Determination of Lethal Doses of Volatile and Fixed Oils of Several Plants

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Objective: **To determine lethal doses of fixed and/or volatile oils extracted from leafs or fruits of *Pimpinella anisum, Foeniculum vulgare, Sesamum indicum, Eugenia caryophyllata, Nigella sativa, Urtica pilulifera, Apium graveolens, Cuminum cyminum, Coriandrum sativum* and *Thymus fallax.***
Method: **Swiss albino mice were injected intraperitoneally with different concentrations of the extract and results were evaluated with the method of probit analysis.**
Results: **Maximum volume of oil administered to mice was kept below 0.5 ml. The oils of *Sesamum indicum* and *Urtica pilulifera* was completely non-lethal even at doses reaching 12.8 ml/kg and considered non-toxic.**
Conclusion: **Lethal doses were determined for all other plants and LD_1, LD_{10}, LD_{50}, LD_{90} and LD_{99} values were given.**

Key words: *Volatile oil, fixed oil, lethal doses, plants*

There is an increasing interest towards medical plants and their active ingradients since 1980's. Some of the underlying motives can be listed as follows:

· Countries lacking developed chemical industries are searching for affordable treatment modalities by using their own plant sources.

· Synthetic drugs show off their dangerous side effects by time but medical plants have generally centuries-long use and little unknown side effects.

· Many commonly used drugs such as steroidal compounds, atropa alkaloids, digitalis glycosides, narcotic alkaloids and ergot alkaloids can be purified from plants much more economically compared to synthetic production.

· Medical plants have multiple actions whereas synthetic drugs have usually only one.

· Additional drugs like vitamins are usually needed to prevent side effects of synthetic drugs. Plant-derived drugs do not necessiate such polypharmacy (1).

Pharmacologic effects of medical plants are investigated by several methods. Appropriate dose of plant extract should be determined by preliminary studies of acute toxicity. Such studies are essential to prevent any overdose of drug which may interfere with results of experiment. They're also helpful in understanding toxicity profiles of plant extracts.

Mice are the preferred animals in toxicity trials as they're widely available and affordable. In these studies plant extracts are administered intraperitoneally (ip) or orally (po) in varying doses. Number of animals in each dose group differs; 5 (2), 10 (2) or 40 (4) aminals can be used. Considering use of 4 dose levels at least, total number of animals needed can be calculated as 20 to 160. Acute toxicity trials are among the highest animal-killing studies since some or all of the animals are to be sacrificed.

We previously performed the acute toxicity trials of many plants and investigated their pharmacological effects. In this article we collected all of our previous lethal- dose studies and presented our findings to help to spare both time and animal life by exchange of information.

In this manuscript lethal doses of fixed or volatile oils exctracted from seeds or leaves of the plants *Pimpinella anisum* L. (Anis seed, anason), *Foeniculum vulgare* Mill. (Fennel seed, rezene), *Sesamum indicum* L. (Sesame oil, susam), *Eugenia caryophyllata* Myrtaceae (Clove, karanfil), *Nigella sativa* L. (Nigella seed, çörek otu), *Urtica pilulifera* L. (Common nettle, 1sırgan otu), *Apium graveolens* L. (Celeriac seeds, kereviz), *Cuminum cyminum* L. (Cumin seed, kimyon), *Coriandrum sativum* L. (Coriander fruit, kişniş) and *Thymus fallax* F. (Thyme herb, kekik) are presented.

Material and Method

Animals

Swiss albino mice (18-24 g) were used in these experiments. The animals were housed in standard cages with food and water ad libitum, at room temparature (20 ±2 ^{0}C) with artificial light from 07 ^{00} am to 07 ^{00} pm. The animals kept under controlled environment following the standard operating procedures of the animal house facility of the Faculty of Medicine (Yüzüncü Yıl University), and provided with pelleted food (Van Animal Feed Factory, Van-TURKEY). The approval of Animal Ethics

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Committee was obtained.

Materials

Pimpinella anisum seeds, *Foeniculum vulgare* seeds, *Sesamum indicum* seeds, *Eugenia caryophyllata* leaves, *Nigella sativa* seeds, *Urtica pilulifera* seeds, *Apium graveolens* seeds, *Cuminum cyminum* seeds and *Coriandrum sativum* seeds used were purchased from a herbal drug store from Van in Turkey. Flowering plants of *Thymus fallax* F. were collected from the vicinities of Özalp-Van (VANF Nr. 5889). Taxonomic identity of the plant was confirmed by Dr. Fevzi Özgökçe, a plant taxonomist in the Department of Biological Sciences, Faculty of Art and Science, Yüzüncü Yıl University, Van, Turkey. The plant specimens with their localities and other required field records were noted and coded. They were pressed, dried according to herbarium techniques and identified by Flora of Turkey (7). All of the plant specimens were kept at the laboratory of Pharmacology, Faculty of Medicine, Yüzüncü Yıl University.

Essential Oil (EO) Extraction of the Plant Material

Dried leaves or seeds were ground in electrical mill and boiled in Clevenger (İldam, Turkey). The volatile oil (essential oil) collected in the instrument was taken into tubes and productivity for volatile oil was calculated.

Fixed Oil (FO) Extraction of the Plant Material

The seeds of the plants were ground in a mixer. Ground plant material was macerated with diethyl ether for 2 hours. The solvent was evaporated (Büchi RE 111 rotavapor and Büchi 461 water bath, Switzerland). The content of the fixed oil of the seeds was calculated.

