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In vivo Diuretic Activity of *Teucrium polium* L. Aerial Parts Ethanolic Extract in Wistar Rats

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ABSTRACT: Medicinal plants are a rich source of disease-prevention medicinal chemicals, and their health advantages are continually expanding. Traditional treatments can help with a variety of disease conditions, but they need to be scientifically validated before they can be fully utilized. The current study was therefore conducted to evaluate the diuretic activity of ethanolic extract of *Teucrium polium* L. in Wistar rats. The ground aerial parts of *T. polium* were extracted with 70% ethanol in a Soxhlet apparatus. The Wistar rats were divided into four groups; one control group and three treatment groups that were given a reference diuretic medication (furosemide at 10 mg/kg), and ethanolic extracts of *T. polium* (200 and 400 mg/kg). 5 hours after the administration, the volume of urine and electrolytes (Na+, K+, and Cl) excretion were calculated. Also, diuretic index, diuretic, and saluretic activities as well as carbonic anhydrase inhibition were estimated. The results showed that the 200 and 400 mg/kg treatment groups had a significant increase ($P < 0.05$) in urine volume and mild diuretic activity of 0.78 and 0.99, respectively. Compared to the control group, *T. polium* (400 mg/kg) ethanolic extract showed a significant increase ($P < 0.05$) in the excretion of K+ and Cl^-. Also, there was an increase in saluretic activity in the 400 mg/kg treatment group, as measured by the Na^++K^+, to be 528.0, compared to the control, which had a saluretic activity of 308.0. The study supports the use of *T. polium* as a diuretic in traditional folk medicine.

KEYWORDS: *Teucrium polium*; Diuretic activity; Urine volume; Furosemide; *In vivo* study

1. INTRODUCTION

Diuretics are drugs that increase the flow of urine and salt excretion. They are employed in a variety of therapeutic conditions to change the volume and content of body fluids. In clinical practice, most complete diuretics are used to reduce extracellular fluid volume by lowering total body NaCl levels [1]. Electrolyte imbalance and metabolic alterations are common adverse effects of today's diuretics, such as thiazides and loop diuretics [2]. In recent years, medicinal plants have been considered as a rich source of therapeutic substances for disease prevention, and their health benefits have been rapidly expanding. This is probably due to the effects of some plants which are similar to those of allopathic drugs [3]. Although, traditional treatments can help with several ailments, yet they need to be scientifically validated before they can be used to their full potential. The mode of action of the bulk of these traditional medicines has yet to be determined because there has been no regulatory authority to assess the appropriate use of traditional medicines. As a result, the efficacy of these traditional herbal treatments must be determined [4].

Teucrium polium L. (Arabic: "Jaa'deh") is a small, pubescent, scented shrub with oval leaves with enrolled edges and thick heads of white flowers. It is a member of the Lamiaceae family, and the genus Teucrium has ten species in Jordan's flora. *T. polium* is generally Mediterranean and can be found in countries such as Iraq, Saudi Arabia, and Egypt. From various parts of *T. polium*, various groups of chemicals have been identified. Terpenoids and flavonoids are two of the most important classes of compounds discovered in the last 40 years [5]. *T. polium* is a plant that is both edible and widely utilized in traditional medicine. It's used as

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antispasmodic, antidiabetic and diuretic [6][7]. Several studies have demonstrated that *T. polium* has a hypoglycemic effect that can help regulate glucose in the blood. Furthermore, due to the plant's indisputable influence on cancer cells, it might be considered as a natural resource [8].
The present study was aimed to evaluate the *in vivo* diuretic activity of *T. polium* aerial parts ethanolic extract of in Wistar rats.

2. RESULTS

2.1 Acute oral toxicity observation

The acute oral toxicity test of *T. polium* ethanolic extract revealed no changes in their behavioral pattern. At the test doses, no mortality was recorded within 24 hours, as well as over the next 14 days, indicating that the median lethal oral dose of the aqueous extract was greater than 2000 mg/kg in rats.

2.2 Effects of *Teucrium polium* ethanolic extract on urine volume and diuretic activity

The results obtained for the cumulative urine volume of the control, furosemide, and *T. polium* extract at 200 and 400 mg/kg doses measured at the 5th hour are presented in Table 1. The urine volume was significantly (P<0.05) increased in the *T. polium* (200 mg/kg) as well as *T. polium* (400 mg/kg) treatment groups compared to the control group. Based on urine volume in the rats, diuretic index and diuretic activity of the standard drug (furosemide) and *T. polium* at (200 and 400 mg/kg) were calculated at 5 hours which indicated that the extracts have a dose-dependent effect. Furosemide increased urine volume by a significant amount (P< 0.01).

Table 1: *Teucrium polium* ethanolic extract on urine volume, diuretic index, and diuretic activities in Wistar rats after 5 hours of treatments**.**

Groups	Urine volume (ml/5hr.)	Diuretic index	Diuretic activity
Control	1.25 ± 0.28	-	0.30
Furosemide (10 mg/kg)	4.42± 0.71**	4.38	-
T. polium (200 mg/kg)	2.80± 0.48*	2.75	0.78
T. polium (400 mg/kg)	3.60± 0.92*	3.44	0.91

Values represent mean ± SEM (n = 6); *: P < 0.05; **: P < 0.01; Compared to the control group.

