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Original article

Anxiety, depression-like behaviors and biochemistry disorders induced by cannabis extract in female mice

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ABSTRACT

Cannabis is an annual herbaceous plant sometimes grown for decoration and used as bird food that looks like flax. The study wanted to determine if a Cannabis extract may have an effect on how anxious and depressed the female mice behaved. forty healthy female mice were divided into four groups. Tap water was administered to the first group (control). Ethanol was administered to second group (positive control). The third and four groups were given 1 and 2 mg/kg cannabis extract respectively. Treatment continued for 14 days. After therapy, the light–dark chamber, forced swimming, tail suspension, plus lamb and open field tests were done to assess anxiety and depressive behavior. The results indicated that the anxiety and depression were increased in treated females significantly compared to control. Biochemical results showed that DA,5-HT, AChE, GSH, GST, CAT and SOD were decreased while TBARS, corticosterone and cortisol were increased. In conclusion, cannabis effects this kind of females' behavior but the mechanisms are not clear yet. We need more researches on this trend.

1. Introduction

Pot, weed, grass, ganja, and skunk are some of the terms commonly used to describe the dried leaves of the Cannabis plant (Okon et al., 2014). Cannabis (Table 1) is an annual herbaceous plant sometimes grown for decoration and used as bird food that looks like flax, found in warm and tropical regions. It is 5 m long with a resin ring, and the leaves are 10 cm long and 1.5 cm wide (Okon et al., 2014; Alagbonsi and Olayaki, 2016). Cannabis is the world's most common illicit substance. The UN Office on Drugs and Crime estimates 2.7–4.9% of adults use cannabis worldwide. In Ghana (21.5%), Zambia (17.7%), Canada (17.0%), the United States of America (12.3%) and New Zealand, the prevalence is particularly high in the case of cannabis usage (13.3%) (Bruijnzeel et al., 2016). It has long been used medicinally for therapeutic uses (treatment of spasticity, movement disorders, asthma, treatment of allergies and as a muscle relaxant). The cancer, multiple sclerosis and chronic pain are also used (Alagbonsi and Olayaki, 2016). However, this is the world's most extensively used illegal drug, which contains about 500 chemicals (Yassa et al., 2010). The main psychoactive component of cannabis is delta-9-tetrahydrocannabinol (Δ9-THC, Fig. 1.1), and its effects have been extensively researched (Bruijnzeel et al., 2016). Including (THC), where the autoradiography shows that (THC) interferes with the normal nerve receptors in the brain, and these receptors are responsible for learning, memory, etc. Resulting in a feeling of sedation, a decrease in body temperature and a lack of movement coordination (Grotenhermen, 2007). THC's pharmacology is due to its partial agonistic activity on the cannabinoid CB1 receptor largely situated in the central nervous system (Pertwee, 2006). It also causes mental health detriments, such as poor memory, poor attention span and addiction (Bruijnzeel et al., 2016).

The results of users' cannabis range from euphoric, anxious and increased risk of depression to paranoia, anxiety and elevated (Kardash et al., 2019). It has also been confirmed that cannabis

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Table 1
Cannabis classification.

Domain	Eukaryota
Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Clade	Rosids
Family	Cannabaceae
Order	Rosales
Genus	Cannabis
Species	Cannabis Sativa

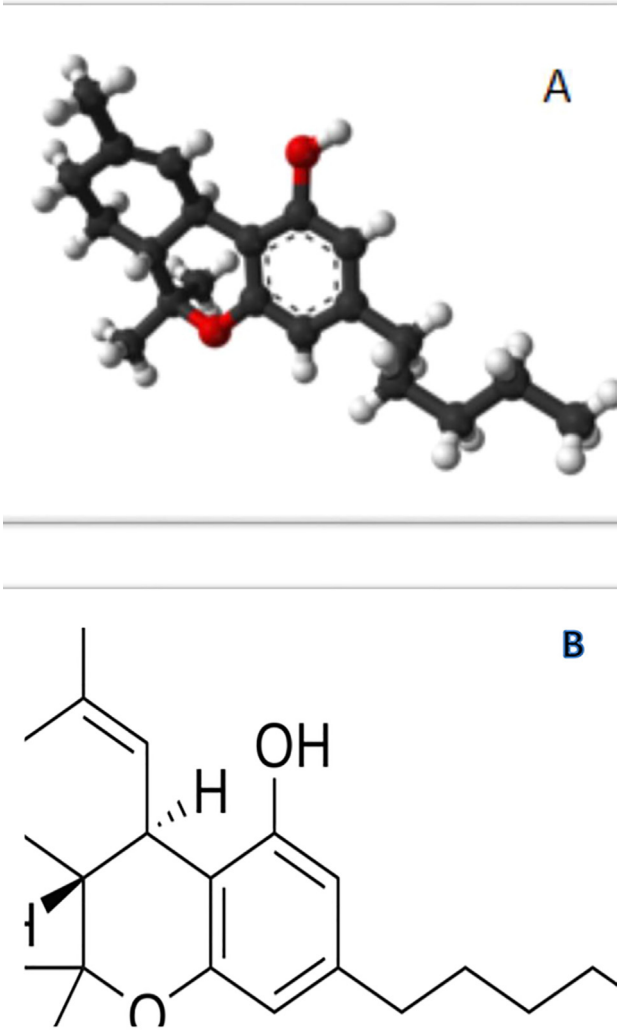


Fig. 1.1. A and B: Tetrahydrocannabinol (THC).

sativa is one of the causes of personal schizophrenia, and that taking cannabis sativa in the long term reduces motor activity and exploration and increases fear (Okon et al., 2014), or increase the causes of anxiety. Some research shows that some male genital parameters such as testosterone secretion, sperm production, motility and vitality have fallen and that the production of LH has fallen.

Cannabis decreases regulation of pituitary and gonadal hyperprolactinemia, according to scientists Alagbosi and Olayki (Alagbonsi and Olayaki, 2016). Effects Cannabis sativa extract also in 2013 have been investigated with oxidative stress and organ tissue damage during systemic inflammation. It increases brain, heart, lung, liver and renal tissue damage (Abdel-Salam et al., 2014). Serotonin (5-HT) and dopamine (DA) are neurotransmitters that are involved in pain, depression and other conditions. Few studies have investigated the influence of 5-HT neurotransmission of CBD in the dorsal raphe nucleus (DRN) of both mood disorders in the brain (De Gregorio et al., 2019). De Gregorio et al (De Gregorio et al., 2019) observed that cannabidiol (CBD) exposure resulted in a decrease in 5-HT. The purpose of this study was to assess the impact of Cannabis extract on the biochemistry, anxiety, and depression behaviors of female mice.

2. Materials and methods

2.1. Animals

Swiss–Webster strains were maintained in opaque plastic cages measuring 30 livres 12 to 12 to 11 cm at biology department, college of science, Jazan University. , Jazan, Saudi Arabia reversed 12-hour lighting conditions and the ambient temperature (18–22 °C). According to ethical criteria adopted by the local ethics and care committee for experimental animals, all methods for treatment and use of laboratory animals have been followed.

