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Anti-inflammatory Activity of *Polygonum bistorta*, *Guaiacum officinale* and *Hamamelis virginiana* in Rats

M. DUWIEJUA†, I. J. ZEITLIN, P. G. WATERMAN* AND A. I. GRAY*

*Department of Physiology and Pharmacology and *Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow, UK*

Abstract—The aqueous ethanolic extracts of *Polygonum bistorta* L. Polygonaceae, *Guaiacum officinale* L. Zygophyllaceae and *Hamamelis virginiana* L. Hamamelidaceae were screened for anti-inflammatory activity. Administered (100 and 200 mg kg^{-1}, p.o.) before the induction of carrageenan rat paw oedema, extracts of *P. bistorta* significantly suppressed both the maximal oedema response and the total oedema response (monitored as area under the time course curve). *H. virginiana* was inactive and *G. officinale* was only active at 200 mg kg^{-1}. At 200 mg kg^{-1} administered before the induction of adjuvant arthritis, *P. bistorta* significantly inhibited both the acute and chronic phases of the adjuvant-induced rat paw swelling, while *G. officinale* and *H. virginiana* were only active against the chronic phase. Further studies on *P. bistorta* (100–800 mg kg^{-1}) revealed a dose-dependent inhibition of the carrageenan-induced rat paw oedema over the dose range 100–400 mg kg^{-1}, the E50 value being approximately 158·5 mg kg^{-1}. The extract (200 mg kg^{-1}), administered after the onset of the inflammatory responses reversed the course of both the carrageenan- and adjuvant- induced rat paw swelling. The results confirm that the extracts of *P. bistorta*, *G. officinale* and *H. virginiana* contain anti-inflammatory substances.

Natural products of plant origin are still a major part of traditional medical systems in developing countries. There is also a resurgence of interest in herbal medicines in western countries (Phillipson & Anderson 1989) as alternative sources of drugs for often intractable diseases such as rheumatoid arthritis. As part of a study to evaluate the efficacy of some of these plants and to search for novel structures with anti-inflammatory actions, we have screened extracts from three plants (*Hamamelis virginiana*, *Guaiacum officinale* and *Polygonum bistorta*) reported in the British Herbal Pharmacopoeia (1983) to be anti-inflammatory.

H. virginiana L. Hamamelidaceae, the subject of the monographs of British Pharmaceutical Codex (1973) and Martindale, The Extra Pharmacopoeia (1972) is included in various preparations used mainly against haemorrhoids. It is also incorporated in a selenium-herbal ointment recommended for the treatment of varicose veins, haemorrhoids, eczema and psoriasis (Berman 1985). Compounds isolated from it include a hydrolysable tannin, hamamelitannin (Maffei-Facino et al 1985), gallic acid and traces of volatile oils (Vanhaelin-Fastre 1983).

Guaiacum wood, the heartwood of *Guaiacum officinale* L. and *G. sanctum* L. Zygophyllaceae, is listed in the monographs of Martindale (1972) as a laxative and diuretic. It is also specifically indicated by the British Herbal Pharmacopoeia (1983) for the treatment of rheumatoid arthritis and gout. It contains a number of lignans including guaiaretic acid, guaiacin, furoguaiacin and dihydroguairetic acid (King & Wilson 1964) and α- and β-guaiaconic acid (Kratochvil et al 1971).

Bistort cuts consist of the dried rhizomes and roots of *Polygonum bistorta* L. Polygonaceae. Reported in the British Herbal Pharmacopoeia (1983) as anti-inflammatory, it is also indicated for the treatment of diarrhoea in children. There are many reports of the abundance of phenolic compounds in *P. bistorta* (Blazej & Buckova 1970). Mityagina et al (1969) reported tannin concentrations of 52·3–61·1% in various water extracts of bistort.

There is no previous pharmacological confirmation of anti-inflammatory or anti-arthritic activity in these plants.

