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The Evaluation of Long-term Effects of Cinnamon Bark and Olive Leaf on Toxicity Induced by Streptozotocin Administration to Rats

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Abstract

The effects of cinnamon bark and olive leaf have been investigated on streptozotocin-induced tissue injury, and some biochemical and haematological changes in rats. The effects on glycaemia were also evaluated.

Long-term administration of olive leaf caused significant improvement in tissue injury induced by streptozotocin treatment; the effect of cinnamon bark was less extent. No effects on blood glucose levels were detected. However, significant decreases in some increased biochemical and haematological parameters of streptozotocin-treated rats were observed. Aspartate aminotransferase, urea and cholesterol levels were significantly decreased by treatment with both plant materials, and alanine aminotransferase by treatment with olive leaf. Cinnamon bark also caused a significant decrease in platelet counts. In addition, any visible toxicity, except decrease in body weight gain, attributable to the long-term use of plant materials was not established in normal rats.

The data indicate that long-term use of olive leaf and cinnamon bark may provide benefit against diabetic conditions. Determination of underlying mechanism(s) of beneficial effects, toxicity to other systems and clinical assessments of related plant materials are major topics requiring further studies.

Many plants and plant extracts have been described as beneficial for diabetic patients. However, several plants have been shown to exert little or no effect on glycaemic control in experimental studies, although some plants possess hypoglycaemic properties (Bailey & Day 1989; Swanston-Flatt et al 1989, 1990; Marles & Farnsworth 1995). Interestingly, some plant materials, whether they have hypoglycaemic activity or not, ameliorated other disturbances of the diabetic conditions (Bailey & Day 1989; Lermioglu et al 1997).

The leaf of *Olea europea* (olive leaf; Oleaceae) and cinnamon, which is the bark of the *Cinnamomum zeylanicum* tree (cinnamon bark; Lauraceae), are among the most popular plant materials used in traditional medicine in Turkey as well as other countries (Baytop 1963, 1984; Zarzuelo et al 1991; Fehri et al 1994). Olive leaf is generally used for its antidiabetic, antihypertensive and diuretic properties (Baytop 1984; Gonzales et al 1992; Fehri et al 1994). Cinnamon is one of the world's oldest spices. In folk medicine it is used for its carminative, stimulant and antiseptic properties. This plant material is generally prescribed of a powder or infusion (5%) and usually used combined with other medicines (Baytop 1984; Bricklin & Trott 1992). Recently it has been suggested that cinnamon has insulin potentiating activity and that it may have a role in glucose metabolism (Khan et al 1990).

Despite wide use of olive leaf and cinnamon as traditional remedies, there is not sufficient knowledge about their effects and toxicities in regard to their long-term use. From this point of view, we aimed to investigate the long-term effects of these two plant materials particularly on tissue disturbance and haematological changes developed in diabetes mellitus, in addition to their antidiabetic activity.

Administration of streptozotocin is the widely used method for the induction of experimental diabetes. Rats treated with streptozotocin display

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many of the features seen in human subjects with uncontrolled diabetes and provide valuable information about the underlying pathophysiological changes that lead to chronic diabetic complications (Tomlinson et al 1992). Therefore, the effects of plant materials on diabetic complications were investigated on streptozotocin-treated rats. For comparison and investigation of their possible toxicities, parallel studies were performed on normal rats.

Previously, we have demonstrated the long-term effect of *Oleum origani* on streptozotocin-diabetic rats (Lermioglu et al 1997). The present study was carried out in our laboratory during the same experimental period and constituted the other part of the study.

Materials and Methods

Animals

Male Swiss albino rats, 180–220 g, were housed in individual cages, at room temperature ($22 \pm 2^{\circ}C$) with a 12-h on/off light schedule. The animals were left to acclimatize for one week before the start of the experiment. They were fed with standard laboratory pellet; tap water or infusions of plant materials, either olive leaf or cinnamon bark, were freely available.

Induction of diabetes mellitus

Rats fasted for 20 h were made diabetic by a single intraperitoneal injection of $45\,mg\,kg^{-1}$ streptozotocin (Upjohn, Kalamazoo, MI; freshly prepared in pH 4·2 citrate buffer). Diabetes induction was confirmed by the presence of hyperglycaemia at three and eight days after streptozotocin injection. The blood glucose levels were estimated by use of glucose reagent strips, read by means of an Accu-check II (Boehringer-Mannheim, Germany). The minimum blood glucose level accepted for a diabetic rat was $> 250\,mg\,dL^{-1}$. Control rats were injected with citrate buffer.

