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Original Article

Acute and Subchronic Toxicity of *Teucrium polium* Total Extract in Rats

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Abstract

Teucrium Polium L. (TP) is widely used in traditional medicine of many countries including Iran. There are various reports about pharmacological properties of TP such as calcium antagonist, anti- ulcer, anti – diabetic. There are a few reports about possible toxicological effects of this plant. In the present study we designed to evaluate the subchronic toxicity of *Teucrium Polium* total extract in rats.

Sprague Dawley rats (40 males, 40 females) were divided into four dose groups (10 animals/dose/sex) and were gavaged daily with either 100, 300 or 600 mg/kg of the total extract for 44 days. Control group was received normal saline. Body weight and food consumption was monitored daily. After 45 days animal was sacrificed and hematological and biochemical parameters, as well as weight of left kidney and liver were measured.

There was no significant difference in hematological parameters in both sexes as compared to their respective Controls. In biochemical parameters, a significant increase ($p<0.05$) was seen in both ALT and AST enzyme activities in female rats receiving 300 mg/kg TP. There was also a significant increase in liver weight of male rats receiving 600 mg/kg. No other significant changes in any other parameter were observed.

Present data suggests that female rats are more sensitive to higher doses of TP and that liver could serve as a target organ toxicity of this extract.

Keywords: *Teucrium Polium*; Acute toxicity; Subchronic toxicity; Rats.

Introduction

Teucrium polium L. (Lamiaceae) is a plant that widely grows in Iran . Some of species of *Teucrium* are used for a great range of action in traditional medicine (1, 2).

There are various reports about pharmacological properties of *Teucrium polium L.* (TP). These include calcium antagonistic (3), anorexic (4), intestinal motility and blood pressure (5), anti-ulcer (6), anti-inflammatory (7), antipyretic and antibacterial actions (8) glycemic and hypolipidemic effects (9-11).

There are few reports about possible toxicological actions of TP. Acute and chronic toxicity of *Teucrium stocksianum* (another species of *Teucrium*) in rats have been studied (12). There is also a report about hepatotoxic effects of TP in rats (13). The present study has evaluated the subchronic toxicity of aqueous extract of TP in rats.

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Experimental

Materials and Methods

Plant Material

The aerial parts of the plant was collected from Central Alborz Kalak at the altitude of 2500 meters and dried in shade in order to protect of enzymes effect (2). The plant was identified by the Department of Pharmacognosy and a sample of the plant was deposited at the Herbarium of the School of Pharmacy (Voucher Specimen No. 675). Powder of dried plant was soaked for 24 h in 1000 ml of boiling water. The infusion was filtered and the filtrate was heated indirectly to obtain dried extract. In this way, 100g of dried plant yielded 20g of extract, which was used for the experiment.

Animals

Sprague-Dawley rats (40 males and 40 females) were purchased from Razi Institute, Karaj. Rats with body weight ranging from 130-170g were housed randomly in stainless steel wire cages and kept on a 12h light/dark cycle at 22+ 3ºC and constant humidity, and were given free access to food and water. All animals were kept for two weeks prior to experimentation.

Acute Toxicity Experiments

In order to study any possible toxic effect or changes in normal behavior, six groups of 10 rats (5 males and 5 females) were used in this experiment. The acute toxicity of the plant was studied by preparing 5 different concentrations of the extract (0.5, 1, 2, 4 and 8 g/kg), and administered orally to five groups of animals. The sixth group was taken as a control and given 1.0 ml normal saline. The behavioral changes, posture and mortality were checked. Animals were kept under observation for 14 days [14].

Subchronic Toxicity Experiments

Animals (40 males and 40 females) were divided into four dose groups (10 animals /dose/sex). The first group was given 1ml normal saline and taken as a control. The second, third and forth group were given a single does of 100, 300, and 600 mg/kg of TP by gavage daily.

Gavage dosing was performed using a curved, ball-tipped intubations needle affixed to a 2.5 ml syringe. All solutions were prepared just prior to dosing and were kept chilled and tightly capped. Body weight, food, and water consumption were monitored daily. Animals were fasted 3h prior to dosing to facilitate administration of the complete dose. Clinical observation was made at least twice/day.

All animals were dosed for 44 days and after this time the animals (10 rats/cage/dose/sex) while fasted for about 16 h after the last dose, sacrificed by decapitation.

Immediately after decapitation blood samples were obtained directly from the neck for hematological analysis and serum chemistry. By thoracic abdominal longitudinal incision the animal abdomen was opend and the liver and left kidney were removed and the wet weights were recorded.

