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Research Article
Studies on Ethanol Extracts of *Olax subscorpioidea* Against Carbon Tetrachloride-Induced Hepatotoxicity in Rats

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Abstract

Background and Objective: Liver disease orchestrated by noxious chemicals are serious health problems the world over. Traditionally, there are claims that ethanol extracts of leaves and stem barks of *Olax subscorpioidea* are used in the treatment of hepatic disorders. Thus, it investigated the impacts of ethanol extract of leaves and stem bark of *Olax subscorpioidea* against carbon tetrachloride (CCl_4)-induced liver damage in rats. **Materials and Methods:** Liver toxicity was induced by intraperitoneal injection of 2.5 mg kg^{-1} b.wt., of CCl_4 in experimental rats. Rats were treated with 200, 400 and 800 mg kg^{-1} dose ethanol leaves and stem bark of *Olax subscorpioidea*, respectively after induction of liver damage. **Results:** Obtained results showed a significant rise in the serum levels of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Malondialdehyde (MDA) and bilirubin as well as decreased Albumin (ALB), Superoxide Dismutase (SOD), Catalase (CAT), reduced Glutathione (GSH) in CCl_4-challenged rats. Treatment with the extracts attenuated serum levels of AST, ALT, ALP, MDA and bilirubin in addition to increased activities of SOD, CAT and the levels of ALB and GSH when compared to the CCl_4 group. Histopathological studies demonstrated that the extracts ameliorated liver necrosis and inflammation due to CCl_4 insult. **Conclusion:** These results concluded that ethanol extract of leaves and stem bark of *Olax subscorpioidea* may reduce hepatic oxidative injury caused by CCl_4 by its antioxidant potentials.

Key words: *Olax subscorpioidea*, antioxidant, curative activity, oxidative injury, hepatoprotective, alanine aminotransferase, bilirubin, reactive oxygen species

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The liver is the major organ for the metabolism of xenobiotics and it is the first target organ in the metabolism of all chemicals[1]. The hepatocyte contains a high amount of toxin metabolizing enzymes that can act on xenobiotics to change them to less harmful product for excretion[2]. Conventional drugs used for the treatment of hepatic ailment like interferon and corticosteroids are exorbitant, especially for third world countries. These drugs may illicit severe reaction and cause further injury[3]. Thus, folklore medicine is crucial for the management of hepatic disorders[4]. Although treatments with herbal medicines have achieved considerable results, several limitations on their usage still exist. These include the complexity of a single herbal component, the variety of fixe-dose combination, the unpredictability of drug formulation, lack of pattern of oral administration and unspecified mode of action. Drugs that are efficacious with a clear mode of action and low incidence of negative actions are earnestly needed[2].

Several studies employing intoxication with carbon tetrachloride (CCl_4) have shown that CCl_4 provokes the production of free radicals in many tissues such as the liver, kidney, brain, heart-lung and blood[5]. CCl_4 is biologically transformed in the hepatocyte by the cytochrome P450 enzymes to trichloromethyl (CCl_3·) radical. This free radical consequently binds covalently to cellular proteins initiating a chain of reactions culminating in cell necrosis[6]. Thus, CCl_4 induces hepatotoxicity by the production of Reactive Oxygen Species (ROS) and depletion of glutathione which culminates in oxidative stress[7].

Olax subscorpioidea is a tree or shrub that belongs to the family *Olacaceae* and is generally spread across West African countries[8]. It has several household names in Nigeria due to its broad usage. It is called Ifon in Western, Aziza in Eastern and Gwano kurmi in the Northern part of Nigeria[9]. A previous study revealed that this plant is used in traditional medicine for the management of several ailments including ulcer treatment[9].

This study qualitatively evaluates the phytochemical components of the ethanol extracts of the leaves and stem bark of *O. subscorpioidea*, Gas chromatography-mass spectrometry (GC-MS) analysis of the extracts to identify the bioactive constituents in addition to the hepatoprotective effect of the extracts on the liver of rats.

MATERIALS AND METHODS

Plant materials and preparation of extracts: *Olax subscorpioidea* leaves and stem bark was collected from farmLand in Umuigboke Ugwulangwu in Ohaozara L.G.A. Ebonyi State, Nigeria and was identified and authenticated by Mr. Alfred Ozioko, a plant Taxonomist at the International Centre for Ethnomedicine and Drug Development Nsukka, Enugu State with voucher specimen no: InterCEDD/208. The study was carried out between April, 2016 and October, 2017. The leaves and stem bark of *O. subscorpioidea* were cut into small pieces, shade-dried and ground into a powdered form using a grinding machine. The powdered form was treated with 98% ethanol and left for 48 hrs. Thereafter, it was filtered using Whatman filter no. 1. The obtained extract was used for both qualitative phytochemical screening, GC-MS analysis of the bioactive components of the leaf and stem bark and treatment of test animals.

