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Pharmacological Activity of Feverfew (*Tanacetum parthenium* (L.) Schultz-Bip.): Assessment by Inhibition of Human Polymorphonuclear Leukocyte Chemiluminescence In-vitro

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Abstract

The bioactivity of feverfew (*Tanacetum parthenium*) leaf extracts has been analysed, by use of a human polymorphonuclear leukocyte (PMNL) bioassay, to assess the relative contributions of solvent extraction and parthenolide content to the biological potency of the extract.

Extracts prepared in acetone–ethanol (system 1) contained significantly more parthenolide (mean ± s.d. 1·3 ± 0·2% dry leaf weight) than extracts in chloroform–PBS (phosphate-buffered saline; system 2; 0·1 ± 0·04% dry leaf weight) or PBS alone (system 3; 0·5 ± 0·1% dry leaf weight). Extract bioactivity, measured as inhibition of phorbol 12-myristate 13-acetate-induced, 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol)-enhanced PMNL chemiluminescence, followed a similar trend. Extracts inhibited phorbol 12-myristate 13-acetate-induced oxidative burst by amounts which, if solely attributable to parthenolide, indicated parthenolide concentrations for the respective solvent systems of 2·2 ± 0·6%, 0·2 ± 0·1% and 0·9 ± 0·1% dry leaf weight. The mean ratio of parthenolide concentration to the parthenolide equivalent/PMNL-bioactivity value, for acetone–ethanol and PBS extracts were both 1 : 1·7.

Parthenolide, although a key determinant of biological activity for *T. parthenium* leaf extracts based on the PMNL-bioassay, seems not to be the sole pharmacologically-active constituent. The identical and elevated bioactivity–parthenolide ratios for both organic and aqueous-phase leaf extracts suggest that a proportion of the other bioactive compounds have solubilities similar to that of parthenolide.

Traditionally, feverfew (*Tanacetum parthenium* (L.) Schultz-Bip., syn. *Chrysanthemum parthenium* (L.) Bernh.; Asteraceae) has been used as a self-medication for the prophylactic treatment of migraine, arthritis and other medical conditions. Its use in the treatment of migraine has been validated in two clinical trials (Johnson et al 1985; Murphy et al 1988); this has prompted extensive pharmacological investigation of the plant and its extracts. These studies have utilized muscle tissues (Hay et al 1994), platelets (Loesche et al 1987; Groenewegen & Heptinstall 1990) and leukocytes, including both monocytes (Krause et al 1990) and polymorphonuclear leukocytes (PMNL; Loesche et al 1988). Other studies have been concerned with effects on arachidonic acid metabolism and associated prostaglandin biosynthesis (Collier et al 1980; Capasso 1986; Sumner et al 1992).

Previous investigations on the effects of feverfew on PMNL activity in-vitro have utilized separated cells stimulated with opsonized zymosan, an assay based on a receptor-based activation process (Loesche et al 1988). In the current study a human PMNL function test was used to assess the biological activity of feverfew extracts but, in contrast with previous work, a whole blood method was used to avoid artifactual results arising from pre-activation of the PMNL by separation techniques (Neilson et al 1992). To measure oxidative burst in response to phorbol 12-myristate 13-acetate, PMNL activity was recorded as luminol-enhanced cellular chemiluminescence. Phorbol 12-myristate 13-acetate, a diacyl glycerol mimic, activates PMNL by a receptor-independent mechanism involving direct activation of protein kinase C, an enzyme associated with biochemical pathways involved in inflammatory responses (Silván et al 1996). A receptor-independent stimulus was utilized in this bioassay because previous work has suggested that sesquiterpene lactones bearing an α-methylene butyrolactone group, such as parthenolide, act on inflammatory processes via inhibition of protein kinase C (Heptinstall et al 1985). This is supported by pharmacological studies of feverfew, and its extracts, on platelet aggregation and secretion of 5-hydroxy-tryptamine, which have implicated possible interactions of such extracts with protein kinase C (Groenewegen & Heptinstall 1990; Marles et al 1992; Silván et al 1996).

