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HEPATOPROTECTIVE ACTIVITY OF HUSK EXTRACT OF *ZEA MAYS* AGAINST CARBON TETRACHLORIDE-INDUCED LIVER INJURY IN RATS

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ABSTRACT: *Zea mays* L (Poaceae) used traditionally by the Ibibios of Southern Nigeria to treat stomach ulcer, malaria, inflammatory diseases and as an antidote, was evaluated for hepatoprotective properties against experimentally-induced liver injuries to ascertain the folkloric claim of its usefulness in the treatment of poisoning. The husk extract of *Zea mays* (187-748 mg/kg) was investigated for hepatoprotective potential against carbon tetrachloride(CCl_4)-induced liver injuries in rats. Assays of liver function parameters as well as histopathological study of the liver were used to assess hepatoprotective activities of husk extract. Administration of the husk extract (187-748 mg/kg) caused significant ($p < 0.05$-0.001) reductions in the levels of liver biomarker enzymes (ALT, AST, and ALP), direct and total bilirubin and elevation of serum level of total protein in all the models. The effects were dose-dependent in most cases. Histology of the liver sections of extract and silymarin-treated animals showed reductions in the pathological features compared to the organotoxic-treated animals. The chemical pathological changes were consistent with histopathological observations suggesting marked hepatoprotective potentials. The results showed that husk extract of *Zea mays* has hepatoprotective potentials against injurious agents which may be due to the activities of its phytochemical components.

Keywords: *Zea mays*, husk, liver, hepatoprotective, antioxidative stress.

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1. INTRODUCTION

Zea mays L.(Poacae) also known as maize or corn, is an annual grass plant cultivated throughout Nigeria primarily for human consumption and as animal feed. The plant is tall with a fibrous root system and has long narrow leaves on opposite side of the stem and bears ears that are enclosed in modified leaves known as husks.[1] In addition to its nutritive values, various parts of the plant are also used in ethnomedicine for the treatment of several ailments. The corn silk is used as an antidiabetic or diuretic, and decoction of the silk is consumed for the treatment of urinary troubles and gallstones.[2],[3],[4] The husks are used for the treatment of pains and arthritis,[5] ulcer,[6] malaria and diabetes in Ibibio traditional medicine.[7] The husk extract has been reported to posses some pharmacological properties which include analgesic, anti-inflammatory,[5] antioxidant,[8] antidepressant,[9] antimalarial and antiplasmodial,[7] hepatoprotective,[10] nephroprotective,[11] antidiabetic and hypolipidaemic,[12] and antiulcer activities.[13] The median lethal dose (LD_{50}) of the ethanol husk extract was determined to be 1874.83 mg/kg.[9] Arabinoxylan, which has immunological effects, has been isolated from the husk extract,[14] while eight phenolic compounds (gallic acid, protocatechuic acid, chlorogenic acid, cafeic acid, femlic acid, rutin, resveratrol, and kaempferol) have also been detected in ethanol husk extract of *Zea mays*.[8] Corn husk has also been reported to be rich in anthocyannins.[15] In this study, we report the hepatoprotective activity of the husk extract against carbon tetrachloride-induced liver injury in rats to confirm its use in the treatment of liver diseases in ethnomedicine

2. MATERIALS AND METHODS

2.1 Collection of plant materials

Fresh husks of *Zea mays* were collected in August, 2018 from Farmland in Uyo, Uyo local Government Area, Akwa Ibom State, Nigeria. The husks were identified and authenticated as *Zea mays* by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Herbarium specimen (FPH, 614) was deposited at the Faculty of Pharmacy Herbarium, University of Uyo, Uyo, Nigeria.

2.2 Extraction

The plant parts (husks) were washed, cut into smaller pieces and air-dried on laboratory table for 2 weeks. The dried husks were pulverized using electric grinder. The powdered husk (1.5 kg) was macerated in 50 % ethanol for 72 hours. The liquid filtrate obtained was concentrated and evaporated to dryness in vacuo at 40 °C using rotary evaporator. The crude extract (yield 2.83 %) was stored in a refrigerator at -4 °C until they were used for the experiments reported in this study.

2.3 Animals

Wistar male rats (150 – 165 g) used for these experiments were gotten from Animal House of Department of Pharmacology and Toxicology, University of Uyo. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) and water *ad libitum*.

