Observed Zonal Labyrinth Trophoblastic Proliferations with Reduced Placental Efficiency from Early intake of Toxic drugs in Wistar Rats

Abstract:

This study investigates the effects of early exposure to tetrahydrocannabinol (THC) and cannabidiol (CBD) on maternal and fetal development in Wistar rats. Female rats (90-120 g) were administered 150 mg/kg of cannabis extract (THC, CBD, or their combination) from gestational day 6 to day 19, with food and water provided ad libitum. Cannabis extracts were prepared through ethanol extraction followed by rotary evaporation to isolate THC and CBD. The effects on body weight, placental morphology, and trophoblast cell count were assessed. Results showed that THC exposure during gestation significantly reduced fetal body weight on gestational day (GD) 19 and postnatal day (PND) 1, with the Early THC group showing a 27.7% reduction in weight at GD 19 and a 17.5% reduction at PND 1. CBD exposure resulted in a similar but less pronounced effect, with a 23.04% reduction in fetal weight at GD 19 and 18.2% at PND 1. The combination of THC and CBD resulted in a fetal weight reduction of 24.5% on GD 19 and 21.39%, falling between the reductions observed for THC and CBD alone. Placental weight and thickness were significantly altered in the THC and CBD groups, with a reduction in fetal-to-placenta weight ratio in all cannabis-exposed groups, indicating compromised placental efficiency. Histological analysis revealed significant reductions in cell counts in the labyrinth zone of the placenta, suggesting impaired trophoblast proliferation. These findings underscore the potential developmental disruptions caused by THC and CBD exposure, with significant effects on placental function and fetal growth. Further investigation is needed to understand the underlying mechanisms and long-term implications of cannabis exposure during pregnancy.

Keywords: Delta-9-tetrahydrocannabinol (THC), cannabidiol (CBD), labyrinth zone, fetal weight, feto-maternal ratio.

1. INTRODUCTION

Cannabis sativa, also known as weed or hemp, defined as an annual dioecious plant with psychoactive and medicinal properties, has been utilized for centuries in various cultural, therapeutic, and industrial contexts [1]. The medicinal use of *Cannabis sativa* was first documented in the ancient Chinese pharmacopeia, Shen Nong Ben Cao Jing (The Divine Farmer's Materia Medica) [2]. This is attributed to the mythical emperor Shen Nong (Chen Nung), who is believed to have lived around 2737 BCE and is revered as a pioneer of herbal medicine in Chinese tradition [3]. Initially passed down orally through generations, the knowledge was eventually compiled into written form, with the earliest surviving versions dated no later than 221 BCE. Researchers have also attributed its origin to central Asia (primarily), southern Caspian region, Siberia or the Himalayas [4]. In recent times, research on *Cannabis sativa* is expanding rapidly, driven by its therapeutic potential and the increasing legalization of cannabis worldwide [5]. Due to its anti-inflammatory properties and demonstrated in-vitro antiviral activity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), cannabidiol (CBD) has been suggested as a potential therapeutic option for managing coronavirus disease 2019 (COVID-19) [6]. Cannabis was described in many literature sources for its therapeutic properties, including pain relief, anti-inflammatory effects, and its use in treating various ailments, such as rheumatism and malaria [7]. *Cannabis sativa* plant contains over 550 natural components, with more than 120 of these being identified as "cannabinoids," which are unique to this plant [8,9]. It is a plant characterized by its active compounds, notably tetrahydrocannabinol (THC) and cannabidiol (CBD), it interacts with the endocannabinoid system to influence neurodevelopment and physiological functions [10]. While it holds promise for medical use, its effects during critical periods, such as pregnancy, remain a subject of concern. Prenatal exposure to *Cannabis sativa* may disrupt normal development, particularly in the brain and placenta, necessitating immediate research to elucidate its safety and long-term consequences [11]. Tetrahydrocannabinol (THC) is the primary compound in cannabis responsible for the psychoactive effects, commonly referred to as the "high" experienced after use [12], while CBD, the main non-psychoactive component, has anxiolytic, antipsychotic, and anticonvulsant properties [13]. Both THC and CBD bind to the Endocannabinoid system to induce their effects. THC has been responsible for various neurodegenerative disorders and CBD has been shown to be ameliorative to THC induced disorders [14]. The growing nature of cannabis use among pregnant women is alarming though it is influenced by a wide range of reasons such as, mental health status, socioeconomic status, age and ethnicity [15]. Grant KS et al. [16] stated that there is a need for a clear message to the entire population about the dangerous nature of cannabis. For example, one study found that out of 100 postpartum women, 11% disclosed cannabis use, whereas 14% tested positive by urinalysis and 28% by hair analysis [17]. In Spain, the typical age of first cannabis use is 18.3 years, emphasizing the urgent need to raise awareness among younger generations about its use [18]. Growing bodies of literatures have linked cannabis use during pregnancy to a range of potential adverse effects on the mother and fetus. Mulligan MK and Hamre KM [19] stated that some pregnant women presented cannabis as an alternative for treating pregnancy related illnesses such as morning sickness. Though peer influence is another reason for cannabis use among pregnant women [20]. Its effects on the fetus is an increased risk of preterm birth, where babies are born earlier than expected, which can lead to various health complications [21]. Additionally, infants exposed to cannabis in utero (via placenta) are more likely to experience low birth weight, which can pose challenges for early development and increase vulnerability to infections and other health issues [22]. Fetal growth restriction (FGR), which occurs in over 10% of pregnant individuals, is a frequent pregnancy complication linked to a higher likelihood of stillbirth, premature birth, and negative outcomes for the newborn [23]. This growth restrictions have been linked to deterred placental development in new born with mechanisms that are not well understood [24]. Another significant concern is the potential for neurodevelopmental issues. Sharapova SR et al. [25] identified links between prenatal marijuana exposure and reduced performance in areas such as memory, impulse control, problem-solving, quantitative reasoning, verbal development, and visual analysis. Conversely, improved performance was observed in attention and global motion perception tests. Prenatal cannabis exposure (PME) is associated with attention deficits, behavioral problems, and a higher risk of ADHD. It may also affect brain connectivity, with lasting impacts on memory and executive functioning [26]. The placenta itself may also be affected by cannabis use, as THC can impair placental function, increasing fetal to placenta weight ratio, placenta weight, and labyrinth layer which can hinder fetal growth [24]. The placenta is a disposable organ, functioning only during pregnancy. It is divided into maternal and fetal parts [27]. The labyrinth zone, basal zone (also referred to as the junctional zone), and yolk sac are components of the fetal part of the placenta, while the decidua and metrial gland are components of the maternal part of the placenta [28]. The labyrinth zone (LZ) is the primary site for maternal-fetal exchange of oxygen, nutrients, and waste products, which are critical for fetal growth and development [28]. It lies on the wide, flat chorionic plate and forms a dense vascular layer that connects to the umbilical cord. It consists of maternal sinusoids, trophoblastic septa, and fetal capillaries [28]. Alterations in the structure or function of the LZ often have direct and measurable impacts on fetal health, making it a crucial area for understanding placental efficiency [29]. Generally, research on the placenta is limited, and the evidence suggests that studies on cannabis' effects on placental health are even more scarce. These gaps highlight the need for further investigation into how cannabis impacts the placenta and its role in mediating fetal development. In the present work, we have analyzed the adverse effects of tetrahydrocannabinol (THC), cannabidiol (CBD), and their combination on fetal weight and placental health. We also investigated the individual and synergistic impacts of these cannabinoids on neurodevelopmental and morphological parameters, using ImageJ software to assess cell proliferation. These findings underscore the importance of understanding the implications of prenatal cannabis exposure on placental function and fetal development.

