological activities of pepino were examined such as antioxidant, diuretic, antitumoral activities and effect on hypertension (5,7,8). Pepino is advertised as having antidiabetic hepatoprotective properties in Turkish market and consumed as fruit. Therefore, this study is to evaluate hypoglycaemic antidiabetic potential of Solanum muricatum fruits by using in vivo and in vitro methods.

#### **EXPERIMENTAL**

Plant material

Fruits of the plant were collected just before ripening (at the end of July 2009) from

Mersin (Turkey) and identified by Prof. Dr. Fatma Ergun.

Preparation of the dried fruit juice

Fruits were cutted into small pieces, weighted (372.05 g) and then squeezed. Fruit juice was filtered and condensed under reduced pressure. Then obtained soft extract was dried by a freeze-dryer until dryness. (Yield of the fruit juice: 4.8 % w/w)

Preparation of the test samples

Each extract was suspended in 0.5% aqueous carboxymethylcellulose (CMC) solution in distilled water prior to oral administration to animals (10 mL/kg, b.w.) [b.w.: body weight]. Animals in the control group received only the vehicle (10 mL/kg, b.w.). Tolbutamide (Sigma T0891) (100 mg/kg, b.w.) was used as the reference drug. The test samples (extracts and tolbutamide) were administered orally by using a gastric gavage needle.

Animals

Male Wistar-albino rats weighing 180-200 g, were purchased from the Gazi University Experimental Animal Research Center. Ankara-Turkey. All rats were kept at room temperature. Throughout the animal experiments were processed following the internationally accepted ethical guidelines for the care of laboratory animals (G.Ü. ET-06.087). Prior to the experiments, rats were fed with standard food for 1 week in order to adapt to the laboratory conditions. Twelve hours before the experiments, they were fasted overnight, but allowed free access to water. Six animals were used in each group. The body weight and fasting blood glucose levels of all the rats were determined before the start of the experiment. Rats were divided into the following groups:

- Group 1: Control, received only vehicle (0.5% CMC) (10 mL/kg)
- Group 2: Reference, tolbutamide was given at a dose of 100 mg/kg
- Group 3: Dried fruit juice was given at a dose of 250 mg/kg
- Group 4: Dried fruit juice was given at a dose of 500 mg/kg

#### Determination of the blood glucose levels

Blood glucose concentration (mg/dl) was determined using an Ascensia-Elite commercial test (Bayer), based on the glucose oxidase method. Blood samples were collected from the tip of tail at the defined time patterns.

### Effect in normoglycaemic rats

Fasting blood sugar level of each rat was determined at the beginning of the experiment, after overnight fasting with free access to water. Blood samples were collected at 30, 60, 120 and 240 min after the oral administration of test samples.

Effect in glucose-hyperglycaemic rats (oral glucose tolerance test, OGTT)

Fasting blood sugar level of each rat was determined at zero time, after overnight fasting with free access to water. Glucose (2 g/kg b.w.) was orally administered 30 min. after the oral administration of the test sample or vehicle (for control). Blood glucose concentrations were measured just before and 30, 60, 120 and 240 min. after the oral administration of the test samples.

Effect in diabetic rats (non-insulin dependent diabetes model-NIDDM)

#### Induction of diabetes

Diabetes was induced in rats by the intraperitoneal (i.p.) injection of streptozotocin (STZ) (Sigma S0130), at a dose of 55 mg/kg b.w. dissolved in distilled water (1 mL/kg b.w.) (9, 10). Seven days after the injection, the blood glucose levels were measured. Each animal with a blood glucose concentration level above 300 mg/dL was considered to be diabetic and used in the experiments.

#### Determination of antidiabetic effect

Test samples were given orally to the fasted diabetic rats. STZ diabetic rats were used in the control group. The blood glucose concentrations of the animals were measured at the beginning of the study and the measurements were repeated 30, 60, 120 and 240 min after the initial of the experiment.

Assay for α-amylase inhibitory activity

The  $\alpha$ -amylase inhibition assay was performed using the chromogenic method used by Ali et al. (11). Porcine pancreatic  $\alpha$ amylase (EC 3.2.1.1, type VI, Sigma) was dissolved in ice-cold distilled water to give a concentration of 4 U/ml solution. Potato starch (0.5%, w/v) in 20 mM phosphate buffer (pH 6.9), was used as a substrate solution. 3,5 dinitrosalicylic acid (DNS) was used as a colouring reagent to react with maltose which occur after the hydrolysis of starch by alphaamylase.  $\alpha$ -amylase activity was determined by measuring the absorbance of the mixture at 540 nm. Experiments were performed with three replicate determinations for each experiment.

