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Impact of mid-gestational δ9-tetrahydrocannabinol exposure on hepatic development in Wistar rat offspring

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ABSTRACT

Cannabis use during pregnancy is increasing globally, raising concerns about fetal exposure to Δ9-tetrahydrocannabinol (THC), the principal psychoactive constituent of cannabis. THC readily crosses the placental barrier and may disrupt the endocannabinoid system, which regulates fetal organogenesis, including hepatic development. This study investigated the effects of oral THC exposure during mid-gestation on liver morphology, growth indices, and selected biochemical markers in Wistar rat offspring. Twelve adult female Wistar rats were mated with proven fertile males, and pregnancy was confirmed by the presence of a vaginal plug and sperm-positive vaginal smear, designated as gestational day (GD) 0. Pregnant rats were randomly assigned to a control group (vehicle only) or a THC-treated group (150 mg/kg, oral gavage) from GD12 to GD21. Offspring were evaluated for body weight, liver weight, histomorphological changes using haematoxylin and eosin and periodic acid–Schiff staining, and serum biochemical parameters. Prenatal THC exposure significantly increased liver weight compared with controls ($p < 0.0001$) and was associated with increased body weight ($p = 0.0004$). Histological examination revealed hepatocellular hypertrophy, cytoplasmic vacuolization, architectural disorganization, and reduced glycogen deposition in THC-exposed offspring. Biochemical analysis showed significant reductions in alkaline phosphatase ($p = 0.012$) and conjugated bilirubin ($p < 0.0001$), with significant increases in total protein ($p = 0.015$) and total bilirubin ($p = 0.014$). Oral THC exposure during mid-gestation disrupts hepatic development in Wistar rat fetuses, primarily affecting liver growth, cellular architecture, and synthetic capacity rather than inducing overt hepatocellular necrosis.

Keywords: Δ9-tetrahydrocannabinol; prenatal exposure; liver development; developmental toxicology

INTRODUCTION

Human fetal development is highly sensitive to the intrauterine environment, and disturbances during critical windows of organogenesis can have lifelong consequences for the structure and function of developing organs. Organogenesis is particularly vulnerable because rapid cell proliferation, migration, and differentiation are underway, and even brief exposures to xenobiotics may permanently alter growth trajectories and metabolic programming. In this context, the increasing use of cannabis among women of reproductive age has raised urgent questions about the effects of its main psychoactive constituent, delta-9-tetrahydrocannabinol (THC), on fetal organ development and long-term health.[1,2]

THC crosses the placenta and binds to cannabinoid receptors (CB1, CB2) expressed in the placenta and multiple fetal organs, including the brain, thereby directly exposing the conceptus to exogenous cannabinoid signaling.[1] Experimental rodent and primate models consistently show that prenatal THC or cannabis exposure can induce fetal growth restriction with reduced organ-to-body weight ratios (heart, liver), followed by postnatal catch-up growth and early tissue remodeling in organs such as the heart and liver.[2,3,4] THC-dominant cannabis smoke or edibles also impair placental development and perfusion, alter placental zonation and trophoblast differentiation markers, and reduce nutrient transport capacity, changes that are closely linked to fetal growth deficits.[2,5,1] Beyond growth, prenatal THC has

been linked to persistent alterations in neurodevelopment, behavior, metabolism, and organ-specific transcriptomes in offspring. Animal studies show long-term neuropsychiatric-like phenotypes, sex-dependent disruption of fatty-acid signaling in the mesolimbic system, and cognitive and social impairments after prenatal THC exposure[6-9]. Parallel primate work demonstrates that chronic prenatal THC exposure alters placental and fetal DNA methylation at genes involved in neurobehavioral development and changes fetal brain growth trajectories and histology, suggesting durable epigenetic and structural programming effects.[1,10]

