Hypotensive Activity, Toxicology and Histopathology of Opuntioside-I and Methanolic Extract of *Opuntia dillenii*

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Methanolic extract of *Opuntia dillenii* cladodes and its pure compound α-pyrone glycoside, opuntioside-I showed potent hypotensive activity in normotensive rats. Both the extract and opuntioside-I showed comparable effect of 44—54% fall in Mean Arterial Blood Pressure (MABP) at the dose of 10 mg/kg. No mortality was observed in rats even at the doses of 1000 mg/kg/d and 900 mg/kg/d per oral of extract and opuntioside-I respectively. However, histopathology revealed adverse effects of high doses on liver and spleen of the experimental animals.

Key words hypotensive activity; *Opuntia dillenii* cladodes; *Opuntiae; α-pyrone glycoside; toxicity; histopathology

In continuation of our search for safe and potent hypotensive constituents from indigenous plants, current work describes the pharmacological and chemical analysis of *Opuntia dillenii*. Opuntia—a large genus of family Cactaceae, is now categorized in *Opuntia*. The plants belonging to Opuntia are succulent shrubs of Western countries, which have been naturalized widely to the warmer regions of world including Pakistan. Medicinal and edible value of Opuntia species is obvious from the fact that they were brought to Eastern countries in eighteenth century as a vegetable by European Travellers to prevent them from scurvy during the long voyages. *Opuntia* species are rich source of dietary fibers, natural colorants and antioxidant vitamins and therefore used as a food and fodder. It is because of their edible fruit that they are known in vernacular as prickly pears. Pharmacological evaluation of *Opuntia* has shown its efficacy as antihyperlipidemic, antiatherosclerotic, antiviral, anti-inflammatory, anti-diabetic, antioxidant and antiuclerogenic agent. It has also been reported to protect nerve cells and used for the treatment of Alzheimer's disease, Parkinson's disease and stroke.

*Opuntia dillenii* (Nagphana), the species under investigation, is not much explored. However, its analgesic, anti-inflammatory, radical scavenging activity and antispermatogenic effect have so far been reported. In folkloric system of medicine *Opuntia dillenii* is considered as a good remedy for asthma, whooping and spasmodic cough and in hepatic congestion. Its cladodes are used in the treatment of scurvy ulcer and ophthalmia. Chemical constituents isolated so far include glycosides of quercetin, rhamnetin and kaempferol; opuntiol and its glucoside.

This is the first time that plant has been studied for its hypotensive activity, toxicity and histopathology. Opuntioside-I which showed potent hypotensive activity in present studies, has been isolated simply by Preparative Thin Layer Chromatography (PTLC) of the extract in 0.327% of weight of cladodes. During the course of chemical studies on its stem (cladodes), isolation of opuntioside-I was reported in 0.078% through solvent fractionation of the extract followed by reversed phase column chromatography. RESULTS AND DISCUSSION

In a bioassay directed hypotensive evaluation of *Opuntia dillenii*, intravenous administration of methanolic extract of its cladodes (OM) showed decrease in blood pressure of normotensive rats in a dose dependent manner. It caused 28% and 54% fall in the Mean Arterial Blood Pressure (MABP) at the doses of 1 mg/kg and 10 mg/kg respectively (Table 1). Hypotensive effect of the former dose lasted for less than one minute while that of higher dose remained for a fairly longer time period of 37 min. A group of rats treated interaperitoneally with 1000 mg/kg/d of OM (*vide* Experimental) caused 32% fall in MABP (Table 2).

Four bands (OM-1—OM-4) obtained by PTLC of OM (*vide* Experimental), showed different potencies of hypotensive action. OM-1 caused maximum decrease of 62% in MABP at 10 mg/kg. However, at higher dose of 30 mg/kg instead of causing more decline, hypotensive effect was reduced to 35% (Table 1). Duration of activity remains same at both doses which was about 7 min. PTLC of band OM-1 (0.084 g) resulted in the isolation of opuntiol (0.070 g, 83.33%) which could not be examined for hypotensive activity due to solubility problem. Isolation of more than 80% of opuntiol from OM-1 suggests that the molecule responsible for the hypotensive activity of band OM-1 may be opuntiol.

