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Research Article
Dichrocephala integrifolia (Linn.f.) O. Kuntze (Asteraceae) Leaves Aqueous Extract Prevents Ethanol-induced Liver Damage in Rats

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Abstract

Background and Objective: *Dichrocephala integrifolia* is used in traditional medicine for the treatment of many diseases including hepatic disorders. The present study was undertaken to evaluate the preventive effects of the aqueous extract of *D. integrifolia* leaves on ethanol induced-hepatoxicity. **Methodology:** In preventive treatment, animals received aqueous extract of *D. integrifolia* at different doses (100 or 200 mg kg^{-1}) or simepar (200 mg kg^{-1}) for one week. A unique dose of 40° ethanol (4 g kg^{-1}) was daily administered orally to animals during 2 weeks. At the end of treatment, transaminases aspartate amino-transferase (AST), alanin amino-transferase (ALT), bilirubin, alkaline phosphatase (ALP), lipid profile, serum and hepatic proteins, superoxide dismutase, catalase, malondialdehyde and nitric oxide were evaluated. Histological analysis of the liver was investigated. **Results:** A unique daily administration of ethanol during 14 days provoked a significant increased of transaminases, ALP, bilirubin, lipid profile, oxidative status and a significant decreased in serum and hepatic proteins. In addition, histopathological analysis revealed hepatic inflammation, steatosis and vascular congestion. The administration of the extract brought transaminases towards normal values. Bilirubin, ALP, lipid profile, protein and oxidative status were improved. The plant extract at all doses prevented the development of different abnormalities on hepatic tissue. **Conclusion:** The aqueous extract of *D. integrifolia* leaves improve the hepatic function in ethanol-induced hepatic damage, probably due to its antioxidant properties. These findings justify the traditional use of this plant in the management of liver problems.

Key words: *Dichrocephala integrifolia*, toxic hepatitis, hepatoprotection, antioxidant

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

INTRODUCTION

Alcoholism is a significant phenomenon in our society. About 3.5% of all health problems are due to the consumption of alcohol[1]. Frequent alcohol ingestion is responsible for excess morbidity and mortality[2]. In fact, the metabolite of ethanol has the ability to generate free radicals causing oxidative stress leading to peroxidation and inflammatory response which in turn could induce some liver diseases[3,4]. The improvement of lifestyle by reducing alcohol consumption, the use of hepatoprotective drugs and vaccination allow people to fight against several types of hepatitis. Discovering news molecules able to fight against alcohol-induced liver diseases will be a great benefit as an alternative therapy. Considering the side effects of synthetic molecules many plants are used in traditional medecin for the treatment of liver diseases. These plants can be hepatoprotective, antihepatotoxic and/or antioxidants thus have the ability to fight the toxic lesions induced on the biological macromolecules[5]. In many Cameroonian regions, one of the plants used in the traditional medicine for the relief of some hepatic disorders is *Dichrocephala integrifolia*. Some researchers have shown that, this plant exhibit anticancer, antimicrobial, anti-inflammatory, antioxidant and antihelmintic[6,7] properties. For the more exploration of the therapeutic properties of this plant, the main objective was to evaluate the preventive effects of the aqueous extract of the leaves of *Dichrocephala integrifolia* against ethanol-induced liver injury in rats.

MATERIALS AND METHODS

Plant material: Fresh leaves of *Dichrocephala integrifolia* were collected in Mendong (Department of Mfoundi, Centre Region of Cameroon) in August, 2013 and was identified at the National Herbarium of Cameroon in comparison with the sample No 65648/HNC.

Preparation of *D. integrifolia* leaves aqueous extract: The *D. integrifolia* leaves were dried at room temperature and crushed. The decoction was carried out by boiling 150 g of powder in 1.5 L of tap water for 10 min following the instruction of the traditional healer. The mixture obtained was filtered, frozen at -20°C and lyophilised at the Institute of Medical Research and Study of Medicinal Plants (IMPM) to yield approximately 11.78% (w/w) which was kept at the room temperature until the use.

