Research Article

Evaluation of acute toxicity of the methanolic extract of Tanacetum parthenium L. in albino wistar rats

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Abstract

Tanacetum parthenium L. commonly known as feverfew and it is belongs to the family Asteraceae. Toxicology may be defined as the study of harmful, poisonous and adverse effects of drugs and other chemicals constituents found in plants, which may increase the chances of mortality or weakness in the general health, physically as well as mentally. The present study has been undertaken to study the adverse or hazardous effects of methanolic extract of T. parthenium and to establish the safety of methanolic extract of T. parthenium in albino wistar rats (180-200g) as per Organization for Economic Cooperation and Development (OECD) guidelines. In acute toxicity study, the oral dose (1000, 2000, 3000 and 4000 mg/kg) of tested plant extract was administered to four groups of animals (GROUP I, II, III and IV) in single dose and for seven days their general behavior, adverse effects and mortality were monitored. In acute toxicity, all treated groups revealed neither mortality nor any significant changes were observed. The result indicates that the oral administration of methanolic extract of T. parthenium plant did not produce any significant toxic effect in albino rats. Hence, the extract can be utilized safely for therapeutic use in pharmaceutical formulations.

Keywords: Tanacetum parthenium, Asteraceae, OECD, acute toxicity.

INTRODUCTION

During the past few decades, traditional system of medicine has received marvelous attention for in vivo studies [1]. Toxicology is the important part of pharmacology which deals with the undesirable effect of phytochemicals on living organisms previous to the use as drug or chemicals in clinical use [2]. Several studies are concentrated on toxicity analysis so as to determine the safety of medicinal plants and their products. Toxicity analysis is essential, as some herbs consumed might have some toxic effects and many reports have been published for toxicity caused due to long term consumption of herbs. The occurrence of toxicity mechanism could differ depending on the cell membrane and chemical properties of the toxicants in human beings. It might happen within the cell membrane or on the cell surface or tissue underneath as well as at the extracellular matrix. According to OECD guidelines, in order to ascertain the protection and effectiveness of a new drug, toxicological studies are extremely significant in animals like mice, rat, guinea pig, dog, rabbit, monkey etc. Toxicological studies aid to extend decision whether a new drug must be adopted for clinical use or not. OECD guidelines such as 401, 423 and 425 do not permit the use of drug clinically without its clinical trial as well as toxicity studies [3].

Depending on the period of drug exposure to animals, toxicological determination could be three types such as acute, sub-acute and chronic toxicological studies. The acute toxicity test in which a single dose is used in each animal on one occasion only for the determination of gross behavior and also LD50 or median lethal dose. The chronic tests in which two species, one rodent and one non rodent are dosed daily for complete six months. The sub-acute tests wherein animals (typically rats and dogs) are dosed daily, beginning at around expected therapeutic level and increasing stepwise every two to three days until toxic symptoms are observed [4].

T. parthenium is a perennial herb belongs to family Asteraceae which is commonly known as feverfew and it is distributed all over the world [5]. The leaves of this plant are eaten raw or used as infusions in conditions like arthritis, migraine and asthma. They are also used for treating various problems in different situations such as tinnitus, vertigo, fever, menstrual disorders, and difficulty in labour, stomach ache, tooth ache and insect bites [6]. According to recent studies, essential oils and extracts of feverfew have anti-inflammatory [7], antioxidant [8], antiviral [9] and anticancer properties [10]. Feverfew also inhibits...
cyclo-oxygenase and arachidonic acid formation\textsuperscript{[11]} and decreases serotonin liberation by collagen and ADP induced platelet aggregating agents in cardiovascular diseases\textsuperscript{[12]}. 

In order to assess the toxic nature of a bioactive compounds present in the plant extract, acute oral toxicity is the first step to be carried out\textsuperscript{[13]}. Acute toxicity testing involves the estimation of lethal dose, the dose that kills 50\% of the tested group of animals. In the present investigation, as a part of safety evaluation, acutetoxic effects of methanolic extract prepared from\textit{T. parthenium} has been studied in albino wistar rats. 

**EXPERIMENTAL**

**Collection and identification of plant**

Fresh leaves of\textit{Tanacetum parthenium} \textit{L.} were collected from kodaikanal hills, Tamil Nadu, India. The taxonomic identity of the plant was confirmed by Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu. The plant materials were rinsed thoroughly under running tap water and then with distilled water to eradicate the surface pollutants. After, the leaves were air dried under shade condition. The dried leaves were powdered and stored at 4°C.

**Preparation of plant extract**

Accurately weighed powder (5g) of leaves of \textit{T. parthenium} was taken and a thimble pack was made using filter paper. The crude drug in the thimble was extracted with 100 ml of methanol in a continuous extraction using Soxhlet system for 24 hours. Afterwards, the extract was filtered using Whatmann No. 1 filter paper. Then the filtrate was evaporated and dried and then used for acute toxicity test.

