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Teratogenic effects of methanolic extract of *Ricinus communis* seed oil on the morphology of foetal wistar rats

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ABSTRACT

The teratogenic effects of the methalonic extract of *Ricinus communis* seed on the morphology of foetal wistar rats was studied, using 15 Cyclic female and 6 male Wistar rats (170-200g) for the study. Vaginal lavage was taken daily to monitor their oestrous cycle for 4 weeks. On the preostrous day of the cycle of each rat, a male rat was introduced into the cage. The presence of spermatozoa in the vaginal lavage was designated as day 1 of conception. The pregnant female Wistar rats were randomly divided into three groups consisting of five pregnant rats each. Group A (control), was gavaged with freshly prepared 2.5%Tween 80 in normal saline (0.2ml)from the 1$^{st\ to}$ the 12th day of gestation. Group B and C, were gavaged with 600mg/kg body weight of Ricinus communis seed extract from the 1stto the 5thday, and from the 8th day to the 12th day of gestation, respectively. And the same volume of 2.5% Tween 80 as in Group A, as vehicle for the extract. On the 20th day of gestation, all pregnant rats were sacrificed, foetuses were examined for malformation and gestational parameters monitored were: number of total implantation, resorption and dead foetuses. Live foetuses were weighed and examined for external malformations and variation. Foetal parameters recorded were: foetal numbers and weight; crown-rump-length; trans-umbilical cord length; and placental weight. The results showed that there was prevention of implantation; abortion; and significant reduction of foetal parameters: crown-rump-length; tail length; and weight, compared to control ($p<0.05$). In conclusion, the above results suggests that the methalonic extract of Ricinus communis seed at 600mg/kg body weight, prevents implantation, induces abortion and significantly reduce foetal growth parameters in wistar rats.

Key words: Methanoic extracts, *Ricinus communis* seed oil, morphology, foetal rats.

INTRODUCTION

Ricinus communis is a species found abundantly in the Mediterranean basin, Eastern Africa and India; places where it arguably took its origin. It is also found abundantly in the southern regions of Nigeria (Oyeleye, 2003). It is a perennial shrub, a member of the Euphorbiaceae family and commonly known as castor oil plant in English, Ogiri-igbo by the Ibos; Eso lara by the Yorubas, Kpanfini-gulu by the Nupes. Internationally as; Palma Christi,Castor bean.

Different parts of Ricinus communis have various medicinal purposes, as they could be used for the treatment of fevers, eczema, warts, cold tumors, indurations of the mammary gland, corns and moles (Gibbbs *et al.,* 2002; Huguet-Termes, 2001; Sathiyariathan *et al*., 2005). Inspite of its wide uses and application, Ricinus communis have been reported to contain among other constituent, toxic substances like ricin and lectin which are chemicals capable of potential poisoning, agglutination heamolysis of the red blood cells and irreparable damage to vital organs like kidney and liver (Wedin *et al*.,1986).

The toxicity reports on the plant have stimulated the investigation of its teratogenic effects when consumed in pregnancy. Due to its

documented reports by several scientists, it has become necessary to investigate the apparent influences it might have on pregnancy.

The use of herbal medicine is popular amongst the populace of Nigeria. This is due to poor financial status, insufficient health care services and hospitals; as most users are poor villagers in remote regions. Therefore, correct doses and mode of action of the constituents of the herbal medicine are not known (Ucheya *et al*., 2007).

In view of the numerous uses of Ricinus communis seed oil, such as contraception; induction of labour; and purgative use. However, following the case report of its teratogenic effects made by El Mauhoub *et al*., (1983), of a young mother who took castor oil seed orally as contraceptive for eight weeks after conception. This study endeavours to further investigate the teratogenic effects of the methalonic extract of Ricinus communis seed on foetal development when consumed by pregnant wistar rats.

Research Methodology

Materials

Wistar rats, Growers mash, watering bowl, Feed bowl, Calibrated Synringes, Permanent Ink Makers, Weighing balance (Electronic), Beakers, Weighing cylinder, Hand gloves, Dissecting set, Plastic buckets, Cages, Water, Tissue paper, Cardboard papers, Protocol notebook, Scale rule, White thread, Normal saline, Microscopic glass slide, Cover slip, Microscope, Pasteur pipette, Chloroform, Disinfectant.

