OPEN ACCESS

Current Research in Complementary & Alternative Medicine

Stephen K, et al. Curr Res Complement Altern Med 7: 201.

www.doi.org/10.29011/2577-2201.100201

www.gavinpublishers.com

GAVIN PUBLISHERS

Research Article

Hepatoprotective Effects of *Tetrapleura tetraptera* (Schum. & Thonn.) Taub. Stem Bark Extracts in Drug-induced Toxicities in Rats

Kusi Stephen[1,2], Christopher Larbie[1*], Frederick Sarfo-Antwi[3]

[1]Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

[2]Department of Science, Prempeh College, Kumasi, Ghana

[3]Department of Biochemistry and Molecular Medicine, School of Medicine, University for Development Studies, Tamale, Ghana

***Corresponding Authors:** Christopher Larbie, Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Citation: Kusi, S., Larbie C, Sarfo-Antwi F (2023) Hepatoprotective Effects of *Tetrapleura tetraptera* (Schum. & Thonn.) Taub. Stem Bark Extracts in Drug-induced Toxicities in Rats. Curr Res Cmpl Alt Med 7: 201. DOI: 10.29011/2577-2201.100201

Received Date: 25 August 2023; **Accepted Date:** 15 September 2023; **Published Date:** 2 October 2023

Abstract

Hepatotoxicity is an issue of global concern because of numerous liver-related deaths over the years. In addition, the conventional hepatoprotective and hepatocurative drugs used are more expensive, not easily accessible, and sometimes have serious adverse effects. These reasons account for why many have resorted to traditional plant medicines. This study evaluated the hepatoprotective effects of aqueous and hydroethanolic stem bark extracts of *Tetrapleura tetraptera,* a commonly used traditional medicinal plant, in paracetamol and carbon tetrachloride-induced hepatotoxicities in rats. Pulverized stem bark samples of 500 g each were soaked in 1000 mL boiled water and 1000 mL 50% hydroethanol respectively for 24 hours and filtered. Filtrates were concentrated over a water bath and designated as aqueous stem bark extract (ASE) and hydroethanolic stem bark extract (HSE). Extracts were evaluated for LD_{50} value, phytochemical constituents, free radical scavenging effects and heavy metals. Fifty-seven male Wistar albino rats weighing 120-180 g were divided into nineteen groups (n=3/group). The LD_{50} for both extracts were found to be above 5000 mg/kg b.wt. This study showed HSE had higher amounts of phytochemicals than ASE. Again, this study also showed that HSE and ASE had greater levels of liver percentage protection than Silymarin. Furthermore, the liver antioxidant profile study proved that HSE and ASE could protect the liver. These results suggest that ASE and HSE, through their antioxidant activities, have hepatoprotective and hepatocurative effects against paracetamol and carbon tetrachloride-induced hepatotoxicities in rats and could be developed as potential liver protective agents.

Keywords: Hepatotoxicity, Hepatoprotective, Hepatocurative, Antioxidant, *Tetrapleura tetraptera*

Introduction

The liver is the largest visceral organ that regulates different bodily functions, such as metabolism, secretion, storage and detoxification [1]. It is continuously and variedly exposed to elevated amounts of xenobiotics like drugs and environmental pollutants which enter the body and are delivered to it via the hepatic portal vein after intestinal absorption.

Xenobiotics undergo biotransformation which is mostly a detoxification process, the many oxidative reactions in this process produce reactive metabolites that are more toxic than the parent compound and can induce lesions within the liver [2]. These metabolites bind covalently to cellular macromolecules resulting in increased lipid peroxidation, reduced cellular ATP production and oxidative damage to cause liver diseases.

Naturally, the liver protects itself from harmful reactive metabolites, and ultimately liver diseases through its antioxidant system. However, it is overpowered by the excessive production of reactive metabolites resulting in liver diseases. Several reports indicate a continuous increase in the incidence rate of hepatic diseases [3,4] despite the advances in modern medicine. Arhoghro *et al.*, [5] reported that conventional drugs used in disease treatment are inadequate and have serious adverse effects. These reasons may partly be responsible for the high incidence rate of liver diseases. Globally, many people, especially those from developing countries, have resorted to medicinal plants since they consider these plants to be natural, have less toxicity, simple accessibility and simple assimilation in the body and are economical [6-8].

Tetrapleura tetraptera (Schum. & Thonn.) Taub. (Family: Leguminosae) is a well-known and indispensable medicinal plant with proven efficacy in the management of numerous health conditions [9,10]. It is known in English as Aidan or Aridan and among the Akans of Ghana as 'prɛkesɛ'. Ethnomedically, all parts of *T. tetraptera* have been reported to be used for medicinal purposes [11]. The stem bark is used to treat cardiovascular disorders, gastrointestinal disorders, malaria and other fevers, low body immunity, reproductive disorders, cancer of the breast and uterus, general body pains and weakness, dental disorders Kemigisha *et al*., [11] and wound Tsala *et al*., [12]. The leaves are used to treat convulsions and epilepsy, malaria and other fevers, reproductive disorders, general body pains and weakness. The fruits have various applications and are extensively used for the management of an array of human ailments including cardiovascular disorders, hypertension, arthritis, diabetes mellitus, epilepsy, convulsion, leprosy, inflammation and rheumatoid pains, gastrointestinal disorders, malaria and other fevers, asthma and chest pain, low body immunity, flu and colds, reproductive disorders, cancer of the breast and uterus, skin disorders of newly born babies, wounds and burns, back and general body pains and weakness, dental disorders, spherocytosis, and iron deficiency anaemia [13-15,11].

The ability of *T. tetraptera* to heal diseases and provide for significant normal body functioning has been attributed to its high number of essential phytochemicals and nutrients including flavonoids, triterpenoid, glycoside, tannins, alkaloids, saponins, steroids and phenolic compounds, potassium, iron, calcium, zinc and phosphorus [16,17]. These phytochemicals and minerals have been identified in the stem bark, leaves and fruits of *T. tetraptera* in varying amounts and have been proven pharmacologically to be anti-inflammatory Onda *et al.,* [18] and antioxidant activities [19,10]. Though literature revealed that anti-inflammatory and antioxidant properties are vital for hepatoprotective activity, no information was found regarding the hepatoprotective activity of stem bark extracts of *T. tetraptera*. This present study, therefore, sought to evaluate the hepatoprotective effects of aqueous and hydroethanolic stem bark extracts of *Tetrapleura tetraptera* in drug-induced toxicities in rats.

Materials and Methods

Materials and reagents

Legalon Flordis, Australia, supplied all solvents used in the extraction process. All chemicals and reagents used in the study were of analytical grade.

Plant collection and identification

The fresh stem bark sample of the *T. tetraptera* plant was obtained in September 2019, from healthy, fully grown plants in Ghana's Ashanti Region (latitude 6° 58'12.0" N and longitude 1°38"23.9" W). The sample was authenticated at the Department of Herbal Medicine, KNUST, with a voucher specimen (KNUST/HM1/2019/SB013) deposited at the Herbarium for reference purposes.

Preparation of Extracts from the Stem Bark

The stem bark was washed, dried in the shade, and milled into powder. The aqueous and hydroethanolic extractions were prepared using 500 g of pulverized stem bark each in 1000 mL boiled water and 50% hydro-ethanol respectively, at room temperature with continued shaking for 24 hours. The extracts were then filtered through cotton wool and concentrated under 60□C pressure using a rotary evaporator (Buchi R205, Switzerland) and designated as aqueous stem bark extract (ASE) and hydroethanolic stem bark extract (HSE).

Qualitative Phytochemical Screening

Phytochemical constituents of *Tetrapleura tetraptera* stem bark

extracts were determined using standard methods as described by Evans [20]. Alkaloids, flavonoids, terpenoids, saponins, coumarins, reducing sugars, sterols, and tannins were the phytochemicals examined.

In vitro Antioxidant Activity

Total Phenol Content (TPC) Determination

The total phenolic content was determined by the Folin-Ciocalteu method Singleton *et al*., [21] with some modifications. About 50 µL of each sample (extracts and gallic acid [0.00, 0.25, 0.50, 0.75 and 1 mM]) was mixed with 3 mL of distilled water and 250 µL of the Folin-Ciocalteu reagent. The mixture was made to stand for a minimum of 5 minutes and 750 µL of 20% Na_2CO_3 was added. The mixture was then vigorously vortexed for 2 minutes followed by 30 minutes of incubation at room temperature. The absorbance of the solutions was measured at 760 nm using a UV-VIS spectrophotometer (Shimadzu Corporation, 1201, Kyoto, Japan). Each sample was tested in triplicate and a calibration curve with six data points for gallic acid was obtained. The results were compared with a gallic acid calibration curve. The total phenolic content was expressed as Gallic Acid Equivalent (GAE) (mg GAE/g extract).

Total Tannins Content (TTC)

The quantity of tannins in extracts was determined by the Folin–Ciocalteu method with slight modifications [22]. One hundred microlitres of extract was added to 5 mL of distilled water, 500 µL of Folin–Ciocalteu reagent and 1 mL of 35% Na_2CO_3 solution. The mixture was shaken well and kept at room temperature for 30 min and absorbance was measured against the blank at 725 nm. The total tannin content was determined from the calibration curve of gallic acid. Results were expressed in terms of mg GAE/100 g. All determinations were performed in triplicates.

Estimation of Total Flavonoids Content (TFC)

The total flavonoid content in the samples was estimated by the aluminium chloride colourimetric assay method Zhishen *et al*., [23] using quercetin as the standard. An aliquot of 500 µL of each extract was mixed with 1.5 mL of 99.9% ethanol, 100 µL of 1 M potassium acetate, 100 µL of 10% aluminium chloride and 3 mL of distilled water. The mixture was vigorously shaken and left to stand in the dark at room temperature. The resulting mixture samples were incubated for 30 min at room temperature and the corresponding absorbance was measured at 415 nm. The total flavonoid content of each extract was determined from the standard curve and the results were expressed as mg QE/100 g.

