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**Research Article**

# Oral Acute and Sub-Chronic Toxicity Studies of the Aqueous and Methanol Leaves Extracts of *Dissotis thollonii* Cogn. (Melastomataceae) in Balb/c Mice and Wistar Rats.

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## Abstract

In Africa, especially in Cameroon, the aqueous extract of the leaves of *Dissotis thollonii* has been used as folk medicine for thousands of years for the diarrhoeal and inflammatory diseases. However, there is no scientific evidence to verify the safety of its use. The aim of this study was to evaluate the toxicological potential of *Dissotis thollonii* through the acute and sub-chronic toxicity tests. Acute and sub-chronic toxicity studies with *Dissotis thollonii* was done on Balb/c mice and wistar albino rats respectively. Anthropometric, hematological and biochemical parameters were measured and histological sections of liver and kidneys were realized. During acute toxicity, a single oral dose of the extracts at 2000 and 5000 mg/kg produced no signs of toxicity such as general behavior change, important variation of body weight and no mortality (LD50 of the extract was ≥ 5000 mg/kg). However, in the sub-chronic toxicity studies, few modifications were observed in hematological, biochemical and histological parameters. We also observed variation in body weight, water and food intake in week 3 and 4 when compared with the control $H_20$ and week 5 and 6 when compared with the satellite control. Histopathology showed the presence of disturbances at the dose of 500 mg/kg in the male. The aqueous and methanolic extracts of the leaves of *D. thollonii* is safe when administered acutely (p.o) and aqueous extract could be moderately toxic at high doses when administered to Albino wistar rats daily for twenty-eight days.

**Keywords:** *Dissotis thollonii;* acute toxicity; sub-chronic toxicity; Balb/c mice; Wistar rats.

## Introduction

Nowadays, there is an increase in medicinal plants and herbal drug research and the demand to use traditional medicine is becoming widely popular in the treatment of a number of diseases including diarrhea, inflammation. Over the last decade, it is possible to observe a significant increase in popular acceptance of natural therapies, both in developed and developing countries[1]. The World Health Organization (WHO) estimated that approximately 80% of the world's population depends on herbal drugs and medicinal plants for their primary health care [2]. However, plants that are consumed by the population, although they have promising pharmacological potential, have not been tested for possible adverse effects [3]. Among the many exploited species, *Dissotis thollonii* (*D. thollonii*) is a species that belongs to the Melastomataceae family, which is distributed in tropical Africa. It is a perennial herb, up to 2 meters high. The stem carries at the end the flowers with pink color. It is found in swampy areas, and at the edges of gallery forests. In Cameroon, the plant is particularly found in the western region. This plant is traditionally used in the treatment of several evils. Thus it is used by traditional healer in Senegal on eczematous wounds and in west region of Cameroon[4]. The leaves are recommended in therapy of the gastrointestinal disorders (obstruction, amoebiasis, diarrhoeas, vomiting, and constipation) inflammation and ulcers. The antidiarroeal and antibacterial activities, anti-ulcer and anti-ulcerogenic activities, are already been demonstrate with this plant[5]. Anti-microbial, antioxidant and anti-inflammatory activities of roots of this plant has been experimentally demonstrated by authors[6]. Phytochemical screening of the aqueous and methanol leaves extract of *D. thollonii* revealed the presence of tannins, flavonoids, anthocyanins, anthraquinones, sterols, phenols and polyphenols [5]. In spite of the wide ethnotherapeutic applications of the plant, the safety of a novel natural extract should be evaluated by more independent studies and more independent researchers, before it would be used in pharmacological applications [7]. We aimed to investigate the oral acute toxicity of the aqueous and methanolic extracts in Balb/c mice and subchronic toxicity of aqueous extract of *D. thollonii* in wistar albino rats.

## Materials and Methods

### *Plant material*

The fresh leaves of *D. thollonii* were collected in September 2016 in the Bafou village, Menoua division, West Region of Cameroon. A sample was identified at the National Herbarium of Cameroon (Yaoundé) by comparison with existing voucher specimen No.13292/SRF Cam.

### *Animals*

Wistar Albino male and female rats of 2 to 2.5 months and weighing on average 130 g were used for sub chronic toxicity whereas male and female (6–8 weeks old) mice, weighing on average 35 g, were used for the acute toxicity. The female rats or mice were nulliparous and non-pregnant. They were bred in the Animal House Facility, Panjwani Center for Molecular Medicine and Drug Research (PCMD), International Center for Chemical and Biological Sciences. Prior to experimental protocol, the rats and mice were acclimatized for 48 h to laboratory conditions for minimizing any nonspecific stress. The treatment of animals was in agreement with the Institutional Animal Care, Use and Standards Committee (IACUC) of ICCBS were followed (Protocol No. 2016-0024), and the study protocols accepted by the ethics committees of ICCBS were followed, University of Karachi, Pakistan.

### *Preparation of plant extract*

The aqueous extract was prepared by boiling 800 g of powder in 5L distilled water for 15 min as indicated by the traditional healer. The decoction was cooled during 1 h and filtered using filter paper (Whatman No. 1), and the filtrate was evaporated in a regulated drying oven at 35 °C to give 51.6 g of the aqueous extract corresponding to an extraction yield of 6.45% (w/w). The other portion of leaf powder (900 g) was macerated in 6.75 L of methanol for 72 h, filtered and the solvent removed from the extract under reduced pressure, using a Büchi (R-200) rotary evaporator at 65 °C. This gave 63.8 g of the methanol extract; corresponding to a yield of 7.09% (w/w).These solid obtained was stored at 4ºC for subsequent experiments.

*Acute oral toxicity*

Acute toxicity assay was carried out according to the Organization of Economic Co-operation and Development, with slight modification [8]. The overnight fasted (water *ad libitum*) animals were divided into 5 groups of 3 animals for each sexes. Male mice were divided into control (group1) which received distilled water, test group which received by oral route, a unique dose of aqueous extract of 2000 and 5000 mg/kg (group 2-3) and the unique dose of methanol extract of 2000 and 5000 mg/kg (group 4-5). The same repartition was done with the female. Animals were kept under laboratory ambient conditions and neither food nor water was given up to 4 h after extract administration. They were observed for the first critical four hours and daily for fourteen days during which the signs of toxicity was observed like body weight change, behavior and mortality.

