Journal of Clinical Toxicology
ISSN: 2161-0495

Dires et al., J Clin Toxicol 2018, 8:3
DOI: 10.4172/2161-0495.1000386

Research Article Open Access

# Evaluation of Acute, Sub-acute and Skin Irritation Toxicity on Essential Oil of *Thymus schimperi* in Ankober, North Shewa, Debre Berhan, Ethiopia

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**Received date:** July 02, 2018; **Accepted date:** July 11, 2018; **Published date:** July 16, 2018



## Abstract

**Background:** The extensive uses of plants as medicines have been reported. However, the use of herbal products should be based on scientific origin; otherwise they would be useless and unsafe. The present study aims to evaluate the toxicity profile of oil of *Thymus schimperi*.

**Methods:** For acute and sub-acute toxicity study, albino mice of both sexes were used. Skin irritation test was conducted on wistar rats. For the acute toxicity study, the essential oil was tested at three dose levels (1500, 1750 and 2000 mg/kg). A total of 10 mice were used for each dose level. Sub-acute toxicity study was done at a dose of 1000 mg/kg with 5 mice. For the skin irritation test, the essential oil was tested at two concentrations of 1% and 5% with 10 rats for both concentrations.

**Results:** The results indicated that *T schimperi* oil did not cause any mortality up to the limit doses of 2000 mg/kg. The essential oil did not cause significant weight change ($p>0.05$). The plant also did not cause significant increase in serum enzyme level of the study mice ($p>0.05$). The histopathological examination on liver and kidney showed that plant did not cause major organ damage. Ointment prepared from *T. schimperi* oil did not cause any abnormal skin reaction up on follow up for 14 days post treatment.

**Conclusion:** It is concluded that the tested medicinal plant is safe as per animal study.

**Keywords:** *Thymus Schimperi*; Acute Toxicity; Sub-acute Toxicity

## Introduction

The extensive uses of plants as medicines have been reported and were initially taken in the form of crude drugs. However, the use of herbal products should be based on scientific origin; otherwise they would be useless and unsafe. Furthermore, the irrational use of these herbal products may cause serious toxicity for humans. Unfortunately, many people underestimate the toxicity of natural products and do not realize that these agents could be as toxic or more than synthetic drugs [1].

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. Many species of Thymus yield the commercially important thyme oil, which exhibits highly antimicrobial effect [2]. 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 [3].

*T. schimperi* is one of the most widely used herbal medicinal plants for treatment of renal diseases, hypertension, inflammation, infections, pain, to wash skin and used as mouth wash in Ethiopia [4]. The volatile oil from thyme was found to contain p-cymene, γ-terpine, carvacrol, rosmarinic acid, eugenol and thymol [5].

The volatile oil not only has carminative action, but also antiseptic, antimicrobial and antifungal activities. Thyme is prepared as infusion to treat spasmodic cough, laryngitis, bronchitis urinary infections, renal diseases, hypertension and tinea capitis. 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 [6].

The rationale behind conducting this study is that some herbs that are used by the community for any purposes may contain harmful chemicals that may cause serious side effects to the host system [7].

*T. schimperi* have been used by the community for many years for a dietary and medicinal purpose without investigating the safety of the plant. So it is necessary to evaluate the toxicity of local medicinal plants which are used by community [8]. Therefore, this study was aimed at investigating the toxic effects of *T. schimperi* in rats and mice.

## Methodology

### Plant material collection and oil extraction

The leaves of the test plant were collected from around Ankober, North Shewa, Ethiopia and the oil was extracted at medicinal plant research laboratory at Ankober by using steam distillation apparatus. Then the oil was kept in the amber glass until used for the study.

### Study animals

For acute and sub-acute toxicity study, albino mice of both sexes aged about 8 weeks old were used. The mice were purchased from Ethiopian public health and research institute. For the skin irritation test, wistar rats were used which were obtained from department of pharmacology at Addis Ababa University. The laboratory animals were housed in standard cages. They were fed and provided water adlibitum [9].

