

## Effect of *Teucrium polium* aqueous extract on mice sperm

**WASNAA H. MOHAMMED  
AND ENASS M. MAHMUD**

**Bio-Chemical technology  
branch, Applied science  
department, Technology  
University, Baghdad, Iraq**

### Abstract

There are several reports about using *Teucrium polium* (TP) in traditional medicine mainly for its antipyretic, antibacterial, anti-inflammatory, antioxidant, antialcerogenic, antispasmodic, anti diabetic, hepatotoxicity and for fertility. Male mice was orally administrated by two concentration (50 mg/kg, 100 mg/kg) of TP aqueous extract to study the effect of this aqueous extract for period of time on fertility (live sperm, motility and abnormality). The result showed that there is significant difference ( $P < 0.05$ ) (in live sperm and motile sperm) between group treated with 100 mg/kg with control, while there was a significant difference in abnormality between the treated groups with the control. The increase in testes weight also indicate the positive effect referring to some TP constituents in the treated groups. The investigation has shown that the extract possesses promising fertility property which can be exploited in fertility therapy.

**Keywords:** *Teucrium polium*, aqueous extract, sperm

### Introduction

The use of plants for medical purposes dates back to the ancient times. For example Lamiaceae or Labiatae, also known as the Mint family, is a family of plants comprises about 210 genera and 3,500 species. One of the most popular species of this family native to the Mediterranean region and the Middle East is *Teucrium polium* (TP) which has been used for

over 2000 years in traditional medicine mainly for its antipyretic (1, 2), antibacterial (3), anti-inflammatory (4), antioxidant (5, 6), antiulcerogenic (7), antinociceptive (8, 9), antidiabetic (10, 11), antispasmodic (12, 13, 14) and hepatotoxicity (15). Phytochemical analyses of TP have identified several constituents comprising mostly flavonoids, sesquiterpenoids, neoclerodane diterpenoids (16, 17), tannin and alkaloids (18, 19), selenium (20) and coumarin (21, 22).

## **Material and Methods**

### ***Preparation of plant extract***

Aerial parts of *Teucrium polium* (TP) was dried for 7–10 days at room temperature. The dried plant material (25 g) was heated in 250 ml of distilled water for 15 min at 95 °C, followed by rapid filtration through a cellulose filter and then Whatman No.1 filter paper and dried on 40 °C (23). The dried extract was dissolved in distilled water to get two different concentrations 50 mg/kg and 100 mg/kg.

### ***Animals***

Healthy adult mice of Swiss albino strain were obtained from animal house of Biotechnology Research Center/ Al-Nahrain University. 60 mice male were used in this study, the age of these experimental animals were in the range between 14 - 16 week old at the beginning of experiment and the weight range was between 20-25 grams. The animals were kept under suitable environmental conditions such as the C and exposed to temperature of room was maintained at about  $24 \pm 2$  14 hour day light program daily. Tap water and food in the form of pellet were accessible freely to them.

The plant extract was administrated orally by micropipette to 20 male mice of group II daily in a dose of 0.1 ml/mice (equivalent to 50 mg plant extract/kg body weight) for 8 consecutive weeks and to 20 male mice of group III daily in a dose of 0.1 ml/mice (equivalent to 100 mg plant powder/kg body weight) for 8 consecutive weeks (10, 11). The control healthy mice (group I, n= 20).

### ***Sperm collection***

The sperm was collected from the male mice after sacrificed and both vas deferens were excised and washed by culture medium, then sperm were collected by flushing of vas deference by sterile syringe G-28 containing 1 ml of tissue culture medium-199 (TCM-199) (24).

### ***Microscopically examination***

Spermatozoa were assessed according to WHO laboratory manual for viability, activity, motility and abnormalities (25), the spermatozoa were diluted in a counting medium (3.2% sodium citrate with 1% formaldehyde) in order to produce a concentration of approximately 20–30 X 10<sup>6</sup> sperm/ml. Diluted sperm suspension was loaded to fill the hemocytometer counting chamber exactly. A total of 5 “large” squares were counted on the hemocytometer, each square being 200 μm<sup>2</sup> and 10 μm in depth.

### ***Statistical analysis***

Statistical analysis was done using SPSS version 7.5 computer software (statistical package for social sciences).

The statistical significance of difference in mean of continuous dependent (normally distributed variable) between more than 2 groups was assessed by ANOVA test (26).

## **Result and Discussion**

### ***Body weight***

There are many reports that show *Teucrium polium* is widely used in traditional and herbal medicine for different medical purposes (27).

Some reports have shown important effects of *Teucrium polium* on body weight and reproductive system in mice (28). Comparison the changes in body weight before and after administration of *Teucrium polium* extract showed the following results that there are significant differences (P<0.05) between non treated groups (initial weight and group I (control)) with the TP treated groups (group II and III) (table 1).

Table 1: weights (g) of animals before and after treatment with TP (mean ± St. Dev).