Importantly, the endocannabinoid system (ECS) is present early in development in the placenta and a wide range of fetal tissues, where it regulates cellular proliferation, differentiation, and metabolic pathways.[11] In the liver and other metabolic organs, prenatal THC exposure has been shown to increase hepatic de novo lipogenesis, alter fatty-acid profiles, and program dyslipidemia and impaired glucose homeostasis in later life.[12,3,13] However, while these metabolic and neurodevelopmental consequences are increasingly recognized, far less is known about how prenatal THC perturbs the structural maturation and cellular organization of specific parenchymal organs such as the fetal liver. The liver is central to metabolism, detoxification, and protein synthesis, and in utero hepatic growth and differentiation are crucial for both fetal survival and postnatal metabolic capacity. Yet, existing THC studies in rodents and primates have rarely provided detailed histomorphometric descriptions of hepatocyte architecture or systematically correlated these with functional indices such as serum transaminases, alkaline phosphatase, albumin, and bilirubin during defined gestational windows. Most work has focused on global liver-to-body weight ratios, lipid accumulation, or later-life metabolic phenotypes rather than on cellular-level changes during organogenesis.[3,12] Moreover, the critical mid-gestational period in Wistar rats, encompassing intense hepatoblast proliferation and initiation of specialized metabolic pathways, has not been comprehensively interrogated for THC-induced micro-architectural and biochemical disturbances.

Given that THC can disrupt other developing organ systems, including the heart, musculoskeletal system, gastrointestinal tract, and vascular tissues, largely via ECS-mediated and inflammatory pathways, similar mechanisms may plausibly operate in the developing liver.[4.14.15.16] However, rigorous anatomical studies focusing specifically on hepatocyte morphology, such as nuclear-to-cytoplasmic ratios, vacuolization, cellular arrangement in cords and plates, and sinusoidal organization during the mid-gestational window, remain scarce.

Wistar rats provide a robust model for developmental toxicology because of their well-characterized organogenesis, short gestation, and suitability for controlled dosing and timing of exposures. Prior Wistar studies of prenatal THC have focused largely on neurobehavioral outcomes, placental morphology, and cardiometabolic endpoints, but have not systematically examined liver histoarchitecture alongside biochemical markers of hepatic function within the same experimental paradigm.[2,17,18] Addressing this gap is essential for clarifying the hepatic component of prenatal THC toxicity and its potential contribution to later-life metabolic disease.

The present study, therefore, aims to evaluate the hepatic morphological and functional consequences of oral THC administration during mid-gestation in Wistar rats. By integrating quantitative histomorphometry of hepatocytes with serum biochemical indices (AST, ALT, ALP, albumin, bilirubin) at a critical window of liver differentiation, this work seeks to provide mechanistic anatomical insight into how prenatal THC exposure perturbs hepatic development, with relevance for understanding the long-term health risks associated with maternal cannabis use.

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.

Acclimatization

Adult female Wistar rats weighing between 150 and 200 g were purchased from a local breeder in Sagamu. The animals were housed in the animal shelter in the Department of Anatomy, Olabisi Onabanjo University. They were kept in clean, well-ventilated cages under a 12-hour light/dark cycle at room temperature and allowed free access to standard rodent feeds from Top Feed Mill Nigeria Ltd, and clean water *ad libitum*. All rats were allowed to acclimatize for 14 days prior to the commencement of the experiment.

Animal mating and grouping

Twelve female rats were housed overnight with six proven fertile male rats that had previously mated successfully with females, and conception was achieved at a 2:1 ratio. The following morning, each female rat was checked for evidence of mating with a visible vagina plug and by obtaining vaginal smears and examining them under a light microscope. The presence of spermatozoa or a copulatory plug in the vaginal smear confirmed successful mating. The day sperm was first detected in the vaginal smear was recorded as gestational day 0 (GD0). Pregnant rats were then separated from males and randomly assigned into control and experimental groups, with each group containing six rats each (n = 6). The dams

in the experimental group were exposed to cannabinoid substance (THC) 150mg/kg per os from gestational day (GD) 12-21, and the control groups were given feed and water *ad libitum* for the same duration; pups were allowed to grow to ensure liver maturity (till postnatal day PND 42).

Extract preparation

Cannabis inflorescences seized by law enforcement were obtained from the National Drug Law Enforcement Agency (NDLEA) office, Abeokuta, Ogun State, Nigeria, at no cost, and authenticated using a U.S.A. Drug Identification Kit (Reference number: NDLEA/SD/2024/2170). Dried plant material was carefully de-shafted to remove stems and coarse debris, then milled to a fine consistency to enhance solvent contact during extraction. Ethanol was selected as the extraction solvent because it is efficient, widely regarded as safe, and commonly used for cannabinoid recovery.[21-23] The comminuted cannabis was macerated in ethanol at room temperature for a defined period with intermittent agitation, following standard dynamic maceration principles described for medicinal cannabis processing.[23,24] After maceration, the mixture was filtered through Whatman filter paper to remove residual biomass, similar to previously reported cold-ethanol extraction procedures.[25] The combined filtrate was concentrated under reduced pressure using a rotary evaporator to obtain a viscous crude cannabis extract rich in cannabinoids.[22,25,26]

