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Acute Toxicity Test of Ten Commercial Essential Oils of Nepalese Origin

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Abstract

Essential oils are originating in aromatic plants and have volatile fractions that are responsible for biological activity, smell, and taste. Present study was designed to explore ten commercial essential oils in toxicological evaluation on mice. The LD50 was 1843, 1606, 1664, 1266, >2000, >2000, >2000, 500, 1347 and 1900mg/kg b.w. of Xanthoxylum Oil , Wintergreen Oil, Eucalyptus Oil, Lemongrass Oil, Hedychium Oil, Sugandhakokila Oil, Jatamansi Oil, Citronella Oil, Anthopogon Oil and Chamomile Oil respectively which reveals that Citronella has highest toxicity than others. Essential oils have wide application and mostly common in cosmetics, drugs and food. They are natural substances, but the results obtained indicate that natural is not synonymous with harmless.

Keywords: *Acute oral toxicity*, *Essential oils, LD50, Median lethal dose*

Introduction

The term "Essential oil" is defined as an odorous product, generally of complex composition, obtained from a botanically defined raw material, either by water vapor extraction , by dry distillation, or by an appropriate mechanical process without heating (Rehman, 2015). The essential oil is most often separated from the aqueous phase through a physical process, which does not involve a significant change in its composition". Dry distillation, without addition of water vapor, is used for wood, bark and roots. The mechanical process is used exclusively for citrus fruit: their essential oils are contained in micro vesicles located in the peel and extracted by pressure or friction.

Plants produce a wide array of secondary metabolites during their growth and development (Ramkrishna, 2011). Essential oils also known as ethereal or volatile oils are among the most important compounds of secondary metabolism of aromatic plants (Rehman, 2015).

Being secondary metabolites, essential oils are not vital for growth and development of the producing plant. Their role has been hypothesized to include protection against pathogens and pests by acting as antifeedants, antibacterial, antivirals, antifungals and insecticides (Ibrahim, 2001). In a number of plants, the essential oils suppress growth of neighboring plants through allopathic effects hence offering the producing plant a competitive advantage (Abad, 2012).

The most common test of potential human toxicity is that of the "LD50" test or the "median lethal dose". This test is routinely applied to laboratory animals (humans do not usually volunteer) in the testing of compounds used in pharmaceuticals, agricultural chemicals, flavors, fragrances and cosmetics, to name a few. In this testing procedure, laboratory animals, usually rats are given measured doses of compounds until approximately half of the test population die. The "median dosages" are then generally given in the ratio of grams of test compound per kilogram of bodyweight. Hence, a LD50 rating of 1.0 represents that 50% of the test animals died on a dosage of 1 gram per kilogram of body weight. Since ancient times, essential oils are recognized for their medicinal value and they are very interesting and powerful natural plant products. They continue to be of paramount importance until the present day. Essential oils have been used as perfumes, flavors for foods and beverages, or to heal both body and mind for thousands of years (Wei & Shibamoto, 2010). Besides that the utilization of

essential oil is very extensive and covers a wide range of human activity some of the important uses as; ingredients in the manufacture of soaps, cosmetics, perfumery, healthcare herbal products, confectionary, aerated water, syrups, disinfectants, insecticides, fungicides. Most essential oil compounds have a "non-specific" toxic effect, whereby the absorption of these lipophilic compounds into cellular membranes can eventually lead to disruption of membrane permeability. The primary toxic outcome is that of the disruption of ion channel function in nerve cells, first affecting the heart and central nervous system, leading to cardiac and respiratory depression (Henary, 1998). To create such effects, however, require huge dosages, in the order of 300mL and beyond. Certain aromatic compounds, most notably 1,8 cineole (as in many Eucalyptus species), camphor (borneone) (as an isolated compound or as in Rosmarinus officinalis CT camphor and Lavandula latifolia) and methyl salicylate (as a synthetically derived compound or as in Gaultheria procumbens) have specific toxic effects at much lower doses. These compounds make up the bulk of both serious and fatal poisonings in children and adults, due not just to their toxicity, but to the common availability of products containing these compounds and their reputed beneficial properties (NDPSC, 1998). With some essential oils or at least with the monoterpenes constituting them, dermal toxicity was observed, among them are the clove, eucalyptus, wintergreen, which are known for their irritability (Hammer, 1999). Bergamot and angelica essential oils cause photosensitivity (Bakkali, 2008), D-limonene produces further irritating transdermal absorption 40 and another that tea-tree oil can cause skin allergies (Rubel, 1998; Rutherford, 2007).

