

Effect of alcoholic extract *Citrullus Colocynthis* fruits in testes function

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Abstract

Effect of ethanolic extract of *Citrullus colocynthis* (CCT) was investigated on testes function ,testosterone ,LH and FSH hormone in albino mature male rats. Twenty four (24) rats weighting (220-280) g were grouped in to 4 groups by (6) animals / day group and treated as follows for (42) days. first group considered control received 2 ml distilled water orally. While the experimental group second group(T1)were received 25 mg/kg B.W. ethanolic extract of CCT,and third group(T2) received 50mg/kg B.W.,while fourth group(T3)received 75mg/kg B.W. The result the animals treated with 75 mg of ethanolic extract of CCT affected the testes by decrease of weight and decrease in hormone testosterone ,LH and FSH .also showed histological change completly absent of spermatogenic cells in certain seminiferous tubules which replacement by large multiple vacuoles and lining by only by Sertoli cells , and marked thickness of interstitial tissue The results showed the presence of a significant decrease ($P < 0.05$) in the proportion of the testicular weight to body weight when compared with the control group. while testes histological study showed (T2, T3) thickness Tunica albuginea ,congested blood vessels with thickness of basement membrane of seminiferous s tubules and vaculation of their spermatogenic cells layer without presences sperms in their lumen. In conclusion the result of this study documented that CCT induced harmful effect on testes functio(50,75 mg/kg B.W.)at 4,6 week of treatment.

تأثير المستخلص الكحولي لفاكهة الحنظل في وظيفة الخصية

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الخلاصة:

تناول هذا البحث تأثير المستخلص الكحولي لفاكهة نبات الحنظل على وظيفة الخصية .حيث استخدم (24) من ذكور الجرذان البالغة يتراوح معدل اوزانها (220-280) غم , قسمت عشوائيا الى اربعة مجاميع وبواقع (6) حيوانات / مجموعة وعوملت يوميا كالاتي لمدة (42) يوما:حيوانات مجموعة السيطرة المجموعة الاولى اعطيت فمويا (2) مل من الماء المقطر ولمرة واحدة يوميا واعتبرت مجموعة اولى (سيطرة) (C) , اما حيوانات المجموعة الثانية فقد اعطيت المستخلص الكحولي لفاكهة الحنظل بجرعة (25ملغم / كغم من وزن الجسم فمويا) ولمرة واحدة يوميا واعتبرت مجموعة (T1) , بينما حيوانات المجموعة الثالثة فاعطيت المستخلص الكحولي لفاكهة الحنظل بجرعة (50ملغم / كغم من وزن الجسم فمويا) ولمرة واحدة يوميا واعتبرت مجموعة (T2)

بينما المجموعة الرابعة اعطيت المستخلص الكحولي لفاكهة الحنظل بجرعة (75ملغم / كغم من وزن الجسم فمويا) ولمرة واحدة يوميا واعتبرت مجموعة (T3). تم جمع عينات الدم في الايام (14,28,42) لغرض قياس تركيز كل من المعايير المذكورة اعلاه وعند نهاية التجربة تم التضحية بالحيوانات وتم حساب نسبة وزن الخصى الى وزن. واخيرا تم دراسة الصفة النسيجية التركيبية الخصوية. بينت النتائج وجود انخفاض معنوي ($P<0.05$) في نسبة وزن الغدة الخصوية الى وزن الجسم عند مقارنتها مع مجموعة السيطرة. كما بينت النتائج فقد وجد انخفاض معنوي ($P<0.05$) في تركيز هرمونات مصل الدم (LF,FSH, testosterone) للمجاميع المعاملة بالمستخلص الكحولي لفاكهة الحنظل مقارنة مع مجموعة السيطرة خاصة T2,T3, وبعد فترة المعاملة 6 اسابيع. كذلك اثبتت الدراسة النسيجية للخصى الى وجود تآخن الغلالة البيضاء, والغشاء القاعدي من الأنابيب المنوية وتقصى ضمن بطانة الخلايا المنوية مع غياب وجود الحيوانات المنوية في تجويفها. يستنتج من هذه الدراسة بان المستخلص الكحولي لفاكهة الحنظل تسببت باثار سلبية على مستوى وظيفة الخصى عند استخدامه بالجرع (50,75ملغم /من وزن الجسم في الجرذان ولمدة 4,6 اسابيع.