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Materials and Methods

Chemicals

5-Amino-2,3-dihydro-1,4-phthalazinedione (luminol), phorbol 12-myristate 13-acetate, tri-sodium citrate, phosphate buffered saline (PBS; 0·01 M phosphate buffer, 0·137 M NaCl, 0·0027 M KCl; pH 7·4), acetone (HPLC grade), chloroform (HPLC grade) and acetonitrile (MeCN; HPLC grade) were purchased from Sigma (Poole, UK). Dimethylsulphoxide (DMSO; spectrosol) and ethanol (analytical reagent) were from BDH (Lutterworth, UK); parthenolide (97% w/w) was obtained from Aldrich, Milwaukee, USA.

Preparation of plant extracts

Clonally-propagated feverfew plants, line JBP/F10, shown previously to accumulate relatively high levels of parthenolide (Brown et al 1996a), were propagated vegetatively and maintained under glass-house conditions, as described previously

(Brown et al 1996b). Six to eight young fully-expanded leaves excized from near the apices of six cloned plants of accession JBP/F10, were combined, ground in liquid nitrogen, and lyophilized (Model SB4 freeze-dryer, ChemLab, Hornchurch, UK) into a fine dry powder. Samples of the powder were extracted with acetone, as employed previously for HPLC and bioassays (Heptinstall et al 1992), chloroform (as used in extraction for HPLC; Dolman et al 1992) or PBS (aqueous phases as used by Gromek et al 1991). The extracts were filtered (Whatman No. 1 filter paper) under reduced pressure to remove tissue debris from the solvent. Organic extraction solvents were evaporated under nitrogen at 35°C, and the residue was re-suspended in either ethanol (99·7–100% v/v; solvent system 1) or PBS (solvent system 2). In addition, the initial PBS extract was used without further preparation (solvent system 3).

Analysis of parthenolide content

Extracts were analysed by HPLC (Brown et al 1996a). Briefly, extract (10 μL) was injected on to a 25 cm × 4·6 mm i.d. Spherisorb S500S2 column eluted with MeCN-water (40 : 60, v/v), and parthenolide (retention time ca 8·5 min) was quantified by reference to calibration curves constructed by injection of standards prepared from commercially-obtained parthenolide.

PMNL bioassay

Blood (25 mL) was obtained by clean venepuncture of healthy human volunteers, and 4·5 mL volumes anti-coagulated with tri-sodium citrate (3·13%, w/v; 0·5 mL). Blood was transferred immediately to a water bath at 37°C and incubated for 30 min before use. Plant extracts (in solvent systems 1–3) were diluted in PBS; extract (18 μL) was added to PBS (312 μL) pre-warmed to 37°C. This solution (10 μL) was added to citrated whole blood (50 μL) and incubated for 2 min. Dilution of solvent system 1 in this manner gave a final ethanol concentration of 1% (v/v); to standardize the other solvent systems an equal amount of ethanol was added to the diluted extracts in solvent systems 2 and 3, to minimize any ethanol-dependent effects on PMNL activity. This incubation mixture (50 μL) was transferred into PBS(1·0 mL) containing a solution of luminol in DMSO (final concentration of luminol: 100 μg mL^{-1}) and incubated for 5 min. The oxidative burst was stimulated by adding phorbol 12-myristate 13-acetate solution (100 μg mL^{-1}, also in DMSO; 20 μL) to the blood, and the luminescence was measured every 2 min for up to 30 min (model 1251 luminometer, LKB, Sweden).

Assay calibration

The PMNL bioassay was calibrated using parthenolide diluted serially in ethanol and added to PBS to give ethanol concentrations equivalent to those in the test samples. Each group of tests also included a control of blood treated with 1% (v/v) ethanol in PBS and an internal standard treated with 80 μM parthenolide to assess reproducibility between test runs.

