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 Distemonathus 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 (CCl₄)-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 CCl₄ 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, α₁-globulin significantly decreased, and DPPH-free radical scavenging activity and TAP were lowered. Pretreatment with plant extracts and silymarin prevent the toxic effects of CCl₄ 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, Distemonathus benthamianus, hepatoprotective activity, histopathology, Olax subscorpioidea

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.[11]

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 Distemonathus benthamianus Baill. (Caesalpiniaceae) are two plants used in traditional medicine to treat respectively jaundice and
hepatitis. Preliminary studies of the aera 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*. 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 vitro* 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 material 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 Houphouët-Boigny 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 (CCl₄) 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 (BUCHI). 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 (LD₅₀) was determined for each extract following the graphic method of Miller and Tainter.

**CCl₄ 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 (CCl₄ group) received solution of NaCl 0.9% (5 mL/kg bw) for 7 days and CCl₄ (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 CCl₄ (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. subscorpioidea* for 7 days and CCl₄ 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 CCl₄ 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 supernatant 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 Fe³⁺ to Fe²⁺ 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 FeCl₃ × 6 H₂O 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 Fe²⁺ in the range of 1.56 to 100 µmol.L⁻¹ were prepared from ferrous sulfate (FeSO₄ × 7 H₂O) 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 toscavenge DPPH radical was calculated by the following equation:

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

where \(\text{Abs}_{control}\) is the absorbance of DPPH radical without deproteinized serum and \(\text{Abs}_{sample}\) 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 (TIVODA). 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 cosin (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 Dunnert’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 (LD₅₀) 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 CCl₄-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, \( \alpha_1 \)-globulin, \( \alpha_2 \)-globulin, \( \beta \)-globulin and \( \gamma \)-globulin contents were more or less modified in \( \text{CCl}_4 \) 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 \( \alpha_1 \)-globulin, \( \alpha_2 \)-globulin, \( \beta \)-globulin and \( \gamma \)-globulin. \( D. \ benthamianus \) 70\% ethanolic extract at 100 mg/kg showed highly significant activity \( (P < 0.05) \) with maximum inhibition of \( \text{CCl}_4 \) 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 \( \text{CCl}_4 \) are summarized in Table 3. There was a significant increase \( (P < 0.001) \) in the levels of TBARS in \( \text{CCl}_4 \)-intoxicated rats compared to control group. Extracts at 25 and 100 mg/kg exhibited an ability to counteract the \( \text{CCl}_4 \) induced toxicity in pretreated groups by decreasing TBARS levels compared to \( \text{CCl}_4 \) 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 \( \text{CCl}_4 \)-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 \( \text{CCl}_4 \)-intoxicated rats (Group 2)

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### 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   | 131.80±10.73 | 36.22±4.17 | 103.20±7.06 | 0.82±0.69 | 0.41±0.03 | 78.90±1.05 |
| NaCl+paraffin oil | | | | | | |
| Group 2   | 470.30±11.52*** | 201.30±0.9*** | 185.20±10.66*** | 2.84±0.31*** | 1.51±0.4*** | 70.04±0.84*** |
| NaCl+\( \text{CCl}_4 \) | | | | | | |
| Group 3   | 282.90±11.07*** | 51.43±3.27*** | 119.90±7.21*** | 1.02±0.17** | 0.67±0.03* | 74.60±0.76** |
| Silymarin (25 mg/kg) +\( \text{CCl}_4 \) | | | | | | |
| Group 4   | 433.10±10.15* | 154.0±6.67*** | 160.50±8.38*** | 2.55±0.47*** | 1.15±0.12* | 70.78±0.71ns |
| EEO (25 mg/kg) +\( \text{CCl}_4 \) | | | | | | |
| Group 5   | 363.60±10.21*** | 125.80±8.82*** | 141.80±10.47*** | 1.02±0.17** | 0.67±0.03* | 74.60±0.76** |
| EEO (100 mg/kg) +\( \text{CCl}_4 \) | | | | | | |
| Group 6   | 427.70±4.19* | 135.30±3.30*** | 158.80±11.41*** | 2.41±0.62ns | 0.97±0.04ns | 72.13±1.02ns |
| EED (25 mg/kg) +\( \text{CCl}_4 \) | | | | | | |
| Group 7   | 353.0±5.53*** | 72.78±4.90*** | 127.0±8.47*** | 1.19±0.13** | 0.79±0.11* | 75.64±1.15*** |
| EED (100 mg/kg) +\( \text{CCl}_4 \) | | | | | | |

