Hepatoprotective and *in vivo* antioxidant activity of *Olax subscorpioidea Oliv*. (Olacaceae) and *Distemonathus benthamianus* Baill. (Caesalpiniaceae)

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ABSTRACT

Background: Liver diseases are on rise and remain a serious health problem. *Olax subscorpioidea* and *Distemonanthus benthamianus* are two plants used in Ivorian traditional medicine in the treatment of many diseases including jaundice and hepatitis. **Objective:** The present study was carried out to assess the hepatoprotective and *in vivo* antioxidant potentials of the hydro-ethanolic leaf extracts of these plants in carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats. **Materials and Methods:** The plant extracts at doses of 25 and 100 mg/kg bw, and silymarin (25 mg/kg bw) were intraperitoneally (i.p.) injected once daily for 7 days to different groups of rats. Hepatotoxicity was induced on the 7th day in all the group animals except control. Rats were sacrificed on the 8th day and blood was collected. Serum biochemical parameters and antioxidant activity were measured using standard procedures. Histopathological examinations of liver rats were also performed. **Results and Discussion:** Hepatotoxicity induced with CCl_4 was well manifested by significant increase in serum activities of GOT, GPT, ALP and GGT, and enhancement of total bilirubin and TBARS levels. On the other hand, the level of total protein, albumin, α_1-globulin significantly decreased, and DPPH-free radical scavenging activity and TAP were lowered. Pretreatment with plant extracts and silymarin prevent the toxic effects of CCl_4 by decreasing serum enzyme activities, total bilirubin and TBARS levels and improving serum TAP and DPPH-free radical scavenging potential. Histopathological observations showed almost normal hepatic cells with a mild degree of inflammation, lesser fatty infiltration and absence of necrosis among the rats treated at 100 mg/kg of extracts of both the plants. **Conclusion:** The results suggest that the hydro-ethanolic leaf extracts of *O. subscorpioidea* and *D. benthamianus* possesses hepatoprotective and *in vivo* antioxidant activity.

Key words: Antioxidant activity, carbon tetrachloride, *Distemonanthus benthamianus,* hepatoprotective activity, histopathology, *Olax subscorpioidea*

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INTRODUCTION

Hepatic system is the major organ system regulating homeostasis in the body. It is involved in the maintenance of metabolic functions and detoxification from the exogenous and endogenous challenges like xenobiotic, drugs, viral infections and chronic alcoholism. This physiological activity is often associated to the generating of highly reactive-free radicals which can attack the liver and lead to hepatic damage.[1]

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In spite of tremendous scientific advances in the field of hepatology in recent years, liver diseases are on the rise and remain a serious health problem. Presently, a few hepatoprotective drugs and that too from natural sources, are recommended for the treatment of liver disorders. Hence, people are looking at the traditional systems of medicine for remedies to hepatic disorders.

Olax subscorpioidea Oliv. (Olacaceae) and *Distemonanthus benthamianus* Baill. (Caesalpiniaceae) are two plants used in traditional medicine to treat respectively jaundice and

hepatitis.[2] Preliminary studies of the aerial parts of both the plants indicated that the polyphenolic and flavonoid contents of the hydro-ethanolic extracts are, respectively, 102.8 ± 0.57 mg Gallic Acid Equivalent/g extract and 30.38 ± 0.32 Quercetin Equivalent/g extract for *D. Benthamianus*; 35.49 ± 0.29 mg Gallic Acid Equivalent/g extract and 22.69 ± 0.69 mg Quercetin Equivalent/g extract for *O. subscorpioidea*.[3] These results showed the presence of relatively high levels of flavonoid and polyphenolic compounds in the hydro-ethanolic extract and a potential antioxidant effect *in vitro* of these plants. Moreover, these plants are used in mixture with palm wine locally called "bandji" for their traditional therapeutic utilization.

The present investigation was carried out to examine the *in vivo* antioxidant and hepatoprotective activity of the 70% ethanolic extracts of the leaves of *Olax subscorpioidea* and *Distemonanthus benthamianus* against carbon tetrachloride-induced hepatotoxicity in albino rats. To assess the degree of liver damage, some serum biochemical parameters were estimated and histopathological liver examinations were carried out.