Band OM-2 which was identified as opuntioside-I by spectroscopic and chemical analysis, caused 25% decrease in...
MABP of rats at the dose of 3 mg/kg through intravenous route. It showed comparable activity at higher doses of 10 mg/kg and 30 mg/kg by reducing 44% and 43% of blood pressure (Table 1). Duration of effect at each dose remained close to one and half minutes. A group of four rats treated orally with 100 mg/kg/d of opuntioside-I for 7 d (vide Experimental) caused 28% fall in MABP (Table 2). Activity of tetraacetyl derivative of opuntioside-I could not be determined due to solubility problem.

Like OM-2 (opuntioside-I), band OM-3 also showed comparable hypotensive effect (27, 23%) at 10 mg/kg and 30 mg/kg, while, band OM-4 caused 15% and 36% reduction in MABP, in a dose dependent manner at the above mentioned doses (Table 1).

None of these substances caused any change in MABP of rats pretreated with 10^-4 m atropine sulphate. The behaviour is indicative of the fact that mode of action is similar to that of acetylcholine and Muscarinic M2 receptors on cardiac muscle. Hypotensive effect in rats.

Fall in the MABP of rats by OM and opuntioside-I by three different routes (intravenous, intraperitoneal, and oral) of administration is significant (Tables 1, 2). However, change in mode of administration, affects the intensity of action. Intraperitoneal administration of the 1000 mg/kg of OM produced hypotensive effect of 32%. Since this effect is comparable to its effect displayed by intravenous administration (at 1 mg/kg, Table 1), it may be tentatively assumed that only 0.1% (1 mg/kg/d) of drug is probably absorbed into blood plasma to lower the blood pressure of rat when it is administered intraperitoneally.

Opuntioside-I also showed significant hypotensive effect through oral route. At the dose of 100 mg/kg/d, it caused 28% decrease in MABP, which is comparable to the effect at 3 mg/kg (i.e., Table 1) and is 63% of the maximum effect recorded by intravenous administration of its 10 mg/kg. Hence it may be supposed that at least 3% of the orally given \( \alpha \)-pyrone glycoside is remaned unaltered and absorbed to produce 28% hypotension. However, if it is metabolized in the acidic atmosphere of stomach, the ultimate hydrolyzed product is \( \alpha \)-pyrone, i.e. opuntiol, which is the main component of active band OM-1 (Table 1). It may therefore be hypothesized that either in glucosidic (opuntioside-I) or agluco-sidic (opuntiol) from, \( \alpha \)-pyrone produces significant hypotensive effect in rats.

**Toxicology** Toxicology of active components, OM and opuntioside-I was carried out in two species of rodents, mice and rats. OM was administered only to rats at the dose of 1000 mg/kg/d both orally and intraperitoneally. Oral administration of OM did not cause any change in the physical behavior or motor activity of animals. However, intraperitoneal administration of the same dose caused hyperemia, red paws, crams, tail erection, enophthalmos, increased respiratory depth, ataxia, decreased motor activity, loss of grip, corner sitting, hind limb abduction and chromatourea in early 2 h of dosing. Like OM, oral administration of opuntioside-I did not produce any apparent change in rats. However, in mice intraperitoneal administration of higher dose (1000 mg/kg/d) caused ataxia, decreased motor activity, corner sitting, tail erection and palpebral ptosis in early 2 h after dosing. OM and opuntioside-I did not cause any mortality in rats. However, one male mouse was expired in each group of mice (group-VII and XI) after taking six oral doses of 100 mg/kg/d

### Table 1. Effect of Methanolic Extract of *Opuntia dillenii* Cladodes and Its Fractions on Mean Arterial Blood Pressure (MABP) of Normotensive Rats Through Intravenous Route

