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The Chronic Effects of *Teucrium polium* on Some Blood Parameters and Histopathology of Liver and Kidney in the Rat

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Abstract: The effects of chronic treatment with small sublethal doses (20 mg/kg and 50 mg/kg) of *Teucrium polium* ethanolic extract were investigated on the hematological and biochemical composition of the blood, histopathology of the liver and kidney, and fertility in the rat. Hematological and biochemical parameters of the blood, as well as sperm count, morphology and motility were normal after 6 weeks of herbal treatment. However, blood urea ($P < 0.05$) and cholesterol ($P < 0.005$) were significantly increased, with marked cytoplasmic vacuolation of the liver and kidney cells after chronic treatment with 50 mg/kg of the plant. These results demonstrate some histopathological effects of *T. polium* on the liver and kidney under long-term administration conditions.

Key Words: *Teucrium polium*, hematology, liver, urea, sperm count

Introduction

The traditional medicinal plant *Teucrium polium* L. (Labiatae) is a perennial shrub widely distributed in the hills and deserts of Mediterranean countries [1-7]. *T. polium* and related species (*T. oliverianum* and *T. mascatense*) are commonly used in folk medicine for various types of pathological conditions as antidiabetic [8-9], anti-inflammatory [10-11], anti-ulcer [12], hypotensive [13], antispasmodic [14-15], anorexic and antipyretic [10,16-17] agents. Phytochemical investigations have shown that the herb contains various compounds such as flavonoids, iridoids and crisiol [10,15,18-22]. However, many herbal medicinal plants including *T. polium* were found to induce fatal hepatic effects [23] and severe acute liver failure with marked hematological and biochemical alterations [24-26] after prolonged administration. The purpose of the present study was to evaluate the medicinal significance of chronic treatment with *T. polium* on the hematological and biochemical composition of the blood, histopathology of the liver and kidney, and reproductive activity in the rat.

Materials and Methods

Preparation of *T. polium* extract

The green aerial parts of *T. polium* were collected locally during May 2000. The plant material was dried at room temperature, ground to a fine powder and

extracted with 95% ethanol under low pressure using a rotary vacuum evaporator. The extract was stored at -20°C .

Experimental animals and treatment

Experiments were performed on adult male albino rats, weighing 230-250g. The extract of the plant was freshly dissolved in olive oil (vehicle) at the corresponding concentration immediately before administration.

Determination of lethal dose of *T. polium*

The lethal dose of *T. polium* ethanolic extract was determined using 6 groups of rats ($n = 8$) injected (i.p.) with 50 mg/kg, 100 mg/kg, 300 mg/kg, 500 mg/kg, 700 mg/kg and 900 mg/kg respectively. A seventh group of rats ($n = 8$), used as the control, was injected with olive oil. After 24 hours, LD_{50} and LD_{100} were calculated [27].

Hematological and biochemical analyses

The hematological and biochemical composition of the blood was measured using four groups of rats ($n = 7$). The first group was the non-treated control, the second group was treated with olive oil, and the third and the fourth groups were treated with 20 mg/kg and 50 mg/kg of *T. polium* respectively. Treatments were made using alternate daily injections (i.p., volume 0.5 ml) for six weeks. Under ether anesthesia, blood samples (2.0-4.0 ml) were withdrawn by cardiac puncture and immediately processed for hematological (Micros RAB 025A automatic

hematology analyzer, France) and biochemical (Spectrophotometer, Clima plus, Spain) analyses in Karak Government Hospital, Karak-Jordan. The hematological parameters were measured including hematocrit, red blood cells count; total, differential and percentage white blood cells count (lymphocytes, monocytes, granulocytes); platelets, mean cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, percentage of red cell distribution width, mean platelet volume, and percentage of platelet distribution width. Biochemical parameters were measured including glucose, urea, uric acid, cholesterol, triglycerides, total protein, Na^+ , K^+ and Ca^{2+} .

