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Mechanism of hepatoprotective potential of aqueous leaves extract of *Eucalyptus obliqua* (Myrtaceae) in carbon tetrachloride intoxicated Wistar rats

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ABSTRACT

This study investigated the protective mechanism of aqueous leaves extract of *Eucalyptus obliqua* (EOAE) in CCl_4-mediated liver damage in Wistar rats. The animals were orally pretreated with either the extract (200 and 400 mg/kg) or vitamin C (200 mg/kg) for 10 days prior to the intraperitoneal administration of 3 mL/kg CCl_4 (30% in olive oil). Subsequently, hepatic function, antioxidant and histological analyses were evaluated. The results showed that the CCl_4-induced increases in the activities of ALT, ALP, AST and the concentrations of bilirubin, oxidized glutathione and malondialdehyde were significantly and dose-dependently reduced in the extract-treated rats. The extract also significantly improved the attenuated activities of superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase as well as total protein, albumin and glutathione concentrations in the hepatotoxic animals. These improvements could be attributed to the phytoconstituents revealed by the GCMS analysis of the extract. The observed activities compared with that of vitamin C and are suggestive of hepatoprotective and antioxidant properties of EOAE and were also supported by the histological results. Overall, these data indicate that EOAE has a significant protective effect against acute CCl_4-induced hepatotoxicity in rats, which may be due to its capability to fortify the antioxidant defense systems.

INTRODUCTION

Carbon tetrachloride (CCl_4) is an experimentally established agent to induce hepatic damage in animals (Adekeye *et al.*, 2014; Balogun and Ashafa, 2016). CCl_4-mediated hepatotoxicity is caused primarily through oxidative stress resulting from free radicals chain events. The free radicals and other reactive metabolites formed subsequent to CCl_4 administration results in impaired endoplasmic reticulum and altered permeability of the mitochondrial membrane of the hepatocytes. This consequently facilitates accumulation of lipids, reduction of protein synthesis and oxidative stress-perturbed hepatocellular necrosis (Weber *et al.*, 2003). While the effectiveness of conventional treatment options (life style changes, medications, and surgery) for hepatic disorders are guaranteed, affordability, non-compliance, limited efficacy and potentially life-threatening side effects have undermined their uses (Ward and Daly, 1999). However, botanicals with significant hepatoprotective potentials are readily available, more affordable, easily accessible and often with minimal side effects and are being explored globally as alternative therapy (Mahmud *et al.*, 2012; Sabiu *et al.*, 2016). The continuous search for hepatoprotective phytoagents might be due in part, to their excellently displayed antioxidant properties in preventing undesirable auto oxidation either by halting harmful influence of reactive metabolites or simply as

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reactive oxygen species scavengers. Interestingly, *Eucalyptus obliqua* is one of the plants that have been advocated in the management of liver ailments.

Eucalyptus obliqua L.Her. (Myrtaceae) Blakely is an evergreen tree of the Australian origin with characteristic fast growing features. It is an exclusive specie of the eucalyptus family where other species derived their common trait of having oblique leaves. Besides being ornamental, *E. obliqua* is used to drain swamps and also very effective as an antimalarial (Barry *et al.*, 2015). In the traditional systems of medicine, leaf infusions of *E. obliqua* are highly ethnomedicinally valued in the treatment of malaria fever, inflammation, diabetes mellitus and liver disorders (Barry *et al.*, 2015; Sabiu and Ashafa, 2016). Additionally, results of our ethnopharmacological survey of medicinal plants of the Local Government Areas of Kwara State, Nigeria, also presented *E. obliqua* as a botanical that is used to manage and treat liver ailments. In view of this and coupled with no previous scientific reports on its potential to treat hepatic diseases, the present study investigated the probable mechanism of the hepatoprotective effect of its aqueous leaves extract in CCl_4-perturbed hepatotoxicity in Wistar rat model. The GC-MS analysis of the extract was also performed.

MATERIALS AND METHODS

Chemicals, reagents and assay kits

All the chemicals, reagents and assay kits used were of analytical grade.

