Toxicological study of *Opuntia elatior* Mill., Fruit (ripen) juice: A folklore medicinal plant

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Abstract

**Objective:** Ripen fruit juice of *Opuntia elatior* Mill, a folklore medicinal plant, is being used by the local people of Gujarat, to treat anemia and general debility. Though used frequently since long, its fruits have not been evaluated for their safety aspects on repeated administration. Hence, the present study was planned to evaluate the acute and long-term toxicity study of *O. elatior* fruit (ripen) juice in rats. **Materials and Methods:** Oral acute toxicity study was carried out by administering the drug once only at the dose of 20.0 ml/kg orally in rats. For long-term toxicity, *O. elatior* fruit juice was administered at three different dose levels of 1.8, 9.0, and 18.0 ml/kg orally for 60 consecutive days in rats following AYUSH 170 guideline/WHO guideline. The effects of the drug on ponderal changes, hematological, biochemical, and histological parameters were noted down. **Results:** No significant behavioral changes and sign symptoms of toxicity were observed during acute oral toxicity study implicating that the sample is relatively safe at 20.0 ml/kg. Long-term toxicity results showed that *O. elatior* fruit juice even at a higher dose of 18.0 ml/kg administered for 60 days, did not affect the parameters studied to a significant level in rats. **Conclusion:** The doses employed for long-term toxicity studies were several folds higher than the clinical dose of *O. elatior* fruit juice. Hence, it is relatively safe for use at a therapeutic dose level.

**Key words:** Acute toxicity, folklore, long-term toxicity, *Opuntia elatior*

INTRODUCTION

Classical texts of *Ayurveda* describe the drugs with regards to their pharmacological properties and actions. The indication, contraindication, and the effect of the drug on *dosha*, *dhatu*, and *mala* are also well-described. On the contrary, in folklore practice, the drugs are prescribed basing on the personal experience of the concerned physician and this tradition passes on from generation to generation. Prolonged and apparently uneventful use of herbal medicines may offer testimony of their safety and efficacy. Experimental evaluation is required to be carried out to provide a scientific basis for their traditional use and to prove that they are safe and efficacious.

*Opuntia elatior* Mill. is a folklore medicinal plant, and its ripen fruits are used by the local people of Gujarat in treating anemia and general debility. Fruit is also a rich source of nutrients and vitamins¹² and are eaten fresh, dried or preserved in jams, syrups or processed into candy-like products.¹³ Fruit of *O. elatior* is reported for its hematinic, analgesic, and antiasthmatic activity including its safety reports during the acute toxic study.¹⁴ Though used frequently, and for a longer duration, for the management of anemia and as a nutritional supplement the fruits of *O. elatior* have not been evaluated for their safety on repeated administration. Hence, the present study was
planned to evaluate the acute and long-term toxicity study of *O. elatior* Mill fruit (ripen) juice in rats.

**MATERIALS AND METHODS**

**Drug and Chemicals**

The ripen fruits of *O. elatior* were collected from its natural habitat from surrounding area of Jamnagar, Gujarat, India. Pharmacognostical studies were carried out for the authentication in Pharmacognosy laboratory, Institute of Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar. Juice was prepared from ripen fruit of *O. elatior* by standard maceration in the Department of Dravyaguna, Institute of Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar. The filtered juice was then preserved in an airtight container until further use. All chemicals used in the study were of analytical grade.

**Animals**

Charles’s Foster albino rats were used for the experimentation. The rats were obtained from animal house attached to Institute of Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC/16/2014/08) in accordance with the guideline formulated by CPCSEA, India. The animals were exposed to 12 h light, and 12 h dark cycle with the relative humidity of 50-70%, and the ambient temperature was 23 ± 2°C. All animals were kept on same environmental conditions. They were fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries, Baroda and drinking water was given *ad libitum*.

**Dose Calculation**

The dose of the test formulations was calculated by extrapolating the human dose (20 ml/day) to rat dose (1.8 ml/kg) based on the body surface area ratio by referring to the standard table of Paget and Barnes. The test drug was administered orally by the oral catheter.

**Acute Toxicity Study**

Acute oral toxicity study for *O. elatior* fruit (ripen) juice was carried out following OECD 425 guideline (modified, adopted 23rd March 2006). Acute toxicity study was conducted using up and down procedure with five animals in each group. The drug was administered once orally to overnight fasted rats at 20.0 ml/kg as highest dose and observed for 14 days. Mortality, Gross behavior, and other parameters were closely observed for first 4 h and up to 8 h on the 1st day and thereafter every 24 h, up to 14 days.

