

... was used as a measure of oxygen radical generation by ... method was performed as previously described (Kharazmi ... ere preincubated with different dilutions of rose hip extract ... with either fMLP or opsonized zymosan. The oxidative ... ctivated cells was measured by a luminometer (1250-LKB ... eers represented by both sexes with a mean age of 47 years ... ere included in this study. All the subjects included were ... ascular, immunological, kidney, liver, allergic, rheumatolog- ... disorders. The volunteers were treated with 45 g of Hyben ... : 28 days (high dose), followed for another 28 days during ... e hip was not taken. At the end of this period, the volunteers ... ent of rose hip at a dose of 10 g daily for 28 days (low dose). ... olunteers went through a screening procedure to assure that ... ntioned diseases were present. Moreover, before inclusion ... ken for C-reactive protein (CRP) measurement to assure that ... olunteers suffered from unknown infectious diseases.

... ium, serum creatinine, alanine aminotransferase, alkaline ... ehydrogenase, bilirubin, hemoglobin and total cholesterol ... fore initiation of the treatment. All measurements were per- ... e conventional laboratory routine. All the above mentioned ... m creatinine and CRP were repeated after 5, 10, 21 and 28 ... days after stopping rose hip therapy and again at the end of ... rum creatinine and C-reactive protein (CRP) were tested be- ... nd 28 days of treatment, 28 days following cessation of the ... t the end of low dose treatment. Rose hip was taken together ... on. On the days of blood sampling rose hip was taken to- ... al at 09.00 a.m., two hours before blood sampling. Blood ... rformed after 15 min at rest sitting in a chair.

Rose hip inhibits chemotaxis and chemiluminescence of human peripheral blood neutrophils *in vitro* and reduces certain inflammatory parameters *in vivo*

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Received 25 May 1999; revised 12 July 1999; accepted 15 July 1999

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

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

Results: Rose hip extract at concentrations higher than 500 µg/ml inhibited the chemotaxis and chemiluminescence of peripheral blood polymorphonuclear leucocytes *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 creatinine and acute phase 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; *Rosa canina*; 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 (Hochberger *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 4°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...

chemical analyses of the commercially available Hyben Vital powder contains proteins, carbohydrates, a low amount of vitamins such as vitamin A, vitamin B, vitamin C, vitamin E and vitamin K. The powder also contains several minerals. Uptake of vitamin C present in the powder was as good as, or even better than, that of vitamin C present in a tablet form. The concentrations and kinetics of uptake through the powder were similar to those of the equivalent of 250 mg vitamin C in rose hip powder was similar to that of vitamin C in tablet form. The better absorption of vitamin C in powder may be due to a larger surface area of rose hip powder as compared to a tablet.

These experiments were performed and it was found that the extract concentrations equivalent to 500 µg/ml and higher inhibited chemotaxis *in vitro*. As shown in Fig. 1, rose hip extract at concentrations of 500 µg/ml inhibited chemotaxis of human peripheral blood neutrophils; the inhibitory effect was as strongly inhibitory as that of rose hip extract. The two major parts of rose hip — shells and seeds — were separated and tested separately for their activity on PMN chemotaxis. It was shown that the inhibitory activity resided in the shells (Fig. 1). The inhibitory activity was higher at both the 1000 µg/ml and 500 µg/ml levels than that of seeds ($p \leq 0.01$ and $p \leq 0.04$, respectively). Rose hip shells with whole powder, there was significantly higher activity at 500 µg/ml ($p \leq 0.03$) but not at 1000 µg/ml.

Chemotaxis

As shown in Fig. 2, rose hip extract inhibited the chemiluminescence of PMNs stimulated with zymosan. Adjustment of pH to physiological values in the assay did not influence the inhibitory effect markedly. Vitamin C in crystalline form did not influence the chemotaxis assay.

Table 1.

Chemical analyses of the commercially available Hyben Vital rose hip powder. The values are given for 100 g of dry powder

Protein	6.2 g
Carbohydrates	39.0 g

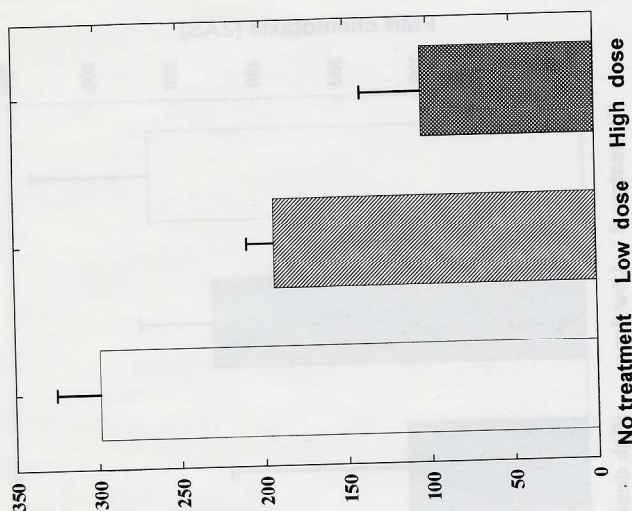


Figure 1. Effect of rose hip extract on polymorphonuclear leukocytes (PMN) chemotaxis *in vitro*. Cells were preincubated with various concentrations of rose hip powder as given in the X-axis for 30 min. The data are presented as percent inhibition of PMN chemotaxis for each subject tested.

