

## POSSIBLE EMBRYOTOXIC AND TERATOGENIC EFFECTS OF A PHYTODRUG (RICOM 1013-J) ON PREGNANT FEMALE WISTAR RATS

Ekwere Okon Ekwere<sup>1,\*</sup>, Tamunotonye Watson Jacks<sup>2</sup>, Iornumbe Usar<sup>3</sup>,  
Ikoni Ogaji<sup>4</sup> & Francis Kanayo Okwuasaba<sup>3</sup>

<sup>1</sup>Department of Anatomy, Faculty of Medical Sciences, University of Jos, PMB 2084, Jos-Nigeria

<sup>2</sup>Department of Human Anatomy, College of Medical Sciences, University of Maiduguri,  
P.O. Box 1069, Maiduguri, Nigeria

<sup>3</sup>Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, PMB 2084, Jos-Nigeria

<sup>4</sup>Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, University of  
Jos, PMB 2084, Jos-Nigeria

\*E-mail: ekweree@yahoo.com, eoekwere@gmail.com

### ABSTRACT

**Objectives:** For centuries, virtually every indigenous culture has utilized plants in an attempt to control its population. Contrary to the belief that African local communities do not care about population growth, the Bassa people of Plateau State of Nigeria are known to have successfully used, for centuries, the seeds of a commonly available plant, *Ricinus communis-linn* (RICOM 1013-J) for birth control. Since the phytodrug, RICOM 1013-J has demonstrated high antifertility efficacy in adult cyclic rats and in women volunteers, there is need therefore to evaluate it for any possible embryotoxic and teratogenic effects in animals.

**Material and Methods:** Forty virgin cyclic female albino rats with weight range of 170-220g were carefully selected for the study. Rat pellets and water were provided *ad libitum*. They were mated with proven males in a 3:1 (female: male) ratio and mating was ascertained the following morning by the presence of clumps of spermatozoa in vaginal smear. Animals were administered subcutaneously with 5mg/kg, 20mg/kg, 2g/kg and 20g/kg of RICOM 1013-J on day 10 of pregnancy and sacrificed on day 20. The foetuses were examined for litter quality (appearance, body weight and crown-rump length).

**Results and Conclusions:** The data obtained did not show any evidence of litter abnormalities or any morphological changes in the pups between the 5mg/kg to 20mg/kg dose range. However, doses between 2g/kg and 20g/kg were embryotoxic.

**Keywords :** *RICOM 1013-J, Antifertility, Embryotoxic, Teratogenic, Litter quality.*

### 1. INTRODUCTION

Drug exposure during pregnancy is increasingly common in modern society, with risks to the developing embryo or fetus. In the United States of America, approximately 200,000 children (3-5% of live births) are born with birth defects each year [1]. Until the appearance of the thalidomide-induced birth defects in the early 1960s, it was generally believed that the uterus provides a protective environment for the fetus. However, improved safety and selectivity of drug therapy in pregnancy has not necessarily been a priority in drug design and development, Holcberg et al, [2].

The placenta is known to be the gatekeeper to the fetus through which drugs and other chemical substances must pass. Placental drug transporters are able to reduce fetal exposure to a wide variety of xenobiotics through their ability to efflux substrates from fetoplacental tissues into the maternal circulation for excretion. It is therefore essential to understand the effect of medications and to know the point in fetal development when drugs are most toxic and when fetal organs are most susceptible. The fetus' susceptibility to injury depends on its period of development. Different organs have different critical periods ranging from gestational day 15 to 60. The heart is considered most sensitive during the third and fourth weeks of gestation, whereas the external genitalia are most sensitive during the eighth and ninth weeks. The brain and skeleton are sensitive from the beginning of the third week to the end of pregnancy and into the neonatal period, [3]. Genetic defects and medications are known to cause similar abnormalities, such as those resulting from warfarin and Happle syndrome. Several studies are related to axial defects in mice, Yasuda et al, [4], Pauken et al, [5]

'*Ricinus*' is a Latin word for 'tick' which describes the shape of castor seed. Castor plant is commonly known for its oil yield and the term 'castor' which means 'beaver' (Latin), seems to have come from the word 'castoreum', a perfume base made from the dried perineal glands of the beaver. *Ricinus communis-linn* is also known as castor bean and is a plant species of the *Euphorbiaceae* [6], the sole member of the genus *ricinus* and of the subtribe

*ricininae*. The plant has been used for wound healing and as a cure for various ailment. This has earned it the name, *Palma Christi* (Palm of Christ) Wedin *et al.*, [7].