**Acute oral toxicity test**

**Experimental animals**

Acute oral toxicity test was performed to determine the LD\textsubscript{50} value of methanolic extract of \textit{T. parthenium}. Experiments were carried out using healthy young adult albino wistar rats weighing 180-200 g. The Institutional Ethical Committee of KMCH College of Pharmacy, Coimbatore, Tamil Nadu, India approved the protocol for these experiments under number KMCRET/Ph.D/14/2014-2015.

**Assignment of animals**

The animals were randomly divided into four groups each containing six rats. They were identified by the markings using a yellow stain. In each group, except a single rat (control), the others were marked on head (GROUP I), body (GROUP II), tail (GROUP III) and head and body (IV) to ease the observation.

**Housing and Diet**

The animals were accommodated in polypropylene cages (55 x 32.7 x 19 cm) with sawdust litter and maintained the temperature of 23 ± 2°C. Lighting was regulated to supply 12 hours of light and 12 hours of dark for each 24 hours period. Each cage was recognized by a card. This card reveals the cage number, number and weight of the animals it contained, test substance code, route of administration and dose level. The animals were fed with standard laboratory animal food pellets with water \textit{ad libitum}.

**Mode of administration and symptoms recorded during study**

Acute oral toxicity studies were performed according to OECD. Albino wistar rats (n = 6/each dose) selected by random sampling technique were used in this study. The animals were fasted for 12 hours with free access to water only. Following the period of fasting, animals were weighed and test extract was administrated orally at a dose of 1000, 2000, 3000, and 4000 mg/kg. After the administration of test extract, food for the animals were withheld for 2 hours. The mortality and clinical signs which included changes in skin, fur, eyes and mucous membranes were noted for the first 4 hours subsequently for 72 hours and thereafter for 7 days of test drug administration. For complete 7 days, the gross behaviors like body positions, locomotion, rearing, tremors and gait were observed and also the effect of plant extract on grip strength, pain response and righting reflex were noted. In addition, the intake of food and water behavior was monitored.

**RESULTS**

Acute toxicity determination is a method for assessing acute oral toxicity that involves the recognition of a dose level that causes mortality. The dose limits were selected on the basis of oral acute toxicity studies in rats according to OECD guidelines. The acute toxicity test was carried out in 24 rats by giving different doses of methanolic extract i.e. 1000 (Group I), 2000 (Group II), 3000 (Group III) and 4000 (Group IV) mg/kg body weight. Parameters such as alertness, grooming, restlessness, touch response, torch response, pain response, tremors, convulsion, righting reflex, gripping, pinna reflex, corneal reflex, writhing, pupils, urination, salivation, skin color, lacrimation, food intake, water intake and mortality were observed (Table 1). All groups of animals showed neither any toxic effect, nor any lethal effect. The present study shows administration of dose up to 4000 mg/kg did not reveal any signs of toxicity or mortality in rats during the entire observation period. Therefore, LD\textsubscript{50} of extract may be considered to be greater than 4000 mg/kg.

**Table 1: Effect of methanolic extract on acute oral toxicity test in Albino Wistar rats**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Response</th>
<th>Before treatment (1000 mg/kg)</th>
<th>After treatment (1000 mg/kg)</th>
<th>After treatment (2000 mg/kg)</th>
<th>After treatment (3000 mg/kg)</th>
<th>After treatment (4000 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alertness</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>Grooming</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>3</td>
<td>Restlessness</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>4</td>
<td>Touch response</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>5</td>
<td>Torch response</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>6</td>
<td>Pain response</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>7</td>
<td>Tremors</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
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</tbody>
</table>
REFERENCES


DISCUSSION

Toxicology tests are used to observe products such as individual compounds, mixture of compounds, crude extract, pesticides, medications, food additives, packing materials or their chemical ingredients. World health organization (WHO) recommends that medicinal herbs would be the dominant source to obtain a range of drugs. Therefore, such medicinal plants must be investigated for better understanding of their medicinal properties, safety and effectiveness.[14]. Safety of plant extract is evaluated mostly by acute oral toxicity analysis. In the present study, even a higher dose of plant extract i.e.4000 mg/kg did not show any signs of toxicity or mortality for animals. Thus, plant extract even at 4000 mg/kg may be considered as safe. This observation is agreed with Pooja et al. (2016) [15] who assessed acute and subacute toxicity of aqueous and ethanol extracts of this plant using two concentrations i.e 1000 mg/kg and 2000 mg/kg and they reported no behavioural changes and no mortality was observed in animals when used both the concentrations.

CONCLUSION

The non toxic nature of methanolic extract prepared from feverfew plant was confirmed by acute oral tacility test conducted as per the OECD guide lines. The normal behavior of animals during the observation of seven days suggests the safety and harmless nature of methanolic extract even up to 4000 mg/kg body weight of animals. Further studies are warranted including sub acute and chronic toxicological evaluations to confirm the safe use of this extract.

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