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# Evaluation of the Long-term Effects of Oleum Origani on the Toxicity Induced by Administration of Streptozotocin in Rats

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## Abstract

Oleum origani, the essential oil of *Origanum onites* L., is a traditional plant material used in Turkey for the treatment of several diseases, including diabetes mellitus. This study has evaluated the effect of oleum origani on streptozotocin-induced tissue injury and haematological changes. The effect of oleum origani on glycaemia was also studied.

Long-term administration of oleum origani resulted in significant improvement of tissue injury induced by streptozotocin treatment. No effect on blood glucose levels was detected. In addition, any visible toxicity or disturbance of haematological parameters and tissue structure attributable to the long-term use of oleum origani were not established in normal rats.

The data indicate that long-term use of oleum origani might be effective in preventing or at least in retarding the development of some complications of diabetes mellitus. Further investigation is required to determine the underlying mechanism(s) of the protective effect against tissue injury induced by streptozotocin-treatment of rats.

Plant materials based on folk medicine are being used in increasing numbers for the treatment of diabetes mellitus throughout the world (Hincer 1978; Bailey & Day 1989; Swanston-Flatt et al 1990). Certain plants, whether or not they have hypoglycaemic activity, have been shown to ameliorate some complications of diabetes mellitus (Bailey & Day 1989). Essential oils of *Origanum* species are used in Turkey as herbal medicine in the therapy of some diseases including diabetes mellitus (Baytop 1963, 1984; Hincer 1978; Baser et al 1986; Cingi et al 1991). Although their analgesic, antispasmodic, antibacterial and antifungal activity has been shown in several studies (VanDen Broucke & Lemli 1980, 1982; Sivropoulou et al 1996) we could find no reference in the literature to research into their long-term effects.

In this study, we have evaluated the long-term effects of oleum origani, the essential oil of *Origanum onites* L. (= *Origanum smyrneum* L.; Labiatae), particularly on tissue disturbance and haematological changes developed in diabetes mellitus, in addition to its antidiabetic activity. For this purpose, we used streptozotocin, the most popular diabetogenic agent, which causes several of the abnormalities seen in man with uncontrolled diabetes mellitus (Tomlinson et al 1992). For comparison and investigation of the possible toxicity of oleum origani, parallel studies were performed on normal rats.

## Materials and Methods

### *Animals*

Experiments were performed on male Swiss albino rats, 180–220 g, which were housed in individual cages, at room temperature (22 ± 2°C) with a 12-h on/12-h off light schedule, and left for one week for acclimization before the start of the experiment. They were fed with standard laboratory pellet; tap water with or without oleum origani was freely available.

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### *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). Three and eight days after streptozotocin injection, tail vein blood glucose levels were estimated by use of glucose reagent strips, read by means of an Accucheck-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.

### *Preparation of oleum origani*

Oleum origani was prepared by vapour distillation of *Origanum onites* L. collected from Izmir. Plant samples were identified as *Origanum onites* by a specialist. A few drops of the distillate are taken daily in drinking water by diabetic patients. Because of the bitter taste, the concentration of oleum origani was adjusted by preliminary experiments to determine the maximum amount tolerated by rats; solution (12 mg per 100 dL, w/v) was then prepared daily by dissolution in drinking water by rapid stirring. The oleum origani container was protected from light by wrapping in aluminium foil.

### *Experimental design*

Eight days after injection of streptozotocin or citrate buffer, rats were divided into four groups: streptozotocin-treated diabetic control rats (D); streptozotocin-treated rats administered oleum origani (D + OO); streptozotocin-untreated control rats (C); and streptozotocin-untreated rats administered oleum origani (C + OO). Administration of oleum origani to rats was terminated after 12 weeks.

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

| | Initial values (week 0) | Terminal values (week 12) |
|---|---|---|
| Streptozotocin-untreated rats administered tap water | 123·4 ± 12·8 | 112·9 ± 7·7 |
| Streptozotocin-untreated rats administered oleum origani | 100·3 ± 12·5 | 98·6 ± 5·8 |
| Streptozotocin-treated rats administered tap water | 412·9 ± 45·0 | 480·5 ± 30·0* |
| Streptozotocin-treated rats administered oleum origani | 424·3 ± 42·5 | 477·5 ± 31·6* |

Values are means ± s.d. (n = 15). *$P < 0.05$, significantly different compared with respective initial levels.

