International Journal of Scientific Research Updates

Journal homepage: https://orionjournals.com/ijsru/

ISSN: 2783-0160 (Online)

(RESEARCH ARTICLE)

In vitro antifertility evaluation of n-hexane seed extract of *Ricinus communis* Var Minor (RICOM 1013-J) on rabbits' fallopian tube

Ayuba Clagba Sanni [1], Olusayo Moritiwon [1], Timothy Olugbenga Ogundeko [2,*], Mamzhi Seljul C. Ramyil [3], Paul Amos Bassi [4] and Francis Kanayo Okwuasaba [1]

[1] *Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria.*
[2] *Department of Pharmacology and Therapeutics, College of Medicine and Allied Health Sciences, Bingham University, Jos Campus, Nigeria.*
[3] *Department of Medical Microbiology and Parasitology, College of Medicine and Allied Health Sciences, Bingham University, Jos Campus, Nigeria.*
[4] *Department of Community Medicine and Public Health, Nile University of Nigeria, Cadastral, Abuja, Nigeria.*

International Journal of Scientific Research Updates, 2024, 08(02), 189-195

Publication history: Received on 16 November 2024; revised on 28 December 2024; accepted on 30 December 2024

Article DOI: https://doi.org/10.53430/ijsru.2024.8.2.0072

Abstract

Natural products used as contraceptive agents among indigenous communities for child spacing are pointers to the ethnopharmacological relevance of medicinal plants, including the seed of *Ricinus communis var minor* (RICOM 1013-J) popularly used by the tribal women in Bassa Local Government Area of Plateau State, Nigeria. Confirmatory studies on the efficacy and safety of RICOM 1013-J) using different models to further authenticate this property scientifically have become imperative. The study aimed to further evaluate the contraceptive mechanism of action of RICOM 1013-J using the rabbit's fallopian tube. The effect of pre-treatment with n-hexane extract of RICOM 1013-J on isolated rabbits' fallopian tube was evaluated after days: 10, 30, 60, and 90 after which uterotonic drugs (Acetylcholine, Oxytocin, Misoprostol, Ergometrine, and KCl) were used to study the activity and response of the isolated rabbits' fallopian tissue. A time-dependent alteration of the contractile profile of the rabbits' fallopian tubes as a result of pre-treatment with a single oral dose of RICOM 1013-J (30 mg/kg) with a remarked abolishment of the normal rhythmicity of the rabbit's fallopian tube.

The antifertility efficacy of RICOM 1013-J is re-established owing to its contraceptive mechanism of action in the fallopian tube activity for over five gestation periods in rabbits.

Keywords: Anti-conceptive activity; Fallopian tube; RICOM 1013-J; Uterotonic drugs; Gestation period; Rabbits

1. Introduction

Medicinal plants have been used over the years as contraceptive agents with increasing interest worldwide [1], [2]. In African societies, the use of plants for purposes of regulating fertility has been practiced for centuries, thereby highlighting their potential as contraceptives [3]. Interestingly, the Rukuba-speaking people of Bassa Local Government Area of Plateau State in North Central Nigeria use the seeds of *Ricinus communis* L. as an anti-conceptive agent [4]. 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 (Guajarati) [5 – 10]. *R. communis* is highly adaptable and can grow in various climates, from warm temperate to tropical regions, including Nigeria [11,12, 13]. The far-reaching benefits of contraception relate

* Corresponding author: Timothy Olugbenga Ogundeko.

to many of the sustainable development goals [14] and reducing unplanned pregnancy is a priority for the global health community [15]. There are now many different contraception products, although access to any or all of these is difficult or impossible in many countries [16]. However, even in countries where contraception is free and widely available, there are many obstacles to access and successful use of contraception [17]. Even though contraception has remarkably far-reaching benefits and is highly cost-effective, women worldwide lack sufficient knowledge, capability, and opportunity to make reproductive choices coupled with the fact that healthcare systems often fail to provide access and informed choice [17], including the use of non-synthetic products.

