

Antifertility Efficacy of n-Hexane Seed Extract of *Ricinus communis* Var Minor in Wistar Rats Uterus In Vitro

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DOI: [10.22178/pos.103-25](https://doi.org/10.22178/pos.103-25)

LCC Subject Category: RS1-441

Received 21.03.2024

Accepted 26.04.2024

Published online 30.04.2024

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Abstract. The seed of *Ricinus communis* var minor (RICOM 1013-J) is a popular contraceptive among the tribal women in Bassa Local Government Area of Plateau State, Nigeria. Several reports have confirmed the efficacy and safety of different fractions of RICOM 1013-J, particularly the n-Hexane fraction. RICOM 1013-J appears to possess a unique contraceptive effect. This study aimed to provide further insight into the impact of RICOM 1013-J on the reproductive organs compared with the activity profile of some uterotonic drugs. A total of 20 adult albino rats (15 females and five males) weighing 150-200 g were initially divided into five groups of 3 female rats each with treatments via the oral route as follows: Groups A (2ml olive oil), others n-Hexane extract of *Ricinus communis* seeds (RICOM 1013-J) B (5 mg/kg), C (10 mg/kg), D (20 mg/kg) and E (30 mg/kg). After three days, the five male rats were introduced into each group (ratio of 3 females to 1 male per cage) till the end of experiments for mating. The effect of the pretreatment with the n-hexane extract of RICOM 1013-J on the rat uterus was evaluated after days 10, 30, 60 and 90. RICOM 1013-J (20 mg/kg and 30 mg/kg) demonstrated potent anti-conceptive effects, protecting female Wister albino rats against pregnancy for over five gestational periods. RICOM 1013-J statistically altered the regular basal activity of the uterus in a time-dependent manner (10, 30, 60 and 90 days pretreatment). Furthermore, pretreatment with RICOM 1013-J decreased the reactivity of the uterus to some uterotonic drugs, including oxytocin (2×10^{-3} iu/ml), ergometrine (1×10^{-3} mg/ml), misoprostol (2×10^{-3} mg/ml), Ach (1×10^{-5} gm/ml) and potassium chloride (1×10^{-3} gm/ml). The contraction to misoprostol and potassium chloride in the uterus was abolished on day 90 of pretreatment. This study has demonstrated the dose-dependent efficacy of RICOM 1013-J in protecting against pregnancy for over five gestation periods in rats. This protective effect may be due to the alterations in the activity profile of smooth muscle quiescence and inertia in the uterus. In addition, the changes in the responsiveness of the uterus to the uterotonic drugs further confirm the anti-conceptive effects of RICOM 1013-J.

Keywords: Peucicap culture; interpretation; breastfeeding mothers.

INTRODUCTION

The increase in population growth worldwide, with a decreased standard of living, increased desertification, decrease in agricultural food production, and increase in movement from rural regions to urban areas, has greatly caused a rise in population in the metropolitan cities, leading

to high rate of crime due to low job opportunities coupled with global economic recession in a country with over 1.8 million people [1]. The world population presently stands at about 8 billion people [2], and its socio-economic impacts cannot be overestimated, particularly in most developing nations. In Africa, particularly Nigeria, the ever-increasing population has

negatively impacted the total health care delivery system and the people's standard of living. Nigeria's population is about 220 million, projected to be over 440 million by 2050 [3]. However, it is noteworthy that decades ago, the Rukuba-speaking people of Bassa Local Government Area of Plateau State in North Central Nigeria used the seeds of *Ricinus communis* var *minor* (RICOM 1013-J) for family planning by exploiting its anti-conceptive properties. Several researchers evaluated such efficacy [4-7], and [4] demonstrated the novel effects of RICOM 1013-J in that administration of 3-4 seeds once orally protected women volunteers against pregnancy for 9-12 months. *Ricinus communis* L. popularly known as the castor oil plant, taxonomically belongs to the family of Euphorbiaceae, native to India and Africa (Ethiopia), though widely distributed in tropical, subtropical, and warm temperate climates of the world also having It is the following local names: Endi (Hindi), Errandi (Marathi), Zurma (Hausa), Jada (Oriya), Kherwal (Saudi Arabia), Diveli (Gujarati) [8-13]. *R. communis* is highly adaptable and can grow in various climates, from warm temperate to tropical regions, including Nigeria [9, 13-15]. Various studies have revealed the rich biological activities of *Ricinus communis*, which authenticates its medicinal properties, including antibacterial [18], Antifungal [18, 9], antioxidants [8, 18], anti-cancer activity [20], antidiabetic activity [17, 19], anti-ulcer properties [6]. The exact mechanism of action of RICOM 1013-J remains elusive, although several mechanisms have been postulated. Authors [4, 21, 22] demonstrated that estrogenic activity altered uterine responsiveness to drugs, histological changes in the ovaries, including atretic ovaries and a disruption of the delicate oestrogen-progesterone balance in the ovaries and uterus as a possible ant-conceptive effect of RICOM 1013-J.

