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Detrimental effects of prenatal combined delta-9-tetrahydrocannabinol and cannabidiol exposure on placental morphometry and neurodevelopment

Dorcas Olubunmi Taiwo-Ola[1*], Isa Adedeji Sulaiman [2], Philemon Dauda Shallie[3], Olatoye Jubril Otulana[1], Omotosho Dhuqarnain Akanji[1], Joseph Ayobamidele Ariyo[1], Ponle Bamidele Fakunle [1], Opeyemi Abel Bamgbose [1], Alli Akorede Aleem[1], Rapheal Olabode Ifarajimi[4].

[1]Department of Anatomy, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ago Iwoye; [2]Department of Anatomy, Crescent University, Abeokuta, Ogun State, Nigeria; [3]University of Kansas, Kansas City, Missouri, United States of America; [4]Department of Veterinary Medicine, Peoples' Friendship University of Russia, Moscow, Russia.

***Corresponding Author**: Email: dorcas.taiwoola@oouagoiwoye.edu.ng; Tel: +234 803 655 2199; ORCID ID- 0000-0001-6939-3496

ABSTRACT

This study evaluates the combined effects of Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD) on the prefrontal cortex, neurodevelopmental indices, placental morphology, and fetal growth in Wistar rats. Pregnant rats received 150 mg/kg of Δ9-THC and CBD from gestational day 6 to day 19 or until delivery. Placental morphometry was assessed using Vernier calipers, while the prefrontal cortex morphology was evaluated histologically using Hematoxylin & Eosin staining. Neurodevelopmental indices were measured using reflex tests. Placental morphometric analysis at gestational day 19 revealed a significant increase in placental length (control: 1.40 ± 0.04 cm; experimental: 1.45 ± 0.22 cm, $P < 0.05$) and a significant reduction in placental area (control: 2.39 ± 0.0153 m²; experimental: 2.23 ± 0.07 m², $P < 0.01$). Fetal weight at gestational day 19 (control: 3.78 ± 0.043 g; experimental: 3.51 ± 0.064 g, $P < 0.001$) and pup weight at postnatal day 1 (control: 5.742 ± 0.081 g; experimental: 3.72 ± 0.039 g, $P < 0.001$) were significantly reduced. Reflex assessment in neonates showed reduction in righting (control: 1.78 ± 0.07; experimental: 1.08 ± 0.15, $P < 0.001$), forelimb grasp (control: 1.88 ± 0.06; experimental: 1.24 ± 0.13, $P < 0.01$), and cliff avoidance tests (control: 1.84 ± 0.06; experimental: 1.15 ± 0.14, $p < 0.05$). Morphology revealed disrupted blood spaces, trophoblast abnormalities, cortical disorganization, and neuronal vacuolation, indicating structural damage and stress. These results suggest that prenatal exposure to combined Δ9-THC and CBD adversely affects placental integrity and neurodevelopmental outcomes, posing potential risks to fetal health.

Keywords: Delta-9-tetrahydrocannabinol, cannabidiol, neurodevelopment, placental morphometry, prefrontal cortex

INTRODUCTION

Cannabinoids are biologically active compounds found in the *Cannabis sativa* plant. Among these cannabinoids, delta-9-tetrahydrocannabinol and cannabidiol are the most abundant and extensively studied, representing key focus areas for understanding cannabis' pharmacological

effects[1,2]. Delta-9-tetrahydrocannabinol (Δ9-THC) and Cannabidiol (CBD), can cross the placental barrier directly and potentially affect fetal neurodevelopment[3]. As important phyto-cannabinoids, they bind to CNR1 and CNR2 receptors to mediate its effects. These receptors are located throughout the placenta and brain [3,4].

The prefrontal cortex (PFC), a highly developed brain area, is responsible for advanced cognitive abilities, decision-making, and behavior regulation. This complex brain region, located in the frontal lobe, serves as a vital hub for higher-order executive functions by integrating and coordinating information from several brain locations [5]. This region contains groups of pyramidal neurons which dynamically encode goal locations that need to be remembered [6]. Dysregulation of this region has been related to neuropsychiatric diseases. Recent findings also suggest that abused drugs disrupt the regulation of PFC pyramidal neurons through perineuronal nets and the fast-spiking, parvalbumin (PV) expressing interneurons that enclose them[7]. Maternal cannabis use during pregnancy is correlated with neurodevelopmental risks in children, including behavioral and cognitive impairments, due to the ability of Δ9-THC to cross the placental barrier [8].

