Acute oral toxicity study of *Thymus serrulatus* and *Thymus schimperi* from Ethiopia

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ABSTRACT

Thymus serrulatus and *Thymus schimperi* both endemic to Ethiopia are used by the public as tea and food additives. They are claimed to have some sort of toxicity. However, no toxicity test has been conducted to date. So the present study aimed to test the acute oral toxicities of their Essential Oils (EOs). *T. serrulatus* was collected from Ofla (Ofl), Alamata (Ala), and Yilmana Densa (Yil) and *T. schimperi* from Tarmaber (Tar), Butajira (Buta), and Bale (Bal). The control group (Group I) mice were administered with calculated amounts of 0.1% Tween-80 in normal saline. Experimental group (Groups II to VI), on the other hand, were delivered with 2000 µL/Kg body weight of Ofl, Ala, Yil, Tar, Buta and Bal EOs respectively. Treated and control mice were observed, and changes were recorded for 14 days. On the 14th day, after mice were humanely killed by heart puncture method, their organs were weighed, organ to body weight ratios were calculated and packed cell volumes (PCVs) were determined. Growth rate decrease was observed in mice treated with Yil and Buta EOs (carvacrol chemotypes) than in those treated with the thymol chemotypes (Ofl, Ala, Tar, and Bal). The organ to body weight ratios of the control group were either significantly higher than or comparable to that of the treatment groups implying that the EOs had no any inflammatory effects on the organs. The % PCVs of mice treated with the EOs were either significantly higher than or comparable to the control mice. The median lethal dose (LD50) of each EO was between 2000 µL/kg to 5000 µL/kg body weight. The LD50 values of the dry weights of thyme were calculated based on their EO yields that were approximated to be around 278g /kg bw (Bal), 313g /kg bw (Yil, Tar, and Buta) and 500g /kg bw (Ofl and Ala). Since the aerial parts, not the EOs, of thyme are used in the form of tea and food additives (not their EOs), this value is so high that these plants are not toxic. However, cautions should be taken for vulnerable groups.

Key words: Acute Toxicity, *Thymus serrulatus*, *Thymus schimperi*, Essential oils

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INTRODUCTION

The genus *Thymus* under *Lamiaceae* is known for its several species and varieties. In Ethiopia, *T. serrulatus* and *T. schimperi* are representatives to this genus. They are locally named as *Tosign* (Amharic) and *Tesni/Thasne* (Tigrigna). These species are endemic to Ethiopian highlands (2200-4000 m. a.s.l.) and are limited to the afromontane and afroalpine zones of the country (IBC, 2008; Destaw Damtie and Yalemtsehay Mekonnen, 2015).

The genus *Thymus* is known for its medicinal value. The medicinal value of the different species of this genus is related to their EO composition. The principal components of *Thymus* EOs are thymol and carvacrol (up to 64% of oils) (Rasooli, 2005; Alekseeva, 2009), along with linalool, *p*-cymol, cymene, thymene, α-pinene and many others (WHO, 1999). Overall, more than 20 EO chemotypes are noticed in different species of *Thymus* genus. These differences in chemotypes can be noticed in species grown in the same habitat which makes the study of this genus interesting

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(De Martino *et al.,* 2009). This chemical diversity indirectly can influence the biological activity of the oils, and it is generally a function of three factors: genetic, physiological and environmental conditions (De Martino *et al.,* 2009).

Differences or similarities in chemotypes can happen either within the same or different *Thymus* species. For example, different research works on the chemical composition of *T. vulgaris* showed different chemotypes: Thymol type (Sharafzadeh *et al.,* 2010), Camphor type (Imelouane *et al.,* 2009), and terpinen-4-ol type (Viuda-Martos *et al.,* 2007). Similarly, *T. daenensis* was found to be thymol (Leila *et al.,* 2008) and carvacrol (Sfaei-Ghomi *et al.,* 2009) chemotypes. On the other hand, different *Thymus* species can be found to have similar chemotypes. For example *T.daenensis*, *T. persicus*, *T. satureoides* and *T. transcaspicus* were carvacrol chemotypes (Jaafari *et al.,* 2007; Sfaei-Ghomi *et al.,* 2009; Tabrizi *et al.,* 2010), *T. vulgaris, T. eriocalyx, T. zygis,* and *T.daenensis* were thymol chemotypes (Jaafari *et al.,* 2007; Leila *et al.,* 2008; Sfaei-Ghomi *et al.,* 2009; Grigore *et al.,* 2010).

