



HISTOLOGICAL ANALYSIS OF FEMALE WISTER RATS OVARIES AFTER 30 DAYS  
ADMINISTRATION OF SUPER7 – A POLY-HERBAL ANTIMALARIAL DRUG FORMULATION

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## ABSTRACT

**Background:** Ovary is one of the major parts of female reproductive system and it produces the ovum under the influence of gonadotropin hormones. Any factor that alters the anatomical structure and the physiological functions of the ovary will consequently affect female fertility. Therefore, this study evaluated the effects of a polyherbal antimalarial drug - Super7 - on female Wister rats' ovarian histology. **Methods:** acute toxicity and lethality studies as well as the phytochemical analysis of Super7 were done. For the histology study, the rats were randomly allocated into four groups I - IV (n = 7). Groups I – III received 507.3 mg/kg body weight, 1,014.6 mg/kg body weight and 2,029.2 mg/kg body weight of Super7 respectively. Group IV rats served as the controls and received 5 ml/kg body weight of distilled water. Treatments were administered daily for 30 days after which two rats were selected from each group and sacrificed by cervical dislocation. The ovaries were quickly isolated and preserved for histological analysis. **Results:** the LD<sub>50</sub> was > 5,000 mg/kg body weight and

flavonoids were mostly abundant in Super7. Sections of ovarian tissue showed normal morphology; the antrum, granulosa cells, primordial follicles and oocytes were consistent with normal ovarian histology. Group II sample 2 showed hyperchromatic and mildly distorted follicular cellular architecture. **Conclusion:** generally, Super7 has no adverse effect on the ovary during the duration of 30 days of its administration; thus supporting an earlier study which reported that Super7 has pro-fertility effects.

## INTRODUCTION

In accordance with the concurrent study done recently on Super7 polyherbal drug formulation, it is commonly sold and consumed in Nigeria and the constituents include: *Azadirachta indica*, *Anthocleista vogelli*, *Aloe vera*, *Antiaris toxicaria*, *Carica papaya*, *Moringa oleifera* and *Xylopi aethiopica*. The label indications of Super7 include: malaria, enteric fever, hepatitis, diabetes mellitus, anti-toxins, sexually transmitted diseases, immune and sperm booster, analgesic, antibacterial and antifungal. Due to the crude nature of most herbal formulations, there is possibility of the drug affecting organs, tissues, or systems other than those targeted for therapy. This study is therefore a follow up of the concomitant study which concluded that Super7 has a positive gonadotropin effects on female Wister rats; which gave an insight on the effects of Super7 on female fertility <sup>[1]</sup>. In order to buttress the profertility properties of Super7, the present study which intends to evaluate the effects of Super7 on ovarian histology was considered absolutely necessary.

The ovaries form part of the female reproductive system. They have two main reproductive functions in the body. These include production of oocytes for fertilisation and production of the reproductive hormones - oestrogen and progesterone and some lesser hormones called relaxin and inhibin <sup>[2]</sup>. The functions of the ovaries are controlled by gonadotropin-releasing hormone (GnRH) released from nerve cells in the hypothalamus which send their messages to the pituitary gland to produce luteinising hormone (LH) and follicle stimulating hormone (FSH) <sup>[3]</sup>. These are carried in the bloodstream to control the menstrual cycle. The ovaries have certain structures that enable them to exercise their functions. These include: follicular cells, oocytes, corpus albicans, stroma, antrum, graafian follicles, granulosa cells and blood vessels among others <sup>[4]</sup>. The ovary is directly involved in ovulation, the process of mature egg release from the ovary into the adjacent fallopian tube; and the hormones involved in this process include: GnRH, FSH, LH, progesterone and estrogen. Both progesterone and estrogen are produced in the ovary by corpus luteum and granulosa cell respectively <sup>[5]</sup>. Any factor, herbal drug formulations inclusive, which alters these anatomical structures of the ovaries and their functions, will influence female fertility. Consequently, histological analyses of Wister rat's ovaries were conducted after 30 days of administration of Super7 polyherbal drug formulation. Histological study implies the study of the microanatomy

of cells, tissues, and organs as seen through a microscope to examine the correlation between structures and functions <sup>[6]</sup>.

## **MATERIALS AND METHODS**

### **Materials**

#### **Animals**

Pubertal female Wistar rats (28) weighing 70 – 100 g were procured from the Animal house of the faculty of Pharmaceutical sciences, Nnamdi Azikiwe University Awka. The animals were acclimatized for seven days under standard conditions of temperature and illumination (12 hours dark: 12 hours light) cycle. The rats were fed with commercially available rats' pellets (Amobyng feeds, Port Harcourt) and given access to drinking water ad libitum. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals (Pub No: 85-23 Revised 1985) and approved by the Nnamdi Azikiwe University's Ethical Committee for the use of Laboratory Animals for Research Purposes.

#### **Chemicals and Reagents**

#### **Drugs**

Super7 (Tozok Natural, Obosi, Anambra State, Nigeria)

#### **Reagents for histological studies**

10 % (v/v) formalin, acetone of histological grade, ethanol of histological grades, isopropyl alcohol of histological grade, chloroform of histological grade, xylene, ethylene glycol, paraffin wax, haematoxylin and eosin dye. All reagents are manufactured by Vector laboratories United States of America.