Seminal fluid analysis

For seminal fluid examination, the right and left epididymos were dissected from the caput to the boundary between the cauda and the first part of vas deferens. For sperm count, the right epididymis was homogenized in 0.5 ml normal saline containing 0.01% Triton X-100, diluted with 1.5 ml of the same solution and examined under the microscope using a Neubauer hemocytometer. For sperm morphology and activity, the left epididymis was placed in buffered normal saline, cut into pieces and squeezed out to remove sperm. Epididymal fragments were removed, the solution diluted (1:10) in the same buffer, mixed thoroughly and examined under microscope. The percentage of motile, sluggish and immotile sperm was calculated [28-30]. The coverslip was removed, the sperm suspension was dried in air, stained with quick panoptic and examined under the microscope. The morphological appearance of abnormal sperm shapes [31] and their percentage [31] were calculated.

Light and electron microscopic preparations

For light microscopic preparation, small tissue samples from the liver and kidney were dissected, fixed in 37% formaline for 3 hours, dehydrated in a graded ethanol series and xylene, infiltrated and embedded in paraffin. Thin sections (10 μm) were cut and stained with hematoxylin and eosin. For electron microscopic preparation, small blocks (about 1.0 mm^3) of tissue samples from the liver and kidney were dissected, fixed for 2 hours in 2.5% gluteraldehyde in 0.1M sodium cacodylate buffer (pH 7.4) at room temperature, postfixed in 1.0% osmium tetroxide in the same buffer for one hour, dehydrated in ascending concentrations of acetone, and embedded in Spurr's medium. Ultrathin

sections (90 nm) were stained with 2.5% uranyl acetate and lead citrate and examined in a Zeiss electron microscope at 60 kV. Data are expressed as means \pm S.E.M., and Student's *t*-test was used at the level of significance of $P < 0.05$.

Results

Lethal dose of *T. polium*

The LD_{50} and LD_{100} of the ethanolic extract of *T. polium* were about 262.0 mg/kg and 700.0 mg/kg respectively. No deaths were recorded among the olive oil treated group.

Hematological and biochemical composition of the rat blood

Table 1 shows the various hematological parameters of the blood in the four groups of rats. There were no significant differences in hematological compositions of the blood parameters between non-treated, olive oil treated, 20 mg/kg and 50 mg/kg *T. polium* treated groups ($P > 0.1$ to just 0.05). Similarly, Table 2 shows the various biochemical parameters of the blood in the four groups of rats. However, blood urea ($P < 0.05$) and cholesterol ($P < 0.005$) were significantly increased after chronic treatment with 50 mg/kg *T. polium* compared to that in other groups.

Effects of *T. polium* on the liver and kidney

Light microscopic observations revealed that chronic treatment with 50 mg/kg *T. polium* induced marked cytoplasmic vacuolation of liver and kidney tubular cells. The ultrastructural appearance also revealed marked cytoplasmic vacuolation of liver cells (Fig. 1) and kidney tubular cells (Fig. 2) after chronic treatment with 50 mg/kg of the herb.

Sperm count, morphology and activity

Seminal fluid examination of the rats showed that the count, morphological appearance and motility of sperm were normal after chronic treatment with 20 mg/kg or 50 mg/kg of *T. polium*. Sperm count in the non-treated group (290.0 ± 22.0 millions/ml) and in the olive oil treated group (280.0 ± 17.0 millions/ml) were not significantly different from that in 20 mg/kg treated group (290.0 ± 12.0 millions/ml) or 50 mg/kg treated group (270.0 ± 16.0 millions/ml), $P > 0.1$. Moreover, the percentage of sperm with normal morphological

