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Effect of *Aloe vera* (L.) Burm. fil. leaf gel and pulp extracts on kidney in type-II diabetic rat models

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Significant degenerative changes were observed in the kidney tissue of untreated neonatal streptozotocin (n0STZ)-induced type-II diabetic rats. These degenerative changes were diminished in the kidney tissue of diabetic animals given glibenclamide and *Aloe* leaf gel and pulp extracts. Kidney lipid peroxidation levels were increased in diabetic rats compared to healthy rats; these levels were higher in rats treated with glibenclamide than in those which received *Aloe* extracts. Serum urea and creatinine levels were higher in diabetic rats in comparison to healthy rats. The administration of *Aloe* gel extract and glibenclamide decreased serum urea and creatinine levels in comparison to diabetic controls. Only *A. vera* leaf gel extract showed improvement both in histological and biochemical parameters suggesting a protective effect of *A. vera* on mild damage caused by type-II diabetes on kidney tissue.

Keywords: *Aloe vera*, Creatinine, Kidney, Lipid peroxidation, Streptozotocin, Type-II diabetes, Urea

Aloe vera (L.) Burm. fil. (Synonym *A. barbadensis* Miller) (Liliaceae) is native of North Africa and also being cultivated in Turkey. *Aloe* species have been used for centuries for their laxative, antiinflammatory, immunostimulant, antiseptic effects[1], wound and burn healing[2,3], antiulcer[4,5] and antitumour[5,6] activities. The antidiabetic activity of *Aloe* extracts has been reported[7,8]. Okyar *et al*.[9] reported blood glucose lowering effect of *Aloe vera* leaf pulp extract on neonatal streptozotocin (n0STZ)-induced type-II diabetic rats. It is well known that STZ causes diabetes by the selective degeneration of pancreatic β-cells. It is assumed that herbal medicine can only be effective, as an alternative to oral hypoglycemic agents, in type-II diabetes, where pancreatic islets are not totally destroyed. Keeping this in view, n0STZ-induced type-II diabetic rats[10] have been used and results compared with glibenclamide, a known hypoglycemic agent.

The effect of diabetes on the kidney has been investigated and the effects of other antidiabetic plant extracts on this tissue, reported[11-14]. The aim of this work is to investigate the effects of *Aloe vera* leaf pulp and gel extracts in comparison with a standart drug glibenclamide on the kidney morphology of type-II diabetic rats and on lipid peroxidation (LPO). Serum urea and creatinine levels have been chosen as biochemical parameters for kidney damage.

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Materials and Methods

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

Preparation of the samples — A. vera leaves (6 big leaves) were washed and cut from the middle, the gel was separated by scratching with a spoon.

Aloe vera leaf pulp extract: The leaves without the gel (pulp) were cut in small pieces (514 g) and homogenized with PBS (*p*H 7; 600 ml) 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 min. 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). Thus 29 g *A. vera* leaf pulp extract was obtained.

Leaf pulp extract (7.5%) was prepared by dissolving the powder in PBS and mixing thoroughly via magnetic stirrer.

A. vera leaf gel extract: The gel (400 g = 2.5 g dry matter) was homogenized in a Waring blender, then diluted with PBS (300 ml) and homogenized for a second time. The extract was kept at 4°C overnight, and then filtered through cloth. The clear filtrate was kept at –20°C in small portions.

Glibenclamide suspension: Glibenclamide (5 mg) was suspended in 21 ml PBS; 4 ml propylene glycol was added and the mixture was kept in an ultrasonic water bath (47.6 kHz.) for 45 min. until a homogenous suspension was obtained.

Animals and treatment

Type-II diabetic model—Wistar pups were injected ip on day 2 after birth with STZ (n0-STZ rats), 100 mg/kg, freshly dissolved in cold citrate buffer (1 mM, *p*H 4.5) according to Bonner-Weir *et al*[10]. The animals were checked for occurence of diabetes after 6 weeks and the diabetics (fasting blood glucose levels 104-170 mg/dl; mean 137 mg/dl, also in accordance with type-II diabetic model) were taken in experiment when they were 2 months old (90-120 g weight). The animals were fed with laboratory pellet diet and water allowed *ad libitum*.

Animal groups—Group I: Healthy (non-diabetic) rats (5) were kept in the same conditions with diabetic rats. Type-II diabetic rats were separated into 4 groups of 5-10 animals. Each group was given the samples as follows: Group II (untreated diabetic control): PBS (6 ml/kg), Group III: *A. vera* leaf pulp extract (500 mg/kg), Group IV: *A. vera* leaf gel extract (10 ml = 63 mg/kg), Group V: 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. Blood was taken by cardiac puncture, sera were separated for urea and creatinine analysis. Kidney tissues were taken for histological evaluation and lipid peroxidation product (LPO) determination.

