

Consequence of *Tetrapleura tetraptera* Leaves on Pro-Oxidants, Hepatic Functions and Histomorphology in Monosodium Glutamate-Intoxicated Rats

Ikechukwu J. Obidike, Chinedum I. Nwankwo and P.O. Ossai

Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria

ABSTRACT

Background and Objective: Medicinal plants contain bioactive components which play important roles in the prevention and management of ailments. *Tetrapleura tetraptera* which is a known medicinal plant contains bioactive components which could play vital roles in the prevention and management of hepatic injuries. Thus, warranting this study aimed at investigating the effects of *Tetrapleura tetraptera* leaves on hepatic architecture and antioxidant activities in monosodium glutamate-intoxicated rats.

Materials and Methods: The liver enzyme activities and antioxidant potential of *Tetrapleura tetraptera* leave on normal and intoxicated rats were determined using standard protocols. The SPSS Version 22 was used for the One-way ANOVA analysis. **Results:** The results of the liver enzymes indicated a significant ($p < 0.05$) decrease across all the co-treated groups as against the monosodium glutamate groups. The co-treated groups also showed significant ($p < 0.05$) improvement in the serum antioxidant activities while also showing higher reducing power than the standard (vitamin C and garlic). The portal veins of the hepatic histopathological examination indicated normal blood flow without congestion in the normal and *Tetrapleura tetraptera* leaves ethanol extract groups while indicating full congestion of the portal vein in the monosodium glutamate group. However, the co-treated groups showed reduced congestion seemingly supporting the serum chemistry results indicating improved renal function.

Conclusion: *Tetrapleura tetraptera* leaves ethanol extract to have high antioxidant properties and reversed hepatic injury induced by monosodium glutamate.

KEYWORDS

Biochemical, hepatic, histopathology, monosodium glutamate, *Tetrapleura tetraptera*, toxicity

Copyright © 2022 Ikechukwu J. Obidike et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Tetrapleura tetraptera is a known medicinal plant and it is a very good source of bioactive components with desirable benefits as food and traditionally for the prevention and treatment of diseases¹ including jaundice, convulsion² and adult-onset type² diabetes mellitus³. Its leaves are popularly used as a seasoning in some areas in Nigeria. *Tetrapleura tetraptera* trees are generally found in the lowland forests of many Tropical African countries including Nigeria and are known to have fruit that consists of fleshy pulp and small brownish-black seeds, a characteristic fragrant and pungent aromatic odour⁴. Apart from its medicinal uses, the fruit is used as a popular seasoning spice in Southern and Eastern Nigeria³.



Most stable molecule species have electrons in their outer orbital, arranged in pairs. Each electron of this pair has an opposite spin, which is crucial for the stabilization of the molecule. Free radicals are molecules with one or more unpaired electrons in their outermost shell, which makes this species very unstable and tend to react with other molecules to pair these electrons and thereby generate more stable species⁵.

An antioxidant is a molecule which can prevent or slow the oxidation of macromolecules. The role of antioxidants is to lower or terminate these chain reactions by removing free radicals or inhibiting other oxidation reactions by being oxidized themselves. So, antioxidants are often reducing agents such as polyphenols or thiols⁶. Although oxidation reactions are vital for cells, they have damaging effects, hence, plants and animals contain various antioxidants, such as vitamins C and E and glutathione, as well as different enzymatic systems which catalyze the antioxidants reactions such as catalase, Superoxide Dismutase (SOD) and peroxidases. The defects in or inhibition of these antioxidant enzymes will lead to oxidative stress and may damage and lyse the cells⁷.

Monosodium glutamate is a sodium salt of non-essential amino acid and a known food additive but has been linked to several diseases including renal assault with associated lower haemoglobin concentration and increased white blood cell⁸ hepatocyte architectural thwart⁹ obesity¹⁰ and neurotoxicity¹¹.

Due to the known pharmacological properties of *Tetrapleura tetraptera*, it could act to replace the use of synthetic drugs in the management of hepatic diseases and prevention of complications attributed to oxidative stress since there has been mistrust in the use of synthetic drugs in the management of ailments leading to the wider call for use of natural bioactive components in the management of diseases. Hence, warranting this study aimed at determining the effects of *Tetrapleura tetraptera* leaves on hepatic architecture and antioxidant activities in monosodium glutamate-intoxicated rats.

MATERIALS AND METHODS

Study area: This study was carried out between January to May, 2019 at the laboratory unit of the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Collection of samples and preparation of extract: Leaves of *Tetrapleura tetraptera* were obtained from the forest in Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The plant sample was identified and authenticated by a Botanist in the Department of Plant Science and Biotechnology, College of Natural Science, Michael Okpara University of Agriculture, Umudike. The leaves were then thoroughly washed to remove dirt and to avoid contamination of the plant sample with clean running water and then air-dried under shade for 28 days. The dried sample was then pulverized using Thomas Laboratory Mill (Crypto model, USA). The fine powder resulting from the sample (500 g) was soaked in 2.5 L of 95% methanol and extracted using filter paper. The crude methanol extract was kept in an air-tight container and stored in a refrigerator and hereafter referred to as *Tetrapleura tetraptera* Leaves Ethanol Extract (TTLEE).

Animals: About sixty adult male albino rats weighing between 110 and 145 g were obtained from the animal house unit of the Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria. The animals were acclimatized for 12 days and thereafter, divided randomly into six treatment groups of ten rats each and then housed in an aluminium cage. All animals had access to food and water *ad libitum* and were maintained under standard laboratory conditions with light and dark cycles of 12 hrs each and room temperature. All guidelines involving the use and care of laboratory animals were duly observed.

Determination of haematological parameter

Determination of Ferric Reducing Antioxidant Power (FRAP): The ferric reducing antioxidant power assay was determined by the standard method of Amarowicz and Pegg¹².

Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay: Qualitative analysis of antioxidant capacity was done using a method based on the reduction of 2, 2-diphenyl-1-picrylhydrazyl¹³.

Determination of Aspartate Aminotransferase (AST) activity: The AST activity was determined by monitoring the concentration of hydrazine formed with 2, 4-dinitrophenylhydrazine¹⁴.

Determination of Alanine Aminotransferase (ALT): The ALT activity was determined by Huang *et al.*¹⁴. This method is based on the principle that Ketoglutarate+L-alanine in the blood react to form -L-glutamate+pyruvate which forms the colour complex and is viewed spectrophotometrically to determine serum activity.

Determination of Alkaline Phosphatase (ALP) activity: The ALP activity was determined by Kanta *et al.*¹⁵.

Determination of Superoxide Dismutase (SOD) activity: The serum SOD activity was determined by Weydert *et al.*¹⁶.

Determination of Catalase (CAT) activity: The serum catalase activity was determined using the standard procedure¹⁶.

Determination of Glutathione (GSH) concentration: The determination of glutathione concentration was done Rahman *et al.*¹⁷.

Determination of Malondialdehyde (MDA) concentration: Lipid peroxidation was measured by determining the concentration of Malondialdehyde (MDA) formed by Mateos *et al.*¹⁸.

Determination of histological parameters: The method described by Slaoui and Fiette¹⁹ was used. The excised organs were rinsed in 0.9% saline solution and preserved in 10% formaldehyde solution. It was embedded in paraffin wax and sectioned into 4-6 microns. The sections were stained with hematoxylin and eosin and photographed.

Study design for *in vivo* biochemical estimation and antioxidant activity and of *Tetrapleura tetraptera* Leaves Ethanol Extract (TTLEE): The 6 groups of experimental animals were treated according to the protocol below:

Group 1: 8000 mg kg⁻¹ b.wt., of MSG and 200 mg kg⁻¹ of TTLEE

Group 2: 8000 mg kg⁻¹ b.wt., of MSG and 400 mg kg⁻¹ of TTLEE

Group 3: 8000 mg kg⁻¹ b.wt., of MSG and 600 mg kg⁻¹ of TTLEE

Group 4: Feed and water only and served as the normal control group

Group 5: 200 mg kg⁻¹ b.wt., of TTLEE only

Group 6: 8000 mg kg⁻¹ b.wt., of Monosodium Glutamate (MSG) only and served as the negative control

At the end of 14 days of treatment, the animals were sacrificed through cervical dislocation and blood was collected by cardiac puncture into plain bottles for the study.

Induction of toxicity: The monosodium glutamate-induced toxicity was achieved using 8000 mg kg⁻¹ body weight of the monosodium glutamate via daily oral administration to the rats for 14 days²⁰.

Calculation of diagnostic ratios and change relative to groups: The diagnostic ratios thus, change relative to control and change relative to MSG groups were calculated using the equation²¹:

$$\text{Change relative to K (\%)} = \frac{(V - K)}{K} \times 100$$

Where:

K = Constant values

V = Variable values

Statistical analysis: Descriptive statistics and tests for significance in mean were carried out on the data generated by One-way Analysis of Variance (ANOVA) with the Statistical Package for Social Sciences (SPSS) Version 22. The Turkey's *post hoc* Test was used to identify the means that differ significantly at $p < 0.05$. Results were expressed as Mean \pm Standard Error of Mean (SEM).

RESULTS

The result of the AST estimation showed that the activity of the MSG group (79.62 ± 0.43) was significantly ($p < 0.05$) higher than that of the control group (60.43 ± 0.22). However, the AST activity of the co-treated groups (68.81 ± 0.33 for low dose, 63.64 ± 0.43 for medium dose, 61.42 ± 0.15 for high dose) all showed significant ($p < 0.05$) dose-dependent reduction in activity compared to that of the MSG group given in Table 1.

The result of the ALT estimation showed that the activity of the MSG group (57.81 ± 0.35) was significantly ($p < 0.05$) higher than that of the control group (34.44 ± 0.10). Meanwhile, the ALT activity of the co-treated groups (38.22 ± 0.15 for low dose, 49.43 ± 0.32 for medium dose, 41.21 ± 0.22 for high dose) all showed significant ($p < 0.05$) reduction in alanine aminotransferase activity across the tested groups compared to that of the MSG group in Table 2.

Table 1: Effects of TTLEE on AST activity of normal and MSG intoxicated rats

Groups	AST (IU L ⁻¹)	Change relative to MSG (%)	Change relative to control (%)
Low extract (MSG 8000+200 mg kg ⁻¹ b.wt., extract)	68.81 \pm 0.33	-13.57	10.86
Medium extract (MSG 8000+400 mg kg ⁻¹ b.wt., extract)	63.64 \pm 0.43	-20.07	5.31
High extract (MSG 8000+600 mg kg ⁻¹ b.wt., extract)	61.42 \pm 0.15	-22.85	1.63
Control (feed+water)	60.43 \pm 0.22	-24.10	0.00
TTLEE (200 mg kg ⁻¹ b.wt., extract)	55.81 \pm 0.16	-30.79	-7.64
MSG (8000 mg kg ⁻¹ b.wt., MSG)	79.62 \pm 0.43	0.00	31.75

Values are Mean \pm SEM for n = 4, the difference is considered statistically significant at $p < 0.05$, +: Denotes higher, -: Denotes lower, MSG: Monosodium glutamate and TTLEE: *Tetrapleura tetraptera* leaves ethanol extract

