A Comprehensive Review of the Chemical Composition and Dermal Toxicity of Essential Oil from Thymus schimperi in Wistar Albino Rats

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ABSTRACT

In Ethiopian traditional medicine, the aerial part of Lymus schimperi is commonly used to cure ailments like fungal skin infections, gonorrhoea, cough, liver and kidney diseases, hypertension, and stomachaches. Since there hasn't been much research on the toxic effects of 1ymus schimperi essential oil, the goal of this study was to evaluate its acute and subacute toxicity in Wistar albino rats. Approach. After hydrodistillation, GC-MS analysis and toxicity tests were performed on the essential oil of T. schimperi's aerial section. For the dermal toxicity study, rats were divided into seven groups (n = 5) at random. The control group received only distilled water with 2% Tween 80, whereas the experimental groups received single doses of 300, 600, 900, 1200, 1500, and 2,000 mg/kg of the oil. Following the experiment, blood samples were obtained for haematological and clinical chemistry examination. Additionally, the gross pathology and histology of the liver and kidneys were evaluated. The in-silico toxicity study revealed that most of the oil's components were safe; some of the compounds showed hepatotoxicity and mitochondrial membrane potential toxicity.

Key Words: Thymus schimperi, Essential oil, Dermal toxicity, Acute toxicity, Wistar albino rats, GC–MS, Histopathology, Hepatotoxicity, Toxicological evaluation, Ethiopian medicinal plant.

1. INTRODUCTION

The genus 1ymus contains more than 350 species that are found in many tropical areas of the world. In many parts of the world, oral T. schimperi (thyme) extracts are used to treat bronchitis, pertussis, tonsillitis, laryngitis, coughs related to colds, dyspepsia, and other gastrointestinal conditions. Topically applied thyme extracts have been used as antimicrobial agents for oral hygiene, small wounds, colds, and oral cavity problems [1]. New products and compounds, particularly those used on a daily basis, should go through the proper toxicological screening before being used in humans or animals. Due to its significant role in the absorption of harmful chemicals and physical substances, the skin is a target organ system that is vulnerable.

Dermal toxicity studies are usually carried out utilising a variety of laboratory animals to describe the cutaneous/systemic toxicity and irritation potential associated with topical administration of new goods or chemicals. Human response is then predicted using the findings from these investigations [2]. The human skin is the principal organ exposed to transdermal therapeutic systems because adherence of such materials to the superficial organs may be a major route of exposure during the administration, utilisation, and disposal of such therapeutic systems [3]. Members of the genus are among Ethiopia's most widely used medicinal plants, mostly because of their antibacterial qualities. Locally known as "Tosign," the leaves of T. schimperi and T. serrulatus are utilised as spices in a range of dishes and drugs. In Ethiopia, T. schimperi is used in a variety of ways, such as tea and condiments to make "shirro" (bean/pea powder) and "berbere" (pepper powder), as well as metata ayb (fermented cottage cheese). Skin fungus, stomach ache, kidney problems, liver disease, renal diseases, cough, gonorrhoea, and hypertension are just a few of the illnesses that T. schimperi is used to treat in traditional medicine [1]. Before using plant compounds to treat human ailments, their toxicity must be tested. When using a herbal product to protect the public's health, toxic property screening is crucial. According to Jain 15, the review processes often address effects that are carcinogenic, reproductive, chronic, subchronic, acute, and subacute [15]. In this study, the cutaneous safety of the plant medication T. schimperi was evaluated using Wister Albino rats and other small animals. The need to assess the dermal safety of plant medicines has been discussed below [4]. When several growth factors, cytokines, and low molecular weight compounds are released from the damaged site, the healing process starts right away. The release of EGF, TGF-α, and FGF can promote the migration and proliferation of epithelial cells and the re-epithelialization of the skin [22].

2. MATERIALS AND METHODS

Research Animals

Wistar albino rats from the Ethiopian Public Health Institute (EPHI) breeding unit that were 8–10 weeks old were used in this study. The female rats were nulliparous, meaning they were not pregnant. Each set of five same-sex rats (n = 5) was housed in a standard cage, divided into experimental and control groups, and maintained under standard conditions (at 20°C, with a regular 12-hour light/12-hour dark cycle). Every experiment was conducted in accordance with internationally accepted guidelines for the handling and care of experimental animals. Additionally, the Institutional Review Board (IRB) at the College of Health Sciences approved the protocol. The animals received food and water during the one-week acclimatisation period before the trial's commencement [1].