Animals: Male Wistar rats of 8-10 weeks of age and weighing between 180-200 g at the beginning of the experiment were used for the study. These animals were bred in the Laboratory of Animal Physiology of the University of Yaounde I. They were maintained under standard laboratory conditions with 12 h light and dark cycle with free access to standard laboratory rat food and tap water. Animal studies were conducted in accordance with the approval of the Cameroon National Ethical Committee (Ref No. Fw-IRb00001954).

Experimental induction of hepatotoxicity in rats: The hepatotoxicity was induced by a daily oral administration of a unique dose of 40° ethanol (4 g kg^{-1}) to the animals during 2 weeks[8].

Preventive effect of *Dichrocephala integrifolia*: Twenty-five normal rats were divided into 5 groups of 5 animals each: One normal control group and one negative control group, treated with distilled water (10 mL kg^{-1}). One positive control group treated with the Simepar as a reference drug at the dose of 100 mg kg^{-1}. Two groups treated with the plant extract at the doses of 100 and 200 mg kg^{-1}, respectively. Different solutions were administered per os once a day for 1 week. Stopping the treatment was followed by a single daily administration of ethanol (4 g kg^{-1}) for 2 weeks.

Assessment of the liver function: On the 22 days of experiment, all animals were anesthetized with diethyl ether vapor and sacrified. The arterio-venous blood was collected into the tubes then centrifuged at 3000 rpm for 15 min at 4°C. The obtained serum (supernatant) was collected and stored at -20°C for the determination of some biochemical parameters (alanine amino-transferase, aspartate amino-transferase, alkaline phosphosphatase, bilirubin, total cholesterol, HDL-cholesterol, triglycerides and proteins) using commercial kits according to procedure provided by the manufacturer. Atherogenic index was calculated using total cholesterol and HDL-cholesterol ratio[9].

Assessment of the liver oxidative stress: Homogenates (20%) of liver samples were prepared in tris–KCl buffer (pH 7.4). Organs were crushed and then the mixture was centrifuged at 3000 g at 4°C for 20 min. The supernatant was collected and stored at −20°C until tissue analysis. The effect of repeated doses of ethanol on some antioxidant parameters including malondialdehyde, reduced glutathione (GSH), catalase and nitrites were assessed.

Histopathological analysis: A remaining part of the liver, of each animal was fixed in 10% buffered formalin. The histological sections (5 µm) of the liver tissues were assessed by haematoxylin and eosin staining method[10,11].

Statistical analysis: The results were expressed as Mean±Standard Error Mean (SEM) and analyzed using one way analysis of variance (ANOVA) followed by DUNNET's test as post-test. Differences were considered significant at $p<0.05$.

RESULTS

Preventive effects of the aqueous extract of *Dichrocephala integrifolia* on some serum parameters of liver function: Table 1 shows that, the administration of a single daily dose of 40° ethanol (4 g kg^{-1}) for 14 days to rats caused a significant increase in serum alanine amino-transferase (ALT), aspartate amino-transferase (AST), alkaline phosphosphatase (ALP) and bilirubin respectively by 65.14, 40.36, 104.92 and 45.29%, compared to normal control. Pretreatment with aqueous extract of the leaves of *Dichrocephala integrifolia* during one week significantly inhibited the increase in transaminases. At the dose of 100 mg kg^{-1}, the levels of ALT and AST were inhibited by 45.10 and 46.42%, respectively, compared to the negative control. At the dose of 200 mg kg^{-1}, the inhibition was 49.51, 51.67%, respectively for ALT and AST. The extract at all doses did not cause a significant change in the rate of ALP and bilirubin compared to the negative control. Simepar, used as a reference drug administered at the dose of 100 mg kg^{-1} for 7 days prior to the ingestion of ethanol resulted in a significant inhibition of 39.72, 40.57 and 25.58%, respectively for ALT, AST and bilirubin compared to the negative control.