Plant materials

A specialist identified the plant samples used in this study. Cinnamon bark was purchased from a retail herbalist in Izmir, Turkey. A sufficient amount of *Olea europea* leaves had been collected in October 1995 in Bornova (a town in the vicinity of Izmir, Turkey). The plant materials were administered to rats as an infusion and was supplied in lieu of drinking water; the concentration (5%; w/v) was based on the literature and preliminary experiments on the tolerance of rats (Hincer 1978; Baytop 1984). Infusions were prepared daily according to the traditional method; briefly, 5 g of each drog was added to 100 mL boiling water and filtered after 15-min infusion (Baytop 1984).

Experimental design

Eight days after injection of streptozotocin or citrate buffer, rats were divided into six groups as follows: 1, streptozotocin-treated diabetic control rats; 2, streptozotocin-untreated control rats; 3, streptozotocin-treated diabetic rats administered cinnamon bark; 4, streptozotocin-treated diabetic rats administered olive leaf; 5, streptozotocin-untreated rats administered cinnamon bark; and 6, streptozotocin-untreated rats administered olive leaf. Administration of plant materials to rats was terminated after 12 weeks.

All rats were observed daily for any visible changes in their general condition. Fluid intake was monitored daily. Body weights and fasting blood-glucose levels (following an 18-h fasting period) were determined weekly. Ketonuria and glucosuria were also tested every two weeks, using diagnostic test strips (Gluketur-Test; Boehringer-Mannheim, Germany). At the end of the experimental period, rats were anaesthetized by diethyl ether, blood samples were collected via the tail vein and animals were killed by cardiac excision. The organs were carefully dissected and relative wet organ weights were calculated as percentage of body weights. Tissue injury was evaluated by biochemical and histopathological studies.

Biochemical determinations

Blood alanine aminotransferase, aspartate aminotransferase and urea were measured in a Reflotron system by use of Reflotron reagent (Boehringer-Mannheim, Germany). Serum creatinine and total protein levels were determined by the Jaffe and biuret methods, respectively (Donald & Zimmer 1967; Peters et al 1982). Serum cholesterol and triglycerides were measured by means of commercial kits (Gokhan Laboratories, Izmir, Turkey).

Haematological determinations

Blood erythrocyte, leucocyte, platelet counts and haemoglobin content were determined by routine techniques (Daice & Lewis 1974).

Histopathological evaluation

Tissue from all groups (n = 5), including thoracic aorta, liver, and kidneys were fixed in 10% buffered formalin solution. After processing in ethanol, xylol, and paraffin, tissues were embedded in paraffin. Multiple sections 5-μm thick were prepared from each block and stained with haematoxylin, eosin and periodic acid–Schiff base for light microscopy studies. Slides were examined by an experienced pathologist unaware of the treatment.

For transmission electron microscopy, tissue samples taken from three of the five rats in each group were fixed in 2·5% glutaraldehyde for 24 h and post-fixed with 1% osmium tetroxide. The tissues were dehydrated through a graded series of alcohol solutions and samples were embedded in pure Araldite epoxy resin (CY 212). Semi-thin sections (2 μm) were cut with a glass knife on an ultra-microtome (LKB Nova, Sweden) and examined by use of a light microscope (Nicon Optiphot, Japan) after staining with methylene blue. Thin sections (60–90 nm) obtained by use of the same ultra-microtome were counter-stained with uranyl acetate and lead citrate and examined by transmission electron microscopy (Jeol JEM 1200 E, Japan).

Statistical analysis

Data were analysed by one-way analysis of variance followed by the Scheffe F-test and Mann–Whitney U-tests. Data are presented as mean ± s.d. Differences were considered significant when $P < 0{\cdot}05$.

Results

Induction of diabetes with streptozotocin was associated with the characteristic development of hyperglycaemia, glucosuria and loss of body weight. Total fluid consumption during the experimental period was significantly higher in streptozotocin-treated rats than untreated control rats (10·39 ± 1·9 and 2·60 ± 0·6 L, respectively; $P < 0{\cdot}001$). Fluid intake was not significantly altered by plant treatments in either streptozotocin-treated or untreated rats (data not shown).

There were no visible signs of toxicity as a result of administration of plant materials, except significant retardation of body-weight gain in streptozotocin-untreated rats as demonstrated below.

