

## ANTIDIABETIC and ANTIOXIDANT EFFECTS of *Vitis vinifera L.* LEAVES in STREPTOZOTOCIN-DIABETIC RATS

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### Abstract

In the present study the acute and subacute antidiabetic activities of the ethanolic extract of *Vitis vinifera L.* leaves were investigated. The acute effect was studied on the normoglycaemic, glucose hyperglycaemic and streptozotocin-induced diabetic rats; and the subacute effect was studied on same diabetic rats for 15 days. The blood glucose levels were measured by using blood glucose measuring strips based on glucose-oxidase method. After the subacute administration, the antioxidant activity of the extracts was investigated on the liver, kidney and the heart tissues of the experimental animals by measuring the tissue GSH and MDA levels. All of the antioxidant parameters were compared with the diabetic control group. According to the obtained data, the ethanolic extract of *V. vinifera* leaves at 250 mg/kg dose was found to possess a high antidiabetic and antioxidant activity. Mainly condensed tannins and flavonoids were suggested to contribute in the activities.

**Key Words:** *Vitis vinifera*, antidiabetic, hypoglycaemia, antioxidant, lipid peroxidation, MDA, GSH

### *Vitis vinifera L.* Yapraklarının Streptozotosin ile Diabet Yapılmış Sıçanlarda Antidiabetik ve Antioksidan Etkileri

Bu çalışmada *Vitis vinifera L.* yapraklarının etanolik ekstresinin akut ve subakut aktivitesi incelenmiştir. Akut etkinin değerlendirilebilmesi için normoglisemik, glukoz hiperglisemik ve streptozotosin ile diyabet oluşturulan sıçanlar kullanılmıştır. Subakut etki ise 15 gün boyunca streptozotosin ile diyabet oluşturulan sıçanlar üzerinde incelenmiştir. Kan glukoz seviyeleri kan glikoz test çubukları kullanılarak glukoz oksidaz metodu ile ölçülmüştür. Subakut uygulama sonrası ekstrelerin antioksidan aktiviteleri karaciğer, böbrek ve kalp dokularının GSH ve MDA seviyeleri ölçüлerek değerlendirilmiştir. Antioksidan parametrelerin hepsi diyabetik kontrol grubu ile karşılaştırılmıştır. *V. vinifera* yapraklarının etanol ekstresi 250 mg/kg dozda antidiyabetik ve antioksidan aktivite göstermiştir. Aktiviteden başlıca kondans tanninlerin ve flavonoidlerin sorumlu olduğu düşünülmektedir.

**Anahtar Kelimeler:** *Vitis vinifera*, antidiyabetik, hipoglisemi, antioksidan, lipit peroksidasyon, MDA, GSH.

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## INTRODUCTION

*Vitis vinifera* L. (Vitaceae) is a perennial, woody vine, usually climbing by tendrils (1). Since ancient times, the different parts of this plant have been used because of many biological activities in folk medicine. The leaves of plant are rich in tannins, flavonoids and procyanidins. Additionally, the leaves also contain organic acids, lipids, enzymes and vitamins (2-4). Grape leaves have been used to stop bleeding, inflammation, and pain, such as the kind brought on by hemorrhoids in the traditional medicine (2, 5).

It is observed that in some regions of Anatolia, the leaves of *V. vinifera* have been used for reducing the blood glucose levels in diabetics.

Diabetes is a major worldwide health problem predisposing to markedly increased cardiovascular mortality and serious morbidity and mortality related to the development of nephropathy, neuropathy and retinopathy. Diabetes mellitus is characterized by abnormal insulin secretion, derangement in carbohydrate and lipid metabolism, and is diagnosed by presence of hyperglycemia (6).

Oxidative stress which is defined as an imbalance between the generation of oxidants and antioxidant defence capacity of the body is suggested as a mechanism underlying diabetes and diabetic complications like many other diseases (7). Several mechanisms seem to be involved in the genesis of oxidative stress in both diabetic patients and diabetic experimental animals like glucose autoxidation, protein glycation, formation of advanced glycation products and the polyol pathway (8). There is also an evidence that higher glucose concentration causes a depression on natural antioxidant defence agents such as glutathione or vitamin C (9). The increase in the levels of reactive oxygen species and free radicals cause damage in the biological structures such as cell wall, genetic material and enzymes. These oxidants also cause microvascular and macrovascular complications, cardiovascular diseases, kidney and nerve damage (10).

