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Research Article

Impact of Cannabidiol oil and prednisolone on liver enzymes, oxidative stress markers and liver histology in cadmium induced toxicity in male Wistar rats

Samuel K. Mobisson*[1], Peter C. Onyebuagu[2], Iheanyichukwu Wopara[3], Desmond Izunwanne[4], Emmanuel C. Madu[1], Augustine C. Emeruem[1], Justin B. Monye[1], Agona O. Obembe[5]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, Madonna University, Elele, Rivers State, Nigeria. [2]Department of Human Physiology, school of Basic Medical Sciences, Federal University of Technology, Owerri, Imo State, Nigeria. [3]Department of Biochemistry, Faculty of Sciences, University of Portharcourt, Choba, Rivers State, Nigeria. [4]Department of Human Physiology, Faculty of Basic Medical Sciences, Imo State University, Owerri, Nigeria. [5]Department of Human Physiology, Faculty of Basic Medical Sciences, University of Calabar, Cross River State, Nigeria.

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* Address for Correspondence:
Email:
samuelmobisson@gmail.com

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ABSTRACT

Background: This study aimed to ascertain the effect of cannabidiol (CBD) oil and prednisolone on serum liver enzyme markers and hepatic oxidative stress markers on cadmium-induced toxicity in male Wistar rats. Forty (40) male Wistar rats weighing between 150g to 200g were assigned into 8 groups (A-H) of five animals each. Group 1 served as control, Groups 2-8 received 1mg/kg body weight of prednisolone; 1.5mg/kg bw of cadmium; 1mg/kg bw of prednisolone + 0.2mg/kg bw of CBD-oil; 0.2mg/kg bw of CBD-oil + 2mg/kg bw of cadmium; 3mg/kg bw of prednisolone + 2mg/kg of cadmium; 0.1mg/kg bw of CBD-oil and 0.2mg/kg bw of CBD-oil respectively. The administration was done using an orogastric tube (gavage) for 14 days. Results revealed a significant decrease in the concentration of aspartate aminotransferase (AST) in all treated groups compared to control. Furthermore, serum alanine aminotransferase (ALT) showed a significant ($p<0.05$) decrease in all treated groups compared to control. There was a significant decrease in the concentration of alkaline phosphatase (ALP) in treated groups compared to the control. Liver catalase significantly increased in rats fed with pred +cadmium compared to control and other treated groups. Liver superoxide dismutase (SOD) significantly decreased in ($p<0.05$) the group treated with cadmium compared to the control and prednisolone groups. Liver malondialdehyde concentration did not reveal any significant change ($p>0.05$). Liver glutathione peroxidase significantly decreased in treated groups than in control. Liver-reduced glutathione significantly decreased across treated groups than the control. Histology of the liver revealed degeneration of hepatocytes and vascular congestion in groups treated with prednisolone, prednisolone+ cadmium, and CBD oil (0.2mg/kg). We conclude that CBD oil, prednisolone, and Cadmium administration at different doses decreased the concentration of serum liver enzyme and oxidative stress markers but caused local inflammation of the liver. If this study is applicable to humans, CBD-oil and prednisolone should be cautiously taken as they may likely present adverse effects, especially in people with liver disease.

Introduction

In southern Nigeria today, there is increasing use of prednisolone and *cannabis* products, especially cannabidiol (CBD) oil as a remedy in the management of some illnesses such as obstructive respiratory disorder, depression, and insomnia associated with hypertension, nausea, and vomiting in cancer chemotherapy, anorexia and cachexia in HIV/AIDS patients, neuropathic pain, allergy, inflammation, and spasticity in multiple sclerosis (Pollmann and Feneberg, 2008). This has raised questions about their possible physiologic adverse effects on the liver as a major metabolic organ. Cannabidiol (CBD) is the major non-psychoactive component of *Cannabis sativa* (Watson *et al.,* 2000). It has been reported that CBD which is an isomer of tetrahydrocannabinol (THC) acts as a balancing force to regulate the strength of psychoactive agent (THC) and regulates the body's metabolism of THC by inactivating cytochrome p450 which is an enzyme that metabolizes drugs (Watson *et al.,* 2000). It was reported in mouse models that CBD-treated mice had a substantial increase in brain concentration of THC as its major metabolites and it was proposed that it may be due to decreased clearance rate of THC from the body (Watson *et al.,* 2000).