Introduction

Citrullus colocynthis is of the nature of the Yearbook of plant that grows in sandy soil. CCT from cucurbitacea family, these is the largest family containing 120 genera and approaching from 825 species (Mabberley 1987). since the 14th century and is still source today. From a medical point it is of value and fame is used in many countries of the world in asia and Africa (Rahimi et al., 2012). Its distributed grows naturally in the western Iraqi (Al- Zahrani et al., 2006). Saharo-Arabian phyto geographic and Sahara Egypt district in Africa and the Mediterranean region until India also the north coast of the Caspian seas. (Kaleem 2006). It has several labels in the world where so-called locally as Handal in Arabic (Al -Ghaithi et al., 2004), is also known Abujahl watermelon in Persian .and called watermelon , bitter goured and bitter cucumber (Zamani et al., 2007). In the English language is known as Bitter Apple (Patrick et al., 1960). The plant CCT is consist from leaf, root, flower (male, female), fruit (pulp, hull, seed) were photochemical analyzed separately and which shows the presence of alkaloids, carbohydrates, and flavonoids. Tannins, gums and mucilages are found to be truant (C. Uma and K.G. Sekar, 2014). The bitter favor that give bitter tast to fruit due to the extract is called colocynthine (Delazar et al., 2006); (Dallak et al., 2010). CCT is used anti-hyperlipedemia (Rahbar and

Nabipour, 2010). anti inflammatory (Marzouk et al., 2011). anti oxidant

(Mohammad Dallak, 2011). analgesic activity (Belsem marzouk et al., 2013). antimicrobial (Belsem marzouk et al., 2011). antihyperglycemic (Omya et al., 2013). (Agarwal et al., 2012). antifertility (Donald et al., 2007). and effect on liver (Farzaneh Dehghani and Mohamad reza, 2006). effect on digestive system (Ismail and Sundus, 2006). Testes functions primarily is produce sperm (spermatogenesis) and to produce androgens, primary testosterone hormone. response to the start of spermatogenesis (Scott, 2000). These tubules are responsible for producing the sperm cells through a process called spermatogenesis. (Elaine 2004; Scott 2000). The spermatogenesis is needed to support presence of both testosterone and Follicle-Stimulating Hormone (FSH) (Michael and Wojciech, 2006; Romer and Parsons, 1977; Skinner et al., 1989). Spermatogenesis can be disrupted cause animals become infertile in case if testes are exposed to either too high or too low levels of estrogens (such as estradiol; E2) (Sierens et al., (2005). significant decreases in cauda epididymal sperm motility, density, number of pups and fertility (Mali et al., 2001). *C. colocynthis* extract showed an ant androgenic nature, reduced reversible infertility in male albino rats. The testes showed degenerative changes in the seminiferous epithelium and

arrest of spermatogenesis at the secondary spermatocyte stage (Chaturvedi et al., (2003). was observed a significant decrease in weight of testes after administration of ethanolic extract of *C. colocynthis* at dose levels of 75mg. also exhibited significant reduction in the level of serum testosterone, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (Aksha Sharma 2014).

Material and methods

Animals

Males adult rats healthy on an average body weight 220-2250 g .the animals were kept in clean cages and maintained under controlled environmental conditions (12h light : 12h dark).and maintained on standard pellet diet. Rats were housed in an animal house at college of Veterinary Medicine, Baghdad University. They were left for two weeks for adaptation with the experimental conditions.

Plant sample

Fresh fruits of *Citrullus Colocynthis* were purchased from the Certified at the Iraq National Herbarium in Kalare , Sulaymania, Fresh fruits were dried at the room temperature and powdered by grinder until it got powder and kept in refrigerator until it used .

Preparation of alcoholic extract of *Citrullus colocynthis* :

Shed dried fruits with seeds of CCT were powdered and extracted with 70% ethyl alcohol was added on the powder which makes the ratio (1/10) (W/V). After that the mixture was shaken by using magnetic stirrer apparatus for (24 hr.), by using whatman No.1 filter paper mixture filtered by using 4 layers of medical gauze then filtered again. This extract was stored in a dark sterile screw bottle in (4 C) until use after using incubator on (40 C) for 72 hr The filtrated mixture was concentrated to obtain crude extract.

