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Toxicological Effects of *Teucrium stocksianum* after Acute and Chronic Administration in Rats

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Abstract

Because of the widespread use of *T. stocksianum* (Boiss) in herbal medicine and reports of the toxicity of *Teucrium chamaedrys* to man, the effects of acute (2 and 4 g kg^{-1}, single dose) and chronic (4% in lieu of drinking water for 48 days) administration of an aqueous extract of *T. stocksianum* has been studied in rats.

After acute administration no change was found in reduced liver glutathione content, plasma total protein concentration or the enzyme activities of aminotransferase or gamma glutamyl transferase. After chronic administration, no change was noticed in the plasma concentrations of total protein, total bilirubin, creatinine, urea, glucose, triglycerides, calcium or phosphorus or the enzyme activities of aminotransferase, alanine aminotransferase, creatinine kinase, gamma glutamyl transferase or lactate dehydrogenase. There was no change in food or water intake or output of urine or faeces; the body weight of the treated animals was, however, slightly reduced. No change was observed in the weight of vital body tissues. Histological examination revealed occasional hepatic 'apoptosis' and cerebral neuronal loss in the cortex and hippocampus in treated animals; focal loss of Purkinje cells in the cerebellum was particularly noticed.

The results did not indicate a major hepatotoxic effect of acute or chronic administration of *T. stocksianum*, unlike other Teucrium spp. We report a neurotoxic effect, however, which warrants monitoring of neurological function in people taking this plant.

Larrey et al (1992) recently, reported several cases of hepatitis characterized by jaundice and a marked increase in serum aminotransferase levels. These symptoms appeared 3–18 weeks after administration of germander (*Teucrium chamaedrys* Family Labiatae). They also reported that hepatocyte necrosis was evident in liver biopsies obtained from three patients and that jaundice disappeared within 8 weeks after discontinuation of germander. Complete recovery was, however, achieved 1·5–6 months after discontinuing treatment with germander and readministration of germander to three cases was followed by prompt recurrence of hepatitis. Pauwels et al (1992) also reported two cases of severe acute hepatocellular injury occurring within 1–2 months of treatment with the same plant species. Because of the reported hepatotoxicity of germander, the French Ministry of Health and Humanitarian Action has suspended marketing authorization for medical products containing extracts from this plant (World Health Organisation 1992).

Teucrium species are traditionally used in many countries world-wide, to treat various ailments (Autore et al 1984; Suleiman et al 1988; Gharaibeh et al 1989; Roman-Ramos et al 1991; Larrey 1994; Loeper et al 1994; Bello et al 1995). *T. stocksianum* (Boiss) is used in the United Arab Emirates (UAE) as a very popular herbal medicine for treatment of diabetes, peptic ulcer and other gastrointestinal ailments (Al Anquar 1987). Although many reports can be found in the literature on various *Teucrium* species, to the best of our knowledge the literature contains no information about the potential toxicity of *T. stocksianum*.

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The questioned safety of *T. chamaedrys* in man has stimulated this study on *T. stocksianum*. We investigated the effect of acute and chronic administration of *T. stocksianum* in normal rats.

Materials and Methods

Animals

Experiments were performed on male adult Wistar rats, 230 ± 5 g. They were housed in an air-conditioned room (25 ± 2°C); standard pellet diet (Abu Dhabi Flour and Animal Feed Factory, Abu Dhabi, UAE) and water were freely available. Animals used for the chronic experiment were housed individually in metabolic cages and were left for 3 days for acclimatization before the start of the experiment.

Plant material

The aerial parts of the plant were collected from the Khorfakan area, UAE, in April 1992. The plant was botanically authenticated and herbarium specimens were deposited at the National Herbarium, Desert and Marine Environment Research Centre, UAE University.

Preparation of the lyophilized extract

Coarsely powdered aerial parts of *T. stocksianum* (500 g) were macerated with distilled water (1500 mL) for 12 h with occasional shaking. The extract was filtered and freeze-dried using a Christ LDC-2 freeze-dryer. The yield was 4·5%. This extract was given to animals in the acute experiment.

Preparation of the aqueous extract

Distilled water (350 mL) was added to coarsely powdered aerial parts of *T. stocksianum* (12·0 g) and marinated for 12 h

with occasional shaking. The filtrate was adjusted to 300 mL with distilled water to make the 4%-extract-containing drinking water for animals. Fresh extracts were prepared daily. This extract was given to animals in the chronic experiment.

Acute experiment

Rats were divided into three groups of six. One group received 2 g kg^{-1}, another 4 g kg^{-1} *Teucrium stocksianum* lyophilized extract and the third group (control group) received water. Twenty-four hours later, animals were decapitated, blood was collected in heparinized tubes and the livers were removed and used for measurement of reduced glutathione content.