The placenta is a vital organ connecting mother and fetus, essential for nutrient and gas exchange, waste elimination, and hormone production [9]. The placenta is composed of several distinct zones, each with specific roles. The decidua basalis is the maternal portion of the placenta, providing the foundation for implantation and interaction with the developing trophoblast. The labyrinth zone is the primary site for nutrient and gas exchange between maternal and fetal blood, ensuring the growing fetus receives essential nutrients and oxygen [10]. Positioned between the labyrinth and the basal plate is the junctional zone, which plays a critical role in hormone production and signaling to support pregnancy [10]. The basal plate, located on the maternal side, contains spiral arteries that supply blood to the labyrinth zone, maternal sinusoids, and fetal blood vessels. Finally, the chorionic plate forms the fetal side of the placenta and houses the fetal blood vessels that connect to the umbilical cord, facilitating the exchange of materials between mother and fetus [12].

Placental morphology refers to measurements such as length and width, along with fetal growth, which have been linked to a higher risk of metabolic disorders, obesity, cardiovascular diseases, and colorectal cancer in adulthood [13]. Early gestation is a crucial phase for placental and fetal development, during which exposure to substances like THC and CBD can greatly impact placental morphology, morphometry, and function. Prenatal cannabis intake has been associated with outcomes such as low birth weight and small-for-gestational-age status, gestational hypertension, preeclampsia, weight gain greater and less than guidelines, alterations in offspring DNA methylation at genes involved in neurodevelopment or general development [14-17].

The effects of prenatal exposure to these substances, particularly on neurodevelopment, placental morphology and placenta morphometry, are poorly understood, with most studies focusing on Delta-9-tetrahydrocannabinol or Cannabidiol individually. This study addresses that gap by examining their combined effects in Wistar rats, aiming to enhance understanding, guide clinical practice, and identify both potential therapeutic targets and risks related to cannabis use during pregnancy. Through the use of a Vernier caliper, this study aims to detect morphometric changes in the placenta. Through neurodevelopmental indices tests, this study also aims to detect changes in neurodevelopmental processes in the offspring of Wistar rats. This study also aims to detect morphological changes in the placenta on day 19 and the cerebrum on day 43.

MATERIALS AND METHODS

Ethical approval

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

Procurement of Wistar rats

A total of twenty-four (24) female and twelve (12) male Wistar rats, weighing between 90–120 g, were obtained from Peter's Farm (Nig.) Enterprises in Sagamu, Nigeria. The animals were transported to the Olabisi Onabanjo University, Sagamu Campus Animal House Facility, where

the research was conducted. The animals were acclimatized for two weeks under proper sanitary conditions and a 12-hour light/dark cycle. The bedding was replaced regularly, and humane handling procedures were strictly followed during the experiment. The animals were fed with guinea feed pellets from Top Feed Mill Nigeria Ltd. and had access to water *ad libitum*.

Extract preparation

Cannabis was obtained from the National Drug Law Enforcement Agency (NDLEA) office in Abeokuta, Ogun State, Nigeria, free of charge. Cannabis identification was confirmed by NDLEA using a U.S.A. Drug Identification Kit (Reference Number: NDLEA/SD/2024/2170). The extraction of THC and CBD from the dried cannabis plant involved de-shafting to remove stems and large plant debris, ground and followed by maceration of the de-shafted plant material in ethanol for 72 hours. The macerated solution was then filtered using Whatman filter paper and concentrated with a rotary evaporator (Model: Premium Rotary Evaporator Yamato RE-601-CW) at reduced pressure to obtain the crude extract. The crude extract or mother liquor was fractionated to isolate THC and CBD components, and confirmation of cannabinoid components was carried out using thin-layer chromatography (TLC) [18].

