

The effect of *Ricinus communis* seeds extract on reproductive activity and blood values of male rabbits

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Abstract

Ricinus communis L. of Euphorbiaceae family is a widespread plant in tropical regions. It is used in traditional medicines as an anti-fertility agent in India and different parts of the world. The ether soluble portion of the methanol extract of *R. communis* var minor possesses anti-implantation, anti-conceptive and estrogenic activity in rats and mice when administered subcutaneously. The study was conducted on 10 local breed male rabbits, 1-2 years old, of 1-2 kg body weight. The animals were divided into two groups, control non-treated group and treated group in which animals were treated with single daily dose of 50 mg/kg b. wt. P.O. of decorticated and defatted castor seeds (DDCS) for 14 days. 28th day post treatment, animals were anesthetized by diethyl ether, sacrificed, abdominal cavity was open. The sexual organ (testes, epididymis, prostate and seminal vesical) weighed. In addition to take a biopsy from each one for histopathological changes. The study also included clinical and hematological parameters, in addition to sperm counts and the changes in sperm morphology. Body weight, body temperature increased significantly in treated males. While in non-treated group there were no significant changes. Respiratory rates and heart rate were none significantly changed in treated and non-treated males. Bleeding time none significantly increased in treated males, but increased significantly in none treated males. Clotting times decreased none significantly in treated and non-treated males. The blood parameters including, total erythrocytes count, hemoglobin concentration, PCV%, MCV, MCH, MCHC, total leucocyte and differential leucocyte counts were either increased or decreased none significantly in both groups. The results revealed that the effects of exposure to extract of ricin for 14 days on reproductive efficiency of rabbits, exhibited Significant decrease in weights of testes, epididymis, tails, heads of epididymis, seminal vesicles and prostate in treated males in comparison with those of non-treated males. While the body of epididymis did not show a significant changes. Significant decrease in live sperm numbers, number of sperms in epididymal head, in addition to deformities in high numbers of sperm, including enlarged or small sperms. breaks head, and its detachment, presence of two heads in one sperm, bifurcation of tail and its breaking, sperm coiling in samples from treated males in comparison with those from non-treated males. Histological changes were hyperplasia of lining epithelial cells and vacuolar degenerative changes, loss of spermatogenesis, and spermatocytes necrosis in those from treated males.