Statistical analysis

Results are expressed as means ± standard deviations (s.d.) and statistical significance was assessed using a Mann–Whitney test. A probability of $P < 0.05$ was considered as indicative of significance.

Results

All feverfew leaf extracts induced inhibition in the human PMNL bioassay, expressed as a reduction of the oxidative burst generated in response to phorbol 12-myristate 13-acetate (Table 1). Although there were quantifiable differences between the plant extracts analysed, extracts prepared in solvent system 1 had the greatest inhibitory activity, mean value of 2·2 ± 0·6% (n = 12) parthenolide equivalents. Parthenolide equivalents, in this context, were units of biological activity calculated assuming that parthenolide was the sole biologically-active feverfew metabolite present in the extract in question, and expressed on a percentage dry leaf weight basis. The extracts in solvent systems 3 and 2 were approximately 50% (0·9 ± 0·1%, n = 12; $P < 0.05$) and 10% (0·2 ± 0·1%, n = 12; $P < 0.05$) as active that in solvent system 1.

The extraction efficacies of the 3 solvent systems, as assessed by HPLC analysis (Table 2), followed the same trend as that from the PMNL bioassay; systems 1–3 extracted, respectively, 1·3 ± 0·2, 0·1 ± 0·04, and 0·5 ± 0·1% dry leaf weight parthenolide (n = 12). Biological activity was consistently greater than that predicted if the effects were solely a result of the parthenolide measured by HPLC (Table 3). This ratio of biological activity, expressed as parthenolide equivalents, to actual parthenolide measured, was consistent at 1·7 ± 0·3 (n = 12) for solvent system 1, and 1·7 ± 0·2 (n = 12) for solvent system 3. Data from solvent system 2 were discounted because they were not reproducible.

Discussion

These results demonstrate that extracts of feverfew leaves inhibit the chemiluminescence of human PMNL induced, in-vitro, by phorbol 12-myristate 13-acetate. The study also demonstrates the crucial importance of the solvent system

Table 1. Mean biological activity in the PMNL bioassay, expressed as parthenolide equivalents, of extracts, obtained using different solvent systems, of leaves of *Tanacetum parthenium*.

Solvent system	Plant number					
	1	2	3	4	5	6
Acetone–ethanol	2·35 ± 0·50	1·62 ± 0·04	1·69 ± 0·31	1·90 ± 0·21	2·92 ± 0·76	2·59 ± 0·29
Chloroform–phosphate-buffered saline	0·11 ± 0·01	0·15 ± 0·01	0·16 ± 0·02	0·21 ± 0·07	0·25 ± 0·12	0·35 ± 0·10
Phosphate–buffered saline	0·81 ± 0·15	0·86 ± 0·13	0·98 ± 0·18	0·71 ± 0·16	0·94 ± 0·14	0·79 ± 0·18

Values are mean ± s.d.; n = 12 throughout.

Table 2. Mean parthenolide content (% dry weight) of leaves of *Tanacetum parthenium* extracted using different solvent systems.

Solvent system	Plant number					
	1	2	3	4	5	6
Acetone–ethanol	1·09 ± 0·03	1·26 ± 0·04	1·02 ± 0·03	1·48 ± 0·04	1·50 ± 0·04	1·36 ± 0·04
Chloroform–phosphate-buffered saline	0·07 ± 0·01	0·13 ± 0·01	0·01 ± 0·01	0·01 ± 0·01	0·05 ± 0·01	0·01 ± 0·01
Phosphate-buffered saline	0·42 ± 0·01	0·45 ± 0·01	0·56 ± 0·02	0·43 ± 0·01	0·69 ± 0·02	0·47 ± 0·01

Values are mean ± s.d.; n = 12 throughout.

Table 3. Mean ratio of parthenolide assessed by bioactivity in the PMNL bioassay and HPLC analysis of extracts, obtained with acetone-ethanol and with phosphate-buffered saline, of the leaves of *Tanacetum parthenium*.