Animal mating and grouping

Twelve healthy adult female Wistar rats were mated with male rats in a 2:1 ratio. Pregnancy was confirmed physically, through the use of a vaginal plug and the presence of sperm cells [19]. The rats were then allocated into two main groups: Control Group (CG) and Experimental Group (EG). The experimental groups (EG & EG2) received 150 mg/kg of delta-9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD) orally from gestational day 6 to day 19

Figure 1: Vaginal plug observed in female Wistar rat, indicative of successful mating.

The rats were weighed on gestational day 19 (GD19) and postnatal day 1 (PND1) to monitor any potential effects of THC/CBD exposure on maternal and pup body weight. Upon reaching the designated endpoints, the rats were euthanized using the isoflurane method. The placental tissues were removed, weighed, measured, trimmed, and fixed in 10% formaldehyde for histological studies. The prefrontal cortex of the pups was carefully isolated from the brain and preserved for further histological analysis.

Tissue processing

The glass slides with paraffin-embedded tissue sections were placed into staining racks, and the paraffin was removed by immersing the slides in three changes of xylene for 2 minutes each. The sections were then rehydrated by transferring them through three changes of 100% ethanol for 2 minutes each, followed by 95% ethanol for 2 minutes, 70% ethanol for 2 minutes, and a rinse in running tap water for at least 2 minutes. Hematoxylin was used to stain the sections for 3 minutes, after which they were washed under

running tap water for at least 5 minutes. Counterstaining was performed using eosin for 2 minutes. The sections were then dehydrated by dipping the slides in 95% ethanol about 20 times, followed by immersion in 95% ethanol for 2 minutes and two changes of 100% ethanol for 2 minutes each. The sections were cleared in three changes of xylene for 2 minutes each. Finally, a drop of Permount was applied, a coverslip was placed over the section, and the slides were observed under a microscope [20].

Statistical analysis: The data obtained from the experiment were analyzed using Microsoft Excel (2018 version). A significance level of ($P < 0.05$) was used for all statistical tests. Significant differences between groups were assessed using an unpaired t-test. Data are expressed as mean ± standard error of the mean (SEM).

RESULTS

Pup body weight at gestational day 19 and post-natal day 1

In this study, the examined fetal and postnatal growth in rats have shown reductions in fetal weight at gestational day 19 and pup weight at postnatal day 1. There was a significant decrease both prenatally and postnatally, indicating that the experimental intervention may disrupt critical developmental processes.

Table 1: Comparison of mean fetal weight and pup weight between control and experimental groups, showing significant reductions in the experimental group.

Weights	CG	EG
Fetal weight at gestational day 19	3.78 ± 0.043	3.51 ± 0.064***
Pup weight at post-natal day 01	5.74 ± 0.081	3.72 ± 0.039***

The data is reported as Mean ± SEM, with statistical significance set at P < 0.001 (***).

Placental morphometry

The results of the placental morphometric analysis revealed differences between the control group (CG) and the experimental group (EG) at gestational day 19 (GD19). While no significant differences were observed in placental weight or thickness, a notable increase in placental length was found in the experimental group. Additionally, placental breadth and area were significantly decreased. These findings suggest potential morphological changes in the placenta due to the experimental conditions, which could influence placental function and impact the neurodevelopment of rat pups.

Table 2: Comparison of placental morphometric values between THC+CBD Group 1 and Control Group 1.

Placenta	CG	EG
Mean weight at GD19 (g)	0.42 ± 0.07	0.42 ± 0.001
Thickness at GD19 (cm)	0.26 ± 0.005	0.26 ± 0.006
Length at GD19 (cm)	1.40 ± 0.04	1.45 ± 0.22*
Breadth (cm)	1.70 ± 0.008	1.27 ± 0.02***
Area(m^2)	2.39 ± 0.0153	2.23 ± 0.07*

GD19: Gestational Day 19; Mean ± SEM; Area calculated from length and breadth measurements; * P < 0.05, *** P < 0.001

Neurodevelopmental indices

The experimental group (EG) showed significant impairments in several neurodevelopmental reflexes compared to the control group (CG), suggesting potential developmental delays or neurological deficits (Table 3).

Table 3: Neurodevelopmental reflex testing results presented as mean ± SEM for each measurement.

