

Hematological and Biochemical Evaluation of *Teucrium Polium* in Albino Rats

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Abstract: This study evaluates the effect of acute (24 h) and subacute (3 weeks) treatment of the *Teucrium polium* decoction on liver and kidney functions and hematological parameters in rats. The toxicity study with this decoction did not produce mortality. No marked adverse alterations were observed in hematological and biochemical parameters during acute toxicity. In the subacute toxicity studies, red blood cells counts, Hemoglobin (Hb) and Packed Cell Volume (PVC) insignificantly decreased. However, other blood indices, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were not changed. There was significant ($p < 0.001$) increase in white blood cells. Serum glucose level change was insignificant, while the level of serum cholesterol and triglycerides were significantly ($p < 0.001$) reduced. The Activity of Serum Transaminases (ALT and AST) showed non significant changes, urea, uric acid and creatinine levels were found non significant changes. In conclusion the statistical analysis of our results indicates that treatment with *T. polium* did not cause any attention that might suggest the presence of pathological damage at the dose investigation.

Keywords: Albino rats, hematological parameters, *Teucrium polium*, traditional medicine

INTRODUCTION

Many plants have been used for their medicinal value in folk medicine for long time, however only minority of them has been studied experimentally or clinically for their actual beneficial or toxic effects. In Jordan 485 species from 99 different families are categorized as medicinal plants (Oran and Al-Eisawi, 1998). A large number of these plants are used in folkmedicine to treat various types of disorders (Aburjai *et al.*, 2007).

Teucrium polium L. is a widespread medicinal plant that belongs to the Lamiaceae family. It has many applications in folkmedicine in many countries including Jordan, because of its hypoglycemic effects (Konuklugil *et al.*, 1997; Hamdan and Afifi, 2004; Afifi *et al.*, 2005; Aburjai *et al.*, 2007), Antiulcerogenic and inflammatory activities (Al-Kofahi and Atta, 1999), Hypolipidemic effects on serum cholesterol and triglycerides (Rasekh *et al.*, 2001) and anti-colitis effect (Abdolgaaffari *et al.*, 2010). Due to its mitotic activity, the plant is used for the treatment of kidney and liver disease and inflammations (Abu-Irmaileh and Afifi, 2003; Shehab, 1980) and has also been reported to have antigenotoxic and antioxidant properties (Sghair *et al.*, 2011; Suboh *et al.*, 2004), Antibacterial effect (Autore *et al.*, 1984).

The bioactive compounds contained in the plant are flavonoids (Kawashty *et al.*, 1999) and neo-clerodane diterpenoids (Bruno *et al.*, 2003). Four major flavonoids

with antioxidant and free radical scavenging activities has been reported (Sharififar *et al.*, 2009)

However, the effect of *T. polium* on hematologic indices has not confirmed experimentally. On this basis the present study was carried out in order to demonstrate the effect of *T. polium* on cell components of the blood and selected biochemical parameters in acute and subacute levels in rats.

MATERIALS AND METHODS

Location and duration of the study: This study was carried out at the animal house of the Faculty of Pharmacy, Al-Zaytoonah University, Amman, Jordan during the period of December 2010 to February 2011.

Plant material: Sample was obtained as dried plant from herbal store, identified by professor Al-Eisaway from biological sciences department at the university of Jordan (personal communication).

Preparation of decoction: A sample of air dried plant used in folkmedicine in Jordan and the dose was similar to the method of preparation used by Jordanian. Five g was boiled in 100 mL distilled water for 10 min, then was filtered through filter paper No. 1 and centrifuged at 1500 g/10 min. The supernatant was used in the experiments.