2.2. Cannabis extract and doses preparation

Calibration was done in GC MS-QP2010, is a single quadrupole gas chromatograph-mass spectrometer offering stable and cost-effective analysis for challenging compounds, for the purpose of determining the quantity of the three main compounds in the extract which are (THC, CBN, and CBD). For this purpose, three standards for the three compounds (THC, CBN and CBD) were used. The concentrations were calculated starting from half a microgram to 10 μg, and calibration is a measure of the area under the curve so that if the curve becomes larger or longer it indicates an increase in the presence of compounds present. Then sample was prepared, where 10 g of cannabis resin were taken and weighed on the scale, and then the sample was dissolved in pure ethanol so that in every milliliter of pure ethanol the sample was completely dissolved, then the filtration action step by a nylon filter measuring 0.45 μm, the sum of the extract was 100 ml. Then the sample was diluted 100 times and two tubes were injected into the GC MS-QP2010 device, and the result for each main compound was obtained with THC = 2.41 mg/ml, CBN = 0.59 mg/ml, CBD = 2.65 mg/ml and the total compounds were 5.6 mg/ml in the filter, then the filter was diluted 20 times to obtain a concentration of 0.28 mg/ml, and from here we can inject the animal with the appropriate dose: the dose low: 1 mg for 1 kg = 0.03 mg for 30 g)Mouse weight(and the dose high: 2 mg for 1 kg = 0.06 mg for 30 g (Mouse weight), whereas extract concentration = 5.6 mg/ml and diluted concentration = 0.28 mg/ml.The dose was extracted for a single mouse in the following way: C1 × V1 = C2 × V2 then compensate first in the low dose(1 mg) :0.28 × ? = 0.03 × 1000 ml = 107 ml for one mouse. Then in the high dose (2 mg) :0.28 × ? = 0.06 × 1000 ml = 214 ml for one mouse (de A. Leite et al., 2018).

2.3. Management and experimental methodology on cannabis

Four groups of ten animals were assigned to the female mice. As a negative control, Group I consumed tap water. Ethanol was administered to Group II (positive control). Cannabis was given in Group III and IV for 14 days at 1 and 2 mg/kg. With the exception

of experimental handling, all animals had free access to food and water. Further grouped all groups of animals into four subgroups:

1. Sub-Group I has undergone anxiety and
2. Anxiety for the light–dark transitional trial of lawnmowers.
3. Enforced swimming testing was conducted on Sub-Group II.
4. Sub-Group I was subjected to an open-field test for depression tail suspension and anxiety.

2.4. Body weight measurement

For every 3 days, the female body weight was recorded.

2.5. Behavioral studies

2.5.1. Depression tests

2.5.1.1. The light–dark chamber test. Female mice underwent the light–dark test with different conditions for this test utilizing two light/dark plywood boxes for 5 min (Mohammad Abu-Taweel and Al-Fifi, 2021; Bourin and Hascoët, 2003). After 5 min, the mouse was taken out of the box and returned to their cage. 70% ethyl alcohol was used for purifying the labyrinth to minimize potential unspecific scents between the testing. The computations included: Dark chamber entry delay, number of transitions, locomotives, rear and wall numbers, posture stretch, tanning, length of time spent in dark and light chambers.

2.5.1.2. Forced swimming test. The Forced Swimming Test (FST) is known as the behavioral despair test, which was conducted by a subgroup of female mouse. It is a test focused on a rodent's response to the threat that it drops into a cylindrical tank of clean water for 6 min whose outcome has been used to assess susceptibility to bad mood and to use several indices to measure animal desperation (Mohammad Abu-Taweel and Al-Fifi, 2021; Petit-Demouliere et al., 2005).

2.5.1.3. Tail suspension test. TST is more sensitive to antidepressants than FST since the TST is longer in motion for the animal than FST the tail suspension test is more sensitive than the FST (Cryan et al., 2005). TST (6 min) is used to quantify stress in rodents and to measure other treatments that should influence depression-related behaviours. TST (6 min) (Mohammad Abu-Taweel and Al-Fifi, 2021; Abu-Taweel, 2020; Abu-Taweel, 2020).

2.5.2. Anxiety tests

2.5.2.1. Plus-maze test. The elevated plus-maze (with two open and two closed arms) is commonly used to examine risk assessment and anxiety responses in ethologically derived animal models. The duration of the test was 300 sec which started by putting the female in middle space his head towards the open arm (Mohammad Abu-Taweel and Al-Fifi, 2021; Wall and Messier, 2001).

2.5.2.2. Open – Field test. Another effective experiment to assess general levels of locomotive activity, anxiety, and readiness to explore in rodents is the open field test (Stanford, 2007). In this study, the female is isolated and maintained for 14 days in groups of 2 or 3. Thereafter, a locomotive activity test for 300 sec will be done for 10 females of each treatment group. Different compartmental aspects reported have been observed (Abu-Taweel, 2020; Mohammad Abu-Taweel and Al-Fifi, 2021; Belovicova et al., 2017).

2.6. Biochemical analyses of brain tissues

The forebrain has been extracted and frozen into liquid nitrogen to determine the neurotransmitters to conduct biochemical inves-

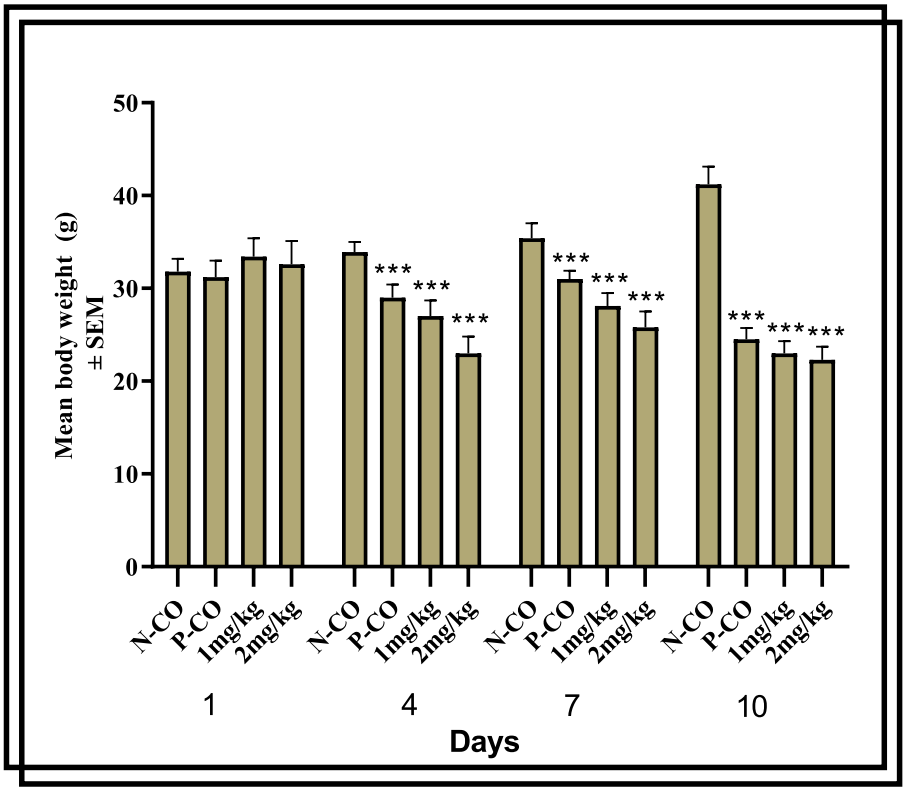


Fig. 3.1. Effect of cannabis exposure on female mice body weight*** represent statistically at $p < 0.001$.

tigations. Dopamine (DA) and serotonin or 5-hydroxytryptamine (5-HT) monoamine transmitters have been evaluated in accordance with Patrick et al (Patrick et al., 1991). The Ellman et al (Ellman et al., 1961) technique has been estimated for acetylcholinesterase (AChE). Non-enzymatic oxidative stress indicators have also been identified. The use of UV–visible sample-photometers (TBARS) was used to determine lipid peroxides (Ohkawa et al., 1979). The Mangino et al (Mangino et al., 1991) approach tested decreased glutathione (GSH) levels. Estimates were made of the activities glutathione-s-transferase (GST), catalase (CAT) and dismutase of superoxide (SOD) (Misra and Fridovich, 1972).

