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Xylopia aethiopica Volatile Compounds Protect Against Panadol-Induced Hepatic and Renal Toxicity in Male Rats

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Abstract: The objectives of this study were to assess *in vitro* and *in vivo* antioxidant activity of aqueous extract of Negro pepper (*Xylopia aethiopica)* fruits, determine its total phenolics content and estimate its volatile constituents by GC-MS as well as investigate its protective effect against panadol-induced hepatic and renal toxicity in male rats. Our results indicated that *Xylopia aethiopica* fruits infusion has high phenolics content and possesses high antioxidant activity *in vitro*. Thirty five volatile compounds were identified. The main volatile compounds are cis-linalool oxide (47.11%), Carveol (13.2%), buten-1-ol (9.05%), iso-borneol (4.91%) and Borneol (4.14%). Twenty eight adult rats were used for studying the effects of the plant infusion on panadol (paracetamol) and hepatic and renal toxicity. Biochemical results showed that Panadol induced significant increase ($P<0.05$) in plasma alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphates (ALP), gamma-glutamyl transferase (GGT), total bilirubin, direct bilirubin, urea and creatinine levels as well as significant decrease ($P<0.05$) in total proteins, albumin and some antioxidant biomarkers; total antioxidant capacity (TAC), catalase (CAT) and cellular glutathione peroxidase (GPx). Administration of aqueous *Xylopia aethiopica* extract decreased the toxic elevation in plasma bio-indicators of liver and kidney functions and increased previous antioxidant biomarkers. The hepatoprotective effect of Negro pepper was confirmed by histopathological and histochemical examinations of the liver tissue of control and treated animals. It could be concluded that the Infusion of *X. aethiopica* fruits possess nutritional and medicinal values. Aqueous extract of *Xylopia aethiopica* fruits rich in phenolics and volatile compounds, act as strong natural antioxidants. The aqueous extract protect against the toxic effect of panadol-induced hepatic and renal toxicity.

Key words: Antioxidant activity · GC-MS · *Xylopia aethiopica* · Liver · Kidney · Panadol

INTRODUCTION

The aromatic plant *Xylopia aethiopica* Dunal (Annonacae), commonly known as Ethiopia or Negro pepper has been used in Europe, Asia and Africa as pepper substitute and spice in local cooking. Various parts of the plant have been traditionally employed in different therapeutic preparations [1, 2]. The mature fruits of green color take a brown-black coloration after drying and are used as spices. The fruit of this plant was used against cough, stomachache, dizziness, amenorrhea, bronchitis, lumbago and neuralgia. It is also used as calmative, purgative, repulsive to pain and in the treatment of boils and skin eruptions [3]. Chemical components of *X. aethiopica* have been helpful in the avoidance and treatment of cancerous tumors [4]. *Xylopia aethiopica* fruits contain alkaloids, flavonoids, terpenoids, fixed oil and volatile aromatic oil. Key constituents are diterpenic and xylopic acids [5, 6]. *X. aethiopica* oil contains carbohydrates, glycosides,

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saponins, tannins and phytosterols [7]. Treatment with *X. aethiopica* ameliorated the radiation-induced decreases in antioxidant status of the animals; *X. aethiopica* could have beneficial effect by inhibiting oxidative damage of exposed rats [8]. Liver diseases are one of the major causes of morbidity and mortality all over world. The manifestations of drug-induced hepatotoxicity are highly variable ranging from asymptomatic elevation of liver enzyme to fulminate hepatic failure [9]. Paracetamol overdose is the most common cause of drug induced liver disease, also known by Panadol, is usually well tolerated in prescribed dose but overdose is the most common cause of drug induced liver disease and acute liver failure worldwide [10]. Initial symptoms of overdose include vomiting, salivation and discoloration of the tongue and gums. Unlike an overdose in humans, liver damage is rarely the cause of death; instead, methaemoglobin formation and the production of Heinz bodies in red blood cells inhibit oxygen transport by the blood causing asphyxiation [11]. In spite of tremendous advances in modern medicine, these are hardly any reliable drugs that protect the liver from damage and/or help in regeneration of hepatic cell. Many active plant extracts are frequently utilized to treat a wide variety of diseases including liver diseases. Therefore, searching for effective and safe drugs for treatment of liver disorder continue to be area of interest.

The aim of this study is to identify volatile compounds present in *Xylopia aethiopica* fruits and determine total phenolic content. Also to evaluate the antioxidant power of its aqueous extract and the protective effect against panadol-induced hepatic and renal toxicity in male albino rats.

MATERIALS AND METHODS

Preparation of Plant Infusion: Dry fruits of *Xylopia aethiopica* were purchased from Omdurman market in Khartoum State, Sudan. The dry fruits under investigation were separately grounded and three grams of plant were infused with 100 ml freshly boiled water for 5 min. followed by filtration. The infusion filtrate of *Xylopia aethiopica* was subjected to the following tests:

- Quantitative determination of total phenolics content (TPC): using Folin-Ciocalteu method [12].
- Quantitative determination of Antioxidant Activity was performed according to β-carotene bleaching method [13] and DPPH free radical scavenging assay [14].