Many internet sites marketing essential oils give the following warnings: "Always keep essential oils out of reach of children". Some oils can irritate sensitive skin. Some oils are phototoxic (angelica, orange, bergamot orange, lemon, etc.) After application of these oils, sun exposure can cause the appearance of marks on the skin. Use of essential oils is definitely not recommended during pregnancy and breastfeeding, except if medically prescribed (Rubel, 1998).

Materials and Methods

Plant Materials

All the essential oils were Nepalese origin and were purchased from different suppliers in Katmandu, Nepal. These essential oils were confirmed with standards oils by CO- TLC.

Acute Oral Toxicity Test

The guidelines for Testing of Chemicals, Acute Oral Toxicity Acute Toxic Class Method 423 of the Organization for Economic Cooperation and Development (OECD), was used. The toxicity of substances was settling several classes as: not classified, dangerous, toxic, very toxic, and highly toxic as shown in Table 1.

Twelve hours before starting the study food was suspended while the body weigh was monitored moments before the administration of the oil. Animals were randomly assigned in two groups one was: a control group treated with physiological saline and the other was experimental group treated with the essential oil at dose of 2000 mg/kg of body weight, using an orogastric tube. Clinical observations of animals were performed four times per day, paying attention to behavior, general physical condition, nasal mucosa, changes in skin and fur, respiratory frequency, somatomotor activity,

Table 1: Classification of substances according to the guideline of the Globally Harmonized system of classification and labeling of chemicals (GHS), third edition

S.N.	Ranges (mg/kg)	Category	Classification	Hazard Statement
1	> 2000mg/kg	Category 5	Not classified	May be harmful if swallowed
2	> 300 ≤ 2000mg/kg	Category 4	Dangerous	Harmful if swallowed
3	> 50 ≤ 300mg/kg	Category 3	Toxic	Toxic if swallowed
4	> 5 ≤ 50mg/kg	Category 2	Very toxic	Fatal if swallowed
5	< 5mg/kg	Category 1	Highly toxic	Fatal if swallowed

Table 2: Median lethal dose (LD50) of Essential Oils

S.N.	Essential Oil	LD50 (mg/Kg BW)	Hazard Statement	Remarks
1	Xanthoxyllum Oil	1843	Harmful if swallowed	Death on Next day
2	Wintergreen Oil	1606	Harmful if swallowed	Sudden death
3	Eucalaptus Oil	1664	Harmful if swallowed	Death on Next day
4	Lemongress Oil	1266	Harmful if swallowed	Death on same day
5	Hedychium Oil	>2000	May be harmful if swallowed	No death at 2000mg/kg
6	Sugandhakokila Oil	>2000	May be harmful if swallowed	No death at 2000mg/kg
7	Jatamansi Oil	>2000	May be harmful if swallowed	No death at 2000mg/kg
8	Citronella Oil	500	Harmful if swallowed	lots of urination
9	Anthopogon Oil	1347	Harmful if swallowed	lots of urination
10	Chamomile Oil	1900	Harmful if swallowed	Death on Next day

and possible occurrence of signs such as tremors, convulsions, diarrhea, lethargy, drooling, low response to stimuli, sleep, photophobia, and coma. Palpation of the abdomen was carried out as well. After 48 hours of clinical observation without any signs of toxicity, the experimental group receives 2000 mg/kg of oil. The statistical test applied was "t-Test for independent groups", implemented in the STATISTIC V. 7.0 for Windows; P values <0.05% were regarded as significant. The animals were humanely euthanized at the end of the study.

Results and Discussion

Obtained data (Table 2) concerning the median lethal dose (LD50) of active principle of essential oils revealed that the doses of the LD50 were 1843, 1606, 1664, 1266, >2000, >2000, >2000, 500, 1347 and 1900mg/kg b.w. of Xanthoxyllum Oil, Wintergreen Oil, Eucalyptus Oil, Lemongress Oil, Hedychium Oil, Sugandhakokila Oil, Jatamansi Oil, Citronella Oil, Anthopogon Oil and Chamomile Oil respectively, for mices under environmental conditions. Obtained data revealed that Citronella Oil is moderately hazardous, while Xanthoxyllum Oil, Wintergreen Oil, Eucalyptus Oil, Lemongrass Oil, Anthopogon Oil and Chamomile Oil are only slightly hazardous. The obtained result indicates that the Hedychium Oil, Sugandhakokila Oil and Jatamansi Oil could be considered safe, might be less harmful orally, showing no in vivo toxicity.

