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Research article

Efficiency of reproduction of female rabbits experimentally treated with *Ricinus communis*

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Abstract

Aim of current study was to demonstrate the reproductive efficiency of female rabbits treated experimentally with Ricinus communis. 32, local breed rabbits, weighing 1- 2 kg, of 1-2 years old, 16 rabbits of either sex. 8 rabbits of either sex were treated with Ricinus extract, 50 mg /kg b. wt. PO, daily for 14 days. The remaining rabbits were left without treatment. In day 14th post treatment, one female was cross to one male in separated cages, as follow: rabbits of the 1st group represented treated females, crossed with treated males. Rabbits of 2nd group represented none treated females, crossed with none treated males Those of 3rd group represented treated females, crossed with none treated males. And those of 4th group represented none treated female crossed with treated males. The mixing continued for 1 week. After crossing with the males, all females were examined by sonar weekly for pregnancy, for 4 weeks. 8 females, 2 from each group, were subjected to euthanasia using chloroform - soaked cotton at day 28 post crossing; each rabbit was lapratomized, and the uterine horns incised, the number of implantation sites was recorded. The remaining females remained, till normal birth and the number of babies delivered by each rabbit were recorded. Body weight significantly increased in both treated and none treated female. Body temperature significantly increased in treated females. Respiratory and heart rates none significantly increased in both treated and none treated females. Bleeding time decreased significantly in treated females. Clotting time not significant changed. Total erythrocytes count and hemoglobin concentration, Packed Cell Volumes (PCV%), Mean Corpuscular Volume (MCV, Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Total leucocytes count not significant changed. Monocytes (M%), Basophils (B%) not significant changed. Eosinophils (E%), Heterophils (H%) significantly decreased in treated females. Lymphocytes (L%) increased significantly in treated females. The results revealed significant decreases in percentage of fertility in females treated with Ricinus extract. As females treated with ricin and crossed with males treated with ricin were none pregnant (0%). The effect of treatment with Ricinus extract for 14 days, on reproductive efficiency of rabbits, did not affect the length of pregnancy, as the period remain 30-32 days. There was significant decrease in fetus numbers in female treated with Ricinus, and those crossed with treated male in comparison with those of none treated female and male.

Keywords: Female rabbits, Ricin, Reproductive efficiency.

Introduction

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 regulators (1). (2) reported the anti-fertility activity of *R. communis* on rats . In

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different societies, the use of the seeds of *R. communis* as oral contraceptive has been documented (3, 4). It is known that 4-5 seeds of this plant in India are orally taken with water during menstrual period to prevent conception for a period of one year (5). In Saudi Arabia; three intact seeds are used on the first day of menstruation as oral contraceptive (4). It has also been the practice that, in the Middle Belt of Nigeria, traditional healers administer to women three seeds of the variety minor as contraceptive for duration of 12 months (6, 7). *R. communis* Linn is tropical plant, distributed widely across the world (8). The plant has many common names as castor bean, castor plant, castor oil plant, wonder boom, dhatura, Eranda, palma Christi (9). Castor oil plant, documented as traditional contraceptive, belongs to Euphorbiaceae family (10). It is terrestrial, flowering, robust perennial shrub with cosmopolitan distribution. Phytosterols, proteins, fatty acids, coumarins, phenolic compound (11), flavonoids (12, 13), alkaloids (12) terpenoid and tocophero; related compounds (14) have already been isolated from different parts of this plant. The seeds, leaves, and stems of the plant contain the glycoprotein ricin, which is poisonous to humans and animals. 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 (15). All parts of plant are important viz, bark, leaves, flowers, seed, oil … etc. The plant is reported to possess antioxidant, anti-implantation, anti-inflammatory, anti-diabetic, central analgesic, antitumor, larvicidal and adult emergence inhibition, and anti-conceptive and anti-asthmatic activity. All these uses are due to the presence of certain phytoconstituent in the plant. The major phytoconstituent reported in this plant are rutin, gentistic acid, quercetin, gallic acid, kaempferol-3-O-beta -d- rutinoside, kaempferol-3-O-beta-d- xylopyranoid, tannins, Ricin A, B, and C, ricinus agglutinin, Indole -3- acetic acid and an alkaloid ricinine (16). 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 (16). Contraceptives, an effective birth control measure, have seen triplicate usage in recent times. So far, male contraceptives are less successful than that of female ones (17). These contraceptives are mostly synthetic and have severe side effects. Different ethnic groups of India use this plant to treat reproductive health related disorders (18). Leave, seeds, roots used for contraception and abortion (19, 20), seed extract decreased chances of pregnancy (21, 22) induce long – term female contraceptive (23), Whereas, ricin –A chain (RAC) from seed extract acts as abortifacient in rabbits (24). Methanolic extract of seed has anti-implantation and anti-conceptive activities (25). As reported petroleum ether fraction of *R. communis* seed (RICOM 1013-J) reduces fertility activity of female reproductive system (26, 6). Many trials have been done to explore the effect of *R. communis* on the reproductive system in both sexes, the first researches had done by (25), 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, (27) found that the treatment of female mice with decorticated and defatted castor seeds lead to decrease fertility of female mice. Earlier, (6) had reported the anti - conceptive and estrogenic effect of the methanol extract of *R. communis* seed in female rats (28). The efficacies of *R. communis* seed as a contraceptive in women and female rodents have been widely reported. A single oral dose of 2.3 g - 2.5 g of the seed prevented conception for a period of 12 months in women volunteers (29) and

