Effects of *Cucumissativus*extract on the histomorphology of the ovaries and hormonal profile of adult female Wistar rats

ABSTRACT

Background: Drugs of plant origin have served through the ages as the mainstay in treating diseases and preserving human health. The cucumber is one of the plants that have nutritional values.
Aim: This study was conducted to investigate the effect of *Cucumissativus*extract on the ovariesand sex hormones of adult female Wistar rats.
Study Design and Methodology: SixteenWistar rats weighing 180-200g were divided into four groups (A-D) of four animals each. Group A(control) received only distilled water and feed, Group B received 250mg/kg body weight of cucumber fruit extract, Group C received 400mg/kg body weight of cucumber fruit extract, and Group D received 1200mg/kg body weight of cucumber fruit extract. All the treatments were given orally daily for twenty-one days. The animals were sacrificed by cervical dislocation and dissected; the ovarieswere harvested and fixed in 10% formal saline for histological studies, while blood was collected for hormonal analysis.
Results: There was a significant increase ($p<0.05$) in theFSH level of group B only when compared to the control groups while a non-significant decrease in the estradiol level in all the treatment groups compared to the control. Histopathological studies show little distortion in the histomorphology of the ovaries in groups C and D.
Conclusion: *Cucumissativus*extract, when consumed in large amount, has deleterious effects on the female reproductive system.

Keywords: *Cucumissativus*, follicle-stimulating hormone, estradiol, ovary, histomorphology

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INTRODUCTION

"Plants have been used as medicinal materials for thousandsof years and remain relevant as a natural sourceof active compounds for the treatments of human diseases" (Cragg and Newman,2013)."Many medicinal plants are used to treatvarious reproductive function ailments, such as female infertility a public health concern in sub-Saharan African countries" (Lienou*et al*., 2010)."Cucumber (*Cucumissativus*L) is a widely cultivated plant in the gourd family, Cucurbitaceae.It is thought to be one of the oldest vegetables cultivated by man, with historical records dating back five (5) millennia"(Gusmini and Wehner, 2008) "The crop is the fourth most important vegetable after tomato, cabbage, and onion in Asia" (Wilcox *et al*., 2015).

"Reproductive toxicity is overwhelmingly becoming identified as an important part of overalltoxicology. Conventional combined oral pills are usually associated with numerous adverse effects, necessitating indigenous drugs. Theuse of plants for medicinal and mythologicalpurposesand for solving problems related to illhealth has been practiced in the African and other societies for centuries" (Mohammed *et al*., 2014). Experts claim that cucumbers contain nutrients that help in male and female fertility (Grubben and Denton,

2004). Cucumbers contain citrulline, which helps to boost fertility in males and females. Citrulline induces blood flow into the penis, which is a way to heal erectile dysfunction in males and increases the number of egg production in females during ovulation(Patil*et al*., 2012).This study investigates the effects of *Cucumissativus*extract administration on the histomorphology of the ovaries and the sex hormones of adult female Wistar rats.

MATERIALS AND METHODS

Location and duration of the study

This study was conducted in Human Anatomy Department, Abia State University, Abia State, Nigeria. The rats were made to acclimatetwo weeks, after which the test substance was administered for 28days; the entire experiment lasted for five weeks.

Preparation of the extract

Fresh*Cucumissativus*(cucumber) fruits were obtained from Nkwo Market in Nnewi, Anambra State of Nigeria. The fruits were properly washed, and sliced. The seeds were discarded and the greenish part of the fruit was air-dried and ground with a laboratory blender.The air-dried, and ground*Cucumissativus*was sifted with a one mm^2 sieve to increase extraction efficiency. After that, about 100g was weighed, then submerged in 1000ml of distilled water and extracted using an inverter condenser on a 70°C water bath for two hours. After that, the extract was filtered using Whatman filter paper No. 1. Then the solvent was removed using a rotary evaporator after setting it at a temperature of 40°C and a pressure of 40 and 140 revolutions per minute until the weight became constant and the extracts condensed (Sultana *et al*., 2009). The extract weight was determined by calculating the difference between the weight of the vessel before and after evaporation. The extract was stored in opaque glass bottles at 4°C until the doses were made.

Experimental design

They were divided into four groups (A to D) and housed in 4 standard cages. The weight of each rat was measured using an analytical weighing balance. They were fed with rat chow and water ad libitum two weeks and were left to acclimatize with their new environment before the experiment began.

Group A-(Control) received only water and chow daily for three weeks

Group B received *Cucumissativus*extract 250mg/kg (0.4ml) body weight daily for three weeks

Group C received *Cucumissativus*extract 400mg/kg (0.8ml) body weight daily for three weeks

Group D received *Cucumissativus*extract 1200mg/kg (2.4ml) body weight daily for three weeks

All administration was done with cannula and syringes orally.

Extraction of the organs and collection of samples

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The rats were sacrificed by cervical dislocation after 28days of administration. The ovaries were then harvested, weighed, and fixed in 10% formal saline to maintain normal physiological conditions for their histological processing while blood was collected for hormonal analysis.

