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Effect of *in utero* exposure to *n*-hexane extract of *Ricinus communis* var. minor (RICOM 1013J) on postnatal foetal weight changes in Sprague-Dawley albino rats

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Abstract
In traditional medicine practice among the Bassa people of central Nigeria, the seeds of *Ricinus communis* (Var. minor) are widely used as anticonceptive agents. This study purposes to evaluate the effect of fetal in-utero exposure to extract of *Ricinus communis* (var. minor) on postnatal growth in rats. Eighteen (18) adult female rats in proestrous phase of the estrous cycle were divided into 3 groups and allowed to mate with males of proven fertility using a male: female ratio 1:3. Group 1 rats were injected subcutaneously on day 2 of pregnancy with 0.2ml of olive oil and served as control, while group 2 animals received 20mg/kg of the extract on day 2 of conception. The rats in group 3 were similarly administered 20mg/kg of the extract subcutaneously on days 2 and 5 of pregnancy. At birth, body weights of pups were taken on days 0, 7, 14 and 28. The results showed that *in utero* exposure to *Ricinus communis* var. minor (RICOM-1013-J) significantly decreased postpartum birth weight ($p < 0.05$) in the first week of life only, while Days 14 and 28 were normal compared to control. The study showed that *in utero* exposure to therapeutic doses of *Ricinus communis* var. minor do not have may have no adverse or deleterious effects on foetal development and postpartum growth in rats.

Keywords: *Ricinus communis*; In-utero; n-Hexane; Postnatal; Foetal; Weight changes

INTRODUCTION

The seeds of *Ricinus communis* var. minor, also known as castor plant, have been used in traditional medicine as anticonceptive agent for centuries by the Bassa people of Plateau State, north central Nigeria [1,2]. The seeds are large, smoothly oval, shiny bean-like and vary widely in size and colour. Generally, they may be brown, black, grey or variable brownish mottling. Based on seed size, *Ricinus communis* has been classified as major, intermediate or minor varieties. The variety minor has been used over the years as an anticonceptive agent by the Rukuba speaking people of Plateau State, in north central Nigeria [1,3]. Usually, the seeds are harvested in dry season, and are made up of 80% kernel and 20% shell. The chemical composition include 20% proteins, ricin (highly toxic), albumin, enzymes (lipase, chymase), nucleoalbumin and amino acids. Other constituents are globulins, glycol proteins, alkaloids and steroids [4,5]. Castor bean also contains magnesium, calcium and manganese in very high amounts, which is said to add up to its toxicity [5]. The stem

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leaves and seed of *Ricinus communis* have been reported to contain the highly toxic glycoprotein ricin, which is absent in the oil [6].

Taken as three seeds (equivalent to 2.3-2.5 g), it prevented conception in women for over a duration of one year, with minimal to no incidence of adverse effects reported among participants [7] This implies that the active principle in RICOM-1013-J may remain in the human body over a long period of time. It is therefore plausible to hypothesize possible effects of RICOM-1013-J beyond the one-year protection time, particularly in the first trimester of pregnancy. It is generally believed that the vast majority of herbs have high safety profile, with little evidence of adverse effect. However, it is known that prenatal exposure to chemical substances of herbal origin could contribute to fetal mortality and morbidity [8-10]. It can therefore be asserted that not all that is natural is indeed safe for pregnancy as often thought by consumers of herbal products [11-13].Thus concerns about outcomes of pregnancies in herbal users have continued to be expressed. Pertinently, studies in both human and experimental animals on foetal origin of adult disease hypothesis, have established a relationship between adverse intra uterine environments and disease in later life [14-16]. Hence, early embryonic exposure to harmful chemical substances may result in permanent organ damage, physiological and metabolic impairments which can be detected through postnatal growth evaluation. Though RICOM-1013-J is reputed to be free of adverse effects, it is still necessary to systematically evaluate its safety claims particularly, since the effect of *in utero* exposure to *Ricinus communis* in offsprings has not been reported. This study therefore aimed to explore the effect of *in utero* exposure to RICOM-1013-J on postnatal growth in rat pups.

EXPERIMENTAL

Plant material. The seeds of *Ricinus communis* var. minor were collected by the consultant herbalist in the Department of Pharmacology, University of Jos, Mrs. O. Azija and authenticated by Professor S. W. H. Huseini of the Department of Botany, University of Jos, Nigeria. A voucher specimen was deposited at the Herbarium of the Department of Pharmacognosy, University of Jos.

Preparation of extract. Sun dried seeds of *Ricinus communis* var. minor were decoated and finely grounded with mortar and pestle. The resulting powder weighing 130.4g was subjected to exhaustive Soxhlet extraction using 250ml of n-Hexane at 67-69^{o} C for 72 h. The oily residue was stored at 4^{0}C until required.