Experimental design

Twenty four adult male rats (220- 280) g were divided randomly in to four groups (six animals per group) and handled daily as follows for 6 weeks:

Control group:Six adult male rats treated orally with distal water daily via Gavage needle.Group T1:Six adult male rats receive orally 25 mg \ kg Body weight of alcoholic extract of *Citrullus colocynthis* fruits daily for 42 days via Gavage needle. Group T2:Receive orally 50 mg \ kg Body weight of alcoholic extract of *Citrullus colocynthis* fruits daily for 42 days via Gavage needle.Group T3:Receive orally 75 mg \ kg Body weight of alcoholic extract of *Citrullus colocynthis* fruits daily for 42 days via Gavage needle.

Blood sample collection

Blood samples were collected from heart animals after anesthetized by the ketamine and Xylazine .whole blood samples were obtained in heparinized tubes to detect hormones concentration.

Measurement of testicular weight to body weight ratio

After sacrificed of animals in the end of the experimental period, animals groups were weighted by electrical balance.

Measurement of hormones testosterone ,LH and FSH in blood

Serum LH, testosterone and FSH concentration (ng/ml) was measured by (IMMUNOTECH SAS)

Statistical analysis

The values were recorded as mean \pm standard error of mean.

Results

Testicular weight

that testicular weight to body weight ratio increase significantly ($P < 0.05$) in group T1 that treated 25 mg /kg B.W. compared with control group and

compared with T2, T3 groups. While showed no significant change between control group and T2 group .the result also show more decrease significantly

(P<0.05) in T3group how treated 75 mg/kg B.W. of CCT compared with T1 group and control, T2 groups.

Table 1. Testicular weight after admenstration of citrullus colocynthis

Group Time	Control group	T1 group 25mg/kgB.W.of cct	T2 group 50mg/kgB.W.of cct	T3 group 75mg/kgB.W.of cct
After 42 day of treatment	0.44±0.004 B	0.52±0.013 A	0.46±0.021 B	0.31±0.011 C

LSD= 0.03

Value are expressed as mean± SE,n =6 each group

-T1: Animals received CCT (75 mg /kg B.W).

-Small letters denote significant differences within groups (P<0.05).

-Capital letters denote significant differences between groups (P<0.05) .

Effect of citrullus colocynthis on serum concentration of testosterone

Treatment of animals with CCT extract caused significant(P<0.05) decreament in testosterone concentrate in all groups and for all periods of experimental compared with control group except T1 group at 14 day only. Also the decrement of this hormone increase gradually with the increase of CCT extract dose table (4-11) of other more there was gradually decrease of testosterone level with the time of treatment.

Table 2. effect of citrullus colocynthis on concentration of testosterone hormone

Group Time	Control group	T1 group 25mg/kgB.W.of cct	T2 group 50mg/kgB.W.of cct	T3 group 75mg/kgB.W.of cct
14 day	3.33±0.41 A	3.43±0.40 A a	1.88±0.31 C ab	1.63±0.20 B a
28 day	3.65±0.33 A	2.61±0.32 B b	2.20±0.20 B a	1.56±0.24 C ab
42 day	3.53±0.19 A	1.85±0.088 B c	1.30±0.26 BC b	0.90±0.13 C b

LSD=0.7

Value are expressed as mean± SE,n =6 each group

-T1: Animals received CCT (75 mg /kg B.W).

-Small letters denote significant differences within groups (P<0.05).

-Capital letters denote significant differences between groups (P<0.05) .

Effect of alcoholic extract of citrullus colocynthis on serum LH hormone (ng/ml) in adult male rats.

LH hormone concentration in serum no significant change in T1 group in day 14 and 28 compared with control group but show significantly decrease (P<0.05) in T1 group in day 42 and in T2,T3 groups compared with control group at the same period. On other hand T1,T3 groups treated of CCT showed significantly decrease (P<0.05) compared with control group at all periods of experimental within group there was significant decrease in LH concentration between last period and 14,28 days.

Table (3) : Effect of alcoholic extract of citrullus colocynthis on serum LH hormone (ng/ml) in adult male rats.