The use of medicinal plant as a therapy for various disease conditions is an age long practice. In regions with rich diversity of flora, it forms an important component of their natural wealth. Herbs and herbal formulations for the treatment of ailments have continued to receive increased attention because of the strong belief that these products are safe (Farnsworth and Soejarto, 1985; Said *et al*., 2002). This assumption to a large extent may have influenced the indiscriminate use of these formulations by many, particularly amongst people living in rural areas. The incidences of adverse effects and sometimes life-threatening conditions that potentially emanate from these herbal medicines have been reported among various ethnic groups (Elvin-Lewis, 2001; Chan, 2003). Consequently, it has become vital to ascertain the toxicity profile of these medicinal herbs.

Research work by Oyewole *et al.,* (2010) on *T. vulgaris* leaf extracts in rats showed no significant signs of toxicity at doses of 100 mg and 200 mg/kg body. At the same time, findings of Tarawneh *et al.,* (2011) revealed no acute oral toxicity of Ivy-*Thyme* syrup purchased from suppliers in rats at dose levels of 3, 6 and 12 ml/kg. Reports show that pure components of thyme EOs like thymol had LD_{50} value much lower than that of the EOs tested. It had been reported by EPA that the LD_{50} values of thymol were 980 mg/kg and 800 mg/kg in rats and guineapigs, respectively. However, the phenols in thymol are considered to be as GRAS (generally recognized as safe) (EPA, 1983). Likewise, the present research work was focusing on acute oral toxicity tests of EOs of *T. serrulatus* and *T. schimperi* in mice since both plants are commonly used as food additives and as traditional medicines in Ethiopia.

Traditionally, *Thymus* species in Ethiopia are used in a variety of forms. The fresh or dried leaves of these species are used locally as condiments and tea, as ingredients in the preparation of *berbere* and *"shirro"* (pepper and bean/pea powder) as well as Metata ayb (a traditional Ethiopian fermented cottage cheese) (Destaw Damtie and Yalemtsehay Mekonnen, 2015). But the side effect of consuming these plants has not yet been studied. Thus, the present study aimed to evaluate the acute oral toxicity of *T. schimperi* and *T. serrulatus* in Ethiopia. Acute oral toxicity is the adverse effect occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24 hours (EPA, 2002). The levels of acute toxicities can be categorized using Globally

Harmonized System of Classification and Labeling of Chemicals (GHS) (UN, 2011). Their LD_{50} value categories vary based on their exposure routes: oral, dermal, gases, vapours, and dusts/mists. Based on GHS, acute oral toxicity of EOs is classified into categories ranging from one to five (Appendix 1).

MATERIALS AND METHODS

Plant material collection and preparation

T. serrulatus and *T. schimperi* for the acute oral toxicity were collected from six localities in Ethiopia. *T. serrulatus* was collected from Ofla District and Alamata District (Southern Tigray Region, North Ethiopia) and from Yilmana Densa district (Amhara Region, North West Ethiopia). *T. schimperi,* on the other hand, was collected from Mojana District of North Shewa (Amhara Region, Central Ethiopia), Meskena Mareko District of Gurage (Southern Nations, Nationalities and Peoples Region, Central Ethiopia), and Sinana Dinsho District (Oromia Region, Southern Ethiopia). These sites were purposely selected making sure that they represent six distantly located areas in the country.

Plants collected (aerial parts) were first washed by tap water and then by distilled water to remove dirt and debris. The collected plant materials were shade dried at room temperature in the biomedical laboratory of Addis Ababa University. The dried parts were then reduced to powder by an electric mill in Ecophysiology laboratory in order to rupture maximum cell walls of oil glands (Ahmad *et al.,* 2006) until the particle sizes were able to pass through 0.6 mm sieve.