2.0 METHODOLOGY

2.1 PROCUREMENT OF RAT

A total of 24 female Wistar rats and 12 male Wistar rats, weighing between 90-120 g, were used for the experiment.

The animals were obtained from Peter's Farm (Nig.) enterprises in Ibadan.

2.2 HOUSING

The animals were kept in wire mesh plastic cages of size (40 cm × 60 cm × 20 cm) in the animal house of the Department of Anatomy, Olabisi Onabanjo University, Sagamu campus, Ogun state, Nigeria. This was done under standard laboratory conditions on a 12-hours light/dark cycle. The animals were left to acclimatize for two weeks before the commencement of the experiment. The animals were given pellet feeds and water (Jafel Agro service, Sagamu, Ogun state) ad libitum. Animals were mated in ratio 1:2 under standard conditions.

2.3 PREPARATION OF EXTRACT

Cannabis sativa was acquired through the National drugs law and enforcement agency (NDLEA); the extract was prepared from the dried leaves of the plant. The identification was done by the NDLEA with reference number NDLEA/SD/2024/2170. The cannabis leaves were dried and their weight was obtained. A mechanical miller was used to grind the leaves into powder. The powdered leaves were then soaked in ethanol for 72 hours or 3 days [30]. This process increased the surface area, facilitating better penetration of the 100% alcohol solvent during the maceration procedure. Solvent-based Agitation was performed manually with paddles and stirrer to increase the contact between the solvent and the cannabis materials [31]. It was then filtered using a filter paper and then taken to the department of pharmacognosy, Olabisi Onabanjo University for solvent-solvent extraction using a rotary evaporator [31] and thin layer chromatography (TLC) procedures. The percentage yield was calculated using this formula Final $\frac{Final\ Weigt}{Initial\ Weight}\ X\ 100$. The initial weight of the Cannabis leaves before extraction was 3300g, and the final weight of the extracted material was 278.64. As much as 8.44% was obtained as the percentage yield through the process of preparation. Reference values gotten for the THC and CBD were compared with journal articles online [32].

2.4 EXPERIMENTAL DESIGN

A total of 36 rats including 12 males were used during the experiment. The study was conducted with two treatments: Control and Experimental groups (receiving THC, CBD and THC/CBD combination.

- **Control Groups:** A total of 6 female Wistar rats were used as controls, provided with food and water ad libitum. They were further divided into two subgroups (n=3): one observed from gestational day 6 to day 19, and the other monitored until gestational day (GD) 21.
- **Early THC Group (E THC):** Six female Wistar rats were provided with food, clean water, and a 150 mg/kg dose of THC for specific durations of gestational days based on the subgroup. One subgroup (n=3) was observed from gestational day 6 to day 19, while the other subgroup (n=3) was monitored until birth.
- **Early CBD Group (E CBD):** Six female Wistar rats which were subdivided into two, n=3. They were provided with food, clean water, and a 150 mg/kg dose of CBD for specific durations of gestational days based on the subgroup, GD6 to GD19 and until birth respectively.
- **Early THC/CBD (E THC/CBD) Group:** Six female Wistar rats were provided with food, clean water, and a 150 mg/kg dose of THC/CBD from gestational day 6 to day 19. One subgroup (n=3) was monitored until birth, while the other subgroup (n=3) was observed from GD 6 to 19.