Chemicals and reagents: All reagents and chemicals used were of analytical grade and were purchased from Sigma Aldrich, St Louis, USA.

Experimental animals: A total of eighty-five male Wistar albino rats, aged 8-12 weeks old were purchased from the Dan Okoro Farms, Abakaliki, Nigeria and were kept under standard environmental conditions. They were safely taken to Animal House, Department of Biochemistry Ebonyi State University and were allowed to acclimatize for 2 weeks and were given access to food and water.

Acute toxicity test: The acute toxicity study was carried out using a modified method of Lorke[10]. Forty rats were divided into two groups. Twenty rats were used for each extract and placed into five sub-groups of four rats each. Group 1 orally received 2 mL distilled water. Groups 2, 3, 4 and 5, respectively orally received a single dose of 800, 1500, 2500 and 5000 mg kg^{-1} b.wt., concentrations of the extract. Behavioural changes and death were observed in the animals for 24, 48, 72 and 96 hrs. Finally, all the animals were observed for toxic symptoms and survivors were recorded on the 14th day of exposure. No mortality was recorded.

Qualitative phytochemical screening: The extracts were investigated by using a standard method by Sofowora[11]. The phytochemical compounds such as tannins, alkaloids, glycosides, saponins, steroids, anthraquinones and polyphenol were determined by the Evans[12] method.

GC-MS analysis: GC-MS analysis of the bioactive components of ethanol extracts of *Olax subscorpioidea* leaf and stem bark was carried out using a piece of GC-MS equipment (Thermo Scientific Co.) Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II. This was done using the procedure described by Kanthal *et al.*[13].

Experimental groups: A total of 45 male Wistar albino rats weighing between 120-140 g were used. Liver toxicity was induced by intraperitoneal administration of 2.5 mL kg^{-1} of CCl_4.

The rats were divided into nine groups of five rats each as follows:

Group 1: Normal control that received distilled water
Group 2: Received only CCl_4
Group 3: Received CCl_4+10 mg kg^{-1} Vit C 24 hrs after CCl_4 administration
Group 4: Received CCl_4+200 mg kg^{-1} ethanol extract of the stem bark of *Olax subscorpioidea* (EESBOS)
Group 5: Received CCl_4+400 mg kg^{-1} EESBOS
Group 6: Received CCl_4+800 mg kg^{-1} EESBOS
Group 7: Received CCl_4+200 mg kg^{-1} ethanol extract of the leaves of *Olax subscorpioidea* (EELOS)
Group 8: Received CCl_4+400 mg kg^{-1} EELOS
Group 9: Received CCl_4+800 mg kg^{-1} EELOS

The plant extracts were given daily through oral intubation for 14 days.

Blood sample and organ collection: On the 14th day of exposure, the animals fasted overnight. The animals were sedated with chloroform, blood samples were collected via vein puncture technique on the left leg into plain container tubes. The serum was obtained by centrifuging the blood at 3,000 rpm for 15 min for biochemical assays. The liver was collected and placed in 10% formalin[14,15].

Biochemical assay: Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were determined using the colourimetric method as described by Reitman and Frankel[16]. Alkaline phosphatase (ALP) was carried out according to the method described by King and Kind[17], while total bilirubin and albumin were determined using methods described by Walter and Gerade[18] and Doumas *et al.*[19]. respectively. Malondialdehyde (MDA) in serum was determined spectrophotometrically by measuring Thiobarbituric Acid Reactive Substance (TBARS) as described by Buege and Aust[20]. Catalase (CAT) activity was assayed by the method described by Sinha[21]. The superoxide dismutase activity was assayed by the method described by Fridovich and Mc-Cord[22] while Glutathione (GSH) concentration was measured according to the method of Ellman[23].

Histopathological analysis of liver: The rats were humanely euthanized and the livers removed with care. After their removal, the livers were fixed for one week in buffered formaldehyde solution (10%). Thereafter, they were embedded in paraffin and were later cut into 3-4 mm slices. Hematoxylin and eosin were used to stain the slices mounted on a glass slide for histopathological study. The examination of the images was carried out under a light microscope[24].

