

Original Research Article

Hepatoprotective effects of partially purified fractions of *Senna occidentalis* ethanolic extract on diethyl nitrosamine-induced toxicity in Wistar rats

Ojochenemi E. Yakubu¹, Elejo B. Ojogbane², Francis O. Atanu^{3*},
Chukwuka S. M. Udeh⁴, Morayo E. Ale¹, Blessing H. Bello¹

¹Department of Biochemistry, Faculty of Pure and Applied Sciences, Federal University Wukari, Taraba State, Nigeria

²Department of Medical Laboratory Science, Taraba State University Jalingo, Taraba State, Nigeria

³Department of Biochemistry, Faculty of Natural Sciences, Kogi State University, Anyigba, Kogi State, Nigeria

⁴Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria

Received: 08 June 2021

Accepted: 03 July 2021

*Correspondence:

Dr. Francis O. Atanu,

E-mail: atanufo@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: Induction of toxicity using nitrosamines provides a reliable animal model for the study of oxidative damage to lipids, cellular membranes, proteins and DNA. In the present report, the effects of partially purified fractions of *Senna occidentalis* leaves on diethylnitrosamine intoxicated rats were studied.

Methods: Fractions obtained from eluting the column with solvents of increasing polarity, n-hexane, chloroform, ethyl acetate, ethanol, methanol and distilled water were subjected to *in vitro* for their ability to scavenge 1, 1-diphenyl 2-picryl hydrazyl (DPPH) radical. Fraction 6a eluted with ethyl acetate:ethanol (50:50) possessed the highest antioxidant activity, this fraction was therefore selected for *in vivo* studies. Twenty rats, each weighing between 150 to 250 g were randomly allocated into four groups of five rats each. Hepatotoxicity was induced using a single intraperitoneal injection of diethylnitrosamine (DEN) at the 200 mg/kg body weight. Treatment was carried out for 3 weeks by oral gavage as follows: group A, normal control, group B, DEN control, group C, DEN+fraction (10 mg/kg), group D, DEN+silymarin (5 mg/kg).

Results: The results showed that DEN toxicity significantly ($p < 0.05$) increased alanine transaminase (ALT) and aspartate transaminase (AST) activities and increased the level of thiobarbituric acid reactive substance (TBARS) in the liver. In contrast, the levels of bilirubin, total protein (TP) and albumin (ALB) decreased. However, treatment of rats with the extract significantly ($p < 0.05$) reduced the concentrations of TBARS, ALT, AST and bilirubin, but increased the concentration of TP and ALB.

Conclusions: These results show hepatoprotective potentials of the fraction. Furthermore, GC-MS fingerprinting of fraction 6a revealed the presence of compounds with anticancer, antioxidant and anti-inflammatory properties confirming its high chance for exploration as a medicinal agent.

Keywords: Hepatoprotective, *Senna occidentalis*, Diethylnitrosamine, Toxicity, Antioxidant

INTRODUCTION

Natural products of plant origin have been used for the treatment of various diseases for centuries. In developing countries where orthodox medicine is still not affordable

to the majority of rural dwellers, herbal medicine is a promising alternative. Consequently, research in phytomedicine (herbal medicine) has led to the discovery of numerous novel therapeutic compounds with prophylactic and curative potentials for several diseases

including cancer, diabetes, infectious diseases and hepatic injuries.¹

The toxic properties of various nitrosamines in animals and humans are well established through numerous adequate experimental investigations. The parenteral or prolonged oral administration of minute quantities of nitrosamines results in severe tissue damage in organs such as the liver arising from intense neutrophilic infiltration, extensive centrilobular hemorrhagic necrosis, bile duct proliferation, fibrosis and bridging necrosis that ends in hepatocarcinogenesis.²

Cancer is a genetic and non-infectious disease resulting in uncontrolled proliferation of cells. In fact advanced stages of cancer are characterised by metastasis of damaged cells to other parts of the body. Cancers affecting the liver resulting from exposure to environmental toxicants such as DEN account for a substantial source of death around the world.³ Though there are orthodox drugs to manage cancer, none can completely treat cancer and are typically accompanied by numerous side effects. Previous several studies have shown that secondary metabolites like polyphenols, terpenes and alkaloids present in plant preparations confer anti-mutagenic and anticancer properties.³

S. occidentalis is an herbal plant belonging to the leguminosae family that is commonly found within the tropical and sub-tropical regions of the world, used traditionally for numerous therapeutic reasons.⁴ Experimental reports published in literature suggest that that *S. occidentalis* has antimicrobial, larvicidal, pupicidal, antitypanosomal activity. Also, there is substantial evidence to show that the plant has antianxiety, antidepressant, antidiabetic and anti-inflammatory properties. The present study investigated the antioxidant and hepatoprotective effects of chromatographic fractions from leaves extract of *S. occidentalis*.