The doses were selected based on previous evaluations in rats [33]. At day GD 19, three animals from each group were sacrificed and placentae were extracted for macro-morphometric calculations and morphological analysis. The remaining three (3) animals in the groups were allowed to litter and their pups were allowed to grow (Figure 1).

Feed used: Top line finisher pellet.

2.5 PHOTOMICROGRAPHY

Image acquisition and analysis: A light microscope (10 - 40x magnification objective) was used. Digital camera – AmScope MD500A attached to P.C - HP was used along with a Java Application Software.

2.5 DATA ANALYSIS

Statistical analyses were conducted using GraphPad Prism. Comparisons between groups were performed using

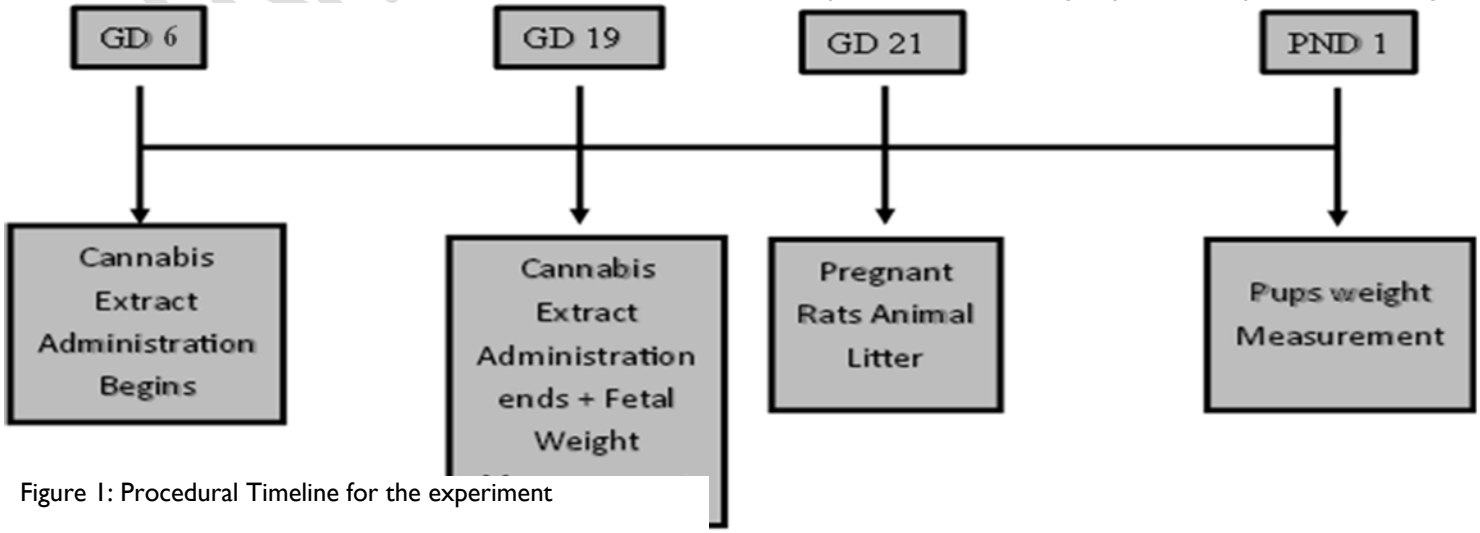

Figure 1: Procedural Timeline for the experiment

Student's t-test for two-group comparisons and one-way ANOVA for multiple groups. Data are expressed as Mean ± S.E.M. For image-based analysis, six images per placenta were taken at 10× magnification, and quantitative analysis of the images was performed using GraphPad Prism. Statistical significance was set at $p < 0.05$.

3.0 RESULT AND ANALYSIS

3.1 Mean Body Weight Reduction at Gestational day 19 and Post Natal Day 1

Notably, cannabis extracts, particularly CBD and THC, both individually and in combination, caused a significant reduction in the body weight of the offspring. Figure 2A shows a significant decrease in fetal body weight on GD 19 in the Early THC group (2.48 ± 0.15 g, $p < 0.001$) compared to the control group (3.42 ± 0.48 g). It has been previously proven that THC at gestational day 19.5 caused an increase in fetal weight, which is not consistent with our findings [24]. On PND 1, the control group had an average pup weight of 4.63 ± 0.075 g, compared to Early THC (3.82 ± 0.81 g, $p < 0.001$). Though results at PND 1 from previous studies were consistent with this article, it was confirmed that THC significantly caused a reduction in fetal weight at PND 1 [34], [35]. This is due to the fact that THC can either penetrate the placenta or bind to receptors prenatally [36]. Postnatally, recent studies support our findings by showing that rat pups from cannabis-induced mothers have lower birth weights. However, after being fed by control mothers, there was an increase in weight, indicating that THC can also penetrate breast milk to impair the fetus [37]. In Eitan A et al. study [38], 30 mg/kg of THC caused a significant weight decrease in mice which corresponds to the findings from this work. Regarding the other groups, the Early CBD group (2.64 ± 0.15 g, $p < 0.05$) experienced a slight significant decrease in weight on gestational day 19. This finding contrasts with previous studies, such as the one of Iezzi D et al. [39], where CBD was shown to increase weight in a sex-specific manner but tallies with the study of Allen S et al. [40] where CBD caused a reduction in fetal weight. It is also important to note that CBD, via the mechanism of THC explained above,

