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Research Article

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Microanatomical and Hormonal Studies of the Effects of Aqueous *Cannabis sativa* Leaf Extract on the Testis of Adult Wistar Rats

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ABSTRACT

Background: *Cannabis sativa* is a psychoactive drug from the green, leafy *Cannabis* plant used in Asian traditional medicine for the treatment of varieties of diseases including inflammation, nausea, headache, hematochesia, diarrhea and alopecia due to its cannabinoid and tetrahydrocannabinol properties.

Methods: Thirty-six adult (36) adult male wistar rats divided into four groups (Groups A, B, C & D) were used for this study. Group A was given normal saline, B 200 mg/kg, C 500 mg/kg & D 800 mg/kg of *C. sativa* orally for 14 days. Ichroma quantitative testosterone method (fluorescence immunoassay) was used to determine testosterone in serum by screening and monitoring androgen levels.

Results: Serum testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels decreased significantly ($p<0.05$) in the experimental groups compared to the control especially for group D (800 mg/kg). Testicular parenchyma showed degeneration of sertoli cells as well as a decrease in spermatogenic cells and sperm motility.

Conclusion: Cannabinoid had negative effects on sertoli cells; sperm function and decreases luteinizing hormone, follicle-stimulating hormone and testosterone levels in adult wistar rats.

Key-words: *C. Sativa*, Cannabinoid, Fertility, Hormone, Sertoli cells

INTRODUCTION

Cannabis, also known as marijuana among other names [1] is a psychoactive drug from the Cannabis plant used primarily for medical or recreational purposes [2]. The main psychoactive component of cannabis is tetra hydro cannabinol (THC), which is one of the 483 known compounds in the plant [3] including at least 65 other cannabinoids. Cannabis can be used by humans by smoking, vaporizing, ingested as food, or as an extract [4]. Cannabis has mental and physical effects on individuals who ingest it ranging from "high", or stoned feeling, general change in thought and perception, difficulty concentrating, impaired short-term memory, altered sense of time, impaired body movement, relaxation [5] and a sharp increase in appetite, otherwise known as "munchies". The onset of effects is felt within few minutes when smoked, and about 30 to 60 minutes when cooked and eaten [6]. The effects usually last for about two to six hours post-ingestion, depending on the quantity or dose consumed [6].

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At high doses, mental effects sometimes include psychosis, delusions, hallucinations, paranoia, and ideas of reference, sometimes with anxiety and panic [7]. Its physical effects include increased heart rate, difficulty breathing, nausea, and behavioral problems in children whose mothers used cannabis during pregnancy. Short-term side effects may include a dry mouth, red eyes, and feelings of paranoia or anxiety [8]. Long-term adverse effects may include addiction, decreased mental ability in those who started regular use as adolescents, chronic coughing, and susceptibility to respiratory tract infections [9]. Heavy, long-term exposure to *C. sativa* may have biologically-based physical, mental, behavioral and social health consequences and maybe "associated with diseases of the liver, lungs, heart, and vasculature" [10].

Drug abuse has been shown to affect gonadal hormones in an unusual physiological phenomenon [11] and quite several studies have examined the effects of cannabis smoking on the reproductive system. Cannabis has been reported to cause downregulation of hypothalamic-pituitary-gonadal axis, endocrine disruption, and hyperprolactinemia [12]. Significant shrinkage of tubular diameter and detrimental changes in the seminiferous epithelium of testis with resulting lowered serum testosterone and pituitary gonadotropins (FSH and LH) levels at low doses [13]. Grossman [14] reported that *Cannabis* affects 'swimming behaviour' of sperm but the fertility effects associated with Cannabis is unclear. Therefore this work is aimed at investigating the histochemical effects of aqueous *C. sativa* leaf extract on the testis of adult wistar rats.

MATERIALS AND METHODS

Plant Materials- The leaves of the plant, *C. sativa* were harvested from plants grown in Kwale, Delta State, Nigeria with approval of the National Drug Law Enforcement Agency (NDLEA). The leaves were identified by a crop scientist (Dr. Ejike Ikenganyia) of the Department of Agronomy and Ecological Management, Faculty of Agriculture and Natural resources Management, Enugu State University of Science and Technology, Agbani, Enugu, Nigeria on October 2019.