Histological analysis

The ovaries of the experimental rats were removed and preserved in labeled bottles containing 10% formal saline. These were allowed to stand for 72hours to achieve good tissue penetration and effective fixation before being placed in ascending grades of ethanol for dehydration. First, they were treated with two changes of 70% ethanol each, lasting for one hour, followed by 95% ethanol and then absolute alcohol for the same duration. Following dehydration, tissues were cleared in three changes of xylene, each lasting for fifteen minutes. Impregnation in molten paraffin wax at 58^{o}C was carried out overnight, and the following morning, the tissues were embedded in wax to form blocks. These tissue blocks were trimmed and sectioned at 5μ thickness using a rotary microtome. The sectionswere floated in warm water (28^{o}C) and then taken up on aluminized glass slides. They were air-dried and stained using the H&E. They were dewaxed in xylene for 2 minutes per 2 changes. Xylene was cleared in 95% alcohol for another minute. The section was washed well in running tap water for 15 minutes, differentiated in 1% alcohol for 5-10 seconds and the section turned blue. They were after that,counter-stainedwith 1% alcohol ascending grades of alcohol, Eosin, for 1 minute. Followed by rapid dehydration through ascending grades of alcohol; cleared in xylene; and mounted with DPX mountant. Stained sections were viewed under a light microscope, and a photomicrograph of the stained tissue was taken.

Statistical analysis

The statistical analysis of this research was done using ANOVA and student's t-test of SPSS version 23 software package and $P \leq 0.5$ was considered as the level of significance.

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RESULTS

Table 1.Comparison offollicle stimulating hormone (FSH) and estradiol on female Wistar rats.

		Mean ±STD	**P**
FSH (ng/ml)	Control	5.60±0.00	
	Group B	6.85±0.07	0.002*
	Group C	6.00±0.14	0.069
	Group D	6.00±0.28	0.069

Estradiol (ng/ml)	Control	26.5±3.53	
	Group B	21±4.24	0.145
	Group C	19.5±2.12	0.083
	Group D	20±1.41	0.099

*P<0.05 means significant. The above data was analyzed using One Way Anova and PostHoc LSD.

The FSH level of the control group averaged 5.60±0.00; that of group B was 6.85±0.07; that of group Cwas 6.00±0.14; and that of group Dwas 6.00±0.28. According to the PostHoc results, compared with the control group, the FSH of the low dose group was significantly higher (p=0.002). The medium-dose group was insignificantly higher (p=0.069). The high dose was also insignificantly higher (p=0.069).

The estradiol level of the control group averaged 26.5±3.53; that of group B was 21±4.24; that of group C was 19.5±2.12; and that of group D was 20±1.41. According to the PostHoc results, compared with the control group, the estradiol of the low-dose group was insignificantly lower (p=0.145), that of medium-dose group was insignificantly lower (p=0.083), and that of thehigh dose was also insignificantly lower (p=0.099).

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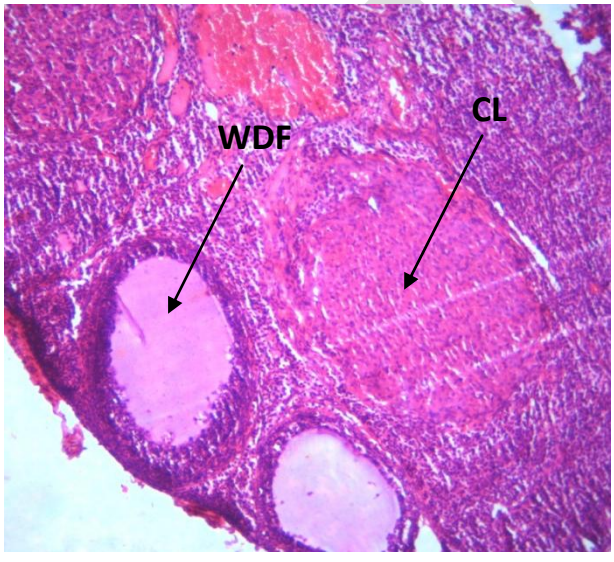

Fig 1.Photomicrograph of group A (Control) section of ovary(x400)(H/E) shows normal ovarian tissue with large corpus luteum (CL) and well-developing follicles (WDF).

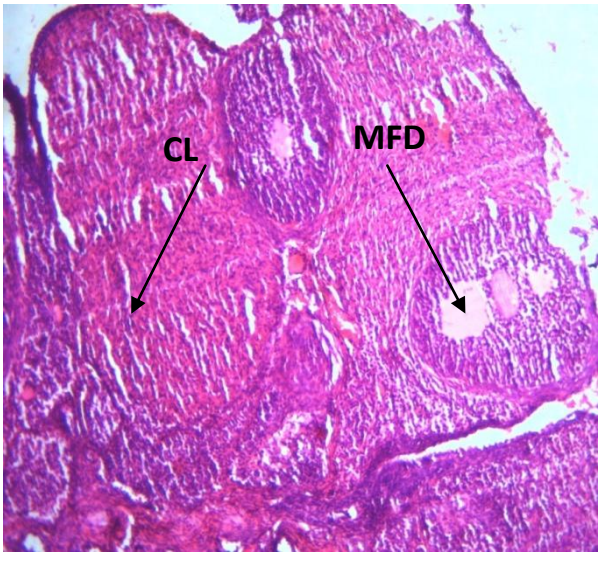

Fig 2.Photomicrograph of group B section of ovary administered with low dose of cucumber (x400)(H&E) shows moderate increase in the follicular development(MFD) and corpus luteum (CL) that appear normal.