Statistical analysis: The data were presented as mean±standard deviation. They were subjected to one-way analyses of variance (ANOVA). The analysis was carried out using Statistical Package for Social Sciences (SPSS), version 20. Test for the significant difference was done using Duncan's Multiple Range test and $p<0.05$) was considered statistically significant.

RESULTS

Acute toxicity: In acute toxicity testing, no abnormal behaviour and mortality were recorded. This indicates that the LD_{50} was greater than 5000 mg kg^{-1} b.wt., of Wistar albino rats. This formed the basis for the selection of the doses of the extracts administered.

Phytochemical screening of the ethanol extracts of leaf and stem bark of *Olax subscorpioidea*: The Phytochemical screening of the ethanol extracts of leaf and stem bark of *Olax subscorpioidea* is shown in Table 1. The results showed that both plant parts contain alkaloids, glycosides, flavonoids, steroids, polyphenol, anthraquinones and tannins except for saponins which are lacking in the ethanol leaf extract.

GC-MS phytochemical constituents of ethanol extract of *O. subscorpioidae* leaves: The results of the GC-MS phytochemical constituents of the ethanol extract of *O. subscorpioidae* leaves are shown in Table 2. The results

Table 1: Qualitative phytochemical screening of ethanol extracts of leaf and stem bark of *Olax subscorpioidea*

Phytochemicals	Ethanol leaf extract	Ethanol bark extract
Tannins	+	+
Alkaloids	++	++
Glycosides	++	++
Saponins	_	++
Steroids	++	++
Flavonoids	+	++
Anthraquinones	++	+
Polyphenols	+	++

++: Highly present, +: Present, -: Absent

Table 2: Phytochemical constituents identified from ethanol extract of leaf from *Olax subscorpioidae* by GC-MS analysis

Retention time	Name of compounds	Molecular formula	Molecular weight	Base peak	Composition (%)
4.51	N-Isobutyl-N nitroso pentylamine	$C_{10}H_{22}N_2O$	186	43	16.09
8.20	2,3-Dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one	$C_6H_8O_4$	144	43	18.47
9.83	4-methyl-4-Hepten-3-one	$C_8H_{14}O$	126	41	31.29
11.05	4,5-Dihydro-2-methylimidazole-4-one	$C_4H_6N_2O$	98	42	3.57
20.31	Eicosanoic acid (arachidic acid)	$C_{20}H_{40}O_2$	312	43	2.98
23.27	9-Octadecanoic acid	$C_{18}H_{34}O_2$	282	55	25.63
27.43	Di-n^{-1}-octyl phtha late (1,2-Benzene dicarboxylic acid)	$C_{24}H_{38}O_4$	390	148	1.93

Table 3: Phytochemical constituents identified from ethanol extract of stem bark from *Olax subscorpioidae* by GC-MS analysis

Retention time	Name of compound	Molecular formula	Molecular weight	Base peak	Composition (%)
3.5	3-ethyl-2,4-dimethylpentane	C_9H_{20}	128	43	1.93
11.95	Dodecanoic acid	$C_{12}H_{24}O_2$	200	60	0.89
14.14	Tetradecanoic acid, myristic acid	$C_{14}H_{28}O_2$	228	60	0.59
15.61	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270	74	1.78
16.20	Ascorbic acid, 2, 6-dihexadecanoate	$C_{38}H_{68}O_8$	652	73	17.83
17.30	9,12-octadecadienoic acid (2,2-methyl ester)	$C_{19}H_{34}O_2$	294	67	16.04
17.51	Octadecanoic acid methyl ester	$C_{19}H_{38}O_2$	298	74	1.18
17.91	9,12-octadecadienoic acid	$C_{18}H_{32}O_2$	280	67	33.28
18.05	Octadecanoic acid, stearic acid	$C_{18}H_{36}O_2$	284	43	4.75
19.09	Docosanoic anhydride	$C_{44}H_{86}O_3$	662	98	19.09
20.52	9,17-Octadecadienoic acid	$C_{18}H_{32}O$	264	67	16.04
22.52	9,12-Octadecadienoic acid, 2-hydroxy l^{-1}-(hydroxyl methyl) ethyl ester linolein	$C_{12}H_{38}O_4$	354	67	1.18

Table 4: Effects of ethanol extracts (leaf and stem bark) of *Olax subscorpioidea* on serum liver function indices in CCl_4-intoxicated rats