### Experimental Protocol

A limit test dose of 2000 mg/kg was used as stipulated in OECD guidelines. Accordingly, for the acute toxicity study, the oil of *T. shimperi* was tested at three dose levels of 1500, 1750, and 2000 mg/kg. A total of 10 mice were used for the three dose levels. The sub-acute toxicity study was done at a dose of 1000 mg/kg with 5 mice. For the skin irritation test, the essential oil of *T. schimperi* was tested at two concentrations of 1% and 5% with 10 rats for each concentration [9].

## Test Procedure

### Acute and sub-acute toxicity

The essential oil was calculated at the desired dose level measured and dissolved in distilled water. The mice were fasted for 12 hours pretreatment. Then the dissolved oil was orally administered to each mouse by using oral needle. For acute toxicity test, the dissolved oil was orally administered at single dose. Then mice were observed for 24 hours for follow up of mortality and any sign of acute toxicity. The mice were also followed up for 14 days for any abnormality manifested from essential oil treatment. For the sub-acute test, repeat-dose oral toxicity was carried out according to OECD guideline 425. The dissolved oil was orally administered daily for a total period of 28 days and the body weight of each mouse was measured and recorded weekly. On day 28, the mice were scarified by anesthesia with ether, blood sample, liver and kidney organs were collected for laboratory analysis. Then serum enzyme level was determined and histopathology examination of both liver and kidney was done [9].

### Skin irritation

For the skin irritation test, ointment was prepared for oil from *T. shimperi* at concentrations of 1% and 5% by using petrolatum as a base. The Ointment was prepared in department of pharmaceutics at Addis Ababa University, Addis Ababa, Ethiopia by addition method using ointment slab, spatula, pestle and mortar. The experimental rats were shaved on the back surface, the ointment was applied, and the rats were monitored for two weeks for any sign of abnormal skin reaction [9].

### Data Analysis

The data were analyzed by using SPSS version 20 and results were considered significant at 95% confidence level. Results were expressed as mean ± standard deviation (SD). The lethal dose in fifty percent of the test animals (LD50) was calculated by using graphical method of LD50 determination.

## Results

### Acute toxicity

The acute toxicity study showed that the oil of *T. schimperi* caused shivering, loss of balance, depression, and abnormal body movements on the study mice. The result of the acute toxicity study on mortality is shown in Table 1. The essential oil of. *T. schimperi* caused 50 percent mortality on the treated mice at the tested dose of 2000 mg/kg indicating that its LD50 is about 2000 mg/kg.

| Plant species | Number of mice dead/treated at each dose | | | |
|---|---|---|---|---|
| | 1500 mg/kg | 1750 mg/kg | 2000 mg/kg | LD50 |
| *T. schimperi* | 0/10 | 43375 | 43378 | About 2000 mg |
| Control (water) | 0/10 | 0/10 | 0/10 | - |

**Table 1:** Result of acute toxicity study

### Sub-acute toxicity

**Effect on body weight :** The effect of the test plant on body weight of the treated mice is shown in Table 2. The test plant did not cause significant weight change ($p>0.05$) during the 28 days study period. The body weight of the treated mice was also not significantly different from that of the control group ($p>0.05$). However, the control group had significantly increased body weight during the study period ($p<0.05$) indicating that the plant prevented weight gain of the study animals.

| Plant species | Weekly average body weight of mice (mg) ± SD | | | | | |
|---|---|---|---|---|---|---|
| | Week 0 | Week 1 | Week 2 | Week 3 | Week 4 | p-value |
| *T.schimperi* | 34.8 ± 5.4 | 34.8 ± 5.4 | 33.8 ± 3.11 | 31.2 ± 4.27 | 31.2 ± 2.86 | p>0.05 |
| Control (water) | 24.6 ± 2.88 | 24.6 ± 2.88 | 29 ± 1.73 | 28.8 ± 1.09 | 28.8 ± 1.09 | p<0.05 |

**Table 2:** Effect of *T. schimperi* essential oil on body weight

**Effect on serum enzyme level :** The effect of the test plant on enzyme level of the treated mice is shown in Table 3 bellow. The essential oil from *T. schimperi* did not cause significant increase in serum enzyme level of the study mice as compared with the control group ($p>0.05$).

