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RESEARCH ARTICLE

SINGLE DOSE ACUTE TOXICITY TESTING AND PRELIMINARY SAFETY EVALUATION OF LEMONGRASS OIL IN MICE

***Abinet Gebremickael**

Department of Biomedical Sciences, School of Medicine, College of Medicine and Health Sciences, Arba Minch University, Arba Minch, Ethiopia

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ABSTRACT

This study was aimed to assess the possible toxic effects and to identify the rough safety margin of lemongrass essential oil-water emulsion after single oral administration in mice. In this study, Swiss albino mice placed in nine groups each containing six animals were used. The eight treatment groups were administered with increasing doses of 0.5ml/kg to 4.0ml/kg body weight test doses spaced by 0.5ml/kg. The control group animals were received vehicle. In the two weeks period of acute toxicity evaluation, test doses below 2.5ml/kg body weight does not produce obvious toxic effects. However, some toxicity signs and deaths of mice were recorded at doses higher than 2.5ml/kg body weight. Generally, the lemongrass oil emulsion was not toxic at doses below 2.5ml/kg after single oral administration in mice.

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INTRODUCTION

Traditional medicine is used throughout the world as it mainly depends on locally available plants, which are easily accessible. It capitalizes on traditional knowledge and it is simple to use and by far affordable. The current account of medicinal plants of Ethiopia shows about 887 plant species are utilized as traditional medicine in Ethiopia. Among these, about 26 species are endemic (Miruts *et al*., 2003). The medicinal plants of Ethiopia and the developing countries play major supplementary roles to the limited modern health care available (Desta, 1988; Miruts, 2007). It is reported that a significant proportion of the Ethiopian population still depends on traditional medicine for its health care services and more than 95% of traditional medical preparations are of plant origin (Flatie *et al*., 2009). Herbs are also origins for the development of several modern drugs. Lemongrass is the common name of *Cymbopogon citratus*, which is an aromatic grass belonging to the family Gramineae, and is generally cultivated almost in all tropical and subtropical countries (Tchinda *et al*., 2009). The essential oil obtained from leaves of this plant has antibacterial and antifungal activities (Suhr and Nielsen, 2003; Wilkinson and Cavanagh, 2005). Its sedative and anticonvulsant properties as well as its use as an anxiolytic agent has been documented (Blanco *et al*., 2009). Parallel with recent increasing interest in using herbal medicines, there is increasing concern about their safety. Literatures have documented severe toxic reactions from the use of herbs and spices for medication because of some toxic bioactive chemicals contained in some plants (Oduola *et al*., 2010; Tyler, 1994). A study conducted in Ethiopian Public Health Institute in collaboration with Addis Ababa University, Faculty of Veterinary Medicine, reported that the formulation comprising essential oil extract of lemon grass (*Cymbopogon citratus*) leaves showed high antiectoparasitic efficacy in both *in-vitro and in vivo* evaluation. However, the investigators reported that the infested animals licked and internalized the emulsion immediately after a topical treatment in a preliminary test. Accordingly, this study was aimed to assess the acute toxicity and ensure the safety of an emulsion comprising lemongrass oil after single oral administration in mice.

MATERIALS AND METHODS

Preparation of Plant Materials

The leaves of lemongrass were collected from Wondogenet Agricultural Research Center (WARC) experimental plot, located in Southern Nations, Nationalities and Peoples' Region (SNNPR), 260 km south of Addis Ababa, and brought to Ethiopian Public Health Institute (EPHI), where the experiment has been carried out. The collected leave specimens were identified and authenticated by a taxonomist,

Corresponding author:* **Abinet Gebremickael,
Department of Biomedical Sciences, School of Medicine, College of Medicine and Health Sciences, Arba Minch University, Arba Minch, Ethiopia.

cleansed from extraneous materials, air dried under shade, and chopped in to smaller pieces. The essential oil was extracted by using hydro-distillation technique in a modified Clevenger-type apparatus (Kawther, 2007), and a 2.5% lemongrass essential oil emulsion was prepared.

Experimental animal preparation

All animals used in this study were bred and reared at the animal house of the EPHI. A total of fifty four healthy adult male and female Swiss albino mice were used in the present experiments. They were kept in separate aluminum cages under standard condition, at a temperature of 22 ± 3^0C and 12 hours light/dark cycles, till the end of the experiment. Before the commencement of the experiment, animals were grouped in to nine groups, one control and eight test groups, each containing six mice. All animals had a free access to standard pellet diets and drunken tap water *ad libitum*. The mice were acclimatized to laboratory conditions for a week prior to the experimental.

Acute toxicity evaluation

All groups of mice were fasted for four hours prior to administration. At the end of the fasting period, the body weight of each mouse was recorded before dosing and the doses were calculated and administered to the mice in the treatment groups based on their fasted body weight. The eight test groups were administered with the designated eight test doses starting with randomly selected low initial test dose (0.5ml/kg body weight to animals in G-I). The additional seven doses were administered to mice in G-II to VIII spaced by 0.5ml/kg body weight dose. The control group (G-IX) received vehicle comprising only 2% Tween-80 and water. The administration procedure was done by gavages using a ball-tipped stainless steel feeding needle. The animals were then observed continuously for 4 hours in 30 minutes interval and in every 24 hours for 14 days for detection and recording of any clinical signs of toxicity and death. All the animals were measured for body weight at the 7th day following the administration. After measuring the final body weight at day 14, all animals were sacrificed by cervical dislocation, and dissected to observe gross pathology of the vital organs such as liver, kidneys, and stomach.