Groups	AST (U L^{-1})	ALT (U L^{-1})	ALP (U L^{-1})	BL (mg dL^{-1})	Alb (g dL^{-1})
Normal control	207.8±7.5	34.0±3.4	191.3±17.5	3.3±0.2	28.1±0.1
CCl_4 control	374.8±3.7*	88.0±0.8*	412.8±11.1*	11.9±0.4*	13.3±0.3*
CCl_4+Vit. C	218.8±7.9$^{\$}$	34.5±2.6$^{\$}$	201.0±11.2$^{\$}$	3.5±0.4$^{\$}$	28.9±0.2$^{\$}$
CCl_4+B200	252.5±13.8$^{\#}$	41.8±2.8$^{\#}$	252.8±8.4$^{\#}$	5.9±0.3$^{\#}$	23.4±0.4$^{\#}$
CCl_4+B400	224.0±12.7$^{\#}$	38.5±2.6$^{\#}$	230.8±17.2$^{\#}$	5.3±0.5$^{\#}$	26.5±0.1$^{\#}$
CCl_4+B800	204.8±7.9$^{\#}$	32.3±2.6$^{\#}$	198.5±12.5$^{\#}$	3.2±0.2$^{\#}$	29.2±0.5$^{\#}$
CCl_4+L200	285.0±4.8$^{\#}$	49.5±6.0$^{\#}$	285.5±7.1$^{\#}$	6.4±0.5$^{\#}$	22.1±0.6$^{\#}$
CCl_4+L400	234.8±11.6$^{\#}$	44.8±2.5$^{\#}$	241.3±3.6$^{\#}$	5.3±0.7$^{\#}$	25.2±0.7$^{\#}$
CCl_4+L800	222.8±9.0$^{\#}$	39.5±2.5$^{\#}$	209.3±8.8$^{\#}$	4.1±0.4$^{\#}$	27.6±0.4$^{\#}$

Values are expressed as Mean±SD (n = 5), CCl_4: Carbon tetrachloride, B200: Stem bark extract (200 mg kg^{-1} b.wt.), L200: Leaf extract (200 mg kg^{-1} b.wt.), BL: Bilirubin, Alb: Albumin. *Significantly different from normal control ($p<0.05$), $^{\$}$Significantly different from CCl_4 ($p<0.05$), $^{\#}$Significantly different from CCl_4 control ($p<0.05$)

showed that seven major compounds were identified in ethanol leaf extract of *O. subscorpioidae* by GC-MS analysis. This includes: 4-methyl-4-hepten-3-one (31.29%), 9-octadecanoic acid (25.63%), 2,3-Dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one(18.47%), N-Isobutyl-N nitroso pentylamine (16.09%), 4,5-dihydro-2-methyl imidazole-4-one (3.57%), eicosanoic acid (arachidic acid) [2.98%] and di-n-1-octyl phthalate (1,2-Benzene dicarboxylic acid) [1.93%].

GC-MS phytochemical constituents of ethanol extract of *O. subscorpioidae* stem bark: The results of the GC-MS phytochemical constituents of the ethanol extract of *O. subscorpioidae* stem bark are shown in Table 3. The results revealed that twelve (12) compounds were identified from the ethanol extract of stem bark from *O. subscorpioidae* by GC-MS analysis which mainly comprises hydrocarbons, saturated and unsaturated fatty acids, fatty acids methyl esters, dihydropyranones and other organic compounds with 9,12-octadecanoic acid (33.28%) having the highest percentage composition.

Effects of ethanol extract of leaves and stem bark of *Olax subscorpioidea* on liver function indices in CCl_4-intoxicated rats: Results of effects of extracts on liver function indices are presented in Table 4. Administration of vitamin C in the CCl_4+Vit. C group significantly ($p<0.05$) modulated the liver function indices compared to CCl_4 control. Interestingly, the stem bark ethanol extract exerted dose-dependent decreases in ALT, AST, ALP activities as well as bilirubin level and increases in albumin. The highest dose of the extracts (800 mg kg^{-1} b.wt.) was more effective. Comparatively, the effect of the extracts at various doses on each of the index is not significant ($p>0.05$).