The present study was designed to provide further insight into the possible mechanism of action of RICOM 1013-J by evaluating its effects on the rat uterus and rabbit fallopian tube.

The *study aims* to provide further insight into the effect of RICOM 1013-J on the reproductive organs and the activity profile of some uterotonic drugs.



Figure 1 – Image of the castor-oil plant [23]

METHODS

Collection and Preparation of Plant Materials. The seeds of RICOM 1013-J were collected from the wild shrubs in Jebbu Bassa, Bassa LGA, Plateau State, Nigeria, between January and March 2016. The seeds were authenticated at the Department of Plant Science University of Jos and the Federal Forestry Research Institute Jos, and the voucher specimen (Voucher No UJ/PCG/HSP/95E25) was deposited in the herbarium of the Department of Pharmacognosy, University of Jos, Nigeria.

Seed Extract Preparation. The dried seeds of RICOM 1013-J were dried and finely grounded with porcelain mortar and pestle. About 130 g was soaked in 250 ml of n-Hexane in a conical flask and agitated using a Gyromax 800-Series Open-Air shaker (medium), Amerex Instruments, Inc., for 96 hours. The mixture was filtered through a glass funnel with filter paper (Whatman No.1), and the residue was soaked in n-Hexane (250 ml) with further continuous agitation for another 96 hours until exhaustive extraction was achieved at room temperature (22 ± 3 °C) [24]. The solvent was evaporated in a fume chamber, and the extract was transferred into a clean, dried specimen bottle and stored at 4-8 °C in a refrigerator before use. The percentage yield was calculated as below:

$$\text{Percentage yield (\%)} = 30.6 \text{ g}/130 \times 100 = 23.4\%$$

Acute Toxicity Studies. Determination of Lethal Dose (LD₅₀). The author [25] described the method for determining LD₅₀ using 12 matured female Wistar rats in two phases. For phase I,

nine rats weighing between 200 g and 250 g were divided into three groups with three animals each and orally administered with 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight RICOM 1013-J – extract, respectively while for the phase II, three animals of same weight range in phase I were grouped into three different cages of one animal each and administered with 1500 mg/kg, 3000 mg/kg and 5000 mg/kg of RICOM 1013-J – extract respectively. The rats were usually fed, allowed access to clean water *ad libitum*, and observed for 24 hours. The animals were monitored for toxicological symptoms and mortality rate within the same period.

The LD₅₀ was calculated using the formula:

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

No mortality was, however, recorded in both phases, indicating the safety of RICOM 1013-J – extract ≤5000 mg/kg.

Phytochemical Screening. RICOM 1013-J extract was screened for secondary metabolites such as flavonoids, alkaloids, tannins, saponins, carbohydrates, anthraquinones and cardiac glycosides according to the methods of [26].

Test for alkaloids. Aliquot of 500 mg of extract was stirred with 3 ml of 1 % aqueous hydrochloric acid on a steam bath and filtered; 1 ml of the filtrate was treated with drops of the following reagents: Mayer's reagent, Picric acid solution and Dragendroff reagent. Precipitation with either of these reagents was taken as preliminary evidence of the presence of the alkaloids.

Test for saponins. An Aliquot of 500 mg extract was shaken with water in a test tube. Frothing that persists during warming was taken as preliminary evidence of the presence of saponins.

Test for tannins. An Aliquot of 500 mg of the extract in a test tube and Ferric chloride solution was added to the test tube. A blue-black, green, or blue-green precipitate was taken as evidence of tannins' presence.

Test for anthraquinones. An Aliquot of 500 mg of extract was put in a dry test tube, and 5 ml of chloroform was added and shaken for 5 minutes.

The filtrate was filtered and shaken with an equal 100% ammonia solution volume. The pink, violet or red colour in the ammoniacal layer (lower layer) indicated the presence of free anthraquinones.

Test for cardiac glycosides (Keller Killani test). An Aliquot of 100 mg of extract in 1 ml of glacial acetic acid containing one drop of Ferric chloride solution and 1 ml of concentrated sulphuric acid was added gently by the side of the test tube. A brown ring formed at the interphase indicated the deoxy sugar characteristics of cardenolides.

Test for steroids (steroidal ring). An Aliquot of 100 mg of the extract was dissolved in 2 ml of chloroform; sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interphase indicated the presence of a steroidal ring.

