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Research Article

Antifertility Effects of Ricinine-Enriched Extract of *Ricinus communis* in Female Wistar Rats

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ABSTRACT
Traditional medicinal plants are widely used for fertility control because of the belief that they have fewer health risks than conventional hormonal contraceptives. *Ricinus communis* is one such plant whose seeds have been used for contraception. The alkaloid Ricinine, isolated from *R. communis*, has shown general antifertility properties, but detailed, dose-dependent effects on implantation and pregnancy maintenance have not been assessed. This study evaluated the antifertility effects of a Ricinine-enriched extract of *R. communis* in female Wistar rats using reproductive outcome, anti-implantation, and abortifacient models. Female rats (n = 20) were treated orally with RCN at 100, 200, or 400 mg/kg and cohabited with males to assess the fertility outcome. In separate experiments, additional pregnant rats (n = 25 per group) received RCN (100–400 mg/kg) from gestational days 1–7 (anti-implantation) and days 8–15 (abortifacient), with a positive control using 8 mg/kg Mifepristone. The number of implantation sites, live foetuses, and resorption sites was recorded. RCN treatment resulted in the complete absence of pregnancy at all doses in the fertility test. In the anti-implantation model, RCN produced a significant, dose-related reduction in implantation sites ($p < 0.05$). In the abortifacient model, treated animals showed a decrease in live foetuses and a significant increase in resorption sites, comparable to mifepristone. These data indicate that RCN exerts both anti-implantation and abortifacient effects in a dose-dependent manner. In conclusion, Ricinine-enriched of *R. communis* extract demonstrates potent antifertility activity in female rats, supporting its traditional use for contraceptive purposes.

Keywords: Abortifacient; Antifertility; Anti-implantation; *Ricinus communis*; Ricinine

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INTRODUCTION
In many parts of Africa, including Nigeria, high fertility rates remain a major demographic and reproductive health challenge (World Bank Group, 2022). Traditional methods for fertility regulation — especially the use of herbal remedies — continue to be widely practiced, often due to limited access to orthodox family-planning services or cultural preferences (Aina & Aina-Pelemo, 2019). Such traditional contraceptive approaches are perceived by many as more natural and less risky than modern hormonal methods (Rabiu & Rufa'i, 2018).

Ricinus communis (castor bean) is among the plants commonly used in various communities for fertility

control, with women consuming seeds, leaves or preparations derived from the plant for anti-conceptive purposes (Aina & Aina-Pelemo, 2019). Previous phytochemical studies have identified several bioactive constituents from *R. communis* seeds, including the alkaloid Ricinine, other steroids, flavonoids, and phenolics (Johnson *et al.*, 2005; Singh & Geetanjali, 2015). These compounds are suspected to contribute to the plant's contraceptive and antifertility properties (Iornmube *et al.,* 2016).

However, these studies used crude or semi-purified extracts, and did not isolate or standardize for specific compounds such as ricinine. Moreover, there is a lack of data on dose–response relationships or mechanistic investigation for ricinine-enriched extracts.

Therefore, the present study was designed to evaluate the antifertility effect of a ricinine-enriched extract of *R. communis* in female Wistar rats. Specifically, we investigated whether oral administration of defined doses of RCN can prevent pregnancy (fertility test), inhibit implantation (anti-implantation model), or cause pregnancy loss (abortifacient model). This fills an important knowledge gap by linking a defined phytochemical constituent to functional reproductive outcomes in a dose-dependent manner.

MATERIALS AND METHODS

Experimental Site

The study was carried out in the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Ahmadu Bello University. The University is located in Zaria, Kaduna State of Nigeria on latitude 11° 15'N to 11°3'N of the equator and longitude 7° 30'E to 7°45'E of Greenwich Meridian.

