In Vitro Assessment of Antihepatotoxic Activity of n-Octacosanol

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n-octacosanol, isolated for the first time from the whole plants of *Fumaria indica*, was characterised and screened for antihepatotoxic activity *in vitro* against galactosamine and thiocetamide induced hepatocytotoxicities. At the concentration of 100 µg/ml, it has exhibited significant activity (P<0.01) against hepatotoxicants.

The whole plant of *Fumaria indica* Pugsley. (parpat/pilpapda/shahterah, *Fumariaceae*), common ingredient of many polyherbal formulations effective against liver disorders, was found to exhibit hepatoprotective activity against different hepatotoxicants that include carbon tetrachloride (CCl₄), paracetamol and rifampicin. The plant was reported to contain glycosides, carbohydrates, fixed oils, phytosterols, phenolic compounds, aliphatic acids such as citric, fumaric, malic, succinic, glycolic and lactic acids, non-nitrogenous compounds like 19-methyl-octacosan-1-ol and 3-methyl octacosan-1, 3-diol. Therefore, in the present investigation, an attempt has been made to isolate and identify the components responsible for the antihepatotoxic activity.

EXPERIMENTAL

Isolation of n-octacosanol

The powered whole plant of F. indica was defatted with petroleum ether (60-80°C, 0.86%). The air dried petroleum ether extract (0.86 g) was fractionated into methanol soluble and insoluble fractions. The methanol insoluble fraction after repeated recrystallisation from acetone gave a colourless, amorphous powder (0.22 g). The purity of the compound was confirmed by TLC using Toluene:Ethyl acetate (93:7) as solvent system and iodine as detecting reagent. By comparing with the reported physicochemical and spectral data, it is characterised as n-octacosanol, (H₃C(CH₂)₂₅ CH₂OH).

In vitro antihepatotoxic activity studies

Albino rats (Wistar strain) of either sex (250-275 g) maintained under controlled conditions were used for all sets of experiments. The rats were allowed to take standard laboratory feed and water *ad libitum*. Galactosamine HCl (40 µg/ml, Sigma Co., USA), thiocetamide (20 µg/ml, National Chemicals, Baroda) were used as hepatotoxins.

Isolation of rat hepatocytes: Hepatocytes were isolated according to the method of Seglen with slight modification as reported by Visen et al by recirculating enzymatic perfusion technique (*in situ*) and were suspended in HEPES (N-2- Hydroxyethyl Piperazine-N-2-Ethane Sulphonic acid) buffer I (HEPES buffer I (sodium chloride: 4.15 g (0.142 M), potassium chloride: 0.25 g (0.0067 M), HEPES: 1.20 g (0.01 M), pH: 7.4, double distilled water to 500 ml); HEPES buffer II (sodium chloride: 1.95 g (0.0667 M), potassium chloride: 0.25 g (0.0067 M), HEPES: 0.50 g (0.0147 M), HEPES buffer II (sodium chloride: 1.95 g (0.0667 M), potassium chloride: 0.25 g (0.0067 M), HEPES:...
Table 1: Effect of n-octacosanol on viability of Isolated Rat Hepatocytes against different Toxicants

<table>
<thead>
<tr>
<th>Group</th>
<th>% Viable Cells</th>
<th>Oxygen Uptake (ul/hr/mg protein)</th>
<th>% Viable Cells</th>
<th>Oxygen Uptake (ul/hr/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>98.05±0.56</td>
<td>4.13±0.13</td>
<td>98.05±0.56</td>
<td>4.13±0.13</td>
</tr>
<tr>
<td>Toxicant n-octacosanol</td>
<td>50.01±0.11</td>
<td>1.98±0.02</td>
<td>24.73±1.14</td>
<td>0.98±0.01</td>
</tr>
<tr>
<td>10 ug/ml</td>
<td>68.39±0.58*</td>
<td>ND</td>
<td>65.66±1.02*</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>(28.89)</td>
<td></td>
<td>(55.82)</td>
<td></td>
</tr>
<tr>
<td>100ug/ml</td>
<td>71.43±0.54*</td>
<td>ND</td>
<td>79.37±0.59*</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>(44.59)</td>
<td></td>
<td>(74.55)</td>
<td></td>
</tr>
<tr>
<td>1000 ug/ml</td>
<td>99.10±1.12*</td>
<td>3.36±0.08*</td>
<td>74.76±1.31*</td>
<td>3.34±0.05*</td>
</tr>
<tr>
<td></td>
<td>(11.55)</td>
<td></td>
<td>(68.24)</td>
<td></td>
</tr>
</tbody>
</table>