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dose (mg/kg)</th>
<th>MABP (mmHg±S.E.M.) before treatment</th>
<th>MABP (mmHg±S.E.M.) after treatment</th>
<th>% fall (mmHg)</th>
<th>Duration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OM</td>
<td>1</td>
<td>119.60±3.75</td>
<td>86.53±8.47</td>
<td>27.65*</td>
<td>0.72±0.23</td>
</tr>
<tr>
<td>(Methanolic extract of <em>Opuntia dillenii</em> cladodes)</td>
<td>10</td>
<td>123.27±4.62</td>
<td>56.56±6.04</td>
<td>54.12*</td>
<td>37.20±1.61</td>
</tr>
<tr>
<td>OM-1 (First band of OM)</td>
<td>10</td>
<td>135.33±1.63</td>
<td>51.67±9.23</td>
<td>61.72*</td>
<td>7.0±2.08</td>
</tr>
<tr>
<td>OM-2 (Opuntioside-I)</td>
<td>30</td>
<td>155.33±2.51</td>
<td>100.66±3.61</td>
<td>35.20*</td>
<td>6.71±1.67</td>
</tr>
<tr>
<td>OM-3 (Third band of OM)</td>
<td>10</td>
<td>108.44±11.98</td>
<td>81.17±10.23</td>
<td>25.15*</td>
<td>1.33±0.22</td>
</tr>
<tr>
<td>OM-4 (Fourth band of OM)</td>
<td>10</td>
<td>121.44±6.95</td>
<td>68.00±9.97</td>
<td>44.00*</td>
<td>1.58±0.28</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>10</td>
<td>99.34±2.3</td>
<td>56.33±2.33</td>
<td>43.29*</td>
<td>1.30±0.12</td>
</tr>
</tbody>
</table>

Values shown represent±S.E.M. of four determinations. a) \( \mu \)g/kg ; *p<0.001.

### Table 2. Effect of OM and Opuntioside-I on MABP of Rats after Oral and Intraperitoneal Administration

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of animals</th>
<th>Dose</th>
<th>Mode of administration</th>
<th>Duration (d)</th>
<th>MABP (mmHg±S.E.M.) of control rats</th>
<th>MABP (mmHg±S.E.M.) of treated rats</th>
<th>% change in MABP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OM</td>
<td>5</td>
<td>1000 mg/kg</td>
<td>Intraperitoneal</td>
<td>15</td>
<td>177.00±8.70</td>
<td>119.60±10.39</td>
<td>-32.43*</td>
</tr>
<tr>
<td>Opuntioside-I</td>
<td>4</td>
<td>100 mg/kg</td>
<td>Oral</td>
<td>7</td>
<td>158.00±4.72</td>
<td>114.00±1.13</td>
<td>-27.85*</td>
</tr>
</tbody>
</table>

− Decrease in MABP  *p<0.001.
and three intraperitoneal doses of 1000 mg/kg/d of opuntioside-I. None of the groups examined showed significant change in body weights.

**Tissue Analysis** No change in the morphology of organs could be detected in rats treated orally with OM and opuntioside-I. However, animals treated intraperitoneally showed many changes. OM treated rats exhibited urinary bladder filled with chromatourea (Figs. 1A, B), red spot on lungs (Figs. 2A, B) and faded and enlarged liver with slight patches on it. One of the mice treated orally with opuntioside-I (at the dose of 100 mg/kg/d) was found to have red spot on liver (Fig. 3) while spleen of some of the mice seemed larger than the control size (Fig. 4).

Opuntioside-I made some significant changes in the weights of vital organs of mice and rats at the doses of 100 mg/kg/d and 900 mg/kg/d respectively (Table 3). It might be interesting to note that irrespective the significance of change, liver and spleen of most of the treated animals showed histopathological changes while the histology of hearts and kidneys of all animals examined seems unaffected.

**Histopathology. Liver** Intraperitoneal administration of 1000 mg/kg/d of OM showed complete disorganization in the structure of liver (Figs. 5A, B). Laminae and sinusoids seem to be disintegrated while cell boundaries are not properly seen. Nuclei have been shrunked and structure inside them is disturbed.

Opuntioside-I also made several changes in the liver of rats and mice. Rats, at the dose of 100 mg/kg/d (p.o.) showed abnormal appearance of surviving liver cells with lot of infiltration, disintegration of many hepatic cells, and irregular shape of cells with nuclei showing atrophy (Fig. 5C). At 900 mg/kg/d (p.o.) these infiltration became more pronounced and further areas of necrosis, hemorrhage, and irregular
Table 3. Effect of Extract (OM) and Pure Opuntioside-I on Weights of Vital Organs of Rats and Mice

