THE EFFECT of Aloe vera (L.) Burm. fil. on the ANTIOXIDANT SYSTEM in LENSES of TYPE-II DIABETIC RATS

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SUMMARY

It is known that diabetes causes damage in lens antioxidant system and leads to opacification of the lens. The present study was carried out to evaluate whether Aloe leaf pulp and gel extracts had any protective effect on the antioxidant parameters for the injury caused by type-II diabetes on the lenses in neonatal streptozotocin (nO-STZ)-diabetic rats. Type-II diabetes was induced by i.p. injection of 100 mg/kg STZ to 2 days old Wistar pups. Two months later, diabetic rats were separated into four groups and each group was given the following samples by gavage, daily for 15 days: 1. phosphate buffered saline (PBS; 6 ml/kg), 2. Aloe leaf pulp extract (500 mg/kg), 3. Aloe leaf gel extract (10 ml/kg), 4. glibenclamide (1 mg/kg). The animals were sacrificed under ether anesthesia on the 15th day. Both lenses of each rat were homogenized in saline. Lens total protein, reduced glutathione (GSH) and lipid peroxidation (LPO) levels, chosen as oxidative stress parameters, were determined spectrophotometrically. The fact that Aloe extracts as well as glibenclamide increase significantly GSH levels in lenses of type-II diabetic rats, shows the beneficial effect of Aloe. Aloe pulp extract and glibenclamide had no significant effect on LPO, whereas a slight increase in LPO is observed with Aloe gel extract.

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It may be concluded that Aloe leaf pulp and gel extracts as well as glibenclamide, if used as hypoglycemic agents, prevent partly the injury caused by type-II diabetes on the lenses.

**Keywords:** Aloe vera, type-II diabetes, streptozotocin, lenses

**ÖZET**

Diabetin lens antioksidan sisteminde hasara neden olarak, lensin opaklaşmasına yol açtığı bilinmektedir. Bu çalışmada, tip II diabette olumlu etkisi gösterilmiş olan Aloe yaprak ekstresinin, sıçanların lenslerinde diabetin neden olduğu oksidatif hasara etkisi incelendiştir. Tip II diabet, 2 günlük Wistar sıçan yavrunlarına 100 mg/kg dozda streptozotosin injeksyonu ile oluşturuldu (n0STZ-diabetik sıçanlar). 2 ay sonra, diabetik olan sıçanlar 4 gruba ayrıldı ve bu gruplara, 15 gün süreyle, güne bir kez, ağızdan, aşağıdaki ekstreler verildi: 1. Kontrol: fosfatla tamponlanmış % 0.9 NaCl (PBS; 6 ml/kg). 2. Aloe yaprak pulpasi ekstresi (500 mg/kg). 3. Aloe yaprak jeli ekstresi (10 ml/kg). 4. Glibenklamid (1 mg/kg). 15. gün, etler anestezisi altında kesilen sıçanların lensleri ayrılarak izotonik tuzlu suda homojenize edildi. Oksidatif stres parametreleri olarak seçilen glutatyon (GSH) ve lipid peroksidasıyonu (LPO) düzeyleri, lens dokusunda spektrofotometrik yöntemlerle tayin edildi. Aloe ekstrelerinin ve glibenklaminin tip-II diabetik sıçanların lens GSH düzeylerini arttırması, Aloe’nin olumlu etkisini göstermektedir. Aloe pulpa ekstresi ve glibenklamidin LPO düzeyine anlamlı bir etkisi olmadığı, Aloe jel ekstresi verilen grupta ise LPO düzeyinde hafif bir artış olduğu gözlemlendi.

Aloe yaprak pulpasi ve jeli ekstrelerinin ve glibenklamidin, hipoglisemik ilaç olarak kullanımları takdirde, diabetin lenslerde oluştuğu hasarı bir ölçüde önlediği sonucuna varıldı.

**INTRODUCTION**

Aloe vera (L.) Burm. fil. (synonym A. barbadensis Miller) (Liliaceae) is native in North Africa and also being cultivated in Turkey. Aloe species have been used for centuries for their various healing properties (1).

In recent years the use of plant extracts in the cure of diabetes has gained interest, especially because oral treatment may be possible particularly in type-II diabetes (2,3). In the last decade there have also been reports on the antidiabetic activity of Aloe extracts (4,5).
In our previous study (6) we have reported the blood glucose lowering effect of *Aloe vera* leaf pulp extract on neonatal streptozotocin (n0STZ)-induced type-II diabetic rats.

It is known that diabetes impairs the function of the lens antioxidant system. The present study was carried out to evaluate whether *Aloe* leaf pulp and gel extracts had any protective effect on the antioxidant parameters for the injury caused by type-II diabetes on the lenses in n0STZ-diabetic rats.

**RESULTS AND DISCUSSION**

It has been demonstrated that tissue antioxidant status is an important factor in the development of diabetic complications (7). Diabetes impairs the function of the lens antioxidant system and reduced glutathione (GSH) is very important for the protection of lenticular proteins against oxidative damage (8,9). Decreased lens glutathione has been reported in experimental diabetes (10). The fact that *Aloe* extracts as well as glibenclamide increase significantly GSH levels in lenses of type-II diabetic rats (Table 1), shows the beneficial effect of *Aloe*.

