



(RESEARCH ARTICLE)



ASSESSMENT OF THE HEPATOPROTECTIVE ACTIVITIES OF ZEA MAYS HUSK EXTRACT AND ITS FRACTIONS ON CCL₄-INDUCED LIVER DAMAGE WISTAR RATS

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ABSTRACT

Introduction: Zea mays is an annual grass plant that was conventionally used to treat various diseases, was assessed for hepatoprotective ability against CCl₄-induced liver injuries in Wistar rats. Hepatoprotective activities of the fractions of maize husk were assessed by determining serum levels of liver function tests, lipid profile pattern and histology of the liver with phytochemical screening of a fraction.

Methodology: Forty (40) Wistar rats of eight (8) groups with five (5) rats each and CCL₄ induced liver damage was established in group 2 to 8. The group 1, 2, and 3 serves as normal, negative, silymarin control while group 4, 5, 6, 7 and 8 represents 200mg/kg each of aqueous, hexane, ethylacetate, butanol and methanol fractions of maize husk.

Results: The husk extract fractions caused significant ($p < 0.05$) decreased in the serum levels AST, ALT and ALP and significant increase in the level of TP and ALB in treated groups against the CCl₄ treated group. The fractions significant ($p < 0.05$) increased TC, TG, HDL and LDL in wistar rats and histology of liver revealed significant reductions in pathological features in the treated rats. The qualitative screening and GC-MS analysis of n-hexane fraction shown the occurrence of phenols and flavonoids, 1-decanol, carbonic acid and 5-eicosene of pharmacological important.

Conclusion: The results of this study indicated that the fractions of maize husk extract has hepatoprotective capacity as a result of some phytochemical components possess.

Keywords: Phytochemical; Phenols; Flavonoids; Pathological

1 INTRODUCTION

Liver, as one of the largest internal organ in the human body situated between the absorptive surface of gastrointestinal tract and offer wide range of functions such as protein synthesis, detoxification and production of biochemical necessary for digestion [1]. It is essential target of the toxicity of drug, xenobiotic and oxidative stress because of an important role it plays in metabolism and relationship to the gastrointestinal tract [2]. The liver's substantial regenerative capacity and crucial role in detoxification processes position it as a critical focus of medical research [3].

Liver diseases represent a substantial health burden globally with high cost for therapy, affecting over 25 % of the population and contributing to millions of cases across diverse liver pathologies [4]. In the United States, annual liver-related healthcare expenses reach \$32 billion, with a 4 % annual increase and a per-person cost of \$22,808. Standing as the eleventh leading cause of mortality worldwide, liver disease accounts for two million deaths annually, underlining its considerable morbidity and mortality [4].

Carbon tetrachloride (CCl₄) is one of the most widely used toxicant for inducing liver injury for mutagenicity and DNA damage study in animals. Carbon tetrachloride when consumed is metabolized by hepatic microsomal enzyme (CYP2E1) enzyme into two degraded metabolites namely, trichloromethyl (CCl₃) and tri-chloromethyl peroxy (CCl₃O₂) which are mainly responsible for hepatotoxicity [6]. These two metabolites are unstable radicals that have strong binding affinity towards protein and lipids in the cell membrane or removing a hydrogen atom from an unsaturated lipid, thereby triggering lipid peroxidation and causing liver damage [6]. In animal models, frequent administration of CCl₄ consistently induces hepatic steatosis (lipid accumulation), dyslipidemia, and significant hepatic metabolic dysfunction [7, 8].

Medicinal plants possess significant socioeconomic and health benefits, especially in developing countries with high poverty rates and limited health care coverage [9]. It continues to be an important element of human healthcare systems in different parts of the world with about 80 % of the world's population depending exclusively on traditional remedies for health care [9] which serves as an important component of global biodiversity [10]. The use of traditional medicine is not restricted to developing countries, over the past two decades, herbal medicine practice and the use of natural therapies have increased significantly in industrialized countries [11, 12, 13]. However, *Zea Mays* (maize/corn) is the most world's widely grown cereal that was domesticated in Central America [14] and the spread of maize from its center of origin in Mexico to various parts of the world has been rapid with respect to its evolution as a crop and its use in a variety of food products [15].