Arachidonic acid metabolites have been shown to exhibit properties similar to compounds found in *Cannabis sativa* (Takayuki *et al.,* 1995). These metabolites are hence referred to as endocannabinoids. These ubiquitous endogenous cannabinoids act as natural ligands for cannabinoid receptors expressed in mammalian tissue, thus constituting an important lipid-signaling system termed the endocannabinoid system (Lombard *et al.,* 2007). The endocannabinoid system is an important biological regulatory system that has been shown to be highly conserved from lower invertebrates to higher mammals (Kaczocha, 2009). Other than the lipid transmitters that serve as ligands for the cannabinoid receptors, the endocannabinoid family also comprises enzymes for the biosynthesis and degradation of ligands. The endocannabinoid system is also involved in immunoregulation. For example, the administration of endocannabinoids or the use of inhibitors of enzymes that break down endocannabinoids led to immunosuppression and recovery from immune-mediated injury to organs such as the liver. Manipulating endocannabinoids and/or using exogenous cannabinoids *in vivo* can constitute a potent treatment modality against inflammatory disorders (Kaczocha, 2009).

Cannabinoid receptors (CB1 and CB2) have been found to be among the G protein-coupled receptor family (Pertwee, 1997). The receptor is found to be present on the cell membrane of both intracellular and extracellular cell membranes. CB1 receptors have been found to be more abundant in the brain while CB2 receptors are structurally different and are mostly found only on the cells of the immune system they are more prevalent on B-cells, natural killer cells, and monocyte, but may be found on polymorphonuclear neutrophil cells, T8 cells, and T4 cells. The binding of Cannabinoids to Cannabinoid receptors decreases adenylyl cyclase activity thereby inhibiting Ca^{2+} and K^{+} channels (Pertwee, 1997).

Prednisone is a synthetic, anti-inflammatory glucocorticoid derived from cortisone (Renner *et al.,* 1986). It is biologically inert and converted to prednisolone in the liver. Prednisone is a delayed-release corticosteroid indicated as an anti-inflammatory or immunosuppressive agent to treat a broad range of disorders such as immunosuppression, rheumatism, collagen, dermatologic, allergic, ophthalmic, respiratory, hematologic, neoplastic, edematous, gastrointestinal, acute exacerbations of multiple sclerosis, and as an anti-inflammatory and an antineoplastic agent (Renner *et al.,* 1986). It works on the immune system to help relieve swelling, redness, itching, and allergic reactions (Bunte *et al*., 2018). Prednisone decreases inflammation via suppression in the migration of polymorphonuclear leukocytes and reversing increased capillary permeability. It also suppresses the immune system by reducing the activity and volume of the immune system (Bunte *et al*., 2018). Cadmium is one of the toxic heavy metals in the environment that induces oxidative stress, dyslipidemia, and membrane disturbances in the heart. Cells undergoing prolonged oxidative stress and inflammation may subsequently lead to tissue injury (Biswas, 2016).

The liver is a vital inner organ that performs numerous functions which include secretion of bile, excretory function, synthetic function, metabolic function, hemopoietic function, hemolytic function, heat production, defense and detoxification

functions (Guyton & Hall, 2004), clotting factors and albumin synthesis (Palmer, 2004). The human liver consists of numerous enzymes that aid to necessitate chemical processes in the body (Palmer, 2004). The most used indicators of liver functions are alanine aminotransferase (ALT), aspartate aminotransferase (AST) (Giboney, 2005), and alkaline phosphatase concentration (Schlaeger, Haux, and Kattermann, 1982). Increased levels of ALT and AST are indications of hepatocellular disease, active cirrhosis, metastatic liver tumor, toxic hepatitis, severe, pancreatitis, myocardial infarction (heart attack), trauma, severe burns, acute hemolytic anemia; crushing injuries and shock (Adeyemi *et al.*, 2015). Alkaline phosphatase is commonly used to access obstruction in the biliary system (Adeyemi *et al.*, 2015). Alkaline phosphatase increase is majorly detected in the biliary tumor, gallstone disease, alcohol abuse, and drug-induced hepatitis (Schlaeger, Haux, and Kattermann, 1982). Hence, this study was aimed to investigate the impact of prednisolone and CBD oil on serum liver enzyme markers and hepatic oxidative stress markers on cadmium-induced toxicity in male Wistar rats.