2.7. Hormone's analysis

After behavioral tests, blood was aspirated from male mice directly from the animal's heart in heparin-coated tubes. Some blood samples were used to estimate the concentration of corticosterone and cortisol. The plasma was separated by using a refrigerated centrifuge at 10000 rpm for 10 min and finally the testosterone and cortisol levels were analyzed using the ECLIA method according to (Abu-Taweel, 2020).

2.8. Statistical analysis

All data were evaluated using one-way ANOVA comparing experimental groups, followed by the student-Newman-Keuls multiple comparison test. The significance values were set at p can be lower than 0.05, 0.01 and 0.001 (Khan et al., 2019; Khan et al., 2015).

3. Results

3.1. Body weight

In Fig. 3.1 demonstrated that cannabis exposure resulted in a substantial ($p < 0.001$) drop in body weight when compared to the control group.

3.2. Behavioral tests

3.2.1. Depression tests

3.2.1.1. The light–dark chamber test. Exposure to cannabis increased ($p < 0.001$) depression in light–dark box elements test (Fig. 3.2,A-I).

3.2.1.2. Forced swimming test. In Fig. 3.3, A – F indicated that the treated females spent less time in swimming than control group do.

3.2.1.3. Tail suspension test. Cannabis exposure increased immobility in treated female mice as compared to their controls (Fig. 3.4, A, B and C).

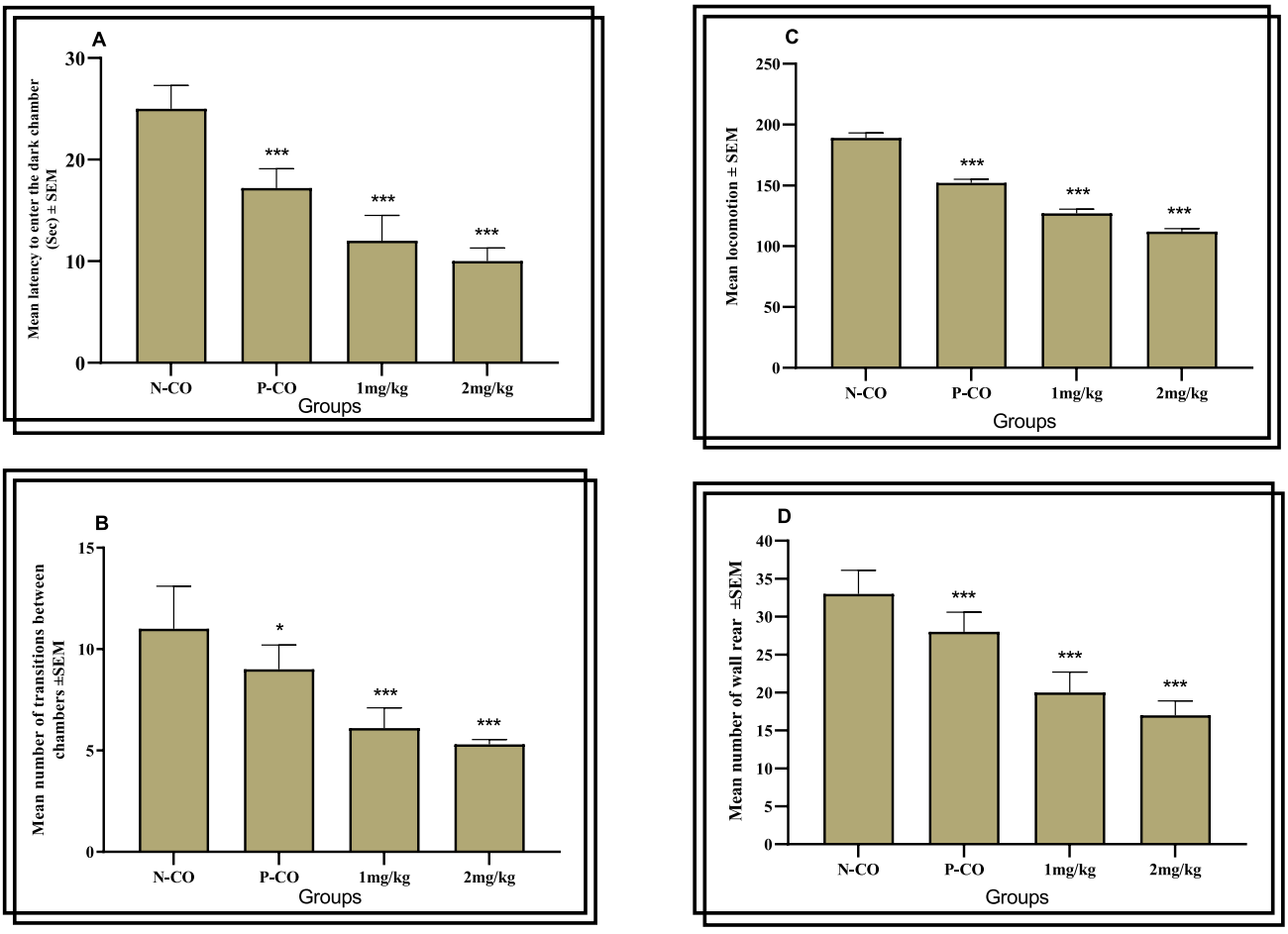
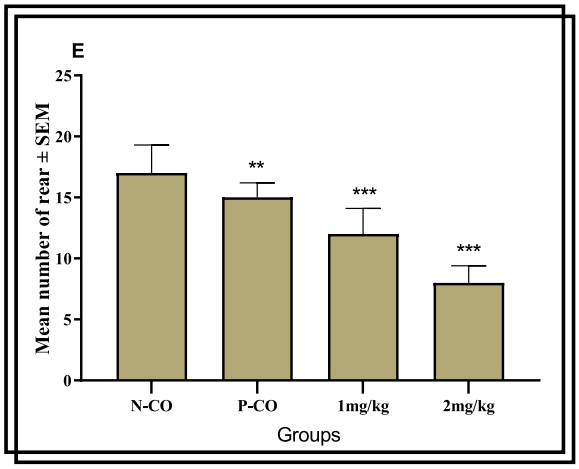
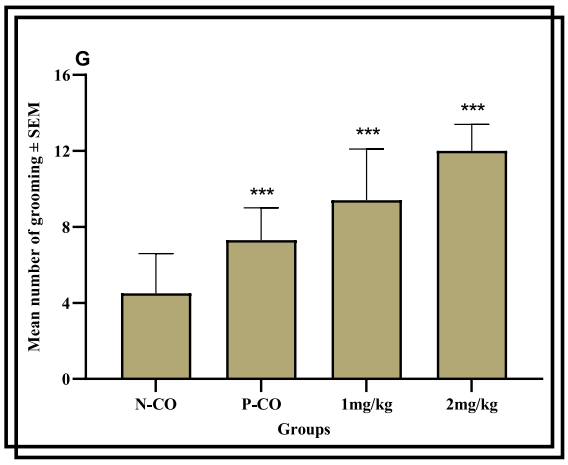
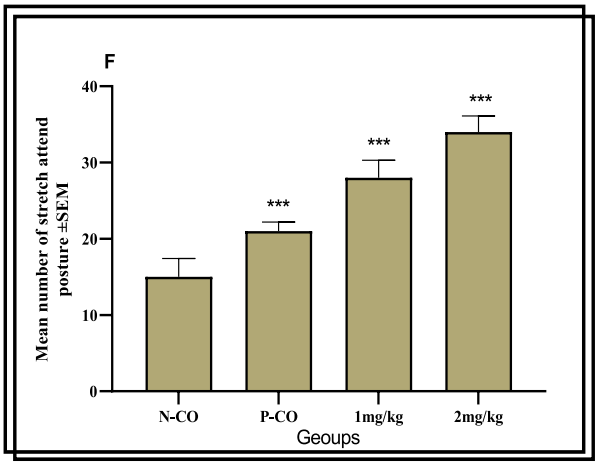
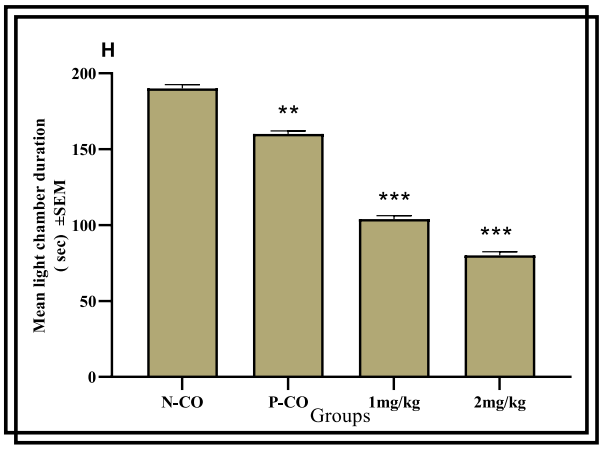


Fig. 3.2. A-I: Effects of cannabis on females in the light–dark chamber test*** represent statistically at $p < 0.001$.