The aim of this study was to evaluate both the antidiabetic activity of the ethanolic extract of *V. vinifera* leaves on normoglycaemic, hyperglycaemic, streptozotocin-induced diabetic rats, and the antioxidant activity by measuring the levels of non-protein sulphhydryl groups (GSH) and malondialdehyde (MDA) in the liver, kidney and heart tissues of the diabetic rats.

## EXPERIMENTAL

### *Material*

The plant material was collected from the Viticultural Research Institute, Faculty of Agriculture, Ankara University (Kalecik) before blooming. The plant was identified and the voucher specimens (GÜEF 2320, GÜEF 2321) are deposited in the Herbarium of Gazi University Faculty of Pharmacy. Plant materials were dried under shade and powdered coarsely before extraction.

### *Preparation of Extract*

The dried and powdered leaves of *V. vinifera* (500 g) were extracted with ethanol 80 % at room temperature (5 L X 6 times). Combined ethanol extract was evaporated to dryness under reduced pressure below 40°C (EtOH Extract, yield 16.07 %).

### *TLC Analysis of Resveratrol*

The ethanol extract of *V.vinifera* was administrated to TLC plate covered with Kieselgel 60F<sub>254</sub> (Merck, 1.05554.001) to evaluate the presence of resveratrol in the ethanolic extract. Chloroform: methanol (8:2) system was used as mobile phase. Sulphuric acid solution prepared in methanol (5 %) was used to determine resveratrol (11).

### *Preparation of the test samples*

The dried extract was suspended in 0.5% aqueous carboxymethylcellulose (CMC) suspension in distilled water prior to oral administration to animals (10 ml/kg, b.w) [b.w.: body weight]. Tolbutamide (100 mg/kg, b.w.) was used as the reference drug. Animals in the control group received only the vehicle (10 ml/kg, b.w.).

### *Animals*

Male Wistar-albino rats (150-200 g) purchased from the Laboratories of Refik Saydam Central Institute of Health (Ankara, TURKEY) were used in the experiments. Prior to the experiments, rats were fed with standard food for one week in order to adapt to the laboratory conditions. 16 h before the experiments, they were fasted overnight, but allowed free access to water. Six animals were used for each group of study.

### *Determination of the blood glucose levels*

Blood glucose concentrations (mg/100 ml) were determined using a Glucometer-elite commercial test (Bayer), based on the glucose oxidase method. Blood samples were collected from the tip of tail at the defined time patterns.

#### *Studies with normal animals*

Animals were divided into three groups and fasting blood sugar level of each was determined at zero-time, after overnight fasting with free access to water. Control group of animal were given 0.5% CMC.

The other two groups were treated with the increasing doses of *V. vinifera* ethanolic extract, 250 mg/kg and 500mg/kg. Blood samples were collected at 0, 1/2, 1, 2, 4 h. after the oral administration of test samples.

#### *Studies with glucose-hyperglycaemic animals (Glucose loaded model, 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 mg/kg b.w) was orally administered 30 min after an oral administration of the test sample. Blood glucose concentrations were measured just before and 1/2, 1, 2, 4 h. after the oral administration of the extract.

*Effects on Streptozotocin-induced 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) at a dose of 55 mg/kg b.w. dissolved in citrate buffer (1 M, pH 4.5) (1 ml/kg) (12,13). Seven days after the injection, the blood glucose levels were measured. Each animal with a blood glucose level above 250 mg/dl was considered to be diabetic. In order to overcome the hypoglycemia which occurred during the first 24 h following the STZ administration, diabetic rats were given 5% glucose solution orally. In all experiments, rats were fasted for 16 h prior to STZ injection.

