Effects of Kolaviron on the Histoarchitecture of the Ovary, Fallopian Tube and Uterus in Female Wistar Rats

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Abstract – This research work determined the effects of kolaviron (a methanolic extract of Garcinia kola seeds) on the histoarchitectures of ovary, fallopian tube and uterus with a view to access its relationship to infertility. A total of thirty adult female Wistar rats were used for this experiment. The animals were randomly divided into six (6) groups: A, B, C, D, E and F with five (5) animals in each group. Group A, were the control group that were given distilled water orally once daily for 14 days; Group B were given kolaviron orally at concentration of 200 mg/kg body weight once daily for 14 days; Group C were given kolaviron orally at concentration of 100 mg/kg twice daily for 14 days; Group D were given clomiphene citrate orally at concentration of 0.50 mg/kg body weight for 5 days; Group E were given kolaviron orally at concentration of 200 mg/kg body weight once daily for 14 days after which clomiphene citrate were administered at concentration of 0.50 mg/kg body weight for 5 days; Group F were given kolaviron orally at concentration of 100 mg/kg body weight twice daily for 14 days after which clomiphene citrate were administered at concentration of 0.50 mg/kg body weight for 5 days. At the end of the experimental period, the animals were anaesthetized by chloroform inhalation. The ovary, fallopian tube and uterus were removed, weighed and fixed in 10% formal saline for Haematoxylin and Eosin staining. The results showed that Relative gonads weight (F=10.700, df=5, P<0.05) were significantly difference across the groups, The histoarchitecthure of the ovary showed that kolaviron ingestion by the female wistar rats (groups B and C) resulted into drastic reduction if not total absence in the number of growing follicles, while uterus and fallopian tube revealed inflammed lumen, groups A and D were normal while groups E and F had evidence recovery from distortion. It was concluded that kolaviron have adverse effects on the ovary, fallopian tube and uterus in female wistar rats and this could add to its role as infertility agent while clomiphene citrate helped in their recovery.

Keywords – kolaviron, ovary, fallopian tube, uterus, infertility, clomiphene citrate.

1. Introduction

The quest for naturally occurring compounds of herbal or plant origin that could be of benefit as contraceptive and fertility control agents, stimulated interest in Garcinia kola (bitter kola) (Heckel) seeds, widely consumed as a stimulant [1]. Traditional African medicinal uses of Garcinia kola include treatment of cough, purgative, anti-parasitic, antimicrobial [2, 3, 4]. The seed is used in the treatment of diarrhea [5] bronchitis and throat infections [6, 7] and liver disorders [8]. The seed, Garcinia kola, enjoys a folk reputation in Africa as a poison antidote [9]. In addition, the plant possesses anti-hepatotoxic [5, 9], antioxidant [10], hypoglycemic [8, 11] and aphrodisiac properties [12]. A Garcinia kola seeds contain biflavonoid (kolaviron) capable of having antiinflammatory properties [13] and is a natural antioxidant [14, 15]. Constituents of the seed of Garcinia kola include 1-3, 8-11 benzophenones, Garcinia biflavonones (GB-1, GB-2) and kolaflavonone. The biological activities of flavonoids include action against allergies, inflammation, free radicals, hepatoxins [15]. Ovulation in rat is brought about by a luteinizing hormone (LH) surge. The circulating level of LH begins to rise on the afternoon of prooestrus, about 2 pm to 3 pm and reaches peak at about 5-7 pm. This rapid surge induces follicular rupture and ovulation. Ovulation can be blocked experimentally by high doses of anti-inflammatory drugs administered before the LH surge because once the levels start to rise it may not be brought down by any drug [16].

The ovary is an ovum-producing reproductive organ, often found in pairs as part of the vertebrate female reproductive system. Ovaries in females are homologous to testes in males, in that they are both gonads and endocrine glands. Ovaries are oval shaped and, in the human, measure approximately 3 cm x 1.5 cm x 1.5 cm (about the size of a Greek olive). The ovary (or a given side) is located in the lateral wall of the pelvis in a region called the ovarian fossa. The fossa usually lies beneath the external iliac artery and in front of the ureter and the internal iliac artery [17].