*Sub-chronic toxicity*

The repeated doses (28 days) procedure for oral toxicity study was carried out in rats according to the OECD test guideline No. 407.[9] . Sixty rats of both sexes were distributed into 6 groups of 10 rats each (5 males and 5 females) as follows: Group I (Control I; distilled water), Groups II, III and IV (extract: 125, 250 and 500 mg/kg, respectively). They received vehicle or extract daily for 28 days. The remaining 20 rats were distributed into 2 satellite groups (control II; Satellite distilled water and Satellite 500 mg/kg extract) that were equally treated for 28 days, but were left for an observation period of 2 more weeks after treatment withdrawal. Body weight, food and water intake were measured after every two days. The animals were observed daily for any death and abnormal clinical signs during the entire study period. At the end of the treatment period, 40 animals (Control I and Groups II, III & IV) were fasted overnight (water *ad libitum*). On day 29, the animals were weighed, and sacrificed (one at a time) using an overdose of ketamin. Each rat was opened up surgically and blood samples were drawn by cardiac puncture. Blood was collected into tubes with and without ethylene diamine tetra acetic acid (EDTA) for hematological and biochemical analysis, respectively. The internal organs such as liver, kidneys, stomach, spleen, lungs and heart were removed and weighed (paired organs were weighed together) to determine relative organs weights. Liver and kidney tissue samples were rinsed in 0.9% saline and preserved in 3,7% neutral buffered formaldehyde solution for histopathological examination. Fourteen days later, the 20 remaining animals (Satellite Control II & Satellite 500 mg/kg) were sacrificed and submitted to the same procedures as above in order to observe reversibility, persistence or delayed occurrence of toxic effects.

*Body weight, food and water intake*

The variation of body weight of rats had been raised throughout the course of experiment. The weight of food and volume of water consumed by rats in each group were measured after every two days as the

difference between the quantity of food and water supplied and the amount remaining after 48 hours respectively.

*Hematological parameters*

Blood samples were collected into sample tubes containing EDTA. The tubes were shaken gently to mix up the blood with EDTA and prevent clotting. Red blood cell count (RBC), white blood cell count (WBC), platelets, hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) were determined using an automatic analyzer (Roche-Hitachi 902 Routine Chemistry Analyzer).

*Biochemical parameters*

Blood samples collected without anticoagulant were immediately centrifuged at 6000 rpm for 10 minutes to obtain serum for analysis of biochemical parameters. The serum was carefully aspirated with a Pasteur pipette into sample bottles for the various biochemical assays. Serum Glutamic-Pyruvic Transferase (SGPT), Serum Glutamic-OxaloaceticTransferase (SGOT), creatinine, alkaline phosphate (ALP), direct bilirubin, total bilirubin and total protein were evaluated using standard analytical kits obtained from Diagnostics Laboratory of Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD), International Center for Chemical and Biological Sciences.

*Histopathological examination*

Tissue samples of liver and kidneys preserved in 3, 7 % neutral buffered formaldehyde solution were dehydrated using upgraded ethanol series (70 %, 90 % and 100 %) and embedded in paraffin blocks. Thin sections (6 μm) were de-waxed by xylene, rehydrated through a degraded ethanol series (100 %, 90 % and 70 %) and stained with hematoxylin and eosin (H&E). The histopathological examination was performed with an optical microscope and microphotographs of the sections were recorded.

*Statistical analysis*

The statistical analyses were performed by GraphPad Prism software version 5.01 for Windows. All values were expressed as mean ± SEM. Data were subjected to one-way Analysis of variance (ANOVA) test, followed by Tukey's Multiple Comparison Test and two-way ANOVA test followed by Bonferroni's Test to establish the differences between the controls and treated groups. Statistical differences were considered at $p < 0.05$; $p < 0.01$ and $p < 0.001$.

## Results and Discussion

*Acute Toxicity studies in Balb/c mice*

Acute toxicity study is usually the first step in toxicological assessment and the index of acute toxicity is the median lethal dose 50. The limit dose of 2000 and 5000 mg/kg did not cause death or any toxic signs in treated male and female mice. In all animals, we did not observe any changes in feces, general appearance and morphological characteristics (fur, skin, eyes and nose) appeared normal and there were no convulsion, salivation and diarrhea until the end of the 14-day period following single oral administration of all selected doses of aqueous and methanol extracts of *D.thollonii*. No lethargy or sleep was observed during the first four hours of extracts administration. The median lethal dose (LD50) of *D.thollonii* leaves extract of aqueous and methanolic was ≥ 5000 mg/kg (p.o.). The variation of body weight remained constant in all groups treated with aqueous and methanol extract at all selected doses, and there were no significant differences compared with the controls. Macroscopic examination did not reveal any changes in organ condition.

*Sub-chronic Toxicity studies in Wistar rats*

During the 28-day treatment period, no deaths, unusual changes in behavior or in locomotors activity were recorded and no signs of intoxication were observed. No significant alteration of body weight gain was recorded for the rats of groups II and III. However, group IV recorded from day 14 to 28, a significant decrease ($P < 0.05$ to $P < 0.001$) in body weight gained as well as food intake (from day 21 to 28) of male compare to control I ( distilled water). We also observed a significant decrease ($P < 0.01$ to $P < 0.001$) in body weight gained from day 14 to 42 as well as food intake of male satellite 500 mg/kg compare to control II (satellite $dH_20$) (Table 1&2).

We noticed a significant decrease ($P < 0.001$) in water consumption with both sexes for the rats of groups III and VI from day 21 to 28 compare to control I (distilled water), and from day 14 to 42 and 28 to 42, male and female respectively in satellite 500 mg/kg compare to control II satellite $dH_20$ (Table 3). The relative organs weights showed no significant differences between treated and control groups on male and female. However, we noticed a significant difference ($P < 0.05$) in the weight of liver of male rats of group IV when compare to control I distilled water (Fig 1). The macroscopic observation of the target organs (liver, lung, heart, spleen and kidney) of the treated animals did not show significant changes in color and texture when compared with the controls.

In the results of hematological parameters of rats obtain after 28 days of daily treatment with the aqueous of the leaves of *D. thollonii* at a sub-chronic concentration of 125, 250 and 500 mg/kg b.wt, there were no significant changes, except on platelet counts. These hematological parameters reveled that in the extract-treated male rats, there is significant decreased ($P < 0.001$) of platelets at the dose of 500 mg/kg ($1013.5 \pm 3.5 \times 10^9$ (l) vs $1048 \pm 1.0 \times 10^9$ (l)) Compare with control I. However, in female rats, platelet counts significantly decreased ($P < 0.05$) at the dose of 125 mg/kg from $1162.5 \pm 2.5 \times 10^9$ (l) to $1155 \pm 1.0 \times 10^9$ (l) , and increased significantly ($P < 0.001$) at the dose of 250 and 500 mg/kg from $1162.5 \pm 2.5 \times 10^9$ (l) to $1215 \pm 3.0 \times 10^9$ (l) and $1260 \pm 5.0 \times 10^9$ (l) respectively, compare with control I. we also observed increasing of platelets in satellite 500 mg/kg from $1159.5 \pm 4.5 \times 10^9$ (l) to $1274 \pm 4.0$ compare with satellite control II (Table 4).