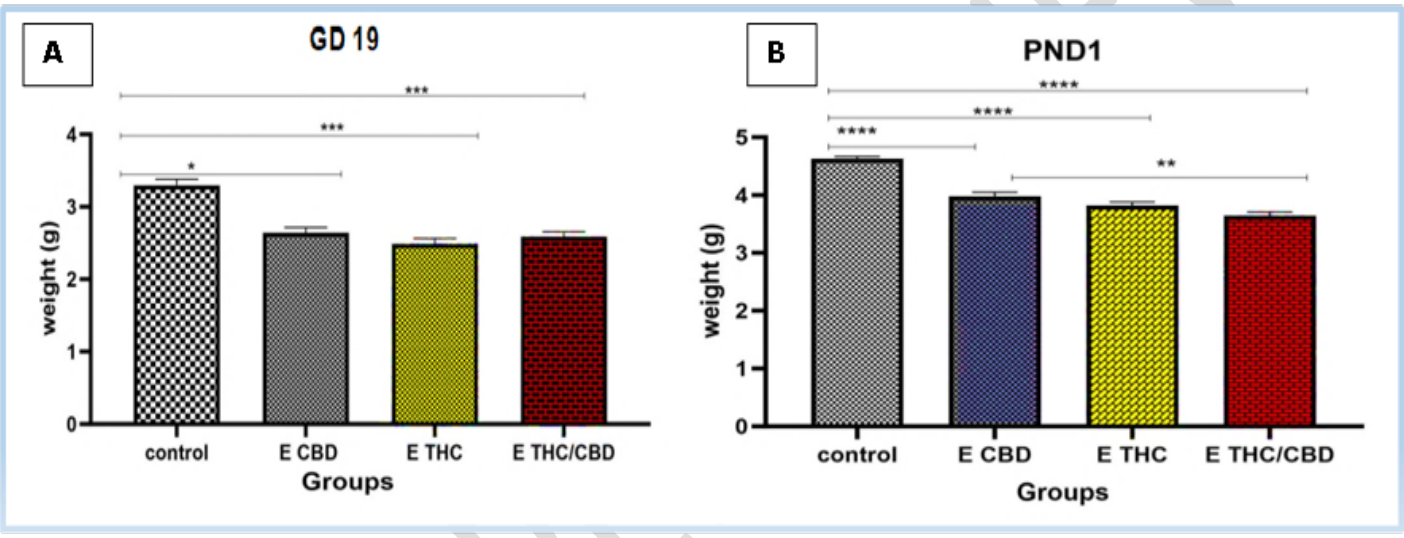

Figure 2: (A) Fetal weight at gestational day 19 (GD 19), Each bar represents Mean ± S.E.M, with a significant difference at $p < 0.05$ (*) and $p < 0.001$ (***) compared with the control. (B) weight at postnatal day 1 (PND 1). Each bar represents Mean ± S.E.M, with a significant difference at $p < 0.01$ (**) and $p < 0.0001$ (****) compared with the control.

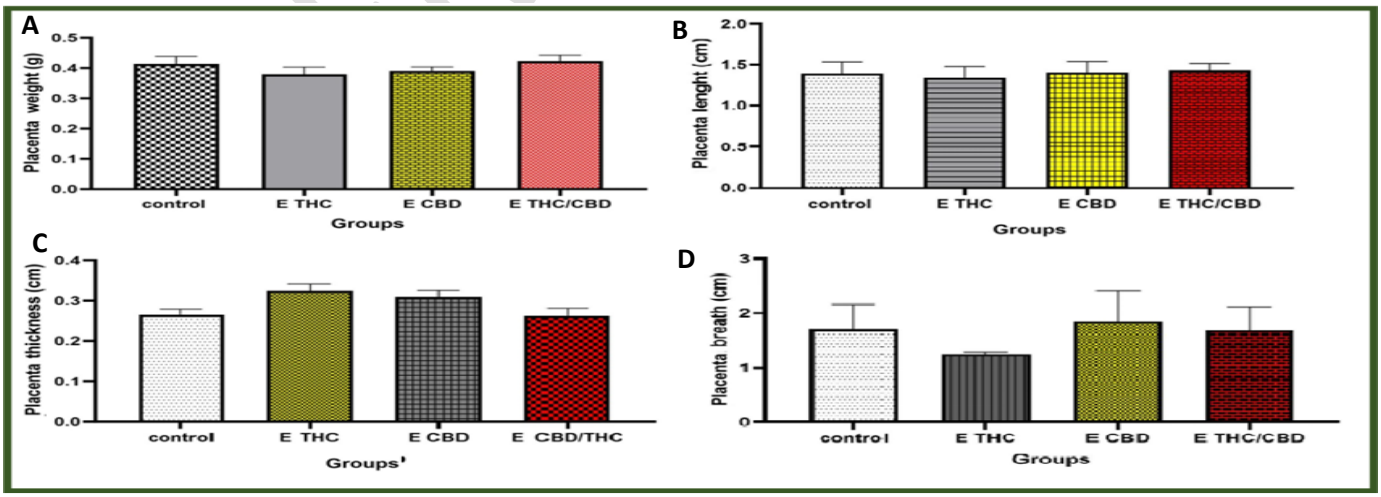

Figure 3: Statistical analysis of placental morphometric parameters among four groups. All values are expressed as mean ± SEM. Statistical significance was determined using one-way ANOVA, with a P-value < 0.05 considered significant. Significant differences were observed in all groups except for placental weight and length in the ETHC/CBD group.

