TOXICOLOGICAL INVESTIGATIONS OF ETHANOLIC LEAVES EXTRACT OF *DISSOTHIS THOLLONII* (MELASTOMATACEAE)

ABSTRACT

Dissotis thollonii is widely used in Cameroon for the treatment of typhoid fever, gastrointestinal disorders, inflammation, kidney diseases, pregnancy control and sinusitis. However there is lack of experimental data on its possible toxicity.

The aim was to investigate acute and subchronic toxicity of the ethanol leaf extract of *Dissotis thollonii* in healthy Wistar rats.

In acute toxicity tests, a single administration of the ethanolic leaf extract (5000 mg/kg) of *Dissotis thollonii* was given orally to 4 female rats. The general behavior, adverse effects, and mortality were recorded for up to 14 days post-treatment. On the 15th day, the rats were weighed and euthanized for necropsy. In sub-chronic toxicity tests, the extract (18.28; 62.50; 250 and 1000 mg/kg/day) was given orally to both male and female rats for 28 days. General behavior, adverse effects, and mortality were observed throughout the experimental period. Food intake, body weight, organ weight, hematological parameters, biochemical parameters and histopathological changes were evaluated.

Dissotis thollonii leaf extract did not cause any death or any hazardous symptoms during acute toxicity, and the LD50 of his was higher than 5000 mg/kg. Sub-chronic administration of *Dissotis thollonii* leaf extract showed significant ($p > 0.05$) variations in some biochemical parameters (serum urea, urinary urea, ALT, AST, and ALP) in the experimental groups at the dose of 250 mg/kg and 1000 mg/kg for both male and female rats. No major morphological changes were observed in the histopathological analysis of liver and kidney. However, a congestion on liver sections at a dose of 1000 mg/kg for females and at 250; 1000 mg/kg for males was observed.

These findings showed that acute or subchronic oral administration of the ethanolic leaf extract of *Dissotis thollonii* may be considered as relatively free of toxicity. However, prolonged use of high doses of the extract orally should be exercised with caution to avoid a possible liver injury.

Keywords: *Dissotis thollonii*, Acute and subchronic toxicity, biochemical parameters, hematological parameters, histopathological changes.

INTRODUCTION

The use of medicinal plants for healing purposes has been increasingly popular as they are believed as beneficial and free of side effects [1]. Therefore, various medicinal plants have been studied using modern scientific approaches because many medicinal plants have a variety of properties and various biological components that can be used to treat various diseases [2]. However, establishing information about the toxicity of herbal medicines has often been overlooked, because many people underestimate the toxicity of natural products and do not realize that these agents could be as toxic or more than synthetic drugs [3].

Dissotis thollonii is a tropical plant which belongs to the Melastomataceae family [4]. It is used in the West Region of Cameroon for the treatment of various diseases such as typhoid fever, gastrointestinal disorders, inflammation, kidney diseases, pregnancy control and sinusitis. Despite the traditional use of *Dissotis thollonii*, no scientific report or information was found in the literature regarding its toxicological profile. Therefore, the aim of this work was to determine the toxicological effects of ethanolic leaves extract of *D. thollonii* on Wistar rats through biochemical, hematological and histopathological assessments.

MATERIALS AND METHODS

Plant material

The fresh leaves of *D. thollonii* were collected in April 2014 in the Fongo-Tongo subdivision, Menoua division, West Region of Cameroon. A sample was identified at the National Herbarium of Cameroon where a voucher specimen was deposited under the reference number 13292/SRF Cam.

Preparation of plant extract

The air-dried and powdered leaves of *D. thollonii* (500 g) was macerated in 05 L of Ethanol for 48 h at room temperature. The extract was filtered using Whatman No. 1 filter paper. The filtrate was then concentrated under reduced pressure on a rotary evaporator at 45 °C and the extract obtained was stored in the refrigerator until further use.

Experimental animals

Wistar Albino rats (aged 8-9 weeks, weighing 145-170 g) of either sex were used for acute and sub-acute toxicity studies. They were bred at the animal house of Department of

Biochemistry, the University of Dschang in the ambient environmental conditions [(23 ± 2) ºC].

Acute toxicity

Healthy young adult nulliparous and non-pregnant female rats were used in this study according to the Organization for Economic Cooperation and Development (OECD) guideline 425 [5]. Female rats were selected for the test because they are frequently more sensitive to the toxicity of test compounds than male. All the animals were fasted to food overnight and weighed before administration of the extract. Animals were randomly divided into two groups. The first group (control) received distilled water orally. The second group was treated as follow: a single dose of 5000 mg/kg was given to the first animal, and signs of toxicity, behavioral changes or mortality were observed for the first thirty minutes and the first hour after treatment, then hourly for 4 h and, finally periodically until 48 h. If this first animal survived, then two additional animals were to be given the same dose sequentially at 48 h intervals. All the experimental animals were individually observed daily for general behavioral and body weight changes, hazardous symptoms and mortality for a period of 14 days post-treatment. The LD50 was predicted to be above 5000 mg/kg if three or more rats survived. At the end of the experimental period, all animals were weighed and sacrificed, and the organs were excised for necropsy.

Sub-chronic toxicity

Sub-chronic toxicity study (28-day repeated oral toxicity study) was carried out according to OECD 407 guidelines [6]. Rats of both sexes were divided into five groups with 8 animals (4 males plus 4 females each). Group I Animal received 10% Dimethyl Sulfoxide (DMSO) daily and served as a control group whereas group II, group III, group IV and group V received the ethanolic *Dissotis thollonii* leaf extract at 18.28 mg/kg, 62.50 mg/kg, 250 mg/kg and 1000 mg/kg body weight, p.o. respectively. All the groups of rats were observed twice daily for mortality and morbidity till the completion of the experiment, and each animal in a group were observed for clinical signs and the time of onset, duration of these symptoms if any were recorded. Body weights of the rats in all groups were recorded once before the start of dosing, daily during the treatment period and finally on the day of sacrifice. The amount of food intake was recorded every day and the data were expressed as 7 days cumulative value.

Sample collection

Rats fasted overnight on the 28th day and urine was collected, centrifuged and stored at +4°C for 24 hours. Upon fasting, the blood samples were collected by cardiac puncture into heparinized and non-heparinized tubes from chloroform anesthetized rats. Animals were further sacrificed and used for gross pathological examinations and relative organ indices determination.

Relative organ weight and preparation of homogenates

After the blood collection, the heart, liver, lungs, spleen, and kidneys, were quickly removed, cleaned with ice-cold saline and weighed. The relative organ weight (organ to body weight ratio) of each animal was then calculated. The homogenates of various organs were prepared at 15% (15 g organ per 100 mL of 0.9% NaCl) as described by Gatsing et al., [7]. This was done by grinding 500 mg of each organ in a mortar containing 3.34 mL saline distilled water (0.9% NaCl). The homogenates were centrifuged at 3000 rpm for 15 min and the supernatants were then used for protein assays.

Haematological assays

White blood cell (WBC) count, red blood cell (RBC) count, hematocrit (Ht), haemoglobin (Hb), platelets (PLT), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), Lymphocytes (L), Monocytes (M), mean corpuscular volume (MCV) and Granulocytes (G) concentration were all determined by use of an automated blood analyzer (QBCAutoread Plus, UK). The heparinized blood samples were first pipetted into QBCcapillary tubes and spurn in a refuge centrifuge (Becton Dickson, UK) for 5 min and read by means of an auto read analyzer.

Biochemical analysis

The non-heparinized blood was allowed for complete clotting and then centrifuged at 3000 rpm for 5 min. The supernatants (serum samples) were aspired and frozen at -20°C. The serum was assayed for creatinine, aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total billirubin, direct bilirubin, total cholesterol, high-density lipoprotein (HDL), triglycerides, urea using commercial kits (IMNESCO GmbH, Germany). The concentration of low-density lipoprotein cholesterol was calculated by Friedewald equation [8]. Urine was assayed for total protein, urea, and creatinine using the same commercial kits (IMNESCO GmbH, Germany). Total serum protein and organ proteins (liver, lungs, heart, kidney, and spleen) were assayed by the Biuret

method [9] while urinary proteins and kidney proteins were quantified by the method of Bradford [10].

