Toxicity Analysis of different medicinal plant extracts in Swiss Albino Mice


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Abstract

Plants and their derivatives played a key role in world health and have long been known to possess biological activity. The importance of biological evaluations of plants derived agents used in the treatment of various diseases. The data of the acute toxicity studies (oral) on medicinal plants obtained in order to increase the confidence in its safety to human, particularly for use in the development of pharmaceuticals. The aim of this work was to study the effects of repeated dosage of two concentration of plant extracts such as 400mg/kg and 600mg/kg were administered to Swiss albino mice daily for 15 days (orally) to check the safety profile of plant extracts. Mice were randomly selected and each group contains three animals, and analyse the physical parameters, biochemical parameters and liver toxicity were studied at the end of the experiment and analyse the histopathological studies to done for 400mg/kg of group animals assess any organ specific toxicity. From the study there was no changes were observed in liver section when compared with treated and control groups.

Keywords: Plant Extracts, Toxicity, Swiss Albino Mice, AST, ALT, Histopathology.

Introduction

Traditional medicines derive the scientific heritage from rich experiences of early civilization [1]. Plants are the source of medication for preventive, curative, protective or promotive purposes [2]. Plant derived foods help in the prevention of lifestyle associated diseases. Several groups of constituents in plants have been identified as potentially health promoting in animal studies, including cholesterol lowering factors, antioxidants, enzyme inducers and others [3]. A thousand years ago an extensive use of plants as medicines have been reported and were initially taken in the form of crude drugs and other herbal formulations[4]. Toxicology is the important aspect of pharmacology that deals with the adverse effect of bio active substance on living organisms prior to
the use as drug or chemical in clinical use [5]. As per the OECD guidelines, in order to establish the safety and efficiency of a new drug, toxicological studies are very essential in animals like mice, rat, pig, dog, rabbit, monkey etc under various conditions of drug. Toxicological studies help to make decision whether a new drug should be adopted for clinical use or not. OECD 401, 423 & 425 does not allows the use of drug clinically without its clinical trial as well as toxicity studies. Depending on the duration of drug exposure to animals toxicological studies may be three types such acute, sub-acute and chronic toxicological studies.

Plants or drugs must be ensured to be safe before they could be used as medicines. A key stage in ensuring the safety of drugs is to conduct toxicity tests in appropriate animal models, and acute toxicity studies are just one of a battery of toxicity tests that are used [6]. Acute toxicity refers to the adverse effects that occur on first exposure to a single dose of a substance. Separate tests are needed to detect the effects of contact with the skin and eye (corrosion, irritancy and sensitisation; topical or local toxicity)

The main aim of our study was to evaluate the extracts for their toxic effects before it can be used for applications that are of importance to the public. Hence the pet ether extract, chloroform extract and methanol extract of plants were analyzed for their acute toxicity profile with reference to behavioural aspects, in Swiss Albino mice. The limit test dose of 400mg/kg and 600mg/kg of body weight was used following OECD guidelines [7] [8].

Materials and Methods

Preparation of drugs

One kg of leaves were dried at room temperature and powdered. The fine powder (200g) was extracted by using different solvents. After extraction, each extract was air dried in vacuum evaporator and resuspended in water before use.

Animal models

The experiment was conducted on healthy Swiss albino mice (male) weighed 25g to 30g with 8 to 10 weeks were obtained from the Animal House. The mice were separated into groups. The experimental procedures relating to the animals were authorized University Ethical committee before starting the study were followed under the internationally accepted principles for laboratory animal use and care.

Acute Toxicity Assay

The mice were housed in cages and randomly selected ones were tagged and marked on the tail for individual identification. All mice were maintained on a 12-h light/dark cycle and located at room temperature approximately 23°C with constant humidity. They were allowed to acclimatize to laboratory conditions for a week before starting the experiment. Drinking water and food were provided ad libitum throughout the experiment period. The acute oral toxicity extract of five medicinal plants extracts was evaluated in mice according to the procedures outlined by the Organization for Economic Co-operation and Development (OECD) [9]. A different dose of 400mg/kg, and 600mg/kg of crude extract was administered to mice in the treatment groups by the oral route. The crude extract was suspended in a vehicle (distilled water). Following the fasting period, body weight of the mice were determined and the dose was calculated in reference to the body weight as the volume of the extracts solution given to the mice is 10 mL/kg. Other mice were allotted distilled water and were regarded as the control groups. Food was provided to the mice approximately an hour after treatment. The mice were observed in detail for any indications of toxicity effect within the first six hours after the treatment period, and daily further for a period of 15 days. Surviving animals were weighed and visual

Plant material Used

*Clitoria ternatea* belongs to family - Fabaceae (*Clitoria ternatea* Blue and White leaf extract).*Solanum nigrum* belong to Solanaceae family (*Solanum nigrum* Blue berries and Red berries leaf extracts). *Aloe vera* belongs to family – Liliaceae.
observations for mortality, behavioral pattern, changes in physical appearance, injury, pain and signs of illness were conducted daily during the period.

