

EFFECT OF ORAL ADMINISTRATION OF *CONVOLVULUS ARVENSIS* CRUDE EXTRACT IN REPRODUCTIVE PERFORMANCE OF ALBINO MALE RATS

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ABSTRACT

This study was conducted to investigate the effect of alcoholic extract of the *Convolvulus arvensis* (*C. arvensis*) on the reproductive performance and fertility of Albino male rats as the following parameters: testes weight, seminal vesicles, prostate gland weights, testosterone concentrations in the serum, some sperm characteristics, structural changes in testicular tissue, as well as the reproductive indices (fertility index). Forty adult male rats (180-200gm) were randomly divided into four equal groups, 1st group was not treated with *C. arvensis* extract and considered as control group while the other three groups were treated with 400, 600 and 800mg/kg B.W of *C. arvensis* orally using special gavage tube for rats and considered as T1, T2 and T3 respectively. Serum testosterone concentrations were determined at zero (before treatments) and after 35 days of experiment. After 35 days of experiment, 8 rats weighted from each group and sacrificed to collect the testicular, epididymis, seminal vesicles, prostate gland weights and examined the concentration, motility, morphology, viability of the sperms. Histological sections of the testes were done for structural changes in testicular tissue. The results showed that treatment with *C. arvensis* led to significant ($P < 0.05$) decrease in serum testosterone concentrations and a significant ($P < 0.05$) decreased in the epididymis, seminal vesicles, prostate gland weights and in sperm concentration, motility, morphology, viability as well as abnormal structural changes in testicular tissue. Reproductive indices examined in non treated females mated with treated rats showed a significant ($P < 0.05$) decrease in the fertility index. In conclusion, negative effect of *C. arvensis* was noticed on testes functions manifested by some histological changes resulting in a decrease of testosterone and inhibition of spermatogenesis.

Key words: testosterone, alcohol extract, antifertility, spermatogenesis.

فاضل وأسحق

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تجريب المستخلص الخام لنبات المديد وتأثيره في الاداء التناسلي لذكور الجرذان البيض

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المستخلص

أستخدم المستخلص الكحولي لنبات المديد لدراسة تأثيره على بعض صفات الكفاءة التناسلية والخصوبة في ذكور الجرذان البالغة وهي: وزن الخصية والبربخ والحويصلات المنوية وغدة البروستات وتركيز الشحوم الخصوي في مص الدم وتركيز النطف وفعاليتها ومظهرها والتشوهات و التغيرات في التركيب النسيجي للخصية وبعض الدلائل التناسلية مثل دليل الخصوبة. أستخدم في هذه الدراسة 40 من ذكور الجرذان البالغة قسمت عشوائيا الى اربع مجاميع متساوية، الاولى لم تعامل بمستخلص نبات المديد وتم اعتبارها كمجموعة مقارنة. أما المجاميع الثلاث الاخرى فقد تمت معاملتها بالمستخلص الكحولي بجرعة 400 و 600 و 800 ملغم/كغم وزن الجسم وتم اعتبارها كمجموعة معاملة أولى (م1) ومجموعة معاملة ثانية (م2) ومجموعة معاملة ثالثة (م3) بالتتابع. تم قياس مستوى هرمون الشحوم الخصوي في مص الدم في الأيام صفر قبل بدء التجريب، وبعد 35 يوم من التجربة. بعد 35 يوم من التجربة وزنت 8 جرذان من كل مجموعة وتم التضحية بها للحصول على أوزان الخصي والبربخ والحويصلات المنوية وغدة البروستات وفحص تركيز النطف وفعالية النطف ومظهر النطف ونسبة النطف الميتة. تم عمل المقاطع النسيجية لمعرفة التغيرات النسيجية في تركيب الخصي. أوضحت نتائج التجربة أن المعاملة أدت إلى انخفاض معنوي في تركيز هرمون الشحوم الخصوي، انخفاض معنوي في وزن الاجهزة التناسلية (الخصي والبربخ والحويصلات المنوية وغدة البروستات)، انخفاض معنوي ($P < 0.05$) في معدل تركيز النطف والنسبة المنوية لحركة النطف والنسبة المنوية للنطف الحية وتغيرات نسيجية في تركيب الخصي. هذه النتائج رافقها ارتفاع معنوي في تشوهات النطف في الثلاث مجاميع المعاملة مقارنة بمجموعة السيطرة. أما بالنسبة للاداء التناسلي (متمثلا بالخصوبة) في أنثى الجرذان الطبيعية (غير المعاملة بمستخلص نبات المديد) بعد تزاوجها من ذكور الجرذان المعاملة فقد أوضحت النتائج أن النسب المنوية للاخصاب أنخفضت معنوياً مقارنة بمجموعة السيطرة. يستنتج من هذه الدراسة أن المعاملة بمستخلص نبات المديد أثر سلبياً على وظائف الخصي في الجرذان وقد أظهرت تغيرات نسيجية أدت إلى انخفاض مستوى الشحوم الخصوي وكافة المعايير الأخرى.

