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THE EFFECT OF RICINUS COMMUNIS L. ON FOETAL DEVELOPMENT IN MICE

BY

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SUMMARY

Ricinus Communis L. was administered at doses of 10 and 20 mg/kg bdoy weight to pregnant mice from the 6th to 15th day of pregnancy. The pregnant mice were sacrificed on the penultimate day of pregnancy and the gravid uterus of each was opend. Morphological examination of each uterine horn revealed 33.33 and 37.5% resorption of foetuses, and 22.22 and 32.5% foetal mortality. Foetal body weight and foetal length were significantly reduced, their mean values being 2.60 ± 0.22 and 1.90 ± 0.16 ($P < 0.05$) gms and 16.20 ± 0.80 ($P < 0.01$) and 14.36 ± 0.71 ($P < 0.01$) mm.respectively. This corresponds to 2.70 ± 0.86 g and 20.70 ± 0.34 mm in the control group.

Viable foetuses were freed from surrounding membranes and half the viable foetuses from each group were immersed in Bouin's fluid prior to sectioning and electron microscopic examination for visceral malformations.

Visceral malformations recorded were, necrosis of brain cells, 10 and 15%, lungs of hard consistency, 5 and 10 %, hepatic damage 40 and 70 %, nephritis in 6 and 8 % respectively.

The other half of the foetuses were fixed in ethyl alcohol prior to staining with alizarine red S for skeletal examination. Skeletal malformations noted were: incomplete ossification of the skull in 3.75 and 5.0% of each dose rate group, absence of one or more of the coccygeal vertebrae 12.5 and 31.25 %; irregular ribs in 7.5 and 12.5% absence of some metatarsal, metacarpal and phalangeal bones 31.25 and 37.5 %.

The results of this investgation indicate that Ricinus Communis L. has a teratogenic effect on mouse embryos when administered orally to pregnant mice from the 6th to 15th day of gestation. It is suggested that the use of Ricinus Communis L. in should be avoided during pregnancy. It must be assumed that the effect of Ricinus Communis L. will be similar in other mamallian species until future investigation shows otherwise.

INTRODUCTION

Recinus Commuis seeds contain a glycoside known as recin and its was considered by the main toxic subatance in the seeds (Catherine & Clask, 1947). The pure neuroto xic substances which was isolted from the leaves of Ricinus Communis *L. produce* the same signs of intoxication as the crude leaves. The compound ricinine was already isolated, identified and its chemical structure obtained (Manske and Holmes, 1950 and Shuraky et al., 1969).

There is a lack of literature on the effects of Ricinus Communis *L. upon* the mouse embryos. This promoted us to investigate the teratogenic effect of this plant seed on these species.

MATERIAL AND METHODS

The dried seeds of Ricinus Communis *L. plant* were exhausted with methanol in a soxhlet. The crude extract was dissolved in water and filtered. The water soluble material was acidified with dilute hydrochloric acid (pH_2) and extracted three time with diethyl ether. The remaining aqueous

phase was made alkaline with 5% solution sodium bicarbonate and then exhausted with chloroform. The two extracts were dried with anhydrous sodium sulphate, dissolved in water and the organic solvent removed under reduced pressure.

Virgin female NMRI strain mice, Weighing 25-30 g were selected in the pro-oestrous phase of their oestrus cycle, indicated by microscopic examination of vaginal smear. The female mice were then placed overnight with proven NMRI fertile male mice. The presence of a vaginal sperm plug, or free spermatazoa in a microscopic examination of vaginal contents the following morning, indicated day 0 of pregnancy.

The pregnant mice were separated into three equal groups of 30 animals each. One group was given saline solution and kept as a control. The other two groups received extract at a dose rate of 10 and 20 mg/kilogram body weight, respectively.

Dosing was carried out, using a stainless steel stomach tube, daily at 12.00 a. m. from the 6th to the 15th day of pregnancy. The project was designed according to the principles of testing drugs for teratogenicity published by the World Health Organisation (1967).

All pregnant mice were sacrificed on the penultimate day of gestation by dislocation of the neck. The uterus was opened and a thorough examination of both uterine horns was carried out. Foetal swellings and sites of resorption were carefully counted. Viable foetuses were freed from their placental membranes and inspected for any external anomalies.

