

# Effect of alkaloid ricinine on fertility and some blood parameters of female mice (*Mus musculus*)

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## Abstract

The present study involved preparation of an alkaloid ricinine from the extract of the castor bean (*Ricinus communis*) seed. Then, these alkaloid administered orally at dose (0.08 mg/gm) to female mice for 30 days to explore their possible influences on fertility (fertility rate, percentage of fertility, fetal number, weight, malformation, mortality of pups), and some blood parameters (R.B.c<sub>s</sub>, W.B.c<sub>s</sub>, Hb. and P.C.V). The results indicated a significant decrease ( $P < 0.05$ ) in number of pups, weight of pups, fertility rate and percentage of fertility, but hadn't preview malformation and mortality states. Also, the results showed a significant decrease ( $P < 0.01$ ) in R.B.c<sub>s</sub>, Hb and P.C.V. value compare with control group, exception in W.B.c<sub>s</sub> parameter have not shown any significant differences.

**Key words** : Ricinine ; fertility; blood parameter.

## Introduction

Many plants and herbs have been reported to have potential anticonceptive properties, and interest in Research in this area is particularly of importance to developing countries [1-4]. *Ricinus communis* (castor bean) is a member of family Euphorbiaceae the common name is a castor plant. It is native of India with about 17 species which have been grouped into shrub and trees that produce large seeds and a second group of annual herbs that produce smaller seeds [5].

Castor bean is one of the most frequently prescribed traditional drugs for the treatment of external affections [6]. Ricinoleic acid, its major hydrolytic product, has served in contraceptive jellies [7]. Isoricinoleic acid, dihydroxystearic, linoleic, oleic acid, and stearic acid are also the hydrolytic products of castor oil although minor components [7].

In addition, there are about 60 mg/Kg uric acid and 7 ppm HCN in one seed. The seed also contains enzymes such as Lipase, amylase, invertase, maltase endotrypsin, oxidase and ribonuclease [7]. However, the castor bean has been well known to the medicinal system in India [8]. The present study aimed to investigate the effect of ricinine as antifertility and hematological active in mice.

## **Material and method**

### **\* Preparation the Ricinine :-**

The alkaloid Ricinine was prepared by using the procedure for isolation the alkaloid from defating crust powder (seeds) which described by [9] and prepared the daily dose (0.08) mg/gm of Ricinine that dissolved in D. W.

### **\* Preparation the animals :-**

20 newly matured females (average body weight  $28 \pm 1.2$  mg) and 10 males (average body weight  $27 \pm 0.8$  mg) of albino Balb/c mice were obtained from the animal house of the Biology Department in Education College, University of Basrah, the animal were caged in special boxes under controlled temperature ( $23 \pm 2$  C°) and a regimen of 14L:10D; sexes were isolated in separate cages until beginning of the experimental, the female were checked for unwanted pre-experimental pregnancy by staining vaginal smears with methylene blue for two successive estrous cycles.

The female mice were divided into two groups each comprising (10) randomly selected animals, group I was treated with 0.08 mg/gm of alkaloid Ricinine, while the group treated with normal saline only (treated for 30 days), In each group, one male introduced to two female ( 1 male : 2 female), after confirmation of matching by visualization of the vaginal copulatory plug.

Six mated female were removed from the cage and each two of them put in same cage. The 4 female remained scarified on day 14 of the gestation period the uterine horne were exposed for estimation of fetal number, females were not allowed to complete gestation period and delivery in order to avoid overcrowding and cannibalism which may lead to miscounting of newborn pups 1 and also to avoid complete embryonal resorption which may progressively be enhanced in the last week of the gestation period [10].

\* **Blood parameters :-**

Sixteen female albino Balb/c mice weighting (22-30) gm. and 9 weeks old were used in this experiment study, mice divided into two groups. The first group given (0.08) mg/gm of Ricinine daily for 30 day. The another group given only normal saline only for 30 days as control group. After experiment period (30 days) female mice were anaesthetized and anatomized then blood samples were with drown directly from their hearts, all blood parameters (Red blood corpuscles count, white blood corpuscles counts, Hemoglobin concentration, packed corpuscular volume) were determined by routine laboratory methods [11-12].

\* **Statistical analysis:-**

Data of antifertility were evaluated by analysis of variance cone was classification followed by Dunnett test [13]. Statistical analysis of the results of blood parameter was performed by SPSS test , data are presented as Mean  $\pm$  stander error.

## **Results**

### **- Reproductive abilities parameter**

The effect of Ricinine administration (0.08 mg/gm) on reproductive abilities of the female mice are illustrated in table(1). The fertility rates and percentage of fertility were decreased significantly  $p < 0.05$  compared with control group. The results also evidenced no malformation and dead pups. The administration of Ricinine caused a reduction of pups weight at 1 and 7 days of old at  $p < 0.05$ , moreover, the number of pups showed highly significant depression compared with control group in  $P < 0.05$ .

Table (1) : Effect of Ricinine on Reproductive ability of female mice.