The crude (mother liquor) was then subjected to fractionation to enrich and separate Δ⁹-tetrahydrocannabinol (THC) and cannabidiol (CBD), in line with solvent-based and chromatographic purification approaches commonly applied to cannabis extracts.[22,26,27] Identity and presence of target cannabinoids in the fractions were confirmed by thin-layer chromatography (TLC), comparing migration patterns with appropriate reference standards, as described in earlier cannabinoid extraction and analytical workflows[21,22].

Table 1: Experimental design

Groups	N	Dosage	Duration
Control (A)	6	Feed and Water ad libitum	GD 12-21
Experimental (B) THC	6	150 mg/kg of THC	GD 12-21

GD= Gestational Day, N= number of dams
THC: Δ⁹-tetrahydrocannabinol

Weight change and percentage of organ weight

The change in weight was calculated as the change in body weight (final – initial)

Percentage of organ body weight ratio was calculated as $\frac{organ\ weight}{body\ weight} x\ 100$

Sample collection and histology

On PND 42, the pups' blood samples for liver function tests were taken, and the rats were euthanized using cervical dislocation. Liver tissues were harvested, weighed, and fixed in 10% formaldehyde for histological studies. Tissues were dehydrated in ascending grades of ethanol (70%,90%, and 100%), cleared in xylene, infiltrated, and embedded in paraffin wax. The tissue blocks were mounted on a wooden block. They were sectioned on a rotatory microtome at 10 µm thickness.

Tissue processing

Haematoxylin and eosin: Paraffin-embedded sections on glass slides were arranged in staining racks and gently

deparaffinized by immersing the slides in three successive changes of xylene, 2 minutes each, to ensure complete removal of wax and optimal dye penetration[28,29]. The sections were then rehydrated through three descending grades of ethanol (100%, 95%, 70%) for 2 minutes per step, followed by rinsing under running tap water for at least 2 minutes to fully restore an aqueous environment[28-31]. Nuclear staining was performed with hematoxylin for 3 minutes, after which slides were washed in running tap water for at least 5 minutes to develop crisp blue nuclei and remove excess stain [28,32]. Counterstaining of cytoplasmic and extracellular components was achieved with eosin for 2 minutes[28,30]. The slides were then dehydrated by rapid dipping in 95% ethanol (approximately 20 dips) and subsequently passed through ascending ethanol concentrations (95%, 100%) for 2 minutes each to completely remove water.[28,30] Clearing was carried out in three fresh changes of xylene, 2 minutes each, to render the tissue optically transparent and compatible with the mounting medium[28,31]. Finally, a drop of resinous mounting medium (Per Mount) was applied, a coverslip was carefully placed to avoid air bubbles, and the preparations were allowed to set before examination under the light microscope[30,31]. This sequence is consistent with established hematoxylin–eosin (H&E) protocols for paraffin sections and yields reproducible, high-quality morphology suitable for detailed histopathological evaluation[28-31].

Periodic Acidic Schiff: Periodic acid–Schiff (PAS) staining was performed on paraffin-embedded sections following established protocols with minor

adjustments[33-37]. Sections were first deparaffinized in xylene and rehydrated through graded ethanol (100%, 95%, 70%), followed by rinsing in running tap water to complete rehydration[35,38]. Slides were then incubated in 1% periodic acid solution for 10–30 min to oxidize carbohydrate vicinal diols and generate aldehyde groups[34,39]. After thorough rinsing in distilled water to remove residual oxidant, sections were immersed in Schiff reagent for 10–30 min in the dark, allowing the formation of the characteristic light pink reaction product as basic fuchsin bound to newly formed aldehydes[34,39,40]. Slides were washed in running tap water for 5–10 min to develop the final dark pink to magenta coloration of PAS-positive structures[34,39]. Nuclei were counterstained with Mayer's hematoxylin for 1–2 min to provide clear nuclear detail, followed by rinsing in water[33,36,41]. Sections were then dehydrated through ascending grades of ethanol (95%, 100%), cleared in xylene, and mounted permanently with a resinous medium for microscopic evaluation[33,36,38,41].

