Research Article

Effect of Ethanolic Extract of *Cannabis sativa* on Progesterone and Estrogen Hormones in Female Wistar Rats

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Abstract

Cannabis sativa have been suggested to disrupt the normal ovulatory cycle and hormonal secretion in animals. In this study, we investigated the changes in progesterone and estrogen levels in female Wistar rats as a result of oral administration of ethanolic extract of *C. sativa* for 7, 14 and 21 days consecutively. A total of 18 female rats were used and selectively divided into three groups (A, B and C) based on their body weight. Group A was control group while Group B and C were test groups and were given 200 ml and 400 ml/kg body weight of the extract respectively. At the end of each week, blood sample was collected from the various groups and subjected to estrogen and progesterone hormonal assay. The estrogen levels observed in Groups B and C were 6.33 ± 5.30 and 2.23 ± 1.07 respectively after 21 days. At the end of the experiment, a significant reduction in progesterone and estrogen levels was observed in the test groups. The present findings demonstrate that medium term (21 days) treatment of female rat with ethanolic extract of *Cannabis sativa* results to a significant decrease in progesterone and estrogen levels thereby leading to female sexual dysfunction directly or indirectly.

Keywords: *Cannabis sativa*; Estrogen; Progesterone; Tetrahydrocannabinol; Wistar rats

Introduction

Cannabis sativa also known as the hemp plant is a diecious, green leafy plant believed to have originated from Central Asia. It has through man's activities been distributed widely around the world. The main psychoactive constituent of Cannabis is tetrahydrocannabinol (THC).

Tetrahydrocannabinol (THC) has mild to moderate analgesic effects. It can be used to treat pain by altering transmitter release on the dorsal root ganglion of the spinal cord and in the periaqueductal grey. The psycho active effects of THC are primarily mediated by its activation of Cannabinoid receptor type-1 CB1 G-protein coupled receptors which result in a decrease in the concentration of cyclic Adenosine monophosphate (cAMP) through inhibition of adenylate cyclase [1].

A principal site of tetrahydrocannabinol action is the hypothalamus [2]. The hypothalamus has a central neuro-endocrine function, most notably by its control of the anterior pituitary which in turn regulates various endocrine glands and hormones [3]. Among the various hormones secreted in the hypothalamus is the Gonadotropin releasing hormone which stimulates the release of Follicle Stimulating hormone (FSH) and Luteinizing Hormone (LH) (known as the gonadotropins) from the anterior pituitary [4]. The female reproductive hormones Estrogen and Progesterone are regulated by the gonadotropins and in turn the gonadotropins are regulated by negative feedback from the estrogen. So, Follicle stimulating hormone and luteinizing hormone stimulate Estrogen and Progesterone [5].

The female sex steroid 17β-estradiol, have long been known to play critical roles in the development of feminine secondary sexual characteristics as well as in the female reproductive cycle, infertility, and maintenance of pregnancy.

Estradiol is also thought to be essential for embryonic and fetal development [6]. Supporting evidence for the crucial role of estrogen is based on pharmacological and genetic data that estrogen antagonists and inhibitors of estrogen synthesis interfere with placental function and cause abortions [6]. There is no doubt that estrogen plays a central role in normal postnatal female physiology and in female pathology [7-9]. Alterations in estrogen level may directly or indirectly lead to female sexual dysfunction.

One of progesterone's most important functions is to cause the endometrium to secrete special proteins during the second half of the menstrual cycle, preparing it to receive and nourish an implanted fertilized egg. If implantation does not occur, estrogen and progesterone levels drop, the endometrium breaks down and menstruation occurs. Progesterone also encourages the growth of milk-producing glands in the breast during pregnancy. Maintaining menstrual cycles, a function of progestins, is important during childbearing years.

Estrogen and Progesterone are 2 crucial ovarian steroids that play vital roles in the female ovarian and menstrual cycle. Estrogen together with Progesterone promotes and maintains the lining of the uterus for implantation of fertilized egg and maintenance of uterus function during gestation. They regulate cellular functions in the brain that control sexual behaviour in female rat [10-12]. It has been suggested that steroid hormone receptors mediate certain neurobehavioral effects of THC [13].