Materials

All drugs, reagents, instruments and devices used for the experiment were of analytical grade. The instruments and devices used include: rat plastic cages**,** rat feeding containers and water bottles, weighing scale**,** mortar and pestle**,** Soxhlet apparatus**,** fractionating flask, 2 ml pipette, 1 ml syringes**,** copper wire**,** microscopic glass slides**,** microscope and oral cannula. The consumables used include: ethanol, chloroform, petroleum ether, hexane and ethyl acetate, used for the extraction and isolation of Ricinine from the *R.communis* seeds. Tween-80 was used as a solubilizer in preparing the extract-water solution prior to administration. Mifepristone 200mg combipack was purchased commercially from M.U.B. Pharmaceutical Enterprises Limited, Sabon Gari, Zaria, Kaduna State with NAFDAC Reg. No: B4-6480 and Batch No.: NR01251A; manufactured by Naari Pharma Private Limited, China with Mfg. Lic. No.: 12/UA/SC/P-2009 and imported by Deep K. Tyagi Foundation Nigeria. Mifepristone, a potent progesterone receptor antagonist, was used to induce pregnancy termination in the female rats (Telleria & Deis, 1996). Ketamine and Diazepam were used for anesthetizing of the rats prior to euthanization.

Collection of plant material

The dry matured *R. communis* seeds were collected during the dry season (February/March) at Samaru, Sabon Gari Local Government of Kaduna State, Nigeria.

Preparation of Ricinine-enriched extract of *R. communis*

The extraction of Ricinine was carried out at the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, using the method described by Gopalasatheeskumar (2018). The extract obtained was washed with chloroform, and further purifications were done using petroleum ether to obtain a Ricinine-enriched compound. Preliminary phytochemical screening was done to confirm the phytochemicals present using the different screening methods. The compound to be administered was prepared by mixing an extract-water solution, using Tween 80 as a solubilizer and water as the vehicle as described by Bawa and Idris, (2021).

Ethical clearance

Ethical clearance for the experiment was obtained from the Animal Use and Care Committee, Ahmadu Bello University, Zaria-Nigeria with approval number: ABUCAUC/2025/017.

Methodology

The stages of the estrus cycle of the rats was determined daily from 9:00 am to 12 noon for 14 days using the Vaginal Smear Examination method as described by Elvis-Offiah *et al.* (2016). The vaginal smear was collected by flushing the vagina with distilled water using a micro-pipette. The swab pipette was gently introduced at a depth of about 0.1

cm into the vagina. The swab pipette was released to flood the vaginal epithelium and a small quantity of vaginal fluid was drawn. The fluid containing the cells was transferred unto a clean glass slide. The smears were fixed with 95% ethyl alcohol and stained with Gentian Violet (GV) for 2 minutes. The GV was rinsed off with water and allowed to dry. The smears were observed under a microscope (magnification X40).

The antifertility activity of the Ricinine-enriched extract was evaluated using three experimental models: the reproductive outcome study, anti-implantation and abortifacient studies (Shah & Jhade, 2018). Twenty (20) female albino Wistar rats and ten (10) albino male Wistar rats were used to study the reproductive outcome of the Ricinine-enriched extract of *R. communis.* Twenty-five (25) female albino and thirteen (13) male albino Wistar rats were used for the anti-implantation study of the effect of Ricinine-enriched extract of *R. communis.* Twenty-five (25) female albino and thirteen (13) male albino Wistar rats were used to study the abortifacient effect of Ricinine-enriched extract of *R. communis.*

Reproductive outcome study

Twenty (20) adult female and ten (10) male Wistar rats were used. Female rats with similar cycle were placed together in a group of five (n=5) rats each. The rats were grouped into 4 groups as follows (Table 1).