†Present address: Department of Pharmacology, Faculty of Pharmacy, University of Science and Technology, Kumasi, Ghana.

Correspondence: I. J. Zeitlin, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW, UK.

Materials and Methods

Preparation of plant extracts

The plants (Hamamelis leaves: B/No 054; guaiacum wood: B/No 1776; and bistort root cuts: B/No 8110), all purchased from Brome and Schimmer Ltd, Hants, UK, were finely powdered and 100 g of each powder was extracted with 70% aqueous ethanol (500 mL) using a Soxhlet extraction apparatus. The extracts were filtered hot using a Whatman No. 1 filter paper and then concentrated under vacuum (40–60°C) and finally freeze-dried to yield extracts of *P. bistorta* (30·0 g), *H. virginiana* (29·8 g) and *G. officinale* (14·0 g).

Preparation of carrageenan suspension

A 1% suspension of carrageenan sodium salt (BDH Chemicals Ltd, Poole, UK, Product No. 38100 5044-630H) was made by sprinkling 100 mg carrageenan powder on 10 mL 0·9% NaCl (saline) and leaving it to soak for 1 h. A homogeneous suspension was then obtained by thorough mixing with a magnetic stirrer.

Induction of carrageenan-induced rat paw oedema

Male Sprague-Dawley rats (200–250 g, Bantin & Kingman bred) maintained on CRM diet were used. Doses of extract suspensions (100 and 200 mg kg^{-1}) and indomethacin (2·5 mg kg^{-1}) in vehicle (10% v/v acacia mucilage and 0·2% Tween 20) were administered orally in a total volume equivalent to 0·1 mL/100 g rat. Control animals received drug vehicle alone. Doses were administered 18 and 2 h before induction of oedema. The oedema was induced by injecting

