Melatonin Mitigates Hormonal Toxicity in Cannabis -Treated Female Wistar Rats

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Abstract

Cannabis which is obtained from the flowering tops, leaves and resin of the female plant of *Cannabis sativa* L. (family *Cannabidaceae*), is the most commonly abused illicit drugs worldwide. It is also one of the commonly abused substances by women of childbearing age. Researchers have shown that Cannabinoids have negative effects on the activity of gonadotropin releasing hormone (GnRH)-secreting neurons by direct and indirect mechanisms. Thus, researches are needed to mitigate these side effects of *Cannabis-sativa*(CS) because of its recreational purposes which are increasing rapidly. This study investigated the effects of CS on hormonal toxicity and the ameliorative role of melatoninin female rats.

CS significantly ($p<0.05$) decreased gonadotropin releasing hormone (GnRH), follicle stimulating hormone (FSH), luteinising hormone (LH), Oestrogen (E), progesterone and prolactin levels of the cannabis-treated female ratsrespectively. However,all these hormones were significantly ($p<0.05$) reducedby co-administration of CS with melatonin to the level comparable to the control.

This study showed the hormonal toxicity caused by CS which could be mediated by endocrine disruption in female rats. However, these effects could be ameliorated by melatonin. Since the consumption of CS is increasing globally because of its medical uses leading to its legalization, therefore, consumption of melatonin as supplement may be recommended for its users.

Key words: *Cannabis sativa*, Melatonin, Reproductive hormones, Toxicity

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Introduction

Cannabis is obtained from the flowering tops, leaves and resin of the female plant of *Cannabis sativa* L. (family *Cannabidaceae*), is the most commonly abused illicit drugs worldwide[1] with medicinal uses.[2] The active component, Δ^9-tetrahydrocannabinol (Δ^9-THC), has been used for treating migraine headache, glaucoma, nausea, and anorexia. However, its detrimental effects on reproductive system have been reported. Marijuana is one of the commonly abused substances by women of childbearing age.[3] It has been reported that about 64-79% of female are cannabis users' nationwide[3] which can lead to pregnancy loss.[4] Cannabinoids have also been reported to have negative effects on the activity of gonadotropin releasing hormone (GnRH)-secreting neurons by direct and indirect mechanisms.[5,6,7] Moreover, it has been shown to have direct oestrogenic effect on the uterus [8] leading to the binding of 3β-estradiol to oestrogen receptors.[9] It also has direct effect on the ovary [10,11]thus, inhibiting ovarian prostaglandin synthesis which is involved in follicular rupturing during ovulation.[11]CBR1 is mostly expressed in the central nervous system (CNS) where it mediates the central cannabinoids actions. Peripherally, its expression has been shown to be present in the reproductive tissues.[12]In addition, CB1 has been found to also be present in the ovary, uterine endometrium, testis, vas deferens, urinary bladder, and other peripheral endocrine and neurological tissues.[13] CB2 receptors, in contrast, have a fairly limited distribution, being found predominantly in immune cells,[14]mast cells,[15] immune cells (B and natural killer cells),[16] hippocampus, striatum, midbrain and cerebellum,[17] but it has now been localized in other tissues, such as central neurons[18], human placenta,[19] myometrium[20] and ovary.[21]

Melatonin (N-acetyl-5-methoxytryptamine) is expressed in the darkness because its highest level always coincides with the dark phase of light/dark cycle.[22] It is secreted in the pineal gland and other extra-pineal sources like retina, gut, skin, bone marrow, lymphocytes, and ovaries.[23] Its ability to scavenge free radicals like hydroxyl radical (OH), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), hypochlorous acid (HOCl), peroxynitrite anion ($ONOO^-$), nitric oxide (NO), and others in many

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conditions [2,4,1]directly by free radical scavenging actions.[25]Its role in reproduction has been contradictory, as both detrimental and beneficial effects have been reported.[6,7,26,27]

This study investigated the effects of cannabis on hormonal toxicity together with the ameliorative effects of melatonin in female rats.

Materials and methods

Animals

Twenty (20) female albino rats (150-180g) were used for this experiment. They were obtained from the Department of Biochemistry, University of Ilorin,Ilorin, Kwara State, Nigeria, were housed at room temperature with unrestricted access to diet and water and maintained on a daily light/dark cycle. Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed.