Treatment	Reproductive ability (Mean $\pm$ S.E)					
	Number of pups	Weight of pups (gm)	Malformation of pups	Mortality of pups	Fertility rate	Percentage of fertility (%)
Control group (normal saline)	8.55 $\pm$ 0.253	1.35 $\pm$ 0.060	—	—	1.000 $\pm$ 0.00	100 %
Treatment group (0.08 mg/gm ricinine)	*2.60 $\pm$ 1.235	*1.10 $\pm$ 0.120	—	—	*0.375 $\pm$ 0.09	*37.5 %

\* There is significant difference compare with control group at  $P < 0.05$ .

### - Blood parameters

Table (2) showed the effect of Ricinine on blood parameters of mice, all blood parameters (R.B.c<sub>s</sub>, Hb. and P.C.V.) were decreased significantly compared with control group at ( $P < 0.01$ ). The results indicated that there no significant difference in W.B.c<sub>s</sub> parameter.

Table (2) : Effect of Ricinine on blood parameters of female mice.

Treatments	Red blood cells count R.B.c <sub>s</sub> 10 <sup>6</sup> / mm <sup>3</sup>	White blood cells count W.B.c <sub>s</sub> 10 <sup>3</sup> / mm <sup>3</sup>	Hemoglobin Hb. gm / dL	Packed cells volume P.C.V. %
Control group (normal saline)	5.792.000 $\pm$ 100.174	10185 $\pm$ 270.235	11.0 $\pm$ 5.324	25.4. $\pm$ 1.50
Treatment group	*5.012.000 $\pm$	9288 $\pm$	*10.0 $\pm$	*24 .0 $\pm$ 3.55

(0.08 mg/gm ricinine)	55.333	341.105 NS	2.508	
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\* : There is significant difference compare with control group at  $P < 0.01$ .

NS : There is no significant difference compare with control group at  $P < 0.01$ .

## Discussion

The using of (0.08) mg/gm as a daily dose for 30 days in the present study was based on the median lethal dose  $LD_{50}$  which is 8.6 mg/kg [14], and the treatment dose is between (1/10 – 1/100) from the  $LD_{50}$ .

The fertility rate and percentage of fertility in female mice were decreased significantly may be because of the active material Ricinine which effect on the metabolic ways and the mean essential representation of decreasing the energy levels. However the poisoning material of Ricinine interfering with enzymes reaction in bodies tissue, such as reproductive system, then decreased the fertility or reproductive performance for these female mice. Moreover, because the effect of Ricinine on the oestrus cycle of female mice, and lag occurring the ovulation which represent the indicator for anticonceptive [15], these result were accepted with [16-18]. A supportive finding for our results comes from the study of [19] who indicated to this toxin had a high target organ and tissue affinity (mostly to parenchymal organ [20] probably including the ovaries). It passes through cell membrane into the cytoplasm by routes originally developed for the uptake of physiological proteins, like certain hormones [21] and the estrogens in appropriate doses prevent conception by inducing hormonal changes which inhibit follicular development and ovulation [22-23].

The study of Sofowora (1984) [24] would suggest that the anticonceptive effect of the *Ricinine communis* seed extract may be due to action at several sites, including direct effects on the endometrial implantation site, on the oviduct and or disruption of the estrogen, progesterone balance.

Ricinine have been reported to reduced the number and weight of pups compared with control group, the mechanism by

which ricinine effects on the embryonal growth is not well demonstrated, but it is well known that the toxin is a potent protein synthesis inhibitor [25-26]. Moreover, the embryonal growth restriction due to effect of ricinine explains the presence of comparatively smaller wrinkled embryos in some uteri, in those with least fetal numbers [27].

Ricinine have been showed decreased in blood parameters such as red blood cells, hemoglobin concentration and packed corpuscles volume, that may be according to its affinity on ability of absorption of folic acid which affected on hemoglobin construction process negatively usually that effect on P.C.V. value. However, Ricinine has blood coagulating property [28]. High dose of Ricinine effect on red blood cells, leading to agglutination and subsequent hemolysis [29-33].

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تأثير قلويد الرزنين في الخصوبة وبعض المعايير الدموية لاثاث الفئران المختبرية

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تضمنت الدراسة الحالية تحضير قلويد الرزنيين من مستخلص بذور نبات الخروج *Ricinus communis* وأعطى فموياً بجرعة 0,08 / غم إلى إناث الفئران لمدة 30 يوم لاستبيان تأثيراته على الخصوبة (معدل الخصوبة والنسبة المئوية للخصوبة وعدد الأجنة وأوزانها والتشوهات وعدد الوفيات للأجنة) وبعض المعايير الدموية (عدد كريات الدم الحمراء وعدد كريات الدم البيضاء وتركيز الهيموكلوبين وحجم الكريات المتراسة). أظهرت الإناث المعاملة بالقلويد انحراف معنوي بعدد الأجنة ومعدل الخصوبة والنسبة المئوية للخصوبة، ولكن لم تظهر أي حالات تشوهات ووفيات بالأجنة، كما أظهرت النتائج انخفاض معنوي في عدد كريات الدم الحمراء وتركيز الهيموكلوبين وحجم الكريات المتراسة ماعدا عدد كريات الدم البيضاء لم تظهر أي اختلاف معنوي مقارنة بحيوانات السيطرة.

الكلمات المفتاحية : الرزنيين ; المعايير الدموية .