Experimental animals: Male wistar albino rats weighing (200-220 g) were obtained from the animal house at university of applied sciences-Jordan. The animals were housed under standard condition of temperature (23±2) and about 70% humidity. They were given diet and water *ad libitum*. The animals were divided into four groups consisting of six rats each as the following:

Acute study: Two groups of 6 male rats each were formed and treated by Intraperitoneal (i.p) route as follows:

- Group 1 (control 24 h) received normal saline (5 mL)
- Group 2 (*T. polium* 24 h) received decoction corresponding 60 mg/kg of dry material (5 mL)

Subacute study: Male rats were divided into two groups of six animals each. The decoction was administered daily (i.p.) for three weeks as follows:

- Group 1 (control 3 weeks) received normal saline (5 mL) daily for three weeks
- Group 2 (*T. polium* 3 weeks) received the decoction (5 mL) daily for three weeks

Blood sample collection and analysis: The animals were anaesthetized with ether then blood was collected from the heart by heart puncture.

The relative blood picture (total red cell, white cell counts, Hemoglobin (Hb) and Packed Cell Volume (PCV) and other blood indices (MCV, MCHC and MCH) were determined using a fully automated coulter counter after the single administration (acute) and after the last treatment (subacute).

Biochemical analysis: At the end of experimental periods, animals were subjected to ether anaesthesia,

blood was collected and allowed to clot at room temperature for 20 min. Serum was obtained by centrifugation at 3000 rpm for 10 min. and kept at 4°C to assay the activities of serum enzymes (AST and ALT), glucose, cholesterol, triglyceride, urea, uric acid and creatinine.

All biochemical parameters were determined on the same day spectrophotometrically using commercially diagnostic kits which rely on colorimetric procedures product (obtained from Boehringer-Germany and Biomerieux-France). The manufacture's protocol was followed.

Statistical analysis: Data were presented as mean±SEM of six separate experiments statistical significance between control and treated groups were assessed by student's t-test. p<0.001 was considered significant.

RESULTS

There was no mortality in any of the studied groups. The results of the hematological and biochemical parameters are presented in Table 1 and 2, respectively.

During the acute study the decoction did not produce any changes in blood picture or biochemical parameters in rats. In subacute studies the results in Table 1 indicate that there were not altered adversely in the red cell count, hemoglobin and hematocrit contents of treated animals after the period of treatment as compared with control group. There were also no change in the values of other blood indices MCV, MCHC and MCH. On other hand, white blood cells exhibit a significant increase in the count (p<0.001) in treated animals with the decoction when compared with control group.

As shown in Table 2, the activity of serum marker enzymes (AST and ALT) showed no significant changes in group tested. The decoction treated rats, indicated a

Table 1: The effect of the *T. polium* decoction on hematological parameters in rats after acute and subacute treatment

Group	RBCs (10 ⁶ /mm ³)	Hb (g/dL)	PCV (%)	MCV% (%)	MCHC (g/dL)	MCH (Pg)	WBCs (10 ³ /mm ³)
Control 24 h	6.4±0.2	14.2±0.4	41.6±0.9	54.2±0.6	29.7±0.9	21.5±0.2	5.7±0.8
<i>T. polium</i> 24 h	6.3±0.2	14.0±0.6	40.8±0.7	52.6±0.5	28.4±0.6	20.7±0.3	6.9±1.2*
Control 3 weeks	7.64±0.1	13.8±0.5	45.5±1.3	58.3±0.7	33.2±0.2	20.3±0.1	7.2±1.2
<i>T. polium</i> 3 weeks	7.20±0.2	15.5±0.7	43.8±0.8	56.6±0.9	34.4±0.3	20.8±0.2	9.9±1.4*
p-value	N.S	N.S	N.S	N.S	N.S	N.S	P< 0.001

The values in the table are means±SEM of 6 separate experiments; *: p<0.001 student's t-test

Table 2: The effect of *T. polium* decoction on biochemical parameters in rats after acute and subacute treatment