METHODS

Sample collection and preparation

Fresh and healthy looking leaves of *S. occidentalis* leaves were collected from Wapan Nngakwu area of Wukari in Taraba State, Nigeria. The leaves were identified at the herbarium of the department of biological science, federal university Wukari, Taraba State. Leaves were washed with copious amount of water and dried to constant weight. The dried leaves were pounded to fine powder with mortar and pestle and then stored and labeled in dry containers until needed. Only healthy plants were used as the leaves were examined to be free from diseases.

Ethanol extraction

The pulverized leaves were soaked in sufficient volume of ethanol for 48 hours at room temperature. It was

stirred at 8 hours interval. After 48 hours, the extracts were filtered out first using a clean white sieving mesh and then using Whatman no. 1 filter paper. The filtrate was then concentrated to dryness at 40°C under reduced pressure on a rotary evaporator, extracts were transferred to air-tight containers, corked and preserved in the refrigerator at 4°C until required. Aliquots of the crude plant extract were weighed and used for phytochemical screening and a portion was given to the Wistar rats of the selected groups.

Animal procurement and treatment

Male and female albino rats (Wistar strain), weighing 150-350 g were obtained from a commercial breeder in Obudu area in Cross Rivers State. The rats were acclimatized for a period of 14 days after randomization, under standard laboratory conditions (25±2°C, relative humidity of 60±5% and 12 hours light/dark cycle). Before and during the experiment, rats were maintained on a commercial feed and drinking water ad libitum.

Fractionation of ethanolic extract

The ethanol extract was subjected to column chromatography using silica gel stationary phase. The column was eluted using varying solvent combinations of increasing polarity.

Packing of column

The packing of the column was done according to the method of Yakubu et al.⁵ The lower part of the glass column was soaked with glass wool with the aid of glass rod. Silica gel slurry was prepared by dissolving 235 g of silica gel (mesh size) in 255 ml of absolute n-hexane. The slurry was packed in 30×400 mm chromatographic column. The chromatographic column (30 mm diameter by 400 mm height) was packed with silica gel and the free flow of the solvent was allowed into a conical flask below. The setup was then seen to be in order when the solvent drained freely without carrying either the silica gel or glass wool into the tap. At the end of the packing process, the tap was locked and the column was then allowed for 24 hours to stabilize after which, the clear solvent at the top of the silica gel was allowed to drain down the silica gel meniscus.

Elution

The method of Yakubu et al was adopted for the elution.⁵ The extract (2 g) was dissolved in 15 ml absolute ethanol and the solution was applied to a chromatographic column (30 mm diameter by 40 mm height). Phytochemicals were eluted from the plant material using solvent combinations of different polarities. They were n-hexane (100:0); n-hexane:chloroform (50:50); chloroform (100:0); chloroform:ethyl acetate (50:50); ethyl acetate (100:0); ethyl acetate:ethanol (50:50); ethanol (100:0);

ethanol:methanol (50:50); methanol (100:0); methanol:distilled water (50:50); distilled water (100:0).

A measured volume (400 ml) of each solvent combination was poured into the column each time using a separator funnel. Eluted fractions were then collected in aliquots of 200 ml using fraction collection tubes.

Determination of antioxidant activity using DPPH free radical activity

The antioxidant activity of fractions of ethanol extract was assayed by the DPPH radical scavenging method described by Karadag et al.⁶

The assay mixture contained 2 ml of 1.0 mM DPPH radical solution prepared in methanol and 1 ml of standard or extract solution of different concentrations (10-500 µg/ml). The solution was rapidly mixed and incubated in dark at 37°C for 20 minutes. The decrease in absorbance of each solution was measured at 517 nm using a spectrophotometer. Ascorbic acid was used as control while 2 ml of 1.0 mM DPPH radical solution with 1 ml ethanol was taken as blank.

Calculation of free radical scavenging (%)

$$\% \text{ free radical scavenging activity} = \frac{A_c - A_s}{A_c} \times 100,$$

where,

A_c=absorbance of control,

A_s=absorbance of sample.

