Investigating the Effect of *Prunus laurocerasus* Fruit Extract in Type II Diabetes Induced Rats

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**Abstract:** *Prunus laurocerasus* (PL) is the fruits of the officinalis species in the Rosaceae family. It is used as diuretic, antidepressive, antiulcer, dermatitis and hemorrhoids. This study investigate the antidiabetic effects of PL fruit extracts. Study was carried out at three stages. First, the effect of PL fruit extract (500 and 1000 mg kg⁻¹ dose levels) was investigated in diabetic rats induced with alloxan and compared with glipizide. Furthermore, the effect of PL extract on postprandial blood levels and hypoglycemia induced by glipizide were investigated. Experimental results showed that PL extract lowered the blood glucose levels significantly at 500 and 1000 mg kg⁻¹ dose levels. On the other hand, PL extract brought the blood levels to a normal after glipizide induced hypoglycemia. The PL extract was found to be an agent with agonist-antagonist properties. The results indicate that PL extract can be used in patients both with hypoglycemia and hyperglycemia.

**Key words:** Rat, diabetes mellitus, experimental, plant extracts

**INTRODUCTION**

The incidence of Diabetes Mellitus (DM) is increasing significantly in the world (Guariguata *et al*., 2011; Shamseddine *et al*., 2011). As a chronic disease, DM can be defined as a decreased sensitivity of the cells against insulin. This event causes an elevation of blood sugar levels and several organ dysfunctions in patients (Pradeepa and Mohan, 2002). Shortening the life expectancy, a need for long term therapy and the cost of treatment make the DM a serious disease. Therefore, for the treatment of DM there is a need for cost effective drugs with higher effectiveness and less side effects. Hypoglycemic drugs are currently used for the treatment of DM (Alarcon-Aguilar *et al*., 2000). In spite of the developments in modern medicine, a better treatment for DM, preventing the complications and stabilising the blood sugar levels can hardly be maintained. Hence, the interest has increased towards botanical extracts and the use of hypoglycemic drugs were restricted (Rao *et al*., 2001). Many treatment methods based on plant extracts are currently being developed for chronic diseases including DM (Malviya *et al*., 2010; Qi *et al*., 2010). There are many studies related to the treatment of DM with plant based medicine (Agarwal *et al*., 2012; Sharma and Garg, 2012). The extract used in this study was obtained from *Prunus laurocerasus* (PL) fruits. PL known as wild cherry which belongs to the Rosaceae family and Prunoideae subfamily. It is distributed in the Eastern Black Sea region of Turkey, the Balkans, Northern Ireland, Western Europe, Southern and Western Caucasus (Beyhan, 2010). The leaves of the plant contains, glucose, tannin, calcium, oxalate and prunasin (Pieroni, 2000; Browicz and Zohary, 1996). Ethnopharmacologically, it is used as diuretic, antidepressive, antiulcer, treatment for bronchitis, dermatitis and hemorrhoids. In folk medicine PL has been promoted as an effective agent against diabetes (Pieroni, 2000). However, so far there has been no scientific proof or indication referring to an active molecule related to the plant or a clinical study showing the effectiveness of this plant. The fruit is consumed directly both fresh and dried as well as in the form of jam. Its most widely known effect is that have been employed externally against pain and feverish symptoms utilized as traditional medicine in Turkey (Ayaz, 1997). In the literature, there was no report related to the antidiabetic activity of the extract. Therefore, the purpose of this study was to investigate the effect of PL fruit extract on hypoglycemia induced with alloxan in rats.

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MATERIALS AND METHODS

Animals: A total of 110 albino wistar rats weighing between 200-220 g were used. The animals were obtained from Ataturk University, Medical and Experimental Research Center. Animals were allowed 14 days to acclimatize before the experiments commenced. They were kept in a 12:12 h light/dark cycle (lights on: 07:00 - 19:00 h) in an air-conditioned constant temperature (22±1°C) colony room, with free access to water and 20% (w/w) protein commercial chow. The rats were supplied with water and standard pelleted diet ad libitum. All studies were performed in accordance with the ethical guidelines set out by the local ethical committee that were fully compatible with the “NIH Guide for the Care and Use of Laboratory Animals”. The study was approved by the Animal Care and Use Committee of Ataturk University, Erzurum (31.05.2013/1-22).

Chemicals: Sodium thiopental (IE Ulagay-Turkey), PL fruit extract (BIOTA, Turkey) and alloxan (Sigma) were obtained and used.

