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·*Complementary Medicine*·

Effect of methanol extract of *Ricinus communis* seed on reproduction of male rats

Yinusa Raji, Ahmed Kolade Oloyo, Ayodele Olufemi Morakinyo

Department of Physiology, College of Medicine, University of Ibadan, Ibadan 23 402, Nigeria

Abstract

Aim: To investigate the effect of methanol extract of *Ricinus communis* seed (RCE) on male rats reproductive functions. **Methods:** Thirty-two male albino rats were divided into four groups. Groups 1, 2 and 3 were gavaged with 0.2 mL of 2.5 % tween 80 (RCE vehicle; control) or 20 mg/(kg·d) and 40 mg/(kg·d) of RCE, respectively, for 30 days, and group 4 was also gavaged with 40 mg/(kg·d) of RCE, but was allowed a recovery periold of 30 days. Five untreated female rats were cohabited with male rats in each group from day 25 of RCE treatment for 5 days, except group 4, where cohabitation began on day 25 of the recovery period. All male rats were sacrificed 24 h after the experiments. The female rats were laparatomized on day 19 of pregnancy and the number and weight of litters were recorded. **Results:** There was a significant decrease ($P < 0.01$) in the weight of the reproductive organs, sperm functions and serum levels of testosterone in RCE treated rats. There was disorganization in the cytoarchitecture of the testes, disruption of the seminiferous tubules and erosion of the germinal epithelium. The number and weight of litters of rats in groups 2 and 4 decreased significantly ($P < 0.05$) but no changes were observed in group 3. RCE caused no changes in liver, kidney, heart or body weights in male rats. **Conclusion:** RCE has a reversible negative impact on male reproductive functions, which appears to be mediated via gonadal disruption in testosterone secretion. *(Asian J Androl 2006 Jan; 8: 115–121)*

Keywords: *Ricinus communis*; sperm; fertility; testosterone; reproduction

1 Introduction

Ricinus communis (Linn), commonly called castor bean, belongs to the family *Euphorbiaceae*. Different parts of the plant have been reported to have several medicinal values. Its seed has antihelmintic, carthartic, emollient, laxative and purgative properties [1]. A decoction of the leaves and roots of *R. communis* plant has antitussive, discutient (disperses tumors), and expectorant activities [2]. The oil from the seed of *R. communis* plant was used in inducing labor at term [3, 4]. Banderjee *et al.* [5] reported that the petroleum ether extract of roots of *R. communis* had an anti-inflammatory effect against carrageenin-, serotonin-, dextran- and bradykinin-induced hind paw edema in rats, the efficacy of which was comparable to that of standard drug prototypes of non-steroidal anti-inflammatory agents. The methanol extract of *R. communis* seed was found to prevent implantation and when implantation occurred, it induced abortion in female guinea pigs [6]. It has also

Correspondence to: Dr Yinusa Raji, Department of Physiology, College of Medicine, University of Ibadan, Ibadan 23 402, Nigeria.
Tel: +234-80-2326-3626, Fax: +234-80-2241-1768
E-mail: raji_ui@yahoo.com, yoraji@yahoo.com
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been shown to prolong the estrous cycle with a marked effect on the diestrous phase [6].

The efficacy of *R. communis* seed as a contraceptive in women and female rodents have been widely reported. A single oral dose of 2.3 g – 2.5 g of the seed prevented conception for a period of 12 months in women volunteers [7] and experimental rodents [8]. Earlier, Okwuasaba *et al.* [9] had reported the anticonceptive and estrogenic effect of the methanol extract of *R. communis* seed in female rats. The antiovulatory and anticonception properties of the methanol extract of *R. communis* seed were achieved by its direct effect on the ovarian tissue and presumably by interfering with the hypothalamic-pituitary-ovarian axis in Sprague–Dawley rats [10].

