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Research Article

Effects of Methanol Extract of *Telfairia occidentalis* Seed on Ovary Antioxidant Enzymes, Serum Hormone Concentration and Histology in Wistar Rats

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ABSTRACT

This study investigated the effects of oral administration of methanol extract of *Telfairia occidentalis* seed (METOS) on ovary antioxidant enzymes, serum hormone concentration and histology in Wistar rats. Twenty (20) female Wistar rats were randomly assigned into 4 groups; Group A, (control) was administered distilled water, group B, C and D were administered 20, 40 and 80 mg METOS /kg bw orally respectively for 30 days. After the completion of treatment, all animals were sacrificed during the proestrus phase of estrous cycle by cervical dislocation. Blood sample was collected and serum was obtained. Ovary was also collected and homogenized. Antioxidant activity and redox status in the ovary were investigated, serum concentration of FSH, LH and Estrogen were also measured, the histology of the ovary was also studied. There was an increase in superoxide dismutase level in all the experimental groups while MDA level decreased in the groups treated with 40 and 80 mg METOS/kg bw when compared with the control group ($p < 0.05$). There was no significant difference in the level of catalase, serum concentrations of FSH, LH and estrogen. The histology of all the experimental groups were similar to that of the control group. In conclusion the result of this study suggests that methanol extract of *Telfairia occidentalis* seed (METOS) has antioxidant activity which is able to protect against lipid peroxidation and cell damage in ovary tissue. It also did not alter sex hormones.

Keywords: *Telfairia occidentalis* seed, ovary, antioxidant enzymes, malondialdehyde, hormones, histology.

INTRODUCTION

The ovaries perform two essential functions namely development of female gamete and the synthesis and release of sex steroid hormones under the influence of gonadotrophic hormones. The process of performing its functions involves the dynamic changes in metabolism and energy consumption[1] as well as participation of inflammatory cells in ovulation[2,3] both of which leads to the generation of reactive oxygen species (ROS). Although, ROS are considered to mediate inter- and intra-cellular signaling, generation of an excess which is determined by the balance between oxidants and antioxidants then results in oxidative stress[1]. Proper functioning of the ovaries may therefore be disrupted by oxidative stress thus leading to infertility. Oxidative stress induces infertility in women through a variety of mechanisms. Increased generation of lipid peroxidation in the ovaries due to oxidative stress can lead to direct damage of oocytes[4]. Nourishment has been linked with animal reproductive success throughout history[5] and reproductive toxicity, for example, *Trichosanthes cucumerina* is known for its gonadotropin lowering effects and its attendant antiovulatory effects thus leading to infertility[6]. However, Food containing high amount of antioxidant play a role in ameliorating the impact of ROS in animal models and in humans[7]. Several herbal diets and fruits have antioxidant effects that can help to ameliorate the antifertility effects of lipid peroxidation, examples of such are Brass*ica Juncea* seed commonly known as brown mustard seed[8], *Tetracarpidium conophhorum* commonly known as African walnut[9] and *Telfairia occidentalis* seed commonly known as pumpkin seed. *Telfairia occidentalis* is a plant belonging to the family of cucurbitaceae, it is largely consumed in Nigeria, Ghana and Sierra Leone[10]. The seed is rich in oil which is used for cooking[11,12]. *Telfairia occidentalis* seed has been reported to possess some essential components such as vitamin A, Vitamin C, carbohydrate, essential minerals such as calcium, phosphorus, magnesium and iron[13]. The oil derived from the seed has very high content of polyunsaturated fatty acid such as linoleic acid, oleic acid and palmitic acid[14]. *Telfairia occidentalis* seed oil has been reported to promote male fertility[15,16]. However similar scientific report on the effects of *Telfairia occidentalis* seed on the female reproductive system is scarce. This study therefore investigated the effects of methanol extract of *Telfairia occidentalis* seed on ovary antioxidant activities, serum hormone concentration and histology in the female reproductive system in Wistar rats.