Test for flavonoids. 200 mg of the extract was detained with acetone. The sample was placed in a hot water bath for all acetone to evaporate. Boiling distilled water was added to the sample arrested. The mixture was filtered while hot. The filtrate was cooled, and 5 ml of 20% sodium hydroxide was added to equal the filtrate volume. A yellow solution indicates the presence of flavonoids.

Test for carbohydrates. Aliquot of 500 mg of the extract was heated with dilute hydrochloric acid. The mixture was neutralized by adding sodium hydroxide solution, and Fehling's solutions 1 and 2 were added and boiled on a hot plate. A brick-red precipitate formed, indicated the presence of flavonoids.

Experimental Animals Procurement and Preparation. A total of 20 adult albino rats (15 females and five males) weighing 150-200 g were obtained from the Animal House Unit of the University of Jos, Nigeria. The rats were housed in plastic cages with stainless steel mesh tops according to groups, maintained under favourable laboratory conditions in a cross-ventilated room (22± 3 °C), lighting (12 hrs light and 12 hrs dark cycle), conducive beddings, fed with standard rat pellets from Grand Cereal Mills, Jos, Nigeria and allowed access to water *ad libitum*. All animals were allowed to acclimatize for 14 days.

Anti-conceptive Studies on RICOM 1013-J. A total of 15 Adult female rats weighing 150-200 g shown to have regular oestrus cycles determined daily by vaginal smear analysis with at least two

successive four days estrus cycles were selected. The rats were randomly divided into five groups (A, B, C, D and E) consisting of 3 rats, each with different treatments as follows:

Group A: administered with olive oil orally using the orogastric tube.

Group B: administered with 5 mg/kg n-Hexane extract of *Ricinus communis* seeds (RICOM 1013-J) orally.

Group C: orally administered with n-Hexane extract of 10 mg/kg *Ricinus communis* seeds (RICOM 1013-J).

Group D: administered with 20 mg/kg n-Hexane extract of *Ricinus communis* seeds (RICOM 1013-J) orally.

Group E: administered with 30 mg/kg n-Hexane extract of *Ricinus communis* seeds (RICOM 1013-J) orally.

Fertile and sexually active male rats were introduced on day three after administration of RICOM 1013-J into each group (ratio of 3 females to 1 male per cage) till the end of experiments for mating according to the methods of [10]. Mating was confirmed by the presence of a whitish vaginal plug with the presence of spermatozoa and is considered to be day '1' of conception [27, 28]. The male rats were in contact with the female rats for the seven gestation periods of the study. The fertility rate was calculated as the number of rats littered per group divided by the total number.

In vitro Study on Rat Uterus. A control group was used.

Control Group. The control Rats were administered olive oil orally at a dose of 30 mg/kg body weight. The effect of the pretreatment with the n-hexane extract of RICOM 1013-J on the rat uterus was evaluated after days 10, 30, 60 and 90. The rats were exsanguinated. The abdomen was dissected, the uterus cut to a size of about 3 cm, placed in a petri dish containing De-Jalon's solution and bubbled with air (oxygen 95% and carbon dioxide 5%) until used.

The tissue was then mounted in a bath containing 25 ml of De-Jalon's solution at 37 °C and aerated using an aerator (student physiograph). A sensitivity of 50 microvolt/cm and speed of 2 mm/sec was used throughout the experiment. The contractile activity of the uterus was recorded via isometric force-

displacement transducer model 707 connected to a 3-way channel student physiograph recorder (model Medicaid, 7013).

The following drugs were used to study the activity and response of the uterine muscle to drugs (Acetylcholine 1×10^{-5} g/ml, Oxytocin 2×10^{-3} iu/ml, Misoprostol 2×10^{-6} g μ /ml, Ergometrine 1×10^{-3} g/ml and KCl 1×10^{-3} g/ml).

Statistical Analysis. Data were expressed as mean \pm SEM.

RESULTS AND DISCUSSION

Phytochemical Screening. The phytochemical screening of the n-Hexane extract of RICOM 1013-J revealed the presence of steroids and carbohydrates only, while alkaloids, saponins, tannins, flavonoids, anthraquinones and cardiac glycoside were not detected (Table 1).

Table 1 – Phytochemical Analysis of RICOM 1013-J

Chemical Constituent	Result
Alkaloid	-
Saponins	-
Tannins	-
Flavonoids	-
Carbohydrates	+
Steroids	+
Anthraquinones	-
Cardiac Glycoside	-

Notes: "-" Not Detected; "+" Detected

Anti-conceptive activity. RICOM 1013-J (20 and 30 mg) protected treated rats against pregnancy for over six weeks following a single oral administration in a dose-dependent manner (Table 2).