Distilling essential oils

One kilogram of fresh T. schimperi leaves was hydrodistilled using a Clevenger-type apparatus. Until it was required for the investigation, the acquired oil was stored in a sealed amber-colored vial at -10°C in a refrigerator.

Analysis of the Essential Oil

GC analysis

The separation was carried out using a gas chromatograph, model GC-14A, fitted with a Supelcowax 10 (30m×0.25mm, 0.2μm film thickness) fused silica column. The oven was set to three different temperatures: 180°C to 240°C (10°C/min), 70°C to 180°C (5°C/min), and 240°C (10 min). With a split ratio of 82:1 and a flow rate of 1 millilitre per minute, helium was used as a carrier gas. The injector and Flame Ionisation Detector (FID) have respective temperatures of 210°C and 260°C.

GC-MS analysis

Qualitative GC-MS analysis was conducted using the Mass Lab VI.1 system with a FI 8000GC. The injector temperature was adjusted to 210°C, and the oven temperature was programmed to 60°C (5 minutes) using a Supelcowax 10 (30m x 0.25mm, 0.2μm film thickness) fused silica column. Electronic integration and flame ionisation detection were used to provide quantitative data without the use of FID response factors; the experiments were not repeated. The compounds were identified by computerised comparison of the resulting mass spectra with library spectra (MS) and injection (GC) with authentic samples.

Acute toxicity

An acute toxicity assessment was carried out in accordance with OECD 425 research standards [21]. Despite fasting for the whole night, seven groups of healthy female Wistar rats were allowed unrestricted access to water. group (n⁽5) at random. Initially, distilled water with 2% Tween 80 was given to the control group. The six classes received oral single dosages of 300, 600, 900, 1200, 1,500, and 2,000 mg/kg of T. schimperi essential oil. Doses were selected after pilot testing. For every treatment, force-feeding was employed.

For a period of 14 days, the animals were observed for signs of poisoning, body weight, and mortality. Within the first three hours of essential oil delivery, toxicological signs and symptoms were recorded in each cage, and they were then routinely evaluated throughout the research [21]. The mortality observed within 14 days was used to derive the LD50 value. On Day 15, all living animals were put to death, their internal organs removed, and their organ weights noted.

Biochemical and haematological analyses

Ethylenediaminetetraacetic acid (EDTA) was used in test tubes to treat blood samples. The haematological parameters were measured using a haematology analyser (SYSMEX XT-1800i, SYSMEX CORPORATION, Japan). Mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLC), haemoglobin concentration (HGB), hematocrit (HCT), white blood cell count (WBC), and red blood cell count (RBC) were all evaluated. In order to prepare for biochemical analysis, blood samples were placed in plain test tubes for three hours to allow for full coagulation before being centrifuged for fifteen minutes at 5000 rpm using a tabletop centrifuge (Humax-k, Human-GmbH, Germany). The serum was kept at -20°C until clinical biochemistry tests were finished after the plasma was drained and transferred to other sterile vials. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, albumin, and creatinine concentrations were automatically measured using the Cobas Integra-400 plus Analyser (Roche Diagnostics, Japan).

Tissue samples and measurements of organ weight

After determining their body weight, all experimental animals were slaughtered on day 29 and their target organs removed. The samples of liver and kidney tissue were placed in a test tube containing 10% formalin, which was buffered for 24 hours before being rinsed under tap water for the entire night. Following dehydration, the fixed tissues were cleaned using xylene and ethanol, respectively. After removing any unnecessary tissues with 1% normal saline for a few minutes, the organs were weighed using a precision balance. Additionally, it was pierced by molten paraffin wax and embedded in paraffin blocks. The blocks were separated into 5–6 µm thick slices using a Leica rotary microtome (Leica RM 2125 RT, China, validated in Germany). The tissue sections were first carefully collected using forceps and placed on the surface of a water bath at 30 to 40°C before being placed over the tissue to facilitate the specimens' fixation on the glass slides. After that, the portions were put on sliding racks and baked for the entire night at 20 to 40 degrees Celsius. Following a series of xylene and alcohol treatments, the thin slices were stained with hematoxylin and eosin [25].

Photomicrography and light microscopy

A binocular compound light microscope (Olympus CX41, Japan) was used to closely examine sections of the liver and stained tissue. Tissue sections from the treatment groups were examined for signs of histological changes. Photomicrographs of a few selected slides from the treated and control groups were taken at magnifications of ×40 and ×20, respectively, using an automatic digital photo camera (Evos XI, China).

Data analysis and processing.