Many studies have shown that the contraceptive efficacy of *R. communis* were exerted through its estrogenic activity [7–10]. A derangement in the serum level of estrogen as a consequence of alteration in its secretion and release was expected to have adverse effects on the normal reproductive functions in the female. Estrogen is a steroid hormone synthesized from cholesterol along the same biosynthetic pathway as male androgens. The rate-limiting step in steroidogenesis is the action of the cytochrome P-450-side chain cleavage enzyme, needed to convert cholesterol to pregnenolone. Other steroid hormones are then formed in step-wise reactions from pregnenolone. Therefore, if the estrogenic effect of *R. communis* was exerted via the steroidogenic pathway, *R. communis* could affect the biosynthesis and release of male androgens, which may in turn impact on male reproductive functions. Although there were many reports on the effects of *R. communis* on female reproductive functions, its effects on male reproductive functions have not been reported. The present study was therefore designed to investigate the impacts of the methanol extract of *R. communis* seed on the male reproductive functions such as sperm function, serum testosterone levels, fertility and histology of the testis.

2 Materials and methods

2.1 Plant materials

The fruits of *R. communis* were collected at the Forest Research Institute of Nigeria (FRIN) in February 2003. The specimen was examined and identified by Mr Usang Felix of the institute, where a voucher specimen number FHI 106 878 was deposited. The fruits with its thorny coat were air-dried, and the seeds were separated from the coats by peeling. The seeds were grounded into powder which was then subjected to Soxhlet extraction. The extract obtained was separated from the solvent by distillation. For the extraction process, 736 g of the mashed seeds were used, which yielded 274 g of oily mass (37.23 % yields); this was then stored in a refrigerator for the study. Fresh solution of the extract was prepared in 2.5 % Tween 80 (Burgoyne, Burbidges & Co., Mumbai, India) when required.

2.2 Animals

A total number of 52 young adult rats (32 male and 20 female rats) whose average weight ranged between 150 g and 170 g (2–2.5 months old) were used for the study. The animals were obtained from the Central Animal House, College of Medicine, University of Ibadan, Ibadan, Nigeria. They were housed in steel cages, and maintained under standard conditions (12 h light/12 h dark). The animals were allowed to acclimatize in the laboratory for a period of 2 weeks before the commencement of the study; feed (Ladokun Feeds Nig. Ltd., Ibadan, Nigeria) and water were provided *ad libitum*. The male rats were certified fertile by isolated mating technique while the female rats were certified to have regular estrous cycles by vaginal smears, before inclusion in the study. All animals were weighed daily, throughout the study.

2.3 Experimental design

Thirty-two male albino rats of proven fertility were divided into four groups of eight rats each. Group 1 was the control and contained rats that were gavaged daily with equal amounts of 2.5 % Tween 80 in normal saline (0.2 mL) for 30 days. Tween 80 in normal saline (0.2 mL) was the vehicle for methanol extract of *R. communis* seed (RCE). Rats in group 2 were gavaged daily with 20 mg/kg of RCE for 30 days, while group 3 received 40 mg/kg of RCE daily for 30 days. Group 4 was the recovery group and contained rats that were gavaged with 40 mg/kg of RCE daily for 30 days, but allowed to recover from the possible effects of the extract for 30 days after the withdrawal of the extract.

2.4 Fertility study

A total of 20 untreated, fertile, prestrous female rats were used for the fertility test. Five untreated female rats were cohabited with each of the four male RCE treated groups from day 25 of treatment except group 4

whose cohabitation commenced on day 25 of the recovery period. All animals were cohabited for 5 days according to Gupta *et al.* [11]. The presence of a vaginal plug was accepted as the index for positive mating and the day of its appearance was recorded as day 1 of pregnancy. A fertility test was calculated using the following formula.

% Fertility Success = Pregnant Females × 100/Mated Females

On day 19 of pregnancy, all female rats were laparatomized. The number of fetuses and their body weights were determined.

