ANTI-INFLAMMATORY ACTIVITY OF TAXIFOLIN

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Received for publication January 11, 1971

Active principles of varied chemical structure isolated from plants possess potent anti-inflammatory activity. The anti-inflammatory activity of triterpenoids, saponins and other natural products has been reported from this laboratory (1-3). The flavonoids viz hesperidin, neohesperidin and naringin have been shown to reduce formalin-induced oedema in the mouse foot (4). The present investigation deals with the study of the anti-inflammatory activity of taxifolin, a flavonoid obtained from Madhuca Butyracea, and the effect of taxifolin on the serum aminotransferase and tissue adenosine triphosphate (ATP) phosphohydrolase activity to elucidate the possible mechanism of its anti-inflammatory activity. The structure of the compound elucidated by Giessmann and Lichner (5) is shown in Fig. 1.

![Taxifolin (Di-hydro quercetin)](image)

**METHODS**

*Anti-inflammatory studies*

The animals used in this study were adult albino rats weighing between 80 and 100 g. They were divided into batches of six animals each except in the case of cotton pellet implantation for which groups of four animals were used.

*Carrageeinin-induced oedema:*

Freshly prepared suspension of carrageeinin, 0.05 ml (1.0% in 0.9% saline) was injected under the plantar aponeurosis of right hind paw of the rats by the method of Winter, Risley and Nuss (6). The animals were pretreated with the test drugs one hour before carrageeinin injection. The volume of the foot was measured before and 3 hours after

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carrageenin treatment by the micro-pipette method described by Buttle, D'Arcy, Howard and Kellet (7).

**Cotton pellet implantation:**

Pellets of surgical cotton weighing 9.0 ± 1 mg were sterilized in an air oven for 2 hours and were implanted in both the axillae and groins under ether anaesthesia according to the method of Meier, Schuler and Desauler (8). The drugs were given intraperitoneally daily for six days. The pellets were dissected out on the 7th day under light ether anaesthesia. They were kept separately in small glass vials, dried for 2 hours at temperature of 150°C and weighed after cooling.

**Formaldehyde-induced arthritis:**

Arthritis was produced by the method of Brownlee (9). 0.1 ml of 2% (v/v) formaldehyde was injected subcutaneously under plantar aponeurosis in each foot on first and third days. The drugs were given intraperitoneally once daily for 10 days. Day-to-day changes in the inflammatory reaction were assessed by measuring the linear cross-section immediately below the ankle joint with a micrometer screw gauge.

The percentage anti-inflammatory effect was calculated for each dose according to the following formula:

\[
\text{Percent anti-inflammatory effect} = \frac{1 - T/C}{C} \times 100
\]

where T and C are the mean volume of oedema, diameter of ankle or weight of granulation tissue in drug treated and control groups respectively.

ED50 values of the test drugs were determined graphically by plotting the percentage protection against log dose and their relative potencies calculated with reference to hydrocortisone.

**Toxicity studies**

Approximate LD50 was determined in albino rats by the method of Smith (10). Adult albino rats weighing between 60 and 65 g were used for this study. The rats were divided into groups of two animals each. The drugs were administered intraperitoneally and the 24 hours mortality was recorded.

**Biochemical studies**

The enzyme estimation were carried out in normal and arthritic albino rats with or without treatment. Serum was obtained from the blood collected after decapitation of the rats. The liver tissues were obtained immediately and pooled.

Serum L-aspartate: 2-oxoglutarate aminotransferase (EC. No. 2.6.1.1, aspartate aminotransferase) and serum L-alanine: 2-oxoglutarate aminotransferase (EC. No. 2.6.1.2, alanine aminotransferase) were estimated by the method of Reitman and Frankel (11). One unit of enzyme activity was the change in the optical density of 0.001/min/ml of serum. Optical density was measured by a Bausch and Lomb spectronic '20' colorimeter at 505 mμ.