The present study was designed to examine the relationship between administrations of *C. sativa* extract and the ovarian steroid levels in female Wistar rats. To this end, we used the levels of these steroids in the rats to examine the time and dose dependent effect of *C.*

sativa extract on ovarian steroid levels. These experiments substantiate the requirement for the relationship between time and dose dependent administration of *Cannabis sativa* extract and decline in the levels of the ovarian steroids.

Materials and Method

18 Female Wistar rats where commercially obtained from the supplier (Abia state, Nigeria). The animals were housed in a wired mesh cage (6 rats per cage). They were kept under hygienic and favorable conditions, and maintained under a 12 h light/12 h dark cycle, with pelletized rat feeds (UAC Vital Feeds®, Nigeria) and water available ad libitum and were in compliance with Federal guidelines for animal care. The animals were allowed to acclimatize for a week before the commencement of administration of extract.

Sample collection

Dried leaves of *Cannabis sativa* were procured from Delta state, Nigeria. It was properly identified at the Herbarium unit of the Plant Science and Biotechnology Department, University of Port-Harcourt, Nigeria.

Chemicals

All chemicals and reagents used were of analytical grade quality. Estrogen (17 β-Estradiol) and Progesterone (HRP Conjugate) were purchased from Randox. Trimethylbenzidine (TMB) reagent was obtained from Sigma Aldrich. Ethanol and Methylated spirit were purchased from Sigma. Estradiol ELISA kit and Progesterone ELISA kit were obtained from Randox.

Ethanolic extraction of *Cannabis sativa*

The Soxhlet extraction method [a] employed for the extraction of the ethanolic extract of *C. sativa.* The extraction method is based on the solubility of the constituents of the Cannabis sample in ethanol. 400 g of dried *Cannabis sativa* leaves was folded into a filter paper and placed into the thimble of the Soxhlet extraction apparatus extraction chamber. The thimble is loaded into the main chamber of the Soxhlet extractor. A flat bottom flask was oven dried for 5 mins and the weight taken. 500 ml of ethanol was poured into the distillation flask which is then attached to the extractor. The sample was extracted for 12 hours at 4 cycles per hour.

After extraction, the solvent was removed by the means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remaining in the thimble was discarded. The weight of the extract was calculated after deducting the original weight of the bottom flask.

Weight of the extract was 26 g.

Derivation of test concentrations

26 g of *Cannabis sativa* was extracted, 1 g was gotten out of the 26 g of the extracted *Cannabis sativa* to obtain a standard concentration. The 1 g was then diluted with 1000 ml of distilled water. This was the stock solution. From the stock solution, two different concentrations were prepared for the 2 experimental groups.

Group B – 200 ml solution with concentration of 0.2 g Cannabis extract

Group C – 400 ml solution with concentration of 0.4 g Cannabis extract.

Dose selection

The dose levels of 200 ml and 400 ml/kg body weight were selected for the present study.

Treatment

Total of 18 rats were divided into 3 groups as control (A), mid dose (B) and high dose (C). Group A was administered water and feed only for the 3 weeks of the experiment. Average body weight of the female rats was 95.18 mg/kg.

The experimental animals were maintained under normal diet and water ad libitum throughout the period of the experiment. During the three-week dosing period, all the animals were observed daily for clinical signs and mortality patterns once daily. The summary of the daily dosage administration plan is given in Table 1 below;

Week	Group B	Group C
1	5.60	5.80
2	5.30	5.64
3	4.91	5.34

Table 1: Daily dosage administration plan.

At the termination of study, the Estrogen and Progesterone levels at Day 7, Day 14 and Day 21 were evaluated in the 3 groups.