*Acute effect of test samples*

The EtOH extract was administered orally by using a gastric gavage needle. Blood glucose levels were determined at 30, 60, 120, and 240 min after the administration of the test samples.

*Subacute effect of test samples*

The EtOH extract was administered fifteen days consecutively. Blood glucose levels were determined at 1<sup>st</sup>, 5<sup>th</sup>, 10<sup>th</sup> and 15<sup>th</sup> days after the administration of the test samples. The effect of each test sample on body weight was also monitored at the same days. On 15<sup>th</sup> day, all animals were sacrificed and then the kidney, liver and heart of each animal were removed for measurement of tissue MDA and GSH levels.

*Lipid peroxidation in liver, kidney and heart tissues*

The method of Ohkawa et al. as modified by Jamall and Smith was used to determine lipid peroxidation in tissue samples (14,15). Rats were sacrificed by an overdose of diethylether. The liver of each rat was immediately excised and chilled in ice-cold 0.9% NaCl. After washing with 0.9% NaCl, 1.0 g of wet tissue was weighted exactly and homogenized in 9 ml of 0.25 M sucrose using a Teflon homogenizer to obtain a 10% suspension. The cytosolic fraction was obtained by a two-step centrifugation first at 1000x g for 10 min and then at 2000x g for 30 min at 4 °C. A volume of the homogenate (0.20 ml) was transferred to a vial and was mixed with 0.2 ml of an 8.1% (w/v) Sodium dodecyl sulphate solution, 1.50 ml of a 0.8% (w/v) solution of TBA and the final volume was adjusted to 4.0 ml with distilled water. Each vial was tightly capped and heated in boiling water bath for 60 min. The vials were then cooled under running water.

Equal volumes of tissue blank or test sample and 10% TCA were transferred into a centrifuge tube and centrifuged at 1000x g for 10 min. The absorbance of the supernatant fraction was measured at 532 nm (Beckman DU 650 Spectrometer). Control experiment was processed using

the same experimental protocol except the TBA solution was replaced with distilled water due to the peroxidative effect of streptozotocin on tissue: livers, kidneys, and hearts of STZ-diabetic rats were used as positive control. 1,1,3,3-tetraethoxypropan was used as standard for calibration of the curve.

#### *Non-protein sulphydryl groups (Cellular GSH) in liver, kidney, and heart tissues (16)*

Liver (200 mg), heart (400 mg), and kidney (400 mg) were homogenized in 8.0 ml of 0.02 M EDTA in an ice bath. The homogenates were kept in the ice bath until used. Aliquots of 5.0 ml of the homogenates were mixed in 15.0 ml test tubes with 4.0 ml distilled water and 1.0 ml of 50% trichloroacetic acid (TCA). The tubes were centrifuged for 15 min at approximately 3000x g. 2.0 ml of supernatant was mixed with 4.0 ml of 0.4 M Tris buffer, pH 8.9, 0.1 ml Ellman's reagent [5,5'-dithiobis-(2-nitro-benzoic acid)] (DTNB) added, and the sample shaken. The absorbance was read within 5 min of the addition of DTNB at 412 nm against a reagent blank with no homogenate. Results were expressed as  $\mu\text{mol GSH/g tissue}$ .

#### *Statistical analysis*

Values are presented as means  $\pm$  SEM. 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.

## **RESULTS AND DISCUSSION**

The data obtained from normal, glucose-hyperglycaemic and diabetic rats were shown in Table 1-4. The ethanolic extract of *V. vinifera* was administrated to normal rats, but no significant effects were observed. Tolbutamide, as used reference, showed significant activity in the same condition (9.5- 31.5%) (Table 1). On the other hand, no effect was observed in the extracts given groups.