#### **Equipment**

#### **Equipment for histological studies**

Rotatory microtome, digital research photographic microscope, slide stainer, histology tissue processor, freezing microtome stage, thermoconductive platforms, automatic slide driers and labelers. All equipments are manufactured by Labcompare United State of America.

## Methods

### Phytochemical analysis

The phytochemical analysis was done according to the method described by Biswal<sup>[7]</sup> and the details were as described in the previous study on Super7<sup>[1]</sup>.

### Acute toxicity studies

The acute toxicities and the median lethal dose (LD<sub>50</sub>) of the test drug were estimated according to the method described by Lorke<sup>[8]</sup>. The details were according to that described in the previous study done on Super7<sup>[1]</sup>.

### Experimental design

In this study, the test animals were randomly allocated into four groups I - IV (n = 7). Groups I – III were the treatment groups and received 507.3 mg/kg body weight, 1,014.6 mg/kg body weight and 2,029.2 mg/kg body weight of the test drug. Group IV rats served as the control and received 5 ml/kg body weight of distilled water. All treatments were administered orally. Treatments were administered daily for 30 days. On day 31, two rats from each group were sacrificed by cervical dislocation. The ovaries were quickly isolated and placed in 10% formalin for three days. After three days, the ovaries were fixed in 10% ethanol until ready for histological analysis. The ovarian tissues were dehydrated in an ascending grade of ethanol, cleared in xylene and embedded in paraffin wax. Serial sections of 7 microns thick were obtained using a rotatory microtome. The deparaffinised sections were stained routinely with haematoxylin and eosin. Photomicrographs of the tissue were obtained with digital research photographic microscope.

## RESULTS AND DISCUSSION

### Results

Photomicrographs of samples 1 and 2 of groups I, III, IV, V and VI revealed that sections of ovarian tissue show normal morphology. The follicular cells, primordial follicles and oocytes were consistent with normal ovarian histology (H&E x100).

Photomicrographs of sample 1 of group II also revealed that sections of ovarian tissue show normal morphology. The follicular cells, primordial follicles and oocytes are consistent with normal ovarian histology. Only sample 2 of group II showed hyperchromatic and mildly distorted follicular cellular architecture (DFC) (H&E x100).

## Discussion

In accordance with the results of the phytochemical analysis, flavonoids are mostly abundant in Super7 and might have contributed to the profertility effects. This is evident in a study done recently on the effects of flavonoids from semen cuscutae (FSCs) on the hippocampal-hypothalamic-pituitary-ovarian sex hormone receptors in female rats exposed to psychological stress. The study reported that FSCs increased estrogen receptor expression in the hippocampus, hypothalamus, and pituitaries, as well as luteinizing hormone receptor expression in the ovaries, but had no effect on follicle stimulating hormone receptor expression in the ovaries; indicating that flavonoids have pro-fertility effects by also enhancing ovulation and are effective medicine in the treatment of ovarian endocrine dysfunction in psychologically stressed rats <sup>[9]</sup>. The results of the acute toxicity tests implied that the drug have high safety profile; however, higher doses should be used with caution <sup>[1]</sup>. Generally, Super7 has no adverse effects on the ovary after 30 days which is an indication of pro-fertility in female Wister rats. The ovaries will be able to perform their physiological functions during the therapeutic use of Super7. Histological analysis has helped in a lot of investigations concerning optimal conditions, structures, functions as well as vitality of the ovaries. A study reported that ovaries stored at 4°C for up to 24 h showed minimal histological changes and the implantation rate and rate of live pups both decrease as storage at 4°C is prolonged <sup>[10]</sup>. Another study provided complete descriptions and illustrative examples of histologic features of the developing rat ovary from postnatal day 3 (PND 3) through puberty and further provided the pertinent neuroendocrine dynamics during development to allow correlations between the morphologic changes and the neuroendocrine parameters <sup>[11]</sup>. A recent histological study provided clear evidence of age-associated physiological changes in peri-gonadal adipose tissue with regards to changes in adipocyte morphology and lipid profiles <sup>[12]</sup>. The researchers recommended that further investigation of the effect of peri-gonadal adipose tissue on the ovarian microenvironment may identify it as a promising target to improve reproductive function in the context of age-related infertility. Another histological study showed that chronic exposure to electromagnetic wave (EMW) frequency of 1,800 MHz caused pathological changes in ovarian tissues ranging from minor to great changes (congestion, decreased ovarian follicle number and development, vacuolation, autophagy, apoptosis and micronuclei formation) <sup>[13]</sup>. The researchers reported that Wister rats exposed to EMW showed separation of granulosa cells from the basement membrane and thinning, irregular zona pellucida as well as ooplasm vacuolation. They also reported that microscopic examination of mice exposed to EMW for 4 h/day during pregnancy revealed that the oocyte's nests were irregularly arranged, and the primordial follicles were undeveloped and ooplasm vacuolation was observed <sup>[13]</sup>. The oocyte changes and the separation of granulosa cells from neighboring cells create the main feature of atretic follicle and characteristic of zona pellucida apoptosis with theca interna vacuolation. This gave an insight about the Global System for Mobile

Communication (GSM) which is one of the operating systems used in cellular phone's communications because our cellular phones are emitting electromagnetic waves (EMW). These studies clarified the extent to which histological analysis can elucidate the effects of structural alterations of organs on their physiological functions.

## Conclusions

Super7 is safe to the ovary when used within 30 days. This supported the previous study which reported that super7 has a positive gonadotropin effects and thus pro-fertility effects.

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