DPPH Scavenging Activity

The DPPH free radical scavenging ability of the extracts was evaluated as described by Oliveira *et al.* [24] with some modifications as previously described by Donkor *et al.* [25]. Two hundred microlitres of each extract were added to 3.8 mL of 0.004% DPPH methanolic solution and incubated at room temperature in the dark for 60 minutes. The absorbance was measured at 517 nm against a methanol blank.

Heavy Metals

Each extract and a raw sample of mass 1 g were weighed into a correspondingly labelled Pyrex digestion tube. Each sample was wet digested using a mixture of 1 mL water, 2 mL HCl, 5 mL 1:1 HNO_3:$HClO_4$, and 2 mL of H_2SO_4 for 20 minutes on a heating block at 150°C. The digest was allowed to cool, transferred to a volumetric flask, and diluted with distilled water to 50 mL. Heavy metals including lead, copper, nickel, zinc, and iron were analysed using an atomic absorption spectrometer (Varian AA 240FS) with a long path air acetylene burner and cathode lamp for respective metals.

Toxicity assessment of *Tetrapleura tetraptera* stem bark extract

Fifty-seven male Wistar albino rats weighing between 120 and 180 grams were obtained from the Animal House of the School of Medical Sciences, University of Ghana, Legon, Accra. They were then kept in propylene cages with wood shavings bedding and allowed to acclimatize for 14 days. Throughout the study, the animals were maintained under standard environmental conditions (room temperature and 12-hour light), fed with standard rat feed (Agricare, Kumasi, Ghana) and given distilled water *ad libitum* except for the overnight fast before the start of the study and termination. Rats were weighed and identified on their tails using a permanent marker to allow for efficient dosing.

Acute oral toxicity

The OECD guidelines 425 [26] was adopted in the acute oral toxicity study of HSE and ASE using six male Wistar albino rats in two groups (120–180g; n=3/group). ASE and HSE at a maximum dose of 5000 mg/kg body weight (b.wt.) were dissolved in normal saline and administered by gavage (p.o.) Following the administration of extracts, the animals were observed for signs of toxicity and mortality for the first critical 4 hours and thereafter daily for 7 days [26,27]. This was used to guide the selection of two doses (100, and 250 mg/kg b.wt) for the hepatoprotective studies.

Evaluation of Hepatoprotective Activity

Hepatotoxicity was induced in animals using carbon tetrachloride (CCl_4; Sigma Aldrich) and acetaminophen (Para, Trade Winds Pharmaceuticals, Kumasi, Ghana). Carbon tetrachloride only treated group received oral administration of 1.0 mL/kg b.wt CCl_4 (1/1 v/v olive oil, intraperitoneal [i.p.]) for two successive days, day 2 and day 3. For the Para (Paracetamol IV Pfizer Solution) only treated group, rats received 500 mg/kg b.wt p.o. daily for 7 days [28].

Table 1: Experimental grouping and treatment of Animals.

S/N.	Group	Treatment
1	Normal (control)	Rats were orally treated with 1.0 mL/kg distilled water each day for 7 days.
Extract only		
2	100 mg/kg HSE only	Rats were orally treated daily with only 100mg/kg HSE for 7 days.
3	250 mg/kg HSE only	Rats were orally treated daily with only 250 mg/kg HSE for 7 days.
4	100 mg/kg ASE only	Rats were orally treated daily with only 100 mg/kg ASE for 7 days.
5	250 mg/kg ASE only	Rats were orally treated daily with only 250 mg/kg ASE for 7 days.
Treatment with CCl_4		
6	CCl_4	CCl_4 i.p (1 ml/kg b.wt, 1:1 v/v olive oil) received on 2nd and 3rd day.
7	CCl_4+Sily	Silymarin p.o (100 mg/kg per day) received for seven days plus a single dose of CCl_4 in olive oil (1:1 v/v, 1.0 ml/kg, i.p.) on the 2nd and 3rd day
8	100 mg/kg HSE + CCl_4	100 mg/kg HSE (per day, p.o.) for seven days plus a single dose of CCl_4 in olive oil (1:0 v/v, 1.0 ml/kg, i.p.) on the 2nd and 3rd day.
9	250 mg/kg HSE + CCl_4	250 mg/kg HSE (per day, p.o.) for seven days plus a single dose of CCl_4 in olive oil (1:0 v/v, 1.0 ml/kg, i.p.) on the 2nd and 3rd day.
10	100 mg/kg ASE + CCl_4	100 mg/kg ASE (per day, p.o.) for seven days plus a single dose of CCl_4 in olive oil (1:0 v/v, 1.0 ml/kg, i.p.) on the 2nd and 3rd day.
11	250 mg/kg ASE + CCl_4	250 mg/kg ASE (per day, p.o.) for seven days plus a single dose of CCl_4 in olive oil (1:0 v/v, 1.0 ml/kg, i.p.) on the 2nd and 3rd day.
Treatment with Para		
12	Para only	500 mg/kg Para (i.p) for 7 consecutive days
13	Para+Sily	Silymarin p.o (100 mg/kg per day) received for seven days plus 500 mg/kg Para (i.p) for 7 consecutive days
14	100 mg/kg HSE + Para	100 mg/kg HSE (per day, p.o.) for seven days plus 500 mg/kg Para (i.p) for 7 consecutive days
15	250 mg/kg HSE + Para	250 mg/kg HSE (per day, p.o.) for seven days plus 500 mg/kg Para (i.p) for 7 consecutive days
16	100 mg/kg ASE + Para	100 mg/kg ASE (per day, p.o.) for seven days plus 500 mg/kg Para (i.p) for 7 consecutive days
17	250 mg/kg ASE + Para	250 mg/kg ASE (per day, p.o.) for seven days plus 500 mg/kg Para (i.p) for 7 consecutive days

Experimental design

On termination, the animals were fasted overnight, and sacrificed under cervical decapitation. The animals were quickly slit at the neck, and blood samples were collected into gel-activated tubes for biochemical analyses and EDTA tubes for haematological analyses. The animals were dissected, and their livers excised, freed of fat, washed with normal buffered saline and blotted with clean tissue paper.

Relative liver weights

The relative organ weights of the liver (ROW) of the rats for each group were determined as:

$$ROW = \frac{AOW}{Body\ weight\ of\ animals\ of\ the\ group\ on\ the\ day\ of\ sacrifice} \times 100\%$$

AOW; Absolute Organ Weight of excised liver

Effect of treatments on haematological parameters of rats

The evaluation of haematological parameters was done using Sysmex XS-1000i Auto Haematology Analyser (Japan). Parameters measured include white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (HGB) concentration, haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet (PLT), lymphocyte (LYM), neutrophils (NEUT), red blood cell distribution width standard deviation (RDW-SD), red blood cell distribution width coefficient of variation (RDW-CV), platelet distribution width (PDW), mean platelet volume (MPV), platelet-large cell rate (P-LCR) and platelet crit (PCT).

Effect of treatments on serum biochemistry of rats

Blood samples in activated gel tubes were allowed to clot and centrifuged at 1500 g for 15 min to obtain blood serum. Biochemical analysis was carried out using Selectra E (Vital Scientific) and reagents from ELITECH. Parameters determined were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (Y-GT), Total protein (TP), albumin (ALB), globulin (Glo), total bilirubin (T-bil), direct bilirubin (D-bil), and indirect bilirubin (I-Bil).

Percentage Protection

Percentage protection was calculated based on the principal indicators of liver protection; AST, ALT, GGT and TBil using the formula below:

$$\text{Percentage Protection} = \frac{\text{Values of Toxin Control} - \text{Values of Test sample}}{\textit{Values of Toxin Control} - \textit{Values of Normal control}} \times 100\%$$

Antioxidant and Oxidative stress biomarkers

After homogenizing the liver (1.0 g) in 10 mL phosphate buffer (0.1 M, pH 7.4) and centrifuging at 10,000 rpm for 20 minutes at 4°C, the post-mitochondrial fraction was extracted (PMF). The supernatant was collected and utilized for antioxidant assays such as reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), and myeloperoxidase (MPO) using methods described by Beutler *et al.* [29], Jollow *et al.* [30] and Oyagbemi *et al.* [31], with modifications as described by Genfi *et al.* [28].

Statistical Analysis

The results were assessed by Two Way ANOVA followed by Tukey's multiple comparisons test and analysed using GraphPad Prism 8.0 (GraphPad Software Inc., La Jolla, CA, USA). Data were presented as mean ± SEM at a 5% significance level.

Results

Preliminary Phytochemical screening

As shown in Table 2, preliminary phytochemical screening of Raw powder, HSE, and ASE revealed the presence of major phytochemical groups.

Table 2: Phytochemical constituents of *T. tetraptera* stem bark extracts.

Phytochemical	HSE	ASE	RAW
Saponins	+	+	+
Coumarins	+	+	+
Reducing Sugars	+	+	+
Tannins	+	+	+
Alkaloids	+	-	+
Terpenoids	+	-	+
Sterols	-	-	+
Flavonoids	+	+	+
Key: present (+);		Absent (-)	

The total phenolic, tannin and flavonoid contents of the ASE and HSE are shown in Figure 1. HSE had the highest amounts of total phenols, flavonoids, and tannins. In addition, the radical scavenging activity levels obtained for HSE, ASE and gallic acid standard were 87.98%, 19.23% and 94.15% respectively.