**Table 1.** Effect of *V. vinifera* EtOH extract on blood glucose levels in normal (NG) rats

| Test sample                | Mean blood glucose concentration $\pm$ SEM (mg/dl)<br>(Inhibition %) |                            |                             |                            |                         |
|----------------------------|--|----------------------------|-----------------------------|----------------------------|-------------------------|
|                            | 0 min.   | 30 min.                    | 60 min.                     | 120 min.                   | 240 min.                |
| Control                    | 77.6 $\pm$ 3.4   | 84.0 $\pm$ 2.8             | 79.5 $\pm$ 3.0              | 79.0 $\pm$ 2.9             | 74.6 $\pm$ 2.2          |
| Tolbutamide<br>100 mg/kg   | 90.2 $\pm$ 3.0   | 69.0 $\pm$ 2.6**<br>(17.9) | 54.5 $\pm$ 5.1***<br>(31.5) | 55.5 $\pm$ 4.0**<br>(29.7) | 67.5 $\pm$ 4.2<br>(9.5) |
| Vv EtOH Extr.<br>250 mg/kg | 75.0 $\pm$ 3.4   | 85.0 $\pm$ 3.6             | 82.0 $\pm$ 3.3              | 71.0 $\pm$ 6.0<br>(10.0)   | 72.5 $\pm$ 4.1<br>(2.8) |
| Vv EtOH Extr.<br>500 mg/kg | 78.0 $\pm$ 2.2   | 83.0 $\pm$ 4.7<br>(1.2)    | 82.0 $\pm$ 2.8              | 73.5 $\pm$ 5.5<br>(7.0)    | 72.0 $\pm$ 2.7<br>(3.5) |

SEM: Mean standard error

\*\*\*  $p < 0.001$ ; \*\*  $p < 0.01$  significant from the control animals

Vv: *Vitis vinifera*

As shown in Table 2, glucose solution was loaded to normal rats just after 30 min measurement. The ethanolic extracts, given in two different doses (250 mg/kg and 500 mg/kg), were found effective at the measurement of 120 min. Tolbutamide showed more potent activity than the extracts (22.2-34.1%).

**Table 2.** Effect of *V. vinifera* EtOH extract on blood glucose levels in 2 g/kg of glucose-loaded hyperglycaemic (OGTT) rats

| Test sample                | Mean blood glucose concentration ± SEM (mg/dl)<br>(Inhibition %) |                           |                        |                         |                         |
|----------------------------|--|---------------------------|------------------------|-------------------------|-------------------------|
|                            | 0 min.   | 30 min.<br>(glucose load) | 60 min.                | 120 min.                | 240 min.                |
| Control                    | 72.0 ± 3.2   | 97.7 ± 2.0                | 145.0 ± 2.9            | 107.0 ± 2.5             | 82.0 ± 2.7              |
| Tolbutamide<br>100 mg/kg   | 80.0 ± 1.5   | 76.0 ± 4.6*<br>(22.2)     | 103.5±3.1***<br>(28.6) | 70.5 ± 2.5***<br>(34.1) | 61.0 ± 5.1***<br>(25.6) |
| Vv EtOH Extr.<br>250 mg/kg | 81.5 ± 2.7   | 86.5 ± 2.6<br>(11.4)      | 158.0 ± 6.2            | 93.0 ± 1.6**<br>(13.1)  | 84.5 ± 1.4              |
| Vv EtOH Extr.<br>500 mg/kg | 78.5 ± 3.2   | 96.0 ± 6.1<br>(1.7)       | 139.6 ± 5.9<br>(3.7)   | 91.0 ± 3.3**<br>(14.9)  | 84.0 ± 4.3              |

SEM: Mean standard error

\*\*\* p<0.001; \*\* p<0.01; \* p<0.05 significant from the control animals

Vv: *Vitis vinifera*

According to the data shown in table 3, the ethanolic extract of *V. vinifera* was possessed significant effect in 30 min at the doses of 500 mg/kg. This effect is more potent than Tolbutamide. However, tolbutamide was found outstanding increasing effective during the experiment (12.9-56.1%). On the other hand, the dose of 250 mg/kg *V. vinifera* extract showed significant activity less than reference drug (7.9-17.4%).

