Study of the effect of decorticated and defatted Castor Seeds (*Ricinus Communis Linn.*) on sperm functions and characters of male mice.

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Receiving Date: 28-12-2010 **- Accept Date:** 23-05-2011

Abstract

This study was designed to investigate the effects of decorticated and defatted castor seeds *(Ricinus Communis)* on the sperms functions and characters of male mice at two consecutive spermatogenic cycles. Eighteen albino Swiss male mice with Ten weeks old were used as animal model, they divided in to three groups (Six male mice of each), 1st group served as control group and received distilled water only, and the 2nd group has been set as treatment group which was treated orally with 1.76 mg/kg. BW from watery suspension of decorticated and defatted castor seeds extract for 38 days (single spermatogenic cycle). The 3rd group has been treated with 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: total sperm count, Sperms viability, abnormal sperm percentage, and the turbidimeteric parameters of sperm motility.

Introduction

During the past few decades sporadic attempts have been made by various investigators to develop male contraceptive agents from various antifertility plants available in their locality or in the market. Various medicinal plant extracts have been tested for their antifertility activity both in male and female (1).

Castor plant *Ricinus Communis* belongs to Euphorbiacaea Family, and grows naturally over a wide range of geographical regions and may be activated under a variety of physical and climatic regimes (2).

Different parts of the plant have been reported to have several medicinal values. Its seed has antihelmintic, carthartic, emollient, laxative and purgative properties (3)

Many trails have been done to explore the effect of *Ricinus Communis* on female reproductive system in order to investigate its ability as an antifretility agent (4-10).

Despite the presence of many studies which ensure the anti- fertility and anti estrogenic effect of *Ricinus Communis*on female reproductive system, but there are little studies about effect of *Ricinus Communis* on male reproductive system or spermatogenesis, (11)and(12). Consequently, our present study was designed to investigate the impacts of the decorticated and defatted castor seed on the sperm characters such as: total sperm count, Sperms viability, abnormal sperm percentage, and the turbidimetric parameters of sperm motility. On male mice and study the possibility of recovery from the resultant effects.

Materials and Methods

The experiment of this research was conducted in the animal house of physiology and pharmacology Department at Veterinary Medicine College of Baghdad University.

The seeds of castor bean *Ricinus Communis* were collected from the middle region of Iraq (Abu Graib, Baghdad province) during August to October 2007. The plant was authenticated at the Ministry of Agriculture/ State Board for Seeds Testing and Certification S.B.S.T.C. in Al-Manns'ur/ Baghdad in number 4229 in 8/11/2007; the seeds were cleaned and washed with tap water then dried in open air and kept in special container till use. The outer husk was removed manually in order to get the white pulp, the decorticated castor seeds were pressed with mechanical hydraulic press for primary castor oil take out, and the result

was friable texture material, this material mixed by the blender with petroleum ether for complete defatting of castor oil, the mixture was filtered by filter paper and special cotton tissue to separate the cake from the castor oil - petroleum ether mixture. The cake was dried using desiccator by utilizing (NaOH) and the final result was dry, whitish – beige, and fine powder kept in special container containing anti-moisture sac to avoid the moisture until use (13).

Eighteen albino Swiss male mice (Ten weeks old) were used as animal model, they divided in to three equal groups; 1st group served as control group and received distilled water only, while the 2nd group has been set as treatment group which was treated orally with 1.76 mg/kg BW from watery suspension of decorticated and defatted castor seeds extract for 38 days (5). (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).

Animals have been sacrificed at the 38th of the experiment, abdominal cavity was opened, testis and epididymus excised and covered with physiological normal saline and cleaned from attached fat and connective tissue. The tail of the left epididymus was taken and immersed in 1 ml of normal saline at 37°C in a watch glass, and then the tail was cut into at least 200 sections by microsurgical scissors.

Sperm count was done under a microscope with the aid of the improved Neubauer hemocytometer. Count was done in five large Thoma square and adjustment was made for volume of the normal saline added. (14).

A viability study (percentage of live spermatozoa) was done using the eosin/nigrosin stain. Semen was squeezed onto a microscope slide and two drops of the stain was added. The live sperm cells were unstained in contrast to the dead sperm cells. The stained and the unstained sperm cells were counted using ×40 objectives of the microscope and an average for each was taken from which percentage viability was calculated. (15)

Sperm morphology was done by staining the sperm smears on microscope slides with two drops of eosin\nigrosin stain and air-dried. The slides were examined under the microscope using ×100 objectives under oil immersion. The abnormal sperm cells were counted and the percentage calculated.(16).

The analysis of sperm motility was done by using the turbidimeteric method described by Sakoloski et al (17) and modified by Al-bayaty (18). It is a spectrophotometric analysis of an absorbed amount of light by injected sperms inside a special masked cuvette when they move toward a determined wave length (454 nm.) of light path.