2. MATERIALS AND METHODS

Drugs: prednisolone used for this study was purchased from Unicure pharmaceutical limited, Lagos, Nigeria. The cadmium chloride was purchased from Sigma-Aldrich Limited Germany with EC number 233-296-7. Cannabidiol (CBD) oil was purchased from TEEMU Premium, California, USA.

2.1 Laboratory animals

Forty (40) male Wistar rats, weighing 150–200g were used for this study. The animals were housed in the Department of Physiology animal house, University of Calabar, Nigeria. Standard animal cages (435 x 290 x150) with wood shavings as bedding were used in housing the animals (n = 5). They were allowed *ad libitum* access to rat chow and clean water, and exposed to 12/12-hr light/dark cycle. The animals were acclimatized for 7 days. Animals were kept in line with laid down principles for animal care as prescribed in Helsinki's 1964 declaration. The animal ethics committee of the University of Calabar approved our study protocol with approval number 040PHY3719.

2.2 Experimental Design and Administration of Drugs

The animals were randomly assigned differently into eight separate groups (n = 5). After seven days of acclimatization, CBD oil, prednisolone, and cadmium administration commenced. The drugs were administered via oral route using an orogastric tube (gavage), once, every day, to animals in treatment groups (2 to 8), using doses outlined in Table 1, while the control group received feed and 0.5ml normal saline as a vehicle. The administration of CBD oil and prednisolone solution lasted for fourteen days, whereas the administration of cadmium chloride solution lasted only two days before rats were killed, and samples were collected for analysis.

TABLE 1: Study Design and Drugs Administration

Groups	No. of rats	Treatment
1(Control)	5	Feed + 0.5ml of normal saline as a vehicle throughout the experimental period.
2	5	1mg/kg bw of prednisolone
3	5	1.5mg/kg bw of Cadmium
4	5	1mg/kg bw of prednisolone + 0.2mg/kg bw of CBD Oil.
5	5	0.2mg/kg bw of CBD oil + 2mg/kg bw of cadmium
6	5	3mg/kg bw of prednisolone + 2mg/kg of cadmium
7	5	0.1mg/kg bw of CBD Oil low dose
8	5	0.2mg/kg bw of CBD oil high dose

2.3 Assessment of serum liver enzyme markers

Alkaline phosphatase was measured according to a standard procedure (Bergmeyer and Bernt 1974). Briefly, P-nitrophenyl phosphate was hydrolyzed to phosphate and p-nitrophenol in the presence of ALP. A calculated amount of sample 0.01ml in a test tube was mixed with reagent (0.5ml) containing substrate p nitrophenyl phosphate and kept at room

temperature. The solution was mixed, and the initial absorbance was read after one minute. The reaction was allowed to stand for three minutes and absorbance read again at 405nm. Alkaline phosphatase activity was calculated from the equation:

UL = 2760 x ΔA nm/minute micro
Where UL = Unit of alkaline phosphatase affinity
ΔA = Change in absorbance

Serum AST and ALT levels were determined using an endpoint colorimetric diagnostic kit (Randox Laboratories, UK) based on Reitman and Frankel's method (Reitman and Frankel, 1957). The pyruvate produced by the transamination reaction between L-alanine and ketoglutarate reacts with 2, 4, dinitrophenyl hydrazine to give a colored hydrazone, and was used to measure alanine aminotransferase activity. The oxaloacetate hydrazone formed with 2, 4 dinitrophenyl hydrazine was used to measure AST. Both ALT and AST were read at 540nm wavelength (Reitman and Frankel, 1957). This method was recently used by Mobisson *et al.,* (2019) and Wopara *et al.,* (2019).