can affect the placenta. Early CBD group (3.79 ± 0.83 g, $p < 0.001$) at PND 1 experienced a significant decrease in weight indicating that cannabis effects persist postnatally. Debates in the research community suggest that THC might retain its efficacy and could be utilized for weight reduction, as it has demonstrated similar effects, as mentioned by Klein C et al. [41]. The combination of THC with the indirect antagonist CBD has been shown to enhance THC's effect in reducing body weight gain [41]. Adolescent rats treated with an equal concentration of THC and CBD maintained the reduced body weight even after a 21-week washout period [41,42]. The Early THC/CBD group (2.59 ± 0.13 g, $p < 0.001$) at gestational day 19 displayed fetal weight outcomes that lay between the extremes observed in the Early THC and Early CBD groups, suggesting a minor effect of THC alongside a potential ameliorating effect of CBD when administered in combination though there are limited research on the combined use of THC and CBD. Early THC/CBD has a weight of (3.64 ± 0.98 g, $p < 0.001$) at PND. So, in summary, the weight reduction on GD 19 was 23.04% in the Early CBD group, 27.7% in the Early THC group, and 24.5% in the Early THC/CBD group. The weight reduction on PND 1 was 18.2% in the Early CBD group, 17.5% in the Early THC group, and 21.39% in the Early THC/CBD group.

3.2 Placental Morphometric Calculations in Induced Wistar Rat.

Placental morphometric parameters, including weight, length, thickness, and breadth, have been utilized as indicators to recognize fetal growth restriction [43]. The impact of tetrahydrocannabinol (THC) on placenta weight and development is a significant concern in maternal-fetal health. Research indicates that THC exposure during pregnancy can lead to reduced placenta weight and compromised placental function, which may adversely affect fetal growth and development. Although comparison bars are not displayed for the data in figure 3, statistical analysis revealed significant differences. In figure 3a, a slight decrease in placental weight was observed in the Early THC (0.38 ± 0.035 g) and Early CBD (0.39 ± 0.023 g) groups compared to the control (0.41 ± 0.036 g). However, placental weight in the Early THC/CBD group (0.42 ± 0.009 g) was similar to the control. These reductions were statistically significant except the ETHC/CBD group where significance was not attained. Interestingly, marijuana use has been reported to increase placental weight in some studies [44]. Some studies observed a slight decrease in placenta weight [45] while others reported an increase due to THC, which has been linked to fetal growth restriction [46]. Lee K and Hardy D [47] further highlighted that FGR can occur via placental insufficiency, implying adverse effects on placental function and development. A recent study reported that CBD exposure led to a reduction in placental weight, which aligns with our findings demonstrating a similar placental weight reduction caused by CBD [48]. It has been suggested that THC and CBD, acting through TRPV2 receptors, can induce weight loss in both the placenta and fetus [36]. Figure 3b shows a significant difference in placental length among groups except ETHC/CBD group. Values were as follows: control (1.40 ± 0.040), Early THC (1.34 ± 0.056), Early CBD (1.41 ± 0.010), and Early THC/CBD (1.43 ± 0.033). Placental thickness (figure 3c) was significantly increased in the Early THC (0.32 ± 0.059) and Early CBD (0.31 ± 0.043) groups compared to the control (0.26 ± 0.022). The Early THC/CBD group (0.26 ± 0.024) exhibited a similar thickness to the control which was not statistically significant. In figure 3d, no significant differences in placental breadth were observed among groups: control (1.71 ± 0.547), Early THC (1.25 ± 0.455), Early CBD (1.86 ± 0.152), and Early THC/CBD (1.69 ± 0.019). However, the Early THC group exhibited a markedly lower breadth compared to the control, suggesting the possibility of a significant difference. Generally, placental thickness was significantly increased in the Early THC (0.32 ± 0.059) and Early CBD (0.31 ± 0.043) groups compared to the control (0.26 ± 0.022). This suggests that early exposure to THC and CBD leads to structural adaptations, possibly due to changes in trophoblast function or vascular development. In contrast, the Early THC/CBD combination group (0.26 ± 0.024) showed no significant difference, indicating a potential neutralizing effect between the two cannabinoids. These findings contrast with the study of Ortigosa S et al. [49], where a decrease in placental thickness was observed, possibly due to variations in dosage, timing, or species.

3.3 Fetal to Placenta Weight Ratio

Placenta weight alone is not a sufficient proof of placenta efficiency [43,44,50]. The fetal-to-placental weight ratios were significantly reduced in the cannabis-exposed groups, indicating compromised placental efficiency and nutrient transfer to developing fetuses [51]. Fetoplacental weight is a true indicator of placenta dysfunction [46,52]. A study reported a trend toward lower fetoplacental weight ratio (FPR) among marijuana-exposed pregnancies, particularly in term stillbirths (6.84 vs. 7.8, $p < 0.001$). However, multivariable analysis from their study showed no significant association ($p = 0.09$) and no differences in placental histology were identified between exposed and non-exposed groups [53]. Results from the study of Metz TD et al. [54] have shown that cannabis generally causes a reduction in the fetoplacental weight ratio. These results are Specific, Natale BV et al. [46] demonstrating that THC can reduce the fetoplacental weight ratio, a finding that is consistent with the data presented in Table 1 below.

Table 1: The fetoplacental weight ratios in pregnancies exposed to Δ9-THC.

Control Group	E THC	*P < 0.05*
8.30 ± 0.52	*6.36 ± 0.166*	*0.040**

The data are displayed as mean ± standard error. Asterisks indicate a significant difference (P <0.05).

CBD was shown to cause a reduction in the fetoplacental weight ratio in the study by Allen S et al. [48]. Similarly, Table 2 demonstrates a reduction in the fetoplacental weight ratio.

Table 2*:* The fetoplacental weight ratios in pregnancies exposed to CBD.