The summary of the results of the biochemical parameters are shown in table 5. These results revealed in male a significant change ($P < 0.001$) of alkaline phosphate (ALP) at all the doses compare with 28 days control (control I), and a significant increased ($P < 0.01$) of Serum Glutamic-Pyruvic Transferase (SGPT) in satellite 500 mg/kg from $40.5 \pm 0.5$ (U/L) to $55.0 \pm 0.0$ (U/L) compare to satellite control II. However, in female, they were no significant change in all parameters except a significant decreased ($P < 0.01$) of alkaline phosphate (ALP) at the dose of 125 mg/kg compared to the control I.

Histology of the kidneys section in all treated groups did not revealed any structural changes after 28 days of treatment as well as satellite 500mg/kg (after 42 days of treatment), except the dose of 500 mg/kg in male where we observed a slight necrosis. (Fig. 4). However, the histopathological study of the liver presented a vascular congestion of the central vein at a dose of 500 mg/kg and satellite 500 mg/kg in both sexes (Fig. 4 & 5).

**Table 1 : Effects of the aqueous extract on the relative body weight (g) in sub-chronic toxicity**

| | Day 0 | Day 7 | Day 14 | Day 21 | Day 28 | Day 35 | Day 42 |
|---|---|---|---|---|---|---|---|
| **Male** | | | | | | | |
| Control ($dH_20$) | 100.00 ± 0.00 | 105.06 ± 0.03 | 114.62 ± 1.60 | 129.06 ± 2.22 | 138.09 ± 0.01 | - | - |
| Extract (125 mg/kg) | 100.00 ± 0.00 | 99.56 ± 2.23 | 108.53 ± 0.11 | 122.19 ± 1.05 | 132.23 ± 1.73 | - | - |
| Extract (250 mg/kg) | 100.00 ± 0.00 | 105.50 ± 0.92 | 116.06 ± 2.56 | 126.18 ± 3.92 | 133.41 ± 4.74 | - | - |
| Extract (500 mg/kg) | 100.00 ± 0.00 | 102.01 ± 5.23 | 105.24 ± 1.49[a] | 113.69 ± 2.55[c] | 117.88 ± 5.34[c] | - | - |
| Satellite ($dH_20$) | 100.00 ± 0.00 | 103.94 ± 1.06 | 117.69 ± 0.30 | 132.16 ± 0.04 | 144.95 ± 2.45 | 147.42 ± 1.81 | 152.71 ± 0.94 |
| Sat. (Extract 500 mg/kg) | 100.00 ± 0.00 | 100.79 ± 0.39 | 104.36 ± 0.87[γ] | 114.17 ± 0.33[γ] | 122.78 ± 1.26[γ] | 123.69 ± 1.19[γ] | 124.46 ± 3.75[γ] |
| **Female** | | | | | | | |
| Control ($dH_20$) | 100.00 ± 0.00 | 103.73 ± 1.01 | 111.94 ± 1.05 | 117.47 ± 0.64 | 119.44 ± 0.38 | - | - |
| Extract (125 mg/kg) | 100.00 ± 0.00 | 106.17 ± 0.52 | 113.73 ± 1.59 | 116.93 ± 1.73 | 123.80 ± 1.61 | - | - |
| Extract (250 mg/kg) | 100.00 ± 0.00 | 100.89 ± 1.67 | 108.43 ± 0.50 | 113.31 ± 0.68 | 116.01 ± 2.98 | - | - |
| Extract (500 mg/kg) | 100.00 ± 0.00 | 107.54 ± 1.88 | 111.99 ± 2.50 | 116.84 ± 1.39 | 117.12 ± 3.13 | - | - |
| Satellite ($dH_20$) | 100.00 ± 0.00 | 106.11 ± 1.29 | 112.56 ± 2.98 | 116.48 ± 1.94 | 122.40 ± 1.90 | 127.32 ± 0.24 | 133.49 ± 0.44 |
| Sat. (Extract 500 mg/kg) | 100.00 ± 0.00 | 106.22 ± 0.81 | 112.85 ± 0.77 | 119.10 ± 1.57 | 122.21 ± 0.65 | 126.97 ± 1.97 | 129.40 ± 0.31 |

Data are reported as the mean ± S.E.M. for n = 5 per group. ANOVA two way followed by Bonferroni posttest. [a]$p<0.05$; [c]$p<0.001$: significant differences compared with control I (distilled water). [γ]$p<0.001$: significant differences compared with control II (satellite $dH_20$)

**Table 2 : Effects of the aqueous extract on the food intake ( g/100g b.w) in sub-chronic toxicity**

| | Day 0 | Day 7 | Day 14 | Day 21 | Day 28 | Day 35 | Day 42 |
|---|---|---|---|---|---|---|---|
| **Male** | | | | | | | |
| Control ($dH_20$) | 0.00 ± 0.00 | 20.36 ± 0.32 | 20.20 ± 0.37 | 20.47 ± 0.91 | 21.00 ± 0.97 | - | - |
| Extract (125 mg/kg) | 0.00 ± 0.00 | 20.22 ± 0.27 | 17.74 ± 2.52 | 17.16 ± 1.39 | 17.94 ± 2.25 | - | - |
| Extract (250 mg/kg) | 0.00 ± 0.00 | 19.52 ± 0.57 | 18.85 ± 0.03 | 17.19 ± 0.58 | 17.41 ± 0.69 | - | - |
| Extract (500 mg/kg) | 0.00 ± 0.00 | 18.00 ± 1.98 | 17.66 ± 1.01 | 15.47 ± 0.51[b] | 15.44 ± 0.45[b] | - | - |
| Satellite ($dH_20$) | 0.00 ± 0.00 | 19.74 ± 0.26 | 20.13 ± 0.17 | 19.25 ± 0.97 | 20.41 ± 0.58 | 21.83 ± 0.19 | 21.98 ± 0.02 |
| Sat. (Extract 500 mg/kg) | 0.00 ± 0.00 | 17.97 ± 0.46 | 17.23 ± 1.77[β] | 16.20 ± 1.01[β] | 15.22 ± 0.58[γ] | 16.11 ± 0.17[γ] | 16.35 ± 0.39[γ] |
| **Female** | | | | | | | |
| Control ($dH_20$) | 0.00 ± 0.00 | 16.32 ± 0.36 | 16.21 ± 0.14 | 16.06 ± 0.02 | 16.23 ± 0.75 | - | - |
| Extract (125 mg/kg) | 0.00 ± 0.00 | 17.60 ± 0.04[b] | 16.13 ± 0.08 | 16.02 ± 0.02 | 15.92 ± 0.07[a] | - | - |
| Extract (250 mg/kg) | 0.00 ± 0.00 | 16.25 ± 0.22 | 16.25 ± 0.15 | 16.52 ± 0.05 | 15.55 ± 0.04 | - | - |
| Extract (500 mg/kg) | 0.00 ± 0.00 | 16.26 ± 0.03 | 15.75 ± 0.09 | 15.54 ± 0.35 | 15.05 ± 0.19 | - | - |
| Satellite ($dH_20$) | 0.00 ± 0.00 | 16.26 ± 0.41 | 16.50 ± 0.07 | 16.29 ± 0.36 | 15.93 ± 0.40 | 15.44 ± 0.17 | 17.56 ± 0.72 |
| Sat. (Extract 500 mg/kg) | 0.00 ± 0.00 | 15.57 ± 0.39 | 15.47 ± 0.33 | 15.53 ± 0.13 | 15.20 ± 0.01 | 15.44 ± 0.29 | 15.48 ± 0.24[α] |