Histological assays—The tissue pieces taken from the kidney of the rats, were fixed by Bouin's solution and subsequently embedded in paraffin after known routine procedures. The sections (5 μm thick) were stained with Masson's tri-dye and Periodic Acid Schiff (PAS), and were examined under Carl Zeiss Ultraphot II microscope.

Biochemical assays—LPO levels were determined as malondialdehyde (MDA) by the method of Ledwozyw *et al*[15]. Kidneys were washed with saline and kept frozen until the day of the experiment. Kidneys were then homogenized in saline (1/10 w/v) by means of Bandelin Sonopuls ultrasonic homogenizer. The homogenates were centrifuged in a Hereaus refrigerated centrifuge (4000 rpm/10 min.) at 4°C. The clear supernatants were used for LPO analysis.

Serum urea and creatinine levels were evaluated by the diacetylmonooxime method[16] and Jaffe reaction[17], respectively.

Statistical analysis—The results were evaluated using an unpaired-*t* test and ANOVA variance analysis using the NCSS statistical computer package.

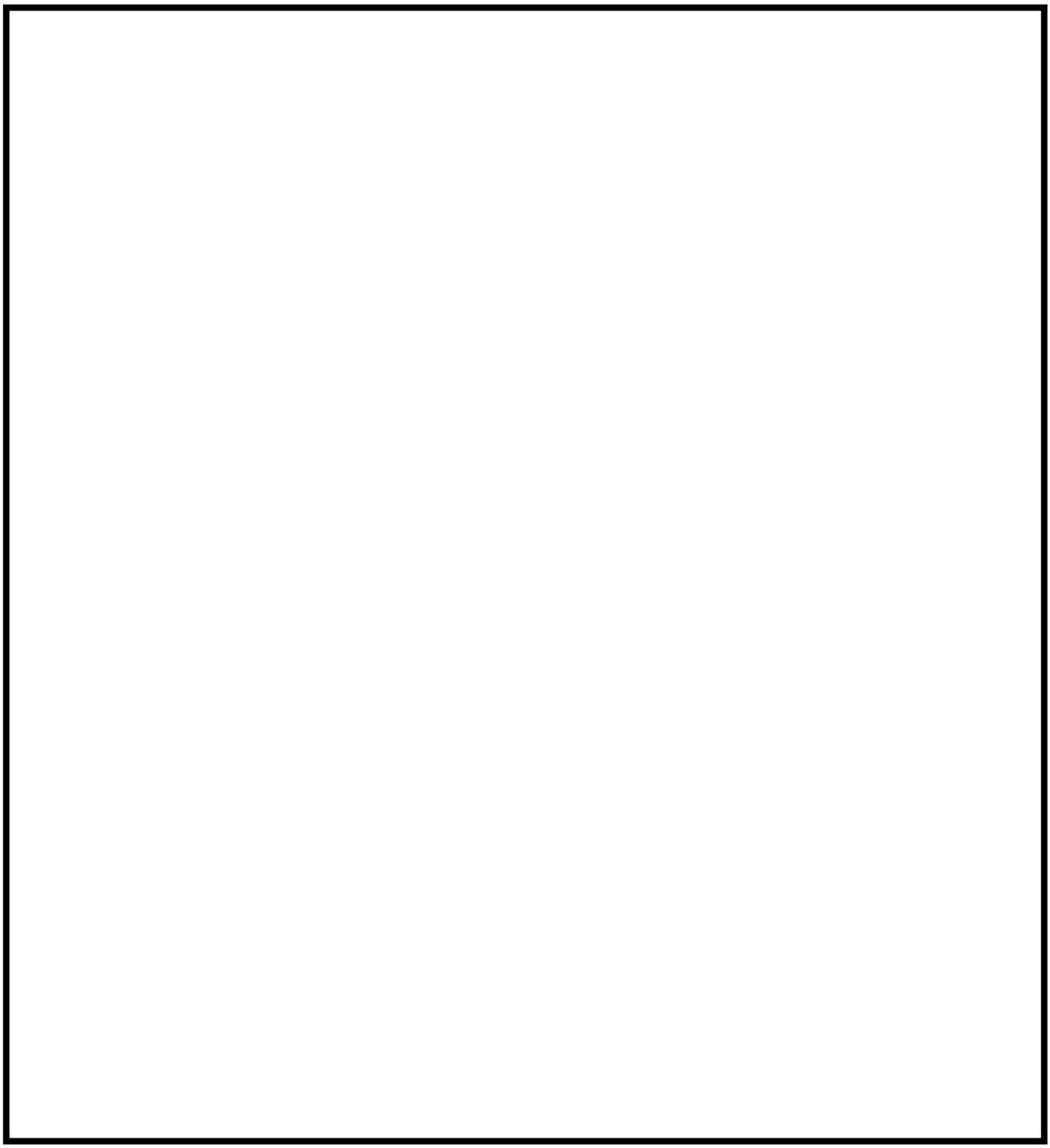
A P value < 0.05 was considered significant.