Lipid peroxidation leads to GSH consumption and is among the major endogenous process which can cause injury to cells and tissues. Increased lipid peroxidation was reported in human diabetic cataract (11,12). In this study, *Aloe* pulp extract and glibenclamide had no significant effect on LPO, whereas a slight increase in LPO is observed with *Aloe* gel extract (Table 1).

**Table 1.** Glutathione (GSH) and lipid peroxidation (LPO) values for all groups in lenses

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>GSH mmole/mg protein*</th>
<th>P&lt;sub&gt;t&lt;/sub&gt; test</th>
<th>LPO mmoleMDA/mg protein*</th>
<th>P&lt;sub&gt;t&lt;/sub&gt; test</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. PBS (control)</td>
<td>5</td>
<td>10.47 ± 0.67</td>
<td>0.011</td>
<td>0.62 ± 0.02</td>
<td>0.590</td>
</tr>
<tr>
<td>II. Aloe pulp</td>
<td>7</td>
<td>12.06 ± 0.99</td>
<td></td>
<td>0.58 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>III. Aloe gel</td>
<td>5</td>
<td>10.47 ± 0.67</td>
<td>0.0001</td>
<td>0.62 ± 0.02</td>
<td>0.009</td>
</tr>
<tr>
<td>IV. Glibenclamide</td>
<td>5</td>
<td>20.76 ± 2.00</td>
<td></td>
<td>0.75 ± 0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10.47 ± 0.67</td>
<td></td>
<td>0.62 ± 0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>27.59 ± 2.30</td>
<td></td>
<td>0.60 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>P&lt;sub&gt;ANOVA&lt;/sub&gt;</td>
<td></td>
<td>0.0001</td>
<td>0.053</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* n: number of animal

* mean ± S.D.
It may be concluded that *Aloe* leaf pulp and gel extracts as well as glibenclamide, if used as hypoglycemic agents, prevent partly the injury caused by type-II diabetes on the lenses.

**EXPERIMENTAL**

**Plant material:** Specimens of *Aloe vera* (L.) Burm. fil. (in Turkish “Sarisabir”) were collected from Kale (Demre) in Antalya, identified by N. Sütlüpinar and cultivated in the greenhouse of the Faculty of Pharmacy. Fresh leaves of this cultivated plant were used in this study.

**Preparation of the samples:** *A. vera* leaves were weighed, washed and cut from the middle, the gel was separated by scratching with a spoon.

a. *Aloe vera* leaf pulp extract: The leaf pulps were cut in small pieces and homogenized with phosphate buffered saline (PBS) by means of Moulinex Masterchief blender. The extract was kept at 4°C overnight, then filtered through cloth and the filtrate centrifuged at 20,000 rpm for 30 mins. at 2°C in a refrigerated centrifuge (Cryofuge 20-3 Heraeus-Christ). The green pellet was discarded and the clear yellow supernatant was taken and lyophilized (Labconco apparatus).

7.5% *Aloe* leaf pulp extract was prepared by dissolving the powder in PBS and mixing thoroughly via magnetic stirrer.

b. *Aloe vera* leaf gel extract: The gel was homogenized in a Waring blender, then diluted with an equal volume of PBS and homogenized for a second time. The extract was kept at 4°C overnight, then filtered through cloth. The clear filtrate was kept at -20°C in small portions.

c. Glibenclamide suspension: 5 mg glibenclamide was suspended in 21 ml PBS; 4 ml propylene glycol was added and the mixture was kept in an ultrasonic water bath for 45 mins. until a homogenous suspension was obtained.

**Animals and treatment:**

**Type-II diabetic model**

Wistar pups were injected intraperitoneally on day 2 after birth, with STZ (100 mg/kg) (13,14). These animals were controlled for occurrence of diabetes after 6 weeks and the diabetics (n0STZ-diabetic rats) were taken in experiment when they were 2 months old.
Animal groups

Type-II diabetic rats were separated into 4 groups of 5-7 animals. Each group was given the samples cited below:

I. Group (control) : PBS (6 ml/kg)
II. Group : Aloe vera leaf pulp extract (500 mg/kg)
III. Group : Aloe vera leaf gel extract (10 ml/kg)
IV. Group : Glibenclamide (1 mg/kg)

Administration of samples

Each group of animal was administered daily during 15 days, the above mentioned extracts orally, by means of a catheter, under mild ether anesthesia. The animals were sacrificed on the 15th day. Intracapsular extracts were prepared from their lenses.

Biochemical assays: Lenses were washed with saline and kept frozen until the day of the experiment. Both lenses of the same animal were then homogenized in saline (1 / 10 w/v) by means of Bandelin Sonopuls ultrasonic homogenizer (8 cycles / 5 mins.). The homogenates were centrifuged in a Hereaus refrigerated centrifuge (4000 rpm / 10 mins.) at 4°C. The clear supernatants were used for biochemical analysis. Lens total protein levels were measured by the Lowry method and reduced glutathione (GSH) levels by using Ellman’s reagent (15). LPO levels were determined by the method of Ledwozyw et al. (16).

Statistical analysis: The results were evaluated using an unpaired-t test and ANOVA variance analysis using the NCSS statistical computer package. A p-value less than 0.05 was considered significant.

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REFERENCES