Essential oil extraction

The fine powder (200 g) of each plant was added to 2L of distilled water (with vegetal material/ extraction solvent rate = 1/10 (w/v) in a 4L round bottom glass flask and subjected to water distillation for 3 h using Clevenger type apparatus in Insect Science Laboratory of Zoological Science Department of Addis Ababa University. Then the volume of each oil was quantified in milliliters (mL), dried over anhydrous sodium sulphate and stored in dark glass at 4°C until used (Imelouane *et al.,* 2009). The EOs, not the dry weights, were tested for toxicity because the EOs are esily administable than the dry weights in terms of time and delivery.

The EOs were named by taking the first letters of the particular area from where plants were collected: Ofla (Ofl), Alamata (Ala), Yilmana Densa (Yil), Tarmaber (Mojana) (Tar), Butajira (Meskena Mareko) (Buta) and Bale (Sinana Dinsho) (Bal).

Phytochemical analysis

The chemical constituents in the EOs were determined using Gas Chromatography Mass Spectrometry (GCMS) in the laboratory of the Institute of Bioorganic Chemistry of Hohenheim University, Germany. The instruments used were TRACE GC ULTRA Gas Chromatograph (Thermo Electron Corporation, USA) coupled with the mass spectrometer POLARIC Q (Thermo Electron Corporation). Even though the EOs contained multiple terpenes, many of which occurred in trace amounts, their chemotypes were defined by their single dominant terpenes (carvacrol and thymol) (Keefover-Ring *et al.,* 2009).

Animal handling

Animals for acute oral toxicity experiments were handled ethically according to the guideline for the Care and Use of Laboratory Animals Developed by the National Academy of Sciences (United States of America) (2011).

Experimental Animals: Animals were selected as per the OECD guideline for testing chemicals 420. Healthy young and nulliparous, non-pregnant female mice weighing from 19 to 27 gm and with age ranges of 8 – 12 week were selected. Female mice were used because literature surveys of conventional LD_{50} tests show that usually there is little difference in sensitivity between the sexes, but in those cases where differences are observed, females are generally slightly more sensitive (OECD, 2001). These animals were randomly grouped, marked, and kept in cages for five days before the experiment to acclimatize to the laboratory conditions at room temperature. The sequence of lighting was 12 hours light and 12 hours dark. Unlimited conventional laboratory diet and drinking water was made available.

Doses were prepared by varying the concentration of EOs in 0.1 percent Tween 80. At the same time each mouse was given the preparations in a single dose by gavage, and the volume to be delivered for each mouse was in 1 mL/100g body weight calculation.

Test procedure followed

Prior to the dosing, the mice fasted for 4 hours from food but not from water. After fasting, the weight of each mouse was determined, and the dose was calculated based on the body weight. After substance administration, food but not water was withheld for a further one hour (OECD, 2001). The procedure of dosing started from 2000 µL/Kg

