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Original Article

Cannabinoid exposure during pregnancy: consequences for growth, memory and prefrontal cortex integrity in Wistar Rats

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ABSTRACT

Background and aim: The increasing use of cannabis among pregnant women raises concerns about its potential impact on prenatal development. Many pregnant women use cannabis for its anti-emetic or anti-nausea effects, often under the belief that it is safe during pregnancy. However, prenatal exposure to the primary cannabinoids, THC and CBD, may result in neurodevelopmental deficits in offspring. This study aimed to assess the effects of late prenatal cannabis exposure on neurodevelopment in Wistar rats.

Materials and methods: Twenty-four female Wistar rats (12 weeks old) were mated, and pregnancy was confirmed by the presence of spermatozoa in vaginal smears on day 1. The animals were divided into four groups: Control (Group A), Late CBD (Group B), Late THC (Group C), and Late THC/CBD (Group D), with six rats per group. From gestational days 15 to 19, Group A received olive oil, Group B received CBD (150mg/kg body weight), Group C received THC (150mg/kg body weight), and Group D received a combination of CBD and THC (150mg/kg body weight each) via oral administration. After birth, the pups were allowed to grow, and at week 7, the adolescent rats were subjected to neurobehavioral tests. Upon completion of behavioral assessments, the rats were euthanized, and their brains were dissected, fixed in 10% formal saline, and processed for histological analysis.

Results: The results showed a significant reduction in birth weight at postnatal day 1 (PND 1) in the CBD, THC, and THC/CBD groups, with an increased percentage of alternate arm return at $p < 0.05$, morphological changes in pyramidal cells in the prefrontal cortex, indicating impaired cognition.

Conclusion: These findings suggest that late prenatal exposure to cannabis, specifically THC and CBD, leads to low birth weight and cognitive impairments in offspring, particularly in the prefrontal cortex.

Keywords:
Cannabis sativa; Cannabidiol; Δ-9 Tetrahydrocannabinol; Prefrontal cortex; Neurobehavioural Assay

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INTRODUCTION

Cannabis (marijuana) use has surged globally over the past decade, particularly in recent years, for both medicinal and recreational purposes (Gilbert, 2020). It is one of the most widely consumed illicit substances worldwide, with increasing prevalence even among pregnant women (Brown *et al*., 2017; Agrawal *et al.,* 2019; Skelton, Hecht, & Benjamin-Neelon, 2021; Leung *et al.,* 2022). Studies indicate a rise in cannabis-derived product usage among pregnant individuals in both North America and the European Union (EU) (Brown *et al.,* 2017; Agrawal et al., 2019). Additionally, the incidence of frequent cannabis consumption in the United States and globally has escalated, largely due to increased legalization and a reduced perception of risk (Hasin *et al.,* 2013; Gregory *et al.,* 2023). In Nigeria, the World Health Organization (WHO) estimates that over 13 million adults smoke cannabis, with a gender ratio of approximately 3:1, where 21.8% are men and 7.0% are women (Fasakin *et al.,* 2022).

Despite the widespread belief that cannabis use during pregnancy poses minimal risk to offspring, accumulating epidemiological and preclinical evidence suggests otherwise, highlighting potential long-term neuropsychiatric consequences (Bayrampour *et al.,* 2019; Sarrafpour et al., 2020; Sarikahya *et al.,* 2023; DeVuono *et al.,* 2024). Pregnant individuals use cannabis to alleviate nausea, pain, stress, appetite changes, and anxiety (Young-Wolff et al., 2020). Moreover, an increasing number of individuals with chronic pathological conditions substitute cannabis for prescription medications, including opioids, anxiolytics, and antidepressants (Corroon

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et al., 2017). However, cannabis is a complex plant containing over 100 individual phytochemicals, with the two primary active cannabinoids being cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC). The distinct effects of isolated cannabinoids, their impact on pregnancy, and their influence on neurodevelopmental outcomes in offspring require further investigation.

A dysregulated placental environment due to cannabis exposure may contribute to maternal and fetal complications, such as pre-eclampsia, spontaneous preterm birth, intrauterine growth restriction (IUGR), and fetal resorption (Black *et al.,* 2023). The endocannabinoid system plays a crucial role in pregnancy coordination and fetal neurodevelopment Costa (2016). A reduction in intrauterine anandamide levels and signaling via cannabinoid receptor 1 (CB1) and receptor 2 (CB2) is essential for successful implantation (Paria, 2001; Black *et al.,* 2023). Disruptions in receptor expression and anandamide levels in reproductive tissues and plasma have been linked to ectopic pregnancy, miscarriage, and pre-eclampsia (Black *et al.,* 2023). Furthermore, CB1 coordinates directional axonal migration, elongation, and the maturation of astrocytes and oligodendrocytes (Bara *et al.,* 2021).