The uterus or womb is a major female hormone-responsive reproductive sex organ of most mammals including humans. One end, the cervix, opens into the vagina, while the other is connected to one or both fallopian tubes, depending on the species. It is within the uterus that the fetus develops during gestation, usually developing completely in placental mammals such as humans and partially in marsupials such as kangaroos and opossums. Two uteruses usually form initially in a female fetus, and in placental mammals they may partially or completely fuse into a single uterus depending on the species. In many species with two uteruses, only one is functional. Humans and other higher primates such as chimpanzees, along with horses, usually have a single completely fused uterus, although in some individuals the uteruses may not have completely fused. The term uterus is used consistently within the medical and related professions,
while the Germanic derived term womb is also common in everyday usage in the English language [18].

2. Materials and Methods

2.1. Methodology

A total of thirty adult female Wistar rats were used for this experiment. The animals were fed with standard rat pellet and given water liberally in the animal holdings of the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife. A total of thirty adult female Wistar rats were used for this experiment. The animals were randomly divided into six (6) groups: A, B, C, D, E and F with five (5) animals in each group. Group A, were the control group that were given distilled water orally once daily for 14 days; Group B were given kolaviron orally at concentration of 200 mg/kg body weight once daily for 14 days; Group C were given kolaviron orally at concentration of 100 mg/kg twice daily for 14 days; Group D were given clomiphene citrate orally at concentration of 0.50 mg/kg body weight for 5 days; Group E were given kolaviron orally at concentration of 200 mg/kg body weight once daily for 14 days after which clomiphene citrate were administered at concentration of 0.50 mg/kg body weight for 5 days; Group F were given kolaviron orally at concentration of 100 mg/kg body weight twice daily for 14 days after which clomiphene citrate were administered at concentration of 0.50 mg/kg body weight for 5 days. At the end of the experimental period, the animals were anaesthetized by chloroform inhalation. The ovary, fallopian tube and uterus were removed, weighed and fixed in 10% formal saline for Haematoxylin and Eosin staining. All animals were handled in accordance with guidelines for animal research as detailed in the NIH Guidelines for the Care and Use of Laboratory Animals [19].

2.1.1. Extraction of Kolaviron

Extraction of Kolaviron was achieved by the procedure previously described by Iwu [20] and modified by Braide [5]. Garcinia kola seeds were peeled and air dried in the laboratory (25-28 °C) and ground into powdered form. The powdered seeds were extracted with n-hexane, in a Soxhlet extractor. The defatted, dried marc was repacked and then extracted with methanol in a Soxhlet extractor. The extract was concentrated and diluted to twice its volume in distilled water and partitioned with chloroform. The concentrated chloroform fraction gave a yellow-brown solid known as kolaviron.

2.1.2. Histological Analysis

The animals were sacrificed by cervical dislocation. The peritoneal cavities were opened so that the ovary, fallopian tube and uterus can be excised and weighed using a Metler sensitive balance. They were fixed in 10% formal saline (pH 7.1), for twenty-four hours for histological procedures. The ovary, fallopian tube and uterus were routinely processed for paraffin wax embedding. Then, 5μm thick paraffin cross sections of the tissues were mounted on slides and stained using routine haematoxylin and eosin method [21]. Microscopy was conducted on an Olympus microscope (Tokyo, Japan) and images were captured and processed by an attached eyepiece camera.

3. Results

3.1. Relative Gonads Weights

There was a significant difference in the relative gonads weight across the group but there was no significant difference when comparing groups C and F which were the groups that received 100 mg/kg body weight likewise no significant difference when compared with the control (group A) that received distilled water (p<0.05). But groups A, B and E differ significantly (p<0.05). Group D differs significantly (p<0.05) with group A. (Table 1).

![Table 1](image)

Table 1. The effect of kolaviron on the relative brain and Gonad weights in female wistar rats.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>GROUP A</th>
<th>GROUP B</th>
<th>GROUP C</th>
<th>GROUP D</th>
<th>GROUP E</th>
<th>GROUP F</th>
</tr>
</thead>
<tbody>
<tr>
<td>RGW</td>
<td>4.80±0.58</td>
<td>3.40±0.24*</td>
<td>5.00±0.45*</td>
<td>4.20±0.37*§</td>
<td>4.40±0.25*§</td>
<td>5.20±0.20*§δ</td>
</tr>
</tbody>
</table>

Values are Mean ± Standard Error of Mean (SEM) of data from the animals. *, §, δ and λ implies significant difference at P<0.05 when compared to group A, B, C, D and E respectively. Significance was verified with ANOVA. SNK test was used for multiple comparisons. Note: RGW means relative gonad weight.

