THE EFFECT OF *RICINUS COMMUNIS-LINN* (RICOM 1013-J) ON SEMEN PARAMETERS: A COMPARATIVE STUDY

Ekwere Okon Ekwere[1]* Rosaleen Thecla McNeil[2] Francis Kanayo Okwuasaba[3]

[1]*Department of Anatomy, University of Jos, PMB 2084, Jos, Plateau State, Nigeria
[2]School of Anatomical Sciences, University of the Witwatersrand, 7 York Road Parktown 193, Johannesburg,
[3]Department of Pharmacology, Faculty of Pharmaceutical Sciences. University of Jos, PMB 2084, Jos, Plateau State, Nigeria

ABSTRACT

Objectives: The seed variety of *Ricinus communis-linn* (RICOM 1013-J) is a popular contraceptive agent among the *Rukuba* women of Central Nigeria. The aim of this study is to compare the effect of RICOM 1013-J to that of an anti-androgen, diane-35 (which contains 0.035g cyproterone acetate and 2mg of ethinyl oestradiol), on testicular histology and function.

Material and Methods: Adult male Wistar rats weighing 200-250g were used for this experiment. The animals were administered orally with 0.41mg/kg of diane-35, RICOM 1013-J (10mg/kg) and10mg/kg of normal physiological saline, for the control group. The rats were then weighed weekly for six weeks.

Results and Conclusions: The semen parameters measured showed marked suppression of testicular function in the groups treated with RICOM 1013-J and diane-35, when compared to that of the control. It can therefore be concluded that RICOM 1013-J suppresses testicular histology and function and is a potential male contraceptive agent.

Keywords: *Ricinus communis-linn*, RICOM 1013-J, castor plant, Diane35, semen analysis

1. INTRODUCTION

RICOM 1013-J

The term *'Ricinus*' is a Latin word for 'tick' which describes the shape of castor seed. Castor plant is commonly known for its oil yield and the term 'castor' which means 'beaver' (Latin), seems to have come from the word 'castoreum', a perfume base made from the dried perineal glands of the beaver. *Ricinus communis-linn* is also known as castor bean and is a plant species of the *Euphorbiaceae* [1], the sole member of the genus r*icinus* and of the subtribe r*icininae*. The plant has been used for wound healing and as a cure for various ailment. This has earned it the name, *Palma Christi* (Palm of Christ), [2]

DIANE-35

Diane-35 is a popular oral contraceptive agent among young women, [3] Functionally, it is an anti- testosterone, each tablet containing 2mg cryproterone acetate and 0.035mg ethinyl oestradiol. The cryproterone acetate component blocks the effect of endogenously produced and exogenously administered androgens at the target organs by means of competitive inhibition. It also has a progestational action, [4] [3]. Diane-35 is also used to induce fertility in poor ovarian conditions such as polycystic ovary syndrome and other medical conditions particularly, acne.[5],Hwang *et al.*,[6]. Combined use of metformin and diane-35 has been recognized as a good choice of drug for polycystic ovary syndrome since they both cause decrease in the serum level of testosterone and free androgen index However, safety concerns have been expressed concerning the use of diane-35 [7],[8].

The current interest in RICOM 1013-J lies in its anti-conceptive property, a relatively new aspect that has been confirmed by many researchers Osunkwo *et al.,* [9]; Okwuasaba *et al.,* [10] Okwuasaba *et al,* [11] ; McNeil *et al.,* [12]. It has been reported that the methanolic extract of the seed has significant anti-fertility property in adult rats (Okwuasaba *et al.,* [11]. It suppresses ovarian function and hence the number of ova released at oestrous, McNeil *et al.,* [12]. Among the Rukuba tribe in Bassa local government area of Plateau State (Nigeria), the use of the seeds for contraception is a widespread practice (Osunkwo *et al.,* [9];Okwuasaba *et al.*,[10] Okwuasaba *et al.*,[11], Kabele-Toge et al, [13] . It is known that 2-3 seeds protected against conception for a whole year. Okwuasaba *et al.,* [11] Kabele-Toge et al, [13] Some other researchers have also reported on its anti-fertility properties (Sani and Sule**,**

[14] Yi-ling Hou et al,[15], [16] . RICOM 1013-J contains a characteristic set of more than100 different polypeptides against which a complex of antiserum has been raised [1]. This study is aimed at determining the effect of RICOM 1013-J on testicular histology and function and comparing with that of an anti-androgen, diane-35.

MATERIALS AND METHODS

This experiment was carried out at the Experimental animal house Unit of the University of Jos, Jos, Nigeria.