Results

Histological examination—In type-II diabetic animals given phosphate buffered saline (PBS), degenerative structural changes were compared to healthy control animals (Fig. 1). Shortening at the brush border, desquamated picnotic nuclei, cytoplasmic debris in the widened lumens and disruption in the integrity of the brush border as a result of the rupturing in the apical membrane of the proximal tubular cells, were noticed. The ruptures at the epithelium and picnotic nuclei in the cytoplasm of distal tubular cells were also observed. Excessive oedema and picnotic nuclei were detected in the cytoplasm of some proximal tubules and especially collecting tubules. In addition, a loss of cells and an expansion in capsular space in the glomeruli, hemorrhage in the capillaries in the glomeruli and in the peritubular area (Fig. 2), as well as an increased PAS positive reaction, were established.

There was a significant regeneration of tubular epithelium of the kidney tissues after the administration of glibenclamide and *Aloe* extracts. Although there were individual differences, the damage to the kidney tissue ranged from moderate to minimal in the diabetic animals administered glibenclamide (Fig. 3), while the tissue damage was only minimal in the diabetic animals administered *Aloe vera* leaf pulp (Fig. 4) and leaf gel (Fig. 5) extracts.

Biochemical parameters—Kidney tissue LPO, serum urea and creatinine levels were significantly elevated in type-II diabetic group given only PBS in comparison to healthy controls (Table 1).



Figs 1-5 — The kidney tissue of healthy control animals. Proximal (P) and distal (D) tubules and glomeruli (G); (2) — The kidney tissue of type-II diabetic rat group given PBS: The shortening (➔) and rupturing (→) at the brush border, desquamated picnotic nuclei (►) and cytoplasmic debris (>) in the widened lumens (L) in the proximal (P) tubules. The ruptures at the epithelium (→) and picnotic nuclei (►) in the distal (D) tubules. The hemorrhage (H) in the capillaries in the glomeruli (G) and in the peritubular areas; (3) — The kidney tissue of type-II diabetic rat given glibenclamide: A reduction in the damage to the kidney tissue. Proximal (P) and distal (D) tubules and glomeruli (G); (4) — The kidney tissue of type-II diabetic rat given *Aloe* pulp extract: A significant reduction in the damage to the kidney in comparison to the diabetic rat given glibenclamide. Proximal (P) and distal (D) tubules and glomeruli (G) and (5) — The kidney tissue of type-II diabetic rat given *Aloe* gel extract: A significant reduction in the damage to the kidney in comparison to the diabetic rat given glibenclamide. Proximal (P) and distal (D) tubules and glomeruli (G). Masson's tri-dye. X 320 for all figures

LPO products levels were higher in rats treated with glibenclamide than even in the untreated diabetic controls. With *Aloe* leaf pulp there was no change in LPO level. But *Aloe* leaf gel extract significantly decreased LPO levels as MDA (1.63±0.05 nmoles/mg protein) in comparison to diabetic controls (1.86±0.05 nmoles/mg protein). The difference between the three groups was also significant (P_{ANOVA}=0.0001; Table 1).

As shown in Table 1, a significant difference was observed in serum creatinine levels between the three treated groups and untreated type-II diabetic rats (P_{ANOVA}=0.0001). Urea levels were significantly decreased in the group given *Aloe* leaf gel extract, and sligthly although significant with glibenclamide, compared to the control group given PBS, whereas *Aloe* leaf pulp extract did not significantly change serum urea level.