Solvent system	Plant number					
	1	2	3	4	5	6
Acetone–ethanol	2·15 ± 0·45	1·29 ± 0·03	1·66 ± 0·31	1·29 ± 0·14	1·94 ± 0·50	1·91 ± 0·21
Phosphate–buffered saline	1·93 ± 0·36	1·93 ± 0·29	1·74 ± 0·32	1·65 ± 0·38	1·37 ± 0·21	1·66 ± 0·38

Values are mean ± s.d., n = 12 throughout.

selected for preparing extracts for the human PMNL bioassay.

Minor fluctuations between individual cloned plants, in terms both of absolute parthenolide concentrations and of biological activity, were detected after extraction with all three solvent systems. Solvent system 1 (acetone–ethanol) consistently extracted more parthenolide (or active compounds) than systems 2 or 3, as assessed by HPLC, although the ratio of biological activity to parthenolide concentration was not significantly different between systems 1 and 3, with both extracts displaying ca 1·7 times the activity expected if it resulted from parthenolide alone.

Extraction of leaf tissues with PBS (system 3) was more efficient than with chloroform–PBS (system 2), in terms both of the amount of parthenolide detected using HPLC and in terms of the biological activity of the resulting extracts. The chloroform–PBS solvent system (system 2) was discounted for reliable extractions, because of the low levels of parthenolide detected by HPLC, and the resulting effects on the parthenolide-activity ratio. These effects were attributed to the poor suitability of PBS for re-dissolving the residue from the initial chloroform extraction of leaf tissues.

These results indicate that, whereas parthenolide is clearly a key component in the biological activity of feverfew leaf extracts, on the basis of the human PMNL bioassay employed in these experiments, it seems not to be the sole active constituent. Other workers (e.g. Barsby et al 1993; Williams et al 1995) have detected biologically active metabolites in feverfew extracts, but in much less abundance than parthenolide. It is possible that such compounds, or metabolites similar to parthenolide, could produce activity in the PMNL-based assay described here, with either additive or synergistic mechanisms enhancing the activity intrinsically associated with parthenolide.

The mean activity–parthenolide ratios for the aqueous and organic extraction systems were very similar, even with the minor variations between individual plants (Table 3). This suggests that the metabolite(s) responsible for the extra bioactivity observed have solubilities similar to that of parthenolide, and that they are, to some extent, soluble in both organic and aqueous phases. If the activity of the organic extract had been greater than that of the aqueous extract, the extra activity could have been ascribed to a predominantly hydrophobic compound, whereas if the opposite were true the extra compounds would, presumably, have been hydrophilic in nature. Work is currently in progress to identify the metabolite(s), in addition to parthenolide, with activity in the PMNL-based bioassay.

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References

Barsby, R. W. J., Knight, D. W., McFadzean, I. (1993) A chloroform extract of the herb feverfew blocks voltage-dependent potassium currents recorded from single smooth muscle cells. J. Pharm. Pharmacol. 45: 641–645

Brown, A. M. G., Lowe, K. C., Davey, M. R., Power, J. B., Knight, D. W., Heptinstall, S. (1996a) Comparison of extraction procedures for parthenolide in feverfew (*Tanacetum parthenium*). Phytochem. Anal. 7: 86–91

Brown, A. M. G., Lowe, K. C., Davey, M. R., Power, J. B. (1996b) Feverfew (*Tanacetum parthenium* L.) Tissue culture and parthenolide synthesis. Plant Sci. 116: 223–232

Capasso, F. (1986) The effect of an aqueous extract of *Tanacetum parthenium* L. on arachidonic acid metabolism by rat peritoneal leukocytes. J. Pharm. Pharmacol. 38: 71–71

Collier, H. O. J., Butt, N. M., McDonald-Gibson, W. J., Saeed, S. A. (1980) Extract of feverfew inhibits prostaglandin biosynthesis. Lancet ii: 992