Preparation of *Cannabis* extract- The sample was dried in air for 7 days and after complete drying was crushed in the form of fine powder with a super Sony Japan electric blender. 200 g of dried plant extract was immersed in 2000 mls of water and was evaporated with a Soxhlet extractor to yield 21.2 g of gel extract, which was stored at room temperature until ready for use.

Animal management and grouping- Thirty-six (36) adult male wistar rats weighing between 220 to 250 g were used for the study and were kept in a plastic cage with iron nettings. They were placed in a well ventilated standard room with twelve hours light and twelve hours darkness. The animals were acclimatized for two weeks before the commencement of administration. During this period, the animals were observed to ensure that they were disease-free and were fed with rat chows, given water ad libitum, at an ambient temperature range of 25–27 maintained at 50% humidity. The animals were weighed with an electronic weighing balance before the commencement and termination of the administration of cannabis. Animals were divided into four groups (A, B, C and D) with nine (9) rats in each.

Animal Treatment/administration- Group A received normal saline, group B received 200 mg/kg of cannabis, group C received 500 mg/kg, while group D received 800 mg/kg of cannabis daily between 8.00–11.00 hours for fourteen (14) consecutive days. Route of administration was via oral.

Table 1: Animal grouping and administration

Groups	Dosage of cannabis	Duration
A	Normal saline	14
B	200 mg/kg	14
C	500 mg/kg	14
D	800 mg/kg	14

Collection of Tissues- Twenty four (24) hours after the last day of administration, the animals were weighed, anesthetized with ketamine (80 mg/kg IP) and xylazine (11 mg/kg IP) and blood sample was taken by cardiac puncture within seventeen (17) minutes. The testis was then removed and preserved in 10% buffered formalin for 48 hours.

Biochemical analysis (Hormonal Assay)- The collected blood was allowed to clot and the serum put in a test tube and centrifuged at 300 rev per minute. The supernatants were collected with the aid of a pasteur pipette as sample.

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Ichroma quantitative testosterone method (fluorescence immunoassay) was used to determine testosterone, follicle stimulating hormone and Luteinizing hormone levels in serum [19]. Testosterone is used as an aid in the screening and monitoring of androgen level.

Statistical Analysis- Statistical testing was carried out using SPSS version 23, with P≤0.05 as level of significance. Newman-Keuls Multiple comparison test was used to compare measurability between experimental groups and control group (one way ANOVA).

Amscope 5.0 digital microscope was used to make the micrographs after processing the testis in the histology laboratory.

Ethical Approval- The study was carried out by the principles of laboratory animal care and standard experimental procedures. It was approved by The Ethical Committee of College of Medicine, Enugu State University of Science and Technology, Enugu, Nigeria.

RESULTS

Table 2 shows how the consumption of *C. sativa* affected the secretion of hormones across groups. The group administered with 800 mg/kg of *C. sativa* had the lowest hormonal secretion level. Thus, cannabis extracts reduced testosterone, FSH and LH levels.

Table 2: Showing the distribution of effect of *C. sativa* on the testicular hormones

	Control	200 mg/kg	500 mg/kg	800 mg/kg	p-value
Testosterone (mμ/ml)	2.16±0.08	1.82±0.10	1.48±0.10	1.23±0.03	0.00
LH (mμ/ml)	2.50±0.05	1.89±0.02	1.02±0.04	0.40±0.06	0.00
FSH (mμ/ml)	1.62±0.07	1.08±0.04	0.49±0.01	0.29±0.02	0.00

There was a decrease in levels of testosterone, LH and FSH across experimental groups compared to the control group. Not statistically significant. Fig. 1 shows that the graphical representation among the relationship between the rate of Testosterone, FSH and LH levels secreted across the groups given various doses of *C. sativa* and comparing it to the control group. Hormonal levels reached a peak of reduction at 800 mg/kg of *C. sativa*.