Cannabis constituents can cross the placental barrier, accumulating in the developing fetus (Baglot *et al*., 2021; Black *et al.,* 2023). Studies report that *in-utero* THC exposure interferes with endogenous cannabinoid signaling, impacting neuronal growth cones, cytoskeletal architecture, and synapse formation (Black *et al.,* 2023). Emerging human data suggest that prenatal cannabis exposure increases the risk of preterm birth, low birth weight, and persistent behavioral and cognitive impairments across the lifespan (Marchand *et al.,* 2022; Koto *et al.,* 2022). However, the long-term consequences remain incompletely understood. Some studies correlate cannabis use with adverse birth outcomes, while others report no significant effects (Pandelides *et al.,* 2020). While THC is known to exert psychoactive effects (Iezzi *et al.,* 2022; Swenson *et al.*, 2023). Existing data on CBD indicates the amelioration of the symptoms of pregnancy such as nausea and anxiety, it usage during pregnancy is increasingly being discussed in social media and online (Sarrafpour *et al.,* 2020). There is still a need for high-quality research studies to fully elucidate the risks and benefits of CBD usage by pregnant patients (Sarrafpour *et al.,* 2020), however, data on CBD safety in pregnancy and postnatal life remain limited.

Given the increasing use of cannabis among pregnant individuals, further investigation into its impact on fetal neurodevelopment is essential. We hypothesize that THC, CBD, and THC/CBD combinations may influence neurodevelopment, particularly in late pregnancy. The prefrontal cortex (PFC), the largest cortical area in the human brain, accounts for approximately 29% of the cerebral cortex. Located in the frontal lobe, anterior to the primary and premotor cortices, the PFC plays a fundamental role in personality formation, behavior regulation, attention maintenance, motor planning, emotional control, speech, memory, temporal perception, and working memory (Chudasama, 2011; Akkoc & Ogeturk, 2017). Given its critical role in cognitive function, examining the effects of prenatal cannabis exposure on the PFC is significant.

The present study evaluated the impact of late prenatal exposure to THC and CBD on the prefrontal cortex of Wistar rat offspring, thereby elucidating the neurodevelopmental consequences of maternal cannabis use during pregnancy.

MATERIALS AND METHODS

Ethical Approval

Ethical clearance was obtained from the Olabisi Onabanjo University Ethical Review Committee (OOU/SCIENG/EC/240924) with affirmation to maintain humane interactions throughout the experiment.

Preparation of Cannabis Extract

Cannabis sativa (marijuana) was obtained from the National Drug Law Enforcement Agency (NDLEA), where its identification was confirmed (Identification Reference Number: NDLEA/SD/2024/21/70). The cannabis leaves were dried and weighed (278.7 g) before being ground into a fine powder using a mechanical grinder. The powdered material was then soaked in ethanol for 48 hours, followed by filtration using Whatman filter paper No 42 diameter 12.5cm (Middlesex, UK).

To extract the active compounds, a liquid-liquid partitioning method was employed using n-hexane and dichloromethane as solvents. The filtrate was poured into a separating funnel and thoroughly mixed with n-hexane. This mixture was agitated until it separated into two distinct layers: ethanol at the bottom and n-hexane at the top. The ethanol layer was carefully discarded, leaving behind the n-hexane solution, which contained Δ9-Tetrahydrocannabinol (THC) and cannabidiol (CBD).

The n-hexane solution was concentrated using a rotary evaporator under reduced pressure to enhance the evaporation process. The water bath was maintained at 40-45°C to facilitate the removal of n-hexane without degrading the cannabinoids. The final extract was collected, and the percentage yield was determined using the formula: $\frac{W2}{W1}X100 = \frac{\text{final weight (W2)}}{initial\ (W1)}$ x 100

initial weight 278.7g and final weight 23.53g; 8.27% obtained as yield.

The crude extract was fractionated to isolate THC and CBD using Thin Layer chromatography (TLC) (Mano-Sousa *et al.,* 2021)

$$\text{Retention factor (Rf)} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

The Rf values are used to identify unknown compounds by comparing them to the known standard run under the same condition (Bele and Khale, 2011, Mano-Sousa *et al.,* 2021),

The Rf values obtained for CBD: 0.271 ± 0.013; and THC: 0.336 ± 0.017 were consistent with standard values as reported by Khan *et al.* (2021). Under ultraviolet light, THC appeared greenish-brown, while CBD exhibited an orange hue.