The concentration of sample required to scavenge 50% of DPPH free radical (IC₅₀) was determined from the curve of percentage inhibitions plotted against the respective concentrations.

Lipid peroxidation assay

Lipid peroxidation was carried out by the method described by Ortuno et al.⁷ Briefly, 2 g of liver samples was homogenized with 10 ml of thiobarbituric acid solution reagent and heated at 95°C for 15 min in a boiling water bath. The mixtures were cooled in ice-cold water and centrifuged at 1,500×g for 15 min at 4°C. Absorbance readings were measured at 532 nm (Shimadzu model UV-1601, Japan). The procedure was repeated three times and results were reported as µg malondialdehyde per ml sample (µg MDA/ml).

Experimental animals

Albino rats were housed in a controlled environment (12 hr light/dark cycle and temperature of ≈25°C) and fed standard commercial rat feed and water ad libitum in the animal facility of the department of biochemistry, federal

university Wukari, Nigeria. Animals were humanely handled according to the guidelines of the national institute of health for the care and use of laboratory animals (NIH publications no. 8023, revised 1978).

Animal grouping and administration

Twenty rats, each weighing between 150 to 250 g were randomly selected into four groups of five rats each. Hepatotoxicity was induced using a single intraperitoneal dose of DEN at the 200 mg/kg body weight. Treatment was carried out for 3 weeks orally as follows: group A was normal control, group B was DEN control, group C was DEN+fraction 6a (10 mg/kg), group D was DEN+silymarin (5 mg/kg).

Blood sample collection and preparation for biochemical assay

At the end of the experimental period (three weeks), blood samples were collected through cardiac puncture from the rats under light chloroform anesthesia and allowed to clot, then centrifuged for 10 minutes at 3000 rpm. Serum was separated and stored in a clinical refrigerator and was used for the determination of liver function parameters.

Biochemical assay

The serum levels of ALT, AST were determined using the method of Reitman and Frankel, serum direct and total bilirubin determined colorimetrically as described by Doumas et al, serum concentration of albumin was determined as described by Bartholomew et al.⁸⁻¹⁰ Total serum protein level was by the method of Fine.¹¹

Statistical analysis

Data collected were subjected to one way analysis of variance (ANOVA) using Dancer's multiple range test (DMRT). Results were expressed in mean±standard deviation and differences at p<0.05 were considered significant.

RESULTS

Total antioxidant capacity (TAC) of fractions of *S. occidentalis*

The TAC of the chromatographic fractions was evaluated against DPPH radical. The IC₅₀ of the various fractions is presented in Figure 2. The values of IC₅₀ were significantly lower in fractions 2a (20.5 mg/ml) and 6a (19.5 mg/ml) which implies high antioxidant capacity as compared to the other fractions as shown in Figure 2. The result showed that fractions 6a and 2a require smaller concentrations to inhibit free radical chain reaction as compared to the other fractions.

Inhibition of lipid peroxidation by fractions of *S. occidentalis*

The anti-lipid peroxidation was assessed by determining the concentration of TBARS in oxidatively stressed tissue homogenates that were pre-treated with the selected fractions. The TBARS values revealed that fraction 6a has the highest anti-lipid peroxidation potential with the lowest concentration of 41.97 µg/ml, followed by fraction 2a (47.07 µg/ml) as represented in Figure 3. Fraction 6a significantly inhibited the generation of TBARS in the tissue homogenates.

Effects of fractions of *S. occidentalis* on liver function

Liver function was assessed by assaying for the activities of ALT, AST. ALB and concentrations of TB, direct bilirubin (DB) and total protein are shown in Table 1. ALT, AST, TB, and DB values increased significantly ($p < 0.05$) in the animals that were induced with DEN but were not treated (group B) as compared to the normal animals whereas, these indices were significantly ($p < 0.05$) reduced to near normalcy in the animals that

were induced but were subsequently treated with the extract fraction and standard drug (silymarin). On the other hand, the albumin and total protein concentrations were significantly ($p < 0.05$) reduced in group B when compared to group A, however, these values were significantly raised compared to the normal animals in groups A and B that were treated with fraction 6a and standard drug (silymarin) respectively.

GC-MS profile of fraction 6a of *S. occidentalis*





The compounds identified from the fraction eluted with ethyl acetate+ethanol are presented in order of decreasing %area as shown in Table 2 with cyclononene having the highest %area. The GC-MS profile of fraction 6a revealed the presence of cyclononene, alpha-D-glucopyranoside, tetra acetyl-d-xylonic nitrile, 8-nonenic acid, beta-D-glucopyranose, (aminomethyl) cyclopropane, isobutyl nonyl carbonate, 4-tetradecene, dichloroacetic acid, 9-oxabicyclo [6.1.0] nonane, n-heptyl acrylate, methanamine, N-methoxy, 3-hexyn-1-ol, cyclopropane, 1,1-dimethyl and 4-nonene, 5-nitro.