Blood glucose levels, and body and organ weights

Administration of cinnamon bark or olive leaf did not cause any decrease in blood glucose levels of streptozotocin-treated or untreated rats (Table 1). Glucosuria and ketonuria were persistent in all diabetic groups, although ketonuria was not observed in four of the 10 rats in the cinnamon bark-administered group and two of the 10 rats in the olive leaf-administered group (data not shown). Glucosuria and ketonuria were not observed in any of the streptozotocin-untreated groups throughout the experiment.

The body and organ weights of the groups after 12 weeks are shown in Table 2. Administration of plant materials caused no significant alterations in the body weights of streptozotocin-treated rats. However, there was a significant fall in body weights of control animals, although it did not seem as streptozotocin did alone. Treatment with plant materials did not cause any significant changes in the weights of organs from either streptozotocin-treated or -untreated rats.

Biochemical and haematological parameters

There were significant differences in most of the parameters measured in streptozotocin-treated rats compared with controls (Table 3). Plant treatments did not cause significant differences in biochemical and haematological parameters of streptozotocin-untreated rats. However, in streptozotocin-treated rats, aspartate aminotransferase, urea and cholesterol levels were significantly decreased by treatment with both plant materials, and alanine aminotransferase by treatment with olive leaf.

Table 1. Blood glucose levels (mg dL^{-1}) of all groups.

Treatment	Initial values (week 0)	Terminal values (week 12)
Streptozotocin-untreated rats + tap water	123·4 ± 12·8	112·9 ± 7·7
Streptozotocin-untreated rats + cinnamon bark	99·0 ± 7·3	99·2 ± 5·8
Streptozotocin-untreated rats + olive leaf	98·1 ± 8·4	99·7 ± 6·6
Streptozotocin-treated rats + tap water	412·9 ± 45·0	480·5 ± 30·0*
Streptozotocin-treated rats + cinnamon bark	419·7 ± 44·5	502·8 ± 16·5*
Streptozotocin-treated rats + olive leaf	412·8 ± 40·8	495·0 ± 38·2*

Values are means ± s.d (n = 15). *$P < 0{\cdot}05$ compared with respective initial levels.

Table 2. The effect of 12-weeks administration of plant materials on body, liver and kidney weight.

Treatment	Body weight (% initial)	Kidney weight (g/100 g body weight)	Liver weight (g/100 g body weight)
Streptozotocin-untreated	177·0 ± 9·7	0·61 ± 0·03	3·00 ± 0·3
Streptozotocin-untreated + cinnamon bark	138·2 ± 7·1*	0·70 ± 0·03	2·91 ± 0·1
Streptozotocin-untreated + olive leaf	130·7 ± 4·7*	0·72 ± 0·03	2·94 ± 0·1
Streptozotocin-treated	87·6 ± 2·8	1·28 ± 0·11	4·18 ± 0·1
Streptozotocin-treated + cinnamon bark	86·2 ± 7·1	1·10 ± 0·08	4·57 ± 0·4
Streptozotocin-treated + olive leaf	88·5 ± 6·5	1·30 ± 0·25	5·07 ± 0·6

All values of streptozotocin-treated rats are significantly different compared with streptozotocin-untreated control rats ($P < 0.001$). Values are means ± s.d. (n = 10–15). *$P < 0.001$ compared with streptozotocin-untreated control rats.

Table 3. The effect of plant materials on biochemical and haematological parameters.

