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Assessment of Hematological, Biochemical and Histopathological Effects of Acute and Sub-chronic Administration of the Aqueous Leaves Extract of *Thymus schimperi* in Rats

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Abstract

Background: *Thymus* species are widely used herbal medicinal plants for various ailments throughout the developing world *Thymus schimperi*. *T. schimperi* is one of the species that is used as spices and traditional medicine for various ailments in Ethiopia.

Objective: This study was designed to assess the acute and subchronic toxic effects of the aqueous leaves extract of *T. schimperi* on blood parameters, histopathology and biochemicals of liver and kidney of rats.

Methods: The aqueous leaves extract of *T. schimperi* was tested for toxicity study. Wistar rats were randomly divided into control and treatment groups. The doses for acute toxicity study were single doses of 300, 2000, 5000 and 10,000 mg/kg body weight of animal. Whereas, subchronic toxicity study daily administration of doses of 200 and 600 mg/kg of the aqueous extract were used for 90 consecutive days. Biomarkers and hematological parameters, microscopic examination of liver and kidney tissue and body weight of rats were evaluated following the test period besides recording the signs of toxicity.

Results: Acute toxicity study did not reveal any signs of toxicity; hence the LD_{50} was higher than 10,000 mg/kg. There was no significant change ($p>0.05$) in general body weight and most of evaluated hematological and biochemical parameters after 90 days of sub-chronic treatment. The kidneys and liver of treatment group appear normal in their texture, shape, size or color compared to the control in gross and histopathological examination. However, the light microscopic examination reveals that there was localized mononuclear lymphocytic infiltration and mild blood congestion within the hepatic portal and central veins in liver at higher dose (600 mg/kg).

Conclusion: The findings revealed that the aqueous leaves extract of *T. schimperi* relatively nontoxic in rats.

Keywords: *Thymus schimperi*; Aqueous extract; Acute toxicity; Sub-chronic toxicity; Biochemicals; Hematology; Histopathology

Introduction

Herbal remedies and alternative medicines are used throughout the world and in the past herbs often represented the original sources of most drugs [1]. Herbal-derived substances remain the basis for a large proportion of the commercial medications used today for the treatment of heart disease, high blood pressure, pain, asthma and other illnesses [2]. Today a great number of modern drugs are still derived from natural sources, and 25% of all prescriptions contain one or more active ingredients from plants [2].

Because herbs are plants, they are often perceived as "natural" and therefore safe [3]. However, as recent reports have shown, in addition to the many benefits there are also risks associated with the different types of Traditional Medicine/Complementary and Alternative Medicine. Although consumers today have widespread access to various TM/CAM treatments and therapies, they often do not have enough information on what to check when using them in order to avoid unnecessary harm [4].

Traditional herbal products are heterogeneous in nature. Side effects are caused from biologically active constituents from herbs, contaminants, and herb-drug interactions. A common toxicity to herbal medicines involves pyrrolizidine alkaloids, which are complex molecules found in certain plants that may be used or inadvertently added to herbal medicines (including comfrey, which is still available in the United States). These alkaloids produce hepatotoxicity through a characteristic veno-occlusive disease that may be rapidly progressive and fatal [5].

Thymus species are among the medicinal plants commonly used in Ethiopia. The genius Thymus includes about 350 species worldwide and is distributed widely in temperate zones [6]. Many species of Thymus yield the commercially important thyme oil, which exhibits

highly antimicrobial effect [7]. Among the various species *T. schimperi* and *T. serrulatus*, are indigenous to Ethiopia locally known as 'Tosign'. The leaves of Thymus are used in Ethiopia as spices to flavor a wide range of food products as well as medicines [8].

Thymus species are one of the widely used herbal medicinal plants for treatment of renal diseases, hypertension, inflammation, infections, pain, to wash skin and used as mouth wash in Ethiopia [9-12]. The volatile oil from thyme was found to contain p-cymene, γ-terpine, carvacrol, rosmarinic acid, eugenol and thymol [8,10]. The volatile oil not only has carminative action, but also antiseptic, antimicrobial and antifungal activities [13]. Thyme is prepared as infusion to treat spasmodic cough, laryngitis, bronchitis urinary infections, renal diseases, hypertension, and Tinea capitis [9]. It is also used as a decongestant, to reduce flatulence and to fight parasites. External uses of thyme include preparations to wash skin wounds or infections [12]. In the Ethiopian traditional medicine the plant has many medicinal applications. Some of the reported applications are for the treatment of gonorrhea, cough, inflammation, spasm, thrombosis, mental illness, eye disease, toothache, urinary retention, stomach problems, leprosy, lung TB, acne and ascariasis [13].

The fresh or dried leaves of both species are used locally as condiments in the preparation of stew, bread and tea. Thyme is often used to flavor meats, soups and stews. It is often used as a primary flavor with lamb, tomatoes and eggs. Even though it's flavorful, but it does not overpower and blends well with other herbs and spices. Thymus contains about 2.5% but not less than 1.0% of volatile oil. The composition of the volatile oil fluctuates depending on the chemotype under consideration. The principal components of Thymus are thymol and carvacrol (up to 64% of oil), along with linalool, p-cymol, cymene, hymene, α- pinene, apigenin, luteolin, and 6-hydroxyluteolin glycosides, as well as di-, tri- and tetra-methoxylated flavones [11].