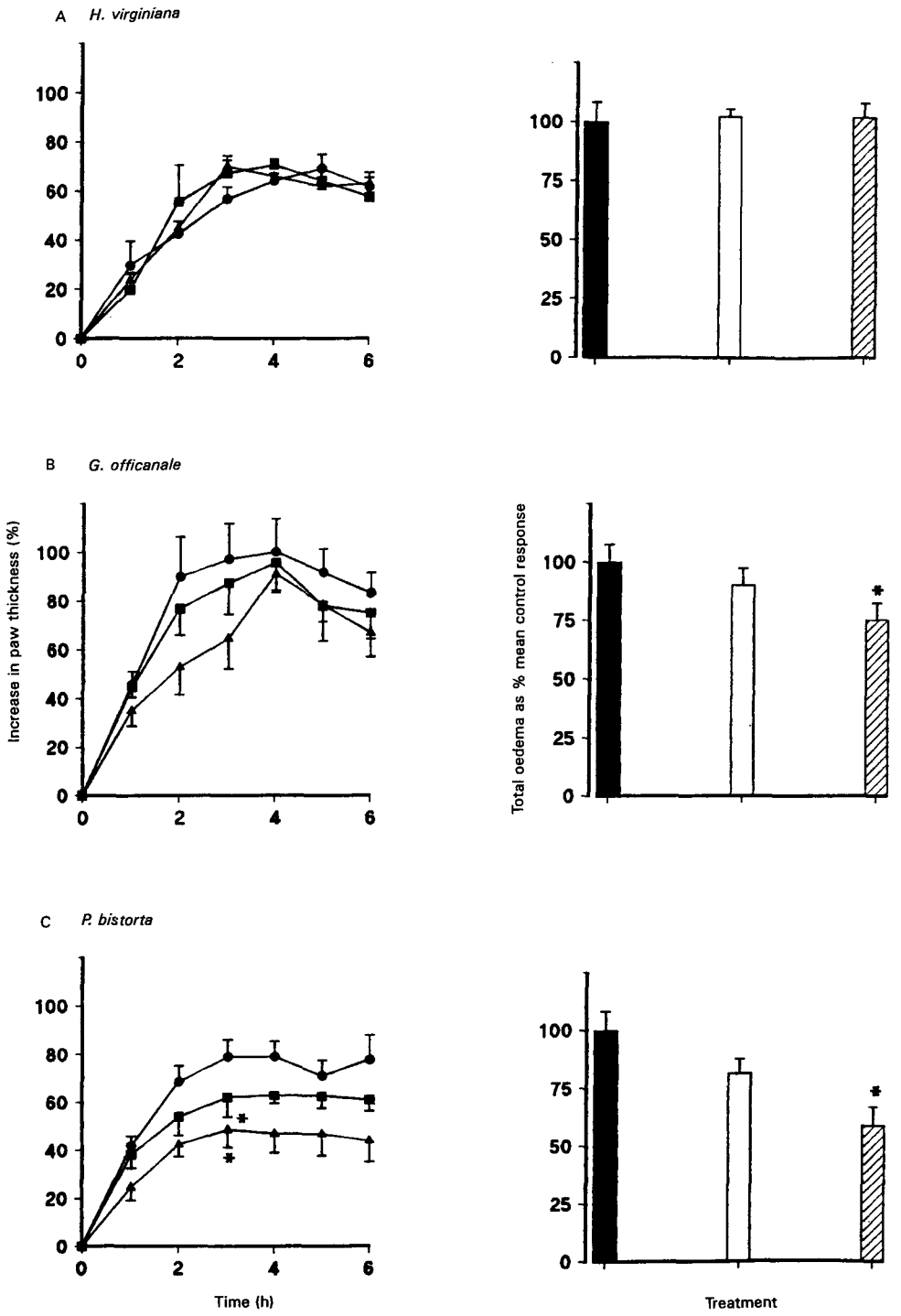

FIG. 1. Effects of extracts of *H. virginiana* (A), *G. officinale* (B) and *P. bistorta* (C) on the time-course of carrageenan-induced rat paw oedema. Solid circles indicate vehicle-treated (control) responses. Effects of 100 and 200 mg kg^{-1} of extracts are represented by solid squares and triangles, respectively. The corresponding bar charts show the effects of the extracts on the total oedema responses as indicated by the areas under the time-course curves. Effects of doses of drug vehicle, 100 and 200 mg kg^{-1} of extracts are indicated by solid, open and hatched bars, respectively. Results are presented as mean values ± s.e.m., n = 6. * $P < 0.05$ compared with vehicle-treated control.

carrageenan suspension (0·1 mL, 1% w/v in saline) subcutaneously into the plantar tissue of the right hindpaw (Winter et al 1962). The contralateral hindpaws were injected with 0·1 mL saline as a control. Oedema, determined as the percentage change in paw thickness (Al-Haboubi & Zeitlin 1983), was monitored at 1 h intervals for up to 6 h. Drug effects were determined using the maximal oedema response attained during 6 h and the total oedema response produced over the 6 h, measured as the area under the time-course curve (AUC). Before each test, the model was validated using indomethacin, 2·5 mg kg^{-1}, administered orally 18 and 2 h before induction of oedema, as a positive control. The mean

maximal and total oedema responses were significantly ($P<0.05$) suppressed by indomethacin to 49·3±4·2 and 56·8±6·1% (n=18) of the mean control (vehicle-treated) responses, respectively.

The influence of bistort extract (200 mg kg^{-1}) administered orally 1 h after the induction of carrageenan-induced oedema on the time-course of the rat paw swelling was also investigated.

Preparation of Freund's adjuvant

Twenty-five milligrams heat-killed *Mycobacterium tuberculosis* cells, strains CDT & PN (mixed) obtained from Ministry of Agriculture, Fisheries and Food, UK, was finely ground using a mortar and pestle. Enough liquid paraffin (BDH Chemicals Ltd) was added and thoroughly triturated to make a 5 mg mL^{-1} suspension. This is referred to as complete Freund's adjuvant. The liquid paraffin alone is referred to in the study as incomplete Freund's adjuvant.