	Streptozotocin-untreated rats			Streptozotocin-treated rats		
	Tap water	Cinnamon bark	Olive leaf	Tap water	Cinnamon bark	Olive leaf
Biochemical parameters						
Aspartate aminotransferase (units L^{-1})	86·28 ± 2·6	86·05 ± 4·0	84·43 ± 4·3	156·42 ± 8·2†††	109·40 ± 4·9***	100·88 ± 3·7***
Alanine aminotransferase (units L^{-1})	55·85 ± 3·8	55·94 ± 3·7	57·33 ± 3·5	82·76 ± 5·5†††	81·64 ± 2·3	74·22 ± 3·9*
Urea (mg dL^{-1})	34·73 ± 3·1	36·46 ± 3·1	36·34 ± 2·2	136·94 ± 6·4†††	56·40 ± 1·2***	60·08 ± 3·3***
Triglycerides (mmol L^{-1})	2·97 ± 0·3	3·05 ± 0·2	2·88 ± 0·2	3·01 ± 0·8	3·08 ± 0·8	2·82 ± 0·5
Cholesterol (mmol L^{-1})	4·19 ± 0·3	4·69 ± 0·1	4·16 ± 0·2	5·76 ± 1·8††	4·32 ± 0·3*	4·24 ± 0·2*
Total proteins (mg dL^{-1})	7·60 ± 0·5	6·48 ± 0·3	7·26 ± 0·7	8·60 ± 1·7	8·03 ± 0·9	7·50 ± 1·1
Creatinine (mg dL^{-1})	0·39 ± 0·07	0·41 ± 0·1	0·41 ± 0·06	0·42 ± 0·07	0·40 ± 0·06	0·42 ± 0·05
Haematological parameters						
Haemoglobin (g dL^{-1})	17·66 ± 0·8	18·08 ± 0·7	18·31 ± 1·1	20·84 ± 0·7†††	23·39 ± 1·5**	19·89 ± 0·6
Red blood cells ($\times 10^6$ mm^{-3})	7·32 ± 0·7	7·74 ± 2·2	7·53 ± 1·4	6·08 ± 0·3†	6·37 ± 0·4	5·62 ± 0·7
White blood cells ($\times 10^3$ mm^{-3})	10·28 ± 0·6	9·34 ± 1·3	9·10 ± 1·5	10·20 ± 0·7	8·40 ± 1·7	8·76 ± 1·9
Platelets ($\times 10^5$ mm^{-3})	3·36 ± 0·3	3·19 ± 0·3	3·21 ± 0·4	5·57 ± 0·33†††	3·22 ± 0·4***	5·78 ± 0·6

Values are means ± s.d. (for biochemistry n = 10; for haematology n = 5). †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ compared with streptozotocin-untreated control group; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with streptozotocin-treated diabetic control group.

Haematological parameters of streptozotocin-treated rats were not effected by treatment with olive leaf. However, treatment with cinnamon bark caused a significant decrease in platelet counts to comparable levels with the control group and also a slight increase in haemoglobin content.

Histopathological evaluation

Light-microscopic and transmission electron-microscopic analysis of all sections from the streptozotocin-untreated rats showed normal histology and treatment with plant materials did not cause any structural and ultrastructural changes (data not shown).

Transmission electron microscopy of diabetic aorta revealed occasional partial detachment of endothelial cells from the underlining matrix, and large areas of relatively smooth, attached endothelial cells (Figure 1A). Thin sections taken from contiguous areas showed large vacuoles and mitochondrial damage in endothelial cells. In aortas from cinnamon bark-treated rats damaged epithelial cells were prominent although some cells retained their integrity (Figure 1B). In olive leaf-treated rats, vacuoles were observed in some endothelial cells of aorta indicating minimum damage and most of the cells were normal in appearance (Figure 1C).

In the renal tubulus of diabetic rats light microscopy revealed cytoplasmic vacuolizations and hyaline arteriolosclerosis associated with erythrocyte cylinders in the tubulus lumen. Certain damaged areas in the epithelium of the medullary proximal tubule were shown by transmission electron microscopy (Figure 2A). The cytoplasm lost most of its organelles and most of the mitochondria were damaged. There was prominent clearing in the subnuclear region. In the cortical region, mild cytoplasmic oedema was observed in the epithelium of the tubule (Figure 2B) and there was reparation