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dose (mg/kg)</th>
<th>Mode of administration</th>
<th>Duration (d)</th>
<th>Animal species</th>
<th>Weight of heart (g ± S.E.M.)</th>
<th>Weight of liver (g ± S.E.M.)</th>
<th>Weight of kidney (g ± S.E.M.)</th>
<th>Weight of spleen (g ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OM</td>
<td>1000</td>
<td>p.o.</td>
<td>15</td>
<td>Rats</td>
<td>Control 0.70 ± 0.02, Treated 0.66 ± 0.02, -5.71</td>
<td>Control 6.14 ± 0.15, Treated 6.40 ± 0.25, +4.23</td>
<td>Control 0.15 ± 0.02, Treated 0.25 ± 0.01, +1.71</td>
<td>Control 0.15 ± 0.02, Treated 0.15 ± 0.02, -3.71</td>
</tr>
<tr>
<td>OM</td>
<td>1000</td>
<td>i.p.</td>
<td>15</td>
<td>Rats</td>
<td>Control 0.75 ± 0.18, Treated 0.67 ± 0.3, -10.67</td>
<td>Control 6.72 ± 0.46, Treated 8.85 ± 1.25, +31.70</td>
<td>Control 0.46 ± 0.05, Treated 0.15 ± 0.05, -6.71</td>
<td>Control 0.15 ± 0.02, Treated 0.25 ± 0.04, +1.25</td>
</tr>
<tr>
<td>Opuntioside-I</td>
<td>1000</td>
<td>p.o.</td>
<td>7</td>
<td>Rats</td>
<td>Control 0.80 ± 0.14, Treated 0.78 ± 0.57, -2.50</td>
<td>Control 6.18 ± 0.37, Treated 5.38 ± 0.38, -13.08</td>
<td>Control 0.05 ± 0.02, Treated 0.05 ± 0.02, -5.33</td>
<td>Control 0.15 ± 0.02, Treated 0.15 ± 0.02, -3.71</td>
</tr>
<tr>
<td>Opuntioside-I</td>
<td>1000</td>
<td>p.o.</td>
<td>7</td>
<td>Mice</td>
<td>Control 0.15 ± 0.01, Treated 0.15 ± 0.01, +13.8*</td>
<td>Control 1.41 ± 0.15, Treated 1.74 ± 0.09, +23.40**</td>
<td>Control 0.02 ± 0.01, Treated 0.02 ± 0.01, +13.8*</td>
<td>Control 0.02 ± 0.01, Treated 0.02 ± 0.01, +13.8*</td>
</tr>
<tr>
<td>Opuntioside-I</td>
<td>900</td>
<td>p.o.</td>
<td>3</td>
<td>Rats</td>
<td>Control 0.82 ± 0.14, Treated 0.72 ± 0.04, -12.20</td>
<td>Control 6.18 ± 0.37, Treated 4.98 ± 0.16, -19.42**</td>
<td>Control 0.06 ± 0.02, Treated 0.06 ± 0.02, +5.33</td>
<td>Control 0.02 ± 0.01, Treated 0.02 ± 0.01, +13.8*</td>
</tr>
<tr>
<td>Opuntioside-I</td>
<td>1000</td>
<td>i.p.</td>
<td>3</td>
<td>Mice</td>
<td>Control 0.12 ± 0.01, Treated 0.15 ± 0.02, +25.0</td>
<td>Control 1.21 ± 0.16, Treated 1.30 ± 0.15, +7.44</td>
<td>Control 0.21 ± 0.02, Treated 0.18 ± 0.02, -14.29</td>
<td>Control 0.33 ± 0.05, Treated 0.25 ± 0.03, -24.24</td>
</tr>
</tbody>
</table>

p.o., per oral; i.p., intraperitoneal; +, increase in weight; −, decrease in weight. *p < 0.05, **p < 0.01.

Fig. 5. Liver of Control Rat (A) and Liver of Rat after Treatment with (B) 1000 mg/kg (i.p.) of OM (×20), (C) 100 mg/kg (p.o.) of Opuntioside-I (×20), (D) 900 mg/kg (p.o.) of Opuntioside-I (×20).
structure of cells are seen (Fig. 5D).