Table 2 – Anti-conceptive Activity of RICOM 1013-J in Female Rats

Dose of RICOM 1013-J	Pregnancy Occurrence	Duration of Protection (months)	Fertility Rate
Control	3/3	0	1
5 mg/Kg	2/3	$5.00 \pm 1.70^*$	0.6
10 mg/Kg	2/3	$5.67 \pm 1.39^*$	0.6
20 mg/Kg	1/3	$6.33 \pm 1.16^*$	0.3
30 mg/Kg	0/3	$6.67 \pm 0.58^*$	0.0

Notes: n = 3; * - P < 0.05

The results obtained from the phytochemical study have demonstrated the presence of steroids and carbohydrates in the n-Hexane extract of RICOM 1013-J.

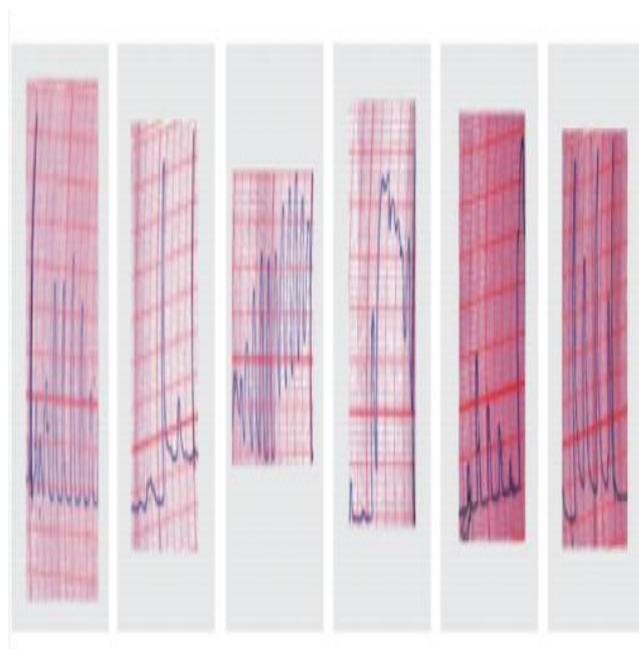
This agrees with the earlier report [22] that *Ricinus Communis* oil contains some steroidal compounds. These findings are significant since some conventional oral contraceptives are of estrogen-progestin combination, and this may contribute in part to the anti-conceptive effect of RICOM 1013-J. Moreover, it is known that plant steroids are converted into animal steroid hormones through synthetic pathways involving steroidogenic enzymes [29].

Effect of RICOM 1013-J pretreatment on the rat uterus. Pretreatment of rats and rabbits with a single oral dose of RICOM 1013-J (30 mg/kg) altered the contractile profiles of the rat uterus in a time-dependent manner (Figure 2).

There was complete quiescence of basal rhythmic contraction on days 10 and 90 (Figure 6) post-treatment. Post-treatment on the rat uteri day 90 (Figure 6) resulted in complete inactivity of the basal rhythmic contraction. There was a marked increase in the frequency of basal rhythmic contraction on days 30 and 60 of the post-treatment Figure 8 and 9, respectively.

Effect of RICOM 1013-J pretreatment on the contractile activity of uterotonic drugs on the rat uterus. Pretreatment with RICOM-1013J altered the uterus response to standard uterotonic agonists [acetylcholine, prostaglandin E₂ (PGE₂), ergometrine and potassium chloride (KCl)]. The responsiveness of the uterus to the uterotonic agonists varied significantly and depended on the pretreatment duration with RICOM 1013-J. The contractile activity of the agonists (Ach, Oxy, Miso, ergot and KCl) was abolished on day 30 of pretreatment with RICOM 1013-J (Figure 4). However, there was recovery of contractile activity on day 60 (Figure 5), while the contractions to misoprostol and KCl were abolished again on day 90 (Figure 6).

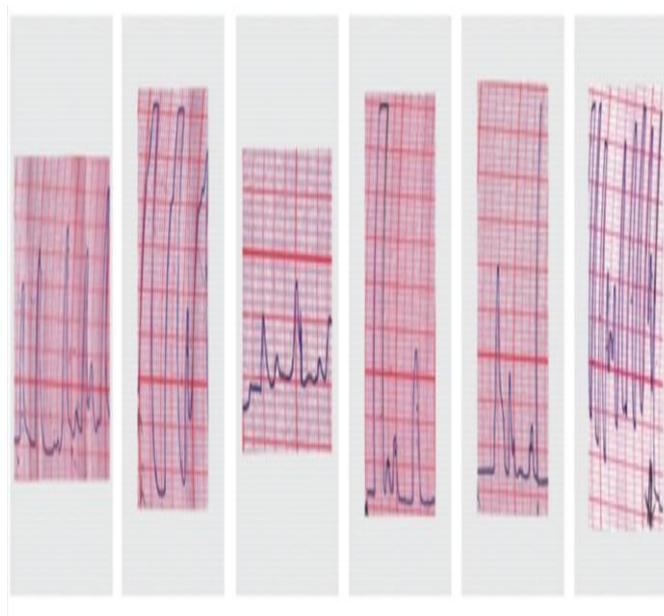
The administration of RICOM 1013-J protected the animals against conception in a dose-dependent manner for six gestational periods. This also bolsters the earlier finding of [4], where he administered different doses of petroleum ether (PE) extract of RICOM 1013-J (5-20 mg/kg), which induced a dose-dependent contraceptive effect over five gestational periods.