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) | \( \alpha_1 \)-globulin (g/L) | \( \alpha_2 \)-globulin (g/L) | \( \beta \)-globulin (g/L) | \( \gamma \)-globulin (g/L) |
| Group 1   | 39.92±1.05 | 14.75±0.3 | 5.75±0.46 | 10.73±0.56 | 7.07±0.18 |
| NaCl+paraffin oil | | | | | |
| Group 2   | 29.22±1.17*** | 12.88±0.27** | 5.02±0.45ns | 14.73±0.69*** | 8.16±0.28ns |
| NaCl+\( \text{CCl}_4 \) | | | | | |
| Group 3   | 34.70±11.07ns | 13.36±0.25ns | 6.20±0.14ns | 13.54±0.31ns | 7.81±0.14ns |
| Silymarin (25 mg/kg) +\( \text{CCl}_4 \) | | | | | |
| Group 4   | 30.82±1.61* | 13.20±0.32** | 6.12±0.56** | 13.12±0.24* | 7.48±0.73* |
| EEO (25 mg/kg) +\( \text{CCl}_4 \) | | | | | |
| Group 5   | 30.98±1.21* | 13.70±0.42** | 6.65±0.31** | 13.65±0.46** | 7.61±0.14** |
| EEO (100 mg/kg) +\( \text{CCl}_4 \) | | | | | |
| Group 6   | 30.66±1.52ns | 13.36±0.25ns | 6.20±0.46** | 13.54±0.31ns | 8.10±0.59* |
| EED (25 mg/kg) +\( \text{CCl}_4 \) | | | | | |
| Group 7   | 34.90±1.68* | 13.75±0.48** | 6.17±0.12** | 13.05±0.31* | 7.55±0.21** |
| EED (100 mg/kg) +\( \text{CCl}_4 \) | | | | | |

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 (CCl₃) which further reacts with oxygen to give trichloromethyl peroxy radical (CCl₃COO). These activated radical bind covalently to the macromolecules and induce peroxidative degradation of polyunsaturated fatty acids present in the cytoplasmic membrane lipids. 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**                   | **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+CCl₃               | 25.48±3.54***                   | 3.77±0.76***                   | 14.33±2.01***                   |
| Group 3 Silymarin (25 mg/kg) +CCl₃ | 10.75±1.12***                  | 9.44±0.78**                    | 30.64±0.96**                    |
| Group 4 EEO (25 mg/kg) +CCl₃    | 21.78±0.92**                    | 6.61±0.94**                    | 25.05±3.44**                    |
| Group 5 EEO (100 mg/kg) +CCl₃  | 20.35±0.63**                   | 8.22±1.31*                     | 28.98±4.21**                    |
| Group 6 EED (25 mg/kg) +CCl₃   | 19.85±0.63**                   | 7.70±1.5**                     | 29.09±4.42**                    |
| Group 7 EED (100 mg/kg) +CCl₃  | 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 present study, administration of CCl₄ caused abnormally higher activities of serum GOT, GPT, ALP, GGT and total bilirubin in CCl₄ 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. 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](https://example.com/image1.png)

**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 CCl₄. 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. 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. A significant decrease of total serum protein, albumin and α₁-globulin levels in CCl₄-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 CCl₄. In fact, histological lesions (necrosis, inflammation) and fatty changes were observed in livers of CCl₄-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 vitro antioxidant activity study, significant increase of TBARS in CCl₄ 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 CCl₄ 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 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 CCl₄-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.

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