Based on existing anecdotal facts, researchers have explored natural resources, including plants, as an alternative to conventional contraceptives [18], [19]. Consequently, the efficacy of RICOM 1013-J has been explored [20,21,22,23], while [11] 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.

This study aimed to further evaluate the contraceptive mechanism of action of RICOM 1013-J using the rabbit's fallopian tube.

Figure 1a Image of the castor-oil plant [24]

Figure 1b Image of the castor-oil plant seed [25]

2. Material and methods

2.1. Collection and Preparation of Plant Materials

2.1.1. Plant Material Collection, Identification and Authentication

Whole Castor plant with fruits of RICOM 1013-J were collected from the wild shrubs in Jebbu Bassa, Bassa LGA, Plateau State, Nigeria, in January, 2016. The plant material was identified and authenticated at the Department of Plant Science University of Jos and the Federal Forestry Research Institute Jos, assigned a voucher number (Voucher No UJ/PCG/HSP/95E25) and the voucher specimen deposited in the herbarium of the Department of Pharmacognosy, University of Jos, Nigeria.

2.1.2. Plant Material Preparation

Seeds of RICOM 1013-J were dried and finely grounded with porcelain mortar and pestle. The powder (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 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) [26]. 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 of RICOM 1013-J extract was calculated as below: Percentage yield (%) = 30.6 g/130 x 100 = 23.4%.

2.1.3. Acute Toxicity Studies

Lethal dose (LD_{50}) of RICOM 1013-J extract was determined use Lorke's method described by [4] and [27] for Phases 1 and 2. These indicated the LD_{50} of RICOM 1013-J extract ≤5000 mg/kg.

Eigth Female rabbits weighing 2 – 3kg were administered orally n-hexane extract of RICOM 1013J at a dose of 30mg/kg body weight each at once.

2.1.4. Procurement and Preparation of Experimental Animals

A total of 8 adult female rabbits weighing 2-3 kg were purchased from the National Veterinary Research Institute (NVRI)Vom, Nigeria. And transported to the Pharmacology Laboratory of the Faculty of Pharmaceutical Sciences, University of Jos, Nigeria. They were housed in stainless steel cages, and maintained under favorable conditions with cross ventilation, room temperature (22± 3 °C), lighting (12 hrs. light and 12 hrs. dark cycle), conducive beddings, fed with standard animal pellets from Grand Cereal Mills, Jos, Nigeria and allowed access to water *ad libitum*. The rabbits were allowed to acclimatize for 14 days prior to the Anti-conceptive Studies on RICOM 1013-J.

2.2. Contraceptive Activity Testing of RICOM 1013-J Extract on Isolated Rabbit Fallopian Tube

2.2.1. Animal Grouping and Pre-treatments

Prior to the Anti-conceptive Studies on RICOM 1013-J. The rabbits were divided into two groups of 4 animals each with pre- treatments as below:

- Group 1 (Control): Administered with olive oil once.
- Group 2 (Control): Administered with n-hexane extract of RICOM 1013-J extract (30 mg/kg b.w) per oral each once.

2.2.2. Tissue Preparation, Test-Drug Administration and Fallopian Tube Activity

The rabbits were humanely killed then the abdomen dissected followed by the isolation of the fallopian tubes, and placed in a petri dish containing De Jalon solution. Approximately 3 cm of the horns were cut mounted in a tissue bath, maintained at 37 °C, aeration (90% O_2 and 5% CO_2), and De Jalon solution. The other end of the tissue was attached to an isometric force transducer model 707 connected to a 3-way channel student physiograph recorder (model Medicaid, 7013) using sensitivity of 50 microvolt/cm and speed of 2 mm/sec) where the contractile activity of the uterus was recorded.