The care and use of animals was conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH, 1996). Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo, Uyo, Nigeria.

2.4 Effects of *Zea mays* husk extract on carbon tetrachloride-induced hepatotoxicity of rat.

A total of 36 rats were used for this experiment and the design was as follows:

Group 1 (Control group): Rats were orally administered 10 mL/kg body weight distilled water per oral for 8 days.

Group 2 (Organotoxic group): Rats were administered 10 mL/kg body weight distilled water orally for 8 days.

Group 3 (Standard group): Rats were administered 100 mg/kg body weight Silymarin per oral for 8 days.

Group 4 (Low dose test group): Rats were administered 187 mg/kg body weight of *Zea mays* husk extract orally for 8 days.

Group 5 (Middle dose test group): Rats were administered 347 mg/kg body weight *Zea mays* husk extract orally for 8 days.

Group 6 (High dose test group): The rats were administered 748 mg/kg body weight *Zea mays* husk extract orally for 8 days.

On the 8^{th} day, the animals in groups 2-6 were administered CCl_4 dissolved in corn-oil mixed in the ratio of 1:3 at a dose of 1.5 mL/kg body weight intraperitoneally. Twenty hours later, all animals were weighed again and sacrificed under light diethyl ether vapour.

2.5 Collection of Blood Samples and Organs

After 8 days of treatment (24 hours after the last treatment) the rats were weighed again and sacrificed under light diethyl ether vapour. Blood samples were collected by cardiac puncture and used immediately. Blood was collected into plain centrifuge tubes and EDTA bottles. The blood in the centrifuge tubes were centrifuged immediately at 2500 rpm for 15 minutes to separate the serum at room temperature to avoid haemolysis and used for biochemical assays. The blood samples collected into EDTA bottles were taken for haematological analysis. The livers were surgically removed, weighed and fixed in 10 % formaldehyde for histological process.

2.6 Haematological Analysis

The following haematological parameters were determined;

1) Haemoglobin level (Hb)
2) Packed Cell Volume (PCV)
3) Total and differential White blood Cell Count (WBC)
4) Platelet Count
5) Full Blood Count

These parameters were determined at Haematology Department of University of Uyo Teaching Hospital (UUTH), Uyo, using automated Haematology analyser.

2.7 Biochemical Analysis

2.7.1. Liver Function Test

The following parameters were determined; Aspartate transaminase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Total plasma protein and Total and direct bilirubin. The determinations were done spectrophotometrically using Randox analytical kits according to standard procedures of manufacturer's protocols[16] at the Chemical Pathology Department of University of Uyo Teaching Hospital.

2.8. Histopathological Analysis

The liver of animals used in the study were surgically harvested and fixed in buffered formalin. They were then processed and stained with haematoxylin and eosin (H&E) for liver study according to standard procedures at Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo. Morphological changes were observed and recorded in the excised organs of the sacrificed animals. Histologic pictures were taken as micrograph

2.9. Statistical Analysis and Data Evaluation

Data obtained from this work was analysed statistically using ANOVA (one –way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means were considered significant at 5 % level of significance ie $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Effect of treatment of Husk extract of *Zea mays* on the blood hematological parameters of rats with carbon tetrachloride-induced hepatotoxicity

The administration of carbon tetrachloride (1.5 mL/kg) to rats was found to decrease significantly ($p<0.05$-0.001) RBC count, platelets count, PCV percentage and Hemoglobin concentration of rats. These reductions were reversed following pretreatment of the animals with husk extract of *Z. mays* and silymarin (Table 1). However, administration of carbon tetrachloride was observed to cause significant ($p <0.005$-0.001) increases in WBC counts, neutrophils, lymphocytes, eosinophils and basophils percentages. Pretreatment of animals with husk extract and silymarin was found to reverse these increases (Table 1).