Extraction of *Cannabis sativa* leaves

Extraction of *Cannabis sativa* (*CS*), which was kindly donated by National Drug Law Enforcement Agency (NDLEA), Nigeria, for research purpose only, was done with Soxhlet apparatus by soaking 800 grams of CS in 98% ethanol for 48 hours. It was filtered and the filtrate was poured into a round bottom conical flask which was fixed with a rotary evaporator. It was then evaporated and cooled. The dried yield of the extract was 62%.

Experimental protocol

After 2 weeks of acclimatization,twenty femalerats were assigned into four groups of five animals each, such that the rats in groups I, II, III and IV received orally 1mL distilled water, 2mg/kg body weight (BW) of EECS, 2mg/kg BW of EECS plus 4mg/kg BW of melatonin and 4mg/kg BW of melatonin, respectively for 14 days. Gonadotropin releasinghormone(GnRH),Luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol (E),progesterone and prolactin were determined using standard methods. Treatment was done orally and once daily in the morning. Animals were sacrificed under ketamine anesthesia on the fifteenth (15th) day.

Drug and assay kits

Melatonin was a product of Sigma Aldrich Company, Mannheim, Germany. The gonadotropin releasing hormone (GnRH), luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol (E),progesterone and prolactin assay kits were products of Monobind Inc., Lake Forest, California, USA. All other chemicals used were productsof Sigma Aldrich Company, Mannheim, Germany.

Preparation of serum

The female rats were **sacrificed under** ketamine anesthesia and blood was collected from the heart puncture into sample bottles. The blood was left for 30 min to clot and thereafter centrifuged at 625×*g* for 10 min using a Uniscope Laboratory Centrifuge (Model SM800B, Surgifield Medicals, Essex, England). The serum was collected into plain bottles with the aid of a Pasteur pipette. Sera were stored in a freezer maintained at -5 °C and used within 12 hours of preparation.

Quantification of reproductive hormones

The serum hormone concentrations of GnRH, FSH, LH, E, progesterone and prolactin were quantified according to the instruction provided by assay kit manufacturers, using microplate immunoenzymometric (EMA/ELISA) assays. The serum hormone concentrations were then interpolated from their respective calibration curves. The analyzer was calibrated and validated for use with rat sera.

Statistical analysis

Results were expressed as the mean ± standard error of mean. Data were analyzed using a one-way analysis of variance, followed by the LSD post-hoc test to determine significant differences in all the parameters with Students Package for Social Science, version 20.0 (SPSS Inc., Chicago, USA). Differences with valuesof $P<0.05$ were considered statistically significant.

Results

EECS significantly ($p<0.05$) decreased GnRH level. However, there was significant ($p<0.05$)increasedin GnRH level when the CBRs were blocked separately. It was evident that administration of EECS with either of the blockers and melatonin significantly ($p<0.05$) increased the GnRH level (fig. 4.1).

EECS significantly ($p<0.05$) decreased FSH level. However, there was significant ($p<0.05$)increasedin FSH level when the CBRs were blocked separately. It was evident that administration of EECS with either of the blockers and melatonin significantly ($p<0.05$) increased the FSH level (fig. 4.2).

EECS significantly ($p<0.05$) decreased LH level. However, there was significant ($p<0.05$)increasedin LH level when the CBRs were blocked separately. It was evident that administration of EECS with either of the blockers and melatonin significantly ($p<0.05$) increased the LH level (fig. 4.3).
EECS significantly ($p<0.05$) decreased estradiol level. However, there was significant ($p<0.05$)increasedin estradiol level when the CBRs were blocked separately. It was evident that administration of EECS with either of the blockers and melatonin significantly ($p<0.05$) increased the estradiol level (fig. 4.4).

