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Original Article

Modulatory role of cannabidiol and sleep recovery on hepato-renal functions and histological alterations triggered by sleep deprivation in adult male Wistar rats

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Abstract

BACKGROUND AND AIM: Sleep deprivation is a significant health concern, affecting various physiological systems, including hepatic and renal functions. Cannabidiol, a compound found in cannabis plants, has gained attention for its therapeutic potential through its antioxidant and anti-inflammatory activities. This study aimed to investigate the effect of cannabidiol and recovery sleep on hepatorenal functions and histological changes in the kidneys and liver of adult male Wistar rats.

MATERIALS AND METHODS: Thirty adult male Wistar rats were divided into five groups (n=6) and were given the following treatments for 21 days: the control group (1ml/kg body weight distilled water), group II [1ml/kg body weight distilled water + 18 hours of sleep deprivation (SD)], group III [10mg/kg body weight of cannabidiol (CBD) + 18 hours SD], group IV [20mg/kg body weight of CBD + 18 hours SD] and group V [1ml/kg body weight distilled water + 18 hours SD + 7 days sleep recovery (SR)]. The modified multiple-platform method was used to induce rapid eye movement sleep deprivation. On completion, the animals were euthanized, and the blood samples were collected via cardiac puncture for biochemical analysis (Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) & Alkaline phosphatase (ALP)], kidney electrolyte, urea and creatinine, serum proteins and oxidative stress biomarker [malondialdehyde (MDA)]. The kidney and liver were harvested, processed, and stained with haematoxylin & eosin for general histoarchitecture.

RESULTS: Results from this study showed no notable differences in CBD and SR groups in the liver enzyme concentrations of AST, ALT and ALP], kidney electrolytes and proteins [potassium ions (K^+), urea and creatinine] and oxidative stress biomarker (MDA), but there was a remarkable ameliorative effect in the histology of the kidneys and liver of CBD-treated and SR groups compared to the SD-alone group.

CONCLUSION: Findings from this study showed that seven days of sleep recovery and treatment with CBD alleviated the detrimental effects of chronic sleep deprivation on the kidneys and liver of the Wistar rats.

Keywords:
Sleep deprivation; sleep recovery; cannabidiol; kidney; liver; biochemical; histopathology

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INTRODUCTION

Sleep is a vital physiological process essential for health and well-being (Walker, 2017). It consists of distinct stages, notably non-rapid eye movement (NREM) and rapid eye movement (REM) sleep, each contributing to various restorative functions (Mogavero *et al.*, 2024). NREM sleep facilitates tissue repair and hormone release, while REM sleep supports cognitive processes like memory consolidation (Rasch & Born, 2013). Adequate sleep is crucial for metabolic homeostasis and immune response, with the liver detoxifying and the kidneys filtering waste during this time (Singh *et al.*, 2024; Lama *et al.*, 2020). The circadian rhythm, regulated by the suprachiasmatic nucleus, helps align sleep-wake cycles with environmental cues (Laposky *et al.*, 2008; Foster, 2020). However, sleep can be disrupted by stress and other factors, negatively impacting physical and mental health (Foster, 2020; Howarth & Miller, 2024; Gottesman *et al.*, 2024).

Sleep deprivation, characterized by inadequate sleep quality or quantity, poses significant health risks (Cort-Blackson *et al.*, 2018). It is associated with metabolic disturbances and liver dysfunction (Jun & Polotsky, 2009; Berman, 2021) and affects a large portion of the population due to societal pressures (Colten & Altevogt, 2006; Godsell & White, 2019). The adverse effects of sleep deprivation include metabolic, immune, and cardiovascular disruptions (Garbarino *et al.*, 2021; Scoditti *et al.*, 2022), along with increased oxidative stress leading to liver injury (Zhang *et al.*,

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2022; 2023). It also negatively impacts renal function, potentially worsening chronic kidney disease (Nigam *et al*., 2018), indicating a cycle of metabolic dysregulation affecting liver and kidney health (Hu *et al*., 2023; He *et al*., 2023).