Plasma was separated by centrifugation for 10 min at 3000 rev min^{-1}, and was then analysed for total protein and the activities of aspartate aminotransferase, acid phosphatase and gamma glutamyl transferase.

Chronic experiment

One group of rats received a fresh, daily-prepared 4% aqueous extract of *T. stocksianum* in lieu of drinking water while another group received tap water. The experimental period lasted for 48 days.

Food and fluid intake were recorded daily. Body weights were recorded and blood samples were obtained from the tail-tips at regular intervals for the determinations of aminotransferase activity, and total bilirubin and total creatinine levels.

At the end of the experimental period the animals were anaesthetized with ether and blood was collected from the posterior vena cava in heparinized tubes. Plasma was separated by centrifugation for 10 min at 3000 rev min^{-1} and was then analysed for the activities of aminotransferase, alanine aminotransferase, creatinine kinase, gamma glutamyl transferase, and lactate dehydrogenase and for the concentrations of glucose, creatinine, urea, total bilirubin, total protein, triglycerides, calcium and phosphorus.

After rinsing in normal saline and blotting, the wet weights of the liver, spleen, stomach, heart, lungs, kidneys (left and right) and testes (left and right) were recorded. Pieces of these organs in addition to pieces from the small and large intestines were prepared for histopathological investigation.

Biochemical determinations

Biochemical analyses were determined by means of a Cobas Fara II autoanalyser (Roche, Switzerland), using appropriate kits supplied by the manufacturer.

Determination of reduced hepatic glutathione

This was measured using the spectrophotometric method described by Sedlack & Lindsay (1968). Liver (200 mg) was homogenized in EDTA solution (0·02 M; 8·0 mL) in an ice bath. Samples (5 mL) of the homogenates were mixed with distilled water (4 mL) and trichloroacetic acid (50%; 1 mL). The tubes were centrifuged at 900 *g* for 15 min at 5°C and the supernatant (2 mL) was mixed with Tris buffer (0·4 M; 4 mL) and 0·1 5,5-dithiobis(2-nitro)benzoic acid. The absorbance was read at 412 nm against reagent blank with no homogenate.

Histopathological evaluation

Tissues from liver, spleen, stomach, heart, lungs, brain, kidneys, testes, and small and large intestine were immersed in 10% buffered formalin solution. Representative sections were processed in Shandon 2LE and Miles VP 1000 tissue processors. These were paraffin embedded, sectioned (5 μm thick), heated at 60°C for 1–2 h and stained using haematoxylin and eosin.

Statistical analysis

The *F*-test was used to examine homogeneity of variance of groups. The difference between two groups was assessed statistically by the unpaired Student *t*-test. P values < 0.05 were considered significant.

Results

Acute experiment

Acute administration of *T. stocksianum* lyophilized extract (2 g kg^{-1} and 4 g kg^{-1}) did not induce any significant change in reduced liver glutathione content, the activities of plasma aminotransferase, alanine aminotransferase and gamma glutamyl transferase, or the total protein content. Values are summarized in Table 1.

Chronic experiment

The body weights, food and fluid intake, the plasma activity of aminotransferase and the concentrations of creatinine and total bilirubin during the chronic experimental period are shown in Fig. 1. The body weights of the *Teucrium*-treated group were consistently less than those of the control group but the differences were of borderline significance except for twice (days 14 and 35) during the experimental period. The pattern of food and fluid intake was more erratic than other parameters and no

Table 1. The effect on rats of acute administration of the lyophilized extract of *Teucrium stocksianum*.

	Control	*Teucrium stocksianum*	
		2 g kg^{-1}	4 g kg^{-1}
Body weight	282 ± 12·3	266 ± 20·2	282 ± 7·1
Liver reduced glutathione*	1·18 ± 0·05	1·22 ± 0·02	1·24 ± 0·04
Aminotransferase (units L^{-1})	119 ± 18·0	124·5 ± 7·7	109·8 ± 9·5
Alanine aminotransferase (units L^{-1})	45·6 ± 2·4	67·5 ± 6·8	44·5 ± 3·4
Gamma glutamyl transferase (units L^{-1})	48·5 ± 3·4	51·0 ± 0·86	53·3 ± 1·03
Total proteins (mg dL^{-1})	6·82 ± 0·2	7·01 ± 0·17	6·94 ± 0·11

*Measured by absorbance at 412 nm. Values are means ± s.d. No significant differences were observed between the groups for any parameter (control group received ordinary tap water).

statistically significant difference was observed between the groups during the experimental period. The activity of aminotransferase and the concentrations of T. bilirubin and creatinine in plasma were similar in both groups. All rats in both groups seemed clinically normal in appearance and behaviour during the experimental period.