Isolation and Characterization of *Xylopia aethiopica* Volatile Compounds: Two hundred grams of the dried fruits were ground to a fine powder using electric grinder; The essential oil was obtained by steam distillation in 3000 ml H_2O for 3 h by Clevenger apparatus. The oil was dried over anhydrous sodium sulphate and filtered. Extraction was carried out in duplicate and the results were averaged. Analysis of the volatile compounds of the plant infusion was done using the chromatographic techniques (GC and GC/MS).

Gas Chromatography (GC): The obtained volatile sample was thermally desorbed, using a modified injector port, directly on the front of a fused silica capillary column (DB5, 60 m x 0.32 mm i.d) in the oven of a Hewlet-packed HP 5890 g as chromatography. Temperature was increased from 45-240°C by the rate of 4°C/min. Kovat's indices were determined by co-injection of the sample with a solution containing homologous series of n-hydrocarbons (C_6-C_{26}). The separated components were identified by matching with NIST mass-spectral library data and by comparison of Kovat's indices with those of authentic components and with published data [15]. The quantitative determination was carried out based on peak area integration. Retention indices (RI) of each compound were calculated from the standard alkane retention time and the peak retention time.

Gas Chromatography-Mass Spectrometry (GC-MS): Analyses were performed on an HP model 6890 GC interfaced to an HP 5791A mass selective detector (GC/MS) was used for mass spectral identification of the GC components at (MS) ionization voltage (70eV. A 30m x 0.25mm i.d). (DF = 0.25 lm) DB wax bonded-phase fused-silica capillary column was used for (GC). The linear velocity of the helium carrier gas was 30 cm/s. The injector and the detector temperatures were 250°C. The oven temperature was programmed from 40-240°C at 4°C/min and held for 50 min.

Biochemical Study

Animals and Experimental Design: Adult male Swiss rats with initial body weight ranging from 120-150g were used. Animals were provided from the Breeding Unit of the National Research Centre (Giza, Egypt). The animals were housed individually in stainless steel wire mesh cages and maintained for one week for acclimatization. Commercial standard pellets and tap water were supplied *ad libitum*. Twenty eight adult rats were used for studying the effects of the plant infusion on panadol (paracetamol) and hepatic and renal toxicity [16]. Rats were equally divided

into four groups, 7 rats in each. Group (1) Normal control, commercial standard pellets and tap water were supplied *ad libitum*. Group (2) rats were orally supplemented with freshly prepared aqueous extract of *Xylopia aethiopica* (3 g/100 ml water) for thirty days instead of tap water, to examine the safety of plant extract. Group (3) commercial standard pellets and tap water were supplied *ad libitum* rats were intoxicated after 4 weeks by a single oral dose of panadol (2g/kg rat B.W) [16]. Group (4) protected rats, where rats were maintained on drinking freshly prepared Sudanese *Xylopia aethiopica* infusion (3g/100 ml boiled water) for 28 days instead of tap water and then rats were intoxicated by single oral administration with panadol (2 g/kg rat B.W). The experiment duration was continued for 30 days. The rats were killed after 48h of a single oral dose of panadol administration.

Blood and Tissue Sampling: Blood samples were withdrawn on heparinized tubes. Plasma was separated and used for determination of liver and kidney functions and antioxidant biomarkers. The RBCs were washed several times with cold saline solution. The packed RBCs were stored at -20°C for determination of Glutathione peroxidase. Liver was excised and rinsed with cold saline and weighed. A portion of the liver tissue was kept into 10% formalin for histological examinations.

Biochemical Assays: Total Plasma antioxidant capacity (TAC), Plasma catalase (CAT) and cellular glutathione peroxidase (GPx) levels were determined using assay kits [17-19]. Total protein, albumin, alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphates (ALP), gamma-glutamyl transferase (GGT), total bilirubin and direct bilirubin were carried out by using assay kits [20-25]. Kidney function (creatinine and urea) was evaluated [26, 27].

Histological Study: Livers were dissected out and fixed instantaneously in 10% formalin saline for 24 hours. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax (melting point 58-60°C). Sections of 6 µm thickness were prepared and stained with Haematoxylin and Eosin [28].

Histochemical Study

Total Proteins: Mercury bromophenol blue method was applied for the histochemical determination of total proteins [29].

The Polysaccharide Inclusions: Periodic acid Schiff method was applied for visualization of the polysaccharide materials [30].

Statistical Analysis: All values obtained were calculated as mean ± standard error and the statistical significance of differences between mean values was determined using the SPSS for Windows version 11.0 statistical program (Chicago, IL, USA). The parametric continuous variables were evaluated with the one-way ANOVA test. Study groups were compared with the control group by applying the t test. For all statistical evaluations, p values<0.05 were recognized as statistically significant [31].