MATERIALS AND METHODS

Plant

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Table 1: Effects of METOS on Antioxidant enzymes and MDA Level in Ovaries in Wistar Rats.

	SOD (U/mg)	CAT (µ/mg tissue)	MDA (nM/mg tissue)
Control	26.00 ± 6.78	7.04 ±0.47	27.06 ± 5.45
20 mg/kg bw	74.00 ± 6.78*	7.8 ± 0.22	27.23 ± 5.99
40 mg/kg bw	78.00 ± 3.74*	8.08 ± 0.77	11.48 ± 0.67*
80 mg/kg bw	82.00 ± 6.33*	10.50 ± 2.71	16.64 ± 2.89*

*Values are expressed as Mean ± SEM (n=5), *P = 0.05 was considered significant compared to control group.*

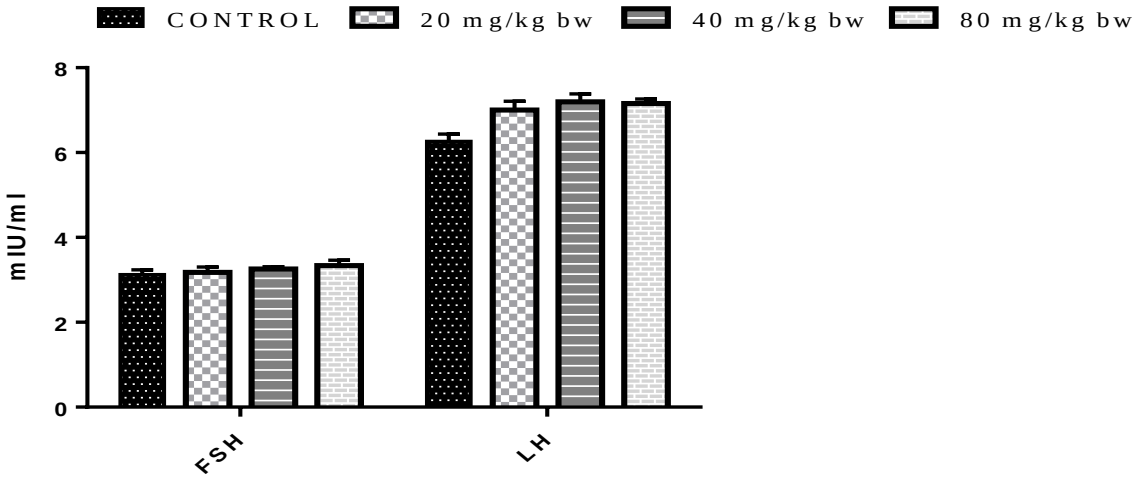

Figure1: Effect of METOS on Serum FSH and LH Concentration in Female Wistar Rat Bars are expressed as Mean ± SEM (n=5), *p<0.05 was considered significant compared to control group.