Our interest in RICOM 1013-J lies in its anti-conceptive property, which has been confirmed by researchers Osunkwo *et al.*, [8]; Okwuasaba *et al.*, [9] Okwuasaba *et al.*, [10]; McNeil *et al.*, [11]. It has been reported that the diethyl fraction of the methanolic extract of the seed has significant anti-fertility property in adult rats, Okwuasaba *et al.* [9] and that it suppresses ovarian function and hence the number of ova released at oestrous, (McNeil *et al.*, [11] Among the Rukuba tribe in Bassa local government area of Plateau State (Nigeria), the use of the seeds for contraception is a widespread practice, Osunkwo *et al.*, [8] Okwuasaba *et al.*, [9], Okwuasaba *et al.*, [10], Kabele-Toge and Igwilo, [12]. Some other researchers have also reported on its anti-fertility properties, Sani and Sule, [13]., Yi-ling Hou *et al.*, [14] RICOM 1013-J contains a characteristic set of more than 100 different polypeptides against which a complex of antiserum has been raised [6]. This study was therefore undertaken in order to assess any possible embryotoxic and/or teratogenic effect of RICOM 1013-J on pregnant female albino rats. It is also important to see if there will be any embryotoxic effect on the foetuses and the final outcome in event of failure of RICOM 1013-J to protect the pregnant woman as an anticonceptive agent.

## 2. MATERIALS AND METHOD

### COLLECTION AND IDENTIFICATION OF PLANTS

The seeds of RICOM 1013-J were collected from the wild in Jos metropolis, Plateau State, North Central Nigeria between January and June, 2003 and supplied by Dr. Oyhu Azija (Consultant traditional medicine practitioner in the Department of Pharmacology, University of Jos, Nigeria). They were identified and authenticated at the Departments of Botany, University of Lagos and Ahmadu Bello University, Zaria and Forestry Research Institute, Jos as described by Okwuasaba *et al.*, [9], with specimen vouchers deposited at the herbarium of the Department of Anatomy, Faculty of Medical Sciences, University of Jos, Nigeria.

### EXTRACTION PROCEDURES

The dried seeds were finely ground and a weighed portion (100g) was subjected to exhaustive soxlet extraction in 350ml of n-hexane for 72h at 30 °C. The extract was concentrated in water bath at 59.0 ± 1 °C until a constant weight of dark, sticky residue was achieved. The mean yield and percentage yield of the extract was calculated. The extract was then kept at -4 °C in the refrigerator until when required. The crude extract was later dissolved in appropriate volumes of corn oil which when tested simultaneously with the extract, was found to have no pharmacological effect on the experimental animals used in the study (Okwuasaba *et al.*, [9])

### ANIMALS

Swiss female Wistar rats were used in this study. They were procured from and acclimatized in the experimental animal house of the University of Jos for 2 weeks under laboratory conditions in a cross-ventilated room (temperature 22±2.5°C, humidity 65±5% and photoperiodicity of 12h light/ 12h darkness). They were well fed with standard mash or rat pellets (Grand Cereals & Oil Mills Limited, Jos) and allowed access to water *ad libitum*.

Forty virgin cyclic Wistar rats weighing between 170-220g were separated for this study. Their oestrous patterns were determined by daily vaginal smear analysis. Animal selection was determined by the presence of at least two consecutive 4-day oestrous cycles. Each animal was smeared daily until at proestrous and then mated with males with proven fertility in 3:1 (female/male) ratio [15]. To ascertain that mating took place, the animals were smeared the following morning. The presence of clumps of spermatozoa in the vaginal smears confirmed mating and the sperm-positive day was considered to be day 0 of pregnancy. The animals were then divided into 8 groups (n=5) and treated as follows: Groups 1 and 2 received 5mg/kg body weight and 20mg/kg on day 10 of pregnancy while groups 3 and 4 were given, 2g/kg and 20g/kg body weight on day 10 of pregnancy respectively. However, groups 5, 6, 7 and 8 received 5mg/kg of corn oil, 20mg/kg of corn oil, 2g/kg of corn oil and 20g/kg of corn oil on day 10 of pregnancy respectively. Groups 5 and 6 served as experimental control for groups 1 and 2 while Groups 7 and 8 was the experimental control for groups 3 and 4 respectively. The animals were observed and later sacrificed. Pregnancy was terminated on day 20 by chloroform inhalation method with the aid of a desiccator. Laparotomy was carried out and the foetuses removed, blotted dry and the following morphological examination was carried out. These include litter/foetal quality (i.e. appearance, weight, crown-rump length) litter size (number per animal) and possible facial, spinal and skeletal, abnormalities.