The liver of mice at the dose of 100 mg/kg (p.o.) of opuntioside-I, was found to contain distended sinusoids (Figs. 6A, B). The red spot observed during tissue analysis, revealed the centrilobular necrosis with hemorrhage and abnormal appearance of surviving cells (Fig. 6B). In the center of necrotic area degenerating liver cells are seen and there is an early fibrous tissue formation around it. At the dose of 1000 mg/kg/d (i.p.) most of the nuclei seem to be disintegrated while others vary in size. Areas of fibrosis are also visible (Fig. 6C).

Spleen At 100 mg/kg/d of opuntioside-I, rats showed severe atrophy of splenic cells destroying whole architecture of splenic cords along with extravasations (Figs. 7A, B). At higher dose of 900 mg/kg/d, it exhibited similar effects with more areas of infiltration and extravasation (Fig. 7C). Spleen of rats treated with OM could not be studied histologically.

In case of mice there is severe hypertrophy of splenic lymphoid cells at the dose of 100 mg/kg/d (p.o.) (Figs. 8A, B). Sinusoids and their lining cells become very prominent. Siderotic area and haemorrhage is also visible. At 1000 mg/kg/d (i.p.) splenic cords of mice though retain their structure but cells became atrophic. Further, disintegration of cords, extravasation of blood and infiltration can be clearly seen in many areas (Fig. 8C).

Effect of OM and Opuntioside-I on Serum Cholesterol, Glucose, Bilirubin and Total Protein Levels of Rats OM and opuntioside-I both decreased the serum cholesterol, glucose and total protein levels (Table 4) however, significant difference was observed only in glucose and total protein lev-
els of rats, treated intraperitoneally with OM. It caused 12% and 14% decrease in glucose and total protein levels respectively (Table 4). The insignificant rise in bilirubin by the administration of OM and opuntioside-I seems independent of routes. Biochemical studies done so far with other species of *Opuntia* also showed decrease in plasma cholesterol[9,10,21–23] and glucose levels.22)

**CONCLUSION**

Methanolic extract of *Opuntia dillenii* cladodes (OM) and opuntioside-I have been emerged as potent hypotensive agents in the present studies. Beside intravenous mode of administration, intraperitoneal and oral modes are also found to be effective in reducing the blood pressure. Further, signifi-

**Table 4. Effect of OM and Opuntioside-I on Cholesterol, Glucose, Bilirubin and Total Protein Levels of Rat Serum**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mode of administration</th>
<th>Dose (mg/kg)</th>
<th>Duration (d)</th>
<th>Cholesterol (mg/dl±S.E.M.)</th>
<th>Glucose (mg/dl±S.E.M.)</th>
<th>Bilirubin (mg/dl±S.E.M.)</th>
<th>Total protein (g/dl±S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OM 1000</td>
<td>p.o. 15</td>
<td>94.84±1.92</td>
<td>46.13±5.01</td>
<td>&lt;4.57</td>
<td>10.37±2.09</td>
<td>104.21±4.39</td>
<td>10.91±2.23</td>
</tr>
<tr>
<td>OM 1000</td>
<td>i.p. 10</td>
<td>98.45±1.03</td>
<td>104.90±3.21</td>
<td>2.21</td>
<td>104.35±1.24</td>
<td>109.25±6.21</td>
<td>6.45±0.20</td>
</tr>
<tr>
<td>Opuntioside-I 100</td>
<td>p.o. 7</td>
<td>104.20±2.02</td>
<td>109.26±3.33</td>
<td>1.34</td>
<td>106.35±1.32</td>
<td>109.35±7.29</td>
<td>10.50±1.23</td>
</tr>
<tr>
<td>Opuntioside-I 900</td>
<td>p.o. 3</td>
<td>104.20±3.33</td>
<td>109.26±4.32</td>
<td>1.15</td>
<td>106.35±1.32</td>
<td>109.35±7.29</td>
<td>10.50±1.23</td>
</tr>
</tbody>
</table>

*p* < 0.05, **p** < 0.001; ND: Not determined.

Fig. 8. Spleen of Control Mice (×20) (A) and Spleen of Mice after Treatment with (B) 100 mg/kg (p.o.) of Opuntioside-I (×20), (C) 1000 mg/kg (i.p.) of Opuntioside-I (×20)
certain reduction in serum glucose level by OM has also been recorded. Since \textit{O. dillenii} is an edible plant, its extract OM, as well as opuntioside-I did not kill any rat during studies, however, they left adverse effects on liver and spleen of experimental animals at higher doses. Opuntioside-I has also caused the expiry of one mouse while survived mice were found to have pathetic liver and spleen. Hence further investigations on lower but effective doses of OM and opuntioside-I in variety of animal species especially in non rodents are required to determine the exact margin of safety for these substances so as to make the study beneficial for human consumption.