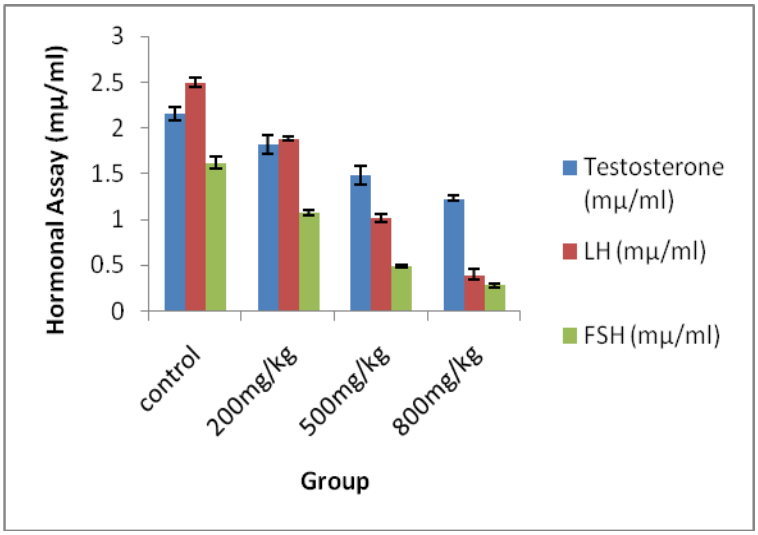

Fig. 1: Comparison of hormonal assay among groups in response to various doses of *C. sativa*

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Serum testosterone, FSH and LH decreased significantly among the experimental groups compared to the control as doses increased (P<0.05), one-way ANOVA shown in Fig. 2.

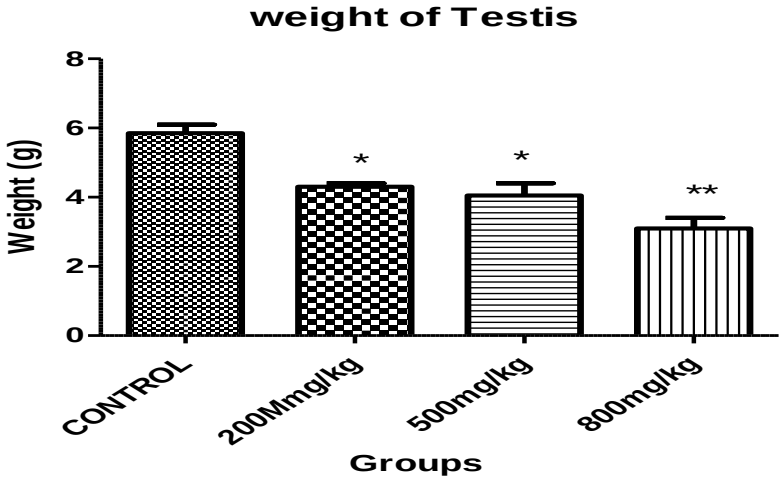

Fig. 2: Testicular weight: Newman-Keuls Multiple comparison test between experimental groups and control group with P≤0.05 (one way ANOVA)

There was a significant difference between the weight of the testis between the control group and all the experimental groups. Level of comparison of weight difference of testis between the control and group D (800 mg/kg of *C. sativa*) was higher than groups B (200 mg/kg) and C (500 mg/kg). P=0.0085 (statistically significant).

Histological findings

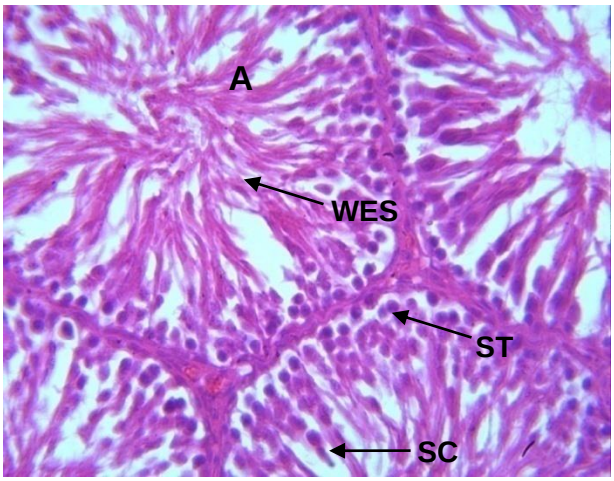

Fig. 3: Photomicrograph of A control section of testis (x400)(H/E) shows normal testicular architecture with seminiferous tubule that lined with sertoli cell (SC) and well-enhanced spermatogenesis (WES).The overall feature appears normal H&E x100