Hematological parameters	Non-treated	Olive oil	20 mg/kg <i>T. polium</i>	50 mg/kg <i>T. polium</i>
RBCs X10 ⁶ /mm ³	4.85±0.43	5.27±0.93	7.70±0.47	6.23±0.82
WBCs X10 ³ /mm ³	10.31±0.57	9.55±1.25	12.19±1.74	15.35±3.50
Platel. X10 ³ /mm ³	644.8±69.82	690.0±71.35	520.3±94.82	479.0±146.11
HCT %	43.08±2.53	49.13±3.19	45.40±1.40	37.52±2.68
Lym X10 ³ /mm ³	5.78±0.49	5.80±0.72	6.36±0.69	5.92±0.71
Mon X10 ³ /mm ³	1.33±0.26	1.08±0.11	2.48±0.85	3.70±1.59
Gran X10 ³ /mm ³	3.30±0.98	4.0±0.97	4.66±0.61	4.94±1.73
% Lymphocytes	56.78±5.40	54.95±4.14	48.16±2.25	48.82±7.89
% Monocytes	13.05±2.02	11.20±1.77	16.80±4.07	21.24±5.09
% Granulocytes	30.18±5.36	33.85±4.69	35.04±4.06	39.94±12.72
MCV (mm ³)	60.82±4.67	57.75±2.95	56.51±0.91	56.28±1.54
MCH (pg)	18.74±0.49	18.50±0.58	18.29±0.39	19.62±0.69
MCHC (g/dl)	31.28±1.45	32.23±1.09	32.41±0.67	34.78±0.68
RDW %	13.0±0.62	13.28±1.27	12.96±0.41	14.20±0.59
MPV (mm ³)	6.88±0.36	6.85±0.29	7.15±0.19	8.27±0.91
PDW %	10.10±0.54	10.23±1.48	12.35±1.54	6.70±3.08

Table 1. Hematological analyses of rat blood after chronic treatment with *T. polium* ethanolic extract. Data are expressed as means ± S.E.M. (n = 7). RBCs (red blood cells count), WBCs (total white blood cells count), Platel. (platelets count), HCT (hematocrit), Lym (lymphocytes count), Mon (monocytes count), Gran (granulocytes count), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), RDW (red cell distribution width), MPV (mean platelet volume) and PDW (platelet distribution width).

Hematological parameters	Non-treated	Olive oil	20 mg/kg <i>T. polium</i>	50 mg/kg <i>T. polium</i>
Glucose (mmol/l)	6.98±0.34	6.20±0.22	7.26±0.54	7.93±0.61
Urea (mmol/l)	6.10±0.31	6.68±0.38	8.86±1.35	14.28±3.33*
Uric acid (µmol/l)	52.73±15.43	41.22±20.95	30.0±9.29	48.78±10.51
Cholesterol (mmol/l)	1.20±0.11	1.50±0.09	1.70±0.11	2.17±0.22**
Triglycerides mmol/l	1.14±0.36	1.40±0.36	1.36±0.24	1.14±0.23
Total protein (g/l)	62.0±11.0	73.33±3.28	72.0±2.94	70.50±2.47
Na ⁺ (meq/l)	150.5±2.5	155.67±3.71	149.5±0.96	150.25±1.03
K ⁺ (meq/l)	5.15±0.05	5.33±0.15	5.20±0.14	4.38±0.20
Ca ²⁺ (meq/l)	1.90±0.50	2.33±0.03	2.53±0.03	2.25±0.23

Table 2. Biochemical analyses of rat blood after chronic treatment with *T. polium* ethanolic extract. Data are expressed as means ± S.E.M. (n = 7). *P < 0.05, **P < 0.005.

appearance (90 - 95%) and active motile (80% - 90%) were found within the same value in all the four groups of rats.

Discussion

In a previous study we demonstrated the immediate and long-term inhibitory effects of the medical plant *T. polium* on the conduction property of sensory nerve fibers, and its anti-inflammatory activity in rat skin [32]. The present study investigated the effects of long-term treatment with small sublethal doses of the herb (which are comparable to that used in folk medicine) on the

hematological and biochemical composition of the blood, histological appearance of the liver and kidney, and reproductive activity in the rat. The results showed that hematological and biochemical parameters, as well as fertility were normal after chronic treatment with the plant. However, the liver and kidney were markedly damaged, and consequently an increase in blood urea and cholesterol were observed under the conditions of prolonged herb administration. These findings are supported by previous case reports that described severe acute liver failure in humans after chronic administration of *T. polium* [24] and its identical genus *T. chamaedrys* [25-26]. The present findings showed that blood glucose