Table 1: Effect of *Zea mays* husk extract on heamatological parameters of rats with CCl_4-induced liver injuries

Treatment	Dose	WBC (L)	Neut. (%)	Lymp (%)	Mono(%)	Eosin (%)	Baso (%)	RBC (L)	Hgb(g/dl)	PCV (%)	Platelets (L)
Control	-	6.12± 0.08	38.75±1.10	41.25±1.65	27.50± 2.02	2.75± 0.47	0.00± 0.00	7.57± 0.37	14.20± 0.33	39.25±1.49	785.25±20.74
CCl4		9.91±0.20^{c}	61.50± .44^{b}	82.25±3.32^{c}	15.25±2.28^{a}	14.75±0.75^{c}	2.00± 0.00^{c}	4.30 ±1.03^{a}	9.37 ±0.74c	21.00±3.62^{b}	294.75±48.90^{c}
Silymarin	100	7.83±0.27^{a}	51.50± .72^{a}	72.75±2.86^{c}	31.25 ±2.92^{f}	5.25 ±0.47^{f}	0.50± 0.28^{f}	5.76 ± 0.57	11.55±0.5^{a}	35.00±4.02^{f}	641.5± 24.25^{f}
Crude extract	187	7.60±0.28	44.50± .44^{e}	65.75±2.86^{a}	24.0±2.34^{f}	2.75±0.50^{f}	0.50 ± 0.28^{f}	6.88± 0.49^{d}	12.85± .40^{b}	37.50±1.32^{f}	783.5 ± 49.61^{f}
	374	7.50±0.63	44.00±1.95^{e}	63.0±3.24^{d}	32.25±1.43^{f}	1.00±0.00^{f}	0.00 ± 0.00^{f}	6.66± 0.63^{d}	12.80 0.88^{b}	42.50 ±1.44^{f}	915.75±24.31^{f}
	784	7.32±0.47	39.00±1.08^{f}	53.25±4.27^{f}	35.50±2.78^{f}	0.25±0.24^{f}	0.00 ± 0.00^{f}	19.21±0.83^{f}	14.95 0.35^{f}	44.25±2.35^{f}	964.75±40.16^{f}

Values are expressed as mean ± SEM. Significant at $^{a}p < 0.05$; $^{b}p < 0.01$; $^{c}p < 0.001$ when compared to control $^{d}p < 0.05$, $^{e}p < 0.01$, $^{f}p < 0.001$ when compared to CCl_4. n = 6

3.2 Effect of Z. mays husk extract on liver function of CCl_4 –induced liver injury in rats

The results in Table 2 show effects of the husk extract in liver function parameters of rats with CCl_4 -induced liver injury. Administration of CCl_4 caused significant ($p < 0.001$) increases in the levels of ALT, AST, ALP, total and direct bilirubin and liver weights of rats when compared with the control. However, significant ($p < 0.05$-0.001) dose-dependent decreases in ALP, ALT, AST, total and direct bilirubin and liver weight were observed following pretreatment with the husk extract. Significant ($p < 0.05$) decrease in total protein level was also observed following CCL_4 administration. This was reversed with pretreatment with the husk extract which caused significant ($p < 0.05$) non dose-dependent increases in the level of total protein of rats.

Table 2: Effect of husk extract of *Zea mays* on CCl_4-induced liver injury in rats

Parameters/ Treatments	Total Protein (g/dl)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)	Alkaline phosphatase (U/L)	ALT (U/L)	AST (U/L)	Liver weight(g)
Normal control	6.47±0.16	0.10±0.01	0.05±0.07	131.0±2.79	37.75±1.93	126.0±1.47	5.87±0.30
CCl_4+Dist water	3.47±0.28^{c}	0.76±0.23^{b}	0.15±0.01^{c}	212.0±9.30^{c}	91.0±5.67^{c}	276.2±11.86^{c}	7.52±0.70^{c}
Silymarin (100 mg/kg)	8.50±0.44^{f}	0.23±0.01^{d}	0.09±0.01^{e}	168.7±4.85^{cf}	76.25±2.46^{c}	239.25±3.75^{cd}	7.24±0.06^{b}
Ext.187 mg/kg	6.65±0.18^{f}	0.20±0.0^{e}	0.09±0.01^{e}	155.25±1.93^{af}	63.0±2.85^{be}	217.75±11.52^{cf}	7.33±1.22^{b}
Ext. 374 mg/kg	6.37±0.28^{f}	0.12±0.0^{e}	0.08±0.01^{f}	150.7±3.79^{f}	64.25±4.60^{be}	213.5±6.91^{cf}	7.10±0.71^{b}
Ext. 748 mg/kg	5.60±0.48^{f}	0.17±0.01^{e}	0.07±0.01^{f}	144.5±3.61^{f}	60.75±4.73^{be}	204.25±5.45^{cf}	7.13±0.04^{b}

Values are expressed as mean ± SEM. Significant at $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$ when compared to control. $^{d}p<0.05$, $^{e}p<0.01$, $^{f}p<0.001$ when compared to CCl_4. n = 6.