RESULTS

Chemical Composition of *X. aethiopica* Fruits

Volatile Compounds: The yield of essential oil from dry fruits was 1.51 ± 0.13 g/100g dry weight. The chemical composition of the investigated essential oil is shown in Figure 1 and Table 1. Gas chromatography/mass spectrometry (GC/MS) analysis revealed the presence of 35 compounds in essential oil. The major constituents of the tested volatiles were (cis-linalool oxide 47.11%), (Carveol 13.2%), (buten-1-ol 9.05%), (iso-borneol 4.91%), (Borneol 4.14%). In addition, it was found that *X. aethiopica* volatiles contained considerable amounts of various minor constituents [7-alpha-hydroxy manool (2.5%), epi-alpha-bisabolol (1.1%), 9-epi-(E)-caryophyllrne (2.19%), sabinene (1.77%), camphor (1.31%), thymol (0.51%), beta-citronellol (0.9%), geriniol (0.73%), dihydrocarveol (0.48%].

Non Volatile Compounds (Total Phenolic Content): Table 2 shows the total phenolic content (TPC) of *Xylopia aethipica* aqueous extract. Results revealed that water extract of *Xylopia aethipica* fruits possess high amount of phenolic compounds (2650±4.0 mg/l). The results are presented as mg of gallic acid equivalent/l.

Antioxidant Activity *In vitro*:

***β*-Carotene/Linoleic Acid Method:** The results in Table 2 indicated that *Xylopia aethiopica* aqueous extract had a strong antioxidant activity towards free radicals (95.0% ± 3.5), when comparing with the standard *Tert*-butyl hydroquinone (TBHQ) (99.5% ±2.7) at 400 µg/ml.

DPPH Free Radical Method: The results in Table 2 indicated that antioxidant activity obtained by DPPH free

Table 1: Total volatile compounds of *Xylopia aethiopica* analyzed by GC-MS

Peak no	K.I	Area %	Identified Compound
1	641	9.05	Buten-1-ol
2	704	0.6	Ethyl propanoate
3	769	0.15	Pentanol
4	795	0.28	3,4-hexanedione
5	980	0.22	Beta-pinene
6	1014	0.03	Delta-3-carene
7	1030	0.21	Limonene
8	1054	1.09	Bergamal
9	1067	1.77	Sabinene
10	1079	47.11	Cis-linalool oxide
11	1098	0.18	Linalool
12	1118	3.31	Cis-hex-3-enyl butyrate
13	1132	0.03	Iso-thujanol
14	1142	1.31	Camphor
15	1157	4.91	Iso-borneol
16	1164	4.14	Borneol
17	1196	13.2	Carveol
18	1224	0.48	Dihydrocarveol
19	1229	0.9	Beta-citronellol
20	1259	0.73	Geriniol
21	1275	0.84	Gerinal
22	1282	1.23	Anethol
23	1290	0.51	Thymol
24	1330	0.07	Cis-piperitol acetate
25	1465	2.19	9-epi-(E)-caryophyllrne
26	1553	0.23	Elemicin
27	1648	0.07	Beta-eudesmol
28	1684	1.1	Epi-alpha-bisabolol
29	1758	0.22	(E)-nuciferol
30	1760	0.27	Beta-bisabolen-12-ol
31	1773	0.15	(E)-alpha-atlantone
32	1797	1.29	14-hydroxy-delta-cadinene
33	1808	0.3	Cryptomeridiol
34	1905	0.17	2-phenylethylphenyl acetate
35	2234	2.5	7-alpha-hydroxy manool

K.I: Kovats index. Compound listed in the order of elution from a DB_5 column, Retention indices relative to C_7–C_{20} *n*-alkanes on the DB-5MS column, identification based on retention index and comparison of mass spectra.

Table 2: Total phenolic content (TPC) and *in vitro* antioxidant activity (A.A.) of *Xylopia aethiopica* extract

		% inhibition at different concentrations							
		A.A by β-carotene/linoleic acid assay				A.A by DPPH free radicals assay			
Item	TPC (mg/l)	50 (µg/ml)	100 (µg/ml)	200 (µg/ml)	400 (µg/ml)	50 (µg/ml)	100 (µg/ml)	200 (µg/ml)	400 (µg/ml)
X. aethipica Extract	2650 ± 4.0	64.8±2.4	73.7±1.9	88.0±2.5	95.0±3.8	62.0±2.30	73.16±1.02	86.06±1.72	96.40±1.59
♦TBHQ	--	75.2± 3.1	85.0± 205	94.0± 3.4	99.5± 2.7	76.53± 2.3	83.75± 2.5	95.36± 2.6	99.73± 2.6

♦TBHQ: Tert –butyl hydroquinone, standard synthetic antioxidant.

Each value represents the mean ± S.E (Standard Error) and mean of three replicates.

radical scavenging assay of *Xylopia aethiopica* aqueous extract exert an excellent antioxidant activity towards DPPH free radicals (96.40% ±1.59) when comparing with the standard *Tert*-butyl hydroquinone (TBHQ) (99.73% ±2.6) at 400 µg/ml). The radical scavenging activity of *Xylopia aethiopica* aqueous extract on β-carotene/linoleic acid and DPPH free radicals increased with increasing concentration of aqueous extract.