Table 1: Effect of fraction 6a of ethanol extract of *S. occidentalis* leaves on biochemical parameters of rats intoxicated with DEN.











Groups	ALT (IU/l)	AST (IU/l)	TP (mg/dl)	ALB (mg/dl)	TB (mg/dl)	DB (mg/dl)
A=normal control	31.91±5.46 ^{ab}	49.64±4.63 ^a	72.20±1.90 ^b	34.56±1.09 ^{ab}	2.60±0.26 ^b	1.33±0.15 ^{ab}
B=DEN control	35.28±4.63 ^b	112.77±45.81 ^c	62.20±1.90 ^a	31.56±1.09 ^a	3.60±0.26 ^c	2.33±0.15 ^c
C=DEN+fraction 6a (10 mg/kg)	24.18±10.28 ^a	52.01±26.86 ^b	84.50±1.56 ^c	36.48±1.76 ^b	2.68±0.13 ^b	1.60±0.10 ^b
D=DEN+silymarin (5 mg/kg)	27.74±3.45 ^a	53.07±34.73 ^b	73.96±3.06 ^b	32.60±1.38 ^a	1.94±0.09 ^a	1.09±0.20 ^a

N=5; values are presented in mean±standard deviation; alanine transaminase, ALT (IU/l); aspartate transaminase, AST (IU/l); total protein, TP (mg/dl); albumin, ALB (mg/dl); total bilirubin, TB (mg/dl) and direct bilirubin, DB (mg/dl); values with different superscript down the column are significantly different at $p < 0.05$.

Table 2: GC-MS profile for fraction 6a of ethanolic extract of *S. occidentalis* leaves.

S. no.	RT	Compound	Area (%)	Chemical structure	Biological function
1.	55.952	Cyclononene	23.70	 C ₉ H ₁₆ 124.227	Cytotoxicity, antimicrobial
2.	56.378	Alpha-D-glucopyranoside, methyl	10.39	 C ₇ H ₁₄ O ₆ 194.18	Anticancer
3.	64.907	8-nonenic acid	6.88	 C ₉ H ₁₆ O ₂ 156.22	Antifungal
4.	50.912	Beta-D-glucopyranose	5.51	 C ₆ H ₁₂ O ₆ 180.16	Anti-diabetic, antihypertensive antihyperlipidemia.

Continued.

S. no.	RT	Compound	Area (%)	Chemical structure	Biological function
5.	35.327	(Amino methyl) cyclopropane	4.02	 C ₄ H ₉ N 71.12	Antidepressant
6.	63.783	Isobutyl nonyl carbonate	3.54	 C ₁₅ H ₃₀ O ₃ 258.40	This ester is mainly used in herbal compositions for cosmetic purposes.
7.	63.046	4-tetradecene	3.25	 C ₁₄ H ₂₈ 196.37	Antibacterial
8.	62.504	Dichloroacetic acid	2.19	 C ₂ H ₂ Cl ₂ O ₂ 128.94	Anticancer, topical chemobalation
9.	61.418	9-oxabicyclo [6.1.0] nonane	2.07	 C ₁₆ H ₂₈ O ₂ 252.39	Antioxidant
10.	17.300	n-heptyl acrylate	1.37	 C ₁₀ H ₁₈ O 170.252	Anti-inflammatory, antibacterial
11.	12.570	Methanamine, N-methoxy	1.14	 CH ₃ NHOCH ₃ 61.08	It is used to illicit production of methamphetamine.
12.	15.206	3-hexyn-1-ol	1.05	 98.14	Antiproliferative
13.	17.067	Cyclopropane, 1,1-dimethyl-	1.04	 C ₅ H ₁₀ 70.13	Antitumor
14.	61.883	4-nonene, 5-nitro	1.02	 C ₉ H ₁₇ NO ₂ 171.24	Antimicrobial