2.4 Liver oxidative stress markers assessment

The liver of each rat was homogenized using a Potter-Elvehjem homogenizer. Twenty percent (1/5 w/v) of tissue homogenate was primed in 50 mm Tris–HCl buffer (pH 7.4) with 1.15% potassium chloride and was centrifuged at 10,000 rpm at 4°C for 10 minutes using a bucket centrifuge (B-Bran Scientific and Instrument Company, England). The supernatant was collected to evaluate catalase (CAT) activity using hydrogen peroxide as substrate. At 412 nm, reduced glutathione (GSH) was evaluated by procedure of Luchese, Pinton, and Nogueira (2009). The activity of glutathione peroxidase (GPx) was examined with the use of hydrogen peroxide as a substrate regarding the procedure of Luchese, Pinton, and Nogueira (2009). The concentration of malonyldialdehyde (MDA) was determined in thiobarbituric acid reacting substances (TBARS) as described by Meenakshi, *et al.,* (2007) and Okhawa, *et al.,* (1979). Thereafter, the mixed reaction produced 0.2 ml of 8.1% sodium dodecyle sulphate, 1.5 ml of 20% acetic acid solution matched to pH 3.5 with sodium hydroxide and 1.5 ml of 0.8% water solution of thiobarbituric acid was introduced to 0.2 ml of 10%(w/v) of homogenate. Using distilled water, the mixture was moved up to 4.0 ml and warmed at 95°C for 60 mins. About 1.0 ml of distilled water and 5.0 ml of a mixture of n-butanol and pyridine (15:1 v/v) were introduced and centrifuged at the end of ice cooling at 4000 rpm. The crude layer was removed and absorbance was calculated at 532 nm and measured up to results obtained from MDA standards. Value levels were measured from absorption values as normal absorption Okhawa, *et al.,* (1979).

2.5 Histological examination of liver

The liver of the control and treated rats were isolated after fixing the whole skull with 10% buffered formaldehyde for 48 hours. Sections were obtained and stained with hematoxylin and eosin (H & E) stains. The microscope slides were labeled appropriately. Photomicrographs were taken at ×500 magnifications using a light microscope (Leica DM 750, Switzerland).

2.6 Statistical analysis

All results are presented as mean ± SEM, n=5. One-way analysis of variance (ANOVA) was utilized in comparing differences within groups, followed by post hoc multiple comparisons. Computer software SPSS version 17.0 and Excel analyzer were used for analysis. The level of significance was set at $p<0.05$.

3 RESULTS

3.1 Comparison of liver enzyme markers of control and experimental groups

Table 2 below revealed a significant ($p<0.05$) decrease in the concentration of Aspartate aminotransferase (AST) across treated groups compared to control. However, groups treated with pred+cadmium and CBD oil (0.1ml) were significantly increased compared to other treated groups. Furthermore, serum Alanine aminotransferase (ALT) showed a significant ($p<0.05$) decrease in all treated groups compared to the control. Though, the group treated with CBD oil (0.1ml) was significantly ($p<0.05$) increased compared to other treated groups. Alkaline phosphatase (ALP) concentration was significantly ($p<0.05$) decreased in treated groups compared to control.

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Table 2: the comparison of liver enzyme markers of control and experimental groups

Parameters	Gp 1 Control	GP 2 pred	GP 3 cadmium	GP 4 pred + CBD oil	GP 5 cadmium+ CBD oil	GP 6 pred+ cadmium	GP 7 CBD oil (0.1ml)	GP 8 CBD oil (0.2ml)
AST u/l	35.67±2.85	23.33±4.18*	17.33±2.33*	12.67±0.88*	32.67±3.38 ^{a,b,c}	18.67±3.38*$^{, a, d}$	35.00±3.79 $^{b, c, e}$	19.00±2.52*$^{, a, d, f}$
ALT u/l	22.00±2.65	6.20±0.62*	4.57±0.30*	4.43±0.23*	4.90±0.23*	7.80±0.35*	18.37±0.32^{x}	12.43±1.29^{y}
ALP u/l	62.67±1.86	50.67±6.12*	54.00±2.31	46.33±3.71*	58.33±2.40^{c}	52.00±1.15*	55.67±2.73	55.67±2.19

Values are expressed in mean ± SEM, n = 5. *=represents values with significant difference. * = p<0.05 vs control; a = p<0.05 vs Pred.; b = p<0.05 vs Cd; c = p<0.05 vs Pred. + CBD oil; d = p<0.05 vs Cd + CBD oil; e = p<0.05 vs Pred. + Cd; f = p<0.05 vs CBD oil (0.1mL) x = p<0.05 vs all groups except with control; y= p<0.05 vs all groups except with CBD oil (0.1mL).