Table 1: Groupings and treatment for Reproductive outcome study

Groups	Treatment
I (Vehicle/normal control)	1 ml distilled water
II	100 mg/kg RCN
III	200 mg/kg RCN
IV	400 mg/kg RCN

RCN: Ricinine-enriched extract of *R. communis*

All administrations were done once daily orally using oro-pharyngeal cannula for 4 days. The experimental female rats were cohabited with male rats at a ratio of 2:1 for a minimum of 10 days (Long & Evans, 1922). The presence of vaginal plug was taken as a sign of positive mating and was taken as the first day of pregnancy (Hamid & Zakaria, 2013). Fertility test was calculated using the formula by Raji *et al*. (2010).

$$\%\text{Fertility success} = \frac{\text{No. of pregnant female rats} \times 100}{\text{No. of mated female}}$$

The pregnant rats were allowed to term (Raji *et al.,* 2010).

Anti-implantation study

The anti-implantation activity is expressed as the percentage of animals showing absence of implantation in uteri when laparotomised on day 10 of pregnancy (Chauhan, 2017)**.** Twenty-five (25) female and thirteen (13) male rats were used for this study. Female Wistar rats in proestrus phase were paired overnight with matured male rats at a ratio of 2:1. Pregnancy was confirmed by the detection of sperm cells in the vaginal smear the following morning (Baker, 1979). Presence of vaginal plug marked day 1 of pregnancy (Lembe, *et al.,* 2014). The pregnant female rats were divided into 5 groups (n=5) as follows (Table 2).

Table 2: Groupings and treatment for anti-implantation study

Groups	Treatment
I (Vehicle/normal control)	1 ml distilled water
II (Positive control)	8 mg/kg Mifepristone (Dao *et al.,*1996)
III	100 mg/kg RCN
IV	200 mg/kg RCN
V	400 mg/kg RCN

RCN: Ricinine-enriched extract of *R. communis*

RCN administrations were done once daily orally using oro-pharyngeal cannula from day 1-7 of pregnancy (Lembe *et al.*, 2014). Mifepristone administration was done once orally on day 7 of

pregnancy (Telleria & Deis, 1996). On the 8th day of pregnancy, the rats were euthanized under anesthesia by intraperitoneal injection of ketamine and diazepam at 75 and 5 (mg/kg) respectively (Molina *et al.*, 2015). Blood was collected by cardiac puncture using syringes and the blood samples were transferred into plain specimen bottles. Sites of implantation and resorption were observed in the uterine horns of the animals (Ugwah-Oguejiofor *et al.,* 2020; Abdu *et al.,* 2023).

Abortifacient effect

The abortifacient activity is expressed as several resorbed implants from the existing number of implants recorded at mid-parturition stage (Megha *et al.,* 2023). Twenty-five (25) female and thirteen (13) male rats were used for this study. Female Wistar rats in proestrus phase were paired overnight with matured male rats at a ratio of 2:1. Pregnancy was confirmed by the detection of sperm cells in the vaginal smear the following morning (Baker, 1979). Presence of vaginal plug marked day 1 of pregnancy (Lembe, *et al.,* 2014). The pregnant female rats were divided into 5 groups (n=5) as follows (Table 3).

Table 3: Groupings and treatment for the abortifacient effect study

Groups	Treatment
I (Vehicle/normal control)	1 ml distilled water
II (Positive control)	8 mg/kg Mifepristone (Dao *et al.,*1996)
III	100 mg/kg RCN
IV	200 mg/kg RCN
V	400 mg/kg RCN

RCN: Ricinine-enriched extract of *R. communis*

RCN treatments were administered once daily orally using oro-pharyngeal cannula from day 8-15 of pregnancy (Lembe *et al.*, 2014). Mifepristone administration was done once orally on day 10 of pregnancy (Telleria & Deis, 1996). On the 16th day of pregnancy, the rats were euthanized under anesthesia by intraperitoneal injection of ketamine and diazepam at 75 and 5 (mg/kg) respectively (Molina *et al.*, 2015). The following parameters were recorded: bleeding sites; number of live and dead fetuses; % survival ratio = (number of live fetuses/numbers of live + dead pups) x 100 (Lembe *et al.*, 2014).

