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Comparative study of *n*-hexane extract and emulsion formulation of a phytodrug (RICOM 1013-J) on the oestrous cycle of female Wistar rats

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Abstract
The seeds of *Ricinus communis var minor* (RICOM-1013-J) have been reported to prevent conception for a year, when taken as single oral dose of 2.5-2.7 g. In order to assess further the potency and anticonceptive effectiveness of RICOM-1013-J and to provide possible insight into its mechanism of action, the *n*-hexane extract and emulsion formulation of RICOM-1013-J was evaluated on the oestrous cycle of the Wistar rats. Both formulations of the drug distorted the oestrous cycle. The *n*-hexane extract distorted the oestrous cycle for a longer period at Dioestrous I (L_I), Dioestrous II (L_{II}) and Proestrous (N), while the emulsion formulation distorted the cycle for a longer period at Oestrous (E). There was also individual animal and group variation, which was phase dependent. It can therefore be deduced that the *n*-hexane extract of RICOM-1013-J had a more potent anticonceptive activity than the emulsion, which was phase dependent, but at varying degrees. This may be attributed to a slow release of the active principle in the emulsion formulation of the phytodrug.

Keywords: *Ricinus communis*; RICOM-1013-J; *n*-hexane; emulsion formulation; anticonceptive; oestrous cycle

INTRODUCTION

Castor plant is commonly known for its oil yield. This plant has been used for the cure of various ailments and hence it has earned the name Palma Christi (Palm of Christ) [1]. It is a species of flowering plant in the spurge family, Euphorbiaceae. The seed coat contains ricin, a toxin that is also present in lower concentrations in the plant. It has been shown to interfere irreversibly with the synthesis of proteins by catalytically inactivating the 60s eukaryotic ribosome subunit in such a manner that a single molecule of ricin is enough to kill a cell [2]

Toxicity

Human data. In adults, the estimated lethal dose in man is 1mg/kg bodyweight [3]. Two to four seeds may cause severe poisoning in an adult and eight are generally fatal [4]. If seeds are swallowed without chewing,

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poisoning is unlikely. However, one to three seeds can be fatal to a child [4].

Animal data. The fatal dose by intravenous injection of ricin in experimental animals (mice) has been reported to be as low as 300ng/kg [3,4]. The toxin, ricin acts as a blood coagulant. The ingestion of 2g undetoxified fibrous residue of castor oil may determine toxic effects in large animals [5]. Repeated small doses result in antibody production. Pretreated animals can withstand up to 800 times the lethal dose [6]. Available data on carcinogenicity, teratogenicity, mutagenicity and its interactions is limited. However, Ekwere *et al*. [7], showed that *n*-hexane extract of RICOM 1013-J produced no embryotoxic and teratogenic effects in Wistar rats and mice. The seed, seed oil, leaves and roots of *Ricinus communis* have great medicinal value. Beyond the health benefits of castor oil, are some commercial purposes, such as soap production, as a lubricant, used in the textile industries, used as stain and dyes [8]. The seed of *Ricinus communis* has long been used as an anticonceptive agent. In a study by Okwuasaba, *et al.* [9], the seed and petroleum ether fraction of RICOM 1013-J demonstrated high antifertility efficacy in both animals and in women volunteers. Another study by Okwuasaba *et al.* [10] also demonstrated the contraceptive efficacy of the ether extract in rodents.

This study aims at investigating the potency and anticonceptive effect of the *n*-hexane extract and emulsion formulation of RICOM-1013-J.

Oestrous cycle. The female rat is a spontaneously ovulating polyoestrous mammal with rapid ovulation cycles occurring at 4–5 days interval (except during pregnancy, pseudo- pregnancy and lactation), [11]. The normal length of the oestrous cycle has been investigated over many decades [12,13,14]. Based on vaginal smears, the duration of the individual components of the oestrous cycle for rats with a four or five-day cycle are Proestrous – twelve to fourteen hours; Oestrous – twenty-five to twenty-seven hours; Metoestrous – six to eight hours; and Dioestrous – fifty-five to fifty-seven hours. However, as noted, above, many authors refer to the day of the cycle, with each period having its own day, and those in a five-day cycle generally showing either an extra day of vaginal cornification (extra day of oestrous) or an extra day of leukocyte infiltration (extra day of dioestrous) [15]. During the oestrous cycle, prolactin, LH and FSH remain and increase in the afternoon of the proestrous phase. Oestradiol levels begin to increase at metoestrous, reaching peak levels during proestrous and returning to baseline at oestrous. Progesterone secretion also increases during metoestrous and dioestrous with a decrease afterwards. Then the progesterone value rises to reach its second peak towards the end of proestrous [16, 17].
The characterization of each phase is based on the proportion among 3 types of cells observed in the vaginal smear, namely epithelial cells, cornified cells and leukocytes [18].