**Long-term Toxicity Study**

The study was carried out as per standard guideline for long-term toxicity test and modified as per experimental need. Rats of either sex weighing 200±20 g were selected. Animals were kept for acclimatization for one week, and thereafter they were randomly divided into four groups of six animals. Group (I) was kept as a control group, received a vehicle as a distilled water in dose of 10 ml/kg, orally. Group (II) to (IV) were administered with test drug, juice of fruit of *O. elatior* at TED (1.8 ml/kg, orally), TED × 5 (9.0 ml/kg, orally), and TED × 10 (18 ml/kg, orally) for 60 consecutive days, respectively. The administration period of the drug for the long-term toxicity study was decided as per WHO guideline from the period of clinical use of *O. elatior*.

The rats were carefully observed daily for any overt and apparent sign and symptoms of toxicity during the entire experimental period. The body weight change of individual rat was noted initially and thereafter weekly during the study period. At the end of experimental periods, blood was withdrawn from the retro-orbital puncture under light ether anesthesia using the capillary tube for estimation of serum biochemical and hematological parameters. The body weight of each rat was noted on the last day, and rats were sacrificed. The abdomen was opened through midline incision to record the autopsy changes followed by dissecting out the important organs.

Hematological analysis was performed using an automatic hematological analyzer (Swelab). The parameters studied were total red blood cell (RBC), hemoglobin (Hb), packed cell volume, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), MCH concentration (MCHC), white blood cell (WBC), neutrophils percentage, lymphocyte percentage, eosinophils percentage, monocyte percentage, and platelet count.

Bone marrow smear from the femur bone was prepared using the standard procedure. All the important internal organs were carefully dissected namely brain, pituitary, liver, heart, thymus, spleen, kidney, lung, stomach, intestine, testis, prostate, seminal vesicle, uterus, ovary, adrenal gland, trachea, aorta, lymph node, and skin. After noting any sign of gross lesion and ponderal changes of major organs, all were transferred to 10% phosphate buffered formalin solution for fixation and later on subjected to dehydrating, wax
embedding, sectioning, and staining with hematoxylin and
eosin for histological evaluation by light microscopy.

**Statistical Analysis**

The data are expressed as mean ± standard error of mean for
six rats per experimental group. Students’ t-test and one-way
analysis of variance were used to compare the mean values
of quantitative variables among the groups followed by
Dunnet’s multiple t-test for unpaired data to determine the
significant difference between groups at \( P < 0.05 \).

**RESULT AND DISCUSSION**

**Acute Toxicity Study**

Acute toxicity test results showed that *O. elatior* did not affect
any behavioral changes and other parameters observed during
the acute toxicity test. *O. elatior* did not produce any sign and
symptoms of toxicity and mortality up to dose of 20.0 ml/kg
in any of the treated rats which suggest that LD50 value may
be much higher than 20.0 ml/kg by the oral route. This dose is
many folds higher than the therapeutic equivalent dose of test
drugs in rats implicating that the test drug is relatively safe
for clinical use at a therapeutic dose level.

**Long-term Toxicity Study**

Effect of *O. elatior* on the percentage change in body weight
[Table 1] showed that weight gain was observed in all three
groups but percentage body weight changes pattern in treated
groups did not differ significantly from the changes observed
in control groups. Body weight change is an important
indicator of gross toxicity. Drastic toxicity or interference
with absorption of nutrients will reflect in the form of body
weight reduction. Since body weight gain pattern in the test,
drug-treated groups did not differ significantly from control
group it can be suggested that the test drug formulation has
no proclivity to produce drastic tissue destruction nor it is
likely to interfere with the absorption of the nutrients. The
results are in conformity with previous toxicity tests of
*O. elatior* fruit extract which revealed no toxic side effect on
the external morphology and the body weights of the mice up
to 600 mg/kg body weight.[25]

Further out of the nine organs for which relative weight were
recorded, *O. elatior* at all dose levels produced non-significant
increase in relative weight of testis in dose-dependent
manner in comparison to control group while non-significant
increase in relative weight of thymus and prostate at TED
× 10 dose level in comparison to control group [Table 2].
Normally decrease in the weight of the organ is indicative
of loss of tissue mass in that organ, the exception being the
secretory organs in which decrease in weight sometimes is
seen along with increased activity. Here, increase in weight
of reproductive organs such as testis and prostate may be
indicative of stimulation of hormone secretion. In the present
study, there were no any remarkable changes observed in
the relative weight of organs at higher doses of test drugs.
Hence, it may be suggested that the test drug does not seem
to produce any serious toxic effect on the relative weight of
important internal organ in long-term toxicity study.