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 (after an 18-h period of fasting) were determined weekly. Ketonuria and glucosuria were also tested every 2 weeks by use of diagnostic test strips (Gluketur-Test; Boehringer-Mannheim, Germany). At the end of the experimental period, the rats were anaesthetized with diethyl ether, blood samples were collected from the tail vein, and animals were killed by cardiac excision. The organs were carefully dissected and relative wet organ weights were calculated as percentages of body weight. 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*

Tissues from all groups (n = 5 each), 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 for light microscopy studies. Slides were examined by an experienced pathologist unaware of the treatment.

For transmission electron microscopy, tissue samples (n = 3 each) 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 (CY212). 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 (Nikon 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 $P < 0.05$.

## Results

Other than significant retardation of body-weight gain in group C + OO ($P < 0.001$ compared with C) there were no visible signs of toxicity as a result of administration of oleum origani.

*Blood glucose levels, body and organ weights*

Administration of oleum origani did not cause any decrease in blood glucose levels in streptozotocin-treated or -untreated rats (Table 1). Glucosuria and ketonuria were persistent in groups D and D + OO, although ketonuria was not observed in 3 rats out of 10 in this group (data not shown). Glucosuria and ketonuria were not observed in groups C and C + OO throughout the experiment.

The body and organ weights of the groups after 12 weeks are shown in Table 2. Administration of oleum origani caused no significant alterations in the body weights of streptozotocin-treated rats or in the weights of organs from streptozotocin-treated or -untreated rats.

*Biochemical and haematological parameters*

Values obtained for all the parameters measured are listed in

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

| Experiment | Body weight (% initial) | Kidney weight (g per 100 g) | Liver weight (g per 100 g) |
|---|---|---|---|
| Control | 177 ± 10 | 0·61 ± 0·03 | 3·0 ± 0·3 |
| Control + oleum origani | 144 ± 15*† | 0·71 ± 0·05† | 2·9 ± 0·2† |
| Streptozotocin-treated | 88 ± 3* | 1·28 ± 0·11* | 4·2 ± 0·1* |
| Streptozotocin-treated + oleum origani | 85 ± 4* | 1·31 ± 0·08* | 4·0 ± 0·05* |

Controls were streptozotocin-untreated rats administered tap water. Values are means ± s.d. (n = 10–15). *$P < 0.001$ compared with control, †$P < 0.001$ compared with rats treated with streptozotocin.

Table 3. The effect of oleum origani on biochemical and haematological parameters.

| | Streptozotocin-untreated rats administered tap water (C) | Streptozotocin-untreated rats administered oleum origani (C + OO) | Streptozotocin-treated rats administered tap water (D) | Streptozotocin-treated rats administered oleum origani (D + OO) |
|---|---|---|---|---|
| Biochemical parameters | | | | |
| Aspartate aminotransferase (units $L^{-1}$)§ | 86·28 ± 2·6 | 84·70 ± 3·4 | 156·42 ± 8·2*** | 125·22 ± 4·3†††,*** |
| Alanine aminotransferase (units $L^{-1}$)§ | 55·85 ± 3·8 | 54·99 ± 2·9 | 82·76 ± 5·5*** | 82·09 ± 3·3*** |
| Urea (mg $dL^{-1}$)§ | 34·73 ± 3·1 | 33·30 ± 2·4 | 136·94 ± 6·4*** | 63·23 ± 3·1†††,*** |
| Triglycerides (mmol $L^{-1}$) | 2·97 ± 0·3 | 2·99 ± 0·1 | 3·01 ± 0·8 | 2·99 ± 0·1 |
| Cholesterol (mmol $L^{-1}$) | 4·19 ± 0·3 | 4·35 ± 0·3 | 5·76 ± 1·8** | 3·75 ± 0·2†† |
| Total proteins (mg $dL^{-1}$) | 7·6 ± 0·5 | 6·70 ± 0·7† | 8·6 ± 1·7 | 7·8 ± 0·5 |
| Creatinine (mg $dL^{-1}$) | 0·39 ± 0·07 | 0·40 ± 0·05 | 0·42 ± 0·07 | 0·38 ± 0·09 |
| Haematological parameters | | | | |
| Haemoglobin (g $dL^{-1}$) | 17·66 ± 0·8 | 18·97 ± 1·1 | 20·84 ± 0·7*** | 22·67 ± 3·2*** |
| Red blood cells ($\times 10^6$ $mm^{-3}$) | 7·32 ± 0·7 | 7·17 ± 0·6 | 6·08 ± 0·3* | 6·10 ± 0·4* |
| White blood cells ($\times 10^3$ $mm^{-3}$) | 10·28 ± 0·6 | 10·04 ± 1·6 | 10·20 ± 0·7 | 10·80 ± 0·9 |
| Platelets ($\times 10^5$ $mm^{-3}$) | 3·36 ± 0·3 | 3·46 ± 0·2 | 5·57 ± 0·3*** | 6·08 ± 0·6*** |