MATERIALS AND METHODS

Plant materials

The plant materiel consists of dried powdered leaves of *Olax subscorpioidea* and *Distemonanthus benthamianus*. The fresh leaves samples were respectively collected in the region of Dimbokro and Daloa, central and west-central of Côte d'Ivoire. The plants specimens were identified and authenticated by Professor Aké-Assiat the National Floristic center, Félix HouphouëtBoigny University of Abidjan. Voucher of these specimens were deposited in the herbarium of the center under the numbers 7616 for *Olax subscorpioidea* and 12473 for *Distemonanthus benthamianus*.

Animals

Studies were carried out using Wistar albino rats and Swiss mice of either sex. The animals were housed in plastic cages and kept at ambient temperature of 30 ± 1°C during the day with 12 h light and 25 ± 1° C in the night with 12 h darkness. All the experimental procedures were approved by the Ethical Committee of Health Sciences, Félix Houphouët Boigny University of Abidjan. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals.

Drugs and chemicals

Silymarin, the reference hepatoprotective drug, 1,1,3,3-tetramethoxypropan (MDA), 2,4,6-tris-pyridyl-s-triazine (TPTZ), and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, USA). Carbon tetrachloride (CCl_4) and 2-thiobarbituric acid (TBA), were from Merck Co. (Germany). All the other reagents and solvents used in this study were of analytical grade.

Extract preparation

The fresh leaves of both the plants were washed and shade at room temperature for 2 weeks. The dried samples were later pulverized using a mechanical grinder (IKAMAG). One hundred grams of powder were separately shaken in ethanol-water (70:30, V/V), (2 L) for 24 hours on an orbital shaker. The hydro-ethanolic solutions were then filtered using a Buchner funnel and Whatman filter paper (3 mm). Each filtrate was concentrated to dryness under reduced pressure at 30°C using a rotary evaporator (BÜCHI). The resultant extracts constitute the hydro-ethanolic extracts.

Acute toxicity

The extracts of both the plants were intraperitoneally (i.p.) administered to two series of five groups of six mice (25-30 g) at doses of 100, 500, 1000, 2000 and 4000 mg/kg body weight (bw), respectively. All the animals were observed for 24 hours. The mortality was recorded at the end of this period and the lethal dose 50 (LD_{50}) was determined for each extract following the graphic method of Miller and Tainter.[4]

CCl_4 induced hepatotoxicity and samples collection

Rats weighing 180 to 200 g were divided into seven groups of six animals. All the drugs were intraperitoneally (i.p.) injected to animals once daily. Group 1 (control) animals received solution of NaCl 0.9% (5 mL/kg bw) for 7 days and liquid paraffin (5 mL/kg bw) on the 7th day. Group 2 (CCl_4 group) received solution of NaCl 0.9% (5 mL/kg bw) for 7 days and CCl_4 (5 mL/kg bw, 20% in liquid paraffin) on the 7th day. Group 3 animals were administered with silymarin (25 mg/kg bw in 50% ethanol) for 7 days and received CCl_4 (5 mL/kg bw, 20% in liquid paraffin) on the 7th day. Groups 4 and 5 received, respectively, 25 and 100 mg/kg bw of ethanolic extract of *O. subcorpioidea* for 7 days and CCl_4 on the 7th day in the above-mentioned doses. Groups 6 and 7 received, respectively, 25 and 100 mg/kg bw of ethanolic extract of *D. benthamianus* for 7 days and CCl_4 on the 7th day in the above-mentioned doses.

On the 8th day, the animals of all groups were anesthetized and sacrificed. Blood was collected and serum was separated by centrifugation at 2500 rpm for 10 min for biochemical investigations and antioxidant activity. Liver of each rat was dissected out and fixed in 10% formalin for histopathological studies.