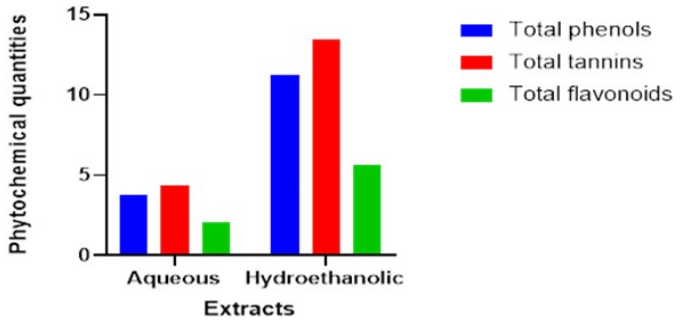

Figure 1: The total phenolic, tannin and flavonoid contents of the stem bark extracts of *T. tetraptera.*

Heavy Metal Content

Table 3 shows the heavy metals content including copper (Cu), zinc (Zn), nickel (Ni), lead (Pb) and iron (Fe) in raw stem samples and extracts.

Table 3: Heavy Metal Content of Stem Bark and Extracts of T. Tetraptera.

Samples	Concentration (mg/L)				
	Cu	Fe	Ni	Pb	Zn
RAW	0.25 ± 0.02	1.87 ± 0.02	B D L	1.33 ± 0.02	0.58 ± 0.02
ASE	0.25 ± 0.03	0.78 ± 0.01	B D L	2.63 ± 0.04	0.40 ± 0.02
HSE	0.55 ± 0.05	8.98 ± 0.12	B D L	2.68 ± 0.04	0.49 ± 0.02

Note: BDL means below the detection limit of 0.00001 mg/L

Toxicity Profile Studies

No death was reported in the acute toxicity investigations within the first 4 hours of continuous monitoring and after the first 24 hours for both extracts. No fatal side effects were found. Hair, skin, eyes, and nose morphological features appeared normal. Drooling, diarrhoea, lethargy, or any other odd behaviour were absent. According to an estimate of the LD_{50} of 5000 mg/kg, it is safe.

Effects of Treatments on the Relative Liver Weights

There were significant increases in the relative organ weights among rats treated with CCl_4 in both HSE and ASE models when compared with the normal group. However, treatment with Para only recorded no significant changes as shown in Figure 2.

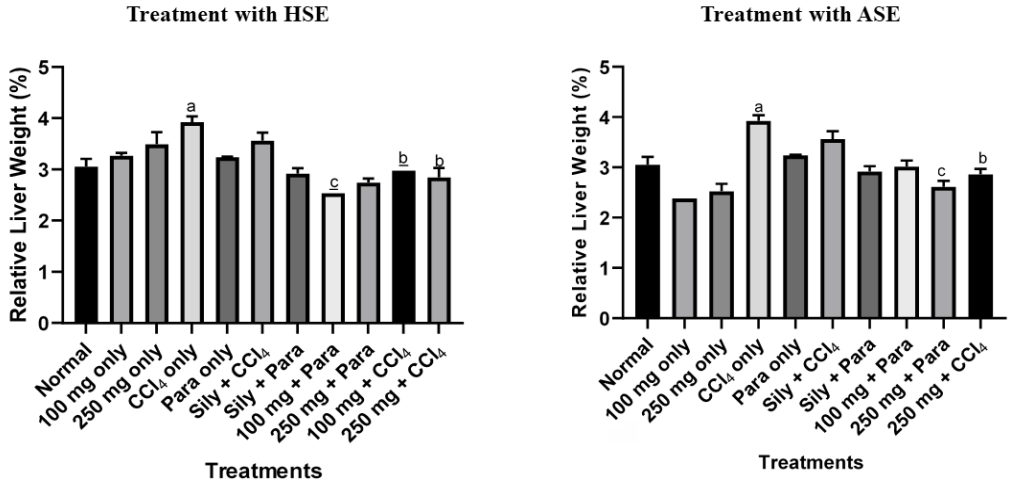

Figure 2: Effects of treatments on Relative Liver Weights. Values are presented as mean ± SEM, n = 3; a = significant difference from normal, b = significant difference between CCl_4 only group, c = significant difference from Para only group; $p<0.05 – 0.0001$.

Effect of treatment on haematological parameters

Tables 4 and 5 show treatment effects of CCl_4, Para, HSE and ASE on haematological parameters.

Table 4: Effect of Stem Bark Hydroethanolic Extract on Haematological Profile.

Parameters	Normal	100 mg only	250 mg only	CCl_4 only	Para only	Sily + CCl_4	Sily + Para	100 mg + Para	250 mg + Para	100 mg + CCl_4	250 mg+ CCl_4
WBC ($x10^3$/ uL)	10.67±0.94	13.13±1.64	11.33±2.42	6.93±0.58	10.50±0.35	8.90±1.96	9.03±0.07	9.47±1.63	11.23±0.94	11.33±0.30	111.57±1.71
RBC($x10^6$/ uL)	6.63±0.75	8.49±0.12	7.59±0.30	9.35±0.37	8.81±0.38	8.85±0.89	7.52±0.20	7.46±0.09	7.58±0.06	7.35±0.18	7.34±0.58
HGB (g/dL)	12.97±0.70	13.73±0.26	12.57±0.38	16.60±0.51	15.20±0.56	16.23±1.21	14.37±0.33	13.77±0.18	14.03±0.27	13.47±0.18	13.73±0.82
HCT (%)	43.10±5.80	48.23±1.13	47.87±2.25	57.80±1.40	53.03±1.87	56.00±4.83	47.67±0.46	47.43±0.67	48.00±1.01	48.80±1.18	48.00±2.69
MCV (fL)	64.73±2.09	56.83±1.05	63.10±1.91	61.93±1.13	60.23±0.49	63.47±1.77	63.47±1.39	63.77±1.43	63.33±1.16	66.73±0.03	64.63±1.52
MCH (pg)	19.87±1.37	16.20±0.15	16.60±0.55	17.77±0.37	17.27±0.13	18.40±0.46	19.10±0.17	18.47±0.24	18.53±0.29	18.47±0.20	18.33±0.35
MCHC (g/ dL)	30.83±2.70	28.47±0.32	26.30±0.50	28.67±0.24	28.63±0.09	29.03±0.71	30.13±0.50	28.97±0.33	29.23±0.07	27.70±0.32	28.57±0.09
PLT ($x10^6$/ uL)	961.33± 137.42	1001.67± 55.32	679.00± 184.91[a]	1846.33± 97.36[a]	834.00± 70.12	681.00± 130.02[ab]	795.00± 34.95[a]	1368.33± 40.04[c]	1197.33± 193.04[ac]	1280.00± 69.86[ab]	1462.67± 201.24[ab]
LYM (%)	69.40±7.54	81.80±6.51	79.97±6.17	52.73±2.30	78.83±3.02	68.87±3.04	79.10±3.04	73.87±1.85	65.10±3.71	67.07±1.82	68.80±5.44
NEUT (%)	30.60±7.54	18.20±6.51	20.03±6.17	47.27±2.30	21.17±3.02	31.13±3.04	20.90±3.04	26.13±1.85	34.90±3.71	32.67±1.79	30.67±5.43
LYM ($x10^3$/ uL)	7.57±1.39	10.83±1.95	9.00±2.01	3.67±0.33	8.30±0.52	6.23±1.64	7.13±0.28	6.93±0.99	7.27±0.55	7.67±0.03	7.67±0.55
NEUT($x10^3$/ uL)	3.10±0.47	2.30±0.75	2.33±0.79	3.27±0.32	2.20±0.25	2.67±0.32	1.90±0.26	2.53±0.64	3.97±0.64	3.73±0.29	5.27±0.29
RDW-SD (fL)	51.80±3.27	37.90±0.87	45.03±2.53	38.27±3.05	24.57 ±10.60	34.80 ±2.23	32.87±1.07	38.33±1.43	36.13±0.80	38.50±1.01	35.57±0.80
RDW-CV (%)	25.77±4.39	20.03±0.20	20.43±1.62	18.30±0.85	16.10±0.26	14.53±1.47	15.20±2.54	16.10±0.44	15.47±0.65	15.03±0.38	14.53±0.03
PDW (fL)	11.77±0.55	9.30±0.38	10.30±0.68	9.53±0.34	8.40±0.15	9.33±0.34	8.53±0.12	8.10±0.21	7.93±0.19	9.20±0.52	9.20±0.06
MPV (fL)	8.07±0.32	7.70±0.21	8.10±0.47	7.90±0.21	7.17±0.12	7.77±0.09	7.23±0.09	6.90±0.06	6.83±0.15	7.60±0.35	7.70±0.06
P-LCR (%)	15.07±1.41	11.37±1.27	13.40±3.19	10.97±1.17	6.33±0.41	10.07±0.79	6.90±0.50	5.90±0.21	5.57±0.72	8.90±1.97	10.00±0.29
PCT (%)	0.77±0.11	1.42±0.04	0.94±0.20	0.54±0.14	0.60±0.06	0.53±0.11	0.57±0.03	0.95±0.04	0.82±0.12	0.96±0.01	1.08±0.16

a = significant difference between normal and others, b = significant difference between CCl_4 only, c = significant difference between para only, $p<0.05$ - 0.0001.

Table 5: Effect of Stem Bark Aqueous Extract on Haematological Profile.