Extract of Prunus laurocerasus fruits: To test the hypothesis of its antidiabetic effect the extract of the whole fruit was obtained. The ripened and dark color fruits were obtained from Rize, Pazar district of Turkey and dried in a special vacuum oven at 45°C to protect the amino acids, enzymes and proteins. The extract was dried to a moisture level of 3-5 percent. The dried extract was ground into a fine flowing powder with a particle size distribution in the range of 250-1000 micrometer. The final product was a dark brown-purple powder with fine flowing properties and was kept at a temperature of 4-8°C throughout its use. The fractioned extracts with ethanol, methanol, acetone and n-hexane were obtained using a Soxhlet apparatus. The extracts were used in analytical studies using GC-MS and HPLC to determine the possible active moieties. For the animal experiments the whole extract was used after evaporating the solvent in a rotary evaporator.

Analysis of extracts with high performance liquid chromatography (HPLC) system: The gradient HPLC method for chlorogenic acid, benzoic acid and gallic acid in PL was as follows (Baziramanegena et al., 1995). The system was Shimadzu (North America) UFLC Prominence with a SPD-M20A PDA Detector, the column was GL Sciences ODS-3 250 m x 4.6 mm x 5 μm Octadecylsilyl silica gel. System conditions were: Mobile phase A, Phosphoric Acid-water (1:1000), mobile phase B, Acetonitrile. Flow: 1.5 mL min⁻¹, injection volume: 5μL, column temperature: 35°C and the wavelength was 330 mM.

Gas chromatography-Mass spectrometry (GC-MS) analysis: The GC-MS method for PL was developed “in house” and was as follows: The extracts in appropriate solvents were injected into the system. The system was Shimadzu GC-MS-QP 2010 Plus ((North America) and the column TRB-1 MS 30 μm 0.25 μm Poly (dimethyl) silicone capillary column. The system conditions were as follows: Injector temperature: 250°C, interface temperature: 2500°C, flow: 2 mL min⁻¹ Helium, injection volume: 5 μL, split ratio: 25, detector voltage: 0.8 eV, scanning range: 40-350 and column temperature: 250°C.

Experimental diabetes model using alloxan: Animals were acclimatized at normal room temperature two days before the experiments. The effect of PL extract on the blood levels was studied. Therefore, the study was carried out at three stages: The first stage was alloxan induced diabetes model (Jaouhari et al., 2000). Alloxan was dissolved in distilled water and injected three consecutive days to the rats at 120 mg kg⁻¹ dose, intraperitoneal (ip). Three days later, after the last alloxan application, the rats were fasted for 12 h. Blood samples were collected from the tail veins of the rats. Blood glucose level were monitored using an auto analyzer. Blood glucose level can be either measured by autoanalyzer or using a commercial blood glucose meter. Autoanalyzer requires more blood sample. Therefore, if only one measurement is required auto analyzer was used, for repeated measurements a commercial blood monitoring devices are more suitable (Cai et al., 2009). Blood glucose level above 250 mg mL⁻¹ considered as diabetic and the rats with blood glucose level below 250 mg dL⁻¹ were not included in the study.

Effects of PL extract and gliclazide on alloxan induced diabetes: In this part of the experimental study, the rats with hyperglycemia were divided into four groups. Group one and two received 500 mg kg⁻¹ and 1000 mg kg⁻¹ PL extract by gastric tube. Group three received distilled water and group 4 was given antidiabetic agent gliclazide at a 30 mg kg⁻¹ dose. The blood glucose levels of the rats were measured at hourly intervals up to 4 h. The effect of PL extract on blood levels was assessed comparing it against the control.
Effects of PL extract postprandial blood glucose levels:
At the second stage of the study, the effect of PL extract on Post Prandial Glucose Levels (PPGL) were investigated along with gliclazide at dose of 30 mg kg⁻¹. The measurements of PPGL were taken two hours after feeding the rats. Immediately after the measurements, 500 and 1000 mg kg⁻¹ PL extract or 30 mg kg⁻¹ gliclazide were administered per orally (p.o.). The PPGL measurements were made up to 4 h. The results of PL extract were compared with the gliclazide.

Effect of PL extract on gliclazide hypoglycemia:
In the last part of the study, the effect of PL extract on gliclazide hypoglycemia was investigated. At first, PPGL measurements were taken, after that all rat groups were given 30 mg kg⁻¹ gliclazide P.O. One hour after gliclazide administration blood glucose levels were measured again. The rats with hypoglycemia were separated into three groups: The first group was given 500 mg kg⁻¹ PL extract, the second group was given 1000 mg kg⁻¹ PL extract and the third group received distilled water as the control. Afterwards, blood glucose levels were measured 1 h apart a total of 4 times. The results were compared with gliclazide group.