3.3 Histopathological studies of rat liver in CCl_4-induced hepatotoxicity

Histopathological examination of liver sections of normal control group showed normal cellular architecture portal triad, bile duct, hepatic artery, hepatic vein, hepatocytes and nucleus, (group 1A and 1B) (Figure 1). Cellular abnormalities including portal and periportal inflammation, vacuolation, presence of kupffer cells, pyknotic nuclei, vascular degeneration, congestion were observed in the CCl_4 treated rats of group 2 when compared to control group (Figure 1, group 2A and 2B). The liver sections of the rats treated with silymarin (100 mg/kg) revealed moderate area of cellular abnormalities including of portal and peri portal inflammation, vacuolation, pyknotic nuclei, vascular degeneration, congestion when compared to control group (Figure 1, group 3A and 3B). Histologic sections of the liver treated with 187 mg/kg (Figure 2, group 4A and 4B) and 374 mg/kg (figure 2, group 5A and 5B) of corn husk extract at magnification of (x100) and (x400) revealed moderate areas of cellular abnormalities including portal and periportal inflammation, vacuolation, pyknotic nuclei, vascular degeneration and congestion when compared to control group, though the group treated with 374 mg/kg was less slightly affected (Figure 2, group 5A and 5B. Histologic section of the liver treated with 748 mg/kg of corn husk (figure 2, group 6A and 6B) extract at magnification (x100) and (x400) revealed reversed cellular architecture with slight area of abnormal cellular integrities when compared to control group.