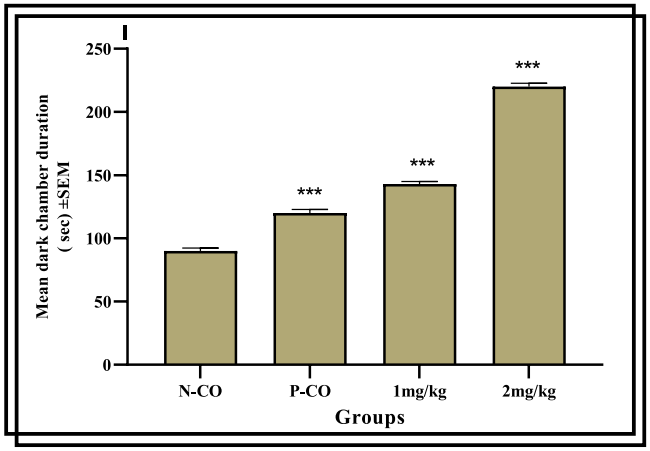


Fig. 3.2. (*continued*)

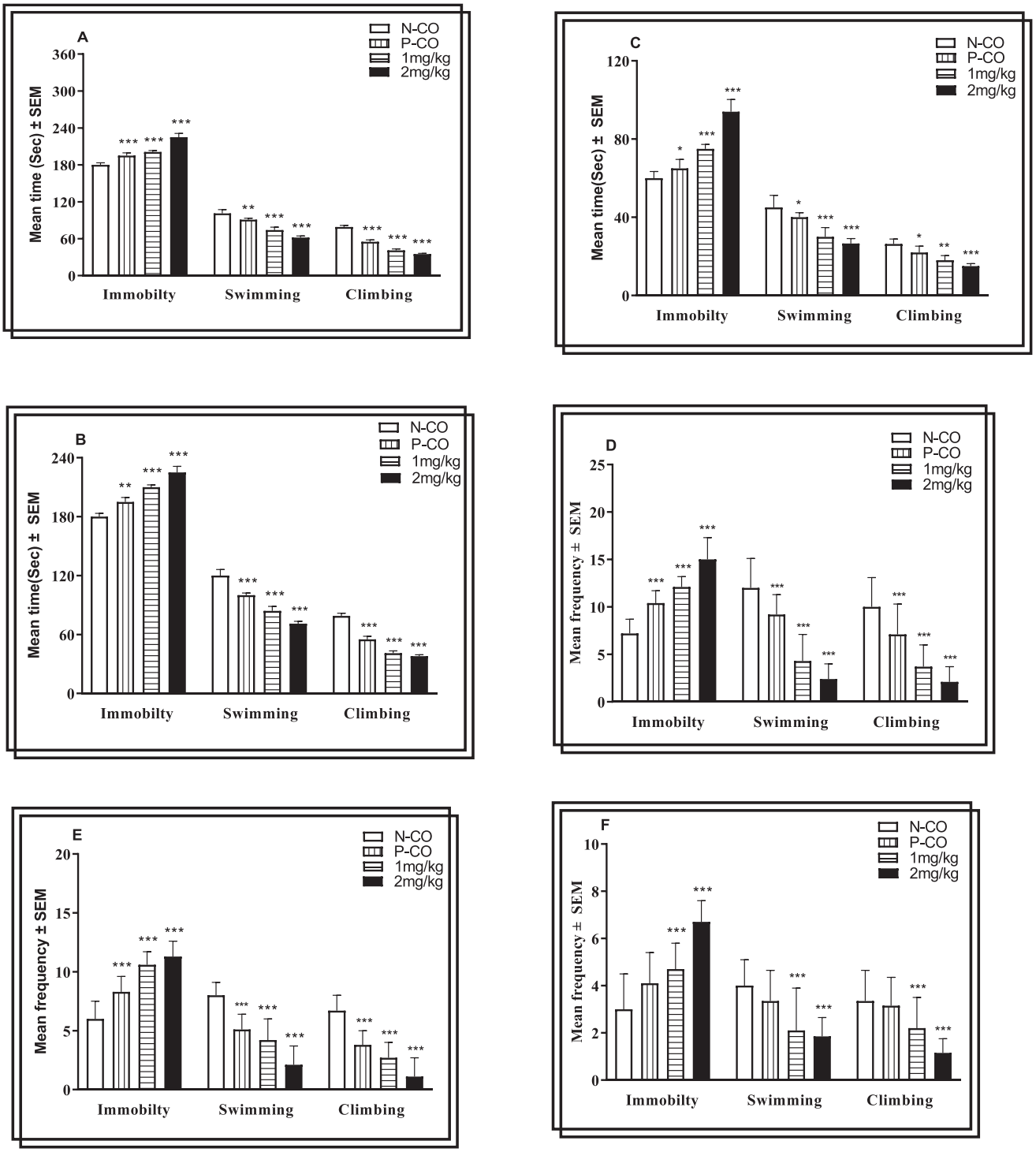


Fig. 3.3. A – F: Forced swimming test*** represent statistically at $p < 0.001$.