Maize is one of the most adaptable crops cultivated in various agro-climatic zones [16]. It is widely distributed around the world and used as either foods, industrial raw material or as animal feed [17]. Beyond its nutritive benefits, the leaves, silks, stalk, maize grain and husk are also used in ethnomedicine for treatment of various illnesses [18]. The maize husk is the multiple leaf-like structure covering an ear of maize and part of the silk [19]. It is the most abundant and least valuable by-product of corn industrial processing, accounting for 10% to 14% of the total fiber content of corn [20]. A study by [19] reported that maize husk contains some natural compounds such as phenols, flavan-3-ol, steroids, flavanone, proanthocyanins and resveratrol that were appraised for biological activities such as antidiabetic, antioxidant, antimicrobial and anticancer [18].

Considering the prevalence of liver diseases, the high cost and side effect associated with the current hepatoprotective therapies underscore the urgent need for novel therapeutic strategies. Due to the important properties of maize husk extract, it is important to evaluate the effects of various fractions of maize husk extract on liver injury. The study prescribed the effects of fractions of maize husk extract on the liver injury which may pave way for its consumption and exploitation into various fields of experimental researches.

2 MATERIALS AND METHODS

All the chemicals and the reagent used were of analytical standard and the chemicals and the reagents for extraction were purchased from Salamat Med Supply Express, Kebbi State.

2.1 Study Location

The study location was the animal house at Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto and Chemical Pathology Laboratory of School of Medical Laboratory, Usmanu Danfodiyo University, Sokoto, Nigeria.

2.2 Ethical Approval

Ethical approval was obtained from the Health and Biomedical Research Ethic Committee (HBREC) of the Usmanu Danfodiyo University, Sokoto with the approved number HBREC/25/035.

2.3 Plant Collection and Identification

Fresh corn husks were collected from the farms around Usmanu Danfodiyo University permanent site, Sokoto, Nigeria and the plant was deposited at herbarium of the Pharmacognosy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto with identification and authentication (PCG/UDUS/POAC/0010).

2.4 Plant Preparation and Extraction

Fresh corn husks collected were carefully separated from the silks, wash with clean water to remove any dust and other unwanted particles. They were allowed to shade dry on a clean surface and then pulverized to powder using a grinder. The cold maceration extraction method was adopted, where by 500 g of ground husks was macerated with 5 L of methanol for 72 hours with intermittent stirring. The mixture was sieved with a clean muslin cloth. Further filtration was carried out with Whatmann filter paper 1. The filtrate was then evaporated to dryness at low temperature (40°C) in a hot air oven to obtain the crude methanol extract (CME) [21]. The dried extract was weighed, kept in sterile container, label and process for fractionation.

2.5 Fractionation of the Crude Methanol Extract

The crude methanol extract (100 g) was suspended in distilled water and filtered; the water-soluble portion was partitioned using n-hexane (1.5 L), ethylacetate (1.5 L) and n-Butanol (1.5 L) to obtain n hexane fraction (HEF), ethylacetate (EAF), n-butanol (NBF) and the residual aqueous (AQF) fractions, respectively [21]. The dried extract of each fraction was weighed, kept in sterile container, label and store in the refrigerator at 8°C.

2.6 Phytochemical screening

Qualitative phytochemical screening and GC-MA analysis of the aqueous husk extract was carried out. The qualitative screening was performed by employing standard procedures and tests as laid down by [22] and adopted by [23] reviewed by [24]. Also GC-MS analysis was carried out as described by [25] using the Agilent 6890 GC-MS system procured from Agilent Technology, No. 5301 Stevens creek Blvd, Santa Clara, CA, USA.

2.7 Experimental Animals

Forty (40) Wistar Rats (150-170g) of either sex of roughly of the same age (8-10 weeks) were purchased from the Animal House, of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. The animals were maintained as described by [26] in clean cage, placed in a well-ventilated room with a temperature of 26°C to 28°C and photoperiods of 12 hours light and 12 hours darkness. The animals were maintained on a pellet feed obtained from Grand cereals oil mills limited, Jos, Nigeria, and were supplied with drinking water *ad libitum*. Cleaning of the animal cages was carried out weekly. All the experimental protocols were in compliance with the Institutional Animal Ethics Committee guidelines as well as internationally accepted practices for use and care of laboratory animals as contained in US guidelines [27], and also in accordance with the recommendation of the International Association for the study of pain (IASP) [28].

