

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

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Abstract – Objective and Design: The objective of this study was to investigate the leucocyte-related anti-inflammatory 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.

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. Inflammatory cytokines such as TNF appear to be involved in the amplification of the disease process. The damage is caused by the release of proteolytic 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.

Materials and methods

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.

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 chemotaxis of the cells towards the chemotactic peptide fMet-Leu-Phe (fMLP) or zymosan activated serum (ZAS) were tested. The migrated cells were counted by a computer-assisted image analysis system.

Chemiluminescence - Chemiluminescence assay was used as a measure of oxygen radical generation by activated PMNs. The method was performed as previously described (Kharazmi et al., 1984). PMNs were preincubated with different dilutions of rose hip extract and then stimulated with either fMLP or opsonized zymosan. The oxidative burst response of the activated cells was measured by a luminometer (1250-LKB Wallace).

Subjects - Thirteen healthy volunteers represented by both sexes with a mean age of 47 years (range 30-59 years) were included in this study. All the subjects included were without known cardiovascular, immunological, kidney, liver, allergic, rheumatological or haematological disorders. The volunteers were treated with 45 g of Hyben vital rose hip daily for 28 days (high dose), followed for another 28 days during which Hyben vital rose hip was not taken. At the end of this period, the volunteers received another treatment of rose hip at a dose of 10 g daily for 28 days (low dose). Before inclusion, all volunteers went through a screening procedure to assure that none of the above mentioned diseases were present. Moreover, before inclusion blood samples were taken for C-reactive protein (CRP) measurement to assure that none of the included volunteers suffered from unknown infectious diseases.

Blood chemistry - Blood potassium, sodium, serum creatinine, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, bilirubin, hemoglobin and total cholesterol were also measured before initiation of the treatment. All measurements were performed according to the conventional laboratory routine. All the above mentioned parameters except serum creatinine and CRP were repeated after 5, 10, 21 and 28 days of treatment, 28 days after stopping rose hip therapy and again at the end of low dose treatment. Serum creatinine and C-reactive protein (CRP) were tested before therapy, after 10 and 28 days of treatment, 28 days following cessation of the

treatment and finally at the end of low dose treatment. Rose hip was taken together with a meal at 12.00 noon. On the days of blood sampling rose hip was taken together with a light meal at 09.00 a.m., two hours before blood sampling. Blood sampling was always performed after 15 min at rest sitting in a chair.

Statistical analysis - Statistical analysis of the data was performed by using Wilcoxon test for matched pairs, p values of < 0.05 were considered significant.

RESULTS

Analysis of rose hip - Table 1 shows the chemical analyses of the commercially available Hyben Vital Rose hip. Rose hip powder contains proteins, carbohydrates, a low amount of fat and several 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 Hyben vital powder was as good as, or even better than, that of vitamin C when given in tablet form. The concentrations and kinetics of uptake through the gastrointestinal tract of the equivalent of 250 mg vitamin C in rose hip powder was similar to 500 mg vitamin C in tablet form. The better absorption of vitamin C in rose hip powder may be due to a larger surface area of rose hip powder as compared to vitamin C tablets.

Chemotaxis Initial dose-response experiments were performed and it was found that the extract of rose hip at concentrations equivalent to 500µg/ml and higher inhibited chemotaxis of PMNs in vitro. As shown in Fig. 1, rose hip extract at concentrations of 500 µg/ml and higher inhibited chemotaxis of human peripheral blood neutrophils; pH-adjusted rose hip extract at these concentrations was as strongly inhibitory as the non-pH-adjusted rose hip extract. The two major parts of rose hip – shells and seeds -- were tested separately for their activity on PMN chemotaxis. It was shown that by far most of the inhibitory activity resided in the shells (Fig. 1). The inhibition of chemotaxis by rose hip shells at both the 1000µg/ml and 500 µg/ml levels was significantly higher than that of seeds ($p \leq 0.01$ and $p \leq 0.04$, respectively). When comparing rose hip shells with whole powder, there was significantly higher inhibition by rose hip shells at 500µg/ml ($p \leq 0.03$) but not at 1000 µg/ml.