Recovery sleep is crucial for restoring functions impaired by sleep deprivation, enhancing metabolic regulation and normalizing hormone levels (Åkerstedt *et al*., 2009; Kim *et al*., 2015). It also strengthens immune function, improving defenses against infections (Walsh *et al*., 2011; Desai *et al*., 2024).

Cannabidiol (CBD), a non-psychoactive cannabis component, is noted for its potential therapeutic effects, particularly on the renal system (Pertwee, 2012). CBD exhibits anti-inflammatory, antioxidant, and nephroprotective properties, suggesting it may mitigate oxidative stress and renal damage (Al Toufaily, 2021; Bartkowiak-Wieczorek & Mądry, 2024). It interacts with the endocannabinoid system, influencing mood, appetite, pain sensation, and immune function (Lowe *et al*., 2021; de Melo Reis *et al*., 2021; Ekpo *et al*., 2023). Research highlights CBD's therapeutic potential through various mechanisms, benefiting conditions such as chronic pain and neurodegenerative diseases (Elsaid *et al*., 2019; Singh *et al*., 2023). However, its effects on the kidneys and liver concerning sleep deprivation remain unclear, prompting this study to explore the modulatory effects of CBD and sleep recovery on hepatic and renal functions, along with related histological changes.

MATERIALS AND METHODS

Ethical clearance

Ethical approval for this was granted by the Ethics Committee on Animal Use and Care at Ahmadu Bello University, Zaria, Nigeria, with the approval number ABUCAUC/2024/006. The study adhered strictly to the guidelines for the care and use of laboratory animals established by the National Research Council (Clark *et al*., 1997).

Drugs and Reagents

Crystalline cannabidiol, 75% full spectrum, with Lot No: FSO7590-0-221103-A1, was obtained from Eurofins Scientific in Luxembourg. It was dissolved in a 1% solution of Polysorbate 80 (Sigma-Aldrich, Steinheim, Germany).

Experimental design

Thirty male Wistar rats, weighing between 160 and 180 g, were utilized in the study. The rats were obtained from and housed in the Department of Human Anatomy animal facility at Ahmadu Bello University, Zaria. They had unrestricted access to a standard rat pellet diet and clean drinking water. The animals were randomly assigned to five groups, with six rats per group. Group I served as the control and received 1 ml/kg of distilled water. Group II was treated with 1 ml/kg body weight of distilled water along with 18 hours of sleep deprivation (SD). Group III was treated with 10 mg/kg body weight of CBD and 18 hours of SD, while group IV was treated with 20 mg/kg body weight of CBD and 18 hours of SD. Group V received 1 ml/kg body weight of distilled water, 18 hours of SD, and sleep recovery (SR). All treatments were administered orally once daily throughout the study using an oropharyngeal cannula and a 1 ml calibrated hypodermic syringe.

The rats underwent sleep deprivation for 18 hours each day (from 1 PM to 7 AM) for 21 days, with a restorative sleep period of 6 hours daily (from 7 AM to 1 PM) (do Lago Godoi *et al*., 2005; da Silva Rocha-Lopes *et al*., 2018). Cannabidiol was administered orally just before the rats were placed in the sleep deprivation tank. Following the 21 days of sleep deprivation, the rats in Group V were allowed a recovery period of 7 days in their home cage (Hipólide *et al*., 2006).

Induction of sleep deprivation

The Modified Multiple Platform (MMP) method was utilized to induce REM sleep deprivation (REM-SD) as described by Machado *et al*. (2004). The sleep deprivation tank, measuring 120 x 46.5 x 44.5 cm, was constructed from plastic and featured ten circular platforms attached to the bottom. Each platform was 6.5 cm high and 8 cm in diameter, with horizontal spacing of 13 cm and vertical spacing of 10 cm. The tank was enclosed with wire mesh to prevent the Wistar rats from escaping. Water was filled to within 1 cm of the upper surface of the platforms, allowing the rats to move by jumping from one platform to another. Due to natural REM muscle paralysis, contact with the water would awaken the rats.