The following drugs were used to study the activity and response of the isolated rabbits' fallopian tissue, including, Acetylcholine 1x10-5 g/ml, Oxytocin 2 x 10-3 iu/ml, Misoprostol 2 x10-6 g µ/ml, Ergometrine 1 x 10-3 g/ml and KCl 1x10-3 g/ml.

The effect of the pre-treatment with the n-hexane extract of RICOM 1013-J on the Rabbit fallopian tube was evaluated after days: 10, 30, 60 and 90.

2.3. Statistical Analysis

Data were expressed as mean ± SEM.

3. Results and discussion

3.1. Effects of N-Hexane extract of RICOM 1013-J on rabbit fallopian tube

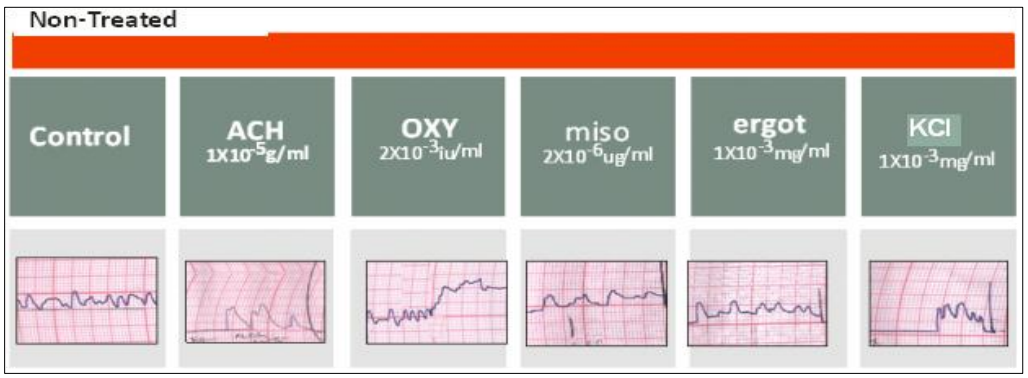

Figure 2 Response to drugs rabbit fallopian tube

3.2. Control (non- treated) Rabbit Fallopian tube

The fallopian tube produced slight contraction as a result of decreased rhythmicity of normal smooth muscle in the presence of acetylcholine. The contraction was slightly higher (not pronounced) in the presence of oxytocin, misoprostol, ergometrine and potassium chloride in the tissue bath – *Figure 3*.

Figure 3 Fallopian tube response to drugs after 10 days post treatment with RICOM 1013-J

Pretreatment with the RICOM 1013J extract (30mg/kg) abolished the normal rhythmicity of the fallopian tube. The responsiveness to acetylcholine (1 x 10^{-5} g/ml) was with a single contraction, a sustained contraction in the presence of oxytocin (2 x 10^{-3} g/ml), produced a sustained contraction, while misoprostol, ergometrine and potassium chloride did not produce any contraction, thus a complete state of inertia was observed – *Figure 4*.