EXPERIMENTAL

The seeds of RICOM 1013-J were collected from the wild in Jos metropolis, Plateau State, North Central Nigeria between January and June, 2014. They were identified and authenticated at the Departments of Botany, University of Jos, Ahmadu Bello University, Zaria and Forestry Research Institute, Jos as described by Okwuasaba *et al*. [19] with specimen vouchers prepared and deposited at the herbarium of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria. The primary emulsion was prepared using 5ml of the *n*-hexane oil extract of *Ricinus communis*, 2.5ml of water, and 1.25g of acacia gum in the ratio of 4:2:1 respectively using the dry gum method. Healthy Swiss female Wistar

rats (45 in all) were used for this study. They were procured from and acclimatized in the Experimental Animal House of the University of Jos for 2 weeks under standard laboratory conditions in a cross-ventilated room (temperature 22±2.5^{o}C, humidity 65±5 % and photoperiodicity of 12h light/ 12h darkness). They were fed with standard mash or rat pellets (Grand Cereals & Oil Mills Limited, Jos) and allowed access to water *ad libitum*, and their beddings changed once weekly. All animal experiments were conducted in accordance with the internationally accepted guidelines on laboratory animal use and care (Based on Helsinki convention) and Institutional Animal Care and Use (IACU) guidelines of the University of Jos, for animal experimentation. The rats were divided into nine groups (n=5).

Groups 1-4 were administered *n*-hexane oil extract of RICOM 1013-J orally with the aid of an orogastric tube. To obtain the required dose of 40mg/kg, serial dilutions were carried out using corn oil, (which has been shown to produce no pharmacological effect [19]). Groups 5-8 received emulsion preparation, while Group 9 received corresponding dose of corn oil. The dose administered in each case was 40mg/kg bodyweight. Vaginal smear analyses were carried out daily between 0700hrs – 0900hrs.

RESULTS

It was observed that there was individual and group variation, which was phase dependent. The experimental group showed that the *n*-hexane extract formulation caused distortion in the oestrous cycle for longer days at L_I, L_{II}, and N, while the emulsion formulation distorted the oestrous cycle for longer days at E.