Plant Materials

The fruits of Ricinus communis was collected from the local farmers at Ekuoma village, Agbor, Delta state. The specimen was authentic by Mrs. A. Adesanya of pharmacognosy Department, University of Benin, Benin city.

The fruit had its thorny coat (endocarp) and seed-coat separated from the seeds by hand peeling. The seeds were sundried for 5 days and grounded into powder and was soaked in 2 litres of methanol for 48 hours. The extract obtained, was separated from methanol by concentrating the filtrate in an oven set at 60oC for 3 days. For the extraction process, 800g of the grounded seeds was used and it yielded 300ml of castor oil.

Sourcingand Caring Forthe Animals

A total of 21 young adults Wistar rats were purchased from Sakpa and Sakpa farms, Benin City: 6 males and 15 females of proven fertility, with average weight of 170-200g were used for the study. They were housed in cages and maintained under standard conditions of room temperature and was allowed to acclimatize in the laboratory for 4 weeks before commencement of the study. Feed and water were given ad libitum. The cages were cleaned every morning during the study.

EXPERIMENTAL

Procedures

During one month, every morning between 8-9 am, vaginal lavage was taken, using Pasteur pipette with 10% normal saline, and smeared on microscopic slides to determine oestrus cycle in female rats (Long and Evans, 1922; Mandl, 1951). One male rat was introduced into the cage, on the preostrous day of the cycle of each female rat for mating overnight. The following morning, vaginal lavage was taken, to detect spermatozoa. Day 1 of pregnancy was taken as the day spermatozoa was seen in vaginal lavage of female rats (Oderinde *et al*., 2002). Pregnant rats were divided into three (3) groups randomly, groups A,B and C of 5 rats each. Group A was the control and was gavaged daily from the 1st day to the 12thday of gestation, with equal amounts of freshly prepared 2.5% Tween 80 in normal saline (0.2ml), serving as vehicle for the extract (Raji *et al.* 2006).Group B, were gavaged from the 1st to the 5th day of gestation with 600mg/kg body weight of Ricinus communis seed extract (implantation studies), with equal volume of 2.5% of Tween 80 as in group A.Group C, were gavaged on 8th-12th day of gestation with 600mg/kg body weight of Ricinus communis seed extract, with equal volume of 25% of Tween 80 as in group A above, (Malformation studies), (Odernde *et al*., 2002).Body weight, Food consumption, gross appearance and behavioural pattern of pregnant wistar rats were monitored daily.

Collectionof Foetuses

On the 20th day of gestation, foetuses were removed from pregnant rats by ventral laparotomy

and examined. The numbers of foetal, implants, resorptions, live and dead foetuses were recorded. Live foetuses were removed from the uterus, weighed; and examined for gross malformations. Foetal parameters such as: foetal numbers, trans-umbilical length, tail length, crown-rump-length, and placental weight were measured.

Statistical Analysis

Calculations of Measures of Dispersion

$$\text{Standard deviation(S)}=\sqrt{\frac{\Sigma\left(X-\overline{X}\right)^2}{n-1}}$$

Standard error of mean $\left(SE\overline{X}\right)=\frac{s}{\sqrt{n}}$

Where

$\sum$ = summation (sum of)

X= observed values

$\overline{X}$ = mean of observed values

n = number of observation

s = Standard deviation

Testing of Hypothesis

To test signicant difference between two samples, two sample unspooled t-test of unequal variations was used.

$$t=\frac{\overline{X}_1-\overline{X}_2}{\sqrt{\left(\frac{S_1^2}{n_1}+\frac{S_2^2}{n_2}\right)}}$$

$$\text{df}=\frac{\left(\frac{S_1^2}{n_1}+\frac{S_2^2}{n_2}\right)^2}{\frac{\left(\frac{S_1^2}{n_1}\right)^2}{n_1-1}+\frac{\left(\frac{S_2^2}{n_2}\right)^2}{n_2-1}}$$

For $n_1 + n_2 > 40$ of independent observations

Where df= degree of freedom

S= Variance of observations

n = number of observations

t = calculated t-test value

x = mean of observations

RESULTS

Effects of methalolic extract of risinus communis oil on the weight of maternal wistar rats

Maternal wistar rats (treatment groups A and B), showed significant body weight changes during and after oral administration of Ricinus communis seed extract, when compared to the control maternal rats (Appendix 1). There were no significant changes in food consumption, behavioural pattern, and gross appearance.