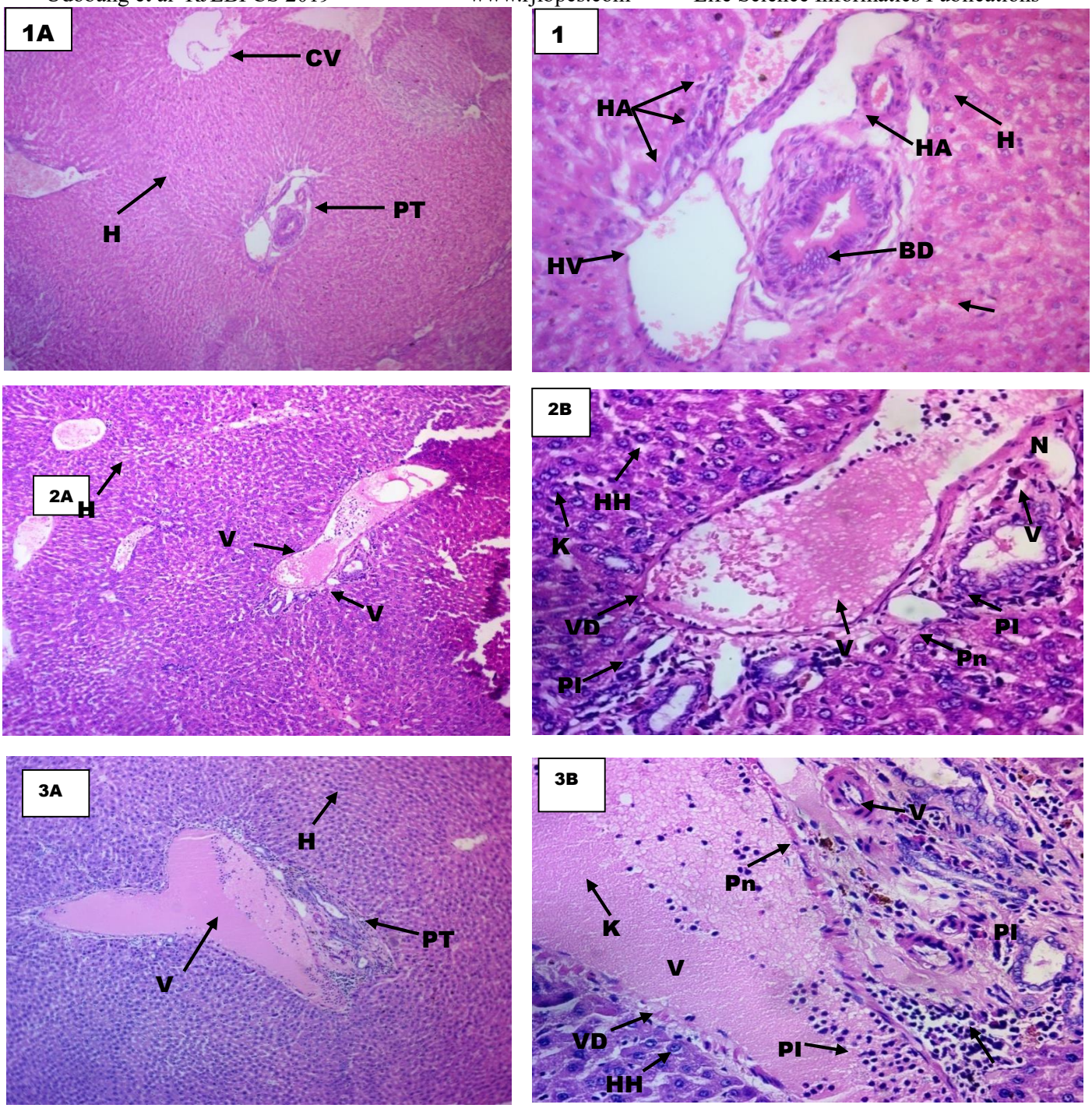

Figure 1: Histological sections of Livers of rats treated with Normal saline 10 mL/kg (1 A/B), CCl_4 1.5mL//kg (2 A/B) and Silymarin 100 mg/kg (3 A/B) at magnification A (x100) and B(x400) stained with H&E method.

Keys: Central vein (CV), Cellular degeneration (CD), Vacuolation (V), Inflammation (I), Hepatocyte (H) and Pyknotic nucleus (Pn) Central vein (CV) Sinusoidal lining (SL), Hepatic vein (HV), Hepatocytic hyperplasia (HH), and Vascular degeneration (VD).