Parameters	Normal	100 mg only	250 mg only	CCl_4 only	Para only	Sily + CCl_4	Sily + Para	100 mg + Para	250 mg + Para	250 mg + CCl_4
WBC (x10^3/uL)	10.67±0.94	10.33±2.33	8.53±1.80	6.93±0.58	10.50±0.35	8.90±1.96	9.03±0.07	9.70±0.06	11.87±0.70	10.33±0.81
RBC(x10^6/uL)	6.63±0.75	7.91±0.37	8.11±0.30	9.35±0.37	8.81±0.38	8.85±0.89	7.52±0.20	7.09±0.27	7.36±0.12	7.49±0.05
HGB (g/dL)	12.97±0.70	14.03±0.38	14.60±0.20	16.60±0.51	15.20±0.56	16.23±1.21	14.37±0.33	13.30±0.31	13.57±0.35	13.47±0.18
HCT (%)	43.10±5.80	51.57±2.17	51.27±0.95	57.80±1.40	53.03±1.87	56.00±4.83	47.67±0.46	47.03±0.20	46.57±1.26	48.43±0.67
MCV (fL)	64.73±2.09	65.23±0.94	63.33±1.92	61.93±1.13	60.23±0.49	63.47±1.77	63.47±1.39	66.50±2.32	63.27±1.35	65.80±0.06
MCH (pg)	19.87±1.37	17.77±0.38	18.07±0.61	17.77±0.37	17.27±0.13	18.40±0.46	19.10±0.17	19.10±0.29	18.43±0.38	18.10±0.06
MCHC (g/dL)	30.83±2.70	27.27±0.43	28.47±0.15	28.67±0.24	28.63±0.09	29.03±0.71	30.13±0.50	28.37±0.50	29.13±0.03	27.53±0.18
PLT (x10^6/uL)	961.33± 137.42	1052.33± 139.85	1079.00± 68.51[a]	1846.33± 97.36[a]	834.00± 70.12[a]	681.00± 130.02[ab]	795.00± 34.95[a]	1241.67± 83.91[ac]	1231.00± 63.26[ac]	1423.33± 95.05[ab]
LYM (%)	69.40±7.54	73.03±7.70	64.63±6.82	52.73±2.30	78.83±3.02	68.87±3.04	79.10±3.04	65.33±3.18	68.03±1.66	75.93±1.74
NEUT (%)	30.60±7.54	26.97±7.70	35.37±6.82	47.27±2.30	21.17±3.02	31.13±3.04	20.90±3.04	34.33±3.18	31.97±1.66	24.43±1.75
LYM (x10^3/uL)	7.57±1.39	7.20±0.75	5.30±0.49	3.67±0.33	8.30±0.52	6.23±1.64	7.13±0.28	6.30±0.31	8.07±0.64	7.87±0.43
NEUT(x10^3/uL)	3.10±0.47	3.13±1.58	3.23±1.33	3.27±0.32	2.20±0.25	2.67±0.32	1.90±0.26	3.27±0.33	3.80±0.10	2.67±0.39
RDW-SD (fL)	51.80±3.27	36.30±2.40	35.33±1.35	38.27±3.05	24.57±10.60	34.80±2.23	32.87±1.07	38.03±1.03	34.73±0.35	38.70±0.21
RDW-CV (%)	25.77±4.39	14.70±1.46	14.17±0.61	18.30±0.85	16.10±0.26	14.53±1.47	15.20±2.54	15.17±0.81	14.80±1.01	16.13±0.29
PDW (fL)	11.77±0.55	8.93±0.32	8.33±0.42	9.53±0.34	8.40±0.15	9.33±0.34	8.53±0.12	7.70±0.26	8.03±0.09	8.60±0.35
MPV (fL)	8.07±0.32	7.47±0.19	7.07±0.19	7.90±0.21	7.17±0.12	7.77±0.09	7.23±0.09	6.77±0.09	6.93±0.12	7.23±0.26
P-LCR (%)	15.07±1.41	8.40±1.15	6.67±0.89	10.97±1.17	6.33±0.41	10.07±0.79	6.90±0.50	5.03±0.41	5.97±0.66	7.47±1.22
PCT (%)	0.77±0.11	0.79±0.11	0.76±0.04	0.54±0.14	0.60±0.06	0.53±0.11	0.57±0.03	0.84±0.07	0.85±0.03	1.03±0.03

a = significant difference between normal and others, b = significant difference between CCl_4 only, c = significant difference between para only, $p<0.05$ - 0.0001.

Effect of treatment on biochemical parameters

Tables 6 and 7 show the treatment effects of CCl_4, Para, HSE and ASE on biochemical parameters. Table 6 reveals significant increases in ALT, AST, GGT and Total Bilirubin levels for the CCl_4-only and Para-only treated groups when compared with the normal. However, HSE treatment resulted in some significant decreases compared with the CCl_4-only treatment. Table 7 also shows that significant increases in ALT, AST, GGT and Total Bilirubin following CCl_4-only and Para-only treatments were restored to near-normal levels with ASE treatment

Table 6: Effect of Stem Bark Hydroethanolic Extract on Biochemical Parameters.

Parameter	Normal	100 mg only	250 mg only	CCl_4 Only	Para only	Sily + CCl_4	Sily + Para	100 mg + Para	250 mg + Para	100 mg + CCl_4	250 mg + CCl_4
ALT (U/L)	54.70±0.42	56.77±0.75	52.57±3.39	636.60± 47.73[a]	109.43± 11.15[a]	326.90± 46.89[abc]	111.53± 13.62	42.83 ±4.42	54.83 ±5.94	75.90 ±0.90[b]	68.65 ±1.05[b]
AST (U/L)	302.47± 8.26	295.87± 1.32	299.50± 6.07	976.57± 73.86[a]	383.67± 2.43[a]	723.53± 43.40[ab]	289.50± 30.77[c]	298.40± 8.16[c]	305.90± 25.85[c]	291.30± 0.80[b]	381.90± 60.50[b]
AST/ALT	5.53±0.12	5.21±0.06	5.76±0.48	1.57±0.13	3.57±0.35	2.33±0.39	2.63±0.23	7.12±0.75	5.78±1.02	3.85±0.05	5.55±0.75
ALP (U/L)	232.20± 5.20	196.30± 49.41	329.60± 37.80[a]	268.20± 15.08	286.93± 80.36	306.80± 31.48[a]	261.27± 10.11	222.13± 14.05[c]	197.77± 26.60[c]	259.70± 1.60	203.20± 50.00[c]
γ-GT (U/L)	1.23±0.07	1.20±0.35	1.13±0.12	10.20±1.48[a]	10.07±0.94[a]	2.73±0.07	2.50±0.51[c]	3.33±0.48[c]	5.10±0.12[c]	8.55±1.25[a]	3.80±0.10[b]
T-bil (µmol/L)	0.59±0.13	0.92±0.10	0.95±0.12	6.41±0.14[a]	2.40±0.31 [a]	2.80±0.11[a]	2.35±0.15 [a]	11.62 ±1.67[a]	5.87±1.28[a]	8.85±0.74[a]	8.41±0.59
D-bil (µmol/L)	0.18±0.05	0.45±0.07	0.54±0.09	1.60±0.37[a]	1.08±0.17	1.22±0.20	0.89±0.03	1.60±0.87	0.79±0.28	1.04±0.31	1.32±0.16
I-Bil (µmol/L)	0.41±0.15	0.47±0.07	0.41±0.04	4.81±0.49[a]	1.31±0.40	1.57±0.10	1.45±0.13	10.03±1.87	5.07±1.01	7.80±0.40	7.05±0.75
TP (g/L)	66.33±2.76	67.07±3.01	57.27±2.98	94.13±2.38[a]	94.47±2.05[a]	96.30±6.21[a]	104.77± 2.80[a]	67.10±2.00[c]	70.23±2.44[c]	66.90±1.00[b]	67.80±0.00[b]
ALB(g/L)	33.37±1.71	31.37±0.29	28.53±2.17	40.07±0.87	39.67±1.63	35.70±3.18	45.43±1.18	34.13±0.88	34.43±1.02	33.30±1.40	35.30±0.50
Glo(g/L)	32.97±1.27	35.70±3.27	28.73±4.49	54.07±1.54	54.80±3.40	60.60±3.76	59.33±2.02	32.97±2.73	35.80±1.53	33.60±0.40	32.50±0.50
A/G	1.00±0.06	0.90±0.10	1.07±0.23	0.73±0.03	0.73±0.07	0.60±0.06	0.77±0.03	1.06±0.12	0.96±0.02	0.99±0.05	1.09±0.03
a = significant difference between normal and others, b = significant difference between CCl4 only, c = significant difference between para only, p<0.05 -0.0001											

Table 7: Effect of Stem Bark Aqueous Extract on Biochemical Parameters.