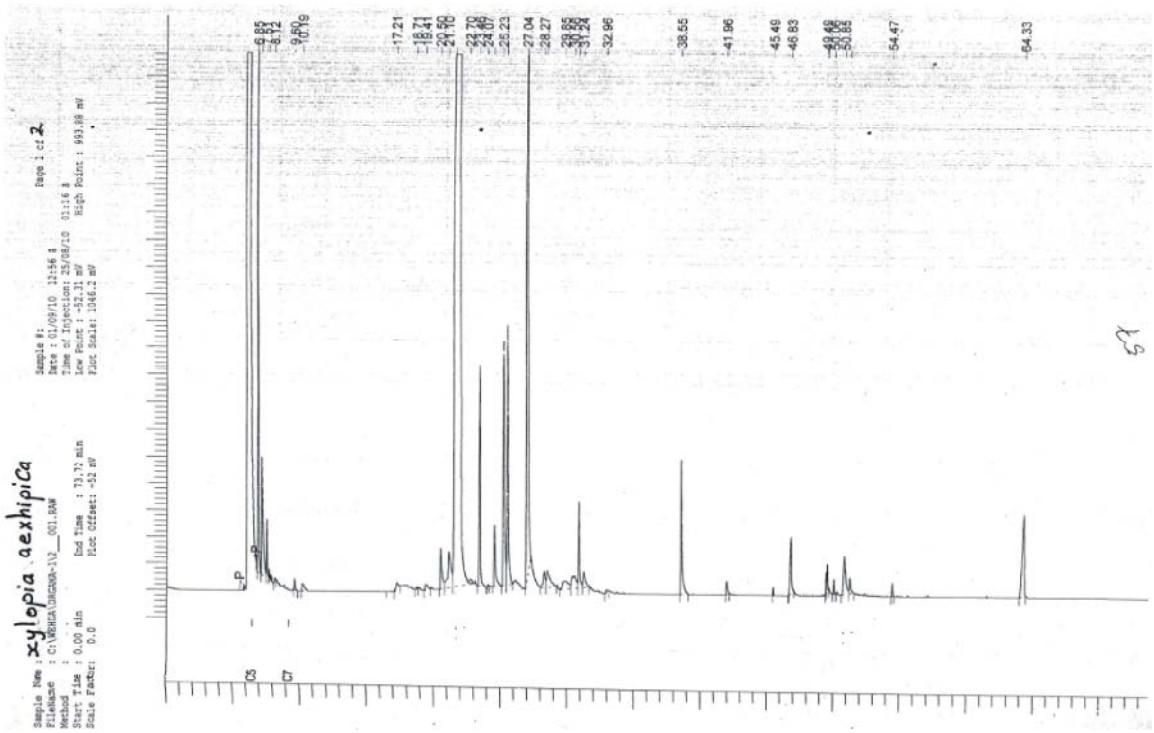

Fig. 1: GC-MS Chromatogram of volatiles in the hydrodistilled oil of Sudanese *Xylopia aethiopica* fruits

Table 3: Effect of panadol and aqueous extract of Sudanese *Xylopia aethiopica* on liver functions.

Parameters									
----------	AST	ALT	ALP	GGT	T. Prot.	Alb	T. bil	D. bil	Ind. bil
Groups	---------- (U/L) ----------				---------- (g/dl) ----------		---------- (mg/dl) ----------		
Control	61.57 ^{a} ±4.28	16.43 ^{a}±1.51	169.09^{a}±7.54	23.20 ^{a}±1.63	6.74 ^{a}±0.39	3.15 ^{a}±0.21	0.34 ^{a}±0.03	0.14 ^{a}±0.01	0.19 ^{a}±0.02
Extract	60.00 ^{a}±3.42	15.43 ^{a}±1.72	161.76 ^{a}±12.49	22.98 ^{a}±1.25	7.05 ^{a}±0.18	3.18 ^{a}±0.18	0.35 ^{a}±0.02	0.15 ^{a}±0.02	0.19 ^{a}±0.03
Panadol	85.00^{b}±5.48	39.57 ^{b}±2.64	240.19 ^{b}±8.49	34.19 ^{b}±1.15	5.97 ^{b}±0.18	2.88 ^{b}±0.19	0.50 ^{b}±0.02	0.24 ^{b}±0.02	0.26 ^{b}±0.02
Panadol + Ext.	73.86 ^{c}±3.24	29.86 ^{c}±2.41	200.59 ^{c}±7.41	29.45 ^{c}±1.31	6.19 ^{b}±0.27	2.90 ^{b}±0.18	0.42^{c}±0.01	0.20 ^{c}±0.01	0.22 ^{c}±0.03

Data presented as mean ± SE

Values in the same column with the same superscripts are not significant at ($P < 0.05$).

Biochemical Results

Liver and Kidney Functions: Table 3 shows plasma activities of alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphates (ALP), gamma-glutamyl transferase (GGT) and plasma levels of total proteins, albumin, total bilirubin, direct bilirubin and indirect-bilirubin of all studied groups. The results of liver function tests of the rats revealed that AST, ALT, ALP, GGT, total bilirubin, direct bilirubin and indirect bilirubin levels of rats treated with panadol were quite higher than that of control group. In contrast, the rats treated with *Xylopia aethipica* extract and panadol had significant reduction in the levels of AST, ALT, ALP and GGT, total bilirubin, direct bilirubin and indirect bilirubin levels when compared with the panadol group (Table 3). A tendency for decreasing concentrations for each of total proteins and albumin were noted in panadol intoxicated- rats as compared to normal control. No significant change was found in total protein and albumin levels in rats treated with *Xylopia aethipica* extract and panadol as compared with panadol intoxicated group. Results in Table 4 showed that panadol induced significant increase in the concentration of plasma urea and creatinine in intoxicated rats as compared to normal control rats. There was no significant difference in creatinine concentration between *X. aethiopica* supplemented rats and protected rats as compared with control. From ANOVA analysis, compared to control, significant decrease in plasma urea concentration of supplemented rats with insignificant difference in protected rats was noted.

