Histological And Functional Study of White Male Mice Testes and Kidney Treated With Tecrium Polium Aqueous Extract

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Abstract
The oral administration of the aqueous extract of Tecrium polium to a mature male mice in daily oral doses of 50 mg/kg body weight (group II) and 100 mg/kg body weight (group III) for eight consecutive weeks were resulted in significant increase (P<0.05) in the testicular weight in the treated groups in comparison with control. The aqueous extract affected the spermatogenesis which demonstrated by an increase in Leydig cell, spermatogonia, spermatocytes and spermatozoa. The hormonal profile was influenced by the aqueous extract. The testosterone level was significantly increased (P<0.05) in the treated groups, but no significant differences (P>0.05) in urea and uric acid levels between control and the treated groups were shown. The kidneys of the group III were affected and showed degenerative changes and necrosis in nucleolus of cell of tubules and there is destruction in glomular by increscent of mesenchymal cells.

Keywords: Tecrium polium, testosterone, testes, urea, uric acid, kidney

Introduction
Tecrium polium (TP) is a member of the Lamiaceae family which is widely grown in Middle East countries. Aerial parts of TP have been used in traditional medicine for various purposes such as anti-hypertensive, antibacterial,
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fertility problems, carminative, anti-
noicceptive, anti-inflammatory, anti-
diarrhea, anti-diabetes, liver 
disorder, kidney stones and anticonvulsant [1, 2, and 3]. Despite 
these huge achievements on the 
successful isolation of some 
important phytochemicals, there is 
very little literature concerning the 
study of the effect of Teucrium polium on vital organs such as the 
testes. We therefore set to elucidate 
the possible effects of aqueous 
e xtract of Teucrium polium on the 
function of the testes as well as 
evaluate the medical significance of 
chronic treatment with TP on the 
biological composition of blood 
and histopathology of kidney. This 
will in a way guide the usage and 
dosage of this important plant [4].

Materials 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 
ºC [5]. The dried extract was dissolved 
in distilled water to get two different 
concentrations 50 mg/kg and 100 
mg/kg mice body weight.

Experimental animals and 
treatment
Healthy adult mice of Swiss albino 
strain were obtained from animal 
house of Biotechnology Research 
Center/ Al-Nahrain University. 60 
amale mice 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 
temperature of room was maintained 
at about 24 ± 2°C and exposed to 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 in two different concentrations 
by micropipette to 20 male mice of 
group II daily in a dose of 0.1 
ml/mice (equivalent to concentration 
of 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 
concentration of 100 mg plant 
powder/kg body weight) for 8 
consecutive weeks [6, 7]. The 
control healthy mice (group I, 
n= 20).

Hormonal and biochemical 
analysis:
Blood samples were collected by 
cardiac puncture and immediately 
was separated by centrifuge at 3000 
rpm for 3 minutes.

Hormonal assay
Bio merieux Italia S.P. a vidia 
camplignano, 58 50015-point A 
EMA (F1) Italia miniVIDAS. Was 
used for the hormonal assay 
(Testosterone hormone).
C. Bio merieux Sa.69230 marcy 
lEtoile-France, testosterone for 30 
sample (test), code No. 09345B.
In testosterone test the assay 
principle combines an enzyme 
immuno assay sandwich method 
with a final fluorescent detection 
(ELFA).
1- only remove the required 
reagents from the refrigerator 
and allow them to come to room
temperature for at least 30 minutes.

2- Use one TES strip and one TES SPR from the kit for each sample, control to be tested.

3- Select TES to enter the code. The calibrator must be identified by "S1", and tested in duplicate. If the control needs to be tested, it should be identified by C1.

4- Mix the samples, the calibrator and/or the control using vortex type mixer.

5- Pipette 200 \( \mu l \) of sample, control into the sample well.

6- Insert the VIDAS SPRs and strips into the positions indicated on the screen. Check to make sure the calibrator strip with the three letter assay code on the SPRs and the reagent strip match.

7- Initiate the assay processing as directed in the operator's manual. All the assay steps are performed automatically by the instrument. The assay will be completed within approximately 60 minutes.

8- After the assay is completed, remove the SPRs and strips from the instrument.

9- After the assay is completed, dispose of the used SPRs and strips into an appropriate recipient.

Biochemical assay
Urea was measured by urease \( \backslash \) salicylate enzyme method in the presence of nitroprusside as coupling agent to yield a blue cromophore. the intensity of the color formed is proportional to the concentration of urea in the sample, read the absorbance (A) of the samples and the standard at 600 nm against the reagent blank [8].

Uric acid measured by kit:CE uric acid, uricase-POD. enzymatic method, SPINREACT, SA.SPAIN. Uric acid is oxidized by uricase to allantoinie and hydrogen peroxide, under influence of peroxidase and 4-aminophenzone-4-aminophenzone and 2-4 dichlorophenol sulfonates from red quinoneimine compound, the intensity of the red color formed is proportional to uric acid concentration in the sample read the absorbance (A) of the samples and the standard at 600 nm against the reagent blank [8].

Histological analysis
Testes and kidney were fixed in Bowin's solution, passed through ascending series of ethanol and then through xylene, and embedded in paraffin wax. Tissues were sectioned at 5 \( m \) and stained with haematoxyline and eosin [9]. The evaluation of the cell population was based on the calculations made for each cell type per cross section of the renal tubules in kidney. Spermatogonia, spermatocytes in testes were counted under 40X and 100Xmagnification [11].