Data are reported as the mean ± S.E.M. for n = 5 per group. ANOVA two way followed by Bonferroni posttest. [a]$p<0.05$; [b]$p<0.01$: significant differences compared with control I (distilled water). [α]$p<0.05$; [β]$p<0.01$; [γ]$p<0.001$: significant differences compared with control II (satellite $dH_20$)

**Table 3: Effects of the aqueous extract on the water intake (ml/100g b.w) in sub-chronic toxicity**

| | Day 0 | Day 7 | Day 14 | Day 21 | Day 28 | Day 35 | Day 42 |
|---|---|---|---|---|---|---|---|
| **Male** | | | | | | | |
| Control ($dH_20$) | 0.00 ± 0.00 | 28.54 ± 0.33 | 30.08 ± 0.74 | 30.12 ± 0.03 | 30.54 ± 0.09 | - | - |
| Extract (125 mg/kg) | 0.00 ± 0.00 | 29.68 ± 0.01 | 30.34 ± 0.46 | 29.94 ± 0.26 | 28.94 ± 0.16$^{a}$ | - | - |
| Extract (250 mg/kg) | 0.00 ± 0.00 | 29.08 ± 0.02 | 28.73 ± 0.08$^{a}$ | 28.02 ± 0.11$^{c}$ | 26.47 ± 0.41$^{c}$ | - | - |
| Extract (500 mg/kg) | 0.00 ± 0.00 | 29.62 ± 0.05 | 28.94 ± 0.04 | 27.06 ± 0.85$^{c}$ | 24.51 ± 0.26$^{c}$ | - | - |
| Satellite ($dH_20$) | 0.00 ± 0.00 | 29.07 ± 0.64 | 30.67 ± 0.83 | 30.10 ± 0.23 | 29.80 ± 0.32 | 31.10 ± 0.17 | 32.07 ± 0.41 |
| Sat. (Extract 500 mg/kg) | 0.00 ± 0.00 | 29.73 ± 0.21 | 28.39 ± 0.11$^{\gamma}$ | 26.72 ± 0.12$^{\gamma}$ | 24.36 ± 0.03$^{\gamma}$ | 25.79 ± 0.46$^{\gamma}$ | 24.33 ± 0.02$^{\gamma}$ |
| **Female** | | | | | | | |
| Control ($dH_20$) | 0.00 ± 0.00 | 29.05 ± 0.64 | 28.67 ± 0.31 | 29.50 ± 0.47 | 30.85 ± 0.02 | - | - |
| Extract (125 mg/kg) | 0.00 ± 0.00 | 29.90 ± 0.08 | 29.63 ± 0.11$^{a}$ | 29.69 ± 0.01 | 28.41 ± 0.31$^{b}$ | - | - |
| Extract (250 mg/kg) | 0.00 ± 0.00 | 28.09 ± 0.00$^{a}$ | 28.90 ± 0.02 | 26.57 ± 0.46$^{c}$ | 27.03 ± 0.51$^{c}$ | - | - |
| Extract (500 mg/kg) | 0.00 ± 0.00 | 28.76 ± 0.00 | 28.95 ± 0.15 | 26.77 ± 0.38$^{c}$ | 26.69 ± 0.05$^{c}$ | - | - |
| Satellite ($dH_20$) | 0.00 ± 0.00 | 28.86 ± 0.13 | 28.70 ± 0.00 | 28.94 ± 0.08 | 30.48 ± 0.32 | 31.34 ± 0.90 | 30.86 ± 0.12 |
| Sat. (Extract 500 mg/kg) | 0.00 ± 0.00 | 28.46 ± 0.07 | 28.22 ± 0.10 | 26.94 ± 0.34$^{\beta}$ | 26.76 ± 0.06$^{\gamma}$ | 25.50 ± 0.10$^{\gamma}$ | 24.93 ± 0.53$^{\gamma}$ |

Data are reported as the mean ± S.E.M. for n = 5 per group. ANOVA two way followed by Bonferroni posttest. $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$: significant differences compared with control I (distilled water). $^{\beta}p<0.01$; $^{\gamma}p<0.001$: significant differences compared with control II (satellite $dH_20$)

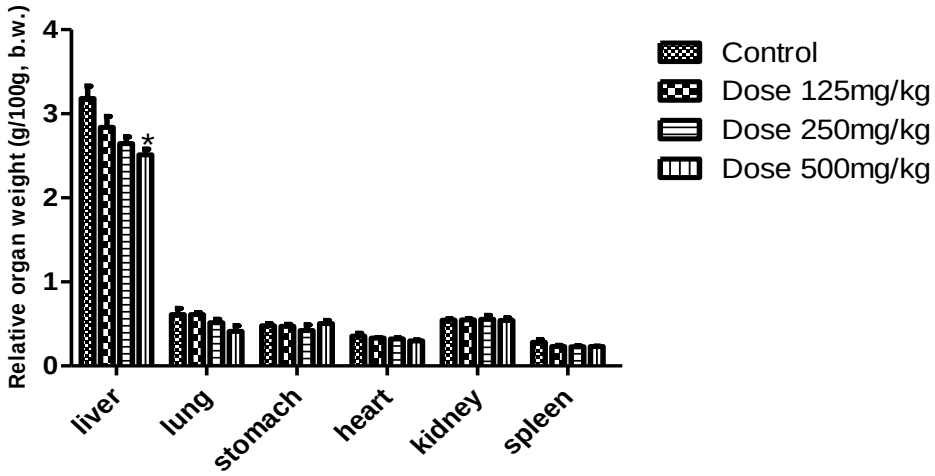


**Figure 1: Effects of the leaves aqueous extract of *D.thollonii* on the relative organ weight (g/100g b.w.) in sub-chronic toxicity on male rat after 28 days of treatment.**

Data are reported as the mean ± S.E.M. for n = 5 per group. ANOVA one way followed by Turkey's posttest. $^{*}p < 0.05$: significant differences compared with control I (distilled water).