EECS significantly ($p<0.05$) decreased

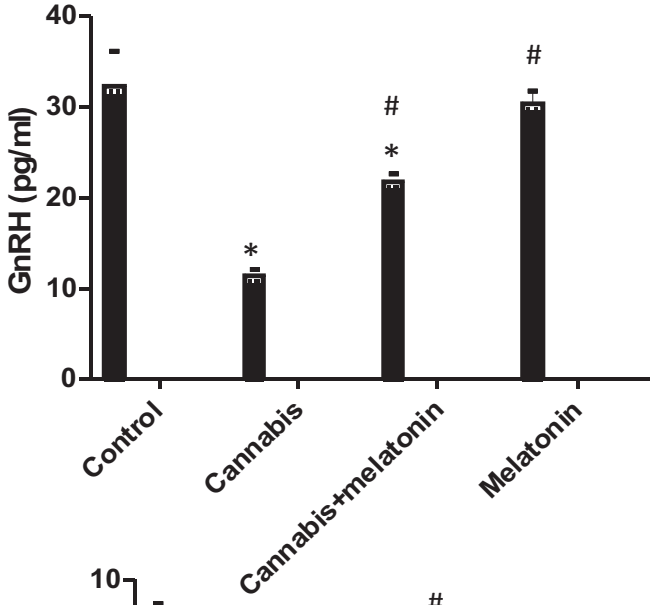


Fig. 4.1: GnRH of rats that received EECS, CBRB1 (rimonabant hydrochloride), CBRB2 (AM630), CBRB1+Cannabis, CBRB2+Cannabis, CBRB1+CBRB2+Cannabis, melatonin, CBRB1+Cannabis+Melatonin and CBRB2+Cannabis+melatonin. Values are expressed as mean±S.E.M;*p<0.05vsControl;#p<0.05vscannabis.

NB: CBRB1–Cannabinoid receptor blocker 1; CBRB2 – Cannabinoid receptor blocker 2, CBRs–Cannabinoid receptors, GnRH-Gonadotropin releasing hormone, mel- melatonin

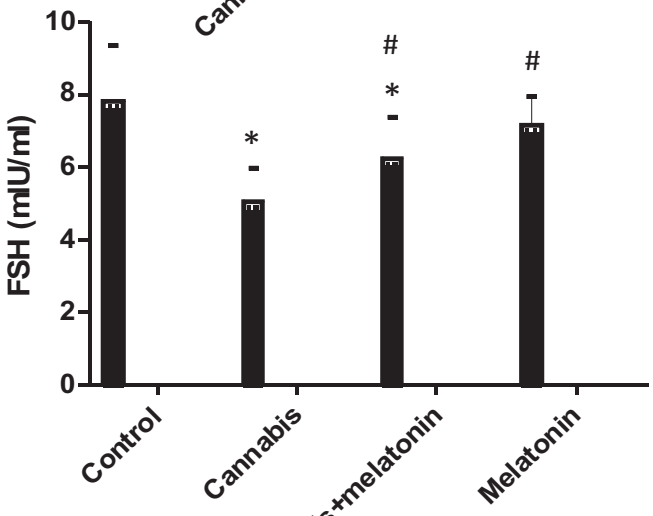


Fig. 4.2:FSH of rats that received EECS, CBRB1 (rimonabant hydrochloride), CBRB2 (AM630), CBRB1+Cannabis, CBRB2+Cannabis, CBRB1+CBRB2+Cannabis, melatonin, CBRB1+Cannabis+Melatonin and CBRB2+Cannabis+melatonin. Values are expressed as mean±S.E.M;*p<0.05vsControl;#p<0.05vscannabis.

NB: CBRB1–Cannabinoid receptor blocker 1; CBRB2 – Cannabinoid receptor blocker2, FSH-Follicle stimulating hormone, mel- melatonin

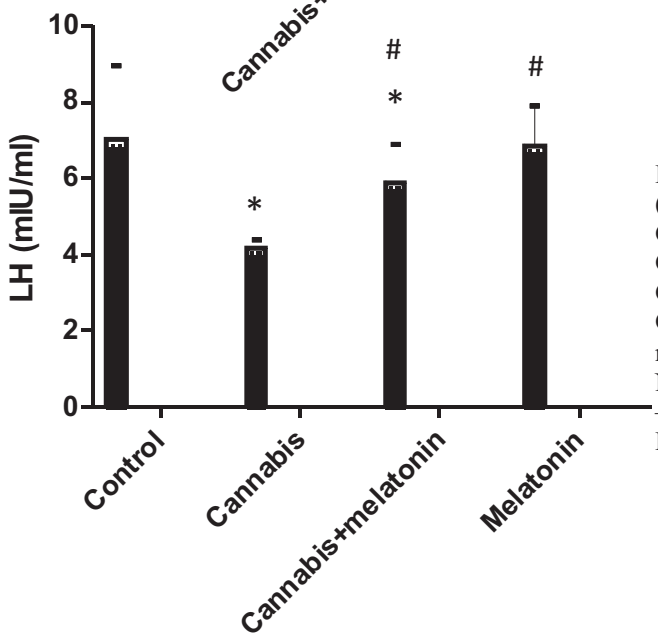


Fig. 4.3:LH of rats that received EECS, CBRB1 (rimonabant hydrochloride), CBRB2 (AM630), CBRB1+Cannabis, CBRB2+Cannabis, CBRB1+CBRB2+Cannabis, melatonin, CBRB1+Cannabis+Melatonin and CBRB2+Cannabis+melatonin. Values are expressed as mean±S.E.M;*p<0.05vsControl;#p<0.05vscannabis.
NB: CBRB1–Cannabinoid receptor blocker 1; CBRB2 – Cannabinoid receptor blocker2, LH-Luteinising hormone, mel- melatonin