**Table 3.** Acute effect of *V. vinifera* EtOH extract on blood glucose levels in STZ-induced diabetic rats.

| Test sample                | Mean blood glucose concentration ± SEM (mg/dl)<br>(Inhibition %) |                         |                       |                          |                          |
|----------------------------|--|-------------------------|-----------------------|--------------------------|--------------------------|
|                            | 0 min  | 30 min                  | 60 min                | 120 min                  | 240 min                  |
| Control                    | 384.5 ± 14.9   | 406.8 ± 13.3            | 398.3 ± 7.4           | 376.0 ± 6.5              | 287.0 ± 7.2              |
| Tolbutamide<br>100 mg/kg   | 394.5 ± 32.2   | 347.0 ± 9.9<br>(14.7)   | 347.0 ± 9.0<br>(12.9) | 209.8 ± 7.8***<br>(44.2) | 126.0 ± 5.2***<br>(56.1) |
| Vv EtOH Extr.<br>250 mg/kg | 384.8 ± 17.3   | 354.5 ± 15.2<br>(12.9)  | 366.8 ± 5.7<br>(7.9)  | 310.5 ± 7.1**<br>(17.4)  | 249.8 ± 6.3*<br>(13.0)   |
| Vv EtOH Extr.<br>500 mg/kg | 392.8 ± 23.7   | 327.5 ± 16.0*<br>(19.5) | 381.5 ± 10.8<br>(4.2) | 324.0 ± 10.7<br>(13.8)   | 292.3 ± 10.6             |

SEM: Mean standard error

\*\*\* p<0.001 ; \*\* p<0.01 ; \* p<0.05 significant from the control animals

Vv: *Vitis vinifera*

The test samples were given to STZ-induced diabetic rats during 15 day. On day 1, 5, 10, 15, blood glucose levels and body weights were determined. The data show that 250 mg/kg of the *V.vinifera* extract has possessed remarkable effect on blood glucose level as equal as reference drug. (11.8-26.0%) (Table 4).

**Table 4.** Subacute effect of *V. vinifera* EtOH extract on blood glucose levels in STZ-induced diabetic rats.

| Test sample                | Mean blood glucose concentration ± SEM (mg/dl)<br>(Inhibition %)) |                        |                          |                          |
|----------------------------|---|------------------------|--------------------------|--------------------------|
|                            | 1 <sup>st</sup> Day   | 5 <sup>th</sup> Day    | 10 <sup>th</sup> Day     | 15 <sup>th</sup> Day     |
| Control                    | 384.5 ± 14.9  | 329.3 ± 11.5           | 425.8 ± 11.6             | 370.8 ± 6.3              |
| Tolbutamide<br>100 mg/kg   | 394.5 ± 32.2  | 309.3 ± 9.5<br>(6.1)   | 370.8 ± 6.3**<br>(12.9)  | 273.0 ± 6.4***<br>(26.4) |
| Vv EtOH Extr.<br>250 mg/kg | 384.8 ± 17.3  | 290.5 ± 6.7*<br>(11.8) | 315.0 ± 12.2**<br>(26.0) | 316.3 ± 8.4**<br>(14.7)  |
| Vv EtOH Extr.<br>500 mg/kg | 392.8 ± 23.7  | 355.0 ± 16.9           | 438.8 ± 4.8              | 362.3 ± 9.9<br>(2.3)     |

SEM: Mean standard error

\*\*\* p<0.001; \*\* p<0.01; \* p<0.05 significant from the control animals

Vv: *Vitis vinifera*

Body weights of the animals in control and reference groups were increased, but the body weights of the other animals, given test samples were decreased. However, on day 15, body weights of the 250 mg/kg extract given animals were remarkably increased.