All biochemical parameters were similar in both groups at the end of the experiment (Table 2). Triglycerides, however, were of borderline significance ($47{\cdot}9 \pm 6{\cdot}4$ compared with $60{\cdot}8 \pm 12{\cdot}8$ mg dL^{-1}, $P < 0{\cdot}0509$, in *Teucrium*-treated and control groups respectively).

There was no difference between organ weights of the two groups whether expressed as absolute values or as the values relative to body weights (Table 3).

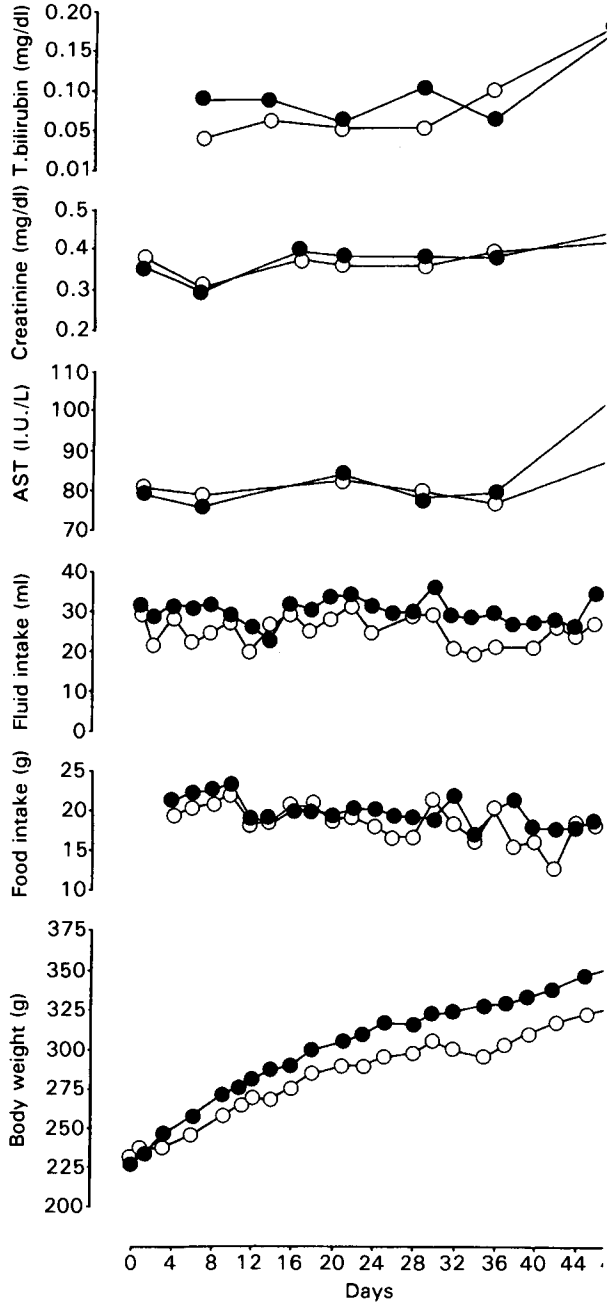

FIG. 1. The effect of *T. stocksianum* extract (4% w/v; obtained by maceration) in place of drinking water on body weight, food and fluid intake and on the plasma activity of aminotransferase and the concentrations of creatinine and total bilirubin, compared with values obtained from rats in a control group which received ordinary tap water. Values are means of six observations in each group. ● *Teucrium*-treated group, ○ control group.

Histopathological results

Tissue sections obtained from the brains of *Teucrium*-treated animals revealed hypoxic changes with a slight neuronal loss in the cerebral cortex and hippocampus. Focal loss of Purkinje cells of the cerebellum tissues and neurons with piknotic nuclei were also noted (Fig. 2). The liver sections showed occasional single-cell necrosis 'apoptosis'. Lymphoid infiltrates were noted in the interstitium of the lungs and the lamina proporia of the small intestine. In sections obtained from control and from *T. stocksianum*-treated animals, the heart showed subendocardial and subepicardial congestion.

Tissue sections of the rest of the organs taken from either the control or the *T. stocksianum*-treated groups showed no marked histological changes, except for a slight thickening of the basement membrane of the somniferous tubules in the *T. stocksianum*-treated group.