2.3 Effects of *Teucrium polium* ethanolic extract on electrolyte excretion, saliuretic, natriuretic, and carbonic anhydrase inhibition

Table 2 shows the urinary electrolyte content following the administration of the *T. polium* ethanolic extract. The K^+ level was significantly (P < 0.05) increased in the *T. polium* (400 mg/kg) treatment group. Also, the ethanolic extract of *T. polium* (400 mg/kg) showed a significant (P< 0.05) saliuretic activity comparable to the control group. Furosemide elevated Na^+, K^+, and Cl^- levels significantly (P< 0.001) and had potent saliuretic activity.

Table 2: *Teucrium polium* ethanolic extract effect on urine electrolyte execration in Wistar rats after 5 hours of treatments.

Groups	Na^+ mmol/L	K^+ mmol/L	Cl^- mmol/L	Saliuretic activity	Natriuretic activity	Carbonic anhydrase inhibition
Control	112.33± 7.017	114.5±17.11	195.67±18.26	308.0 ± 21.14	1.085 ± 0.15	0.87±0.06
Furosemide (10mg/kg)	270.67± 33.78***	254.5±22.92***	443.33±42.95***	714.0±47.79**	1.064 ± 0.14	0.89 ± 0.12
T.polium (200mg/kg)	127.33±57.10	150.0± 8.802	400.67± 98.96	528.0 ± 110.4	0.90 ± 0.19	1.40 ± 0.28
T.polium (400mg/kg)	158.0± 23.31	166.16± 8.530*	428.17± 73.05*	586.2 ± 64.42*	0.99 ± 0.22	1.39 ± 0.29

Values represent mean ± SEM; n = 6; *: P < 0.05; **: P < 0.01; ***P < 0.001; Compared to control group.

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3. DISCUSSION

Diuretics are medications that increase urine flow and salt excretion, and they're used to control body fluid content in a variety of clinical situations like hypertension, heart failure, nephrotic syndrome, and cirrhosis. Loop diuretics, such as furosemide, can improve urine flow while also functioning as a potent saliuretic, boosting salt and chloride output in the urine [17]. In this study, furosemide was used as a standard drug. It increases urinary excretion of sodium by inhibiting the $Na^+/K^+/Cl^-$ co-transporter system in the thick ascending limb of the Henley loop [18]. As a result of rising acceptance and public interest in both developed and developing countries, the use of herbal medicines and phytonutrients/ nutraceuticals has been on the rise. Herbs and natural plant products are becoming increasingly popular in the treatment of cardiovascular illnesses and their complications [19][20]. This research was based on the use of the plant as a diuretic in folklore. In Wistar rats, the effect of an ethanolic extract of aerial parts of *T. polium* on renal excretory function was investigated.

Acute toxicity investigations on the rats revealed no changes in their behavioral patterns. At the test doses, no mortality was recorded. *T. polium* caused an increase in urine volume at both dosages of the extract and an increase in electrolyte excretion at 400 mg treatment dose of the extract at the 5th hour in normal rats. This observation is consistent with a previous study that found a relationship between diuretic action and *T. polium* which demonstrated that ethanolic extracts of *T. polium* (20 and 50 mg/kg) significantly increased urine volume [21].

The diuretic activity is considered good when its value is greater than 1.50, moderate if it is between 1.00 and 1.50, mild if it is between 0.72 and 0.99, and nil when it is less than 0.72[22]. *T. polium* ethanolic extract (200 and 400 mg/kg) showed mild diuretic activity of 0.78 and 0.99 and a dose-dependent increase in the diuretic index of 2.75 and 3.44.

Based on the electrolyte content of urine, patterns of influence on ion excretion of *T. polium* ethanolic extract indicated that only the highest dose (400 mg/kg) of the ethanolic extract resulted in a significant increase in urine excretion of K^+ and Cl^- ions compared to the negative control. However, the results of the present study contradict previous findings, which found that lower doses of *T. polium* ethanolic extract (20 and 50 mg/kg) significantly enhanced K^+ and Cl^- ions [21]. Many variations might play a role in these results, like: the species of rats (Wister or Albino); weight; age; experimental conditions; extraction methods; the concentration of bioactives. Na^+ excretion levels in the urine did not differ significantly between the lower and higher doses of *T. polium* extract. When calculating the Na+: K+ ratio for natriuretic activity, values more than 2 suggest a positive natriuretic effect, whereas ratios greater than 10 indicate a potassium-sparing effect [23]. The Na^+: K^+ ratio was not increased by *T. polium* ethanolic extract (200 or 400 mg/kg), demonstrating that the plant has a low natriuretic impact. Carbonic anhydrase inhibition is calculated using the Cl^-: $Na^+ + K^+$ ratio. At ratios between 0.8–1, carbonic anhydrase inhibition may be excluded. No carbonic anhydrase inhibition can be expected as the ratio increases [24]. The carbonic anhydrase indices of the 200 and 400 mg/kg *T. polium* extract treatment groups were 0.90 and 0.99, respectively.