Values are means ± s.d. (for biochemistry n = 10; for haematology n = 5). *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$, significantly different from group C; †$P < 0·05$, ††$P < 0·01$, †††$P < 0·001$, significantly different from group D; §$P < 0·001$, results for group C + OO were significantly different from results for D + OO.

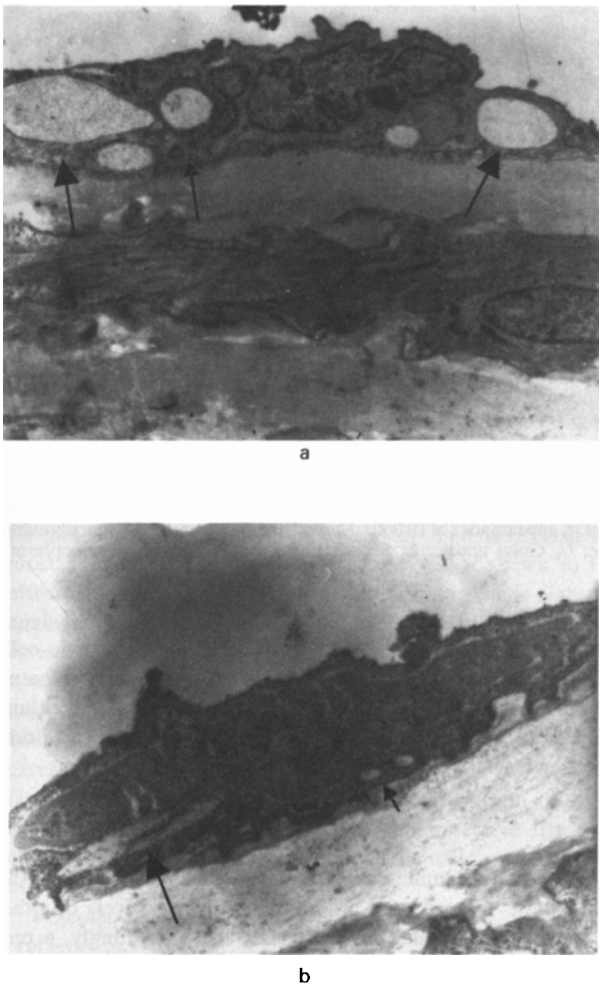

FIG. 1. Transmission electron micrographs of sections of thoracic aorta: (a) damaged endothelial cells with vacuoles in streptozotocin-treated controls (×4000); (b) fewer damaged endothelial cells in streptozotocin-treated rats administered oleum origani (×12 000). Large and small arrows show vacuoles.

Table 3. Administration of oleum origani resulted in large reductions in aspartate aminotransferase, urea, and cholesterol levels of the rats in group D + OO. There were no significant differences between the values of the biochemical and haematological parameters measured for rats in groups C and C + OO.

*Histopathological results*

Light-microscopic and transmission-electron-microscopic analysis of all sections from the rats in group C showed normal histology. Treatment with oleum origani did not cause any structural and ultra-structural changes in tissues of normal rats (data not shown).

Transmission electron microscopy of diabetic aorta revealed occasional partial detachment of endothelial cells from the underlining matrix, and there were large areas of relatively smooth, attached endothelial cells (Fig. 1a). Thin sections taken from contiguous areas showed large vacuoles and mitochondrial damage in endothelial cells. In aortas from rats in group D + OO the extent of damaged endothelial cells was less, and most retained their integrity (Fig. 1b).