Plant collection, authentication and extract preparation

Fresh leaves of *Eucalyptuc obliqua* (EO) were collected from the Kwara State Ministry of Agriculture, Ilorin, Nigeria, in the month of April, 2014. Taxonomical authentication was done by Mr. Bolu (Chief Taxonomist) of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria and a voucher specimen (Reference no. UIH001/1178) was deposited in the Departmental herbarium. The leaves of EO were carefully rinsed under running water tap to remove foliar contaminants prior to air-drying to constant weight. Subsequently, the leaves were pulverized into coarse powder using an electric blender (model MS-223; Blender/Miller III, Taiwan, China). A portion (300 g) of the powdered sample was exhaustively extracted with continuous agitation in distilled water. The resulting infusion was filtered (Whatman no. 1 filter paper) and lyophilized using Virtis Bench Top lyophilizer (SP Scientific Series, USA). This yielded 37.8 g crude extract (EOAE) corresponding to 12.6% of the powdered sample.

Experimental animals

Subsequent to Departmental Ethical clearance (KSU/IECCULA/003/02/016) for the study, a total of thirty male Wistar rats (mean weight: 120 ± 5.2 g) were collected from the Animal Facility of the Biochemistry Laboratory, Kwara State University, Malete, Nigeria. The rats were housed in clean metallic cages, maintained under standard laboratory conditions and had *ad libitum* access to standard pellets and potable water. They were allowed ten days of acclimatization before being used for experimentation and the whole treatment was in accordance with the guidelines of National Research Council Guide for the Care and Use of Laboratory Animals (NRC, 2011).

Experimental protocol

Induction of hepatotoxicity

Adopting the modified method of Lu *et al*. (2002) using 30% carbon tetrachloride dissolved in olive oil, hepatic damage was induced in rats. Briefly, the animals were intraperitoneally administered with a single dose (3 mL/kg) of the 30% CCl_4 solution.

Animal grouping and treatments

The animals were randomized into 6 groups of 5 rats for this experiment. Rats in group 1 were given sterile placebo (distilled water) and designated as control. Group 2 comprised animals (hepatotoxic control) induced with hepatotoxicity but not treated. Animals in group 3 received 400 mg/kg b.w. of EOAE only, while rats in groups 4, 5 and 6 were hepatotoxic rats pre-treated with EOAE (200 and 400 mg/kg b.w.) and vitamin C (200 mg/kg b.w.) respectively. Pre-treatments with the standard drug and extract were done once daily via oral intubation and lasted for 10 days prior to hepatotoxicity induction. A transition period of 24 h was observed after the last oral pre-treatment with the graded doses of EOAE and vitamin C before CCl_4 administration. Prior to the termination of the studies, the rats were fasted over night but had *ad libitum* access to water.

Serum preparation and excision of liver

Forty eight hours after CCl_4 treatment, the rats were humanely sacrificed by halothane anaesthetization and blood was collected by cardiac puncture into non-heparinized tubes. Serum was subsequently prepared, carefully aspirated and used for liver function tests. The rats were also quickly dissected and the liver isolated, blotted with clean tissue paper, cleaned of fat and sliced into two portions. A portion was homogenized in ice-cold Tris-HCl buffer (0.05 mol/L Tris-HCl and 1.15% KCl, pH 7.4) for antioxidant analyses, while the other portion was used for histological examination.

Liver function indices and antioxidant analyses

Liver function parameters were evaluated according to manufacturer's instructions on the assay kits. Serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) as well as albumin, bilirubin and protein concentrations were assayed. While the homogenate activities of glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GRx) were assayed using the methods of Rotruck *et al.* (1973), Marklund and Marklund (1974), Aebi (1984), and Thabrew *et al.* (1987)

respectively, level of malondialdehyde (MDA) was evaluated adopting the methods of Reilly and Aust (2001). The reported protocols of Ellman (1959) and Hissin and Hilf (1976) were employed in the determination of reduced glutathione (GSH) and oxidized glutathione (GSSG) concentrations, respectively.

Histopathological examination

The reported method of Drury and Wallington (1980) was adapted for the histopathological examination of the harvested liver sections. Microscopic features of the hepatocytes of EOAE and vitamin C-treated rats were compared with both normal and hepatotoxic control groups. Based on the degree of hepatic damage observed, the liver sections were further evaluated and scored by the histopathologist who was unaware of the experimental treatments. Scores were assigned on a 0 to 4 scale as earlier reported (Sabiu *et al.*, 2016).

