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Mbatchou Adolphe
Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, Yaoundé, Cameroon

Ngueguim Tsofack Florence
Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, Yaoundé, Cameroon

Donfack Jean Hubert
Department of Pharmaceutical Sciences, Faculty of Medicine and Pharmaceutical Science, University of Dschang, Dschang, Cameroon

Gounoue Kamkumo Raceline
Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, Yaoundé, Cameroon

Fifen Ngapout Rodrigue
Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, Yaoundé, Cameroon

Dimo Theophile
Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, Yaoundé, Cameroon

Corresponding Author:
Dimo Theophile
Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, Yaoundé, Cameroon

Protective potential of aqueous extract from *Dichrocephala integrifolia* (Linn. f.) O. Kuntze (Asteraceae) on blood and biochemical constituents in ethanol-induced hepato-nephrotoxicity in rats

Mbatchou Adolphe, Ngueguim Tsofack Florence, Donfack Jean Hubert, Gounoue Kamkumo Raceline, Fifen Ngapout Rodrigue and Dimo Theophile

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Abstract
Dichrocephala integrifola is a plant used empirically to manage hepatic affections. This study evaluates the preventive effects of the aqueous leave extract of *Dichrocephala integrifolia* on the blood and biochemical parameters in ethanol-induced hepato-nephrotoxicity in rats. To achieve this, the animals received different treatments (p.o): a normal control group and a negative group receiving respectively distilled water (10 mL/kg) and ethanol 40° (4 g/kg) with distilled water simultaneously; 3 other groups receiving the two doses of the plant extract or Simepar (100 mg/kg) and ethanol simultaneously. At the end of the experimental period, haematological and biochemical analyses were performed. The ingestion of ethanol provoked a decrease (at least $p< 0.01$) in the liver relative weight. The concentration of RBC, HGB, HCT; MCV; PLT; WBC; LYMP; NEUT; MONO dropped while the levels of WBC raised. Ethanol administration caused an ($p< 0.001$) increase in malondialdehyde even SOD and catalase activities whereas a decreased in nitrites and GSH was noticed. Transaminase levels, ALP, bilirubin and lipid profile were significantly altered associated with a micro-architectural disorganization materialized by hepatic cytolysis, diffuse infiltration of periportal inflammatory cells and dilation of sinusoids capillaries as well as an architectural disorganization of the kidney parenchyma with leukocyte infiltration. The extract protected the alteration of these parameters. It prevented the amplification of alterations induced by ethanol on hepatic and renal micro-architecture tissue whatever the doses. Data suggest the antioxidant and hepatoprotective activities of the extract, attesting the use of this extract in the treatment of liver affection in traditional medicine.

Keywords: Hepato-nephrotoxicity, ethanol, *Dichrocephala integrifolia*, hepatoprotection, antioxidant

1. Introduction
Chronic alcohol ingestion causes several abnormalities such as gastro-intestinal damage, pancreas inflammation, neurons and liver dysfunctions [1]. Alcohol consumption is responsible for about 3.5% of health problems [2]. Liver Pathologies due to excessive consumption of alcohol lead to liver cirrhosis and liver cancer. In these conditions, the oxidative stress generated is incriminated in the amplification of liver damage through the toxicity of acetaldehyde [3]. Large quantity of ethanol induces kidney damage [4]. Moreover, Alcohol consuming causes adverse effects on haematological parameters such as anemia, neutropenia and thrombocytopenia [5, 6]. Lifestyle improvement, the use of hepatoprotective drugs, by reducing alcohol consumption, vaccine administration are some strategies to manage hepatic pathology. The pharmacological molecules used in the treatment of these liver damage are very expensive, inaccessible, and the treatment takes a long time [7]. This can justify the renewal interest in research on medicinal plants with hepatoprotective properties. *Dichrocephala integrifolia* is used in Cameroon to manage several type of hepatitis. Different groups of compounds of this plant include saponins, alkaloids, phenols, flavonoids, glycosides, terpenoids, and tannins [8]. Compounds such as stearic acid (fatty acid), stigmasta-7, 22-diene-3-ol (sterol), α-amyrine (triterpene), epifriedelanol (derivative of frideline, Triterpene), methyl stearate (methylated derivative of stearic acid, fatty acid), and tritetracontane (aliphatic compound) where isolated from this plant [9]. Previous studies have shown that *D. integrifolia* possess anticancer, antibiotic, anti-inflammatory, anti-oxidant properties [10]; anxiolytic and

sedative activities [11]; hepatoprotective properties against ethanol-induced hepatotoxicity [12]. Thus, the possible activities of *D. integrifolia* aqueous extract against ethanol-induced hepato-nephrotoxicity in rats was investigated.

2. Materials and Methods

2.1 Plant material

Fresh leaves of the plant were collected in Cameroon (Mendong-Yaounde) in August, 2018. Botanical identification was done in the National herbarium, Yaoundé, (Cameroon) using an existing voucher specimen No 65648/HNC.