Histopathological analysis

Tissue cross sections were prepared and analyzed using conventional techniques described by Mosaid et Alferah [11]. After sacrificing the animals, small pieces of liver were fixed in 10% formalin, dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissue was embedded in paraffin wax and sectioned into five micrometers thick with the rotary microtome, then stained with hematoxylin and eosin. Then the sections were examined with a light microscope and photographed using a microscopic camera.

Statistical analysis

Data obtained were expressed as the mean ± standard deviation (S.D) and were statistically analyzed using One-way ANOVA. The Waller Duncan test was used to compare means of different groups. A P-value of < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS for Window software version 21.

Ethics

This work was carried out with respect for the welfare of animals, as recommended by WHO

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

[12].

RESULTS

Acute Toxicity Study

No mortality or morbidity was observed up to the 14-day period following single oral administration of ethanolic leaf extract of *D. thollonii* at the dose of 5000 mg/kg. The animals did not show any changes in general appearance during this period. Morphological characteristics (fur, skin, eyes, and nose) were normal. No tremors, convulsion, diarrhea, reduction of locomotion and unusual behavior were observed. In addition, no abnormality was found in organs at necropsy (Figure 1).

]

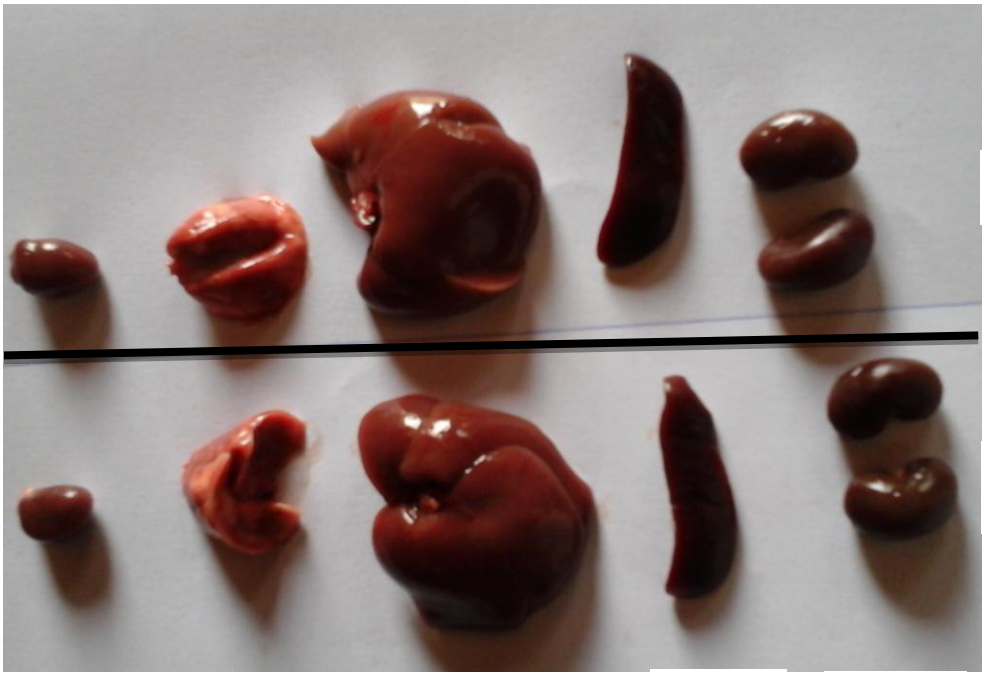

Figure 1: Morphological structure of organs after treating with *D. thollonii* extract in acute toxicity study after 14 days.

Sub-chronic toxicity

General signs

No death or significant change in general behavior or other physiological activities were observed during the treatment period either in the controls group or in the test groups.

Food intake, weight gain, and organ indices

The food consumption and Weight gain of an animal of both sexes decreased significantly ($p<0.05$) throughout the study period generally at the doses of 250 and/or 1000 mg/kg (Table 1, 2). The results of the effects of *D. thollonii* extract on relative organ indices of both male and female rats are summarized in Table 3. There were no significant variations ($p \geq 0.05$) between treated groups and control group in all organs in both sexes.

Table 1. Effect of daily intake of the ethanolic extract of *D. thollonii* on food consumption in rats.

Sex	Dose (mg/kg)	Food consumption (g)			
		Week 1	Week 2	Week 3	Week4
Female	0	35.46 ± 1.13^{b}	37.68 ± 2.52^{b}	36.50 ± 1.77^{d}	36.89 ± 0.76^{b}
	18.28	35.07 ± 1.37^{ab}	34.54 ± 2.97^{b}	34.61 ± 0.63^{cd}	35.39 ± 2.10^{b}
	62.50	34.18 ± 0.95^{ab}	34.21 ± 0.44^{b}	33.46 ± 0.36^{c}	33.54 ± 2.10^{b}
	250	33.11 ± 1.60^{a}	28.61 ± 4.12^{a}	27.61 ± 2.13^{b}	27.54 ± 2.09^{a}
	1000	33.25 ± 0.87^{a}	25.54 ± 1.50^{a}	24.71 ± 1.35^{a}	24.29 ± 3.43^{a}
Male	0	39.18 ± 1.25^{b}	39.07 ± 1.43^{b}	36.00 ± 1.91^{b}	37.60 ± 2.49^{cd}
	18.28	36.75 ± 2.87^{ab}	38.96 ± 0.50^{b}	36.64 ± 0.44^{b}	38.96 ± 0.82^{d}
	62.50	37.36 ± 2.34^{ab}	36.93 ± 1.02^{b}	34.39 ± 0.32^{ab}	34.82 ± 2.01^{bc}
	250	35.11 ± 2.79^{ab}	32.82 ± 1.08^{a}	33.89 ± 1.90^{ab}	30.79 ± 0.14^{a}
	1000	33.82 ± 3.17^{a}	31.04 ± 1.76^{a}	30.54 ± 4.50^{a}	32.64 ± 2.60^{ab}

Along each column and same-sex, values with the same letter superscripts are not significantly different. Waller-Duncan ($p< 0.05$).

Table 2. The body weight gain trend of rats fed with ethanolic extract of *D. thollonii* for 28 days.

Sex	Dose (mg/kg)	Body weight gain			
		Week 1	Week 2	Week 3	Week4
Female	0	22.11 ± 2.03^{bc}	32.60 ± 6.21^{c}	34.76 ± 2.25^{b}	38.91 ± 4.10^{b}
	18.28	22.56 ± 1.99^{c}	31.72 ± 5.91^{c}	35.46 ± 5.01^{b}	41.94 ± 3.49^{b}
	62.50	19.30 ± 2.11^{b}	26.48 ± 2.42^{bc}	34.82 ± 3.53^{b}	39.54 ± 2.78^{b}
	250	15.10 ± 1.80^{a}	23.96 ± 1.43^{ab}	28.36 ± 2.389^{a}	36.02 ± 2.93^{b}
	1000	14.18 ± 0.70^{a}	18.25 ± 2.78^{a}	25.07 ± 1.33^{a}	27.66 ± 3.66^{a}
Male	0	18.72 ± 1.48^{a}	31.00 ± 2.36^{b}	39.09 ± 4.13^{b}	46.13 ± 2.96^{b}
	18.28	16.78 ± 3.71^{a}	31.99 ± 4.50^{b}	43.74 ± 4.47^{b}	48.55 ± 5.32^{b}
	62.50	19.16 ± 1.99^{a}	28.55 ± 3.72^{b}	36.99 ± 5.80^{b}	44.68 ± 4.58^{b}
	250	17.90 ± 2.48^{a}	31.71 ± 4.39^{b}	39.07 ± 5.73^{b}	47.41 ± 4.42^{b}
	1000	18.82 ± 2.44^{a}	20.28 ± 1.87^{a}	21.89 ± 1.51^{a}	31.05 ± 4.70^{a}

Along each column and same-sex, values with the same letter superscripts are not significantly different. Waller-Duncan ($p<0.05$).