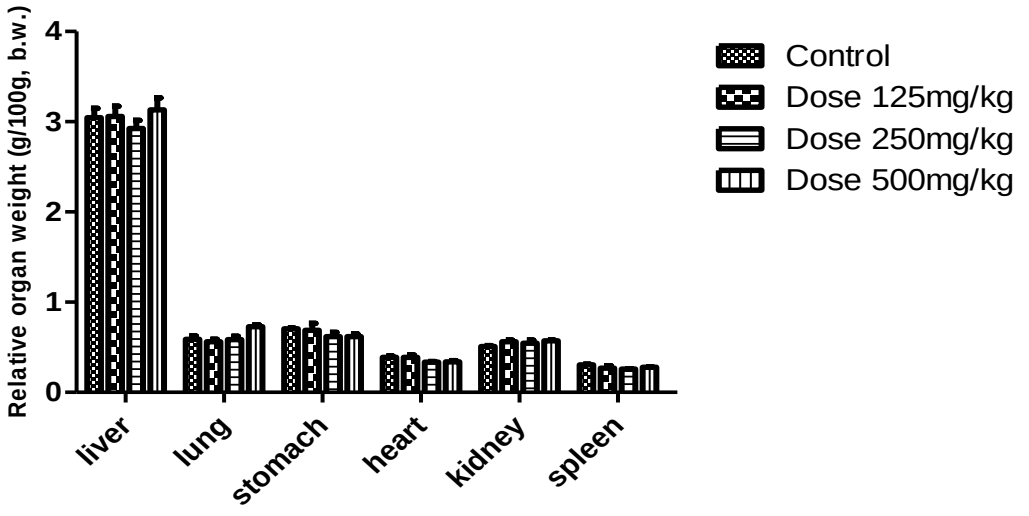


**Figure 2: Effects of the leaves aqueous extract of *D.thollonii* on the relative organ weight (g/100g b.w.) in sub-chronic toxicity on female rat after 28 days of treatment.**

Data are reported as the mean ± S.E.M. for n = 5 per group.

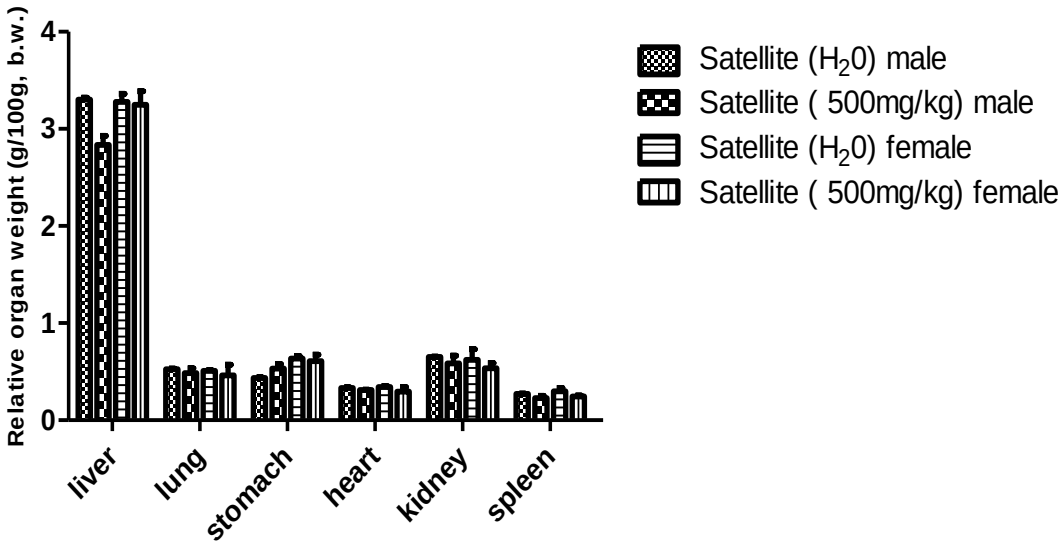


**Figure 3: Effects of the leaves aqueous extract of *D.thollonii* on the relative organ weight (g/100g b.w.) in sub-chronic toxicity on Satellite male and female rat after 42 days of treatment.**

Data are reported as the mean ± S.E.M. for n = 5 per group.