2.5 Organ and blood sample collection

On day 31 of the experiment, all the male rats in groups 1, 2 and 3 were killed, while rats in group 4 were sacrificed on day 61 of the study. Blood sample from each rat was collected via cardiac puncture into a sterilized sample bottle and was allowed to clot at room temperature. The clot was retracted and sample centrifuged at 2 647 × *g* for 15 min and the serum separated. The serum samples were stored frozen at –20 °C. The heart, liver, kidney, testes, seminal vesicle, prostate glands and epididymes were carefully removed, cleared of adherent tissues and weighed immediately.

2.6 Sperm collection and analyses

Each testis was removed along with its epididymis. The epididymis was carefully separated from the testis and the cauda severed from its remaining part. The cauda was quickly transferred to a pre-warmed slide (27 °C) and lacerated with a razor. Sperm characteristics analyses were done as previously described [12].

Progressive motility was tested immediately. The semen was squeezed onto the microscope slide and two drops of warm 2.9 % sodium citrate was added. This was then covered with a cover slip, examined and scored under the microscope using the ×40 objective of the microscope. A viability study (percentage of live spermatozoa) was done using the eosin/nigrosin stain. Semen was squeezed onto a microscope slide and two drops of the stain was added. The motile (live) sperm cells were stained. The stained and the unstained sperm cells were counted using ×40 objectives of the microscope and an average for each was taken from which percentage viability was calculated. Sperm morphology was done by staining the sperm smears on microscope slides with two drops of Walls and Ewas stain and air-dried. The slides were examined under the microscope using ×100 objectives under oil immersion. The abnormal sperm cells were counted and the percentage calculated. The epididymis was immersed in 5 mL normal saline in a measuring cylinder and the volume of fluid displaced was taken as the volume of the epididymis. Sperm count was done under a microscope with the aid of the improved Neubauer hemocytometer. Count was done in five large Thoma square and adjustment was made for volume of the normal saline added.

2.7 Testosterone assay procedure

An enzyme-based immunoassay (EIA) system was used to measure testosterone level in serum samples collected. The EIA kit was obtained from Immunometrics (London, UK), and contained a testosterone EIA enzyme label, testosterone EIA substrate reagent and EIA quality control sample. A quality control was carried out at the beginning and the end of the assay to ascertain the acceptability with respect to bias and within batch variation. The EIA kit used had a sensitivity level of approximately 0.3 nmo/L (0.l g/mL) of testosterone. The intra- and inter-assay variations were 10.02 % and 10.12 %, respectively.

2.8 Histological study

This was carried out according to the instructions detailed in a previous study [12]. The testes from each rat were fixed in 10 % formalin, so as to preserve the various constituents in their normal micro-anatomical positions and prevent them from autolytic changes. A thin section (0.05-mm thick) of the tissue was made. The section was stained with hematoxylin-eosin dye. Each slide was clean-blotted and mounted in Canada balsam under a cover slip. A photomicrograph of the slide preparation was taken after examination under the microscope.

2.9 Statistical analysis

Data were expressed as mean ± SEM. Statistical significance between the various groups was determined using unpaired *t*-test and ANOVA.

3 Results

3.1 Effects of RCE on body weight of rats

There were no significant differences ($P > 0.05$) in the mean body weight of RCE treated rats, before and during the treatment period compared with the vehicle-treated control group. However, there was a significant weight gain of 20.76 %, 21.11 %, 19.66 % and 20.68 %

in groups 1 (control), 2, 3 and 4 (recovery group), respectively, when the final weight of each group after the treatment was compared with the initial weight before the treatment (Table 1). *R. communis* seed extract did not affect normal increases in body weight.

3.2 Effects of RCE on organ weight of rats

There were no significant differences ($P > 0.05$) in mean weight of the heart, kidney and liver in RCE-treated groups when compared with the control group. However, the mean weights of the testis, epididymis, prostate gland and seminal vesicle of the RCE-treated rats were significantly reduced ($P < 0.05$) when compared with those of the control group. There was a significant recovery ($P < 0.05$) in the mean weight of these organs in the recovery group (Table 2).