#### Assay for α-glucosidase inhibitory activity

 $\alpha$ -Glucosidase activity was assayed according to the reported method by Lam et al. (12). The enzyme solution (3 U/mL) was prepared by dissolving  $\alpha$ -Glucosidase type IV (Sigma Co., St. Louis, USA) from *B. stearothermophilus* in 0.5 M phosphate buffer (pH 6.5). The increment of absorbance at 405 nm due to the hydrolysis of *p*-nitrophenyl- $\alpha$ -d-glucopyranoside (NPG) by  $\alpha$ -glucosidase was measured by an ELISA microtiter plate reader.

# Estimation of total phenol content

Total phenol content of pepino was determined by using Folin-Ciocalteu's method adapted to a 96 well-plate by Zongo et al. (13). 100 µL Folin Ciocalteu's reagent was mixed with 20 µL of extract (100 µL/ml) in 96 well-plate. After incubation for 5 min, 80 uL of sodium carbonate solution was added to the wells. Plate was slightly shaken and incubated for 30 min. The absorbance was measured at 735 nm utilizing a 96-well **ELISA** microplate reader (VersaMax, Molecular Devices, USA). The measurements and calculations were evaluated by using Softmax PRO 4.3.2.LS software. Gallic acid was used as standard and the equation was y=5.3471x+0.0672 ( $r^2=0.9986$ ), where y is the absorbance read at 735 nm, x is the concentration of gallic acid (µl/mL). Total phenol content of the extracts was expressed as gallic acid equivalent per gram of extract.

Estimation of radical scavenging activity

The DPPH radical scavenging activity of the extracts was determined in a 96 well-plate according to the method reported by Jung et al (14).  $160~\mu\text{L}$  of extract was mixed with  $40~\mu\text{L}$  of DPPH solution and incubated in darkness for 30 min. Then the absorbance was measured at 520 nm utilizing a 96-well ELISA microplate reader (VersaMax, Molecular Devices, USA). The measurements and calculations were evaluated by using Softmax PRO 4.3.2.LS software. BHT was used as a positive control at 0.05, 0.1 and 0.2 mg/mL concentrations.

#### Statistical analysis

Data of *in vivo* studies are presented as means  $\pm$  S.E.M. Statistical differences between the treatments and the controls were tested by one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test using the "Instat" statistic computer program. A difference in the mean values of p<0.05 was considered to be statistically significant. All experiments *in vitro* studies were done in triplicate. Mean of

three results, standard deviation (S.D.) and standard error of the mean (S.E.M.) were calculated.

#### RESULTS

In the present study, antidiabetic and hypoglycaemic activities of the fruit juice of S. muricatum (pepino) were investigated by using in vivo techniques in order to evaluate the usage of the plant in Turkey. Fruit juice was obtained according to the method described above and administrated to normal, glucose-hyperglycaemic and STZ induced diabetic rats at two different doses to determine the acute effects on blood glucose concentrations. Changes in the blood glucose level of each group of animals were followed during a 4 h period. Hypoglycaemic effect of pepino fruit juice on normoglycaemic rats was examined and the results were shown in Table 1. While the reference drug, tolbutamide, possessed potent activity during experiment (19.4-39.5 %), no remarkable effect was observed at fruit juice given groups.

**Table 1.** Effect of *S. muricatum* fruit juice on blood glucose levels of normoglycaemic rats.

Groups	Dose mg/kg	Blood Glucose Concentration (mg/dL) ± S.E.M (Inhibition %)					
		Initial	30 <sup>th</sup> min.	60 <sup>th</sup> min.	120 <sup>th</sup> min.	240 <sup>th</sup> min.	
Control	I	$77.8 \pm 1.7$	$85.8 \pm 1.9$	$86.3 \pm 1.8$	$79.7 \pm 1.5$	$81.2 \pm 2.2$	
Tolbutamide	100	$87.0 \pm 2.9$	$69.2 \pm 4.3^{**}$ (19.4)	$55.39 \pm 2.6^{***}$ (35.9)	$48.2 \pm 1.9^{***}$ (39.5)	$60.8 \pm 5.2^{***} $ (25.1)	
Dried Fruit Juice	250	$80.0 \pm 2.5$	$96.5 \pm 3.7$	$90.3 \pm 2.2$ (2.2)	$83.8 \pm 3.3$	$83.5 \pm 2.8$	
	500	$85.5 \pm 2.0$	$101.3 \pm 3.2$	$97.5 \pm 2.5$	$84.3 \pm 1.1$	$86.5 \pm 1.6$	

number of animals in each group=6, \* p<0.05, \*\* p<0.01, \*\*\* p<0.001 results compared to control group, S.E.M: Standard error of the mean