The rationale of conducting this study is that some herbs that we are using for any purposes may contain harmful chemicals that may cause serious side effects to the host system [14]. *T. schimperi*, have been used by the community for many years for a dietary and medicinal purpose without investigation of the safety of the plant. Therefore, it is necessary to investigate the toxicity of local medicinal plants which are employed by herbalists or communities for therapeutic or diagnosis purposes by gross observation and histopathological examination of liver and kidney. The liver and kidney are major organs of early screening efforts in the preclinical research and a major target organ in the repeated-dose non-clinical safety studies used to support clinical trials [15]. Therefore, the aim of conducting this study was to investigate the toxic effects of using *T. Schimperi* that is used as spices and traditional medicine for various ailments.

Material and Methods

Plant material collection and extraction

The fresh leaves of *Thymus schimperi* were collected from south eastern Ethiopia around Dinsho, about 400 km far from Addis Ababa on March 2014. The plant material was authenticated by a botanist in the Ethiopian Public Health Institute and a voucher number HH-001 was deposited in the herbarium for future reference.

About 520 g of the powdered leaves of *T. schimperi* were macerated with distilled water for 2 hours with intermittent agitation by orbital shaker. Then, the supernatant part of agitated materials were decanted and filtered with 0.1 mm^2 mesh gauze from the undissolved portion of the plants. The filtrates of the plants were freeze-dried at lower temperature and reduced pressure, and then lyophilized to form crude extract. A yield of 60.2 g (11.57% w/w) of *T. schimperi* was obtained. Then it was kept in a desiccator at room temperature until used.

Preparation of experimental animals

For this study male and female healthy Wistar rats of 9 to 10 weeks of age obtained from the animal breeding unit of Ethiopian Public Health Institute were employed. Females were nulliparous and non-pregnant.

Animals of the same sex were grouped into experimental and control groups in a standard cages with six animals per group (n=6) and were kept under standard conditions (at a temperature of 20°C (± 2°C), with natural 12 hrs light/12 hrs dark cycle). For feeding, conventional rodent laboratory diets was used with an unlimited supply of drinking water. The animals were acclimatized to laboratory conditions for one week prior to the experiment to alleviate any non-specific stress [16].

Method of extract administration

Each group of animal was given different doses of aqueous leaf extracts of *T. schimperi* orally using intra-gastric catheter. For acute toxicity study, the extracts were given once after the animals were fasted of food for 18 hours with a free access for water. After the period of fasting, the animals were weighed and the dose was calculated according to the body weight, then the test substance was administered accordingly [17]. For the sub-chronic toxicity study, the animals were given the aqueous leaves extract of *T. schimperi* for 90 consecutive days. Whereas, animals in the control group were given distilled water. All equipment used were cleaned and placed in an oven after each administration to prevent any contamination.

Acute toxicity study

The lethal dose for fifty percent (LD_{50}) for aqueous leaf extracts of *T. schimperi* was determined using female Wistar rats according OECD protocol [16]. Total of five animals (n=5) per group were used for each dose level investigated. Four test and one control groups were used for the study. The time interval between dosing of each animal at each level was determined by the onset, duration, and severity of toxic signs. A period of 24 hours was allowed between the dosing of each animal. All animals of each level were observed for 14 days. All experimental animals were observed closely for any acute toxicity responses. Treatment of animals at the next dose was given after the previously dosed animals survive. A period of 3 days was allowed between dosing at each dose. According to fixed dose test, the selected starting test dose was 300 mg/kg, because of no available evidences of the toxicity of the plant. The following doses, 300 mg/kg for group-1, 2000 mg/kg for group-2, 5000 mg/kg for group-3, and 10,000 mg/kg for group-4 were given for the experimental animals. The animals in the control group were received distilled water.

Sub-chronic toxicity study

The study was carried out by using 30 Wistar rats (15 male and 15 female). The rats were randomly grouped in to two experimental and one control groups, containing 10 rats per group (5 male and 5 female in separate cage). Animals of different sexes were placed in the separate cages.

The animals in the experimental group were treated with the aqueous leaves extract of *T. schimperi* at doses of 200 mg/Kg and 600 mg/kg with the intervals of 24 hours for 90 days. All animals have a free access to determined standard pellet and tap water. All groups were closely observed for any physical, food intake, behavioral alterations and signs of abnormalities throughout the study.

Body weight measurement

Body weight of all experimental animals was taken by using digital electronic balance before commencing the first oral administration and then weekly till last day of oral administration of the extract.

Cage side observations

For the acute toxicity study animals were observed individually at least once during the first 30 minutes after dosing and periodically during the first 24 hours (with special attention given during the first 4 hours [16]. Whereas, for sub-chronic toxic study, animals were observed daily in group immediately before and after administration of extract.