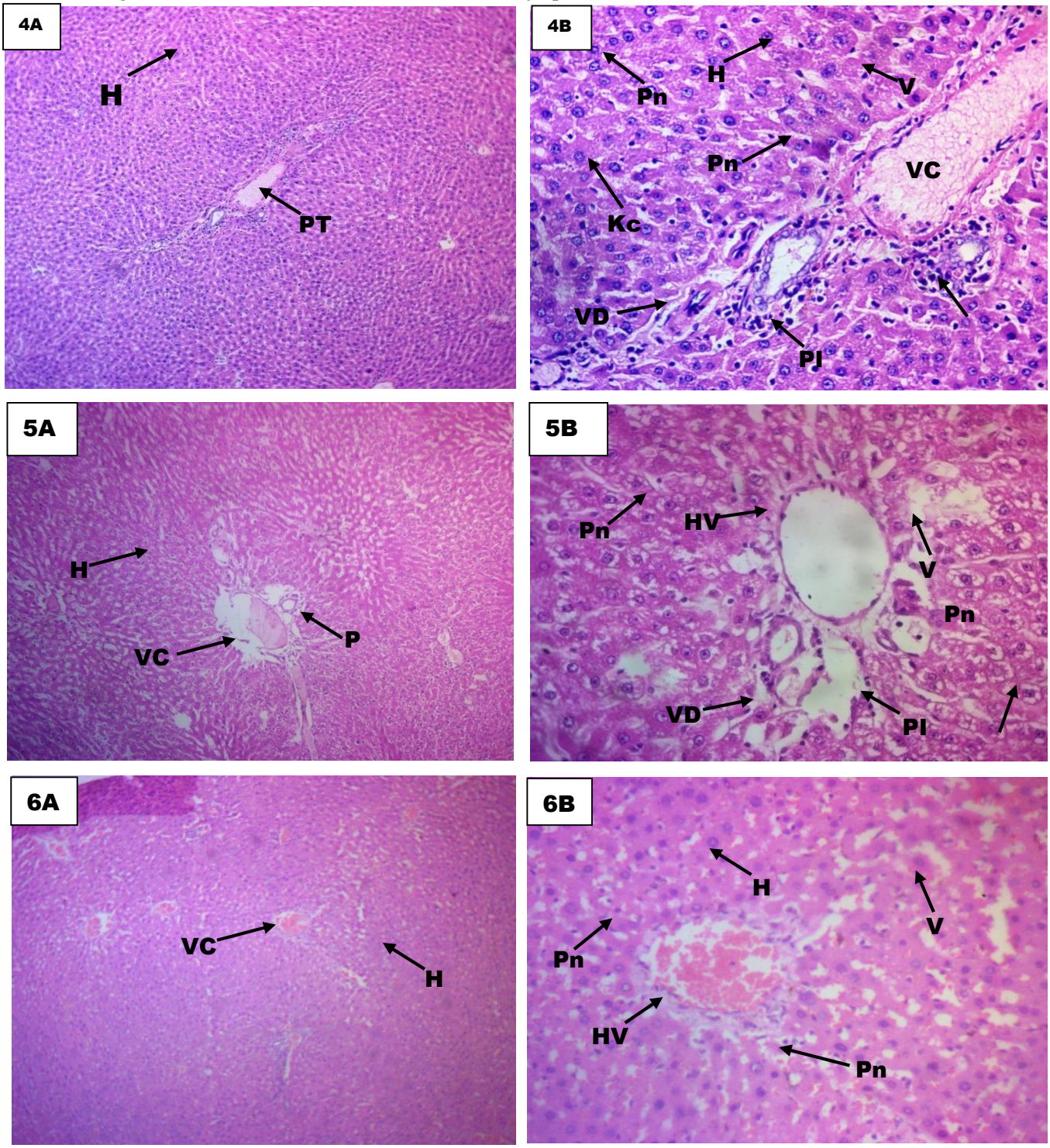

Figure 2: Histological sections of Livers of rats treated with HE 187 mg/kg and CCl4 (4 A/B), HE 374 mg/kg and CCl4 (5 A/B) and HE 784 mg/kg and CCl4 (6 A/B) at magnification A (x100) and B(x400) stained with H&E method.

Keys: Central vein (CV), Cellular degeneration (CD), Vacuolation (V), Inflammation (PI), Hepatocyte (H) and Pyknotic nucleus (Pn) Central vein (CV), Hepatic vein (HV), and Vascular degeneration (VD).

DISCUSSION

Carbon tetrachloride (CCl_4) is widely used for experimental induction of liver damage.[17] Carbon tetrachloride (CCl_4) induces hepatic damage in lipid peroxidation and decreases activities of antioxidant enzymes. It is well established that CCl_4 induces hepatotoxicity by metabolic activation,[18] selectively causes toxicity in liver cells, while maintaining semi normal metabolic function.[19] CCl_4 is biotransformed by cytochrome P_{450} system in the endoplasmic reticulum to produce trichloromethyl free radical (CCl_3). Trichloromethyl free radical when combined with cellular lipids and proteins in the presence of oxygen form trichloromethyl peroxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical.[20] Thus, trichloromethyl peroxyl radical elicits lipid peroxidation. The destruction of Ca^{2+} homeostasis finally results in cell death.[21] Lipid peroxidation as well as altered levels of some endogenous scavengers is taken as indirect *in-vivo* reliable indices for oxidative stress.[19] CCl_4 is metabolised by mixed – function oxidase system in the endoplasmic reticulum of the liver to the highly reactive trichloromethyl radical, and this reactive metabolite leads to auto – oxidation of the fatty acids present in the cytoplasmic membrane phospholipids and causes both functional and morphological distortion of the cell membrane.[21] The hepatocyte membrane distortion is associated with membrane leakage of the hepatocyte cytosolic contents which is manifested by significant elevation of the serum marker enzymes of acute hepatocellular damage namely, ALT and AST, and ALP as markers for hepatobiliary damage[22] as evident in this study. However, of these marker enzymes, ALT is the most reliable. AST is known to be present in abundance in the cardiac muscle, skeletal muscle, kidneys and testes, and ALP is abundant in the growing bone. Thus, any disease state affecting any of these extrahepatic tissues significantly elevates the serum levels of these enzymes.[23] In the carbon tetrachloride study, CCl_4 significantly increased WBC values compared to the control group. The elevated WBC can be due to the stimulation of immune defence system.[24] Similarly, literatures have shown that increased concentration of antigen in the body results in high values of WBC.[25] In this study, it was observed that the administration of ethanol extract of *Z. mays* husk caused significant decrease in values of WBC, lymphocyte, monocyte, basophils and eosinophils when compared to the organotoxic group. This trend agrees with earlier results obtained by Adisa, Ajayi, Awujo, and Thomas (1999)[26] and Ezekiel and Onyeyili (2007).[27] This maybe due to ability of the plant to counter delirious effect of CCl_4. CCl_4 has been known to produce hepatic damage by generation of highly reactive trichloromethyl (CCl_3) and trichloromethyl peroxy radicals when metabolized by cytochrome P_{450} .[28],[29] Pretreatment with husk extract of *Z. mays* in this study was observed to slightly improve the PCV, RBC, Hb and eosinophils values when compared to the CCl_4 group. As shown by the results, CCl_4 doses induced acute hepatic damage as evidenced by a marked elevation in the serum levels of the liver enzymes ALT, AST and ALP, and a significant decrease in the circulatory level of total protein, which is in conformity with earlier report of the