For comparison among groups, the one way ANOVA method was followed by utilizing means and standard error.

Results and Discussion

The results obtained from this study were indicated to the deleterious effect of the decorticated and defatted castor seeds on sperms characters of mice, this effect can most probably attributed to the action of ricin (5).

Ricin belongs to a class of proteins known as ribosomal inactivating proteins (RIPs). Ribosomal Inactivating proteins. As their name suggests, are proteins which interfere with the function of ribosomes, halt protein synthesis and thus induce cellular death. (19).

The significant reduction in the sperm count and sperm viability both ($P< 0.05$) (table1) 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 (20), 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 (21).

Our results displayed an increment of abnormal sperm morphology ($P< 0.05$) (table1) that may be due to the direct effect of ricin on spermatogenic cells and immature spermatozoa due to the glycoconjugate–ricin complex (20) and ribosomal impairment (19). Deformity in division might cause the double parts of spermatozoa structure (double head and double tail) (figure A and B).

The conductive system role of the Epididyms is very important part in spermatozoa maturation, transport and storage during the period of spermatozoa develop motility (22). The direct effect of ricin on the epidydimis is due to presence of the glycoconjugates in the epididymis of mammalian animals (23) may explain the high percentage of abnormalities in the sperm morphology of the treatment and recovery groups 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 (figure, C and D).

Motility of spermatozoa is one of the most important parameters which used to assessing the quality of sperm suspension. Since this parameter is most closely related to conception rate (24). The turbidiametric assay is providing a rapid, reproducible and quantitative analysis of the sperm suspension in normal and abnormal (17, 25). Motility index (MI) parameter of turbidiametric assay reflects of relative ratio of motile spermatozoa to number of viable sperm. (18).

Motility index (MI) was significantly decrease ($P< 0.01$) (table 2) in treatment and recovery groups as compared with control group of experiment.

Ricin decrease Ca^{+2} ion up take (26), this evidences will cause disturbance in the Calcium homeostasis (27, 28) and lead to reduce sperm motility.

Sperm velocity of treatment and recovery groups also decreased significantly ($P< 0.01$) (table 2). Yanagimachi and Usui (29) and others (30 – 32) reported that the importance of Ca^{+2} ion which exist in seminal fluid in sperm motility. Since Ca^{+2} ion has a regulatory role in the context of axonemal function (33). Mainly Ca^{+2} affect the velocity of sliding and bending of axonemal microtubules (34, 35)

Also presence of glycoconjugates on the sperm tail too (36) may be presumably led to appearance of possible tail deformity due to effect of ricin on the sperm tail which affect the sperm motility.

Moreover, reduction of sperm motility index can be speculated to reduction of both spermatozoal concentration and viability of spermatozoa (24, 37)

Lag time represent the time required for the faster fraction of spermatozoa to leave the suspension and swim into the light path (18).

Sakolski et al. (17) and Levin et al. (25) reported that the value of lag time changed independently with dose and time, furthermore the lag time is a quantitative parameter. The lag time was significantly increased ($P< 0.01$) (table 2) in treatment and recovery groups. This increment in lag time may be attributed to loss of part of rapidly movement of sperm(18), which also affected in treatment and recovery groups ($P< 0.01$) (table 2).

The results of recovery group of the experiment which revealed to the extended harmful effect of the ricin on the reproductive parameters may attributed to mRNA persist miscoding effect on the reproductive enzymes and cells dysfunction mechanisms that may be attributed to the direct effect of ricin on the DNA helix because ricin has the ability to depurinate the adinine which possessed by DNA as well as in rRNA (38, 39) in our speculation this evidence may led to desynchronization in the DNA helix that may led to false or misreading in protein synthesis at the levels of protein elongation and as a sequent protein translation. These foundations provoke the injurious effect of decorticated and defatted castor seeds in testicular function and their components which play a role of permanent negative effects on the measured parameters of the recovery group.

Table (1): Effect of decorticated and defatted castor seeds suspension on sperm characters in response to different duration of treatment.