Photomicrograph

Photomicrographs were captured using a light microscope (Olympus CX31) with the aid of an Am scope MD 500A digital camera (5.1 MP, 2592 x 1944 resolution).

Statistical analysis

Mean ± SEM was calculated, and comparisons were made using an unpaired t-test, with a significant difference set at $P \leq 0.05$ using GraphPad Prism version 10.0. The cell count was done using Image J (1.54d version).

RESULTS

Effect of mid-prenatal exposure to THC on the body weight and organ weights

The result of the body weight of the rats shows a significant change in body weight when comparing the control group, 88.05±1.223, and the THC group, 96.80±0.860. The relative organ weight for liver results reveals a significant increase in the organ weight when comparing the control (3.792 ± 0.0797 %). The mid THC group (5.424 ± 0.0981%) at $p < 0.0001$ as observed in Figure 1.

Effect of mid-prenatal exposure to THC on the liver enzymes

Figure 2 shows the effect of THC exposure on selected liver function parameters (Figure 2). Serum albumin (ALB) levels were reduced in the THC-treated group (12.0 ± 0.577) compared with the control group (13.5 ± 0.577), although this difference was not statistically significant ($p = 0.288$). Alkaline phosphatase (ALP) levels were significantly decreased in the THC group (30.0 ± 0.8165) relative to the control (35.0 ± 1.080) ($p = 0.012$). Likewise, conjugated bilirubin (CBL) levels showed a highly significant reduction in the THC-treated group (0.2 ± 0.004) compared with the control (0.3 ± 0.004) ($p < 0.0001$). In contrast, total protein (TPRO) levels were significantly increased in the THC group (45.0 ± 0.913) compared with the control group (40.0 ± 0.408) ($p = 0.015$), while total bilirubin (TBL) also showed a significant increase in the THC-treated group (0.5 ± 0.020) relative to the control (0.4 ± 0.004) ($p = 0.0144$). However, alanine aminotransferase (ALT) levels showed no statistically significant difference between the control (6.75 ± 0.479) and the THC-treated group (6.25 ± 0.479) ($p = 0.48$).

Figure 3A shows the histological architecture of the liver in both control and THC-treated groups. The control group demonstrates well-defined hepatic lobules with an orderly arrangement of hepatocytes. The hepatocytes appear polyhedral in shape with centrally placed spherical nuclei, while the sinusoids are clearly delineated, and the central vein is distinctly visible. In contrast, the THC-treated group exhibits notable cytoplasmic disruption, characterized by congested sinusoids, congestion of the central vein, and distortion of the normal hepatic architecture, including irregular radiating plates of hepatocytes.

Fig. 3B illustrates the glycogen distribution within hepatocytes of both control and THC-treated groups. The control group (A) shows abundant hepatocytes with intense cytoplasmic staining, indicating a high presence of glycogen granules. Conversely, the THC-treated group (B) reveals hepatocytes with sparse glycogen content, suggesting glycogen depletion likely resulting from metabolic disruption induced by THC exposure.

Effect of mid-prenatal exposure to THC on the cytometry of the liver.

The cell count was performed to determine the number of liver cells in both the control group and the experimental group. There was a significant decrease in the cell count when comparing the control group, 450.4±3.52, to the THC group, 277±5.38. The hepatic duct is 13 µm, the central vein is 67 µm in the control group, while in the experimental group, the hepatic duct is 15 µm, central vein is 76 µm.