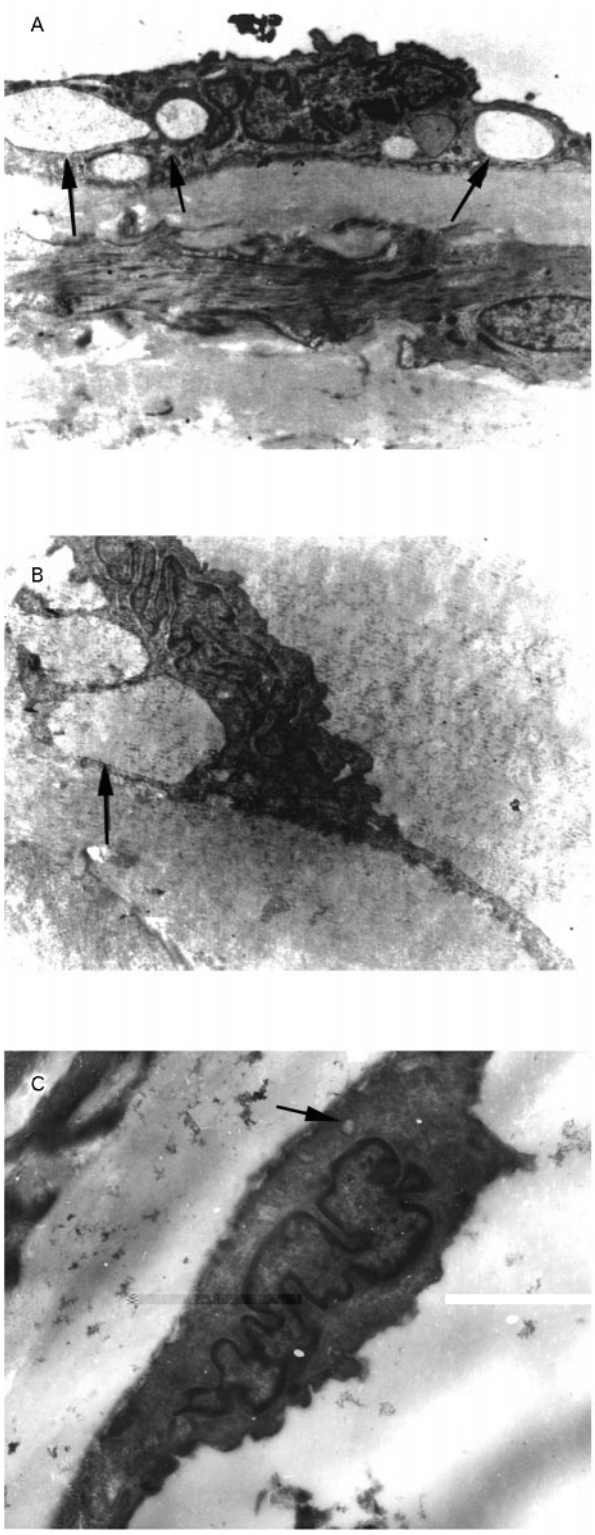

Figure 1. Transmission electron micrographs of sections of thoracic aorta: (A) damaged endothelial cells with vacuoles in streptozotocin-treated controls (× 4000); (B) damaged endothelial cells from streptozotocin-treated rats administered cinnamon bark (× 4000); (C) endothelial cells with minimum damage in streptozotocin-treated rats administered olive leaf (× 2500). Big and small arrows show vacuoles.

of the endothelium. The basal membrane of the glomerulus was thickened and the pores were absent in some areas of the endothelium. There was also fusion in foot-processes and vacuoles in the cytoplasm of endothelium owing to oedema. In cinnamon bark-treated rats, the damage in the epithelial cells of the tubules of the medullary region still remained (Figure 2C). There was granular appearance of the cytoplasm. The oedema was easily observed. The mitochondria were normal. In the cortical region, similar results as in the medulla were found.

In olive leaf-treated rats, damage to kidneys was less. In the medullary region, there was conspicuous oedema in the epithelium of the cells of the proximal tubule and less oedema was found in the mitochondria. In epithelial cells of the distal tubule less damage was found (data not shown).

In the liver of streptozotocin-treated rats, there was prominent widening in the middle layer of the trilaminar nuclear membrane of cells (Figure 3A). Large vacuoles occurred in the cytoplasm; most of these represented the widened granular endoplasmic reticulum. Mitochondria without cristae were observed rarely. The cytoplasm lost its normal characteristics in the subnuclear region. The structures in the cytoplasm had become thin and granular in appearance. In cinnamon bark-treated rats, the damage in the nucleus membrane persisted (Figure 3B). The damage in granular (rough) endoplasmic reticulum decreased but was still observed to same extent. The vacuoles were still present. The mitochondria were near to normal. In the liver of olive leaf-treated rats, the nucleus appeared normal (Figure 3C). Only a few nuclei showed separated nucleolemma. The mitochondria were also normal with their cristae. The glycogen deposits were normal. Some granular endoplasmic reticulum cisternae were enlarged.

Discussion

The principal finding in this study was the prominent improvement of tissue injury observed in streptozotocin-treated rats by administration of olive leaf, and to a lesser extent by cinnamon bark. Tissue antioxidant status is suggested to be an important factor in the development of diabetic complications (Wohaieb & Godin 1987). Olive leaf contains flavonoids such as luteolin, apigenin, oleuropein and flavonoid glycosides in addition to tannins, triterpens, alkaloids, resin, volatile oil, and organic acids (Baytop 1984; Mericli & Yilmaz 1993; Fehri et al 1994; Marles & Farnsworth 1995; Heimler et al 1996). Flavonoids have strong antioxidant and free radical scavenging properties