Preventive effects of the aqueous extract of *Dichrocephala integrifolia* on lipid profile: Table 2 illustrates the preventive effects of the aqueous extract of the leaves of *D. integrifolia* on lipid profile. It shows that poisoning of animals with ethanol resulted in a significant increase in total cholesterol, LDL cholesterol, triglycerides, atherogenic index and a significant decrease in HDL-cholesterol respectively of 31.17% ($p<0.01$), 149.76% ($p<0.01$), 80.84% ($p<0.01$), 83.23% ($p<0.01$) and 28.16% ($p<0.001$) when compared to normal control. However, compare to the negative control, the pretreatment of animals with the plant extract at the dose of 100 mg kg^{-1}, prevented the increase of total cholesterol, LDL-cholesterol and the atherogenic index by 24.11% ($p<0.01$), 71.80% ($p<0.001$) 44.50% ($p<0.001$), respectively. Furthemore, at the same dose, the plant extract also prevented the decrease of HDL-cholesterol by 40.79% ($p<0.05$).

At the dose of 200 mg kg^{-1} the plant extract inhibited the increase of the total cholesterol (27.37%, $p<0.01$), LDL-cholesterol (81.07%, $p<0.001$), triglycerides (38.95%, $p<0.05$), the atherogenic index (53.10%, $p<0.001$) and a decrease in HDL-cholesterol (56.74%, $p<0.01$) as compared to negative control. Simepar administered to animals in the same conditions as the extract significantly prevented the increase in the total cholesterol (24.64%, $p<0.01$), LDL-cholesterol (61.70%, $p<0.001$), triglycerides (48.11%, $p<0.01$), atherogenic index (44.50%, $p<0.01$) and the decrease HDL-cholesterol (40.79%, $p<0.051$) compared to negative control.

Preventive effects of aqueous extract of *Dichrocephala integrifolia* on some oxidative stress markers of liver tissue: Hepatoprotective effect of *D. integrifolia* aqueous extract against alcohol induced oxidative stress is shown in Table 3. The administration of ethanol (4 g kg^{-1}) to normal rats prior to distilled water for 7 days induced a significant increase in hepatic levels of SOD by 195.47%, MDA by 134.68% and catalase by 229.60% compared to normal control. In addition, ethanol ingestion provoked a no significant decrease in nitric oxide. The administration of the aqueous extract of the leaves

Table 1: Preventive effects of aqueous extract of *Dichrocephala integrifolia* (Di) on some serum parameters of liver function

Parameters	Normal control	Negative group	Simepar (100 mg kg^{-1})	Di (100 mg kg^{-1})	Di (200 mg kg^{-1})
ALT (UI L^{-1})	17.56±1.18	29.03±1.33***	17.48±1.24$^{\$\$\$}$	15.92±2.22$^{\$\$\$}$	14.64±1.29$^{\$\$\$}$
AST (UI L^{-1})	59.71±3.33	83.87±4.60***	49.88±1.58**$^{\$\$\$}$	44.90±4.52*$^{\$\$\$}$	40.50±3.69*$^{\$\$\$}$
ALP (UI L^{-1})	0.91±0.17	1.87±0.22*	1.53±0.19	1.41±0.17	1.05±0.33
Bilirubin (mg dL^{-1})	3.93±0.11	5.71±1.34***	4.25±0.12$^{\$\$}$	4.95±0.14	4.88±0.18

Values are Mean±SEM, n = 5, *p<0.05, **p<0.01, ***p<0.001 significantly different from normal control, $^{\$\$}$p<0.01, $^{\$\$\$}$p<0.001 significantly different from the negative control, Di: *Dichrocephala integrifolia*

Table 2: Preventive effects of aqueous extract of *D. integrifolia* on lipid profile

Parameters	Normal control	Negative group	Simepar (100 mg kg^{-1})	Di (100 mg kg^{-1})	Di (200 mg kg^{-1})
Total cholesterol (mg dL^{-1})	62.13±4.34	81.50±3.76**	61.41±3.80$^{\$\$}$	61.84±4.57$^{\$\$}$	59.18±3.61$^{\$\$}$
Cholesterol-HDL (mg dL^{-1})	38.58±1.44	27.71±1.34*	39.01±5.19$^{\$}$	39.89±1.25$^{\$}$	43.43±2.96$^{\$\$}$
Cholesterol-LDL (mg dL^{-1})	16.24±4.90	40.56±2.72**	15.53±3.31$^{\$\$\$}$	11.43±4.40$^{\$\$\$}$	7.67±3.00$^{\$\$\$}$
Triglycerides (mg dL^{-1})	36.55±4.71	66.11±9.49**	34.30±3.30$^{\$\$}$	52.58±4.80	40.35±4.05$^{\$}$
Atherogenic index	1.610±0.12	2.96±0.14***	1.64±0.13$^{\$\$\$}$	1.55±0.12$^{\$\$\$}$	1.38±0.10$^{\$\$\$}$