COLLECTION AND IDENTIFICATION OF PLANTS

The seeds of RICOM 1013-J were collected from the wild in Jos metropolis and supplied by Dr. Oyhu Azija – the consultant traditional practitioner attached to the Department of Pharmacology, University of Jos. They were identified and authenticated at the Departments of Botany, University of Lagos and Ahmadu Bello University, Zaria and Forestry Research Institute, Jos as described by Okwuasaba et al,[11] with specimen vouchers deposited at the herbarium of the Department of Anatomy, Faculty of Medical Sciences, University of Jos.

EXTRACTION PROCEDURES

The dried seeds were finely ground and a weighed portion (100g) was subjected to exhaustive soxlet extraction in 350ml of n-hexane for 72h at 30 ^{o}C. The extract was concentrated in water bath at 59.0 ±1 ^{o}C until a constant dark, sticky residue was achieved. The mean yield and percentage yield of the extract was calculated. The extract was then kept at -4 ^{o}C in the refrigerator until when required.

ANIMAL GROUPINGS

Fifteen adult male Wistar albino rats (200-250g) were used for the experiment and divided into 3 groups (n=5). Food and water was provided *ad libitum*.

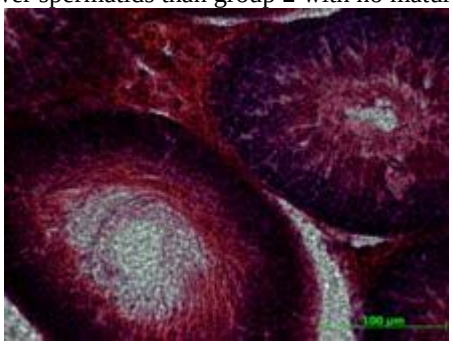
Group 1 received 0.41mg/kg of diane-35; Group 2, 10mg/kg of RICOM 1013-J and Group 3 (control) administered with 10mg/kg normal saline respectively.

The drugs were administered orally as a single dose and the rats were weighed once weekly for six weeks. They were sacrificed on the 7th week under chloroform anaesthesia. The caudal epididymis was excised; multiple fenestrations were made in each one using the tip of a new scalpel blade and were then placed in 1ml of physiological saline for semen analysis.

RESULTS

A *Histology*

Group 1 (Diane-35): The group treated with Diane-35 also showed normal testicular structure but the seminiferous tubules contained fewer spermatids than group 2 with no mature spermatozoa found.



× 100

Group 2: The testicular tissues in the group treated with RICOM 1013-J showed normal histological appearance under light microscopy. The seminiferous tubules showed active meiotic division as indicated by the numerous primary spermatocytes. The lumen of the tubules also contained spermatids but no mature spermatozoa.

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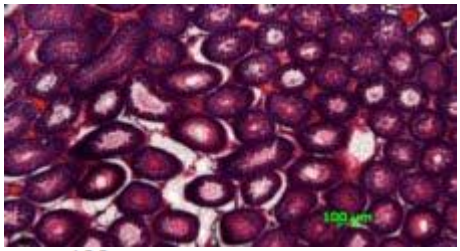

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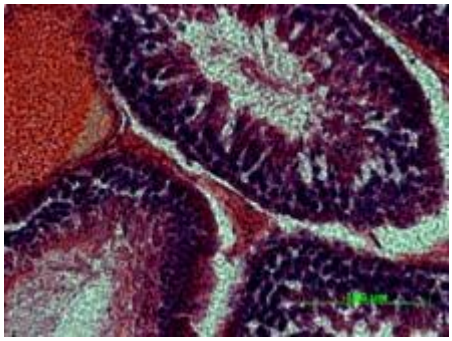
Group 3 (Control): Testicular tissues from the control group showed histological appearance with numerous seminiferous tubules containing sperms cells at all stages of spermatogenesis; there were more primary spermatocytes and spermatids than the specimens from Group 1 and 2.



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OLYMPUS model XSZ107BN Optical microscope was used in all cases to read the slides

B. Semen Analysis

The morphology of the sperm cells in both treated and control groups had 100% normal forms. These mean motility were 11%± and 15%± in groups 1 and 2 respectively, whereas the mean sperm count was grossly reduced (38.6 ±) and (25 ±) in groups 1 and 2 respectively compared with the control (80%).

Table Showing Effect Of Diane-35 And Ricom 1013-J On Semen Parameters

GROUP	**% Normal Morphology**	**Mean Liquefaction**	**Mean Consistency**	**% Mean Motility** ±SEM	**Mean Count** (x106/ml) ±SEM
(1) DIANE 35	100	Normal	Normal	55	25
(2) RICINUS COMMUNIS	100	Normal	Normal	11	38.6
(3) CONTROL	100	Normal	Normal	82	30

2. DISCUSSION

This study however revealed that the semen parameters in the treated groups showed marked suppression of testicular function in both groups when compared with control. There was no change in cell morphology in both the treated groups. The lowest sperm motility and highest count was observed in the group treated with extract of

RICOM 1013-J. This suggests that the effect of RICOM 1013-J on testicular tissue is reversible. Recovery which can be inferred by the presence of immature spermatocytes (primary spermatocytes and spermatids) occurred 7 weeks after treatment.