Table 4: Effect of panadol and aqueous extract of Sudanese *Xylopia aethiopica* on kidney function

Parameters --- Groups	Urea (mg/dl)	Creatinine (mg/dl)
Control	37.78 ±1.59 [a]	0.71 ±0.04 [a]
Extract	33.20±2.22[b]	0.71 ±0.04 [a]
Panadol	42.69 ±1.08 [c]	0.76±0.03 [b]
Panadol + extract	38.970[a]±4.16 [a]	0.73 ±0.05 [a]

Data presented as mean ± SE

Values in the same column with the same superscripts are not significant at ($P < 0.05$).

Table 5: Effect of panadol and *X. etheapia* extract on some antioxidant biomarkers

Biomarker --- Groups	Total antioxidant capacity (TAC) mM/L	Catalase (CAT) U/ml	Glutathione peroxidase (GPx) U/ml
Control	1.15± 0.13[a]	386.17±28.02[a]	0.16±0.03[a]
Extract	1.80±0.15[b]	385.27±38.07[a]	0.22± 0.04[b]
Panadol	0.92±0.06[c]	198.91±19.54[b]	0.12±0.02[c]
Panadol + Ext.	1.80± 0.15[a]	282.04±41.60[c]	0.15±0.02[a]

Data presented as mean ± SE

Values in the same column with the same superscripts are not significant at ($P < 0.05$).

Antioxidant Biomarkers: The effect of aqueous extract of *Xylopia aethiopica* fruits and the administration of panadol on some antioxidant biomarkers (plasma total antioxidant capacity (TAC), catalase activity (CAT) and cellular glutathione peroxidase activity (GPx)) are shown in Table 5. ANOVA analysis indicated that rats which were supplemented with aqueous extract (group 2) recorded a significant increase in TAC and GPx with non significant change in CAT activity compared to control. Oral administration of panadol recorded significant decrease in TAC, CAT and GPx compared to normal control rats. Rats supplemented with aqueous extract and then administrated with panadol showed a significant increase in TAC, CAT and GPx levels compared with panadol intoxicated rats. On the other hand, compared to normal control, the values of all parameters in the groups given aqueous extract (protected group) return back to near control values.

Histological Results: The hepatic lobules are the structural units of the liver; each is formed of cords of hepatocytes and blood sinusoids in-between (Fig. 2-A). The hepatocytes are polyhedral cells with one or rarely two spherical nuclei and abundant cytoplasm. The cytoplasm of such cells is granular and strongly eosinophilic. The nuclei of the hepatocytes are large with peripherally dispersed chromatin and prominent nucleoli. The cell boundary has three domains: perisinusoidal, intercellular and pericanalicular. Hepatocytes are oriented in cords composed of a single row of cells separated from vascular sinusoids by endothelial cells. Photomicrographs of liver of rats treated with *Xylopia aethiopica* showed normal structure (Fig. 2-B). Administration of Panadol showed several apoptotic cells, focal necrosis associated with lymphocytic infiltration (Fig.2-C). On the other hand, liver of rats treated with Panadol showed portal tracts with dilated and congested veins. Periportal necrosis of the hepatocytes that surround the portal areas and inflammatory infiltration were also seen (Fig. 2-D). Liver of rats treated with *Xylopia aethiopica* and panadol showed normal structure (Fig. 2-E). In some rats focal necrosis associated with lymphocytic infiltration was noticed (Fig. 2-F).

Histochemical Results

Liver Total Proteins: Examination of sections of the liver of the control rats displayed the proteinic inclusions in the hepatocytes as grayish blue irregular particles of various sizes against weakly to moderately stained ground cytoplasm. The nuclear chromatin and the nucleoli are densely stained indicating their rich content of proteinic constituents (Figure 3-A). Oral administration *of Xylopia aethiopica* extract *showed* normal distribution of the protein inclusions in the hepatocytes (Fig. 3-B). Treatment with oral dose of panadol caused the protein inclusions to become smaller in size than those of the control rats (Fig. 3-C). Oral administration of panadol plus *Xylopia aethiopica* displayed diffuse stainability. A few number of the hepatocytes display dense stainability than the others (Fig. 3-D).