Values are Mean±SEM, n = 5, *p<0.05, **p<0.01, ***p<0.001 significantly different from control, $^{\$}$p<0.05, $^{\$\$}$p<0.01, $^{\$\$\$}$p<0.001 significantly different as compared to negative group, Di: *Dichrocephala integrifolia*

Table 3: Preventive effects of the aqueous extract of *D. integrifolia* on some oxidative stress markers in liver tissue

Parameters	Normal control	Negative group	Simepar (100 mg kg^{-1})	Di (100 mg kg^{-1})	Di (200 mg kg^{-1})
SOD (U mg^{-1} proteins)	14.920±2.08	44.080±3.88***	15.72±2.30$^{\$\$\$}$	14.24±2.08$^{\$\$\$}$	20.06±1.75$^{\$\$\$}$
Catalase (mM mg^{-1} proteins)	1.140±0.24	4.860±0.51***	1.23±0.20$^{\$\$\$}$	1.05±0.09$^{\$\$\$}$	1.68±0.06$^{\$\$\$}$
MDA (μM mg^{-1} proteins)	1.580±0.38	3.700±0.69*	2.08±0.69	1.43±0.28$^{\$}$	1.92±0.31
Nitric (μM)	0.062±0.009	0.042±0.002	0.05±0.006	0.04±0.00	0.06±0.007

Values are Mean±SEM, n = 5, *p<0.05, **p<0.01, ***p<0.001 significantly different from control, $^{\$}$p<0.05, $^{\$\$}$p<0.01, $^{\$\$\$}$p<0.001 significantly different from the negative group, Di: *Dichrocephala integrifolia*

of *D. integrifolia* at the dose of 100 mg kg^{-1} for 7 days prior to the ingestion of ethanol significantly inhibited the increase of hepatic levels of SOD, MDA and catalase by 67.68% (p<0.01), 61.28% (p<0.05) and 78.31% (p<0.01), respectively. At the dose of 200 mg kg^{-1} of the extract the percentage of inhibition was 54.49% (p<0.001) and 65.45% (p<0.001) respectively for SOD and catalase. The administration of Simepar at the dose of 100 mg kg^{-1} in the same conditions significantly (p<0.001) prevented the increase in hepatic levels of SOD and catalase to 64.33 and 74.70%. The extract at the dose of 200 mg kg^{-1} and Simepar did not significant change the hepatic levels of MDA. The animals pretreated with the plant extract (100 or 200 mg kg^{-1}) or Simepar for a week and then intoxicated with ethanol for 2 weeks showed a non significant increase in liver level of nitrites.

Preventive effect of the aqueous extract of the leaves of *Dichrocephala integrifolia* on serum and hepatic protein levels: Figure 1 shows the effects of the extract in preventive treatment on serum and hepatic protein levels (Fig. 1a, b). It is clear from this figure that the administration of distilled water for 7 days and alcohol intoxication for 14 days to normal rats induced a significant decrease in serum and liver protein levels respectively by 30.13% (p<0.001) and 57.96% (p<0.01), compared to normal control. Administration of the extract (100 or 200 mg kg^{-1}) during 1 week prior to ethanol for 2 weeks showed a significant increase in serum and hepatic protein levels compared to negative control. At the dose of 100 mg kg^{-1}, the plant extract induced an inhibition of serum and liver protein by 35.65 and 178.30%, respectively compared to the negative control, while the dose of 200 mg kg^{-1} induced the increase by 38.56% for the serum proteins and 111.21% for liver proteins. The administration of Simepar for 7 days prior to the ingestion of ethanol resulted in a significant increase by 37.63 and 140.56%, respectively for the serum and liver protein levels compared to the negative control.