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 [10].
Results And Discussion
The results (table 1) showed that there are significant differences (P<0.05) in testes weight among the three groups. The oral administration of *Teucrium polium* (TP) aqueous extract resulted in a significant increase (P<0.05) in testicular weight, which is known to be mostly related to the number of spermatids and spermatozoa present in the tissue [4]. The increase in the weight of the testes as compared with the control group might be related to the proliferative and differentiation changes in the surface epithelium of the testes which enhance the active role of the extract in spermatogenesis [11, 12]. Spermatogenic cells and Leydig cells might be the consequence of intracellular redistribution of water and ions [13]. The diminished mitochondrial function in the degenerating cells resulted in reduction in ATP and ATPase causing failure of the active transport system. So accumulation of sodium will retain water intracellularly. It led to the swelling of the cell [14]. The inactive spermatozoa travel through the straight ducts of testes to reach to the epididymis for maturation under the influence of various enzymes and factors released by Sertoli and epididymal mucosal cells [13]. They pass to the vas deferens to get further maturation and motility under the influence of various enzymes secreted by the mucosal cells of vas deferens. These mature and active spermatozoa stay in the vas deferens till they are ejaculated along with prostatic and seminal vesicle secretions in the semen. Any abnormalities in the functioning of mucosal cells of epididymis and vas deferens may result in a defective maturation of spermatozoa leading to male infertility. The administration of TP (50 mg/Kg body weight and 100 mg/kg body weight) daily for two months has shown histological changes in testis (Figure 1, 2, 3 and 4). The spermatogenesis in mammals depends on testosterone production by Leydig cells in response to stimulation by FSH and LH. FSH increases Sertoli cell synthesis of an androgen binding protein needed to maintain high concentrations of testosterone [15].

The administration of various doses of the plant extract resulted in a significant increase (P<0.05) in serum testosterone concentration between control with treated groups (table 1). Although a number of alkaloids and coumarins have been isolated from TP [16, 17], this might enhance the secretion of the accessory organs by affecting the neural control of the secretory process and/or by increasing the amount of secretory epithelium in the glands. Although Al-Ashban et al. (2006) [14] indicated that the ethanolic extract of TP reduced testosterone on the contrary the results of the present investigation indicate an increase in testosterone level. This discrepancy could be attributed to the difference in the TP extraction procedure. Therefore the crude aqueous extract cause a testosterone stimulatory effect on the
target organs, which effect on motility and morphology [18].

As shown in table (2) the various biochemical parameters of blood in the three group of mice

Urea is the principal end product of protein catabolism. No significant differences (p>0.05) in urea level in serum in treated groups with TP. In comparison, an elevated level of urea in rat exposed to TP an increase in the level of urea might be the result of impairment of the normal kidney function [19, 20]. Malfunctioning in glomerular filtration result in the retention of substances including urea and this might be responsible for high level of urea [21].

On the other hand the elevated urea levels in serum may due to destruction of the RBCs [22].

No significant differences (p>0.05) in uric acid in serum in the treated group. Uric acid is the end product of catabolism (42 h) the level of uric acid in blood can build up in the body, the increasment in uric acid concentration may be due to degradation of purines or to an increase of uric acid levels by either over production or inability of kidney remove it from blood normally [19].

The result of histopathological studies as shown in figure 5, 6 and 7. The section from group 1 showed normal histology of the kidney of mice, the tubules and renal corpuscles with glomerulus’s and glumerular capsule are very clear and promint. Kidney of mice treated with TP concentration (50mg/kg), were not much effected while kidney of treatment group with high dose of herb concentration (100mg/kg) shows vacuolation, moderativ degenerative changes and necrosis in nucleolus of cell of tubules and there is destruction in glumerular by increscent of mesenchyma cells. This study is agreement with [19 and 20] which they accentual phytotoxic effect of medicinal plant TP on kidney. In conclusion the stimulatory effect of aqueous extract of TP seem to be mediated the physiological events of spermatogenesis and also renal dysfunction in mice treated with aqueous extract of TP.

References


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Ethnopharmacol, 24 (1): 93-99, 1988


[20] M. Iriadam, D. Musa, H. G.m.<span class="redacted" data-redacted-not-redacted="false" data-redacted-redacted="true">3</span>han3 and F. sun Baba, Effects of two Turkish medicinal


Table (1) Testes weight and testosterone level (mean±SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testes weight (mg)</td>
<td>71.80 ± 2.94</td>
<td>79.89 ± 2.98</td>
<td>87.59 ± 3.90</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>0.41 ± 0.06</td>
<td>0.50 ± 0.07</td>
<td>0.57 ± 0.08</td>
</tr>
</tbody>
</table>

P < 0.05 (significantly different).

Table (2) Effect of aqueous extract of TP on some biochemical parameters (Urea and Uric acid) (mean±SD)

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Group I (control)</th>
<th>Group II (50mg/kg)</th>
<th>Group III (100mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mmol/l)</td>
<td>70.68±4.78</td>
<td>69.80±7.00</td>
<td>70.76±3.96</td>
</tr>
<tr>
<td>Uric acid (μmol/l)</td>
<td>50.70±10.4</td>
<td>40.73±10.00</td>
<td>44.50±10.50</td>
</tr>
</tbody>
</table>

P > 0.05 (not significantly different).
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Figure (1) Histological section of male mice testes not treated with T.P (group I= normal)

Figure (2) Histological section of testes treated with T.P conc. 50mg/kg body weight (group II)

Figure (3) Histological section in testes treated with T.P conc. 100mg/kg body weight (group III)

Figure (4) Effect of Teucrium polium extract on spermatogonia and spermatocytes
Figure (5) Histological section of kidney not treated with T.P (group I)

Figure (6) Histological section of kidney that treated with T.P conc. 50mg/kg body weight (group II)

Figure (7) Histological section of kidney treated with T.P conc. 100mg/kg body weight (group III)