progesterone level. However, there was significant (p<0.05)increasedin progesterone level when the CBRs were blocked separately. It was evident that administration of EECS with either of the blockers and melatonin significantly (p<0.05) increased the progesterone level (fig. 4.5).

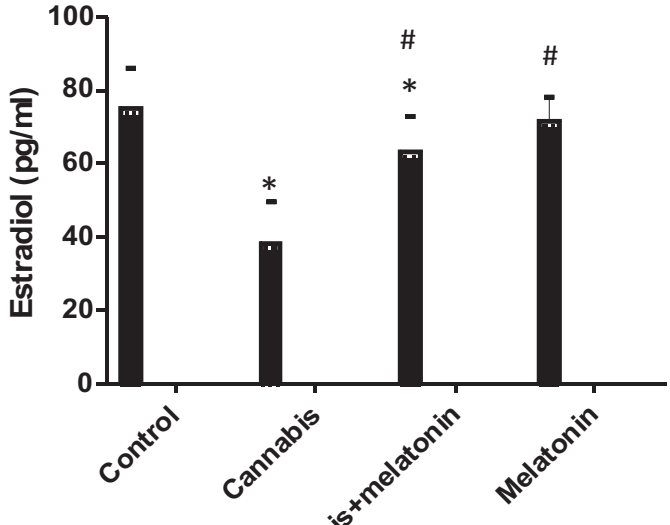


Fig. 4.4: Estradiol of rats that received EECS, CBRB1 (rimonabant hydrochloride), CBRB2 (AM630), CBRB1+Cannabis, CBRB2+Cannabis, CBRB1+CBRB2+Cannabis, melatonin, CBRB1+Cannabis+Melatonin and CBRB2+Cannabis+melatonin. Values are expressed as mean±S.E.M;*p<0.05vsControl;#p<0.05vscannabis.

NB: CBRB1–Cannabinoid receptor blocker 1; CBRB2 – Cannabinoid receptor blocker 2,

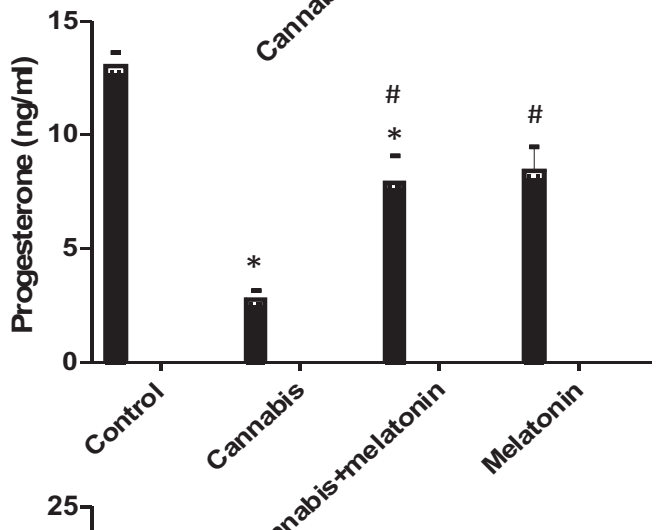


Fig. 4.5: Progesterone of rats that received EECS, CBRB1 (rimonabant hydrochloride), CBRB2 (AM630), CBRB1+Cannabis, CBRB2+Cannabis, CBRB1+CBRB2+Cannabis, melatonin, CBRB1+Cannabis+Melatonin and CBRB2+Cannabis+melatonin. Values are expressedasmean±S.E.M;*p<0.05vsControl;#p<0.05 vscannabis.