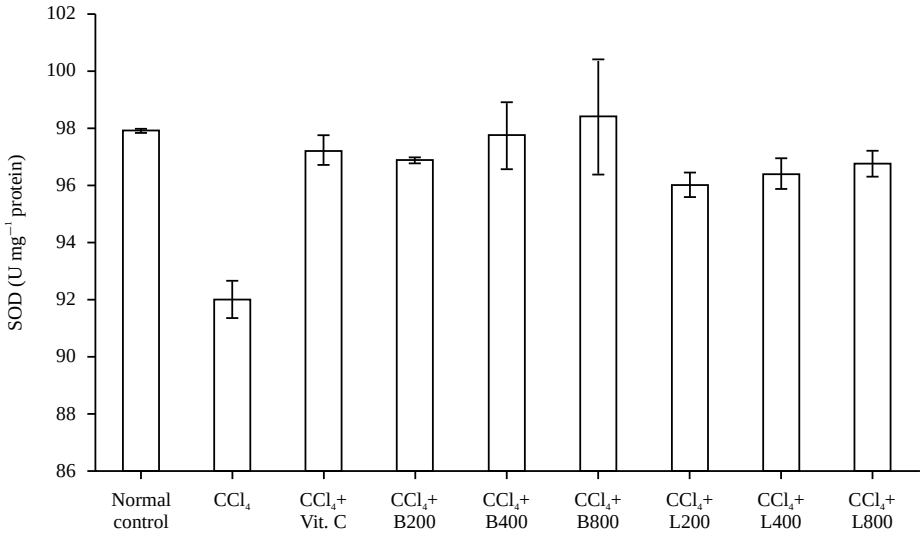

Fig. 1: Effects of EESBOS and EELOS on serum superoxide dismutase (SOD) activity in CCl_4-intoxicated rats

Values are expressed as mean±SD (n = 5), B200: Stem bark extract (200 mg kg^{-1} b.wt.), L200: Leaf extract (200 mg kg^{-1} b.wt.)

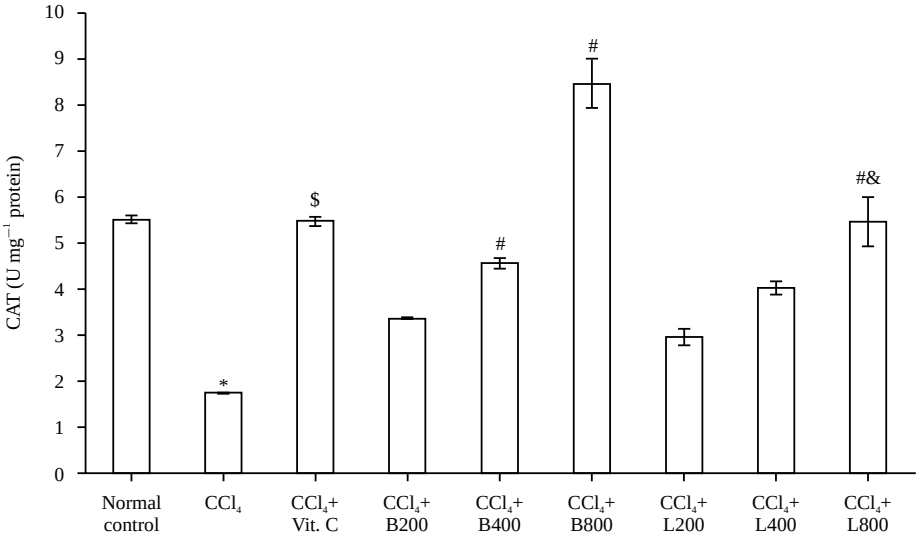

Fig. 2: Effects of EESBOS and EELOS on serum catalase (CAT) activity in carbon CCl_4-intoxicated rats

Values are expressed as mean±SD (n = 5), B200: Stem bark extract (200 mg kg^{-1} b.wt.), L200: Leaf extract (200 mg kg^{-1} b.wt.), *Significantly different from normal control ($p<0.05$), $^{\$}$Significantly different from CCl_4 ($p<0.05$), $^{\#}$Significantly different from CCl_4 ($p<0.05$), $^{\&}$Significantly different from CCl_4+B800 group

Effects of EESBOS and EELOS on oxidative stress indices in CCl_4-intoxicated rats: The data of Fig. 1-4 revealed the effects of the two extracts on oxidative stress indices in CCl_4-intoxicated rats. In Fig. 1, administration of vitamin C and various doses of EESBOS and EELOS up-regulated SOD activity insignificantly compared to the CCl_4 group. In Fig. 2, treatment with vitamin C and the extracts markedly increased CAT activity when compared with the CCl_4 group. Likewise, in Fig. 3, vitamin C and the administration of the extract significantly ($p<0.05$) increased the level of GSH relative to the CCl_4 group. On the other hand, in Fig. 4, different doses of both extracts significantly ($p<0.05$) decreased MDA levels in comparison to the CCl_4 group. Different doses of both extracts significantly improved CAT, GSH and MDA levels ($p<0.05$) in this study.