Group Time	Control group	T1 group 25mg/kgB.W.of cct	T2 group 50mg/kgB.W.of cct	T3 group 75mg/kgB.W.of cct
14 day	1.43±0.15 A	1.53±0.20 A a	0.77±0.22 B a	0.63±0.20 B a
28 day	1.50±0.17 A	1.65±0.16 A a	0.61±0.08 B ab	0.46±0.05 B ab
42 day	1.46±0.23 A	0.33±0.06 B b	0.33±0.03 B b	0.20±0.02 B b

LSD=0.4

Value are expressed as mean± SE,n =6 each group

-T1: Animals received CCT (75 mg /kg B.W).

-Small letters denote significant differences within groups (P<0.05).

-Capital letters denote significant differences between groups (P<0.05) .

Effect of alcoholic extract of citrullus colocynthis on FSH hormone (ng/ml) in adult male rats.

FSH hormone concentration in serum significantly decrease (P<0.05) in T1 ,T2,T3 groups at day 14 ,28 and 42 of treatment compared with control group at the same period except period 14 day at T1 group . But in day 14 in T1 group no significant change compared with control group. Also there a significant decrease (P<0.05) in FSH concentration within time between 28,42 and first period. On other hand the that a dose of CCT (50,75 mg /kg B.W.) caused high decrement in concentration of this hormone.

Table (4): Effect of alcoholic extract of citrullus colocynthis on FSH hormone (ng/ml) in adult male rats.

Group Time	Control group	T1 group 25mg/kgB.W.of cct	T2 group 50mg/kgB.W.of cct	T3 group 75mg/kgB.W.of cct
14 day	7.75±0.22 A	7.20±0.94 A a	6.01±0.45 B a	5.48±0.45 B a
28 day	7.68±0.26 A	5.18±0.50 B b	2.85±0.28 C b	2.11±0.31 C b
42 day	7.70±0.46 A	4.28±0.34 B b	2.26±0.29 C b	1.55±0.12 C b

LSD=1.1

Value are expressed as mean± SE,n =6 each group

- T1: Animals received CCT (75 mg /kg B.W).
- Small letters denote significant differences within groups (P<0.05).
- Capital letters denote significant differences between groups (P<0.05) .

Testicular histology

In the histological study of testes of control animals showed clear organization of cells at various stages of spermatogenesis figure 1.and on show clear leasion in group treated with 25 mg /kg B.W. of ethanolic extract of CCT (figure 2) also can showed in group of treated animals with 50 mg /kg B.W. of ethanolic extract of citrullus colocynthis ,the histological of testes show thickness Tunica albuginea ,congested blood vessels with thickness of basement membrane of seminiferous s tubules and vaculation of their lining spermatogenic cells layer without presences sperms in their lumen figure 3). also show in group received 75mg/kg B.W. extract of CCT appearance completly absent of spermatogenic cells in certain seminiferous tubules which replacement by large multiple vacuoles and lining by only by Sertoli cells , and marked thickness of interstitial tissue (figure 4,5).

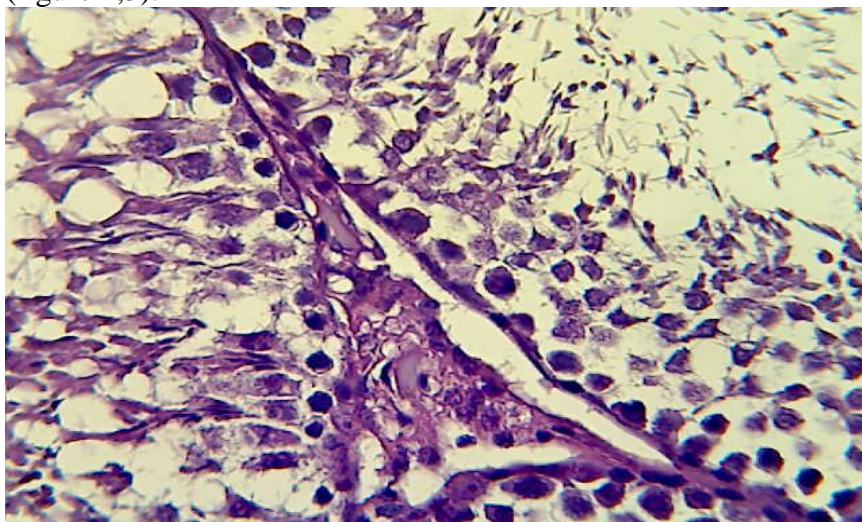


Figure 1:Histopathological section in the testis of control animal shows no clear