The median lethal dose (LD50) of THC has been reported in various animal species, ranging from 800-900 mg/kg (Terance *et al*., 2023) to 1270 mg/kg (Bioquest, 2024). The LD50 of CBD is approximately 212 mg/kg bw (Gingrich *et al*., 2023)

Experimental Design

Twenty-four (24) female Wistar rats (10 weeks old), weighing between 160-180 g, were obtained from a breeder (Peter's Farm (Nig.) Enterprises, Ibadan, Nigeria). The animals were housed in wire mesh plastic cages under standard laboratory conditions in the Department of Anatomy, Olabisi Onabanjo University, Ago-Iwoye, Nigeria. They were allowed to acclimatize for two weeks with free access to water and commercial rat chow (Jafel Agro Services, Sagamu, Ogun State).

At 12 weeks, following acclimatization, the estrous cycle of the female rats was monitored, and those in the proestrus phase were introduced to male rats for mating in a ratio of 2 to 1 per cage, the mating was done in the evening time at done at 17:00.

Timed and multiple pairing mating was employed to ensure multiple pregnancies at the same time. The presence of spermatozoa in a vaginal smear confirmed successful mating, marking gestational day (GD) 0. The pregnant rats were randomly assigned to four groups (n=6 per group) the estrous cycle was determined for a period

Group A (Control) received 0.5 ml of olive oil orally from GD 15 to GD 19. Group B (THC) received 150 mg/kg body weight/day of THC in 0.5 ml olive oil from GD 15 to GD 19. Group C (CBD) received 150 mg/kg body weight/day of CBD in 0.5 ml olive oil from GD 15 to GD19 and Group D (THC/CBD) received a combined dose of THC and CBD 150 mg/kg body weight/day each in 0.5 ml of olive oil from GD 15 to GD 19.

After delivery, the first day was regarded as postnatal day (PD) 0, and the pup's weight was recorded at PD1. The reduction in weight of the pup was estimated as

$$\frac{\text{mean (weight of control pup} - \text{weight of cannabinoid exposed pups)} \quad \text{x100}}{\text{mean weight of control pups}}.$$

The offspring were allowed to develop naturally. Pups were weaned at 5 weeks, and at 6 weeks (PD 42), they underwent behavioral studies for memory and cognitive tests. The weights of the rats were recorded at the end of the studies

Neurobehavioral Tests

The Elevated Plus-Maze (EPM)

Anxiety-like behavior was assessed using the Elevated Plus Maze (EPM) test (Szkudlarek *et al*., 2019; Sotoudeh *et al.,* 2020). The apparatus consisted of two open arms (50 × 10 cm) with 5 cm-high edges and two closed arms (50 × 10 cm) with 40 cm-high walls, extending from a central platform (5 × 5 cm). The maze was elevated 50 cm above the floor.

Each rat was placed at the central platform facing an open arm, and its behavior was recorded for 5 minutes using a video camera placed above the maze in a way that all of its arms were fully recorded. Time spent in the open arms and the number of entries into the open arms were used as indicators of anxiety-like behavior. The maze was cleaned with 70% ethanol between trials to prevent olfactory bias. Entry into an arm was defined as all four paws crossing into the respective area (Serafim et al., 2012; Sudakov *et al.,* 2013; Sotoudeh *et al.,* 2020).

Y-maze

The Y-maze can be used to determine short-term memory, general locomotor activity, and stereotypic behavior. This apparatus consisted of three arms made of plywood joined in the middle to form a ''Y'' shape at a 120° angle from one another. The walls of the arms were 8 cm high, allowing the rat to see distal spatial landmarks. The rats were introduced at the center and allowed to move freely exploring the three arms. The arms of the Y maze were labeled as A, B, and C. The maze was cleaned with alcohol and allowed to dry before subsequent use. The position was maintained throughout the Y maze test of all the rats. The rat was placed into one of the arms of the maze (start arm) and allowed to explore the maze with one of the arms closed for 15 min (training trial). After a 1-h inter-trial interval, the rat was returned to the Y maze by placing them in the start arm, the rat was allowed to explore freely all three arms of the maze for 5 min (test trial). The number of entries into and the time spent in each arm, and the first choice of entry was recorded with a video that was placed above the maze in a way that all of its arms were fully recorded (Yinka *et al*.,2023; Melbiarta *et al.,* 2023).