Parameters	Normal	100 mg only	250 mg only	CCl_4 only	Para only	Sily + CCl_4	Sily + Para	100 mg + Para	250 mg + Para	250 mg + CCl_4
ALT (U/L)	54.70±0.42	64.90±5.51	63.90±6.84	636.60± 47.73[a]	109.43± 11.15[a]	326.90± 46.89[abc]	111.53± 13.62[b]	78.70±4.10[c]	61.87±3.90[c]	70.00±5.20[b]
AST (U/L)	302.47± 8.26	284.97± 23.54	312.20± 33.28	976.57± 73.86[a]	383.67± 2.43[a]	723.53± 43.40[ab]	289.50± 30.77[c]	299.70± 37.70[c]	294.57± 32.37[c]	266.80± 0.90[b]
AST/ALT	5.53±0.12	4.40±0.46	4.89±0.14	1.57±0.13	3.57±0.35	2.33±0.39	2.63±0.23	3.80±0.30	4.73±0.25	3.83±0.27
ALP (U/L)	232.20± 5.20	289.20± 120.57	233.20± 11125.65	268.20± 15.08	286.93± 80.36	306.80± 31.48[a]	261.27± 10.11	270.60± 60.50	183.20± 11.89[c]	297.80± 20.30
γGT (U/L)	1.23±0.07	4.47±1.07	3.57±0.44	10.20±1.48[a]	10.07±0.94[a]	2.73±0.07[b]	2.50±0.51[c]	4.75±0.05[c]	4.70±0.35[c]	4.25±0.35[b]
T-bil (µmol/L)	0.59±0.13	1.53±0.38	1.64±0.90	6.41±0.14[a]	2.40±0.31[a]	2.80±0.11[a]	2.35±0.15[a]	2.29±0.40[a]	2.91±1.23[a]	2.05±0.78[a]
D-bil (µmol/L)	0.18±0.05	1.37±0.06[a]	1.01±0.16[a]	1.60±0.37[a]	1.08±0.17[a]	1.22±0.20[a]	0.89±0.03[a]	0.47±0.01[a]	0.44±0.05[a]	0.66±0.00[a]
I-Bil	0.41±0.15	0.16±0.06	0.63±0.08	4.81±0.49[a]	1.31±0.40[a]	1.57±0.10[a]	1.45±0.13[a]	1.80±0.30[a]	2.47±0.20[a]	2.47±0.80[a]
TP (g/L)	66.33±2.76	71.93±4.68	74.30±1.50	94.13±2.38[a]	94.47±2.05[a]	96.30±6.21[a]	104.77±2.80[a]	75.15±0.35	70.43±1.51	79.10±7.30
ALB(g/L)	33.37±1.71	32.33±1.23	36.03±1.19	40.07±0.87	39.67±1.63	35.70±3.18	45.43±1.18	35.00±0.30	35.37±1.42	35.65±2.55
Glo(g/L)	32.97±1.27	39.60±5.90	38.27±0.38	54.07±1.54	54.80±3.40	60.60±3.76	59.33±2.02	40.15±0.05	35.07±2.70	43.45±4.75
A/G	1.00±0.06	0.86±0.14	0.94±0.02	0.73±0.03	0.73±0.07	0.60±0.06	0.77±0.03	0.87±0.01	1.03±0.12	0.82±0.03
a = significant difference between normal and others, b = significant difference between CCl4 only, c = significant difference between para only, p<0.05 -0.0001.										

Percentage Protection of the Stem Bark Extracts

Treatments with HSE and ASE showed protection against Para and CCl_4, comparable to that of standard silymarin (Figure 3). The HSE showed the highest protection level (80.70 %) against CCl_4 group at 250 mg; the same dose was protective against Para at 44.59%. This was followed by the Sily + Para group (49.49%). For the ASE, the 250 mg treatment showed high protection against CCl4 (63.7%) and Para (58.84%).

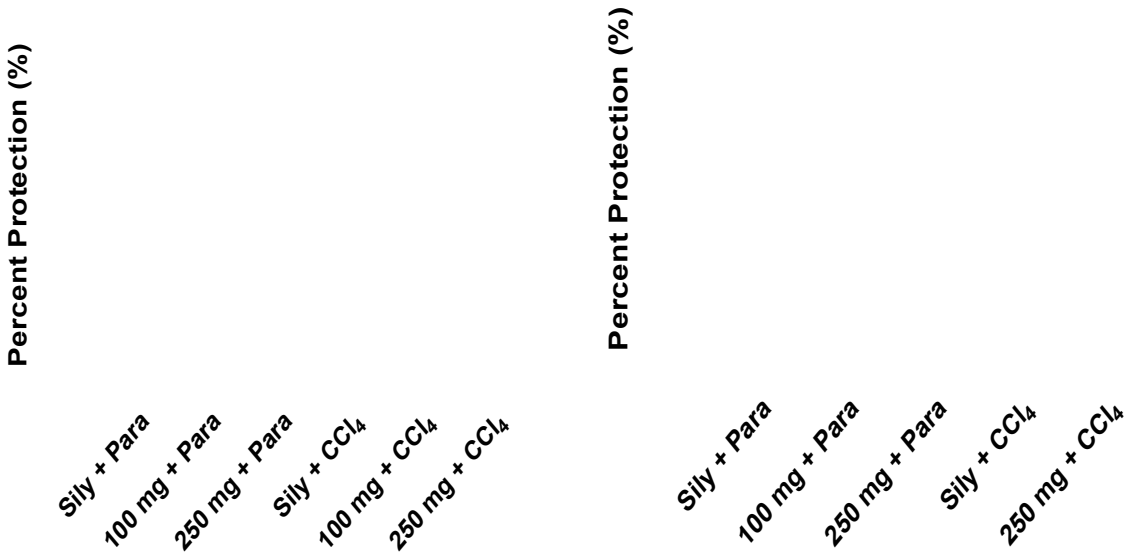

Figure 3: Percentage Protection of HSE and ASE. Values are presented as mean ± SEM, n = 3; a = significant difference from normal, b = significant difference between CCl_4 only group, c = significant difference from Para only group; $p<0.05 – 0.0001$.

Effect of treatments on Antioxidant and Oxidative stress biomarkers

Tables 8 and 9 show the effect of treatments on antioxidant and oxidative stress biomarkers.

The CCl_4-only treatment group caused significant decreases in GPX and GSH but significant increases in GST and MDA when these were compared with the normal (Tables 8 and 9). However, co-treatments of CCl_4 with HSE and ASE caused significant increases in GPX and GSH to near-normal levels while causing decreases in MDA, also to near-normal levels. Para-only treatment also resulted in significant decreases in GPX and GSH levels above Normal but was significantly increased to near-normalcy by co-treatments with HSE and ASE.

Table 8: Effect of Stem Bark Hydroethanolic Extract on Oxidative stress parameters.

	Normal	100 mg only	250 mg only	CCl_4 only	Para only	Sily + CCl_4	Sily + Para	100 mg + Para	250 mg + Para	100 mg + CCl_4	250 mg + CCl_4
GPX	58.36 ±4.16	57.87 ±1.94	59.96 ±2.79	41.30 ±1.49[a]	41.80 ±2.61[a]	40.60 ±0.90[a]	41.94 ±2.38[a]	55.77 ±2.97[c]	50.13 ±1.72[c]	52.40 ±1.91[b]	55.76 ±2.22[b]
GSH	172.94 ±12.68	120.64 ±7.07[a]	186.33 ±17.49	133.31 ±15.84[a]	141.15 ±7.93[a]	154.12 ±11.93[a]	184.83 ±18.01	127.12 ±17.99[a]	195.32 ±9.91[c]	197.58 ±3.72[c]	152.86 ±20.42[b]
GST	0.07±0.02	0.07±0.01	0.13±0.03	0.26±0.03[a]	0.09±0.03	0.18±0.03	0.18±0.04	0.13±0.04	0.06±0.02	0.19±0.06	0.00±0.00
SOD	7.37±0.50	5.54±0.39	7.73±0.65	6.16±0.60	6.29±0.49	7.08±0.56	8.79±0.86	7.57±0.28	9.55±0.57[ac]	10.42±0.49[b]	8.31±0.88
H_2O_2	29.98 ±1.43	60.43 ±10.26[a]	57.83 ±7.11[a]	40.40 ±4.53	36.27 ±4.96	34.36 ±1.37	47.38 ±2.74c	25.68 ±2.63	24.95 ±1.70	23.58±0.70	27.12 ±2.18
MDA	1.45±0.36	0.93±0.06	1.31±0.19	2.57±1.14	1.91±0.72	0.68±0.06	1.30±0.13	1.88±0.74	1.06±0.21	0.52±0.03	0.61±0.03

a = significant difference between normal and others, b = significant difference between CCl_4 only, c = significant difference between Para only, $p<0.05$ -0.0001.

Table 9: Effect of Stem Bark Aqueous Extract on Oxidative stress parameters.

	Normal	100 mg only	250 mg only	CCl_4 only	Para only	Sily + CCl_4	Sily + Para	100 mg + Para	250 mg + Para	250 mg + CCl_4
GPX	58.36 ±4.16	59.46 ±2.40	60.45±1.77	41.30 ±1.49[a]	41.80 ±2.61[a]	40.60 ±0.90[a]	41.94 ±2.38[a]	52.24 ±1.51[c]	60.49 ±2.58[c]	63.47 ±5.66[b]
GSH	172.94 ±12.68	127.81± 15.41[a]	128.14 ±11.96[a]	133.31 ±15.84[a]	141.15 ±7.93[a]	154.12 ±11.93	184.83 ±18.01[c]	162.84 ±10.83	130.64 ±16.14[a]	137.29 ±16.88[a]
GST	0.07±0.02	0.07±0.02	0.11±0.03	0.26±0.03[a]	0.09±0.03	0.18±0.03	0.18±0.04	0.10±0.01	0.09±0.01	0.11±0.01
SOD	7.37±0.50	7.11±0.62	6.96 ±0.44	6.16±0.60	6.29±0.49	7.08±0.56	8.79±0.86	8.80±0.70	6.38±0.82	7.63±0.41
H_2O_2	29.98± 1.43	34.77 ±4.90	32.93 ±1.74	40.40 ±4.53	36.27 ±4.96	34.36 ±1.37	47.38± 2.74[a]	28.31 ±3.10	34.39 ±1.73	32.95 ±3.28
MDA	1.45±0.36	0.60±0.07	0.56±0.04	1.57±0.30	1.91±0.72	0.68±0.06	1.30±0.13	0.57±0.04	0.63±0.06	0.59±0.05

a = significant difference between normal and others, b = significant difference between CCl_4 only, c = significant difference between para only, $p<0.05$ -0.000.

Discussion

Drugs like paracetamol and carbon tetrachloride induce varied degrees of injury to the liver, which is the primary organ for xenobiotic processing. To protect the liver against these injuries, medicinal plants, and other materials with hepatoprotective properties are utilized. The hepatoprotective effect of aqueous (ASE) and hydroethanolic (HSE) stem bark extracts of *Tetrapleura tetraptera*, a medicinal plant, was tested in rats against liver injury induced by paracetamol and carbon tetrachloride.