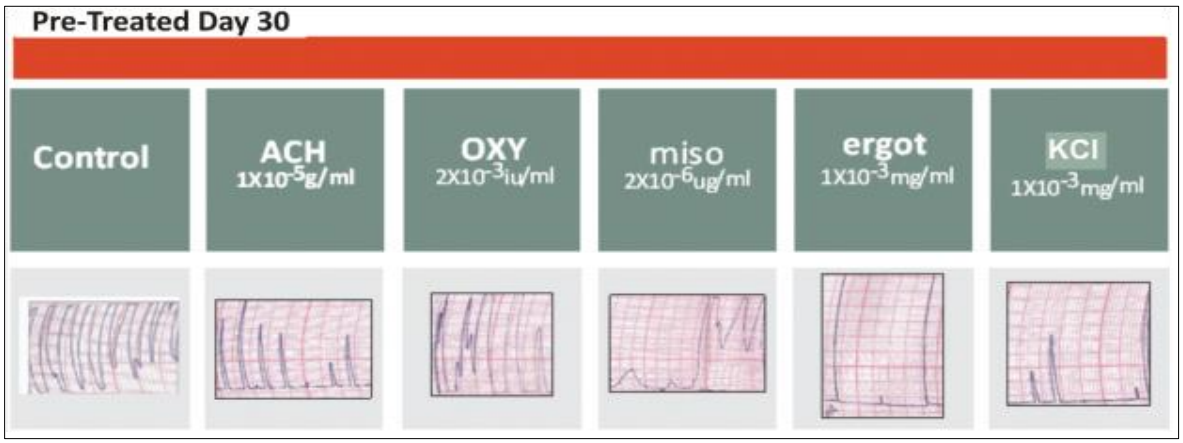

Figure 4 Fallopian tube response to drugs after 30 days post treatment with RICOM 1013-J

There was a complete recovery of contraction with increased amplitude of the isolated fallopian tube. A significant increase in responsiveness compared to pretreated day 10 in the presence of acetylcholine (1 x 10^{-5} g/ml), both oxytocin (2 x 10^{-3} g/ml), and misoprostol (1 x 10^{-6} g/ml) produced a contractile a response, while ergometrine (1 x 10^{-3} g/ml), and potassium chloride (1 x 10^{-3} g/ml) produced a partial contraction characterized by partial recovery of contraction – *Figure 5*.

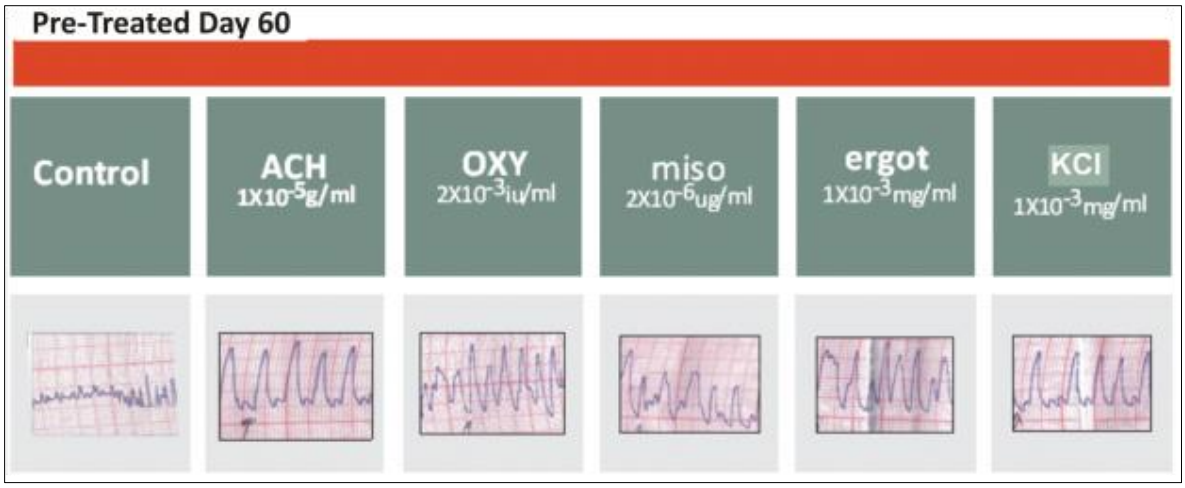

Figure 5 Fallopian tube response to drugs after 60 days post treatment with RICOM 1013-J

Sixty days pre-treatment with RICOM 1013-J extract altered the normal rhythmicity of the Rabbit's fallopian tube. The isolated tissue produced a significant contraction in the presence of all the drugs acetylcholine (1 x 10^{-5} g/ml), oxytocin (2 x 10^{-3} g/ml), misoprostol (1 x 10^{-6} g/ml), ergometrine (1 x 10^{-3} g/ml), and potassium chloride (1 x 10^{-3} g/ml) – *Figure 6*.