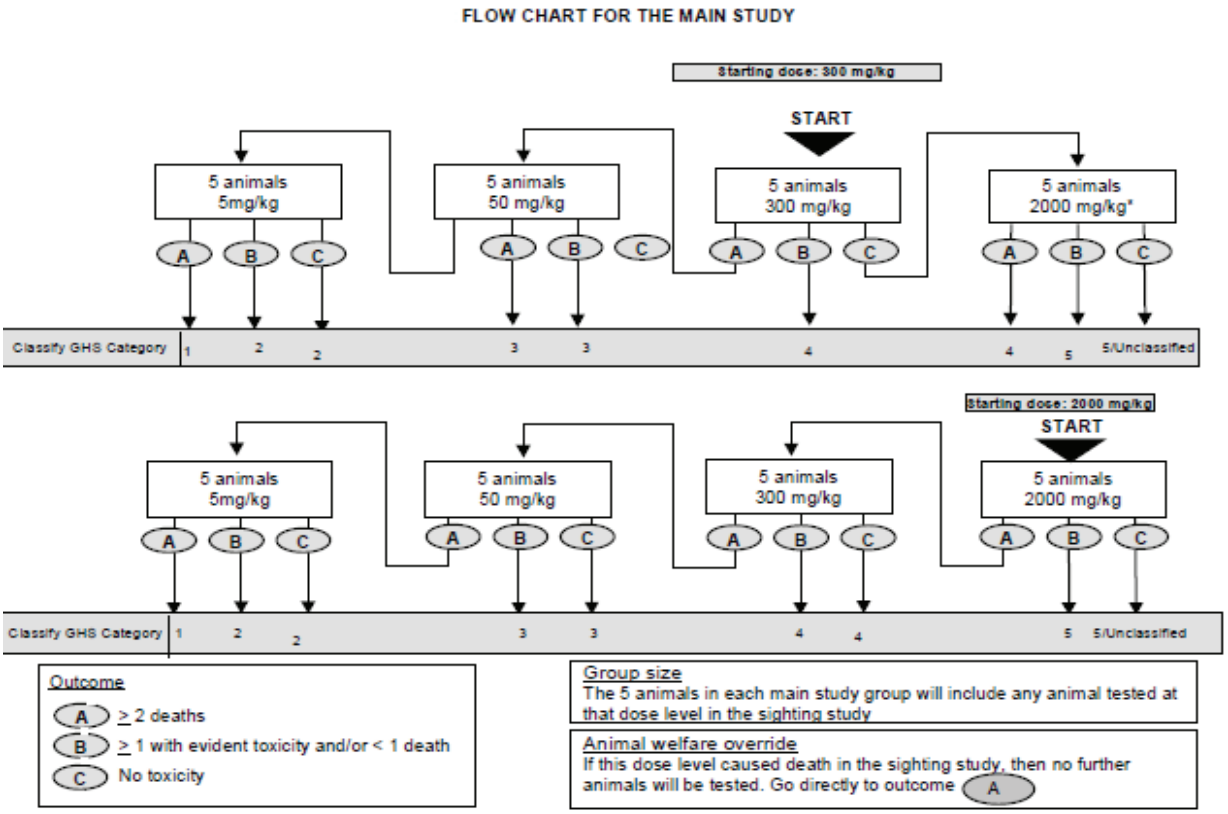

Figure 1: Procedure for acute oral toxicity testing, adapted from OECD (2001)

body weight in accordance with OECD guideline 420 (Figure 1). This 2000 µL/Kg body weight was selected due to the fact that *T. serrulatus* and *T. schimperi* are used as human food additives in different localities of Ethiopia (UN, 2011).

Thirty-five female mice were randomly assigned into seven groups, each group containing five animals. Mice in Group I (control group) were administered with calculated amounts of 0.1% Tween-80 in normal saline, the vehicle for EO administration (Grespan *et al.,* 2014; Pinho *et al.,* 2014). Groups II –VII were given 2000 µL/Kg body weight of Ofl, Ala, Yil, Tar, Buta and Bal EOs respectively. The EO dose 2000 µL/Kg body weight of the EOs was made in a vehicle (normal saline containing 0.1% T-80 (Grespan *et al.,* 2014; Pinho *et al.,* 2014).

Observations made: Animals were observed individually after dosing once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. Toxic reactions, time of onset and length of recovery period were noted. All observations were systematically recorded with individual records being maintained for each animal.

Individual weights of animals were determined shortly before the test substance was administered, on the 24th and the 48th hours and on the 7th and the 14th days. Weight changes were calculated and recorded. At the end of the test surviving animals were weighed and then killed using chloroform anaesthetizing. The hearts, kidneys, livers, brains, lungs, and spleen of the killed mice were weighed so that body to organ weight ratios were calculated and compared with that of the control mice (OECD, 2001). These organs were selected according to the recommendation given by Sellers *et al.* (2007). In addition, Packed Cell Volumes (PCVs) of each mouse were measured by taking blood from the tails of mice before they were killed.

Observations included changes in skin and fur, eyes, mucous membranes and behavioral pattern. Attention was given to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and mortality (OECD, 2001).

Evaluation of the LD_{50} values: The LD_{50} values of the EOs were determined based on the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) (UN, 2011) (Appendix 1).

Statistical analysis

Data were expressed as mean ± SEM and analyzed statistically using One Way Analysis of Variance (ANOVA) followed by LSD Post Hoc Multiple Comparisons. The minimum level of significance was set at $P<0.05$. The statistics were computed using SPSS program version 20.

