EFFECT OF AN AQUEOUS EXTRACT OF *Salsola collina* ON THE COURSE OF EXPERIMENTAL CHOLELITHIASIS IN RABBITS

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The herbs of hill-growing saltwort (*Salsola collina* Pall. of Chenopodiaceae family) are known to be a source of substances possessing hepatoprotector properties. With respect to experimental hepatitis, the effect of active substances from *Salsola collina* exceeds that of preparations based on *Silibum marianum*, which are widely used in clinical practice [1 – 3].

The purpose of this study was to evaluate the possibility of using an aqueous extract from *Salsola collina* for the prophylaxis of cholelithiasis.

EXPERIMENTAL PART3

The experiments were performed on a group of 33 chinchilla rabbits weighing 2.0 – 2.2 kg divided into four groups. Animals in group 1 (3 rabbits) were killed under ethaminal (sodium pentobarbital) narcosis prior to the main experiment. Biochemical characteristics of their gallbladder bile served as the initial data. Animals in group 2 (10 rabbits) received the usual combined fodder and water. Group 3 (10 rabbits) received the same combined fodder with a 2% cholesterol additive and water. Animals in group 4 (10 rabbits) were fed with the combined fodder containing a 2% cholesterol additive and received water containing a freshly prepared aqueous extract from *Salsola collina* (100 ml per day). The extract was prepared as follows: 30 g of the above-ground part of the herbs (containing 0.7% glycinebetaine [4]) was poured overnight with 1 liter of drinking water. Next morning, the extract was boiled for 30 sec, cooled, and filtered.

The test animals were observed over a period of six months, with monthly weighing and taking blood for biochemical analyses from the otic vein. During this period of time, all experimental groups showed spontaneous lethality of animals. However, no statistically reliable differences in the number of lost animals between groups 1 to 4 were observed. After 2 and 6 months, two to three animals from each of groups 2 – 4 were taken out of the experiment and samples of bile (for biochemical analyses) and biopsy materials from liver and gallbladder (for histological analyses) were taken. The experimental data were statistically processed in terms of the Student $t$-criterion. The analyses of gallbladder lipids after 2 and 6 months included data on the levels of cholesterol, total lipids, bile acids, and phospholipids.

The biochemical analyses were performed on a Corona Clinicon automated analyzer (LKB Sweden). The blood lipids were analyzed by enzymatic techniques at 37°C using reagent kits from Boehringer-Mannheim and Human compa-

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![Micrograph of the gallbladder bile of a rabbit not treated with *Salsola collina* extract, showing numerous crystals of calcium allodeoxycholate and separate crystals of cholesterol monohydrate (magnification, × 250).](image-url)
nies (Germany). The cholesterol level was determined using the cholesterol esterase enzyme test, with spectrophotometric determination of the equivalent amount of hydrogen peroxide. Triglycerides were determined by means of enzymatic hydrolysis (glycerol release) and phospholipids, with the aid of cholinesterase (hydrogen peroxide formation) [5, 6].

RESULTS AND DISCUSSION

Visually, the gallbladder bile of rabbits from group 1 appeared as a transparent golden-brownish fluid, readily spreading on the glass plate surface. Phase-contrast microscopy examination revealed no inclusions. The gallbladder bile of animals from group 3 appeared as a viscous dark-brown liquid with floccules, in contrast to the bile of animals from groups 2 and 4. Phase-contrast microscopy of the bile from group 3 showed the presence of a large number of separate vesicular aggregates, numerous crystals of calcium allodeoxycholate, and separate crystals of cholesterol monohydrate. The vesicular aggregates contained calcium bilirubinate granules, forming massive associated bodies (Figs. 1–3).

Thus, the phase-contrast microscopy data are indicative of the formation of a biliary slough and gallstone precursors in the bile of animals of group 3, since calcium allodeoxycholate crystals are known to be the base component of gallstone in rabbits.

The bile of rabbits of group 4 showed only separate regions with vesicular aggregates of relatively small size and contained no calcium allodeoxycholate crystals. Calcium bilirubinate and calcium carbonate granules were observed as single inclusions (Figs. 4 and 5). The gallbladder bile from the rabbits of group 2 appeared intermediate between that of groups 1 and 4.

The results of histological investigation of the gallbladder walls in the animals of group 3 revealed thickening of the mucous membrane, proliferation of the intraepithelial cells, and increase in the number of intraepithelial lymphocytes. In the samples taken from rabbits of groups 2 and 4, these changes were manifested only slightly (Figs. 6 and 7).

The results of biochemical analyses showed that the gallbladder bile of rabbits in all three experimental groups contains excess cholesterol as compared to the preliminary control (group 1). This implies that even feeding with combined fodder favors an increase in the cholesterol level. As can be seen from the data in Tables 1 and 2, the concentrations of

<table>
<thead>
<tr>
<th>Test group</th>
<th>n</th>
<th>m</th>
<th>[Ch], μmole/ml</th>
<th>[PL], μmole/ml</th>
<th>[BA], μmole/ml</th>
<th>[TL], mg/ml</th>
<th>CSI</th>
<th>[TP], mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>0</td>
<td>3.36 ± 0.57</td>
<td>4.57 ± 1.09</td>
<td>41.49 ± 3.20</td>
<td>2.51 ± 0.21</td>
<td>2.08 ± 0.09</td>
<td>3.12 ± 0.66</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>2</td>
<td>7.70 ± 0.73*</td>
<td>4.10 ± 0.78</td>
<td>30.47 ± 2.71</td>
<td>2.11 ± 0.17*</td>
<td>6.24 ± 1.25</td>
<td>2.24 ± 0.51*</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>2</td>
<td>11.13 ± 0.75*</td>
<td>4.19 ± 1.18</td>
<td>26.21 ± 1.61</td>
<td>2.04 ± 0.15*</td>
<td>8.57 ± 1.34</td>
<td>2.51 ± 0.35*</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>2</td>
<td>7.82 ± 1.25*</td>
<td>4.46 ± 0.69</td>
<td>33.89 ± 2.64</td>
<td>2.31 ± 0.13*</td>
<td>5.02 ± 0.80</td>
<td>2.59 ± 0.50*</td>
</tr>
</tbody>
</table>

Notes: Here and in Table 2, n is the number of observations; m is the month of observation; Ch = cholesterol; PL = phospholipids; BA = bile acids; TL = total lipids; CSI = cholesterol saturation index; TP = total protein; * differences reliable for $p < 0.01$. 

Fig. 2. Micrograph of the gallbladder bile of a rabbit not treated with *Salsola collina* extract, showing well-developed crystals of calcium allodeoxycholate and a large vesicular aggregate in the central region (magnification, × 250).

Fig. 3. Micrograph of the gallbladder bile of a rabbit not treated with *Salsola collina* extract, showing large vesicular aggregates with calcium bilirubinate inclusions (magnification, × 250).