تأثير مستخلص بذور الخروع على الفعالية التناسلية وقيم الدم لذكور الارانب

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الخلاصة

نبات الخروع *Ricinus communis* L. من العائلة Euphorbiaceae واسع الانتشار في المناطق الاستوائية ، و يستخدم في الطب البديل كعامل مضاد للحمل في الهند وأجزاء مختلفة من العالم. يمتلك الجزء الذائب في الايثر من مستخلص الكحول الميثيلي لبذرة نبات الخروع نوع *R. communis var minor* تأثيرات مضادة للالتهاب ، مضادة للأخصاب ، وتأثيرات استر وجينية في الجرذ والفران عند حقنها تحت الجلد. أجريت الدراسة على 10 ذكور أرانب محلية، بعمر 1-2 سنة، ووزن 1-2 كغم. قسمت الحيوانات الى مجموعتين: مجموعة سيطرة لم تعالج بالمستخلص ، ومجموعة معالجة عولجت بمستخلص بذور الخروع وبجرعة 50 ملغم / كغم من وزن الجسم عن طريق الفم يوميا ولمدة 14 يوم. في اليوم 28 بعد العلاج ذبحت الحيوانات وتم فتح البطن ، وتم وزن الاعضاء التناسلية والاعضاء الملحقة بها ، وعد النطف في راس البربخ ، وحساب اعدادها الطبيعية والمشوهة والحية والميتة . ثم اخذت نماذج للفحص النسيجي . اظهرت نتائج الدراسة ان وزن الجسم ، ودرجة حرارة الجسم ارتفعا معنويا في الذكور المعالجة ، لكنهما لم يتغيرا معنويا في الذكور غير المعالجة . معدل التنفس وضربات القلب لم يتغيرا معنويا في كل من الذكور المعالجة وغير المعالجة. زمن النزف طال بشكل غير معنوي في الذكور المعالجة لكن الاطالة كانت معنوية في الذكور غير المعالجة . زمن التخثر هبط بشكل غير معنوي في كل من الذكور المعالجة وغير المعالجة. قيم الدم والتي شملت العدد الكلي لمستخلص بذور الحمرة، تركيز خضاب الدم، النسبة المئوية لحجم الخلايا المرصوصة ، معدل حجم الكرية، معدل خضاب الكرية، معدل تركيز خضاب الكرية، والعدد الكلي والتفريقي للخلايا البيض اظهرت اما زيادة او نقصان غير معنوية في كلا المجموعتين . كشفت نتائج الدراسة ان التعرض لمستخلص بذور الخروع لمدة 14 يوم اظهر تأثيرا على أوزان الأعضاء التناسلية والأعضاء الملحقة بها في الذكور المعالجة مقارنة بتلك غير المعالجة . كما اظهرت النتائج هبوط معنوي لاعداد النطف الحية ، فضلا عن زيادة اعداد النطف المشوهة في الذكور المعالجة . اظهرت الفحوصات النسيجية للخصى تدهور كبير وشديد في عملية تكوين النطف (تتخر النطف) وشوهت النبيبات الناقلة للمني بأحجام مختلفة . وظهرت البروستات في الذكور المعالجة تتخن في سدى بين النبيبات، كما لوحظ كميات كبيرة من الكولاجين ، حلم مفرطة التنسج للبطانة الطلائية ، وجود اجسام نشوية في جوف بعض النبيبات . يمكن الاستنتاج من الدراسة ان زيت بذور الخروع له تأثير سلبي على كفاءة التكاثر لذكور الارانب.

الكلمات المفتاحية : الكفاءة التناسلية ، ارنب ، نبات الخروع

Key words: Reproductive activity, Rabbit, Ricinus communis

Introduction

In the last few decades there has been an exponential growth in the field of herbal medicine. One of such medicinal plant is *R. communis* L. which belongs to the Euphorbiaceae family (1). The plant has many common names such as castor plant, castor oil plant, castor bean plant, wonder boom, dhatura, eranda, palma Christi (2). *R. communis* grow naturally over a wide range of geographical regions and may be activated under a variety of physical and climatic regions (3). Many plants are indicated as folklore medicine as anti - fertility agents and efforts are being made to look into the practicability of employing these herbs as commercial fertility

Sandhyakumary *et al.*, (5) reported the anti - fertility activity of *R. communis* on rats. Sperm function and sperm immobilization study on male rats conducted with seed extract of the plant (6-8). The seeds are more commonly classified into three groups that include the large seeds (Variety major), medium seeds (variety intermediate) and the small seeds (variety minor) Sani and Sule (9). *R. communis* is tropical plant, known as castor bean that is distributed widely across the world (10). The castor oil makes up 35% to 55% of the weight of the seeds (11) and the oil contains 85% to 90% ricinoleic acid (12). Literature revealed that castor seeds

contain about 42-55% oil (13) which can be extracted by variety of processes or combination of processes, such as hydraulic pressures, continuous screw presses and solvent extraction. If the castor seed is swallowed without chewing and there is no damage to the seed husk, it passes harmlessly through the digestive tract. However, if it is chewed and then swallowed, the intestine absorbs the ricin toxin (14). Many trials have been done to explore the effect of *R. communis* on the reproductive system in both sexes. The first research had done by Okwuasaba *et al.*, (15), who refer to anti-implantation and contraceptive activities effect of ether – soluble fraction of a methanol extract of *R. communis* var. minor seeds administered subcutaneously at a doses 1.2 g / kg and 600 mg / kg, respectively, in divided doses on adult female rats and rabbits (16). There is little information about effect of *R. communis* on male reproductive system or spermatogenesis, one of these studies revealed that the 50% ethanol extract of the roots of *R. communis* produced reversible anti-fertility effects on male rats. There was a drastic reduction in the epididymal sperm counts, alteration in the motility, mode of movement and morphology of the sperms were observed. Reductions the fructose and testosterone levels were suggestive of reduced reproductive performance. Reversibility tests showed that the anti-fertility effects of *R. communis* was completely reversible on withdrawal of the extract (5). The most recent study was done by Raji *et al.*, (7) who suggested that the methanol extract of *R. communis* has a deleterious effect on male reproductive functions in rats, sufficient to cause reversible infertility in the male rats.