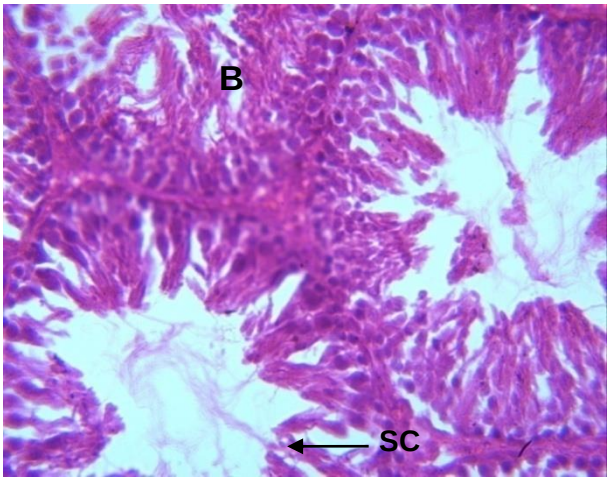

Fig. 4: Photomicrograph of B2 section of testis administered with 200 mg/kg marijuana for 2 weeks (x400)(H/E) shows mild to moderate damage on the spermatogenic area with mild arrest of spermatogenesis (MAS) H&E x100

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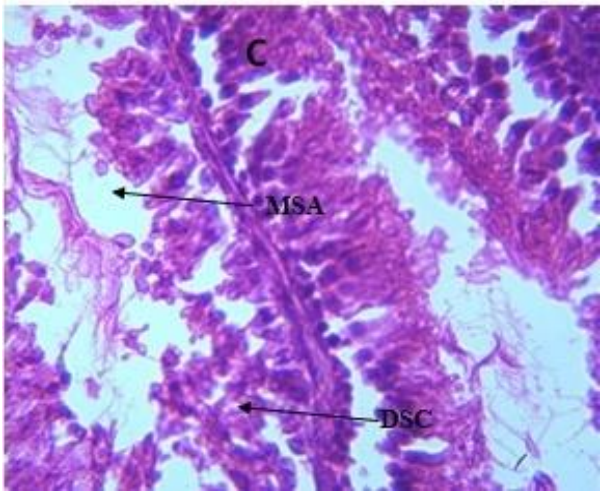

Fig. 5: Photomicrograph of CT section of testis administered with 500 mg/kg marijuana for 2 weeks (x400)(H/E) shows moderate damage on the testicular tissue with moderate degeneration of sertoli cell (DSC) and moderate spermatogenic arrest (MSA) H&E x100

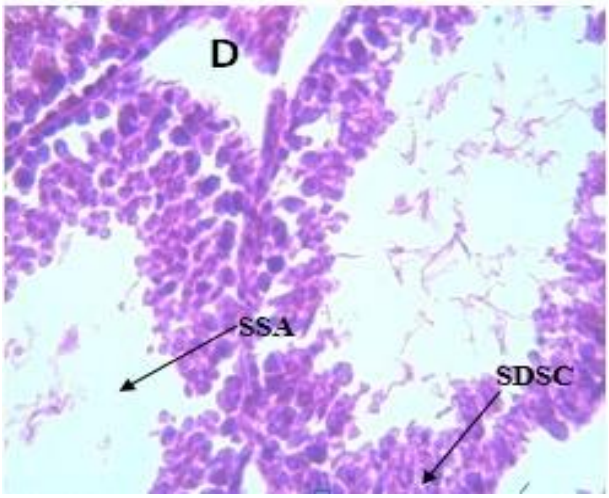

Fig. 6: Photomicrograph of DL section of testis administered with 800 mg/kg marijuana for 2 weeks (x400)(H/E) shows severe damage on the with degeneration of sertoli cell (DSC) and sever spermatogenic arrest (SSA) H&E x100