Preventive effects of aqueous extract of *Dichrocephala integrifolia* on liver histology: Figure 2a shows the histology of the liver of a normal rat. This section shows hepatic bile duct and hepatocytes separated by the sinusoids. The histology of the liver of animals treated with ethanol for 14 days (Fig. 2b) has a disorganized parenchyma, vascular congestion and steatosis. Histological analysis of the liver of animals pretreated with Simepar (Fig. 2c) showed normal architecture. Animals pretreated with the plant extract at the doses of 100 mg kg^{-1} (Fig. 2d) and 200 mg kg^{-1} (Fig. 2e) for 1 week prior to the administration of ethanol for 2 weeks has very close architecture as compared to that of the normal rat. However, it was noted a slight dilation of sinusoids in the liver of animals treated with Simepar or plant extract.

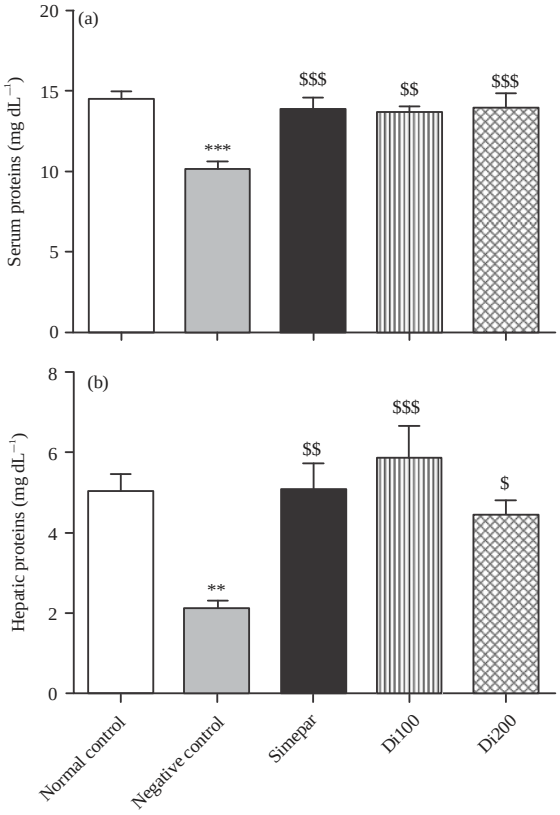

Fig. 1(a-b): Preventive Effects of aqueous extract of *D. integrifolia* on (a) Serum protein levels and (b) Liver. Each bar represents the Mean±SEM, n = 5, **p<0.01, ***p<0.001 significantly different from normal group, $^{\$}$p<0.05, $^{\$\$}$p<0.01, $^{\$\$\$}$p<0.001 significantly different from the negative control and Di: *Dichrocephala integrifolia*