In the renal tubulus of group D 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 (Fig. 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 (Fig. 2b) and there was reparation 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 the rats of group D + OO, damage to the kidneys was minimal. In the medullary region the cytoplasm of the proximal tubule

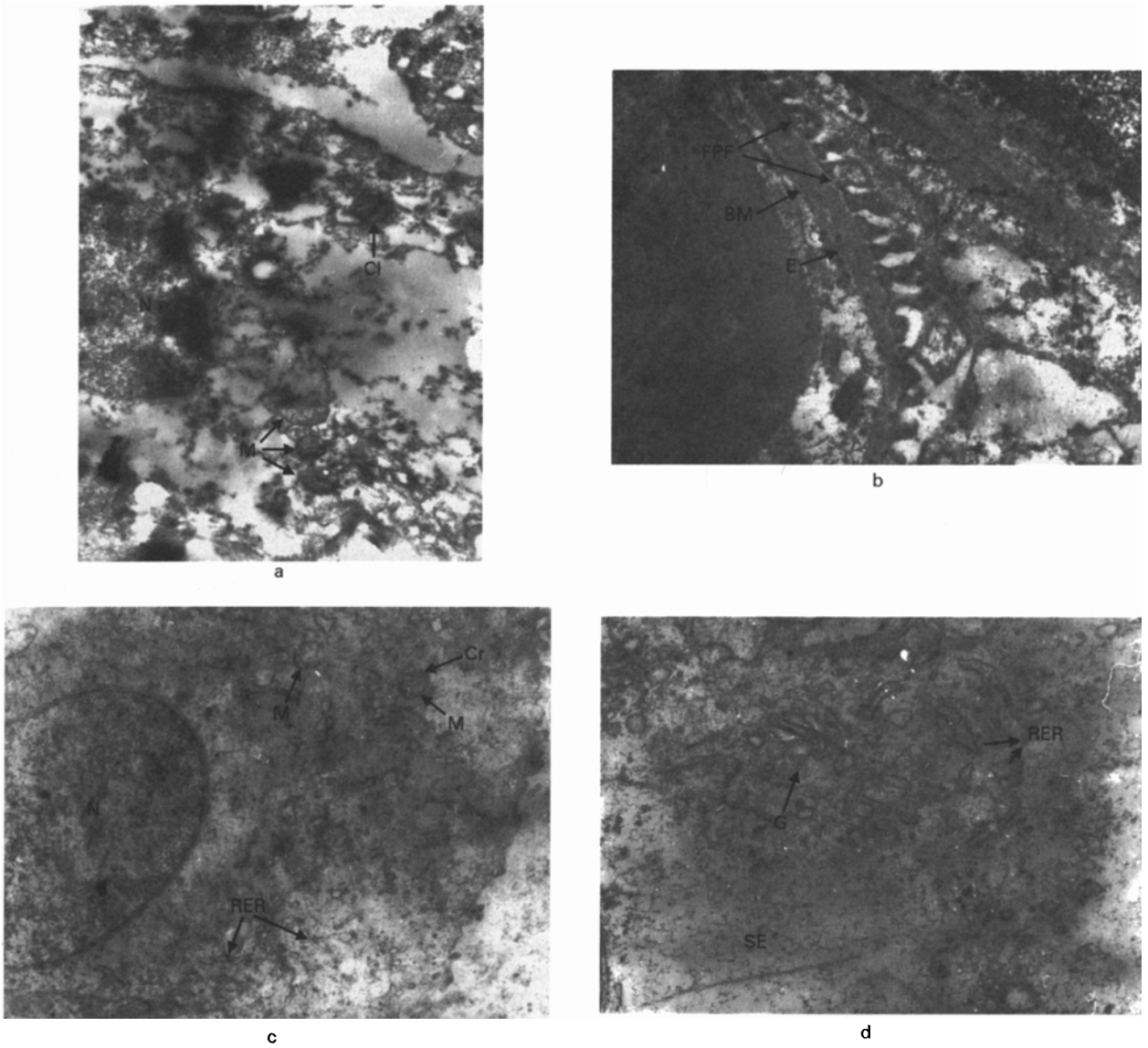


FIG. 2. Transmission electron micrographs of kidney tissue sections. There was prominent damage in the medullary (a, × 10 000) and cortical (b, × 15 000) proximal tubule epithelium of streptozotocin-treated controls ( × 10 000). The damage was minimal in streptozotocin-treated rats administered oleum origani; c and d show medullary epithelium mostly normal in appearance ( × 10 000). N, nucleus; Cl, clearing; M, mitochondria; FPF, foot process fusion; BM, basal membrane; E, erythrocyte; Cr, cristae; G, Golgi apparatus; SE, slight oedema; RER, rough endoplasmic reticulum.

epithelium was more homogenous than for the rats of group D (Fig. 2c). The mitochondria were oedematous in some areas, but generally the cristae were seen. There was slight oedema in the cytoplasm of some cells, but it was not prominent. The organelles, e.g. Golgi apparatus, changed to normal (Fig. 2d). In the cortex the epithelium of the proximal tubule, the basal membrane and the organelles had normal appearance with no damage. There was no clearing in the subnuclear region (data not shown).