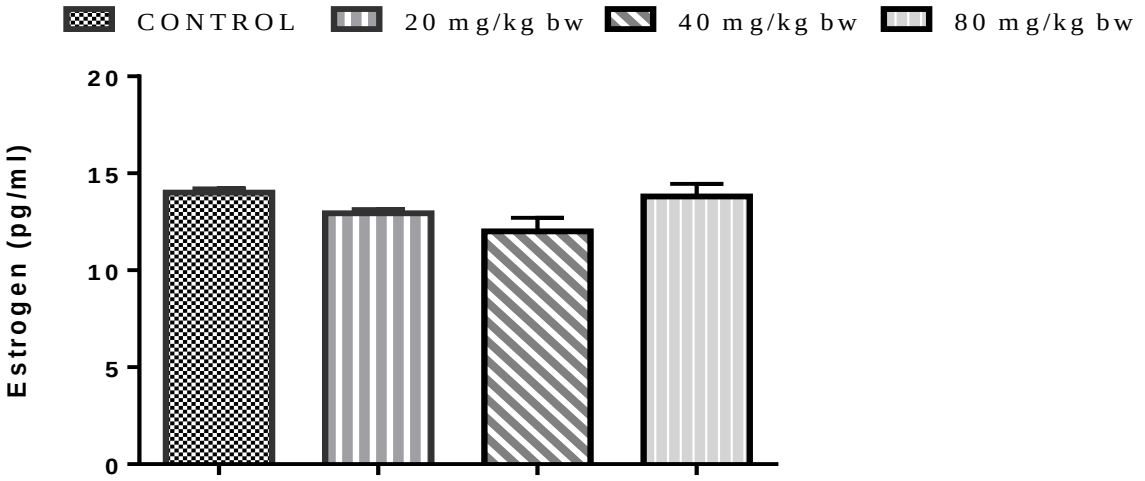

Figure2: Effect of METOS on Serum FSH and LH Concentration in Female Wistar Rat Bars are expressed as Mean ± SEM (n=5), *p<0.05 was considered significant compared to control group.

Fluted Gourds of *Telfairia occidentalis* were purchased from Orie Ugba market in Umuahia, Abia State. The whole plant was authenticated at the herbarium of Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The sample of the plant was assigned with the voucher number: 108846.

Method of Extraction

The plant seeds were obtained from the gourd after it was cut open. The seed was decocted and air dried until a consistent weight (1,678 g) was obtained at about the fourth week. The seeds were then blended into powdery form using Flourish electrical blender CA/BD912, New york. The weight of the powder was 1,645 g. It was then soaked in six liters of methanol for 48 hours, during which period it was frequently agitated and filtered at the end of the duration. The filtrate was concentrated by freeze drying. The methanol extract of *Telfairia occidentalis* seed (METOS) obtained appeared brownish, sticky and oily with a percentage yield of 4.6. It was then stored at room temperature throughout the experiment.

Experimental Animals

Twenty (20) female Wistar rats weighing between 165 - 200 g were purchased from Central Animal House, Igbinedion University, Okada, Nigeria. The animals were kept in well ventilated plastic cages and maintained under standard laboratory conditions. They were fed with rat chow and water *ad libitum*. The animals were acclimatized for two weeks before the study commenced.