Data Analysis

Data collected were analyzed using One-way Analysis of Variance (ANOVA) followed by Tukey's *post hoc* test for multiple comparisons. Results are expressed as mean ± Standard Error of Mean (SEM) and presented as appropriate. Values with $p<0.05$ were considered significant. SPSS version 27 was used for the analysis.

RESULTS

Phytochemical investigations

Preliminary screening methods showed the presence of phytochemicals such as alkaloids, anthraquinones, flavonoids, steroids and glycosides in different proportions (Table 4).

Effect of RCN on reproductive outcome (fertility test) in female Wistar rats

There was a statistically significant ($p<0.05$) difference in the reproductive outcome of the distilled water group (0) when compared with RCN 100, 200 and 400 mg/kg (33.3, 33.3 and 33.3 % respectively) treatment groups for the non-pregnant rats. Also, for the pregnant rats, there was statistically significant ($p<0.05$) difference in the reproductive outcome of the distilled water group (100%) when compared with the RCN 100, 200 and 400 mg/kg (0, 0 and 0 % respectively) treatment groups as shown in Table 5.

Effect of RCN on number of implantation sites (Anti-implantation study) in female Wistar rats

There was statistically significant ($p<0.05$) decrease in the mean number of implantation sites of the distilled water group (7.00±1.00) when compared with the Mifepristone treated group (8.00±1.15) and RCN 100, 200 and 400 mg/kg (2.33±0.88, 2.67±1.76 and 4.33±1.20 respectively) treatment groups. Similarly, there was statistically significant ($p<0.05$) decrease between Mifepristone treated group (8.00±1.15) and the RCN 100, 200 and 400 mg/kg (2.33±0.88,

2.67±1.76 and 4.33±1.20 respectively) treatment groups (Figure 1).

Table 4: Results of Phytochemical Screening

S/No	Phytoconstituents	Test	Inferences
1	Alkaloids	Dragendorff test	+
2	Cardiac Glycosides	Keller-Kiliani test	+
3	Saponins	Frothing test	-
4	Phenolic compounds	Lead acetate test	+
5	Tannins	Ferric Chloride test	-
6	Steroids	Salkowiski test	+
7	Carbohydrates	Molisch test	+
8	Flavonoids	Shinoda test	+
9	Terpeniods	Liebermann Burchard test	+
10	Anthraquinones	Bontragers test	-

Keys: + = PRESENT; - = ABSENT

Table 5: Assessment of the Association between Reproductive outcomes in RCN Treated female Wistar rats

Treatment	Total *N* = 20 N (%)	Pregnant *n* = 5 n (%)	Non-pregnant *n* = 15 n (%)
Group 1 (1 ml/kg DW)	5 (25)	5 (100)	0 (0)
Group 2 (RCN 100 mg/kg)	5 (25)	0 (0)[a]	5 (33.3)[a]
Group 3 (RCN 200 mg/kg)	5 (25)	0 (0)[a]	5 (33.3)[a]
Group 4 (RCN 400 mg/kg)	5 (25)	0 (0)[a]	5 (33.3)[a]

[a]: the distilled water group was statistically significant compared with the treatment groups {χ^2 (3, 200) = 20.00, p = 0.000}, std residuals >1.96}. DW: Distilled Water, RCN: Ricinine-enriched extract