In the liver from group D rats there was prominent widening in the middle layer of the trilaminar nuclear membrane of cells (Fig. 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 characteristics in the subnuclear region. The structures in the cytoplasm had become thin and granular in appearance. In group D + OO rats most of the hepatic cells showed a minimum of damage (Fig. 3b). The nuclear membrane was almost normal. The granular endoplasmic reticulum, the mitochondria and the glycogen in the cytoplasm had normal characteristics. Rare vacuoles were observed.

## Discussion

In this study, administration of oleum origani for 12 weeks did not cause any reduction in the blood glucose levels of either streptozotocin-treated or -untreated rats. Interestingly, a prominent improvement was observed in tissue injury induced by streptozotocin treatment. In addition, toxic effects, except for retardation of body weight gain, were not observed in streptozotocin-untreated normal rats.

It has been demonstrated that tissue antioxidant status is an

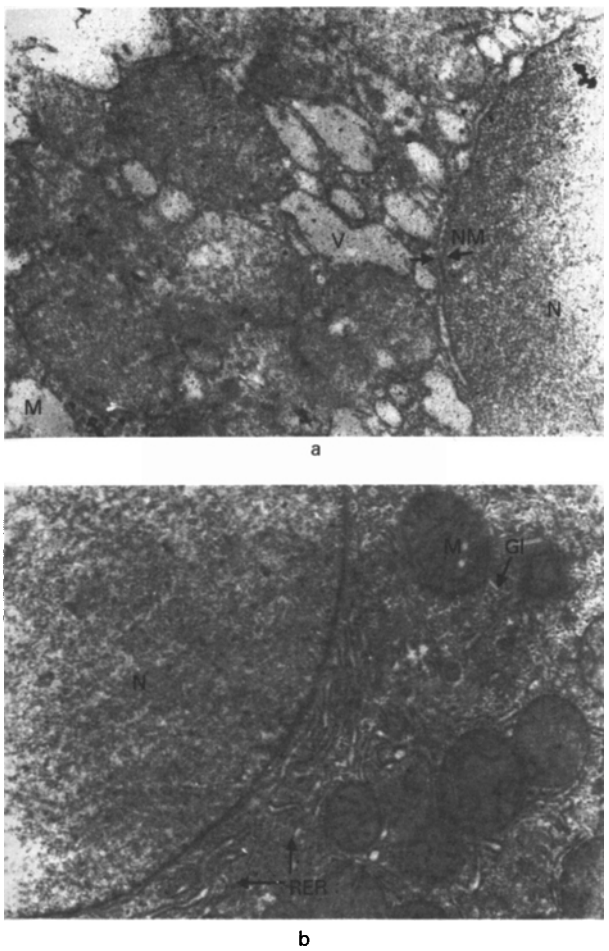


FIG. 3. Transmission electron micrographs of liver tissue sections: (a) damaged hepatic cells from streptozotocin-treated diabetic controls (×15 000); (b) hepatic cells with minimal damage from streptozotocin-treated rats administered oleum origani (×15 000). N, nucleus; NM, nuclear membrane; M, mitochondria; V, vacuole; RER, rough endoplasmic reticulum; Gl, glycogen.

important factor in the development of diabetic complications (Wohaieb & Godin 1987). Inhibition of lipid peroxidation, prostanoid synthesis and platelet activity were shown to be effective in the prevention of tissue injury and diabetic angiopathy (Sensaki et al 1985; Somova et al 1989). The major essential oil components carvacrol and thymol are antioxidant compounds; it has been suggested that these are responsible for most of the effects of essential oils (VanDen Broucke & Lemli 1982; Aeschbach et al 1994; Sivropoulou et al 1996). Because carvacrol, the main component of oleum origani obtained from *Origanum onites*, is present at levels ranging from 66·5 to 80·4% of total oil (Baser et al 1993), the protective effect of oleum origani observed in this study might be related to its carvacrol and thymol content.

Vascular and renal complications are among the major problems in long-term diabetes mellitus (Cotran et al 1989). Our results suggest that long-term use of this plant material might be useful in preventing, or at least retarding, structural tissue disturbance developed in diabetes mellitus. Clinical assessment of oleum origani, determination of the underlying mechanism(s) of the protective effect, and investigation of toxicity to other systems are interesting topics requiring further study.

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