Rose hip inhibits chemotaxis and chemiluminescence of human peripheral blood neutrophils \emph{in vitro} and reduces certain inflammatory parameters \emph{in vivo}

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Abstract—Objective and Design: The objective of this study was to investgate the leucocyte-related antiinflammatory properties of rose hip.

Materials and Methods: The effect ofrose hip on a number of inflammatory parameters was evaluated using the following models: (1) The effect of rose hip extract or chemotaxis and chemiluminescence of peripheral blood polymorphonuclear leucocytes (PMNs) from healthy subjects \emph{in vitro}; (2) The effect of rose hip administered to healthy subjects on serum levels of creatine and C-reactive protein and on chemotaxis and chemiluminescence of peripheral blood PMNs.

Results: Rose hip extract at concentration higher than 500 \textmu g/ml inhibited the chemotaxis and chemiluminescence of peripheral blood polymorphonuclear leucocytes \emph{in vitro}. Daily intake of rose hip powder at doses of 45 grams or lower by healthy subjects resulted in reduced chemotaxis of peripheral blood PMNs and reduced the level of serum creatine and acutephase protein CRP.

Conclusions: These results indicate that rose hip possesses antiinflammatory properties and might be used as a replacement or supplement for conventional drug therapies in some inflammatory diseases such as arthritis.

Key words: Rose hip; \emph{Rosacanina}; neutrophil; chemotaxis; CRP; antiinflammatory.

1. INTRODUCTION

Inflammatory diseases such as arthritis involve a broad spectrum of different clinical manifestations. Inflammatory cells such as polymorphonuclear leucocytes have been shown to be involved in the inflammatory process and tissue damage.
and hydrolytic enzymes as well as toxic reactive oxygen radicals from these cells activated in the tissue and joints (Harris, 1988). Therapy of inflammatory diseases involves alleviation of the symptoms associated with the disease, such as relief of pain, reduction of inflammation and increase of motion. Acetylsalicylic acid (aspirin) and other non-steroid anti-inflammatory drugs such as ibuprofen, methotrexate and naproxen, and glucocorticoids have been used for the treatment of arthritis (Hochberg et al., 1995a; b; Ridker, et al., 1997). Control of the symptoms with these drugs requires long term daily treatment. These drugs have a variety of toxic and other side effects, such as gastric erosion and adverse effects on kidneys and liver. Some of these drugs, particularly the glucocorticoids, inhibit the immune response to infections. Therefore, there is a great need for alternative therapies for the management of arthritis which can eliminate the need for conventional drugs and their side-effects, particularly for prolonged daily use. In a short communication we have reported on the anti-inflammatory activity of rose hip in four subjects suffering from mild osteoarthritis (Winther et al., 1999). The purpose of this study was to investigate in more detail the anti-inflammatory property of the natural product rose hip, utilizing in vitro methods in a larger number of healthy subjects.

2. MATERIALS AND METHODS

2.1. Rose hip

The extract was prepared by incubating 80 mg of Hyben Vital rose hip (Langeland, Denmark) dry powder from Rosa canina with 4 ml of minimal essential medium (MEM) containing 50 units/ml of penicillin and 0.05 mg/ml of streptomycin, for 19 h at 37°C. The extracts were prepared from either the whole fruit powder, the shells or the seeds. The shells and the seeds were separated from each other by splitting the dried fruit and separating the shells from the seeds manually. They were then ground in a mortar. Chemical analyses of Hyben Vital rose hip was performed by Steins Laboratorium A/S, Holstebro, Denmark. Following incubation of the powders in MEM, the mixtures were centrifuged at 4000 rpm for 10 min. The supernatants were collected, sterile filtered and diluted further. The pH of extract preparations was adjusted to pH 7.2 before use.

2.2. Chemotaxis

The chemotaxis assay was performed using a modified Boyden chamber technique as previously described (Jensen and Kharazmi, 1991). PMNs isolated from peripheral blood of healthy subjects were preincubated with different dilutions of rose hip extract for 30 min at 37°C. Following preincubation, the chambers from the Boyden chambers were filled with 1 ml of fresh RPMI containing 5% FCS. The upper and lower chambers were separated by a polycarbonate filter with 5 μm pores. The lower chamber was then filled with 0.1 ml of 10% serum RPMI. Supernatant was collected and the lower chamber was discarded after 1 h incubation. The white blood cells were counted in a hemocytometer and the chemotaxis was expressed as chemotaxis index (CI).

### Table 1

<table>
<thead>
<tr>
<th>Protein</th>
<th>6.2 g</th>
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</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>23.0 g</td>
</tr>
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</table>
of peripheral blood polymorphonuclear leukocytes (PMN) from subjects cessation of treatment with 45 g (high dose) or 10 g (low dose) daily intake no treatment. The chemotaxis is determined towards the chemotactic peptide given as mean ± SEM number of cells migrate from 13 subjects. The mean with low dose and high dose were significantly lower than that for no treatment (p < 0.001, respectively).