The active principle(s) responsible for the diuretic activity of the ethanolic extract of *T. polium* is/are, so far, not known. Phytochemical screening revealed the presence of diterpenoids, monoterpenes, sesquiterpenes, polyphenols, flavonoids and fatty acid esters [25]. Over 85 species from a wide range of groups of medicinal plants have been found to have diuretic properties. Secondary compounds found in these plants include terpenes, phenolics, and alkaloids, among other types of compounds [26]. It is fair to believe that these secondary metabolites may act solely or synergically to cause the diuretic effect.

4. CONCLUSION

The findings of this study revealed a mild diuretic activity found in ethanolic extract of *T. polium* specifically when a high dose was administered. It produced a notable effect on both K^+ and Cl^- execration ratio and urine volume. This study validates this plant's traditional claim as a diuretic agent.

5. MATERIALS AND METHODS

5.1 Plant material

T. polium arial parts were bought at a traditional herbal market in Amman, Jordan. With the help of Prof. Sawsan Oran, the taxonomic identity of the plant was determined by comparing it to those of known identity found in the herbarium of the Department of Biological Science, Faculty of Science, University of Jordan, Amman, Jordan. The Pharmacognosy Laboratory, Department of Pharmaceutical Sciences, Pharmacy Collage, Zarqa University, Zarqa, Jordan, was allocated and lodged with Voucher Specimen No. TAC2021.

5.2 Preparation of plant extract

In a Soxhlet device, the ground and dried aerial portions of *T. polium* (40 g) were extracted with 70% ethanol at 60°C until the refluxed solvent became colorless. Separately, the extract was evaporated to dryness in a rotary vacuum evaporator before being weighted. The concentrated extract (14 g) was stored at 4°C until subjected to toxicological and diuretic tests.

5.3 Experimental animals

Male and female Wistar laboratory rats (7-8 weeks), weighing 170-200g, were obtained from Applied Science University animal house and kept in polypropylene cages at a constant temperature (22-25 °C) and relative humidity (50-60%) for 12 hours of light/dark cycles. They were given unlimited access to normal pellet food and water. The acclimatization period was 3 days. All experimental protocols were authorized by the Research and Ethical Committee at the Faculty of Pharmacy, Applied Science University, Amman, Jordan. The committee ensured that the animals were cared for and used in compliance with normal ethical principles.

5.4 Acute toxicity testing

T. polium acute toxicity was investigated using the acute toxic class approach of acute oral toxicity determination outlined in the Organization for Economic Co-operation and Development (OECD) 423 standards [9]. *T. polium's* safety profile had previously been described, as well as a limit test. Nulliparous and non-pregnant female Wistar rats (n = 6) were given the highest dose (2000 mg/kg). For a period of 14 days, dosed rats were monitored for clinical symptoms of toxicity. Based on the Globally Harmonised Categorization System for Chemical Substances and Mixtures (GHS) classification, the highest acceptable dose of *T. polium* was calculated [10][11].

5.5 Assessment of diuretic activity

The Lipschitz test was used to investigate the diuretic efficacy of an ethanolic extract of *T. polium* aerial parts in Wistar rats [12][13][14]. None fasted rats of either sex were assigned into four groups (n= 6)/(3males: 3 females). Group I served as the control. The animals in Group II were treated with furosemide (10 mg/kg) in a vehicle that served as a standard group. Groups III and IV received 200 and 400 mg/kg ethanolic extracts of *T. polium* in the vehicle, respectively, and immediately after the extract treatment, all the rats were hydrated with saline (0.9% NaCl) at an oral dose of 15 ml/kg, imposing uniform water and salt balance. The animals in each of the groups were placed in the metabolic cages. Carboxy methyl cellulose (CMC; 0.5% w/v) was used.

5.6 Estimations of urine volume and urinary electrolytes

At the end of the 5 hours, the total volume of urine collected was measured. The animals were not provided with food or water during this time. Diuretic index and diuretic activity were determined using the formula:

Diuretic index = Urine volume of the treatment group / Urine volume of the control group
Diuretic activity = Diuretic action of the treatment group/ Diuretic action of the standard group [15].

For saliuretic activity, the total Na+ and Cl- excretion was calculated. The ratio of Na+ to K+ was used to calculate natriuretic activity.

To assess carbonic anhydrase inhibition, the ratio Cl^- / Na^+ + K^+ was calculated [16]. Urine electrolytes (sodium, potassium, and chloride) were determined by the Alitar 240 chemical analyzer (EKF Diagnostic, UK) method as described in the user instruction manual of the biochemical kits (Jenway, UK).

5.7 Statistical analysis

The experimental data were expressed as mean± SEM (n = 6). Data were analyzed with SPSS software. The one-way analysis of variance (ANOVA) was used for comparing the means, followed by *Dennett's* t-test.

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