Estimation of serum hepatic markers

Hepatic parameters like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline

phosphatase (ALP), gamma-glutamyl transferase (GGT), total protein and total bilirubin were measured with an automatic analyzer (Roche/INTEGRA) using experimental kits (COBAS INTEGRA). Serum-specific proteins were also assessed by electrophoresis on a semi-automatic analyzer (HYDRASYS/SEBIA) using Hydragel 7 protein kits.

Estimation of lipid peroxidation

Lipid peroxidation, a major indicator of oxidative stress, was estimated by Thiobarbituric Acid Reactive Substances (TBARS) assay.

At 0.5 mL of serum, 2.5 mL of TCA 20% (m/V) was added to precipitate the sample proteins. The mixture was then centrifuged at 3000 rpm for 10 min, and 2.5 mL of sulfuric acid (0.05 mol.L^{-1}) and 2 mL TBA (0.2%) were added to the sediment which was shaken and incubated for 30 min in a boiling water bath. Four milliliter of *n*-butanol was then added, and the solution was centrifuged again, cooled and the supernatant absorption was recorded at 532 nm using a spectrophotometer (Spectronic Genesis 5). The calibration curve was obtained using different concentrations of 1,1,3,3-tetramethoxypropane (2.4-38 μmol.L^{-1}) as standard to determine the concentration of TBA-MDA adducts in samples.[5]

Total antioxidant power assay

The total antioxidant capacity of serum was determined by measuring its ability to reduce Fe^{3+} to Fe^{2+} by the Ferric Reducing Ability of Plasma (FRAP) test. The FRAP assay measures the change in absorbance at 593 nm due to the formation of a blue-colored Fe (II) tris-pyridyltriazine compound from Fe (III) by the action of electron-donating antioxidants. The FRAP reagent consists of 300 mmol.L^{-1} acetate buffer (pH = 3.6), 10 mmol L^{-1} TPTZ in 40 mmol.L^{-1}HCl and 20 mmol.L^{-1} $FeCl_3 \times 6\ H_2O$ in the ratio of 10:1:1.

Thus, 20 μL of serum was added to 300 mL freshly prepared and pre-warmed (37°C) FRAP reagent in a test tube and incubated at 37°C for 10 min. The absorbance of the blue-colored complex was then read against a reagent blank (300 mL FRAP reagent +20 μL distilled water) at 593 nm. Standard solutions of Fe^{2+} in the range of 1.56 to100 μmol.L^{-1} were prepared from ferrous sulfate ($FeSO_4 \times 7\ H_2O$) in water. The data were expressed as μmol of ferric ions reduced to ferrous form per liter (FRAP value).[6]

DPPH radical scavenging activity

Serum ability to inhibit DPPH free radical was measured following the method of Yokozawa *et al*.[7] with a few modifications.

Serum aliquots (200 μL) were mixed with 200 μL of acetonitrile (60% in distilled water), incubated for 2 min at room temperature and then centrifuged at 4000 rpm for 10 min. Supernatant samples (200 μL) were immediately added to 200 μL of DPPH solution (100 μmol L^{-1} in methanol) which was first completed with 1 mL of methanol, and the reaction mixture was shaken vigorously. After incubation at room temperature for 10 min, the absorbance of this solution was determined at 517 nm. Acetonitrile solution without serum samples were used as the control.

The ability of serum to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH inhibition}\,\% = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

where $\text{Abs}_{\text{control}}$ is the absorbance of DPPH radical without deproteinized serum and $\text{Abs}_{\text{sample}}$ is the absorbance of DPPH radical with deproteinized serum.

Histopathological studies

Sections were taken from liver tissues of each rat, dehydrated in gradual ethanol, cleared in toluene and infiltrated in paraffin using an automatic tissue processor (TIYODA). They were then embedded in molten paraffin. Thick sections were cut at 4 μm with a rotary microtome, processed in toluene-ethanol series and stained with hematoxylin and eosin (H-E) dye. They were finally observed under photonic microscope for histopathological changes.

Statistical analysis

The results were expressed as mean ± standard error of means (SEM). Statistical analysis was performed by one way analysis of variance (ANOVA) and differences between samples were determined by Dunnett's Multiple Comparison test using Graph Pad Prism 5.0 program. $P < 0.05$ were considered as significant.

