Acute toxicity study of ethanolic extract of Apium leptophyllum pers in swiss albino mice

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Histopathology

ABSTRACT

The recent study was planned to determine the acute toxicity study of *Apium leptophyllum pers*. A single dose of 400,1200,2000 mg/kg of ethanolic extract of *Apium leptophyllum pers* according to the OECD guidelines and the dose level was administrated orally into swiss albino mice. Oral administration of maximum level of ethanolic extract of *Apium leptophyllum pers* upto 2000 mg/kg body weight to experimental group of albino mice was evaluated. Biochemical, haematological and histopathological parameters were checked throughout the study. Signs of toxicity, mortality and body weight were monitored for 14 days post treatment of *Apium leptophyllum pers*. There is no substantial variations were noticed in control and treated groups. Result revealed that the ethanolic extract of *Apium leptophyllum* is safe and no toxicity was caused.

INTRODUCTION

The role of natural herbal plants and preparations obtained from them as dietary supplements have become more widely accepted in developing countries. It is essential to evaluate these plants and their preparations to check for adverse effects and enhance the confidence of mankind regarding its safety. The scientific evaluation of these time-tested plant drugs is essential for their use in the development of pharmaceutical products. 80% of the world population is mainly used natural herbal drugs in developing countries for an essential system of human health care (Kamboj, 2000). Recent research provides information about toxicity, isolation of active principle and claimed therapeutic properties. (Caparroz-Assef et al., 2005)

Based on the USA survey report, the usage of natural herbal medicines had increased up to 13% population in 1993 as complementary and other drug sources. (Eisenberg et al., 1998, 1993). Since, the Vedic system of medicine is mainly utilized on a huge level. Now the major concentration of research people to perform safety and efficacy of herbal drug analysis for toxicity studies. (Chen et al., 2009) The legend of toxicology Paracelsus demonstrated the concept of toxicity studies based on the dosage level of herbal drug. The correct dose of natural herbal medicines which distinguishes prescription from a toxin” (Hunter, 2008). Recent trends of the drugs are derived from natural herbal compounds in large numbers. (Rizvi et al., 2009)

*Apium leptophyllum* (Pers) F. Muell, ex Benth belongs to the family Apiaceae (Umbelliferae). It
is a tap-rooted, green leaf and branching herb arrangements. (Asameneew et al., 2008) Ethiopian Traditional Medicine reported that the leaves of *Apium leptophyllum* is an essential role for different clinical conditions identified, such as loss of appetite, inflammation, and sweat. (Asameneew et al., 2008; Park and Sutherland, 1969). Seed part of *Apium leptophyllum* has antispasmodic, anti-inflammatory, antiseptic, analgesic and diuretic properties. (Sharma et al., 2001) The seed extract of *Apium leptophyllum* oil shows potent antifungal activity against *Candida albicans*. The essential oil consists of methyl esters of thymol, thymoquinone, and carvacrol used as carminative. (Khare, 2007)

**MATERIALS AND METHODS**

**Authentication of Plant Material**

Collected dried seed part of the plant *Apium leptophyllum* pers was authenticated from the Botanical Survey of India (BSI) TNAU, Coimbatore. Tamil Nadu. The voucher number is BSI/SRC/ 5/23/ 2019 /Tech/140.

**Preparation of plant extract**

The ethanolic plant extract was prepared by soaking 25gm of *Apium leptophyllum* pers powdered seed sample in 250 ml of ethanol separately and agitated manually, and allowed to extract for 48 hours. The ethanolic extract was then filtered using Whatman No-1 Filter paper. The extract was stored at 5ºC until further processing.

**Animals**

Swiss albino mice were used for acute toxicity studies. Mice weighing 24 - 30 gram (Age 4-6 weeks) were placed in stainless-steel wire cages in a well-ventilated room at temperature 23ºC (± 3º C). Humidity 55± 5% and under 12 hrs Light/dark cycle. The animals were bought from Kovai Medical College of Pharmacy, Coimbatore, and were fed on a standard pelleted diet and water *ad libitum*. Each cage contained 4 to 5 mice of the same gender with bedding of husk. All experimentations were done as per CPCSEA guidelines. [Approval No – SAC / IAE/C/BC/2016/Ph.D-005].