Chemiluminescence - As shown in Table 2, rose hip extract inhibited the chemiluminescence of PMNs activated by opsonized zymosan. Adjustment of pH to physiological values in the extract did not influence the inhibitory effect markedly. Vitamin C in crystalline 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 1µg/ml inhibited chemiluminescence.

Protein	6.2 g
Carbohydrate	39.0 g
Fat	4.0 g
Vitamin C	560 mg
Energy	916 kJ

Table 1. Chemical analyses of the commercially available Hyben Vital rose hip powder. The values are given for 100 g of dry powder

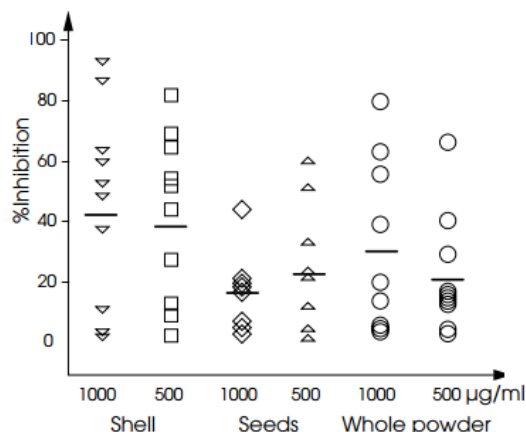


Figure 1. Effect of rose hip extract on polymorphonuclear leukocytes (PMN) chemotaxis in vitro. Cells were preincubated with various concentrations of rose hip powder as given in the X-axis for 30 min. The data are presented as percent inhibition of PMN chemotaxis for each subject tested.

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

ND: Not determined.

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

Ex vivo studies

Blood chemistry - No significant changes occurred in potassium, sodium, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, bilirubin, haemoglobin or total cholesterol comparing values from before intake to values obtained after 5, 10, 21 and 28 days of high dose therapy, values obtained 28 days after stopping intake and those obtained at the end of low dose therapy (data not shown).

Serum creatinine, however, declined significantly compared with initial value given as mean $4 \pm \text{SEM}$ ($90.0 \pm 2.1 \mu\text{mol/l}$) to values obtained after 10 days: ($87.4 \pm 1.8 \mu\text{mol/l}$) 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 levels significantly increased ($93.2 \pm 1.9 \mu\text{mol/l}$) ($p < 0.001$) and were similar to values obtained before intake.

The 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 mean $4 \pm \text{SEM}$ values of CRP were $5.38 \pm 0.4 \text{ mg/l}$ and declined to $3.31 \pm 0.49 \text{ mg/l}$ and $4.31 \pm 0.47 \text{ mg/l}$, after 10 and 28 days of intake respectively ($p < 0.05$). After stopping therapy for 28 days, the levels increased to $5.75 \pm 0.54 \text{ mg/l}$ ($p < 0.051$) as compared with that previously.

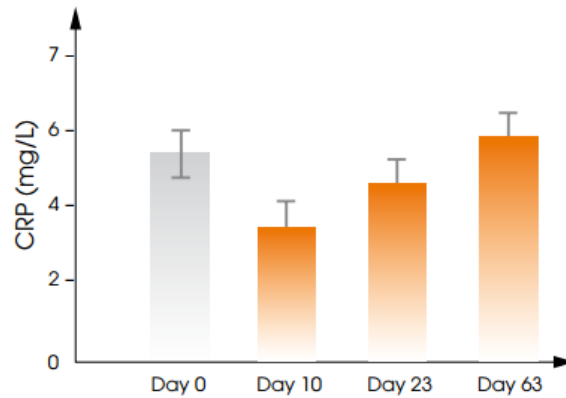


Figure 2. Levels of serum C-reactive protein (CRP) as given in mg/l from subjects on the start (Day 0), 10 days and 28 days during intake of rose hip and 35 days after cessation of treatment (Day 63) with 45 g daily intake of rose hip. The results are given as mean $4 \pm$ SEM values from 13 subjects. The mean value on day 10 was significantly lower than that on day 0 and day 63 ($p \leq 0.05$).