NB: CBRB1–Cannabinoid receptor blocker 1; CBRB2 – Cannabinoid receptor blocker2,

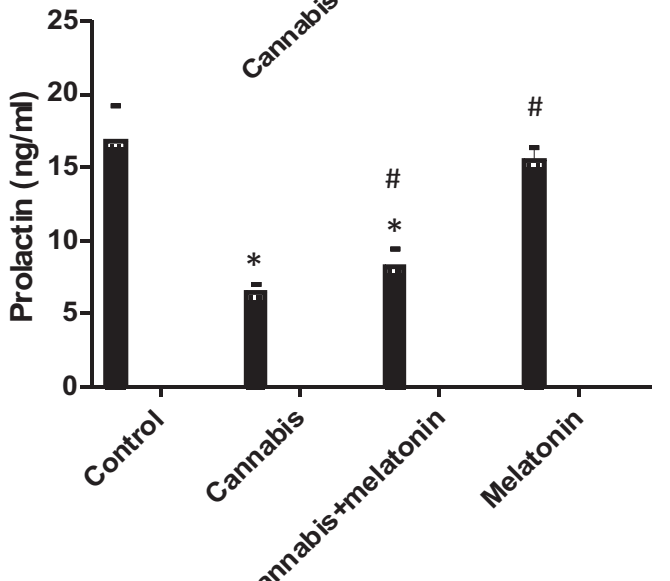


Fig. 4.6: Prolactin of rats that received EECS, CBRB1 (rimonabant hydrochloride), CBRB2 (AM630), CBRB1+Cannabis, CBRB2+Cannabis, CBRB1+CBRB2+Cannabis, melatonin, CBRB1+Cannabis+Melatonin and CBRB2+Cannabis+melatonin. Values are expressed as mean±S.E.M;*p<0.05vsControl;#p<0.05vscannabis**.**

EECS significantly ($p<0.05$) decreased prolactin level. However, there was significant ($p<0.05$)increasedin prolactin level when the CBRs were blocked separately. It was evident that administration of EECS with either of the blockers and melatonin significantly ($p<0.05$) increased the prolactin level (fig. 4.6).

Discussion

Study has shown that the entire endocannabinoid system is active at the ovarian level and CB1R and CB2R and anadamide (AEA), have been identified in ovarian tissue[19]. Immunostaining shows expression of CB1R and CB2R in the medulla and cortex of the ovary. In the cortex, the receptors are

expressed in the granulosa cells of primordial, primary, secondary and tertiary follicles and in the theca cells of secondary and tertiary follicles. Both receptors have also been observed in the corpus luteum and corpus albicans. In vertebrates, many authors have reported inhibitory effects exerted by endocannabinoids on the reproductive physiology in both sexes.[28]In this study, there were significant decrease ($p<0.05$) in GnRH, FSH, LH, estradiol and progesterone levels in the cannabis-treated rats which were partially abolished when CB_1 and CB_2 receptors were blocked separately but the effects were more in the former than in the latter. This could be due to the inhibitory effect of cannabis on the GnRH neurons in the hypothalamus.[29]These effects were ameliorated by melatonin when the cannabiniod receptors (CBRs) were stimulated and blocked. Some studies have reported increase in prolactin level following the administration of cannabis to both male and female rats.[30,31]However, in this study, there was significant($p<0.05$) decrease in the prolactin level in the cannabis-treated rats which was partially abolished when CB_1 and CB_2 receptors were blocked separately but the effect was more in the former than in the latter. This could be due to the stimulation of the activity of dopaminergic neurons in the hypothalamus by the cannabis.[32] This effect was ameliorated by melatoninwhen the cannabiniod receptors (CBRs) were stimulated and blocked.In contrast, pathological changes such few acidophils (the cells that contain the polypeptide hormones such as prolactin) and basophils (the cells that contain glycoproteins hormones such as LH and FSH) were found in pituitary gland of the rats treated with CS. Also, the hypothalamus of cannabis-treated rats showed few nissl bodies (which are proteinious in nature and are also essential for the production of GnRH hormones) compared to other groups. These pathological changes were ameliorated by melatonin.

Conclusion

This study concluded that the gonadotoxic effects of CS could be mediated by endocrine disruption. In addition, both cannabinoid receptors 1 and 2 contribute to CS-induced hormonal toxicity, though that of former is more than the latter. However, these effects could be ameliorated by melatonin. Since the consumption of CS is increasing globally because of its medical uses leading to its legalization, therefore, consumption of melatonin as supplement is recommended for its users to prevent its gonadotoxic effects.

Conflict of Interests

The authors declare no conflict of interests. The authors alone are responsible for the writing and content of the papers.

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