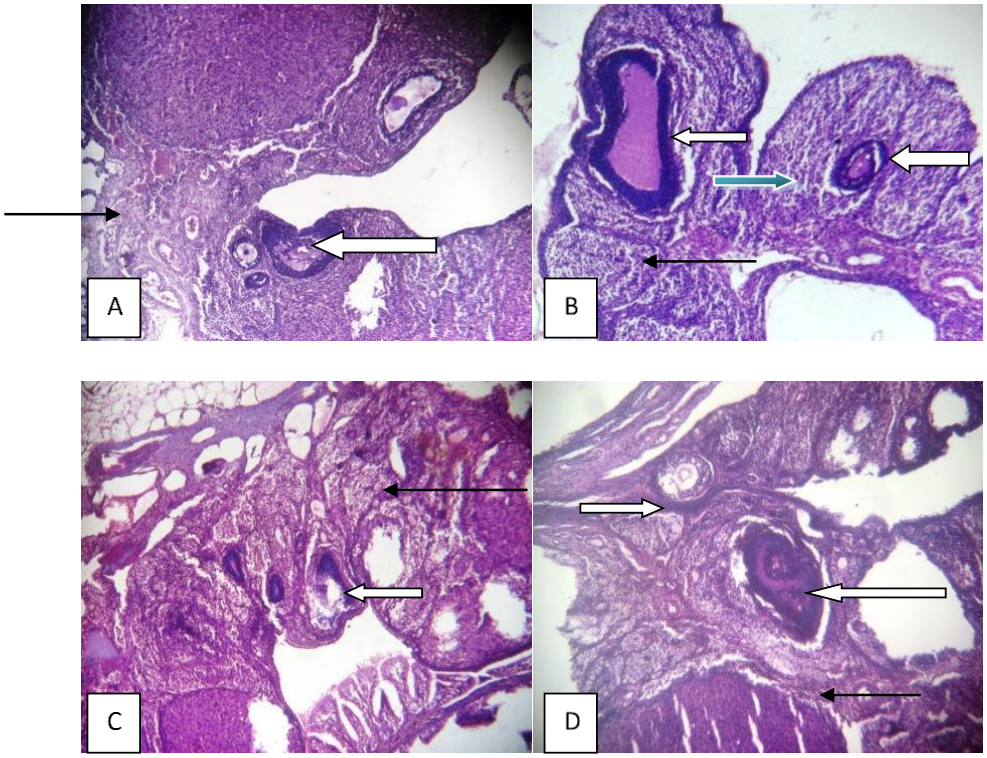

Figure 3: Photomicrograph of ovary section (x100) (A) shows normal ovarian stroma with leutinization within the granular cells (slender arrow). There are several follicles with differential stages of maturation consisting of premodial and antral follicles (white arrow) at the cortical region of the ovary. and the stroma shows normal connective tissues and no inflammatory cells. (B) shows normal ovarian stroma with leutinization within the granular cells (blue arrow). There are several follicles with differential stages of maturation consisting of premodial and antral follicles (white arrow) at the cortical region of the ovary. The vessles appear normal, and the stroma shows normal connective tissues and no inflammatory cells (slender arrow). (C) shows normal ovarian stroma with leutinization within the granular cells (slender arrow). There are several follicles with differential stages of maturation consisting of premodial and antral follicles (white arrow) at the cortical region of the ovary. The vessles appear normal, and the stroma shows normal connective tissues and no inflammatory cells. (D) photomicrograph of an ovary section shows several normal follicles with differential stages of maturation (white arrow) and ovarian stroma with leutinization within the granular cells, the stroma appear mildly fibrotic (slender arrow).

Experimental Design

The animals were randomly distributed into four groups of five animals each. The extract was orally administered for a period of 30 days as follows; Group A, (control) was administered distilled water, group B, C and D were administered 20, 40 and 80 mg METOS /kg bw respectively.

Tissue Collection

The animals were sacrificed by cervical dislocation after the last day of administration, (day 31) and dissected through the linea alba. Blood was collected by cardiac puncture and serum was obtained from it. The ovaries were harvested, freed from every adherent tissue, weighed and the right ovary was preserved in 10 % formalin for histology while the left ovary was homogenized in 2 ml of phosphate buffer saline and its supernatant was used for the estimation of superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA).

Antioxidant Enzyme Assay

Superoxide dismutase (SOD) level

This was estimated according to the method of Misra and Fridovich[17]. The principle is based on rapid auto-oxidation of adrenaline in aqueous solution to adrenochrome due to the presence of superoxide anions. The concentration was determined with a spectrophotometer at 420 nm.

Catalase level

This was determined according to the method of Aebi[18]. Upon the addition of 30 mM H_2O_2 in 50 mM of phosphate buffer (pH 7.4) to sample, it is converted to oxygen and water. This action was stopped after three minutes by the addition of H_2SO_4 to the mixture, followed by 7.0 ml of $KMnO_4$. Catalase (CAT) activity was estimated by decrease in absorbance of H_2O_2 at 520 nm.

Thiobabituric Acid (TBARS) Assay

This was done according to the method of Rice-Evans *et al*,[19]. The principle is based on the reaction of Malondialdehyde, a product of lipid peroxidation with thiobarbituric acid to give a red species that can be detected at 535 nm.

Histology of Ovary

Each right ovary was harvested and immediately fixed in 10 % formalin for at least 5 hrs. Each sample was dehydrated using ascending grades of alcohol. It was cleared with two changes of xylene, embedded in paraffin wax, trimmed, nicked and sectioned using a microtome

and stained using haematoxylin and eosin (H&E) for the purpose of determining the general morphology.

