

Effects of *Cucumis sativus* 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 main stay in the treatment of diseases and preservation of human health. Cucumber is one of the plants that have nutritional values.

Aim: This study was conducted to investigate the effect of *Cucumis sativus* extract on the ovaries, and sex hormones of adult female Wistar rats.

Study Design and Methodology: Sixteen Wistar rats weighing about 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 while 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 ovaries were 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 FSH 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 when compared to the control. Histopathological studies show little distortion in the histomorphology of the ovaries in groups C and D.

Conclusion: *Cucumis sativus* extract when consumed in large amount has deleterious effects on the female reproduction system.

Keywords: *Cucumis sativus*, follicle stimulating hormone, estradiol, ovary, histomorphology

INTRODUCTION

Plants have been used as medicinal materials for thousands of years and remain relevant as a natural source of active compound for treatments of human diseases (Cragg and Newman, 2013). Many medicinal plants are used to treat various reproductive function ailments such as female infertility which is a public health concern in sub-Saharan African countries (Lienou *et al.*, 2010). Cucumber (*Cucumis sativus* 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 overall toxicology. The commonly used conventional combined oral pills are usually associated with numerous adverse effects, necessitating indigenous drugs. The use of plants for medicinal and mythological purposes, and for solving problems related to ill health 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 male and female. Citrulline induces blood flow into the penis which is a way to heal erectile dysfunction in males and also increases the number of egg production in females during ovulation (Patil *et al.*, 2012). This study investigates the effects of *Cucumis sativus* extract administration on the histomorphology of the ovaries and on the sex hormones of adult female Wistar rats.

MATERIALS AND METHODS

Location and duration of the study

This study was carried out in Human Anatomy Department, Abia State University, Abia State, Nigeria. The rats were made to acclimatize for a period of two weeks after which the test substance was administered for 28 days; the entire experiment lasted for five weeks.

Ethical approval

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

Preparation of the extract

Fresh fruits of *Cucumis sativus* (cucumber) 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 *Cucumis sativus* was sifted with a 1 mm² sieve to increase extraction efficiency. After that, about 100g was weighed and then submerged in 1000ml of distilled water and extracted using an inverter condenser on a 70°C water bath for two hours. Thereafter, the extract was filtered using Whatmann filter paper No. 1 and then the solvent 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 becomes 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 are made.

Experimental design

They were divided into 4 groups (A to D) each and were 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 for a period of 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 only 250mg/kg (0.4ml) body weight daily for three weeks

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

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

All administration was done with canula and syringes, orally.

Extraction of the organs and collection of samples

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

Histological analysis

The ovaries of the experimental rats were removed and preserved in labelled bottles containing 10% formal saline. These were allowed to stand for 72 hours to achieve good tissues 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°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 5 μ thickness using rotary microtome. The section were floated in warm water (28°C) and then taken up on aluminized glass slides. They were air-dried and stained using the H&E. They were dew axed in xylene for 2 minutes per 2 changes. Xylene was cleared in 95% alcohol for another minute. The section were washed well in running tap water for 15 minutes, differentiated in 1% alcohol for 5-10 seconds section turned blue. They were thereafter counter stained with 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 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.

RESULTS

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

		Mean \pm STD	P
FSH (ng/ml)	Control	5.60 \pm 0.00	
	Group B	6.85 \pm 0.07	0.002*
	Group C	6.00 \pm 0.14	0.069
	Group D	6.00 \pm 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 C was 6.00±0.14; and that of group D was 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); that of medium dose group was insignificantly higher (p=0.069), and that of 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 insignificantly lower (p=0.145); that of medium dose group was insignificantly lower (p=0.083), and that of high dose was also insignificantly lower (p=0.099).