ATP phosphohydrolase (EC. No. 3.6.1.4) activity was assayed in 10 per cent (w/v) homogenates of pooled liver prepared in 0.25 M sucrose by Potter Elvehjem homogenizer. The reaction mixture consisted of 0.05 M Tris, pH 8.1, 1 mM ATP and 0.1 ml of 10 per
cent tissue homogenates in a final volume of 2 ml. Release of Pi (inorganic phosphorus) from ATP was measured according to Fiske and SubbaRow (12). The split of 1 µm of Pi/100 mg of tissue in 15 minutes at 37° was considered as one unit of the enzyme activity.

RESULTS

The effects of taxifolin (40 mg/kg i.p.) and hydrocortisone (10 mg/kg i.p.) were studied on carrageenin induced oedema (Table 1), formaldehyde induced arthritis (Table 2) and on granulation tissue formation by cotton pellet implantation (Table 3) in albino rats. Taxifolin showed significant anti-inflammatory activities (P<0.001) similar to hydrocortisone (P<0.01) in all the three tests.

TABLE 1. Effect of hydrocortisone and taxifolin against carrageenin induced oedema in albino rats.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Dose mg/kg i.p.</th>
<th>Mean volume of oedema ml ± S.E.</th>
<th>Percent anti-inflammatory effect</th>
<th>'P'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>0.5 ml</td>
<td>0.93 ± 0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(Control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>10</td>
<td>0.53 ± 0.03</td>
<td>43.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Taxifolin</td>
<td>40</td>
<td>0.66 ± 0.04</td>
<td>29.1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

TABLE 2. Effect of hydrocortisone and taxifolin on the weight of granulation tissue formed by cotton pellet implantation in rats.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Dose mg/kg i.p.</th>
<th>Mean average weight of granulation tissue in mg ± S.E.</th>
<th>Percent anti-inflammatory effect</th>
<th>'P'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>0.5 ml</td>
<td>16.3 ± 0.42</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(Control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>10</td>
<td>11.1 ± 0.23</td>
<td>31.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Taxifolin</td>
<td>40</td>
<td>9.1 ± 0.3</td>
<td>44.2</td>
<td>&lt;0.001</td>
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</tbody>
</table>

TABLE 3. Effect of hydrocortisone and taxifolin against formaldehyde induced arthritis in albino rats.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Dose mg/kg i.p.</th>
<th>Average initial diameter in mm ± S.E.</th>
<th>Average 10 days diameter in mm ± S.E.</th>
<th>Percent anti-inflammatory effect</th>
<th>'P'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>0.5 ml</td>
<td>5.11 ± 0.02</td>
<td>7.0 ± 0.08</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(Control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>10</td>
<td>5.16 ± 0.03</td>
<td>6.07 ± 0.05</td>
<td>51.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Taxifolin</td>
<td>40</td>
<td>5.06 ± 0.06</td>
<td>6.20 ± 0.05</td>
<td>34.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The anti-inflammatory ED₅₀ was determined by testing graded doses of taxifolin and hydrocortisone against carrageenin induced oedema. The approximate LD₅₀ and anti-inflammatory ED₅₀ values for taxifolin and hydrocortisone are shown in Table 4. The ratio of LD₅₀/ED₅₀ (therapeutic index) for taxifolin was 12.2 as compared to 11.6 for hydrocortisone.

The effects of hydrocortisone and taxifolin on serum aminotransferases in normal
and arthritic rats are shown in Table 5. Both aspartate and alanine aminotransferases activities were significantly increased in the serum during inflammation. Taxifolin prevented the increase in the enzyme activities due to the inflammatory reaction similar to that obtained with hydrocortisone.

The effects of taxifolin and hydrocortisone on ATP phosphohydrolase activity in pooled liver homogenates obtained from normal and arthritic rats are shown in Table 5. Both aspartate and alanine aminotransferases activities were significantly increased in the serum during inflammation. Taxifolin prevented the increase in the enzyme activities due to the inflammatory reaction similar to that obtained with hydrocortisone.

