Study of the effect of decorticated and defatted Castor Seeds (*Ricinus communis Linn.*) on Testosterone level and testicular architecture of male mice

Mustafa A. Jasim[1] and Farid J. O. Al-Tahan[2]
[1]Dept. Physiology & Pharmacology- College of Veterinary Medicine-University of Diyala
[2]Dept. Physiology & Pharmacology- College of Veterinary Medicine-University of Baghdad

KeyWords: Castor Seeds, Testosterone

Correspondence: Mustafa A. Jasim

Dept. Physiology & Pharmacology- College of Veterinary Medicine-University of Diyala

Email: 1m9a8j3@gmail.com

Abstract

The aim of this study is to investigate the effect of decorticated and defatted castor seeds *(Ricinus communis)* on the testis of male mice hormonally and histopathologically at two consecutive spermatogenic cycles. Eighteen albino Swiss male mice were used as animal model, they divided in to three groups equally, 1st group served as control group, while the 2nd group was treated orally with 1.76 mg/kg. B.W. from watery suspension of decorticated and defatted castor seeds extract for 38 days (single spermatogenic cycle). The 3rd group also treated by the same protocol of the 2nd group but was allowed a recovery period (free from the treatment) of another 38 days (double spermatogenic cycles). The measured parameters were: the ratio of testicular weight to the body weight, Levels of testosterone hormone and histopathological study of testicular architecture. The results of the present study showed that the treatment with decorticated and defatted castor seeds caused a significant decrease ($P< 0.05$) in the ratio of weight of testis to the body weight and the Levels of testosterone hormone, in treatment group and these effects have been sustained in the recovery group. The histopathological sections of testis of treated mice showed sever changes in testicular architecture in both periods of the experiment. This was attributed to the lipolytic and cytotoxic activity of ricin, a toxic protein present in castor seeds. In conclusion, the decorticated and defatted castor seeds have negative hormonal and histopathological effects on the testis of mice and this effect was irreversible at the level of two spermatogenic cycles.

دراسة تأثير بذور الخروع المقشرة والمزال منها الزيت على مستوى الهرمون الذكري ونسيج الخصية لذكور الفئران

مصطفى احمد جاسم[1] و فريد جميل عمر الطحان[2]
[1] فرع الفسلجة والادوية– كلية الطب البيطري– جامعة ديالى
[2] فرع الفسلجة والادوية– كلية الطب البيطري– جامعة بغداد

الخلاصة

الهدف هذه الدراسة هو للتحري عن تأثير بذور الخروع المقشرة والمزال منها الزيت على خصى ذكور الفئران هورمونيا ونسيجيا و خلال دورتين انطافييتين. استعمل ثمانية عشر فأرا ذكرا سويسريا كنموذج للتجربة وقسمت هذه الفئران إلى ثلاثة مجاميع بالتساوي (ستة لكل مجموعة). عوملت المجموعة الأولى كمجموعة سيطرة وجرعت فمويا بالماء المقطر فقط بينما عولجت المجموعة الثانية فمويا بـــ 1.76 ملغ/ كلغ من وزن الجسم الحي من المعلق المائي لبذور الخروع المقشرة والمزال منها الزيت ولمدة 38 يوما (دورة انطافية واحدة) وعولجت المجموعة الثالثة بنفس نظام المجموعة الثانية ولمدة 38 يوما أيضا ولكن سمح لها بالبقاء بدون تجريع لمدة 38 يوما أخرى (دورتين انطافيتين).وتم قياس المعايير الآتية: نسبة وزن الخصى الى وزن الجسم و قياس مستوى الهرمون الذكري ودراسة نسيجية للخصى. وأظهرت نتائج الدراسة إن المعالجة ببذور الخروع المقشرة والمزال منها الزيت قد سبب انخفاضا معنويا ($P<0.05$) في كل من نسبة وزن الخصية إلى نسبة وزن الجسم وكذلك في مستوى الهرمون الذكري في مجموعة العلاج وقد استمرت هذه التغيرات في مجموعة العلاج أيضا. أظهرت الدراسة النسيجية المرضية تغيرات نسيجية شديدة في نسيج الخصية في كل من مجموعتي العلاج والشفاء. وقد عزي هذا التأثير الى الفعالية الخلوية السامة والحالة للدهون لبروتين الريسين الموجود في بذور الخروع. وأستنتج من الدراسة الحالية إن لبذور الخروع المقشرة والمزال منها الزيت تأثيرا سلبيا على مستوى الهرمون الذكري ونسيج الخصية لذكور الفئران وان هذا التأثير غير رجعي وعلى مستوى دورتين انطافيتين.