Hematology

The following hematological parameters were determined by pathobiology laboratory: leukocyte count (WBC), hemoglobin (Hb), hematocrit (Hct) and mean corpuscular Hb concentration (McHc).

Serum chemistry

Serum prepared from blood samples and was assayed for the following constituents by pathobiology laboratory: Glucose (Glu), creatinin (Crea), urea (Urea), cholesterol (Chol), triglyceride (Trig), total protein (Total Pro), lactate dehydrogenase (LDH), low density lipoprotein (LDL), high density lipoprotein (HDL), alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Statistical analysis

Mean±SEM were calculated for body weights, food and water consumption, organ weights and organ/body weight ratios. Differences between dose groups with controls were evaluated for males and females separately by performing a one-way analysis of variance (ANOVA) followed by Tucky's post test. P values of 0.05 or less were taken as significant.

Results and Discution

Acute Toxicity Experiments

All rats treated with different concentrations of the total extract of TP were alive during the 14 days of observation. The animals did not show visible signs of acute toxicity. It suggested that the LD_{50} of the total extract was higher than 8g/kg.

Subchronic Toxicity Experiments

Mortalities and clinical findings: One male rat at 600 mg/kg died on d9 of the experiment. But survival in other groups was 100%.

Hematological values: After 44 days (table 1) of treatment, there were no significant differences between treated groups and control group in both sexes.

Biochemical values: After 44 days of treatment in female rats there was a significant increase in AST and ALT concentration, respectively, at 300mg/kg but all remained unchanged in both sexes (table 2).

Body and organ weight and food consumption:

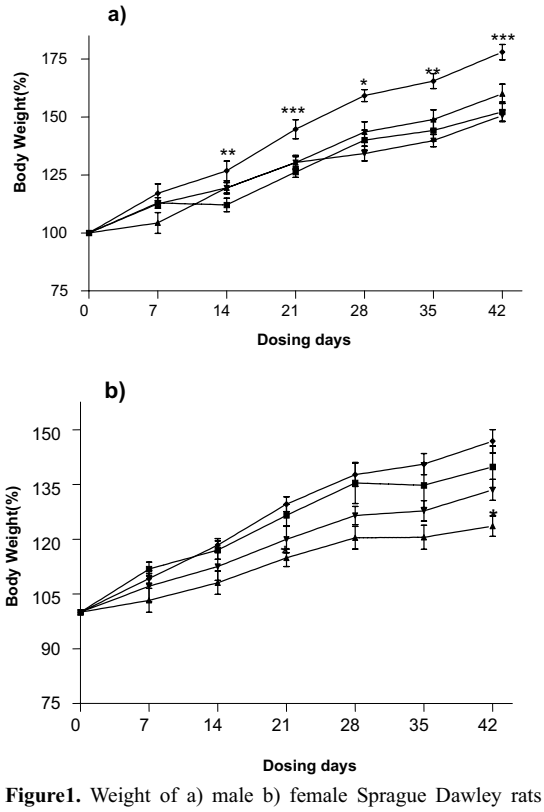

Figure1. Weight of a) male b) female Sprague Dawley rats after oral gawage dosing of TP total extract for 44 days.
Note: All The groups compare to control

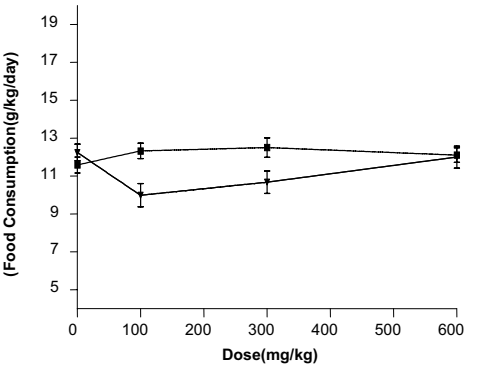

Figure 2. Food consumption of Sprague Dawly rats after oral gawage dosing of TP extract for 44 days.

There was statistically significant increase in male body weight at 600mg/kg in 14-44 days compared to control. Female body weight at 100mg/kg had decreased significantly ($p<0.05$) at 21, 44 days of study (figure 1).

Also the weight of liver (percent of body weight) was significantly increased ($p<0.01$) in male rats that received 600 mg/kg of the extract but in other groups no differences were observed (table 3).

No significant changes in food consumption were observed (figure 2).

Acute Toxicity Experiment

The obtained result in the study of acute toxicity indicated that the high dose of TP (8g/kg) did not produce any symptoms of toxicity and none of the rats died after 14 days of observation. Also in the study about antipyretic and antibacterial action of ethanolic extract of TP had no effect in acute study (8).