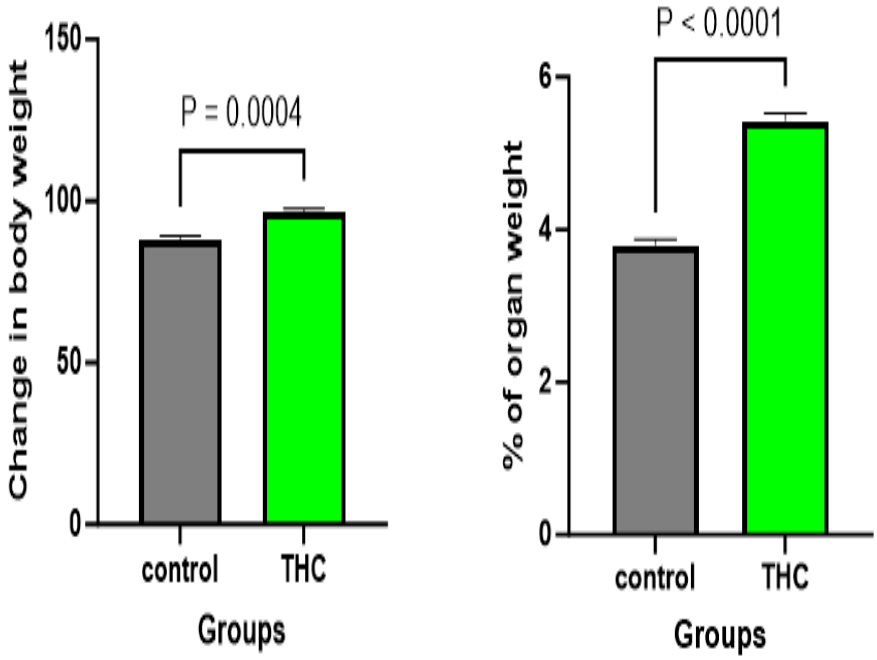

Figure 1: The effect of mid-prenatal exposure to THC on the rat body weight and relative organ weight of Wistar rats at PND 42.

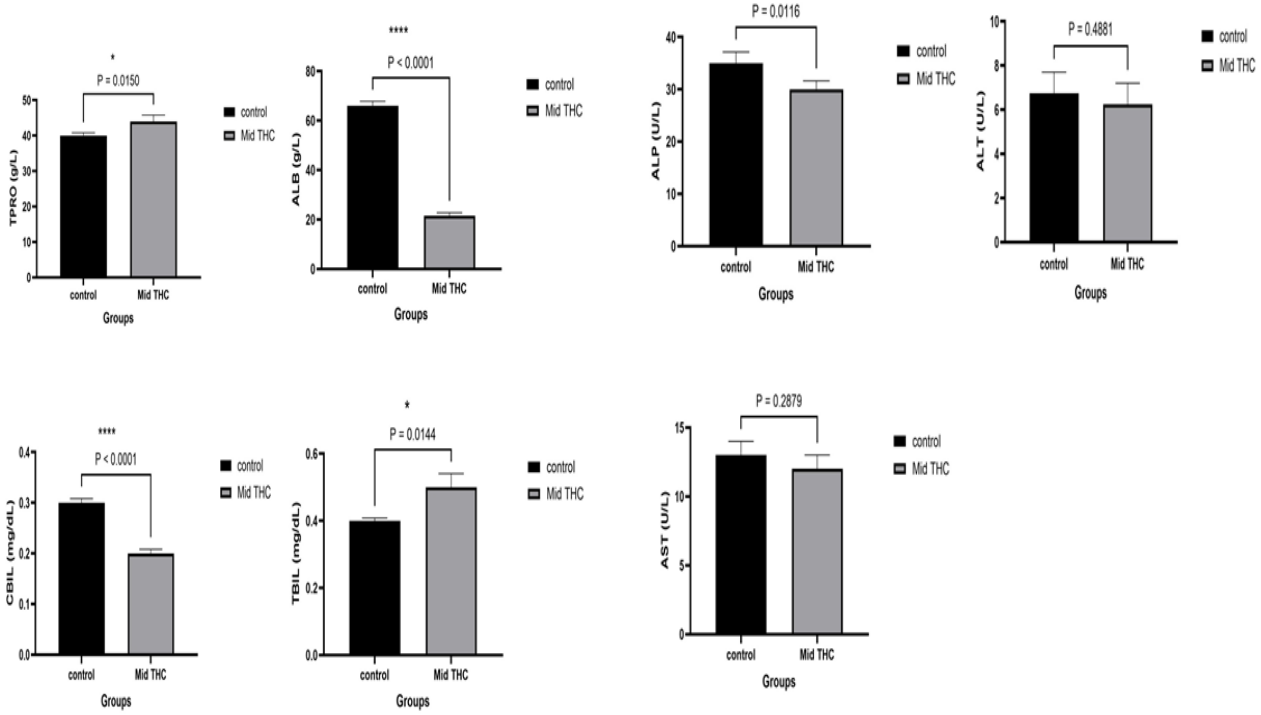

Figure 2: The effect of mid-prenatal exposure to THC on the liver enzymes (Aspartate transferase, Alanine aminotransferase, Alkaline phosphate, Albumin, Total protein, Total bilirubin, and Conjugated bilirubin).