Discussion

In the chronic experiment, the measured live-phase parameters such as food and water intake or urine and faeces output were not significantly different in the control and *Teucrium*-treated groups, although the body weight of the treated animals tended to be less than that of the control animals throughout the chronic experimental period (Fig. 1) and reached statistical significance at two points (days 14 and 35; $P < 0{\cdot}05$). Such an effect might indicate a genuine though modest weight-reducing effect of *T. stocksianum* water extract. Indeed, Germander was marketed and used in France to reduce body weight (Loeper et al 1994). Because the reported reduction in body weight was not associated with a parallel effect on food intake, this is suggestive of a mechanism of action other than anorexia. The observed weight reduction was, presumably, a result of an effect on food absorption or an effect on energy expenditure by body tissues, or both.

In the study by Larrey et al (1992), patients who ingested Germander (*T. chamaedrys*) showed liver injury which was mainly characterized by jaundice and high serum aminotransferase. Histologically, a non-specific acute cytolytic hepatitis was noticed in some patients. It was concluded that *T. chamaedrys* might induce non-specific liver injury. In our study, treatment of rats with *T. stocksianum* did not alter any of the measured biochemical parameters including bilirubin and aminotransferase. Histologically, however, we observed occasional hepatocyte 'apoptosis'. Our inability to show changes in bilirubin plasma levels might be explained by one or more of the following: *T. stocksianum* lyophilized water extract might not be as hepatotoxic as that from *T. chamaedrys*, or hepatoxic at all; the rat might be more resistant to the hepatotoxic effect of this plant extract than man; *T. stocksianum* is hepatotoxic but in our experiment the degree of induced hepatotoxicity might have not been high enough to induce significant changes in plasma bilirubin or hepatic enzyme levels.

Investigating the effects of *T. stocksianum* after acute

Table 2. The effect of chronic administration of *T. stocksianum* extract (4% w/v; obtained by maceration) in lieu of drinking water for 48 days) on some biochemical parameters in rats.

	Control	*Teucrium stocksianum*
Aminotransferase (units L^{-1})	105 ± 43	87 ± 16
Alanine aminotransferase (units L^{-1})	52 ± 32	25 ± 2
Creatinine kinase (units L^{-1})	687 ± 495	648 ± 329
Gamma glutamyl transferase (units L^{-1})	1·89 ± 6·88	0·94 ± 0·30
Lactate dehydrogenase (units L^{-1})	1525 ± 779	1628 ± 697
Creatinine (mg dL^{-1})	0·44 ± 0·07	0·42 ± 0·09
Urea (mg dL^{-1})	47 ± 4	50 ± 7
Total bilirubin (mg dL^{-1})	0·18 ± 0·07	0·18 ± 0·06
Total proteins (mg dL^{-1})	7·7 ± 0·3	7·7 ± 2·20
Glucose (mg dL^{-1})	120 ± 7	105 ± 24
Triglycerides (mg dL^{-1})	60·8 ± 12·8	47·9 ± 6·4
Calcium (mg dL^{-1})	11·2 ± 0·37	10·9 ± 0·27
Phosphorus (mg dL^{-1})	8·1 ± 1·9	7·4 ± 0·93

Values are means ± s.d. (n = 6 for both groups). No significant differences were observed in any parameter between the two groups.

administration was undertaken to test whether or not the plant extract would alter hepatic reserve of glutathione as a measure of hepatic capacity to assimilate xenobiotic cytotoxic agents. This parameter was considered because of the findings of Kouzi et al (1994) who reported that in mice a bioactive metabolite of teucrine A (a diterpene constituent of *Teucrium chamaedrys*) is the compound responsible for *T. chamaedrys* hepatotoxicity. These authors provided evidence that teucrine A causes such an effect by being bioactivated via an oxidative pathway involving depletion of glutathione liver stores. Although we administered *T. stocksianum* to rats in a very high dose (approximately 10 times the chronic daily consumption and the equivalent of a full dose in man), it did not cause a change in hepatic glutathione content. No change was, furthermore, noticed in aminotransferase, gamma glutamyl transferase or alanine aminotransferase activities. Together, these observations indicate minimal hepatotoxic potential of *T. stocksianum* in rats. The reasons for the apparent discrepancy between our results and those of Kouzi et al (1994) is not clear. It might, however, be hypothesized that the teucrine A content of the lyophilized water extract of *T. stocksianum*, at the dose used in our experiment, is less than that needed to cause hepatotoxicity. Teucrine A, being a diterpene, has low water solubility and thus the water extract might not contain an appreciable amount of this compound.

Histologically, except for brain and liver all the examined tissues from both groups, showed almost no or similar changes. The observed congestion of the cardiac tissue and the dark

Table 3. The effect on body and organ weights of chronic administration of *T. stocksianum* extract (4% w/v; obtained by maceration) in lieu of drinking water for 48 days).