RESULTS

The major compounds in Ofl EO were thymol (49.55%), carvacrol (36.34%), and p-cymene (3.06%). In Ala EO, thymol was the dominant component (65.63%) followed by carvacrol (6.68%) and thymol methyl ether (6.55%). Yil EO, on the other hand, had carvacrol (80.84%), thymol (6.52%), and p-cymene (3.65) as its major components. Tar was the EO with thymol (48.84%), carvacrol (42.12%), and linalool (2.97%) as its

major components. In the same way, the major components of Buta EO were carvacrol (71.83%), thymol (15.77%), and p-cymene (3.75%). The predominant components of the last EO Bal, were thymol (53.57%), carvacrol (34.55%), and p-cymene (3.20%). Four of the EOs (Ofl, Ala, Tar, and Bal) were found to be thymol and the rest two (Yil and Buta) carvacrol chemotypes.

Observations of mice dosed with EOs at 2000 µL/Kg body weight

Observation of body weight changes: As can be seen from Figure 2, the control mice grew continuously in the duration of 14 days. On the other hand, mice treated with EOs at 2000 µL/Kg body weight resulted in reduction of body weight within the first 24 to 48 hours. This in turn may be due to the burning sensations of the essential oils on their oropharengial tract. After that, improvement in body weight was observed except on those treated with Buta. Mice that were given Buta, however, continued to waste through the first seven days after which they started to improve. These mice reached their initial weight by day 14. Yil was the next EO which resulted in slow growth of the treated mice.