.The percentage of spontaneous alternation was calculated using the following formula

Spontaneous alternation (SAP) % =

$$\frac{\text{Number of spontaneous alternation}}{\text{Total number of arm entries - 2}} \text{ x } 100$$

The percentage of alternate arm return was calculated using the following formula

Alternate arm return (AAR) % = $\frac{\text{Number of alternation arm return}}{\text{Total number of arm entries - 2}}$ x100

(Biobserve behavioral research)

Histological assessment

Animals were sacrificed by cervical dislocation; the brain was removed and the prefrontal cortex was collected and fixed in Neutral buffered formalin (NBF). Tissues were dehydrated in ascending grades of ethanol, cleared in xylene, infiltrated, and embedded in paraffin wax. The tissue blocks were mounted on a wooden block and trimmed to size 20µ thick. They were sectioned on a rotatory microtome at 10µ thick. The sections were stained with Haematoxylin and Eosin (H &E) (Sahar, 2009). Photomicrographs were taken using the Evos FL Auto 2 microscope for histopathological evaluation.

Statistical Analysis

Data were expressed as mean ± SEM and analyzed using GraphPad Prism version 10.01. The body weights and Y-Maze data were analyzed with One-way ANOVA, while the Elevated Plus Maze data were analyzed with Two-way ANOVA followed by Dunnett's multiple comparison test was used for comparisons between groups with a significance threshold set at $p<0.05$.

RESULTS

Prenatal maternal exposure to THC and CBD in late pregnancy significantly impacts offspring body weight at birth, with effects persisting until PND 42.

Figure 1 depicts a significant decrease in pup body weight on Postnatal Day (PND) 1. The control group had an average body weight of 4.63 ± 0.095 g, whereas pups in the Late CBD (3.53 ± 0.180 g, p = 0.0001), Late THC (3.58 ± 0.173 g, p = 0.0001), and Late THC/CBD (3.44 ± 0.177 g, p = 0.0001) groups exhibited markedly lower weights. No significant differences were observed among the cannabis-exposed groups. The reductions in body weight relative to controls were 23.1% for Late CBD, 22.7% for Late THC, and 25.7% for Late THC/CBD.

Figure 2 demonstrates that by PND 42, body weight deficits persisted in the cannabis-exposed groups. The control group weighed 76.6 ± 3.444 g, whereas the Late CBD (66.5 ± 0.957 g, $p < 0.05$), Late THC (66.1 ± 1.961 g, $p < 0.05$), and Late THC/CBD (64.3 ± 1.84 g, $p < 0.05$) groups remained significantly lighter. This sustained reduction in body weight may be linked to intrauterine growth restriction (IUGR) or fetal growth restriction (FGR), both of which have been associated with prenatal cannabis exposure. IUGR is commonly characterized by birth weights below the 10th percentile (Hoyert *et al.,* 2006; Harris *et al.,* 2001).

Prenatal maternal exposure to cannabis in late pregnancy did not affect anxiety levels in the offspring at PND 42, as assessed by the Elevated Plus Maze.

Elevated Plus Maze (EPM) analysis, assessed using Dunnett's multiple comparisons post hoc test, revealed no significant differences in the average time spent in the closed arm across groups (Figure 3). In the open arm, the average time spent was 30.9s for the control group, 27.2s for the Late CBD group, 16s for the Late THC group, and 27.6s for the Late THC/CBD group. The Late CBD group tended to spend more time in the closed arm, whereas the Late THC and Late THC/CBD groups showed a slight decrease. Time spent in the open arm was reduced in all cannabis-exposed groups compared to controls, with the Late THC group displaying the most pronounced reduction (Figure 3).

The mean number of entries into the closed arm did not differ significantly among groups. However, while control animals had fewer closed-arm entries, exposure to THC, CBD, and THC/CBD resulted in an increased number of entries, particularly in the Late THC and Late THC/CBD groups. Conversely, open-arm entries were reduced in all exposed groups relative to controls, although the Late THC and Late THC/CBD groups exhibited a slight increase compared to the Late CBD group (Figure 4).

Prenatal exposure to cannabis during late pregnancy significantly impaired offspring spatial memory at PND 41, as measured by the Y-maze test.

Prenatal maternal exposure to cannabis in late pregnancy significantly impaired offspring spatial memory at PND 41, as assessed by the Y-maze.

A significant increase in memory impairment was observed in the Late THC group (36.5 ± 5.36%; p = 0.001), Late CBD group (43.3 ± 3.33%; p = 0.01), and Late THC/CBD group (40.5 ± 13.28%; $p \leq 0.05$) compared to the control group (11.9 ± 4.28%) (Figure 5).