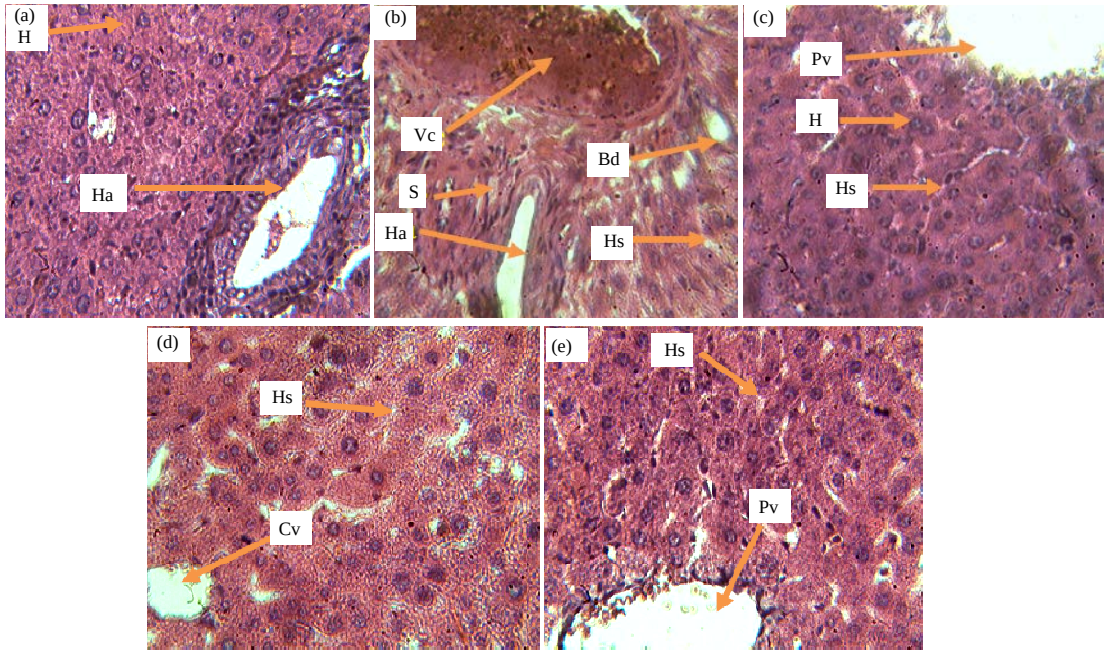

Fig. 2(a-e): Histology of the liver of (a) Normal rats (b) Negative control, (c) , Simepar those pretreated with the plant extract at doses o f (d) 100 mg kg^{-1} and (e) 200 mg kg^{-1} during one week prior to the administration of ethanol for 14 days (HEX400), Ha: Hepatic artery, Bd: Bile duct, H: Hepatocyte, Cv: Centro-lobular vein, S: Steatosis, Vc: Vascular congestion, Hs: Hair sinusoid and Pv: Portal vein

DISCUSSION

The results of this study showed that a single daily administration of the 40° ethanol at the dose of 4 g kg^{-1} for 14 days to rats induced a significant increase ($p<0.001$) in serum ALT, AST and biluribin. The increase in these parameters is conventional sign of liver injury[2] resulting from a loss of hepatocytes membrane integrity[12]. In this study, the plant extract prevented ethanol-induced elevation of ALT, AST and biluribin levels. It is possible, that the extract protects cell membranes or counteracts the deleterious effects of ethanol. These results could suggest the presence of some compounds (tannins, alkaloids) which could inhibit the cytochrome P_{450} responsible for the ethanol metabolism or boost the gene responsible for the regeneration of liver cells, or scavenged free[8,13]. Animals treated with the Simepar (100 mg kg^{-1}) in the same conditions showed significant decrease in transaminases. Simepar is a drug used for the treatment of some liver diseases. The active principle of this drug is silymarin which is a flavonoid[14]. Simepar has the ability to fight against lipid peroxidation, regulating the membrane permeability and increase membrane stability[15]. The intoxication of rats with ethanol caused a significant increase of ALP and bilirubin. This increase in serum ALP and bilirubin could be due to the liver damage resulting from the liver inflammation and disruption of the synthesis of the bile salts induced by ethanol[14]. Bilirubin is a pigment generated from the degradation of erythrocytes[16]. The glucuroconjugated bilirubin present in liver cells is actively excreted in the bile ducts. Inflammation or cholesterol deposition in the bile ducts can induce the partial or complete obstruction of bile ducts. This results in an increase of the concentration of direct bilirubin and ALP in the liver. This bile constituent reaches the plasma by passage through tight junctions located between the bile canaliculi and sinusoids or by diffusion hence, the increase of these parameters[16]. The administration of the extract inhibited the increase in serum bilirubin and ALP at all doses though non significant. These results suggest that the plant extract could protect the liver against inflammation and disorders in bilirubin excretion. This results show that ethanol intoxication resulted in a significant decrease ($p<0.001$) in serum protein levels. This could be due to the oxidation of proteins by ethyl alcohol-induced free radicals. In fact, several proteins synthesized in the liver are found in the blood. The oxidation of these proteins could cause protein denaturation thus reduced their liver and blood concentrations[1,3]. The ingestion of the extract maintained the serum protein around the normal probably due to its antioxidant character. Ethanol