Acute toxicity study

Male and female Swiss albino mice were randomly assigned to 7 groups with 8 animals in each group. First group was treated with normal saline and considered as control and the other six groups were treated with herb extract given intraperitoneally (i.p.) in increasing dosages of 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 ml/kg body weight. Maximum volume of oil administered to mice was kept below 0.5 ml. The mortality in each cage was ssessed 72 h after administration of extract. The percentage mortalities were converted to probits. Regression lines were fitted by the method of least squares and confidence limits for the LD_1, LD_{10}, LD_{50}, LD_{90} and LD_{99} values were calculated by the method of Litchfield and Wilcoxon and Kouadio et al (5,6).

Results

The Yields of the Extracts

The yields of the plant extracts are showed Table I. The yield of the essential oil of *Nigella sativa* L. was the lowest among the plants. The yield of the fixed oil of *Sesamum indicum* L. was the highest.

The Lethal Dose Levels of the plant extracts

The lethal dose (LD) levels of the plant extracts are showed Table 2. The fixed oils of *Sesamum indicum* and *Urtica pilulifera* was completely non-lethal even at doses reaching 12.8 mL/kg and considered non-toxic. The essential oil of *Nigella sativa* L. was the most toxic extract.

Table I. The yields of the essential oil or fixed oil exracts of the plants.

Essential or Fixed Oil Extract	% Yield
Apium graveolens (EO)*	0.96
Apium graveolens (FO)*	4.40
Coriandrum sativum (EO)	0.27
Coriandrum sativum (FO)	1.85
Cuminum cyminum (EO)	1.50
Cuminum cyminum (FO)	3.50
Eugenia caryophyllata (EO)	1.06
Eugenia caryophyllata (FO)	6.50
Foeniculum vulgare (EO)	1.00
Foeniculum vulgare (FO)	10.00
Nigella sativa (EO)	0.40
Nigella sativa (FO)	22.00
Pimpinella anisum (EO)	1.43
Pimpinella anisum (FO)	14.00
Sesamum indicum (FO)	42.00
Thymus fallax (EO)	1.60
Urtica pilulifera (FO)	24.00

**EO: Essential oil.*
**FO: Fixed oil.*

Discussion

In this study lethal doses and yields of fixed oil or volatile oil exctracted from seeds or leaves of the plants *Pimpinella anisum* L., *Foeniculum vulgare* Mill., *Sesamum indicum* L., *Eugenia caryophyllata* Myrtaceae, *Nigella sativa* L., *Urtica pilulifera* L., *Apium graveolens* L., *Cuminum cyminum* L., *Coriandrum sativum* L. and *Thymus fallax* F. are presented.

The volatile oils were more toxic than the fixed oils while the fixed oils of some plants like *Sesamum indicum* L. and *Urtica pilulifera* L. were completely non-toxic as seen in Table 2. Fixed oils are formed by the esterification of fatty acids with glycerine. By that means different fixed oils are formed from the essentially similar compounds (linoleic acid, oleic acid, etc.) and these fixed oils like olive oil, corn oil, nut oil etc. constitute some of the basic elements of the human diet. In contrast volatile oils are quite distinct compounds except for those from plants of close kinds. They are called as oil only for their appearance, in fact they have nothing in common with the fixed oils (8). The dissimilarity may explain lower toxicity of the fixed oils compared to the volatile oils.

Determination of the appropriate dose is a very important issue in studies of plant extracts. For instance in an animal study of a behavioral model could get futile if the dose of extract exceeds toxicity limits. Therefore

Table II. The lethal dose levels of the essential oil or fixed oil exracts of the plants.

	Lethal doses (mL/kg)				
Plant extract	LD_1	LD_{10}	LD_{50}	LD_{90}	LD_{99}
Apium graveolens (EO)*	0.706	1.261	2.568	5.228	9.333
Apium graveolens (FO)*	0.789	1.274	2.291	4.120	6.647
Coriandrum sativum (EO)	1.114	1.530	2.257	3.331	4.574
Coriandrum sativum (FO)	4.779	7.568	13.300	23.375	37.016
Cuminum cyminum (EO)	0.162	0.440	0.780	1.121	1.399
Cuminum cyminum (FO)	0.449	0.655	1.039	1.650	2.404
Eugenia caryophyllata (EO)	0.200	0.330	0.613	1.137	1.880
Eugenia caryophyllata (FO)	0.238	0.425	0.863	1.753	3.123
Foeniculum vulgare (EO)	0.449	0.654	1.038	1.648	2.402
Foeniculum vulgare (FO)	1.100	2.270	5.519	13.417	27.680
Nigella sativa (EO)	0.057	0.157	0.542	1.866	5.111
Nigella sativa (FO)	2.517	5.060	11.915	28.058	56.403
Pimpinella anisum (EO)	0.156	0.334	0.847	2.151	4.596
Pimpinella anisum (FO)	0.886	1.566	3.152	6.340	11.209
Sesamum indicum (FO)			Non-toxic		
Thymus fallax (EO)	0.205	0.365	0.741	1.504	2.679
Urtica pilulifera (FO)			Non-toxic		

**EO: Essential oil.*
**FO: Fixed oil.*

before starting a plant study researchers should determine the dose of extract by referring the previous toxicity trials or do the toxicity workup by themselves. In the latter condition performing a toxicity trial for every new study of the same plant results in loss of a significant number of animal as well as time and money. In conclusion we thought that compilation and review of previous toxicity trials may help other researchers to save their sources.

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