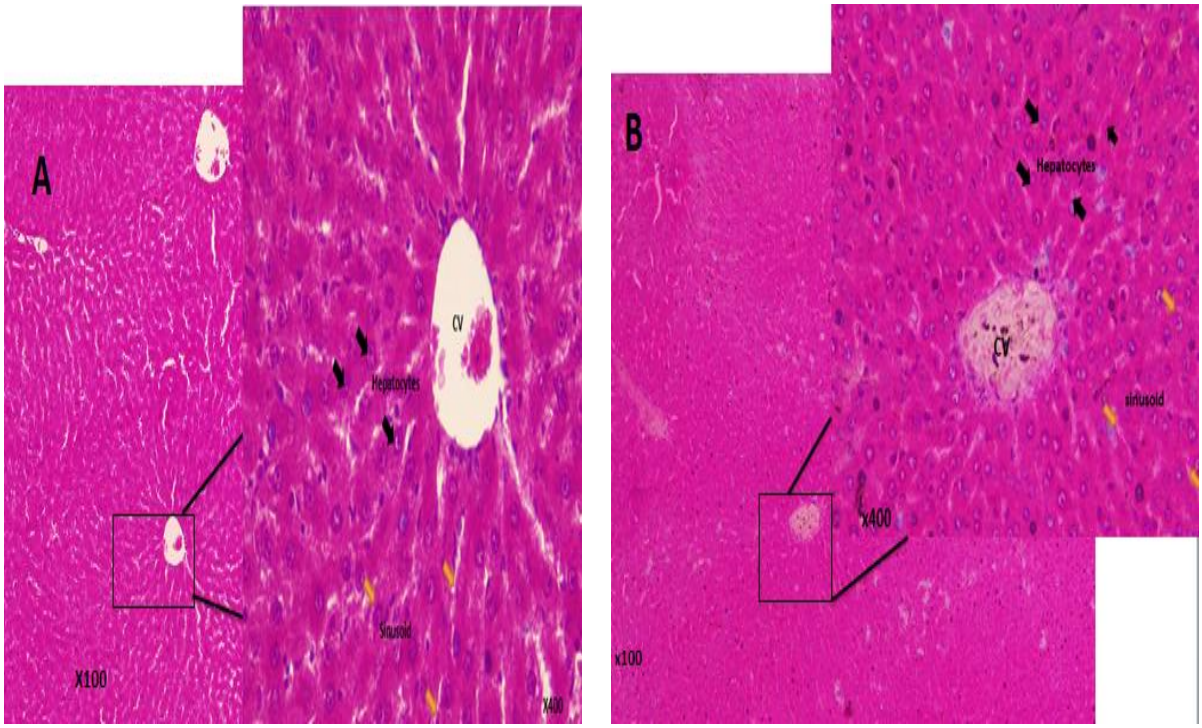

Figure 3: Photomicrograph of a section of the liver, H&E Staining at 100 & 400 magnifications. Control(A) and Experimental group(B). Legends: black arrow- hepatocytes, CV: hepatic central vein, orange arrow-sinusoid.