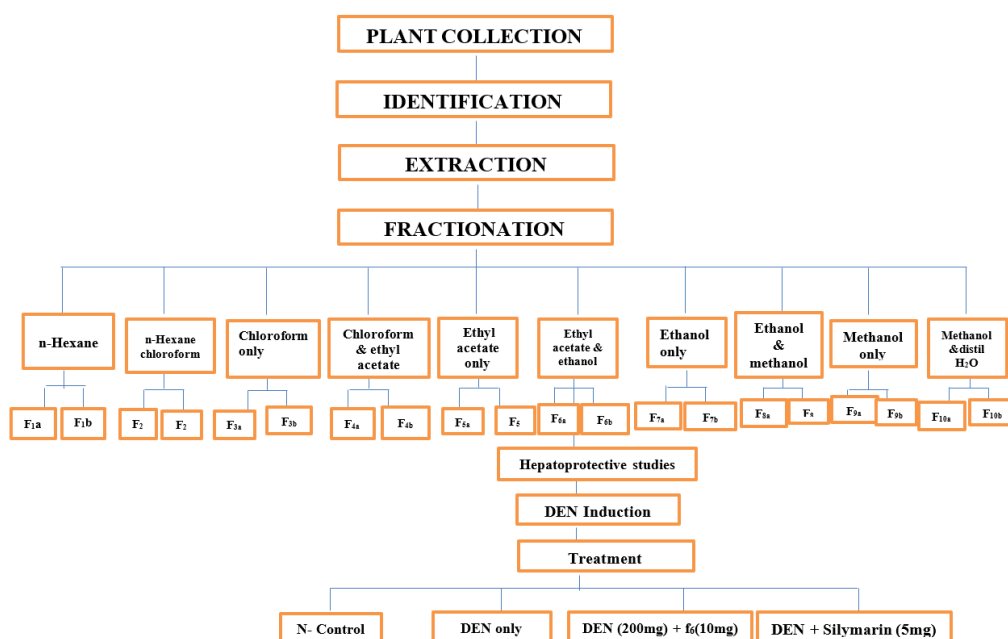


Figure 1: Experimental design for the extraction, fractionation and biological evaluation of *S. occidentalis* leaves.

