

ACUTE ORAL TOXICITY OF ESSENTIAL OILS OF *Cymbopogon citratus* Stapf. IN MICE

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Abstract:

The purpose of this study was to assess the acute oral toxicity profile of the essential oils of lemon grass that is used as a natural food and medicinal flavor. The oil was obtained by steam distillation of 4.6 kg of fresh lemon grass leaves using a Clevenger apparatus, dried using anhydrous sodium sulphate and its net weight taken and this was found to be 14.2 g (0.31% as yield).

A stock solution of 200 g/ml was made by dissolving 4 ml of the essential oil in 20 ml of DMSO. A total of 36 (18 male and 18 Female) health mice were selected for the toxicity studies. Five different doses (4000 mg, 2000 mg, 1500 mg, 1000 mg, and 500 mg)/kg BW were administered to the grouped animals randomly in the preliminary phase of the study. Results from the preliminary phase were used to determine the dosages used in acute toxicity (500 mg, 750 mg, 1000 mg, and 1500 mg) and finally, 5 ml of the vehicle DMSO were administered to the control group. The animals were observed continuously for first 4 hours for behavioural changes and signs of toxicity and mortality within 36 hours. The values obtained were plotted against the corresponding log dose to determine the

LD₅₀. Hyper-urination, standing hair, rattling sound, hard breathing, dizziness and defecation of loose or greasy stools were some of the observable effects of the essential oil after administration.

Total mortality was observed in 2,000 mg/kg BW dosage level and there was no mortality at 500 mg/kg B/w just like in control group.

KEY WORDS: Essential oils, Acute Oral toxicity, *Cymbopogon citratus* Stapf.

Introduction:

Essential oils of many plant sources have been used today in many Over the Counter (OTC) therapeutic products, mosquito repellants and for flavoring food products like tea, however there are no standard dosage rates used in formulation of such products.

Cymbopogon citratus Stapf (family Poaceae), commonly known as lemon grass, is a tropical plant species traditionally used in as an aromatic crop for flavoring tea.

Lemongrass oil is used in manufacturing perfume, soaps, cosmetics and mosquito repellants. Beta-ionone derived from lemon grass has been the source of commercial Vitamin A (Schery 1972). In Uganda, lemon grass oil has been chiefly

used for flavoring tea which has turned to be a lucrative business in Kampala the capital city where most dwellers have resorted to consuming this lemon scented Tea because of its aromatic sweet taste.

The lemongrass essential oils dominated the essential oils market over twenty years but suffered from the harsh competition of synthesized essential oils and the Chinese essential oil made from Litsea cubeba, another raw material for citral, the principle essential oil in Lemon grass (Africabiz Online.2003).

Citral-rich oils derived from lemon grass and lemon scented tea tree have been shown to inhibit *Candida albicans* more than four times the rate of tea tree oil (Williams and Home 1995).

Many phytochemical compounds should be viewed as pharmaceutical compounds because although they occur naturally, they still require the same proof of efficacy and safety in use as synthetic pharmaceutical agents. Like many nutrients, phytochemicals can have adverse effects as well as beneficial effects on human health depending on the biochemical nature of the compound; whether it is absorbed and excreted rapidly in the urine or retained (Michael et al., 2003).

Essential oils may not necessarily be safe to humans and the environment as some may be quite toxic. Toxicological studies aimed at assessing their safety should be done before they are used to avoid possible dangers to non-target organisms and the environment (Belmain et al., 2001). This study was carried out to determine the acute oral toxicity levels of essential oils of lemon grass hence preceding recommendations for their

rational use by the public, herbalists and manufacturers of therapeutic agents and Tea blending companies who are using this oil to blend tea for human consumption.

MATERIALS AND METHODS

PLANT MATERIAL COLLECTION

Fresh leaves of lemon grass were obtained from a medicinal garden in Mayuge district, Baitambogwe Sub County-Wagana village, 136 km from, and East of Kampala, the capital of Uganda.

The dry leaves were sorted out of the fresh leaves and dry weight of the fresh leaves was obtained using a digital scale and this was 4.6 kg.

EXTRACTION OF ESSENTIAL OILS

The fresh leaves were cleaned and chopped to increase surface area for distillation

The oil was obtained by steam distillation of the leaves using a Clevenger apparatus

PREPARATION OF ANIMALS

A total of 36 (18 male and 18 Female) health mice bought from the animal breeding house of college of Veterinary medicine Animal resources & Biosecurity (COVAB) were selected for the toxicity studies. Their individual weight was taken using a digital weighing scale and this ranged from 21.4 g to 37.9 g.

Plastic cages with white papers laid on surface were labelled and used to accommodate the different groups. The animals were divided into 5 groups each of 6 animals, 12 animals were used in preliminary phase of the study and 24 in the final phase of the toxicity studies.

The animals were fasted for 8 hours, and then different doses were administered to the different groups randomly using an intra-gastric tube (gavage) with Dimethyl sulphoxide (DMSO) as the vehicle and the animals were observed for 4 hours, clinical observations were made and recorded.