CONTROL 1x10⁻⁵g/ml ACH 2x10⁻²iu/ml OXY 2x10⁻⁴ ug/ml MISO 1x10⁻³mg/ml ERGOT 1x10⁻³mg/ml KCl

Notes: ACH – Acetylcholine, OXY – Oxytocin, MISO – Misoprostol, ERGOT – Ergometrine, KCl – Potassium

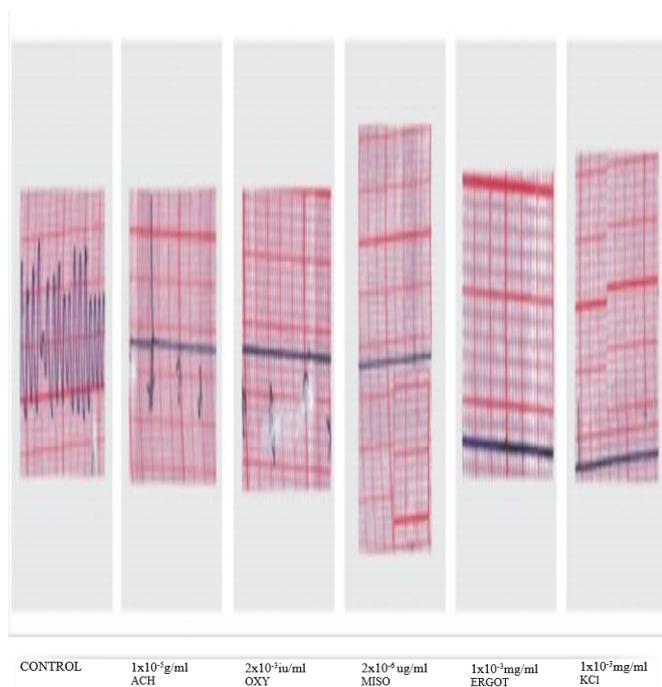
Figure 2 – Rat uterus response to drugs (Non treated)



CONTROL 1x10⁻⁵g/ml ACH 2x10⁻²iu/ml OXY 2x10⁻⁴ ug/ml MISO 1x10⁻³mg/ml ERGOT 1x10⁻³mg/ml KCl

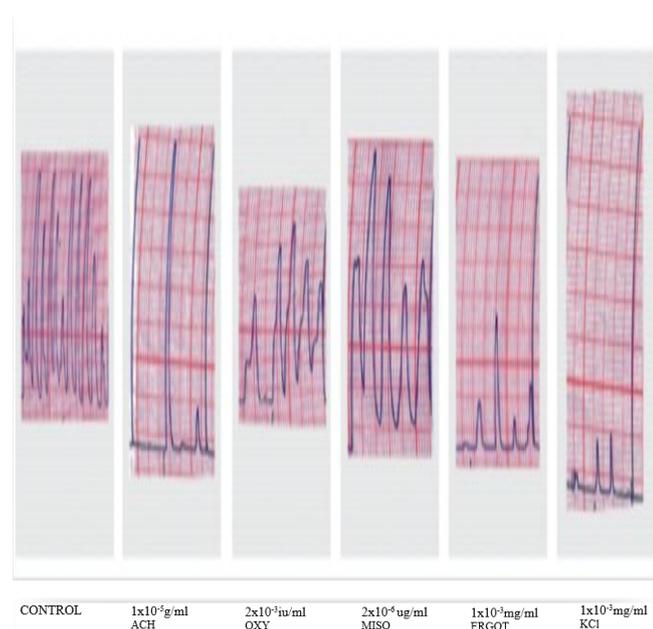
Notes: ACH – Acetylcholine, OXY – Oxytocin, MISO – Misoprostol, ERGOT – Ergometrine, KCl – Potassium

Figure 3 – Rat uterus response to drugs after ten days of pretreatment with RICOM 1013-J