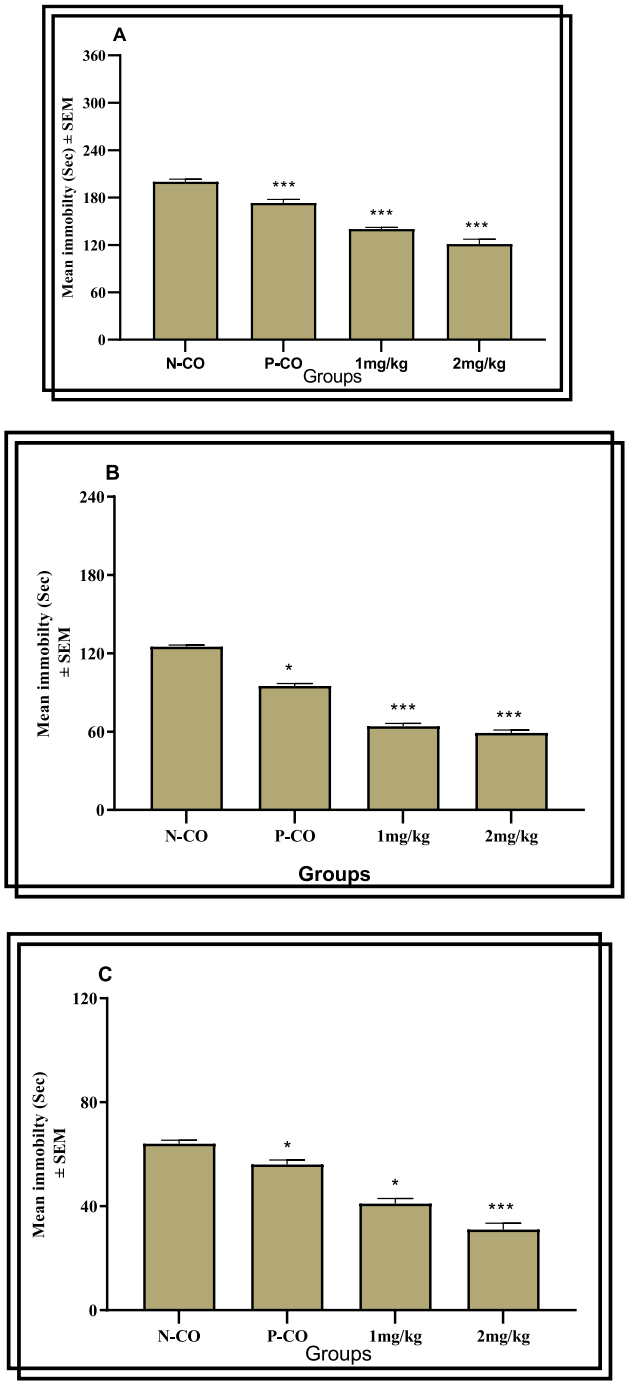


Fig. 3.4. A, B and C: Effects of cannabis on immobility of female mice*** represent statistically at $p < 0.001$.

3.2.2. Anxiety tests

3.2.2.1. Plus-maze test. In Fig. 3.5, A and B Showed that the anxiety was increased in treated females compared to control group.

3.2.2.2. Open – Field test. The treated females showed less locomotor activity compared to the control (Fig. 3.6, A-G).

3.3. Biochemical analyses of brain tissues

In Fig. 3.7, A-C, indicated that the treated females showed a decreasing ($p < 0.001$) in DA, 5-HT, AChE, GST, CAT, SOD (Fig. 9A-C) and GSH (3.8,A) while TBARS (Fig. 8,B) was increased ($p < 0.001$) as compared to the control group.

3.4. Hormones

Corticosterone and cortisol levels in treated females increased considerably ($p < 0.001$) (Fig. 10, A and B).

4. Discussion

Cannabis is the world's most common illicit drug (Alagbonsi and Olayaki, 2016). Some users have seen diminished motor abilities, anxiety and acidity in different mental health disorders, such as impaired memory and focus (Degenhardt et al., 2010).

Present results indicated that exposure to Cannabis reduced body weight, DA,5-HT, AChE, GSH, GST, CAT and SOD while the anxiety, depression, TBARS, corticosterone and cortisol were increased in treated female mice. Results are agreeing with (Alagbonsi and Olayaki, 2016).

The continuous use of medical cannabis can lead to numerous detrimental neurological effects depending on the dosage of THC and THC related cannabinoids, according to verified studies (Bruijnzeel et al., 2016). The list of neurological signs that have been found following chronic exposure to THC is broad and includes convulsions, epileptic seizures, headache - symptoms similar to that of medicinal cannabis. In addition, THC can cause not only to chronic, but also to acute, mental events, such as anxiety, temporary hallucination and delusion (Solimini et al., 2017). Earlier studies have also showed some long-term unfavorable mental health impacts such as attention impairment, psychomotor task capacity, shorter-term memory, an elevated psychotic risk, depression, and anxiety disorders (González-Pinto et al., 2016).

The brain monitors depression and fear (Lago et al., 2017). A number of macro and microscopic structural problems may lead to higher risk of depression and anxiety in the hippocampal, cortex, and subcortical brain regions of patients with general developmental disorders. In both early- and late-life depression, the volume reduction of hippocampal was reported. An increased incidence of white matter lesions in late life depression was observed only (Janssen et al., 2004). Okon et al. (2014) showed that amygdala seems to mediate anxiety and depression.

Exposure to cannabis in the current study reduced body weight and motor activity in the treated females. Administration of THC produces dilation of the peripheral blood arteries, causing changes in blood pressure and reflection, accelerating the cardiac rates. If the cardiac velocity is inadequate, blood flow to organ such as the kidney can decline and tiny arteries can be thrombosed (Yassa et al., 2010). The decreased motor activity may be the result of the muscle relaxation that causes exposure to cannabis (Okon et al., 2014).

Over the last four decades, our knowledge of pathogenesis and therapy of depression is greatly contributed to the focus on brain monoaminergic systems containing serotonin (5-hydroxytryptamine, 5-HT), norepinephrine, and dopamine (DA) (Żmudzka et al., 2018). There has been a considerable reduction in dopamine (DA), serotonin (5-HT) and acetylcholinesterase (AChE) levels in cannabis treated animals' forebrain (Owolabi et al., 2017).

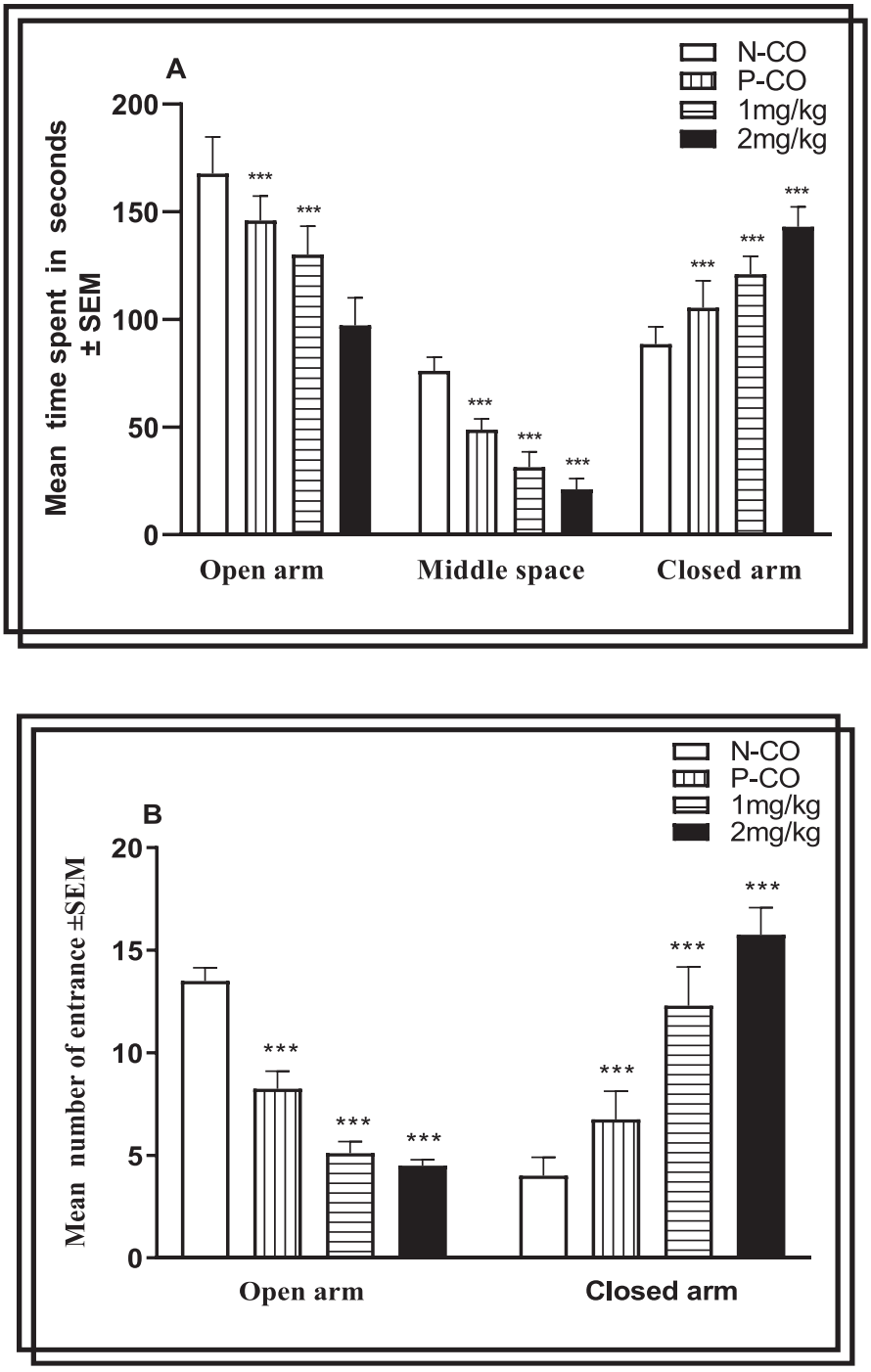


Fig. 3.5. A and B: Plus-maze test. *** represent statistically at $p < 0.001$.