Induction of adjuvant-induced arthritis

Male Wistar rats (200–250 g, Bantin and Kingman bred) were used. Adjuvant arthritis (Pearson 1956) was induced by subcutaneous injection of complete Freund's adjuvant (0·1 mL) into the plantar tissue of the right hindpaw of each rat. Rats in this group were known as the inflamed control group. The non-inflamed control group consisted of rats injected with 0·1 mL liquid paraffin. The test groups consisted of complete Freund's adjuvant-injected rats challenged with doses of test drugs (plant extracts 200 mg kg^{-1}; indomethacin 2·5 mg kg^{-1}) administered orally 18 and 2 h before the induction of arthritis. The drug administrations were continued daily at the same time of the day for 19 more days. Development of the adjuvant-induced swelling in the paws of both the injected and non-injected limbs of each rat were monitored daily as the percentage increase in paw thickness.

In a separate experiment, bistort extract (200 mg kg^{-1}) was administered orally daily starting from the 5th day after the induction of arthritis and the time-course of adjuvant-induced swelling in the rat paw was studied.

Statistics

Results are expressed as the mean value±standard error (n=6). Results have been analysed using a one-way analysis of variance. Significant differences were further analysed using the Newman-Keuls range test at 5% significance.

Results

Carrageenan-induced oedema

The aqueous ethanolic extract of *H. virginiana* (100 and 200 mg kg^{-1}, p.o.) produced no anti-inflammatory effects (Fig. 1). Some activity was only detected in the extract of *G. officinale* at 200 mg kg^{-1} at which it significantly ($P<0.05$) reduced only the AUC to 76·5±7·2% of the mean control (vehicle-treated) response (Fig. 1).

Administration of *P. bistorta* extract at 100 and 200 mg kg^{-1}, significantly ($P<0.05$) suppressed the maximal oedema responses attained during 6 h, to 79·6±5·3 and 61·5±9·5% of the mean control values, respectively (Fig. 1). The total oedema responses attained over the 6 h were also significantly ($P<0.05$) reduced to 81·5±6·3 and 58·9±8·2% of the mean control values, respectively (Fig. 1). On the basis of its higher activity, further tests on bistort were carried out and showed a dose-dependent anti-inflammatory activity over the dose range 100–400 mg kg^{-1} on carrageenan-induced rat paw oedema (Fig. 2). A dose of 800 mg kg^{-1} produced no further suppression of the oedema. The E50 value was approximately 158·5 mg kg^{-1} for both the maximal and total oedema responses produced over the 6 h.

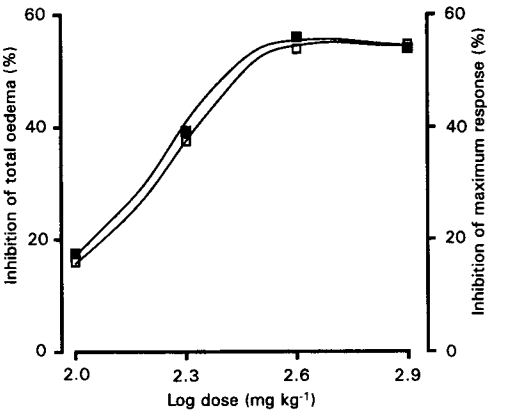

FIG. 2. Dose-effect relationship for percentage inhibition of carrageenan-induced rat paw oedema by bistort extract. Extract doses (100–800 mg kg^{-1}) were administered orally 18 and 2 h before induction of oedema. ■ Inhibition of the maximal paw swelling attained during 6 h; □ inhibition of the total oedema response over 6 h. (Each point on the graph is calculated from the mean of six responses.)