Rats from the same group were placed in the sleep deprivation tank for 18 hours, during which they had unrestricted access to standard rat chow and water, provided via hanging food baskets and water bottles mounted on the top of the wire mesh. The water in the tank was changed daily after the rats were removed. Following each 18 hours of sleep deprivation, the rats were returned to their home cages for 6 hours of restorative sleep. The modified multiple platforms were chosen for this study to reduce psychosocial, immobilization, and separation stress (Suchecki and Tufik, 2000; Machado *et al*., 2004). The rats were habituated to the modified platforms for 2 days, spending one hour each day on them before the sleep deprivation phase began.

Animal Sacrifice and sample collection

At the end of the 21-day period, Wistar rats from groups 1–4 were sacrificed, while group 5 was given a 7-day sleep recovery period. The animals were euthanized via cervical dislocation. The thoracic cavities of the rats were opened, and blood was collected through cardiac puncture. The blood samples were then centrifuged at 3000 rpm for 10 minutes to separate the plasma for biochemical analysis. The kidneys and liver were carefully dissected and fixed in 10% formol saline for histological assessment. These analyses

were conducted in the Neuroscience and Bioinformatics laboratory of the Department of Human Anatomy at Ahmadu Bello University, Zaria.

Determination of Serum Alkaline Phosphatase Activity

Alkaline phosphatase activity was assessed using the procedure established by Bessey *et al*. (1946). The samples were incubated with p-nitrophenyl phosphate as the substrate for 15 minutes at 37°C. The reaction was terminated by adding 5.0 mL of 0.5 M NaOH, and absorbance was measured at 405 nm with a RA-50 spectrophotometer.

Determination of Alanine Transferase (ALT) and Aspartate Transferase (AST) Activities

The activities of ALT and AST were measured using the method described by Reitman and Frankel (1957). Initially, 500 µL of ALT and AST substrates were preincubated at 37°C for 5 minutes. Next, 100 µL of samples and controls were added, and the mixture was incubated for an additional 30 minutes for ALT and 60 minutes for AST. The reaction was stopped by adding 500 µL of 1 mmol 2,4-dinitrophenylhydrazine, and the mixture was allowed to sit at room temperature for 20 minutes. Color development was achieved by adding 5 mL of 0.4 M NaOH, and the absorbance was measured at 505 nm using a RA-50 spectrophotometer.

Determination of Potassium, Urea, and Creatinine

Serum potassium levels were measured using the Corning 410 Clinical Flame Photometer method. Urea and creatinine concentrations were assessed by spectrophotometry, following the protocols established by Coloumbe and Farreau (1963) and Taussky (1956), respectively.

Light Microscopy

Fixed kidney and liver tissues were processed using standard histological techniques. This included fixation, dehydration, clearing, infiltration, and embedding in paraffin wax. Hematoxylin and eosin staining was employed to evaluate the overall histology. A pathologist, who was not part of the research team, examined the stained sections under a light microscope, and photomicrographs were captured using an AmScope MD 900 digital microscope camera at a magnification of 250X.

Data Analysis

The data collected at the end of the experiment were presented as mean ± SEM (Standard Error of Mean). A one-way analysis of variance (ANOVA) was employed to compare the mean differences, followed by a Tukey *post-hoc* test, with a P-value of 0.05 or less considered statistically significant. All results were analyzed using the Statistical Package for the Social Sciences (SPSS version 25).

RESULTS

Serum Alkaline Phosphatase (ALP), Alanine Transferase (ALT) and Aspartate Transferase (AST) Activities

There were no statistically significant differences in ALP, AST, and ALT levels among the groups ($p > 0.05$) (Figure 1).

Serum Potassium, Urea, and Creatinine Levels

There were no significant differences in potassium, urea, and creatinine levels between the groups ($p > 0.05$) (Figure 2).