Table 1: Showing Foetal number of the control and treatment Groups

Experimental groups	Number of maternal rats (n)	Number of foetal rats (n)
Group A (control)	5	46
Group B	5	9
Group C	5	-

Table 2: Showing Foetal parameters of control and treatment groups

Foetal parameter	Group A (control) n=5	Group B n=5	Group C n=5
Foetal weight (g)	2.48±0.002	*1.43 ±0.03	-
Crown-rump-length (cm)	2.82± 0.01	*1.77 ±0.03	-
Tail length (cm)	1.25± 0.005	*0.68 ±0.02	
Trans-umbilical cord length (cm)	2.1 ±0.01	2.1± 0.05	-
Placental weight (g)	0.56± 0.001	0.54 ±0.02	-

Mean± SEM, *Statistically Significance P<0.05

Effects of methanolic extract of risinus communis oilon implantation

Obvious vaginal bleeding in four rats of group B, on the second and third day of oral administration of Ricinus communis seed extract was observed.

Observations on abortifacient effect

Obvious vaginal bleeding in all the rats of group C, from the second day and third day of administration was observed. Lifeless foetuses were seen on the floor of the cages on the third day of administration.

Effects on gestational parameters

9 foetuses in group B, 46 foetuses in group A and no foetus in group C. There were no dead foetus in all the groups, no external malformations and no resorptions, in all foetuses of groups A, B and C (Table 1).

Effects on foetal parameters

Data on foetal parameters are presented in Table 2. There was significant reduction in foetal weight, crown-rump-length, but no significant reduction in trans-umbilical length and placental weight ($P<0.05$) (Table 2).

DISCUSSION

Teratogens are substances that induce the occurrence of significant abnormalities, either structural or functional in the offspring after being administered to either parent, before or after conception (Schardein and Keller, 1993). The most common effects of teratogens, are spontaneous abortion, congenital malformation, intrauterine growth retardation, functional abnormalities, carcinogenesis and mutagenesis (Briggs, 1994).

Table 1 shows that there is reduced number of foetuses per group (group B), the obvious vaginal bleeding in four pregnant rats (group B) on the second and third day of administration of 600mg/kg body weight of Ricinus *communis* seed extract. We suggest it is an early sign of prevention of implantation; which is in consistence with the report by Jonathan *et al.,* (1995), and population council, (2005), that some substances cause the existence of post – fertilization mechanisms including prevention of implantation and destruction of blastocysts.

This study has revealed that the methalonic extract of Ricinus communis seed prevents implantation and is in line with earlier findings of Makonem *et al*., (1999), on female guinea pigs.The obvious vaginal bleeding and lifeless foetuses observed on the floor of the cages on the second day of administration, is apparently an indication of abortion. And on the sacrifice of maternal rats, no foetuses were seen (table 1 and 2). Though the mechanism of action that might have led to the signs of an apparent spontaneous abortion cannot be explained as it was not within the purview of this study. But it is apparently in consistence with report that abortifacient effect may be mediated by the oestrogenic contractility effects of Ricinus communis seed extract, by interfering with the hypothalamic pituitary axis (Okwuasaba *et al.,* 1999; Mcneil *et al.,* 2003).Indices used in accessing foetal growth retardation includes body weight (Davies, 1968); Crown-rump-length (Goldman and Yakorak, 1965); Trans-umbilical cord length (Cambel, 1974) and placental weight (Hill, 1974). In the present study, the teratogenic potential of the mathalonic extract of Ricinus communis seed have been demonstrated as most of the above named parameterswere significantly reduced ($P<0.05$) when compared to the animals in the control group (Table 2). This finding is in line withthe case report by El Mauhoub et al., (1983) in which consumption of castor seeds oil by a young pregnant mother for eight weeks caused foetal growth retardation, on the other hand it contradicts some findings of El Mauhoub *et al.,* (1983) as no external malformations such as congenital malformation of the limbs were recorded.

CONCLUSION

These present findings shows that the methanolic extracts of Ricinus communis when consumed during pregnancy, might cause spontaneous abortion and reduced foetal parameters such as foetal weight; foetal Crown-rump-length; and foetal tail length in wistar rats. Therefore, we should be wary of the consumption of castor seed oil.

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