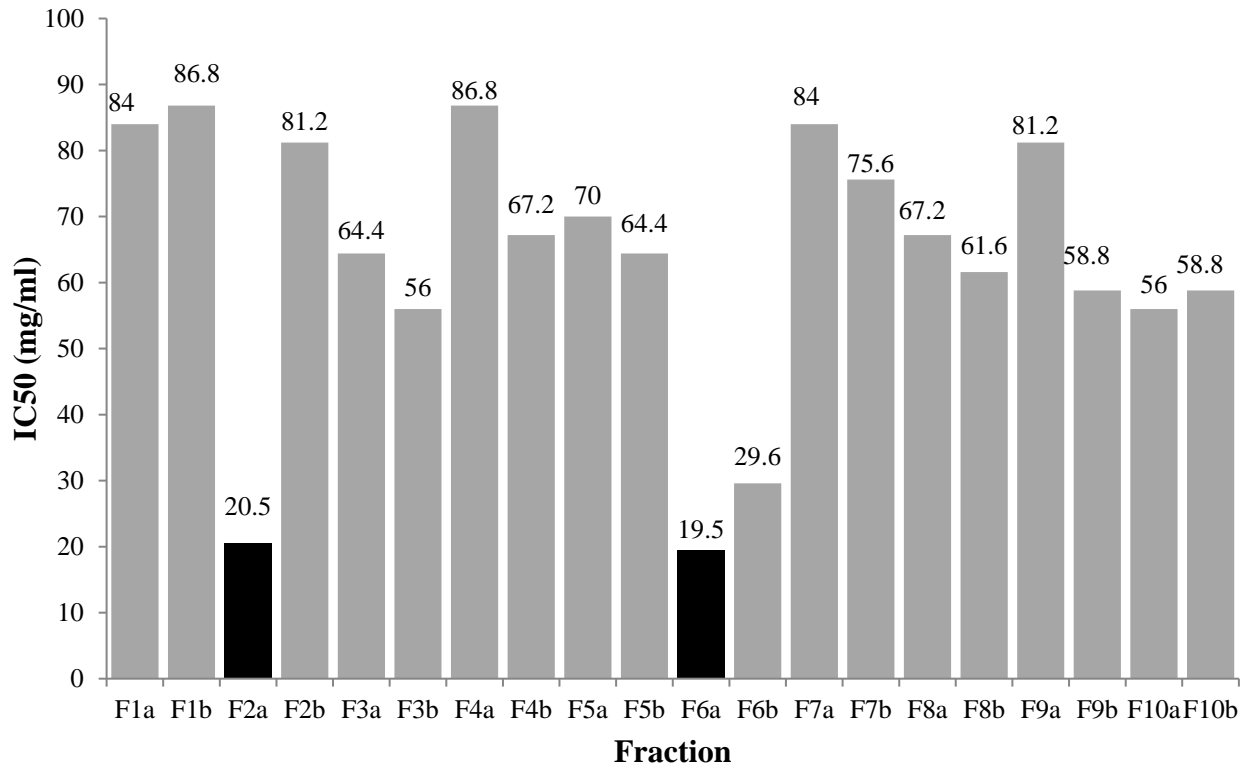


Figure 2: Total antioxidant capacity of solvent fractions of ethanolic extract of *S. occidentalis* leaves; the most active fractions are shaded in black.

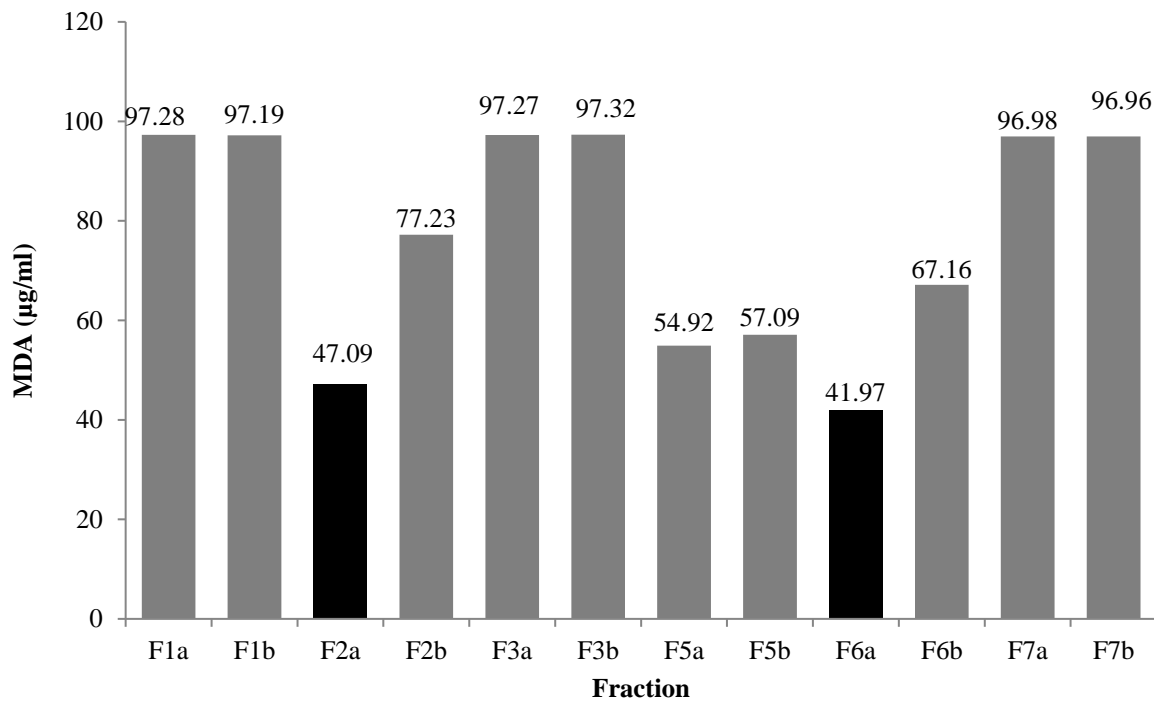


Figure 3: Effect of selected solvent fractions of ethanolic extract of *S. occidentalis* on total lipid peroxides in liver; the most active fractions are shaded in black.

DISCUSSION

The total antioxidant capacity was evaluated using the free radical scavenging activity of the various fractions. The DPPH method was a sensitive means to evaluate the antioxidant potential of a given substance or plant extracts in terms of free radical scavenging ability.¹² DPPH is a stable nitrogen-centered free radical whose colour changes from violet to yellow when it is reduced by either the addition of hydrogen or electron, thus, compounds that are capable of reducing DPPH can be termed antioxidants due to their radical scavenging activity.¹³ It was found that all the chromatographic fractions exhibited radical scavenging activity with fraction 6a showing the highest hydrogen donating tendency than the other fractions as evident in its low IC₅₀ value which represented a high inhibitory percentage. The antioxidant properties determined, showed that the solvents were able to extract substances with antioxidant potency. However, water was less efficient in the extraction than other solvents that were employed. This finding was in agreement with the earlier findings of Fatma et al and Vivek et al where antioxidant properties of plant materials were evaluated using DPPH with considerable inhibitory activity against free radicals.^{14,15}