Half of the viable foetuses in each group were immersed in Bouin's fluid prior to measurement and subsequent sectioning for visceral examination by electron microscope (Wilson, 1965). The rest of the foetuses were immersed in ethyl alcohol for tissue clearing procedures followed by staining with alizarine red S. (Dawson, 1926; Kotb, 1973).

RESULTS AND DISCUSSION

Teratogenicity of Ricinus Communis L. was determined in this investigation by foetal morphological visceral and skeletal malformations following oral administration to pregnant mice from the 6th to the 15th day of gestation. This time period was chosen as it is the time of embryonic development when oragongenisis occurs and the embryo is most susceptible to drug-induced malformation. It is also easier to determine the sensitivity of the embryo to a drug and recognize the pattern of embryonic abnormality (Tuchamann-Duplessis,

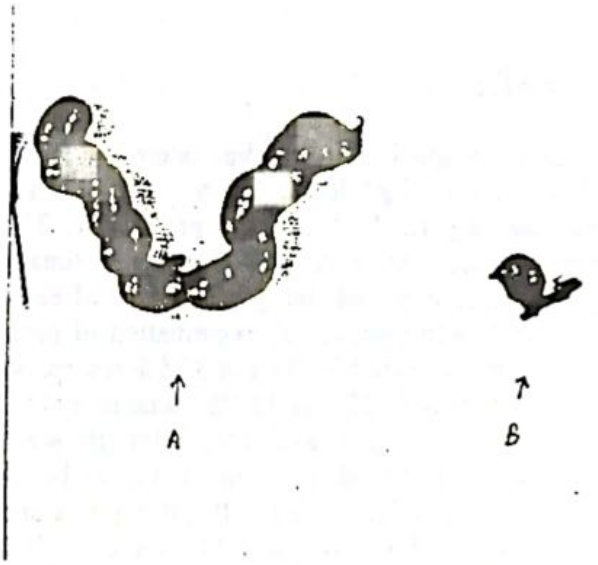

Fig. 1: Mouse uterus showing foetal resorption after oral administration of 20 mg/kg b. wt. Ricinus Communis L. to pregnant mice from the 6th-15th day of gestation (control: left side).

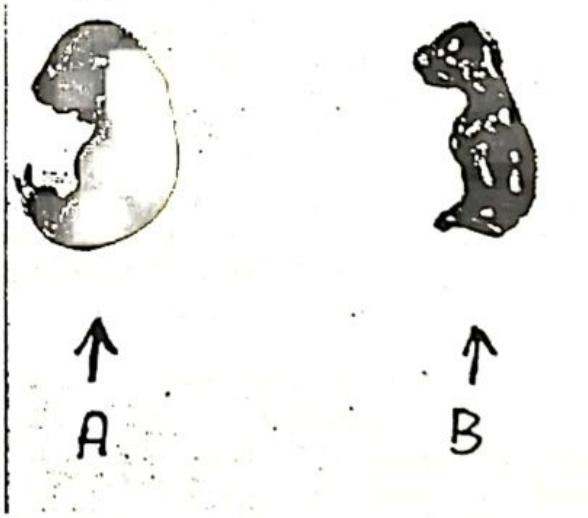

Fig. 2: Showing foetal body length of mice after oral administration of 20 mg/kg b. wt. Ricinus Communis L. to pregnant mice from the 6th - 15th day of gestation (Control left side).

1971).

Ricinus Communis *L.* in doses of 10 and 20 mg/kg body weight caused 33.33 and 37.5% resorption; 22.22 and 32.5% foetal mortality of examined foetuses. Moreover the body weight and length of examined foetuses were significantly reduced, as the mean values were 2.60 ± 0.22 and 1.90 ± 0.16)P < 0.05) gms and 16.20 ± 0.80 (P < 0.01) and 14.36± 0.71 (P < 0.01) mm respectively, corresponding to 2.70 ± 0.86 g 20.70 ± 20.70 ± 0.34 mm respectively in the control group. (Table, 1 Figures 1 and 2).

Other investigators have noted the toxicity of ricinine in vitro studies, revealed its inability to inhibit the activity of cytochrome oxidase. This indicates that ricinine did not act in away similar to prussic acid in tissue respiratory enzymes. However, the in vitro effects of ricinine on liver and brain tissue homogenates made it possible to speculate on the probable mechanism of its action. On the basis, ricinine impaired tissue respiration, as judged by the reduction of their uptake of oxygen. This indicates the inhibitory effect of ricinine somewhere in the chain of reactions

Table (1):Morphological malformations in mouse embryos after oral administration of Ricious Communis L. to pregnant mice.