Fig 1: Fruit of *Ricinus communis*

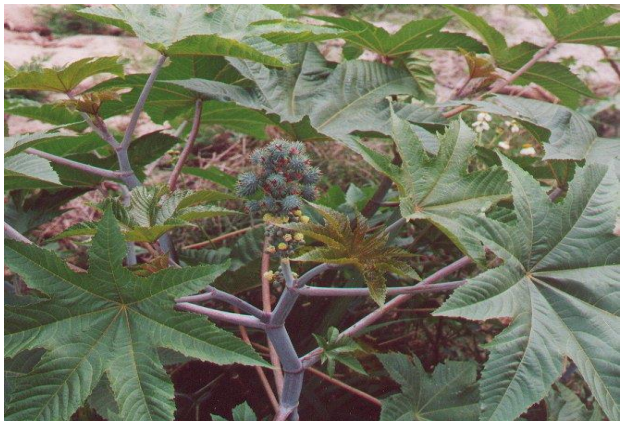

Fig.2: Plant of *Ricinus communis*

Fig. 3: Seeds of *Ricinus communis*

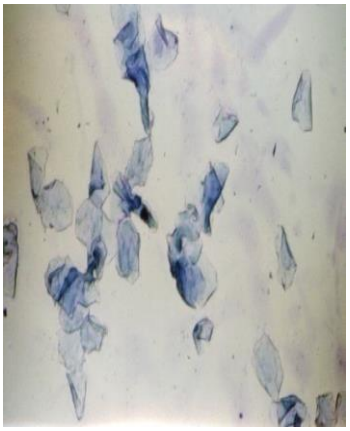

Fig. 4: Oestrous

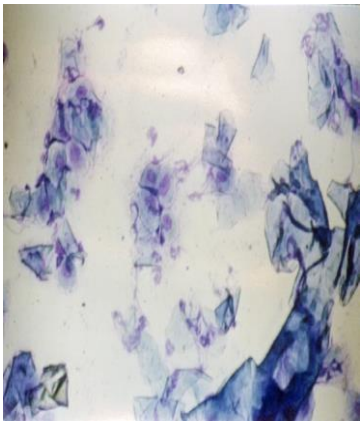

Fig. 5: Transition from oestrous to Metoestrous

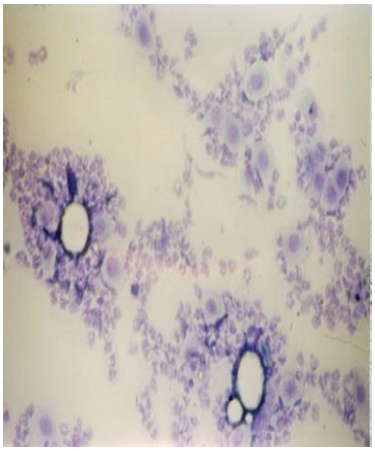

Fig. 6: Metoestrous

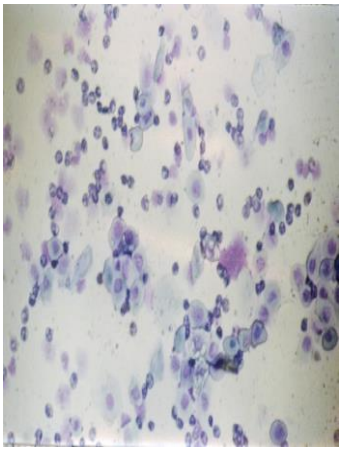

Fig. 7: Metoestrous

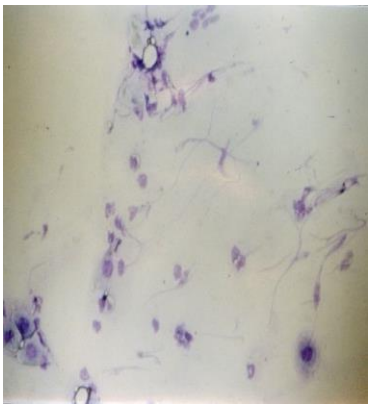

Fig. 8: Transition from Dioestrous I to Dioestrous II

Fig. 9: Dioestrous II

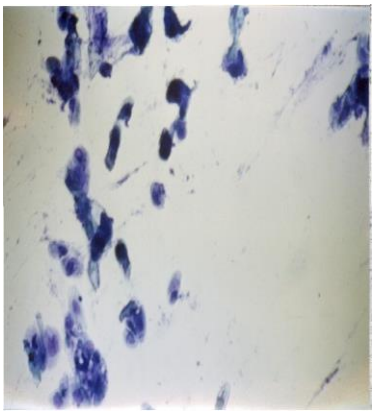

Fig. 10: Transition from Dioestrous II to Proestrous

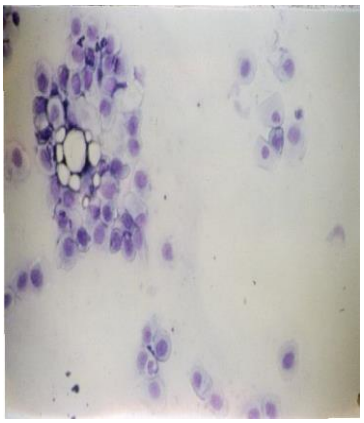

Fig. 11: Proestrous

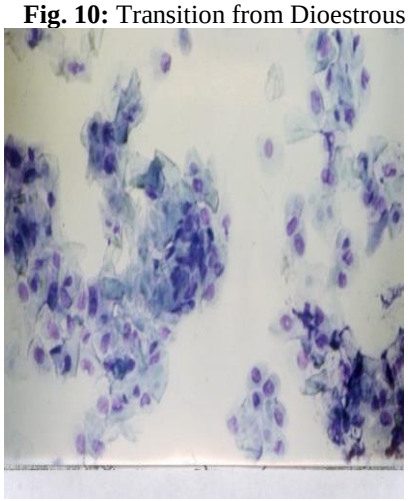

Fig. 12: Transition from Proestrous to oestrous

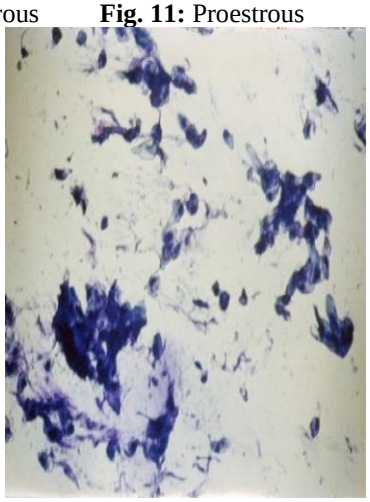

Fig. 13: Anoestrous

Fig 4-13: Pictomicrographs of the various phases of the oestrous cycle

Table 1: n-Hexane extract formulation

Phase	Oestrous (E)	Dioestrous (L_I)	Dioestrous (L_{II})	Proestrous (N)
Average days of distortion	24	28	27	24

Table 2: Emulsion formulation

Phase	Oestrous (E)	Dioestrous (L_I)	Dioestrous (L_{II})	Proestrous (N)
Average days of distortion	26	26	25	22

DISCUSSION

The administration of *n*-hexane extract of RICOM 1013-J caused alteration of the oestrous cycle: At E, there was alteration of this phase to L_{II} → E → N → L_{II} → L_I… up to E. This continued for 24, 25, 23 days for each of the animals in the group with an average of 24 days. Thereafter, there was restoration of the regular cycle. At L_I, there was alteration of this phase to E → E → L_{II} → N → E… up to N. This continued for 28 days for each of the animals in this group with an average of 28 days. Thereafter there was restoration of the cycle. At L_{II} there was alteration of this phase to N → L_{II} → L_I → L_I → E… up to L_I. This continued for each animal for 27, 26, 29 days, with the average of 27days. At N, there was alteration of this phase to L_{II} → L_I → L_I → E → N ... up to N. This continued for 24 days for each of the animals in this group with the average of 24 days. Thereafter, the cycle was restored. (Table 1)