الكلمات الدالة :
بذور الخروع ، الزيت ، الهرمون الذكري

للمراسلة :
مصطفى أحمد جاسم
فرع الفسلجة والادوية– كلية الطب البيطري–جامعة ديالى

ايميل:
1m9a8j3@gmail.com

Introduction

Many trails have been done to investigate the antifertility effect of many medicinal plants in male animals. (Deshpande, *et al*., 1980); (Zhen, *et al*., 1995); (Lohiya, *et al*., 2002); (Mdhluli, and Van der Horst, 2002) and (Coutinho, 2002)Some of these plants had spermicidal effects; others caused reduction in the sperm counts and altered the mobility of the sperms. Some of them caused testicular change and altered hormone levels. (Bhargava, 1984) and (Madhusudhana, *et al*., 1997)Castor bean plant *Ricinus communis* (Linn), belongs to the family *Euphorbiaceae*. Different parts of the plant have been reported to have several medicinal values. (Chiej, 1984).Different parts and extraxts of Castor bean has been examined to detect its capability to be used as antifertility agent among different species of animal females (Okwuasaba, *et al*., 1991); (Al-Tahan, 1994); (Makonnen, *et al*., 1999-); (Okwuasaba, *et al*., 1999); (Isichei, *et al*., 2000); (Das, *et al*., 2000) and (McNeil, *et al*., 2003).Sandhyakumary, *et al*., (2003) and Raji, *et al*., (2006) studied the antifertility effect of different alcoholic extracts of *Ricinus communis* on male rats. Both of them found a reversible negative impact on male reproductive functions.Al-Tahan, and Jasim, (2011) concluded that the decorticated and defatted castor seeds have an injurious effects on sperm characters of male mice in particular on sperms mobility of the examined sperms and these effects have been continued for two consecutive spermatogenic cycles.Here we are try to investigate if there were a possible effect of the decorticated and defatted castor seeds extract on the testicular architecture and testosterone level and explore if this effect will be reversible or not.

Materials and Methods

This study has been achieved in the animal house of the college of veterinary medicine / Baghdad University, during January – March in 2008.Decorticated and defatted castor seeds extract has been prepared as previously mentioned (Al-Tahan, and Jasim, 2011) and (Al-Tahan and Al-Shaha, 1990).Eighteen albino Swiss male mice were used as animal model, they divided in to three equal groups; 1st group served as control group and received distilled water only, the 2nd group has been treated orally with 1.76 mg/kg (Al-Tahan, 1994). from the watery suspension of decorticated and defatted castor seeds extract for 38 days (single spermatogenic cycle). The 3rd group also treated by the same protocol of the 2nd group but was allowed a recovery period (free from the treatment) of another 38 days (double spermatogenic cycles).After treating periods, animals were weighed, and after sacrifice testis were removed and weighed by sensitive balance after being cleaned from the accessory connective and adipose tissues. Testicular weight to body weight ratio was calculated as in the following equation:*Testicular wt-to-body wt ratio = (Wt. of testis (gm) / Wt. of animal (gm)) X 100* .(Al- Janabi, 2007).Blood samples were obtained via cardiac puncture from each anesthetized animal using disposable insulin syringes. Samples were centrifuged at 2500 RPM for 15 minutes, Levels of the hormone testosterone were measured by using Radio-immunoassay (RIA) kit, after treating the samples with I^{125} (Labeled testosterone tracer), by employing Gamma Counter the connection between I^{125} with testosterone hormone were measured in ng/ml unit (Al- Janabi, 2007). Testis were excised and cleared off the attached fat and connective tissue. Testis preserved in formalin 10% and Histological sections were prepared for histological study (Luna, 1968).For comparison among groups, the one way ANOVA method was followed by utilizing means and standard error.