**Acute Toxicity Study and Dose Selection**

An acute toxicity test was performed as per guidelines. The overnight fasted mice were randomly circulated into one control group and experimental groups containing six animals per group. The control group of animals received vehicles alone. Oral administration of the maximum level of ethanolic extract of *Apium leptophyllum* pers 2000 mg/kg of body weight to an experimental group of albino mice. After the administration of the ethanolic plant extract, the experimental mice were monitored constantly for initial 2 hours and 24 hours to find variations and 14 days for any signs of behavioral changes, such as lethargy, sleep, toxicity, mortality, and changes in body weight.

**Biochemical Analysis**

Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP), Glucose, Total protein, A/G Ratio, Cholesterol, Bilirubin, Urea, and Creatinine were quantitatively analyzed by using kit methods.

**Hematological analysis**

Blood specimens from experimental mice were collected in EDTA containing tubes for hematological observations. Hemoglobin (Hb), Total RBC, White Blood Cell (WBC), Granulocytes, and Agranulocytes (Monocytes and Lymphocytes) were evaluated with humalyzer.

**Histopathological Study**

Essential organs such as Liver and Kidney were isolated from sacrificed mice were entrapped with 10% formalin solutions then after processing embedded in paraffin wax. The section of paraffin was made at 10 minutes and stained with eosin and hematoxylin. The slides were observed under a light microscope and taken the expanded images of tissue structure for further analysis.

**Statistical Analysis**

All the experimental results were stated as Mean = SEM. The statistical examination was done using the student –t-test. P<0.05, P<0.01 AND P<0.001 were measured statistically relevant.

**RESULTS AND DISCUSSION**

The results revealed that the group administrated with 2000 mg/kg body weight of ethanolic seed extract of *Apium leptophyllum pers* observed a slight increase in body weight when compared to the control group. The percentage increase in body weight of treated mice was observed weekly on days 7 and 14. Consumption of water and food were analyzed daily from the volume of water and food distribution and the amount remaining after 24 hrs. Water and food consumption were found to be normal throughout the dosing period of 14 days when compared to the treatment of animals with control.

**Observation of Bodyweight**

Body weight of experimental albino mice of both control and *Apium leptophyllum pers* extract-treated
Table 1: Effect of acute toxicity study of the ethanolic extract on body weight of swiss albino mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body Weight (gm) 1st Day (gm)</th>
<th>Body Weight (gm) 7th Day (gm)</th>
<th>Body Weight (gm) 14th Day (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25.32± 0.321</td>
<td>26.54 ± 0.429</td>
<td>27.58 ± 0.587</td>
</tr>
<tr>
<td>2000 mg/kg EEAL</td>
<td>25.72 ± 0.734</td>
<td>27.24 ± 0.478</td>
<td>28.49 ± 0.578</td>
</tr>
</tbody>
</table>

EEAL: Ethanolic extract of Apium Leptophyllum pers; Values are represented as mean ± SEM; N=5

Table 2: Effect of ethanolic extract of Apium leptophyllum pers on hematological parameters after 14 days of acute toxicity testing in Swiss albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hb % (gm)</th>
<th>RBC (mil/cu.mm)</th>
<th>WBC (cell/cu.mm)</th>
<th>Differential Leukocytes count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.92 ± 2.13</td>
<td>11.32 ± 1.73</td>
<td>6.84 ± 0.47</td>
<td>74</td>
</tr>
<tr>
<td>2000 mg/kg EEAL</td>
<td>15.34 ± 3.24</td>
<td>12.74 ± 1.59</td>
<td>7.12 ± 1.79</td>
<td>79</td>
</tr>
</tbody>
</table>

EEAL: Ethanolic extract of Apium Leptophyllum pers; Values are represented as mean ± SEM; N=5