Statistical analyses:
Repeated measures analysis of variance were used to find the significance of study parameters between groups of samples and post hoc Least Significant Difference (LSD) option test was used to find the pair-wise significance by using Statistical Package for Social Sciences 18.0 (Armonk, NY, USA) software. Differences among groups were obtained using the least significant difference option and significance was declared at p≤0.05. The results are expressed as Mean±SEM.

RESULTS

Some of the compounds of PL were found by GC-MS and HPLC were as follows: Anisaldehyde, arabinose, cholecalciferol, dodecanoic acid, glyceryl acetate, glyceraldehyde, furfuryl alcohol, cyclomannor, manitol, heptose, 2-deoxy-D-galactose, xanthosine, oleic acid, docosanoic acid, chlorogenic acid, galactose, glycerol, 2,2-bioxiran, hydroxy acetone.

Effects of PL extract and gliclazide on alloxan induced experimental hyperglycemia:
The average blood glucose levels before and after alloxan administration were given in (Table 1) and (Fig 1). The PL extract 1000 mg kg⁻¹ significantly lowered blood glucose levels according to alloxan control. The blood sugar level decreased to 280.5±26.9 mg dL⁻¹ from 431.3±28.6 mg dL⁻¹ in PL1000 group, 371±35.5 mg dL⁻¹ from 450±29.6 mg dL⁻¹ in PL500 group and 292.6±7.3 mg dL⁻¹ from 446±4.8 mg dL⁻¹ in gliclazide group. The difference was not significant when compared with gliclazide group (p>0.05).

Effect of PL extract and gliclazide on postprandial blood glucose levels:
The effect of 500 and 1000 mg kg⁻¹ PL extract and 30 mg kg⁻¹ gliclazide on postprandial blood glucose levels at 1, 2, 3 and 4 h were given in (Table 2). The blood sugar level decreased to 118±1.3 mg dL⁻¹ from

<table>
<thead>
<tr>
<th>Groups (n=10)</th>
<th>Before administration of alloxan (mg/kg)</th>
<th>6 days after alloxan administration (mg/kg)</th>
<th>Time (h)</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>77.9±2.66</td>
<td>440.0±29.1</td>
<td>447.0±28.9</td>
<td>432.8±24.2</td>
<td>437.9±21.9</td>
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</tr>
<tr>
<td>Gliclazide</td>
<td>79.1±2.2</td>
<td>446.4±8.0</td>
<td>457.0±8.1</td>
<td>462.5±10.2</td>
<td>293.7±6.4</td>
<td>292.6±7.3</td>
<td>0.03±*</td>
</tr>
<tr>
<td>PL 500</td>
<td>79.0±1.8</td>
<td>450.0±29.6</td>
<td>465.0±31.2</td>
<td>419.9±33.7</td>
<td>354.0±33.7</td>
<td>371.0±35.5</td>
<td>0.491</td>
</tr>
<tr>
<td>PL 1000</td>
<td>74.3±1.9</td>
<td>431.3±28.6</td>
<td>388.0±28.6</td>
<td>370.5±28.2</td>
<td>265.6±27.3</td>
<td>280.4±26.9</td>
<td>0.015</td>
</tr>
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</table>

According to repeated measures ANOVA post-hoc LSD analysis of the gliclazide PL500 and PL1000 groups vs. control group, PL500: 500 mg kg⁻¹ Prunus laurocerasus extracts, PL1000: 1000 mg kg⁻¹ Prunus laurocerasus extracts, Gliclazide: The group given 30 mg kg⁻¹ gliclazide; n: No. of animals, Glucose levels were determined as mg dL⁻¹, *p<0.05 is significant.

Table 2:

<table>
<thead>
<tr>
<th>Groups (n=10)</th>
<th>PPEG (mg/kg)</th>
<th>Time (h)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gliclazide</td>
<td>137.6±1.6</td>
<td>76.7±8.1</td>
<td>54.4±1.3</td>
<td>47.6±1.5</td>
<td>43.3±1.1</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>PL 500</td>
<td>138.0±1.3</td>
<td>126.0±1.4</td>
<td>119.9±9.5</td>
<td>118.4±1.1</td>
<td>112.7±9.4</td>
<td>0.009*</td>
<td></td>
</tr>
<tr>
<td>PL 1000</td>
<td>135.5±2.3</td>
<td>110.0±1.9</td>
<td>108.7±3.8</td>
<td>124.3±1.8</td>
<td>118.0±1.3</td>
<td>0.009*</td>
<td></td>
</tr>
</tbody>
</table>