Preliminary phytochemical analysis showed the presence of saponins, coumarins, reducing sugars, tannins, and flavonoids in both ASE and HSE. In addition, the HSE had terpenoids and alkaloids. These findings are consistent with the results of Larbie *et al.,* [10], concerning their aqueous and hydroethanolic stem bark extracts of *T. tetraptera*. Saponins, coumarins, tannins, terpenoids and flavonoids are strong antioxidants and account for the observed levels of DPPH radical scavenging activity, percentage protection and antioxidant activity of HSE and ASE in this present study. These are confirmed by Reiter [32], who reported that antioxidants scavenge free radicals and help to protect cells and tissues from damage and diseases. These phytochemical antioxidants found in HSE and ASE may also contribute to their levels of hepatoprotection against Para and CCl_4. This is by the suggestion by Gupta *et al.,* [33] that extracts with antioxidants may have hepatoprotection through the elimination of deleterious effects of toxic metabolites from paracetamol or CCl_4.

Furthermore, quantitative analyses revealed the HSE to have higher phenol, tannins, and flavonoids than the ASE. These findings indicate that 50% hydroethanol, being an organic solvent, is better at extracting these phytochemicals which are also organic. This may account for HSE having a greater hepatoprotection in albino rats in this study than ASE. It is therefore not surprising the HSE had a higher DPPH scavenging activity than the ASE. This is confirmed by Koma *et al.* [34] who stated that phenols donate hydrogen ions to react with reactive oxygen and nitrogen species. Phenols, tannins and flavonoids are powerful antioxidants and according to Parr and Bolwell [35] and Cos *et al*., [36], they can chelate metal ions involved in the production of free radicals and also inhibit some enzymes involved in the generation of radicals. The HSE's DPPH radical inhibition level (87.98%) is comparable to the level (28.74% to 85.26%) obtained by Famobuwa *et al.* [37] in a similar DPPH scavenging activity by the ethanolic stem bark extract of *T. tetraptera*, where they concluded that the extract's efficacy in some of their bioactivities could be attributed to its favourable antioxidant potential.

The quantities of Zn, Cu and Fe were all found to be below the tolerable upper intake (TUIL) limits (mg/L) of 40, 10 and 45 respectively for powdered samples and both extracts [38]. This suggests that both extracts and powdered samples are safe for consumption. Copper is a vital trace element that prevents damage to cells due to its antioxidant action and as a component of many enzymes; it helps in the production of energy from carbohydrates, protein, and fat [39]. It is essential for iron utilization, and melanin production Ullah *et al*., [40] as well as for the formation of bone, connective tissues, and red blood cells [41]. Zinc, a very useful medicinal trace element for the human body, offers a natural protective mechanism against viruses that cause respiratory tract infections [42]. It is extensively used in warfare against HIV and it is believed to delay the integration of HIV in the blood [43]. These may confirm the belief that the plant cures colds, HIV, and other viral infections. Zinc is also described to play another vital role in the development and functioning of the pituitary gland, the gonads and the reproductive organs [44]. This may confirm the use of stem bark extracts in wound healing and as an aphrodisiac. Zinc is involved in the function of enzymes, improves immune function, helps blood clotting, maintains a sense of taste and smell, keeps skin healthy, and facilitates bone formation, wound healing, brain development and normal growth and development processes [41, 45]. Iron is another important trace element needed as a component of haemoglobin and as such said to be involved in the regeneration of lost blood. Iron in haemoglobin binds to oxygen and then transports it from the lungs to all parts of the body. It forms part of many enzymes and is essential for the growth, healing, immune function and synthesis of DNA [41]. Lead, a non-essential metal, was found in both stem bark extracts at levels higher than the RDA of 0.10 mg/L [38]. High amounts of it have been related to liver and kidney damage, reduced gestation in humans, mental retardation, and decreased hearing [46]. Severe anaemia, lasting brain damage,

neurological disorders, reproductive issues, reduced intelligence, and a variety of other ailments have all been linked to high levels [47]. The high levels of lead in these extracts therefore suggest a likelihood of exposure to poisoning. Further determination of different plant material sources as well as on the fruits will be required to rule out the species as a source of lead poisoning.

The toxicity profile of a drug or extract is established through acute toxicity studies. The purpose of acute toxicity is to determine the nature and extent of the adverse reactions to a single dose or an overdose of a drug [48]. In this study, a single dose of 5000 mg/kg b.w.t of HSE and ASE of *T. tetraptera,* based on OCED guidelines [26], proved safe based on the absence of mortality, or signs of toxicity after seven days. This, according to Lorke, [49] is indicative of low toxicity. This suggests that both extracts of *T. tetraptera* have a very high margin of safety and hence tolerable.

Kharasch [50] reported that organ toxicity occurs when changes in cellular structure or function persist beyond the administration and elimination of a substance. Such cellular structural changes affect organ morphology as well as its function. This is confirmed by Piao *et al.* [51] who reported that organ weight is one of the most sensitive drug toxicity indicators and its changes often precede morphological changes. This suggests that the increases in liver weight by Para and CCl_4-only treatments are strongly indicative of hepatotoxicity. The death of all rats in the 100 mg + CCl_4 stem bark aqueous group further confirmed the strength of CCl_4-induced hepatotoxicity. However, there were significant decreases in liver weight with extract co-treatment in both CCl_4 and Para-treated animals, a clear indication of hepatoprotection by hydroethanolic and aqueous stem bark extracts of *T. tetraptera* based on liver weight.

Observations in haematological assessment of normal and treated animals showed only significant differences in PLT levels for some treatment groups. Platelets control blood loss through blood clotting following vessel damage; they are also crucial to wound healing. A high number of platelets in the blood is known as thrombocytosis and may result in thrombosis while a low level is thrombocytopenia. Thus, the significant increases in PLT levels for CCl_4 only, and other treated groups when compared with normal suggests a possible thrombocytosis. Thrombocytosis is caused by chronic inflammatory diseases, haemorrhage, iron deficiency anaemia, etc. [52]. It can be inferred from the above that the hepatotoxic drug (i.e., CCl_4 treatment group) induced inflammation responsible for the significantly high platelet levels. The observed decreases in PLT levels following silymarin, HSE and ASE co-treatments suggest these extracts had antioxidant activities against CCl_4. Thrombocytopaenia represents significantly reduced platelet count which may be caused by issues in platelet making or faster destruction of platelets than normal. According to Cheesbrough [52], these may be caused by infections, immune destruction of platelets and drugs like aspirin or deficiency of folate or vitamin B_{12}. In this study, Para caused insignificantly low PLT levels for HSE and ASE when compared to the normal. Thus, Para might have caused some level of liver injury and hence thrombocytopenia. This is confirmed by the report that Para causes thrombocytopenia which correlates with the severity of liver dysfunction and fibrosis in many studies [53-55]. The liver is a major organ that secretes thrombopoietin to stimulate platelet production so damage to the liver means decreased thrombopoietin secretion and hence a low platelet count.

WBC differential parameters namely neutrophils and lymphocytes function in phagocytosis and antibody or toxin production respectively to protect an animal from diseases. Their increases and decreases in the blood are usually an indication of a bacterial infection or tissue or organ damage Cheesbrough [52] caused by drugs among others. In the current study, all extract-treated groups non-significant changes in total WBC and its differential parameters including neutrophils and lymphocytes when compared with the normal group, implying that the treatment groups including the extract were safe.

The degree of liver damage induced by Para and CCl_4 intoxication shows parallelism with the serum level of liver biomarkers. In this study, both CCl_4 only and Para only caused significant increases in ALT, AST, GGT and Total Bil levels when compared with the normal group. ALT is a more specific and sensitive liver biomarker than AST [56]. The current study revealed that CCl_4 and Para-only treated groups experienced liver necrosis with significantly high AST levels indicative of excessive levels of liver toxicity though other organs like the heart, muscles and kidneys also produce AST and hence it is not specific to the liver alone [56]. Thus, AST alone cannot confirm liver damage. Though GGT has a low concentration in the liver as compared to other organs Ozer *et al.,* [57], it is described to be a more specific biomarker of cholestasis and other biliary defects [58]. Thus, the significantly high level of GGT in CCl_4 and Para-only treated groups suggests the incidence of cholestasis and other biliary defects caused by the CCl_4 and Para. Increased levels of bilirubin could be due to decreased hepatic clearance and ultimately, cholestasis. This goes on to show that the Para and CCl_4 caused cholestasis and demonstrate the extent of liver damage being associated with Para and CCl_4 treatment.

In this current study, significant decreases in ALT levels were observed following HSE and ASE treatments when compared with CCl_4 only proved an indication of hepatoprotection. These near-normal levels in ALT given by HSE and ASE treatment groups mean these extracts were restoring liver function to normal levels. The percentage protection studies confirm the above deductions for the hepatoprotective levels of HSE and ASE.

HSE and ASE had greater levels of protection than Silymarin. HSE had the highest protection level of 80.70% at 250 mg/kg b.wt as against the CCl_4 group compared with the ASE protection level of 63.7% against the CCl_4 group at 250 mg/kg b.wt. For Para, the HSE level of protection was 44.59% at 250 mg/kg b.wt. while ASE had 58.84% at 250 mg/kg b.wt. Sily only had a protection level of 49.49%.