2.7.1 Induction of Liver Disease in Rats.

Liver damage was induced using carbon tetrachloride (CCl₄) administration of 30% CCl₄ (dissolved in olive oil, v/v) at 1.0 mL/kg b.w. twice a week for 6 weeks as described by [29] and [30].

2.7.2 Experimental design and Treatment

Forty (40) Wistar rats were randomly grouped into eight (8) groups comprising of 5 rats per group and they were treated as follows:

- Group 1 (n = 5): Normal control rats (NC), untreated/ received clean water and standard rodent chow *ad-libitum*.
- Group 2 (n = 5): Negative control rats (NegC), received 1mL/kg CCl₄ intraperitoneal without treatment
- Group 3 (n = 5): Positive control rats (PosC), received 1mL/kg CCl₄ with 100 mg/kg Silymarin
- Group 4 (n = 5): Aqueous test group, received 1mL/kg CCl₄ and 200 mg/kg of maize husk aqueous extract.
- Group 5 (n = 5): Hexane test group, received 1mL/kg CCl₄ and 200 mg/kg of maize husk hexane extract
- Group 6 (n = 5): Ethylacetate (EthylAce) test group, received 1mL/kg CCl₄ and 200 mg/kg of maize husk ethylacetate extract
- Group 7 (n = 5): Butanol test group, received 1mL/kg CCl₄ and 200 mg/kg of maize husk butanol extract
- Group 8 (n = 5): Methanol test group, received 1mL/kg CCl₄ and 200 mg/kg of maize husk methanol extract

The appropriate doses of the treatment regimens were calculated based on a study by [19] and were administered intragastric to the animals according to their body weight by intubation using intravenous cannula tube for 14 days.

2.8 Blood Samples Collection and processing

After 2 weeks, the rats were fasted for 12 hours, and anaesthetized with 75mg/b.w ketamine injection. Blood sample was collected from the animals through cardiac puncture into clean and plain bottles. The rats were later sacrificed through lumbar dislocation, the liver tissue was removed and place in buffer formalin. The sample collected in the plain tubes was allowed to clot at room temperature and later centrifuged at 4000 revolution per minute (rpm) for 10 minutes. The sera were transferred into labelled sterile serum bottles with tighten cap and stored at -20°C until assayed for the serum levels of liver function tests and lipid profile assessment.

2.9 Biochemical Parameters

The biochemical parameters were determined as markers of liver function (AST, ALT, ALP, TP, ALB, TBIL and DBIL and serum level of lipid profile (total cholesterol, high density lipoprotein (HDL), triglyceride (TG), low density lipoprotein (LDL)) using Randox diagnostic kits with Chm 535 semi-auto analyzer.

2.10 Identification of the Compounds

The identification of components present in the aqueous fraction of the plants' extract was based on direct comparison of the retention times and mass spectral data with those for standard compounds, and by computer matching with the Wiley and Nist Libraries [31, 32].

2.11 Histopathological examination

The liver of each animal that was used in the study were surgically harvested and fixed in buffered formalin. They were then processed and stained with haematotoxylin and eosin (H&E) for liver study according to standard procedures at Histopathology Department, Usmanu Danfodiyo University Teaching Hospital, Sokoto. Histologic pictures were taken as micrographs.

2.12 Data Evaluation

The data obtained were compute using Microsoft Office Excel 2007 and analyse with Statistical Package for Social Sciences (SPSS) version 23.0. The results were expressed as mean±standard error and group comparisons were made using one-way analysis of variance (ANOVA) followed by a posthoc test. Value of equal to or less than 0.05 ($P \leq 0.05$) was considered as statistically significant.