الكلمات المفتاحية: التستوستيرون، المستخلص الكحولي، مانع الخصوبة، تكوين الحيامن.

INTRODUCTION

Several plants are reported to enhance reproductive processes but, on the other hand, to also hinder reproductive functions (1, 2, 3). Several plants and plant products are reported to impede all functions of reproductive system in many different animal species such as rats, dogs, humans and monkeys (4, 5, 6). The effects of plants on reproductive system functions could be rightly compared with those of a double-edged sword. Field bindweed is native to Europe and Asia (7) and widely distributed in the Middle East (8). Toxicity of medicinal plants such as field bindweed may be the result of several factors, including a combination of secondary products present in the plant, heavy metals that accumulate in the plant (9, 10). Toxicity of field bindweed in mice had been investigated many years ago related to several tropane alkaloids and pyrrolidine alkaloids including tropine, pseudotropine and calystegins (11). Biological control of vertebrate pests defined, reduction in number or density of pests through biological processes such as fertility control, predation, pathogens, and habitat modification. The goal of vertebrate pest control is not to just reduce pest populations, but rather to alleviate damage (12). Rats are frequently blamed for damaging food supplies and other goods, or spreading disease including Weil's disease, typhus, salmonella and bubonic plague (13, 14). They are very harmful and they are causing big financial losses for example in 1994, a localized outbreak of plague in Surat, India, led to 50 deaths (15) and national and international fear resulting in a collapse of tourism and trade in at a national level at an estimated cost of US\$600 million (16). Albino rat's reproductive toxicology has recently become a rapidly extended area of research and testing. In the last decade there has been concern over the effects of either synthetic or natural products on the Albino Rat's reproductive performance (17). Mice fed exclusively *C. arvensis* ate the plant readily. In high dose trials, the mice showed no abnormalities until day 6, histological lesions were found in stomach and liver (18). This study is the first attempt to use *C. arvensis* as a way to cause infertility in male rat to control reproduction and is one of the means of

biological control. We are thinking of a biological control of rats by different ways, therefore, the aim of present study is find out:

1. The effect of *C. arvensis* crude extract on Albino male rat's reproductive performance and fertility includes: Body weight, testicular, epididymis, seminal vesicle, and prostate gland weights to body weight ratio; absolute weights of the testes, epididymis, seminal vesicles, prostate gland; serum testosterone concentration, sperm concentration, sperm motility sperm morphology and sperm viability.

2. Structural changes in testicular tissue.

3. The effect of different doses of *C. arvensis* on the fertility and pregnancy indices of non-treated Albino female rat's.

MATERIALS AND METHODS

Animals

Sixty albino rats (*Rattus norvegicus*) of Wister strain (40 males and 20 females) were used in this study. The animal's age was between 10-12 weeks; of 180-200g body weight. The animals used were obtained from the College of Pharmacy, University of Baghdad and maintained in the animal house of the same college. These animals were kept under conditions of controlled temperature 21-25 °C and photoperiod of 12 hours daily. The feed was given as pellets of freshly prepared ration. The animals were kept for two weeks for adaptation before starting the experiment.