Reflexes	CG (Mean ± SEM)	EG (Mean ± SEM)
Righting on PND3	1.78 ± 0.07	1.08 ± 0.15***
Forelimb Grasp on PND3	1.88 ± 0.06	1.24 ± 0.13**
Hindlimb Grasp on PND3	1.82 ± 0.07	1.31 ± 0.16
Cliff Avoidance on PND4	1.84 ± 0.06	1.15 ± 0.14*
Hindlimb Placing on PND 4	1.74 ± 0.08	1.15 ± 0.14*
Gait on PND6	23.33 ± 0.92	25.00 ± 0.91
Auditory Startle on PND10	1.31 ± 0.18	1.00 ± 0.04
Posture on PND 12	1.75 ± 0.15	1.42 ± 0.03
Eye Opening	1.41 ± 0.16	0.58 ± 0.03*
Accelerated Righting	1.59 ± 0.14	1.35 ± 0.03

Mean ± SEM reported; significance: P < 0.05 (*), P < 0.01 (**), P < 0.001 (***)

Placenta morphology

Histological evaluation at GD19 showed a well-organized placenta in the control group with distinct decidual, junctional and labyrinth zones, and abundant intervillous spaces. The experimental group revealed structural distortions, reduced intervillous spaces, and trophoblast alterations, suggesting potential placental dysfunction.

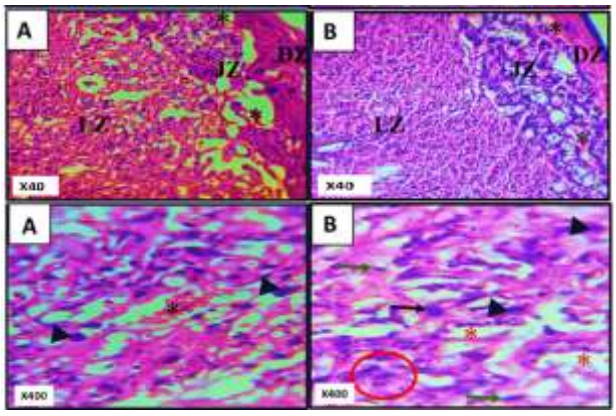

Figure 2: [A] Control group (CG) showing well-defined labyrinth zone, blood spaces (asterisks) and trophoblast cells (triangle). [B] Experimental group (EG) showing trophoblast cells(arrows) with acidophilic cytoplasm, pale elongated nuclei (triangles), clumped nuclei (red ring), and disrupted blood spaces (asterisks). (H&E).

Prefrontal cortex morphology

Histological analysis of the prefrontal cortex in the control group revealed well-defined cortical layers with normal cytoarchitecture. The granular layer displayed intact neurons with deeply stained nuclei and minimal vacuolation. In contrast, sections from EG showed notable disruptions in cortical layers,

particularly in Layers 4–6, with evidence of neuronal vacuolation and nuclear clumping. These findings suggest structural alterations and cellular stress in the experimental group compared to the control.