Table 3: Comparison of liver oxidative stress markers in control and experimental groups

Parameters	Gp 1 Control	GP 2 pred	GP 3 cadmium	GP 4 pred + CBD oil	GP 5 cadmium + CBD oil	GP 6 pred+ cadmium	GP 7 CBD oil 0.1ml)	GP 8 CBD oil (0.2ml)
CAT u/g	6.48±0.72	5.5±0.77	4.98±0.13	5.93±0.57	5.07±0.32	8.98±0.33^{Z}	5.57±1.66	6.28±0.25
SOD ug/ml	0.40±0.01	0.64±0.14	0.31±0.07^{a}	0.57±0.05	0.44±0.18	0.52±0.10	0.44±0.02	0.50±0.02
MDA umol/ml	0.45±0.01	0.32±0.09	0.48±0.06	0.30±0.03	0.43±0.14	0.37±0.10	0.42±0.02	0.34±0.01
GPx ug/ml	0.07±0.01	0.06±0.01	0.03±0.00*$^{, a}$	0.04±0.00*^{,a}	0.07±0.01^{b,c}	0.06±0.00^{b,c,d}	0.03±0.00*^{,a,d,e}	0.04±0.00*^{,a,d,e}
GSH ug/ml	1.58±0.12	1.37±0.19	0.48±0.11*^{,a}	0.88±0.05*^{,a}	1.58±0.16^{b,c}	1.26±0.05^{b,c}	0.50±0.07*^{,a,c,d,e}	0.84±0.04*^{,a,b,d,e,,f}

Values are expressed in mean ± SEM, n = 5. *=represents values with a significant difference. * = p<0.05 vs control; z=P<0.05 VS all other groups; a=P<0.05 vs pred; b = p<0.05 vs Cd; c = p<0.05 vs Pred. + CBD oil; d = p<0.05 vs Cd + CBD oil; e = p<0.05 vs Pred. + Cd; f = p<0.05 vs CBD oil (0.1mL)

3.2 Comparison of liver oxidative stress markers in control and experimental groups

Liver catalase significantly (p<0.05) increased in the group treated with pred +cadmium compared to the control and other treated groups. Liver superoxide dismutase (SOD) significantly (p<0.05) decreased in the group treated with cadmium compared to the control.

Liver malondialdehyde concentration did not reveal any significant change statistically. Liver glutathione peroxidase significantly (p<0.05) decreased in treated groups compared to control. However, the group treated with Cd+CBD oil was significantly (p<0.05) increased compared to other treated groups. Furthermore, the group treated with CBD oil (0.2ml) was significantly increased compared to the CBD oil (0.1ml) treated group. Liver-reduced glutathione (GSH) significantly decreased across treated groups compared to control except for Cd+CBD oil-treated group. Furthermore, the group treated with CBD oil (0.2ml) was

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significantly increased compared to the CBD oil (0.1ml) treated group.