Analysis of the effect of *O. elatior* on hematological
parameters [Table 3] revealed that out of the twelve parameters
studied none of the parameters were found to be affected at
a significant level in comparison to control group. In TED
dose level non-significant decrease in WBC while increase
in neutrophil and monocyte count was observed. In TED
× 5 dose level non-significant decrease in WBC count and
eosinophil. In TED × 10 dose level non-significant increase
in the neutrophil count was seen, but all the values are within

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>TED</th>
<th>TED×5</th>
<th>TED×10</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>233.33±4.94</td>
<td>216.67±11.45</td>
<td>218.33±10.46</td>
<td>196.67±9.55</td>
</tr>
<tr>
<td>7</td>
<td>240.83±5.54</td>
<td>211.67±12.76</td>
<td>227.50±13.15</td>
<td>213.33±9.46</td>
</tr>
<tr>
<td>14</td>
<td>246.67±7.38</td>
<td>225.00±18.03</td>
<td>246.67±10.85</td>
<td>220.00±6.83</td>
</tr>
<tr>
<td>21</td>
<td>255.83±7.90</td>
<td>230.00±17.75</td>
<td>245.00±12.97</td>
<td>226.67±11.52</td>
</tr>
<tr>
<td>28</td>
<td>259.17±8.51</td>
<td>227.50±20.36</td>
<td>242.50±10.47</td>
<td>223.33±9.55</td>
</tr>
<tr>
<td>35</td>
<td>261.67±8.72</td>
<td>237.00±23.11</td>
<td>245.00±9.31</td>
<td>216.67±12.29</td>
</tr>
<tr>
<td>42</td>
<td>261.67±8.72</td>
<td>259.00±17.49</td>
<td>254.17±10.20</td>
<td>229.00±14.87</td>
</tr>
<tr>
<td>49</td>
<td>265.00±8.56</td>
<td>266.00±18.33</td>
<td>256.67±9.97</td>
<td>238.00±11.58</td>
</tr>
<tr>
<td>56</td>
<td>253.33±16.67</td>
<td>248.00±22.00</td>
<td>253.33±8.82</td>
<td>224.00±6.78</td>
</tr>
<tr>
<td>60</td>
<td>253.33±12.23</td>
<td>245.00±22.02</td>
<td>253.33±8.91</td>
<td>218.75±5.15</td>
</tr>
</tbody>
</table>

% change to initial | 8.57↑ | 13.07↑ | 16.03↑ | 11.22↑

Data: Mean±SEM, ↑: Increase, SEM: Standard error mean
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**Table 2: Effect of *O. elatior* fruit juice on relative weights of organs in rats during long-term toxicity study**

<table>
<thead>
<tr>
<th>Organ weight</th>
<th>Control</th>
<th>TED</th>
<th>TED×5</th>
<th>TED×10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen (mg/100 g)</td>
<td>194.74±8.98</td>
<td>198.68±6.79</td>
<td>171.21±6.05</td>
<td>191.08±12.83</td>
</tr>
<tr>
<td>Thymus (mg/100 g)</td>
<td>166.24±7.01</td>
<td>136.99±8.40</td>
<td>147.54±7.48</td>
<td>193.98±63.53</td>
</tr>
<tr>
<td>Uterus (mg/100 g)</td>
<td>229.37±62.69</td>
<td>247.38±39.39</td>
<td>211.09±62.69</td>
<td>253.62±119.71</td>
</tr>
<tr>
<td>Kidney (mg/100 g)</td>
<td>713.23±35.0</td>
<td>745.69±15.03</td>
<td>699.37±36.33</td>
<td>695.90±22.94</td>
</tr>
<tr>
<td>Heart (mg/100 g)</td>
<td>265.89±12.79</td>
<td>270.03±10.50</td>
<td>277.49±8.71</td>
<td>296.13±21.87</td>
</tr>
<tr>
<td>Testis (mg/100 g)</td>
<td>680.28±167.02</td>
<td>795.82±267.82</td>
<td>795.77±162.33</td>
<td>822.12±55.85</td>
</tr>
<tr>
<td>Prostate (mg/100 g)</td>
<td>112.17±8.35</td>
<td>86.71±17.41</td>
<td>99.73±1.15</td>
<td>164.8±63.91</td>
</tr>
<tr>
<td>Seminal vesicle (mg/100 g)</td>
<td>253.84±20.94</td>
<td>233.99±70.72</td>
<td>253.29±60.97</td>
<td>234.65±36.47</td>
</tr>
<tr>
<td>Liver (g/100 g)</td>
<td>3.13±0.037</td>
<td>2.96±0.082</td>
<td>2.77±0.10</td>
<td>2.99±0.084</td>
</tr>
</tbody>
</table>