The anxiety and depression are associating with hormones like corticosterone and cortisol (Alagbonsi and Olayaki, 2016). Cannabis exposure disrupts corticosterone hormone (Lago et al., 2017) and cortisol (Yassa et al., 2010) which increase the anxiety and depression in females.

The effect of cannabis depends on the dose taken, how the user has used cannabis before, what drug use the user has, what the user is expected of, the attitude to the effect of cannabis, their mood and the social environment in which it is used (Yassa et al., 2010; Kardash et al., 2019). Kardash et al. (2019) have indicated that the effects of cannabis depend on individuals may require attention in respect to the usage of cannabis in medical therapy. We argue that personality differences in behavior should be seen as an essential component of medical cannabis treatment, and that the proper doses of THC contained in patients should also be prescribed and selected.

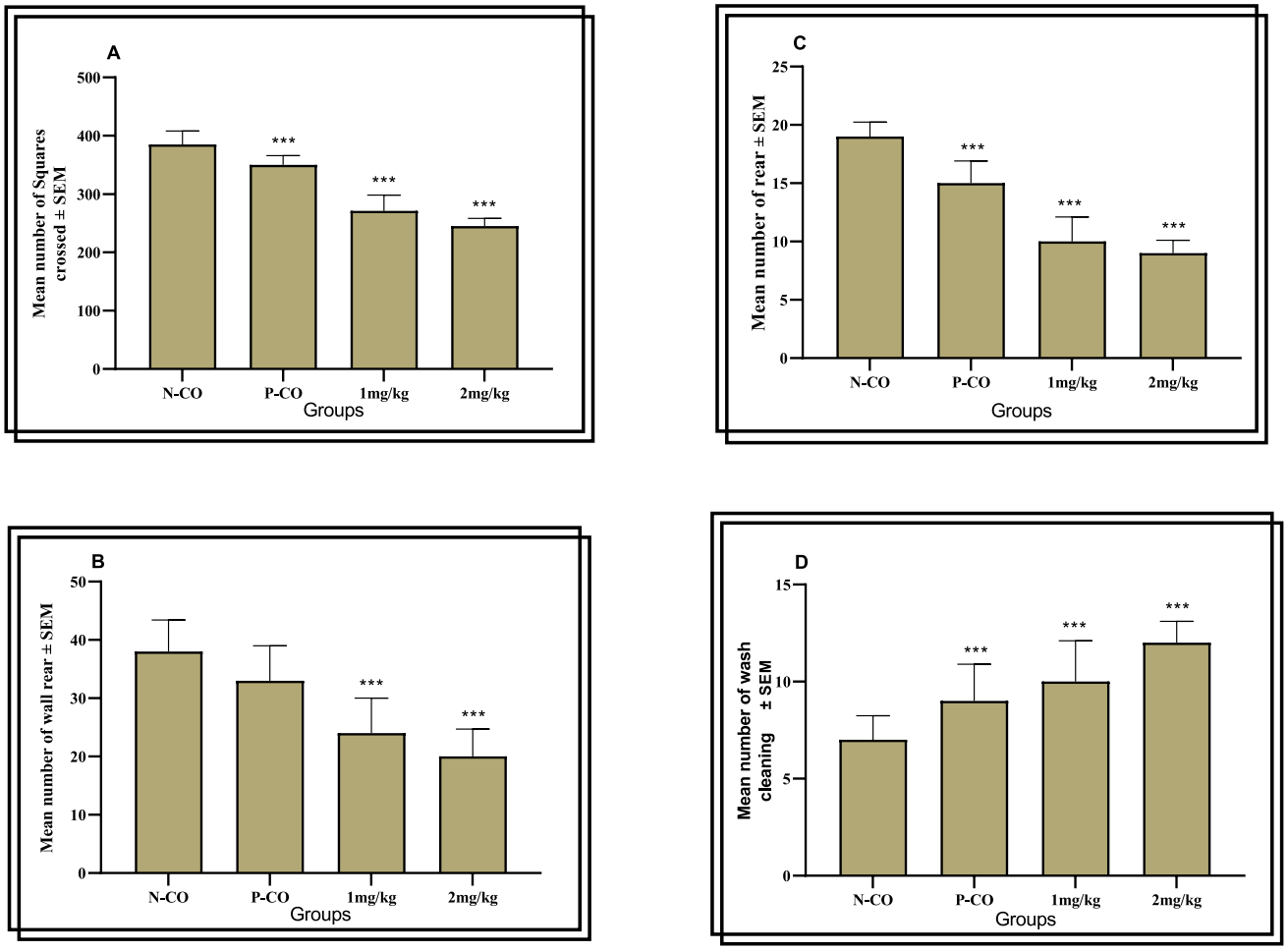
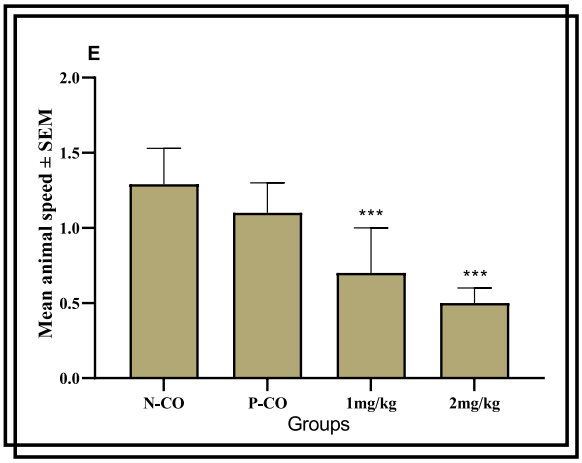
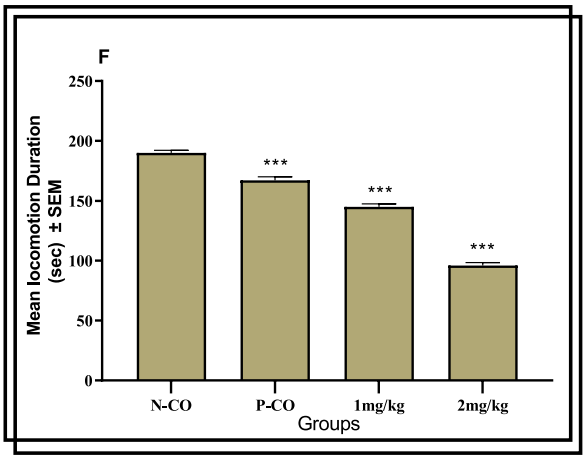


Fig. 3.6. A-G: Locomotor activity test. *** represent statistically at $p < 0.001$.