## 3. RESULTS

In the treated groups 1 and 2 there were no marked changes on the litter quality (appearance, weight and crown-rump length). However, groups 3 and 4 showed foetal resorption. Morphological examination revealed no facial, spinal and limb abnormalities (Table 1).

TABLE 1: The Effect of Extract on Litter Quality and Litter Size

|                               | LITTER QUALITY<br>(Mean $\pm$ SEM) |                                 |                                 | LITTER SIZE<br>(pups/dam)/activity       |
|-------------------------------|------------------------------------|---------------------------------|---------------------------------|--|
|                               | Appearance<br>(Colour)             | Weight (g)                      | C-R length (cm)                 | Mean $\pm$ SEM                           |
| <b>5mg/kg</b>                 | <b>PINK</b>                        | <b>5.7 <math>\pm</math> 1.3</b> | <b>4.4 <math>\pm</math> 0.9</b> | <b>4.5 <math>\pm</math> 1.1 /ACTIVE</b>  |
| <b>20mg/kg</b>                | <b>PINK</b>                        | <b>4.0 <math>\pm</math> 1.2</b> | <b>4.2 <math>\pm</math> 1.3</b> | <b>2.8 <math>\pm</math> 0.8 /ACTIVE</b>  |
| <b>*2g/kg</b>                 | -                                  | -                               | -                               | -  |
| <b>CONTROL<br/>(Corn oil)</b> | <b>PINK</b>                        | <b>4.4 <math>\pm</math> 1.2</b> | <b>3.3 <math>\pm</math> 0.9</b> | <b>7.0 <math>\pm</math> 1.9 /ACTIVE</b>  |
| <b>CONTROL<br/>(Corn oil)</b> | <b>PINK</b>                        | <b>5.7 <math>\pm</math> 1.2</b> | <b>4.6 <math>\pm</math> 1.0</b> | <b>10.0 <math>\pm</math> 2.2 /ACTIVE</b> |

\* Total foetal resorption

#### 4. DISCUSSION

This study shows that the n-hexane extract of RICOM 1013-J in a single therapeutic dose produced no embryotoxic effects on the embryos. But with the higher doses of 2g/kg and 20g/kg body weight (100 times and 1000 times the therapeutic dose of 20mg/kg body weight), there was resorption of foetuses. Since there was resorption in the higher doses (2g/kg and 20g/kg), this explains why no statistical analysis was carried out.

It is clear that any drug or chemical substance administered during pregnancy is able to cross the placenta to some extent unless it is metabolized or altered during passage or if its molecular size/weight and low lipid solubility pass the limit for a transplacental transfer [16]. Several studies indicate that human fetuses are widely exposed to prescription and non-prescription drugs prenatally or during labor and delivery [17]. Nonetheless, the placenta is still an effective barrier for the developing fetus, preventing the entry of various xenobiotics from the mother to the fetus and facilitating the passage of other xenobiotics from the fetus to the mother, Ganapathy et al, [18]. Placental transport from the fetus to the mother is established only in the fifth week of fetal life. Several drugs rapidly cross the placenta and pharmacologically significant concentrations equilibrate in maternal and fetal plasma.

In this study, the low doses of 5mg/kg and 20mg/kg body weight did not produce any form of embryotoxicity, but the higher doses of 2g/kg and 20g/kg body weight were toxic to the embryos. The n-hexane extract of RICOM 1013-J is lipid soluble and subject to placental transfer, which may explain the foetal resorption observed with higher doses.

The wide margin of safety of ether-soluble fraction of RICOM 1013-J reported previously by Das et al, [19] has been further established with the n-hexane extract (5-20mg/kg body weight) in this study. N-hexane is known to be a non-poisonous solvent widely acceptable and used industrially in the production of vegetable oils and certain drugs due to its non-toxic properties compared with other solvents. This study further establishes the safety of n-hexane extract of RICOM 1013-J on embryos and foetuses of rodents at the therapeutic doses of 5-20mg/kg.

If taken in pregnancy, it will not cause any embryotoxicity. This is however important and shows that in event of RICOM 1013-J failure, as a contraceptive agent, there will be no embryotoxic effect on the foetuses in its final outcome.