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experimental rodents (30). The anti - ovulatory and anti- conception properties of the methanol extract of *R. communis* seed were achieved by its direct effect on the ovarian tissue and presumably by interfering with the hypothalamic pituitary-ovarian axis in Sprague -Dawley rats (31). Aim of current study was to demonstrate the reproductive efficiency of female rabbits treated with *R. communis* extract under experimental conditions.

Materials and Methods

1. The source of plant:

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

2. Extraction

Seeds are cleaned and washed with tap water and then dried. The outer coating (husks) of the seeds was manually removed and the residual wet flesh was grounded. The wet ground pulp was pressed with mechanical hydraulic press. For primary castor oil take out, the whitish scum was mixed by the blender with petroleum ether for complete defatting of the castor oil, the mixture was filtered by filter paper and special cotton tissue to separate the cake from the castor oil and petroleum ether. 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 (32).

3. Anti- fertility study

3.1. Experimental animals:

Thirty- two, local breed rabbits, weighing 1- 2 kg, of 1-2 years old, 16 rabbits of either sex. 8 rabbits of either sex, were treated with Decorticated and Defatted Caster Seeds (DDCS) at a dose of, 50 mg /kg b. wt. PO, daily for 14 days. The remaining rabbits were left without treatment (Control). In day 14th post treatment, one female was cross to one male in separated cages, as follow: rabbits of the 1st group represented none treated females, crossed with none treated males. Rabbits of 2nd group represented treated females, crossed with treated males Those of 3rd group represented treated females, crossed with none treated males. And those of 4th group represented none treated female crossed with treated males. The mixing continued for 1 week.

3.2. Experimental Protocol

After crossing with the males, all females were examined by sonar weekly for pregnancy, for 4 weeks. 8 females, 2 from each group, were subjected to euthanasia using chloroform – soaked cotton at day 28 post crossing; each rabbit was lapratomized, and the uterine horns incised, the number of implantation sites was recorded. The remaining females remained, till normal birth and the number of babies delivered by each rabbit were recorded. Blood samples were collected, for complete blood pictures: Total erythrocytes counts (TEC), Hemoglobin concentration (Hb), Packed cell volumes (PCV%), Total and differential leucocytes counts (TLC, and DLC), in addition to Bleeding and Clotting times, according to (33). In addition to samples from genital organs, for histopathological examination according to (34). The dependent parameters also include clinical examination (heart rates, Respiratory rates, Body temperature and Body weight).

4. Statistical analysis

The data were analyzed by one way analysis of variance, and then SD the least significant difference was applied, with use Duncan test for finding the significant differences (35), the level of significance was at level of ($P<0.05$).