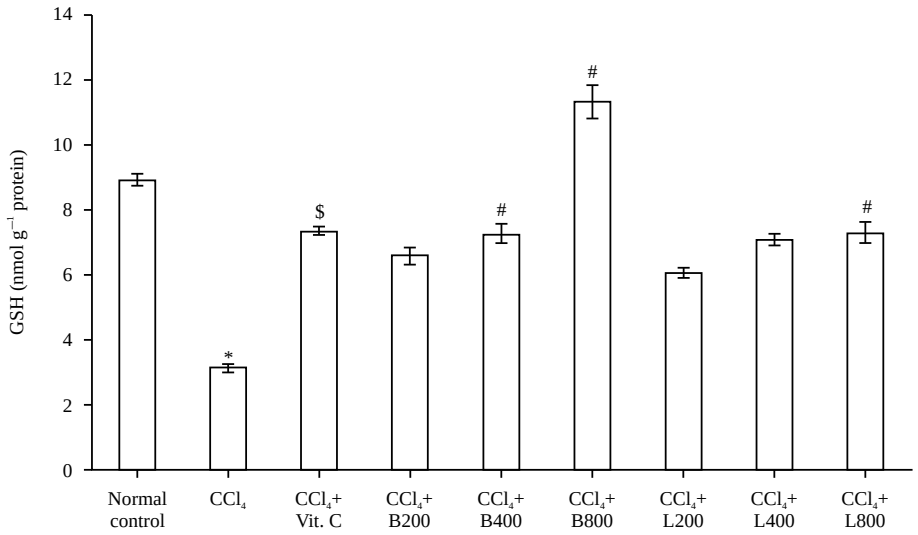

Fig. 3: Effects of EESBOS and EELOS on serum GSH level in CCl_4-intoxicated rats

Values are expressed as mean±SD (n = 5), B200: Stem bark extract (200 mg kg^{-1} b.wt.), L200: Leaf extract (200 mg kg^{-1} b.wt.), *Significantly different from normal control ($p<0.05$), $^{\$}$Significantly different from CCl_4 ($p<0.05$), $^{\#}$Significantly different from CCl_4 ($p<0.05$)

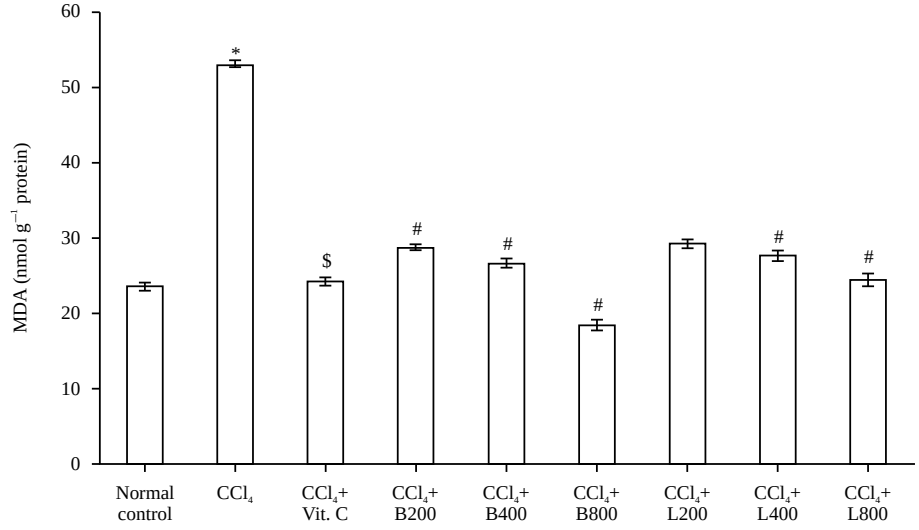

Fig. 4: Effects of ethanol extracts (leaf and stem bark) of *Olax subscorpioidea* on serum malondialdehyde (MDA) level in carbon tetrachloride (CCl_4)-intoxicated rats

Values are expressed as mean±SD (n = 5), B200: Stem bark extract (200 mg kg^{-1} b.wt.), L200: Leaf extract (200 mg kg^{-1} b.wt.), *Significantly different from normal control ($p<0.05$), $^{\$}$Significantly different from CCl_4 ($p<0.05$), $^{\#}$Significantly different from CCl_4 ($p<0.05$)

Histopathological assessment of liver: The result of a histopathological assessment of liver biopsy is presented in Fig. 5a-e. The result in Fig. 5a indicates that the normal control group showed normal liver architecture/Normal Hepatocyte (NH) with distinct hepatic cells, well-demarcated sinusoid and sinusoidal space. However, CCl_4-challenged rats (CCl_4) without treatment (Fig. 5b) showed drastic histopathological alterations, such as liver Necrosis (N), fatty degeneration and changes (FC), no distinct appearance of hepatocytes, sinusoid not well demarcated and Inflammatory Mediation (IM). In Fig. 5c, CCl_4-challenged rats treated with vit C showed Normal Hepatocytes (NH) with Mild Fatty Changes (MFC). In Fig. 5d, CCl_4-challenged rats treated with stem bark extract also showed normal hepatocyte and mild fatty changes. In the same vein, CCl_4-challenged rats treated with leaf extract showed normal hepatocyte with mild fatty changes (Fig. 5e). Thus, treatment with the extracts ameliorated the altered hepatic changes.