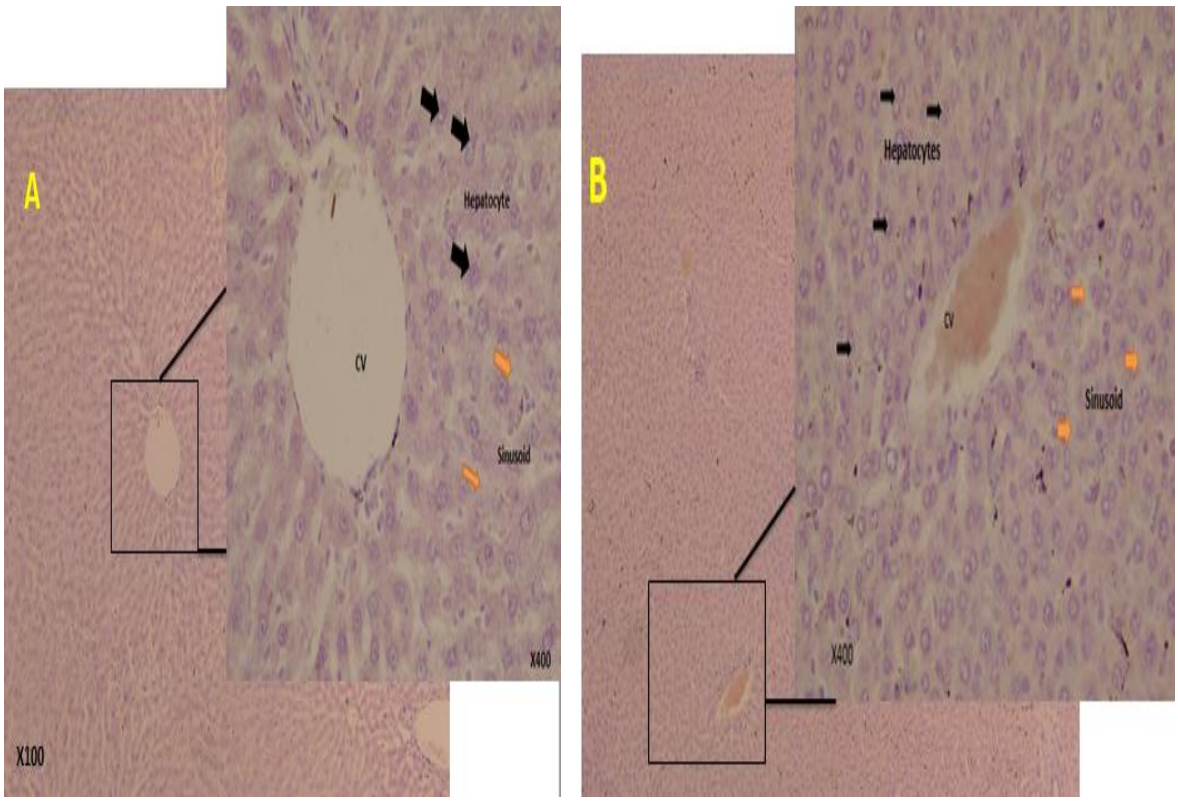

Figure 4: Photomicrograph of a section of the liver, PAS Staining at 100 & 400 magnification, Control(A) and Experimental group(B). Legends: black arrow- hepatocytes, CV: hepatic central vein, orange arrow-sinusoid.

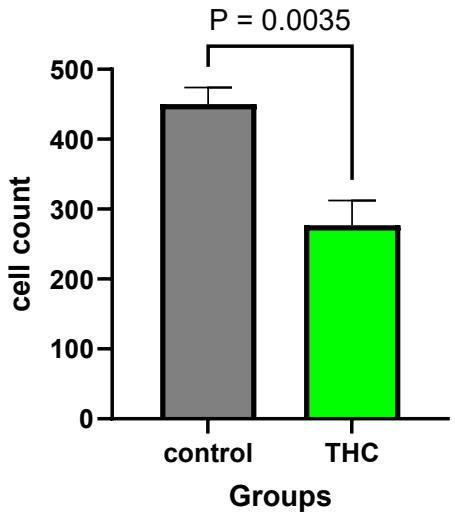

Figure 5: The effect of mid-prenatal exposure to THC on the cell count in the liver of Wistar rats at PND 42. There is a significant difference at p≤ 0.05 when compared to the control

DISCUSSION

This study investigated the hepatotoxic effects of mid-gestational delta-9-tetrahydrocannabinol (THC) administration on the hepatocytes of Wistar rat offspring, providing insights into the potential risks of cannabis exposure during pregnancy. The research builds on a foundation of prior studies[42], particularly focusing on the liver, a critical organ for metabolism and detoxification, but exposure during the mid-gestational phase is novel for research.

The Prenatal THC exposure triggers placental insufficiency, and this will restrict fetal nutrient supply and cause symmetrical intrauterine growth restriction (IUGR) [10]. The exposure to THC in utero is associated with a lower birth weight and a lower liver-to-body weight ratio. Pups in the exposed group underwent rapid postnatal catch-up growth [2]. The compensatory trajectory growth drives systemic, rapid weight gain that often exceeds baseline physiological norms. At the organ level, this phase is characterized by accelerated cellular proliferation as the liver attempts to rapidly restore functional hepatic mass to meet escalating postnatal metabolic demands. THC is highly lipophilic and readily crosses the placental barrier, acting as an exogenous ligand that overstimulates fetal endocannabinoid receptors, particularly CB1 [10]. The disruption of the developing ECS heavily influences both systemic and hepatic metabolism.