## Table 4. Effects of the extract on biochemical parameters in sub-chronic toxicity

| | Control ($dH_20$) | Extract (125 mg/kg) | Extract (250 mg/kg) | Extract (500 mg/kg) | Satellite ($dH_20$) | Sat. (Extract 500 mg/kg) |
|---|---|---|---|---|---|---|
| **Male** | | | | | | |
| creatinin (mg/dl) | 0.45 ± 0.05 | 0.60 ± 0.00 | 0.90 ± 0.00 | 0.95 ± 0.05 | 0.55 ± 0.01 | 0.57 ± 0.02 |
| ALP (U/L) | 197.5 ± 7.5 | 134.5 ± 4.5$^{c}$ | 135.0 ± 5.0$^{c}$ | 217.5 ± 0.5$^{c}$ | 198.5 ± 1.5 | 204.0 ± 1.0 |
| SGPT (U/L) | 33.5 ± 3.5 | 35.5 ± 2.5 | 32.0 ± 0.0 | 38.0 ± 0.0 | 40.5 ± 0.5 | 55.0 ± 0.0$^{\beta}$ |
| SGOT (U/L) | 141.0 ± 1.0 | 145.0 ± 5.0 | 148.5 ± 8.5 | 151.5 ± 1.5$^{a}$ | 142.5 ± 2.5 | 147.5 ± 7.5 |
| Total Bilirubin (mg/dl) | 0.15 ± 0.01 | 0.16 ± 0.00 | 0.21 ± 0.08 | 0.20 ± 0.01 | 0.11 ± 0.00 | 0.2 ± 0.00 |
| Direct Bilirubin (mg/dl) | 0.04 ± 0.01 | 0.06 ± 0.03 | 0.05 ± 0.03 | 0.06 ± 0.03 | 0.05 ± 0.01 | 0.04 ± 0.00 |
| Total protein (g/dl) | 7.7 ± 0.2 | 7.5 ± 0.0 | 12.5 ± 0.2 | 9.8 ± 0.9 | 7.0 ± 0.5 | 7.47 ± 0.02 |
| **Female** | | | | | | |
| creatinin (mg/dl) | 0.65 ± 0.05 | 0.4 ± 0.2 | 0.8 ± 0.0 | 0.9 ± 0.2 | 0.7 ± 0.1 | 0.65 ± 0.10 |
| ALP (U/L) | 147.5 ± 2.5 | 134.0 ± 4.0$^{b}$ | 146.5 ± 1.5 | 154.0 ± 6.0 | 157.5 ± 2.5 | 152.5 ± 2.5 |
| SGPT (U/L) | 35.0 ± 2.0 | 33.0 ± 3.0 | 45.0 ± 5.0 | 45.0 ± 5.0 | 35.0 ± 0.0 | 45.0 ± 0.0 |
| SGOT (U/L) | 140.0 ± 7.0 | 137.5 ± 2.5 | 145.0 ± 5.0 | 150.0 ± 5.0 | 142.5 ± 2.5 | 147.5 ± 7.7 |
| Total Bilirubin (mg/dl) | 0.21 ± 0.04 | 0.16 ± 0.00 | 0.23 ± 0.07 | 0.19 ± 0.02 | 0.18 ± 0.02 | 0.13 ± 0.02 |
| Direct Bilirubin (mg/dl) | 0.04 ± 0.01 | 0.05 ± 0.02 | 0.03 ± 0.01 | 0.05 ± 0.02 | 0.04 ± 0.01 | 0.04 ± 0.01 |
| Total protein (g/dl) | 8.8 ± 0.5 | 11.7 ± 0.3 | 8.75 ± 0.45 | 7.7 ± 1.0 | 6.7 ± 0.3 | 7.25 ± 0.25 |

Data are reported as the mean ± S.E.M. for n = 5 per group. ANOVA two way followed byBonferroniposttest. $^{a}p<0.05$; $^{b}p<0.01$:$^{c}p<0.001$: significant differences compared with control (distille water).$^{\beta}p<0.01$: significant differences compared with control (satellite

## Table 5. Effects of the extract on hematological parameters in sub-chronic toxicity

| | Control ($dH_20$) | Extract (125 mg/kg) | Extract (250 mg/kg) | Extract (500 mg/kg) | Satellite ($dH_20$) | Sat (Extract 500 mg/kg) |
|---|---|---|---|---|---|---|
| **Male** | | | | | | |
| Haemoglobin (g/dl) | 13.8 ± 0.0 | 14.35 ± 0.35 | 14.40 ± 0.30 | 13.45 ± 0.15 | 13.75 ± 0.15 | 14.1 ± 0.0 |
| RBC (million/µl) | 7.66 ± 0.34 | 8.11 ± 0.25 | 7.73 ± 0.36 | 7.44 ± 0.23 | 8.0 ± 0.01 | 8.09 ± 0.30 |
| Hematocrit (%) | 47.1 ± 1.5 | 50.65 ± 1.35 | 48.75 ± 1.85 | 45.90 ± 0.70 | 48.55 ± 0.15 | 47.45 ± 0.15 |
| MCV (fl) | 61.5 ± 0.8 | 62.4 ± 0.3 | 63.05 ± 0.55 | 61.7 ± 1.0 | 54.65 ± 0.05 | 58.7 ± 2.0 |
| MCH (pg) | 18.05 ± 0.85 | 17.7 ± 0.1 | 18.6 ± 0.5 | 18.1 ± 0.8 | 16.05 ± 0.05 | 17.45 ± 0.65 |
| MCHC (g/dl) | 29.35 ± 0.95 | 28.35 ± 0.05 | 29.6 ± 0.5 | 29.3 ± 0.8 | 29.2 ± 0.1 | 29.7 ± 0.1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| WBC x$10^9$ (l) | 5.7 ± 0.9 | 9.1 ± 0.8 | 7.0 ± 2.3 | 8.05 ± 0.05 | 6.4 ± 0.1 | 4.45 ± 0.35 |
| Platelet x $10^9$ (l) | 1048.0 ± 1.0 | 1053.0 ± 2.5 | 1055.5 ± 6.5[a] | 1013.5 ± 3.5[c] | 1051.0 ± 1.0 | 1052.0 ± 4.0 |
| **Female** | | | | | | |
| Haemoglobin (g/dl) | 12.95 ± 0.05 | 13.15 ± 0.95 | 13.65 ± 0.15 | 13.5 ± 0.1 | 12.95 ± 0.75 | 13.85 ± 0.05 |
| RBC (million/µl) | 6.55 ± 0.21 | 7.01 ± 0.49 | 7.39 ± 0.23 | 7.45 ± 0.01 | 7.07 ± 0.23 | 6.22 ± 1.26 |
| Hematocrit (%) | 41.35 ± 1.05 | 44.7 ± 4.4 | 43.5 ± 0.1 | 45.35 ± 0.05 | 41.85 ± 2.05 | 45.6 ± 1.1 |
| MCV (fl) | 63.15 ± 0.45 | 63.6 ± 1.8 | 58.9 ± 7.0 | 60.85 ± 0.05 | 59.15 ± 0.95 | 59.05 ± 0.35 |
| MCH (pg) | 19.75 ± 0.55 | 18.75 ± 0.05 | 18.5 ± 0.4 | 18.0 ± 0.0 | 18.30 ± 0.50 | 17.95 ± 0.45 |
| MCHC (g/dl) | 31.35 ± 0.65 | 29.5 ± 0.8 | 31.4 ± 0.3 | 30.0 ± 0.5 | 30.95 ± 0.25 | 30.4 ± 0.6 |
| WBC x$10^9$ (l) | 5.75 ± 1.45 | 5.5 ± 2.1 | 6.5 ± 0.9 | 6.05 ± 0.55 | 5.85 ± 1.75 | 6.45 ± 1.05 |
| Platelet x $10^9$ (l) | 1162.5 ± 2.5 | 1155.0 ± 1.0[a] | 1215.0 ± 3.0[c] | 1260.0 ± 5.0[c] | 1159.5 ± 4.5 | 1274.0 ± 4.0[γ] |