3.3 Effects of RCE on sperm functions in rats

There was a significant decrease ($P < 0.01$) in sperm count, motility and viability of RCE-treated groups in a dose-dependent manner when compared with the vehicle-treated control group. However, there was a significant recovery ($P < 0.01$) in the sperm count, motility and viability of the recovery group towards the vehicle-treated control group. There was also a dose-dependent and significant decrease ($P < 0.01$) in the number of normal spermatozoa in RCE-treated groups when compared with the control group. However, there was a significant recovery ($P < 0.01$) in the number of normal spermatozoa in the recovery group towards the vehicle-treated control group (Table 3). More than 90 % of the morphological abnormalities in the spermatozoa of RCE-treated rats were of secondary forms: bent tail, curved mid piece, bent mid piece and headless tail.

Furthermore, the epididymal volume significantly decreased in RCE-treated rats when compared with the control. However,epididymal volume returned to normal control value in the recovery group (Table 4).

3.4 Effects of RCE on serum levels of testosterone in rats

There was a significant decrease ($P < 0.01$) in serum levels of testosterone of the RCE-treated group when compared with the vehicle-treated control group. However,

Table 1. Body weights of male rats treated with methanol extract of *R. communis* seed (RCE).

Groups (n = 8)	Body weight (g)				
	Before treatment	Week 1	Week 2	Week 3	Week 4
1 (control)	159.60 ± 5.32	182.85 ± 6.67	189.80 ± 7.59	191.13 ± 8.85	207.18 ± 8.32
2 (20 mg/kg RCE)	160.29 ± 9.76	181.08 ± 10.77	193.09 ± 12.23	196.07 ± 10.71	207.63 ± 10.69
3 (40 mg/kg RCE)	162.86 ± 11.01	183.44 ± 11.77	192.37 ± 11.72	196.07 ± 10.91	207.63 ± 6.95
4 (40 mg/kg RCE, recovery)	158.29 ± 6.79	180.73 ± 8.73	187.25 ± 8.45	191.19 ± 7.56	204.96 ± 6.95

Table 2. Organ weights of male rats treated with methanol extract of *R. communis* seed (RCE). $^{b}P < 0.05$, compared with the corresponding controls.

Groups (n = 8)	Heart (g)	Kidney (g)	Liver (g)	Testis (g)	Epididymis (g)	Seminal vesicle (g)	Prostate gland (g)
1 (control)	0.66 ± 0.02	0.82 ± 0.05	7.42 ± 0.30	1.23 ± 0.07	0.43 ± 0.02	0.63 ± 0.08	0.38 ± 0.01
2 (20 mg/kg RCE)	0.65 ± 0.03	0.83 ± 0.06	7.46 ± 0.20	1.08 ± 0.06^{b}	0.36 ± 0.03^{b}	0.56 ± 0.10^{b}	0.33 ± 0.03^{b}
3 (40 mg/kg RCE)	0.64 ± 0.30	0.84 ± 0.06	7.50 ± 0.36	1.03 ± 0.08^{b}	0.31 ± 0.03^{b}	0.51 ± 0.02^{b}	0.30 ± 0.02^{b}
4 (40 mg/kg RCE, recovery)	0.66 ± 0.02	0.79 ± 0.03	7.40 ± 0.29	1.13 ± 0.06	0.37 ± 0.05	0.53 ± 0.05	0.35 ± 0.02

Table 3. Sperm function of rats treated with RCE. $^{b}P < 0.05$, $^{c}P < 0.01$, compared with the corresponding controls.

Groups (n = 8)	Progressive motility (%)	Live spermatozoa (%)	Total sperm count ($\times 10^{6}$/ mL)	Epididymal volume (mL)	Abnormal sperm morphology (%)
1 (control)	92.00 ± 1.05	96.50 ± 0.67	101.3 ± 5.37	5.19 ± 0.01	3.17 ± 0.25
2 (20 mg/kg RCE)	74.25 ± 2.02^{c}	82.86 ± 1.00^{c}	77.00 ± 2.10^{c}	4.14 ± 0.02^{b}	4.36 ± 0.09
3 (40 mg/kg RCE)	57.44 ± 1.85^{c}	77.80 ± 3.08^{c}	62.57 ± 4.75^{c}	4.10 ± 0.02^{b}	5.57 ± 0.32
4 (40 mg/kg RCE, recovery)	81.30 ± 4.10	85.50 ± 1.71	80.12 ± 3.02	5.16 ± 0.02	3.63 ± 0.14

Table 4. Serum testosterone levels of male albino rats treated with methanol extract of *Ricinus communis* seed (RCE). $^{c}P < 0.01$, compared with the control group; $^{f}P < 0.01$, compared with groups 2 and 3.