Blood collection

At the end of the experiment, all experimental animals were fasted overnight, cervically dislocated, and blood samples were collected by cardiac puncture into tube with anticoagulant ethylene di-amine tetra acetic acid (EDTA) for hematology and into a tube without anticoagulant for blood chemistry. Blood samples in test tubes containing EDTA were immediately processed for hematological parameters using Automated Hematological Analyzer, SYSMEX XT-1800i (SYSMEX CORPORATION, Japan). White blood cell count (WBC), red blood cell count (RBC), the hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count (PLC) were determined. For biochemical analysis, the blood samples in the plain test tubes were allowed to stand for 3 hours for complete clotting and then centrifuged at 5000 rpm for 15 minutes using a bench top centrifuge (HUMAX-K, HUMAN-GmbH, Germany). The plasma was withdrawn and transferred into other clean vials. The sera were kept at -20°C until analysis for clinical biochemistry measurements. The concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, albumin and creatinine were automatically determined using COBAS INTEGRA-400 plus Analyzer (ROCHE DIAGNOSTICS, Japan).

Organs weight measurements and tissue sample

All experimental animals were sacrificed at the end of 90 days treatment after body weight had been measured. Then the animals were dissected and the target organs in the study were removed. After the organs has been removed from the body they were kept in 1% normal saline for a few minutes to clean off any extraneous tissues, further the organs were weighted with the precision balance; and tissue samples were taken from liver and kidney. Sample tissues were placed in a test tube containing 10% buffered formalin for 24 hours and thoroughly rinsed over running tap water overnight. Then the fixed tissues were dehydrated and cleared in a graded series of ethanol and xylene respectively. Then the tissues were infiltrated with molten paraffin wax and embedded in paraffin blocks. The blocks were sectioned at thickness of 5-6 µm using Leica rotary microtome (LEICA RM 2125 RT, China, checked in Germany). Ribbons of the tissue sections were gently collected using a forceps and laid onto the surface of a water bath heated at 30-40°C. After the sections were thoroughly spread on the water bath, they were placed over tissue glass slides. The slides were then arranged in slide racks and placed in an oven at a temperature of 20-40°C overnight to facilitate the fixation of the specimens onto the glass slides. The thin sections then were undergoes through different stages of xylene and alcohol, being stained with heamatoxylin and eosin [18].

Light microscopy and photomicrography

Stained tissue sections of the liver and kidney were carefully examined under binocular compound light microscope (OLYMPUS CX41, Japan). Tissue sections from the treated groups were examined for any evidence of histopathologic changes with respect to those of the controls. After examination, photomicrograph of selected slides from both the treated and control groups were taken under a magnification of x40 and x20 objective by using (EVOS XI, China) automated built-in digital photo camera.

Data processing and analysis

All data which are represented by numbers were packed and analyzed by SPSS statistical software. All values of parameters were expressed in mean ± SEM (standard error of mean). Treatment over time were compared between control and treated groups by using one-way analysis of variance (ANOVA), followed by Dunnett's t-test to determine their level of significance. Differences at $p<0.05$ were considered statistically significant.

Ethical consideration

The study was conducted after the ethical and clearance letter was obtained from Ethiopian Public Health Institute. Animals those were used in this study were kept from any unnecessary painful and terrifying situations and handled humanely throughout the study period [16].

Results

Acute toxicity

Intragastric administration of extract at different doses of 300, 2000, 5000, and 10,000 mg/kg did not produce any sign of morbidity and mortality in female animals during the period of experiment for acute toxicity. This result indicates that the LD_{50} was above 10,000 mg/kg for the aqueous leaf extract of *T. schimperi*.

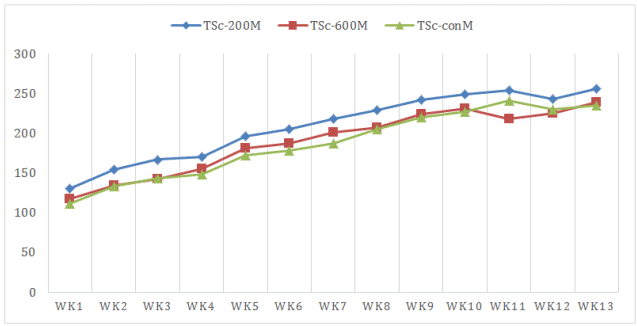
Sub-chronic toxicity

Effects of extract on the body weight: The changes in the mean values of the initial and final body weights of male rats treated with 200 mg/kg and 600 mg/kg of aqueous extract of *T. schimperi* is displayed in Table 1.

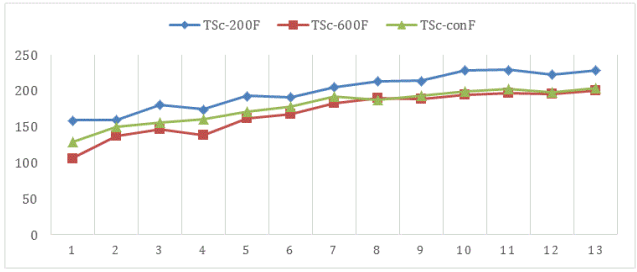
The progressive body weight gains were recorded in nearly all groups of rat with time over the period of the experiment. In respect to male rats exposed to *T. schimperi* at the dose of 200 mg/kg compared to control caused a significant increase ($p<0.05$) in change in mean body weight on the 7th to 10th week and 13th week (Table 1, Graph 1, Graph 2).