**Mode of administration**
The test substance was administered in a single dose by oral gavage (18 gauge).

**Experimental Design**
Group I: Control (Water)
Group II: *Clitoria ternatea* Blue leaf extract (400mg/kg, 600mg/kg)
Group III: *Clitoria ternatea* white leaf extract (400mg/kg, 600mg/kg)
Group IV: *Solanum nigrum* blue berries extract (400mg/kg, 600mg/kg)
Group V: *Solanum nigrum* Red berries extract (400mg/kg, 600mg/kg)
Group VI: *Aloe vera* extract (400mg/kg, 600mg/kg)

**Signs recorded during acute toxicity studies**
Direct observation parameters include touch response, salivation and skin color.

**Experimental animals observation throughout the period of study**
(i) Body Weight: Weight of each mice was recorded on day 0 to day 15.
(ii) Food and water Consumption: Food and water consumed per animal was calculated for control and the treated dose groups.
(iii) Clinical signs: All animals were observed daily for clinical signs or symptoms.
(iv) Mortality: All animals were observed twice daily for mortality during entire of study.

**Biochemical Parameters analysed**
Biochemical parameters were performed in serum. Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase activities (SGPT), serum levels of alkaline phosphatase (ALP), cholesterol, triglycerides, total protein and total albumin levels were estimated.

**Histological Examination of Liver**
At the end of treatment, animals were sacrificed and liver were collected for histological examinations. The organs were immediately fixed in 10% formalin and processed for histology with H&E staining.

**Result and Discussion**
The present study conducted as per the OECD guidelines 423 revealed that the extracts did not produce any mortality throughout the study period of 15 days even when the limit dose was maintained at 400mg/kg and 600mg/kg of body weight. So, testing the extracts at a minimum dose were practically non-toxic. **Table 1** indicates the direct parameters observed before and after the administration of the test substance for the five different plant extracts. The results of current study showed no adverse changes in physical parameters throughout the dosing period. There was no significant change in the mean body weight of the animals in treated groups as compared to vehicle treated control group at the end of treatment (Table 2). The food and water intake of all the treated groups were comparable to control group without having significant alteration in body weight and growth rate. We did not find any abnormality and behavior of mice treated with plant extracts and compared with control group. There was no significant difference in the initial and final weights between the control and treated mice. 400mg/kg and 600mg/kg of drug treatment in mice for 15 days did not show any significant changes in body weights. The different groups like Normal control, *Clitoria ternatea* Blue extracts (400 and 600mg/kg), *Clitoria ternatea* white extracts (400 and 600mg/kg), *Solanum nigrum* Blue berries (400 and 600mg/kg), *Solanum nigrum* red berries (400 and 600mg/kg), *Aloe vera* (400 and 600mg/kg) treated animals were drawn all the blood, serum and liver analysed for the biochemical parameters shown in **Table 2** and following was further confirmed by histopathological analysis shown in **Figure 1** where the liver section of control and experimental animals showed intact central vein,
well preserved hepatocytes, Sinusoidal veins and vacuoles. No damage was observed in the liver section of treated animals as compared to control animals. Serum analysis were done in all the control and treated groups this data shows no significant changes not observed at dose level in serum glucose, SGPT and SGOT activities in all the treated groups as compared to respective control group. However cholesterol, triglycerides, total protein albumin and alkaline phosphatase level were found elevated at highest dose level of treated group and control groups. Histological examination was done and there were no significant changes were observed in organs of all the treated groups. There was no mortality found till the completion of study.

Table: 1 Direct parameters observed before and after the administration of the test substance