The aim of current study was to investigate the impacts of the decorticated and defatted castor seed on the male rabbit's reproductive functions including weight changes of sexual organs, sperm function and morphology.

Material and methods

An amount of seeds of *R. communis* were collected from farms distributed in Baquba city, Diyala, Iraq.

Extraction

Seeds are cleaned and washed with tap water and then dried. The outer coating (husks) of the seeds were manually removed and the residual wet flesh ground into pulp. The wet ground pulp was pressed with mechanical hydraulic press, then treated with ether to dissolve and get rid as much as possible from the oil. The cake was dried using desiccators by using NaOH and the final result was dry, whitish – beige, and fine powder kept in special container till use (17)

Anti – fertility study

The study was conducted on 10 local albino male rabbits, 1-2 years old, of 1-2 kg b. wt. after acclimatization for 2 weeks at room temperature of 25- 27 °C, and 12 hr light, 12 hr dark conditions. The animals divided into two groups: Control non treated with extract, and treated group in which the animals were treated by single daily dose of 50 mg /kg b. wt. P.O. of decorticated and defatted castor seeds (DDCS) for 14 days. Animals were weighed then anesthetized by diethyl ether, sacrificed in the 28th day of treatment, abdominal cavity was opened. Testis and epididymis excised and rinsed with physiological normal saline, and cleaned from attached fat and connective tissue. Both testes left and right were weighed individually. The tail of the left epididymis was taken and immersed in one ml of normal saline at 37 °C in Petri dishes, and then the tail was cut into at least 200 sections by microsurgical scissors, to perform the dependent examinations including sperm character (18). Calculation sperm content in epididymal head Method of Sakamoto and Hashimoto (18) was applied for counting the sperms in epididymal head. As the head of left

epididymis weight, minced to small pieces in Petri dish, 9.8 ml of neutral formalin buffer, and 0.1 ml of eosin stain 5% were added. A clean hemocytometer slide prepared. One drop of the prepared solution put under the cover slide in hemocytometer, the slide left for 5 minutes to settle the sperms in squares. The sperms numbers were counted in 5 squares, in center and in corners of the slide (in 80 small squares). The total number of sperms in epididymis head was accounted according to (19). Calculation of live sperm ratio and the ratio of abnormalities in sperms. The tail of left epididymis cut after put it in 2 ml of normal physiological saline at 37°C, then one drop took, put on slide to which one drop of necrosin – eosin added, The two drops were mixed gently for half minute, then in border of slide a sample was taken and spread on the new slide at acute angle, then dry, examined by x 100 oil lens (20). Number of live sperm those not stained, in addition to ratio of deformity of sperms were calculated. Additional parameters were included in this study which was clinical, hematological according to (21).

Statistical analysis

Results are expressed as Mean \pm SE. The data were analyzed by t- test (22) the level of significant was at level of $P < 0.05$.

Results

Body weight increased significantly in treated males, but in non- treated males non- significantly decreased. Body temperature increased significantly in treated males, but non- significantly increased in non- treated males. Respiratory rates non- significantly increased in treated and non- treated males. Heart rates none significantly decreased in treated males, but increased none significantly in non-treated males. Bleeding time none significantly increased in treated males, but increased significantly in non-treated males. Clotting time decreased none significantly in both treated

and non-treated males (Table -1-).