Histological Features of the Liver of Control and Treatment Groups

Histopathological analysis of liver tissue sections revealed that the control group exhibited normal histoarchitecture, characterized by intact hepatocytes, Kupffer cells, central veins, and sinusoids. In contrast, liver sections from Wistar rats subjected to 18 hours of sleep deprivation showed significant distortions, including pyknosis, karyorrhexis, dilated central veins, and vacuolation. The liver sections from group III (10 mg/kg CBD + 18 hours of sleep deprivation) displayed mild improvement, though with some distortions. The liver sections from group IV (20 mg/kg CBD + 18 hours of sleep deprivation) demonstrated a marked improvement in histoarchitecture. Finally, liver sections from the sleep recovery group exhibited restoration of normal architecture, including central veins, hepatocytes, Kupffer cells, and sinusoids (Figure 3).

Histological Features of the Kidney of Control and Treatment Groups

Histopathological investigations of the outer renal cortex revealed normal histoarchitecture in the control group, including Bowman's space, glomeruli, and podocytes. In contrast, the renal cortex sections from Wistar rats deprived of sleep for 18 hours exhibited distortions such as shrunken glomeruli, excessively dilated Bowman's space, and pyknosis. The sections from group III (10 mg/kg CBD + 18 hours of sleep deprivation) showed mild improvement, with identifiable glomeruli, mildly dilated Bowman's space, and podocytes. In group IV (20 mg/kg CBD + 18 hours of sleep deprivation), there was a notable improvement in renal histoarchitecture, including well-defined glomeruli, Bowman's space, and podocytes. Lastly, the renal cortex sections from group V (Sleep Recovery) demonstrated restoration of the histoarchitecture, showcasing glomeruli, Bowman's space, and podocytes (Figure 4).

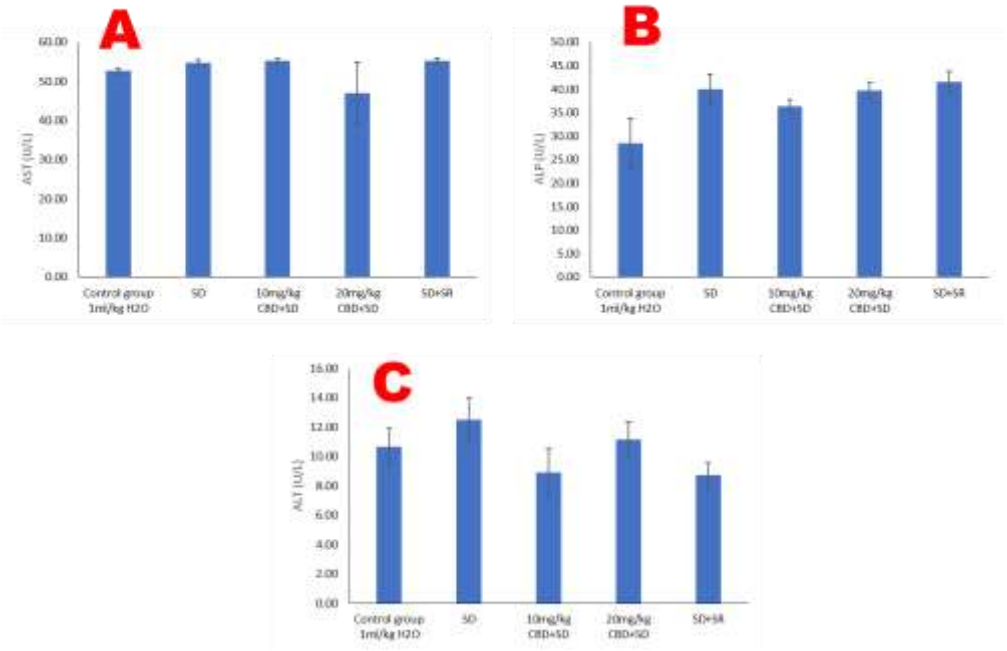

Figure 1: Liver function tests of Wistar rats following chronic sleep deprivation and treatment with CBD. (A) Aspartate aminotransferase levels (AST) (B) Alkaline phosphatase levels (ALP). (C) Alanine transaminase levels (ALT). n=6, mean ± SEM, one-way ANOVA, p< 0.05. SD= Sleep Deprivation; CBD= Cannabidiol; SR= Sleep Recovery.