GCMS analysis of the extract

Using an Agilent Technologies 6890 Series gas chromatograph coupled with an Agilent 5973 Mass Selective detector driven by Agilent Chemstation software, the GC-MS analysis of EOAE was performed. The operating conditions included: An eHP-5MS capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness), ultra-pure helium (carrier gas) at a flow rate of 1.0 mL/min and a linear velocity of 37 cm/s, injector temperature (300°C), initial oven temperature (10°C with flexibility of lower sensitivity) programmed to increase to 300°C at the rate of 10°C/min with a hold time of 4 min at each increment and the extract (2 µL) was injected in the splitless mode with a split ratio of 20:1. The mass spectrometer (MS) was operated in the electron ionization mode (70 eV) and electron multiplier voltage (1859 V). Other operating parameters for the MS were as follows: ion source temperature 230°C, quadruple temperature 150°C, solvent delay 4 min and scan range 50-700 amu. For the identification of the constituents of EOAE, direct comparison of the retention times, mass spectral data and fragmentation pattern of the compounds were made with those in the National Institute of Standards and Technology (NIST) library and Wiley libraries having more than 75,000 compounds. The name, structure, molecular weight and %composition of each constituent having super impossible factor value of greater than 90 was subsequently ascertained.

Data analysis

Results were subjected to one-way analysis of variance (ANOVA) using SPSS 16.0 (SPSS Inc., South Wacker Drive, Chicago, USA) and expressed as mean ± standard error of mean (SEM) (n = 5). Significant difference between the treatments mean were determined at $p < 0.05$, $p < 0.001$ and $p < 0.0001$ confidence levels using Duncan's Multiple Range Test.

RESULTS

The results of EOAE pre-treatments on the liver function indices of the animals are shown in Tables 1 and 2. While intraperitoneal injection of CCl_4 (3 mL/kg) caused significant ($p < 0.05$) elevation in the specific activities of AST, ALT, ALP and concentration of bilirubin, the levels of albumin and total protein were significantly reduced ($p < 0.05$) when compared with the normal control. However, the observed alterations in these liver function indices were significantly ($p < 0.05$) and dose-dependently reversed in the extract pre-treated rats. It is also noteworthy that the most prominent effect that compared well with the vitamin C treated animals were observed at the highest investigated dose (400 mg/kg b.w.) of EOAE (Tables 1 and 2). The rats placed on 400 mg/kg dose of the extract alone showed no traces of hepatotoxic tendencies as they compared well with the normal control for these parameters.

Table 1: Effect of *Eucalyptus obliqua* aqueous leaf extract on serum activities of liver function enzymes of CCl_4-treated rats.

Treatments	ALP (U/I)	ALT (U/I)	AST (U/I)
Sterile placebo (Control)	175.09 ± 1.32^a	23.15 ± 0.03^a	30.89 ± 0.05^a
CCl_4 treated	333.21 ± 1.08^b	70.99 ± 0.05^b	100.76 ± 0.05^b
400 mg/kg b.w. EOAE	173.45 ± 1.35^a	22.87 ± 0.05^a	29.99 ± 0.03^a
200 mg/kg b.w. EOAE+ CCl_4	222.21 ± 1.75^c	45.12 ± 0.03^c	50.89 ± 0.03^c
400 mg/kg b.w. EOAE+ CCl_4	174.21 ± 1.45^a	22.99 ± 0.04^a	32.09 ± 0.01^a
200 mg/kg b.w. vitamin C+ CCl_4	173.92 ± 1.09^a	31.09 ± 0.03^d	33.33 ± 0.08^a

(n = 5, Mean ± SEM). [a] represents not significantly ($p>0.05$) different from the normal control. [b] represents a significant difference at $p < 0.0001$ when compared to normal control, while [c] and [d] represent significant differences at $p < 0.001$ and $p < 0.0001$, respectively, when compared to the CCl_4-treated group alone. EOAE= *Eucalyptus obliqua* aqueous extract, ALT= alanine aminotransferase, AST= aspartate aminotransferase, ALP= alkaline phosphatase.

Table 2: Effect of *Eucalyptus obliqua* aqueous leaf extract on serum levels of albumin, bilirubin and total protein of CCl_4-treated rats.