3.2. Histological Studies of the Ovaries, Fallopian Tube and Uterus

The histological findings in the ovaries showed various follicular stages, graafian follicles and congestion of the stroma and intact blood vessels in group A while groups B and C showed no developing follicle, and degenerated luteum with the pool of intact blood vessels. It was also evident that there were some follicles springing up in section D despite the presence of numerous corpus albican. Groups E and F showed evidence of corpus albican with few matured follicles. Simple ciliated cuboidal epithelium of the fallopian and uterus were seen in group A and D, the muscular layer, active stages of follicular growth were also seen, but groups B and C showed bulging of the epithelium into the lumen and the gland in the subcutaneous tissues were distorted, this were also evidenced in groups E and F but with clear appearance of recovery.
PLATE 1. Photomicrograph of tranverse sections showing ovaries in groups A-F. Note the various follicular stages-f, intact blood vessels-v and well organised stroma-s in (A). In (B) & (C), the degenerated follicles-d, corpus albican-c, varicose blood vessel-v; in (D), the various follicular stages-f, corpus albican-c, normal blood vessel-v. In (E) & (F), the few matured follicles-f, corpus albican-c. H & E. X 100.
PLATE 2. Photomicrograph of tranverse section showing fallopian tubes in groups A-F. Note, the lumen-L, Epithelium-E and glands (red arrow) were clearly represented in (A) & (D). In (B) & (C), the lumen-L were inflamed, epithelial lining-E bulged into the lumen and glands were inactive; in (E) & (F), the lumen-L were inflamed, epithelial lining-E bulged into the lumen and degenerated glands (arrows) recovering. H & E. X 100.
4. Discussion

The quest for naturally occurring compounds of plant origin that could be of benefit as contraceptive and fertility control agents stimulated the interest in kolaviron. Plant-based *in vivo* research has made significant rewarding progress in many important areas such as development of antibiotics[22], cancer[23] and is still contributing to research in reproductive medicine including hastening fetal delivery, prenatal development, pre- and post-coital contraceptives.

Antifertility and abortifacient activities of phenolics, phytosteroids and saponins have also been confirmed in animal models [24]. Therefore, alkaloids, phenolics and saponins in the kolaviron may have a long way to go either by acting alone or in combination may be partly responsible for the observed antifertility effects in this study.

There was a significant decrease when relative brain and gonads weights of the control female wistar rats were compared with 200 mg/kg body weight of kolaviron treated female rats but significantly increase with 100 mg/kg body weight of kolaviron treated female rats. This
opposes Uko [25] who reported that G. kola extract had no significant effects on the relative organ mass of the rats used in his study.

Plates 1. Group A showed the basic structure of the normal ovary of a mammal with the ovarian stroma well laid, the numerous follicles are seen with their various follicular stages, graafian follicles , corpus luteum. In groups B and C, there is not a single developing follicle but old degenerated corpus luteum (corpus albican), these are showed that the ovaries were not active thereby showing that Kolaviron is prevented ovulation by acting as an anti-inflammatory agent. Group D showed old degenerated corpus luteum but different stages of follicles making it active, while groups E and F were with degenerated follicles but showing recovery states of the ovarian stroma. Blood vessels were all intact in all the groups showing that the ovaries in all the sections were well nourished and still functional. Groups A and D of the fallopian tube is well shown, the simple ciliated columnar epithelium, lumen, subcutaneous layer, and muscular layer were clearly seen. Groups B, C, E and F showed edema in their epithelial lining and their subcutaneous layers are inflammed but removery features are seen in sections E and F. Groups A and D of the fallopian tube is well shown, the simple ciliated columnar epithelium , lumen, subcutaneous layer, and muscular layer are clearly seen. Groups B, C, E and F showed edema in their epithelial lining and their subcutaneous layers are inflammed but removery features are seen in groups E and F. This study is corroborated by work of Iranloye [26] that confirmed that Garcinia kola seed extract made the uterine endometrial gland inactive, stopped maturation of the follicles, caused acute inflammation of the tubes but all the organs showed full recovery after administration of garcinia kola seed extract was stopped. garcinia kola seed extract may be used used as a contraceptive with a possible advantage of reversibility.

5. Conclusion
In conclusion, Kolaviron successfully caused damage to the reproductive tissues as evidenced with the reduction in follicular growth, inflammed uteri and fallopian tubes which were recovered with the administration of clomiphene citrate. Thus, kolaviron may be used as a contraceptive having the advantage of reversibility.

References