consumption enhanced lipogenesis, which leads to excessive de novo fatty acids and triglycerides synthesis that contributes to the development of liver fat accumulation[17,2]. It also inhibits the activity of enzymes like lipases involved in the transformation of lipoproteins and triglycerides. The inhibition of these enzyme induced the modification of lipid profile (serum accumulation) observed in this study[18]. The administration of the extract maintains serum lipid profile parameters around the normal range. This suggests that, the extract might possess compounds able to inhibit the adverse effects of ethanol on lipoproteins and triglycerides processing enzymes. Acute or chronic exposure of alcohol induced generation of Reactive Oxygen Species (ROS) and depletion in cellular antioxidant levels causing oxidative stress[19,2]. However, in this study, there was an elevation of cellular superoxide dismutase (SOD) and catalase. The increased levels of these enzymes are the response of ethanol-induced oxidative stress[20,21]. The administration of the extract whatever the dose prevents the increase in these enzymes, suggesting the antioxidant character of the extract which already has been proven[6]. Thus, the extract could protect the liver against free radicals or trap them when they are already generated, restoring the balance prooxidant/antioxidant. This ability of the extract to protect the liver is probably due to the presence of compounds such as carotenoids, tannins and triterpenes which possess antioxidant activity[22,23] in the plant extract. Lipid peroxidation is one of the processes by which the ethanol-induced hepatotoxicity[2,24]. This study has shown a significant increase of malondialdehyde (MDA) in the liver of animals intoxicated with ethanol. This increase in MDA levels suggests an increase in lipid peroxidation leading to the destruction of liver tissue and a reduction in antioxidant defenses to neutralize ROS[25]. The production of free radicals reacts with ethanol to give a hydroxyethyl radical, which is highly toxic to cell membranes. The administration of the extract (100 or 200 mg kg^{-1}) inhibited the increase of hepatic MDA levels induced by alcohol ingestion. These results suggest that the aqueous extract of the leaves of *D. integrifolia* exerts its hepatoprotective effects by inhibiting lipid peroxidation of membranes of liver cells. Poisoning rats to ethanol resulted in a decrease in the hepatic levels of nitrites. Nitric Oxide (NO) is generated by the L-arginin in the presence of NO synthase. The NO can slowly react with O_2 to form the nitric or nitrate or react rapidly with the superoxide anion (O^{2-}) to form a highly reactive compound, peroxynitrite ($ONOO^-$) which is an agent capable to induce lipid peroxidation[26]. The decrease of hepatic levels of nitrites is in agreement with the study of Aboubakar *et al.*[18]. For these authors, the decline would be the result of endothelial dysfunction which is strengthened by the increase in the atherogenic index observed in rats receiving ethanol. The administration of the aqueous extract of *D. integrifolia* has reduced or maintains the nitric levels around the normal value. These results suggest that the extract has a protective role against the endothelial dysfunction induced by ethanol. In this study the oxidant character of ethanol-induced free radicals contributing to damage cells is confirmed in histological section by the presence of vascular congestion, steatosis, diffuse infiltration of inflammatory cells and dilatation of periportal sinusoids. These different abnormalities attest the sign of liver tissue inflammation[27]. Animals treated with aqueous extract of *D. integrifolia* in preventive treatment showed normal architecture. This result justifies the improvement of oxidant status of animals, due to the hepatoprotective and antioxidant character of the plant extract.

CONCLUSION

It is clear from this work that, the administration of a single daily dose of 40° ethanol (4 g kg^{-1}) for 14 days to rats results in an increased in transaminases, PAL and bilirubin, hypercholesterolemia, hypertriglyceridemia, oxidative stress and liver injury. *Dichrocephala integrifolia* leaves aqueous extract protected rats against oxidant character of ethanol-induced liver dysfunction but also prevents its deleterious effect. This is demonstrated by the ability of the extract to improve transaminases activities, lipid profile, oxidant status and to prevent the severity of liver damage. These results suggest that *D. integrifolia* leaves aqueous extract possess hepatoprotective and antioxidant effect. More experiments are required to better understand the molecular mechanism involved in the pharmacological effects observed.

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