Extract preparation

The fresh aerial parts of *Convolvulus arvensis* (field bindweed) were collected from the gardens of College of Agriculture and were authenticated by Prof. Ali Hussein Al-Mousawi (College of Sciences, University of Baghdad). Alcohol extract of *C. arvensis* (field bindweed) was prepared according to the method of the Todd et al. (18). The aerial parts (107g wet wt.) were extracted at room temperature for 24 hr with methanol (CH₃OH). The mixture was filtered and methanol was evaporated to leave about 26 g gummy residue as a crude extract. Different dilutions of *C. arvensis* (field bindweed) crude extract were freshly prepared from the concentrated gummy stock residue to insure administration of (400, 600, and 800 mg/kg B.W) for each group respectively. These stock solutions have a concentration of 2.4, 3.7 and

4.8 g were suspended in 30 ml of 5% carboxymethyl cellulose (CMC) respectively. Forty adult male rats 180-200g were randomly divided into four equal groups, 1st group was not treated with *C. arvensis* (field bindweed) extract and considered as control group where as the other three groups were treated with 400, 600 and 800mg/kg B.W of *C. arvensis* (field bindweed) orally using special gavage tube for rats and considered as T1 Group, T2 Group and T3 Group respectively.

Blood collection

At zero time and after 35 days of experiment, animal were anesthetized with ether; blood samples were obtained via cardiac puncture technique from each anesthetized animal using disposable insulin needles. Samples were centrifuged at 2500 rpm for 15 minutes, and then serum samples were separated and stored at -20 °C until use.

Determination of testosterone concentrations

Serum testosterone concentration in blood was measured at zero time and after 35 days of experiment by Radioimmunoassay (RIA) using Double antibody technique. The kit was provided by Immunotech, A Beckman coulter Company, de Lattre de Tassigny, Marseille, France. Testosterone Analysis was performed based on the steps taken by the company, testosterone concentration (ng/ml) was measured in tubs using Gamma Counter system, Spain 1470, Wallace, Perkin-Elmer.

Fertility test

After 35 days of treatment, two animals (2 rats) leftover from each group were used to measure the fertility index by using 20 female rats (5 females per group).

Testes and reproductive organs weights

At the end of 35 days of experiment, eight animals (8 rats) from each group were sacrificed on the next day after the last dosing. At autopsy, selected organs were removed and weighed (testes, epididymis, seminal vesicles and prostate gland).

Sperm analysis

Sperm count was assessed in caudal epididymis according to the method described by World Health Organization (19) while Sperm motility was assessed according to the method reported by Robb et al. (20). The assessment of live and dead sperms was carried out according to the method of

Chemineau et al. (21), while the method of Siegmund, (22) was used to evaluate the abnormal sperm morphology.

Histological preparation

Testes of each rat were fixed in Formalin's fluid, passed through ascending series of ethanol and then through xylene and, embedded in paraffin wax (23). Tissues were sectioned at the thickness of 5µm and stained with haematoxyline and eosin (24). After that, the histological sections examined by light microscope to study the histological changes may be occur in these sections prepared from the testes of animal of control and three treated groups.

Statistical analysis

Data obtained during the experiment was statistically analyzed using a completely randomized design (CRD) procedure by SAS (25) to study effect of treatments in different parameters. Duncan's multiple range tests was used to determine the significance of differences between treatments means Duncan (26).

RESULT AND DISCUSSION

Testosterone concentrations

The effect of three different doses of field bindweed on serum testosterone concentration is showed in Table (1). Results revealed no significant differences among the four groups in testosterone level. At the end of the treatment period (day 35), the results showed a significant ($P<0.05$) decrease in the testosterone level in T2 and T3 groups as compared with control group. On the other hand, there was no significant difference in mean value of testosterone concentration between control and T1 groups. The significant decrease in testosterone level may be due to the effects of field bindweed on serum cholesterol level, a precursor of testosterone synthesis by its action on the Leydig cells. Similar result was given by Das et al. (27) in rats treated with *Aegle mermelos* extract. Numerous studies suggest that field bindweed lowered serum cholesterol level (28, 29, 30, 31). The secretion of testosterone is under the control of luteinizing hormone (LH) (32, 33). Therefore, the decrease in testosterone concentration produced by the action of field bindweed could be explained either by inhibition of the enzymatic pathways

of its synthesis in the testes or the adrenal cortex. Or, may be interfering with LH release, all of that leading to decrease testicular testosterone synthesis and release (34). Furthermore, field bindweed may be act directly on the hypothalamic-pituitary-testicular axis, as a result serum testosterone level would decrease. Prasad and Rajalakshmi, (35) reported that spermatogenesis occurs in the seminiferous tubules while the interstitial cells secrete the testicular hormone, mainly testosterone, therefore any alteration in the seminiferous tubules as observed in the histological study would have its consequential effect on spermatogenesis and hormone secretion.

