An evaluation of toxicity in essential oils of Geraniol, Geranial acetate, Gingerol and Eugenol in rats
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Abstract
The present study was carried out to investigate the effects of essential oils geraniol, geranial acetate, eugenol and gingerol on biochemical parameters in rats. Male rats were treated with 5 mL/Kg/day of essential oil in ethanol as vehicle for 14 consecutive days. Rats in the treated groups showed a more pronounced increase in body weight in comparison to control rats after 14 days and the biochemical (glucose, urea, creatinine, SGOT and SGPT) were observed and the rat was sacrificed and its stomach was taken out, opened and examined for macroscopic haemorrhagic gastric lesions. The results suggested that the biochemical parameters geraniol and geranial acetate caused a significant increase in the levels of Glucose and Urea whereas there is a decreased level of glucose and urea in Gingerol and Eugenol and The level of creatinine differ slightly between the rats of control and treated groups and no haemorrhagic lesions were found.

Keywords: Geraniol, Geranial acetate, Gingerol, Eugenol, Biochemical parameters

Introduction
In recent years there has been an increasing interest in the use of natural substances. Essential oils, odors and volatile products of plant secondary metabolism, have a wide application in folk medicine as well as in fragrance industries. Essential oils are complex natural mixtures of volatile secondary metabolites, isolated from plants by hydro or steam-distillation. The main constituents of essential oils, for example, monoterpenes and sesquiterpenes and phenylpropanoids including carbohydrates, alcohols, ethers, aldehydes and ketones, are responsible for the fragrant and biological properties of aromatic and medicinal plants[9]. Various essential oils and their components possess pharmacological effects, demonstrating anti-inflammatory, antioxidant and anti-carcinogenic properties [6]. In addition to inducing resistance, antibiotics are sometimes associated with opposing effects such as hypersensitivity, immune-suppression and allergic reactions [1]. Therefore, there is a need to develop alternative drugs for the treatment of infectious diseases [2]. Various essential oils and their components have been shown to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties [3,8]. Some oils has shown to possess anti-carcinogenic properties [10]. Some other oils have been used in food preservation [5], aromatherapy [4] and fragrance industries [11]. Essential oils are a rich source of biologically active compounds. Also, the resurgence of interest in natural therapies and increasing consumer demand for effective, safe, natural products means that quantitative data on plant oils and extracts are required.

The main advantage of essential oils is that they can be used in any foods and are considered generally recognized as safe (GRAS) as long as their maximum effects is attained with the minimum change in the organoleptic properties of the food [7]. The present work evaluated the oral toxicities of essential oils in male rats and the results obtained from this study will provide the safety information of this oils before processing for further studies on natural therapies.

Materials and Methods

Essential oil compounds

Four essential oil compounds obtained from sigma & co (P) Ltd, India (commercial producers of plant essential oils and aromatic substances) were used in this study. Quality of the oils was ascertained by GC to be more than 98% pure.

Animals

Male rats (10 weeks old and between 220 and 260 g in body weight) were used for acute toxicity and pharmacological studies. The animals were maintained at room temperature and fed with standard pellet diet (Lipton India Ltd.) and tap water, ad libitum.
The studies were approved by the Institutional Animals Ethical Committee.

**Subacute toxicity**

The animals were divided randomly into six groups were treated with 5mg/kg for 14 consecutive days, while the control rats received distilled water only. Toxicity signs and mortality were monitored daily, whereas body weight changes, and food and water consumptions were monitored weekly. At the end of the study, animals were fasted overnight, anesthetized with mild ether anaesthesia, and the blood was taken from tail. The heparinised blood samples were used for determining haematological parameters. Meanwhile, the non-heparinized tube was used for blood glucose determination. Following, the rats were sacrificed and stomach was removed and examined for macroscopic hemorrhagic gastric lesions.

**Chemicals**

Kits for glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), glucose, urea, uric acid, creatinine used for biochemical studies were supplied by Trace Scientific, Melbourne, Australia.

**Body weight analysis**

The body weight of each rat was assessed using a sensitive balance during the acclimatization period, once before commencement of dosing, once weekly during the dosing period and once on the day of sacrifice.

**Mortality and clinical signs**

During the dosing period, the rats were observed for clinical signs of acute toxicity or stress. They were daily observed for overt signs of toxicity or stress during the period of treatment.

**Preparation of Test Serum**

The animals were exsanguinated under mild ether anaesthesia and blood samples were drawn from the tail of each animal. The samples were collected in plastic test tubes and allowed to stand for 3 h to ensure complete clotting. The clotted blood samples were centrifuged at 3000 rpm for 10 min and clear serum samples were aspirated off and stored frozen.

**Biochemical analysis**

In biochemical analysis, on the 14th day of the post-treatment, blood was taken out from the tail under mild ether anaesthesia. The serum was separated out. The following parameters were determined colorimetrically by employing the standard ready-to-use kits Glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), glucose, urea, uric acid, creatinine. The manufacturer’s instructions for each biochemical parameter were strictly followed in the course of the investigations.

**Results and Discussion**

**Subacute toxicity Studies**

The rats showed no clinical signs of acute toxicity or stress. There were no changes in the nature of stool, urine and eye colour of all the animals. No mortality was observed in the rats that received essential oils orally after 72 hours.

**Weekly body weight**

There were variable changes in the body weight of rats in all the groups. The control rats gained weight throughout the duration of treatment and the treated rats showed a slight increased weight in the duration of treatment.

**Table-2 Macroscopic hemorrhagic gastric lesions in animals tested for toxicity with essential oil compounds**

<table>
<thead>
<tr>
<th>Source of essential oil</th>
<th>Haemorrhagic Gastric lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geraniol</td>
<td>Negative</td>
</tr>
<tr>
<td>Geranial acetate</td>
<td>Negative</td>
</tr>
<tr>
<td>Gingerol</td>
<td>Negative</td>
</tr>
<tr>
<td>Eugenol</td>
<td>Negative</td>
</tr>
</tbody>
</table>

**Haemorrhagic and Biochemical Changes**

The Glucose content showed a increase in Geraniol and Geraniol acetate and a sight decrease is seen in Gingerol and Eugenol compared to the control (figure 1)
Figure 1 Analysis of Glucose content (mg/ml) in rats
The Urea content showed a slight increase in all the essential oils (figure 2).

Figure 2 Analysis of Urea content (mg/ml) in rats
The Creatinine content showed an increase in Gingerol whereas there is a steady decrease in Geraniol and Eugenol and Geranial acetate showed a constant result similar to control (figure 3).
Figure 3 Analysis of Creatinine content (mg/ml) in rats
In SGOT and SGPT content there was a steady increase in all the essential oils (Figure 4 and 5).

Figure 4 Analysis of SGOT content (mg/ml) in rats
Conclusion

The essential oil Geraniol, Geranial acetate, Eugenol and Gingerol could be categorized as NOAEL crude drug, as it acts harmlessly under the current normal usage, and this phenomenon is considered to be of no toxicological concern. However, this is the first study to investigate the toxicity of essential oil in rats, and a subchronic toxicity test should also be conducted to establish the adverse effects of a repeated response of essential oil.

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