Table 3: Effect of ethanolic extract of Apium leptophyllum pers on biochemical markers after 14 days of acute toxicity testing in Swiss albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (D.W)</td>
<td>44.82 ± 2.85</td>
<td>15.20 ± 0.55</td>
<td>25.43 ± 0.64</td>
<td>55.02 ± 1.87</td>
<td>0.52 ± 0.51</td>
</tr>
<tr>
<td>2000 mg/kg EEAL</td>
<td>46.82 ± 2.35</td>
<td>16.28 ± 2.09</td>
<td>24.56 ± 0.89</td>
<td>54.69 ± 1.54</td>
<td>0.52 ± 0.35</td>
</tr>
</tbody>
</table>

EEAL: Ethanolic extract of Apium Leptophyllum pers; Values are represented as mean ± SEM; N=5

Table 4: Effect of ethanolic extract of Apium leptophyllum pers on biochemical markers after 14 days of acute toxicity testing in Swiss albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose (mg/dl)</th>
<th>Total Protein (G/dl)</th>
<th>A/G Ratio</th>
<th>Total Bilirubin (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (D.W)</td>
<td>276.04 ± 3.54</td>
<td>7.23 ± 0.08</td>
<td>2.74 ± 0.05</td>
<td>0.87 ± 0.03</td>
<td>102 ± 0.03</td>
</tr>
<tr>
<td>2000 mg/kg EEAL</td>
<td>272.05 ± 4.35</td>
<td>7.89 ± 0.05</td>
<td>2.58 ± 0.03</td>
<td>1.04 ± 0.05</td>
<td>124 ± 0.09</td>
</tr>
</tbody>
</table>

EEAL: Ethanolic extract of Apium Leptophyllum pers; Values are represented as mean ± SEM; N=5

groups were elevated gradually throughout the study period was observed. Table 1

**Hematological Analysis**

Hematological results revealed no significant changes in the levels of hemoglobin, enumeration of RBC, WBC, Granulocytes, and Agranulocytes. (Lymphocytes and Monocytes). Table 2

**Biochemical Analysis**

Biochemical studies revealed that there is mild changes in total cholesterol and total protein level from the normal level in the acute toxicity group. There were no remarkable (p < 0.05) changes in biochemical markers of liver function test as summarized in Table 3.

Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Alkaline phosphatase (ALP) levels were retained within the normal limits.

No remarkable changes were showed in total bilirubin, creatinine, Albumin and Globulin ratio, glucose and urea level were observed. Tables 3 and 4

**Histopathology Report**

Histopathology indicated no abnormalities in pathological examinations during microscopical examinations of liver and kidney as compared to control animals. Figures 1, 2, 3 and 4. Body weight serves as an indicator of fat deposition in the body. (Teo et al., 2002) No changes in animal behaviour was observed and hence it is clear that EEAL is non toxic at the dose level administrated. Acute toxicity study
also revealed no significant changes in hematological parameters, biochemical parameters and Liver marker enzymes.

**Histopathological report**

Liver

![Liver of normal control mice showing normal](image1)

**Figure 1: Liver of normal control mice showing normal**

![Liver of mice treated with ethanolic extract of Apium leptophyllum pers(2000 mg/kg) showing Sinusoidal lymphocytic infiltration. No necrosis.](image2)

**Figure 2: Liver of mice treated with ethanolic extract of Apium leptophyllum pers(2000 mg/kg) showing Sinusoidal lymphocytic infiltration. No necrosis.**

Increase in the granulocytes may be attributed to some hypersensitivity reaction or infection. (Nancy, 2004) The plant drug administered did not induce such reactions and hence may be considered non-toxic.

The histopathological examinations also did not reveal any marked changes in the histoarchitecture and hence it becomes evident that the plant is non-toxic.

**CONCLUSIONS**

Based on the results obtained, no toxic effect was observed after the administration of ethanolic extract of Apium leptophyllum pers up to 2000 mg/kg bodyweight for 14 days in Swiss albino mice. Hence, the drug may be considered as safe for consumption.

**REFERENCES**