90 ± 26.2 when blood samples were taken 28 days after cessation (p < 0.001). The mean ± SEM values for PMN chemotaxis activated serum (ZAS) which contains the biologically active 25a was 218 ± 60.0 as compared to 529.9 ± 39.9 when tested of treatment with rose hip (p < 0.001). The decline in IMLP was 65% in 12 out of 13 volunteers: a considerable response. The decline in chemotactic response to ZAS was variable decline in 12 out of 13 volunteers. I was the same subject nd of therapy in both assays.

![Graph showing inhibition of chemotaxis and chemiluminescence](image)

**Figure 1.** Effect of rose hip extract on polymorphonuclear leukocytes (PMN) chemotaxis in vitro. Cells were preincubated with various concentrations of rose hip powder as given in the X-axis for 3 min. The data are presented as percent inhibition of PMN chemotaxis for each subject tested.

**Table 2.**
Effect of rose hip extract on human peripheral blood polymorphonuclear leucocyte (PMN) chemiluminescence. The data are presented as percent inhibition as compared with control.

<table>
<thead>
<tr>
<th>Rose hip extract (mg/ml)</th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>2500</td>
<td>37</td>
<td>27</td>
<td>57</td>
</tr>
<tr>
<td>1000</td>
<td>8</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>500</td>
<td>0</td>
<td>0</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: Not determined.

form used as control up to a concentration of 5000 µg/ml had almost no effect on PMN chemiluminescence when the pH of vitamin C solution was adjusted to the physiological pH 7.2. Vitamin E (alpha-tocopherol) was also used as a known antioxidant control. Vitamin E at a concentration of over 1 µg/ml inhibited chemiluminescence.

4. EX VIVO STUDIES

4.1 Blood chemistry
of high dose therapy, values obtained 28 days after stopping intake and those
obtained at the end of low dose therapy (data not shown).

creatinine, however, declined significantly compared with initial values
as mean ± SEM (90.0 ± 2.1 μmol/l) to values obtained after 10 days
18 μmol/l) and 28 days (84.9 ± 1.9 μmol/l) of intake, respectively.
(0.001). When treatment had been stopped for 28 days the serum creatinine
significantly increased (93.2 ± 1.9 μmol/l) (p < 0.001) and were similar to
values obtained before intake.

Data on C-reactive protein are given in Fig. 2. Similar to the findings on serum
creatinine, CRP values were also decreased during intake of rose hip. The initial
± SEM values of CRP were 5.38 ± 0.4 mg/l and declined to 3.31 ± 0.49 mg/l
(3.31 ± 0.47 mg/l, after 10 and 28 days of intake respectively (p < 0.05). After
therapy for 28 days, the levels increased to 5.75 ± 0.54 mg/l (p < 0.05)
compared with that previously.

5. DISCUSSION

The studies described in this communication demonstrate that the extract from rose
hip inhibited, in vitro, the chemotaxis and oxidative burst response of the human
peripheral blood polymorphonuclear leukocytes, important and abundant inflammatory
cells involved in the pathogenesis of arthritis. Furthermore, administration of rose
hip to healthy volunteers for a period of 28 days inhibited the chemotactic
response of neutrophils by approximately 60% or higher. Moreover, rose hip
lowered the level of serum creatinine and the acute phase protein C-reactive protein
in volunteers with values within normal range, which is below 10 mg/l. Serum
creatinine levels were within the normal range in all the volunteers (males 55–125
and females 45–100 μmol/l). However, the decline was statistically significant and
might indicate enhanced glomerular filtration. The blood chemistry data presented in
this study showed that intake of rose hip had no harmful effect on any of the liver
functions determined in this study.

Studies on the inhibition of neutrophil oxidative burst response by rose hip extract.
extract from shells, seeds and the whole powder were prepared and tested in PMN chemotaxis assay. As shown in Fig. 1 the major inhibitory activity was found to reside in the shells. It will be interesting to identify the compound(s) responsible for the anti-inflammatory activity of rose hip.

The inhibition of chemotaxis observed in our study was comparable to that observed with acetylsalicylic acid as reported by Matzner et al. (1984). On the other hand Kemp et al. (1982) showed that incubation of neutrophils in vitro with sodium salicylate increased the chemotaxis of these cells. Similar increased response was observed in normal individuals after ingestion of sodium salicylate (Kemp et al., 1982). Some non-steroid anti-inflammatory drugs such as ibuprofen at attainable concentrations during therapy has been shown to inhibit neutrophil locomotion by 50%; a finding which is similar to our findings with rose hip (Rivkin et al., 1976; Kaplan et al., 1984; Maderazo et al., 1984).

6. CONCLUSION

Rose hip possesses anti-inflammatory and anti-oxidant properties. These properties are important in alleviation of tissue damage in the inflammatory sites. As a natural product, rose hip has no side effects, is safe and can be administered easily. It can be designed for daily consumption as supplemental part of a therapeutic regimen for some inflammatory diseases, or as a prophylactic regimen for individuals having a genetic or environmental predisposition to these diseases. A large scale placebo-controlled clinical study will be required to extend confirmation of the anti-inflammatory effect of rose hip described in this report.

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REFERENCES