2.2 Animals

Male Wistar rats (8-10 weeks of age; between 180-200 g) were used. They were bred in the Animal house of Physiology Laboratory of the University of Yaoundé I. All the experiments were conducted in accordance with the approval of the Cameroon National Ethical Committee (Ref No. Fw-IRb00001954).

2.3 Aqueous leaves extract preparation

The *D. integrifolia* leaves were harvested, dried under shade then, crushed. The decoction was obtained as described by Ngueguim [12]. Briefly, the powder (150 g) was boiled into 1.5 L of tap water for 10 min following the traditional healer procedure. The dried extract (11.78% yield) was obtained.

2.4 Evaluation of hepatoprotective activity of *Dichrocephala integrifolia*

2.4.1 Experimental design of the study

The suppressive effect of *Dichrocephala integrifolia* was conducted on twenty-five healthy rats. To induce alcoholic hepatitis, animals received a unique daily dose of 40° ethanol (4 g/kg) for 21 days by gastric intubation [13]. To achieve the experimentation, animals (5rats/ group) received different treatments: a normal control group receiving distilled water only (10 mL/kg), a ethanol group receiving simultaneously distilled water (10 mL/kg) and ethanol 40° (4 g/kg) and 3 other groups receiving the plant extract (100 and 200 mg/kg) or (Simepar, 100 mg/kg) as the reference drug and ethanol simultaneously. At the end of the treatment, experimental rats were anesthetized (diazepam (10 mg/kg) and ketamine (50 mg/kg)) and sacrificed. Haematological parameters were analyzed after blood sample collecting. Some serum biochemical parameters were measured. The organs (liver and kidney) were weighted.

2.4.2 Hematological analysis

Some hematological constituents including red blood cell (RBC) count, hemoglobin (Hb) level, hematocrit (HCT) rate, white blood cell (RBC) count; mean corpuscular volume (MCV), platelets (PLT), lymphocytes, monocytes, and granulocytes were analyzed using an haematological analyzer (Sysmex XP 300, Germany).

2.4.3 Serum biochemical analysis

Serum biochemical analysis including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) activities, total bilirubin, creatinine, total cholesterol, triglycerides and HDL-cholesterol levels were assessed using commercial diagnostic kits (SGM, Italia). A spectrophotometer HACH DR 3900 (Germany) was used to determine absorbance and to calculate values. Atherogenic index and LDL-cholesterol were determined as indicated by diagnostic kits.

2.4.4 Evaluation of some oxidative stress biomarkers

A section of 0.4 g from each organ was homogenized with a Tris-HCl buffer, pH 7.4 (liver and kidney). After a cold (4 °C) centrifugation for 25 min, the supernatant was collected. Parameters including reduced glutathion level (GSH), Catalase activity, Nitrites concentration, Superoxide dismutase level (SOD) and Malondialdehyde level (MDA) were analyzed. All the parameters were evaluated by using the standard methods as respectively described by Ellman [14], Sinha [15], Fermor [16], Misra and Fridovish [17] and Wilbur [18].

2.4.5 Histopathological analysis

A section of liver and kidney were fixed in buffered formaldehyde (10%). The histology was performed using hematoxylin-eosin staining [19].

2.5 Statistical analysis

Data were expressed as Mean ± Standard Error of Mean (SEM). Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Turkey post-test using Graphpad Prism 8.01 software. Difference was considered as significant for $p < 0.05$.

3. Results

3.1 Effects the aqueous extract leaves of *Dichrocephala integrifolia* on the relative weight of liver and kidney

Figure 1 shows the effects of *D. integrifolia* on relative weight of liver and kidney. Twenty one days of alcohol administration induced significant ($p < 0.001$) decrease of relative weight of the liver compared to non-intoxicated rats. The co-administration of the aqueous leaves extract of *D. integrifolia* and ethanol significantly prevented to the decrease ($p < 0.001$) of the liver's relative weight by 23.91% and 29.09% at the doses of 100 and 200 mg/kg respectively as compared to intoxicated rats. Simultaneous administration of reference drug and ethanol resulted in significant increase of the liver by 32.21% ($p < 0.001$). No significant change was recorded in relative weight of the kidney.

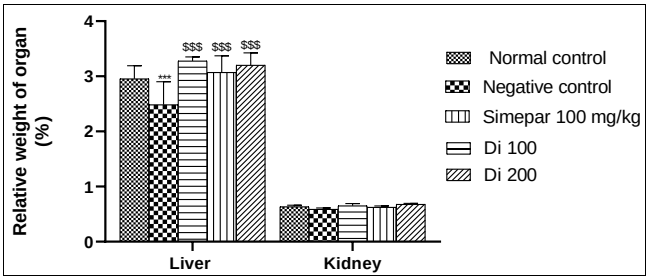

Fig 1: Aqueous leaves extract of *Dichrocephala integrifolia* improves the relative weight of liver

Values represent the mean ± SEM, n = 5, ***$p < 0.001$: versus normal control, $$$$p < 0.001$: versus negative control, Di: animal receiving simultaneously ethanol and the plant extract (100 mg/kg or 200 mg/kg).