Malnutrition, alcoholism, and the action of certain drugs lead to alterations in spermatogonia, resulting in decrease in the production of spermatozoa. X-irradiation and cadmium salts are quite toxic to cells of the spermatogenic lineage, causing the death of those cells and sterility in animals. Drugs like busulfan acts on the germinal cells. When administered to pregnant female rats, it promotes the death of germinal cells of their offspring and cause sterility, with their seminiferous tubules containing only Sertoli cells. Junqueira and Carneiro [17];Gossypol inhibits human sperm motility and its effect varies in severity depending on the analogue of the drug used. Tanphaichitr et al, [18]. It has been noted that pure gossypol in addition interacts with enzymes and hydrophilic macromolecules which are involved in sperm motility. Kalla et al,[19] ; Tanphaichitr et al,[18].

Furthermore, exposure of young male rats to acetic acid analogue of gossypol led to reduction in the number of microtubules of spermatocytes and spermatids, with the tubulins of the spermatids being more susceptible. This effect was reversed within 8 weeks of withdrawal of the drug. Kalla et al, [19]. It is possible that RICOM 1013-J may be acting in the same way. Unlike gossypol, not much work has been done using RICOM 1013-J on male fertility.

Most commonly, male factor infertility is described in terms of abnormal sperm concentration (oligospermia), impaired sperm motility (asthenospermia) or teratospermia (abnormal sperm morphology). Oligospermia is the presence of less than 20 million sperm per cc. in a semen specimen. According to the World Health Organization Criteria, impaired sperm motility refers to sperm motility <50% and the term impaired sperm morphology is applied if normal forms < 30% or < 14% (Kruger strict criteria). Such findings are associated with impaired fertility.

The morphology of the sperm cells in both treated groups with 100% normal forms was grossly reduced (40%) compared with the control (80%) which is below the W.H.O standard. In addition their mean sperm count was also reduced when compared with that of the control group.

The mean percentage of sluggish cells and non motile cells in this group was greater than that of the control group. The group treated with Diane 35 showed testicular section with several seminiferous tubules separated by interstitial connective tissue in which are seen interstitial cells (of Leydig). Efferent ductules were located in the midst of the seminiferous tubules and contained very few spermatids. The tissue from the control group had the conventional histological appearance with apparently more primary spermatocytes than the specimen from both treated groups and the seminiferous tubules contained more spermatids than those of the treated groups. No apparent pathological features were observed in tissues of any of the treated groups.

Diane-35 (an anti- testosterone, which inhibits the influence of androgens via its cyproterone acetate component), is known to have a pronounced progestational action. The combination with ethinyl oestradiol ensures control of the ovarian cycle. This action could explain the effects of diane-35 observed in the present study.

In the same way, the group treated with RICOM 1013-J, suppressed sperm parameters; the mean motility of cells was very low (11%) which was by far lower than that of the control (82%) and Diane-35 (15%) groups. Moreover, the mean percentage of sluggish and abnormal cells in the pretreated groups was higher than for the control. This portends the less likelihood of these abnormal sperm cells to fertilise the oocytes at the ampulla of the uterine tube as normal cells and motility remain important factors associated with fertilisatition.

Testicular histology in the group treated with RICOM 1013-J, showed several seminiferous tubules separated by interstitial connective tissue. Some of the seminiferous tubules showed active meiosis as indicated by the presence of primary spermatocytes. The lumen of the tubules also contained spermatids. No mature spermatozoa were observed. This effect may be due to observations that in rats, RICOM 1013 –J showed oestrogen-like effect and induced vaginal opening in immatured rats Okwuasaba et al, [10,11] and altered oestrous cycle as well as reduced the number of ova at ovulation McNeil et al, [12]. Normally, the apical plasma membrane of Sertoli cells form complexes around spermatids and spermatozoa until they are matured enough to be released. Disruption of Sertoli cells will cause the release of immature cells as found in the testicular tissue sections It is possible that the alterering of semen parameters by RICOM 1013-J could be due to the disruption of the supporting function of Sertoli cells and/or direct Leydig cell function, This finding is similar to that of gossypol, which has been reported to cause disorganization of early germ cells and reduction in the number of these germ cells Tanpharchita et al, [18]

3. CONCLUSION

This work showed that n-hexane extract of RICOM 1013-J suppresses testicular function in a reversible manner and may be a possible male contraceptive agent. The exact mechanism of action, however, cannot be delineated from this study.

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