ORIGINAL ARTICLE

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 the 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.[1]

Opuntia elatior Mill. is a folklore medicinal plant, and it's ripen fruits are used by the local people of Gujaratin treating anemia and general debility. Fruit is also a rich source of nutrients and vitamins[2,3] and are eaten fresh, dried or preserved in jams, syrups or processed into candy-like products.[4,5] Fruit of *O. elatior* is reported for its hematinic, analgesic, and antiasthmatic activity including its safety reports during the acute toxic study.[6] 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

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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.[7] 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).[8,9] 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.[1,10] 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), respectively, 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 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.

Serum biochemical parameters were estimated using fully automated biochemical random access analyzer (BS-200, Lilac Medicare Pvt. Ltd., Mumbai). The parameters were blood sugar,[11] total cholesterol,[12] triglycerides,[13] high density lipoprotein (HDL)-cholesterol,[14] blood urea,[15] creatinine,[16] serum glutamic pyruvic transaminase (SGPT),[17] serum glutamic oxaloacetic transaminase (SGOT),[18] total protein,[19] albumin, globulin,[20] alkaline phosphatase,[18] total bilirubin,[21] direct bilirubin,[22] uric acid,[23] and calcium.[24]

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' 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 that 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 the normal range.[26] 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

Table 1: Effect of test drug on body weight of rats during different intervals of long-term toxicity study

Days	Body weight (g)			
	Control	TED	TED×5	TED×10
0	233.33±4.94	216.67±11.45	218.33±10.46	196.67±9.55
7	240.83±5.54	211.67±12.76	227.50±13.15	213.33±9.46
14	246.67±7.38	225.00±18.03	246.67±10.85	220.00±6.83
21	255.83±7.90	230.00±17.75	245.00±12.97	226.67±11.52
28	259.17±8.51	227.50±20.36	242.50±10.47	223.33±9.55
35	261.67±8.72	237.00±23.11	245.00±9.31	216.67±12.29
42	261.67±8.72	259.00±17.49	254.17±10.20	229.00±14.87
49	265.00±8.56	266.00±18.33	256.67±9.97	238.00±11.58
56	253.33±16.67	248.00±22.00	253.33±8.82	224.00±6.78
60	253.33±12.23	245.00±22.02	253.33±8.91	218.75±5.15
% change to initial	8.57↑	13.07↑	16.03↑	11.22↑

Data: Mean±SEM, ↑: Increase, SEM: Standard error mean

Table 2: Effect of *O. elatior* fruit juice on relative weights of organs in rats during long-term toxicity study

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

O. elatior: Opuntia elatior

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

Parameters	Control	TED	TED×5	TED×10
TWBC (10^3/Cumm)	9300±1218.47	8540.00±823.77	7816.67±974.82	9375.00±2220.13
Neutrophil (%)	27.83±2.52	30.80±5.11	28.00±1.63	31.00±2.27
Lymphocyte (%)	67.00±2.61	63.80±5.51	67.67±1.52	64.00±2.52
Eosinophil (%)	3.00±0.00	2.80±0.37	2.33±0.21	2.75±0.25
Monocyte (%)	2.17±0.17	2.60±0.25	2.00±0.00	2.25±0.25
RBC (10^3/µL)	7.88±0.10	8.05±0.32	8.19±0.10	7.99±0.19
Hb (g%)	14.05±0.13	14.20±0.53	14.45±0.17	14.63±0.08
PCV (%)	43.32±0.41	44.16±1.78	45.10±0.48	44.50±0.57
Platelet (10^3/µL)	1050.17±86.30	1128.20±54.83	1154.00±67.54	1116.50±65.53
MCV (fl)	55.05±0.38	54.86±0.34	55.12±0.54	55.80±0.63
MCH (pg)	17.87±0.30	17.62±0.24	17.65±0.32	18.33±0.40
MCHC (g/dL)	32.45±0.35	32.16±0.28	32.03±0.39	32.83±0.36

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*

indicates that the test formulation is not likely to produce any serious hematological changes. This clearly indicates 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.[27] 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

Table 4: Effect of *O. elatior* fruit juice on biochemical parameters during long-term toxicity study

Parameters	Control	TED	TED×5	TED×10
Blood glucose (mg/dl)	96.50±5.02	85.20±2.22	94.33±4.72	105.50±3.40
Cholesterol (mg/dl)	53.83±4.11	60.60±2.80	60.33±7.17	56.75±1.11
Triglycerides (mg/dl)	50.67±9.37	54.40±9.71	48.00±7.56	56.50±8.87
HDL-cholesterol (mg/dl)	32.33±4.04	37.20±3.60	38.17±4.82	37.00±1.08
Blood urea (mg/dl)	57.17±2.59	60.80±3.57	50.83±3.77	49.0±01.47
Creatinine (mg/dl)	0.60±0.03	0.58±0.05	0.57±0.02	0.50±0.07
SGPT (IU/dl)	67.67±2.70	64.00±3.79	68.17±4.11	75.75±11.85
SGOT (IU/dl)	131.67±10.88	128.80±13.53	133.17±13.05	146.25±28.20
Total protein (g/dl)	6.63±0.26	6.90±0.11	7.10±0.25	7.05±0.22
Albumin (g/dl)	3.13±0.13	3.08±0.15	3.65±0.38	3.43±0.10
Globulin (g/dl)	3.50±0.26	3.82±0.12	3.38±0.28	3.63±0.23
Alkaline phosphatase (IU/dl)	211.17±28.86	188.80±20.91	173.17±24.44	207.75±61.60
Total bilirubin (mg/dl)	0.47±0.06	0.500±0.08	0.40±0.04	0.48±0.05
Direct bilirubin (mg/dl)	0.15±0.03	0.14±0.02	0.10±0.00	0.15±0.03
Uric acid (mg/dl)	0.85±0.18	0.70±0.09	0.73±0.08	0.80±0.09
Calcium (mg/dl)	11.20±0.24	10.90±0.26	10.90±0.27	11.28±0.19

O. elatior: Opuntia elatior, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase, HDL: High-density lipoprotein

suggested that *O. elatior* fruit (ripen) juice is relatively safe for use at a therapeutic dose level.

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