Results and discussion

The results of our work revealed to irreversible negative impact of decorticated and defatted castor seeds on testosterone level and ratio of testis weight to the body weight both of two parameters were decreased significantly ($P< 0.05$) (table1). This effect was credited to presence of the lectins in particular the toxic moiety (Ricin) as a major continent of the decorticated and defatted castor seeds (Al-Tahan, 1994); (Al-Tahan and Al-Shaha, 1990) and (Gruenwald, 2000). lipolytic activity of ricin on neutral lipids has been mentioned (Gruenwald, 2000) and (Lumbard *et al*., 2001). Cholesterol is one of the neutral lipids (Lumbard *et al*., 2001), cholesterol is considered as a precursor in androgenic biosynthesis process (Murray *et al*., 2003). Consequently the disturbance in testosterone hormone level (table1) was negatively reflects on the ratio of testis weight to body weight (table1) (Raji, *et al*., 2006). Testosterone is necessary for the development, growth and normal functioning of the testis and male accessory reproductive glands (Kamtchouing *et al*., 2002) and (Setty, *et al*., 1977). The abnormal histopathological view of the testis that treated with decorticated and defatted castor seeds 1.76 mg/kg in treatment group (Figure,2) and even sustained severely in the recovery group (figure,3) display a thickness of basement membrane of the seminiferous tubules, vacculation of Sertoli cells and mono-nuclear cells and cellular debris inside the lumen of the seminiferous tubules in compare with normal histopathological section of normal testis of control group (figure1) , Sertoli cells represent a good target to ricin molecule due to presence of the glycoconjugates on its' surface (Arenas, *et al*., 1998) and (Gheri, *et al*., 2004). Reduction of Sertoli cells led to depletion in its'

function of secreting of androgen binding protein (ABP) which enhance accumulation of testosterone and dihydrotestosterone in high concentrations within the seminiferous tubules and the interstetium of the testis (Cunningham, 2002). This find out led to adverse effects on the structure of the testis and that might due to reduction of serum testosterone levels (Prins, *et al*., 1991) which consequently caused this persistent and negative effect of decorticated and defatted castor seeds on the testis even on the recovery group.

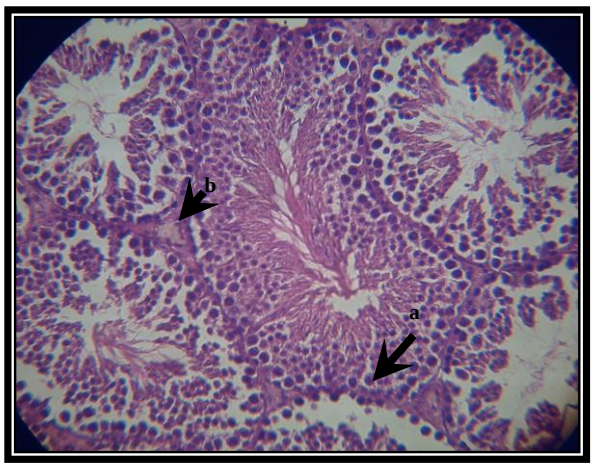

Figure (1): Cross-section in testis male mouse (Control group) shows (a)-Sertoli cell number, (b)-Leydig cell number. (H&E X 400).

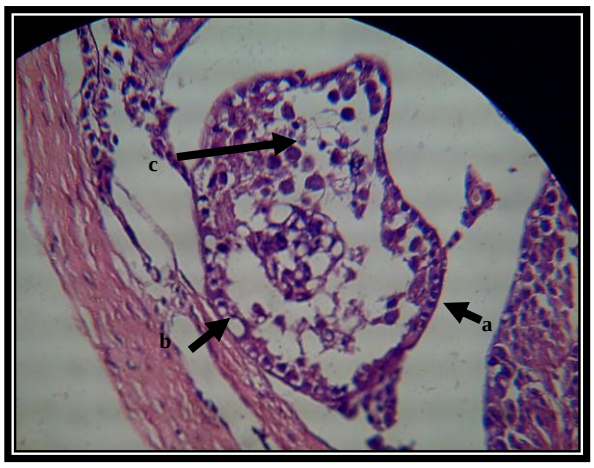

Figure (2): Cross-section in testis male mouse treated with DDCS 1.76 mg/kg (daily dose 38th day) shows:- (a) Thickness of basement membrane (b) vacculation of Sertoli cells (c) mono-nuclear cells and cellular debris (H&E X 400).