3.2 *Dichrocephala integrifolia* extract improves haematological parameters

The oral administration of alcohol to rats for 21 days significantly dropped of erythrocytes (RBC) count ($p < 0.05$), haemoglobin (HGB) ($p < 0.001$), hematocrit (HCT) ($p < 0.001$), mean corpuscular volume (MCV) ($p < 0.01$) and thrombocytes count ($p < 0.001$) in negative control versus

normal group (Table 1). However, it was observed an increase in white blood cells (WBC) count ($p< 0.01$) and the monocyte percentage ($p< 0.001$). The percentage of lymphocytes, neutrophils and platelets decreased in intoxicated rats by 37.99 ($p< 0.05$), 51.74% ($p< 0.001$) and 35.61% ($p< 0.001$) respectively, compared to the normal control. At the respective doses of 100 and 200 mg/kg of the plant extract as well as Simepar there was an increased of HGB level by (82.11%, 80.89% and 74.80%) ($p< 0.001$); HCT rate by (169.75%, 185.71% and 164.7%) ($p< 0.001$) and thrombocytes level by (39.36%, 40.48% and 53.26%) ($p< 0.001$). In addition, the treatment resulted to the decrease ($p< 0.05$) in WBC count, and some leucocytes species (lymphocytes and neutrophils) percentages versus negative control.

Table 1: *Dichrocephala integrifolia* aqueous extract improves haematological parameters in rat

	Treatments				
Parameters	**Normal control**	**Negative control**	**Simepar 100 mg/kg**	**Di 100**	**Di 200**
RBC (10^3/µL)	8.46 ± 0.57	6.36 ± 0.47*	7.91 ± 0.28	7.76 ± 0.10	8.11 ± 0.07$^{\$}$
HGB (g/dL)	14.67 ± 0.52	8.20 ± 0.17***	14.33 ± 0.23$^{\$\$\$}$	14.93 ± 0.49$^{\$\$\$}$	14.83 ± 0.28$^{\$\$\$}$
HCT (%)	33.37 ± 0.32	12.53 ±1.39***	33.17 ± 0.03$^{\$\$\$}$	33.80 ± 0.46$^{\$\$\$}$	35.80 ± 0.81$^{\$\$\$}$
MCV (fL)	42.90 ± 0.92	32.87 ± 0.71**	39.47 ± 3.04	40.27 ± 2.9	40.83 ± 3.45
PLT (10^3/µL)	603.67 ± 6.69	388.60± 10.48***	546.00 ± 20.40$^{\$\$\$}$	595.60± 15.9$^{\$\$\$}$	541.67±11.17$^{\$\$\$}$
WBC (10^3/µL)	7.10± 1.62	16.30 ± 1.31**	7.97± 1.31$^{\$\$}$	7.77± 0.77$^{\$\$}$	8.73± 0.52$^{\$\$}$
LYMP (%)	23.33 ± 1.76	14.47± 1.73*	16 ± 2.08*	18.33 ± 0.67	20.33 ± 0.88
NEUT (%)	63 ± 2.52	30.40 ± 1.73***	56.5 ± 0.87$^{\$\$\$}$	49.00 ± 3.00$^{*\$}$	51.00 ± 1.53$^{*\$\$}$
MONO (%)	10.67 ± 0.33	27.60 ± 1.51***	15.33 ± 1.33$^{\$\$\$}$	18 ± 1.15$^{*\$\$}$	18 ± 1.15$^{*\$\$}$

Data are Mean ± SEM, n = 5, *$p< 0.05$, **$p< 0.01$, ***$p< 0.001$ versus normal control, \$\$$p< 0.01$, \$\$\$$p< 0.001$ versus negative control. Normal control = healthy animals receiving distilled water (10 mL/kg). Negative group = rats receiving distilled water (10 mL/kg) and simultaneously ingesting ethanol (4 g/kg); Positive group = rats receiving Simepar (100 mg/kg) and simultaneously ingesting ethanol (4 g/kg); Di (100 and 200) = rats receiving the extract and simultaneously ingesting ethanol 40° (4 g/kg).

3.3 Effects of the aqueous extract of *Dichrocephala integrifolia* on some liver and kidney parameters functions

The effects of *D. integrifolia* on some liver and kidney function markers are summarized in Table 2. Daily administration of ethanol to rats significantly raised the serum ALT, AST, ALP, total bilirubin and creatinine respectively by 127.11% ($p< 0.05$), 130.17% ($p< 0.01$), 166.91% ($p< 0.001$), 84.02% ($p< 0.001$) and 360.02% ($p< 0.001$) while total serum protein concentration significantly decreased by 54.12% ($p< 0.001$) versus normal control. The plant extract administration significantly decreased the level of ALT ($p< 0.05$), AST ($p< 0.01$), ALP ($p< 0.001$), total bilirubin ($p< 0.001$), creatinine ($p< 0.001$) whereas serum proteins increased ($p< 0.001$) whatever the dose. The reference drug administered under the same conditions as the extract significantly dropped ALT level of 57.74% ($p< 0.05$); AST by 39% ($p< 0.05$); ALP by 57.76% ($p< 0.001$); total bilirubin of 33.7% ($p< 0.01$) and creatinine by 49.13% ($p< 0.001$); the concentration of total serum protein increased by 111.9% ($p< 0.001$).