All of the data that was displayed as numbers was examined using SPSS statistical software. All numbers have been expressed using the standard error of the mean, or mean±SEM. Treatments were compared over time using a one-way analysis of variance (ANOVA) between the treated and control groups, and the significance level was determined using Dunnett's t-test. Statistical significance was considered at $P<0.05$.

Ethical Consideration

The College of Health Sciences, AAU, and EPHI Institutional Review Boards authorised every technique utilised in this study.

3. RESULT

Analysis of chemical composition

With a yield percentage of 1.39% (w/w), the essential oil of fresh T. schimperi leaves extracted by hydro distillation had a strong spicy scent and a dark yellowish colour. GC/MS and GC qualitative and quantitative assays revealed 57 compounds, or 88.75% of the total essential oil. displays the GC chromatogram for the oil and describes the findings of the GC/MS study. According to Table 1, the main constituents of the oil were carvacrol (49.90%), thymol (10.64%), o-cymene (8.54%), α-terpinene (4.5%), linalool (2.51%), and 3-octanol (2.48%). The result of single doses of oral T. schimperi essential oil is severe toxicity. The rats started to show signs of toxicity, such as piloerection, convulsions, hypoactivity, and even death, after receiving 900 mg/kg of the essential oil. There were 20%, 20%, 80%, and 100% mortality in the 900, 1200, 1500, and 2000 mg/kg groups, respectively. An approximate LD50 of 1,284.2 mg/kg was found in the acute toxicity study. The groups treated with 900 mg/kg and 1200 mg/kg experienced a significant decrease in body weight on day 7 of the acute toxicity study when compared to the control group ($P<0.05$). Additionally, by day 14, the body weight of the 600 mg/kg, 900 mg/kg, and 1200 mg/kg treatment groups had significantly dropped in comparison to the control group ($P<0.05$). Similarly, compared to the group treated with 300 mg/kg, the group treated with 1200 mg/kg had a significantly lower body weight ($P<0.05$). Nevertheless, the treatment groups (1500 mg/kg and 2000 mg/kg) were not included in the study because they had too few or no living rats, respectively. Furthermore, the kidney and liver weights in the treatment groups (900 mg/kg and 1200 mg/kg) were considerably higher than in the control group.

Haematological and Biochemical Parameters

Haematological analysis showed that the high-dosage group (260 mg/kg) had significantly higher MCV and lower WBC counts than the control group. There was no significant difference in RBC, HB, HCT, MCH, MCHC, or PLT levels between the experimental groups and the control group. Three markers of liver impairment, ALT, AST, and ALP, did not differ substantially between the treatment groups and the control group. There were also notable changes in blood urea and creatinine levels, which are indicators of kidney impairment. Similarly, HDL and LDL levels did not significantly differ between the treatment and control groups. Finally, electrolyte measurements revealed no appreciable changes in blood electrolyte levels, such as potassium and sodium, between the treatment and control groups.

Analysis of morphology

The selected organs showed no obvious abnormalities according to the gross pathological examination. Furthermore, the histological analysis revealed no appreciable abnormalities in either the treatment or control groups.

4. DISCUSSION

Worldwide, a wide range of ailments have been treated mostly with herbal therapies [20]. To evaluate a medication's safety and find any potential negative effects, general preclinical toxicity tests are conducted, primarily in animal kidneys and livers [21]. The permeability of the cell membrane will significantly increase if it is found that these organs are slightly damaged and inflamed, resulting in the presence of cytoplasmic enzymes like AST and ALP in the blood. Similarly, inflammation results in the production of mitochondrial ALT and AST [22, 23]. Toxicity screening models offer valuable preliminary data that could help identify natural remedies with potential health benefits [24].

O-cymene, carvacrol, thymol, and α-terpinene were the principal constituents of T. schimperi leaf essential oil. The chemical composition of the oil was similar to that previously reported by Asfaw et al. [25], who discovered that the primary components of the oil were carvacrol, p-cymene, c-terpinene, and thymol. The LD50 of T. schimperi essential oil, as found in this study utilising probit analysis, was 1284.2 mg/kg in accordance with WHO guidelines for pesticide regulations. When taken orally, this level of LD50 is thought to be somewhat dangerous [26]. The LD50 value of 2000 mg/kg found in a study conducted in Debre Berhan, Ethiopia, was slightly higher than this result [27]. Wistar albino rats were used in this study, while mice were used in the previous one, which could account for the little discrepancy. The current acute toxicity study also revealed that T. schimperi essential oil caused hypoactivity, piloerection, convulsions, and irregular body movements in the animals under examination. The outcomes are reliable.