The mechanism of defence of the liver against free radicals requires GPX, GSH, GST, and SOD. GPX is a selenium cytosolic enzyme that catalyzes the reduction of hydrogen peroxide to water and oxygen and peroxide radicals to alcohol and oxygen [59,60]. GSH is a substrate for glutathione-related enzymes like GST and GPX where it acts to remove free radicals. The hepatotoxic drugs, Para and CCl_4, produced harmful intermediate metabolites to consume GSH. The significant decreases in GPX and GSH levels for CCl_4 and Para-only groups suggest liver damage caused by these two hepatotoxic drugs. However, co-treatment with HSE and ASE treatment gave near to normal but insignificant values of GPX and GSH indicating their ability to reduce oxidative stress caused by the toxicants.

Acetaminophen (paracetamol) has been reported to be safe and non-hepatotoxic in low doses since its metabolism produces a minor amount of the harmful metabolite N-acetyl-p-benzoquinoneimine (NAPQI) which is electrophilic and binds to the sulfhydryl group of reduced glutathione (GSH) to form APAP-GSH, and ultimately excreted in the urine as cysteine and mercapturic acid conjugates (APAP-cys) [61]. However, the continuous administration of a dose of 500 mg/kg b.wt Para for 7 days might have resulted in the production of excess NAPQI. This inflammation caused by excess NAPQI may be responsible for the high levels of PLT in some Para-treated groups. The inflammation of the liver coupled with its damage caused an increase in ALT, AST, GGT, ALP, Tbil and TP in blood circulation. Thus, significantly high levels of ALT, AST, GGT, T-bil and TP are due to inflammation by NAPQI. According to Liu *et al.* [62], the development of therapeutic agents to shorten the injury phase for inflammatory targets by HMGB1 is a potential clinical strategy to improve liver injury caused by Para. Considering this, it may be said that the phytochemicals such as flavonoids, tannins, phenols, and saponins and heavy metals like Cu and Zn in ASE and HSE increased the antioxidants in rats to prevent oxidative stress and inflammation caused by NAPQI. It has been reported that antioxidant and anti-inflammatory properties are responsible for hepatoprotection [63,64]. Thus, ASE and HSE of *T. tetraptera* have a hepatoprotective activity where their phytochemical antioxidants act through antiinflammation by mopping up NAPQI.

Carbon tetrachloride toxicity has been described to be dependent on dosage and duration of exposure. According to Knockaert *et al.* [65], covalent binding and lipid peroxidation result in hepatocyte swelling and may account for the significant increase in the relative liver weight of rats administered CCl_4 only in this study. It is also reported that some products of lipid peroxidation such as 4-HNE may induce inflammations by activating NF-κB pathways which may lead to cell apoptosis [66]. These inflammations are responsible for the significantly high PLT level for CCl_4 only and confirmed by Cheesbrough [52], who stated that inflammations cause high PLT levels. Furthermore, high levels of biochemical parameters are associated with cell damage. Thus, for CCl_4, the cell damage is caused by covalent binding and lipid peroxidation which resulted in the significantly increased levels of ALT, AST, GGT, TP and T-Bil in this present study. ASE and HSE acted against CCl_4-induced liver damage by mediation through its antioxidant phytochemicals. The radicals and the products of lipid peroxidation consume antioxidants to result in oxidative stress. In this study, the significantly low levels of GSH, and GPX for the CCl_4-only group are due to the excessive building of oxidants in liver cells. Oxidative stress resulted in the depletion of GSH required for the proper functioning of GPX and GST. As realized from the above discussion, the covalent binding of carbon tetrachloride radical plays the most significant role in liver damage by lipid peroxidation. This suggests that the prevention of carbon tetrachloride radical production or its covalent binding will prevent liver damage. It is therefore suggested that extracts of *T. tetraptera* moped up the radicals generated, modulated the synthesis of GSH for effective function of GPX, inhibited related inflammation and further improved liver function.

In conclusion, this study showed that the aqueous and hydroethanolic stem bark extracts of *Tetrapleura tetraptera* have hepatoprotective effects against paracetamol and carbon tetrachloride (CCl_4) induced hepatotoxicities in rats through their antioxidant phytochemicals and metals mopping up or inhibiting free radicals' production by Para and CCl_4 respectively, modulating the synthesis of reduced glutathione and function of glutathione peroxidase and reducing inflammation.

References

1. Delgado-Montemayor C, Cordero-Pérez P, Salazar-Aranda R, Waksman-Minsky N (2015) Models of hepatoprotective activity assessment. Medicina Universitaria 17:222-228.
2. Hodgson E, Levi PE (2004) Hepatotoxicity: In A Textbook of Modern Toxicology. (Third Edition, p. 269). Essay, Hoboken, New Jersey: John Wiley & Sons, Inc.
3. Méndez-Sánchez N, Villa AR, Chávez-Tapia NC, Ponciano-Rodriguez G, Almeda-Valdés P, et al., (2005) Trends in liver disease prevalence in Mexico from 2005 to 2050 through mortality data. Annals of Hepatology 4:52-55.
4. Agyei-Nkansah A, Taylor-Robinson S (2021) The state of Ghanaian liver medicine. Pan African Medical Journal 39:148.
5. Arhoghro EM, Ekpo KE, Anosike EO, Ibeh GO (2009) Effect of

aqueous extract of bitter leaf (*Vernonia amygdalina* Del) on carbon tetrachloride (CCl_4) induced liver damage in albino Wistar rats. Eur J Sci Res. 26:122-130.

6. da Costa Lopes L, Albano F, Augusto Travassos Laranja G, Marques Alves L, Fernando Martins e Silva L, et al., (2000) Toxicological evaluation by in vitro and in vivo assays of an aqueous extract prepared from *Echinodorus macrophyllus* leaves. Toxicology Letters 116:189-198.

7. Mukherjee A, Kandhare AD, Bodhankar SL (2016) Effect of Chrysin on Gentamicin-induced Nephrotoxicity in Laboratory Animals. Pharmacologia 7:296-307.

8. Fatima T, Bashir O, Naseer B, Hussain ZS (2018) Dandelion: Phytochemistry and clinical potential. Journal of Medicinal Plants Studies 6:198-202.

9. Enema OJ, Umoh UF, Thomas PS, Adesina SK, Eseyin OA (2019) Phytochemical and Antioxidant Studies of Leaf of *Tetrapleura tetraptera* (Schum and Thon) Taubert (Mimosaceae). CERN European Organization for Nuclear Research - Zenodo.

10. Larbie C, Robertson FCM, Quaicoe EB, Opoku R, Kabiri NC, et al., (2020) *Tetrapleura tetraptera* of Ghanaian Origin: Phytochemistry, Antioxidant and Antimicrobial Activity of Extracts of Plant Parts. Journal of Pharmaceutical Research International 32:78-96.

11. Kemigisha E, Owusu E, Elusiyan CA, Omujal F, Tweheyo M, et al., (2018) *Tetrapleura tetraptera* in Ghana, Nigeria and Uganda: households use and local market. Forests, Trees and Livelihoods 27: 243-256.

12. Tsala D, Habtemariam S, Simplice F, Ndzana M, Jital A, et al., (2014) Topically Applied Tetrapleura tetraptera Stem-Bark Extract Promotes Healing of Excision and Incision Wounds in Rats. J Intercult Ethnopharmacol 3:63-67.

13. Okwu DE (2004) The potentials of *Ocimum gratissimum*, *Penrgularia extensa* and *Tetrapleura tetraptera* as a spice and flavouring agents. Nigeria Agricultural Journal 34:143-148.

14. Ojewole JA, Adewunmi CO (2004) Anti-inflammatory and hypoglycaemic effects of *Tetrapleura tetraptera* (Taub) [fabaceae] fruit aqueous extract in rats. Journal of Ethnopharmacology 95:177-182.

15. Chibunna Igwe M, Ifeanyi Obeagu E (2018) Determination of the Effect of Methanol Extract of *Tetrapleura tetraptera* Fruit Osmotic Fragility of Erythrocytes, Platelet Aggregation and Phospholipase A2 Activity. Annals of Clinical and Laboratory Research 06:250.

16. Maillard M, Adewunmi C, Hostettmann K (1992) A triterpene glycoside from the fruits of *Tetrapleura tetraptera*. Phytochemistry 31:1321-1323.

17. Akin-Idowu PE, Ibitoye DO, Ademoyegun OT, Adeniyi OT (2011) Chemical Composition of the Dry Fruit of Tetrapleura tetraptera and its Potential Impact on Human Health. Journal of Herbs, Spices & Medicinal Plants, 17:52-61.

18. Onda EE, Sonibare MA, Ajayi AM, Umukoro S (2017) Antiinflammatory and antioxidant effects of *Tetrapleura tetraptera* (Schumach & Thonn.) Taub. Fruit extract in Carrageenan/Kaolin-induced acute monoarthritis in rats. Nigerian Journal of Pharmaceutical Research 13:157-166.

19. Badu M, John Mensah, Nathaniel Owusu Boadi (2012) Antioxidant Activity of Methanol and Ethanol/Water Extracts of *Tetrapleura tetraptera* and *Parkia Biglobosa*. International Journal of Pharma and Bio Sciences 3:312-321.

20. Evans WC (2009, June 10). Trease and Evans' Pharmacognosy (16th ed.). Saunders Ltd.

21. Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology 299:152-178.

22. Tambe VD, Bhambar RS (2014) Estimation of total phenol, tannin, alkaloid and flavonoid in Hibiscus tiliaceus Linn wood extracts. Journal of Pharmacognosy and Phytochemistry 2:41-47.

23. Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry 64:555-559.

24. Oliveira I, Sousa A, Ferreira IC, Bento A, Estevinho L, et al., (2008) Total phenols, antioxidant potential and antimicrobial activity of walnut (Juglans regia L.) green husks. Food and Chemical Toxicology 46:2326-2331.

25. Donkor S, Larbie C, KomLaga G, Emikpe BO (2019) Phytochemical, antimicrobial and antioxidant profiles of Duranta erecta L. parts. Biochemistry Research International 2019:8731595.