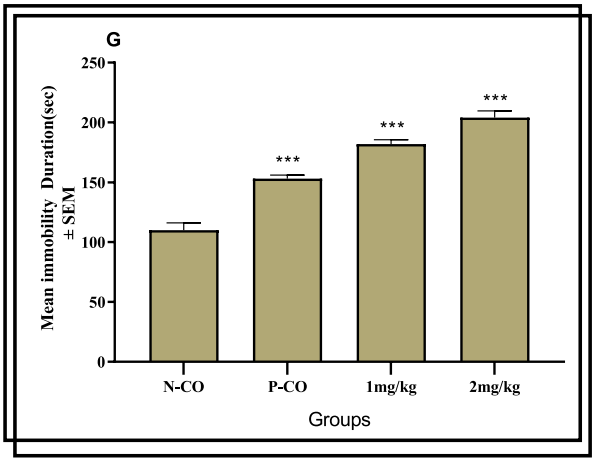
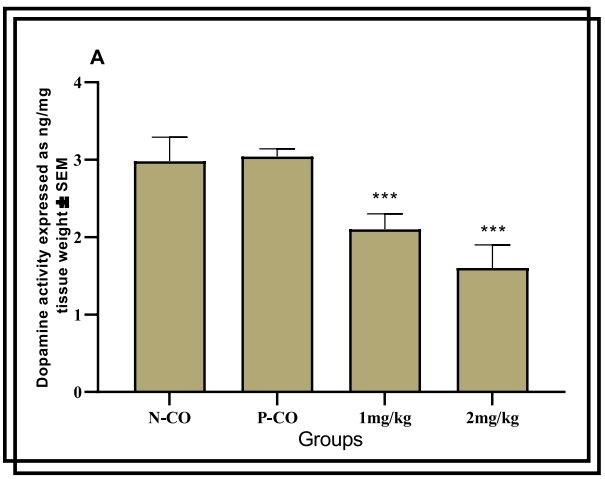
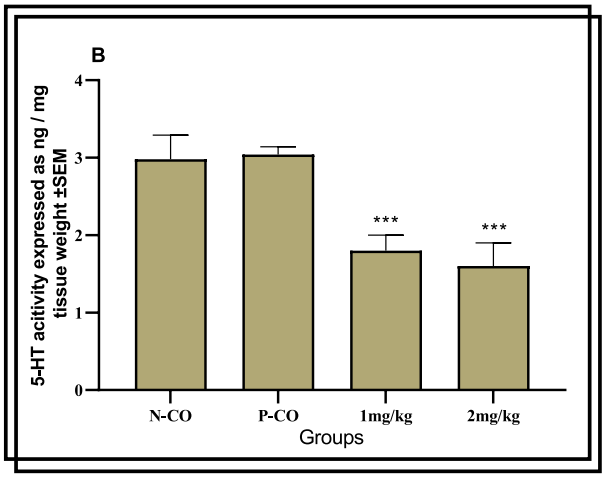


Fig. 3.6. (*continued*)





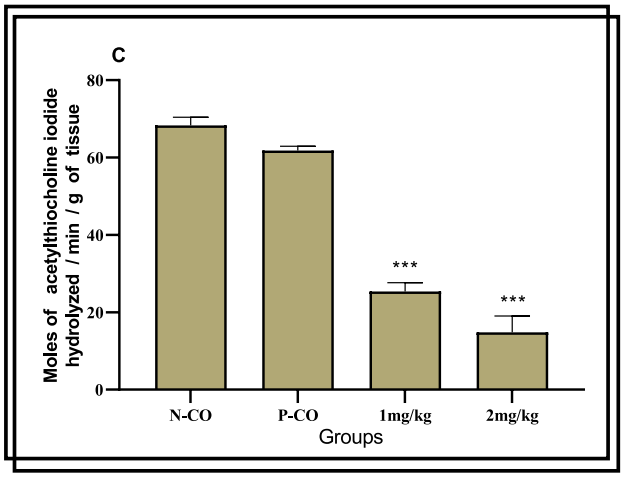


Fig. 3.7. A-C: Effect of cannabis on neurotransmitters. A, DA. B,5-HT.C, AChE*** represent statistically at $p < 0.001$.

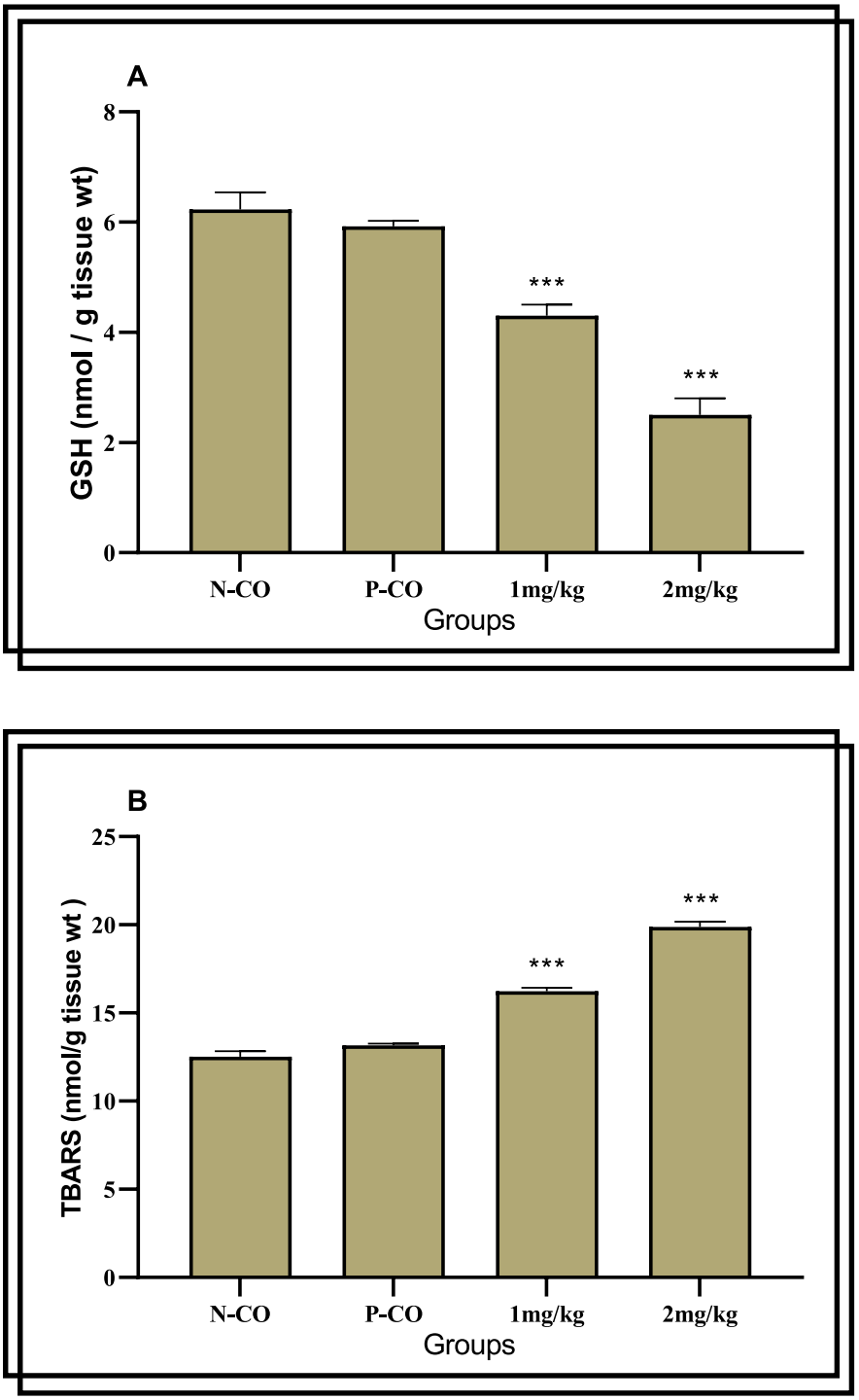


Fig. 8. A and B: Cannabis effects on non –enzymatic parameters. A, GSH.B, TRBARS. *** represent statistically at $p < 0.001$.