A dose of 200 mg kg^{-1} bistort extract administered 1 h after the onset of carrageenan oedema reversed the course of an oedema response already in progress (Fig. 3). At 6 h the oedema was significantly ($P<0.05$) reduced to 62·0±9·1% of the control value. In comparison, administration of the extract before the induction of oedema reduced the paw swelling at 6 h, to 51·4±4·9% of the control value. This result

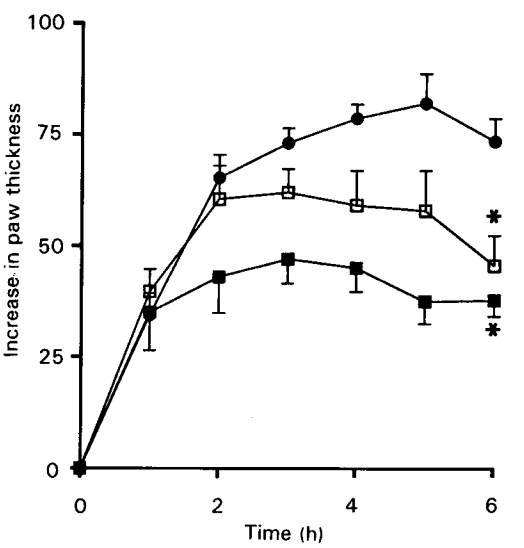

FIG. 3. Influence of bistort extract (200 mg kg^{-1}) on the time-course of the rat paw swelling when administered orally 18 and 2 h before (■) and 1 h after (□) injection of carrageenan. The results are presented as mean values±s.e.m., n=6. *$P<0.05$ compared with control (●).

was not statistically different from the effect of the extract when administered after the onset of oedema.

Adjuvant-induced arthritis

When administered at 200 mg kg^{-1} *H. virginiana* produced no significant inhibition of the adjuvant-induced rat paw swelling in the injected limbs, whilst the extracts of *G. officinale* and *P. bistorta* significantly reduced the maximal swelling attained during 19 days to 76·2±4·9 and 63·3±6·3% of the inflamed control response, respectively (Fig. 4A). The acute phase of the adjuvant-induced response, measured as the increase in paw thickness of the injected limb over the first 7 days after injection of complete Freund's adjuvant was significantly ($P<0·05$) suppressed by only *P. bistorta*.

On the non-injected (contralateral) limbs, all the extracts significantly ($P<0·05$) inhibited both the maximal and the total adjuvant-induced paw swellings during the 19 days (Fig. 4B). The inhibitory effects of *P. bistorta* (36·6%) and *G. officinale* (33·0%) on the maximal swelling induced in the non-injected paws were significantly ($P<0·05$) greater than that produced by the extract of *H. virginiana* (24·7%). There was no statistical difference between the inhibitory effects of *G. officinale* and *P. bistorta*.