26. Organisation for Economic Co-operation and Development (2008) OECD Acute oral toxicity: up and down procedure. Guideline for the Testing of Chemicals, 425 (2008), pp. 1-2, OECD Publishing, Paris, France\.

27. Arthur FKN, Woode E, Terlabi EO, Larbie C (2011) Evaluation of acute and subchronic toxicity of Annona muricata (Linn.) aqueous extract in animals. European Journal of Experimental Biology 1:115-124.

28. Genfi AKA, Larbie C, Emikpe BO, Oyagbemi AA, Firempong CK, et al., (2020) Modulation of Oxidative Stress and Inflammatory Cytokines as Therapeutic Mechanisms of *Ocimum americanum* L Extract in Carbon Tetrachloride and Acetaminophen-Induced Toxicity in Rats. Journal of Evidence-Based Integrative Medicine 25:2515690X2093800.

29. Beutler E, Duron O, Kelly BM (1963) An improved method for the determination of blood glutathione. J Lab Clin Med 61:882-888.

30. Jollow DJ, Mitchell JR, Zampaglione N, Gillette JR (1974) Bromobenzene-induced liver necrosis, the protective role of glutathione, and evidence for 3,4-bromobentene oxide as the hepatotoxic metabolite. Pharmacology 11:151-169.

31. Oyagbemi AA, Omobowale TO, Asenuga ER, Adejumobi AO, Ajibade TO, et al., (2016) Sodium fluoride induces hypertension and cardiac complications through the generation of reactive oxygen species and activation of nuclear factor-kappa beta. Environ Toxicol 32:1089-1101.

32. Reiter RJ (2003) Melatonin: clinical relevance. Best Pract Res Clin Endocrinol Metab 17:273-285.

33. Gupta M, Mazumder UK, Kumar ST, Gomathi P, Kumar SR (2004) Antioxidant and Hepatoprotective Effects of Bauhinia racemosa against paracetamol and carbon tetrachloride-induced liver damage in rats. Iranian Journal of Pharmacological Therapeutics 3:12-20.

34. Koma O, Olawumi O, Godwin EU, Theophilus O (2016) Phytochemical Screening, In-vitro Antimicrobial Activity and Antioxidant Characteristics of Tetrapleura tetraptera Extracts. European Journal of Medicinal Plants 17:1-10.

35. Parr AJ, Bolwell GP (2000) Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. Journal of the Science of Food and Agriculture 80:985-1012.

36. Cos P, Ying L, Calomme M, Hu JP, Cimanga K, et al., (1998) Structure−Activity Relationship and Classification of Flavonoids as Inhibitors of Xanthine Oxidase and Superoxide Scavengers. J Nat Prod 61:71-76.

37. Famobuwa O, Lajide L, Owolabi B, Osho I, Amuho U (2016) Antioxidant Activity of the Fruit and Stem Bark of *Tetrapleura tetraptera* Taub (Mimosaceae). British Journal of Pharmaceutical Research, 9:1-4.

38. Edori OS, Marcus AC (2017) Phytochemical screening and physiologic functions of metals in seed and peel of *Citrullus lanatus* (Watermelon). International Journal of Green and Herbal Chemistry 6:35-46.

39. Akintola OO, Bodede AI, Ogunbanjo OR (2015) Nutritional and medicinal importance of *Tetrapleura tetraptera* Fruits (Aridan). African Journal of Science and Research 6:33-38.

40. Ullah R, Khader JA, Hussain I, Talha NMA, Khan N (2012) Investigation of macro and micro-nutrients in selected medicinal plants. African Journal of Pharmacy and Pharmacology 6:1829-1832.

41. Sukhsaley Batra DM (2015) Importance of trace elements in the human body.

42. Rowland TC (2004) Zinc, ions and Sars. Zn news, Iza, 63:82-91.

43. Sadler KF (2004) Neuropsychopharmacology. Biopsychiatry 28:181190.

44. Pamplona-Roger GD (2004) Encyclopedia of Foods and Their Healing Power (Volume 1: The Science of Foods). Editorial Safeliz/Review & Herald Publishing.

45. Jabeen S, Shah MT, Khan S, Hayat MQ (2010) Determination of major and trace elements in ten important folk therapeutic plants of Haripur basin, Pakistan. Journal of Medicinal Plants Research 4:559-566.

46. Iwegbue CMA (2010) Composition and Daily Intakes of Some Trace Metals from Canned Beers in Nigeria. Journal of the Institute of Brewing 116:312-315.

47. Nkansah MA, Amoako CO (2010) Heavy metal content of some common spices available in markets in the Kumasi metropolis of Ghana. American Journal of Scientific and Industrial Research 1:158-163.

48. Klein R (1996) Toxicology and herbs: an introduction. Australian Journal of Medical Herbalism 8:100-110.

49. Lorke D (1983) A new approach to practical acute toxicity testing. Archives of Toxicology, 54:275-287.

50. Kharasch ED (2007) Volatile Anesthetics: Organ Toxicity. Complications in Anesthesia, 66-68.

51. Piao Y, Liu Y, Xie X (2013) Change Trends of Organ Weight Background Data in Sprague Dawley Rats at Different Ages. Journal of Toxicologic Pathology, 26:29-34.

52. Cheesbrough M (2006) District Laboratory Practice in Tropical Countries, Part 1. Cambridge University Press. 287, 318.

53. van der Meer AJ, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, et al., (2012) Association Between Sustained Virological Response and All-Cause Mortality Among Patients with Chronic Hepatitis C and Advanced Hepatic Fibrosis. JAMA 308:2584-2593.

54. Giannini EG, Zaman A, Kreil A, Floreani A, Dulbecco P, et al., (2006). Platelet Count/Spleen Diameter Ratio for the Noninvasive Diagnosis of Esophageal Varices: Results of a Multicenter, Prospective, Validation Study. The American Journal of Gastroenterology 101:2511-2519.

55. Lu SN, Wang JH, Liu SL, Hung CH, Chen CH, et al., (2006) Thrombocytopenia as a surrogate for cirrhosis and a marker for the identification of patients at high risk for hepatocellular carcinoma. Cancer 107:2212-2222.

56. Giannini EG, Testa R, Savarino V (2005) Liver enzyme alteration: a guide for clinicians. Canadian Medical Association Journal 172:367-379.

57. Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S (2008) The current state of serum biomarkers of hepatotoxicity. Toxicology 245:194-205.

58. Deneau MR, Mack C, Abdou R, Amin M, Amir A, et al., (2018) Gamma Glutamyltransferase Reduction Is Associated with Favorable Outcomes in Pediatric Primary Sclerosing Cholangitis. Hepatol Commun 2:1369-1378.

59. Fanucchi MV (2004) Development of Antioxidant and Xenobiotic Metabolizing Enzyme Systems. The Lung – Development, Aging and the Environment 177-185.

60. Tabet F, Touyz RM (2007) Reactive Oxygen Species, Oxidative Stress, and Vascular Biology in Hypertension. Comprehensive Hypertension, 337-347.

61. Hodgman MJ, Garrard AR (2012) A Review of Acetaminophen Poisoning. Critical Care Clinics 28:499-516.

62. Liu J, Jiang M, Jin Q, Wu YL, Cui ZY, et al., (2021) Modulation of HMGB1 Release in APAP-Induced Liver Injury: A Possible Strategy of Chikusetsusaponin V Targeting NETs Formation. Front Pharmacol 12.

63. Singh D, Arya P, Aggarwal V, Gupta R (2014) Evaluation of Antioxidant and Hepatoprotective Activities of Moringa oleifera Lam. Leaves in Carbon Tetrachloride-Intoxicated Rats. Antioxidants 3:569-591.

64. Njoku P, Akumefula M (2007) Phytochemical and Nutrient Evaluation of Spondias Mombin Leaves. Pakistan Journal of Nutrition 6:613-615.

65. Knockaert L, Berson A, Ribault C, Prost PE, Fautrel A, et al., (2011) Carbon tetrachloride-mediated lipid peroxidation induces early mitochondrial alterations in mouse liver. Laboratory Investigation 92:396-410.

66. Yin G, Wang Y, Cen X, Yang M, Liang Y, et al., (2015) Lipid Peroxidation-Mediated Inflammation Promotes Cell Apoptosis through Activation of NF-κB Pathway in Rheumatoid Arthritis Synovial Cells. Mediators of Inflammation 2015:460310.

67. Ellman M (1959) A spectrophotometric method for determination of reduced glutathione in tissues. Analyt Biochem 74:214-226.

68. Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics, 125:189-198.

69. Hodges DM, DeLong JM, Forney CF, Prange RK (1999) Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207:604-611.

70. Maroso M, Balosso S, Ravizza T, Liu J, Bianchi ME, et al., (2011)

Interleukin-1 type 1 receptor/Toll-like receptor signalling in epilepsy: the importance of IL-1beta and high-mobility group box 1. Journal of Internal Medicine 270:319-326.

71. Misra HP, Fridovich I (1972) The Role of Superoxide Anion in the Autoxidation of Epinephrine and a Simple Assay for Superoxide Dismutase. Journal of Biological Chemistry 247:3170-3175.

72. Şenoğlu M, Kurutaş EB, Altun I, Yalçın ATLI, Nacitarhan V (2009) Myeloperoxidase activities in polymorphonuclear leukocytes in lumbar disc disease. Journal of Neurological Sciences 26:292-297.

73. Sinha AK (1972) Colorimetric assay of catalase. Analytical Biochemistry 47:389-394.

74. Wise RR (1995) Chilling-enhanced photooxidation: The production, action and study of reactive oxygen species produced during chilling in the light. Photosynthesis Research 45:79-97.