Treatments	Albumin (g/L)	Bilirubin (µmol/l)	Protein (g/L)
Sterile placebo (Control)	5.95 ± 0.05^a	12.01 ± 0.05^a	35.88 ± 0.05^a
CCl_4 treated	0.23 ± 0.03^b	53.99 ± 0.04^b	13.24 ± 0.01^b
400 mg/kg b.w. EOAE	5.81 ± 0.02^a	19.25 ± 0.09^c	34.55 ± 0.05^a
200 mg/kg b.w. EOAE+ CCl_4	2.24 ± 0.02^c	33.73 ± 0.05^d	25.17 ± 0.04^c
400 mg/kg b.w. EOAE+ CCl_4	6.01 ± 0.06^a	11.57 ± 0.25^a	33.24 ± 0.04^a
200 mg/kg b.w. vitamin C+ CCl_4	5.89 ± 0.10^a	11.60 ± 0.25^a	33.56 ± 0.01^a

(n = 5, Mean ± SEM). [a] represents not significantly ($p>0.05$) different from the normal control. [b] represents a significant difference at $p < 0.0001$ when compared to normal control, while [c] and [d] represent significant differences at $p < 0.001$ and $p < 0.0001$, respectively, when compared to the CCl_4-treated group alone. EOAE= *Eucalyptus obliqua* aqueous extract.

Tables 3 and 4 show the effects of oral pre-treatments with EOAE and subsequent intraperitoneal CCl_4 treatment on the antioxidant biomarkers (SOD, CAT, GPx, GRx, GSH and GSSG) and MDA in the treated rats. CCl_4 treatment caused significant ($p < 0.05$) decreases in the hepatic tissue SOD, CAT, GPx, GRx, and GSH while causing significant ($p < 0.05$) increases in the GSSG and MDA. Oral pre-treatment with 200 and 400 mg/kg b.w. doses of EOAE significantly ($p < 0.05$) induced the hepatic activities of SOD, CAT, GPx, and GRx as well improved the GSH level in a dose-related manner.

The levels of GSSG and MDA were also significantly attenuated in the extract treated animals. These effects (particularly at 400 mg/kg b.w. of the extract) competed favourably with that of the vitamin C administered rats. The antioxidant status of the animals given 400 mg/kg b.w. of EOAE alone was also significantly ($p < 0.05$) boosted. Macroscopic examination of the liver sections from normal control group revealed that they were normal with characteristic dark maroon colour and smooth texture. While the liver from the CCl_4-intoxicated rats showed changes in colour from maroon to brown with characteristic uneven texture, those of the extract-treated animals revealed mild spots of brown colour changes. Furthermore, detailed histoarchitectural examination of the liver of the control rats showed normal morphological features with well-preserved cords of hepatocytes, well demarcated sinusoids and no area of infiltration by inflammatory cells (Figure 1a). This is in contrast to the hepatic centrilobular vacuolation and vascular congestion indicative of early hepatic necrosis observed in the untreated CCl_4 injected animals (Fig. 1b). However, oral pre-treatments with vitamin C and the extract for 10 days revealed significantly preserved and attenuated CCl_4-induced histological lesions of hepatic necrosis (Figs. 1c-f). Furthermore, the histopathological grading of the liver tissues of the extract-administered groups showed that the obvious necrosis, inflammation, and haemorrhage present in the hepatocytes of the CCl_4-intoxicated rats were significantly and dose-dependently prevented in a manner comparable to the vitamin C administered animals (Table 5). Results from the GC-MS analysis of the extract revealed the presence of eucalyptol, ursolic acid, thymol, 3-hexen-1-ol, triterpenoic acid, isopinocarveol, 1,8-cineole, α-pinene, quercetin and procyanidins as major identifiable adaptogenic constituents (Table 6).