RESULTS

Acute toxicity

The extracts at the doses of 500-4000 mg/kg caused behavioral changes and death in mice. Signs of acute toxicity such as agitation, aggressiveness, body twisting, convulsion and diarrhea were observed at the high doses of extracts. The lethal dose 50 (LD_{50}) was estimated at 1000 mg/kg bw for *O. subscorpioidea* and 1148.15 mg/kg bw for *D. benthamianus*.

Effect of extracts on biochemical parameters

The effect of extracts on biochemical parameters in serum is shown in Table 1. In the CCl_4-intoxicated group, the

levels of SGOT, SGPT, ALP, GGT and total bilirubin were significantly increased ($P < 0.001$) when compared with control group (Group 1), but the content of total protein decreased. Table 2 showed result of serum protein electrophoresis where albumin, α_1-globulin, α_2-globulin, β-globulin and γ-globulin contents were more or less modified in CCl_4 group compared to control group. Pretreatment with *O. subscorpioidea* and *D. benthamianus* 70% ethanolic extracts at the doses of 25 and 100 mg/kg decreased serum enzyme levels and increased total protein and albumin contents in a dose-dependent manner. However, there were no significant changes in level of α_1-globulin, α_2-globulin, β-globulin and γ-globulin. *D. benthamianus* 70% ethanolic extract at 100 mg/kg showed highly significant activity ($P < 0.05$) with maximum inhibition of CCl_4 effects and this was comparable to that of silymarin (25 mg/kg).

In vivo antioxidant activity of extracts

The results of antioxidant activity after administration of plant extracts and CCl_4 are summarized in Table 3. There was a significant increase ($P < 0.001$) in the levels of TBARS in CCl_4-intoxicated rats compared to control group. Extracts at 25 and 100 mg/kg exhibited an ability to counteract the CCl_4 induced toxicity in pretreated groups by decreasing TBARS levels compared to CCl_4 group. Pretreatment of rats with extracts at the same doses significantly increase ($P < 0.01$) the serum DPPH scavenging potential as well as the serum TAP when compared with CCl_4-intoxicated group.

Histopathological observations

Histology of the liver sections of control animals (Group 1) showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus and visible centrilobular vein [Figure 1a]. The liver sections of CCl_4-intoxicated rats (Group 2)

Table 1: Effects of the different treatments of rats on the serum biochemical parameters

Treatment	Biochemical parameters					
	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	GGT (U/L)	Total bilirubin (mg/L)	Total protein (g/L)
Group 1 NaCl+paraffin oil	131.80±10.73	36.22±4.17	103.20±7.06	0.82±0.69	0.41±0.03	78.90±1.05
Group 2 NaCl+CCl_4	470.30±11.52***	201.30±4.0***	185.20±10.66***	2.84±0.31***	1.51±0.4***	70.04±0.84***
Group 3 Silymarin (25 mg/kg) +CCl_4	282.90±11.07***	51.43±3.27***	119.90±7.21***	1.02±0.17**	0.67±0.03*	74.60±0.76**
Group 4 EEO (25 mg/kg) +CCl_4	433.10±10.15*	154.0±6.67***	160.50±6.83^{ns}	2.55±0.47^{ns}	1.15±0.12^{ns}	70.78±0.71^{ns}
Group 5 EEO (100 mg/kg) +CCl_4	363.60±10.21***	125.80±8.82***	141.80±10.47**	1.58±0.13^{ns}	1.09±0.46^{ns}	72.93±0.88^{ns}
Group 6 EED (25 mg/kg) +CCl_4	427.70±4.19*	135.30±3.30***	158.80±11.41^{ns}	2.41±0.62^{ns}	0.97±0.04^{ns}	72.13±1.02^{ns}
Group 7 EED (100 mg/kg) +CCl_4	353.0±5.53***	72.78±4.90***	127.0±8.47***	1.19±0.13**	0.79±0.11*	75.64±1.15***