Animals. Mature Sprague-Dawley rats bred at the animal house of the University of Jos, were used for this study. They were housed in a well-ventilated room of temperature 22 ± 2.5^{0}C, relative humidity of 65% ± 5% and 12 h light/dark cycle. The animals were fed standard diet (Vital Feed, Jos) and tap water *ad libitum.* Eighteen (18) adult female rats in proestrous phase of the estrous cycle were divided into 3 groups of 6 rats each and allowed to mate with males of proven fertility using a male: female ratio 1:3. The presence of plugs of spermatozoa in a vaginal smear the next morning confirmed that mating had occurred and termed day-1of pregnancy. Group 1 rats were injected subcutaneously on day 2 of pregnancy with 0.2ml of olive oil and served as control, while group 2 animals received 20mg/kg of the extract on day 2 of conception. The rats in group 3 were similarly administered 20mg/kg of the extract subcutaneously on days 2 and 5 of pregnancy. All animal groups were closely observed and allowed free access to food and water until parturition. At birth, birth weight of pups

were taken and repeated at 7, 14 and 28 days postpartum.

Oestrous cycle. In rats, as in all other mammals, ovarian activity in the oestrous phase of the cycle is regulated by anterior pituitary gonadotropins [19]. Female rats show a 4 or 5-day oestrous cycle, consisting of proestrous, oestrous and diestrous. A 4-day cycle has a two day diestrous (diestrous 1 and 2), while a 5-day has a three day diestrous (diestrous 1, 2 and 3), distinguishable by vaginal smear observation of the cell population in the smear. On this basis, in proestrous nucleated cells predominate, while the major cell population in estrous is swollen cornified cells, lacking nuclei. Predominance of nucleated cells and leukocytes typify diestrous [17].

Statistical analysis. The results are expressed in terms of Mean ± SEM. Statistical difference was determined by simple ANOVA approach and a probability level of equal to or less than 5% was considered significant.

RESULTS

The effects of *in utero* exposure to *Ricinus communis* var. minor (RICOM-1013-J) on growth of pups, measured by changes in birth weight showed no statistically significant difference in birth weight at birth. However, statistically significant changes in postnatal weight of pups between treated rats (Groups 2 and 3) and their control (Group 1) occurred by day 7 postpartum. This significant association was not sustained through days 14 and 28 of delivery. In addition, postnatal weight gain between treatment groups relative to control rats was statistically significant only in the first week of life. No difference occurred in weight gain beyond the 7th day of birth. A similar pattern of change was demonstrated when postnatal weight gains were expressed in percentages (Tables 1-3).

Table 1: Effect of RICOM-1013-J administration in pregnant rats on postnatal weight changes of pups

Group	At birth	Day 7	Day 14	Day 28
1	6.1±0.07	13.66±0.17	20.16±0.09	49.38±0.27
2	6.1±0.05	14.1±0.10*	20.38±0.19	49.2±0.26
3	6.1±0.12	14.04±0.08*	20.14±0.21	48.96±0.11

Values are Mean ± SEM * Statistical significance P<0.05 against Group 1 (control)

Table 2: Postnatal weight gain (g) of pups exposed to RICOM-1013-J *in utero*

Group	Day 7	Day 14	Day 28
1	7.57±0.15	14.07±0.09	43.29±0.30
2	8.01±0.06*	14.59±0.22	43.11±0.24
3	7.98±0.12*	14.08±0.20	42.9±0.11

Values are Mean ± SEM * Statistical significance P<0.05 against Group 1 (control)

Table 3: Postnatal percentage (%) weight gain of pups exposed to RICOM-1013-J *in utero*

Group	Day 7	Day 14	Day 28
1	124.36±2.73	231.16±3.28	711.16±11.23
2	131.53±0.58*	238.69±4.88	707.95±4.41
3	131.98±4.08*	232.71±4.59	708.99±14.03

Values are Mean ± SEM. * statistically significant change in % weight gain relative to group 1.

DISCUSSION

This is the first study of the *in utero* exposure of fetuses to RICOM-1013-J during pregnancy and provides useful insight into the safety profile of the phytomedicine. The evidence presented appears to suggest that RICOM-1013-J is not deleterious to fetal development, even in the first trimester of gestation, a most critical period when fetal organogenesis takes place [10,18], and the

potential for structural and teratogenic consequences are high [19-21]. These findings add to the overall safety profile of RICOM-1013-J in animals and human models as reported by earlier authors [9,22].

A major strength of this study lies in its significance for health care delivery, since medicinal plants will remain central in health care delivery in Nigeria and Africa for some time to come. This is particularly so in the face of growing usage of herbs globally and wide spread poverty and high costs of pharmaceutical products [13,23]. In addition to the problems of accessibility and affordability highlighted, product adulteration and counterfeiting are further factors that push up the consumption of herbal therapeutics [24]. The strength is tempered by the inherent narrow range of extract dose regimen used and non-screening of pups for biochemical functioning, given that herbal extracts contain numerous active molecules that may elicit permanent structural, physiological and metabolic impairments [25,26]. In addition, the duration of the study was short for the nature of phenomenon studied: some harmful effects may not be apparent until long after discontinuation of use, or may only manifest with cumulative intake.

The study concludes that *in utero* exposure to therapeutic doses of *Ricinus communis* var. minor may have no adverse or deleterious effects on foetal development and postpartum growth in rat offsprings.

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