<table>
<thead>
<tr>
<th>Parameter observed</th>
<th>Control</th>
<th>Clitoria ternatea (CBL)</th>
<th>Clitoria ternatea (CWL)</th>
<th>Solanum nigrum (SBB)</th>
<th>Solanum nigrum (SRB)</th>
<th>Aloe vera (AV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>400mg/kg</td>
<td>600mg/kg</td>
<td>400mg/kg</td>
<td>600mg/kg</td>
<td>400mg/kg</td>
</tr>
<tr>
<td>Initial body weight of animals(g)</td>
<td>25± 2</td>
<td>25± 2</td>
<td>28.5±0.2</td>
<td>26±2</td>
<td>27.8±2</td>
<td>25.3±2</td>
</tr>
<tr>
<td>Final body weight of animals(g)</td>
<td>28± 2</td>
<td>28.4±0.2</td>
<td>29.7±0.2</td>
<td>29±0.2</td>
<td>28.5±2</td>
<td>28±0.2</td>
</tr>
<tr>
<td>Food consumption of animal daily(g)</td>
<td>2±0.5</td>
<td>1.5±0.2</td>
<td>1.8±0.2</td>
<td>1.3±0.5</td>
<td>1.9±0.2</td>
<td>1.4±0.2</td>
</tr>
<tr>
<td>Water consumption of animal daily (ml)</td>
<td>3±0.2</td>
<td>5±0.3</td>
<td>3±0.2</td>
<td>4±0.4</td>
<td>5±0.4</td>
<td>4±0.2</td>
</tr>
<tr>
<td>Clinical signs</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Mortality</td>
<td>Not observed</td>
<td>Not observed</td>
<td>Not observed</td>
<td>Not observed</td>
<td>Not observed</td>
<td>Not observed</td>
</tr>
</tbody>
</table>

Conclusion

In the present study, the mice in the control and treated groups were administrated with vehicles and crude extract, respectively. The mice were monitored daily until day fifteen for any toxic signs and mortality. During the 15 days of period acute toxicity evaluation, mice which are orally administrated with different plant solvent extract at two dose of 400 mg/kg and 600mg/kg showed no overt signs of distress, and there were no observable symptoms of neither toxicity nor death. All of the mice gained weight and displayed no significant changes in behavior. Apart from that, the physical appearance such as skin, fur and eyes were found to be normal and whilst the body weight of the mice showed as increase, this indicates that the administration of the crude extract does not posses any toxicity on the growth of the animals. Furthermore, determination of food intake and water consumption is important in the study of safety of a product with therapeutic purpose. The histopathology study of mice liver also showed no toxic effect to animal.
Table 2: Biochemical Parameters

<table>
<thead>
<tr>
<th>Test name</th>
<th>Control</th>
<th>Clitoria ternatea Blue (CBL)</th>
<th>Clitoria ternatea White (CWL)</th>
<th>Solanum nigrum (SBB)</th>
<th>Solanum nigrum (SRB)</th>
<th>Aloe vera (AV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>400mg/kg</td>
<td>600mg/kg</td>
<td>400mg/kg</td>
<td>600mg/kg</td>
<td>400mg/kg</td>
<td>600mg/kg</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>99.47</td>
<td>115.0±0.2</td>
<td>118±0.2</td>
<td>113.9±0.4</td>
<td>124.4±0.4</td>
<td>108.9±0.2</td>
</tr>
<tr>
<td>Triglycerids(mg/dl)</td>
<td>129.30±0.5</td>
<td>145.30±0.2</td>
<td>132±0.2</td>
<td>139.70±0.4</td>
<td>147.8±0.2</td>
<td>133.02±0.2</td>
</tr>
<tr>
<td>Aspartate transaminase (AST) U/L</td>
<td>146.38±0.4</td>
<td>142.60±0.5</td>
<td>148±0.2</td>
<td>135.2±0.52</td>
<td>143±0.4</td>
<td>113.31±0.3</td>
</tr>
<tr>
<td>Alanineaminotransferase (ALT) U/L</td>
<td>62.45±0.2</td>
<td>69.97±0.52</td>
<td>70.24±0.2</td>
<td>58.62±0.52</td>
<td>64.6±0.2</td>
<td>53.07±0.52</td>
</tr>
<tr>
<td>Alkaline phosphate U/L</td>
<td>72.6±0.52</td>
<td>65.3±0.2</td>
<td>70.1±0.2</td>
<td>71.7±0.2</td>
<td>73.5±0.2</td>
<td>97.9±0.2</td>
</tr>
<tr>
<td>Total Protein (g/dl)</td>
<td>7.22±0.5</td>
<td>7.08±0.2</td>
<td>7.38±0.3</td>
<td>6.91±0.2</td>
<td>7.21±0.2</td>
<td>6.09±0.52</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.63±0.2</td>
<td>3.85±0.2</td>
<td>3.92±0.3</td>
<td>4.02±0.5</td>
<td>4.58±0.2</td>
<td>3.22±0.4</td>
</tr>
</tbody>
</table>

Figure: 1 Histopathological images for toxicity study in liver

Control, CBL - Clitoria ternatea Blue leaf extract, CWL - Clitoria ternatea white leaf extract, SBB - Solanum nigrum blue berries extract, SRB - Solanum nigrum Red berries extract, AV - Aloe vera extract
Acknowledgement

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Reference