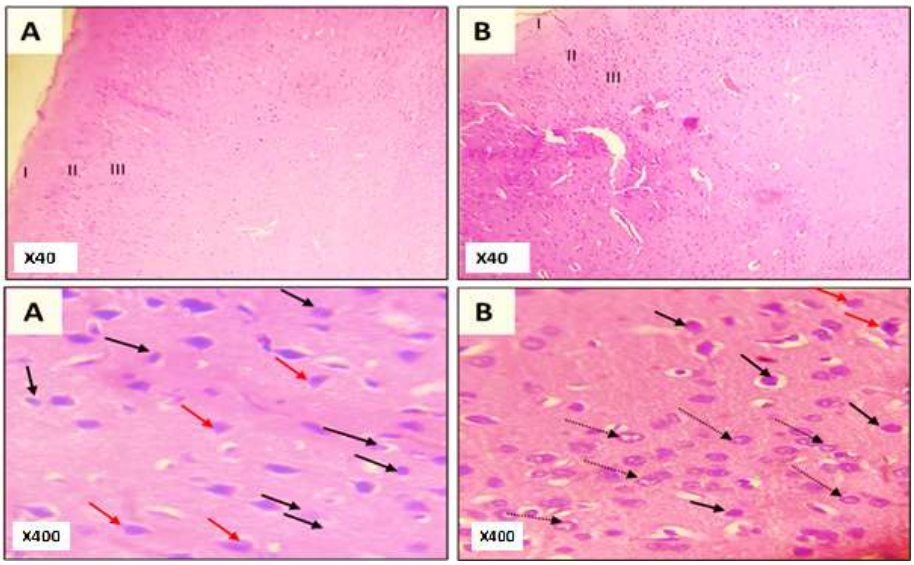

Figure 3: (A) Granular layer in the control group showing normal granular neurons (deeply stained nuclei) and minimal vacuolation (red arrows: pyramidal cells). (B) The granular layer in EG shows vacuolated neurons (dark arrows) and nuclear vacuolation (dotted arrows), suggesting cellular stress. Red arrows indicate pyramidal cells. (H&E).

DISCUSSION

Cannabis intake has been associated with decreased weight both prenatally and postnatally, although some studies have noted an increase in weight following cannabis use [21]. Delta-9-tetrahydrocannabinol (THC) alone led to an unexplained increase in fetal weights at GD 19.5, whereas CBD resulted in a decrease in fetal weights at gestational day 19.5 [22,23]. There is significant weight decrease after the combined use of both THC and CBD. This decrease is still persistent after birth indicating that there is a fetal growth restriction due to combined THC and CBD use.

Placental morphometric analysis at GD19 showed a significant increase in length but reduced breadth in the experimental group. These findings suggest impaired placental growth and potential functional deficits following combined THC and CBD exposure.

Prenatal cannabinoid exposure affected neuromotor development which was reflected by delayed reflexes and altered gait patterns. Reduced righting reflex on post-natal day 3 and cliff avoidance on post-natal day 4 indicate impaired vestibular and proprioceptive function, while delayed eye-opening is a potential marker of developmental delay. However, some analyses revealed significant associations between prenatal marijuana exposure and reduced performance in memory, impulse control, problem-solving, quantitative reasoning, verbal development, and visual analysis tests. Conversely, improved performance was observed in attention and global motion perception assessments [24]. There is limited evidence on the long-term neurodevelopmental outcomes associated with prenatal cannabis exposure extending into early childhood.

The placenta is considered a window to the brain, and exposure to THC or CBD may disrupt its normal function and development [25]. Disrupted blood spaces were observed in the experimental group, indicating possible placental stress or injury. This disruption in blood spaces aligns with other studies, which also reported that delta-9-tetrahydrocannabinol (THC) exposure affected blood spaces in the labyrinth layer, suggesting compromised placental structure and function [22].

Nuclear vacuolations in the prefrontal cortex at post-natal day 43 is a marker of tissue stress due to cannabis extract consumption. The functional significance of nuclear vacuoles in the prefrontal cortex (PFC) is multifaceted, involving ion homeostasis, metabolic stress response, and potential implications for cognitive functions. Recent studies highlight the role of vacuolar H+-ATPase (V-H+-ATPase) in regulating nuclear pH, which is crucial for maintaining electrochemical gradients and facilitating substrate transport across nuclear membranes [20]. Furthermore, the dynamic remodeling of nucleus-vacuole junctions during metabolic stress suggests that these structures play a role in cellular adaptation and communication, potentially influencing neuronal health and function in the PFC [21].

Conclusion

Prenatal delta-9-tetrahydrocannabinol and cannabidiol exposure significantly affects placental morphometry and compromises neurodevelopmental outcomes in Wistar rat offspring. There are potential risks associated with combined THC and CBD use during pregnancy on fetal growth and overall development.

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Conflict of Interest: The authors have no conflict of interest to declare.

Authors' contributions:

DOT: Research conception, design and writing of the manuscript; IAS: Design modification, data collection, co-drafting the manuscript; PDS: Research conception, design and Supervisor: OJO: Drafting and revising the manuscript: ODA: Data acquisition, analysis and interpretation

JAA: Data acquisition, analysis, interpretation, drafting the manuscript; PBF: Revised the manuscript; OAB: Data acquisition, analysis and

interpretation; AAA: Result interpretation, drafting the manuscript; ROI: Drafting and revising the manuscript.

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