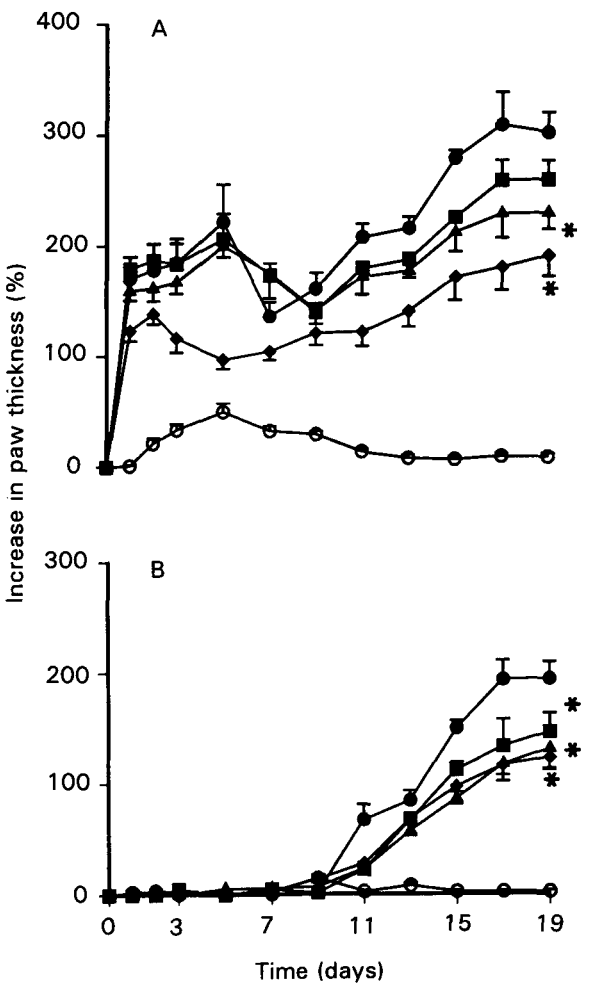

FIG. 4. Effects of extracts (200 mg kg^{-1}, p.o.) of *H. virginiana* (■), *G. officinale* (▲), and *P. bistorta* (◆) on the time-course of adjuvant-induced swellings in the injected paws (A) and non-injected paws (B) of male Wistar rats. The extracts were administered 18 and 2 h before induction of arthritis and repeated daily for 19 days. Results are presented as mean values±s.e.m., n=6. ● Inflamed and ○ non-inflamed control groups. *$P<0·05$ compared with inflamed control.

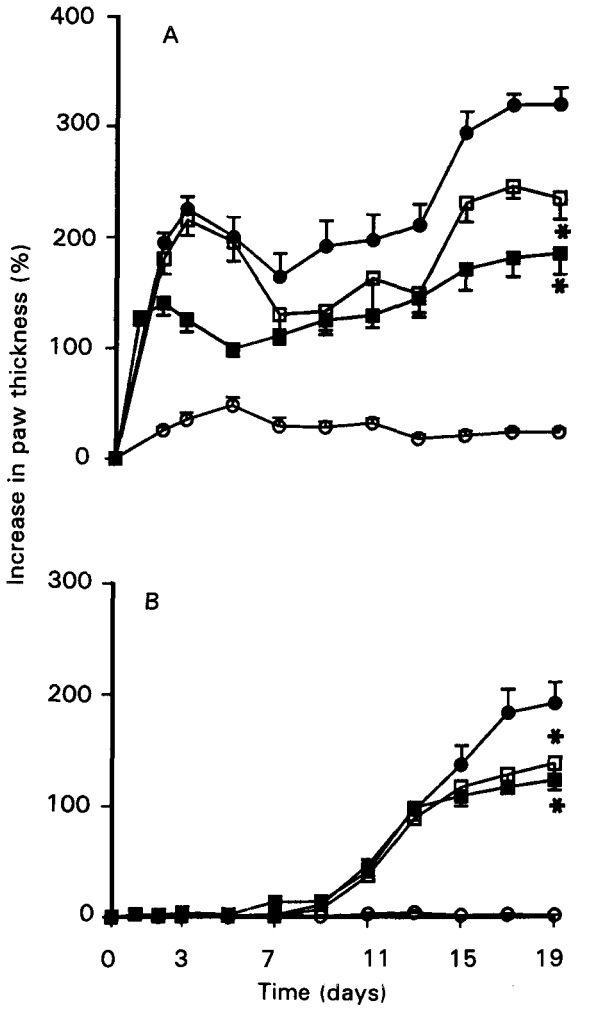

FIG. 5. Influence of bistort extract (200 mg kg^{-1}, p.o.) when administration commenced before induction (■) and when administered daily commencing day 5 (□) of rat adjuvant-induced arthritis, on the time-course of the increase in thickness of the injected paw (A) and non-injected paw (B). ● Inflamed and ○ non-inflamed control groups. *$P<0·05$ compared with inflamed control.