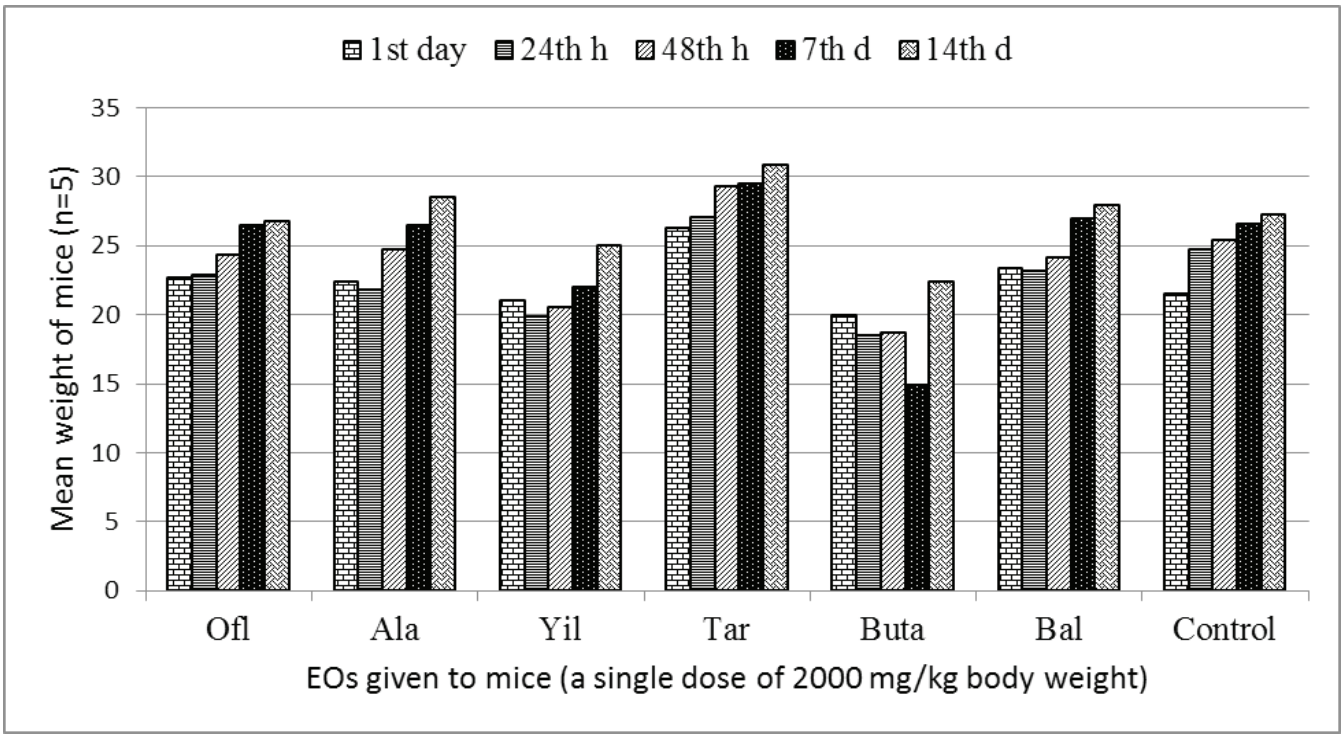

Figure 2: Mean body weight changes during acute oral toxicity test period [starting from day zero to 14 days after single dose (2000µL/kg) application). Ofl (Ofla), Ala (Alamata), Yil (Yilmana Densa), Tar (Tarmaber), Buta (Butajira), Bal (Bale), Cont (control)

Organ weight to body weight ratio (bw/ow): The organ weight to body weight ratios and packed cell volume (PCV) were calculated and are presented in Table 1.

Table 1: Organ to body weight ratio and %PCV in mice 14 days after treatment with 2000µL/kg bw of *T. serrulatus* and *T. schimperi* EOs (n=5)

EO	% Organ to body weight ratio (Mean ± SEM)						%PCV (Mean ± SEM)
	Heart	Kidneys	Liver	Brain	Lung	Spleen	
Ofl	0.53 ± 0.03 [b]	1.45 ± 0.06[a]	6.98 ± 0.31 [a]	1.48 ± 0.06 [a]	0.21 ± 0.01 [c]	0.58 ± 0.02 [b]	52.85 ± 0.98 [b]
Ala	0.72 ± 0.01 [ab]	1.27 ± 0.01[b]	6.65 ± 0.06 [a]	1.50 ± 0.06 [a]	1.05 ± 0.14 [a]	0.57 ± 0.01 [bc]	39.86 ± 0.08 [c]
Yil	0.63 ± 0.08 [ab]	1.37 ± 0.01[ab]	6.81 ± 0.13 [a]	1.66 ± 0.03 [a]	0.82 ± 0.03 [ab]	0.74 ± 0.01 [a]	44.29 ± 0.15 [c]
Tar	0.72 ± 0.05 [ab]	1.39 ± 0.02[ab]	6.64 ± 0.16 [a]	1.45 ± 0.02 [a]	0.73 ± 0.01[b]	0.56 ± 0.03 [bc]	43.62 ± 1.01 [c]
Buta	0.73 ± 0.03 [a]	1.27 ± 0.06[b]	7.00 ± 0.33 [a]	1.71 ± 0.11 [a]	0.19 ± 0.01 [c]	0.46 ± 0.04 [c]	64.28 ± 4.46[a]
Bal	0.59 ± 0.02 [ab]	1.26 ± 0.07[b]	6.19 ± 0.32[a]	1.45 ± 0.06 [a]	0.24 ± 0.01 [c]	0.54 ± 0.04 [bc]	54.36 ± 0.64 [b]
Control*	0.69 ± 0.04 [a b]	1.34 ± 0.02[ab]	6.01 ± 0.09[a]	1.54 ± 0.02[a]	1.00 ± 0.02 [a]	0.51 ± 0.02 [bc]	39.39 ± 0.19 [c]

a, b, c,……means with the same letters in columns are not significantly different ($p > 0.05$); * Control-given only 0.1 % T-80 in 0.9% normal saline; PCV= Packed cell volume. Ofl (Ofla), Ala (Alamata), Yil (Yilmana Densa), Tar (Tarmaber), Buta (Butajira), Bal (Bale)

DISCUSSION

Observation immediately after EO administration: Mice that were given single doses of thyme EOs (2000 µL/Kg body weight (bw) showed immediate responses after administration. Such responses included burning sensations in their oral cavities, esophagus or gastrointestinal tracts. Some mice tried to cool themselves by climbing on the water teat, eating straw bedding, and some others tried to hide under the straw bedding and produced pain sounds. This may be due to the irritating ability of the phenolic monoterpenes thymol and carvacrol (Soni, 2012). The irritating symptoms were more pronounced in Ala EO than in the rest of EOs. This may be due to the irritating effect of thymol in this EO (Fachini-Queiroz *et al.,* 2012). It contained over 65% thymol which is much higher than that of Ofl (49.6%), Tar (48.8%), Bal (53.6%) Buta (15.8%) and Yil (6.5%).