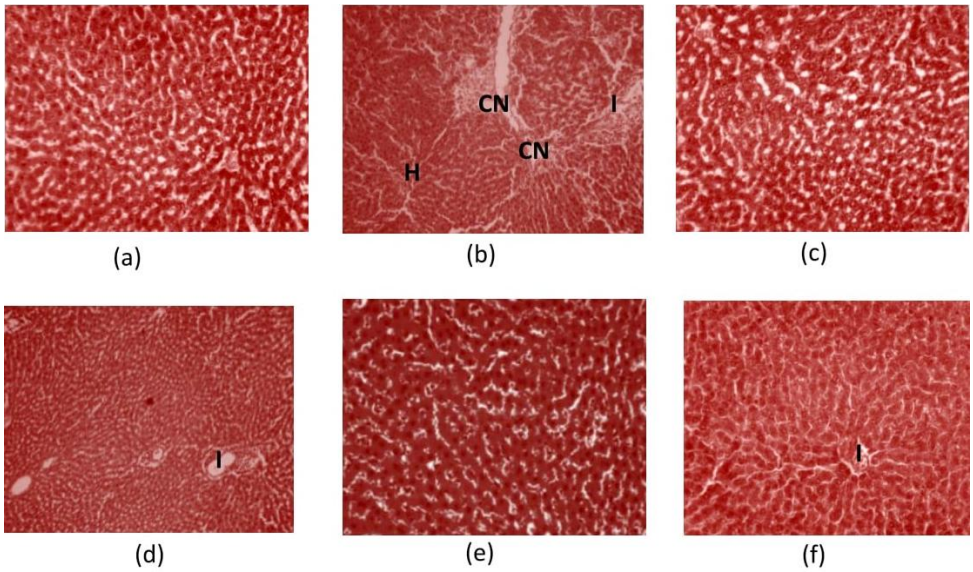

Fig. 1: Liver micrograghs (x 400, Haematoxylin and eosin stained) of (a) Normal control rat, (b) Hepatotoxic rat, (c) EOAE (400 mg/kg b.w) treated rat, (d) Hepatotoxic rat pre-treated with EOAE (200 mg/kg b.w), (e) Hepatotoxic rat pre-treated with EOAE (400 mg/kg b.w), (f) Hepatotoxic rat pre-treated with vitamin C (200 mg/kg b.w). EOAE= *E. obliqua* aqueous extract, I= inflammation, CN= coagulative necrosis, H= haemorrhage

Table 3: Effect of *Eucalyptus obliqua* aqueous leaf extract on specific activities of enzymic antioxidant system of CCl_4-treated rats.

	Antioxidant enzymes (nmol min^{-1} mgprotein^{-1})			
Treatments	**SOD**	**Catalase**	**Glutathione Rx**	**Glutathione Px**
Sterile placebo (Control)	55.03 ± 1.25^a	44.11 ± 1.05^a	68.01 ± 1.25^a	249.99 ± 1.45^a
CCl_4 treated	23.09 ± 1.30^b	13.99 ± 1.11^b	27.09 ± 1.30^b	100.01 ± 1.09^b
400 mg/kg b.w. EOAE	70.09 ± 1.21^c	59.99 ± 1.09^c	70.44 ± 1.23^a	269.45 ± 1.12^c
200 mg/kg b.w. EOAE+ CCl_4	45.23 ± 1.09^d	32.98 ± 1.05^d	43.01 ± 1.44^c	210.99 ± 1.10^d
400 mg/kg b.w. EOAE+ CCl_4	53.67 ± 1.23^a	43.56 ± 1.22^a	69.45 ± 1.23^a	247.99 ± 1.09^a
200 mg/kg b.w. vitamin C+ CCl_4	52.01 ± 1.25^a	42.99 ± 1.26^a	68.89 ± 1.45^a	236.19 ± 1.99^e

(n = 5, Mean ± SEM). [a] represents not significantly ($p>0.05$) different from the normal control. [b] represents a significant difference at $p < 0.0001$ when compared to normal control, while [c, d] and [e] represent significant differences at $p < 0.05$, $p < 0.001$ and $p < 0.0001$, respectively, when compared to the CCl_4-treated group alone. EOAE=*Eucalyptus obliqua* aqueous extract, SOD= superoxide dismutase, Rx= reductase, Px= peroxidase.

Table 4: Effect of *Eucalyptus obliqua* aqueous leaf extract on the levels of non-enzymic antioxidant system and MDA of CCl_4-treated rats.

Treatments	Reduced glutathione(X)	Peroxidized glutathione (X)	GSH: GSSG ratio	MDA (nmol mgprotein^{-1})
Sterile placebo (Control)	45.12 ± 0.09^a	0.16 ± 0.05^a	282.00^a	10.42 ± 0.13^a
CCl_4 treated	12.09 ± 0.05^b	2.90 ± 0.07^b	4.17^b	28.43 ± 0.12^b
400 mg/kg b.w. EOAE	33.42 ± 0.04^c	0.13 ± 0.10^c	257.08^c	10.22 ± 0.12^a
200 mg/kg b.w. EOAE+ CCl_4	43.99 ± 0.04^a	0.19 ± 0.02^a	231.53^d	14.73 ± 0.13^c
400 mg/kg b.w. EOAE+ CCl_4	44.01 ± 0.05^a	0.17 ± 0.07^a	258.88^c	10.53 ± 0.12^a
200 mg/kg b.w. vitamin C+ CCl_4	45.19 ± 0.05^a	0.18 ± 0.03^a	251.06^c	10.45 ± 0.14^a