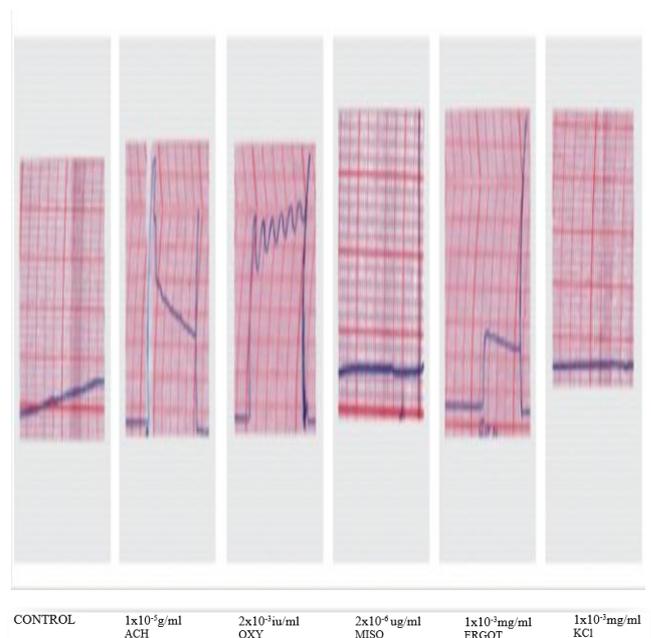
Notes: ACH – Acetylcholine, OXY – Oxytocin, MISO – Misoprostol, ERGOT – Ergometrine, KCl – Potassium

Figure 4 – Rat uterus response to drugs after 30 days of pretreatment with RICOM 1013-J



Notes: ACH – Acetylcholine, OXY – Oxytocin, MISO – Misoprostol, ERGOT – Ergometrine, KCl – Potassium

Figure 5 – Rat uterus response to drugs after 60 days of pretreatment with RICOM 1013-J



Notes: ACH – Acetylcholine, OXY – Oxytocin, MISO – Misoprostol, ERGOT – Ergometrine, KCl – Potassium chloride

Figure 6 – Rat uterus response to drugs after 90 days of pretreatment with RICOM 1013-J

Authors [30] demonstrated the possible contraceptive efficacy of Ricinus communis by exerting its regulatory role over luteinizing hormone (LH), primary ovarian cell function and secretion activity. All those processes could disrupt the estrogen/progesterone balance, leading to an unfavourable environment in the endometrium that prevents implantation of the fertilized ovum.

Pretreatment of rats and rabbits with RICOM 1013-J alters the activity profile of the uterus as well as their responsiveness to uterotonic agents in a characteristic pattern. The uterine inertia after days 30 and 90 pretreatment mimics the effect of progesterone [31, 32]. The disordered uterine quiescence and inertia seen in this study may contribute to the antifertility property of RICOM 1013-J [33]. When all the effects of RICOM 1013-J on the uterus are considered together, it is not unreasonable that changes in estrogen/progesterone balance may partly be responsible for the antifertility efficacy of RICOM 1013-J.

CONCLUSIONS

This study has demonstrated the dose-dependent efficacy of RICOM 1013-J in protecting

against pregnancy for over five gestation periods in rats. This protective effect may be due to the alterations in the activity profile exhibited in smooth muscle quiescence and uterine inertia. In addition, the changes in the responsiveness of the uterus to the uterotonic drugs further confirm the anti-conceptive effects of RICOM 1013-J.

Ethical Considerations

Ethical clearance was obtained from the Research and Ethical and Animal Research

Committee of the Faculty of Pharmaceutical Sciences, University of Jos, Nigeria.

Acknowledgements

The authors acknowledge technical assistance rendered by Mr Thomas Philip Yakubu, Mr Sunday Azi, Mr Pwajok Dung, and Mr Amah, all of the faculty of pharmaceutical sciences at the University of Jos, Nigeria.