Results

The results of the study revealed that body weight significantly increased in both treated and none treated females. Body temperature significantly increased in treated females. Respiratory rates and heart rates not significantly increased in both treated and

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none treated females Table (1). Bleeding time decreased significantly in treated females. while Clotting time not significantly changed Table (2). The results revealed that, total erythrocytes count, hemoglobin concentration, PCV % and MCV, MCH, MCHC were not significantly changed Table (2). The results revealed that total leucocytes count, Monocytes%, and Basophils %, were not significantly changed. Eosinophils decreased significantly in treated female. Heterophils significantly decreased in treated females. Lymphocytes increased significantly in treated females Table (3).

Table 1. body weight, body temperature, heart rates, respiratory rates, bleeding time and clotting time of treated and none treated female with *R. communis* seeds extracts

Parameter	Treated group		None treated group	
	Days		Days	
	0	15th	0	15th
Body weight Kg	1.386±0.030	1.572±0.045*	1.433±0.088	1.744±0.058*
Body temp.° C	37.94±0.31	38.94±0.12*	38.23±0.37	38.8±0.24
Resp. rate / min	117.6±18.74	188±19.56	151.33±5.93	155.2±21.7
Heart rate / min	206±12.47	225.6±12.28	216.67±27.32	217.6±14.32
Bleed. time / sec.	19±1.84	32±4.05*	31.67±6.02	51±8.56
Clott. time / sec.	19±1.87	33±7.34	45±17.43	32±5.60

The values are M+ SEM.* the value significant at level of P<0.05

Table (2). Total erythrocytes count, Hemoglobin concentration, PCV % and erythrocytes indices (MCV, MCH, MCHC) in treated and none treated females to *R. communis* seeds extract

Parameter	Treated group		None treated group	
	Day		Day	
	0	15th	0	15th
RBC x10^6/ µl	3.44±1.54	3.93±0.65	5.01±0.87	5.52±0.87
Hb gm/dl	11.12±0.49	11.44±0.26	11.73±0.63	12.18±0.38
PCV %	32.8±1.61	33.8±4.33	34.67±1.67	35.8±1.16
MCV ft	78.32±8.26	87.12±18.17	72.07±8.54	77.33±19.87
MCH pg	24.78±1.63	23.01±6.03	24.35±2.77	26.67±7.36
MCHC gm/ dl	33.91±0.18	33.84±0.51	33.82±0.20	34.03±0.09

The values are M+ SEM.* the value significant at level of P<0.05

Table (3). Total and differential leucocytes counts in rabbits treated and those none treated with *R. communis* seeds extract

Parameter	Treated group		None treated group	
	Days		Days	
	0	15th	0	15th
WBCx10^3/µl	4.914±0.691	3.395±0.626	3.681±0.362	3.270±0.507
H%	50.6±.61	34.0±3.08*	45±5.01	40.0±6.96
L%	40.2±7.29	57.6±4.28*	41.33±3.53	52.6±6.97
E%	4.0±0.71	2.8±1.02*	6.0±2.31	1.8±0.49*
M%	3.8±1.11	3.4±1.03	6.67±0.34	3.8±0.73
B%	1.6±0.46	2.0±0.45	1.0±0.58	1.0±0.32

The values are M+ SEM.* the value significant at level of P<0.05

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Table (4): Information about the fetuses

Parameter	Group; none treated both males and females	Group; treated either male, or female or both
Average weight of fetuses gm	18.07 ±1.87	4.58± 0.26
Length of skull from nose to top of skull mm	19±0	10.75 ±2.75
Whole length from top of skull to end of tail mm	75.4±1.4	45.25± 0.25
Width of skull between eyes mm	11.4± 1.4	6.25± 0.25
Diameter of placenta mm	21.1 ± 0.5	21.25 ± 2.5
Thickness of placenta mm	3.6±0.6	5.5±2.5