Data are reported as the mean ± S.E.M. for n = 5 per group. ANOVA two way followed by Bonferroni posttest. [a]p<0.05; [c]p<0.001: significant differences compared with control I (distilled water). [γ]p <0.001: significant differences compared with control II (satellite $dH_20$)

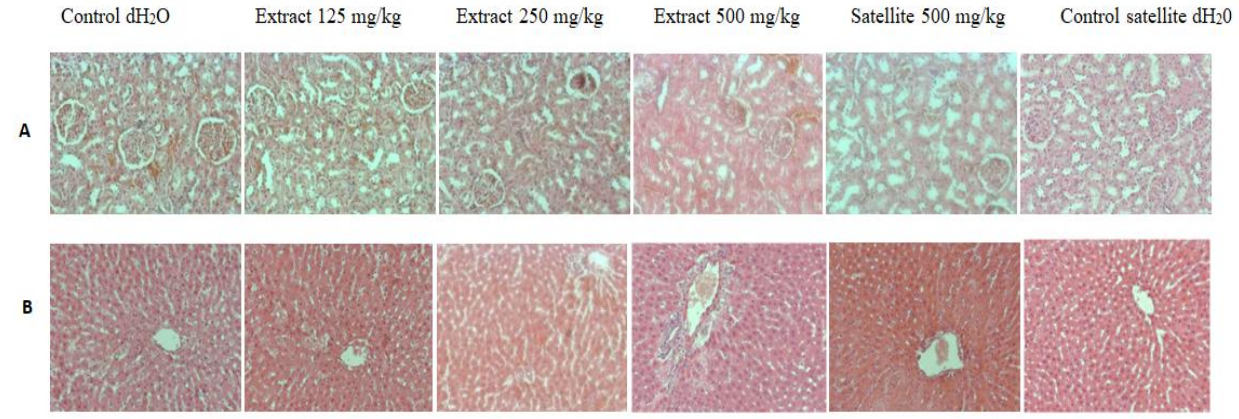


**Figure 4. Effects of the extract on histology of kidneys (A) and liver (B) in male rats (H&E × 200).**

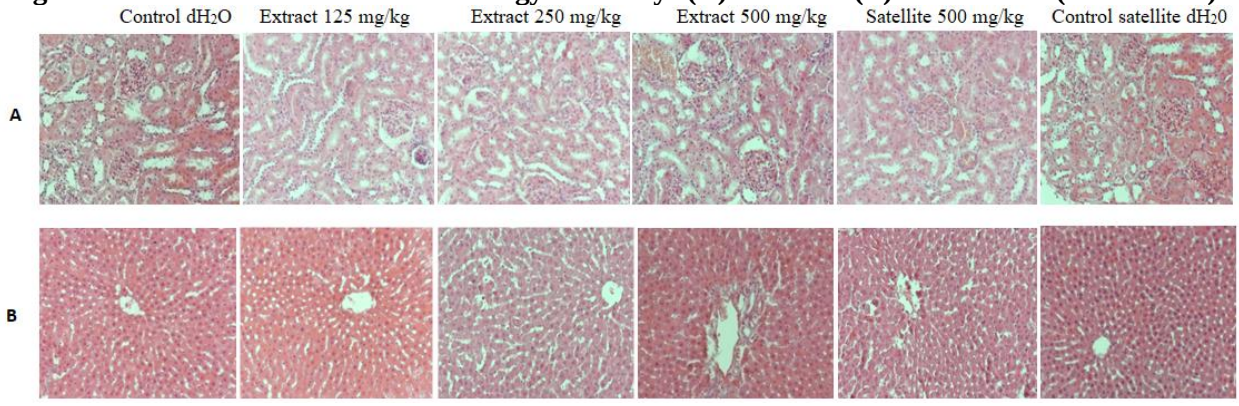


**Figure 5. Effects of the extract on histology of kidneys (A) and liver (B) in female rats (H&E × 200).**

The results of the acute toxicity study demonstrated that aqueous extract of *D. thollonii* administered by oral route up to the highest dose 5000 mg/kg did not produce death or any sign of toxicity in the mice indicating that the LD50 of the extract is greater than 5000 mg/kg of body weight. Therefore it may be considered to be substantially safe if incorporated into an oral administration for the treatment of diseases. According to [8], substances with LD50 values higher than 5000 mg/kg, ingested by oral route, are regarded as being relatively safe or practically nontoxic and does not probably contain toxic compounds. In line with the chemical labeling and classification of acute systemic toxicity, the aqueous extract of *D. Thollonii* can be assigned to the lowest toxicity class (class 5; no label; unclassified) [9].

The sub-chronic toxicity study was carried out for evaluation of long-term effects of the orally administration of *D.thollonii* at doses of 125, 250 and 500 mg/kg. This study did not present mortality, alterations in animal behavior, no noticeable changes were observed in body weight and food consumption of rats. The body weight change serves as a sensitive indication of the general health status of animals [10]. However, loss in body weight has been used as an indicator of adverse effects of drugs and chemicals [11]. Significant differences in body weight as well as food intake were noted only in the male (satellite controls 500 mg/kg and dose 500 mg/kg) which loss body weight from weeks 3 to 6 compared with the control distilled water and satellite control distilled water. This reduction of food consumption and loss of body weight could be suggested that the extract of *D.thollonii* have the capacity to reduced appetite. Another important index of toxicity is the changes in organ weights which are readily determined in long term toxicity tests. There is a possibility that herbal products, when ingested into the body may be toxic to important organs such as the kidneys, liver, spleen, stomach, and lungs because of their diverse roles in the human body [12]. Macroscopic observation of these organs showed no abnormalities in morphology and appearance in the rats treated for 28 days with the extract.

Since blood is the main carrier of substances in the body, its components are very sensitive to toxins and, so, hematological parameters represent an important clinical response to toxic compounds[13]. In our experiments, all the differences reported were within physiological limits found in the literature[14, 15] except in platelets. The significant decrease of platelet counts in male rats at the dose of 500 mg/kg may lead to clinical implications such as high risk of hemorrhage due to thrombocytopenia. Platelets play an essential role in blood clotting that takes place in plasma following the rupture of blood vessel or lesion of their epithelium. Some medicines such as diuretics, ibuprofen, aspirin and chloramphenicol can slow the production of platelets [16]. This reduction of platelets may be attributed to the presence of saponins and cardiac glycosides in the extract [17, 18].