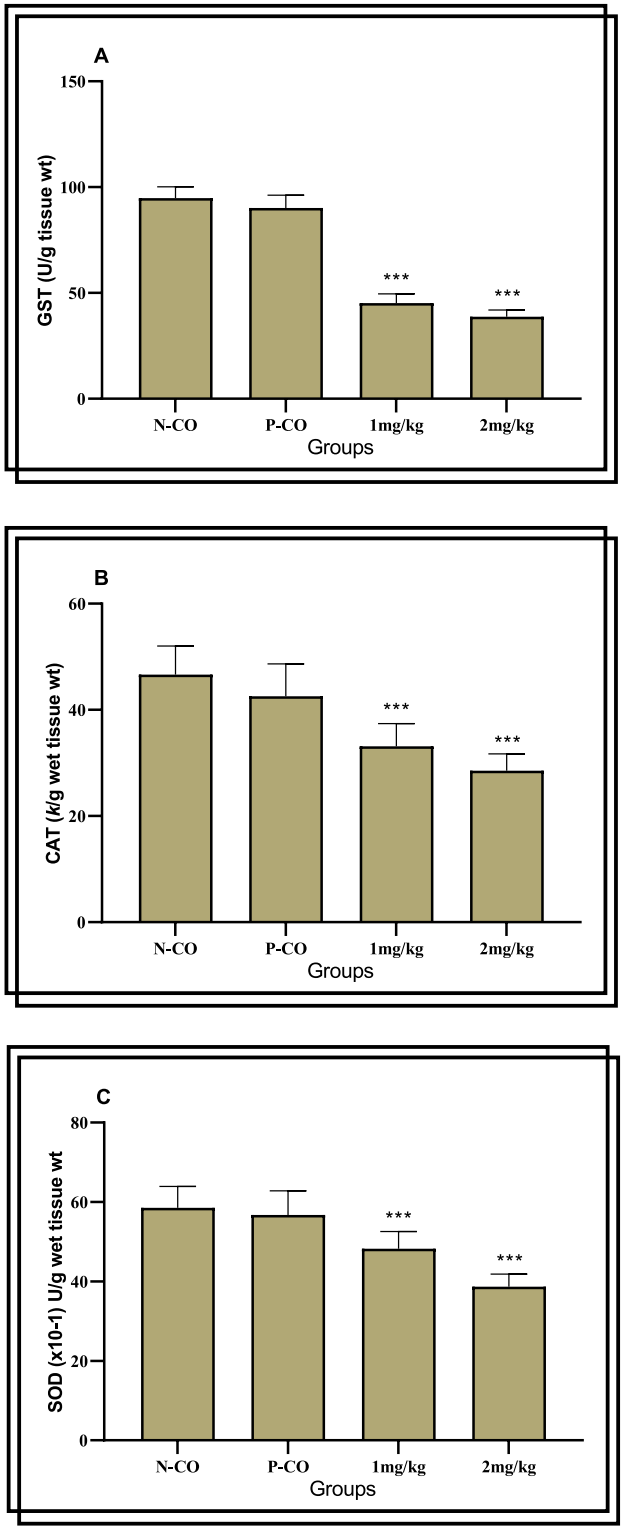


Fig. 9. A-C B : Cannabis effects on enzymatic parameters. A,GST.B,CAT.C,SOD. *** represent statistically at $p < 0.001$.

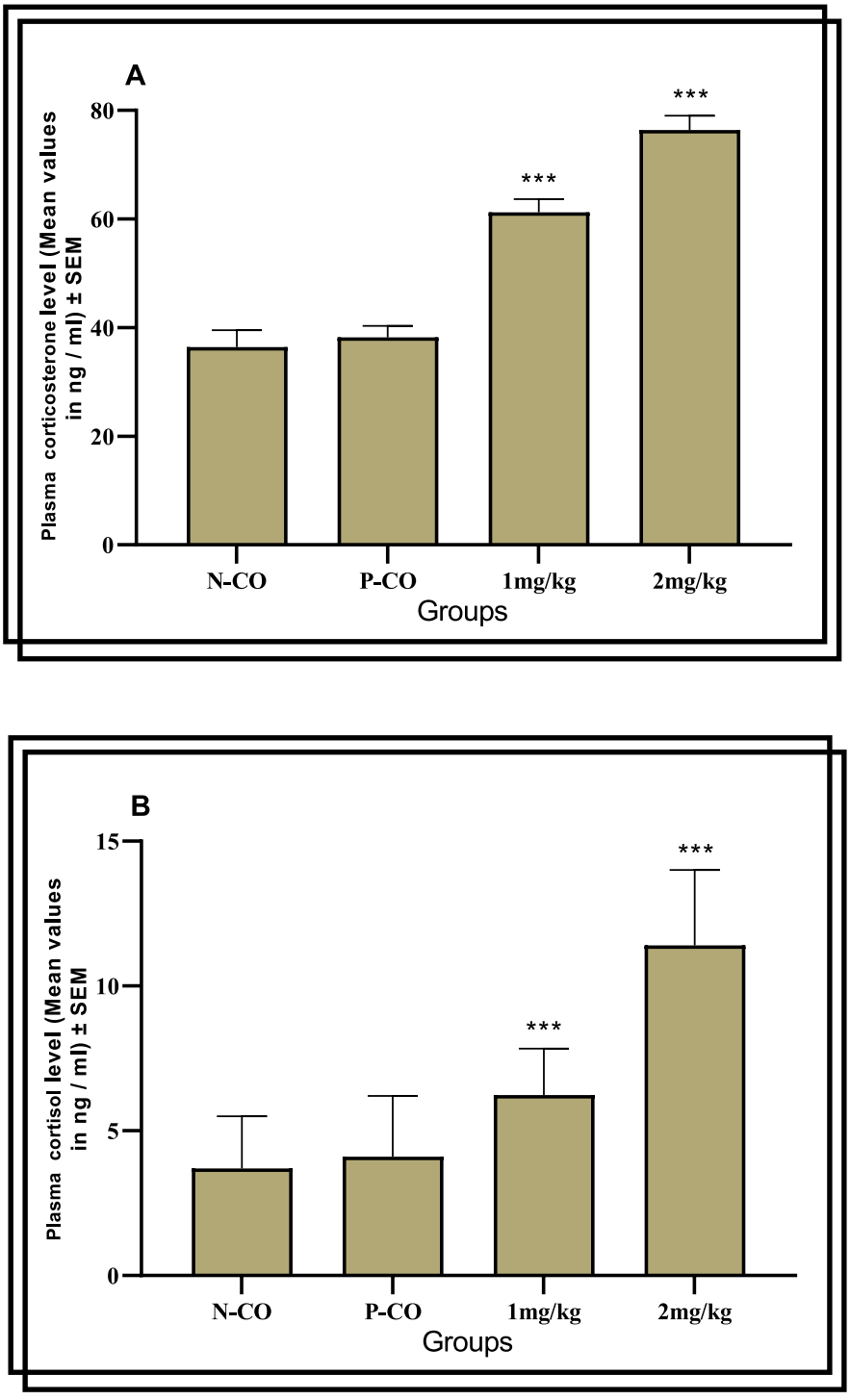


Fig. 10. A and B : Effects of cannabis on A, corticosterone. B, cortisol. *** represent statistically at $p < 0.001$.

5. Conclusion

Cannabis exposure has caused to anxiety disturbance and sadness in female mice. The results of the current study illustrate the dangers of cannabis use on general manifestations of behavior, including anxiety and depression behavior, and this leads to the need to conduct more behavioral studies on this drug, especially during pregnancy and lactation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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