Gr. I.: Treated females crossed with treated males: no pregnancy Gr. II.: none treated female crossed with none treated male: full pregnancy, total numbers 10 fetuses. Gr. III.: Treated female crossed with none treated males, no pregnancy in some, but some pregnant, total fetuses 6. Gr. IV.: None treated female crossed with treated male, more females none pregnant, one pregnant with two fetuses. The results revealed significant decreases in percentage of fertility in females treated with Ricinus extract. As females treated with ricin and crossed with treated males, were none pregnant (Gr.I.): 0% Figure (1). Group II: Female none treated with ricin crossed with none treated male: full pregnant 5 fetuses; 3 in right horn and 2 in left horn. Figure (2). Group III: Female treated with ricinus crossed with none treated male: pregnant 4 fetuses. Group IV: Female none treated with ricinus crossed with treated male: pregnant 2 fetuses. Figure (3). The results revealed that treatment with Ricinus extract for 14 days showed that there was no effect on the length of pregnancy in female treated with Ricinus extract as the periods remain 30-32 days. The results revealed significant decrease in % of fetus numbers in female treated with extract of Ricinus, and those crossed with treated male in comparison with those of none treated female and male. The results also revealed decreased body weight of fetuses in uterine in day 28 and in day of birth in female treated with ricinus in comparison with female none treated Figure (4).

Figure (1): showed a uterus in female treated with ricin crossed with treated male (None pregnant uterus).

Figure (2): a uterus of female none treated crossed with none treated male (uterus with 5 fetuses).

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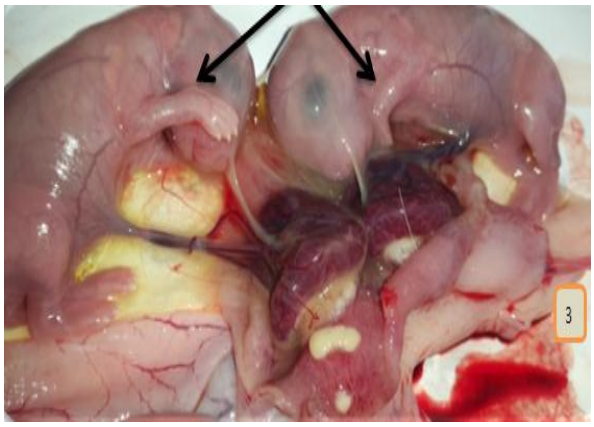

Figure (3): a uterus of none treated females crossed with treated male (Gr. IV).

Figure (4): fetuses from none treated female crossed with none treated male.

Figure(5): a fetuses from none treated female crossed with treated male

Histopathology

Treated female:

Uterus: Thickening of endometrium (due to proliferation of granulation tissue). Many newly blood vessels congested with newly small endometrial glands. Infiltration of inflammatory cells. Mammary glands: Cystic dilation of acini and filled with proteincious materials. The interlobular septa infiltrated with inflammatory cells (mild). Fibro muscular hyperplasia of interlobular septa and hyalinization; vacuolar degeneration of lining cuboidal epithelium of acini and their lumen filled with foamy (empty) proteincious material (loss of material) and other acini appeared with hyperplasia occluded their lumen. Infiltration of inflammatory cells Figure (6, 7). Placenta and regressed fetus: extensive necrosis of trophoblastic cells of placenta (curuncles), and undeveloped chondroid ostoid and other tissue of regressed fetus. None treated females**:** Ovary: No significant lesions, only different follicular cells Figure (8, 9).

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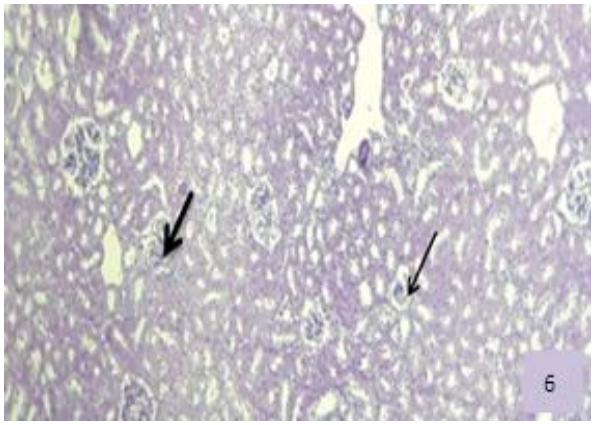

Figure (6): Ovary few follicles in tunica albujinia, perivascular infiltration of inflammatory cells,40X.