Statistical analysis

Data's were organized and analyzed by using Statistical Package for Social Sciences (SPSS) version-16 software packages. Values of the body weight were analyzed and the results were expressed as M ± SEM (Mean ± standard error of the mean). Differences between the treated and control groups were compared 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.

RESULTS AND DISCUSSION

The acute toxicity evaluation was aimed to assess treatment related toxic effects and identify rough safety margin of the emulsion. Accordingly, Gross physical and behavioral observation revealed no visible signs of toxicity following oral administration of the emulsion at 0.5, 1.0, 1.5, 2.0 and 2.5ml/kg body weight test doses. However, in mice treated at 3.0ml/kg and in mice survived after receiving 3.5 and 4.0ml/kg body weight test doses, some toxicity signs like piloerection, reduced food and water consumption, yellowish watery diarrhea, and gather together for more than two hours after administration of the emulsion was observed. Moreover, there was no noticeable gross pathological alteration in post mortem examination of the vital organs in all mice. Death of mice was recorded at 3.5 and 4.0ml/kg body weight doses (Table 1).

Table 1. Death rate record and corresponding doses of an emulsion

Doses administered	Group	Number of mice	Death rate	Death rate in %
0.5ml/kg	I	6	0	0%
1.0ml/kg	II	6	0	0%
1.5ml/kg	III	6	0	0%
2.0ml/kg	IV	6	0	0%
2.5ml/kg	V	6	0	0%
3.0ml/kg	VI	6	0	0%
3.5ml/kg	VII	6	2	33.3%
4.0ml/kg	VIII	6	4	66.67%
Vehicle	IX	6	0	0%

Table 2. Comparison of body weight changes of mice treated with different test doses of the emulsion and the control during the two weeks observation

Group	Dose (ml/kg)	Initial body weight (at 1st day)	Body weight at 7th day	Body weight at 14th day
I	0.5	24.3 ± 1.14 (1.72)	24.6 ± 0.95 (1.72)	27 ± 0.7 (1.65)
II	1.0	25 ± 1.7 (1.00)	25.6 ± 1.5 (1.00)	26.8 ± 1.6 (1.65)
III	1.5	26.1 ± 0.7 (1.00)	26.2 ± 0.92 (1.00)	26.4 ± 0.68 (1.00)
IV	2.0	23.8±0.23 (0.88)	24.25 ± 0.25 (0.81)	26 ± 0.49 (1.00)
V	2.5	25.45 ± 1.1 (1.00)	25.87 ± 1.29 (1.00)	27.9±1.14 (0.96)
VI	3.0	24.7±1.66 (1.00)	24.6 ± 1.8 (0.93)	26.17 ± 1.7 (1.00)
VII	3.5	24.6 ± 1.53 (1.00)	24.87 ± 1.47 (0.97)	25.77 ± 1.63 (1.00)
VIII	4.0	24.6 ± 1.09 (1.00)	24.65 ± 1.13 (0.93)	26.85 ± 0.86 (1.00)
IX	Vehicle	25.55 ± 1.025	26.17 ± 0.88	26.57 ± 0.85

Values are expressed as Mean ± SEM. The figures in brackets indicate the calculated p values of the treatment groups as compared to the control.

After 14 days of observation, no significant ($p>0.05$) body weight differences as compared to the control was observed in all groups of treated mice, even in survived mice treated with 3.5 and 4.0ml/kg doses (Table 2). Body weight is one of the sensitive indices of toxicity assessment after exposure to substances (Vahalia *et al*., 2011). In this study, overall body weight gain was recorded in all groups, and there was no

significant difference (P>0.05) between treated and control mice. This indicates positive health status of the animals or safety of the emulsion at low doses (Lu, 1996; Tofovic and Jackson, 1999; Fandohan *et al*., 2008; Blanco *et al*., 2009). Moreover, the emulsion did not result in any mortality of the treated mice after oral administration of single doses up to 3.0ml/kg body weight test doses. Nevertheless, in all mice treated at 3.0ml/kg and in animals survived after administration of 3.5ml/kg and 4.0ml/kg body weight test doses, some toxicity signs like piloerection, reduced food and water consumption, yellowish watery diarrhea, and gather together were observed. Two of the six mice in a group treated with 3.5ml/kg and, four of the six animals in a group treated with 4.0ml/kg body weight test doses of the emulsion were died following the administration. Hence, the oral route LD_{50} of the emulsion falls between 3.5ml/kg and 4.0ml/kg body weight doses.

Conclusion and Recommendations

It can be concluded from the findings of the present study that, doses up to 2.5ml/kg body weight of 2.5%lemongrass essential oil emulsion does not produce significant acute toxic effect after single oral administration in mice and is generally safe. Yet, further toxicological evaluation is recommended to confirm this.

Conflict of interest

The author reports there is no conflict of interest in this work.

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