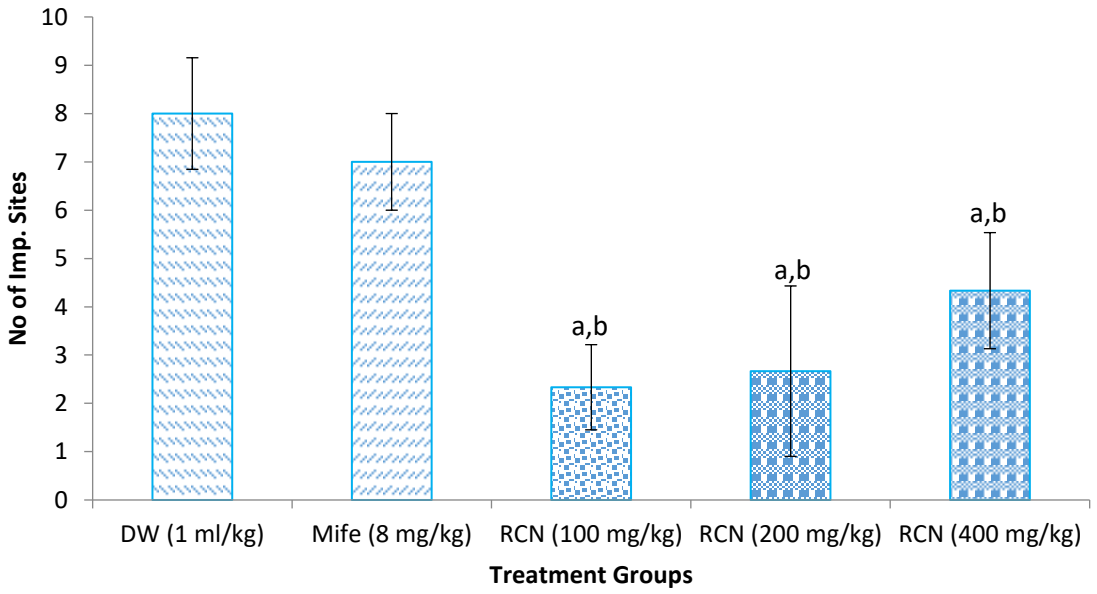

Figure 1: Effect of Ricinine-enriched extract on number of implantation sites in female Wistar rats
Results presented as mean ± SEM using one-way ANOVA. a= statistically significant (p<0.05) compared to DW; b= statistically significant (p<0.05) compared to Mife; DW: Distilled Water, Mife: Mifepristone, RCN: Ricinine-enriched extract

Abortifacient effect of RCN in female Wistar rats

Effect of RCN on number of fetuses (survival ratio) in female Wistar rats

The result shows a statistically significant (p<0.05) decrease in the number of fetuses in the Mifepristone 8 mg/kg (standard drug) (0.00 ±1.00) and RCN 100, 200 and 400 mg/kg (2.00±2.00, 0.00±00 and 0.00±0.00 respectively) treatment groups when compared with the distilled water group (7.00±1.00). There was no statistically significant (p<0.05) difference between the Mifepristone group (0.00±0.00) and the RCN 100, 200 and 400 mg/kg (2.00±2.00, 0.00±00 and 0.00±0.00 respectively) treatment groups (Table 6).

Effect of RCN on number of resorption sites in female Wistar rats

The result shows a statistically significant (p<0.05) increase in the number of resorption sites of Mifepristone 8 mg/kg (standard drug) (8.67 ±0.67) and RCN 200 and 400 mg/kg (9.33±0.67 and 9.67±1.20 respectively) treatment groups when compared with the distilled water group (0.00±0.00). However, no statistical significance (p<0.05) was observed between the distilled water group (0.00±0.00) and RCN 100 mg/kg (4.67±2.40). There was no statistically significant (p<0.05) difference between the Mifepristone group (8.67 ±0.67) and the RCN 100, 200 and 400 mg/kg (4.67±2.40, 9.33±0.67 and 9.67±1.20 respectively) treatment groups (Table 7).