Groups (n = 8)	Testosterone (nmol/mL)
1 (control)	5.74 ± 1.42
2 (20 mg/kg RCE)	3.35 ± 0.9^{c}
3 (40 mg/kg RCE)	1.23 ± 0.68^{c}
4 (40 mg/kg RCE, recovery)	4.15 ± 1.14^{f}

Table 5. Fertility test in male albino rats treated with methanol extract of *Ricinus communis* seed (RCE). $^{b}P < 0.05$, compared with the corresponding controls.

Groups (n = 5) (female rats)	Litter number	Litter weight (g)	Fertility %
1 (control)	8 ± 1.00	5.5 ± 0.05	80
2 (20 mg/kg RCE)	4 ± 0.00^{b}	4.6 ± 0.08^{b}	20
3 (40 mg/kg RCE)	0.00^{b}	0.00^{b}	0
4 (40 mg/kg RCE, recovery)	5 ± 1.00^{b}	5.15 ± 0.07^{b}	40

there was a significant increase ($P < 0.01$) in serum levels of testosterone in the recovery group when compared with groups 2 and 3 (Table 4).

3.5 Effects of RCE on litter number, litter weight and percentage fertility success

There was a significant decrease ($P < 0.05$) in the number and weight of litters in group 2 when compared with the vehicle-treated group. Female rats mated with male rats from group 3 did not conceive throughout the duration of the study. Although there was a significant decrease ($P < 0.05$) in the number and weight of litters in group 4 (recovery), these parameters had higher values than those of the group 2 and 3. Four out of the five female rats cohabited with male rats from the control groups conceived (Table 5).

3.6 Effects of RCE on histology of the testis in rats

There was a disruption in the seminiferous tubule cytoarchitecture in the testis of RCE-treated rats when compared with that of the control (Figure 1A, B, C). The severity of germinal epithelium and cell erosion, and the disruption of the interstitium were dose-dependent. However, there was regeneration of the germinal epithelium and restructuring of the interstitium towards normal in the recovery rats in group 4 (Figure1D).

4 Discussion

The results of the present study suggested that RCE have a deleterious effect on male reproductive functions in rats, sufficient to cause reversible infertility in the male rats. Previous studies [6–10] suggested that *R. communis* seed extract had estrogenic activities, which may reside in its steroid components [10]. It was possible that decreased testosterone levels alone caused the decrease in testis, epididymis, seminal vesicle and prostate gland weights. Therefore part of the estrogenic activities of the *R. communis* seed extract was inhibition of pituitary gonadotrophins secretion and release. Gonadal steroid was the most potent negative feedback for pituitary gonadotrophins secretion and release [13].

The present study showed for the first time that *R. communis* seed extract impair reproductive activities in male rats possibly by decreasing testosterone secretion. Testosterone was necessary for the development, growth and normal functioning of the testes and male accessory reproductive glands [14, 15]. Low serum testosterone levels have been reported to adversely affect the structure, weight and functions of the testes [14], epididymis [14] and prostate gland [15]. The reduction in weight of the testis, epididymis, seminal vesicle and prostate gland could be associated with the decrease in serum levels of testosterone in the RCE treated rats. A major reproductive role for testosterone involved development of the sperm cell and maintenance of normal testosterone levels was essential for this development [16]. A derangement in the serum level of testosterone in a RCE-treated rat could have affected these reproductive functions.