Table 2: Aqueous extract of *Dichrocephala integrifolia* improves some serum parameters of liver and kidney function

	Groups				
Parameters	**Normal control**	**Negative control**	**Simepar 100 mg/kg**	**Di 100**	**Di 200**
ALT (UI/L)	28.25 ± 7.97	64.16 ± 10.52*	27.11 ± 5.85$^{\$}$	30.67 ± 5.28$^{\$}$	31.25 ± 5$^{\$}$
AST (UI/L)	41.00 ± 5.52	94.37 ± 10.49**	57.56 ± 5.94$^{\$}$	43.74 ± 7.78$^{\$\$}$	47.97 ± 9.1$^{\$\$}$
PAL (UI/L)	210.90 ± 23.6	563.13 ± 31.5***	237.8 ± 19.7$^{\$\$\$}$	263.5 ± 28.1$^{\$\$\$}$	248.5 ± 15.9$^{\$\$\$}$
T. Bilirubin (mg/dL)	1.27 ± 0.11	2.34 ± 0.27***	1.41 ± 0.33$^{\$\$}$	1.35 ± 0.19$^{\$\$\$}$	1.28 ± 0.06$^{\$\$\$}$
Total Protein (mg/dL)	14.38 ± 1.36	6.69 ± 0.58***	14.18 ± 0.48$^{\$\$\$}$	14.12 ± 1.33$^{\$\$\$}$	15.89 ± 0.40$^{\$\$\$}$
Creatinine (mg/dL)	0.23 ± 0.02	1.01 ± 0.10***	0.51 ± 0.03$^{**\$\$\$}$	0.40 ± 0.03$^{\$\$\$}$	0.30 ± 0.02$^{\$\$\$}$

Values express Mean ± SEM, n = 5, *$p< 0.05$, **$p< 0.01$, ***$p< 0.001$ versus normal control; \$$p< 0.05$, \$\$$p< 0.01$, \$\$\$$p< 0.001$ versus negative group; Normal control = healthy animals receiving distilled water (10 mL/kg). Negative group = rats receiving distilled water (10 mL/kg) and simultaneously ingesting ethanol (4 g/kg); Positive group = rats receiving Simepar (100 mg/kg) and simultaneously ingesting ethanol (4 g/kg); Di (100 and 200) = rats receiving the extract and simultaneously ingesting ethanol (4 g/kg). Total Bilirubin; *D. integrifolia*: *Dichrocephala integrifolia.*

3.4 Effects of the aqueous extract of *Dichrocephala integrifolia* on lipid profile

The single daily administration of ethanol to rats resulted in significant increase in the levels of total cholesterol ($p< 0.01$), triglycerides ($p< 0.05$), LDL-cholesterol ($p< 0.001$), atherogenic index ($p< 0.001$) respectively by 31.47%, 40.52%, 50.85%, 154.44% and significant decrease ($p< 0.001$) of serum HDL-cholesterol level by 51.58% compared to normal control (Table 3). The simultaneous administration of ethanol 40° (4 g/kg) with the aqueous leaves extract of *Dichrocephala integrifolia* at the doses of 100 and 200 mg/kg prevented the increase of LDL-cholesterol respectively, by 17.33% ($p< 0.05$) and 32.12% ($p< 0.01$); atherogenic index by 52.46% ($p< 0.001$) and 55.38% ($p< 0.001$);total cholesterol by 15.97% ($p > 0.05$) and 23.55% ($p< 0.01$) and triglycerides by 22.70% ($p > 0.05$) and 24.66% ($p< 0.05$); whereas the extract protected from the decrease of HDL-cholesterol by 89.43% ($p< 0.01$) and 78.64% ($p< 0.05$) as compared to negative control.

The Simepar (100 mg/kg) administration significantly decreased LDL-cholesterol concentration by 15.83%, ($p< 0.05$), atherogenic index by 52.67% ($p< 0.001$) and enhanced HDL-cholesterol of 78.42% ($p< 0.05$) compared to the animal receiving ethanol.