PREPARING OF ESSENTIAL OIL SOLUTIONS AND DOSAGE DETERMINATION

The oil obtained was dried using anhydrous sodium sulphate, its net weight taken and this was found to be 14.2 g. A stock solution of 200 g/ml was made by dissolving 4 ml of the essential oil in 20 ml of DMSO. DMSO was used as vehicle because it was miscible with essential oils of *Cymbopogon citratus* unlike water. According to Stoughton and Fritsch 1964, DMSO was found also to enhance percutaneous absorption (Weyer 1967) on addition to being miscible in organic solvents.

The different doses were determined basing on the weight of the animals and the oil was administered using gavage tube and syringe.

Preliminary Toxicity studies

The preliminary phase of the study involved selecting 12 mice and grouping them into 5 groups each of 2 animals with 2 animals in control group.

A stock solution of 200g/ml was prepared by diluting the oil with DMSO. Five

different doses (4000mg, 2000mg, 1500 mg, 1000 mg, and 500 mg)/kg Body Weight were administered to the grouped animals randomly.

Results from the preliminary phase were used to determine the dosages used in acute toxicity (500 mg, 750 mg, 1000 mg, and 1500 mg) and finally, 5 ml of the vehicle (Dimethyl sulphoxide solution) were administered to the control group.

The animals were observed continuously for first 4 hours for behavioural changes and signs of toxicity and mortality within 36 hours. The values thus obtained were plotted against the corresponding log dose.

PROCEDURE OF ACUTE ORAL TOXICITY TESTING AND OBSERVATIONS MADE

The essential oils were obtained by hydro-distillation, and then administered orally. The oil was assigned thirty-six mice.

These were subdivided into 5 groups of 6 animals each and 5 different dosage levels were prepared and administered to the different groups randomly.

A group of 6 rats were kept as a control group and special amount of the carrier (5 ml) substance (DMSO) was administered orally to this group.

RESULTS AND DISCUSSION

Toxicity studies are relevant in scrutinising the safety profile of exogenous substance introduced or exposed to living substance.

Dose (mg/kg)	N=6	No: Dead	Percentage mortality (%)	Log dose	Probits
500	6	0	0	2.67	3.04
750	6	1	16.7	2.88	4.19
1000	6	2	33.3	3.00	4.64
1500	6	4	66.7	3.18	5.56
2000	6	6	100.0	3.30	6.96

Most herbal remedies have been claimed to be safe, cheap and readily available for use in most local communities in the world however, their safety profiles have not been documented.

This study determined the LD50 value of *Cymbopogon citratus* essential oils. The LD50 value was calculated to be 1071.5 mg/kg. According to OECD 423 guidelines (2008) and WHO, (2002), classification of toxic substances this essential oils could be said to be slightly toxic for use.

In addition the signs of toxicity observed in higher doses were, standing hair, hyper-urination, and loose stool and rattling sound, this could be associated to the effect of this oil on different body systems. For instance, the increase in hyper urination observed could be associated with the oil diuretic activity in the body, hence making it applicable in hypertensive cases.

Defecation of loose stools was due to increased activity of the Gastro-intestinal tract and irritation caused standing of hair. There was no mortality in the control group where the vehicle (0.5 ml, 1:1, DMSO 4: Distilled water) was administered hence the mortality obtained was due to the oil.

The percentage yield was determined by getting the net weight of the oil and dividing it by the weight of fresh plant materials (leaves) used multiplied by 100.
 Weight of collected samples = 4600 g
 Weight of dried essential oil = 14.2 g
 Percentage yield $(14.2/4600)100 = 0.31\%$
 Unlike in the control group, the animals in the experimental group exhibited the following physiological responses after administration of the oil: hyper-urination, standing hair, rattling sound, hard breathing and Defecation of loose stools. Synoptic effects were also observed in the experimental group.

Total mortality was observed in 2,000 mg/kg BW dosage level and there was no mortality at 500mg/kg B/w just like in control group as seen below.

Table of results showing percentage mortality and log dose

CONCLUSION

The essential oils of *Cymbopogon citratus* Stapf are practically toxic in mice models at dose level of 750 mg/kg BW following oral administration. The signs of toxicity observed within 30 minutes of oil administration included hyper urination, rattling sound, standing hair and defecation of loose stool.

Although there has been rampant use of these essential oils in flavouring tea, there could be cumulative detrimental effects following chronic oral consumption of beverages flavoured by the essential oils of *Cymbopogon citratus*.

RECOMMENDATIONS

There is need to carry out sub-chronic and chronic toxicity studies in other animal models particularly rats and rabbits so as to clearly predict the possibility of human intoxication that may result from oral consumption of the oil.

Toxicity studies based on intra-dermal and intra-nasal administration should also be carried out since the oil has been used for topical application as a cosmetic and in perfumery.

The plant showed a good yield (3.1%) hence the government through the ministry of agriculture should encourage its commercial production by the rural poor provided its ready market in the growing food and cosmetic industries.

There is need to study about the metabolism, bioavailability and mode of action of the essential oils from Lemon grass.

Intensive research should be done on the individual compounds of lemon grass oil responsible for the observed physiological changes.

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