Table 6: Effect of RCN on number of fetuses in female Wistar rats

Groups	No. of fetuses
DW (1 ml/kg)	7.00±1.00
Mife (8 mg/kg)	0.00±0.00[a]
RCN (100 mg/kg)	2.00±2.00[a]
RCN (200 mg/kg)	0.00±0.00[a]
RCN (400 mg/kg)	0.00±0.00[a]

Results presented as mean ± SEM using one-way ANOVA. a= statistically significant when compared with DW group at p<0.05. DW: Distilled Water, Mife: Mifepristone, RCN: Ricinine-enriched extract

Table 7: Effects of RCN on number of resorption sites in female Wistar rats

Groups	No. of resorption sites
DW (1 ml/kg)	0.00±0.00
Mife (8 mg/kg)	8.67±0.67[a]
RCN (100 mg/kg)	4.67±2.40
RCN (200 mg/kg)	9.33±0.67[a]
RCN (400 mg/kg)	9.67±1.20[a]

Results presented as mean ± SEM using one-way ANOVA. a= statistically significant when compared with DW group at p<0.05. DW: Distilled Water, Mife: Mifepristone, RCN: Ricinine-enriched extract

DISCUSSIONS

The fertility test result showed a total absence of pregnancy in all the animals in the treatment groups. Nazar (2019) had a similar result of significant decrease in percentage fertility in female rabbits treated with *Ricinus* extract. Also, Raji *et al.* (2010) and Kaur *et al.* (2022) had similar results which they related to the decreased estradiol levels which act to down regulate the brain's hypothalamic mechanisms. The result for the anti-implantation study shows a significant, dose-dependent reduction in uterine implantation sites. This suggests that RCN interferes with early uterine events necessary for nidation. This agrees with the result by Sani & Sule (2007), where they observed that the *R. communis* extract induced anti-implantation in mice subcutaneously treated with a dose of 200 mg/kg body weight once and before mating. Implantation requires a well-coordinated interplay between embryo and maternal endometrium, mediated by precise hormonal balance (mainly estrogen and progesterone), uterine receptivity, and molecular signals (e.g., cytokines, adhesion molecules, growth factors) (Megha *et al.,* 2023). The presence of bioactive alkaloids (like

ricinine), steroids and phenolics in *R. communis* may disrupt endocrine signalling, uterine differentiation, or endometrial receptivity (Sani & Sule, 2007). Phytosterols or plant-derived steroidal compounds may bind to estrogen or progesterone receptors, acting as antagonists or partial agonists, thereby altering the uterine milieu. Similar mechanisms have been proposed for other plant-derived antifertility agents (Bhatt & Deshpande, 2021).

Abortifacient effects: In rats treated during gestational days 8–15, RCN caused substantial fetal loss and increased resorption sites. This suggests that, in addition to preventing implantation, RCN may impair maintenance of early pregnancy. Potential mechanisms include:

Hormonal disruption: RCN components might reduce circulating progesterone or disturb the estrogen/progesterone ratio, which is critical for sustaining pregnancy and maintaining uterine quiescence.

Uterine toxicity or decidual destabilization: Alkaloids or other constituents may exert cytotoxic effects on decidual/uterine tissue, leading to degeneration of embryo-supportive structures and subsequent resorption.

Interference with placental or embryonic development: RCN may impair early placentation, vascularization, or nutrient exchange, causing embryo death.

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CONCLUSION

This study demonstrates, for the first time to our knowledge, that a ricinine-enriched extract of *R. communis* exerts potent antifertility effects in female Wistar rats, characterized by complete prevention of pregnancy, a dose-dependent reduction in implantation sites, and increased fetal resorption when administered during gestation. These findings provide strong experimental support for the traditional use of castor bean preparations as contraceptives. Given the clear anti-implantation and abortifacient activities, *R. communis* (ricinine) has potential as a basis for developing herbal contraceptive agents. However, before any consideration for human application, further research is needed to elucidate the precise mechanism(s) of action, evaluate long-term safety, define the minimal effective and non-toxic doses, and assess reversibility.

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Author's contribution

BU and IGB contributed to the study design. All authors participated in the data collection. BU and IMM participated in data analysis and manuscript preparation. All authors collaborated in drafting the initial manuscript and provided critical revisions. IGB and FAD reviewed and approved the final version of the manuscript.

Competing interest

There is no conflict of interest between the authors.

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