			Control	*Teucrium stocksianum*
Body weight		(g)	352 ± 21	326 ± 21
Liver		(g)	12·2 ± 1·5	11·5 ± 0·2
		(%)	3·5 ± 0·4	3·5 ± 0·2
Kidneys	Left	(g)	1·26 ± 0·18	1·28 ± 0·09
		(%)	0·36 ± 0·05	0·4 ± 0·02
	Right	(g)	1·29 ± 0·14	1·31 ± 0·13
		(%)	0·37 ± 0·03	0·40 ± 0·02
Testes	Left	(g)	1·72 ± 0·14	1·66 ± 0·11
		(%)	0·49 ± 0·04	0·51 ± 0·04
	Right	(g)	1·70 ± 0·15	1·68 ± 0·07
		(%)	0·48 ± 0·04	0·49 ± 0·04
Spleen		(g)	0·60 ± 0·04	0·54 ± 0·19
		(%)	0·17 ± 0·01	0·19 ± 0·01
Stomach		(g)	1·76 ± 0·14	1·64 ± 0·20
		(%)	0·50 ± 0·09	0·50 ± 0·07
Heart		(g)	1·05 ± 0·02	1·04 ± 0·07
		(%)	10·30 ± 0·02	0·32 ± 0·01
Lung		(g)	1·39 ± 0·14	1·46 ± 0·17
		(%)	0·41 ± 0·06	0·44 ± 0·06
Brain		(g)	1·72 ± 0·09	1·68 ± 0·07
		(%)	0·49 ± 0·05	0·51 ± 0·04

Values are mean ± s.d (n = 6 for both groups; control group received ordinary tap water). No significant difference was observed in any parameter between the two groups. Values given are absolute wet weight of organ (g) and weight per cent relative to body weight (%).

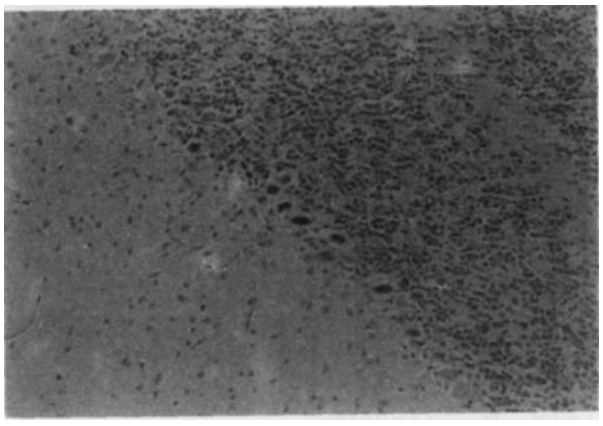

FIG. 2. Photograph demonstrating the neuronal loss, mainly Purkinje cells, in a histological section obtained from brains of rats treated with *T. stocksianum*.

nucleated Purkinje cells apparent in both groups might be a result of ether anaesthesia or the handling of animals during tissue removal, or both. Other histopathological observations, such as the lymphoid infiltrate in the lungs and intestine, might not be of great pathological importance, because they can take place under normal conditions. Most importantly, we observed neuronal loss manifested as a low density of neurons in the cerebrum and loss of Purkinje cells in the cerebellum. The physiological significance of this observation is still to be investigated. Examination of muscular coordination is one function that should probably be studied after treatment with this plant, because Purkinje cells are concerned with this physiological process (Guyton 1991). Larrey et al (1992) or Pauwels et al (1992) did not specifically mention any symptoms of CNS toxicity. Our findings give good cause for monitoring the appropriate physiological indicators of neurotoxicity in future clinical investigations related to the consumption of *Teucrium* species.

The reported cytotoxicity could either be a result of a direct cellular effect or have been induced secondarily as a result of tissue ischaemia caused by the *T. stocksianum* treatment. The presence of occasional hepatocyte 'apoptosis', which could only have been caused by direct cellular action, makes secondary induction unlikely and outweighs the possibility of direct neurocytotoxic action of *T. stocksianum* extract. Although Larrey et al (1992) were unable to determine the mechanism of *T. chamaedrys*-induced hepatotoxicity they suggested an immunoallergic mechanism. No evidence is provided in our report to support or undermine such a mechanism. More evidence is, nevertheless, needed before such action can be confirmed.

In conclusion, we report a neurocytotoxicological effect of *T. stocksianum* which requires further investigation. Also, our results warrant further clinical assessment of patients using *Teucrium* species in order to investigate its potential neurotoxicity in man.

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