Table 2: Effects of TTLEE on ALT activity of normal and MSG intoxicated rats

Groups	ALT (IU L ⁻¹)	Change relative to MSG (%)	Change relative to control (%)
Low extract (MSG 8000+200 mg kg ⁻¹ b.wt., extract)	38.22 \pm 0.15	-33.88	10.97
Medium extract (MSG 8000+400 mg kg ⁻¹ b.wt., extract)	49.43 \pm 0.32	-14.45	43.52
High extract (MSG 8000+600 mg kg ⁻¹ b.wt., extract)	41.21 \pm 0.22	-28.71	19.66
Control (feed+water)	34.44 \pm 0.10	-40.42	0.00
TTLEE (200 mg kg ⁻¹ b.wt., extract)	35.62 \pm 0.25	-38.38	3.42
MSG (8000 mg kg ⁻¹ b.wt., MSG)	57.81 \pm 0.35	0.00	67.85

Values are Mean \pm SEM for n = 4, the difference is considered statistically significant at $p < 0.05$, +: Denotes higher, -: Denotes lower, MSG: Monosodium glutamate and TTLEE: *Tetrapleura tetraptera* leaves ethanol extract

Table 3: Effects of TTLEE on ALP activity of normal and MSG intoxicated rats

Groups	ALP (IU L ⁻¹)	Change relative to MSG (%)	Change relative to control (%)
Low extract (MSG 8000+200 mg kg ⁻¹ b.wt., extract)	15.24±0.13	-32.59	13.56
Medium extract (MSG 8000+400 mg kg ⁻¹ b.wt., extract)	14.23±0.11	-37.06	6.03
High extract (MSG 8000+600 mg kg ⁻¹ b.wt., extract)	15.43±0.39	-31.75	16.54
Control (feed+water)	13.42±0.15	-40.64	0.00
TTLEE (200 mg kg ⁻¹ b.wt., extract)	13.61±0.25	-39.80	1.41
MSG (8000 mg kg ⁻¹ b.wt., MSG)	22.61±0.38	0.00	68.47

Values are Mean±SEM for n = 4, the difference is considered statistically significant at p<0.05, +: Denotes higher, -: Denotes lower, MSG: Monosodium glutamate and TTLEE = *Tetrapleura tetraptera* leaves ethanol extract

Table 4: Effects of TTLEE on GSH concentration of normal and MSG intoxicated rats

Groups	GSH (m dL ⁻¹)	Change relative to MSG (%)	Change relative to control (%)
Low extract (MSG 8000+200 mg kg ⁻¹ b.wt., extract)	3.17±0.08	46.75	-13.38
Medium extract (MSG 8000+400 mg kg ⁻¹ b.wt., extract)	4.44±0.48	105.55	-3.89
High extract (MSG 8000+600 mg kg ⁻¹ b.wt., extract)	4.64±0.42	114.81	0.43
Control (feed+water)	4.62±0.73	113.88	0.00
TTLEE (200 mg kg ⁻¹ b.wt., extract)	6.72±0.22	211.11	45.45
MSG (8000 mg kg ⁻¹ b.wt., MSG)	2.16±0.10	0.00	-53.24

Values are Mean±SEM for n = 4, the difference is considered statistically significant at p<0.05, +: Denotes higher, -: Denotes lower, MSG: Monosodium glutamate, TTLEE: *Tetrapleura tetraptera* leaves ethanol extract and PCV: Packed cell volume

Table 5: Effects of TTLEE on SOD activity of normal and MSG intoxicated rats

Groups	SOD (U L ⁻¹)	Change relative to MSG (%)	Change relative to control (%)
Low extract (MSG 8000+200 mg kg ⁻¹ b.wt., extract)	11.38±0.03	0.61	-0.61
Medium extract (MSG 8000+400 mg kg ⁻¹ b.wt., extract)	11.48±0.02	1.50	0.28
High extract (MSG 8000+600 mg kg ⁻¹ b.wt., extract)	11.48±0.03	1.50	0.28
Control (feed+water)	11.45±0.05	1.23	0.00
TTLEE (200 mg kg ⁻¹ b.wt., extract)	11.48±0.04	1.50	0.28
MSG (8000 mg kg ⁻¹ b.wt., MSG)	11.31±0.03	0.00	-1.22

Values are Mean±SEM for n = 4, the difference is considered statistically significant at p<0.05, +: Denotes higher, -: Denotes lower, MSG: Monosodium glutamate, TTLEE: *Tetrapleura tetraptera* leaves ethanol extract and RBC: Red blood cell

The result of the ALP estimation showed that the activity of the MSG group (22.61±0.38) was significantly (p<0.05) higher than that of the control group (13.42±0.15). Meanwhile, the ALP activity of the co-treated groups (15.24±0.13 for low dose, 14.23±0.11 for medium dose, 15.43±0.39 for high dose) all showed a significant (p<0.05) reduction in alkaline phosphatase activity across the tested groups compared to that of the MSG group in Table 3.

The result of the glutathione estimation showed that the glutathione concentration of the MSG group (2.16±0.10) was significantly (p<0.05) lower than that of the control group (4.62±0.73). However, the glutathione concentration of the co-treated groups (3.17±0.08 for low dose, 4.44±0.48 for medium dose, 4.64±0.42 for high dose) all showed significant (p<0.05) dose-dependent increase in concentration across the tested groups compared to that of the MSG group in Table 4.

The result of the serum antioxidant activity showed that the SOD activity of the MSG group (11.31±0.03) was lower than that of the control group (11.45±0.05). Meanwhile, the SOD activity of the co-treated groups (11.38±0.03 for low dose, 11.48±0.02 for medium dose, 11.48±0.03 for high dose) all showed a dose-dependent increase in superoxide dismutase activity across the tested groups compared to that of the MSG group shown in Table 5.