REFERENCES

1. National Population Commission. (2018). *Nigeria Demographic and Health Survey*. Retrieved from <https://ngfrepository.org.ng:8443/jspui/bitstream/123456789/3145/1/NDHS%202018.pdf>
2. UN. (2022). *UN Population Prospects 2022*. Retrieved from https://www.un.org/development/desa/pd/sites/www.un.org.development.desa.pd/files/wpp_2022_summary_of_results.pdf
3. UN. (2018). *World Urbanization Prospects. The 2018 Revision*. Retrieved from <https://population.un.org/wup/Publications/Files/WUP2018-Report.pdf>
4. Okwuasaba, F. K., Das, S. C., Isichei, C. O., Ekwenchi, M. M., Onoruvwe, O., Olayinka, A. O., Uguru, V. E., Dafur, S. J., Ekwere, E. O., Parry, O. (1997). Pharmacological studies on the antifertility effects of RICOM-1013-J from *Ricinus communis* var minor and preliminary clinical studies on women volunteers. *Phytotherapy Research*, 11, 547–551.
5. Salhab, A. S., Issa, A. A., & Alhougog, I. (1997). On the Contraceptive Effect of Castor Beans. *International Journal of Pharmacognosy*, 35(1), 63–65. doi: 10.1076/phbi.35.1.63.13268
6. Hou, Y.-L., Ding, X., Duan, S.-Q., Yang, Z.-R., & Gao, P. (2010). Microencapsulation and pharmacological evaluations for the antifertility effect of castor bean extract in female mice. *International Journal of Pharmacy and Pharmacology*, 5(10), 001-008.
7. Salami, S. A., & Raji, Y. (2014). Oral *Ricinus communis* oil exposure at different stages of pregnancy impaired hormonal, lipid profile and histopathology of reproductive organs in Wistar rats. *Journal of Medicinal Plant Research*, 8(44), 1289–1298.
8. Elkousy, R. H., Said, Z. N. A., Ali, M. A., Kutkat, O., & Abu El Wafa, S. A. (2023). Anti-SARS-CoV-2 in vitro potential of castor oil plant (*Ricinus communis*) leaf extract: in-silico virtual evidence. *Zeitschrift Für Naturforschung C*, 78(9–10), 365–376. doi: 10.1515/znc-2023-0075
9. Abdul, W., Hajrah, N., Sabir, Jamal S. M., Al-Garni, S., Sabir, M., Kabli, S., Saini, K., & Bora, R. (2018). Therapeutic role of *Ricinus communis* L. and its bioactive compounds in disease prevention and treatment. *Asian Pacific Journal of Tropical Medicine*, 11(3), 177. doi: 10.4103/1995-7645.228431
10. Okwuasaba, F. K., Osunkwo, U. A., Ekwenchi, M. M., Ekpenyong, K. I., Onwukeme, K. E., Olayinka, A. O., Uguru, M. O., & Das, S. C. (1991). Anticonceptive and estrogenic effects of a seed extract of *Ricinus communis* var. minor. *Journal of Ethnopharmacology*, 34(2–3), 141–145. doi: 10.1016/0378-8741(91)90031-8
11. Akpan, G. J., Udongwo, A. M., & Obodom, M. I. (2022). Extraction, Characterization And Applications Of Castor Seed Oil From Wild *Ricinus communis*. *Journal of Chemical Society of Nigeria*, 47(4). doi: 10.46602/jcsn.v47i4.795