Control Group	*E CBD*	*P < 0.05*
8.30 ± 0.52	*6.95 ± 0.59*	*0.002**

The data are displayed as mean ± standard error. Asterisks indicate a significant difference (P <0.05).

The fetoplacental weight ratio in the combined THC/CBD effect (Table 3) is much lower, despite the ameliorative influence of CBD on THC, as described by Freeman AM et al.

[55]. However, it cannot be definitively concluded that CBD fully mitigates the negative effects of THC on the fetoplacental ratio. While CBD has shown potential in reducing some of THC's adverse effects, the overall impact of their combination may still lead to disruptions in placental function or fetal development, as indicated by the reduced fetoplacental weight ratio. Further studies would be needed to clarify the precise interactions between THC and CBD in this context. Though, the dose dependency was addressed by Solowij N et al. [56], where a high dose of CBD reduced the intoxicating effects of THC, a low dose of CBD combined with THC enhanced the intoxicating effects of CBD, this interaction does not appear to be directly related to the fetoplacental weight ratio. While the dose of CBD influences the effects of THC on maternal and fetal outcomes, it seems that the fetoplacental weight ratio is not significantly impacted by these combined doses of THC and CBD in the manner observed in other developmental markers. Further research is necessary to explore the complex relationships between these compounds and placental function.

Table 3: The fetoplacental weight ratios in pregnancies exposed to THC/CBD.

Control Group	*ETHC/CBD*	*P < 0.05*
8.30 ± 0.52	*6.17 ± 0.143*	*0.018**

The data are displayed as mean ± standard error. Asterisks indicate a significant difference (P <0.05).

3.3 Cell Counts

Exposure to 150 mg/kg of CBD, THC, and a combination of CBD/THC from early gestational day 6 to day 19 resulted in a significant reduction in the cell count of the Labyrinth Zone (LZ) of the placenta compared to the control group. Image analysis of the control group showed a mean LZ cell count of 1663 ± 213.25. In contrast, the group exposed to CBD had a mean count of 552.7 ± 7.45, with a p-value of 0.007, indicating a significant reduction in trophoblast cell count (Figure 4). A similar significant reduction was observed in the THC-exposed group (Figure 5), which had a mean count of 361.67 ± 54.69 (p-value = 0.004). The combined CBD/THC group (Figure 6) also exhibited a significant reduction, with a mean count of 637.67 ± 17.57 (p-value = 0.013). A general reduction was observed

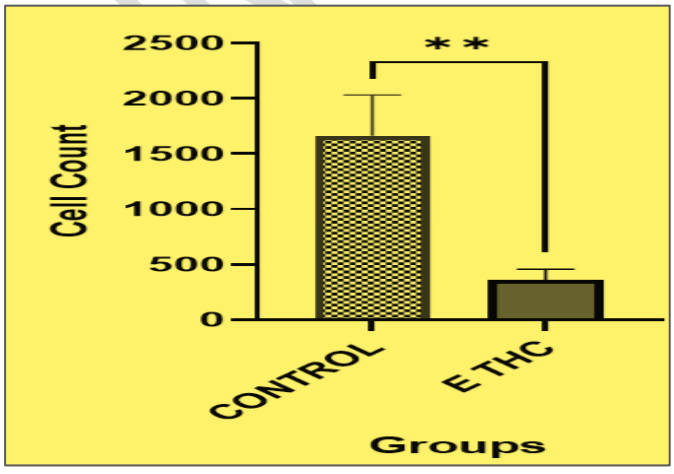

Figure 4: Statistical analysis of the l labyrinth zone cell counts in the placenta of rats between the control and E THC groups. All values are expressed as mean ± SEM. Statistical significance was determined using an independent t-test, with a P-value < 0.05 being considered significant.

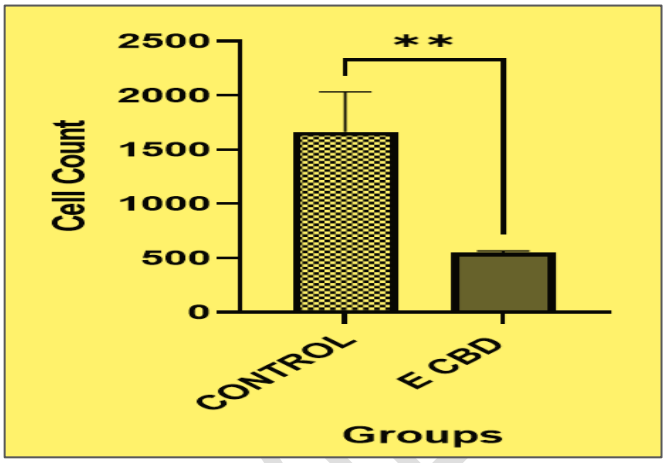

Figure 5: Statistical analysis of the labyrinth zone cell counts in the placenta of rats between the control and E CBD groups. All values are expressed as mean ± SEM. Statistical significance was determined using an independent t-test, with a P-value < 0.05 being considered significant.

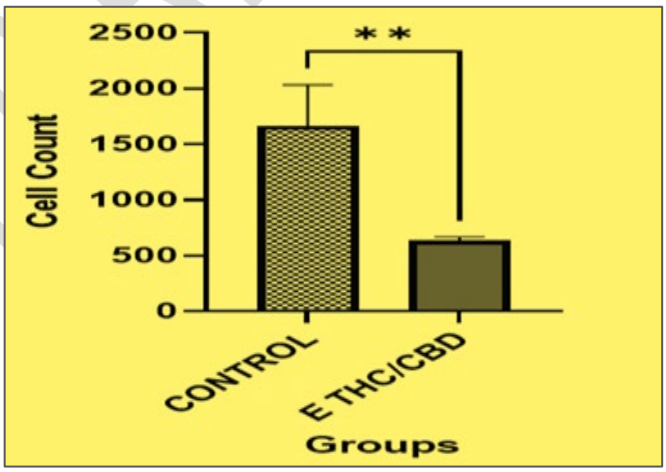

Figure 6: Statistical analysis of the labyrinth zone cell counts in the placenta of rats between the control and E CBD/THC groups. All values are expressed as mean ± SEM. Statistical significance was determined using an independent t-test, with a P-value < 0.05 being considered significant.