Table 6: Effects of TTLEE on CAT activity of normal and MSG intoxicated rats

Groups	Hemoglobin (g dL ⁻¹)	Change relative to MSG (%)	Change relative to control (%)
Low extract (MSG 8000+ 200 mg kg ⁻¹ b.wt., extract)	16.66±0.13	-49.33	-43.35
Medium extract (MSG 8000+ 400 mg kg ⁻¹ b.wt., extract)	27.87±0.11	-15.23	-5.23
High extract (MSG 8000+ 600 mg kg ⁻¹ b.wt., extract)	29.10±0.15	-11.49	-1.05
Control (feed+water)	29.41±0.10	-10.55	0.00
TTLEE (200 mg kg ⁻¹ b.wt., extract)	19.81±0.14	-39.75	-32.64
MSG (8000 mg kg ⁻¹ b.wt., MSG)	32.88±0.22	0.00	11.79

Values are Mean±SEM for n = 4, the difference is considered statistically significant at p<0.05, +: Denotes higher, -: Denotes lower, MSG: Monosodium glutamate and TTLEE: *Tetrapleura tetraptera* leaves ethanol extract

Table 7: Effects of TTLEE on MDA concentration of normal and MSG intoxicated rats

Groups	MDA (mg dL ⁻¹)	Change relative to extract (%)	Change relative to MSG (%)
Low extract (MSG 8000+200 mg kg ⁻¹ b.wt., extract)	0.29±0.10	-6.25	15.36
Medium extract (MSG 8000+400 mg kg ⁻¹ b.wt., extract)	0.28±0.04	0.00	23.07
High extract (MSG 8000+600 mg kg ⁻¹ b.wt., extract)	0.27±0.01	-9.37	11.53
Control (feed+water)	0.26±0.01	-18.75	0.00
TTLEE (200 mg kg ⁻¹ b.wt., extract)	0.26±0.02	-18.75	0.00
MSG (8000 mg kg ⁻¹ b.wt., MSG)	0.32±0.01	0.00	23.07

Values are Mean±SEM for n = 4, the difference is considered statistically significant at p<0.05, +: Denotes higher, -: Denotes lower, MSG: Monosodium glutamate, TTLEE: *Tetrapleura tetraptera* leaves ethanol extract and WBC: White blood cell

The result of the serum catalase activity showed that the CAT activity of the MSG group (32.88±0.22) was higher than that of the control group (29.41±0.10). However, the CAT activity of the co-treated groups (16.66±0.13 for low dose, 27.87±0.11 for medium dose, 29.10±0.15 for high dose) all showed significantly (p<0.05) dose-dependent decrease activity across the tested groups compared to that of the MSG group and even the control group in Table 6.

The result of the lipid peroxidation showed that the MDA activity of the MSG group (0.32±0.01) was higher than that of the control group (0.26±0.01). However, the MDA activity of the co-treated groups (0.29±0.10 for low dose, 0.28±0.04 for medium dose, 0.27±0.01 for high dose) all showed a significant (p<0.05) dose-dependent decrease in concentration across the tested groups compared to that of the MSG group in Table 7.

The result of the FRAP assay revealed the presence of high antioxidant activity in the extract. The result showed that the extract of *Tetrapleura tetraptera* leaves has strong reducing power (40.47) as compared to the standard (gallic acid) (18.35) at 1000 µg mL⁻¹ while at 500 µg mL⁻¹, it was 13.41 for TTLEE as against 6.10 for gallic acid and at 125 µg mL⁻¹, 4.61 for TTLEE and 1.18 for gallic acid in Fig. 1.

The result of the DPPH showed methanolic extract of *Tetrapleura tetraptera* leaves showed a significant (p<0.05) higher percentage of inhibition as compared to ascorbic acid (vitamin C) across all concentrations except at 1000 µg mL⁻¹. The result showed that re DPPH for TTLEE was 95.82 for TTLEE and 62.61 for vitamin C at 15.63 µg mL⁻¹, 85.88 for TTLEE and 63.30 at 125 µg mL⁻¹, 67.83 for TTLEE and 64.13 for vitamin C at 250 µg mL⁻¹, 74.19 for TTLEE and 62.78 for vitamin C at 500 µg mL⁻¹ in Fig. 2.

Histopathological examinations

Plates of a photomicrograph of rat liver section (Hematoxyline and Eosin (H&E)) stained ×400: The Photomicrograph of the liver section of group A rats suggests a normal flow of blood with partial congestion of the central vein as shown in Fig. 3a.