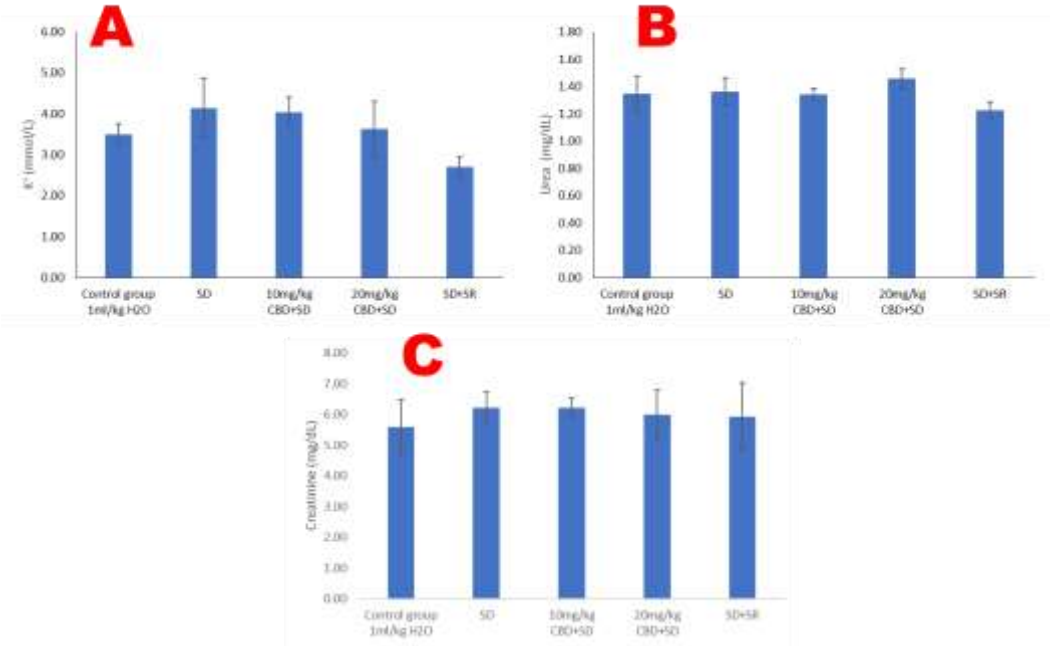

Figure 2: Electrolyte and proteins levels following chronic sleep deprivation of and treatment with CBD. (A) Potassium. (B) Urea (Ur). (C) Creatinine (Cr). n= 6, mean ± SEM, one-way ANOVA, a=p< 0.05. SD= Sleep Deprivation; CBD= Cannabidiol; SR= Sleep Recovery.