Total erythrocytes count increased none significantly in treated males, but decreased none significantly in non-treated males. Hemoglobin concentration increased none significantly in both treated and non-treated males. PCV showed no changes in treated males, but increased none significantly in non-treated males. MCV, MCH, MCHC increased none significantly in treated males but decreased none significantly in non-treated males (Table -2).

Total leucocytes count increased none significantly in both treated and non-treated males. Eosinophils, Basophils none significantly decreased in both treated and non-treated males. Monocytes increased none significantly in both treated and non-treated males. Heterophils decreased none significantly in treated male, but increased none significantly in non-treated males. Lymphocytes increased none significantly in treated males, but decreased none significantly in non-treated males (Table-3). Table -3- Showed total leucocytes and differential leucocytes of animals used in the study.

The results revealed that the effect of treatment with extract of ricinus for 14 days on reproduction of rabbits included, significant decrease in weight of testis, epididymis, tails, heads of epididymis of treated males in comparison with non-treated males. The body of epididymis did not show a significant changes, the seminal vesicle and prostate of males treated with extract also showed decrease in weight in comparison with those of non-treated males. The results also showed significant decrease in live sperm numbers in treated males in comparison with those of non-treated males (Table -4-).

The results showed increase in numbers of deformed sperms from treated males. Deformity represented by deformity of head, included enlarged or small size. Breaks head, and its detachment, presence of two heads in one sperm, bifurcation of tail and its breaking, sperm coiling (picture-1-).

Table -1 Showed body weight, body temperature, respiratory rates, heart rates, bleeding time and clotting time of animals used in study.

Parameters	Treated		Non treated	
	Day		Day	
	0	15 th	0	15 th
Heart rate / min	220±11.38	205.6±11.95	191.5±8.10	228±18.51
Resp. rate/ min	145.6±18.34	155.2±15.49	147.75±18.68	175.2±22.42
Body tem. °C	37.58±0.22	39.1±0.2*	38.7±0.19	39.0±0.13
Body weight kg	1.244±0.065	1.716±0.086*	1.553±0.123	1.474±0.129
Bleeding time / seconds	27±2.99	34±7.64	29.5±4.01	51±8.11*
Clotting time / seconds	40±8.20	27±3.74	58.75±17.12	48±18.04

The values are M + SE. * Significant at level of P < 0.05

Table -2- Showed total erythrocytes count, hemoglobin concentration, PCV% and erythrocytes indices (MCV, MCH, MCHC) of animals used in the study.

Parameters	Treated		Non treated	
	Days		Days	
	0	15 th	0	15 th
RBC X10 ⁶ /µl	4.81±0.80	5.00±0.82	4.51±0.55	5.32±0.63
Hb gm/ dl	12±1.04	12.14±0.17	11.28±0.39	11.54±0.35
PCV%	35.4±3.10	35.6±1.49	33±1.08	34±1.05
MCV ft	80.87±11.48	81.46±16.46	76.61±8.68	67.61±9.05
MCH pg	27.01±3.89	27.81±5.64	26.18±2.99	22.97±3.13
MCHC gm/ dl	33.90±0.08	34.10±0.11	34.16±0.10	33.94±0.09

The values are M + SE.