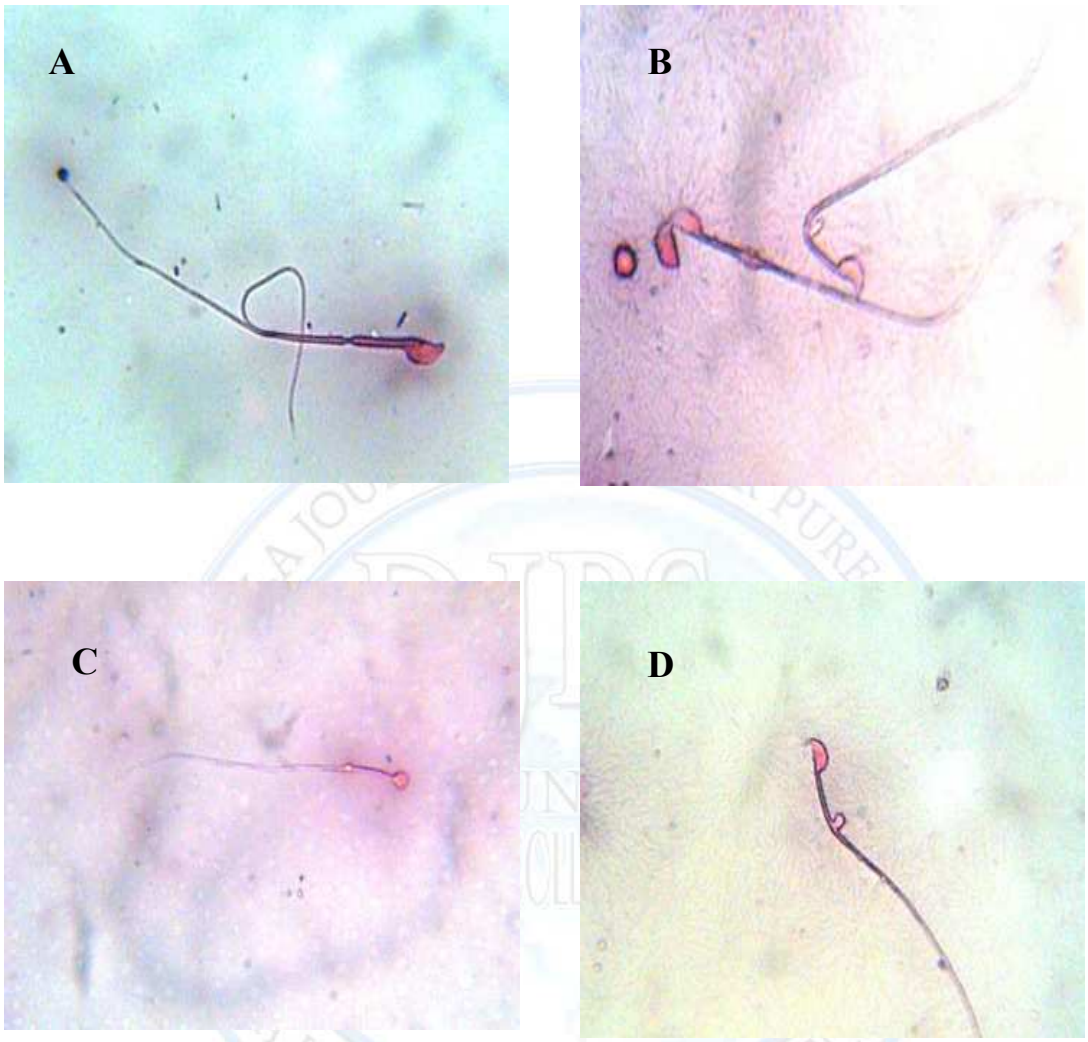
Parameters / Groups	Sperms concentration X 10^4/ml	Sperms viability (%)	Abnormal sperms Morphology (%)
Control Group	310 ±10.8 **A**	78 ±1.24 **A**	19.58 ±1.13 **A**
Treatment group treated with decorticated and defatted castor seeds *Ricinus Communis* (1.76 mg\kg) at 38th day	187 ±6.67 **B**	18.42 ±3.57 **B**	88.25 ±2.66 **B**
Recovery group at 76th day	113 ±9.3 **B**	17.67 ±4.67 **B**	58.83 ±5.47 **B**

P < 0.05 (n=6). M ± SE
*Difference in letters refers to significant differences among groups.

Table (1): Effect of decorticated and defatted castor seeds suspension on sperm characters in response to different duration of treatment.

Parameters / Groups	Lag time (Sec.)	Motility index (%)	Sperms Velocity (µm/Sec.)	Fraction of rapidly moving sperm x 10^6/ml
Control Group	145.8 ±25.2 **A**	0.540 ±0.0372 **A**	77.15 ±4.84 **A**	0.0064 ±0.00058 **A**
Treatment group treated with decorticated and defatted castor seeds *Ricinus Communis* (1.76 mg\kg) at 38th day	0 **B**	0 **B**	0 **B**	0 **B**
Recovery group at 76th day	0 **B**	0 **B**	0 **B**	0 **B**

P < 0.01 (n=6). M ± SE
*Difference in letters refers to significant differences among groups.



Different Types of sperm Abnormalities in treatment and recovery groups (100X): A- Double tail sperm, B- Double head sperm, C-Microhead sperm And D- Cytoplasmic droplets

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