Subchronic Toxicity Experiment

There are no report about subchronic toxicity of TP extract and only in a study of toxicological effect of *T. stocksianum* (another species of T.) after acute and chronic administration (48 days) in rats, it was concluded that this plant had a neurotoxic effect but did not indicate a hepatotoxic effect (12).

In current study no significant changes were observed in hematological parameters after 44 days of treatment. As shown in the result the ALT and AST enzyme in female rats at the middle dose group and weight of liver in male rats at the highest dose group significantly increased

Table 1. Hematological parameters of Sprague Dawley rats after 44 days treatment with *Teucrium Polium*

	Males				Females			
	Control	100	300	600	Control	100	300	600
WBC (U/L)	8980 (1115)	9140 (815.2)	8820 (848.8)	9225 (1035.5)	7980 (685.6)	9040 (1066.1)	8920 (2190)	9225 (1035)
Hemoglobin (g/di)	13.86 (0.41)	13.94 (0.28)	14.12 (0.30)	14.30 (0.27)	13.32 (0.52)	12.60 (0.29)	12.30 (0.29)	13.72 (0.37)
Hematocrit (%)	42.20 (1.20)	42.60 (0.81)	43.20 (0.97)	43.75 (0.75)	40.60 (1.60)	38.40 (1.03)	37.60 (0.87)	41.40 (1.08)
MCHC (%)	32.84 (0.19)	32.74 (0.09)	32.68 (0.21)	32.70 (0.07)	32.82 (0.13)	32.82 (0.16)	32.72 (0.09)	33.16 (0.05)

Note: Data is given as mean (SEM) for 10 animals.
Animals were dosed orally for 44 days.

($p<0.05$). In another study cytoplasmic changes in 1/3 to 2/3 of the liver lobule in perivenular and midzonal areas was shown in TP–treated rats [13]. Therefore more experiment must be designed for assessment of hepatic effect of this plant.

There were no significant differences in food consumption and variation in the body weight was negligible. Present data suggests that female rats are more sensitive to higher dose of TP and liver could serve as a target organ in oral toxicity of the extract. This may be due to presence of several neoclerodane diterpenoids in TP extract (15, 16). In Summary, since a proposed pharmacological used of TP in condition of hypercholesterolemia, hepatic effects of the extract in chronic uses and high doses could be concern. More studies are underway to evaluate the efficacy and safety of different fractions of TP.

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Table 2. Biochemical parameter of Sprague Dawley rats after 44 days treatment to *Teucrium Polium*.

	Males				Females			
	Control	100 (mg/g)	300 (mg/g)	600 (mg/g)	Control	100 (mg/kg)	300 (mg/kg)	600 (mg/kg)
Glucose (mg/dl)	73.60 [a] (2.16)	74.6 (2.01)	78 (4.16)	76 (2.16)	79.8 [a] (3.83)	75.4 (4.42)	76.2 (5.78)	70.6 (0.24)
Creatinine (mg/dl)	1.26 (0.09)	1.12 (0.024)	0.94 (0.08)	1.23 (0.10)	1.06 (0.13)	1.22 (0.09)	1.46 (0.06)	1.24 (0.06)
Urea (mg/dl)	22.6 (1.08)	20.0 (0.84)	22.6 (1.57)	23.7 (2.17)	24.6 (3.30)	23.2 (1.46)	25.4 (0.93)	21.4 (0.75)
Cholesterol (mg/dl)	52.60 (3.01)	48.60 (3.68)	67.40 (6.05)	53.75 (4.96)	54.40 (5.01)	55.40 (5.22)	60.75 (2.06)	60.40 (2.80)
Triglycerides (mg/dl)	145.0 (5.39)	103.75 (23.29)	135.6 (12.18)	92.75 (22.85)	144.0 (11.08)	100.6 (13.50)	147.2 (2.91)	116.6 (15.33)
IIDL (mg/dl)	36.75 (0.75)	40.40 (1.78)	36.40 (2.18)	37.00 (2.53)	32.40 (1.81)	37.00 (1.51)	37.80 (1.80)	43.40 (2.94)
LDL (mg/dl)	14.05 (1.85)	12.25 (1.93)	16.20 (0.66)	13.50 (1.71)	18.60 (0.68)	17.80 (2.37)	14.40 (1.57)	15.60 (1.17)
Total Pr (mg/dl)	7.50 (0.29)	6.88 (0.18)	7.48 (0.35)	7.35 (0.36)	7.42 (0.19)	6.90 (0.09)	7.02 (0.06)	7.46 (0.33)
AST (IU/L)	67.00 (3.36)	57.40 (6.31)	55.00 (2.47)	60.00 (3.51)	59.57 (7.23)	90.00 (8.68)	128.70 * (12.30)	124.70 (26.27)
ALT (IU/L)	51.40 (2.29)	54.20 (3.18)	58.40 (3.06)	65.00 (6.81)	60.50 (4.25)	83.20 (6.43)	144.70 ** (14.50)	118.50 (20.40)
LDII (IU/L)	4238.0 (406.0)	4353.2 (498.1)	4784.2 (294.6)	5218.7 (219.4)	4755.0 (227.3)	4424.2 (482.0)	3025.0 (647.3)	4129.0 (706.9)