Cannabis active constituents, including THC and CBD, have been linked to trophoblast proliferation at both the cellular and molecular levels [57]. The Labyrinth Zone (LZ) is the primary site for maternal-fetal exchange of oxygen, nutrients, and waste products, which are critical for fetal growth and development. Alterations in the structure or function of the LZ often have direct and measurable impacts on fetal health, making it a crucial area for understanding placental efficiency. The observation of a general reduction in cell count aligns with findings by Natale BV et al. [46], who reported that THC exposure led to an increase in labyrinth area but a reduction in EpCAM expression. EpCAM is a marker for trophoblast progenitors, and its reduced expression indicates impaired renewal and differentiation of these progenitor cells.

Together, these findings suggest a specific disruption in labyrinth development due to THC exposure. Allen S et al. [48] reported that CBD exposure did not affect the expression of markers for labyrinth progenitors (EpCAM) or junctional zone progenitors (Ascl2) and suggested that

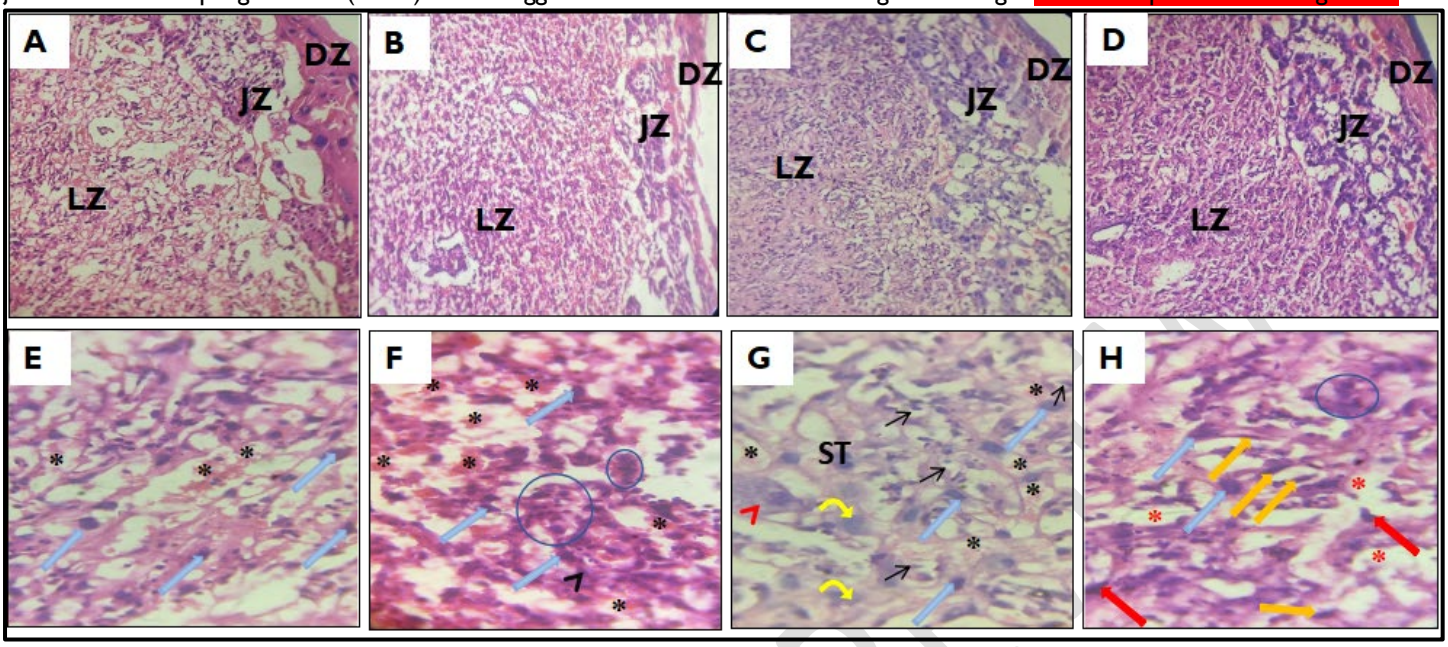

Figure 7: Representative photomicrographs of hematoxylin and eosin-stained placental sections of the experimental groups showing;
[A]: Three-layered placenta structure showing an intact junctional zone (JZ), labyrinth zone (LZ), and decidual zone (DZ) of the control group (X100 magnification). [B]: Three-layered placenta structure from the THC induced group, showing a distorted decidual zone, with alterations in the labyrinth and junctional layers (X100 magnification). [C]: Three-layered placenta structure from the CBD group, showing the three zones, with alterations in the labyrinth and junctional layers. [D]: The placenta section from the ETHC/CBD group, showing the junctional zone (JZ), labyrinth zone (LZ), and decidual zone (DZ) of the placenta (X100 magnification). [E] The control group of the placenta shows normal labyrinth cell morphology, characterized by giant trophoblast cells (blue arrows) and intact blood vessels (asterisks) (X400 magnification). [F] The labyrinth zone of THC induced group features visible maternal spaces containing blood cells with loss of blood compartmentalization (asterisks). Giant trophoblast cells (blue arrows) are present, with basophilic cytoplasm (black arrowhead). Blue circles indicate clusters of cells with tiny nuclei and basophilic cytoplasm (X400 magnification). [G] Photomicrographic section of the labyrinth zone of the CBD-induced group: Spongiotrophoblast cells exhibit highly vacuolated cytoplasm and darkly stained nuclei (ST). The giant trophoblast cells display prominent nuclei (thick blue arrow), lightly stained nuclei (curved yellow arrow), nuclear vacuolations (arrowhead), and disrupted plasma membranes (arrowhead). Glycogen-filled cells with shrunken nuclei are indicated by thin black arrows (X400 magnification). [H] This section of the labyrinth zone, treated with a combined dose of THC and CBD, contains spongiotrophoblast cells with basophilic cytoplasm (red arrow) and giant trophoblast cells (blue arrows), including flat giant trophoblast cells (orange arrows). Irregular maternal blood spaces lack blood cells (red asterisks), and clusters of cells with basophilic cytoplasm are visible (blue circle) (X400 magnification).

trophoblast progenitor populations were maintained. However, our data reveal a reduction in trophoblast cell count, highlighting a disconnection between gene expression stability and cellular population outcomes. In addition, Allen S et al. [48] reported that in utero exposure to 3 mg/kg CBD resulted in reduced populations of labyrinth endothelial cells and SynTII cells at E19.5, a critical time point in placental and fetal development. This is in line with our findings because we observed a decrease in trophoblast cell count, particularly in the labyrinth zone, suggesting that CBD exposure adversely affects trophoblast differentiation and function.

3.4 PLACENTA MORPHOLOGY