Spontaneous alternation was significantly reduced in the Late THC (36.7 ± 7.16%) and Late THC/CBD (32.2 ± 13.7%) groups (p = 0.001) compared to the control group (88.1 ± 4.28%). However, the Late CBD group (67.5 ± 5.95%) did not show a significant difference relative to controls (p = 0.218). Additionally, the Late THC (36.7 ± 7.16%) and Late THC/CBD (32.2 ± 13.7%) groups exhibited a significant decrease in spontaneous alternation ($p < 0.05$) compared to the Late CBD group (67.5 ± 5.95%) (Figure 6).

Prenatal maternal exposure to cannabis in late pregnancy altered offspring Prefrontal Cortex Structural Integrity.

The photomicrograph of the prefrontal cortex in the control group **(A)** displays normal structural architecture, with the internal pyramidal layer clearly showing pyramidal neurons with visible soma and pyramidal cells, as well as stellate neurons with visible soma and granular cells.

In the **L CBD group (B)**, chromatolysis changes in the Nissl bodies, indicative of neuronal degeneration, were observed in the pyramidal neurons. This group also showed an abundance of granular cells, with the presence of **perivascular cuffing**, a hallmark of neuroinflammation.

The **THC group (C)** showed pyramidal neurons with visible somas, but some pyramidal cells exhibited morphological changes, including **pericellular spaces** and **vacuolation** in the ground **In the** Combination (THC and CBD) **groups (D)** displayed pyramidal neurons with visible somas, and some pyramidal cells showing altered morphology. These cells exhibited **pericellular spaces**, indicating loss of cytoplasmic detail, and vacuolation in the ground substance around blood capillaries.

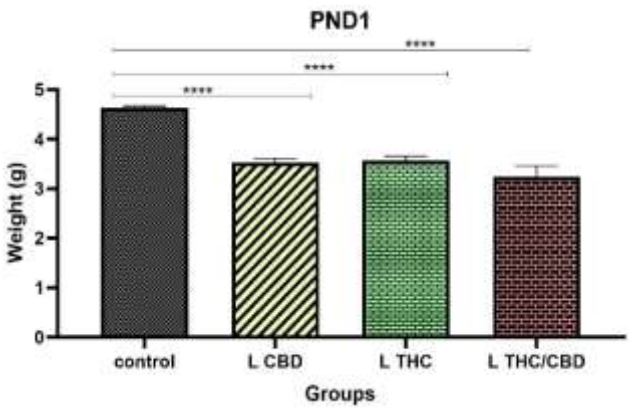

Figure 1: showing the body weight of pups at PND 1. Each bar represents Mean ± S.E.M, with a significant difference at p< 0.05 (*) compared with the control

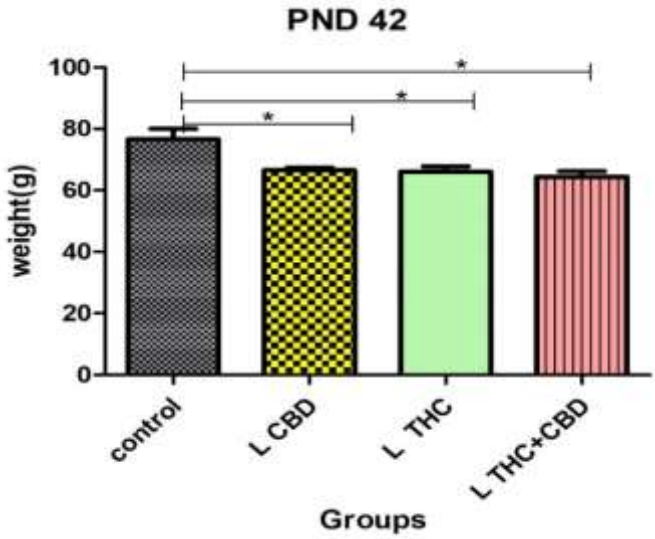

Figure 2: showing the body weight of pups at PND 42. Each bar represents Mean ± S.E.M, with a significant difference at p < 0.05 (*) compared with the control

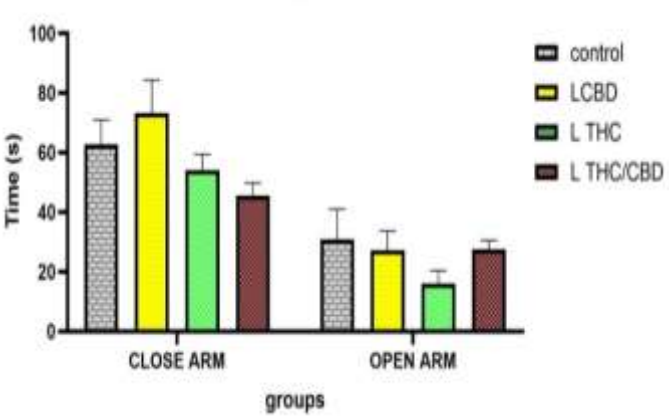

Figure 3 shows the amount of time spent in the arms. Each bar represents Mean ± S.E.M, with no significant difference.