3 RESULTS

3.1 Liver function test parameter in CCl₄ induced liver damage treated with fractions of maize husk extract.

The serum level of AST, ALT, ALP and TBIL were significantly decreased while TP and ALB were significantly increased in normal control and the maize husk fractions ($p < 0.05$) compared with negative control. There were no significantly different in the serum level of AST, ALT, ALP, TP, ALB, TBIL and DBIL in positive control compared to maize husk fraction by only butanol indicated significantly increased in AST level ($p < 0.05$) while haxane, butanol and methanol fractions significantly decreased ALP level ($p < 0.05$). Also, when the fractions were compared, only the methanol fraction presented significant decreased in ALP ($p < 0.05$) and significant increase in TP and ALB compared to other fractions ($p < 0.05$).

Table 1: Liver function test parameter in CCL4 induced liver damage treated with fractions of maize husk extract.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	TP (g/dL)	ALB (g/dL)	TBIL (mg/dL)	DBIL (mg/dL)
NC	7.33 ± 0.58	6.00 ± 0.58	90.33 ± 1.20	67.33 ± 0.88	37.67 ± 0.32	0.65 ± 0.03	0.24 ± 0.03
NegC	36.33 ± 1.02 ^{ac}	48.67 ± 1.20 ^a	127.33 ± 3.18 ^{ab}	58.33 ± 1.20 ^a	34.00 ± 0.57 ^a	1.12 ± 0.04 ^a	0.51 ± 0.07
PosC	10.00 ± 1.00 ^b	9.67 ± 0.33 ^{ab}	103.00 ± 1.53 ^{ab}	66.00 ± 0.52 ^b	37.33 ± 0.90 ^b	0.93 ± 0.03 ^{ab}	0.23 ± 0.03 ^b
Aqueous	14.00 ± 1.00 ^{ab}	12.33 ± 0.88 ^{ab}	94.33 ± 1.86 ^b	67.33 ± 0.67 ^b	38.67 ± 0.33 ^b	0.85 ± 0.03 ^{ab}	0.23 ± 0.03 ^b

Hexane	11.33 ± 1.53 ^b	11.00 ± 0.57 ^{ab}	90.00 ± 1.15 ^{bc}	67.00 ± 0.58 ^b	37.33 ± 0.89	0.93 ± 0.04 ^{ab}	0.37 ± 0.12
EthylAcet	13.33 ± 0.58 ^{ab}	11.27 ± 0.67 ^{ab}	98.33 ± 0.88 ^b	66.33 ± 1.45 ^b	36.33 ± 0.88	1.03 ± 0.04 ^{ad}	0.45 ± 0.02
Butanol	15.00 ± 1.00 ^{abc}	11.67 ± 0.88 ^{ab}	91.00 ± 1.00 ^{bc}	65.00 ± 0.45	37.33 ± 0.13 ^b	0.83 ± 0.03 ^{ab}	0.33 ± 0.19
Methanol	11.33 ± 0.56 ^b	9.55 ± 0.33 ^{ab}	84.00 ± 1.73 ^{bcd}	69.66 ± 1.10 ^{bd}	38.66 ± 0.33 ^{bd}	0.83 ± 0.03 ^{ab}	0.23 ± 0.09 ^b

Values are expressed as mean ± standard errors; ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; TP: Total protein; ALB: Albumin; TBIL: Total bilirubin; DBIL: Direct bilirubin; Grp 1: Normal control; Grp 2: Negative control (CCL4 only); Grp3: Positive control (Silymarin); Grp 4: 200mg/kg aqueous maize husk extract; Grp5: 200mg/kg hexane maize husk extract; Grp6: 200mg/kg ethylacetate maize husk extract; Grp7: 200mg/kg Butanol maize husk extract; Grp 8: 200mg/kg methanol maize husk extract Value differs significantly when $p < 0.05$.

^ap mean significance against NC, ^bp mean significance against NegC, ^cp significance against PosC, ^dp significance among the fractions

3.2 Lipid profile parameters in CCL₄ induced liver damage treated with fractions of maize husk extract.

As presented in table 2 below, the serum level of TC, TG and LDL were significantly decreased in negative control compared to normal ($p < 0.05$) while there was no significant different in HDL level in normal control compared to negative control ($p > 0.05$). Also, the aqueous and methanol fractions indicated significant increase in the level of TC and LDL compared to negative control ($p < 0.05$). Similarly, all the fractions of maize husk extract significantly increased TG level compared to negative control ($p < 0.05$). However, the treatment groups indicate increased in HDL but only the methanol fraction show significant increase compared to negative control ($p < 0.05$).