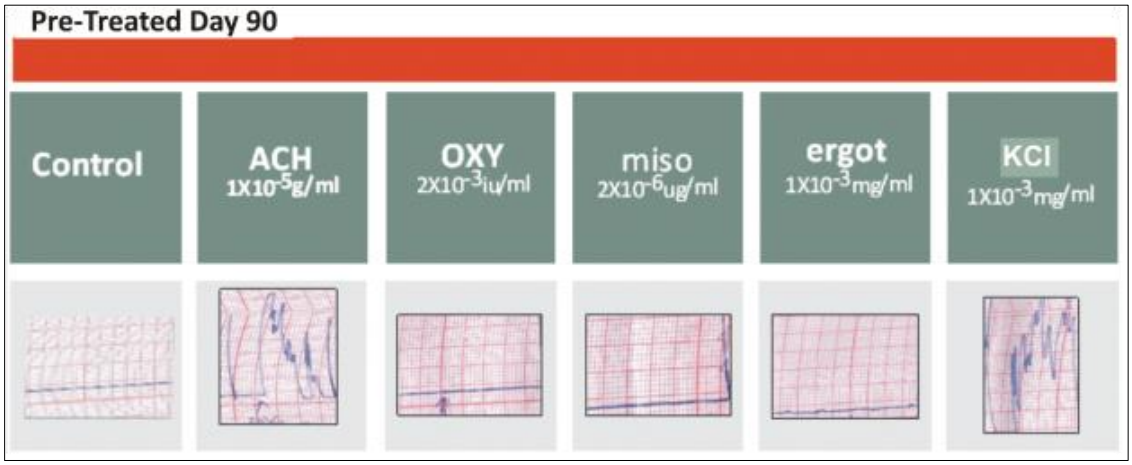

Figure 6 Fallopian tube response to drugs after 90 days post-treatment with RICOM 1013-J

The presence of the RICOM 1013-J extract after 90 days pre-treatment completely abolished the normally rhythmicity of the isolated rabbit's fallopian tube similar to pre-treated day 10. On administration of acetylcholine (1 x 10^{-5} g/ml), the tissue responded with a sustained contraction, likewise potassium chloride (1 x 10^{-3} g/ml).

The fallopian tube exhibited a prolonged state of inertia characterized by absence of responsiveness of the tissue to oxytocin (2 x 10^{-3} g/ml), misoprostol (1 x 10^{-6} g/ml) and ergometrine (1 x 10^{-3} g/ml) – *Figure 7*.

The presence of alkaloid, saponins tannins flavonoids carbohydrates, steroids anthraquinones, and cardiac glycoside profiling of RICOM 1013-J, which further reiterates the anti-conceptive activity of RICOM 1013-J in female rats as reported by [4] together with that of [21], that Ricinus Communis oil contains some steroidal compounds are important pointers 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 [28]. A time-dependent alteration of the contractile profile of the rabbits' fallopian tubes as a result of pre-treatment with a single oral dose of RICOM 1013-J (30 mg/kg), while complete quiescence of basal rhythmic contraction on days 10 and 90 on post-treatment with marked increase in the frequency of basal rhythmic contraction on days 30 and 60 were observed from our study. Our result is in tandem with earlier reports that the observed disordered uterine quiescence and inertia may contribute to the antifertility property and changes in estrogen/progesterone balance may partly be responsible for the

antifertility efficacy of RICOM 1013-J [4], [29], thus a remarked abolishment of the normal rhythmicity of the rabbit's fallopian tube.

4. Conclusion

The antifertility efficacy of RICOM 1013-J is re-established from its contraceptive mechanism of action in the fallopian tube activity for over five gestation periods in rabbits.

Compliance with ethical standards

Acknowledgments

The authors acknowledge various assistance rendered by the technical staff of the Faculty of Pharmaceutical Sciences at the University of Jos, Nigeria.