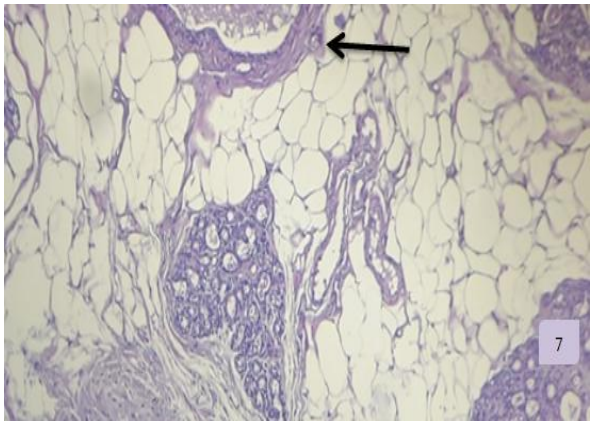

Figure (7): Hyperplasia of mucosal epithelium (papillary projection), infiltration of inflammatory cells in sub mucosa and presence of eosinophilic, foamy appearance of necrotic epithelial cells and hyalinized, 40X.

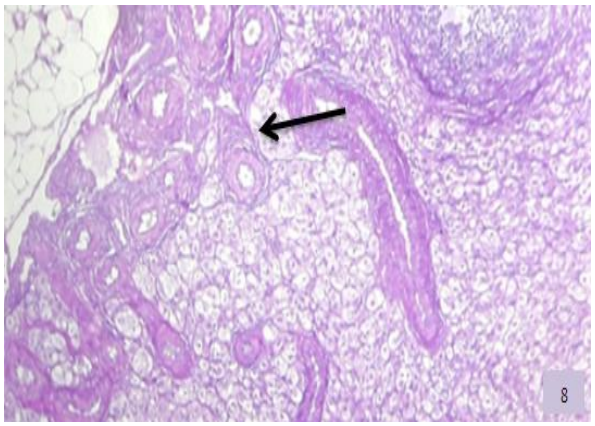

Figure (8): Un ovulatory follicles in tunica albujinia inflammatory cells,40X

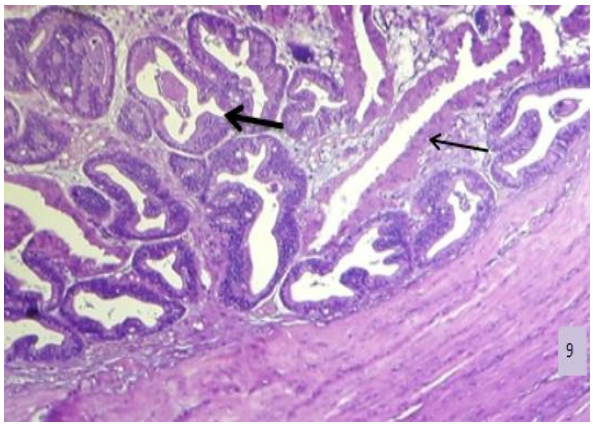

Figure (9): Oviduct: cystic dilation of epithelial mucosa filled with sever vacuolar cells, hyperplastic papillae of mucosa,40X.

Discussion

The reduced of pregnancy efficiency in females crossed with males exposed to *R. communis* can resulted from many variants in fertility, the first of reduced in sperm counts, increased dead sperm numbers, and the percent of sperm deformity, in addition to disturbance of epididymus functions under the effects of androgens (36). The reduction in fertility also increased due to disturbances of functions of accessory sexual organs which supplied seminal plasma which is important for continuity of sperms life (36; 37).The increased in embryonic resorption and reduced weight of embryos can attributed to sperm deformity, as many of these deformities can lead to inhibition of embryonic development, or can results from deformity of endometrium functions before arriving of embryos (38). This improved by the histological changes in section took from uterus which showed a hyperplasia of uterine glands, and degeneration with thickening of myometrium, the intoxication of females can lead to embryonic resorption and reduced their weights and their deaths (39).

Conclusions:

R. communis seeds extract did not affected on length of pregnancy of treated rabbits, with significant reduction in fertility of treated female. The clinical and hematological dependent parameters did not show a significant change. Thus, Ricinus extract has a contraceptive effect without any side effects on health of animals.

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