Table 3: Aqueous extracts from *Dichrocephala integrifolia* improves lipid profile

	Groups				
Parameters	**Normal control**	**Negative control**	**Simepar 100 mg/kg**	**Di 100**	**Di 200**
Total Cho (mg/dL)	209 ± 11.47	275.1 ± 8.79*	232.57 ± 3.54	231.14 ± 11.73	210.3 ± 15.91$^{\$\$}$
HDL-Cho (mg/dL)	19.9 ± 2.65	9.63 ± 0.4**	17.19 ± 0.4$^{\$}$	18.26 ± 1.83$^{\$\$}$	17.22 ± 1.41$^{\$}$
LDL-Cho(mg/dL)	137.49 ± 6.35	207.4 ± 1.36***	174.55 ± 2.4$^{*\$}$	171.44 ± 11.1$^{*\$}$	140.76 ± 9.18$^{\$\$\$}$
Triglycerides (mg/dL)	180.18± 7.09	254.32 ± 20.79	204.11 ± 13.05	207.23 ± 10.15	191.58 ± 10.15$^{\$}$
Atherogenic index	11.25± 1.48	28.62 ± 1.96***	13.54 ± 0.29$^{\$\$\$}$	13.61 ± 2.47$^{\$\$\$}$	12.77 ± 1.94$^{\$\$\$}$

Values represent Mean ± SEM, n = 5, *$p< 0.05$, **$p< 0.01$, ***$p< 0.001$ versus normal control; \$$p< 0.05$, \$\$$p< 0.01$, \$\$\$$p< 0.001$ versus negative group; Normal control = healthy animals receiving distilled water (10 mL/kg). Negative group = rats receiving distilled water (10 mL/kg) and simultaneously ingesting ethanol (4 g/kg); Positive group = rats receiving Simepar (100 mg/kg) and simultaneously ingesting ethanol (4 g/kg); Di (100 and 200) = rats receiving the extract and simultaneously ingesting ethanol (4 g/kg). Di: *Dichrocephala integrifolia*. Total Cho: Total Cholesterol, HDL-Cho = high density lipoprotein cholesterol, LDL-Cho = low density lipoprotein-cholesterol

3.5 Effects of the extract on some oxidative stress markers

The daily intake of ethanol 40° (4 g/kg) to rats during 21 days exhibited significant ($p< 0.001$) decrease of 54.75% and 49.73% in reduced glutathione level (Fig 2A) and 40.68% and 29.72% in nitric level (Fig 2E). However, an increase of 114.52% and 310.03% in catalase activity (Fig 2B), 110.05% and 139.38% in SOD activity (Fig 2C), 103.96% and 110.08% in MDA rate (Fig 2D)was recorded respectively, in the liver and kidney of intoxicated rats. The simultaneous ingestion of the extract and Simepar with alcohol significantly increased the GSH level in liver ($p< 0.01$) and in kidney ($p< 0.001$) and the concentration of nitric oxide ($p< 0.001$) while the catalase activity decreased ($p< 0.001$) in the liver and kidney; as well as the SOD activity ($p< 0.01$) and the MDA level ($p< 0.001$) as compared to intoxicated animals.

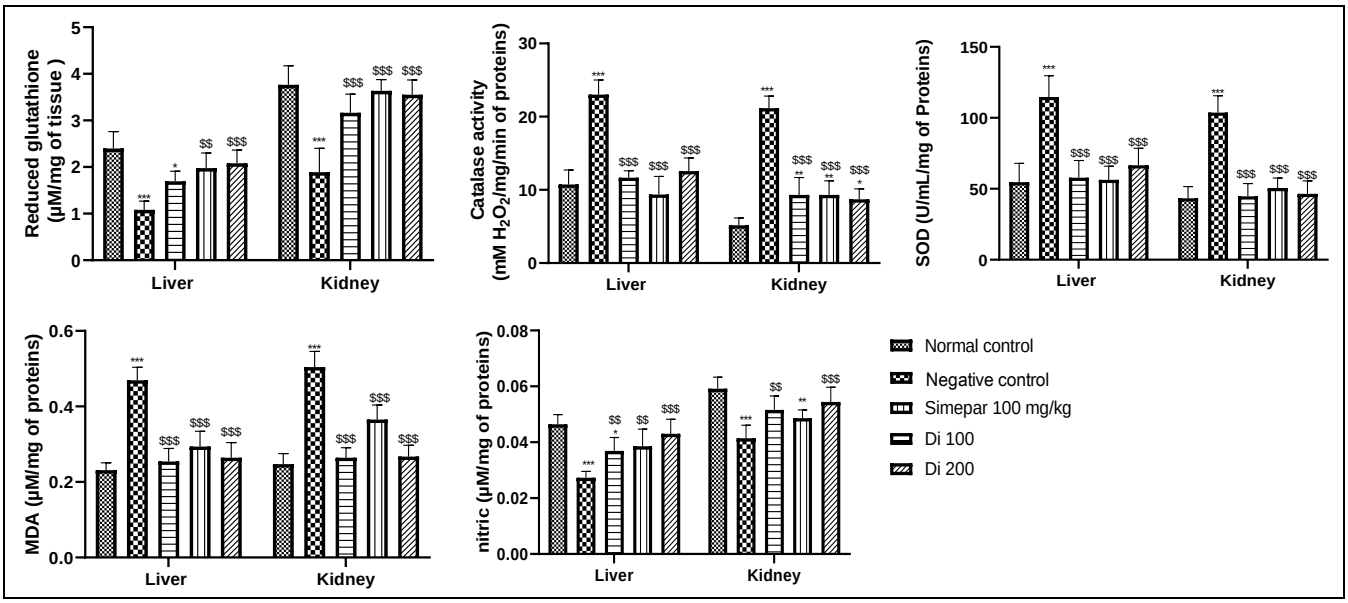

Fig 2: Effects of the aqueous extract of *D. integrifolia* on the reduced glutathione (A), catalase activity (B), SOD activity (C), MDA activity (D) and nitric (E) levels of liver and kidney.