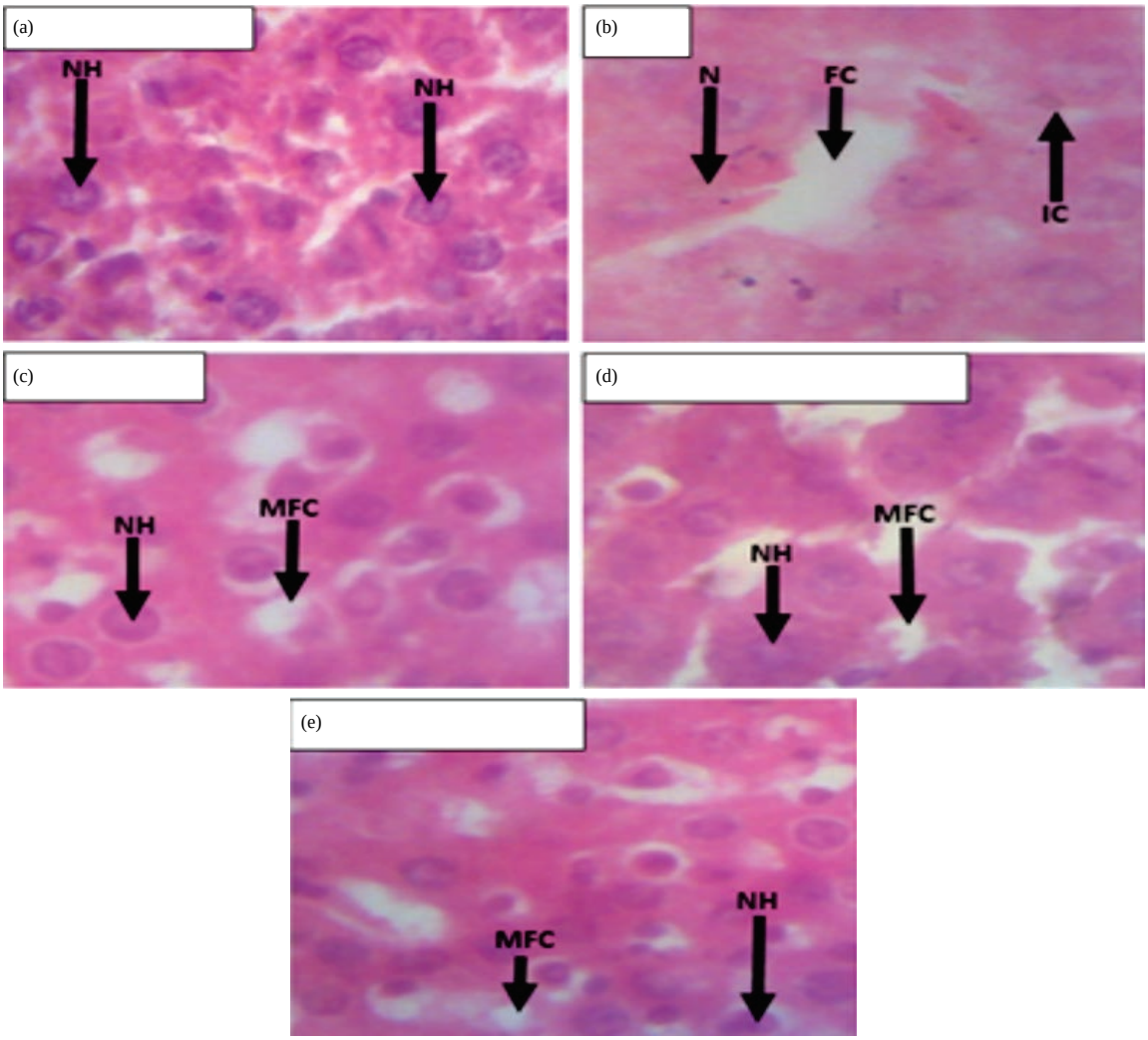

Fig. 5(a-e): Representative photomicrographs of hepatic sections stained with H and E (H and E mag×100)