Groups	Body weight of male rats (g)			Body weight of female rats (g)		
	Initial	Final	Change	Initial	Final	Change
TS-200 M	130 ± 11.7	255.6 ± 11.01	125.6	158.4 ± 21.3	229.0 ± 9.28	70.6
TS-600 M	117 ± 18.8	239.0 ± 14.3	122	106.4 ± 16.4	200.6 ± 4.41	94.2
TS-conM	111 ± 29.08	235.2 ± 12.63	124.2	129.6 ± 12.5	204.4 ± 14.01	74.8

Table 1: The mean body weight of rats treated with *T. schimperi* at different weeks and different doses. Values are expressed as mean ± SEM; *=p<0.05. TSc-200M: rats that received extract at dose of 200 mg/kg; TSc-600M: rats that received extract at dose of 600 mg/kg; TSc-conM: rats that received distilled water (control group).



Graph 1: Effect of aqueous leaves extract of *T. schimperi* on mean body weight of male rat. The changes in the mean values of the initial and final body weights of female rat treated with 200 and 600 mg/kg of aqueous extract of *T. schimperi* is displayed in Table 1. Concerning female rats, compared to control, *T. schimperi* at the dose of 200 mg/kg caused significant increase (p<0.05, 0.03) in change in body weight on weeks 10th and 11th. At the dose of 600 mg/kg, the herbal remedy does not elicit any significant effect.



Graph 2: Effect of aqueous leaves extract of *T. schimperi* on mean body weight of female rat of different groups.

Effects of extract on hematological and biochemical parameters

Effect of *T. schimperi* on hematological parameters of female and male rats was listed in Table 2. The aqueous extract of the *T. schimperi* did not produce any significant effect on hematological parameters after the 90 days administration except in respect of basophils count in which there was a significant decrease (p<0.05) in the female group treated at the dose of 200 mg/kg (0.003 ± 0.003 × 10^3/µl) when compared with the group treated with distilled water (0.016 ± 0.002 × 10^3/µl) (Table 2). There was also a significant decrease (p<0.05) in the proportion of basophils in the male groups treated with *T. schimperi* at doses of 200 mg/kg (0.01 ± 0.004 × 10^3/µl) and 600 mg/kg (0.01 ± 0.002) compared to control group (0.02 ± 0.01 × 10^3/µl) (Table 2).

Hematological Parameters	Female rats group			Male rats group		
	TS-200	TS-600	TS-conF	TS-200	TS-600	TS-conF
WBC (x10^3/µL)	3.85 ± 1.29	3.15 ± 0.17	3.648 ± 0 .58	4.07 ± 0.65	5.56 ± 1.14	5.66 ± 0.32
RBC (x10^6/µL)	8.9 ± 0.075	8.79 ± 0.18	8.67 ± 0.13	9.32 ± 0.06	9.7 ± 0.20	9.64 ± 0.33
HGB (g/dL)	17.4 ± 0.49	16.8 ± 0.37	16.72 ± 0.25	17.4 ± 0.22	18.2± 0.35	18.05 ± 0.95
HCT (%)	51.4 ± 0.78	50 ± 1.12	49.42 ± 0.78	52 ± 0.64	54.6 ± 1.04	53.55 ± 2.05
MCV (fL)	57.8 ± 0.61	56.8 ± 0.12	57.02 ± 0.32	55.7 ± 0.35	56.3 ± 0.16	55.55 ± 0.25
MCH (pg)	19.5 ± 0.45	19.1 ± 0.05	19.26 ± 0.08	18.6 ± 0.12	18.8 ± 0.14	18.75 ± 0.35
MCHC (g/dL)	33.8 ± 0.44	33.6 ± 0.05	33.84 ± 0.13	33.4 ± 0.05	33.4 ± 0.25	33.7 ± 0.5
PLT (x10^3/µL)	856 ± 61.86	831± 0.00	772 ± 45.9	804 ± 45.21	716 ± 43.81	839.5 ± 7.5

Table 2: The subchronic effect of aqueous leaves extract of *T. schimperi* on hematological parameters in rats; Values are expressed as mean ± SEM; *=p<0.05.

Effect of *T. schimperi* on serum biochemical parameters of female rat after the 90 days administration is displayed in Table 5. There was a significant decrease (p<0.05) in the concentration of albumin in the female group treated with the extract at the dose of 200 mg/kg (5.02 ± 0.27707 g/dl) compared to the group treated with distilled water (5.75 ± 0.559) (Table 3). On the other side there was also a significant increase (p<0.05) in the concentration of creatinine level in the female groups treated with *T. schimperi* at doses of 200 mg/kg (0.86 ± 0.14) compared to the group treated with distilled water (0.635 ± 0.081).