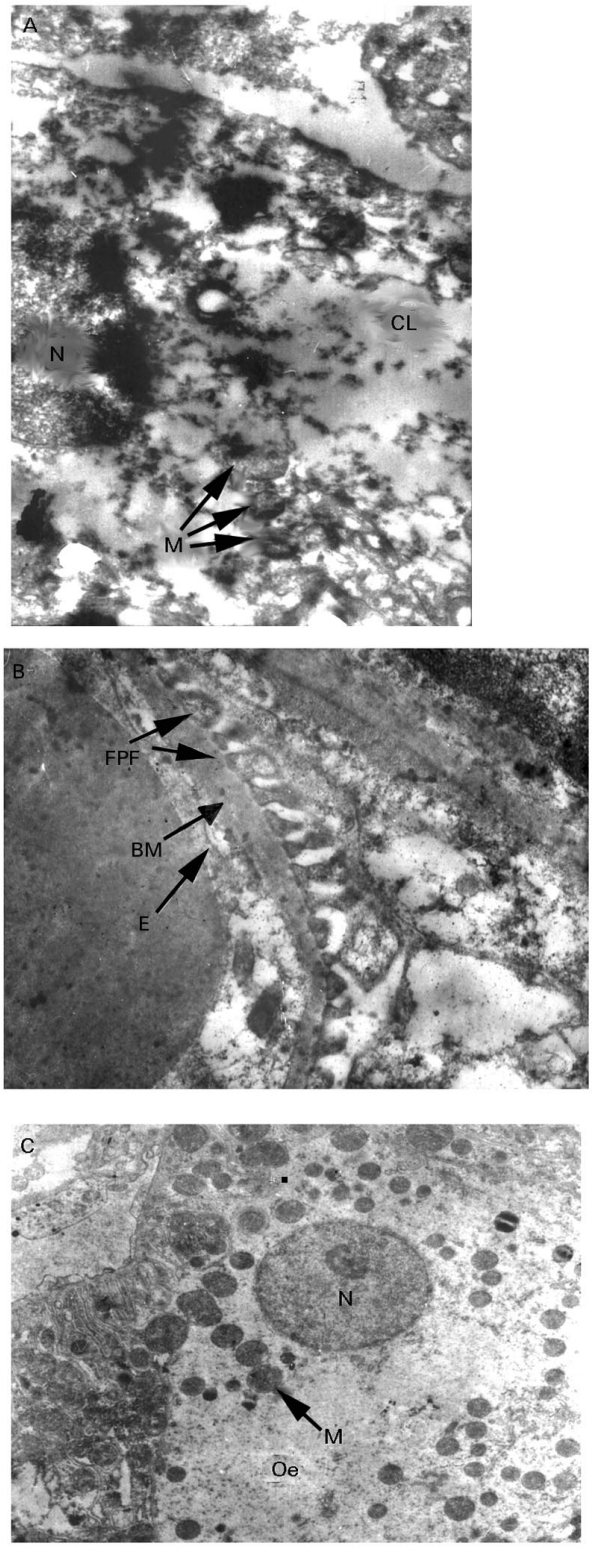

Figure 2. Transmission electron micrographs of kidney tissue sections. There was prominent damage in the medullary (A; $\times 10\,000$) and cortical (B; $\times 15\,000$) proximal tubule epithelium of streptozotocin-treated controls. C. The cytoplasma and intracytoplasmic structures in medulla of kidney from streptozotocin-treated rats administered cinnamon bark ($\times 4000$). Cl, clearing; M, mitochondria; FPF, foot process fusion; E, erythrocyte; N, nucleus; BM, basal membrane; Oe, oedema.