The decrease in sperm functions of the RCE-treated rat supported the dose-dependent reduction in serum testosterone levels and its consequent effects on the testis, epididymis and the male accessory reproductive organs. Epididymis normally provided a favorable *milieu* for acquisition of fertilizing ability and viability of spermatozoa [16, 17]. Reduction in the activities of the epididymis probably led to a decrease in progressive motility of sperm in RCE-treated rats. The highest abnormalities in the sperm morphology of the extract-treated rats were of the secondary form (bent tail, curved mid-piece, bent mid-piece and tailless head), which were associated with epididy-

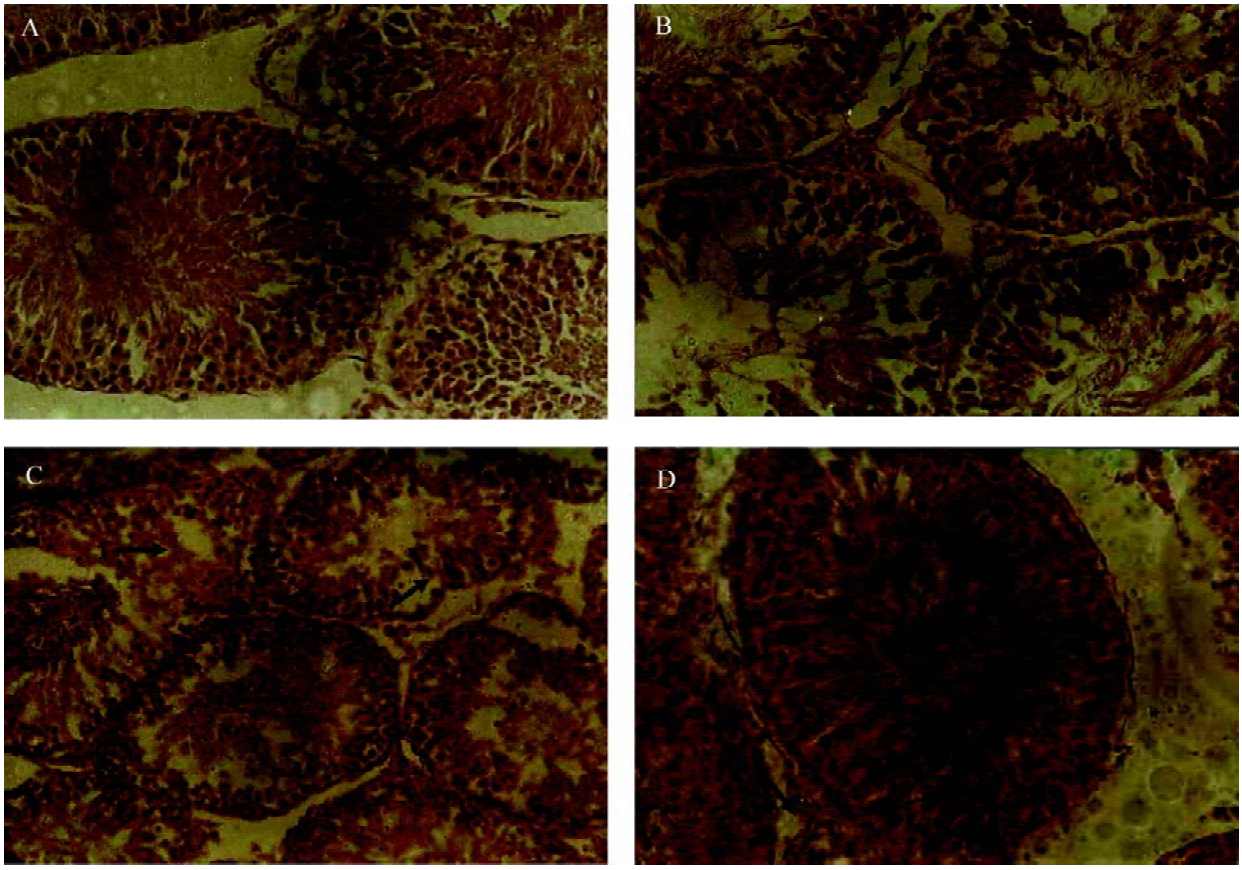

Figure 1. The photomicrographs of the testis of control (group1) and RCE-treated rats (groups 2–4). (A): arrows 1 and 2 show the normal germ cell and Leydig cell population and the seminiferous tubule interstitium, respectively (group 1, control); (B) the eroded germ cell and Leydig cell population and the disrupted seminiferous tubule, respectively (group 2, 20 mg/kg RCE-treated rat); (C) greater erosion of germ cell and Leydig cell population and the disruption of the seminiferous tubule interstitium, respectively (group 3, 40 mg/kg RCE-treated rat); and (D) regeneration of the germ cell and Leydig cell population and the reorganization of the interstitium, respectively (group 4, recovery rats). Magnification, ×40.

mal functions of transportation, maturation and storage of sperm cell during which period the spermatozoa develop motility [16]. Semen qualities were often used as a measure of sperm production, testicular function and/or male fertility. Low sperm count and motility and high percentage abnormal spermatozoa level each have been associated with reduced fertility [18].

Reduction in the total number of epididymal sperm count in RCE-treated rats could also be a result of disruption of seminiferous tubules as observed in the histological section of the testis. Unless other circumstances are at play (e.g. tumors, edema and inflammation), there was a strong correlation between the weight of the testis and the number of germ cells presented in the testis [18]. The arrest of spermatogenesis possibly occurred as a consequence of reduction in serum levels of testosterone, which had been shown to be essential for the completion of meiotic division during spermatogenesis [19, 20]. The disruption of testicular cytoarchitecture by RCE could have adversely affected Leydig cell number and function and probably led to a decrease in the serum levels of testosterone. Furthermore, regulation