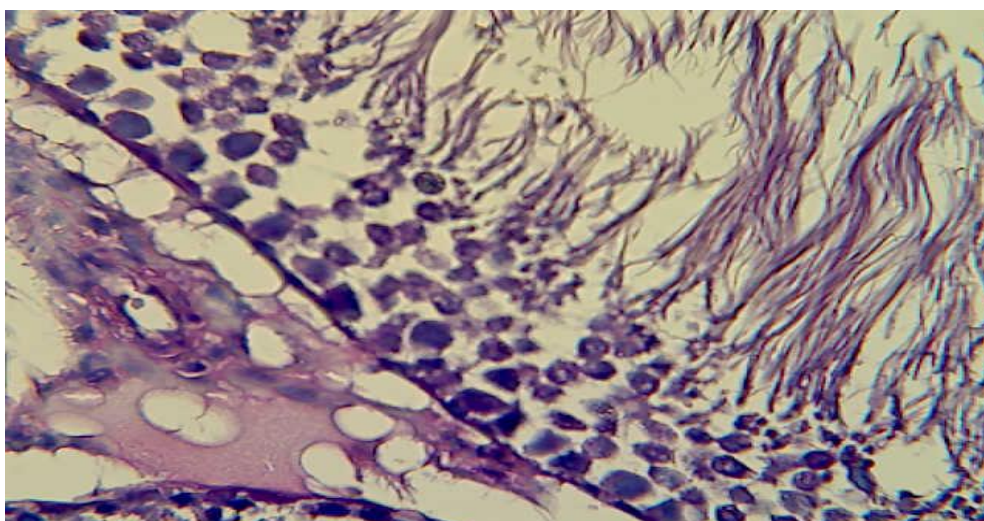
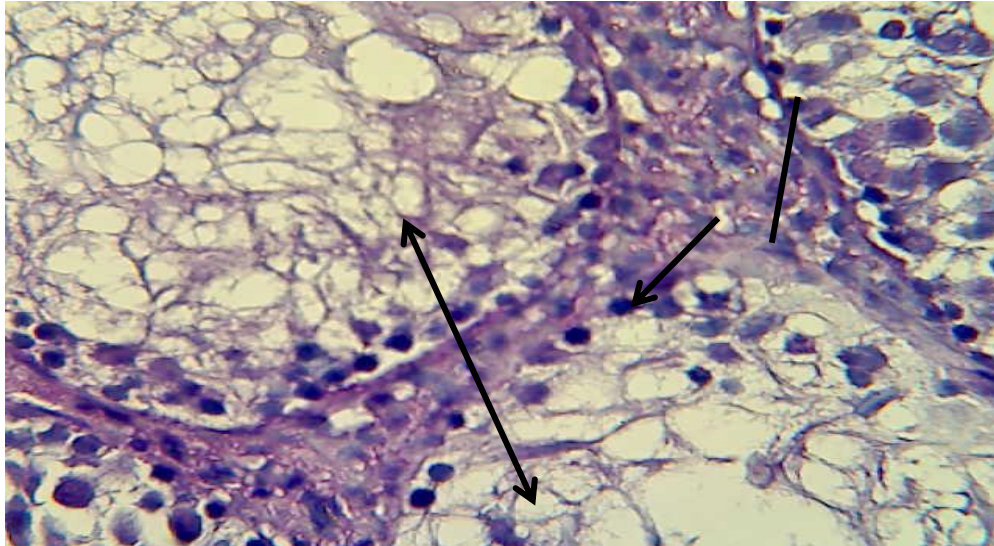


figure 2: Histopathological section in the testis of animal treated with twenty five

mg/kg of (T1) shows no clear lesions (H&E stain 400X)



3: Histopathological section in the testis of animal treated with fifty mg/kg of (T2) show thickness Tunica albuginea →, congested blood vessels ↔ with figure 2: thickness of basement membrane of seminiferous tubules ↕ and vaculation of their spermatogenic cells layer without presences sperms in their lumen (H&E stain lining 100X)



Figuar 4 : Histopathological section in the testis of animal treated with seventy five mg/kg of CCT show completely absent of spermatogenic cells in certain seminiferous tubules replacement by large multiple vacuoles ↔ and lining by only by Sertoli → which cells, — and marked thickness of interstitial tissue