(n = 5, Mean ± SEM). [a] represents not significantly ($p>0.05$) different from the normal control. [b] represents a significant difference at $p < 0.0001$ when compared to normal control, while [c] represents significant difference at $p < 0.001$ when compared to the CCl_4-treated group alone. EOAE=*Eucalyptus obliqua* aqueous extract, X= nmol mg protein^{-1}

Table 5: Histopathological grading of liver tissue sections of *Eucalyptus obliqua* aqueous leaf extract treated animals.

Treatments	Scores				
	0	**1**	**2**	**3**	**4**
Control	(5)	(0)	(0)	(0)	(0)
CCl_4 treated	(0)	(0)	(1)	(1)	(3)
400 mg/kg of EOAE.	(5)	(0)	(0)	(0)	(0)
200 mg/kg b.w. EOAE+ CCl_4	(2)	(2)	(1)	(0)	(0)
400 mg/kg b.w. EOAE+ CCl_4	(4)	(1)	(0)	(0)	(0)
200 mg/kg b.w. vitamin C+ CCl_4	(4)	(1)	(0)	(0)	(0)

(n= 5; figure in parenthesis represents number of rats affected in the group). EOAE=*Eucalyptus obliqua* aqueous extract

Table 6: Bioactive constituents of *E. obliqua* aqueous leaf extract as revealed by GC-MS chromatogram.

Peak	Constituent	RT (Min)	Area %	MF	MW (g/mol)
1	Drimenol	2.63	6.97	$C_{15}H_{26}O$	222.37
2	Eucalyptol[#]	3.53	1.25	$C_{10}H_{18}O$	154.25
3	Ursolic acid[#]	4.20	1.49	$C_{30}H_{48}O_3$	456.70
4	Lactone acetate	4.68	2.40	$C_{24}H_{36}O_4$	59.04
5	Thymol[#]	5.13	1.25	$C_{10}H_{14}O$	150.22
6	Cis-nerolidol*	5.38	2.52	$C_{15}H_{26}O$	222.37
7	Cis-geranoil*	6.05	4.82	$C_{10}H_{18}O$	154.25
8	3-hexen-1-ol	6.58	6.40	$C_6H_{12}O$	100.16
9	Eucalyptanoic acid*	7.05	2.94	$C_{30}H_{46}O_3$	354.31
10	Triterpenoic acid[#]	7.30	4.95	$C_{30}H_{48}$	456.71
11	Spathunelol	7.57	1.80	$C_{15}H_{24}O$	220.35
12	Isopinocarveol*	7.77	5.35	$C_{10}H_{18}O$	154.25
13	α-pinene[#]	8.42	8.15	$C_{10}H_{16}$	136.24
14	1,8-cineol[#]e	9.13	5.99	$C_{10}H_{18}O$	154.25
15	P-cymene*	9.42	2.34	$C_{10}H_{14}$	134.21
16	Linalool*	9.73	3.99	$C_{10}H_{18}O$	154.25
17	γ-terpinene*	10.13	4.77	$C_{10}H_{16}$	136.23
18	Quercetin[#]	10.85	7.01	$C_{15}H_{10}O_7$	302.24
19	α-terpineol[#]	11.10	2.55	$C_{10}H_{18}O$	154.25
20	Terpinen– 4– ol*	11.38	3.10	$C_{10}H_{18}O$	154.25
21	Euglobals*	12.05	5.46	$C_{23}H_{30}O_5$	386.48
22	Aromadendrene*	12.66	7.60	$C_{15}H_{24}$	204.35
23	Bicyclogermacrene*	13.25	2.43	$C_{15}H_{24}$	204.35
24	β-phellandrene*	13.98	3.06	$C_{10}H_{16}$	136.24
25	β-bisabolene*	14.93	1.05	$C_{15}H_{24}$	204.36
26	Procyanidins[#]	15.42	0.34	$C_{30}H_{26}O_{12}$	578.52

*compounds with known antioxidant potential, [#]compounds with known antioxidant and hepatoprotective potentials. RT= retention time, MF= molecular formula, MW= Molecular weight.