Table 2: Lipid profile parameters in CCL₄ induced liver damage treated with fractions of maize husk extract.

Groups	TC (mmol/L)	TG (mmol/L)	LDL (mmol/L)	HDL (mmol/L)
NC	5.37 ± 0.23	1.13 ± 0.19	3.37 ± 0.29	1.23 ± 0.09
NegC	3.73 ± 0.21 ^a	0.33 ± 0.10 ^a	1.90 ± 0.25 ^a	0.87 ± 0.03
PosC	4.07 ± 0.09	0.60 ± 0.10	2.73 ± 0.89	1.13 ± 0.12
Aqueous	5.10 ± 0.10 ^b	1.03 ± 0.19 ^b	3.03 ± 0.23 ^b	1.57 ± 0.33
Hexane	3.83 ± 0.09 ^a	1.02 ± 0.09 ^b	2.00 ± 0.10 ^a	1.30 ± 0.15
EthylAcetate	4.40 ± 0.40	1.05 ± 0.13 ^b	2.23 ± 0.45 ^a	1.30 ± 0.12
Butanol	4.57 ± 0.56	1.10 ± 0.10 ^b	2.53 ± 0.47	1.40 ± 0.10
Methanol	5.23 ± 0.24 ^b	1.07 ± 0.12 ^b	3.10 ± 0.10 ^b	1.63 ± 0.08 ^b

Values are expressed as mean ± standard errors; TC: Total cholesterol; TG: Triglyceride; HDL: High density lipoprotein; LDL: Low density lipoprotein; Grp 1: Normal control; Grp 2: Negative control (CCL4 only); Grp3: Positive control (Silymarin); Grp 4: 200mg/kg aqueous maize husk extract; Grp5: 200mg/kg hexane maize husk extract; Grp6: 200mg/kg ethylacetate maize husk extract; Grp7: 200mg/kg Butanol maize husk extract; Grp 8: 200mg/kg methanol maize husk extract Value differs significantly when $p < 0.05$. ^ap mean significance against NC, ^bp mean significance against NegC, ^cp significance against PosC, ^dp significance among the fractions

3.3 Histopathological findings

The photomicrographs demonstrate the general hepatic architecture and the histological features identified across the liver sections of group 1 to group 8. Normal control shows hepatocytes (HC) arranged around a central vein (CV), with preserved hepatic architecture. Negative control demonstrates hepatocytes with more conspicuous cytoplasmic vacuolation and rarefaction together with arterial (ART) structures like with prominent cytoplasmic vacuolation (VAC) accompanied by apparent disruption of the normal tissue pattern. The silymarin-treated shows hepatic parenchyma with hepatocytes (HP), arterial structures with cytoplasmic vacuolation. Aqueous extract retained hepatocytes despite focal vascular congestion with a vascular region comprising a central venous structure. Hexane treated group shows marked alteration of the hepatic parenchyma, with prominent vascular structures, hepatic portal structures, and arterial structures. Ethyl acetate demonstrates hepatocellular parenchyma containing hepatic portal structures and sinusoidal structures, with areas of cytoplasmic vacuolation. Butanol treated shows a vascular region containing venous, arterial, and hepatocellular components, with vascular congestion while methanol group demonstrates hepatic parenchyma with hepatic portal structures, arterial structures and less cytoplasmic vacuolation.