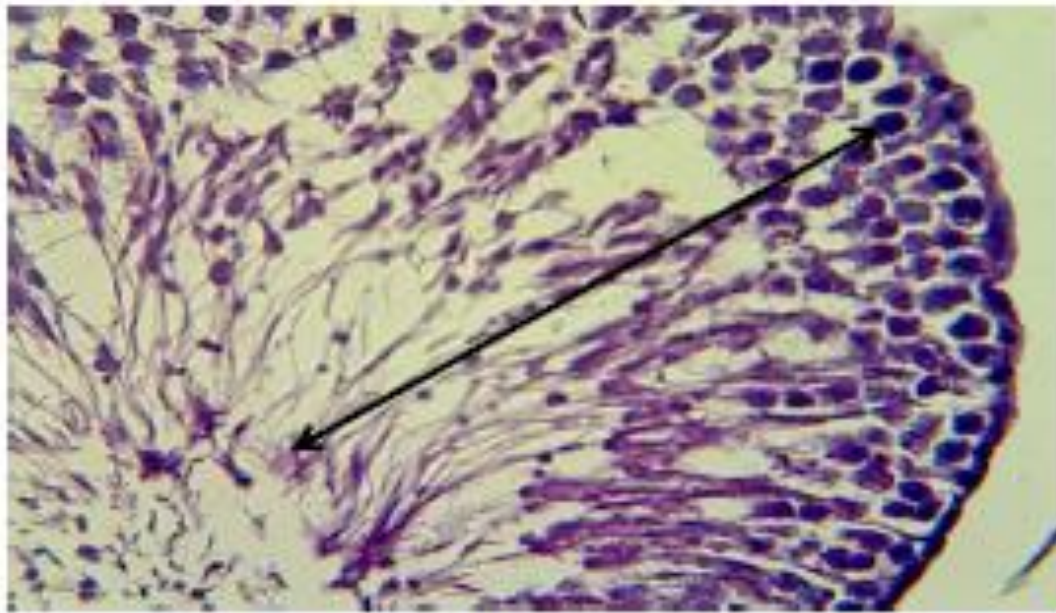


figure 4 :Histopathological section in the testis of animal treated with seventy five mg/kg CCT (T3) show completely spermatogenesis process in the seminiferous tubules with sperm in their lumen vacuolation of Leydig cells (H&E stain 400X)

Discussion

In this study oral administration of ethanolic extract of *Citrullus colocynthis* at the dose 25,50,75 mg/kg B.W. orally for 42 days showed reduction in weight of testes due to low of level of androgen, which was not enough to maintain the weight of gonads (Sharma and Jacob,2001).the decreasing weight of the testes in the extract –treated male rats clearly indicated that the extract caused structural and functional alteration in the reproductive organ. (Sakar et al.,(2000)play an important role in spermatogenesis concentration of testosterone ,LH and FSH (Zitzmann 2008). in the testes are FSH and LH hormone are regulate the testosterone hormone (Wannang et al., 2008) and also act to maturation ,spermatogenesis and the maintenance of accessory sex organs and play active role in development and growth of male reproduction organs (Keel

and Abney ,1980;Joshi et al .,2012;Thejashwini et al., 2012). The indicate reported the CCT causes of low in weight testes, epididymus ,seminal vesical completely absent of spermatogenic cells in certain seminiferous tubules which replacement by large multiple vacuoles and lining by only by Sertoli cells , and marked thickness of interstitial tissue and decrease in serum testosterone and the production of the sperm cells (spermatozoa) that indicate pituitary gonadotropins will be effected by the extract and inturn in the testosterone in the testosterone biosynthesis in the testes and reproductive organs. (Borhade Pravin et al.,2013). (Connel et al .,(1968);Hnsnson et al., (1987)show the may be the decrease of serum level of FSH ,LH which are essential for the gonadal development and steroidogenesis in rats that lead to reduction in the serum level of testosterone this reduction may be the reason is due to its

content of this plant from toxic substances such as cucurbitacis, saponin (Abdel Hassan et al., 2000; Diwan et al., 1996; Elizzi et al., 1990).). histological change of the testes and process of spermatogenesis may be resone to this and decrease in weight of epididymis ,seminal vesicle ,and prostate and decrease in the level of serum testosterone (Chaturvedi et al., 2003) , (Borhad Pravin et al., 2013).

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