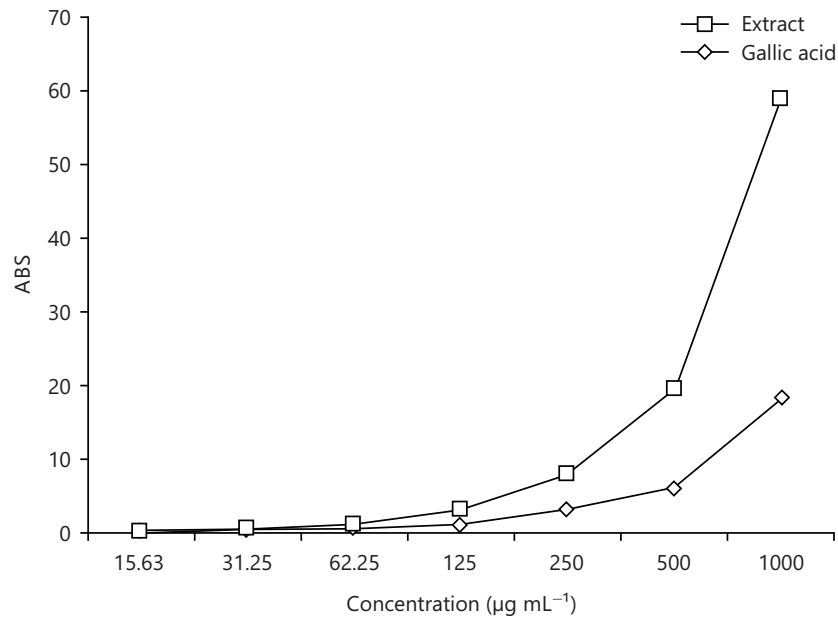


Fig. 1: Ferric reducing antioxidant power of *Tetraptera tetraptera* leaves

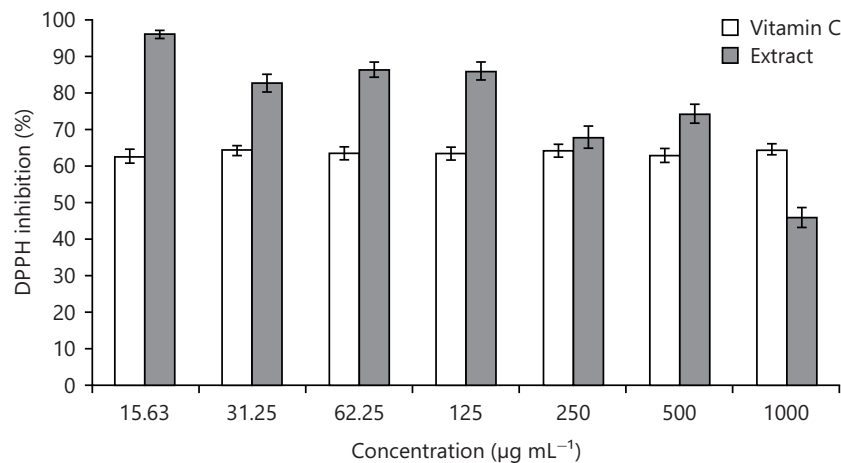


Fig. 2: DPPH activity of *Tetraptera tetraptera* leaves

The photomicrograph of the liver section of group B rats suggests a normal flow of blood with mild congestion of the central vein as shown in Fig. 3b.

The photomicrograph of the liver section of group C rats suggests a normal flow of blood with mild congestion of the central vein as shown in Fig. 3c.

The photomicrograph of the liver section of group D (control) rats showed well-preserved architecture with no histopathological lesion and a normal central vein as shown in Fig. 3d.

The photomicrograph of the liver section of group E rats suggests a normal flow of blood with no congestion of the central vein and mild steatosis as shown in Fig. 3e.

The photomicrograph of the liver section of group F rats suggests full congestion of the central vein as shown in Fig. 3f.