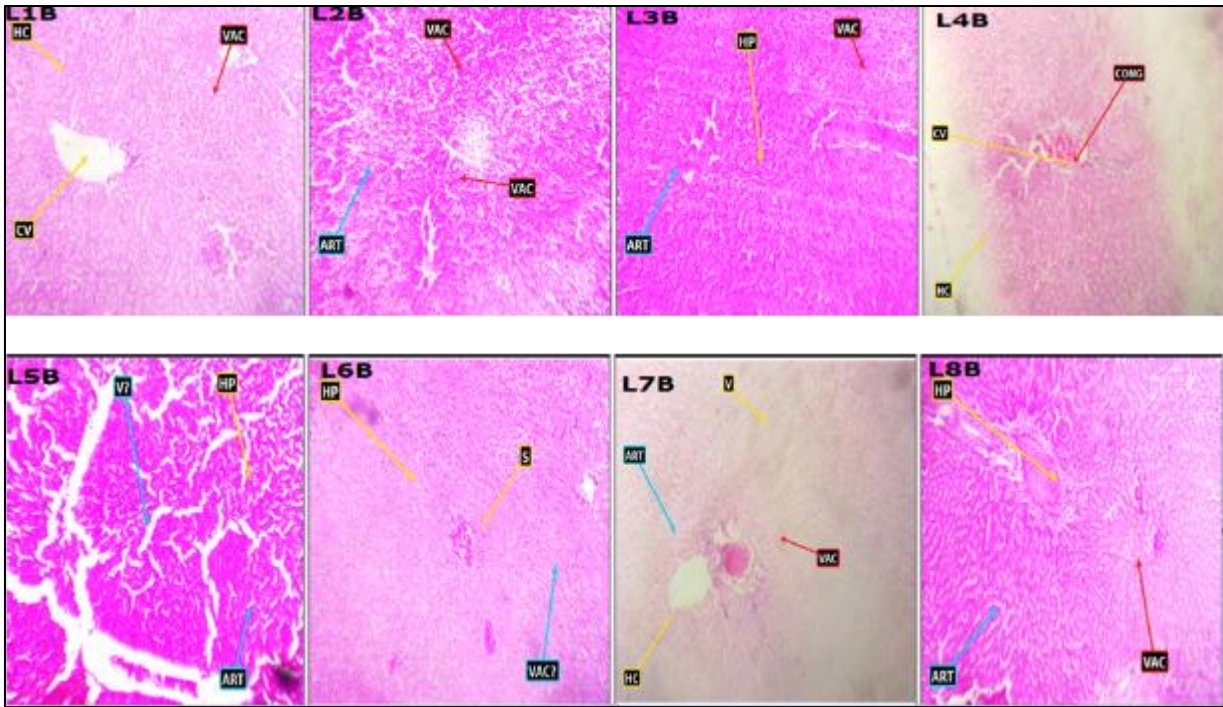


Plate A: Histomorphological features of the liver at ×40 magnification following H&E staining. Representative sections (group 1-8) showing hepatocytes (HC/HP), central venous structures (CV), venous channel (V) arterial structures (ART), hepatic portal structures (HP), hepatic cords (HC) sinusoids (S), cytoplasmic vacuolation (VAC), and vascular congestion (CONG), as indicated by the arrows. The micrographs demonstrate variable preservation of hepatic architecture with differing degrees of hepatocellular vacuolation and vascular alterations.

3.4 Qualitative analysis of the phytochemical constituents in aqueous extract of Maize husk

The qualitative analysis of the phytochemical constituents in aqueous extract fraction of maize husk reveal the significant presence of flavonoid, carbohydrate and phenol, moderate presence of cardiac glycosides, steroids, alkaloids, presence of tannins, saponins and absence of protein and anthraquinine.

Table 3: Qualitative analysis of the phytochemical constituents in aqueous extract of Maize husk

Compounds	Test	Result
Tannins	Ferric chloride test	+
Saponins	Froth test	+
Flavonoids	Ferric chloride test	+++
Cardiac glycosides	Alkaline (Ammonia) test	+++
	Keller-Killiani test	++
Steroids	Salkowski test	++
Alkaloids	Meyers test	++
	Wagner test	++
Carbohydrate	Molich' test	+++
Phenols	Ferric chloride test	+++
Protein	Xanthoproteic test	-
Anthraquinine	Borntrager's Test	-

Key: + = Present; ++ = Moderate; +++ = Significantly present;

3.5 Gas Chromatography-Mass Spectroscopy (GC-MS) analysis of the phytochemical constituents in aqueous extract of maize husk