Table 3. Effects of ethanolic extract of *D. thollonii* on the relative organ weight of Wistar rats.

Sex	Dose (mg/kg)	Relative organ weight				
		Liver	**Kidneys**	**Lungs**	**Heart**	**Spleen**
Female	0	3.53 ± 0.30[a]	0.65 ± 0.03[a]	0.68 ± 0.14[a]	0.36 ± 0.05[a]	0.35 ± 0.04[a]
	18.28	3.25 ± 0.44[a]	0.66 ± 0.09[a]	0.60 ± 0.14[a]	0.31 ± 0.03[a]	0.32 ± 0.08 [a]
	62.50	3.31 ± 0.29[a]	0.64 ± 0.06[a]	0.65 ± 0.07[a]	0.32 ± 0.02 [a]	0.33 ± 0.06[a]
	250	3.31 ± 0.24[a]	0.61 ± 0.05[a]	0.62 ± 0.12[a]	0.32 ± 0.02[a]	0.34 ± 0.05[a]
	1000	3.06 ± 0.02[a]	0.60 ± 0.06[a]	0.63 ± 0.06[a]	0.35 ± 0.01[a]	0.28 ± 0.05[a]
Male	0	3.15 ± 0.30[a]	0.61 ± 0.05[a]	0.52 ± 0.05[a]	0.30 ± 0.03[a]	0.28 ± 0.05[a]
	18.28	3.04 ± 0.03[a]	0.56 ± 0.03[a]	0.56 ± 0.06[a]	0.28 ± 0.02[a]	0.23 ± 0.02[a]
	62.50	3.03 ± 0.11[a]	0.58 ± 0.06[a]	0.517 ± 0.08[a]	0.31 ± 0.02[a]	0.31 ± 0.02[a]
	250	3.03 ± 0.05[a]	0.56 ± 0.04[a]	0.57 ± 0.06[a]	0.28 ± 0.02[a]	0.26 ± 0.10[a]
	1000	3.04 ± 0.13[a]	0.62 ± 0.03[a]	0.59 ± 0.10[a]	0.32 ± 0.03[a]	0.23 ± 0.05[a]

Along each column and same-sex, values with the same letter superscripts are not significantly different. Waller-Duncan ($p<0.05$).

Hematological parameters

Hematological analysis indicated that, red blood cell (RBC), hematocrit (Ht), haemoglobin (Hb), platelets (PLT), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), Lymphocytes (L), mean corpuscular volume (MCV), Mean corpuscular volume (MCV) and Granulocytes (G) were not affected in males (Table 4). However, total White blood cell (WBC) significantly increased in females treated at doses of 250 and 1000 mg/kg. Similarly, Monocytes (M) significantly increased in the male at doses of 62,5; 250 and 1000 mg/kg.

Table 4. Haematological parameters following 28-day of oral administration of rats with ethanolic extract of *D. thollonii*.

Sex	Dose (mg/kg)	WBC (10^3/µl)	LYM (%)	GRAN (%)	RBC (10^3/µl)	HGB (g/dl)	HCT%	MCV (fl.)	TCMH (pg)	CCMH (g/dl)	PLT (10^3/µl)	VPM (fl.)	Mon%
Female	0	4.50 ± 0.22^{a}	81.68 ± 2.75^{a}	13.78 ± 1.62^{a}	6.96 ± 0.91^{a}	15.50 ± 0.39^{a}	36.28 ± 2.36^{a}	49.55 ± 1.63^{a}	21.15 ± 0.44^{a}	42.83 ± 2.11^{a}	349.00 ± 32.33^{a}	6.18 ± 0.53^{a}	4.23 ± 0.81^{ab}
	18.28	4.50 ± 0.62^{a}	80.90 ± 4.915^{a}	14.48 ± 3.65^{a}	6.41± 0.87^{a}	14.60 ± 1.43^{a}	34.85 ± 2.00^{a}	51.20 ± 1.75^{a}	20.40 ± 1.27^{a}	42.15 ± 1.11^{a}	310.75 ± 15.84^{a}	6.18 ± 0.91^{a}	3.88 ± 0.53^{a}
	62.50	4.68 ± 0.21^{a}	82.35 ± 1.955^{a}	13.28 ± 1.85^{a}	6.97 ± 0.79^{a}	15.23 ± 0.78^{a}	36.03 ± 1.26^{a}	52.20 ± 4.52^{a}	21.98 ± 2.05^{a}	42.20 ± 1.49^{a}	294.00 ± 20.78^{a}	6.15 ± 0.34^{a}	5.05 ± 0.57^{b}
	250	6.35 ± 0.33^{b}	83.90 ± 3.32^{a}	13.43 ± 1.90^{a}	6.85 ± 0.77^{a}	15.83 ± 0.46^{a}	34.45 ± 2.11^{a}	50.90 ± 5.30^{a}	22.13 ± 0.78^{a}	45.83 ± 2.62^{a}	319.00 ± 45.95^{a}	6.03 ± 0.59^{a}	3.68 ± 0.43^{a}
	1000	7.58 ± 1.26^{c}	85.28 ± 3.24^{a}	12.35 ± 3.05^{a}	7.15 ± 1.20^{a}	15.27 ± 0.77^{a}	36.53 ± 1.95^{a}	49.70 ± 3.52^{a}	21.55 ± 2.59^{a}	43.45 ± 3.37^{a}	330.75 ± 26.46^{a}	6.18 ± 0.59^{a}	4.10 ± 0.81^{ab}
Male	0	5.23 ± 0.56^{a}	84.15 ± 3.40^{a}	12.60 ± 2.87^{a}	7.46 ± 0.51^{a}	15.90 ± 0.88^{a}	37.98 ± 1.92^{a}	47.35 ± 0.53^{a}	19.75 ± 0.73^{a}	41.85 ± 1.77^{a}	312.50 ± 20.27^{ab}	6.10 ± 0.24^{a}	3.35 ± 0.25^{a}
	18.28	5.40 ± 1.06^{a}	83.15 ± 0.99^{a}	13.20 ± 1.20^{a}	7.39 ± 0.29^{a}	14.85 ± 1.19^{a}	35.90 ± 2.28^{a}	48.98 ± 2.11^{a}	21.00 ± 0.58^{a}	43.70 ± 2.07^{a}	290.75 ± 49.79^{a}	6.55 ± 0.61^{a}	3.65 ± 0.37^{a}
	62.50	6.13 ± 1.39^{a}	80.98 ± 5.27^{a}	14.20 ± 4.61^{a}	7.04 ± 0.70^{a}	15.05 ± 0.70^{a}	37.70 ± 0.80^{a}	50.73 ± 6.20^{a}	21.58 ± 2.46^{a}	42.28 ± 2.34^{a}	336.75 ± 54.17^{ab}	6.42 ± 0.28^{a}	4.83 ± 0.90^{b}
	250	5.48 ± 0.93^{a}	81.55 ± 6.23^{a}	15.38 ± 4.53^{a}	7.87 ± 0.88^{a}	15.75 ± 0.84^{a}	37.38 ± 2.58^{a}	49.85 ± 3.73^{a}	23.58 ± 4.68^{a}	42.83 ± 2.69^{a}	381.00 ± 24.37^{b}	6.03 ± 0.46^{a}	5.58 ± 0.38^{b}
	1000	6.08 ± 0.82^{a}	81.40 ± 5.46^{a}	13.25 ± 3.95^{a}	7.63 ± 0.53^{a}	15.48 ± 1.25^{a}	36.60 ± 1.45^{a}	50.55 ± 5.98^{a}	24.48 ± 3.91^{a}	43.40 ± 3.01^{a}	367.25 ± 43.17^{b}	6.60 ± 0.52^{a}	5.55 ± 0.30^{b}