Table1. Effect of different oral doses administration of methanolic extract of *C. arvensis* on serum testosterone level (ng/ml), in male rats (Mean±S.E.).

Time	Trts.			
	Control n=8	T1 400mg/kg n=8	T2 600mg/kg n=8	T3 800mg/kg n=8
Pre-treatment	1.92±0.06a	1.89±0.07a	1.97±0.06a	1.95±0.06a
After 35 days of Treatment	1.85±0.05a	1.65±0.03ab	1.51±0.06c,b	1.30±0.18c

Means with different letters within the same row are significantly different ($P<0.05$).

Sperm characteristics

All of the andrological parameters investigated in field bindweed treated groups (count, motility, viability: death-live ratio) in Table (2) showed a significant ($P<0.05$) reduction in mean values when compared with the control group. While the percentage of abnormal sperms increased significantly ($P<0.05$) in field bindweed- treated groups as compared with control group. The sperm count was reduced significantly ($P<0.05$), which is an indication that the crude extract of field bindweed reduced or inhibited spermatogenesis. Sperm count is considered to be an important parameter which assesses the effects of chemicals on spermatogenesis (36). Spermatogenesis is influenced by the hypothalamic- adenohipophysial -Leydig cell system relating gonadotrophin releasing hormone, luteinizing hormone and androgen. This implies that the decrease in sperm count

caused by the extract in treated rats could be as a result of decrease in plasma level of testosterone, because this hormone has been reported to be important in the initiation and maintenance of spermatogenesis (37). A significant increase in the percentage of abnormal sperm cells morphology after treatment of rats with the field bindweed crude extract this could be due to the ability of the extract to either interfere with the spermatogenic processes in the seminiferous tubules, epididymal functions or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropins which may result in alteration of spermatogenesis (38, 39).

Table2. Effect of different oral doses administration of methanolic extract of *C. arvensis* on the sperm characteristics of male rats (Mean±S.E.).

Groups	Control n=8	T1 400mg/kg n=8	T2 600mg/kg n=8	T3 800mg/kg n=8
M Sperm/ml	64.62±1.45a	51.62±0.75b	42.50±1.11c	36.25±1.04d
Motility%	77.75±1.53a	61.37±1.79b	53.37±1.29c	48.25±1.61d
Dead sperm%	15.75±1.14d	20.00±0.84c	27.25±0.88b	36.37±0.73a
Abnormality%	10.25±0.92d	15.00±0.80c	21.37±0.73b	30.50±0.53a

Means with different letters within the same row are significantly different ($P<0.05$).

Testes, epididymis, seminal vesicles and prostate gland weights

Administration of the field bindweed crude extract for 35 days caused significant decrease ($p<0.05$) in means testes, epididymis, seminal vesicles and prostate gland weights of rats in the three treated groups T1, T2 and T3, respectively, as compared with the control group (Table 3). The decrement of testicular weight in the treated rats as compared with the control group may be due to the decline of testosterone level, where testosterone has an important role in increasing the weight of the reproductive organs including the testes and seminal vesicles, etc. (40), or may be due to the decreased production of seminiferous tubular fluid, which contributes to the weight of testes (41). This decreasing in testicular weight may be due to direct action of field bindweed on testicular tissue which cause degenerate and necrosis of spermatogonia and vaculation of sertoli cells and degeneration of interstitial tissue, finally leading to decrement in testicular weight.

Table 3. Effect of different oral doses of methanolic crude extract of *C. arvensis* given to male rats, on the weights of Testes, Epididymis, Seminal vesicles, Prostate gland (gm).(Mean±S.E.).