3.3 Histological examination of liver in control and treated groups after administration of drugs

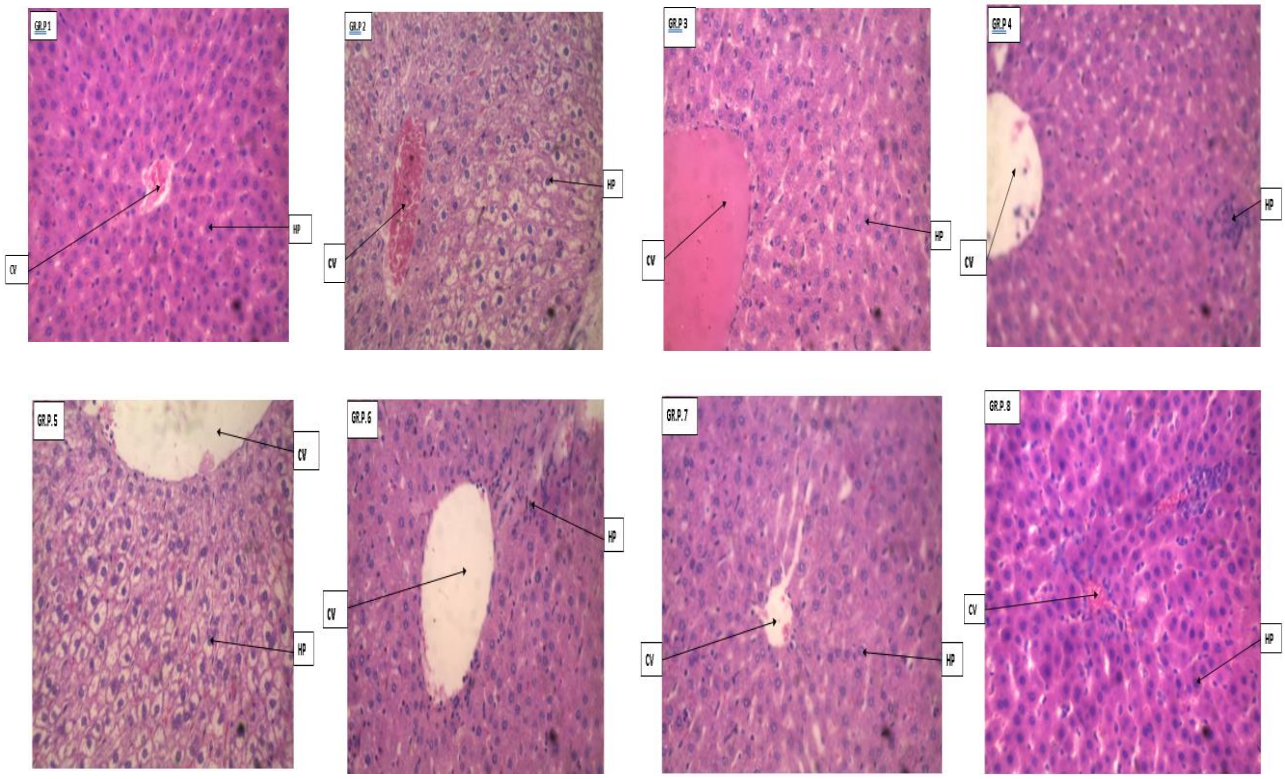

Figure 1. Groups 1 to 8: Photomicrographs of the liver of different experimental groups after administration of CBD oil, prednisone, and cadmium. Magnification: x500. Key: CV: Central vein, HP: Hepatocyte

Plate 1 above shows a photomicrograph of the control (group 1) with a central vein in the hepatic lobule. No visible pathologic change was seen. Plate 2 (group 2) revealed a photomicrograph of prednisolone-treated rats with degeneration of hepatocytes and vascular congestion. Plate 3 (group 3) showed cadmium-treated rats with vascular congestion. Plate 4 (group 4) revealed pred+CBD oil-treated rats with portal expansion. Plate 5 (group 5) showed cadmium+ CBD oil-treated rats with no pathologic change. Plate 6 (group 6) revealed pred+cadmium treated rats with lymphocytic infiltration in sinusoidal spaces adhering to endothelium causing portal expansion (a sign of local inflammation). Plate 7 (group 7) revealed CBD oil (0.1ml) treated rats with no visible pathologic change after drug administration.

Plate 8 (group 8) showed CBD oil (0.2ml) treated rats with vascular congestion, the presence of lymphocytes in sinusoidal spaces, and endothelial adhesion.

4 DISCUSSION

The result has shown that consumption of CBD-oil, prednisolone, and cadmium at different doses causes the reduced concentration of serum liver enzyme markers, liver oxidative stress markers, and mild alteration in the cytoarchitecture of the liver.