*O. elatior: Opuntia elatior*

**Table 3: Effect of *O. elatior* fruit juice on hematological parameters during long-term toxicity study**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>TED</th>
<th>TED×5</th>
<th>TED×10</th>
</tr>
</thead>
<tbody>
<tr>
<td>TWBC (10³/Cumm)</td>
<td>9300±1218.47</td>
<td>8540.00±823.77</td>
<td>7816.67±974.82</td>
<td>9375.00±2220.13</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>27.83±2.52</td>
<td>30.80±5.11</td>
<td>28.00±1.63</td>
<td>31.00±2.27</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>67.00±2.61</td>
<td>63.80±5.51</td>
<td>67.67±1.52</td>
<td>64.00±2.52</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>3.00±0.00</td>
<td>2.80±0.37</td>
<td>2.33±0.21</td>
<td>2.75±0.25</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>2.17±0.17</td>
<td>2.60±0.25</td>
<td>2.00±0.00</td>
<td>2.25±0.25</td>
</tr>
<tr>
<td>RBC (10⁹/μL)</td>
<td>7.88±0.10</td>
<td>8.05±0.32</td>
<td>8.19±0.10</td>
<td>7.99±0.19</td>
</tr>
<tr>
<td>Hb (g%)</td>
<td>14.05±0.13</td>
<td>14.20±0.53</td>
<td>14.45±0.17</td>
<td>14.63±0.08</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>43.32±0.41</td>
<td>44.16±1.78</td>
<td>45.10±0.48</td>
<td>44.50±0.57</td>
</tr>
<tr>
<td>Platelet (10⁹/μL)</td>
<td>1050.17±86.30</td>
<td>1128.20±54.83</td>
<td>1154.00±67.54</td>
<td>1116.50±65.53</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>55.05±0.38</td>
<td>54.86±0.34</td>
<td>55.12±0.54</td>
<td>55.80±0.63</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>17.87±0.30</td>
<td>17.62±0.24</td>
<td>17.65±0.32</td>
<td>18.33±0.40</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>32.45±0.35</td>
<td>32.16±0.28</td>
<td>32.03±0.39</td>
<td>32.83±0.36</td>
</tr>
</tbody>
</table>

MCHC: Mean corpuscular hemoglobin concentration, MCH: Mean corpuscular hemoglobin, MCV: Mean corpuscular volume, PCV: Packed cell volume, Hb: Hemoglobin, RBC: Red blood count, WBC: White blood cells, *O. elatior: Opuntia elatior*

The test drug at all dose level did not affect the RBC related parameters. If the overall picture is taken into consideration, the data profile clearly indicates that the test formulation is not likely to produce any serious hematological changes. This clearly indicates that at all dose levels, the test drug do not affect the both cellular and non-cellular elements of the blood to a significant extent.

Out of 16 biochemical parameters (Table 4), none were significantly affected by *O. elatior* at TED, TED × 5, and at very high dose of TED × 10, even after repeated administration for 60 days in rats. However, administration of test drug at TED dose level resulted in non-significant increase in total cholesterol, triglyceride, and HDL-cholesterol while at TED × 5 dose level produced non-significant increase in total cholesterol and HDL-cholesterol level in comparison to control group. Test drug at TED × 10 dose level resulted in non-significant increase in triglyceride, HDL-cholesterol, SGOT, SGPT, total protein, and albumin level in comparison to control group, but values are still within the normal range. There were no any drastic changes observed in the biochemical parameters in the test drugs treated groups. Hence, it may be suggested that the test drug does not seem to produce any serious toxic effect during long-term toxicity study.

The result of histopathological studies revealed that *O. elatior* fruit juice at higher dose level of TED × 10 level did not produce any changes in cytoarchitecture of brain, pituitary, liver, heart, thymus, spleen, kidney, lung, stomach, intestine, testis, prostate, seminal vesicle, uterus, ovary, adrenal gland, trachea, lymph node, and skin in comparison to control group.

**CONCLUSION**

From the present study, it can be concluded that *O. elatior* fruit (ripen) juice did not produce any sign and symptoms of acute toxicity and mortality up to dose of 20.0 ml/kg in any of the treated rats which suggest that LD50 value may be much higher than 20.0 ml/kg by oral route in rat. The result of long-term toxicity concluded that test drug at therapeutic dose and...
even at TED × 10 dose level, equivalent of which are not likely to be ever employed in clinical conditions, for longer duration of 60 days has not produced any drastic or significant toxic effect on ponderal, hematological, biochemical, and histopathological parameters in rats. Overall, it can be suggested that Opuntia elatior fruit (ripen) juice is relatively safe for use at a therapeutic dose level.

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