#### 5. REFERENCES

- [1]. Centers for Disease Control and National Birth Defects Prevention Network (NBDPN). Preventing Birth Defects. Available at <http://www.nbdpn.org/current/2008pdf/PrevBDBroch.pdf> (Accessed 15/1/2008)
- [2]. Holcberg, G, Tsadkin-Tamir, M., Sapir, O, Mahmoud H., Moshe, M, and Ben, Z New Aspects in Placental Drug Transfer. *IMAJ*; 2003 5: 873-876

- [3]. Brent RL Teratology in the 20th century: environmental causes of congenital malformations in humans and how they were established. *Neurotoxicol Teratol.* 2004.26(1):1-12.
- [4]. Yasuda Y, Okamoto M, Konishi H, Matsuo T, Kihara T and Tanimura T. Developmental anomalies induced by all-trans retinoic acid in fetal mice: I. Macroscopic findings. *Teratology.* 1986 Aug ; 34(1):37-49.
- [5]. Pauken CM, LaBorde JB., Bolon B Retinoic acid acts during peri-implantational development to alter axial and brain formation. *Anat Embryol (Berl).* 1999./200(6):645-55.
- [6]. Evans. Trease and Evans Pharmacognosy 14th ed. W. B. Saunders Co. Ltd., London, Philadelphia, 1998. Toronto. 612p.
- [7]. Wedin, GP., Neal, JS., Everson, GW., Krenzelok, EP. Castor bean poisoning. *Am J Emerg Med.* 1986. 4(3): 259-61
- [8]. Osunkwo, MM., Ibu, J., Okwuasaba, FK. Anticonceptive and oestrogenic effects of a medicinal plant (*Ricinus communis*) in rats. Abstract of 17th Annual Conference of the West African Society of Pharmacology, Port Harcourt, 1988 pp 41-44.
- [9]. Okwuasaba, FK., Osunkwo, UE., Ekwewchi, MM ., Ekpenyong KI., Onwukeme, K., Olayinka, AO., Uguru, VE., Das, SC Anticonceptive and oestrogenic effect of a seed extract of *Ricinus communis* var. minor. *J. Ethnopharm* 1991.: 34: 141-145.
- [10]. Okwuasaba, FK., Das, SC., Isichei, CO., Ekwewchi, MM., Onoruvwe, O., Olayinka, AO., Uguru, VE., Dafur, SJ., Ekwere, EO., Parry O. Pharmacological Studies on the Antifertility Effects of Ricom-1013-J from *Ricinus communis* var minor and Preliminary Clinical Studies on Women Volunteers. *Phytother Res* .1997. 11: 547-551
- [11]. McNeil, RT, Noronha, CC., Kusemiju, TO and Okanlawon, AO. The Anti-ovulatory effect of a seed extract of *Ricinus communis*-linn. *Nig J Hlth Biomed Sci* 2003 2: (1): 31-34
- [12]. Kabele-Toge, BB., Igwilo, FT., Tayo, F., Isamah, GK., Eniojukan, JF. Some toxicological and pharmacological studies on the extract of *Ricinus communis* var. minor seeds with contraceptive properties. *Nig. Cet. J. Hosp. Med.* 1997. 7(4)
- [13]. Sani, UM. and Sule, MI. Anti-fertility activity of methanol extracts of three different seed varieties of *Ricinus communis* linn (euphorbiaceae) *Nig J Pharm Scs.* 2007.6 (2):21-27
- [14]. Yi-ling Hou, Xiang Ding, Shuang-quan Duan, Zhi-rong Yang., Ping Gao Microencapsulation and pharmacological evaluations for the anti-fertility effect of castor bean extract in female mice. *Afr J Pharm Pharmacol* 2010 4(10) : 700-707
- [15]. UFAW. The UFAW Handbook on the Care and Management of laboratory animals. Churchill Livingstone, London. 1976.: 5th ed.: 635p..
- [16]. Yaffe, SJ. Introduction. In: Briggs G, Freeman RK, Yaffe SJ, eds. *Drugs in Pregnancy and Lactation.* Williams & Wilkins. Baltimore. 1998.: xiii-xix.
- [17]. Sastry, BV Techniques to study human placental transport. *Adv Drug Deliv Rev.* 1999: 38:17-39.
- [18]. Ganapathy VV, Prasad PD, Ganapathy ME, Leibach FH Drugs of abuse and placental transport. *Adv Drug Deliv Rev.* 1999. 38:99-110.
- [19]. Das, SC., Isichei, CO., Okwuasaba, FK., Uguru, VE., Onoruvwe, O., Olayinka, AO., Ekwere, EO., Dafur, SJ., Parry, O. Chemical Pathological and Toxicological Studies of the Effects of RICOM-1013-J on women volunteers and rodents. *Phytother Res* 2000. 14: 15-19.