Bars represent the Mean ± SEM, n = 5, *$p< 0.05$, **$p< 0.01$, ***$p< 0.001$ compared to Normal control, \$\$$p< 0.01$, \$\$\$$p< 0.001$ compared to negative group (ethanol 40°: 4 g/kg) and *D. integrifolia*: *Dichrocephala integrifolia*. Normal control = healthy animals receiving distilled water (10 mL/kg). Negative group = rats receiving distilled water (10 mL/kg) and simultaneously ingesting ethanol (4 g/kg); Positive group = rats receiving Simepar (100 mg/kg) and simultaneously ingesting ethanol (4 g/kg); Di (100 and 200) = rats receiving the extract and simultaneously ingesting ethanol (4 g/kg).

3.6 *Dichrocephala integrifolia* activity on liver micro-architecture

In normal rat treated with distilled water, liver architecture revealed a normal hepatic parenchyma with distinct hepatocytes, sinusoid capillaries and a portal space (consisting of the portal vein, the bile canaliculus and the hepatic artery) (Fig. 3A). Daily administration of ethanol for three weeks resulted in a disorganized architecture of the hepatic parenchyma with hepatic cytolysis, diffuse infiltration of periportal inflammatory cells and dilation of sinusoidal capillaries (Fig. 3B). The liver section of the rat treated with the extract or simepar showed well organized structure close to that of the normal control (Fig. 3D-3E and 3C).

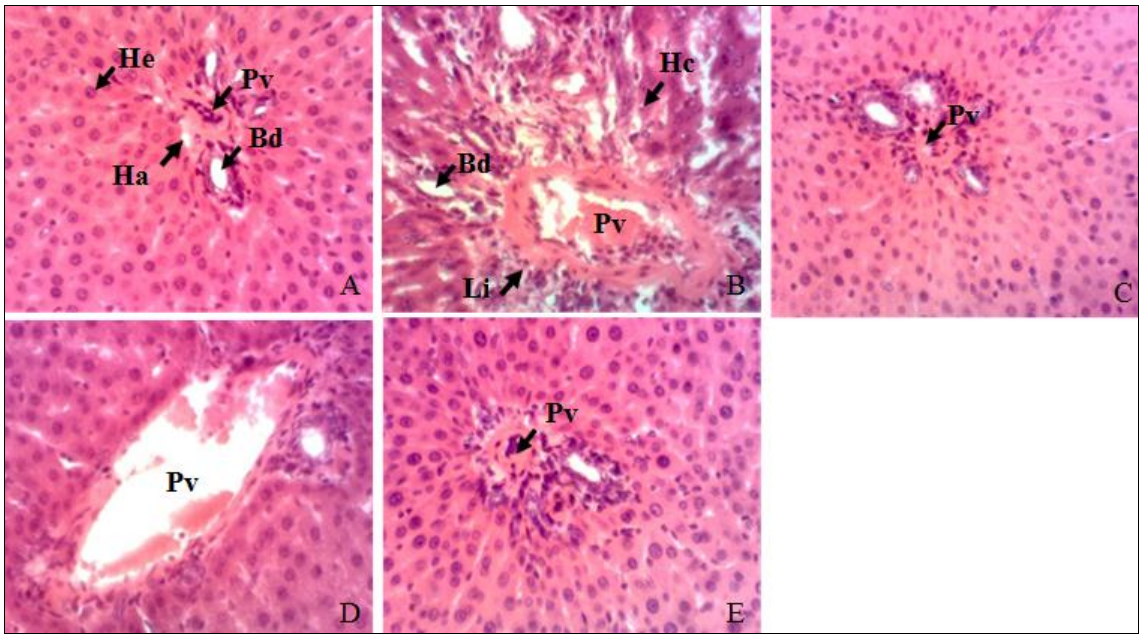

Fig 3: Aqueous leaves extract of *Dichrocephala integrifolia* improves liver micro-architecture in ethanol-induced hepatotoxicity (HE x 200)

A = Normal control; B = Negative control; C = Simepar 100 mg/kg; D = Di 100; E = Di 200. Normal control = healthy animals receiving distilled water (10 mL/kg). Negative group = rats receiving distilled water (10 mL/kg) and simultaneously ingesting ethanol (4 g/kg); Positive group = rats receiving Simepar (100 mg/kg) and simultaneously ingesting ethanol (4 g/kg); Di (100 and 200) = rats receiving the extract and simultaneously ingesting ethanol (4 g/kg) He: Hepatocyte; Pv: Portal vein; Ha: Hepatic artery; Hc: Hepatic cytolysis; Bd: Bile duct; Li = Leucocytes infiltrations.