In oral glucose tolerance test (OGTT), effect of pepino juice on glucose absorption was investigated and the results were given in Table 2. Effect of pepino fruit juice was not

promising (4.3-9.3 %) after loading glucose but the effect of tolbutamide was found to be highly effective (22.7-41.3 %).

**Table 2.** Effect of on *S. muricatum* fruit juice blood glucose levels of glucose-loaded rats (OGTT)

Groups	Dose mg/kg	Blood Glucose Concentration (mg/dL) ± S.E.M (Inhibition %)					
		Initial	30 <sup>th</sup> min. (+ 2 g/kg glucose)	60 <sup>th</sup> min.	120 <sup>th</sup> min.	240 <sup>th</sup> min.	
Control	-	$93.3 \pm 1.7$	$103.7 \pm 4.5$	$136.0 \pm 5.9$	$103.5 \pm 4.8$	$94.8 \pm 2.0$	
Tolbutamide	100	$94.3 \pm 1.8$	$80.2 \pm 3.5^{**}$ (22.7)	95.4 ± 5.0*** (29.9)	$79.0 \pm 5.2^{**}$ (23.7)	$55.2 \pm 2.4^{***}$ (41.8)	
Dried Fruit Juice	250	$95.7 \pm 1.5$	$112.5 \pm 4.9$	$126.2 \pm 8.6$ (7.2)	$106.2 \pm 8.3$	$96.2 \pm 5.5$	
	500	$80.5 \pm 1.9$	$99.2 \pm 3.0$ (4.3)	$123.3 \pm 2.6$ (9.3)	$104.5 \pm 3.3$	$103.0 \pm 2.6$	

number of animals in each group=6, \* p<0.05, \*\* p<0.01, \*\*\* p<0.001 results compared to control group, S.E.M: Standard error of the mean

In order to evaluate antidiabetic effect of pepino, diabetes was induced by i.p. injection of streptozotocin. Changes in blood glucose levels after oral administration of pepino fruit juice were monitored for 4 h and the results were given in Table 3. In STZ-induced

diabetic rats, the effect of pepino fruit at 500 mg/kg was found more potent than the reference drug tolbutamide. The antidiabetic effect of pepino fruit was promising (2.2-27.1%) however the effect of reference drug tolbutamide was not remarkable (3.1-8.2%).

**Table 3.** Effect of *S. muricatum* fruit juice on blood glucose levels of streptozotocin-induced diabetic rats

Groups	Dose mg/kg	Blood Glucose Concentration (mg/dL) ± S.E.M (Inhibition %)				
		Initial	30 <sup>th</sup> min.	60 <sup>th</sup> min.	120 <sup>th</sup> min.	240 <sup>th</sup> min.
Control	-	$381.5 \pm 4.4$	$397.8 \pm 6.4$	$418.3 \pm 4.8$	$393.8 \pm 6.9$	$354.3 \pm 8.9$
Tolbutamide	100	$392.0 \pm 4.6$	$409.8 \pm 2.9$	$405.3 \pm 21.9$ (3.1)	$376.3 \pm 3.4$ (4.4)	$325.2 \pm 4.0$ (8.2)
Dailed Emit	250	$373.8 \pm 5.1$	$397.8 \pm 3.7$	$410.8 \pm 4.0$ (1.8)	$358.3 \pm 3.0$ (9.0)	$336.8 \pm 3.5$ (5.0)
Dried Fruit Juice	500	$381.8 \pm 2.8$	$397.8 \pm 2.7$ (2.2)	400.8±21.4 (4.2)	. ,	$   \begin{array}{c}     (3.0) \\     \hline     209.3 \pm 9.5 *** \\     (27.1)   \end{array} $

number of animals in each group=6, \* p<0.05, \*\*\* p<0.001 results compared to control group, S.E.M: Standard error of the mean

 $\alpha$ -Amylase and  $\alpha$ -glucosidase inhibitory activities of fruit juice were evaluated to have an idea about the effect of pepino juice on carbohydrate digestive enzymes. Pepino has

no inhibitory activity on both  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes at tested concentrations (30, 100, 300, 1000, 3000  $\mu$ g/mL).