EEO: Hydro-ethanolic extract of O. *subscorpioidea*; EED: Hydro-ethanolic extract of *D. benthamianus*. Values are the means± SEM of six rats. Symbols represent statistical significance. Group 2 is compared with group 1; Groups 3, 4, 5, 6 and 7 are compared with Group 2. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ and ^{ns}: Not significant

Table 2: Effects of the different treatments of rats on the serum-specific proteins

Treatment	Serum-specific proteins				
	Albumin (g/L)	α_1-globulin (g/L)	α_2-globulin (g/L)	β-globulin (g/L)	γ-globulin (g/L)
Group 1 NaCl+paraffin oil	39.92±1.05	14.75±0.3	5.75±0.46	10.73±0.56	7.70±0.18
Group 2 NaCl+CCl_4	29.22±1.17***	12.88±0.27**	5.02±0.45^{ns}	14.73±0.69***	8.16±0.28^{ns}
Group 3 Silymarin (25 mg/kg) +CCl_4	34.70±11.07^{ns}	13.07±0.24^{ns}	6.09±0.14^{ns}	12.85±0.33**	7.39±0.10^{ns}
Group 4 EEO (25 mg/kg) +CCl_4	30.82±1.61^{ns}	13.20±0.32^{ns}	6.12±0.56^{ns}	13.12±0.24*	7.48±0.73^{ns}
Group 5 EEO (100 mg/kg) +CCl_4	30.98±1.21^{ns}	13.70±0.42^{ns}	6.65±0.31^{ns}	13.65±0.46^{ns}	7.81±0.14^{ns}
Group 6 EED (25 mg/kg) +CCl_4	30.66±1.52^{ns}	13.36±0.25^{ns}	6.20±0.46^{ns}	13.54±0.31^{ns}	8.10±0.59^{ns}
Group 7 EED (100 mg/kg) +CCl_4	34.90±1.68*	13.75±0.48^{ns}	6.17±0.12^{ns}	13.05±0.31*	7.55±0.21^{ns}

EEO: Hydro-ethanolic extract of *O. subscorpioidea*; EED: Hydro-ethanolic extract of *D. benthamianus*. Values are the means±SEM of six rats. Symbols represent statistical significance. Group 2 is compared with group 1; Groups 3, 4, 5, 6 and 7 are compared with Group 2. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ and ^{ns}: Not significant

showed intense centrilobular necrosis, inflammation and micro and macro-vesicular steatosis [Figure 1b]. The sections of liver of the rats treated with standard drug silymarin (Group 3) showed architecture quite similar to that of control [Figure 1c]. In the group of rats treated with plant extracts at high dose (100 mg/kg), the histological architecture of liver sections showed almost normal hepatic cells with a mild degree of inflammation, lesser fatty infiltration and absence of necrosis [Figure 1e and g]. Moderate lobular inflammation and micro and macro-vesicular steatosis were noticed in the liver sections of rats treated at low dose (25 mg/kg) of plant extracts [Figure 1d and f].

DISCUSSION

Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver diseases. It is converted by the liver drug-metabolizing enzyme system (cytochrome P450 2E1) into trichloromethyl radical (CCl_3) which further reacts with oxygen to give trichloromethyl peroxy radical (CCl_3COO^-). These activated radical bind covalently to the macromolecules and induce peroxidative degradation of polyunsaturated fatty acids present in the cytoplasmic membrane lipids.[8] This leads to the formation of lipid peroxides and affects the cellular permeability of hepatocytes.

Table 3: Effect of the different treatments of rats on the serum antioxidant activity

Treatment	Serum antioxidant activity		
	Lipid peroxidation (TBARS) (μmol/L)	Total antioxidant power (μmol/L)	DPPH radical scavenging activity (% inhibition)
Group 1 NaCl+paraffin oil	7.89±0.48	12.71±0.72	34.59±0.88
Group 2 NaCl+CCl_4	25.48±3.54***	3.77±0.76***	14.33±2.01***
Group 3 Silymarin (25 mg/kg) +CCl_4	10.75±1.12***	9.44±0.78**	30.64±0.96**
Group 4 EEO (25 mg/kg) +CCl_4	21.78±0.92^ns^	6.61±0.94^ns^	25.05±3.44^ns^
Group 5 EEO (100 mg/kg) +CCl_4	20.35±0.5^ns^	8.22±1.31*	28.98±4.21**
Group 6 EED (25 mg/kg) +CCl_4	19.85±0.63^ns^	7.70±1.5^ns^	29.09±4.42**
Group 7 EED (100 mg/kg) +CCl_4	15.35±0.69***	9.88±1.0**	29.92±2.47**