**Table 5.** Effect of *V. vinifera* EtOH extract on body weights in STZ-induced diabetic rats.

| Test sample                | Mean body weight ± SEM (g)<br>(increase from the initial weight%) |                        |                       |                       |
|----------------------------|---|------------------------|-----------------------|-----------------------|
|                            | 1 <sup>st</sup> Day   | 5 <sup>th</sup> Day    | 10 <sup>th</sup> Day  | 15 <sup>th</sup> Day  |
| Control                    | 161.5±6.8   | 162.0±8.1<br>(+0.3)    | 172.3±9.6<br>(+ 6.7)  | 165.3±8.2<br>(+ 2.4)  |
| Tolbutamide<br>100 mg/kg   | 168.3±7.0   | 174.0±9.5<br>(+ 3.4)   | 183.3±11.4<br>(+ 8.9) | 178.0±9.2<br>(+ 5.8)  |
| Vv EtOH Extr.<br>250 mg/kg | 186.3±16.9  | 186.5±16.6<br>(+ 0.1)  | 186.0±17.7<br>(- 0.2) | 203.5±10.7<br>(+ 9.3) |
| Vv EtOH Extr.<br>500 mg/kg | 176.5±11.8  | 153.5±12.3<br>(- 13.0) | 163.8±13.2<br>(- 7.2) | 164.5±12.8<br>(- 6.0) |

As shown in Table 6, a significant elevation in the concentration of MDA is observed in kidney and heart of diabetic rats. The ethanolic leaf extract of *V. vinifera* given orally at dose of 250 mg/kg produced a significant reduction in LPO in kidney (26.9 %) and liver (16.5 %) tissues, while the heart LPO were not recovered with both ethanolic extracts (at 250 and 500 mg/kg doses). These findings suggest that 250 mg/kg dose of the ethanolic extract may protect the liver and kidney from lipid peroxidation.

**Table 6.** Malondialdehyde (MDA) contents in the heart, kidney and liver of STZ-induced diabetic rats after treated with *V. vinifera* EtOH extract

| Test sample                             | Tissue MDA $\pm$ SEM (nmol/g wet wt)<br>(Change %) |                                 |                                 |
|---|--|---------------------------------|---------------------------------|
|   | Liver  | Kidney                          | Heart                           |
| Control                                 | 326.7 $\pm$ 13.5                                   | 227.1 $\pm$ 22.6                | 169.4 $\pm$ 22.9                |
| Diabetic control <sup>a</sup>           | 371.1 $\pm$ 16.2**<br>(+ 13.6)                     | 426.3 $\pm$ 24.2***<br>(+ 87.7) | 285.3 $\pm$ 23.3***<br>(+ 68.4) |
| Tolbutamide <sup>b</sup><br>100 mg/kg   | 361.1 $\pm$ 11.3<br>(- 2.7)                        | 412.3 $\pm$ 24.5<br>(- 3.3)     | 299.1 $\pm$ 20.1<br>(+ 4.9)     |
| Vv EtOH Extr.<br>250 mg/kg <sup>b</sup> | 309.7 $\pm$ 17.7***<br>(- 16.5)                    | 311.8 $\pm$ 20.9***<br>(- 26.9) | 292.7 $\pm$ 22.7<br>(+ 2.6)     |
| Vv EtOH Extr.<br>500 mg/kg <sup>b</sup> | 440.0 $\pm$ 18.9***<br>(+ 18.6)                    | 346.9 $\pm$ 21.0<br>(- 18.6)    | 294.2 $\pm$ 14.7<br>(+ 3.1)     |

[SEM: Mean standard error].

<sup>a</sup> Compared to vehicle control (0.5% CMC), <sup>b</sup> Compared to diabetic control

(+) represents percentage of increase and (-) represents decrease in each value when compared to either control or diabetic control.

\*\* p&lt;0.01; \*\*\* p&lt;0.001 significant from control or diabetic control

Vv: *Vitis vinifera*

We have observed a decrease in the level of GSH in liver, kidney and heart tissues in diabetic control group (Table 7). But, the administration of *V. vinifera* ethanolic extracts at both 250 mg/kg and 500 mg/kg doses significantly increased the content of GSH in especially liver tissue, respectively (79.5 % and 48.7 %). But, in heart tissues, these extracts were found to be ineffective to protect from the oxidative damage.