Group	Concentration (mg/mL)	Inhibition %	± S.D.
BHT	0.2	76.1	0.3
	0.1	72.5	1.3
	0.05	61.8	2.7
Pepino	2	29.2	10.0
	1	42.2	6.0
	0.5	15.7	2.7

**Table 4.** DPPH radical scavenging activities of *S. muricatum* fruit juice.

SD:Standard deviation

Additionally, DPPH radical scavenging activity of pepino juice was determined. BHT was used as positive control and antioxidant activity of the fruit juice was found insufficient compared to BHT.

Total phenol content of the pepino juice was measured to have an idea about the chemical profile. Pepino juice was not found to be rich in terms of phenolic substances, total phenol content of the dried juice were  $0.96 \pm 0.17$  (mg gallic acid equalent/g dried juice).

#### **DISCUSSION**

In our experiments, a moderate decrease was observed on the blood glucose levels of diabetic rats. The dried fruit juice of pepino did not reduce the blood glucose levels of normoglycaemic and glucose loaded rats. As known, fibers play a key role in the inhibition of carbohydrate absorption from intestine. While pepino fruits are rich in soluble and unsoluble fibers, pepino fruit juice contains only water soluble fibers (15). Since we administrated pepino juice instead of total fruit, unsoluble fibers were not contributed to inhibition of glucose absorbtion from intestine in OGTT. This might be reason of ineffectiveness of fruit juice of pepino in the glucose loaded experiments.

According to our *in vitro* studies, pepino did not possess any inhibitory effect on carbohydrate digestive enzymes at tested concentrations.

In the literature survey, we realized that Hsu et al. (16) examined the protective effects of pepino aqueous extract (PAE) in diabetic mice for 5 weeks. They found that PAE treatments at 2 and 4% significantly lowered

plasma glucose level; additionally at 4% elevated plasma insulin level at week 5 significantly. They also revealed that PAE treatments decreased levels malonyldialdehyde and reactive oxygen species, reduced oxidized glutathione formation, increased glutathione level, and retained renal glutathione peroxidase and catalase activities. PAE treatments significantly decreased aldose reductase activity and sorbitol production in kidney. Nevertheless, there is no positive control in this study and the results are not clearly declared. Even so, data obtained from this study confirm our results (16).

In another study, Sudha et al. (17, 18) investigated the content of total phenols and flavonoids in ethyl acetate extract of raw and ripe pepino fruit. They also examined the level of antioxidant potential of the extracts DPPH. **ABTS** (2,2'-azino-bis by ethylbenzothiazoline-6-sulphonic acid)), and OH radical scavenging, reducing power, ferric reducing antioxidant power and chelating effects on ferrous ions. In all methods extracts of both raw and ripe fruits showed good scavenging activity. Substantial amount of phenol and flavonoids was noticed. Although pepino juice have low radical scavenging potential and low phenolic content in our study, fruits of the plant includes much more phenolics and other effective compounds than juice and shows antioxidant activity.

On the other hand, many studies were conducted on the nutritional value of pepino. It is 92 % water and only 7 % carbohydrates. It is a good source of vitamin C containing about 35 mg (per 100 g) and also supplies a fair amount of vitamin A. Additionally, aqueous and ethanol extracts of pepino are

rich in phenolic acids such as ferulic, cinnamic, rosmarinic, coumaric acid, and flavonoids including quercetin, naringenin, myrcetin, and rutin (3,19).

#### **CONCLUSION**

Present study demonstrated that pepino juice was not found to have high hypoglycaemic effect on normoglycaemic and glucose loaded animals but a significant antidiabetic activity was observed on diabetic animals. According to previous literatures, pepino fruit, that is marketed as an antidiabetic fruit in Turkey, rich in phenolics and dietary fibers might be effective as it is claimed. As an advice, pepino fruit should be consumed by eating the whole fruit and it may be recommended to diabetic patients as a beneficial fruit with its low sugar content, high dietary fibers and its high antioxidant properties.

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