deleterious biochemical effects of CCl_4 on hepatic injury.[30] Extract treatment significantly attenuated the acute elevation of these enzymes by CCl_4. This is an indication that the extract preserved hepatic protein synthesis. CCl_4 induction was also associated with significant decrease in the serum level of total protein. However, treatment with ethanol husk extract of *Z. mays* protected the liver from the deleterious effect of the toxin by ameliorating the decrease in the circulatory level of total protein and thus stabilizing the endoplasmic reticulum. CCl_4 induction causes degeneration of hepatocytes and blockade of the bile ducts which resulted into significant increase in the serum levels of total bilirubin, direct bilirubin and ALP.[31] Treatment with *Z. mays* husk extract reduced the elevated serum levels of total bilirubin, direct bilirubin and ALP. Therefore, reduction in the levels of ALT and AST towards the normal value is an indication of regeneration process from hepatocellular damage. Reduction in the levels of ALP, total bilirubin and direct bilirubin suggests the stabilisation of the biliary function. An increase in the serum level of total protein suggests the regeneration of endoplasmic reticulum, leading to protein synthesis. This may be due to presence of phytochemical compounds present in the extract. CCl_4 -induced hepatotoxicity produced in rats leading to hepatic injury triggers the generation of toxic radicals which can be masked by using a correct antioxidant in adequate amounts.[29] The presence of flavonoids and other polyphenolic compounds in the plant explain its role in hepatoprotection by inhibiting the free radicals mediated damage.[29] The hemorrhage caused by CCl_4 in the liver was minimized by use of the plant extract as flavonoids are known to be vasculo protectors. On the basis of results obtained, it can be suggested that the ethanol husk extract of *Z. mays* seems to possess hepatoprotective activity in rats. Furthermore, histological damage to the rat liver induced by carbon tetrachloride administration support other well established study that intoxication with CCl_4 leads to severe necrosis in the liver centrilobular regions around the central veins[32] and fatty infiltration.[33] Interestingly, the microscopic examination also revealed the potential ability of the extract in pre-treated group to reduce inflammation, steatosis and necrosis as indicated by decrease in histological scoring. Relatively, the silymarin did not show more histologically hepatoprotective effect than the extracts at the doses administered. It is also worthy of note that tissue features in animals that received the highest dose of the extract (748 m/kg) were more secured and organized with more evidence of hepatocyte regeneration and recovery when compared to the mimimum and middle dose groups. The result suggests that the plant may protect against tissue microtubules damage and leakages, altered homoestasis as well as preventing oxidative lipid peroxidation caused by the reactive metabolite of CCl_4 by acting as an effective scavenger of reactive oxygen species (ROS). This positive effect may be similar to established substances as silymarin, Vitamin E, Vitamin C and other free radical scavengers such as black tea which have been reported to reduce the toxic effects of CCl_4, especially on the liver.[29]

4. CONCLUSION

The results of this study indicate that the husk extract of *Zea mays* possesses hepatoprotective potential against carbon tetrachloride-induced liver injury. This therefore confirms it use in the treatment of poisons in traditional medicine.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo.

HUMAN AND ANIMAL RIGHTS

The care and use of animals in this study was in accordance with the National Institute of Health Guide for the Care and Use of laboratory Animals (NIH, 1996).

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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