**Table 7.** GSH contents in the heart, kidney and liver of STZ-induced diabetic rats after treated with *V. vinifera* EtOH extract.

| Test sample                             | Tissue GSH ( $\mu$ mol/g) $\pm$ SEM<br>(Change %) |                             |                              |
|---|---|-----------------------------|------------------------------|
|   | Liver   | Kidney                      | Heart                        |
| Control                                 | 78.7 $\pm$ 1.2                                    | 84.9 $\pm$ 1.4              | 47.2 $\pm$ 1.7               |
| Diabetic control <sup>a</sup>           | 72.1 $\pm$ 7.8*<br>(- 8.4)                        | 78.5 $\pm$ 1.4**<br>(- 7.6) | 44.7 $\pm$ 1.4<br>(- 5.3)    |
| Tolbutamide <sup>b</sup><br>100 mg/kg   | 122.5 $\pm$ 6.6***<br>(+ 69.9)                    | 82.9 $\pm$ 2.7<br>(+ 5.6)   | 45.5 $\pm$ 1.5<br>(+ 1.8)    |
| Vv EtOH Extr.<br>250 mg/kg <sup>b</sup> | 129.4 $\pm$ 6.9***<br>(+ 79.5)                    | 83.1 $\pm$ 3.2<br>(+ 6.0)   | 43.0 $\pm$ 2.9<br>(- 4.0)    |
| Vv EtOH Extr.<br>500 mg/kg <sup>b</sup> | 107.2 $\pm$ 0.7***<br>(+ 48.7)                    | 81.9 $\pm$ 0.8<br>(+ 4.3)   | 41.0 $\pm$ 2.0***<br>(- 8.3) |

[SEM: Mean standard error].

<sup>a</sup> Compared to vehicle control (0.5% CMC), <sup>b</sup> Compared to diabetic control

(+) represents percentage of increase and (-) represents decrease in each value when compared to either control or diabetic control.

\* p&lt;0.05; \*\* p&lt;0.01; \*\*\* p&lt;0.001 significant from control or diabetic control

Vv: *Vitis vinifera*

Qualitative analysis revealed that flavanoid, tannin, cardioactive glucoside, reducing or nonreducing sugar, organic acids, terpenes, lipids, coumarine, anthocyanin glucosides are found as active principle in leaves (17).

In previously, several chromatographic studies established the presence of flavan-3-ols like (+)-catechin, (-)-epicatechin- ( $\pm$ )-epigallocatechin and (-)-epicatechin-3-O-gallate and of flavonols like quercitrin, traces of quercetin and kaempferol; rutin, isoquercitrin and luteolin were also identified in leaves of plant. Flavanols and flavanol oligomers and proanthocyanidins (condensed tannins) have been proven to possess powerfull antioxidant activities (free radical scavenging activity and the inhibiting effect of the lipid peroxidation). The radical scavenging properties of condensed tannins have been demonstrated *in vitro* on different biochemical models mimicking preconditions of pathological situations such as ischemia, inflammation and diabetic conditions (18). Therefore, in this study, mainly condensed tannins and flavonoids were suggested to contribute in the antilipoperoxidant activity of *V.vinifera* leaves.

On the other hand, the phytoalexin resveratrol which is known as antioxidant, can also be found in *V.vinifera* leaves. But, healthy leaves of *V.vinifera* are free from resveratrol and derivatives (2). Indeed, TLC analysis indicated that *V.vinifera* leaves do not contain resveratrol. Therefore, in this study, it can be proposed that resveratrol is not the major antioxidant active constituent of the ethanolic extract .

Additionally, studies have found that a crude alcohol extract of *Pterocarpus marsupium* that contains epicatechin and catechin derivatives, has regenerated functional pancreatic beta cells in diabetic rats. Also the antidiabetic activity of these compounds has been confirmed by some studies on different experimental models (19).

It is known that *V.vinifera* leaves contain catechin and catechin derivates (2) which may be responsible for antidiabetic effect and further studies should be focused on this goal.

As a conclusion, this study shows that the ethanolic extract of *V.vinifera* leaves at 250 mg/kg dose has a remarkable antidiabetic effect. In liver and kidney tissues, 250 mg/kg dose of the ethanolic extract also was found effective to protect from the oxidative damage. But, the ethanolic extract increased the GSH content of especially liver tissue at 250 and 500 mg/kg doses.

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