Hormone Assay

Determination of the serum concentration for luteinizing hormone, follicle stimulating hormone and estrogen was done using Enzyme-linked immunosorbent assay (ELISA) technique. The kit used is a product of Calbiotech Inc. (Spring Valley, California). Basically, the procedure for running the three of them is the same and was stated in the manufacturer's mannual. After running the assay, the optical density obtained was used to find the concentration of each hormone.

Statistical Analysis

Data from each group were expressed as mean ± standard error of mean (Mean ± SEM). The data were analyzed with one-way analysis of variance (ANOVA) followed by Waller-Duncan's post hoc test. *p* - value less than 0.05 was considered significant. The statistical packages used were Microsoft excel and graph pad prism 6.

RESULTS

Effects of METOS on Biochemical Assay in female Wistar Rats.

Effects of METOS on Antioxidant enzymes and MDA Level in Ovaries in Wistar Rats.

There was an increase in SOD level in the groups treated with 20, 40 and 80 mg/kg of METOS when compared with the control ($p < 0.05$), but there was no significant difference in catalase level in all the experimental groups when compared with the control group. There was a decrease in level of MDA in groups treated with 40 and 80 mg/kg of METOS when compared with control group ($p < 0.05$).

Effects of METOS on Hormone profile in female Wistar Rats.

Effect on Serum Concentration of FSH and LH

There was no significant difference in the serum concentration of FSH in all the experimental groups when compared with the control group. There was also no significant difference in the serum concentration of LH in all the experimental groups when compared with the control group.

Effect of METOS on Serum Concentration of Estrogen

There was no significant difference in all the experimental groups treated when compared with control group.

Effects of METOS on Ovary Histology in female Wistar Rats.

DISCUSSION

This study investigated the effects of methanol extract of *Telferia occidentalis* seed (METOS) on the levels of ovary antioxidant enzymes, serum hormone concentration and histology in Wistar rats. The observed increase in SOD level in this study suggests that METOS has antioxidant activity. *Telfairia occidentalis* seed has been reported to contain significant amount of Vitamins A and C[13] which are potent antioxidant. Antioxidants are important in the detoxification of reactive oxygen species generated during the preovulatory gonadotrophin surge in order to make oocyte maturation and embryo development possible[20,1]. The SOD result is similar to the result of Ajani and Akinyemi[21] in which both aqueous and ethanolic extract of *Telfairia occidentalis* seed increased SOD level in prostate tissue of testosterone induced benign prostatic hyperplasia in rats. The reduced MDA level observed in this study is in agreement with the work of Ajani and Akinyemi[21] and it is consistent with the SOD result observed. ROS is normally generated during ovulation due to the presence of inflammatory cells such as Macrophages and neutrophils in the ovary[3,2]. However, gametes are extremely sensitive to damage by ROS. The availability of low molecular weight antioxidants such as Vitamins A and C convert them to harmless compounds[1]. The presence of significant amount of vitamins A and C in *Telfairia occidentalis* seed as reported by Agatermor[13] may have also contributed to the observed reduction in MDA level in this study. In this study, the level of FSH, LH and Estrogen in all the experimental groups were comparable with the control. Rats have four different phases of estrous cycle[22] and the serum concentration of each hormone differ from one phase to another based on the different reproductive activities that occur in the respective phases. The similarity in serum concentration of FSH, LH and estrogen observed in this study may be as a result of the fact that all groups were sacrificed during the proestrous phase of the cycle. Similarly, the ability of METOS to cause a reduction in MDA level is an indication that *Telfairia occidentalis* seed does not cause direct oxidative damage on the oocyte. This suggests that METOS at the dosage administered is well tolerated by the ovaries and does not interfere with the synthesis and release of these hormones. The histoarchitecture of the ovaries in all the experimental groups is comparable with that of the control group. This further buttress the hormone results as well as the MDA result observed in this study.

CONCLUSION

In conclusion the result of this study suggests that methanol extract of *Telfairia occidentalis* seed (METOS) has antioxidant activity which is able to protect against lipid peroxidation and cell damage in ovary tissue. It also did not alter sex hormones.

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