Table (1): Effect of decorticated and defatted castor seeds suspension on Testosterone level and testicular weight/body weight ratio in response to different duration of treatment.

Parameters / Groups	Testicular weight / body weight ratio (%)	Testosterone level (ng/ml)
Control Group	0.28 ±0.014 **A**	0.016 ±0.0122 **A**
Treatment group treated with decorticated and defatted castor seeds *Ricinus communis* (1.76 mg\kg) at 38th day	0.22 ±0.010 **B**	0.010 ±0.0054 **B**
Treatment group at 76th day (Recovery group)	0.21 ±0.009 **B**	0.011 ±0.0067 **B**

$P < 0.05$ (n=6). M ± SE

*Difference in letters refers to significant differences among groups.

Figure- (3): Cross-section in testis male mouse treated with DDCS 1.76 mg/kg (daily dose 76th day) shows (a) thickness of basement membrane (b) cellular debris and proteinus material (c) mono-nuclear cells (H&E X 400).

References

Al- Janabi, O. S. (2007). Possible Improvement of Reproductive Parameters By *Zingiber Officinale* Rhizome After use of Cimetidine In Male Mice. Master thesis College of Veterinary Medicine - University of Baghdad.

Al-Tahan, F. J. (1994). Antifertility effect of castor bean on mice. Fitoterapia 1: 34–37.

Al-Tahan, F. J., and Al-Shaha, O. M. S. (1990). A Primary Study on Castor Beans Cultivated in Iraq and it's Content of the Toxic Substance Ricin. The Proceeding of the 2nd Technical Education Conference, Baghdad, Iraq. Pp. 227-240. (In Arabic).

Al-Tahan, F. J., and Jasim, M. A.(2011). Study of the effect of decorticated and defatted castor seeds *Ricinus communis Linn.* on sperm functions and characters of male mice. Diyala journal of pure sciences 7 (3):192-204.

Arenas, M. I.; Madrid, J. F.; Bethencourt, F. R.; Fraile, B. and Paniagua, R. (1998) Lectin histochemistry of the human testis. J. Histochemistry. 21: 332-342.

Bhargava, S.K. (1984). Effects of plumbagin on reproductive functions in male dog. Indian J. Exp. Biol. 22: 153–156.

Chiej, R. (1984). "Encyclopedia of Medicinal plants" London: MacDonald.Pp1–5.

Coutinho, E.M. (2002).Gossypol: a contraceptive for men. Contraception 65 (4): 259–263.

Cunningham, G. J. (2002) Textbook of Veterinary Physiology. 3rd edition, Saunders, Elsevier health science, USA. P. 374-390.

Das, S. C.; Isichei, C. O.; Okwuasaba, F. K.; Uguru, V. E.; Onoruvwe O.; Olayinka, A. O.; Dafur, S. J.; Ekwere, E. O. and Parry, O. (2000). Chemical pathological toxicological studies of the effects of RICOM-1013-J of Ricinus Communis var minor on women volunteers and rodent. Phytother. Res. 14: 15–19.

Deshpande, V.Y.; Mendulkar, K.N. and Sadre, N.L. (1980). Male antifertility activity of Azadirachta Indica in mice. J Postgrad Med. 26 (3): 167–170.

Gheri, G.; Sgambati, E.; Thyrion, G. D.; Vichi D. and Orlandini G. E. (2004). The oligosaccharidic content of the glycoconjugates of the prepubertal descended and undescended testis: lectin histochemical study. Ital. J. Anat. Embryol. 109 (2): 69-84.

Gruenwald J.; Brendler T. and Jaenicke C. (2000). "PDR for herbal medicines".(Book) Medical Economics Company, Inc. P. 159.