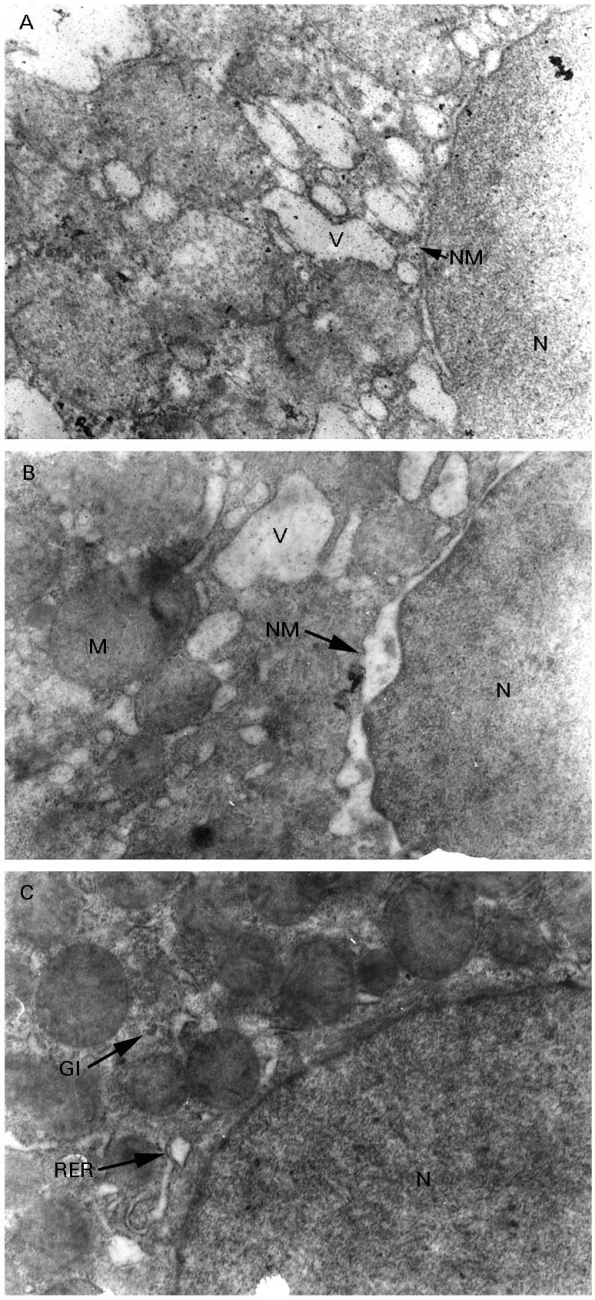

Figure 3. Transmission electron micrographs of liver tissue sections: A. Damaged hepatic cells from streptozotocin-treated diabetic controls ($\times 15\,000$); B. hepatic cells from streptozotocin-treated rats administered cinnamon bark ($\times 15\,000$); C. hepatic cells with nearly normal appearance from streptozotocin-treated rats administered olive leaf ($\times 15\,000$). N, nucleus; NM, nuclear membrane; M, mitochondria; V, vacuole; Gl, glycogen; RER, rough endoplasmic reticulum.

(Hertog et al 1993). Therefore, the prominent improvement of the tissues might be possibly related to the flavonoid content of olive leaf.

Considerable changes were also seen in other parameters measured in streptozotocin-treated rats (Table 3). Olive leaf-treatment has been shown to

reduce blood urea (Fehri et al 1994) and cholesterol levels (De Pasquale et al 1991). Oleuropein has been suggested to be responsible for the hypocholesterolaemic effect of the plant material (De Pasquale et al 1991). In contrary to our results, serum cholesterol concentrations were not decreased by administration of *Cinnamomum zeylanicum* to hypercholesterolaemic male Wistar rats, at about five times the normal human intake (Sambaiah & Srinivasan 1991). In contrary to other studies, hypoglycaemic activity of plant materials was not observed in this study (Khan et al 1990; Gonzales et al 1992; Bricklin & Trott 1992; Fehri et al 1994). The variations of flavonoid content and other compounds as well as the activity of the olive leaf have been shown (Gonzales et al 1992; Heimler et al 1996). The differences in the results might be possibly related to the quantitative variations of active ingredients as well as the design of the experiments, and the form of the plant materials used.

Plant treatments caused significant decrease in body weight gain of control rats. However, body weight loss did not seem worse in streptozotocin-treated rats as streptozotocin did alone. This discrepancy and its underlying mechanism remain to be elucidated.

This is the first study, at least to our knowledge, showing the effects of long-term administration of both olive leaf and cinnamon bark on streptozotocin-diabetic rats. Although the possibility of the direct effect of streptozotocin cannot be excluded, tissue injury and other disturbances observed after streptozotocin treatment have been suggested to occur as a result of the development of diabetes mellitus (Wohaieb & Godin 1987; Tomlinson et al 1992). Therefore, we can speculate that long-term use of plant materials tested in this study might offer some protection against diabetic conditions and may be used as adjuncts to conventional therapy. Determination of underlying mechanism(s) of the protective effect, and toxicity to other systems as well as clinical assessments of the related plant materials are major topics requiring further study and could be of interest in the future.