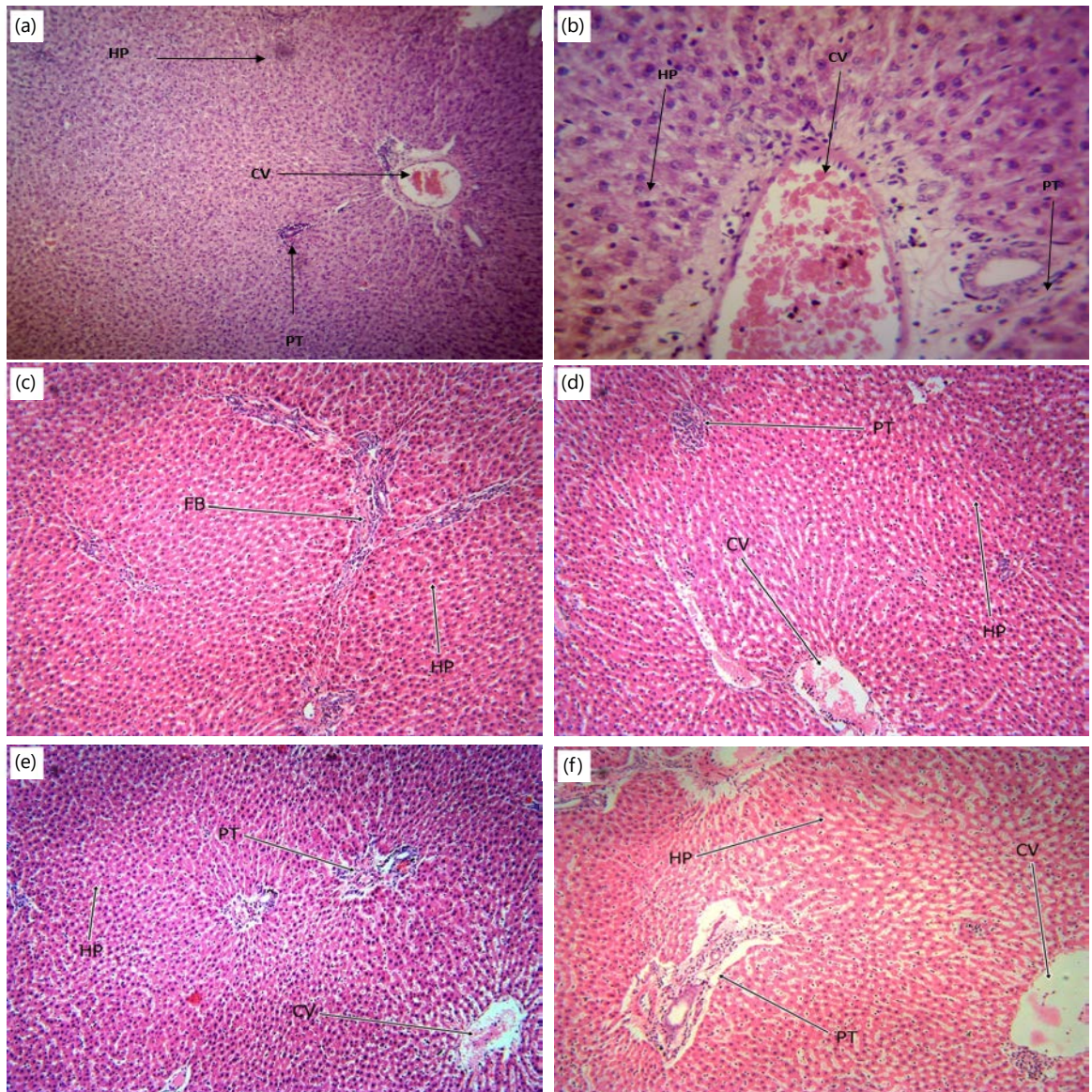


Fig. 3(a-f): Photomicrograph of the liver section of MSG intoxicated rats co-treated with, (a) Low dose TTLEE, (b) Medium dose TTLEE, (c) High dose TTLEE, (d) Normal control rats, (e) Rats treated to dose of TTLEE and (f) MSG intoxicated rats (untreated group)

PT: Portal triad, HP: Hepatocyte, CV: Central vein, MSG: monosodium glutamate, TTLEE: *Tetrapleura tetraptera* leaves ethanol extract and F: Fibrosis

DISCUSSION

Varying degrees of phytochemical constituents may confer different levels of antioxidant activity on the studied plant extracts especially, polyphenolic components which have been implicated in recent studies as antioxidants via other mechanisms to prevent disease processes²². They are capable of removing free radicals, chelating metal catalysts, activating antioxidant enzymes, reducing α -tocopherol and inhibiting oxidases²³.

The DPPH result shows significantly ($p < 0.05$) higher percentage of inhibition as compared to ascorbic acid across all concentrations except at $1000 \mu\text{g mL}^{-1}$. Antioxidant power has been reported by some investigators to be concomitant with the development of reducing power²⁴. Reductones, which have strong reducing power, are generally believed not only to react directly with peroxides but also to prevent peroxide formation by reacting with certain precursors²⁵. The result of the reducing power of

Tetrapleura tetraptera (40.47) as compared to (18.35) for gallic acid showed that *Tetrapleura tetraptera* has strong reducing power suggesting that it can act as an electron donor, reacting with free radicals and converting them to more stable products, which can terminate radical chain reaction.

Measurement of activities of various enzymes in tissue and the body fluids plays a significant role in disease investigation and diagnosis and determination of the extent of toxicity of a drug²⁶ and aid in the determination of cellular damage long before it is picked up by the conventional histological technique.

The common parameters used to indicate hepatocellular damage are AST, ALT and ALP activities²⁷. The AST is an enzyme found in the cytoplasm and mitochondria of tissues such as the heart, skeletal muscles, liver kidney and erythrocytes²⁸. An increase in AST activity when accompanied by a concomitant increase in ALT as seen in the MSG group could be indicative of liver injury thus, agrees with the older reports^{29,30}. The significant ($p < 0.05$) decrease in the extract group indicated that *Tetrapleura tetraptera* could contain hepatoprotective molecules which are further buttressed by a significant ($p < 0.05$) reduction in the co-treated groups and confirmed by the negative percentage change relative to MSG.

The ALT which is localized primarily in the cytosol of the hepatocytes is a liver-specific enzyme³¹. It is a more sensitive marker for liver damage when compared to AST and can provide a quantitative evaluation of the extent of the damage sustained by the liver³². The treatment groups showed a significant ($p < 0.05$) decrease in the ALT activity compared with the MSG group. This could suggest the ameliorative potential of the extract and hence, could be indicative of some hepatoprotective potentials of *Tetrapleura tetraptera* and agrees with the report of Obidike and Egbuonu⁹ that administration of extracts with hepatoprotective potential reduces the ALT serum activity in intoxicated animals.

Alkaline phosphatase which serves as a marker enzyme for endoplasmic reticulum and plasma is usually used to determine the integrity of the plasma membrane³³. Its increase is usually attributed to *de novo* synthesis of enzymes, loss of proteins from the tissue or congestive/obstruction of the biliary system which may occur within the liver duct³⁴. The increase in the ALP activity of the MSG group when compared with the control group could be indicative of liver labilization and agrees with the result of previous study²¹. The significant ($p < 0.05$) decrease in ALP activity of the treatment groups shows that *Tetrapleura tetraptera* could have some plasma membrane protective potential hence, ameliorating the toxicity of the MSG. Since ALP is intracellular, repair of the distorted hepatic architecture by the extract restored the membrane integrity and hence reduced the extracellular concentration of the enzyme leading to reduced serum activity.

Catalase and GSH reduced oxidative stress and lipid peroxidation either by protecting the detoxifying enzymes by increasing the efficacy of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) or by helping in the elimination of compounds which produce peroxidation in the cell membranes³⁵. The result of the glutathione concentration showed a significant decrease in GSH concentration. This could be a result of the decreased amount of NADPH as a result of oxidative stress thus, leaving a high quantity of glutathione in its oxidized state (GS-SG). However, the significant ($p < 0.05$) increased GSH in a dose-dependent manner suggests that *Tetrapleura tetraptera* possibly terminated the excessive reactive species and restored the glutathione molecules to their reduced state leading to increased serum concentration. This agrees with the result of other study³⁶ that administration of *Tetrapleura tetraptera* extract reversed oxidative stress leading to increased GSH concentration in an animal model.

The result of the catalase activity increased CAT activity in the MSG group. This increase could be a result of the decreased amount of NADPH or an increase in erythrocyte CAT activity through the increased generation of ROS that activates the enzyme translation³. The dose-dependent decrease in the CAT

activity of the co-treated groups could be indicative that *Tetrapleura tetraptera* reduced the effects of the pro-oxidant. The reason for this reduction could be that TTLEE contains high antioxidant phytochemical composition such as polyphenols and tocopherol which has been shown to boost the activity of endogenous antioxidant enzymes³⁷.