DISCUSSION

The liver is naturally endowed with the major task of drug detoxification and excretion. Many toxicants have been studied and established to induce varying degrees of hepatic injury (Sabiu *et al.*, 2016). One of such potent hepatotoxicant is the CCl_4. The CCl_4-induced hepatic injury is a classical and reliable model

of xenobiotic-induced hepatotoxicity that has been well studied and adopted to screen drugs for their possible hepatoprotective potential (Balogun and Ashafa, 2016). It is metabolically activated by the hepatic CYP2E1 to form CCl_3^* radical, which spontaneously reacts with molecular oxygen, forming trichloromethyl peroxy radical (CCl_3OO^*), that subsequently interacts with important macromolecules (lipids, proteins and DNA) to induce auto-oxidation (Risal *et al.,* 2012). This results in ROS formation that promotes functional loss of membranal integrity, alters enzyme activities, and consequently results in hepatic injury or necrosis (Rajib *et al.,* 2009). When these happen, cytosolic AST, ALT and ALP are released into systemic circulation and their measurement can be used to assess the extent of drug-induced hepatotoxicity (Jaeschke *et al.,* 2003). In this study, the elevated activities of these marker enzymes in the CCl_4-intoxicated rats may be indicative of liver damage and cell necrosis resulting from formation of CCl_3^* in excess of GSH detoxification capacity. This is in agreement with previous studies (Surendra *et al.,* 2012; Balogun and Ashafa, 2016), where CCl_4 administration proved toxic to hepatocytes. However, the significantly and dose-dependently reduced specific activity of these enzymes in rats treated with EOAE suggests that it was able to prevent the harmful effects of CCl_4. This observation indicates hepatoprotective potential of the EOAE at the tested doses.

While the biological value of bilirubin has been employed to assess the excretory role of the liver (Tietz, 1995), the metabolic alterations in the serum concentrations of albumin and total protein are used to monitor its secretory capability (Oloyede and Sunmonu, 2009). In the present study, the significantly increased serum level of bilirubin in the untreated hepatotoxic rats could be associated with CCl_4-mediated defect in the carrier-mediated saturable system at the sinusoidal surface of the hepatocytes that consequently obstruct bilirubin uptake and secretion into bile (Sabiu *et al.,* 2014). Similarly, the CCl_4-mediated significant reduction in the levels of albumin and total protein may be suggestive of diminished synthetic function of the liver (Sabiu *et al.,* 2015). Conversely, the dose-dependent and significant improvements in albumin and total protein levels coupled with the reduced concentration of bilirubin in the extract-treated rats is indicative of aided secretory and excretory functions of the hepatocytes facilitated by the EOAE. This does not only suggest that the extract is endowed with phytonutrients capable of stabilizing the plasma membrane of the hepatocytes but also supportive of its hepatoprotective attribute. This assertion agrees with previous submissions (Guo-Cai *et al.,* 2012; Selvaraj *et al.,* 2016), where normalization of the hepatic protein systems in CCl_4-intoxicated rats was attributed to the bioactive constituents of plant extracts.

The role of oxidative stress in the pathogenesis of hepatic disorders is well documented (Cesaratto *et al.,* 2004; Sabiu *et al.,* 2016). The free radicals generated as a result of reductive halogenation of CCl_4, in the presence of oxygen, bind covalently to membrane macromolecules and abstract hydrogen atoms. This consequently initiates auto-oxidative chain reactions that cause functional and morphological alterations in the hepatocyte cell membrane (Basu, 2003). Hence, prevention and/or inhibition of free radical generation and augmentation of the body's antioxidant defense system are germane to annihilating deleterious influence of CCl_4-induced hepatic injury (Wang *et al.,* 2005). The decreased tissue activities of the assayed antioxidant enzymes (SOD, CAT, GRx and GPx) could be due to their excessive mobilization towards detoxification of CCl_3* and free radicals during CCl_4 hepatotoxicity. This might have led to uncontrolled oxidative attack on cellular macromolecules that consequently results in necrosis (Sabiu *et al.,* 2014). This finding is consistent with the report of Lu *et al.* (2002) where similar reductions in activities of radicals detoxifying enzymes were associated with formation of CCl_3* and free radicals in CCl_4-mediated hepatotoxicity in rats. Thus, the dose-dependent significant reversion of the CCl_4-induced reduction in the activities of these detoxifying enzymes by EOAE is informative of its antioxidant potential. This may be adduced to the tendency of the extract to either scavenge CCl_3* and other free radicals or induce and optimize radicals detoxifying enzymes. Similarly, the observed attenuation in the level of GSH might be due to depletion of GPx and GR, as well as formation of reactive metabolites in excess of GSH detoxification capacity (Gini and Muraleedhara, 2010).