The concentration of urea were significantly increased (p<0.001) in the female groups that received *T. schimperi* at a dose of 600 mg/kg (44.2 ± 2.28) compared to the group treated with distilled water (32 ± 5.83). Moreover the concentration of other parameters such as ALP, ALT and AST did not show a significant change in the female groups that received *T. schimperi* at a dose of 200 mg/kg and 600 mg/kg compared with the group treated with distilled water (Table 3).

Regarding the male groups that receive the same dose as the female groups the extract did not cause significant effect at all doses compared to control, except for AST level which significantly decrease in the male groups that received *T. schimperi* at doses of 200 mg/kg (216.8 ± 3.85) and 600 mg/kg (227.2 ± 8.51) compared to a group treated with distilled water (520.4 ± 2.55) (Table 4).

	Parameters					
Groups	**Creatinine (mg/dl)**	**Albumin (g/dl)**	**ALP (U/ L)**	**ALT (U/ L)**	**AST (U/L)**	**Urea (mg/dl)**
TSc-200 F	0.86 ± 0.14*	5.02 ± 0.27*	93.4 ± 22.27	84.8 ± 14.86	190 ± 2.98	37.8 ± 2.48
TSc-600 F	0.694 ± 0.083	5.19 ± 0.47	75.6 ±19.14	87.2 ± 7.563	232.6 ± 5.35	44.2 ± 2.28*
TSc-conF	0.635 ± 0.081	5.75 ± 0.56	89.2 ± 9.99	141.4 ± 10.26	335.2 ± 2.14	32 ± 5.831

Table 3: The sub-chronic effect of aqueous leaves extract of *T. schimperi* on serum biochemical parameters of female rats. Values are expressed as mean ± SEM; *=p<0.05.

	Parameters					
Groups	**Creatinine (mg/dl)**	**Albumin (g/dl)**	**ALP (U/ L)**	**ALT (U/ L)**	**AST (U/L)**	**Urea (mg/dl)**
TSc-200 M	0.75 ± 0.03	4.76 ± 0.88	155.2 ± 34.71	99.8 ± 10.26	216.8 ± 3.85*	44.4 ± 15.04
TSc-600 M	0.694 ± 0.11	5.02 ±.55	187 ± 101.80	95.6 ± 11.72	227.2 ± 8.51*	35.6 ±1.14
Tsc-conM	0.76 ± 0.14	4.64 ± 0.21	104.4 ± 44.53	161.2 ± 71.26	520.4 ± 2.55	35.2 ± 2.77

Table 4: The sub-chronic effect of aqueous leaves extract of *T. schimperi* on some serum biochemical parameters of male rats. Values are expressed as mean ± SEM; *=p<0.05.

Effects of extract on the weights of liver and kidney

Effect of aqueous leaf extract of *T. schimperi* did not produce any significant effect on weights of liver and kidneys of rats after the 90 days administration in the group treated with the preparation at the doses of 200 mg/kg and 600 mg/kg compared with the group treated with distilled water. The values are indicated in Table 5.

Groups	**Male organ weight (g)**		**Female organ weight (g)**	
	Liver	**Kidney**	**Liver**	**Kidney**
TS-200	6.78 ± 0.32	0.65 ± 0.07	7.14 ± 0.85	0.66 ± 0.06
TS-600	7.04 ± 0.67	0.612 ± 0.04	6.52 ± 0.37	0.60 ± 0.07
TS-con	6.52 ± 0.76	0.624 ± 0.04	6.52 ± 0.76	0.64 ± 0.04

Table 5: The sub-chronic effect of aqueous leaves extract of *T. schimperi* on the weight of liver and kidneys of male and female rats. Values are expressed as mean ± SEM; *p<0.05.

Gross Pathologic Observations

On the examination of the gross appearance of internal organs of treated rat (Figures 1A-1F), such as liver and kidney did not show any abnormal changes in texture, shape, size or color compared to the control. There was no sign of necrosis or lesion was appreciated on the organs of all treated groups.

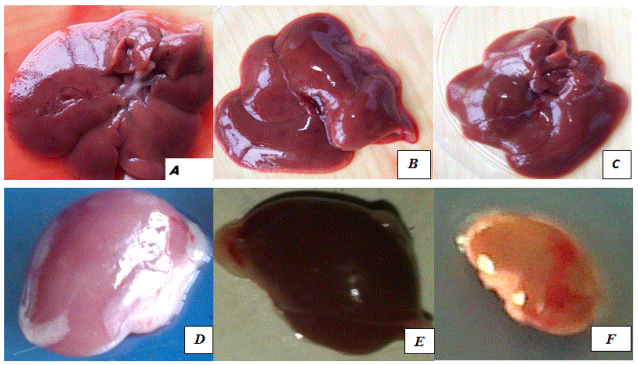


Figure 1: The gross appearance of liver (control rat (A), rat treated with 200 mg/kg (B), and rat treated with 600 mg/kg (C) and of kidney (control rat (D), rat treated with 200mg/kg (E), and rat treated with 600 mg/kg (F).

Light microscopy of liver

The microscopic examination of liver sections of control group rat (Figures 2A and 2B) showed the normal architecture of structural units of the liver, the hepatic lobules, formed by cords of hepatocytes separated by hepatic sinusoids.