3.7 *Dichrocephala integrifolia* activity on kidney micro-architecture

The effects of administration of ethanol (4 g/kg) and *Dichrocephala integrifolia* extract on kidney tissue are summarized in the Figure 4. Kidney histology of normal control presents a normal parenchyma with glomerulus; urinary space; distal convoluted tubule and proximal convoluted tubule quite distinct (Fig. 4A). In ethanol-treated rat, the presence of leucocyte infiltration with tubular clarification and an increase in urinary space (Fig. 4B) was noticed. The plant extract and Simepar alleviated ethanol-induced alterations in the kidney (Fig.4 C-D).

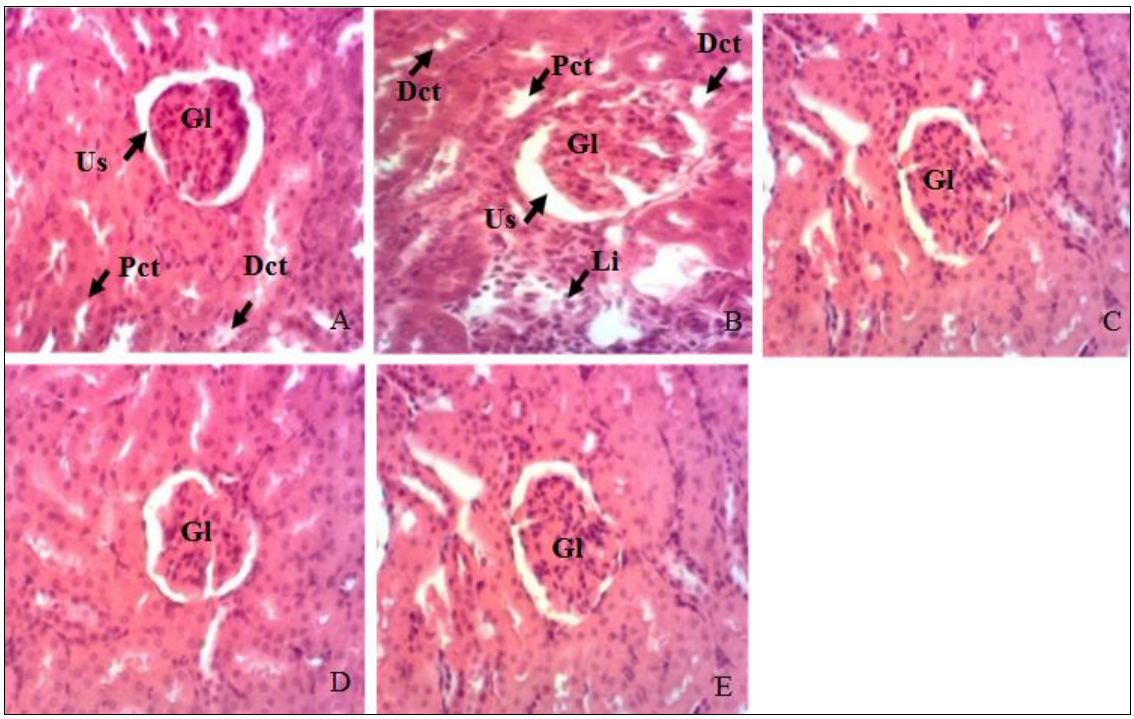

Fig 4: Aqueous leaves extract of *Dichrocephala integrifolia* improves kidney micro-architecture in ethanol-induced hepatotoxicity (HE x 200)

A = Normal control; B = Negative control; C = Simepar 100 mg/kg; D = Di 100; E = Di 200. Normal control = healthy animals receiving distilled water (10 mL/kg). Negative group = rats receiving distilled water (10 mL/kg) and simultaneously ingesting ethanol (4 g/kg); Positive group = rats receiving Simepar (100 mg/kg) and simultaneously ingesting ethanol (4 g/kg); Di (100 and 200) = rats receiving the extract and simultaneously ingesting ethanol (4 g/kg); Gl

= Glomerulus; Us = Urinary space; Dct = Distal convoluted tubule; Pct = Proximal convoluted tubule; Li= Leucocytes infiltration.

4. Discussion

Alcohol consumption abuse is incriminates in liver and kidney damages. In this study, the preventive effects of *Dichrocephala integrifolia* aqueous leave extract on ethanol-induced hepatic damage in rat was evaluated. A daily single ingestion of ethanol 40° (4g/kg) for 21 days significantly decreased the liver relative weight. These results could express the massive destruction of hepatocytes caused by alcohol that disrupts the metabolism of sugars and fats, resulting in fatty liver disease [20]. Moreover, acetaldehyde, a reactive toxic metabolite of alcohol is incriminated in inflammation process, mitochondria dysfunction related to fibrosis and cell death [21]. This result is in agreement with the micrograph of the liver where some alterations of the hepatocytes in the animals receiving ethanol were mentioned. The administration of *D. integrifolia* prevented the decrease in liver relative weight indicating the hepato-protection effect of the extract against ethanol-induced hepatotoxicity. This properties would be assigned to the presence flavonoids that the capacity to boost regeneration of hepatocytes and to improve hepatic functions were reported [22].