The result showed that rats treated with CBD oil, prednisolone, and cadmium at different doses had significantly lowered serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) concentrations compared with the control group. The decreased serum liver enzyme markers in treated rats compared to control may likely be indicating a healthy liver and nontoxic effect of the dose-dependent drugs. However, it was reported that CBD may cause dose-related liver damage (O'Connell *et al.,* 2017; Chen *et al.,* 2019). Furthermore, confirmed the study that was conducted by McPartland *et al.,* (2015), whereby they reported that CBD is non-intoxicating and has little affinity for CB1 and CB2 receptors but acts as a negative allosteric modulator of CB1 with pharmacologic effects on other receptor systems such as GPR55, TRPV1, 5-$HT_{1A,}$ adenosine A2A and non-receptor mechanisms (Laprairie *et al.*, 2015). In contrast, Tal *et al.,* (2020), reported that CBD-rich extract may cause a pro-inflammatory effect in the liver which is linked to an unfavorable change in microbiota profile and suggested that the effect may be mediated by mechanisms other than an increased expression of endocannabinoid receptor. Furthermore, Rolando *et al.,* (2014), reported that prednisolone did not repair liver damage in brain-dead rats. The mechanism through which liver ALT, AST, and ALP decreased with prednisolone in this study is not well ascertained but could be linked to the anti-inflammatory properties of prednisolone (Rolando *et al.*, 2014). The most commonly used indicators of liver functions are alanine aminotransferase (ALT), aspartate aminotransferase (AST) (Giboney, 2005), and alkaline phosphatase concentration. Variations in concentrations of these enzymes have many implications. Increased levels of ALT and AST are indications of hepatocellular disease, active cirrhosis, metastatic liver tumor, toxic hepatitis, severe, pancreatitis, myocardial infarction (heart attack), trauma, severe burns, acute hemolytic anemia, crushing injuries, and shock (Adeyemi *et al.*, 2015). Alkaline phosphatase is commonly used to signify obstruction in the biliary system. Its increase is majorly detected in the biliary tumor, gallstone disease, alcohol abuse, and drug-induced hepatitis (Schlaeger, Haux, and Kattermann, 1982).

The results of this study a revealed significant ($p<0.05$) increase in liver catalase activity in rats treated with prednisolone and cadmium compared to all other groups. There was a significant decrease in liver SOD activity in rats treated with cadmium compared to prednisolone and other groups which did not show a significant increase. Liver MDA activity did not show any significant change statistically when compared with the control. There was a significant decrease in liver GPx and GSH activities across treated groups compared to control. The alterations in the concentration of liver oxidative stress markers in this study especially in cadmium-treated rats may likely be an indication of hepatotoxicity associated with cadmium. The decreased GPx and GSH levels suggest a link between drugs and hepatic oxidative stress and decrease antioxidant levels in the liver (Anbarasu *et al.,* 2011; Anand *et al.*, 2014). The liver is a major organ targeted usually in drug toxicity. Radical species production, particularly reactive oxygen species (ROS) and reactive nitrogen species (RNS) is suggested as an early event of drug hepatotoxicity and as an indicator of hepatotoxic potential (Sodhi *et al.*, 1998). It has been documented that many drugs may likely induce oxidative stress by altering cellular oxidants levels and lipid peroxidation, (Wopara *et al.,* 2021; Mobisson *et al.,* 2022). The histology of the liver revealed slight pathologic changes including, vascular congestion and lymphocyte infiltration which are signs of inflammation that may have released enzymes like neutral proteases, acid hydrolases, or phosphatases that resulted in hepatocyte damage (Collins 1999). These changes could be linked to the direct toxic effects of the drugs.

CONCLUSION

In conclusion, the administration of CBD oil, prednisolone, and cadmium altered the concentration of serum liver enzyme markers, liver oxidative stress markers, and cytoarchitecture of the liver leading to direct toxicity of hepatocytes.

Author Contributions

Mobisson Samuel Kelechi and Agona Odeh Obembe designed the study and wrote the study protocol. Mobisson Samuel Kelechi, Desmond Izunwanne, Madu Emmanuel Chibuikem, and Augustine Chidera Emeruem performed laboratory

experiments and literature searches. Mobisson Samuel Kelechi drafted the manuscript; Iheanyichukwu Wopara and Onyebuagu Peter Chukwuma worked on data analysis. Iheanyichukwu Wopara performed the statistical analysis. All authors read and approved the final manuscript.

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