Drug	Dose mg/kg body weight	Number of implantation sites	Foetuses						Foetal weight in gms. mean ± SE	Foetal length in mm. mean ± SE
			Resorbed		Dead		Viable			
			No.	%	No.	%	No.	%		
Control	-	190	6	3.16	0	0	184	96.84	2.70±0.86	20.70±0.34
Ricinus Communis L.	10	180	60	33.33	40	22.22	80	44.44	2.60±0.22	16.20±0.80**
	20	200	75	37.5	65	32.5	60	30	1.90±0.16*	14.36±0.71**

* Significant at P < 0.05
** Significant at P < 0.01

Table (2): Visceral malformations in mouse embryos after oral administration of Ricinus Communis L. to pregnant mice.

Drug	Dose mg per kilogram body weight	Number of foetuces examinued	Malformations											
			Brain		Plate		Heart		Lung		Liver		Kidney	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Control	-	100	-	-	-	-	-	-	-	-	-	-	-	-
Ricinus Communis L.	10	100	10	10	2	2	0	0	5	40	40	6	6	-
	20	100	15	15	3	3	0	0	10	10	70	70	8	8

which are necessary for tissue respiration. It appears that the gradual and persistent histotoxic anoxia of brain and other tissues could have been a mode by which ricinine produced its lethal action (Buck et al., 1976).

The extract of Ricinus Communis *L. stimulates* intestinal motility of rabbits when added at the

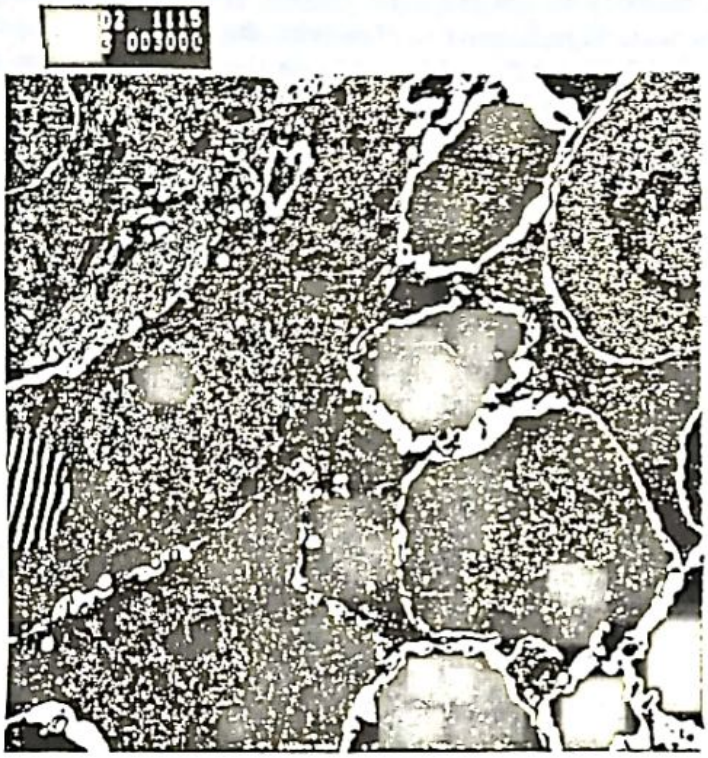

Fig. 3: Necrosis of liver cells of the mouse foetus after oral administration of 20 mg/kg b. wt. Ricinus Communis L. to pregnant mice from the 6th - 15th day of gestation. Electron micrograph (x 6000).

Table (3): Skeletal malformations in mouse embryos after oral administration of Ricinus Communis L. to pregnant mice.