It is well known that excessive alcohol acts on the hematopoietic system either by direct toxic effects on bone or blood cells or through indirect pathway via nutritional deficiencies or structural abnormalities that compromise their function resulting to anemia and leukocytosis [23]. In this study, significant decrease in erythrocytes (RBC) count, HGB, HCT and MCV express the signs of anemia probably caused by direct or indirect toxic effects on blood synthesis induced by alcohol consumption [24]. Moreover, the enhancement in monocyte-macrophage system could be due to the presence of invading microorganisms or defective proteins [24]. The increase of monocyte percentage due to administration of ethanol clearly showed liver inflammation justified by the presence of leucocyte infiltration in liver structure. The co-treatment with the *D. integrifolia* extract as well as simepar significantly inhibited the ethanol–induced blood cell deterioration.

In this study, the ethanol intoxication of rats is characterized by the decrease in serum protein level while serum level of transaminases, alkaline phosphatase and bilirubin increased. These disturbances express conventional signs of liver injury [12]. Ethanol in the body is a source of free radicals which damages biomolecules such as proteins causing their denaturation resulting to the fall of their concentrations in the liver and blood as observed in this work [2]

These effects could be incriminated to the tannins and alkaloids within the plant acting through inhibition of the cytochrome P_{450} or are capable to scavenge free radical [13, 25, 8]. Likewise, the increase of ALP and bilirubin levels could result from ethanol-induced liver inflammation and disruption of bile salt synthesis [26]. Animals treated with *D. integrifolia* extract exhibited significant reduce in the level of transaminases, ALP and bilirubin, indicating the protective effect of the plant against liver impairment. This effect result from the ability of the plant to thwart lipid peroxidation thus stabilizing membrane function [27].

The significant increase in creatinine level caused by alcohol consumption. It has been demonstrated that chronic alcohol intake increases blood pressure consequently disturbs kidney function [4] justified by the increase in serum creatinine level in negative group. The treatment with the extract of *D. integrifolia* as well as Simepar prevented the serum creatinine enhancement. These effects indicate that the extract could inhibit ethanol-induced kidney damage interfering on the toxic mechanisms of ethanol.

Likewise, the lipid profile disorder as observed in this study testified the ethanol intoxication, due to the expression of β-hydroxymethylglutaryl CoA, whose activation by ethanol promotes the biosynthesis of cholesterol in the tissues while the excess could spill into blood [28]. Ethanol consumption enhances lipogenesis. Ethanol also directly acts on lipoprotein lipase and triglyceride lipase by reducing their activity resulting to a drop in the breakdown of triglycerides in serum, hence its accumulation [28]. However, the administration of *D. integrifolia* extract as well as Simepar regulates serum lipid profile due to the presence of compounds such as alkaloids and flavonoids found in the extract which could inhibit the adverse effects of ethanol [29].

Alcohol-induced hepatotoxicity is well documented and caused by the production of toxic acetaldehyde generating reactive oxygen species (ROS), responsible to the breakdown of some antioxidant defense species [30]. Significant decrease of gluthatione, proteins and nitrite levels with increase of MDA level as observed in this study express the oxidative stress occurrence. Glutathione is the most abundant endogenous antioxidant, considered as a non-enzymatic and direct extractor of ROS. The decrease in hepatic and renal nitrite levels could be attested the endothelial dysfunction caused by chronic ingestion of ethanol [31, 32]. In addition, the increase of the level of MDA also confirm hepatic and renal tissue damages and alteration of the antioxidant defenses to neutralize the ROS [33]. Lipid peroxidation is a process by which ethanol induces hepatotoxicity [12, 34]. These disturbances in the antioxidant status is accompanied by tissue damages as observed in the investigated organs. Meanwhile, the increase in enzymatic antioxidant in the chronic alcohol ingestion suggests the increase of antioxidant defenses [35, 1]. *D. integrifolia* extract treatment as well as Sylimarine significantly prevented the elevation of MDA level, the decrease in the nitrite levels, and prevented the tissue damage, suggesting the hepatoprotective properties of the plant extract. This property to restore antioxidant balance is probably attributed to some secondary metabolites such as carotenoids, flavonoids, tannins and triterpenes in the plant extract that are able to scavenge ROS [36, 1, 37].

5. Conclusion

The investigation of the potential bioactivity of *D. integrifolia* leaves aqueous extract showed that the plant extract prior to the alcohol ingestion significantly prevented the drop in the liver weight, blood disturbance, liver and kidney function failure. The plant also restored the antioxidant status and protected from the liver and kidney damage as recorded in affected rats. The results showed that *Dichrocephala integrifolia* leaves aqueous extract is a source of active biomolecules necessary to control hepato-nephrotoxicity that could justify its use as traditional remedies in the treatment of hepatitis.

6. References

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