Measurement of progesterone and estrogen (17β-estradiol)

The blood samples were centrifuged for 10 mins using 40 revolutions/minute with a bucket centrifuge. After centrifugation, serums were isolated using a micropipette. Serum Progesterone (ng/ml) and Estradiol (ng/ml) were determined by ELISA using MAP LAB PLUS (Biochemical Systems International, RM 2060) according to manufacturer's direction.

Statistical analysis

The mean ± S.E.M was calculated for each parameter. Total variations present in a set of data were estimated by one way analysis of variance (ANOVA), followed by Dunnet's test. $P \leq 0.05$ was considered as statistically significant when compared to control.

Results and Discussions

Concentration (ml/kg body weight)	Estrogen (ng/ml) Day 7	Estrogen (ng/ml) Day14	Estrogen (ng/ml) Day21
0.00	7.87 ± 0.61	8.23 ± 0.21	2.00 ± 1.73
200	8.03 ± 0.06	8.00 ± 0.02	6.33 ± 5.30
400	7.79 ± 0.15	8.40 ± 0.40	2.23

Table 2: Effect of ethanolic extract of *Cannabis sativa* on estrogen level of female Wistar rats.

In the present study a time dependent reduction in Estrogen level was observed in the 3 groups as shown in Table 2. In the control group, the estrogen levels for Day 7, 14 and 21 were 7.87 ± 0.61, 8.23 ± 0.21, 2.00 ± 1.73 and 2.00 ± 1.73 ng/ml respectively. A sudden rise in Estrogen (17β-estradiol) level was recorded in the Day 14 of the control group. This may be attributed to the onset of the ovulatory phase.

Estradiol concentration measured in rat serum on days 7, 14 and 21 of administration of ethanolic extract of Cannabis sativa were higher when compared to Sprague Dewey rats that were administered Atrazine, Zearalenone and a combination of Atrazine and Zearalonone [14]. In the test groups B and C, there was no significant difference in the estrogen (17β- Estradiol) level in the female Wistar rats when compared to the control group (A) after 14 days of Cannabis sativa extract administration. However, a significant reduction was observed in the two test groups B and C with estrogen concentration of 6.33 ± 5.3 and 2.23 ± 1.07 respectively after 21 days. This is in line with previous studies on the action of Cannabis sativa on female reproductive hormones [15-17].

From the results in Table 3, it was observed that the female Wistar rats administered 200 mg and 400 mg/kg body weight of the ethanolic extract respectively showed a significant increase in the level of serum Progesterone after 14 days when compared to the control group. The percentage increases were B (68.17%) and C (72.76%) for Day 7 while B (61.28%) and C (66.43%) were observed in Day 14 of the experiment.

Concentration (ml/kg body weight)	Progesterone (ng/ml) Day 7	Progesterone (ng/ml) Day 14	Progesterone (ng/ml) Day 21
0.00	2.89 ± 0.26	3.33 ± 0.28	2.67 ± 1.53
200	9.08 ± 1.24	8.06 ± 0.60	2.07 ± 0.50
400	10.61 ± 2.73	9.92 ± 0.81	5.13 ± 3.40

Table 3: Effect of ethanolic extract of *Cannabis sativa* on progesterone level of female Wistar rats.

However, within the test groups B and C, there was a time dependent reduction in the serum concentration of Progesterone. The percentage reduction from Day 7 to Day 21 in group B (77.20) was higher compared to that of group C (51.65). This suggests that the 200 mg/kg body weight extract administration was more effective in reducing the Progesterone level compared to the 400 mg/kg body weight extract administration. The hormones estrogen and progesterone need to be in balance as they both enhance the action as well as offset the action of the other [18].

Conclusion

Cannabis sativa have been shown to disrupt the normal ovulatory and hormonal secretion in both animals and humans. However, similar to findings in males and females tolerance may develop over time [18]. It therefore could be inferred from the result that long term administration or use of the *Cannabis sativa* extract can bring about estrogenic and progesteronic effects as evidenced in the result. These effects may directly or indirectly affect female menstral cycle and ultimately undermine the reproductive health in female.

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