Group	AST 4/L	ALT 4/L	Glucose (mg/dL)	Cholesterol (g/dL)	Triglyceride (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)	creatinine
Control 24 h	192.4±11.3	47.2±2.6	146±5	87.3±5.2	139±14	46±1.8	5.8±1.2	0.52±0.1
<i>T. polium</i> 24 h	186.8±12.2	44.6±2.3	133±4	65.5±3.8	112±12	43±1.7	6.2±1.1	0.50±0.1
Control 3 weeks	220.7±12.2	43.13±2.1	110.5±5.2	168.8±12.9	148.7±7.2	38±2.1	6.7±1.1	0.61±0.2
<i>T. polium</i> 3 weeks	209.5±15.1	40.26±1.6	93.7±4.9	105.3±8.3*	83.5±4.4*	40±2.3	7.2±1.3	0.58±0.1
p-value	N.S	N.S	N.S	p< 0.001	p<0.001	N.S	N.S	N.S

The values in the table are means±SEM of 6 separate experiments; *: p<0.001 student's t-test

significantly ($p < 0.001$) decreased total cholesterol and triglycerides in serum compared with normal control. Serum glucose level was insignificantly decreased. The mean values of urea, uric acid and creatinine were insignificantly changed in treated rats when compared with control group.

DISCUSSION

In recent years, extensive applications of medicinal plants have been reported to be employed in folkmedicine in the treatment of anemia (Dina *et al.*, 2006). Hematological analysis of plant extract in animals is one of the important methods of assessing the toxicity of plant extract (Ashafa *et al.*, 2009). It is possible that the extract contain constituent that can stimulate the hematopoietic systems (Ita *et al.*, 2007; Aka *et al.*, 2009; Odesanm and Lawal, 2010; Kolawoles *et al.*, 2011).

In the present study, there were no significant changes in the values of blood picture, this may result from the action of the *T. polium* decoction content especially the presence of flavonoids (Kawashty *et al.*, 1999) which has been reported to have antioxidative effects (Suboh *et al.*, 2004). Flavonoids inhibit peroxidation of polyunsaturated fatty acids in cell membrane (Middleton, 1996).

Moreover, reports have shown that flavonoids inhibit the formation of superoxide ions and hydroxyl radicals, which are two strong peroxidation agents (Facino, 1990).

However, it is noticed that hemoglobin content and hematocrit values were directly correlated with RBC's count. There are no changes in the value of MCV and MCHC; this indicates that Hb-content of RBC's is normal. The absolute values of MCV and MCHC have widely used in the classification of anaemia (Daci and Lewis, 1995).

Data recorded in the present study revealed that *T. polium* treated rats was not anaemic. This can be explained that the *T. polium* decoction treatment was devoid of the inhibition of erythropoietic activity of the bone marrow. On the other hand white blood cells exhibit a significant increase ($p < 0.001$). This can be attributed to the reactive response due to stress.

Serum AST and ALT are the most sensitive hepatic markers employed in the diagnosis of hepatic damage (Sallie *et al.*, 1991). In the present investigation, there were no significant difference between the treated and control group. Previous study has shown extraction of *T. polium* to be an effective antioxidant under *in vitro* conditions. No significant change in the levels of AST and ALT is suggestive of the fact that decoction is successful in quenching the free radicals inhibiting lipid peroxidation and protecting the membrane lipids from

oxidative damage in the liver of rats (Suboh *et al.*, 2004). Rasekh *et al.* (2001) have demonstrated no changes in these enzymes in male rats but elevated and more sensitive in female rats in response to the extract of the plant.

In the current study, no significant decrease in serum glucose was observed in *T. polium* administered rats; therefore do not support the folkore claim that it is anti-diabetic agent. This result was in agreement with (Hamdan and Afifi, 2004; Afifi *et al.*, 2005). The decoction decreased the mean values of cholesterol and triglycerides significantly. These results of our investigation are in accordance with (Rasekh *et al.*, 2001) who has reported the antilipidemic activity of alcoholic *T. polium* leaf extract used i.p method.

Stabilization of serum urea, uric acid and creatinine levels through the administration of the decoction is further a clear indication of the protection of the functional status of kidney and liver cells.

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