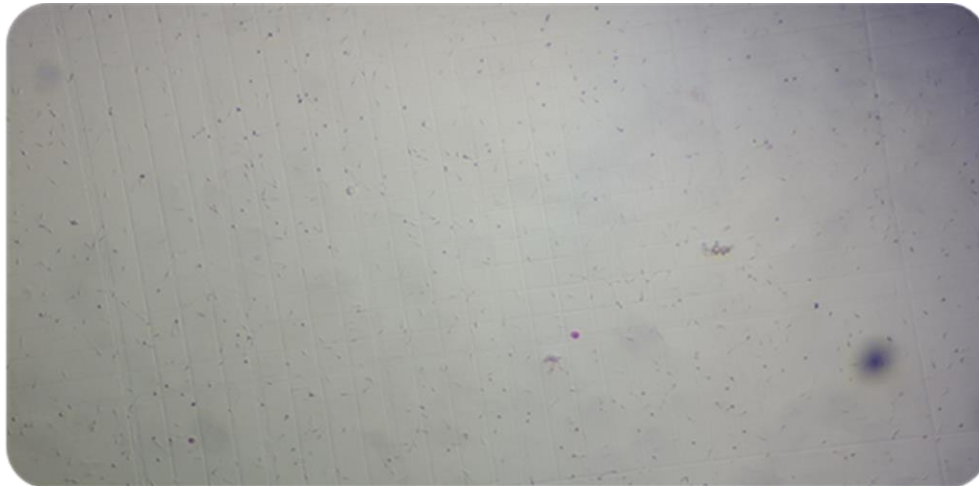
Table -3- Showed total leucocytes and differential leucocytes of animals used in the study.

Parameters	Treated		Non treated	
	Days		Days	
	0	15 th	0	15 th
WBCX10³/ μl	3.088±0.52	4.070±0.62	2.822±0.65	3.422±0.44
H%	50±5.29	39.2±5.2	43±3.08	46.4±4.24
L%	39.8±4.59	54.4±5.77	50.5±4.11	47.4±4.84
E%	5.2±0.97	1.8±0.49	2.25±0.75	2.8±0.58
M%	3.2±0.97	3.8±0.86	2.5±1.5	2.8±0.73
B%	1.8±0.91	0.8±0.2	1.75±0.25	0.6±0.4

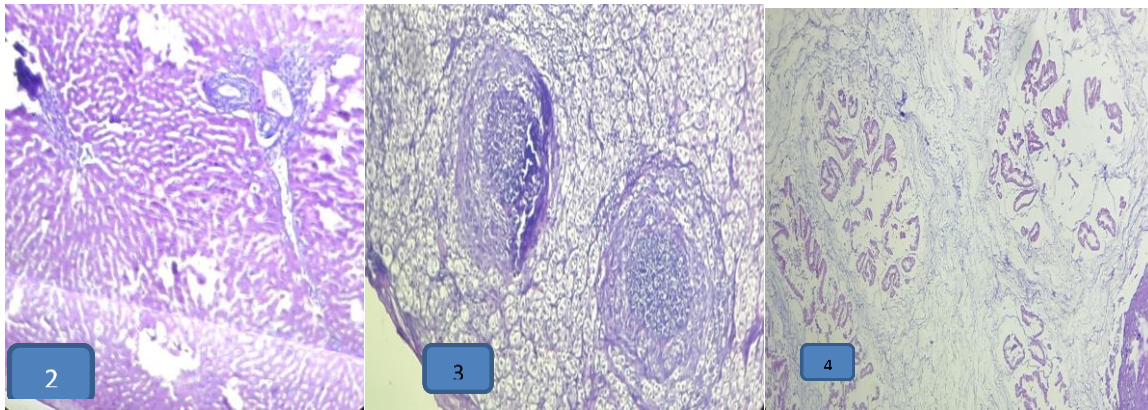
The values are M + SE.

Table -4- Showed weights of sexual organs in grams with The morphology deformity and counts of sperms

Part			Non treated	Treated
Testis	left		1.41	1.06
	right		1.31	1.02
Epididymis	head	Left	0.18	0.14
		right	0.3	0.18
	body	Left	0.26	0.03
		Right	0.24	0.09
	tail	Left	0.23	0.14
		Right	0.325	0.21
Prostate			0.1	0.12
Seminal vesicle			0.55	0.18
Sperm count			47681.16	297222.22
Morphology	dead		7.5	0
	normal		49	50
	head		10	6
	tail		6.5	2
	double		1	2



picture-1- Showed a method of sperm counts in hemocytometers



Picture -2- Showed a histopathological change in testis of male treated with ricinus.

Picture -3- Showed a histopathological change in seminiferous tubules of males treated with ricinus

Picture -4- Showed histopathological changes in testis of male none treated with ricinus.