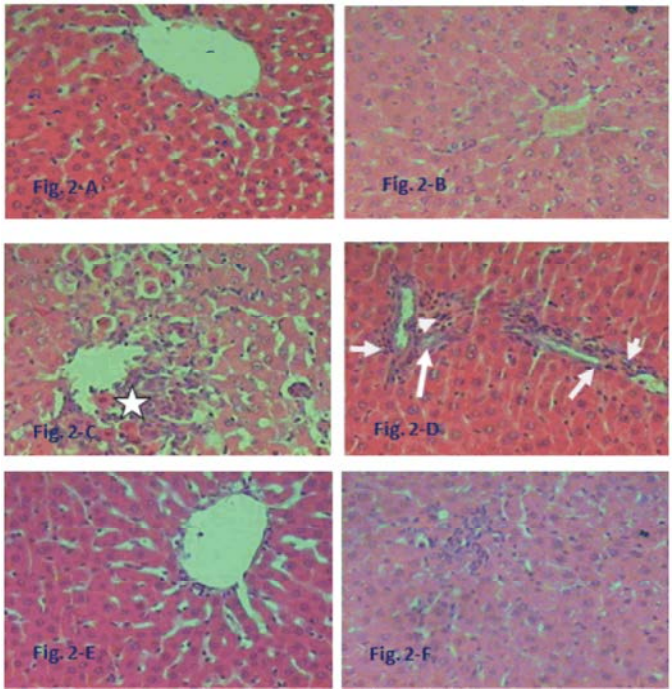

Fig. 2: Sections of liver of A) control rat shows the normal architecture of a hepatic lobule, B) rat treated with *Xylopia aethiopica* shows normal structure, C) rat treated with Panadol shows several apoptotic cells (arrows) focal necrosis (arrowheads) associated with lymphocytic infiltration (asterisk), D) rat treated with Panadol shows a portal tract with dilated and congested vein (arrow). Notice the periportal necrosis of the hepatocytes that surround the portal area (long arrow) and the inflammatory infiltration (arrowhead), E) rat treated with *Xylopia aethiopica* and Panadol shows normal structure, F) rat treated with *Xylopia aethiopica* and Panadol shows normal structure. Notice focal necrosis associated with lymphocytic infiltration (H & E stain-X 300)

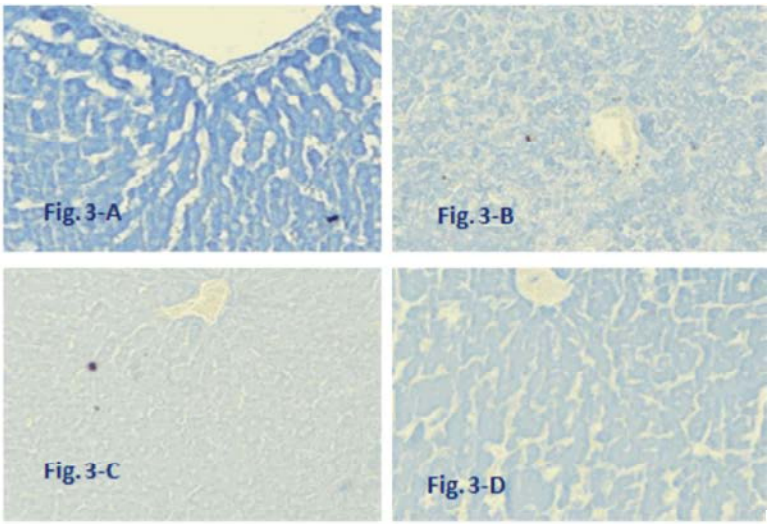

Fig. 3: Sections of liver of A) control rat shows the normal distribution of proteinic contents, B) rat treated with *Xylopia aethiopica* shows the stainability of the proteinic inclusions that relatively diffused in both of the cytoplasm and nucleus, C) rat treated with Panadol shows the proteinic inclusions distribution. Notice that these inclusions are diminuted and acquire pale stainability in the hepatocytes, D) rat treated with *Xylopia aethiopica* and Panadol showing the stainability of the proteinic inclusions that relatively diffused in both of the cytoplasm and nucleus (Bromophenol blue reaction- X 400)

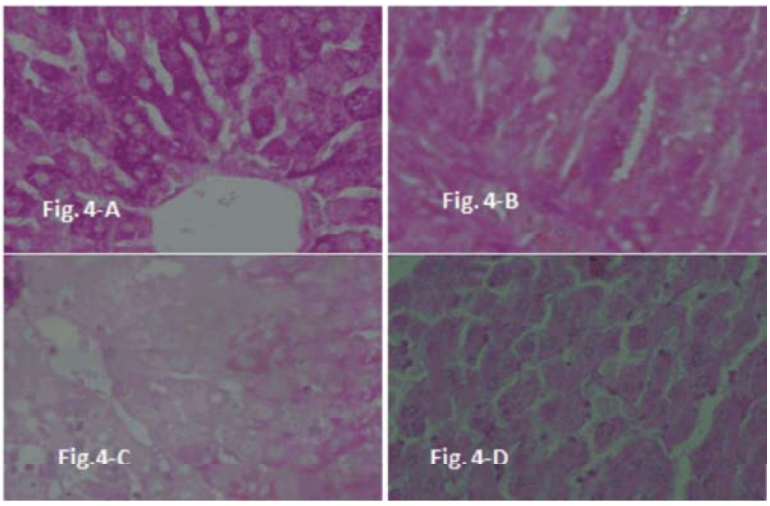

Fig. 4: Sections of liver of A) control rat shows the normal abundance of glycogen in the cell of the hepatic lobule, B) rat treated with *Xylopia aethiopica* showing normal distribution of the polysaccharide inclusions in the hepatocytes, C) rat received Panadol a single shows marked depletion of the polysaccharide inclusions, D) rat treated with *Xylopia aethiopica* and panadol showing the polysaccharides inclusions. Notice that such inclusions displayed diffuse stainability. A few number of the hepatocytes display dense stainability than the others (PAS/H- X 300)