Gas chromatography-mass spectroscopy analysis of the phytochemical analysis of one the fraction (aqueous) of *Zea mays* revealed the presence of 20 compounds (Table 4). The compounds present include Carbonic acid, Eicosyl vinyl ester, Octadecyl vinyl ester, Octadecyl prop-1-en, 5-Octadecene, Octadecyl prop-1-en-2-yl ester, Cetene, Tetracontane, 1-Decanol, 5-Eicosene, 3-Eicosene, Cycloeicosane, Hexadecyl prop-1-en, 7-Hexadecene, 1-Dodecanol, 2-hexyl-Octatetracontane, Z-5-Nonadecene, Tetracontane, Octatetracontane and 1-Dodecanol

Table 4: GC-MS analysis of the phytochemical constituents in aqueous extract of Maize husk

Peak	Retention Time	Area%	Compound Name	Quality
1	4.474	1.46	Carbonic acid, Eicosyl vinyl ester, Octadecyl vinyl ester	90% 90% 90%
2	5.183	8.12	Octadecyl prop-1-en	90%
3	5.475	1.18	5-Octadecene, Cetene	96% 90%
4	5.801	0.63	Tetracontane	90%
5	6.145	12.01	1-Decanol, 5-Eicosene	91% 90%
6	6.723	5.20	3-Eicosene, 5-Octadecene Cycloeicosane	95% 95% 91%
7	8.021	2.99	Hexadecyl prop-1-en	91%
8	8.256	3.16	7- Hexadecene	94%
9	8.571	4.22	1-Dodecanol, 2-hexyl-Octatetracontane	91% 90%
10	8.920	3.67	Z-5-Nonadecene	91%
11	10.968	5.71	Tetracontane	91%
12	11.552	2.22	Octatetracontane	90%
13	12.399	0.79	1-Dodecanol	91%

4 DISCUSSION

Herbal drugs are gaining intense consideration of the researchers due to safe, efficient, and non-toxic effects through which they are considered as a basic part of health care by the people of developing countries [33]. Medicinal plants possess medicinal properties as they retain biologically active organic compounds such as terpenoids, steroids, saponins, flavonoids, alkaloids and phenolic compounds. Researchers are taking interest to evaluate these compounds due to their better pharmaceutical performance. Hence, investigation and isolation of bioactive compounds from medicinal plants can be interesting for pharmacologists and researchers [34]. *Zea mays* and its parts are used in the treatment of malaria, diabetes, dyslipidemia, liver and kidney diseases [19]. This work was focused on the assessment of fractions of *Zea mays* husk extract for hepatoprotective potentials in CCl₄ induced liver injury wistar rats.

The pattern of derangement recorded in the negative control (CCl₄ induced only) in this study indicate marked elevation of AST, ALT and ALP alongside reduced total protein and albumin and elevated bilirubin. This is reported in a well-established biochemistry of CCl₄-induced hepatocellular injury as the reactive species covalently bind to membrane lipids and proteins and initiate a chain reaction of lipid peroxidation that compromises hepatocyte membrane integrity, leading to leakage of cytosolic enzymes such as AST and ALT into the circulation and impairment of the liver's synthetic and excretory functions [35]. Conversely, following the treatment with the fractions of maize husk extract, the fractions considerably reduced the level of AST, ALT, TBIL and substantially increased the level of TP and ALB. Though, methanol fraction of the maize husk extract exhibit more potential in relation to silymarin treated group and other fraction.

Likewise the pronounced improvement in liver function indices and among treated rats was also reported in the study by [36]. Silymarin's own hepatoprotective action is generally attributed to a combination of direct free-radical scavenging, glutathione-sparing activity, hepatocyte membrane stabilisation and stimulation of hepatocyte regeneration [37]. The improvement observed in the fraction-treated group may be due to protective role of the fraction in alleviating the effect of free radicals generated by CCl₄ due to the presence of free radical scavenging activities of the phytoconstituents such as phenolic compounds present in the husk extract fractions [38].