In addition, CCl_4-mediated elevation in the level of GSSG may be ascribed to either GSH auto-oxidation or its mobilization towards formation of GPx. The reduction in the GSH/GSSG ratio caused by CCl_4 intoxication reveals possible oxidative onslaught on the hepatocytes. However, the significantly and dose-dependently improved GSH level coupled with the corresponding high GSH/GSSG ratio and low GSSG levels in the liver of the extract-treated rats relative to the untreated CCl_4-intoxicated rats is suggestive of the probable antioxidant activity of EOAE and further supports that it offered considerable level of hepatoprotection at the investigated doses. This was also evidently supported by the optimized antioxidant status of the rats placed on 400 mg/kg b.w. of EOAE alone. Furthermore, CCl_4 has been linked with lipid peroxidation and may facilitate elevated level of peroxidized products like MDA in hepatotoxicity (Balogun and Ashafa, 2016). Therefore, the significantly increased levels of these products may depict inherent oxidative routs of CCl_4 on membrane-bound lipids which might have disrupted membrane fluidity and orientation. The attenuation of CCl_4-mediated increase in the MDA level by EOAE is suggestive of considerable level of protection on the membrane lipids. This could be adduced to ability of the extract to enhance detoxification of reactive metabolites, which could have initiated and promoted peroxidation of polyunsaturated lipids of the hepatocyte membrane. Our submissions are in conformity with previous reports (Gini and Muraleedhara, 2010; Ajani *et al.,* 2014) that administration of plant extracts resulted in improved antioxidant status in xenobiotic-mediated hepatic injury in rats.

Accompanying these biochemical changes are histological changes of the hepatocytes which may also give clues on how therapeutically potent an agent is against hepatic injury.

The apparently annulled degenerative threats posed by CCl_4 on the architectural features of hepatocytes in the extract-administered rats possibly suggest that EOAE significantly protected and stabilized the overall histoarchitectural integrity of the liver. It is noteworthy that, the hepatocyte regeneration progress and architectural organization of some of the hepatocytes of the EOAE-treated rats was almost normalized with increasing number of viable cells as evidently shown by hepatocyte scores. The effects noticed compared favourably with vitamin C and is consistent with the results of the biochemical assays in this study. Our report agrees with the submissions of Fukao *et al*. (2004) and Adeneye *et al*. (2015), where recovery towards normalization of serum enzymes and liver histological architecture caused by CCl_4 in rats was attributed to treatment with plant extracts.

Overall, the elicited antioxidant and hepatoprotective properties of EOAE in this study could be attributed to its constituents as revealed from the GCMS data. Apart from the preventive and chain-breaking antioxidant properties of these compounds, significant hepatoprotective effects of triterpenoic acid, 1,8-cineole, α-terpene and quercetin have been reported (Santos, 2001; Jian-Guo *et al.*, 2016; Karacaa *et al.*, 2016; Selvaraj *et al.*, 2016). Studies have also lent credence to the capabilities of ursolic acid, thymol, eucalyptol and procyanidins to enhance regeneration of hepatocytes following exposure to chemical hepatotoxins (Janbaz *et al.*, 2003; Ciftci *et al.*, 2011; Gabriel *et al.*, 2016; Min *et al.*, 2016).

In view of the foregoing, a tentative mechanism of antioxidative and hepatoprotective potentials of EOAE may be idealized. This could be proposed to involve induction and optimization of chain-breaking (SOD, GRx) and preventive (CAT, GPx) antioxidants that conversely improved the cellular GSH level, scavenged CCl_3* and CCl_3OO*, and significantly inhibited oxidative radicals. This eventually modulated membrane fluidity and protected the membrane of the liver cells.

CONCLUSION

This study suggests that EOAE significantly elicited hepatoprotective property in CCl_4-mediated hepatic injury in rats. This property may be attributed to its antioxidant potential as evidently shown by inducing reactive metabolites detoxifying enzymes and scavenging free radicals generated by CCl_4. These findings enrich biochemical and histological data supporting the use of the extract in the management and treatment of drug-induced hepatic disorders.

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