Along each column and same-sex, values with the same letter superscripts are not significantly different. Waller-Duncan ($p<0.05$). RBC: Red Blood Cell, WBC: White Blood Cell, LYM: Lymphocytes, GRAN: Granulocytes, HGB: hemoglobin, HCT: hematocrit, MCV: Mean corpuscular volume, MCHC: Mean Corpuscular Haemoglobin Concentration, PLT: platelets, Mon: Monocytes

Biochemical parameters

Biochemical values of rats treated with the ethanolic extract from *D. thollonii* are shown in Tables 5, 6, 7 and 8. This extract did not affect serum and urinary creatinine of animal of both sexes, although, a decrease in the urine creatinine level was noted at the dose of 1000 mg/kg for a male. urinary urea decreased significantly in both males and females at doses of 62.50; 250; 1000 mg/kg, while serum urea increased significantly at doses of 250; 1000 mg/kg for females and at doses of 62.50; 250; 1000 mg/kg for males. Total cholesterol and urinary proteins were not affected in both sexes, although, a significant decrease was noted in total cholesterol at the dose of 250 and 1000 mg/kg for females and males respectively. The increase in urinary proteins at the dose of 1000 mg/kg was noted in female rats. Triglycerides, HDL-cholesterol and LDL-cholesterol were not affected in both sexes. ALT, AST, and ALP levels significantly increased in both sexes generally at the doses of 250; 1000 mg/kg. Total and direct bilirubin were not affected by both sexes. The level of spleen proteins, heart proteins, lung proteins, and serum proteins were not significantly affected in both sex while liver proteins increased significantly at the dose of 1000 mg/kg in both males and females and kidney proteins decreased significantly at the dose of 1000 mg/kg for female and at the doses of 250, 1000 mg/kg for male.

Table 5. Effect of aqueous leaf extract of *D. thollonii* on liver function parameters

Sex	**Dose (Dose (mg/kg)**	Liver function parameters				
		ALT (UI/L)	**AST (UI/L)**	**ALP (UI/L)**	**T Bil (mg/dl)**	**D Bil (mg/dl)**
Female	0	31.57 ± 6.44^{a}	51.79 ± 3.00^{a}	46.19 ± 3.03^{a}	0.28 ± 0.07^{a}	0.21 ± 0.05^{bc}
	18.28	33.97 ± 1.31^{a}	48.34 ± 4.53^{a}	44.55 ± 3.58^{a}	0.27 ± 0.09^{a}	0.22 ± 0.03^{bc}
	62.50	30.2 ± 6.72^{a}	49.64 ± 3.34^{a}	47.91 ± 6.39^{a}	0.32 ± 0.07^{a}	0.24 ± 0.03^{c}
	250	48.38 ± 4.94^{b}	$54.26 \pm 4.52^{a.\ b}$	66.10 ± 3.98^{b}	0.37 ± 0.033^{a}	0.17 ± 0.06^{ab}
	1000	48.82 ± 6.75^{b}	59.73 ± 2.52^{b}	105.54 ± 6.70^{c}	0.29 ± 0.06^{a}	0.12 ± 0.01^{c}
Male	0	42.49 ± 4.92^{a}	46.86 ± 2.81^{a}	63.10 ± 3.28^{b}	0.40 ± 0.09^{a}	0.19 ± 0.06^{a}
	18.28	54.31 ± 1.74^{b}	43.53 ± 1.34^{a}	49.97 ± 5.57^{a}	0.40 ± 0.08^{a}	0.18 ± 0.07^{a}
	62.50	$48.58 \pm 6.92^{a.b}$	58.10 ± 5.10^{b}	$56.10 \pm 5.63^{a.\ b}$	0.28 ± 0.07^{a}	0.21 ± 0.08^{a}
	250	97.30 ± 7.53^{c}	64.49 ± 3.26^{c}	81.94 ± 5.57^{c}	0.40 ± 0.07^{a}	0.22 ± 0.06^{a}
	1000	98.77 ± 6.24^{c}	64.74 ± 2.69^{c}	102.70 ± 2.39^{d}	0.32 ± 0.08^{a}	0.24 ± 0.04^{a}

Along each column and same-sex, values with the same letter superscripts are not significantly different. Waller-Duncan ($p<0.05$). ALT: alanine aminotransferase, AST = aspartate aminotransférase ; ALP = alkaline phosphatase; T Bil: total bilirubin; D Bil: direct bilirubin.

Table 6. Effects of ethanolic extract of *D. thollonii* on some lipid profiles after 28 days of oral administration

Sex	Dose (mg/kg)	Lipidic parameters (mg/dl)			
		Triglyceride	Total Cholesterol	HDL- Cholesterol	LDL- Cholesterol
Female	0	32.90 ± 3.68 ^{a}	122.77 ± 4.40^{c}	56.98 ± 4.93^{a}	59.22 ± 3.95 ^{a}
	18.28	31.46 ± 1.52 ^{a}	118.66 ± 5.40^{bc}	56.98 ± 5.69^{a}	55.40 ± 7.75 ^{a}
	62.50	36.25 ± 4.07 ^{a}	113.86 ± 2.03^{ab}	53.69 ± 1.24^{a}	52.92 ± 1.84 ^{a}
	250	32.07 ± 2.36 ^{a}	117.00 ± 1.58^{bc}	50.57 ± 3.17^{a}	60.02 ± 4.85 ^{a}
	1000	32.29 ± 2.62 ^{a}	109.57 ± 2.00^{a}	50.18 ± 3.69^{a}	52.94 ± 5.38 ^{a}
Male	0	43.10 ± 1.52 ^{a}	118.25 ± 2.46^{b}	42.44 ± 5.79^{a}	67.01 ± 7.96 ^{a}
	18.28	44.68 ± 1.63 ^{a}	114.63 ± 2.94^{ab}	44.31 ± 2.49^{a}	61.38 ± 3.09 ^{ab}
	62.50	40.96 ± 5.50 ^{a}	118.23± 4.24^{b}	45.05 ± 3.08^{a}	64.99 ± 2.18 ^{ab}
	250	43.16 ± 6.90 ^{a}	109.90 ± 4.70^{a}	46.19 ± 3.86^{a}	55.08 ± 7.24 ^{b}
	1000	43.47 ± 1.87 ^{a}	111.62 ± 4.30^{ab}	42.27 ± 2.62^{a}	60.66 ± 6.71 ^{ab}

Along each column and same-sex, values with the same letter superscripts are not significantly different. Waller-Duncan ($p<0.05$).

Table 7. Effects of ethanolic extract of *D. thollonii* on serum creatinine, urinary creatinine, serum urea, urinary urea and urinary proteins of rats after 28 days of oral administration.