The flavonoids have been reported to possess potent anti-inflammatory activity (4). In the present study taxifolin which is a flavonoid was found to possess significant anti-inflammatory activity against carrageenin induced oedema involving the exudative phase of inflammation and also on formaldehyde induced arthritis and cotton pellet implantation which involve the proliferative phase of inflammatory reaction. Though taxifolin was one-eighth as active as hydrocortisone, its therapeutic index (ratio of LD$_{50}$: ED$_{50}$) was almost equal to that of hydrocortisone.

Taxifolin prevented the increase in serum aspartate and alanine aminotransferase activities due to the inflammatory reaction and stimulated liver ATP phosphohydrolase activity. However, in our study liver ATP phosphohydrolase activity was unaltered during inflammation. This indicate that increase in liver ATP phosphohydrolase activity by taxifolin and hydrocortisone is not related to their anti-inflammatory effect. Whereas, the inhibitory effect of taxifolin and hydrocortisone on serum aminotransferases may be

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Approximate LD$_{50}$ (mg/kg)</th>
<th>Anti-inflammatory ED$_{50}$ (mg/kg)</th>
<th>Relative Potency</th>
<th>Therapeutic index LD$<em>{50}$/ED$</em>{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocortisone</td>
<td>150</td>
<td>12.9</td>
<td>1</td>
<td>11.6</td>
</tr>
<tr>
<td>Taxifolin</td>
<td>1200</td>
<td>98.0</td>
<td>0.13</td>
<td>12.2</td>
</tr>
</tbody>
</table>

**TABLE 5.** Effect of hydrocortisone and taxifolin on serum aminotransferases and liver ATP phosphohydrolase activities in normal and arthritic rats.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Serum aspartate aminotransferase $^{*}$</th>
<th>Serum alanine aminotransferase $^{*}$</th>
<th>Liver ATP phosphohydrolase $^{**}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28.2 ± 1.2</td>
<td>32.1 ± 1.5</td>
<td>10.73</td>
</tr>
<tr>
<td>Arthritic</td>
<td>50.4 ± 1.3</td>
<td>39.2 ± 1.5</td>
<td>10.72</td>
</tr>
<tr>
<td>Arthritic + Hydrocortisone</td>
<td>28.3 ± 1.3</td>
<td>24.5 ± 1.1</td>
<td>16.09</td>
</tr>
<tr>
<td>Arthritic + Taxifolin</td>
<td>28.0 ± 1.0</td>
<td>23.0 ± 1.1</td>
<td>13.41</td>
</tr>
</tbody>
</table>

* Enzyme activity in Karmen Unit; one unit = change in optical density of 0.001/min/ml of serum.
** Expressed in pmoles of Pi split for 100 mg tissue in 15 minutes at 37°C.
responsible for inhibition of mucopolysaccharide synthesis which is mainly concerned with the proliferative phase of inflammation. An inhibition of aminotransferases (13–17) and stimulation of ATP phosphohydrolase (18–21) have also been observed with salicylates, phenylbutazone, corticoids, indomethacin, glycyrrhetic acid and other anti-inflammatory drugs.

SUMMARY

Taxifolin, a flavonoid obtained from *Madrhuca Butracea* was found to possess potent anti-inflammatory activity on the exudative and the proliferative phases of inflammation in albino rats. Taxifolin was one-eighth as active as hydrocortisone on carrageenin-induced oedema; however, its “therapeutic index” was almost equal to that of hydrocortisone.

Taxifolin prevented the increase in serum aminotransferase activity during inflammation. Similar findings were also obtained with hydrocortisone control. ATP phosphohydrolase activity in liver homogenate remained unaltered during inflammation but was significantly elevated by these agents. The significance of these biochemical changes is discussed.

**Acknowledgement:** The authors wish to express their thanks to Mr. O.S. Tewari for the technical assistance and Indian Council of Medical Research for the financial assistance.

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