Liver Polysaccharides: Examination of liver thin sections of control rat stained according to Periodic Acid Schiff`s technique (PAS) showed the abundance of polysaccharide materials in the hepatocytes. The nuclei of the hepatocytes a negative Periodic Acid Schiff`s reaction indicating the absence of polysaccharides (Fig. 4-A). Oral administration of *Xylopia aethiopica* plant caused normal distribution of the polysaccharide inclusions in the hepatocytes (Fig. 4-B). Daily treatment with an oral dose of panadol induced faint homogeneous stainability of the polysaccharide inclusions in the hepatocytes of the administrated rats (Fig. 4-C). Oral administration with panadol plus *Xylopia aethiopica* displayed diffuse stainability. A few number of the hepatocytes display dense stainability than the others (Fig. 4-D).

DISCUSSION

It has long been recognized that naturally occurring substances in higher plants have antioxidant activity. Among those substances, the phenolic compounds that are widely distributed in plants have the ability to scavenge free radicals, superoxide and hydroxyl radicals by single-electron transfer. Our results revealed that water extract of *Xylopia aethipica* fruits possesses high amount of phenolic compounds (2650±4.0 mg GAE/L). Antioxidants exert their activity through various mechanisms, including chelating ferrous iron, degrading peroxide and scavenging free radicals [32]. The free radical-scavenging activity of water extract of *X. aethiopica* was determined by β- carotene and DPPH tests. These tests are used as a measure of the ability of the extract, or any other antioxidant, such as TBHQ, to scavenge any free radical [33]. Our results showed excellent radical scavenging activity of the water extract of *X. aethiopica* compared with the synthetic antioxidant TBHQ. The results obtained in the present study are consistent with previous results reported, Konan *et al*. [34]. In the present study, thirty five volatile compounds were identified in *X. aethiopica* fruits. The main chemical compounds are (cis-linalool oxide 47.11%), (Carveol 13, 2), (buten-1-ol 9.05%), (iso-borneol 4.91%) (Borneol 4.14%) with a minor constituents of (sabinene 1.77%), (β-pinene 0.22%) and (linalool 0.18%). The composition of fruit essential oil of *X. aethiopica* has given in the literature, shows that it is constituted of monoterpenes hydrocarbon. These compounds are represented mainly by β-pinene which is the major constituent of the sample plant extract as reported by Rodolfo and Kwon [35], Karioti and Hadjipavlou-Litina [36], Koba and Sanda [37] and Poitou *et al.* [38]. More than 60 compounds were identified in four samples of *X. aethiopica* essential oils which show the complexity of this natural extract, the main chemical compounds are: β-pinene, β-phellandrene, 1,8-cineole, α-pinene, terpinen-4-ol and germacrene D present in different concentration in sample [39]. It was possible

using GC-MS and NMR investigations to unambiguously identify the 13-epi manoyl oxide, a diterpene which is reported for the first time in *X. aethiopica* essential oils. Nevertheless due to its very low concentration in all analyzed hydrodistillates it is not established that this molecule could play a significant role in the essential oil activity [40]. Keita *et al.* [41] reported that volatile compounds of *Xylopia aethiopica* from dried fruits and the powder obtained from crushed dried fruits analyzed by combined GC and GC/MS, contain mainly (beta-pinene 19.1%), (gamma-terpinene 14.7%), (trans-pinocarveol 8.6%) and (p-cymene 7.3%). Regarding, the volatile compounds from the powder, the major constituents were (beta-pinene 9.9%), (alpha-cadinol 6.9%), (Trans-pinocarveol 4.6%), (alpha-pinene 4.1%) and (1, 8-cineole 4.0%). According to Poitou *et al*. [38] Germacrene D is the most important sesquiterpene and the oxygenated compounds are mainly 1, 8-cineole and terpinen-4-ol. A survey undertaken on *X. aethiopica* volatile compounds from Egypt, showed very particular composition with more than two third of oxygenated compounds (23.4% of terpinen-4-ol, 16.3% of 1,8-cineole and 1% of α-terpineol) [42]. Similar composition with oxygenated monoterpenes (15.1% of 1, 8-cineole, 6.6% of terpinen-4-ol) has been reported in essential oil from Nigeria [43]. The essential oil has been well characterized with linalool, trans-ocimene, α-farnesene, α-pinene, β-pinene, myrtenol, phellandrene and 3-ethylphenol as the major volatile constituents [3]. Researchers noted that the intense 'pepperish note' of the oil of the fruit is largely due to the presence of linalool which provides the characteristic aroma of the ground, dried, smoked fruit.