### Organ toxicity

The toxicity of the test plant on histopathology is shown in Figure 1. The histopathological examination of this medicinal plant on liver and kidney showed that the tested plant did not cause major organ damage.

| Plant species | Average enzyme level ± SD | | |
|---|---|---|---|
| | SGOT | SGPT | ALP |
| *T. schimperi* | 354 ± 163 | 50.8 ± 22.2 | 264 ± 159 |
| Control (water) | 348 ± 123 | 80.8 ± 26.08 | 267 ± 33 |

**Table 3:** Effect of *T. schimperi* essential oil on serum enzyme level.

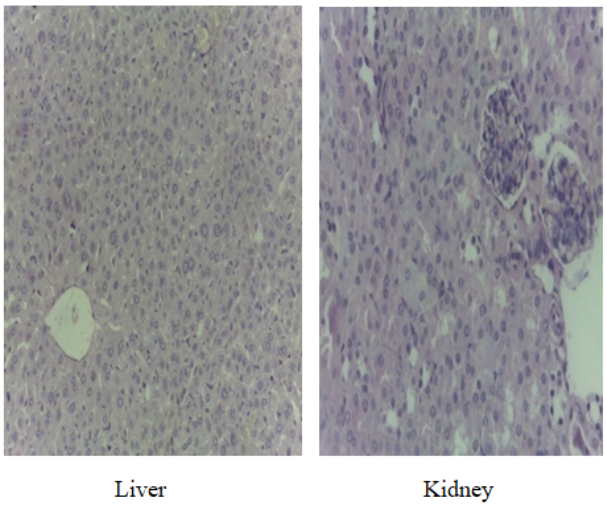


**Figure 1:** Histopathological effect of essential oil of *T. schimperi* on liver and kidney.

**Skin irritation:** The ointment prepared from essential oil of *T. schimperi* did not cause any abnormal skin reaction up on follow up for 14 days post treatment.

## Discussion

Various medicinal plants and botanical drugs have been widely adapted as primary therapeutic agents or supplements for treating various human diseases [10].The safety study is accomplished by the implementation of general pre-clinical toxicity experiments to uncover potential poisonous effects of any drug mainly in liver and kidney of animals [11].

From the current acute toxicity study, the LD50 of essential oil of T. shimperi is about 2000 mg/kg. A previous research conducted on aqueous extract of leaves from *T. schimperi* showed that the LD50 was greater than 10,000 mg/kg [12] indicating that the essential oil of *T. schimperi* is relatively toxic than the aqueous extract of leaves from the same plant.

The current acute toxicity study also showed that the oil of *T. schimperi* caused shivering, loss of balance, depression, and abnormal body movements on the study mice and this finding is not consistent with the previous studies [12-14].

The essential oil from *T. schimperi* did not cause significant weight change ($p>0.05$) during the 28 days study period. The body weight of the treated mice was also not significantly different from that of the control group ($p>0.05$). However, the control group had significantly increased body weight during the study period ($p<0.05$) indicating that the plant prevented weight gain of the study animals. Previous study done on aqueous extract of *T. schimperi* showed that low dose of the tested medicinal plant increased significantly the weight of treated rats but the high dose did not show significant effect on body weight of rats [12] i.e. this finding is supported by such a study.

In the present study, the toxicological effect of 1000 mg/kg dose of the essential oil of *T. schimperi* on liver biochemical parameters was investigated in mice. In liver function test, there were no significant changes in the serum level of SGOT, SGPT and ALP in most animal groups treated with essential oil of *T. schimperi* in comparison to the control group. The non-significant change of these enzymes between the control and test animals after 4 weeks of treatment shows that the essential oil did not cause adverse toxic effect or hepatic damage on the liver and this finding is consistent with other studies [12-14].

From this study, it can be concluded that the essential oil of *T schimperi* is safe for topical and systemic use as per animal study.

## Acknowledgement

We would like to thank Debre Berhan University for providing necessary budget to carry out the study. Ethiopian public health institute is also appreciated for providing experimental animals.

## Funding

This work was funded from Debre Berhan University core budget.

## Ethical considerations

Ethical clearance for experimental animals was obtained from the Ethics Review board of Debre Berhan University. The care and handling of animals including the scarification was as per the internationally accepted ethical guidelines [9].

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