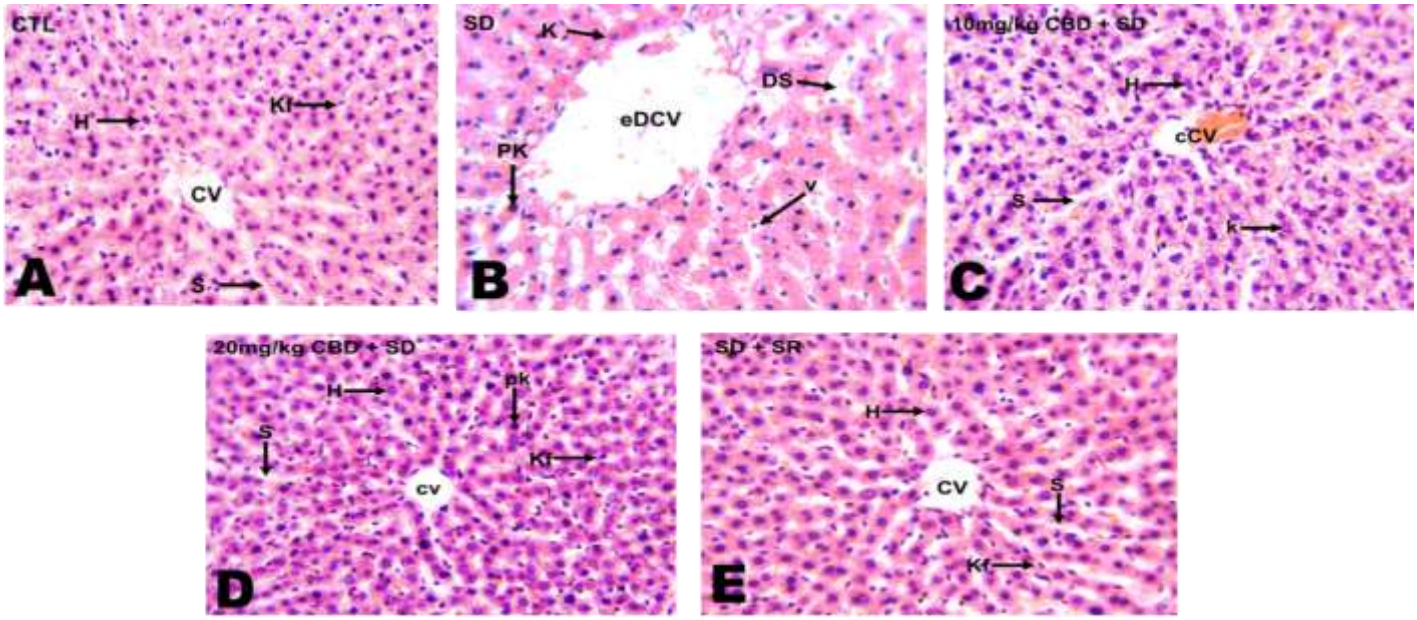

Figure 3: Photomicrograph of the liver section. (A) Control group (1 ml/kg H_20) showing normal histoarchitecture. (B) Group II (18 hours of Sleep Deprivation) showing distortions of the histoarchitecture. (C) Group III (10 mg/kg CBD +18 hours of Sleep Deprivation) showing mild improvement of the histoarchitecture. (D) group IV (20 mg/kg CBD +18 hours of Sleep Deprivation) showing improvement of the histoarchitecture. (E) Group V (SD + SR) showing restoration of the histoarchitecture. H and E x250. Hepatocytes (H), Kupfer cells (Kf), Central vein (CV), Sinusoid (S). Pyknosis (py), Karyorrhexis (K), Excessive Dilated Central Vein (eDCV), Dilated Sinusoid (DS), Vacuolation (V), Sleep Deprivation (SD), Sleep Recovery (SR), Congested Central Vein (cCV),