EEO: Hydro-ethanolic extract of *O. subscorpioidea*; EED: Hydro-ethanolic extract of *D. benthamianus*. Values are the means±SEM of six rats. Symbols represent statistical significance. Group 2 is compared with group 1; Groups 3, 4, 5, 6 and 7 are compared with Group 2. **P* < 0.05; ***P* < 0.01; ****P* < 0.001 and ns: Not significant

In the assessment of liver damage, the determination of serum biochemical parameter levels such as glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), bilirubin and total protein is used. SGOT and SGPT are the most commonly used markers of hepatocellular necrosis. Serum ALP, GGT, bilirubin and total protein levels on the other hand are related to the function of hepatic cells. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver. Moreover, abnormal increase of serum bilirubin indicates hepatobiliary disease and severe disturbance of hepatocellular architecture.[9]

In the present study, administration of CCl_4 caused abnormally higher activities of serum GOT, GPT, ALP, GGT and total bilirubin in CCl_4 group when compared to the control. This is an indication of the development of hepatic injury which is responsible for leakage of cellular enzymes into the blood. In fact, when liver plasma membrane gets damaged, a variety of enzymes normally located in the cytosol are released into the circulation.[10] The reduction in levels of serum GOT and GPT by the hydro-ethanolic extracts of *O. subscorpioidea* and *D. benthamianus* indicates a stabilization of plasma membrane as well as a repair of

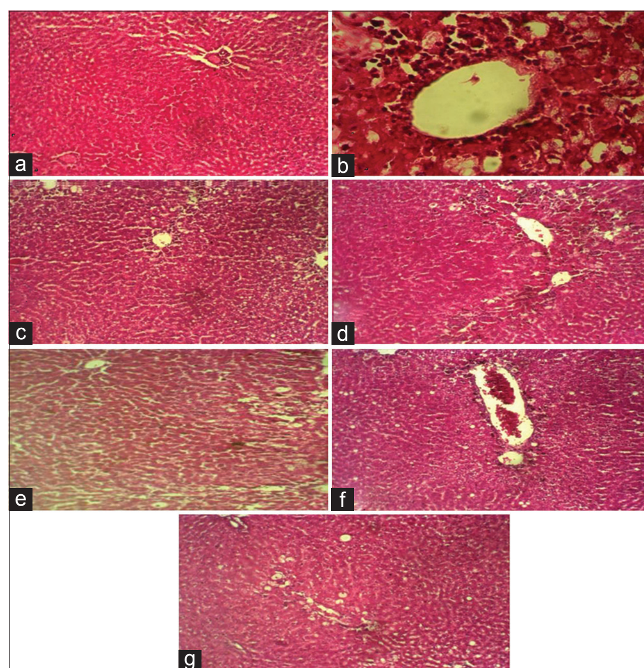

Figure 1: Histology of liver section of rats (a) Liver section of normal control group rats (H-E ×100); (b) Liver section of CCl4 intoxicated group rats (H-E ×400); (c) Liver section of Silymarin treated group rats (H-E ×100); (d) Liver section of EEO (25 mg/kg) treated group 4 rats (H-E ×100); (e) Liver section of EEO (100 mg/kg) treated group rats (H-E ×100); (f) Liver section of EED (25 mg/kg) treated group rats (H-E ×100); (g) Liver section of EED (100 mg/kg) treated group rats (H-E ×100)

hepatic tissue damage caused by CCl_4. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes.[11] These extracts also caused decrease of serum ALP, GGT and bilirubin levels in a dose-dependent manner.