The MDA is the final product of peroxidation of membrane lipids and accumulates when the animals are subjected to oxidative stress. Therefore, the MDA level is routinely used as an index of lipid peroxidation under stress conditions. Generally, free radical generation and membrane damage would be low in tolerant animals and thereby formation of lower levels of MDA content. The MDA of the co-treated groups indicated reduced MDA concentration and this reduction could be attributed to the bioactive composition of TTLE which can reduce oxidative damage by breaking the pro-oxidative chain³⁸.

The biochemical parameters estimated in this study are liver function parameters and antioxidant parameters which are used to determine oxidative stress and liver function, respectively. The application of this study is that *Tetrapleura tetraptera* leaves ethanol extract has shown to possess antioxidant properties and hepato-protective potentials and thus could be used as a cheap alternative way of management of oxidative stress and liver dysfunction since they could possess bioactive components to that effect.

CONCLUSION

Tetrapleura tetraptera leaves ethanol extract reversed the hepatic thwairt induced by monosodium glutamate and reversed the oxidative stress induced by the same toxicant. This is complemented by the reversed abnormal architecture of the hepatocytes to a normal state and thus, it can be concluded that TTLEE has strong antioxidant potential and could reverse hepatic injuries caused by toxicants.

SIGNIFICANCE STATEMENT

This study discovers the antioxidant potential and hepato-protective potential of TTLEE which can be beneficial for oxidative stress and hepatic assault induced by monosodium glutamate in rats. This study will help the researcher to uncover oxidative stress and hepatic injuries that many researchers were not able to explore. Thus a new theory on hepato-protective potentials and antioxidant properties of *Tetrapleura tetraptera* leaves ethanol extract in monosodium glutamate intoxication could be arrived at.

REFERENCES

1. Altemimi, A., N. Lakhssassi, A. Baharlouei, D.G. Watson and D.A. Lightfoot, 2017. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, Vol. 6. 10.3390/plants6040042.
2. Udourioh, G.A. and M.F. Etokudoh, 2014. Essential oils and fatty acids composition of dry fruits of *Tetrapleura tetraptera*. *J. Appl. Sci. Environ. Manage.*, 18: 419-424.
3. Odesanmi, O.S., R.A. Lawal and S.A. Ojokuku, 2011. Effects of ethanolic extract of *Tetrapleura tetraptera* fruit on serum lipid profile and kidney function in male Dutch-white rabbits. *Niger. Q. J. Hosp. Med.*, 21: 299-302.
4. Aladesanmi, A.J., 2007. *Tetrapleura tetraptera*: Molluscicidal activity and chemical constituents. *Afr. J. Traditional Complementary Altern. Med.*, 4: 23-36.
5. Guetens, G., G. de Boeck, M. Highley, A.T. van Oosterom and E.A. de Bruijn, 2002. Oxidative DNA damage: Biological significance and methods of analysis. *Crit. Rev. Clin. Lab. Sci.*, 39: 331-457.
6. Pandey, K.B. and S.I. Rizvi, 2009. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid. Med. Cell. Longevity*, 2: 270-278.
7. Albasha, M.O. and A.E.S. Azab, 2014. Effect of cadmium on the liver and amelioration by aqueous extracts of fenugreek seeds, rosemary, and cinnamon in guinea pigs: Histological and biochemical study. *Cell Biol.*, 2: 7-17.