Discussion

In recent years, various plant extracts have been claimed to be useful for the cure of diabetes mellitus[11-14,18-21] but few of them were tested for their effects on tissues of diabetic animals[11,13,14,22]. Acute treatment with *Aloe* leaf pulp resulted in 30 and 34% decrease in blood sugar levels of n0STZ-diabetic rats, after 2 and 3 hr of administration of the extract respectively[9], whereas chronic treatment with the same extract leads to 7% decrease in blood glucose on the 7th day of administration[25]. On the other hand, 11 and 14% reduction in blood glucose levels were observed 3 and 4 hr after administration of *Aloe* leaf gel in acute studies[9] and only 3 and 9% decrease in blood sugar was seen in chronic studies on the 7th and 15th days respectively, after oral administration of the gel extract[25], whereas 14 and 13% reduction was observed with glibenclamide in the same conditions[25].The present study was undertaken in order to examine morphologically and by three biochemical parameters, whether the *Aloe* extracts have a beneficial effect on kidney tissue of type-II diabetic rats.

Kidney is a major organ involved in diabetic complications[26]. As known, the function and structure of kidney may be affected by changes in the levels of insulin[13,23]. Diabetic kidney exhibits characteristic changes leading to renal insufficiency or complete kidney failure[13] . Histological studies indicate the characteristic changes in the kidney[11,27]. Shukla *et al*.[14] have shown that plant extracts reverse the damage in the kidneys of diabetic animals. In the present study, morphologic findings in the type-II diabetic group were in agreement with the results of other studies[12,23], although concerned with STZ-induced type-I diabetes. In the present study, the major alteration was observed especially in the proximal tubules of the kidney tissue in the diabetic animals. The rupturing of the brush border, shows that the structural integrity of the membrane was disrupted. This disruption leads to the functional disorders in membrane-dependent functions. On the other hand, an increased PAS positive reaction in the glomeruli may be associated with basement membrane thickening of glomerular capillaries.

Lipid peroxidation has been implicated in the pathogenesis of many degenerative disorders including chemically induced diabetes[28,29]. In diabetes, increase in lipid peroxidation can cause tissue damage; indeed, lipid peroxide level was increased in STZ-diabetic rat kidney[30,31]. In the present study, LPO values (Table 1) were decreased significantly in the groups given *Aloe* gel extract compared to diabetic controls whereas it was increased in the group treated with the hypoglycemic drug glibenclamide, effective in lowering blood sugar of the same rats[9]. *Aloe* gel extract was also found effective in reducing urea and creatinine levels (Table 1). *Aloe* gel contains glycoproteins, polysaccharides and other constituents (enzymes etc.) and is essentially used for the treatment of various skin conditions[32]. The effectiveness of *Aloe* may be due to its well known wound healing effect[2,3].

It was observed that the kidney tissue is partially protected by *Aloe* leaf pulp extract and glibenclamide, used as an antidiabetic drug, as judged by histology

Table 1 — Lipid peroxidation product (LPO) and mean levels of serum urea and creatinine of type-II diabetic rats given *Aloe* extracts and glibenclamide for all groups.

[Values are mean ± SD]

Group	LPO nmol MDA/mg protein*	Urea (mg %)*	Creatinine (mg %)*
I. Healthy (control)	1.17 ± 0.07	23.06 ± 0.77	0.52 ± 0.01
II. Untreated diabetic (PBS control)	1.86 ± 0.05 P_t=0.0001[a]	33.77 ± 1.63 P_t=0.0001[a]	0.80 ± 0.03 P_t=0.0001[a]
III. *Aloe* pulp	1.89 ± 0.11 P_t=0.503[b]	34.62 ± 1.29 P_t=0.166[b]	0.67 ± 0.02 P_t=0.0001[b]
IV. *Aloe* gel	1.63 ± 0.05 P_t=0.001[b]	24.03 ± 1.02 P_t=0.001[b]	0.70 ± 0.05 P_t=0.007[b]
V. Glibenclamide	2.30 ± 0.23 P_t=0.003[b]	31.25 ± 0.76 P_t=0.003[b]	0.66 ± 0.02 P_t=0.0001[b]
P_{ANOVA}**	0.0001	0.0001	0.0001

**P_{ANOVA} comparison of groups II-V.
[a]compared to group I
[b]compared to group II

and serum creatinine, but not by LPO and serum urea levels. *Aloe* leaf gel extract is more effective by histology and also in reducing tissue LPO, serum urea and creatinine levels, in comparison to *Aloe* leaf pulp extract and glibenclamide. It may be concluded that only *Aloe vera* leaf gel extract showed improvement both by histological and all the three biochemical parameters and could have a protective effect on the mild damage caused by type-II diabetes on kidney tissue.

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