According to repeated measures ANOVA post-hoc LSD analysis of the PL500 and PL1000 groups vs., the gliclazide group, PL500: 500 mg kg⁻¹ Prunus laurocerasus extracts, PL1000: 1000 mg kg⁻¹ Prunus laurocerasus extracts, Gliclazide: 30 mg kg⁻¹ gliclazide; n: No. of animals, PPEG: Post-Prandial Blood Glucose, Glucose level was determined as mg dL⁻¹, *p<0.05 is significant.
Table 3: Effect of *Prunus laurocerasus* extract on gliclazide-induced hypoglycemia

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gliclazide</td>
<td>132±1.9</td>
<td>55.9±1.3</td>
<td>40.5±0.7</td>
<td>42.1±1.2</td>
</tr>
<tr>
<td>PL 500</td>
<td>133±1.9</td>
<td>52.6±1.3</td>
<td>53.1±0.7</td>
<td>56.8±1.2</td>
</tr>
<tr>
<td>PL 1000</td>
<td>128±1.9</td>
<td>51.0±1.3</td>
<td>67.5±0.7</td>
<td>67.5±1.2</td>
</tr>
</tbody>
</table>

According to repeated measures ANOVA post hoc LSD analysis of the PL 500 and PL1000 groups vs. the gliclazide group. PL 500: 500 mg kg⁻¹ *Prunus laurocerasus* extracts, PL 1000: 1000 mg kg⁻¹ *Prunus laurocerasus* extracts, Gliclazide: 30 mg kg⁻¹ gliclazide, n: No. of animals, PPG: Post-Prandial Blood Glucose. Glucose level was determined as mg dl⁻¹. 30 mg kg⁻¹ gliclazide group blood glucose levels measured after 1 h: PL extract was given 1, 2, 3 and 4 h after blood glucose levels were measured, *p*<0.05 is significant.

Fig. 1: Comparison of mean blood glucose levels between groups in an alloxan-induced diabetes model. FBG: Fasting blood glucose, h: hour, PL 500; the group given 500 mg kg⁻¹ *Prunus laurocerasus* extracts, PL 1000; 1000 mg kg⁻¹ *Prunus laurocerasus* extracts, Gliclazide; 30 mg kg⁻¹ gliclazide

133.5±2.2 mg dl⁻¹ in PL 1000 group, 112.7±0.94 mg dl⁻¹ from 138±1.3 mg dl⁻¹ in PL 500 group and 43.8±1.1 mg dl⁻¹ from 137.6±1.6 mg dl⁻¹ in gliclazide group at 4 h. Gliclazide significantly lowered the blood glucose level in comparison with PL extract (p<0.05).

**Effect of PL extract on gliclazide induced hypoglycemia:**

The effects of PL extract at 500 and 1000 mg kg⁻¹ dose on gliclazide induced hypoglycemia and the comparison of the groups were given in (Table 3) and (Figure 2). The PL extract significantly improved the blood glucose levels after gliclazide induced hypoglycemia (p<0.05).

**DISCUSSION**

In this study, PL extract was studied in experimentally induced diabetic rats. Besides, the effects of PL extract on postprandial blood glucose level and also on hypoglycemia induced by gliclazide were investigated. In general, the PL extract at 1000 mg kg⁻¹ lowered the blood glucose level in comparison with gliclazide (Scherthaner et al., 2004). The blood glucose lowering effect of gliclazide as the most significant in 2 h after alloxan administration. Especially 1000 mg kg⁻¹ dose of extract used in the PL, show similar effect gliclazide. On the contrary, in the experimental hypoglycemia model induced with gliclazide, the PL extract at a dose level of 500 mg kg⁻¹ and 1000 mg kg⁻¹ elevated the blood glucose levels. Elevation in blood glucose level was significant in especially 1000 mg kg⁻¹ PL groups. Unlike gliclazide, it was observed that PL extract has lowered the postprandial blood glucose levels to a normal value. These effects
.similar, 500 mg kg⁻¹ and 1000 mg kg⁻¹ dose of extract for
given PL. Lowering the high blood glucose and
showing a tendency towards normalizing the low blood
[...]

ACKNOWLEDGMENT

This research received no specific grant from any
funding agency in the public, commercial, or not-for-profit
sectors.

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[...]

CONCLUSIONS

In summary, results presented in this study confirmed
that the PL extract significantly prevented hyperglycemia
in alloxan induced diabetic rats by a still unknown
mechanism. It was observed that PL extract elevated
blood sugar to a normal level in hypoglycemic rats and
crowed the blood glucose to a normal level after feeding
the rats. It was concluded that PL extract is an antidiabetic
agent with agonist and antagonist properties, having
such a property it can be used both in hypoglycemic and
hyperglycemic patients.

Determination of organic acids in soil extracts by ion
Beyhan, O., 2010. A study on selection of promising
[...]