Chemotaxis - PMN chemotaxis in the period during which the volunteers had not taken any rose hip powder was compared with values obtained in the preceding 28 days (Figs 3 and 4). The mean \pm SEM value of PMN chemotaxis towards the chemotactic peptide fMLP was 103.6 ± 60.0 when tested on day 28 of treatment with rose hip as compared with 298.9 ± 26.2 when blood samples were taken 28 days after cessation of rose hip intake ($p < 0.001$). The mean \pm SEM values for PMN chemotaxis towards zymosan activated serum (ZAS) which contains the biologically active chemotactic factor C5a was 218 ± 60.0 as compared to 529.9 ± 39.9 when tested 28 days after cessation of treatment with rose hip ($p < 0.001$). The decline in chemotaxis response to fMLP was 65% in 12 out of 13 volunteers: a considerable decline of chemotaxis response. The decline in chemotactic response to ZAS was 59%, also a considerable decline in 12 out of 13 volunteers. It was the same subject who did not respond to therapy in both assays.

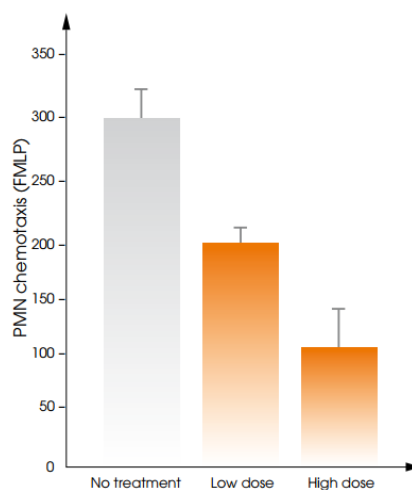


Figure 3. Chemotaxis of peripheral blood polymorphonuclear leukocytes (PMN) from subjects during and 28 days after cessation of treatment with 45 g (high dose) or 10 g (low dose) daily intake of rose hip for 28 days or no treatment. The chemotaxis is determined towards the chemotactic peptide FMLP. The results are given as mean \pm SEM number of cells migrated from 13 subjects. The mean chemotaxis values for both low dose and high dose were significantly lower than that for no treatment group ($p \leq 0.01$ and $p \leq 0.001$, respectively).

Clinical findings - No allergic reactions or any other side-effects were observed during therapy. Only two volunteers complained of mild gastrointestinal gas disturbances at the end of the study, while on the high dose

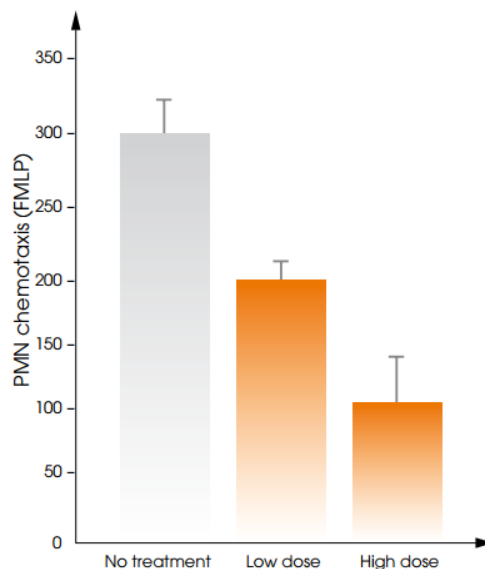


Figure 4. Chemotaxis of peripheral blood polymorphonuclear leukocytes (PMN) from subjects during and 28 days after cessation of treatment with 45 g (high dose) or 10 g (low dose) daily intake of rose hip for 28 days or no treatment. The chemotaxis is determined towards zymosan activated serum (ZAS). 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 ($p \leq 0.001$)

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 showed that this effect was not due to vitamin C content of the extract. This is shown by the inability of pH-adjusted vitamin C to inhibit chemiluminescence whereas pH adjusted rose hip extract was still as inhibitory as non-pH-adjusted extract. In order to determine which part of rose hip exhibited the inhibitory effect on chemotaxis the 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).

CONCLUSION - Rose hip possesses anti-inflammatory and antioxidant 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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