of testicular secretion occurred via a negative feedback system involving the hypothalamic-pituitary-testicular axis. The hypothalamus released pulses of gonadotrophin releasing hormone, which stimulated the anterior pituitary to secrete and release luteinizing hormone (LH) and follicle stimulating hormone (FSH), in a pulsatile manner. LH acted on the Leydig cell to activate the synthesis of pregnenolone from cholesterol. Synthesized testosterone had

a paracrine effect on the Sertoli cells, where it played an essential role in the facilitation of spermatogenesis. Disruption of this pathway could deprive the Leydig cells of LH, and its stimulatory action leading to reduction in the secretion and release of testosterone. Noteworthy was the significant increase in the serum level of testosterone in the recovery group, following the withdrawal of the extract. This could probably be due to release of the inhibitory impact of RCE on the pituitary gonadotrophin secretion and release as the rats recover from the effects of the extract. After withdrawal of the extract for a period of 30 days, the mean weight of testis, epididymis, prostate gland, the mean volume of epididymis, sperm count, motility, viability, morphology, serum levels of testosterone and the cytoarchitecture of the RCE-treated male rats were similar to those of the vehicle-treated control group, which suggested that the impacts of *R. communis* seed extract on male reproductive functions were reversible. At the dose employed no mortality was recorded and there was no adverse effect on the mean weight of the liver, kidney, heart and the body weight which was consistent with previous studies [7, 8].

The active principle in RCE with these antisteroidogenic and antifertility activities was not known. Phytochemical analysis of the extract revealed that the major component of *R. communis* seed extract is ricinoleic acid (12-hydrixy-(cis)-9-octadecenoic acid). The use of ricinoleic acid as contraceptive jelly in folk medicine had earlier been reported [1]. It was therefore possible that ricinoleic acid had a spermicidal capacity, and probably exerted this effect on spermatozoa in the testis and epididymis via inhibition of steroidogenesis. In conclusion, the results of the present study suggested that RCE possesses reversible antifertility and anti-androgenic properties. The mechanism involved in these activities may reside in the hypothalamic-pituitary-gonadal axis. *R. communis* seed extract has the potential to be developed into a male contraceptive agent.

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