Disclosure of conflict of interest

All authors hereby declare no conflict of interest.

Statement of ethical approval

Ethical clearance was obtained from the Research and Ethical and Animal Research Committee of the Faculty of Pharmaceutical Sciences, University of Jos, Nigeria.

References

[1] Jain S, Choudhary GP, Jain DK. Medicinal plants with potential anti-fertility activity: A review. Int. J. Green Pharm. 2015; 9:223–228.

[2] Zaman W, Ahmad M, Zafar M, Amina H, Ullah F, Bahadur S, Ayaz A, Saqib S, Begum N, Jahan S. The quest for some novel antifertility herbals used as male contraceptives in district Shangla, Pakistan. Acta Ecol. Sin. 2020; 40:102–112. doi: 10.1016/j.chnaes.2019.05.017.

[3] Moroole MA, Materechera SA, Otang-Mbeng W, Aremu AO. African indigenous contraception: A review. Afr. J. Reprod. Health. 2020; 24:173–184.

[4] Sanni AC, Moritiwon O, Ogundeko TO, Ramyil MSC, Bassi AP, Okwuasaba FK. Antifertility Efficacy of n-Hexane Seed Extract of Ricinus communis Var Minor in Wistar Rats Uterus In Vitro. Path of Science. 2024; 10:4. 8001-8009. ISSN 2413-9009 DOI: 10.22178/pos.103-25.

[5] Elkousy, RH, Said ZNA, Ali MA, Kutkat O, Abu El Wafa SA. Anti-SARS-CoV-2 in vitro potential of castor oil plant (Ricinus communis) leaf extract: in-silico virtual evidence. Zeitschrift Für Naturforschung C. 2023; 78 (9–10): 365–376. doi: 10.1515/znc-2023-0075

[6] Abdul W, Hajrah N, Sabir Jamal SM, 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. 2018; 11(3): 177. doi: 10.4103/1995-7645.228431

[7] Okwuasaba FK, Osunkwo UA, Ekwenchi MM, Ekpenyong KI, Onwukeme KE, Olayinka A O, Uguru MO, Das SC. (1991). Anticonceptive and estrogenic effects of a seed extract of Ricinus communis var. minor. Journal of Ethnopharmacology. 1991; 34(2–3):141–145. doi: 10.1016/0378-8741(91)90031-8

[8] Akpan GJ, Udongwo AM, Obodom MI. (2022). Extraction, characterization and applications of castor seed oil from wild Ricinus communis. Journal of Chemical Society of Nigeria. 2022; 47(4).

[9] Sandford EC, Muntz A, Craig JP. Therapeutic potential of castor oil in managing blepharitis, meibomian gland dysfunction and dry eye. Clinical and Experimental Optometry. 2021; 104(3): 315–322. doi: 10.1111/cxo.13148

[10] Sharma R, Goyal A, Bhat R. Antifertility activity of plants extracts on female reproduction: A Review. 2013. Retrieved from https://www.researchgate.net/publication/262938502_Antifertility_Activity_of_Plants_Extracts_on_Female_Reproduction_A_Review

[11] Salhab AS, Issa, AA, Alhougog I. On the contraceptive effect of castor beans. international journal of pharmacognosy. 1997; 35(1): 63–65. doi: 10.1076/phbi.35.1.63.13268

[12] Salihu BZ, Gana AK, Apuyor BO. Castor oil plant (Ricinus communis L.): Botany, Ecology and Uses. International Journal of Science and Research. 2014; 3(5): 1333–1334.

[13] Patel VR, Dumancas GG, Viswanath LCK, Maples R, Subong BJJ. Castor Oil: Properties, Uses, and Optimization of Processing Parameters in Commercial Production. Lipid Insights. 2016; 9, LPI.S40233. doi: 10.4137/lpi.s40233

[14] Dockalova B, Lau K, Barclay Het al.. Sustainable Development Goals and Family Planning 2020. London: International Planned Parenthood Federation; 2016. Available from: https://www.ippf.org/sites/default/files/2016-11/SDG%20and%20FP2020.pdf [accessed 7 October 2024].