The photomicrographs in Figure 4 illustrate notable histological changes in placental morphology across experimental groups treated with THC, CBD, or their combination. The control group displays a normal three-layered structure with intact labyrinth cell morphology, suggesting efficient maternal-fetal exchange and healthy placental function. In the THC group, the decidual layers show distortion, indicating structural disruptions that may impair nutrient and gas exchange. Maternal spaces containing blood cells remain visible, but abnormalities like loss of blood compartmentalization similar to the lipid peroxidation caused by aspartame …. [58]. Both aspartame and THC can induce oxidative stress and lipid peroxidation, but their impacts on blood compartmentalization and mechanism of action in the placenta differ [59]. At GD 19.5, the presence of proliferating cells is typically low; however, proliferation can be altered in response to placental stress, potentially leading to changes in cell division and tissue development. We observed a decrease in cell count, which may indicate impaired cellular proliferation due to such stress [60]. In the CBD group, less pronounced cellular stress is observed in spongiotrophoblast cells, likely due to improved blood compartmentalization. However, some histological changes, such as vacuolated cytoplasm, nuclear vacuolations, and glycogen-filled cells with shrunken nuclei, may indicate localized metabolic dysfunction and possible compromises in placental efficiency [61]. Additionally, a reduction in cellular proliferation was noted, as reflected in

the cell count data. These results suggest that while CBD may alleviate some stress through enhanced vascular dynamics, it does not completely prevent cellular and metabolic disturbances in the placenta. The THC group contains numerous plasma cells, indicative of neuroinflammation. A gradual decrease in plasma cells is observed in the CBD group, with a complete absence in the THC/CBD combination group. Given that CBD is known for its anti-inflammatory properties [62], its presence in combination with THC might have mitigated any potential inflammatory response, thus preventing the accumulation of blood cells in the placental tissue [63]. These findings suggest a compounded impact on placental structure, likely reducing its ability to support normal fetal development.

The disruptions to the fetal micro-environment from maternal factors can negatively impact these processes and impair fetal brain development, leading to prenatal programming of neurodevelopmental disorders [64], as THC or CBD can penetrate the placenta barrier and reach the fetus [52]. Overall, the observed histological changes highlight the detrimental effects of cannabinoid exposure on placental integrity, with potential consequences for fetal growth and development.

We might raise the question as to why the oral route was chosen for cannabis administration when intravenous (IV) administration offers 100% bioavailability. We selected the oral route because it is the most commonly used method, especially among people in Nigeria. The oral route reflects real-world patterns of cannabis consumption, making it more relevant for studies aiming to understand typical use and its effects on health [65]. Since the oral route of administration, despite being affected by first-pass metabolism, resulted in similar plasma concentrations of THC in the treated animals, it emphasizes the importance of simulating a more human-like environment in animal studies. This approach would help to ensure that the results more accurately reflect the human experience of cannabis use [66].

CONCLUSION

This study demonstrates that cannabis (THC, CBD or its combination) exposure during early gestation significantly impairs fetal weight, reduces placental efficiency, and disrupts trophoblast cellular proliferation in the labyrinth zone of the placenta. The findings of this study unequivocally underline the detrimental effects of prenatal cannabis exposure on fetal weight and placental health. Both THC and CBD, independently and in combination, were shown to result in a significant reduction in fetal growth and adverse changes in placental morphology and function. These outcomes suggest possible disruptions in nutrient transfer, cellular proliferation, and overall placental efficiency, which are critical for healthy fetal development. The research highlights the urgent need for further comprehensive studies to elucidate the mechanisms of cannabis-induced developmental perturbations and their long-term consequences. A deeper understanding is critical for informing public health policies and clinical guidelines concerning cannabis use during pregnancy, aiming to mitigate risks and safeguard maternal and fetal health.

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