Lipid peroxidation was extensively implicated in primary toxicological effects and was caused by the generation of reactive species from varying sources including organic peroxides, redox cycling and iron-containing substances. Lipid peroxidation is a free radical-induced oxidative chain reaction in which lipid molecules become oxidized to form lipid peroxides and this reaction can be terminated when the substrate is depleted or by when an antioxidant is added, which makes hydrogen available to reduce peroxy radicals.¹⁶ The TBARS assay has been previously used to determine the extent of lipid peroxidation in biological systems and in food materials. TBA reacts particularly with malondialdehyde (MDA), a secondary product of lipid peroxidation to produce a red chromogen that can be determined using a spectrophotometer.¹⁷ In this study, all the fractions were capable of reducing the concentration of MDA. But the highest anti-lipid peroxidation activity was observed in fraction 6a. This finding indicated that the fractions of the extracts might prevent reactive radical species from damaging biological and food systems. The result obtained in this study collaborate with the result of Tuyen et al that showed a significant reduction in the concentration of MDA of minced pork upon treatment with the leaves extract of *Phyllanthus acidus*.¹⁸

Administration of DEN significantly elevated the values of liver biomarker enzymes. These enzymes were mostly present in low concentrations in the serum and usually in high concentrations in the liver of animals with intact liver architecture. But once the liver architectural integrity was compromised as a result of lipid peroxidation arising from oxidative stress or viral

infections such as hepatitis, the enzymes thus escape from the hepatocytes into the bloodstream where they are not known to play any specific role.¹⁹ The hike in levels of these enzymes could be a result of hepatic necrosis, damage to the bile duct or even fibrosis which precludes hepatic carcinogenesis. However, the administration of the selected fraction 6a was able to normalise the levels of these indices compared to the normal rats. The normalisation corroborates the total antioxidant capacity of the fraction 6a which was further confirmed by its ability to inhibit lipid peroxidation. Similarly, liver damage affected other normal hepatic physiological mechanisms such as the metabolism of bilirubin and protein.²⁰ This was evident in the significant increase in the concentration of bilirubin and the decrease in total protein and albumin whose metabolisms are being regulated by the liver. However, the administration of the extraction fraction considerably reversed the conditions to normalcy.

Gas chromatography-mass spectroscopy (GC-MS) is a very compatible and one of the best methods to identify pure compounds present at less than 1 ng in biological specimens for quantification purposes. The unknown organic compounds in a complex biological mixture can be identified by the interpretation and matching of the test sample spectra with the reference spectra.²¹ In this present study, the GC-MS profile of fraction 6 of *S. occidentalis* leaves ethanolic extract, fraction eluted with ethyl acetate+methanol were presented in order of decreasing % area, cyclononene having the highest % area as shown in the above table. Compounds identified from fraction 6 which have antioxidant, anticancer and anti-inflammatory properties are alpha-D-glucopyranoside, methyl, dichloroacetic acid, 9-oxabicyclo [6.1.0] nonane, n-heptyl acrylate, 3-hexyn-1-ol and many others. The GC-MS observation was consistent with the hepatoprotective effect and antioxidant potency of the fraction as demonstrated in this study, most especially that many earlier studies have always implicated the hepatoprotective effect of plant materials to their antioxidant and anti-inflammatory properties.²¹

CONCLUSION

In conclusion, the findings from this work portray fractions of ethanol extract of *S. occidentalis* leaves as possible antioxidants and anti-hepatotoxic agents that are capable of preventing tissue damage that may be arising from oxidative stress imposed by certain chemicals agents such as DEN. The foregoing properties were collaborated by the presence of some very important bioactive components in the fraction 6a that exhibited a much more promising activity as compared to the other fractions. However, it will be very useful to understand the molecular mechanism of these tissue-protective activities of this fraction.