The main constituents of *Xylopia aethiopica* volatiles were differ found to be from what is present in the literature [39, 40]. This is believed to be due to many external and internal factors that affect the constituents of the volatile compounds, affecting the plant such as: environmental and climate conditions, season of collection, age of plants, the stage of ripening of the fruits or genetic data [44] It is well known that oxygenated terpenes exhibited a higher antioxidant power in comparison to the other identified classes [45]. Presence of many volatile compounds that posses strong antioxidant activity beside the high concentration of total phenolic content in *X. aetheopica* extract may be responsible for enhancing the antioxidant activity *in vivo* as well as protection of the kidney and liver cells through scavenging free radicals produced from panadol overdose. It has been well established that elevated levels of AST, ALT, ALP and GGT are indicative of cellular leakage and loss of functional integrity of hepatic cell membranes implying hepatocellular damage. Plasma total protein and bilirubin levels on the other hand are related to the function of the hepatic cells [46]. Therefore, we investigated the hepatoprotective activity of aqueous extract of *X. aetheopica* by measuring all the previous parameters in panadol intoxicated rats. Some antioxidant parameters as CAT, GPx, TAC as well as kidney function; (urea and creatinine concentration) were also measured. Paracetamol (panadol) is a common antipyretic agent which is safe in therapeutic doses but can produce fatal hepatic necrosis in toxic doses [47]. It was observed that oral administration of panadol produced liver damage in rats as manifested by decreasing in plasma total proteins and albumin with increasing in plasma bilirubin concentration and activities of liver marker enzymes. Our results are in agreement with others reported in the literature which found increased plasma AST, ALT, ALP and GGT activities, in animals treated with hepatotoxic dose of panadol [48, 49]. The marked elevation of plasma marker enzyme activities observed in rats treated with panadol alone may be explained in terms of liver injury mediated by increased lipid peroxidation. It is well established that administration of toxic doses of panadol produces a large amount of a highly reactive metabolite called N-acetyl-pbenzoquinone imine (NAPQI), through the activation of hepatic cytochrome P-450 enzymes [50]. By the process of one electron reduction, NAPQI may further be converted to semiquinone radicals, which possess a remarkable ability of attacking polyunsaturated fatty acids, causing hepatic lipid peroxidation with resultant liver injury [49]. The protection of rats with *water extract of X. aethiopica* appears to produce a mitigating effect on panadol-induced liver injury. Evidence for this observation lies in the fact that *X. aethiopica* extract was able to produce a significant reduction ($p < 0.05$) in paracetamol-induced increases in plasma bilirubin concentration and activities of AST, ALT, ALP and GGT. As shown in the present study water extract of *X. aethiopica* contain high concentration of total phenolic compounds and oxygenated volatile compounds such as cis-linalool oxide, Carveol, buten-1-ol, iso-borneol, Borneol, thymol, beta-citronellol, geriniol, dihydrocarveol, linalool, which have strong antioxidant activity. These strong antioxidants might be responsible for the observed anti-hepatotoxic potential of *X. aethiopica* extract. On the basis of the fact that the phenolic compounds and terpens are known for their properties to trap the free radicals [34, 51], it is well-documented that polyphenoles are good hepatoprotective

agents because they can effectively inhibit lipid peroxidation, scavenge free radicals and enhance antioxidant enzyme activities [52] as well as it is well known that oxygenated volatile compounds exhibited a high antioxidant power [45]. Phytochemicals in plants have received a great deal of attention mainly due to their role in preventing diseases caused as a result of oxidative stress [53]. The fruits of *X. aethiopica* contain many photochemical compounds. These photochemical compounds exhibit a wide range of biological effects as a consequence of their antioxidant properties [54]. Excessive use of panadol can damage kidneys, causes renal tubular damage, uremia and produces acute tubular necrosis [55, 56]. The kidneys are organs with several functions; they serve the body as a natural filter of the blood and remove wastes which are diverted to the urinary bladder. In producing urine, the kidneys excrete wastes such as urea and ammonium; the kidneys also are responsible for the reabsorption of water, glucose and amino acids. Panadol caused severe nephrotoxicity, Abdul Hamid *et al*. [57] pointed out that panadol toxicity enhance the production of its reactive metabolite, N-acetyl-p-benzoquinoneimine (NAPQI).

The accumulated NAPQI induces greater formation of free radicals, which are responsible in mediating cellular damages and renal toxicity. Kidney function tests help to determine if the kidneys are performing their tasks adequately [58]. Our results showed significant increase in plasma urea and creatinine in panadol toxicated group that was in accordance with Mandal *et al.* [55] and Sharma and Sharma [59]. The results of this study showed that renal dysfunction in panadol toxicity were attenuated by the use of *X. aethiopica* water extract. The extract played an important role to turn back urea and creatinine levels to near normal control level. The amount of antioxidant compounds present in *X. aethiopica* contributes significantly to its antioxidant property. The antioxidant properties of the plant *X. aethiopica* may suggest the potential applications as an alternative antidote against panadol-induced nephrotoxicity, as well as a novel antioxidant source. *X. aethiopica* can be used to reduce renal damage and may serve as an alternative medicine in protection of kidneys.

CONCLUSION

Aqueous extract of *Xylopia aethiopica* fruit is rich in phenolics and volatiles compounds, which act as strong natural antioxidants. The aqueous extract normalized the toxic effect of panadol-induced hepatic and renal toxicity. *X. aethiopica* possesses nutritional and medicinal values.

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