

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 Teucrium 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 glomerular by increscent of mesenchymal cells.

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

دراسة نسيجية ووظيفية لخصى وكلى ذكور الفئران البيض المعاملة بالمستخلص المائي لنبات الجعده

الخلاصة

تم التجريب اليومي لمجاميع ذكور الفئران البالغة لمدة ثمانية اسابيع بتراكيز 50 ملغم/كغم من وزن الجسم (المجموعة II) و 100 ملغم/كغم من وزن الجسم (المجموعة III) من المستخلص المائي لنبات الجعده (Teucrium polium) وتم تسجيل زيادة معنوية ($P < 0.05$) في وزن الخصى في المجاميع المعاملة مقارنة مع مجموعة السيطرة، كما اظهرت النتائج تأثير المستخلص على عملية نشأة النطف (Spermatogenesis) من خلال وجود زيادة في خلايا لايدك، امهات المنى، الخلايا المنوية و الحيوانات المنوية فضلاً عن وجود زيادة معنوية في مستوى هرمون الشحمون الخصوي (testosterone) لدى المجاميع المعاملة، ولم توجد فروقات معنوية لنسبه اليوريا وحامض اليوريك بين مجموعة السيطرة والمجاميع المعاملة، ولكن اظهرت الدراسة تأثر انسجة الكلى في المجموعة المعاملة الثالثة بالتراكيز العاليه من المستخلص المائي للنبات مما أدى الى تحلل ونخر للانوية الموجوده داخل الانسجة وتدمير النبيبات الكلويه عن طريق زياده حجم الانسجة.

Introduction

Teucrium 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,

fertility problems, carminative, anti-nociceptive, 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 Tecrium polium on vital organs such as the testes. We therefore set to elucidate the possible effects of aqueous extract of Tecrium polium on the function of the testes as well as evaluate the medical significance of chronic treatment with TP on the biochemical 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 Tecrium 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 [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 male 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 campligliano, 58 50015-point A EMA (F1) Italia miniVIDAS. Was used for the hormonal assay (Testosterone hormone).

C. Bio merieux Sa.69230 marcy l'Etoile-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 µl 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 color labels 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. Dipose of the used SPRs and strips into an appropriate recipient.

Biochemical assay

Urea was measured by urease \ salycliate enzymate 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-aminophenazone-4-aminophenazone 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 µm 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 100X magnification [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 I 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.

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

Parameter	Group I	Group II	Group III
Testes weight (mg)	71.80 ± 2.94	79.89 ± 2.98	87.59 ± 3.90
Testosterone (ng/ml)	0.41 ± 0.06	0.50 ± 0.07	0.57 ± 0.08

P < 0.05 (significantly different).

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

biochemical parameters	Group I (control)	Group II (50mg/kg)	Group III (100mg/kg)
Urea (mmol/l)	70.68±4.78	69.80±7.00	70.76±3.96
Uric acid (µmol/l)	50.70±10.4	40.73±10.00	44.50±10.50

P > 0.05 (not significantly different).

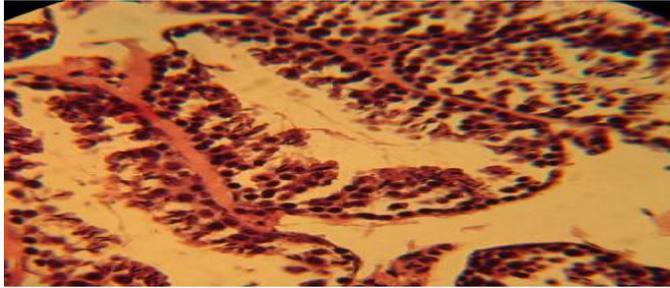


Figure (1) Histological section of male mice testes not treated with T.P (group I= normal)

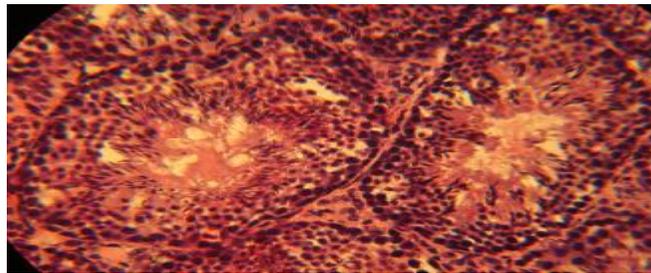


Figure (2) Histological section of testes treated with T.P conc. 50mg/kg body weight (group II)

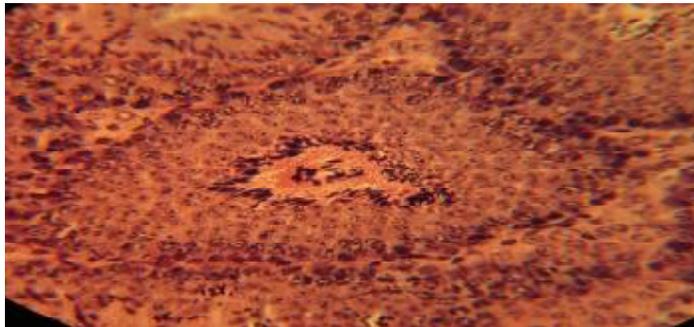
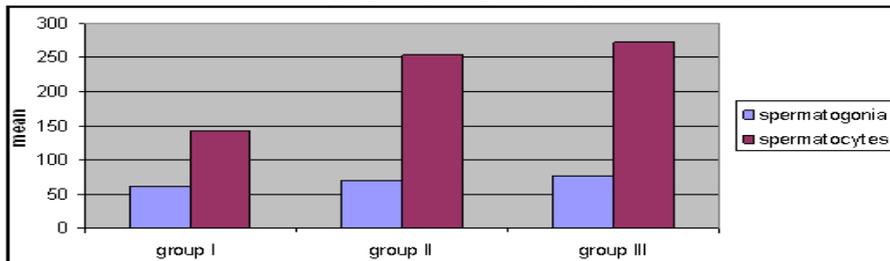


Figure (3) Histological section in testes treated with T.P conc. 100mg/kg body weight (group III)



Figure(4) Effect of Teucium polium extract on spermatogonia and spermatocytes

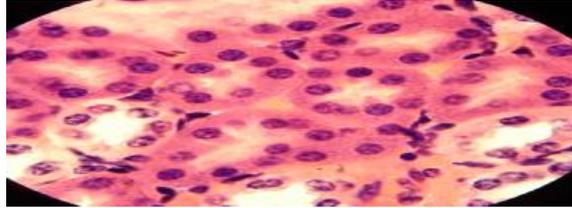


Figure (5) Histological section of kidney not treated with T.P (group I)



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)