<b>Parameters</b>	<b>Group I control</b>	<b>Group II treated with 50mg/kg body weight</b>	<b>Group III treated with 100mg/kg body weight</b>
<b>Initial weight (g)</b>	22.44 ± 1.19	22.66 ± 1.58	22.34 ± 1.57
<b>Final weight (g)</b>	27.30 ± 0.99	30.65 ± 1.64	30.98 ± 1.82

Total n= 60



P<0.05 (significantly different between non treated groups and treated groups) The significant differences between group II, group III with the control shows that TP contain materials have very important roles in metabolism such as amino acids and alkaloids (18, 19, 29). Our result similar to (28) there is increase in body weight after treated with TP, while (23, 30) showed that the body weight did not affected after treated with TP.

Comparison of the testes weight among the three groups showed that there are significant differences between the groups. This significant differences in testes weight related to coumarin (one of the teucrium components) which cause accumulation of water in testes (31, 32), and the increasement in the weight and volume of the testes with the control group might be related to the proliferative and differentiating changes in the surface epithelium of the seminiferous tubules which enhance the active role of the extract in spermatogenesis (33, 34). Our result similar to (28, 35), while the results not agree with (30), which said there is a reduction in weight of testes of male mice.

Seminal fluid examination of the mice showed that the count, morphological appearance, live, abnormality and motility of sperm, were no significant differences in live sperm between group II and control, while there are significant differences (P<0.05) between group III with control and group II (table 2).

Table 2: testes weight (mg), sperm (live, motility and abnormality) of treated and control animals (mean  $\pm$  stdev).

<b>Parameters</b>	<b>Group I control</b>	<b>Group II treated with 50mg/kg body weight</b>	<b>Group III treated with 100mg/kg body weight</b>
<b>*Testes weight (mg)</b>	71.80 $\pm$ 2.94	79.89 $\pm$ 2.98	87.59 $\pm$ 3.90
<b>**Live sperm</b>	65 $\pm$ 4.63	70 $\pm$ 3.78	75.63 $\pm$ 4.17
<b>**Sperm motility</b>	65.63 $\pm$ 5.63	70.66 $\pm$ 7.29	76.88 $\pm$ 5.30
<b>***Sperm abnormality</b>	33.91 $\pm$ 4.03	24.08 $\pm$ 3.23	20.33 $\pm$ 2.08

Total n= 60

Control n= 20

\*P<0.05 (significantly different in testes weight among groups)

\*\* P<0.05 (significantly different between group III with the group I and II)

\*\*\* P<0.05 (significantly different between control with the treated groups)

The observed increase in live sperm in the treated groups was dose dependant. There is no significant difference ( $P>0.05$ ) between group I and group II, while there is significant difference between these groups with group III. Although the mechanism of action of the extract for the increased live sperm is yet to be elucidated, it may however be connected with the induction of the germinal epithelial cells called spermatogonia to continuously proliferate to replenish themselves to differentiate through definite changes of developments (28, 36). Our result similar to (28) in the increasement of live sperm.

There were no significant differences in sperm motility between group I and group II, while there are significant differences ( $P<0.05$ ) between group III with previous groups (table 2).

Data for sperm motility and progressively (table 2) showed a significant increase in group III (treated with 100 mg/kg) with group I & II this could be due to the influence of the extract of the mitochondria in the body of the tail of the spermatozoon to synthesize energy in the form of triphosphate (36).

Also there were no significant differences in sperm abnormality between group II and group III, while there are significant differences ( $P<0.05$ ) between these groups with control (table 2).

The results agree with (20) that the abnormalities reduced according to presence of selenium in the composition of TP (contained 0.020-0.055 mg selenium for each kg of TP) (37).

In conclusion, this plant has been demonstrated to have a promising profertility property which can be exploited in fertility therapy.

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### الخلاصة

استخدم نبات الجعدة *Teucrium polium* في الطب البديل وذلك لفعاليتيه كمضاد للبرد والحمى ومضاد بكتيري ومضاد للالتهابات ومضاد للاكسدة وفي علاج القرحة وعلاج المغص المعوي وعلاج السكري ويزيد الخصوبة. تم تجريع ذكور الفئران بتركيزين مختلفين ( 50 mg/kg, 100 mg/kg) من المستخلص المائي لنبات الجعدة وقد اظهرت النتائج وجود فروق معنوية ( $P<0.05$ ) في نسبة الحيوانات المنوية الحية وفعاليتها الحركية بين مجموعة السيطرة والمجموعة المعاملة بالمستخلص ذو التركيز (100 mg/kg) وايضاً سجلت الدراسة وجود فروق معنوية ( $P<0.05$ ) في انخفاض تشوهات الحيوانات المنوية للمجاميع المعالجة بالمقارنة مع مجموعة السيطرة. لقد اظهرت هذه الدراسة وجود زيادة في وزن الخصى بعد معاملتها بالمستخلص المائي وقد فسرت هذه الزيادة بناءً على مكونات المستخلص الحيوية في زيادة الخصوبة. اظهرت التجارب والتحريات بان المستخلص يملك صفات اخصابية واعدة والتي يمكن ان تستخدم في علاج الخصوبة.