Isichei, C. O.; Das, S. C.; Ogunkeye, O. O.; Okwuasaba, F. K.; Uguru, V. E.; Onoruvwe, O.; Olayinka, A. O.; Dafur, S. J.; Ekwere, E. O. and Parry, O. (2000). Preliminary clinical investigation of the contraceptive efficacy and chemical pathological effects of RICOM-1013-J of Ricinus communis var minor on women volunteers. Phytother. Res. 14: 40–2.

Kamtchouing, P.; Mbongue,; Fandio, G.Y.; Dimo, T. and Jatsa, H.B. (2002). Evaluation of androgenic activity of Zingiber officinale and Pentadiplandra brazzeana in male rats. Asian J. Androl. 4: 299-301.

Lohiya, N.K.; Manivannan, B. and Mishra, P.K. (2002). Chloroform extract of Carica papaya seeds induces long-term reversible azoospermia in langur monkey. Asian J. Androl. 4 (1): 17–26.

Lumbard, S.; Helmy, E. M. and Pieroni, G. (2001) Lipolytic activity of ricin from Ricinus sanguineus and Ricinus communis on neutral lipids. Biochem. J. 358: 773–781.

Luna, L. G. (1968). Manual of histology staining. Methods of armed forces. Institute of Pathology. 3rd ed. McGraw-Hill Book Company, New York and London.

Madhusudhana, R. C.; Rama, K. M. D. and Saraswati, B. P. (1997). Antispermatogenic and androgenic activities of various extracts of Hibiscus rosasinesis in albino mice. Indian J. Exp. Biol. 35: 1170–1174.

Makonnen, E.; Zerihun, L.; Assefa, G. and Rostom, A. (1999). Antifertility activity of Ricinus Communis seed in female guinea bigs. East African Medical J. 76 (6): 335-337.

McNeil R. T.; Noronha C. C.; Kusemiju T. O. and Okanlawon O. (2003). The anti-ovulatory effect of a seed extract of Ricinus communis (linn). Nig. J. Health Biomed. Sci. 2: 31–34.

Mdhluli, M.C. and Van der Horst, G. (2002). The effect of oleanolic acid on sperm motion characteristics and fertility of male Wistar rats. Lab. Anim. 36 (4): 432–437.

Murray K. R., Granner K. D., Mayes A. P. and Rodwell W. V., (2003) Harper's Illustrated Biochemistry, 26th Edition. Lange Medical Books, McGraw-Hill Boston, Toronto, New Jersey P. 111.

Okwuasaba, F. K.; Osunkwo, U. A.; Ekwenchi M. M.; Ekpenyong, K. I.; Onwukeme, K. E.; Olayinka, A. O.; Uguru, M. O. and Das, S. C. (1991). Anticonceptive and estrogenic effects of a seed extract of Ricinus communis var. minor. J. Ethnopharmacol. 34: 141–145.

Okwuasaba, F. K.; Osunkwo, U. A.; Ekwenchi, M. M.; Ekpeyong, K. I.; Onwukeme, K. E.; Olayinka, A. O.; Uguru, V. E.; Dafur, S. J.; Ekwere, E. O. and Parry, O. (1999). Anticonceptive and estrogenic effect of a seed extract of Ricinus communis var minor. J Ethnopharmacol; 34: 141–5.

Prins, S.G.; Birch. L. and Greene, G.L. (1991) Androgen receptor localization in different cell types of the adult rat prostate. Endocrinol. 129: 3187–3199.

Raji, Y.; Oloyo, k. A. and Morakinyo, O. A. (2006). Effect of methanol extract of Ricinus

communis seed on reproduction of male rats. Asian J. Androl. 8 (1): 115–121.

Sandhyakumary, K.; Bobby, R. G. and Indira M. (2003). Antifertility Effects of Ricinus communis (Linn) on Rats. Phytother. Res. 17: 508–511.

Setty, B.S.; Riar, S.S. and Kar, A.B. (1977). Androgenic control of epididymalfunction in rhesus monkey and rabbit. Fert. Steril. 22: 674–681.

Zhen, Q.S.; Ye, X. and Wei, Z.J. (1995) Recent progress in research on Tripterygium: a male antifertility plant. Contraception 51 (2): 121–129.