DISCUSSION

This study investigated the biochemical, morphological and histological effect of *C. sativa* on the testis of albino wistar rats. Testosterone, follicle-stimulating and luteinizing hormone levels were checked. In this study, *C. sativa* extract caused significant decrease in hormonal levels ($p<0.05$), when compared with the control in all the hormones assayed, testosterone (2.16±0.08, 1.23±0.03), luteinizing hormone (2.50±0.05, 0.40±0.06) and follicle-stimulating hormone (1.62±0.07, 0.29±0.02) especially at 800 mg/kg. This may be due to the temporary impairment of cannabinoids in the pituitary function reflected in decreased LH and FSH levels and hence reduced testosterone levels. The decreased serum concentrations of testosterone and LH in our findings, was consistent with results of findings from Elazeem *et al.* [11] and also concurs with studies of Adu [15] and Kolodny [16]. The decrease in testosterone could be attributed to inhibition of the gonadotrophin releasing hormone in the hypothalamus by 9-tetrahydrocan nabinol (THC) [17]. Thus, 800 mg/kg of *C. sativa* caused significant decrease in hormonal level in serum.

C. sativa extracts also had significant effects in the weight distribution of the testis of animals used for this study. Fig. 2 revealed that the weight of the testis of animals in group D (800 mg/kg) reduced drastically (SEM±3.1 g) against the weight of the control group (SEM±5.85). That of group B (200 mg/kg) weighed an average of ±4.3, while that of group C (500 mg/kg) weighed an average of ±4.03. Payne and his group of researchers conducted a research on effects on *C. sativa* extract on sperm parameters and testis and also observed that there was slight decrease in testicular weight of wistar rats used for the study [18].

Histological findings showed that 500 mg/kg and 800 mg/kg of *C. sativa* caused cellular damages in the spermatogenic area of the seminiferous tubules of the testis of adult wistar rats as revealed by Fig. 5 and 6. Also, sertoli cell population was reduced in the germinal epithelium of the seminiferous tubules as shown in Fig. 6 administered with 800 mg/kg of *C. sativa*. In the study of Mandal and Nas [13], this was a prognosis of testicular regeneration and is consistent with the histological findings of a significant reduction in tubular diameter and detrimental changes in the seminiferous epithelium of the testes, resulting in lower serum testosterone and pituitary gonadotropin (LH and FSH) levels in the study.

CONCLUSIONS

The present research has shown that *C. sativa* has an adverse effect on the serum concentration of gonadotrophic hormones and also reduced sperm population in the germinal area of the seminiferous tubules of the testis of adult wistar rats. This exposure of an individual to *C. sativa* could lead to a significant

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reduction of testosterone levels and subsequently cause sertoli cells dysplasia. Thus, could lead to sub-fertility issue in a man. Also, testicular weight reduction in animals used for this study indicates effects of ingestion of *C. sativa* extracts and finally corroborating that extract can has significant effect on testicular weight, which constitutes part of the total body weight of an individual. Thus, can resultantly contribute to body weight loss, hence more research should advocate screening of gonadal hormones among acute drug abusers in addition to other metabolic and psychiatric support. Further studies should also be carried out on any possible effect of *C. sativa* on the interstitial cells of the testis.

CONTRIBUTION OF AUTHORS

Research concept- Dr. Ignatius Ikemefuna Ozor

Research design- Dr. Ignatius Ikemefuna Ozor, Onyinye Mary Ozioko, Uche Sebastine Ozioko, Christian Ejuiwa Mba, Dr. Ifeanacho Abireh, Offor Chukwuebuka Blessed

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Data collection- Dr. Ignatius Ikemefuna Ozor, Christian Mba

Data analysis and Interpretation- Dr. Ignatius Ikemefuna Ozor, Christian Mba

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Article editing- Dr. Ignatius Ikemefuna Ozor, Christian Mba, Ozioko Onyinye

Final approval- Dr. Ignatius Ikemefuna Ozor

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