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

Rose hip extract concs (µg/ml)	Subject 1	Subject 2	Subject 3
2500	37	27	57
1000	8	8	12
500	0	0	ND

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

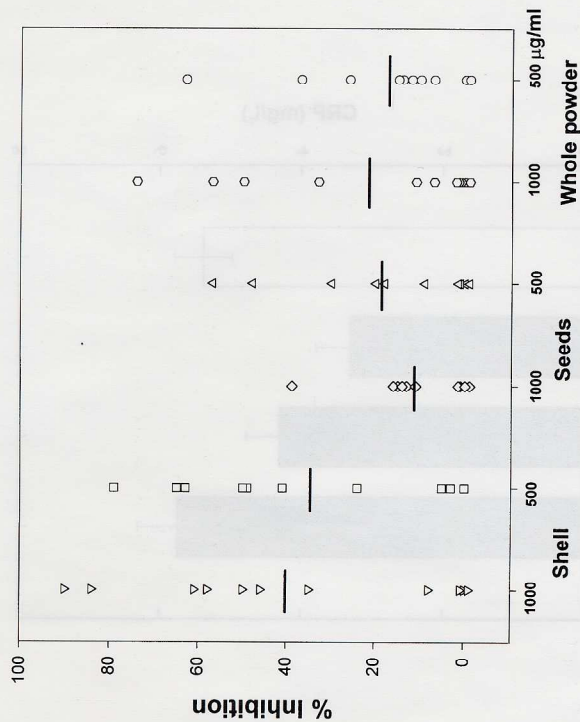


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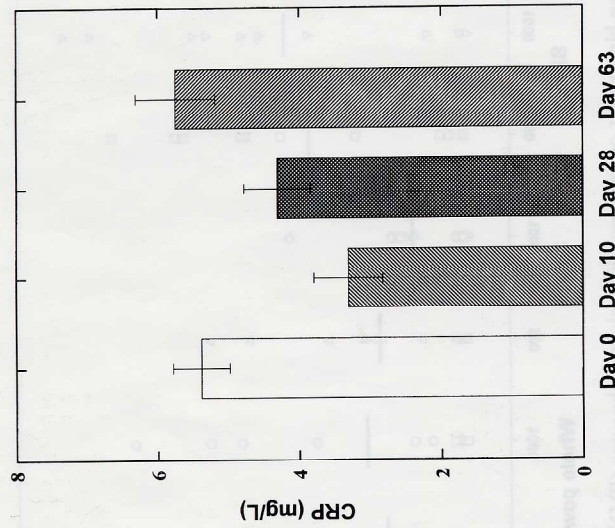
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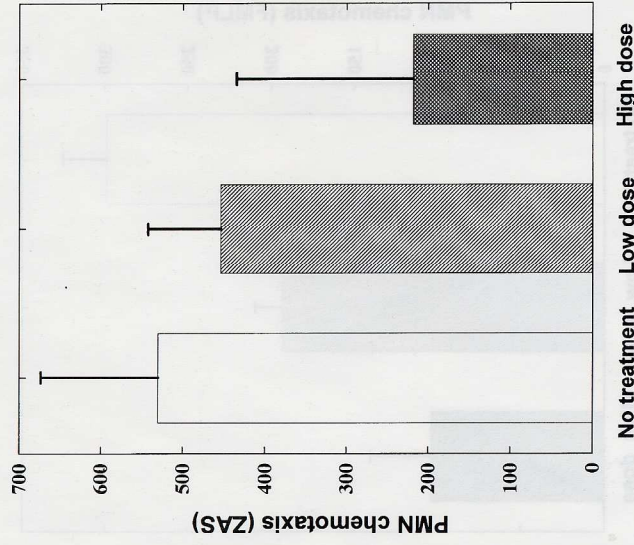


2. Levels of serum C-reactive protein (CRP) as given in mg/l from subjects on the start, 10 days and 28 days during intake of rose hip and 35 days after cessation of treatment (Day 63) after 45 g daily intake of rose hip. The results are given as mean \pm SEM values from 13 subjects. The value on day 10 was significantly lower than that on day 0 and day 63 ($p \leq 0.05$).

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

um creatinine, however, declined significantly compared with initial values as mean \pm SEM ($90.0 \pm 2.1 \mu\text{mol/l}$) to values obtained after 10 days $\pm 1.8 \mu\text{mol/l}$) and 28 days ($84.9 \pm 1.9 \mu\text{mol/l}$) of intake, respectively ($p < 0.001$). When treatment had been stopped for 28 days the serum creatinine significantly increased ($93.2 \pm 1.9 \mu\text{mol/l}$) ($p < 0.001$) and were similar to those 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 \pm SEM values of CRP were $5.38 \pm 0.4 \text{ mg/l}$ and declined to $3.31 \pm 0.47 \text{ mg/l}$. After 28 days of intake respectively ($p < 0.05$). After 35 days of intake respectively ($p < 0.05$). After 35 days of intake respectively ($p < 0.05$). After 35 days of intake respectively ($p < 0.05$).



4. Chemotaxis of peripheral blood polymorphonuclear leukocytes (PMN) from subjects during and 28 days after cessation of treatment with 45 g (high dose) or 10 g (low dose) daily intake of rose hip for 28 days or no treatment. The chemotaxis is determined towards zymosan activated serum (ZAS). The results are given as mean \pm SEM number of cells migrated from 13 subjects. The mean chemotaxis value for high dose was significantly lower than that for no treatment group ($p \leq 0.001$).

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 $\mu\text{mol/l}$). 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.

Acknowledgements

Expert technical assistance of Anne Asanovski and Mounir Ainouz is acknowledged. This work received partial financial support from The Danish Rheumatism Association.

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