Sex	Dose (mg/kg)	Urinary creatinine	Serum creatinine	Urinary urea	Serum urea	Urinary proteins
Female	0	75.62 ± 4.42^{a}	0.60 ± 0.07^{a}	314.88 ± 2.87^{c}	42.06 ± 2.22^{ab}	0.18 ± 0.04 ^{a}
	18.28	75.00 ± 4.04^{a}	0.57 ± 0.07^{a}	308.46 ± 6.78^{c}	41.67 ± 3.69^{a}	0.20 ± 0.02 ^{a}
	62.50	73.93 ± 4.65^{a}	0.54 ± 0.10^{a}	255.44 ± 4.35^{a}	47.08 ± 3.06^{bc}	0.20 ± 0.06 ^{a}
	250	74.96 ± 5.11^{a}	0.61 ± 0.05^{a}	266.67 ± 5.77^{b}	47.36 ± 2.10^{c}	0.23 ± 0.04 ^{ab}
	1000	78.48 ± 3.26^{a}	0.53 ± 0.06^{a}	256.00 ± 4.49^{a}	49.28 ± 3.82^{c}	0.32 ± 0.07 ^{b}
Male	0	126.55 ± 6.02^{bc}	0.51 ± 0.05^{a}	361.11 ± 5.56^{c}	35.00 ± 2.94^{a}	0.41 ± 0.09^{a}
	18.28	134.76 ± 3.12^{c}	0.50 ± 0.03^{a}	351.17 ± 11.94^{bc}	36.81 ± 2.42^{a}	0.57 ± 0.19^{a}
	62.50	128.46 ± 9.10^{bc}	0.53 ± 0.06^{a}	341.67 ± 3.80^{b}	42.50 ± 3.99^{b}	0.52 ± 0.19^{a}
	250	122.73 ± 6.45^{b}	0.54 ± 0.09^{a}	322.22 ± 6.42^{a}	42.08 ± 2.66^{b}	0.41 ± 0.05^{a}
	1000	95.24 ± 3.51^{a}	0.53 ± 0.05^{a}	311.11 ± 9.07^{a}	45.94 ± 2.43^{b}	0.47 ± 0.09^{a}

Along each column and same-sex, values with the same letter superscripts are not significantly different. Waller-Duncan ($p<0.05$).

Table 8. Effects of ethanolic extract of *D. thollonii* on proteins profiles of liver, kidney, heart, spleen, lungs, and serum after 28 days of oral administration.

Sex	Dose (mg/kg)	Liver (mg/g)	Spleen (mg/g)	Heart (mg/g)	Kidney (mg/g)	Lungs (mg/g)	Serum (g/dL)
Female	0	303.78 ± 13.53^{a}	271.11 ± 52.23^{a}	217.12 ± 33.54^{a}	289.96 ± 12.55^{b}	202.62 ± 29.45^{a}	6.78 ± 0.91^{a}
	18.28	324.69 ± 16.12^{a}	288.41 ± 50.27^{a}	249.65 ± 38.77^{ab}	289.78 ± 20.82^{b}	191.80 ± 28.31^{a}	6.80 ± 0.71^{a}
	62.50	307.19 ± 17.02^{a}	269.29 ± 51.65^{a}	267.30 ± 47.26^{ab}	300.22 ± 19.05^{b}	227.99 ± 44.72^{a}	6.58 ± 0.51^{a}
	250	303.85 ± 6.65^{a}	394.60 ± 69.00^{b}	251.81 ± 39.43^{ab}	301.58 ± 8.38^{b}	186.58 ± 30.36^{a}	6.84 ± 0.82^{a}
	1000	346.90 ± 5.33^{b}	343.81 ± 26.71^{ab}	299.82 ± 34.11^{b}	204.63 ± 14.02^{a}	203.20 ± 18.25^{a}	6.24 ± 0.24^{a}
Male	0	296.92 ± 12.36^{bc}	337.74 ± 28.98^{a}	230.44 ± 56.96^{ab}	375.68 ± 30.87^{b}	205.55 ± 18.52^{a}	6.59 ± 0.22^{ab}
	18.28	301.99 ± 2.28^{c}	380.66 ± 39.61^{a}	241.90 ± 28.92^{ab}	383.35 ± 27.49^{b}	194.59 ± 42.11^{a}	6.18 ± 0.89^{a}
	62.50	282.79 ± 15.13^{ab}	380.44 ± 11.29^{a}	281.55 ± 17.18^{b}	396.40 ± 17.71^{b}	186.10 ± 27.23^{a}	6.64 ± 0.37^{ab}
	250	289.97 ± 8.35^{abc}	369.82 ± 26.54^{a}	236.02 ± 21.04^{ab}	243.63 ± 8.08^{a}	205.50 ± 38.14^{a}	7.11 ± 0.22^{b}
	1000	274.81 ± 10.56^{a}	375.35 ± 16.14^{a}	207.15 ± 54.71^{a}	233.36 ± 8.90^{a}	166.43 ± 11.61^{a}	7.21 ± 0.44^{b}

Along each column and same-sex, values with the same letter superscripts are not significantly different. Waller-Duncan ($p<0.05$).

Histopathology analysis

Microscopic examination of rat liver tissues in the control group revealed that they were normal (normal hepatic cells with well-preserved cytoplasm, prominent nucleus, and visible central veins) for both sexes. Sections from an animal treated with *D. thollonii* leaves extract did not reveal any significant change from normal histological features generally. Meanwhile, we observed a slight change from normal histological features (congestion) at a dose of 1000 mg/kg for females and at doses of 250; 1000 mg/kg for males.

At the level of the kidney, histopathological examination of the kidney sections in the control group showed normal renal tubules and normal glomerular capillaries. In the treated group of both sexes, the tissues appeared normal as compared with a control group and showed no special pathological changes. Except the male treated at the dose of 1000 mg/kg which show a slight mesengial expansion.

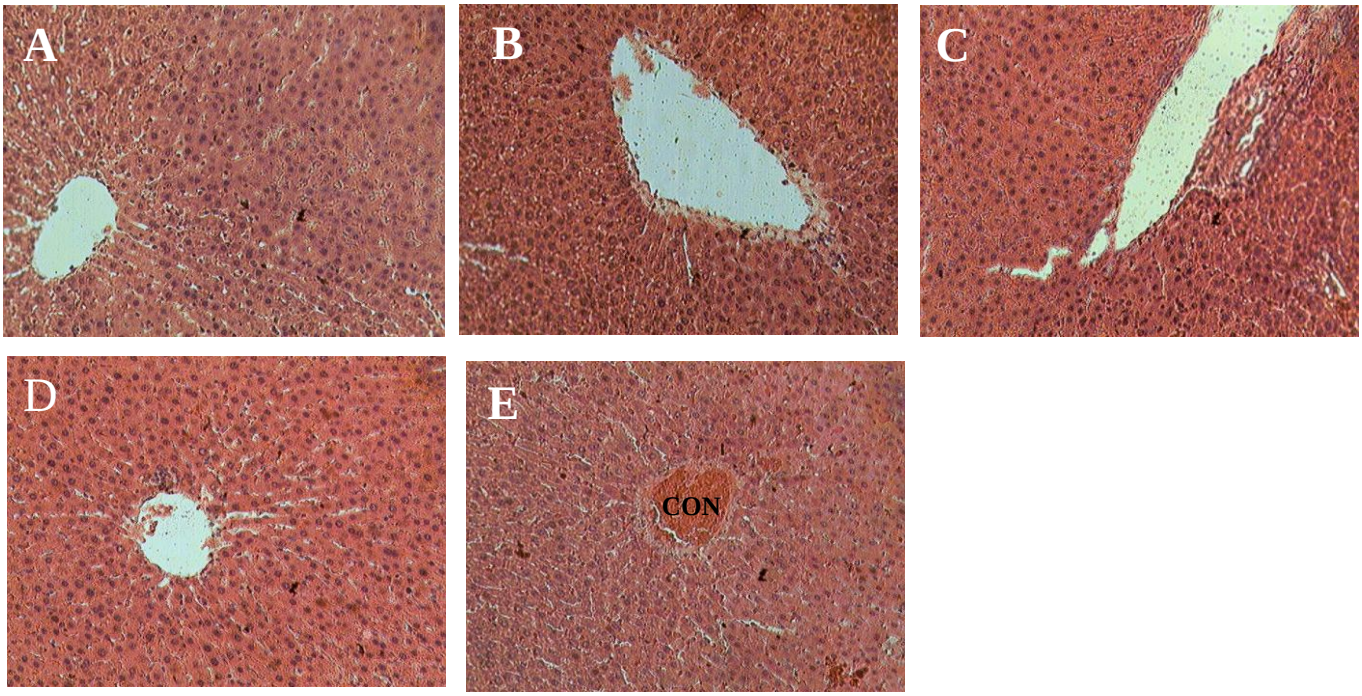

Figure 2. Histopathological changes in liver of rats after 28 days of treatment in female rats (400X). (A) control. (B) wistar rats treated with 18,28 mg/kg leaves extracts of *D. thollonii*. (C) wistar rats treated with 62,50 mg/kg leaves extracts of *D. thollonii*. (D) wistar rats treated with 250 mg/kg leaves extracts of *D. thollonii*. (E) wistar rats treated with 1000 mg/kg leaves extracts of *D. thollonii*. CON : congestion.