Prostate presence of corpora amylacea in the lumen of some acini.

Histologically showed a histopathological change in testis of treated with ricinus, loss of spermatogenesis in seminiferous tubules; vacuolar degeneration of spermatocytes in others, loss of spermatogenesis in seminiferous tubules; vacuolar degeneration of spermatocytes in others (Picture-2-). Histologically change in seminiferous tubules: hyperplasia of lining epithelial cell and vacuolar degenerative changes (Picture -3-). histopathological changes in testis of male none treated with ricinus, Prostate presence of corpora

amylacea in the lumen of some acini(Picture -4-)

.Discussion

Results of current study indicated that there were deleterious effects of the decorticated and defatted a castor seeds on male reproductive system of male rabbits.

In study done by (23) there were deleterious effects of the decorticated and defatted a castor seeds on male reproductive system of mice, this effect can most probably attributed to the action of ricin (16), since the another lectin (Agglutinine) is not significantly absorbed

from the gut (24).

The exposure to *R. communis* extract lead to important changes in weight of sexual organs, spermatogenesis, in addition to histological changes in testis of exposed animals, these can attributes to many factors from which an important one are hormones (25). The decreases in weights of testes, epididymis, and accessory glands, and the histological changes may attribute to reduce in level of LH and testosterone levels, as there are indications that reduce in weight of these organs and their function can result from decreased level of these two hormones (26). The reduced content of head of epididymis from sperms in males expose to plant extracts can attributed to reduced level of testosterone hormone, as (27, 28) referred to presence of receptors for testosterone in sperms in their early stages and the primary sperm cells and they strongly referred to the importance of this hormone in all stages of sperm genesis only in stage of spermatogenesis. Also they referred to possibility of continuity of spermatogenesis in make from which their pituitary gland removed by androgens injection. The significant reduction in the sperm count can be attributed to the direct effect of ricin on the spermatozoa, since the spermatozoa has sugar residues like galactose, acetylgalactosamine, and D-mannose known by the Glycoconjugates or glycocalyx (29), these residues form a target to the ricin toxin B chain (RTB) which considered the binding domain of ricin to the surface of the eukaryotic cells (30). There sequences of interactive binding presumably prevent protein synthesis which led to cellular death (31).

The decrement in the ratio of testis weight to body weight may be attributed to the disturbance of testosterone levels (7). On the other hand, this drop of the ratio of testis weight attributed with results of spermatogenic cells destruction can be returned to presence of the glycoconjugates in the testis itself (32), and this decrement are coincided with the mathematical

decrement in the body weight changes.

Mustafa (23) referred that results of his study displayed an increment of abnormal sperm morphology, due to the direct effect of ricin on spermatogenic cells and immature spermatozoa due to the glycoconjugate- ricin complex and ribosomal impairment and deformity in division caused the double parts of spermatozoa structure (double head and double tail) and the deformity of the immature cells and that correlated to phagocytic action.

The conductive system role of the epididymis is very important part in spermatozoa maturation; transport and storage during the period of spermatozoa develop motility (33). the direct effect of ricin on the epididymis is due to presence of abnormalities in the sperm morphology of treated group at 28th day which associated with presence of sperm surface glycoconjugates may promote alteration of cell membrane and promote loses of elasticity and fluidity of head and tail and confirm mainly head abnormalities and made micro irregular head due to shrinkage of head and irregular shape.

The decrease in eight of epididymis and testes and the accessory organs may attribute to LH and testosterone as the decrease in these two hormones lead to decrease in weight of sex organs and their functions (26).

The decrease in head of epididymis count from sperm in male exposed to ricinus the cause of it may be decrease in testosterone hormone level. As (27, 28) refer to presence of receptors

The exposure of males to *R. communis* lead to increase the percent of dead sperms in epididymis tail and this may be due to presence of spermicidal material (34).

Conclusions:

The extract of *Ricinus communis* has negative influences on reproductive performance of male rabbits in current study.

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