*Significant reduction compared to: toxicant = * (p<0.01)

ND = Not done, Values in parentheses = % Protection

12.00 g (0.1 M), collagenase type IV (630 units/mg solid): 1.6x10^5 g/ml, pH:7.6, double distilled water to 500 ml; collagenase was added to buffer II (10 ul/50 ml) just before the isolation. Buffer I was bubbled with oxygen (O_2) for 10 min, pH was adjusted with 1N sodium hydroxide.

**Hepatocytotoxicity studies**: n-octacosanol was tested for Hepatocytotoxicity at 10, 100 and 1000 ug/ml on isolated normal rat hepatocytes. After 24 h of incubation at 37°C, the percent viable hepatocytes were determined using the trypan blue exclusion method and oxygen uptake was determined using a Gilson's oxigraph according to the method of Estabrook.

**Antihepatotoxic activity**

Hepatocytotoxicity was induced with galactosamine (40 ug/ml) and thioacetamide (20 ug/ml). The hepatocyte suspension was distributed into various culture tubes 0.1 ml each and were divided into control, toxicant and test (test sample + toxicant) groups each in triplicate. To the control and toxicant groups 0.1 ml of double distilled water was added. While to the test groups, 0.1 ml of test suspension was added. To the toxicant and test groups, 0.1 ml of toxicant solution was added and the volume in each tube was made to 1ml with HEPES buffer I. All the tubes were mixed well and incubated in a carbon dioxide incubator for 24 h at 37°C. Then the hepatocytes were subjected to viability and O_2 uptake tests.

**Statistical analysis**: The mean value ± was calculated for each parameter. Percentage reduction against the hepatocytotoxin by the test samples was calculated by considering the viability difference between the hepatocytotoxin treated and the control group as 100% level of reduction. For determination of significant intergroup differences each parameter was analysed separately and one way analysis of variance was carried out. After, individual comparisons of group mean values were done using Dunnett's test.

**RESULTS AND DISCUSSION**

The whole plant of *Fumaria indica* was selected for further investigation since this plant has docu-
mented use in the traditional systems of medicine. n-octacosanol, a known compound was isolated for the first time form the whole plant of F. indica using a new method and was characterised by comparing with the literature values\(^4\). It was found to be non hepatocytotoxic. It showed the maximum, significant (P<0.01) increase in % viable cells at 100 ug/ml and increase in oxygen uptake at 1000 ug/ml against galactosamine- and thioacetamide-induced hepatic cytotoxicities (Table 1).

Hepatotoxicity of galactosamine has been reported to be due to its metabolism in the liver which lowers the levels of uracil nucleotides (UTP, UDP-glucose and UDP-galactose) resulting in inhibition of RNA synthesis leading to necrosis\(^1\).

Thioacetamide has been reported to cause inhibition of the respiratory metabolism of the liver due to the uncontrolled entry of calcium ions into the hepatocytes resulting in inhibition of oxidative phosphorylation. Early metabolic disturbances caused by thioacetamide include considerable increases in the ribonucleic acid and the protein content of the nuclear fraction of hepatocytes. Thioacetamide has widely been used to produce varying grades of liver damage in rats including nodular cirrhosis, liver cell proliferation, production of pseudolobules and parenchymal cell necrosis\(^12\).

Antihapatotoxic activity of n-octacosanol may be due to prevention of inhibition RNA synthesis or by altering the membrane permeability or by stimulation of hepatic regeneration.

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REFERENCES