Organs weight gm	Control n=8	T1 400mg/kg n=8	T2 600mg/kg n=8	T3 800mg/kg n=8
Testes	1.44±0.04a	1.27±0.008b	1.12±0.008c	0.83±0.04d
Epididymis	0.60±0.01a	0.54±0.01b	0.45±0.008c	0.36±0.007d
Seminal vesicle glands	0.57±0.01a	0.52±0.01b	0.43±0.01c	0.39±0.006d
Prostate gland	0.53±0.02a	0.49±0.008b	0.41±0.007c	0.36±0.005d

Means with different letters within the same row are significantly different ($P<0.05$).

Histopathology of testicles of rats

Figure1. Control group: There was no visible lesion observed and normal histological structure of seminiferous tubules.

Figure2. T1 Group: Section revealed degeneration and necrosis among spermatogenic cells in the seminiferous tubules.

Figure3. T2 Group: There was shrunk distorted seminiferous tubules.

Figure4. T3 Group: There was marked different damage of seminiferous tubule.

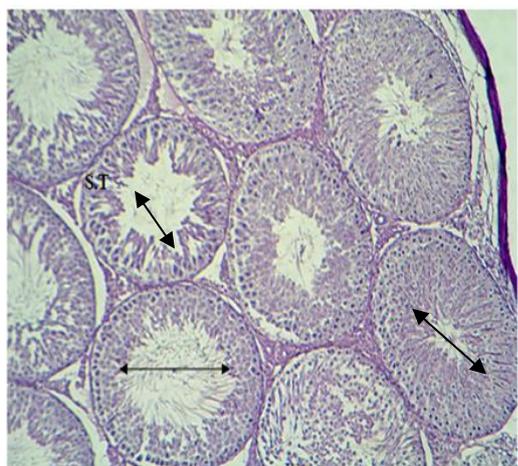


Figure 1: Cross section of rat testes (control group) showed the normal histological structure of seminiferous tubules (S.T.) and large number of sperm (↔) in their lumen (H&E X100).

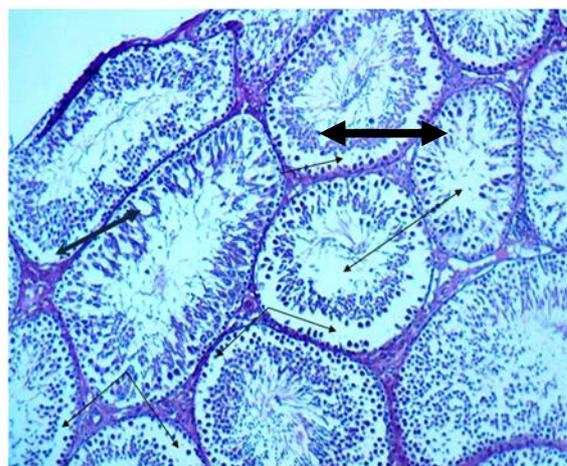


Figure 2: Cross section of rat testes treated with 400mg/kg crude extract of *C. arvensis* showed degeneration and necrosis among spermatogenic cells in the seminiferous tubules (↔) and decreasing in number of sperm (↔) and sertoli cells (↔) (H &E X 100).

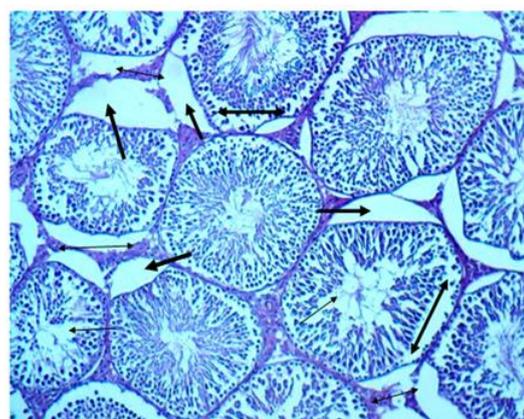


Figure 3: Cross section of rat testes treated with 600mg/kg *C. arvensis* showed regression and shrunk distorted seminiferous tubules (↔) containing detached spermatogenic cells (↔) and few sperm (↔) and atrophic state in leydig cells (↔) (H&E X100)