8. Johnlouis, O.I. and E.A. Cemaluk, 2020. Some biochemical effect of cocoa (*Theobroma cacao*) pod ethanol extract on renal histo-morphology and function in monosodium glutamate (MSG)-intoxicated rats. *Int. J. Recent Res. Appl. Stud.*, 7: 17-28.
9. Obidike, I.J. and A.C. Egbuonu, 2019. Effects of ethanol extract of cocoa (*Theobroma cacao*) pod on normal and monosodium glutamate-intoxicated rats' hepatic histo-morphology, serum bio-functional parameters and serum antioxidant activities. *Int. J. Recent Res. Appl. Stud.*, 6: 1-13.
10. Gobatto, C.A., M.A.R. de Mello, C.T. Souza and I.A. Ribeiro, 2002. The monosodium glutamate (MSG) obese rat as a model for the study of exercise in obesity. *Res. Commun. Mol. Pathol. Pharmacol.*, 111: 89-101.
11. Park, C.H., S.H. Choi, Y. Piao, S.H. Kim and Y.J. Lee *et al.*, 2000. Glutamate and aspartate impair memory retention and damage hypothalamic neurons in adult mice. *Toxicol. Lett.*, 115: 117-125.
12. Amarowicz, R. and R.B. Pegg, 2019. Natural Antioxidants of Plant Origin. In: *Advances in Food and Nutrition Research*, Ferreira, I.C.F.R. and L. Barros (Eds.), Elsevier, Netherlands, ISBN: 978-0-12-816567-6, pp: 1-81.
13. Garcia, E.J., T.L.C. Oldoni, S.M. de Alencar, A. Reis, A.D. Loguercio and R.H.M. Grande, 2012. Antioxidant activity by DPPH assay of potential solutions to be applied on bleached teeth. *Braz. Dent. J.*, 23: 22-27.
14. Huang, X.J., Y.K. Choi, H.S. Im, O. Yarimaga, E. Yoon and H.S. Kim, 2006. Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) detection techniques. *Sensor*, 6: 756-782.
15. Kanta, P., T. Ghosh, A. Kaur and T. Muthukumarappa, 2021. An innovative and cost-effective way to estimate alkaline phosphatase activity in *in vitro* cellular model systems. *Int. J. Biochem. Mol. Biol.*, 12: 1-7.
16. Weydert, C.J. and J.J. Cullen, 2010. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. *Nat. Protoc.*, 5: 51-66.
17. Rahman, I., A. Kode and S.K. Biswas, 2006. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. *Nat. Protocols*, 1: 3159-3165.
18. Mateos, R., E. Lecumberri, S. Ramos, L. Goya and L. Bravo, 2005. Determination of malondialdehyde (MDA) by high-performance liquid chromatography in serum and liver as a biomarker for oxidative stress: Application to a rat model for hypercholesterolemia and evaluation of the effect of diets rich in phenolic antioxidants from fruits. *J. Chromatogr. B*, 827: 76-82.
19. Slaoui, M. and L. Fiette, 2010. *Histopathology Procedures: From Tissue Sampling to Histopathological Evaluation*. In: *Drug Safety Evaluation*, Gautier, J.C. (Ed.), Humana Press, Totowa, New Jersey, ISBN: 978-1-60327-186-8, pp: 69-82.
20. Thomas, M., K.S. Sujatha and S. George, 2009. Protective effect of *Piper longum* Linn. on monosodium glutamate induced oxidative stress in rats. *Indian J. Exp. Biol.*, 47: 186-192.
21. Egbuonu, A.C.C., C.I. Opara, D. Akachukwu and U.B. Onyedikachi, 2018. Effect of ethanolic extract of avocado pear (*Persea americana*) seed on normal and monosodium glutamate-compromised rats' hepatic histo-morphology and serum bio-functional parameters. *Res. J. Environ. Sci.*, 12: 53-62.
22. Oboh, G. and J.B.T. Rocha, 2007. Polyphenols in red pepper [*Capsicum annum var. aviculare* (Tepin)] and their protective effect on some pro-oxidants induced lipid peroxidation in brain and liver. *Eur. Food Res. Technol.*, 225: 239-247.
23. Cheplick, S., Y.I. Kwon, P. Bhowmik and K. Shetty, 2010. Phenolic-linked variation in strawberry cultivars for potential dietary management of hyperglycemia and related complications of hypertension. *Bioresour. Technol.*, 101: 404-413.
24. Sultana, B., F. Anwar and M. Ashraf, 2009. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*, 14: 2167-2180.
25. Oh, P.S. and K.T. Lim, 2008. Antioxidant activity of *Dioscorea batatas* Decne glycoprotein. *Eur. Food Res. Technol.*, 226: 507-515.
26. Nafiu, M.O., M.A. Akanji and M.T. Yakubu, 2011. Effect of aqueous extract of *Cochlospermum planchonii* rhizome on some kidney and liver functional indices of albino rats. *Afr. J. Traditional Complementary Altern. Med.*, 8: 22-26.

27. El Hilaly, J., Z.H. Israili and B. Lyoussi, 2004. Acute and chronic toxicological studies of *Ajuga iva* in experimental animals. *J. Ethnopharmacol.*, 91: 43-50.
28. Aniagu, S.O., F.C. Nwinyi, B. Olanubi, D.D. Akumka and G.A. Ajoku *et al.*, 2004. Is *Berlina grandiflora* (Leguminosae) toxic in rats? *Phytomedicine*, 11: 352-360.
29. Onyema, O.O., E.O. Farombi, G.O. Emerole, A.I. Ukoha and G.O. Onyeze, 2006. Effect of vitamin E on monosodium glutamate induced hepatotoxicity and oxidative stress in rats. *Indian J. Biochem. Biophys.*, 43: 20-24.
30. Egbuonu, A.C.C., O. Obidoa, C.A. Ezeokkonkwo, L.U.S. Ezeanyika and P.M. Ejikeme, 2009. Hepatotoxic effects of low dose oral administration of monosodium glutamate in male albino rats. *Afr. J. Biotechnol.*, 8: 3031-3035.
31. Gwaltney-Brant, S.M., 2016. Nutraceuticals in Hepatic Diseases. In: *Nutraceuticals: Efficacy, Safety and Toxicity*, Gupta, R.C. (Ed.), Academic Press, United States, ISBN: 978-0-12-802147-7, pp: 87-99.
32. Al-Mamary, M., M. Al-Habori, A.M. Al-Aghbari and M.M. Baker, 2002. Investigation into the toxicological effects of *Catha edulis* leaves: A short term study in animals. *Phytother. Res.*, 16: 127-132.
33. Yakubu, M.T., A.A. Adesokan and M.A. Akanji, 2006. Biochemical changes in the liver, kidney and serum of rat following chronic administration of cimetidine. *Afr. J. Biomed. Res.*, 9: 213-218.
34. Rajesh, M.G. and M.S. Latha, 2004. Preliminary evaluation of the antihepatotoxic activity of Kamilar, a polyherbal formulation. *J. Ethnopharmacol.*, 91: 99-104.
35. Nandi, A., L.J. Yan, C.K. Jana and N. Das, 2019. Role of catalase in oxidative stress- and age-associated degenerative diseases. *Oxid. Med. Cell. Longevity*, Vol. 2019. 10.1155/2019/9613090
36. Atawodi, S.E.O., O.E. Yakubu, M.L. Liman and D.U. Iliemene, 2014. Effect of methanolic extract of *Tetrapleura tetraptera* (Schum and Thonn) Taub leaves on hyperglycemia and indices of diabetic complications in alloxan-induced diabetic rats. *Asian Pac. J. Trop. Biomed.*, 4: 272-278.
37. Catalá, A., 2012. Lipid peroxidation modifies the picture of membranes from the "fluid mosaic model" to the "lipid whisker model". *Biochimie*, 94: 101-109.
38. Mazumder, P.M., P. Rathinavelusamy and D. Sasmal, 2012. Role of antioxidants in phytomedicine with special reference to antidiabetic herbs. *Asian Pac. J. Trop. Dis.*, 2: S969-S979.