There were no significant histopathological presentations observed in the groups treated with distilled water and treatment groups. The liver appeared normal with preserved hepatic architecture, hepatocytes arranged as radial plates, and having eosinophilic cytoplasm and basophilic central nuclei. No cytoplasmic inclusions were seen and no portal inflammation (Figures 2A-2F). Mild blood congestion was observed in the rat liver at dose of 600 mg/kg within the hepatic portal and central veins (Figures 2E and 2F).

Light Microscopy of Kidney

On the microscopic examination of kidney sections of rats there were no adverse histopathological presentations observed in all the treatment groups. Normocellular glomerular tufts were displayed on a background containing tubules. Examination of kidney sections of rat treated with the aqueous extract of *T. schimperi* (Figures 3C-3F) at doses of 200 mg/kg and 600 mg/kg doses indicated no structural difference compared to the control groups (Figures 3A and 3B) rat. The microscopic architecture of sections of kidney in treated groups had a similar appearance to that of the controls in which renal corpuscles maintaining their normal size of urinary space and normal tubular structures are examined. No necrosis was observed.

Discussion

Worldwide, various medicinal plants and botanical drugs have been widely adapted as primary therapeutic agents or supplements for treating various human illnesses [19]. The safety study is accomplished by the implementation of general pre-clinical toxicity experiments to uncover potential poisonous effects of any drug majorly in liver and kidney of animals [20]. If there is mild inflammation and tissue damage to these organs, the permeability of the cell membrane will increase and release cytoplasmic enzymes such as LD, ALP, and AST, while necrosis will release mitochondrial ALT as well as AST leaking

into the blood and increase in levels [21,22]. Toxicity screening models provide important preliminary data to help select natural remedies with potential health beneficial properties for future work [23]. The first toxicity test performed on the formulation was the evaluation of acute toxicity determined from the administration of a single exposure. It was conducted to determine the safety margin of the formulation [19]. Accordingly, the aqueous leaf extracts from the *T. schimperi* did not induce lethality in rats when administered orally up to doses of 10,000 mg/kg. Therefore, this result suggests the LD50 of aqueous extract of *T. schimperi* was estimated to be greater than 10,000 mg/kg because of no morbidity and mortality were recorded up on oral administration of this dose. Hence, the herbal preparation can be said to be safe when orally administered substance does not produce lethality up to 10, 000 mg/kg [24]. Visible signs of acute or delayed toxicity were not observed in acute toxicity study. This result goes in line with the previous study [25,26]. Observations by the naked eye on organs like the liver and kidneys of the treated animals after 2 weeks did not show any gross pathological change such as in color, organ swelling, texture and atrophy or hypertrophy after single administration of the formulation when compared with the control group.

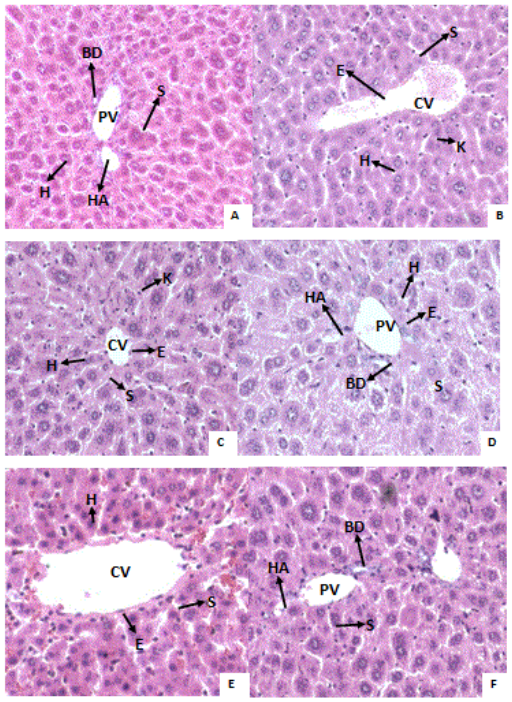


Figure 2: Photomicrographs of liver sections of control group rats (A & B), rats treated with 200 mg/kg (C & D), rats treated with 600 mg/kg body weight/day (E & F) of the aqueous extracts of *T. schimperi*. (CV=Central vein, E=Endothelial cells, PV=Portal vein, BD=Bile duct, H = Hepatocytes, S=Sinusoids, HA=Hepatic artery, K=Kupffer cells, I=Infiltration. (Sections were stained with H and E).

Daily treatment with both doses of the aqueous leaf extracts of *T. schimperi* to male and female Wistar rats for a period of 12 weeks did not show any toxicity related morbidities and mortalities. Hematological parameters were also evaluated to obtain further toxicity related information which is not only detected by direct examination of organs and body weight analysis. Studies on hematological parameters can easily reveal abnormalities in body metabolic processes, and the blood profile usually provides important information on the response of the body to injury or lesion, deprivation and stress [27]. Therefore, the extent of toxic effect of drugs and/or plant extracts can be determined by assessment of hematological parameters [28].