ACKNOWLEDGEMENTS

The authors are grateful to the laboratory staff of department of biochemistry, federal university Wukari, Nigeria for their technical assistance.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the institutional ethics committee

REFERENCES

- Gupta M, Mazumder UK, Kumar RS, Kumar T. Antitumour activity and antioxidant role of *Bauhinia racemosa* against Ehrlich ascites carcinoma in swiss albino mice. *Acta Pharmacol Sin.* 2004;25(8):1070-6.
- Tolba H, Moghrani H, Benelmouffok A, Kellou D, Maachi R. Essential oil of Algerian *Eucalyptus citriodora*: Chemical composition, antifungal activity. *Journal de Mycologie Médicale.* 2015;25(4):128-33.
- Sylla BS, Wild CP. A million Africans a year dying from cancer by 2030: what can cancer research and control offer to the continent. *Int J Cancer.* 2012;130(2): 245-50.
- Veronique JE, Gabriel NM. Sub-chronic toxicity of the beverage made from *Cassia occidentalis* seeds in mice. *Int J Nutrit Food Sci.* 2013;2(5):237-42.
- Yakubu OE, Nwodo OFC, Joshua PE, Ugwu MN, Odu AD, Okwo F. Fractionation and determination of total antioxidant capacity, total phenolics and total flavonoids contents of aqueous, ethanol and n-Hexane extracts of *Vitex doniana* leaves. *Afr J Biotechnol.* 2014;13(5):693-8.
- Karadag A, Ozcelik B, Saner S. Review of methods to determine antioxidant capacities. *Food Analyt Method.* 2009;2:41-60.
- Ortuno J, Serrano R, Jordan MJ, Banon S. Relationship between antioxidant status and oxidative stability in lamb meat reinforced with dietary rosemary diterpenes. *Food Chem.* 2016;190:1056-63.
- Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clinic Pathol.* 1957;28(1):56-63.
- Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica Chimica Acta.* 1997;258(1):87-96.
- Bartholomew RJ, Delaney AM. Sulphonphthaleins as specific reagents for albumin: determination of albumin in serum. *Proc Aust Assoc Clin Biochemists.* 1966;1:214-8.
- Fine J. Quantitative determination of serum proteins by colorimetric method. *Biochem J.* 1935;29:799.
- Koleva II, Beek TAV, Linssen JPH, DeGroot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochem Analysis.* 2002;13(1):8-17.
- Engoor SA, Mangalanandan SL, Paul HC, Jagathala MS. *In vitro* antioxidant, anti-lipid peroxidation activities and HPLC analysis of methanol extracts from bark and stem of *Mahonia leschenaultia* takeda. *Asian J Plant Sci Res.* 2013;3(2):116-26.
- Fatma A, Sokindra K, Shah AK. Estimation of total phenolic content, in-vitro antioxidant and anti-inflammatory activity of flowers of *Moringa oleifera*. *Asian Pac J Trop Biomed.* 2013;3(8):623-7
- Vivek KB, Yong-Ha P, Pooja, A. Studies on phytochemical analysis, antioxidant and lipid peroxidation inhibitory effects of a medicinal plant, *Coleus forskohlii*. *Frontiers Life Sci.* 2015;8:2:139-47.
- Richa U, Jitendra KC, Kavindra NT, Karuna S. Antioxidant property of aerial parts and root of *Phyllanthus fraternus* webster, an important medicinal plant. *Scient World J.* 2014:692392.
- Coppen PP. In: Allen JC, Hamilton RJ, eds. Rancidity in foods. New York, USA: Applied Science Publishers; 1983: 67.
- Tuyen TKN, Chamroon L, Montinee T, Komkhae P. Potential antioxidant and lipid peroxidation inhibition of *Phyllanthus acidus* leaf extract in minced pork. *Asian Australas J Animal Sci.* 2017;30(9):1323-31.
- Balu M, Sangeetha P, Haripriya D, Panneerselvam C. Rejuvenation of antioxidant system in central nervous system of aged rats by grape seed extract. *Neurosci Letter.* 2005;383:295-300.
- Patrick-Iwuanyanwu KC, Wegwu MO, Okiyi JK. Hepatoprotective effects of African locust bean (*Xylopiya aethiopica*) in CCl4 induced liver damaged Wistar albino rats. *Int J Pharmacol.* 2010;6:744-9.
- Hites AR. Gas Chromatography Mass Spectroscopy: Handbook of Instrumental Techniques for Analytical Chemistry. Upper Saddle River, NJ: Prentice Hall PTR; 1997: 609-11.

Cite this article as: Yakubu OE, Ojogbane EB, Atanu FO, Udeh CSM, Ale ME, Bello BH. Hepatoprotective effects of partially purified fractions of *Senna occidentalis* ethanolic extract on diethyl nitrosamine-induced toxicity in Wistar rats. *Int J Sci Rep* 2021;7(8):374-81.