Observation at 30 minutes after dosage: At this time, most of the mice showed no interest in feeding and drinking. However, some mice (for example, those delivered with Yil EO) remained dormant for the next two hours after dosage. Furthermore, in the first 30 minutes after dosing, all the mice exhibited symptoms of toxicity like convulsions, tremors, morbidity, pilo-erection and depression. Some mice, for instance two that were treated with Bal and one with Ofl EOs, had spasm in hind legs. They were unable to walk during this time, and the spasm level decreased through time. This agrees with the finding of Elhabazi *et al.* (2012) where mice treated with *T. broussonetii* and *T. leptobotrys* EOs remained immobilized for some time. This is also supported by another report which explains that a concentrated ethanol extract of *T. vulgaris* produced decreased locomotor activity and slight slowing down of respiration in mice in an acute toxicity test (EMA, 2014).

Observation at 4th hour after dosing: Convulsion, pilo-erection and depression continued to the 4th hour after dosing in all mice while signs of morbidity continued in nearly 80% of the mice by the 4th hour of observation. On the other hand, the control mice (delivered with 0.1% T- 80 in normal saline) remained normal starting from the time of delivery except showing some symptoms of discomfort which may be due to the stress they faced from handling during administration.

Unlike mice in the control group, those treated with EOs at 2000 µL/Kg body weight resulted in reduction of body weight within the first 24 to 48 hours. After this time, there was a progressive increase in body weight of mice treated with EOs from Ofl, Ala, Tar, and Bal. This suggests that these EOs did not affect normal physiological functioning implying that they did not affect general growth and body weight.

Mice treated with Buta were exceptional in that their body weights decreased till day seven and showed improvements afterwards. At the same time, the trend of growth in mice treated with Yil EO was slower throughout the 14 days. Thus it is possible to conclude that mice were more sensitive to the EOs of Yil and Buta (carvacrol chomotypes) than to the other four. The EOs of Yil and Buta possessed carvacrol with respective percentages of 80.8% and 71.8%, whereas the EOs Ofl, Ala, Tar and Bal contained predominantly thymol 49.6%, 65.6%, 48.8% and 53.6% respectively. It can be supposed that carvacrol is mainly responsible for this toxic effect. Similar trends of toxicity were seen in two Moroccan endemic species: *T. broussonetii* (36.7% thymol and 90% carvacrol) and *T. leptobotrys* (96.8% carvacrol and trace amounts of thymol) (Elhabazi *et al.,* 2012).

The heart to body weight ratio, kidneys to body weight ratio, liver to body weight ratio and brain to body weight ratio in the mice tretead with all the EOs showed no statistically significant difference from the control group. This verifies that all the EOs had no negtive effect on the mentioned organs. On the other hand, statistically significant differences were observed among groups with respect to the lung to body weight ratio, spleen to body weight ratio and PCV.

Mice treated with Ofl, Buta and Bal had lung to body weight ratios significantly lower than those treated with Ala, Yil, Tar, and the control group. The mice treated with Yil had spleen to body weight ratios significantly higher than that of the remaining groups including the control. Thus, spleen enlargement observed in Yil treated group may have resulted from the toxic effects of carvacrol (Elhabazi *et al.,* 2012). In all the cases, none of the EOs was found to be responsible for organ inflammation. Anatomical observtion of these organs from each group also showed no signs of toxicity.

With regard to %PCV, mice treated with 2000 µL/Kg bw EOs (Ala, Yil, and Tar) had %PCVs, which were not significantly different from that of the control group. The %PCVs of mice treated with 2000 µL/Kg bws of Ofl, Buta, and Bal EOs, however, were higher than the control group. Generally, it is possible to argue that all the EOs had no toxicity effects on red blood cells since this finding contradicts with cases of toxicities where the %PCVs decrease (Husna *et al.,* 2013).