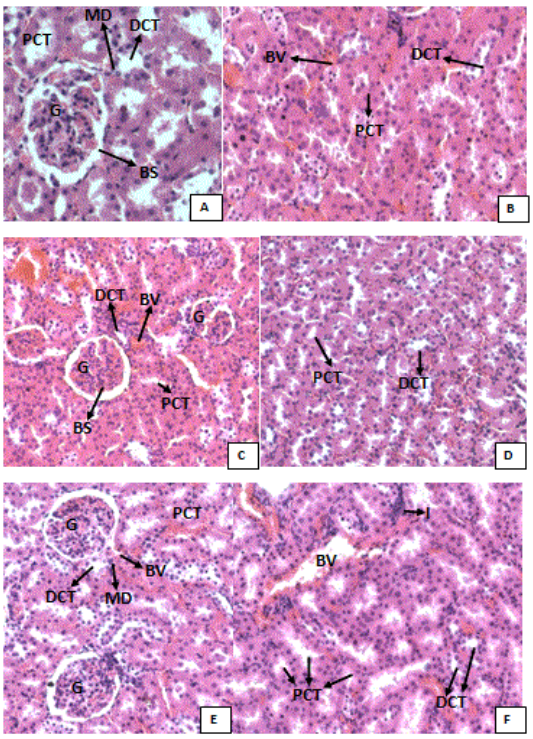


Figure 3: Photomicrographs of kidney sections of control group rats (A & B), rats treated with 200 mg/kg (C & D), Rats treated with 600 mg/kg body weight/day (E&F) of the aqueous leaves extract of *T. schimperi*. G=Glomeruli, BS=Bowman's space, DCT=Distal convoluted tubule, PCT=proximal convoluted tubule, I=Infiltration, MD=Macula densa, BV=Blood vessel. (Sections were stained with H & E).

Red blood indices such as the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) are the most useful indicators in the diagnosis of anemia in most animals [29]. As indicated on (Table 2) the effect of the aqueous leaf extract of *T. schimperi* on MCV, MCH, and MCHC were insignificant in treated group compared to the control. These observations demonstrate that the aqueous extract of the leaves in this study did not cause significant toxic effect on the levels of calculated red blood cell (RBC) indices at both doses. Hence, subchronic treatment with the aqueous leaf extract of *T. schimperi* has shown no effect on the size of RBCs and in hemoglobin weight per RBC in rats. This effect clearly suggested that the aqueous leaf extracts did not cause macrocytic and microcytic anemia [30]. This finding is in agreement with other findings in which the values of the various RBC parameters of extract treated rabbits were found to be comparable with those of the control group [31]. The changes in RBC count,

average hemoglobin (Hgb) and hematocrit (HCT) levels of treated group animals were also insignificant ($p>0.05$) compared with that of vehicle received rats.

In the hematological analysis the white blood cell (WBC) count also was performed. In herbal toxicity studies, increase in WBC may indicate the impact of plant extracts in inducing the immune response of treated animals [32]. On the other hand, significant decrease in the WBC of the blood indicates a decline in the production of leukocytes called leukopenia, means that the body is less able to fight off infections. However, the hematological analysis in this study demonstrated that the estimated total WBC count after sub-chronic administration of the formulation was not significantly changed in response to the administered aqueous leaf extract of *T. schimperi* at doses of 200 mg/kg and 600 mg/kg compared to the control. This result may indicate that the aqueous leaf extract in this study does not possess chemicals capable of inducing leukocytosis, which is an abnormally high number of WBC in the blood circulation or in suppression of normal production of WBC [29]. Also, similar results were obtained by previous study that found no changes in the WBC count with different groups compared with control groups [31].

In platelet count, thrombocytopenia is a condition of abnormally low number of platelets in the circulation, may result from decreased production or increased destruction of platelets [32]. Some drugs provoke platelet antibodies and platelet destruction, resulting in thrombocytopenia [29]. On the other hand, thrombocytosis is an abnormal increase in the number of circulating platelets [33]. However, in this study the change in the platelets count as compared to the control group is insignificant in both treated group of rats. This result suggests that the aqueous leaf extract at both doses used in this study has no effect in inducing neither thrombocytopenia nor thrombocytosis.

Elevated serum levels of enzymes produced by the liver or nitrogenous wastes to be excreted by the kidney might be indications to their spillage into the blood stream as a result of necrosis of the tissues [34]. Because of the liver's strategic location between intestinal tract and rest of body it's the first organ to encounter ingested nutrients, vitamins, metals, drugs, and environmental toxicants as well as waste products of bacteria that enter portal blood [19]. So the liver is a primary destination for any toxic substance entered to the body, especially through gastrointestinal route, the liver suffers first. Because of its wide range of functions, any abnormal change in liver will definitely affect complete metabolism of an animal [35]. Liver chemistry tests include several serum chemistries that reflect liver function. The most commonly used serum liver chemistry tests include serum transaminases (alanine aminotransferase (ALT), aspartate aminotransferase (AST)), serum alkaline phosphatase (ALP), Gamma-glutamyl transpeptidase (GGT), bilirubin and albumin. The major intracellular enzymes of the liver are alanine aminotransferase (ALT) and aspartate aminotransferase (AST). However, injuries of liver cells (hepatocytes) allowing for escape of these enzymes into the bloodstream raises their levels in the blood [36]. The levels of ALP and GGT in the serum are important parameters for evaluation of hepatobiliary route. For hepatocellular evaluation, measurement of a minimum of two scientifically appropriate blood tests is recommended, e.g ALT, AST, sorbitol dehydrogenase, glutamate dehydrogenase, or total bile acids [28].