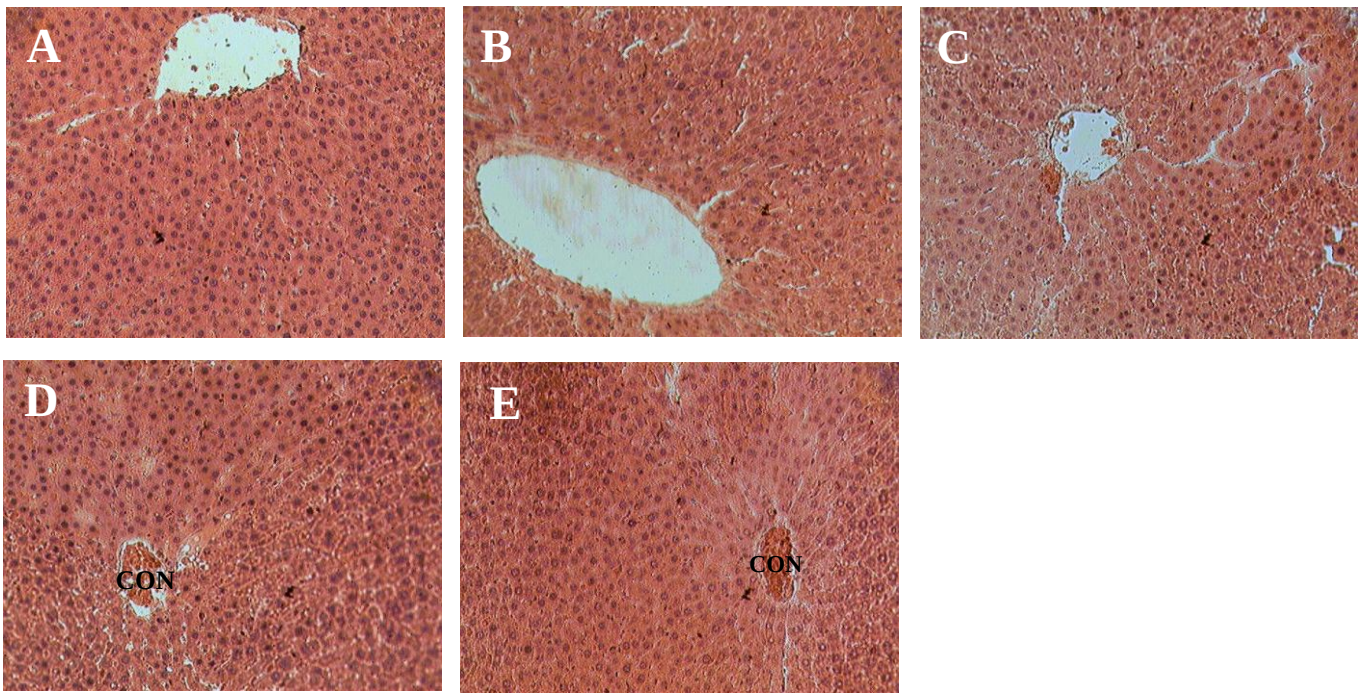

Figure 3. Histopathological changes in liver of rats after 28 days of treatment in male rats (400X). (A) control. (B) wistar rats treated with 18,28 mg/kg leaves extracts of *D. thollonii*. (C) wistar rats treated with 62,50 mg/kg leaves extracts of *D. thollonii*. (D) wistar rats treated with 250 mg/kg leaves extracts of *D. thollonii*. (E) wistar rats treated with 1000 mg/kg leaves extracts of *D. thollonii*. CON : congestion.

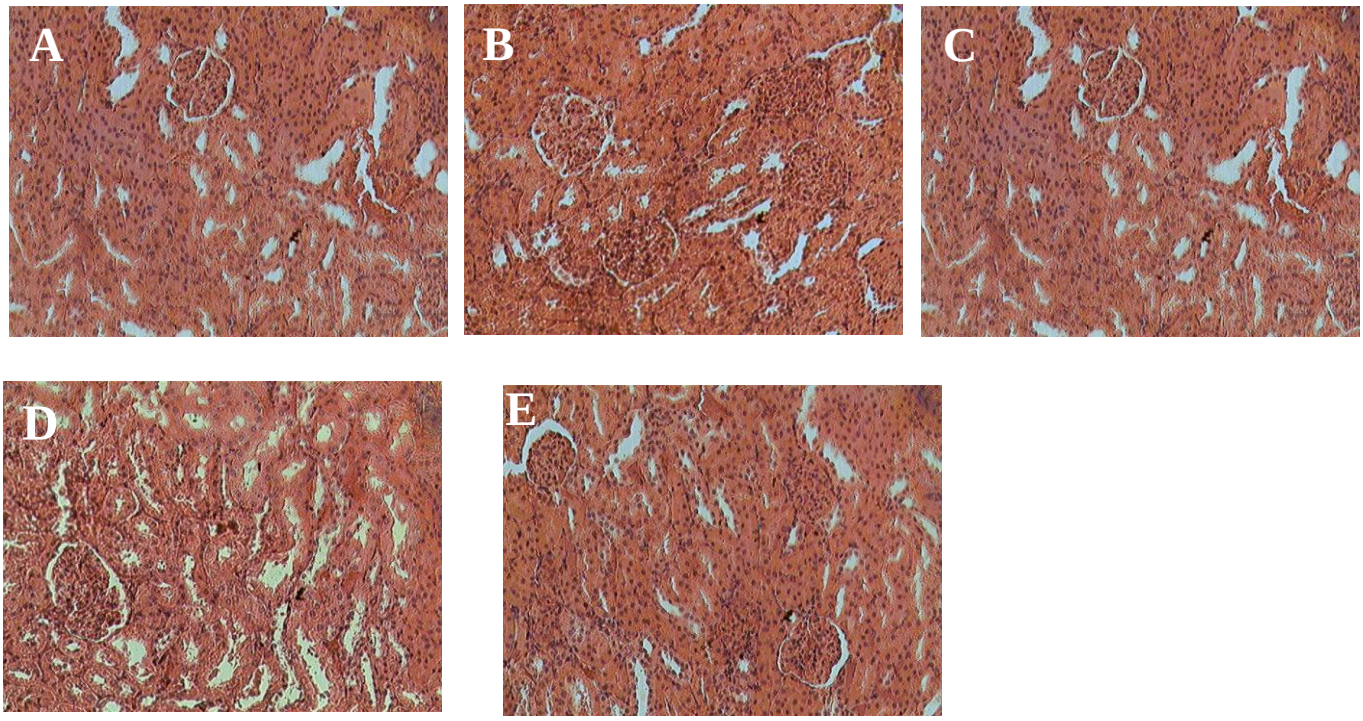

Figure 4. Histopathological changes in kidney of rats after 28 days of treatment in female rats (400X). (A) control. (B) wistar rats treated with 18,28 mg/kg leaves extracts of *D. thollonii*. (C) wistar rats treated with 62,50 mg/kg leaves extracts of *D. thollonii*. (D) wistar rats treated with 250 mg/kg leaves extracts of *D. thollonii*. (E) wistar rats treated with 1000 mg/kg leaves extracts of *D. thollonii*.