MATERIALS AND METHODS

\textbf{Chemistry. Plant Material} Cladodes of \textit{Opuntia dillenii} were collected in the month of October, 2001 from University of Karachi campus and authenticated and deposited by Dr. Surraya, Dept. of Botany, University of Karachi with voucher specimen No. KUH GHS 68218.

\textbf{Instrumentation} Ultraviolet spectra were recorded in MeOH on Hitachi-U3200 and infrared spectra were measured in CHCl$_3$ on JASCO-A-302, spectrophotometers. The electron impact (EI) mass spectra were recorded on a Finni- 

gmat-112 instrument while recording of High-resolution mass spectra were carried out on a JMS HX-10 spectrometer. The $^1$H- and $^{13}$C-NMR spectra were run in CDCl$_3$ on a Bruker Aspect AM-500 spectrometer operating at 500 MHz for $^1$H and 125 MHz for $^{13}$C nuclei, with spectra referenced to residual solvent signals. For PTLC silica gel 60 GF$_{254}$ (Merck) was used.

\textbf{Extraction and Isolation of Chemical Constituents from \textit{Opuntia dillenii}} Fresh and undried cladodes of \textit{Opuntia dillenii} (3.34 kg) were cut into small cubes and extracted four times with methanol at room temperature for 3 d. The combined extracts were concentrated on rotavapour under reduced pressure to give a residue (OM, 203 g). Extract (OM, 6.74 g) was subjected to preparative thin layer chromatography (PTLC) (silica gel, CHCl$_3$ : MeOH 8.0 : 2.0) which gave four bands (OM-1—OM-4). The band OM-2 (0.36 g) showed single spot on TLC (CHCl$_3$ : MeOH 8.2 : Rf 0.44) and identified as opuntioside-I through detailed spectral studies including UV, IR, MS, NMR, 2D-NMR and chemical transformation.

The band OM-1 (0.084 g) was further purified through PTLC (silica gel, CHCl$_3$ : MeOH, 9.5 : 05, Rf 0.36) to give pure opuntiol (0.070 g). Spectral data of both opuntioside-I and opuntiol were comparable to the literature values. Opuntioside-I furnished its acetyl derivative on treatment with acetic anhydride and pyridine at room temperature. The remaining bands OM-3 (0.32 g) and OM-4 (0.83 g) were found to be the mixture of several compounds on TLC.

\textbf{Acetylation of Opuntioside-I} To the solution of opuntioside-I (0.04 g) in pyridine (1 ml) acetic anhydride (1 ml) was added and left overnight at room temperature. After usual work up of reaction mixture, it gave pure tetraacetyl derivative of opuntioside-I (0.05 g).

\textbf{Pharmacology. Animals and Drugs} Animals used in this study were Sprague–Dawley rats (200—250 g) and NMRI mice (20—30 g). They were housed at the Animal House of Dr. HMI Institute of Pharmacology & Herbal Sciences, Hamdard University and were given a standard diet and tap water ad libitum. Drugs used were Acetycholine and Sodium chloride from E. Merck, Atropine sulfate from Boehringer Ingelheim, and Pentothal® sodium from Abbott Karachi. Acetycholine (1 µg/kg) and saline (0.9% NaC1) were used as positive and negative controls respectively.

\textbf{Hypotensive Activity} Normotensive Sprague–Dawley rats (either sex) were anaesthetized with pentothal® sodium (50 mg/kg i.p.). The trachea was exposed and cannulated to facilitate spontaneous respiration. Drugs were injected (vol. 0.2—0.25 ml) through a polyethylene cannula inserted into the right external jugular vein followed by a saline flush (0.2 ml). The arterial blood pressure was recorded from the carotid artery via polyethylene arterial cannula connected to a Research Grade Blood pressure Transducer (Harvard, 60-3003) coupled with four channel Harvard Universal Oscillograph (Curvilinear, 50-9307). The temperature of the animals was maintained at 37 °C by use of over head lamp. Animals were allowed to equilibrate for at least 15 min before administration of any drug. Mean arterial blood pressure (MABP) was calculated as sum of the diastolic blood pressure plus one-third pulse width. Changes in blood pressure were expressed as the percent of control values, obtained immediately before the administration of test substance.