Hepatotoxins impair the capacity of liver to synthesize albumin.[12] A significant decrease of total serum protein, albumin and α_1-globulin levels in CCl_4-intoxicated rats may be attributed to impaired protein synthesis by damaged liver tissue. Pretreatment with the plant extracts dose dependently increased the concentration of these parameters in serum.

Histopathological studies confirmed the capacity of the extracts to reduce the harmful effects and restore the normal hepatic physiology that has been disrupted by CCl_4. In fact, histological lesions (necrosis, inflammation) and fatty changes were observed in livers of CCl_4-intoxicated rats while those of pretreated rats by extracts at 100 mg/kg exhibited, as silymarin, an almost normal architecture with a mild degree of inflammation and lesser fatty infiltration when compared to control groups.

In the *in vivo* antioxidant activity study, significant increase of TBARS in CCl_4 group suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. The significant inhibition of serum DPPH radical scavenging activity and decrease of total antioxidant power in CCl_4 group confirmed that. Pretreatment with the hydro-ethanolic extracts of *O. subscorpioidea* and *D. benthamianus* dose dependently raised the serum antioxidant ability by reversing these changes. These results are in agreement with those of our recently published paper[3] where *O. subscorpioidea* and *D. benthamianus* 70% ethanolic extracts have been proved to be rich in flavonoids and phenolic compounds and exhibit strong antioxidant activity *in vitro*.

CONCLUSIONS

This study has demonstrated a potential hepatoprotective effect of the *O. subscorpioidea* and *D. benthamianus* 70% ethanolic extracts as well as antioxidant activity in rats with CCl_4-induced hepatotoxicity. This finding justifies the use of both plants in traditional medicine for the treatment of liver diseases. Further researches are needed to isolate and purify the active principles involved in hepatoprotection of these plants.

REFERENCES

1. Wolf PL. Biochemical diagnosis of liver diseases. Indian J Clin Biochem 1999;14:59-90.
2. Burkill HM. The useful plants of West Tropical Africa. 2nd ed. Vol. 3. United Kingdom: Families JL Royal Botanic Gardens, Kew, Richmond; 1995. p. 857.
3. Kouassi K, N'guessan JD, Méité S, Yapi A, Yapi HP, Djaman AJ. *In vitro* antioxidant activity and phenolic contents of the leaves of *Olax subscorpioidea* and *Distemonanthus benthamianus*. Res J Pharm Biol Chem Sci 2013;4:1419-30.
4. Miller LC, Tainter ML. Estimation of the ED50 and its error by means of logarithmic-probitgraph paper. Proc Soc Exp Biol Med 1944;57:261-4.
5. Satho K. Serum lipid peroxidation in cerebrovascular disorders determited by a new colorimetric method. Clin Chim Acta 1978;90:37-43.
6. Benzie IF, Strain JJ. Ferric reducing ability of plasma (FRAP) as a measure of an antioxidant power: The FRAP assay. Anal Biochem 1996;239:70-6.
7. Yokozawa T, Chen CP, Dong E, Tanaka T, Nonaka GI, Nishioka I. Study on the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl-2 picrylhydrazyl radical. Biochem Pharmacol 1998;56:213-22.
8. Recknagel RO, Glende EA, Dolak JA, Walter RL. Mechanism of carbon tetrachloride toxicity. Pharmacol Ther 1989;43:139-54.
9. Martin P, Friedman LS. Assessment of liver function and diagnostic studies. In: Freidman LS, Keefe EB, editors. Hand Book of Liver Disease. Philadelphia: Churchill Livingstone; 1992. p. 1-14.
10. Mitra SK, Venkataranganna MV, Sundaram R, Gopumadhavan S. Protective effect of HD-03, a herbal formulation, against various hepatotoxic agents in rats. J Ethnopharmacol 1998;63:181-6.
11. Thabrew M, Joice PD, Rajatissa W. A comparative study of the efficacy of *Pavetta indica* and *Osbeckia octanda* in the treatment of liver dysfunction. Planta Med 1987;53:239-41.
12. Dubey GP, Agrawal A, Dixit SP. Effect of Liv-52 on different biochemical parameters in alcoholic cirrhosis. Antiseptic 1994;91:205-8.

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