The highly significant decrease in lipid profile parameter was observed after CCl₄ administration (table 2) and the decreased level observed could be the result of liver damage because liver produces 80% cholesterol in the body [39]. When the fractions of maize husk extract was given in combination, it caused highly significant increase in serum level of TC, TG, LDL and HDL most especially the aqueous and methanol fractions. The result of this study concurred with the previous studies of [36, 40] who independently reported that administration of maize husk extract resulted to a significant improvement in overall lipid profile. The presence of flavonoids and other polyphenolic compounds in the plant explain its role in hepatoprotection by inhibiting the free radicals mediated damage [41]. On the basis of the results obtained, it can be suggested that the fractions of maize husk extract seems to possess hepatoprotective activity in rats. Furthermore, treatment with maize husk mitigated the hepatic damages, which was associated with the improvement of liver function.

This study shows that carbon tetrachloride induced histological damage to the rat liver and this was also reported in other studies which concluded that CCl₄ intoxication leads to severe liver damage [42]. Remarkably, the microscopic investigation revealed the potential ability of the maize husk extract in treated group to reduce vacuolations, steatosis and necrosis compared with the CCl₄ induced group. Comparatively, the extract treated groups and silymarin did not show any difference effect in histologically observed. The methanol extract indicate more protective property with hepatocyte regeneration and recovery when compared to the other extract groups. The result of our study was in line with a study by [43] who reported that the maize husk plant may protect against tissue microtubules damage and leakages, altered homeostasis as well as preventing oxidative lipid peroxidation caused by the reactive metabolite of CCl₄ by acting as an effective scavenger of reactive oxygen species (ROS). This positive effect may be related to known compound such 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 CCl₄, especially on the liver [41].

Moreover, the qualitative screening of the aqueous fraction in this study confirmed an abundance of flavonoids, phenols and carbohydrates, alongside cardiac glycosides, steroids, alkaloids, tannins and saponins; a profile broadly consistent with recent phytochemical surveys of *Zea mays* husk and other maize by-products which possess similarly identified polyphenols, flavonoids and tannins as principal secondary metabolites underlying the plant's reported antioxidant, anti-inflammatory and hepatoprotective activities [44, 45]. Flavonoids in particular are recognised to protect hepatocytes against free-radical-mediated injury by donating hydrogen atoms to, and thereby neutralising, reactive oxygen- and chlorine-centred radicals such as those generated during CCl₄ metabolism, in addition to chelating pro-oxidant transition metals [46]. It is plausible that the flavonoid and phenol-rich constituents of the extract contributed to the attenuation of AST, ALT, ALP and bilirubin observed across the treated groups in the present study through a broadly similar radical-scavenging mechanism.

Notably, this study presented the GC-MS analysis of the aqueous fraction which identified a chemical profile dominated by long-chain aliphatic hydrocarbons (eicosene, octadecene and nonadecene isomers) and carbonic acid ester derivatives rather than other classical constituents such as polyphenolic and polyflavonoid, which were nonetheless confirmed present by the complementary qualitative screening. The Carbonic acid an eicosyl vinyl ester, by virtue of its activity, was among the phytocomponents identified from *Cakile maritima* Scop extracts with antioxidant activities [47] that may contribute to the hepatoprotective potential exhibit by these fractions consequently reduced liver enzymes activities, increased total protein and albumin.

5 CONCLUSION

The findings of this study reveal that the fractions of maize husk extract possess hepatoprotective capability against CCl₄-induced liver damage in Wistar rats. The treatment with the various fractions was accompanying with improvements in the biochemical and histological arbitrations induced by CCl₄ intoxications. This protection was fraction-dependent, with the Methanol fraction producing the most complete restoration of liver function indices, comparable to other treated group. These findings lend biochemical support to the ethnomedicinal use of maize husk in the management of hepatic disorders and are consistent with the phytochemical profile of the plant, which is rich in flavonoids, phenolic compounds and other secondary metabolites with recognised antioxidant and hepatoprotective properties.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

No possible conflict of interest relevant to this article was observed.

Statement of ethical approval

The research was carried out in line with the experimental protocols which is in compliance with the Institutional Animal Ethics Committee guidelines as well as internationally accepted practices for use and care of laboratory animals as contained in US guidelines, and also in accordance with the recommendation of the International Association for the study of pain.

Author's contribution

- Jimoh A. A.: Conceptualization, editing and carried out data curation and statistical analysis.
- Wali U.: Conceptualization and Proofreading
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