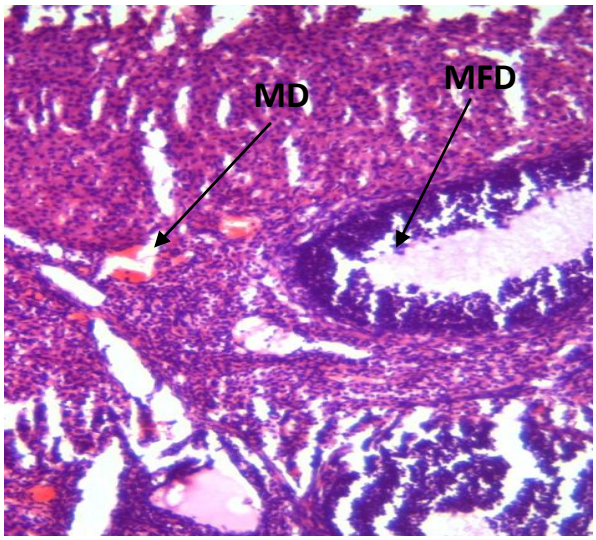

Fig 3.Photomicrograph of group C section of ovary administered with mild dose of cucumber (x400)(H&E) shows amild increase in the follicular development (MFD) and moderate distortion of the architecture.

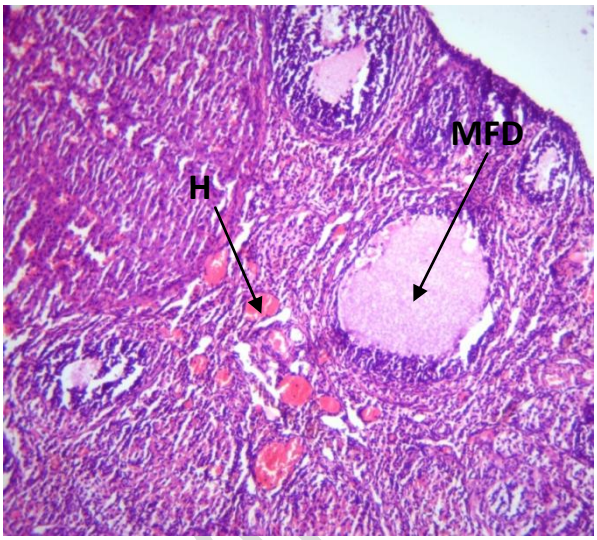

Fig 4.Photomicrograph of group D section of ovary administered with a high dose of cucumber (x400)(H&E) shows a moderate increase in the follicular development (MFD) and mild areas of hemorrhage (H) otherwisenormal.

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DISCUSSION

"Evidence abounds that plant derivatives have therapeutic potential against vast human, animal, and plant diseases and thus, plants have become indispensable to human and animal existence" (Ogbonnia et al., 2008). "Consisting mostly of water and important electrolytes, cucumber was shown to lower stress, and fight against the aging process, has a cleansing action within the body by removing accumulated pockets of waste materials and chemical toxins and has good antioxidant activitysource" (Kaur, 2010).

This study used 16 adult female Wistar ratsas working models to test the effect of cucumber fruit extract on the ovaries and sex hormones.Result from Table 1 shows that *Cucumissativus*administration causes asignificant increase in the follicle-stimulating hormone level in group B and a non-significant increase in groups C and D when compared to the control. In the estradiol level, there was a non-significant decrease in the hormone level in all the treatment groups compared to the control group. These differences in action can be attributed to the estrus cycle of the female Wistar rats as each cycle affects the hormone level in the rats corresponding to the cycle or phase. *Cucumissativus*has been shown to have numerous beneficial active phytochemicals,including alkaloids, flavonoids, tannins, phlorotannins, steroids, saponins, and phytonutrients such as Vitamin C (Gupta and Prakash, 2009; Agte*et al*., 2000; ofoego*et al*., 2019).

Histopathological examination of the ovaries shows amild increase in the follicular development with corpus luteum that appears normal. This is due to the protective effects of *Cucumissativus*,which is rich in phytonutrients and free radical scavenging properties and

agrees with the findings of Obeten*et al*. (2019), Soliman*et al*. (2015), Andreia*et al.* (2013), and Kumar *et al*. (2010).

CONCLUSION

This study indicates the near-toxic effects of *Cucumissativus.* Itshows that it has beneficial effects on the female reproductive organs and hormones when consumed appropriately but disastrous when consumed in large amounts.

Ethical approval

Ethical approval was obtained from the Ethical Committee, Faculty of Basic Medical Science, Abia State University,UturuAbia State.

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