A single oral dose of T. schimperi essential oil causes toxicity symptoms like hypoactivity, piloerection, and convulsions, which may have been brought on by abnormalities in the activity of the autonomic nervous system (ANS) and central nervous system (CNS), according to a study by Dires et al. [27].

In an acute toxicity study, T. schimperi essential oil significantly reduced body weight at higher dosages, which may be connected to the adverse symptoms that caused the rats to become anorexic [28]. The current study's rise in liver and kidney weight is most likely due to oedema [29]. The haematological system can be a helpful indication for detecting physiological changes in both humans and animals since it is sensitive to dangerous substances [30]. A blood sample usually provides crucial information on how the body reacts to stress, hunger, and disease, and haematological testing can quickly identify physiological changes in the body [31].

Thus, it is possible to determine the extent of the negative impact of drugs and/or plant extracts by evaluating haematological parameters [32]. At a dose of 260 mg/kg, the mean white blood cell (WBC) count in the current investigation was considerably lower than that of the rats in the control group. The primary bioactive components of T. schimperi essential oil, such as thymol and carvacrol, may cause cellular apoptosis, cell proliferation, and cell cycle arrest in the sub-G0/G1 phase [33, 34]. The mean corpuscular volume (MCV), a measure that helps determine the size of erythrocytes, also increased at a dosage of 260 mg/kg. This may be due to the fact that any substance that either directly or indirectly affects the synthesis of cellular DNA might trigger macrocytic changes. Elevated MCV indicates changes in DNA biosynthesis [35].

A previous study found that carvacrol inhibits DNA synthesis [36]. Biochemical measurements are crucial indicators in toxicological assessment because they respond to clinical signs and symptoms caused by toxins.

An extensive assessment of liver and renal function is necessary to identify the potentially harmful properties of extracts and drugs [37]. The use of T. schimperi essential oil did not significantly alter any of the biochemical measurements in the animals used in this study. Blood levels of both ALT and AST are produced by any liver injury, and these levels may be considered the first sign of the damage [37]. The main function of glomerular filtration is to control the plasma concentration of creatinine, which is generated naturally and steadily released into body fluids. As a result, both plasma concentration and kidney clarity were used to calculate the glomerular filtration rate [38].

The measurement in this analysis, which was within the reference value, was supported by the lack of histological changes in the kidneys. The rise in total serum protein is caused by both an increase in the concentrations of one or more different proteins in the plasma and a change in the amount of plasma water. Histopathological exams provide information on haematological and biochemical parameters that require improvement [32]. Hepatocyte, the general structure of the liver. When compared to controls, hepatic sinusoids, portal triads, central veins, and morphology are all normal. Furthermore, when compared to the control, the general histological architecture of none of the treatment groups was affected. Following four weeks of therapy, there was no appreciable difference in the liver histological parameters of the test animals and control animals, indicating that the essential oil did not have any detrimental toxic effects or induce hepatic damage, which is consistent with earlier studies [27,33].

5. CONCLUSION

The yield of the essential oil from the aerial portion of T. schimperi was found to be 1.39% v/w, and a GC-MS study of the oil enables the identification of 57 components. Carvacrol accounted for 49.90% of the essential oil, with thymol coming in second at 10.64%. An acute toxicity study found that the oil's LD50 was 1284.2 mg/kg. Similarly, a subacute toxicity investigation revealed that, although the MCV was dramatically increased and the WBC count was significantly decreased at 260 mg/kg, T. schimperi oil did not adversely affect body weight, biochemicals, or other haematological markers at the tested levels. Additionally, there were no signs of damage in the liver and kidney portions of the treated rats. None of the components of T. schimperi essential oil showed any cytotoxicity, Ames Mutagenicity, or cardiac toxicity (h-ERG Blocker), according to ADMET and vNN-ADMET toxicity predictions. However, just 1.75% of the compounds had the potential to be toxic to the mitochondrial membrane, 8.6% of the compounds were hepatotoxic, and only 3.45% of the compounds caused drug-induced liver injury. This study indicates that it is safe to take T. schimperi essential oil orally up to 130 mg/kg. However, in the high-dose (260 mg/kg) group, the WBC count sharply decreased, and the MCV significantly increased. The in-silico toxicity study revealed that most of the oil's components were safe, despite a few compounds exhibiting hepatotoxicity and mitochondrial membrane potential toxicity. Therefore, a chronic toxicity study on the essential oil and its constituents that showed toxicity in the in-silico analysis must be done before using compositions including T. schimperi essential oil as pharmaceuticals.

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