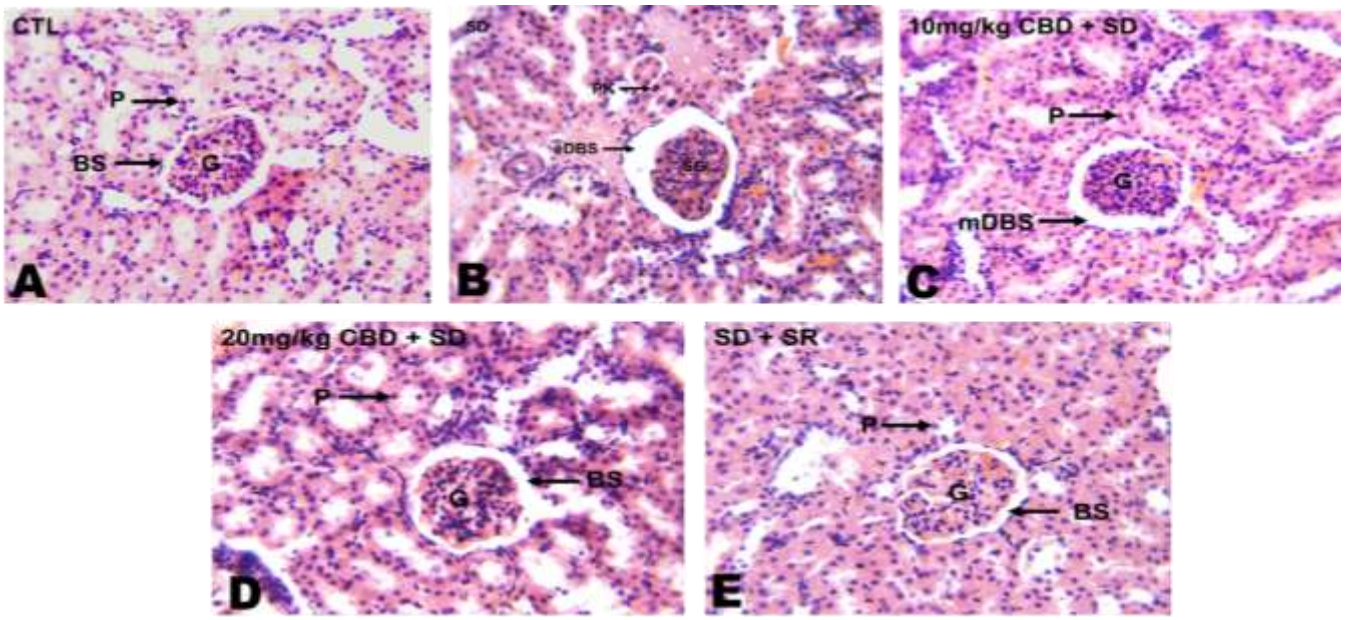

Figure 4: Photomicrograph of the kidney section. (A) Control group (1 ml/kg H_20) showing normal histoarchitecture. (B) Group II (18 hours of Sleep Deprivation) showing shrunken glomerulus (G), excessive dilated Bowman's space (eDBS), pyknosis (PK). C) Group III (10 mg/kg CBD +18 hours of Sleep Deprivation) showing mild improvement of the histoarchitecture. (D) group IV (20 mg/kg CBD +18 hours of Sleep Deprivation) showing improvement of the histoarchitecture. (E) Group V (SD + SR) showing restoration of the histoarchitecture. H and E x250. Control (CTL), Glomerulus (G), Bowman's space (BS), Podocytes (P), mild dilated Bowman's space (mDBS), Sleep Deprivation (SD), Cannabidiol (CBD), Sleep Recovery (SR).

DISCUSSION

Sleep deprivation is increasingly recognized as a significant public health issue that can lead to various physiological disturbances

(Altevogt & Colten, 2006). The liver and kidneys play crucial roles in metabolic regulation and detoxification, and their functions can be significantly impacted by sleep patterns (Gachon & Firsov, 2011). Previous studies have indicated that sleep deprivation can lead to increased oxidative stress and inflammation, which may compromise liver and kidney function (Ghouri *et al*., 2010; Wang *et al*., 2019). Specifically, sleep deprivation has been associated with elevated levels of inflammatory markers and oxidative stress, which can adversely affect the integrity and function of these organs (Liu *et al*., 2016). Furthermore, the disruption of normal sleep patterns can lead to metabolic dysregulation, heightening the risk of developing conditions such as obesity and diabetes. These metabolic disorders further strain liver and kidney health, as they are closely associated with increased fat accumulation and insulin resistance, which can exacerbate the risk of liver disease and renal impairment (McEwen, 2007; Wang *et al*., 2019).