(a) Normal control: showing the normal structure of hepatocytes, (b) CCl_4: showing necrotic cells, fatty changes and inflammatory mediation, (c) CCl_4+Vit C: showing near normal hepatocytes with mild fatty changes, (d) CCl_4+Stem bark extract: showing near normal hepatocytes with moderate fatty changes and (e) CCl_4+Leaf extract: showing near normal hepatocytes with moderate fatty changes

DISCUSSION

The results from this study demonstrated that CCl_4 insults caused liver injury reflected by marked elevation in the activities of ALT, AST and ALP which is indicative of cellular leakage and compromise in the function of the cell membrane of the hepatocytes[25]. The decline in liver function is further supported by the reduction in serum albumin which is suggestive of injury to a synthetic capacity of the liver. This could be because intoxication with CCl_4 culminates in interference and detachment of polyribosomes from the endoplasmic reticulum which brings about the reduction of protein synthesis[26].

CCl_4-induced hepatic damage is mediated by lipid peroxidation, reduction in the activity of antioxidant enzymes and generation of free radicals[27]. In this study, treatment with EESBOS and EELOS attenuated the gravity of hepatic damage caused by the CCl_4 challenge and improved the level of albumin. The activities of the liver function enzymes were brought to near normal control indicating stabilization of hepatic membrane and prevention of enzymes leakage by the extracts.

Several studies have been carried out with the CCl_4 model for hepatotoxicity and it has been proven that CCl_4 assembles in liver parenchymal cells and is metabolically activated by cytochrome P-450 dependent monooxygenases to produce a

trichloromethyl free radical ($CC1_3$'). This $CC1_3$` introduces alkyl radical into cellular proteins inclusive of cytochrome P450 and other macromolecules accompanied by a simultaneous attack on polyunsaturated fatty acids in the presence of oxygen to produce lipid peroxides which culminate in hepatic injury[28]. Bilirubin which is a major product obtained from the degradation of haemoglobin is elevated when there is damage or injury to the hepatocyte[29]. The rise in total bilirubin which emanates from reduced uptake and conjugation of bilirubin by the hepatocyte is brought by the disturbance in the functioning of the liver cell[29]. The extracts probably shield the liver cell from damage, thus increasing the uptake of bilirubin and its conjugation by the liver and subsequent secretion into the bile ducts. The elevation in MDA levels is suggestive of enhanced lipid peroxidation culminating in tissue damage and compromise of the antioxidant defence mechanisms[30]. In the present study, the marked rise in the serum levels of MDA in CCl_4-challenged rat was reversed by treatment with the extract.

Moreover, physiological mechanisms in the body operate to prevent the formation of free radicals via radical scavengers and chain breakers such as vitamins C and E and antioxidants such as Glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx)[31]. The diminution of serum GSH, CAT, SOD and GPx following the CCl_4 challenge may be linked to free radical production. However, it was observed that EESBOSL and EEOSL significantly boosted the activities of CAT, SOD, GPx and GSH level which may be attributed to the extracts scavenging of free radicals provoked by lipid peroxidation probably due to high their content of phenol and flavonoids.

The ability of EESBOSL and EEOSL to ameliorate liver damage in CCl_4-challenged rats were further substantiated by histopathological studies of the liver, which mainly corroborated the results from the serum assays. Histopathological studies of the hepatocytes revealed that the liver architecture of the rats treated with the extracts was reversed to a greater extent near-normal control.

CONCLUSION

EESBOSL and EEOSL are not toxic even at a higher dose of 5000 mg kg^{-1}. The extracts attenuated the activities of the liver enzymes while the antioxidants were significantly improved. The histopathological evaluation indicated that the extracts attenuated liver injury. The results of this study show that EESBOSL and EEOSL have hepatoprotective and antioxidant potentials.

SIGNIFICANCE STATEMENT

This study discovered that ethanol extracts of *Olax subscorpioidea* leaves and stem bark attenuated liver damage caused by CCl_4 in albino Wistar rats. Thus, extracts of *Olax subscorpioidea* leaves and stem bark can be beneficial for the treatment of rising cases of hepatic disorders. The baseline data from this study will help other researchers know the medicinal potentials of *Olax subscorpioidea* and the need to exploit the novel phytochemical compounds inherent in the plant for new potent drug development. This suggests that a new treatment regime using bioactive phytochemicals with no adverse effects from *Olax subscorpioidea* may be developed for hepatotoxic disorders.

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