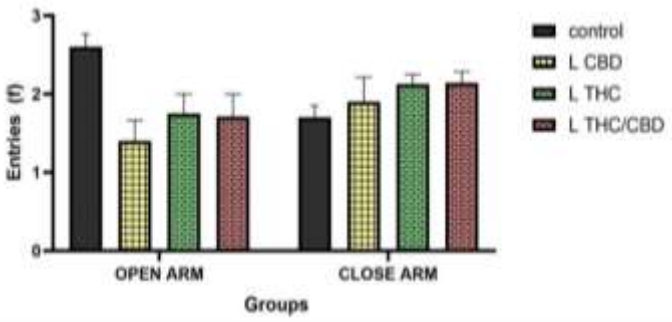

Figure 4 shows the frequency of entries in the arms. Each bar represents Mean ± S.E.M, with no significant difference

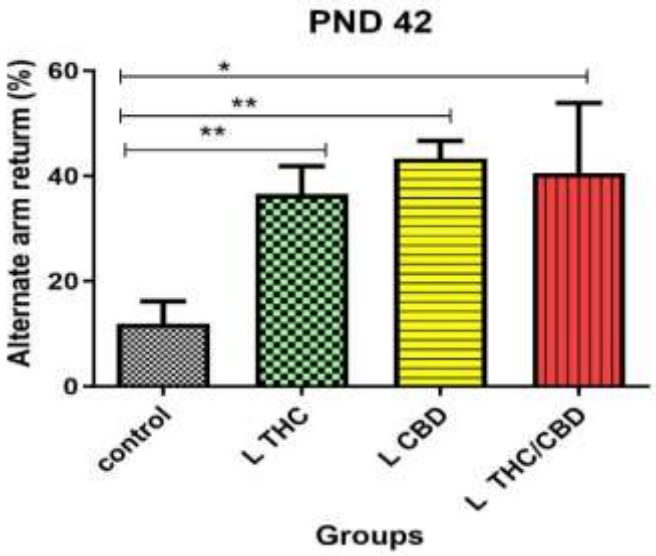

Figure 5: showing the percentage of Alternation arm return in the Y maize. Each bar represents Mean ± S.E.M, with a significant difference at p< 0.05 (*) compared with the control

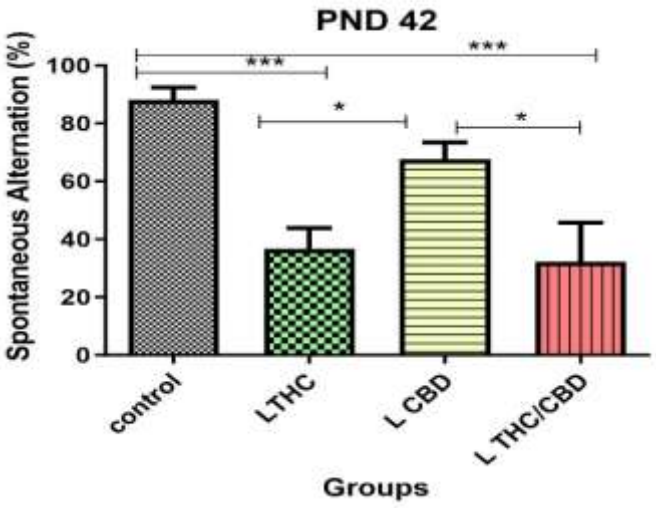

Figure 6: Showing the percentage of spontaneous Alternation in the Y maize. Each bar represents Mean ± S.E.M, with a significant difference at and p< 0.05 (*).