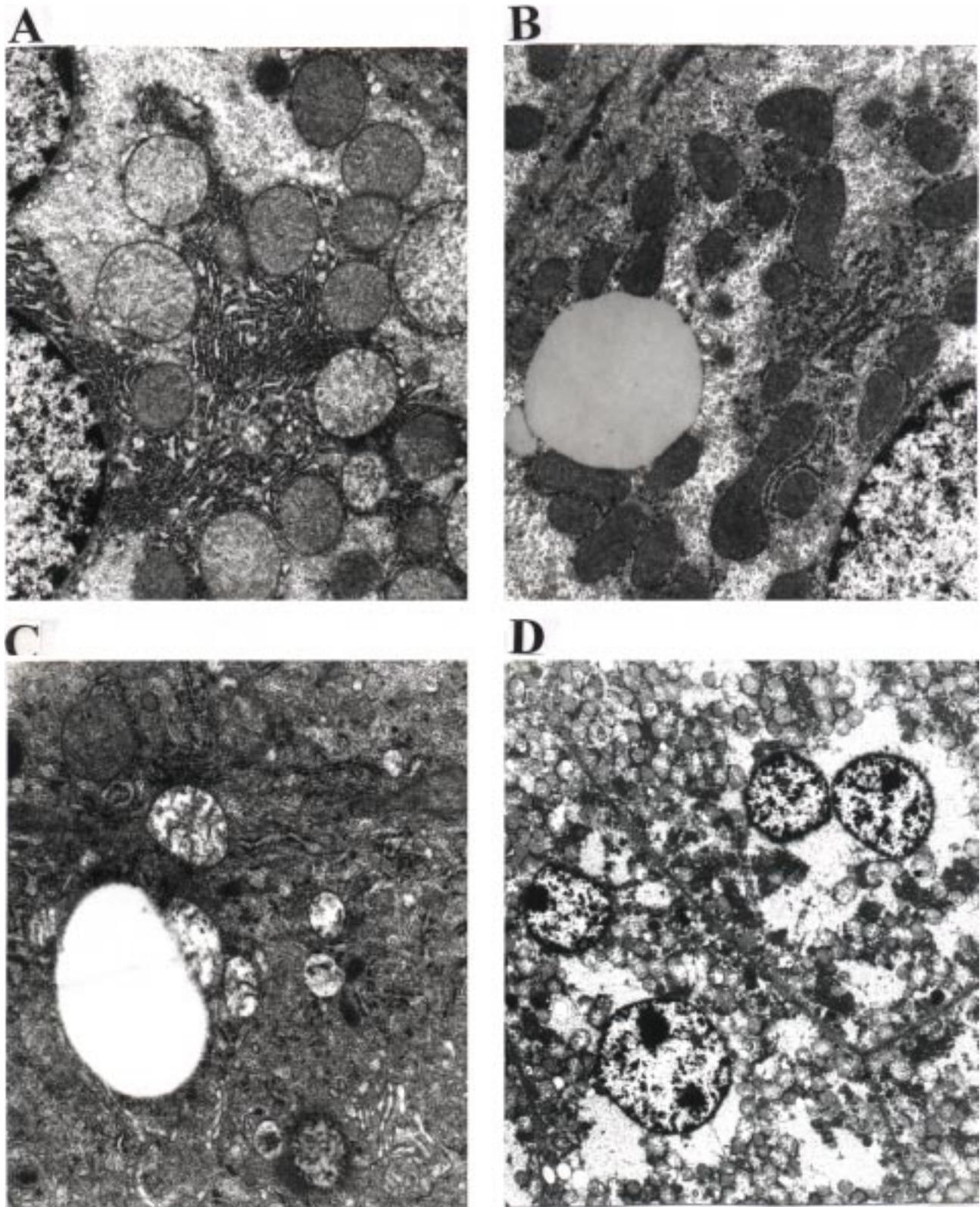


Fig. 1. Electron micrographs of the rat liver in non-treated (A), olive oil (B), and after chronic treatment with 20 mg/kg (C) and 50 mg/kg (D) of *T. polium* ethanolic extract. Uranyl acetate and lead citrate. X12500 (A and B), X20000 (C) and X3125 (D)