Daily administrations of *P. bistorta* extract at 200 mg kg^{-1} starting 5 days after the induction of adjuvant arthritis caused a signficant reduction in the paw swelling in the injected and non-injected limbs to 73·5±2·5 and 71·9±6·8% of the mean control values, respectively, on the 19th day. In comparison, administration of the extract before the induction of arthritis produced significantly ($P<0·05$) higher inhibitions of the paw swellings in both the injected and non-injected limbs to 57·8±5·9 and 64·2±4·8% of the mean control responses, respectively (Fig. 5).

Discussion

The British Herbal Pharmacopoeia (1983) describes extracts of *Polygonum bistorta*, *Guaiacum officinale* and *Hamamelis virginiana* as anti-inflammatory, the extracts of *G. officinale*

being specifically indicated for the treatment of rheumatoid arthritis and gout. The failure of *H. virginiana* extract to inhibit the carrageenan-induced rat paw oedema and the weak anti-inflammatory actions shown by *G. officinale* extract when administered at 100 and 200 mg kg^{-1} may be due to the doses being too low, suggesting that these extracts lack high activity against acute inflammatory responses.

Although there are no reports on the anti-inflammatory actions of *P. bistorta*, such activity has been reported for other species of the genus *Polygonum* on the carrageenan-induced rat paw oedema. Singh et al (1987) reported the anti-inflammatory actions of *P. glabrum* and Furuta et al (1987) showed that polygonolide, an isocoumarin, isolated from *P. hydropiper* possessed anti-inflammatory properties. On adjuvant-induced arthritis, all the extracts studied here produced significant inhibitions of the polyarthritis phase as measured by the paw swellings on the non-injected limbs. However, only *P. bistorta* inhibited the acute phase of the adjuvant-induced response (measured by the response during the first 7 days following the injection of the complete Freund's adjuvant), confirming its greater potency relative to the extracts of *G. officinale* and *H. virginiana* against acute inflammatory responses.

The events in the primary phase of adjuvant arthritis and carrageenan-induced oedema correspond to those in the early exudative phase of inflammation, an important feature of inflammatory pathology (Vinegar et al 1976). Therefore, the demonstrated activities of bistort extract against the primary phase of adjuvant arthritis and the carrageenan-induced oedema, indicates its ability to exert anti-inflammatory effects, at least in the acute phase of the inflammatory process.

During adjuvant arthritis, especially at the peak of secondary lesion development, an alteration in the pattern of plasma protein synthesis by the liver occurs resulting in a pronounced fall in albumin levels (Billingham & Gordon 1976). A consequence of the reduction in albumin levels is a decrease in the available binding sites for highly albumin-bound drugs (Paulus & Whitehouse 1973). It is, therefore, conceivable that the fall in albumin levels coupled with the reduction in liver metabolizing capacity associated with severe inflammatory stress (Beck & Whitehouse 1974) caused increased and sustained high plasma levels of the free unbound anti-inflammatory principles in the extracts. This could account for the potency of the extracts during adjuvant arthritis, especially on the polyarthritis phase. Alternatively, the results may be an indication of a selective capacity of the extracts (especially *H. virginiana* and *G. officinale*) to inhibit the cell-mediated mechanisms of adjuvant arthritis in contrast to a lesser influence on the contact-activation processes responsible for the acute inflammatory response induced by carrageenan in the rat paw.

Suppression of the inflammatory response following administration of bistort extract after the onset of both adjuvant- and carrageenan-induced swellings indicates an ability to suppress established inflammation, both the acute and the cell mediated chronic responses. An inhibitory effect when administered after the onset of inflammation is more likely to be due to a genuine anti-inflammatory mechanism as opposed to a demonstration of activity only when administered before the initiation of the inflammatory stimulus, a standard practice when drug screening.

The present findings provide pharmacological support for the British Herbal Pharmacopoeia's indication of the extracts of *P. bistorta*, *H. virginiana* and *G. officinale* as anti-inflammatory agents and also provides preliminary evidence to justify a search for anti-inflammatory compounds, especially from extracts of *P. bistorta*.

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