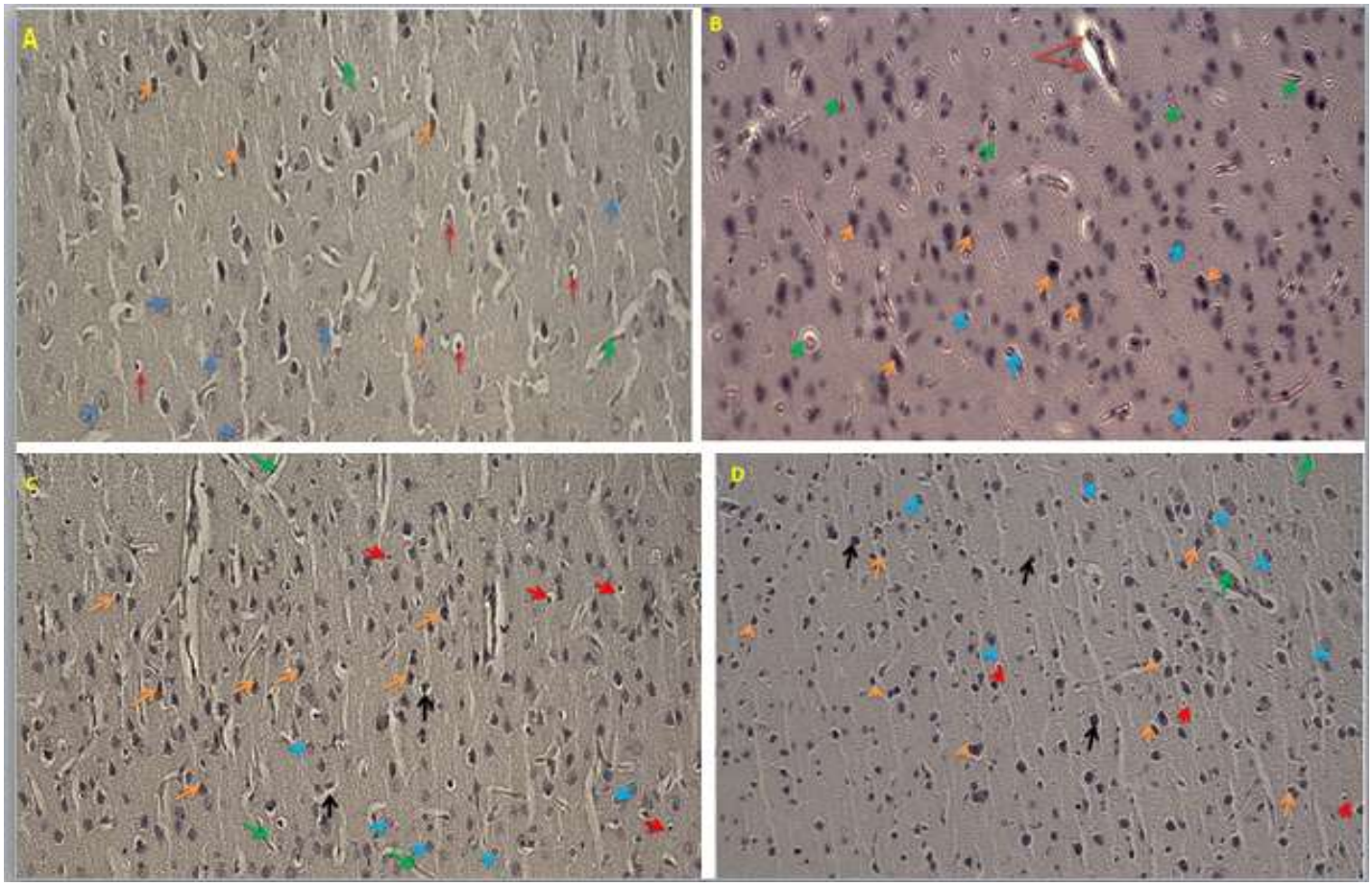

Figure 7: Photomicrographs of prenatal exposure of cannabis sativa (150mg/kg THC; 150 mg/kg CBD and combined 150mg/kg THC & CBD) of the prefrontal cortex. (A) control, (B) LCBD, (C) L THC, and (D) L THC/CBD with H&E stain, 40×.

Legend: Red arrow- neuroglia, Green arrow-blood capillaries, Blue arrow- granular cell, Orange arrow- pyramidal cell, Black arrow – change in morphology of the pyramidal cell, V- vacuolation, Brown arrow- perivascular cuffing.

DISCUSSION

Cannabis use during pregnancy is often perceived as harmless by the general public, with little awareness of the potential long-term neuropsychiatric risks to offspring, despite growing epidemiological and preclinical evidence highlighting its detrimental effects (DeVuono *et al.,* 2024). Cannabis contains two primary cannabinoids—tetrahydrocannabinol (THC), the psychoactive compound responsible for most of cannabis' mind-altering effects, and cannabidiol (CBD), which is typically recognized for its anxiolytic and neuroprotective properties. Both cannabinoids interact with the endocannabinoid system, leading to widespread physiological and behavioral changes.