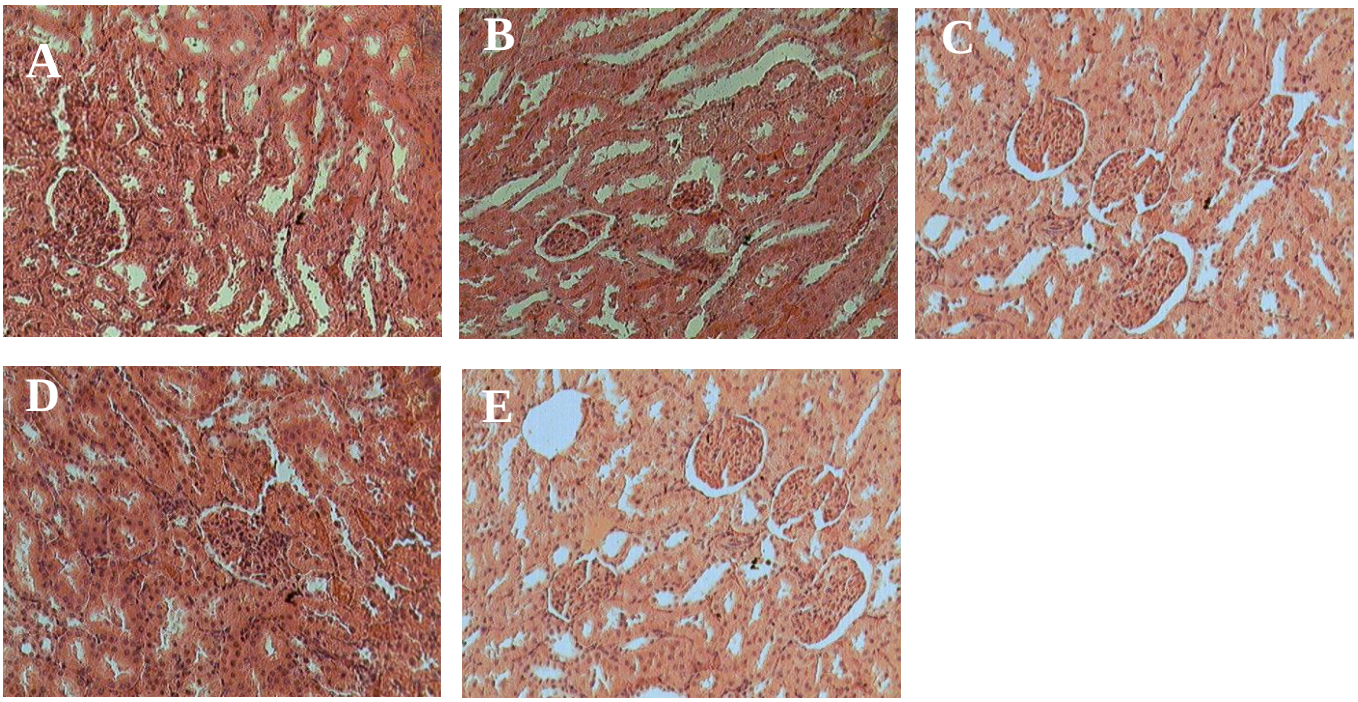

Figure 5. Histopathological changes in Kidney of rats after 28 days of treatment in female rats (400X). (A) control. (B) wistar rats treated with 18,28 mg/kg leaves extracts of *D. thollonii*. (C) wistar rats treated with 62,50 mg/kg leaves extracts of *D. thollonii*. (D) wistar rats treated with 250 mg/kg leaves extracts of *D. thollonii*. (E) wistar rats treated with 1000 mg/kg leaves extracts of *D. thollonii*.

DISCUSSION

Medicinal plants are an important source of bioactive compounds and are used worldwide in traditional medicine for the treatment of various ailments. Although medicinal plants may have biological activities that are beneficial to humans, the potential toxicity of these bioactive substances has to be well established [13]. Moreover, despite the widespread use, few scientific studies have been undertaken to ascertain the safety and efficacy of traditional remedies [14]. Thus, the safety of these plants must be studied to avoid their potential toxicity in human. To achieve this objective, the present investigation was performed to evaluate the possible acute toxicity and 28 days sub-chronic toxicity effects of ethanolic leaf extract of *D. thollonii*, used as a natural medicine in Cameroon to alleviate many pathological conditions by traditional healers. Data obtained from this study showed that *D. thollonii* in an acute dose of 5000 mg/kg, by the oral route, did not produce any sign of toxicity in general behavior or death. All animals treated with the extract survived until the end of the 14 days observation period. No abnormality was found in organs at necropsy at the end of the experimental period. This suggests that the median acute toxicity value (LD50) of the extract is above 5000 mg/kg body weight. According to the chemical labelling and classification of acute systemic toxicity recommended by OECD [5,6], the crude extract of *D. thollonii* extract was assigned class 5 status (LD50 > 5000 mg/kg), which was the lowest toxicity class according to Kennedy et al. [15], substances that present LD50 higher than 5000 mg/kg via oral route may be considered practically non-toxic. Moreover, as per the Hodge and Sterner [16] scale, its toxicity index was 5 thus almost non-toxic. Since no toxic effects were found during the acute toxicity study, further study was conducted to evaluate the subchronic toxicity of ethanolic extract from *D. thollonii* up to 28 days to prepare inclusive toxicological records on this plant.

Sub-chronic treatment did not produce any death or clinical signs of toxicity. There was a concomitant significant decrease in food intake and body weight gain at high doses (250 and/or 1000 mg/kg) in both male and female rats. This weight decreases might have been as a result of dietary palatability problem when the concentration of *D. thollonii* increased. Loss of appetite is often associated with weight loss due to carbohydrate, protein or fat metabolisms disorders [17]. Furthermore, at higher doses, crude plant extracts may metabolize to a toxic end product which may interfere with gastric function efficiency [18]. An important index to diagnose whether an organ has been exposed to injury is to calculate

the organ-to-body weight ratio [13]. Hence, if rats are exposed to toxic substances the weight of the damaged organ will either increase or decrease as will the organ-to-body weight ratio. In the present study, there were no significant changes in the relative weights of organs between the control and treated rats. This suggests no glossy toxic effect from the extract. The analysis of blood parameters is relevant to risk evaluation as any changes in the hematological and biochemical systems have a higher predictive value for human toxicity when data are translated from animal studies [19]. In this study, the hematological profile of treated rats showed no significant difference with the control group, except white blood cells and monocytes amount which increased in females and males treated with high doses (250 and/or 1000 mg/kg) of the ethanolic leaf extract of *D. thollonii*. This elevation in white blood cells and monocytes suggests that the extract contains biologically active compounds that have the ability to boost the immune system [14]. Estimation of renal excretion of waste metabolites and histological changes in the kidney has provided useful information on the health status of the kidneys [20]. In fact, abnormally high levels of serum creatinine, and urea are biomarkers of a possible malfunction of the kidneys [21]. Moreover, a significant increase in the urinary total protein is an evidence of inflammation of the glomerular capillaries [22]. In this study, there was a significant decrease of urinary urea with a significant increase of serum urea in both males and females at high doses (250; 1000 mg/kg) and a significant increase of urinary proteins in males at doses of 1000 mg/kg. These results suggest that the kidney functions are altered in animals treated with high doses of extract. Meanwhile, further microscopic examination of kidney sections shows that the plant extract has no marked effect on kidney of rats of both sex. Numerous studies have pointed out the increased risk of coronary disorders with elevated levels of TC, TG, and LDL-cholesterol [23]. Besides, High level of serum TC and LDL-cholesterol have been shown to pose a significant risk in ischemic stroke, whereas high levels of HDL-cholesterol indicated beneficial effects on the atherosclerotic process [24]. In the present study, it was observed that administration of aqueous extract of *D. thollonii* to rats did not induce significant changes in the serum levels of TG, LDL-cholesterol and HDL-cholesterol in both sexes, while the levels of TC decrease significantly at the dose of 250 and 1000 mg/kg for females and males respectively. This result suggests that *D. thollonii* contains component which may have no risk of cardiovascular and coronary diseases. The increase in the serum levels of ALT, AST, ALP or bilirubin are associated with damage of hepatic cells indicates hepatic toxicity [14]. These changes occur in the blood when the hepatic cellular permeability is changed or when necrosis and cellular injury occurs [25]. In the present investigation, sub-chronic