It is also known that the formation of platelets is controlled by a hormone called thrombopoietin [19]. So, significant increase in platelet level at dosage 250 mg/kg, 500 mg/kg and satellite 500 mg/kg in female compared to controls could be due to the fact that leaf aqueous extract of *D.thollonii* would stimulate the production of thrombopoeitin.

Serum biochemical analyses were carried out to evaluate the effect of the extract on hepatic and renal functions. AST and ALT are considered sensitive markers of hepato-cellular toxicity and its increased activity indicate liver damage [20]. Analysis of some basic liver enzymes activities such as ALAT and ASAT in the plasma or serum can be used to indirectly assess the integrity of tissues after being exposed to certain

pharmacological agents [21].Theses enzymes are releases into circulation when there is some damage or Necrosis. So, they can be measured in the serum. ASAT appears in higher concentrations in the tissues like liver, kidneys, heart and pancreas and is released slowly in comparison to ALAT [12]. This enzyme is considered a more sensitive marker of liver inflammation or damage than ASAT and within limits can provide a quantitative assessment of the degree of damage sustained by the liver [22].

Our study revealed that in sub-chronic treatment with *D. thollonii*, there is an increased of SGOT (500 mg/kg) and SGPT (satellite 500 mg/kg) in male compare with controls (distilled water). However, we observed a significant decreased (125 and 250 mg/kg) and increased (500 mg/kg) of ALP in males rats, while female rats presented a significant decreased at 125 mg/kg. In all other parameters, there is no significant difference. The slight increase in the SGOT (500 mg/kg) and SGPT (satellite 500 mg/kg) activities in male rats could suggests that the extract would have nevertheless caused a cytolysis of a certain proportion of hepatic tissue.

Creatinine is mostly derived from endogenous sources by tissue creatine breakdown. The plasma creatinine concentrations in normal individuals are usually affected by a number of factors such as the muscle mass and high protein diet. Creatinine serves as a good indicator of the renal function [22].Any rise in the blood creatinine levels is only observed if there is marked damage of functional nephrons [23]. The leaves of *D.thollonii* did not change significantly the serum creatinine in this study.

Bilirubin is formed by the breakdown of hemoglobin in the liver, spleen and bone marrow. Levels of serum bilirubin reflect the liver's ability to take up, process, and secrete bilirubin into the bile[12]. An increase in tissue or serum bilirubin concentrations occurs as a result of increased breakdown of RBC (hemolysis) or liver damage e.g., hepatitis or bile duct obstruction [24].In our study, there is no significant difference in Bilirubin with all treated groups compare to controls (distilled water)

Tissue protein rate is one of the most widely used means of measuring hepatocellular injury. The measurement of these proteins can reflect nutritional status and may be used to screen for and help diagnose kidney disease, liver disease, and many other conditions. Low levels of protein can suggest a liver disorder, a kidney disorder, or a disorder in which protein is not digested or absorbed properly. High total protein levels may be seen with chronic inflammation or liver infections[12]. in treated groups, total protein were similar to the control groups suggesting that the extract of *D.thollonii* would not have impeded on these organs, which corroborate with the absence of significant variation of relative organ weight of kidney, but in contrary with organ weight of liver of male rats which present a significant decreased at the dose of 500 mg/kg compare to control I (distilled water). This result can be due to the fact that, extract at higher dose causes a slight damage at certain point of liver, but, without any perturbation in the digestion and absorption of proteins. So, histopathological examination of the sub-chronic study showed that the extract did not cause liver and kidney damage in female, but slight damage in male at the dose of 500 mg/kg. Liver is the main organ responsible for the biotransformation of xenobiotics in the organism and, therefore, is one of the major targets of the adverse effects caused by toxic substances.

Histopathological condition of the liver can be characterized by a congestion and dilation of central vein filled with blood, but also by the rupture of blood vessel or lesion of their epithelium. This slight lesion and obstruction

by clotting blood of central veins could be due to a slowing down action of the extract on venous return which would have induced an accumulation of blood in the central vein. This could be justifying by the decrease of weight of the liver and platelets in male rats at the higher dose (500 mg/kg). However, reversibility was observed with satellite 500 mg/kg. According to [25] , the reversibility observed in the liver damage suggests that the plant extract is considered to be non toxic. In the same way, histopathological examination of kidney sections showed that high doses of extract (500 mg/kg) produced slight epithelial cell necrosis associated with the presence of small injury. Generally in toxic injury, renal epithelial cell necrosis is accompanied by massive tissue damage leading to rapid collapse of internal homeostasis of the cell characterized by cell swelling, loss of plasma membrane integrity, major changes to organelles, and swelling of the nucleus [12]. Glomeruli play important functions in the maintaining of homeostasis. These include removal of macromolecules from circulation, plasma ultrafiltration, regulation of blood pressure and tubular metabolism. Many drugs can induce glomerular damage by a variety of pathogenic mechanisms which in turn, can lead to a loss of nephron function and significantly affect blood flow to other nephron segments [26]. Creatinine is considered to be effective markers of kidney function and the increase of these parameters may be associated with renal damage [27]. The results of this work showed normal cortical architecture with normal glomerulus in all treated groups when compared with the controls, except slight epithelial cell necrosis associated with the presence of small injury observed in male treated at dose of 500 mg/kg. The presence of these small injuries in the kidney of males at the higher dose does not indicate kidney dysfunction, since the satellite 500 mg/kg did not present any alterations.

The establishment of a satellite group is essential in order to observe reversibility, persistence, or delayed occurrence of toxic effects related to the substance tested [28] . In this study, we observed constancy decreased in water intake in both sexes, but the reversibility was observed in body weight and food consumption. There was no statistical difference in any biochemical and hematological parameters between the treated animals when compared to the satellite control, except the slight increased of SGPT in male rats and platelets in female rats. The absence of any toxicity sign in the satellite group at the end of the 42-day treatment period indicates that the aqueous extract of *D. thollonii* did not show any delayed toxic effect in animals tested.

## Conclusion

Our results demonstrate that the aqueous extract of *D. thollonii* leaves can be considered safe, as it did not cause either lethality or adverse changes in general behavior when administered orally to male and female mice in acute toxicity study up to the dose of 5000 mg/kg. The middle dose used in our study (250 mg/kg) which is the dose used in traditional medicine through oral route had not affected biochemical, hematological and histopathological parameters. This may justify the use by traditional healers and provide supportive evidence of the safety of aqueous extract of *D. thollonii* leaves. Nevertheless, in these parameters, slight abnormalities were found with the dose of 500 mg/kg. So, caution should be exercised in its use especially at high doses. However, clinical studies of this plant are required with a view to further studies.

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