The histological findings reveal significant morphological alterations in the liver of adolescent rats at PND 42. There was congestion in the sinusoid space and central vein in the experimental THC group. This indicates obstruction, and this could result in hypertrophy of the liver and potential hepatocellular degeneration, thus underscoring the vulnerability of the developing liver to THC exposure during the critical mid-gestational period. This effect is largely mediated through the endocannabinoid system, particularly the activation of cannabinoid receptor 1 (CB1) in hepatocytes [43,44]. THC acts as a partial activator of Peroxisome Proliferator-Activated Receptor (gamma), which can remodel adipocyte lipid handling[44]. THC in the liver can provoke an inhibition of certain enzymatic systems, including the autodigestion of glycogen, contributing to increased organ weight. THC-induced CB1 receptor activation leads to the down-regulation of carnitine palmitoyltransferase-1 (CPT-1), an enzyme required for fatty acid oxidation, further increasing lipid accumulation [45].

This study aligns with a previous study that reported increased visceral adiposity and higher hepatic triglycerides following gestational THC exposure in male offspring[2]. These observations are consistent with the findings that prenatal THC exposure can impair cellular organization in developing organs [46]. The reduced glycogen stores observed in PAS-stained sections suggest that THC may interfere with glycogen metabolism, potentially through interactions with the endocannabinoid system, which is present in the developing liver and implicated in regulating metabolic functions [46]. This metabolic disruption could have long-term implications for glucose homeostasis in offspring. This present finding reveals a significant decrease in liver cell count, despite the significant increase in both liver weight and total body weight, which perfectly illustrates a pathological shift in tissue architecture.

The mid-prenatal exposure to (THC) over-activates the developing fetal endocannabinoid system (CB1 receptors), which has been linked to severe mitochondrial dysfunction in the liver [2]. This dysfunction results in the massive overproduction of Reactive Oxygen Species (ROS). The resulting oxidative stress damages hepatocellular DNA and lipid membranes, triggering the intrinsic apoptotic pathway. This leads to programmed cell death of hepatocytes, directly causing a reduction in total cell count. The decreased hepatocyte count indicates impaired proliferation or increased apoptosis; THC may slow down cell growth or cause more cells to die. These results are similar to findings from a study that showed that THC exposure during pregnancy can stunt growth and change the liver's structure in rat offspring[44].

A notable finding was the significant reduction in serum albumin concentration in the THC-treated group, indicating compromised hepatic synthetic

function. Albumin, exclusively synthesized by hepatocytes, is a critical plasma protein, and its reduction reflects functional impairment. rather than overt necrotic damage. This observation aligns with studies that reported detrimental effects of prenatal cannabinoid exposure on organ development, including impaired synthetic capacity[2]. The lack of significant transaminase elevation suggests that the hepatotoxicity observed is sub-lethal, primarily affecting metabolic and synthetic functions rather than causing widespread cell death. This is in contrast to studies using higher THC doses or different administration routes, where acute liver injury and elevated transaminases were more pronounced. The absence of significant changes in alkaline phosphatase (ALP), total bilirubin (TBIL), or conjugated bilirubin (CBIL) suggests that THC's effects are primarily metabolic rather than cholestatic or haemolytic.

The mid-gestational period, characterized by active hepatocyte proliferation and differentiation, is particularly sensitive to teratogenic insults. THC's ability to cross the placenta and interact with cannabinoid receptors in the developing liver likely underlies the observed effects. These findings corroborate with animal studies, that prenatal THC exposure disrupts organogenesis, particularly in organs with high endocannabinoid receptor expression [47].

Compared to human studies, the results are consistent with reports of adverse fetal outcomes associated with maternal cannabis use, including low birth weight and developmental delays [48]. However, human studies often face challenges like underreporting due to social stigma, making animal models critical for controlled investigations [49]. The oral administration of THC in this study provides a more relevant model for human exposure compared to older studies using intraperitoneal or intravenous routes, which do not reflect typical cannabis consumption.

CONCLUSION

Mid-gestational oral THC exposure induces structural and functional liver changes in Wistar rat fetuses, including hepatomegaly, histological disruption, reduced glycogen, and impaired albumin synthesis. These effects highlight the fetal liver's sensitivity to THC during development. The findings underscore the potential risks of cannabis use during pregnancy and support the need for increased public health awareness and further research.

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

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Authors' contributions: DOT: Research conception, design, and writing of the manuscript; IAS: Design modification, data collection, and co-drafting the manuscript; ROI: Drafting and revising the manuscript; RMA: Data acquisition, analysis, interpretation, drafting the manuscript

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