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References

Bailey, C. J., Day, C. (1989) Traditional treatments for diabetes. Diabetes Care 12: 553–564

Baytop, T. (1963) Turkiye 'nin Tibbi ve zehirli bitkileri. Istanbul Universitesi, Eczacilik Fakultesi Yayinlari, No: 1039. Istanbul

Baytop, T. (1984) Turkiye 'de bitkiler ile tedavi. Istanbul Universitesi, Eczacilik Fakultesi Yayinlari, No: 3255. Istanbul

Bricklin, M., Trott, M. (1992) The spice of longer life. Prevention 44: 37–38

Daice, J. V., Lewis, S. M. (1974) Basic hematological techniques I & II. In: Practical Hematology. Fourth edn. Churchill Livingstone, London

De Pasquale, R., Monfoile, M. T., Trozzi, A., Raocuia, A., Tommasini, S., Ragusa, S. (1991) Effect of leaves and shoots of *Olea europaea* L. and oleuropein on experimental hypercholesterolemia in rat. Plantes Medicinales Phytotherapie 25: 134

Donald, M., Zimmer, A. (1967) Atlas of Clinical Laboratory Procedures. Vol. 1, The Blakiston Division, McGraw-Hill Book Company, New York

Fehri, B., Aiache, J. M., Memmi, A., Korbi, S., Yacoubi, M. T., Mrad, S., Lamaison, J. L. (1994) Hypotension, hypoglycemia and hypouricemia recorded after repeated administration of aqueous leaf extract of *Olea europaea* L. J. Pharm. Belg. 49: 101–108

Gonzales, M., Zarzuelo, A., Gamez, M. J., Utrilla, M. P., Jimenez, J., Osuna, I. (1992) Hypoglycemic activity of olive leaf. Planta Med. 58: 513–515

Heimler, D., Cimato, A., Alessandri, S., Sani, G., Pieroni, A. (1996) Seasonal trend of flavonoids in olive (*Olea europaea* L.) leaves. Agr. Med. 126: 205–209

Hertog, M. G. L., Feskens, E. J. M., Hollman, P. C. H., Katan, M. B., Kromhout, D. (1993) Dietary antioxidant flavonoids and risk of coronary heart disease: the zutphen elderly study. Lancet 342: 1007–1011

Hincer, I. (1978) Seker hastaligini iyilestiren halk ilaclari. Turk. Folklor Arastirmalari. 348: 8381–8383

Khan, A., Bryden, N. A., Polansky, M. M., Anderson, R. A. (1990) Insulin potentiating factor and chromium content of selected foods and spices. Biol. Trace Elem. Res. 24: 183–188

Lermioglu, F., Bagci, S., Onderoglu, S., Ortac, R., Tugrul, L. (1997) Evaluation of the long-term effects of *Oleum origani* on the toxicity induced by administration of streptozotocin in rats. J. Pharm. Pharmacol. 49: 1157–1161

Marles, R. J., Farnsworth, N. R. (1995) Antidiabetic plants and their active constituents. Phytomedicine 2: 137–189

Mericli, F., Yilmaz, F. (1993) Halk arasinda tansiyon dusurucu olarak kullanilan zeytin (*Olea europaea* L.) yapraklari uzerinde bir calisma. 10. Bitkisel Ilac hammaddeleri toplantisi bildiri özetleri, PB-40, Istanbul Universitesi, Istanbul

Peters, T., Biamonte, G. T., Doumas, B. T. (1982) Protein (total protein) in serum, urine and cerebrospinal fluid; albumin in serum. In: Faulkner, W. R., Meites, S. (eds) Selected Methods of Clinical Chemistry. Vol. 9, American Association for Clinical Chemistry, Washington, D. C.

Sambaiah, K., Srinivasan, K. (1991) Effect of cumin, cinnamon, ginger, mustard and tamarind in induced hypercholesterolaemic rats. Nahrung 35: 47–51

Swanston-Flatt, S. K., Day, C., Bailey, C. J., Flatt, P. R. (1989) Evaluation of traditional plant treatments for diabetes studies in streptozotocin diabetic mice. Acta Diabetol. Lat. 26: 51–55

Swanston-Flatt, S. K., Day, C., Bailey, C. J., Flatt, P. R. (1990) Traditional plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. Diabetologia 33: 462–464

Tomlinson, K. C., Gardiner, S. M., Hebden, R. A., Bennett, T. (1992) Functional consequences of streptozotocin-induced diabetes mellitus, with particular reference to the cardiovascular system. Pharmacol. Rev. 44: 103–150

Wohaieb, S. A., Godin, D. Y. (1987) Alterations in free-radical tissue-defense mechanisms in streptozotocin-induced diabetes in rat. Effects of insulin treatment. Diabetes 36: 1014–1018

Zarzuelo, A., Duarte, J., Jimenez, J., Gonzales, M., Utrilla, M. P. (1991) Vasodilator effect of olive leaf. Planta Med. 57: 417–419