Note: Data given as mean (SEM)[a] 10 animals .
*,**: Significantly different from control (*: $P<0.05$, **: $p<0.01$) .

Table 3. Effect of Subchronic Toxicity of *Teucrium Polium* on Organ Weight of Rats.

Animal Groups	Males	Males	Females	Females
	Liver (%)	Kidney (%)	Liver (%)	Kidney (%)
Control	3.18[a] (0.12)	0.36 (0.01)	3.18 (0.12)	0.36 (0.10)
100 mg/kg	2.98 (0.11)	0.33 (0.01)	3.25 (0.09)	0.38 (0.10)
300 mg/kg	3.26 (0.05)	0.36 (0.01)	3.41 (0.07)	0.40 (0.02)
600 mg/kg	3.53* (0.11)	0.37 (0.02)	3.39 (0.07)	0.40 (0.01)

Note: Data are given as percent of organ weight /body weight a: Mean (SEM) 10 Animals. *: Significantly different from control at $p<0.05$

References

(1) Zargari A. Medicinal Plants. University of Tehran Press, (1997) 130

(2) Aynehchi Y. Pharmacognosy and Medicinal Plants of Iran. University of Tehran Press, (1991) 1079

(3) Agel MB, Gharaibeh MN and Salhab AS. Calcium antagonist effect of the volatile oil of *Teucrium polium*. *Int. J. Crude Drug Res.* (1990) 28: 201-207

(4) Gharaibeh MN, Elayan HH and Salhab AS. Anorexic effect of *Teucrium polium* in rats. *Int. J. Crude Drug Res.* (1989) 27: 201-210

(5) Suleiman MS, Abdul-Ghani AS, Al-Khalil S and Amir R. Effect of *Teucrium polium* boiled leaf extract on intestinal motility and blood pressure. *J. Ethnopharmacol.* (1988) 22: 111-116

(6) Twaij HA, Albadr AA and Abdul-Khalil A. Anti ulcer activity of *Teucrium polium. Int. J. Crude Drug Res.* (1987) 25: 125-128

(7) Capasso F, Cerri R, Morrica P and Senatore L. Boll Soc. Chemical composition and anti inflammatory activity of an alcoholic extract of *Teucrium polium*. *Ital Biol. Sper.* (1983) 59: 1639-1643

(8) Autore G, Capasso F, De-Fusco R, Fasulo MP, Lembo M, Mascolo N and Menghini A. Anti pyretic and anti bacterial actions of *T. polium. Pharmacol. Res. Commun.* (1984) 16: 21-26

(9) Gharaiben MN, Elayan HH and Salhab AS. Hypoglycemic effect of *Teucrium polium*. *J. Ethnopharmacol.* (1988) 24: 93-99

(10) Konuklugil B, Deniz G, Yildiz O, Senoz S and Saygi S. Hypoglycemic effect of *Teucrum polium. Fitoterapia* (1997) 68: 39-41

(11) Rasekh HR, Khoshnood- Mansourkhani MJ and Kamalinejad M. Hypoglycemic effect of *Teucrium polium. Fitoterapia* (2001) 72: 937-939

(12) Tanira MO, Wasfi IA, Homsi MA, Bashir AK. Toxicological effects of *T. Stocksianum* after acute and chronic administration in rats. *J. Pharm. Pharmacol. (*1996) 48: 1098-1102

(13) Zal F, Vasei M, Rasti M and Vessal M. A Hepatotoxicity associated whit hypoglycemic effects of *T. polium* in diabetic rats. *Rch. Irn. Med.* (2001) 4: 188-192

(14) Rhodes C. Principles of testing for acute toxic effects. In: Ballantyne B, Marrs T, Syversen T, editors. General and Applied Toxicology. Bath Press, 1999; 1: 39-42

(15) Malakov P and Papanov GP. Faranoid diterpenes from *T. polium phytochem*. (1983) 22: 2791-2793

(16) Fernandez P, Rodriguez B and Savona G. Neoclerodane Diterpenoids from *T. polium Phytochem.* (1986) 25:181-184

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