The reduction in the prostate gland and seminal vesicle weight in the field bindweed treated groups may be due to the lowered availability of pituitary luteinizing hormone/ Interstitial cell-stimulating hormone (LH/ ICSH) as the conversion of cholesterol to pregnanalone is dependent upon pituitary LH/ICSH (42, 43). The loss in primary spermatocytes and secondary spermatocytes and spermatids in treated groups reflected non-availability of androgen binding protein (ABP) from sertoli cells (44). ABP is required to maintain intra-testicular androgen concent-

ration and transformation of advance stages of germ cells. Meiotic and post-meiotic germ cells were highly sensitive to androgen concentration (45, 46). The atrophic state of Leydig cells in the testes of treated animals may be due to declined LH secretion (47, 48). A reduction in the number of Sertoli cells will adversely affect spermatogenesis as Sertoli cells provide all or most nutritional and physical support for the developing germ cells (49).

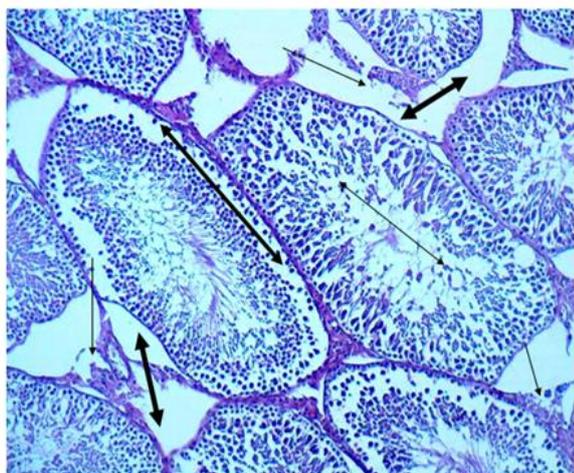


Figure 4: Cross section of rat testes treated with 800mg/kg *C. arvensis* showed different damage of seminiferous tubule such as regression (↔) shrunken and disorder of systemic arrangement of the stages of spermatogenesis and loss of one or more stages of spermatogenesis (↔) and few numbers of leydig cells with atrophic state (→) and few number of sperms (↔) (H&E X100).

Field bindweed crude extract exerted a strong inhibitory effect on epididymis, seminal vesicle and prostate gland as evidence by decrease in their weights. A reduction in the weight of accessory reproductive organs suggested the reduced availability of androgens (50, 51). Rat prostate gland contain some androgen receptors which are the direct target of androgen action (52) and mainly dependent on testicular androgen (53).

Fertility index

The effect of three doses of field bindweed crude extract given to male rats on the fertility

index by using non treated females showed that, when treated males mated with untreated females, there were a significant changes (P<0.05) in the percentage of fertility index among the four groups. The results refer to 100% in control group, (80%) in T1 group, (40%) in T2 group, and (20%) in T3 group (Table 4). Testosterone hormone is necessary for development and divisions of spermatogonea (54). The sperm morphology is also an important characteristic of sperm for evaluating male fertility (55). The sperm motility is the most important parameter in determining fertilization rate (56). Therefore, all of these parameters leading to decrease the fertility capacity in treated rats.

Table 4. Effect of different oral doses of methanolic crude extract of *C. arvensis* given to male rats, on the fertility percentage of non-treated female rats

Groups	Control	T1 400mg/k g	T2 600mg/k g	T3 800mg/k g
No. of female mated Successfully	5	5	5	5
No. of pregnant animal	5	4	2	1
No. of non-pregnant Animals	0	1	3	4
Fertility (%)	100	80	40	20

T1: group of females mated with males received 400mg/kg of field bindweed crude extract.

T2: group of females mated with males received 600 mg/kg of field bindweed crude extract.

T3: group of females mated with males received 800 mg/kg of field bindweed crude extract.

Eventually, it can be concluded that the administration of the methanolic crude extract of field bindweed aerial parts caused decreased mean values of testosterone concentration and andrological parameters as a result of lesions of the seminiferous tubules. This indicates that administration of this extract has provided evidence to suggest an antifertility potential of field bindweed crude extract on male albino rats.

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