Drug	Dose mg/kg body weight	Number of foetuces examinued	Malformations									
			Shull		Coccygeal vertebrae		Ribs		Rternebrae		Limbs	
			No.	%	No.	%	No.	%	No.	%	No.	%
Control	-	80	-	-	-	-	-	-	-	-	-	-
Ricinus Communis L.	10	80	3	3.75	10	12.5	6	7.5	0	0	25	31.25
	20	80	4	5.0	25	13.25	10	12.5	0	0	30	37.5

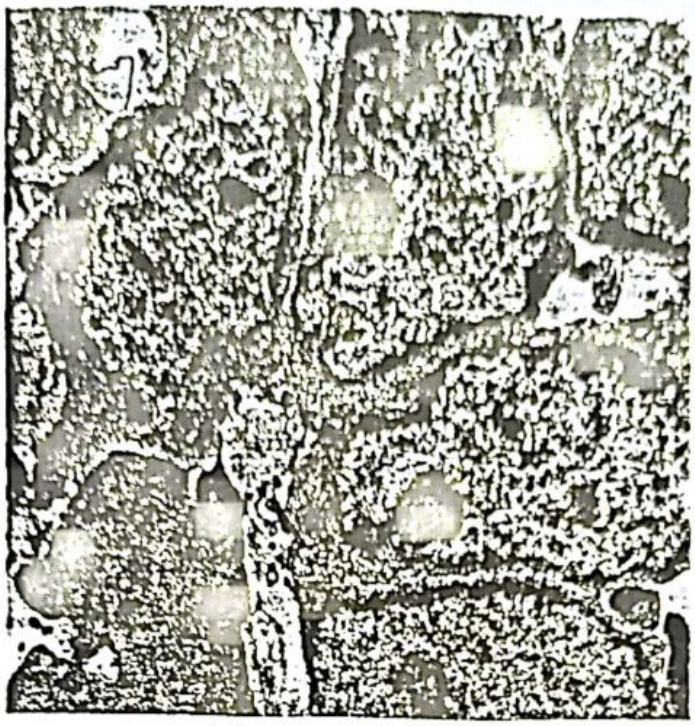

Fig. 4: Nicrosis of tubular cells of the kidney in the mouse foetus after oral administration of 20 mg/kg b. wt. Ricinus Communis L. to pregnant mice from the 6th - 15th day of gestation. Electron micrograph (X 12900).

Fig. 5: Showing absence of coccygeal vertebrae, some phalangen and limbs in mouse foetus after oral administration of 20 mg/kg b. wt. Ricinus Communis L. to mothers at 6th-15th day of gestation (control right side).

Fig. 6: Showing absence of some coccygeal vertebrae and some phalanges and one for limb in mice foetuses after oral administration 20 mg/kg b. wt. Ricinus Communis to mothers at 6th-15th day of gestation.

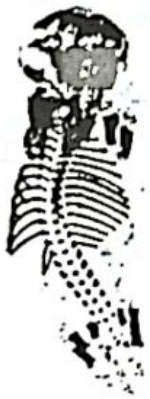

Fig. 7; showing irrigular diversion of sids and absence of hind limbs, coccygeal vertebrae in mice foetuses after oral administration 20 mg / kg b. wt. Ricinus communis L. to mother at 6th - 15 th day of gestation.

used level. It was proved also the the effect is myogenic in nature since the alkaloid produced its effect after nicotinisation and atropinization of intestinal strip. This finding could explain the cause of colicy pains observed in mice during acute toxicity study. It stimultes the uterine motility of rats of various stages of sex cycle, therefore leaves of Ricinus Communis plant are considered harmful to pregnant animals as they could induce abortion. The cardioinhibitory effect of the plant could be attributed to its direct toxic effect on cardiac muscles as it impaire oxygen of tissues as shown in homogenates of liver and brain tissues in this investigation. Moreover, its hypotensive action could be explained by the inhibitory effect on the heart (Farah et al., 1987).

Oral administration of Ricinus Communis to pregnant mice produced some foetal visceral organ malformations such as necrosis of brain cells 10 and 15%, lungs of hard consistency 5 and 10%, hepatic damage 40 and 70%, nephritis in 6 and 8%, respectively (Table 2, figures 3 and 4).

The skeletal malformations recorded were: incomplete ossification of the skull 3.75 and 5.0 %, absence of one or more of the coccygeal vertebrae 12.5 and 31.25 %, irregular ribs 7.5 %, and absence of some metatarsal, metacarpal and phalangeal bones 31.25 and 37.5% respectively. (Table 3, Figures 5-7).

The administration of Ricinus Communis L. to pregnant mice caused morphological skeletal and visceral malformations in the mouse foetuses. It is advised on the basis of these data, that the use of Ricinus Communis during pregnancy should be avoided until further testing on different species proves otherwise.

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