For emulsion formulation of RICOM 1013-J: At E there was alteration of this phase to N → L_{II} → N → N → E…. up to N. This continued for 24, 27, 27 days for each animal with the average of 26 days. Thereafter the cycle was restored. At L_I, there was alteration of this phase to L_I → E → E → L_{II} → E → N … Up to L_{II}. This continued for 26, 26, 27 days for each animal with an average of 26 days. Thereafter, the cycle was restored. At L_{II}, there was alteration of this phase to E → L_I → E → L_I → L_I … up to L_I. This continued for 25, 25, 26 days for each animal with an average of 25 days. Thereafter, the cycle was restored. At N, there was alteration of this phase to L_I → L_{II} → E → E → L_I … up to E. This continued for 23, 22, and 21 days with an average of 22 days. Thereafter, the cycle was restored (Table 2).

The results showed that both the *n*-hexane extract and emulsion formulation of RICOM 1013-J caused a distortion in the oestrous cycle. The number of days for the distortion varied and the mode of alteration varied depending on the phase of the cycle. At E, the distortion of the cycle was more prolonged with the emulsion formulation than with the *n*-hexane extract formulation. At L_I, the distortion of the cycle was prolonged with *n*-hexane extract than with the emulsion. At L_{II}, the distortion of the cycle was slightly prolonged with *n*-hexane extract than with emulsion. At N, the distortion was prolonged with *n*-hexane extract than with emulsion (Tables 1 and 2).

Several studies carried out previously have shown that RICOM-1013-J contains phytoestrogens [1,7,9,10,19]. Oestrogen in appropriate doses prevents conception by inducing hormonal changes that inhibit follicular development and ovulation [16,20,21]. This work further confirms the findings of Osunkwo *et al*. [22], Okwuasaba *et al.* [9,10,19], and McNeil *et al.* [23].

It can be observed that, the oestrogen-like activities exhibited by the extract and emulsion formulation was phase-dependent and the anti-conceptive effect may be due at least in part to such oestrogenic action. Similar findings have been made on the effect of *n*-hexane extract of RICOM-1013-J on cyclicity in mature female Wistar rats [24]. This is further confirmed by this study.

It can therefore be deduced that the *n*-hexane extract formulation of RICOM-1013-J has a more potent activity on the cyclicity than the emulsion preparation, possibly because the emulsion formulation had a slow release of the active principle. This study further confirms the anticonceptive activity of this phytodrug, and its folkloric use.

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