Furthermore, even though some of these mice treated at 2000 µL/Kg body weight manifested symptoms of toxicity like pilo-errection, weight loss (Buta), and irritability during administration,

none of them died at the end of the 14th day. Thus the EOs can be categorized as category 5 according to the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) (OECD, 2001; UN, 2011). Thus all the six EOs tested are relatively of low acute toxicity hazards but which, under certain circumstances, may present a danger to vulnerable populations (UN, 2011). Paracelsus (1493-1541) argued that all substances are poisons; there is none which is not a poison but the right dose differentiates a poison and a remedy (Lahlou, 2004). Thus it is necessary to determine the median lethal doses of substances to be taken as medicaments.

The EOs in this study have an oral LD_{50} values in a range of 2000-5000 µL/Kg bodyweight and converted acute toxicity point (LD_{50}) estimate of 2500 µL/Kg body weight (bw) (UN, 2011) (Appendix 1). The acute oral toxicity was done using Eos, and EOs are known to be highly concentrated substances. The yield of EOs from dry materials was found to be 0.5% (Ofl and Ala), 0.8% (Yil, Tar, and Buta) and 0.9% (Bal). Thus the LD_{50} values of the dry weights of thyme were approximated to be around 278g /kg bw (Bal), 313g /kg bw (Yil, Tar, and Buta) and 500g /kg bw (Ofl and Ala).

This extrapolation is very important since people in these localities use the dry materials in the form of tea or food additives, not the EOs. Thus, the high LD_{50} values of *T. serrulatus* and *T. schimperi* suggest that these plants are relatively safe and nontoxic. The results from this work agree with the statement that "thyme is known to be a nonpoisonous plant" (Yürüktümen *et al.,* 2011) and with the fact that the phenols in thymol are considered to be as GRAS (generally recognized as safe) (EPA, 1983).

CONCLUSION

The entire EOs tested for acute oral toxicity showed burning sensations in mice with Ala (thymol chemotype) being the most irritant than the rest of the EOs. The carvacrol chemotypes (Yil and Buta) resulted in reduced growth of mice than did the thymol chemotypes (Ofl, Ala, Tar, and Bal). The LD_{50} of the EOs are in the range of 2000 µL/Kg to 5000 µL/Kg body weight of the test mice. Since the aerial parts, not the EOs, of thyme are used in the form of tea and food additives and since the EO yield of thyme ranged from 0.5 to 0.9% (v/w), consumption of a big amount of these herbs could be poisonous to vulnerable populations such as children and pregnant women, and thus cautions should be taken.

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Appendix 1: Conversion from experimentally obtained acute toxicity range values (or acute toxicity hazard categories) to acute toxicity point estimates for use in the formulas for the classification of mixtures

Exposure routes	Classification category or experimentally obtained acute toxicity (LD_{50}) range estimate	Converted acute toxicity point (LD_{50}) estimate
Oral (mg/kg bodyweight)	0 < Category 1 ≤ 5	0.5
	5 < Category 2 ≤ 50	5
	50 < Category 3 ≤ 300	100
	300 < Category 4 ≤ 2000	500
	2000 < Category 5 ≤ 5000	2500
Dermal (mg/kg bodyweight)	0 < Category 1 ≤ 50	5
	50 < Category 2 ≤ 200	50
	200 < Category 3 ≤ 1000	300
	1000 < Category 4 ≤ 2000	1100
	2000 < Category 5 ≤ 5000	2500
Gases (PpmV)	0 < Category 1 ≤ 100	10
	100 < Category 2 ≤ 500	100
	500 < Category 3 ≤ 2500	700
	2500 < Category 4 ≤ 20000	4500
	Category 5	
Vapours (µL/L)	0 < Category 1 ≤ 0.5	0.05
	0.5 < Category 2 ≤ 2.0	0.5
	2.0 < Category 3 ≤ 10.0	3
	10.0 < Category 4 ≤ 20.0	11
	Category 5	
Dust/Mist (µL/L)	0 < Category 1 ≤ 0.05	0.005
	0.05 < Category 2 ≤ 0.5	0.05
	0.5 < Category 3 ≤ 1.0	0.5
	1.0 < Category 4 ≤ 5.0	1.5
	Category 5	

Source (UN, 2011 page 113)