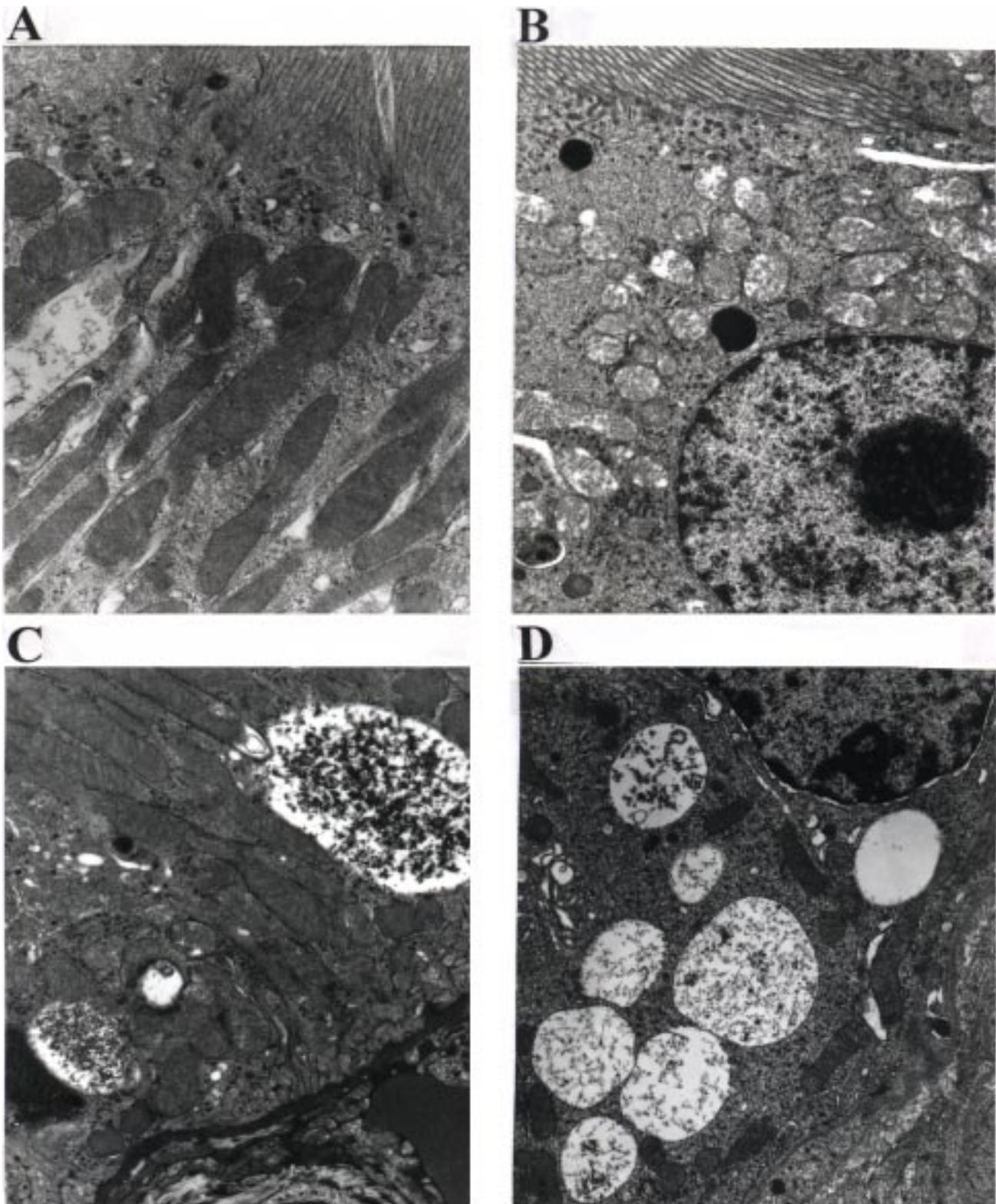


Fig. 2. Electron micrographs of the rat kidney proximal convoluted tubule in non-treated (A), olive oil (B), and after chronic treatment with 20 mg/kg (C) and 50 mg/kg (D) of *T. polium* ethanolic extract. Uranyl acetate and lead citrate. X 12500 (A, B, C and D).

was unaffected by chronic treatment with *T. polium*, which is also in agreement with previous findings following the chronic administration of *T. mascatense* [17]. However, the hypoglycemic effect that has been described for *T. polium* [9] and *T. oliverianum* [8] were found after short-term herbal administration, which is

probably due to a transient effect on the homeostatic regulatory carbohydrate metabolism. In conclusion, the present study demonstrates the phytotoxic effect of the medicinal plant *T. polium* on the liver and kidney that resulted in a rise in urea and cholesterol levels after prolonged herbal administration.

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