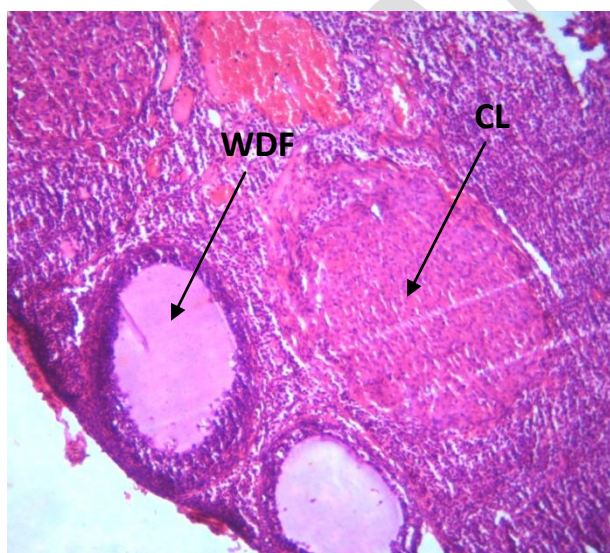


Fig 1. Photomicrograph of group A (Control) section of ovary (x400)(H/E) shows normal ovarian tissue with large corpus luteum (CL) and well developing follicles (WDF).



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.

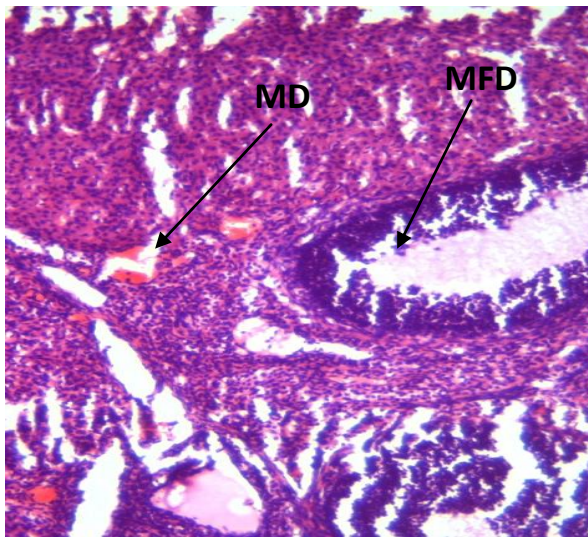


Fig 3. Photomicrograph of group C section of ovary administered with mild dose of cucumber (x400)(H&E) shows mild increase in the follicular development (MFD) and moderate distortion of the architecture.

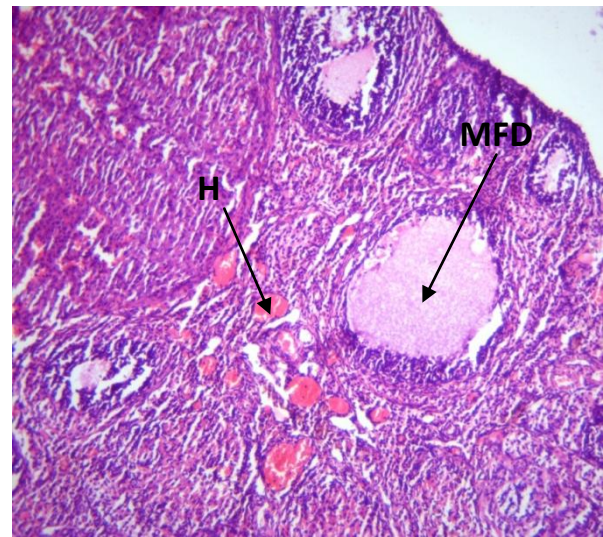


Fig 4. Photomicrograph of group D section of ovary administered with high dose of cucumber (x400)(H&E) shows moderate increase in the follicular development (MFD) and mild areas of haemorrhage (H) otherwise

DISCUSSION

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

In this study, 16 adult female Wistar rats were used as working models to test the effect of cucumber fruit extract on the ovaries and sex hormones. Result from table 1 shows that *Cucumis sativus* administration causes significant 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 when compared to the control group. This differences in action can be attributed to the estrus cycle of the female Wistar rats as each cycle affect the level of each hormone in the rats corresponding to the cycle or phase. *Cucumis sativus* has been shown to have numerous beneficial active phytochemicals including alkaloids, flavonoids, tannins, phlobatannins, 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 mild increase in the follicular development with corpus luteum that appears normal. This is due to the protective effects of *Cucumis sativus* 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 *Cucumis sativus* and shows that it has beneficial effects in the female reproductive organs and hormones when consumed appropriately but disastrous when consumed in large amount.

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