In this study, the results indicated that there were no statistically significant differences in serum levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) among groups subjected to sleep deprivation, treatment with Cannabidiol (CBD), and sleep recovery in the rats. Similarly, serum potassium, urea, and creatinine serum levels showed no significant differences across these experimental conditions. These findings suggest that the duration of sleep deprivation (18 hours) may not have been sufficient to induce measurable hepatic or renal dysfunction, as previous research has shown that the effects of sleep deprivation on liver enzymes can vary based on the severity and duration of deprivation (Periasamy *et al*., 2015). Additionally, the potential protective effects of CBD against oxidative stress and inflammation may have played a role in stabilizing liver and kidney function, preventing significant alterations in these biomarkers (Gopalakrishnan *et al*., 2004). This aligns with other studies that have reported no significant changes in liver and kidney function markers following acute sleep deprivation, indicating a degree of resilience in these organs under short-term stress conditions (McEwen, 2007).

The liver and kidney are susceptible to damage from prolonged sleep deprivation, which can induce oxidative stress and inflammation, leading to cellular and structural changes in their histology (Abuyassin, 2018; Liu *et al*., 2022). In this study, the histological examination of liver and renal cortex sections from the Wistar rats subjected to 18 hours of sleep deprivation revealed significant cellular distortions indicative of acute organ stress and damage. In the liver, notable findings included pyknosis (nuclear shrinkage), karyorrhexis (nuclear fragmentation), dilated central veins, and vacuolation of hepatocytes. These alterations suggest a state of cellular injury likely driven by increased oxidative stress and inflammation, which are known consequences of sleep deprivation. The dilated central veins may reflect impaired venous return and increased portal pressure, while vacuolation indicates lipid accumulation or cellular swelling due to metabolic disturbances (Kang & Elia, 2016; Huang *et al*., 2019; Ekpo *et al*., 2023). Similarly, the renal cortex exhibited shrunken glomeruli, excessively dilated Bowman's space, and pyknosis, indicative of glomerular injury and tubular atrophy. These changes can be attributed to the disruption of renal hemodynamics and increased susceptibility to ischemic damage due to sleep loss (Toba & Lindsey, 2019). The observed renal distortions align with findings from other studies that have reported similar histopathological changes in renal tissues following sleep deprivation, emphasizing the detrimental effects of inadequate sleep on kidney function (Periasamy *et al*., 2015; Mohsin & Kamoona, 2024).

Cannabidiol (CBD) has emerged as a potential therapeutic agent due to its anti-inflammatory and neuroprotective properties. Studies have shown that CBD can mitigate oxidative stress and inflammation, which are key contributors to the organ damage associated with sleep deprivation (Booz, 2011; Atalay *et al*., 2019). In this study, rats administered with CBD and subjected to sleep deprivation showed improvement in the histology of the liver and the kidneys. Also, seven days of sleep recovery after a period of sleep deprivation showed restoration of the histoarchitecture of the kidneys and the liver. In the liver, CBD has been reported to reduce inflammatory cytokine levels and oxidative stress markers, thereby protecting hepatocytes (Jiang *et al*., 2022). This protective effect may be mediated through the activation of the endocannabinoid system, which plays a crucial role in regulating inflammation and cellular stress responses (Paloczi *et al*., 2018). Furthermore, studies indicate that CBD can improve blood flow and reduce vascular permeability, potentially addressing issues such as dilated central veins seen in sleep-deprived rats (Su *et al*., 2015; Benyó *et al*., 2016).

Similarly, in the kidneys, CBD may help restore normal histological architecture by preventing glomerular injury and tubular atrophy associated with sleep deprivation. Research has demonstrated that CBD exerts diuretic effects and promotes renal blood flow, which could counteract the shrunken glomeruli and dilate Bowman's space as observed in sleep-deprived rats (Park *et al*., 2017).

Moreover, sleep recovery plays a crucial role in reversing histological changes by facilitating the restoration of cellular functions and promoting tissue repair in affected organs. Adequate sleep allows the body to repair and regenerate, restoring normal cellular function and reducing inflammation (Everson *et al*., 2014). In conclusion, this study's findings demonstrated the modulatory role of cannabidiol and sleep recovery in alleviating the histological alterations in the liver and the kidneys triggered by sleep deprivation in adult male Wistar rats.

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