administration of the extract caused significant increase of ALT, AST and ALP levels in both sexes at the doses of 250 and 1000 mg/kg in generally. This result suggests that the extract may caused liver damages at high doses. It was further strengthened by histological studies of liver section which showed that the plant extract induce slight liver damage. The assessment of histopathology in the body tissues is the gold standard for evaluating treatment-related pathological changes in tissues [1]. The kidney sections from animals of both sex showed normal renal morphology with normal renal tubules and normal glomerular capillaries. While histological examination of the liver of rats treated with *D. thollonii* at a higher dose (250 ; 1000 mg/kg) for both sex revealed some histopathological changes. The changes in the liver were characterized by congestion. This may be explained by the fact that at relatively high doses, the extract had the ability to induce liver damage. However, the apoptosis or necrosis of hepatocytes remains one of the major signs of liver damage due to toxic compounds [26], and this was not observed in this study.

CONCLUSION

The ethanol extract of leaf of *D. thollonii* is not likely to produce any severe toxic effects. Its median lethal dose (LD50) of greater than 5000 mg/kg body weight justifies its safety. Besides, daily oral administration of extract for a period of 28 days did not cause mortality and observed adverse-effect in female or male rats. However, the prolonged oral administration at a high dose may cause observable changes in biochemical parameters (ALT, AST, ALP) with slight liver damage. Hence, the extract should be used with caution at high doses. Chronic toxicity tests are recommended to determine the long-term effects of the extracts to further support the safe use of this plant.

REFERENCES

[1] Attanayake AP, Jayatilaka KA, Pathirana C, Mudduwa LK. 2015. Toxicological investigation of Spondias pinnata (Linn. F.) Kurz. (Family: Anacardiaceae) bark extract in Wistar rats. *Int. J. Green Pharm.*, **9**: 26- 31.

[2] Prime C, Penlap V, Nkedoum B, Taziebou C, Tekwu E, Etoa F, Ngongang J. 2006. Evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of Senna alata (L.) roxb (Ceasalipiniaceae). *African Journal of Biotechnology*, **5**: 283-289.

[3] Atsafack Serge Secco, Kuiate Jules-Roger, Mouokeu Raymond Simplice, Koanga Mogtomo Martin Luther, Tiabou Tchinda Alembert, Tamokou Jean De Dieu, Magnifouet Nana Huguette, Ebelle Etame Rébecca Madeleine, Biyiti Lucie, Ngono Ngane Rosalie Annie. 2015. Toxicological studies of stem bark extract from Schefflera barteriHarms (Araliaceae). *BMC Complementary and Alternative Medicine*, **15**: 44.

[4] Loigier HA. 1994. Descriptive flora of Puerto Rico and Adjacent islands. *Spermaphyta*, 13.

[5] OECD. 2008a. Organization for Economic Cooperation and Development (OECD) guidelines for the testing of chemicals Acute Oral Toxicity – Up-and-Down-Procedure (UDP). OECD, 1-27.

[6] OECD. 2008b.Organization for Economic Cooperation and Development guidelines for the Testing of Chemicals/Draft Updated Test Guideline 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents.2008.

[7] Gatsing D, Aliyu R, Kuiate JR, Garba IH, Tedongmo N, Tchouanguep FM. 2005. Toxicological evaluation of the aqueous extract of Allium sativumbulbs on laboratory mice and rats. *Cameroon J. Exp. Biol*., **1**: 39-45.

[8] Friedewald WT, Levy RI, Fredrickson DS. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.,* **18**: 499- 502.

[9] Gornall AG, Bardawill CJ, David MM. 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem*., **177**: 751-66.

[10] Bradford MM. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*., **72**: 248-254.

[11] Mosaid AZ, Alferah. Toxicity Induced Histological Changes in Selected Organs of Male (Wistar) Rats by Lawsonia inermis Leaf Extract. Eur J Med Plants. 2012;2(2):151–158.

[12] World Health Organization. Research guidelines for evaluating the safety and efficacy of herbal medicines. Geneva: World Health Organization; 1993. [Online] Available from: http://apps.who.int/medicinedocs/en/d/Jh2946e/ [Accessed on 20th May, 2014]

[13] Rosidah YMF, Sadikun A, Ahmad M, Akowuah GA, Asmawi MZ. 2009. Toxicology evaluation of standardized methanol extract of Gynura procumbens. *J. Ethnopharmacol*., **123**(2): 244-249.

[14] Kokou Idoh, Amegnona Agbonon, Yao Potchoo, Messanvi Gbeassor. 2016. Toxicological assessment of the hydroethanolic leaf extract of clerodendrum capitatum in Wistar rats. *Pan African Medical Journal,* **24**:66.

[15] Kennedy GL, Ferenz, Burgess BA. 1986. Estimation of acute oral toxicity in rats by determination of the approximate lethal dose rather than the LD50. *J. Appl. Toxicol*., **6**(3): 145-148.

[16] Hodge AC, Sterner JH. 1980. In études de toxicité: quelques données fondamentales (A. K. DONE) TEMPO MEDICAL Afrique N°7, 18p.

[17] Klaassen CD, Casarett, Doull's. 2013. *Toxicology: the Basic Science of Poisons*. Vol. 1236. McGrawHill : New York (NY).

[18] Chokshi D. 2007. Subchronic oral toxicity of a standardized white kidney bean (Phaseolus vulgaris) extract in rats. *Food and Chemical Toxicology,* **45**(1): 32-40.

[19] Olson H, Betton G, Robinson D et al. 2000. Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regul. Toxicol. Pharmacol*., **32**(1): 56-67.

[20] Fodouop SPC, Tala SD, Lunga PK, Yemele MD, Gatsing D, Nwabo KAH, Nji-kah B, Kodjio No, Tchoumboue J. 2017. Effects of *Vitellaria paradoxa* (C.F. Gaertn.) aqueous leaf extract administration on Salmonella typhimurium-infected rats. *BMC Complementary and Alternative Medicine* 17:160

[21] Palm M, Lundblad A. 2005. Creatinine concentration in plasma from dog, rat, and mouse: A comparison of 3 different methods. *Vet. Clin. Pathol*., 34: 232–236.

[22] Dare JO, Rufus OA, Abubarkar AS, Christian EI, Olaoluwa SO, Ayowole AO. 2016. Effects of two weeks administration of Ocimum gratissimum leaf on feeding pattern and

markers of renal function in rats treated with gentamicin. *Egyptian Journal of Basic and Applied Sciences*, **3**: 219-231.

[23] Kapur NK, Ashen D, Blumenthal RS. 2008. High density lipoprotein cholesterol: an evolving target of therapy in the management of cardiovascular disease. Vasc. Health Risk Manag, **4**(1): 39-57.

[24] Uddin MJ, Alam B, Jabbar MA, Mohammad QD, Ahmed S. 2009. Association of lipid profile with ischemic stroke. *Mymensingh Med. J*., **18**(2): 131-5.

[25] Hyder MA, Hasan M, Mohieldein AH. 2013. Comparative levels of ALT, AST, ALP and GGT in liver associated diseases. *Eur. J. Exp. Biol*., **3**(2): 280-4.

[26] Eroschenko VP, Di Fiore. 2000. *Atlas of Histology with Functional Correlations*. 9th ed. Philadelphia: Lippincott. Williams and Wilkins;.