The present study investigated the effects of THC and CBD exposure during late gestation in rats, administered at a dose of 150 mg/kg. Our results showed a significant reduction in the body weight of pups at postnatal day 1 (PND1) (Figure 1), a hallmark of low birth weight associated with exposure to THC and CBD. Specifically, pups exposed to L CBD, L THC, and L THC/CBD exhibited weight reductions of 23.1%, 22.7%, and 25.7%, respectively. Birth weight below the 10th percentile is indicative of intrauterine growth restriction (IUGR), a condition linked to placental dysfunction and impaired nutrient supply to the developing fetus. This low birth weight, a result of placental dysregulation caused by cannabinoid exposure, could contribute to late embryonic death or the onset of postnatal diseases (Elmore *et al.,* 2022).

At PND 42, although the animals reached a more mature state, they continued to show growth deficits (Figure 2), suggesting that prenatal exposure to THC and CBD had a long-lasting impact on postnatal growth. The observed growth catch-up was insufficient to fully overcome the effects of prenatal low birth weight. The impact of CBD on growth could be related to its potential to decrease food intake and alter metabolic functions, although the exact mechanisms remain unclear. Kaplan *et al.* (2021) reported that CBD's weight-reducing effects were more pronounced in females, suggesting sex-specific differences in cannabinoid-induced metabolic alterations.

Neurobehavioral assessments using the elevated plus maze (EPM) and Y-maze further revealed cognitive deficits and behavioral alterations due to cannabinoid exposure. The EPM showed no significant changes in anxiety-related behaviors, though there was an increase in the time spent in the closed arm and a decrease in the time spent in the open arm. In the Y-maze

task, which assesses spatial working memory, we observed significant impairments in spontaneous alternation and alternate arm return in the L THC, L CBD, and L THC/CBD groups (Figures 5 and 6). These deficits highlight the detrimental effects of THC and CBD on cognition and memory, potentially due to disruptions in the central nervous system. Carone *et al.* (2024) reported similar cognitive deficits in animals exposed to high doses of THC, and Yinka *et al*. (2023) found alterations in neurotransmitter levels and spatial memory following cannabis exposure, along with neuronal degeneration and astrogliosis in the prefrontal cortex (PFC). Our findings align with these studies and emphasize the negative impact of THC and CBD on brain function.

Histological analysis of the PFC revealed structural changes, with evidence of neuronal degeneration and altered morphology in response to THC, CBD, and the combination of both cannabinoids (Figure 7). These changes included the loss of cytoplasmic detail and vacuolation in the pyramidal cells, which may be attributed to oxidative stress and hypoxia. This cellular insult is likely caused by placental dysfunction during embryogenesis, which disrupts nutrient and oxygen supply to the developing fetus and results in long-term neurological deficits in adulthood. The ability of THC and CBD to cross the placenta and affect fetal development has been well-documented, with exposure leading to placental insufficiency and fetal growth restriction (FGR) (Natale *et al.,* 2020; DeVuono *et al.,* 2024).

Our findings also corroborate studies that suggest cannabis exposure, particularly THC, can significantly alter body weight and growth. Allen *et al.* (2024) reported reduced fetal weight following CBD exposure at 3 mg/kg, and Cluny *et al*. (2015) observed a decrease in weight gain in mice following chronic THC exposure. Cannabis, especially THC, has been shown to reduce maternal weight gain and disrupt fetal development, possibly through alterations in appetite regulation and metabolic processes mediated by the endocannabinoid system. THC activates CB1 receptors, enhancing appetite and food intake, but chronic exposure can disrupt metabolic regulation, contributing to poor growth and low birth weight in offspring (Pintori *et al.,* 2023).

The PFC, a region heavily impacted by cannabinoid exposure, particularly during adolescence, is critical for cognitive functions such as working memory, decision-making, and emotional regulation. During adolescence, the brain undergoes significant maturation, including synaptic pruning and neural circuit refinement. THC exposure during this developmental window disrupts these processes, resulting in long-term cognitive and emotional deficits (Gabaglio *et al*., 2021). Our study supports this notion, as we observed significant alterations in cortical morphology, synaptic density, and neuronal function in response to THC and CBD exposure. Chronic THC exposure has been shown to reduce cortical thickness and impair neuroplasticity in rodent models, which are associated with deficits in cognitive and emotional processing and increased vulnerability to stress-related disorders (Testai *et al.,* 2022).

In conclusion, our study provides strong evidence that prenatal exposure to THC and CBD results in significant neurodevelopmental and growth impairments, which are likely mediated by placental dysfunction, oxidative stress, and alterations in metabolic and neuroplastic processes. These findings underscore the need for further research into the long-term effects of cannabis use during pregnancy, particularly on cognitive and emotional health in offspring.

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