12. Sandford, E. C., Muntz, A., & Craig, J. P. (2021). Therapeutic potential of castor oil in managing blepharitis, meibomian gland dysfunction and dry eye. *Clinical and Experimental Optometry*, 104(3), 315–322. doi: 10.1111/cxo.13148
13. Sharma, R., Goyal, A., Bhat, R. (2013). *Antifertility Activity Of Plants Extracts On Female Reproduction: A Review*. Retrieved from https://www.researchgate.net/publication/262938502_Antifertility_Activity_of_Plants_Extracts_on_Female_Reproduction_A_Review
14. Salihu, B. Z., Gana, A. K., & Apuyor, B. O. (2014). Castor oil plant (*Ricinus communis* L.): Botany, Ecology and Uses. *International Journal of Science and Research*, 3(5), 1333–1334.
15. Patel, V. R., Dumancas, G. G., Viswanath, L. C. K., Maples, R., & Subong, B. J. J. (2016). Castor Oil: Properties, Uses, and Optimization of Processing Parameters in Commercial Production. *Lipid Insights*, 9, LPI.S40233. doi: 10.4137/lpi.s40233
16. JU, I., Nnamdi, N. C., PC, N., PO, A., & FK, O. (2023). Studies on Acute and Sub-Chronic Toxicities of N-Hexane Seed Extract of Ricom-1013-J in Wistar Rats. *Saudi Journal of Biomedical Research*, 8(05), 63–68. doi: 10.36348/sjbr.2023.v08i05.003
17. Basiri, N., & Ibraheem O. (2014). Assessing *Ricinus communis* L.(castor) whole plant parts for Phenolics and Saponins constituents for Medicinal and Pharmaceutical applications. Retrieved from [https://www.semanticscholar.org/paper/Assessing-Ricinus-communis-L.-\(-castor\)-whole-for-Inayor-Ibraheem/c6e611fd7d3e0d1bba266d355cd3f05c068e2394](https://www.semanticscholar.org/paper/Assessing-Ricinus-communis-L.-(-castor)-whole-for-Inayor-Ibraheem/c6e611fd7d3e0d1bba266d355cd3f05c068e2394)
18. Vandita, P., Nirali, A., Kyati, P., & Monisha, K. (2013). Effect of phytochemical constituents of *Ricinus communis*, *Pterocarpus santalinus*, *Terminalia bellerica* on antibacterial, antifungal and cytotoxic activity. *International Journal of Toxicological and Pharmacological Research*, 5(2), 47–54.
19. Chouhan, H. S., Swarnkar, G., & Jogpal, B. (2021). Medicinal properties of *Ricinus communis*: A review. *International Journal of Pharmaceutical Sciences and Research*, 12(7), 3632–3642.
20. Abbas, M., Ali, A., Arshad, M., Atta, A., Mehmood, Z., Tahir, I. M., & Iqbal, M. (2018). Mutagenicity, cytotoxic and antioxidant activities of *Ricinus communis* different parts. *Chemistry Central Journal*, 12(1). doi: 10.1186/s13065-018-0370-0
21. Raji, Y., Oloyo, A. K., & Morakinyo, A. O. (2006). Effect of methanol extract of *Ricinus communis* seed on reproduction of male rats. *Asian Journal of Andrology*, 8(1), 115–121. doi: 10.1111/j.1745-7262.2006.00055.x
22. Desouky, M. M. A., Abd El-Atti, M. S., Elsheakh, A. A., & Elgohary, W. S. (2022). Effect of *Eucalyptus globulus* oil and *Ricinus communis* methanolic extract as potential natural molluscicides on the reproductive biology and some antioxidant enzymes of the land snail. *Theba pisana. Heliyon*, 8(12), e12405. doi: 10.1016/j.heliyon.2022.e12405
23. Britannica. (2024). *Castor-oil plant*. Retrieved February 28, 2024, from <https://www.britannica.com/plant/castor-oil-plant>
24. Sbihi, H. M., Nehdi, I. A., Mokbli, S., Romdhani-Younes, M., & Al-Resayes, S. I. (2018). Hexane and ethanol extracted seed oils and leaf essential compositions from two castor plant (*Ricinus communis* L.) varieties. *Industrial Crops and Products*, 122, 174–181. doi: 10.1016/j.indcrop.2018.05.072
25. Erhirhie, E. O., Ihekwereme, C. P., & Ilodigwe, E. E. (2018). Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. *Interdisciplinary Toxicology*, 11(1), 5–12. doi: 10.2478/intox-2018-0001
26. Odebiyi, O. O., & Sofowora, E. A. (1978). *Phytochemical screening of Nigerian medicinal plants II*. Retrieved from <https://pubmed.ncbi.nlm.nih.gov/672462/>
27. Kaur, S., & Prabha, V. (2012). Infertility as a Consequence of Spermagglutinating *Staphylococcus aureus* Colonization in Genital Tract of Female Mice. *PLoS ONE*, 7(12), e52325. doi: 10.1371/journal.pone.0052325

28. Sari, G. P., Hilario, P. L. L., Yuri, S., Honda, A., & Isotani, A. (2022). Scheduled simple production method of pseudopregnant female mice for embryo transfer using the luteinizing hormone-releasing hormone agonist. *Scientific Reports*, *12*(1). doi: [10.1038/s41598-022-26425-2](https://doi.org/10.1038/s41598-022-26425-2)
29. Green, N., Stout, G., & Taylor, D. (1995). *Biological Sciences* (2nd ed.) Cambridge: Cambridge University Press.
30. Nath, S., Kadasi, A., Grossmann, R., Sirotkin, A. V., Kolesarova, A., Talukdar, A. D., & Choudhury, M. D. (2015). Ricinus communis L. stem bark extracts regulate ovarian cell functions and secretory activity and their response to Luteinising hormone. *International Journal of Impotence Research*, *27*(6), 215–220. doi: [10.1038/ijir.2015.19](https://doi.org/10.1038/ijir.2015.19)
31. Conneely, O. M. (2002). Reproductive Functions of Progesterone Receptors. *Recent Progress in Hormone Research*, *57*(1), 339–355. doi: [10.1210/rp.57.1.339](https://doi.org/10.1210/rp.57.1.339)
32. Graham, J. D., & Clarke, C. L. (1997). Physiological Action of Progesterone in Target Tissues. *Endocrine Reviews*, *18*(4), 502–519. doi: [10.1210/edrv.18.4.0308](https://doi.org/10.1210/edrv.18.4.0308)
33. Kaluwa Kaingu, C., Oduma, J. A., & Kanui, T. (2012). Preliminary investigation of contractile activity of Ricinus communis and Euclea divinorum extracts on isolated rabbit uterine strips. *Journal of Ethnopharmacology*, *142*(2), 496–502. doi: [10.1016/j.jep.2012.05.026](https://doi.org/10.1016/j.jep.2012.05.026)