The toxicity of essential oils varies according to their composition, which itself varies with the plant, which itself may vary with the soil where it grows (chemotype). Their composition may be ascertained with precision by gas chromatography. For example, the essential oil of the Salvia officinalis L. leaf is richer in toxic thujone in Estonia than in other parts of Europe (Raal, 2007). Toxicity varies according to the period of the year where the plant is harvested (Murbach, 2006; Amin, 2007). It varies with the route of administration (oral, cutaneous or airborne), with the general health of the exposed person (penetration and toxicity are maximized by damaged skin) and with eventual additives associated with the oil (surface active potpourris for instance) (Richardson, 1999). It varies according to the species of the recipient and its level of development.

First aid measures for ingestion of significant amounts of particularly toxic essential oils (such as more than 2mL of high-cineole Eucalyptus oils in young children) is straightforward: take the child to the nearest hospital emergency room or at least call or a Poisons Information Centre for instructions. The vast majority of accidental essential oil ingestion in children result in few, if any symptoms and resolve safely with no medical intervention (Webb, 1993).

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References

Abad, M.J., Bedoya, L.M., Apaza, L. & Bermejo, P. (2012). The *Artemisia* L. Genus: A review of bioactive essential oils. *Molecules*, *17*, 2542-2566.

Amin, G., Sourmaghi, M.H., & Jaafari, S.(2007). Influence of phonological stages and method of distillation on Iranian cultivated Bay leaves volatile oil. *Pak J Biol Sci*, *10*, 2895-9.

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils - A review. *Food Chem. Toxicol*, *46*, 446–75.

Hammer, K.A., Carson, C.F. & Riley, T.V. (1999). Antimicrobial activity of essential oils and other plant extracts. *J. Appl. Microbiol*, *86*, 985–900.

Henry, J. A., & Cassidy, S. L. (1998). Acute Non-Specific Toxicity NDPSC Working Party on Essential Oils Toxicity monographs.

Ibrahim, M.A., Kainulainen, P., Aflatuni, A., Tiilikkala, K. & Holopainen, J.K. (2001). Insecticidal, repellent, antimicrobial and phytotoxicity of essential oils: with special reference to limonene and its suitability for control of insect pests. *Agricultural and Food Science, 10*, 243-259.

Murbach Freire, C.M., Marques, M.O.M. & Costa, M. (2006). Effects of seasonal variation on the central nervous system activity of *Ocimum gratissum* L. essential oil. *J Ethnopharmacol*, *105*, 161-6.

NDPSC. (1998), Compilation of Poisons Information Centre Reports Working Party on Essential Oils Toxicity monographs.

Raal A., Orav A. & Arak E. (2007). Composition of the essential oil of *Salvia officinalis* L. from various European countries. *Nat Prod Res, 5*, 406-11.

Ramakrishna, A. & Ravishankar, G.A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling & Behavior*, *6*(11), 1720-1731.

Rehman, R., Hanif M.A., Mushtaq Z. & Al-Sadi, A.M. (2015). Biosynthesis of Essential Oils in Aromatic Plants: A Review. *Food Reviews International*, *32*(2), 117-160.

Richardson, JA. (1999). Pots pourris hazards in cats. *Vet Med*, *4*, 1010-2.

Rubel, D.M., Freeman, S. & Southwell, I.A. (1998). Tea tree oil allergy: What is the offending agent? Report of three cases of tea tree oil allergy and review of the literature. *Aust. J. Dermatol*, *39*, 244–47.

Rutherford, T., Nixon, R., Tam, M. & Tate, B. (2007). Allergy to tea tree oil: retrospective review of 41 cases with positive patch tests over 4.5 years. *Aust. J. Dermatol*, *48*, 77-83.

Webb, N. J. & Pitt, W. R. (1993). *Eucalyptus* oil poisoning in Childhood: 41 Cases in SE Queensland. *J. Paediatr. Child Health*, 368-371

Wei, A. & Shibamoto, T. (2010). Antioxidant/ Lipoxygenase Inhibitory Activities and Chemical Compositions of Selected Essential Oil. *J. Agric. Food Chem*., *58*, 7218-7225.