[15] Obare F, Kabiru C, Chandra-Mouli V. Family planning evidence brief – reducing early and unintended pregnancies among adolescents: WHO/RHR/17.10. Geneva: World Health Organization, 2017. [accessed 7 October 2024].

[16] Alkema L, Kantorova V, Menozzi C, Biddlecom A. National, regional, and global rates and trends in contraceptive prevalence and unmet need for family planning between 1990 and 2015: a systematic and comprehensive analysis. Lancet. 2013;381(9878):1642-1652. doi:10.1016/S0140-6736(12)62204-1

[17] D'Souza P, Bailey JV, Stephenson J, Oliver S. Factors influencing contraception choice and use globally: a synthesis of systematic reviews. The European Journal of Contraception & Reproductive Health Care. 2022; 27(5): 364–372. https://doi.org/10.1080/13625187.2022.2096215

[18] Shehab NG, Abu-Gharbieh E. Phenolic profiling and evaluation of contraceptive effect of the ethanolic extract of Salsola imbricata Forssk. in male albino rats. Evid.-Based Complementary Altern. Med. 2014; 2014:695291. doi: 10.1155/2014/695291.

[19] Moroole MA, Materechera SA, Otang-Mbeng W, Aremu AO. Medicinal plants used for contraception in South Africa: A review. J. Ethnopharmacol. 2019; 235:19–27. doi: 10.1016/j.jep.2019.02.002.

[20] Okwuasaba FK, Das SC, Isichei CO, Ekwenchi, 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. Phytotherapy Research. 1997; 11: 547–551.

[21] Hou YL, Ding X, Duan SQ, Yang ZR, Gao P. Microencapsulation and pharmacological evaluations for the antifertility effect of castor bean extract in female mice. International Journal of Pharmacy and Pharmacology. 2010; 5(10): 001-008.

[22] 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. 2014; 8(44): 1289–1298.

[23] Raji Y, Oloyo AK, Morakinyo AO. (2006). Effect of methanol extract of Ricinus communis seed on reproduction of male rats. Asian Journal of Andrology. 2006; 8(1): 115–121. doi: 10.1111/j.1745- 7262.2006.00055.x

[24] Image of castor-oil plant https://www.istockphoto.com/photos/castor-plant [accessed 11 December 2024].

[25] Image of the castor-oil plant seed https://www.sciencephoto.com/media/34041/view/castor-oil-plant-seeds-ricinus-communis [accessed 11 December 2024].

[26] Sbihi HM, Nehdi IA, Mokbli S, Romdhani-Younes M, Al-Resayes SI. Hexane and Ethanol Extracted Seed Oils and Leaf Essential Compositions from Two Castor Plant (Ricinus communis L.) Varieties. Industrial Crops and Products. 2018; 122: 174-181. https://doi.org/10.1016/j.indcrop.2018.05.072

[27] Erhirhie EO, Ihekwereme CP, Ilodigwe EE. Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. Interdiscip Toxicol. 2018;11(1):5-12. doi:10.2478/intox-2018-0001

[28] Tarkowská D. Plants are Capable of Synthesizing Animal Steroid Hormones. Molecules. 2019 Jul 16;24(14):2585. doi: 10.3390/molecules24142585. PMID: 31315257; PMCID: PMC6680614

[29] Nath S, Kadasi A, Grossmann R, Sirotkin AV, Kolesarova A, Talukdar AD, Choudhury MD. Ricinus communis L. stem bark extracts regulate ovarian cell functions and secretory activity and their response to Luteinising hormone. Int J Impot Res. 2015 Nov-Dec;27(6):215-20. doi: 10.1038/ijir.2015.19. Epub 2015 Aug 27. PMID: 26311247.