AST is not specific for liver. AST is also present in red blood cells, cardiac muscle, skeletal muscle, kidney and brain tissue, and may be elevated due to damage to these sources as well. AST is defined as a biochemical marker for the diagnosis of acute myocardial infarction [37]. Therefore, it is an absolute prerequisite not to take in consideration of extra-hepatic tissue damage as a possible source of serum AST when evaluating the enzyme in relation to the liver. Beside this, only about 20% of AST is cytosolic and the rest is found in mitochondria of hepatocytes and other cells [38]. Unlike AST, ALT is fairly specific being found largely in the liver and it is commonly used as a biomarker for liver problems [39]. ALT is purely cytosolic and is more specific for hepatocytes. Elevation of serum levels of both AST and ALT can occur with states of altered hepatocellular membrane permeability. Because ALT is located only in the cytosol, serum levels tend to be relatively higher than AST. Since majority of AST is found within mitochondrial of cells, it is released slowly in comparison to ALT [37]. Hence, serum transaminases, especially ALT, are the most important markers of hepatic injury [39].

In the present study, the toxicological effects of 200 mg/kg and 600 mg/kg body weight doses of the aqueous leaf extracts of *T. schimperi* on liver biochemical parameters were investigated in rats. In liver function test, there were no significant changes in the serum level of ALT and AST in most animal groups treated with both doses of the aqueous leaf extracts of *T. schimperi* in comparison to the control group. The non-significant change of these enzymes between the control and treated group animals after 12 weeks administration indicates that the aqueous leaf extract did not cause adverse toxic effect or hepatic damage on the liver. There was also significant decrement in the level AST was observed in male rats treated at both doses compared with the control group. The result signifies that Thymus species had no liver damage effect on AST. AST levels are usually not as high in chronic liver injury, often less than 4 times the highest normal level [38]. This result was supported by previous study conducted on rats states that administration of 500 mg/kg of the extract of *T. vulgaris* could significantly improve altered enzyme levels as well as the histological architecture of liver [40].

Kidney is a sensitive organ, whose function is known to be aected by a number of factors such as drugs including phytochemicals of plant origin that ultimately lead to renal failure. Kidney function test is a collective term for a variety of individual tests and procedures that can be done to evaluate how well the kidneys are functioning [41]. Accordingly, renal function can be assessed by measuring the levels of plasma creatinine, urea and uric acid concentrations [41]. Assessment of possible renal damage due to aqueous leaf extract of plants in this study was made by assaying plasma urea and creatinine levels [42]. Results show in most of the groups of animals no significant alteration in the blood urea nitrogen (BUN) and creatinine levels due to *T. schimperi* treatment. The female rat treated at 200 mg/kg had shown significant increased creatinine values compared with control group. Even if the values has shown significant increment but still all values are under the normal range (i.e 0.2-0.8 mg/dl) [43].

Histopathological examinations provide information to strengthen the findings on biochemical and heamatological parameters [44]. The present histological examination indicated that liver sections of rats treated with aqueous leaf extract of *T. schimperi* at both 200 mg/kg and 600 mg/kg doses did not show focal necrosis and pyknosis. In addition, the general histological architecture and its functions were not affected in any of the treatment group rats compared to the control. This was strengthened by study done in rats over 8 weeks indicated that the altered renal tissue could be assisted by the aqueous extract of Thyme [45].

Moreover, there were no eect on the levels of AST and ALT, which is considered to be sensitive indicators of hepatocellular damage and within limits, can provide a quantitative evaluation of the degree of damage to the liver [37]. It is reasonable to deduce, therefore, that *T. schimperi* did not induce any damage to the liver or kidneys. This is further confirmed by the histological assessment of these organs [46]. No dierence was observed in the weight and structure of the liver and kidney between the control and the treated groups. In agreement with these results, the findings of another study demonstrated no changes of liver weight were reported in broiler chickens fed 1 g/kg thyme powder [47]. Altogether, the sub-chronic study indicates that *T. schimperi* ingestion did not induce detrimental changes and morphological alterations in these organs.

Conclusion

The acute toxicity test suggests that oral single dose (10,000 mg/kg) administration of *T. schimperi* is practically non-toxic to Wistar rats. The sub-chronic toxicity study suggests that *T. schimperi* are do not caused a significant effect in liver, kidney and blood parameters when administered orally at doses of 200 mg/kg and 600 mg/kg in Wistar rats. Therefore, with respect to results from liver and kidney, the aqueous leaf extract of *T. schimperi* can be considered as relatively nontoxic in rats.

Conflict of interest

The authors declare that they have no competing interests.

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