\textbf{Toxicology} Toxicity of OM was measured in four groups (I—IV) of rats (either sex) containing 10 animals each group. The dose of 1000 mg/kg/d of OM was given orally (p.o.) and intraperitoneally (i.p.) to group-I and II respectively for fifteen consecutive days. Group-III and IV serving as control were given saline through oral and intraperitoneal routes respectively. Toxicology of opuntioside-I was studied in both rats and mice. Two sets of rats (Group-V, VI) and mice (Group-VII, VIII) containing 10 animals (either sex) each group were treated orally for 7 d. Group-V and VII were treated with opuntioside-I at the dose of 100 mg/kg/d while Group-VI and VIII serving as corresponding control were given saline.

Two more sets of rats (Group-IX, X) containing 4 animals each group and two groups of mice (Group-XI, XII) containing 10 animals (either sex) were subjected to toxicology for 3 d. Group-IX was treated orally with opuntioside-I at the dose of 900 mg/kg/d while group-X served as its control. Group-XI of mice was given opuntioside-I intraperitoneally at the dose of 1000 mg/kg/d and group-XII serving as its control was injected (i.p.) with saline. Number of animals in group-IX, and duration of toxicology for groups-IX—XII have been reduced due to the paucity of opuntioside-I. Volume of each dose in rats and mice were 0.6—0.8 ml and 0.20—0.35 ml respectively.

All the animals were kept under observation for early 2 h after the administration of dose, for any change in behaviour or physical activities. Number of expired animals were noted at the end of study period. Survived animals of both rats and mice were anaesthetized with 1.5—2.0 ml and 0.04 ml respectively of pentothal sodium (50 mg/ml, i.p.) for cannula- tion, withdrawing of blood and tissue analysis. Blood collected from rats was incubated and centrifuged for biochemical study, while heart, liver, kidneys, and spleen of both rats and mice were removed, blotted and weighed immediately on electronic balance.

\textbf{Effect of OM and Opuntioside-I on MABP of Rats}
after Oral and Intraperitoneal Administration All survived animals of groups II, IV and VI at the end of their toxicity trials were anaesthetized with pentothal sodium and cannulated through trachea and carotid artery in the same manner as described earlier to record their blood pressure.

Effect of OM and Opuntioside-I on Some Biochemicals of Rat Serum Blood drawn (2—4 ml each rat) from all treated and control rats was left at room temperature for 20 min. Then incubated at 37 °C for 30 min and centrifuged separately in (BHG) Herml Z230 (Germany) at the speed of 3000 rpm for 20 min.

Serum obtained (1—1.5 ml) was subjected for the study of serum cholesterol, glucose, bilirubin and total protein levels by using commercial assay kits. Kits used were Ecoline® 25 by CHOD-PAP method for cholesterol, Ecoline® 1000 by GOD-PAP method for glucose, Mercko test® for bilirubin and Merck test® by biuret method for total protein. All these kits were purchased from diagnostica Merck (Germany). U-2000 spectrophotometer (Hitachi) was used to measure the absorbance of light.

Histology Heart, liver, spleen and kidneys were fixed in 10% formalin. After usual processes of dehydration, clearing and infiltration, tissues were embedded in paraffin wax and sectioned into 7-μm slices through Leica RM 2145-Rotation Microtom. The tissues were stained with haematoxylin and eosin. The slides were studied and photographed through Nikon Advance Trincocular Research Microscope OP-TIPHOT Model X2T-21E equipped with Nikon Microphot. The tissues were sectioned into 7-μm slices through Leica RM 2145-Rotation Microtom. The tissues were stained with haematoxylin and eosin. The slides were studied and photographed through Nikon Advance Trincocular Research Microscope OP-TIPHOT Model X2T-21E equipped with Nikon Microphot.

Statistical Analysis Changes in blood pressure, serum biochemical levels, body weights and weights of vital organs were compared using analysis of variance followed by Student’s t-test. Values of p<0.05, p<0.01 and p<0.001 were considered to be significant.

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