Dolman, D. M., Knight, D. W., Salan, U., Toplis, D. (1992) A quantitative method for the estimation of parthenolide and other sesquiterpene lactones containing α-methylenebutyrolactone functions present in feverfew, *Tanacetum parthenium*. Phytochem. Anal. 3: 26–31

Groenewegen, W. A., Heptinstall, S. (1990) A comparison of the effects of feverfew and parthenolide, a component of feverfew, on human platelet activity in-vitro. J. Pharm. Pharmacol. 42: 553–557

Gromek, D., Kisiel, W., Stojakowska, A., Kohlmünzer, S. (1991) Attempts of chemical standardization of *Chrysanthemum parthenium* as a prospective antimigraine drug. Pol. J. Pharmacol. Pharm. 43: 213–218

Hay, A. J. B., Hamburger, M., Hostettmann, K., Hoult, J. R. S. (1994) Toxic inhibition of smooth muscle contractility by plant-derived sesquiterpenes caused by their chemically reactive α-methylenebutyrolactone functions. Br. J. Pharmacol. 112: 9–12

Heptinstall, S., White, A., Williamson, L., Mitchel, J. R. A. (1985) Extracts of feverfew inhibit granule secretion in blood platelets and polymorphonuclear leucocytes. Lancet i: 1071–1073

Heptinstall, S., Awang, D. V. C., Dawson, B. A., Kindack, D., Knight, D. W., May, J. (1992) Parthenolide content and bioactivity of feverfew [*Tanacetum parthenium* (L.) Schultz-Bip.]. Estimation of commercial and authenticated feverfew products. J. Pharm. Pharmacol. 44: 391–395

Johnson, E. S., Kadam, N. P., Hylands, D. M., Hylands, P. J. (1985) Efficacy of feverfew as prophylactic treatment of migraine. Br. Med. J. 291: 569–573

Krause, S., Arese, P., Heptinstall, S., Loesche, W. (1990) Influence of substances affecting cell sulphydryl/disulphide status on adherence of human monocytes. Arzn. Forsch. 40: 689–692

Loesche, W., Mazurov, A. V., Heptinstall, S., Groenewegen, W. A., Repin, V. S., Till, U. (1987) An extract of feverfew inhibits interactions of human platelets with collagen substrates. Thromb. Res. 48: 511–518

Loesche, W., Michel, E., Heptinstall, S., Krause, S., Groenewegen, W. A., Pescarmona, G. P., Thielmann, K. (1988) Inhibition of the behaviour of human polynuclear leukocytes by an extract of *Chrysanthemum parthenium*. Planta Med. 54: 381–384

Marles, R. J., Kaminski, J., Arnason, J. T., Pazos-Sanou, L., Heptinstall, S., Fischer, N. H., Crompton, C. W., Kindack, D. G., Awang, D. V. C. (1992) A bioassay for inhibition of 5-HT release from bovine platelets. J. Nat. Prod. 55: 1044–1056

Murphy, J. J., Heptinstall, S., Mitchell, J. R. A. (1988) Randomized, double-blind, placebo-controlled trial of feverfew in migraine prophylaxis. Lancet ii: 189–192

Neilson, C. P., Crowley, J. J., Vestal, R. E., Connolly, M. J. (1992) Impaired beta-adrenoreceptor function, increased leukocyte respiratory burst, and bronchial hyper-responsiveness. J. Allergy Clin. Immunol. 90: 825–832

Silván, A. M., Abad, M. J., Villar, A. (1996) Inhibition by hydroxyachillin, sesquiterpene lactone from *Tanacetum microphyllum*, of phorbol 12-myristate 13-acetate-induced mouse ear oedema. Inflamm. Res. 45: 289–292

Sumner, H., Salan, U., Knight, D. W., Hoult, J. R. S. (1992) Inhibition of 5-lipoxygenase and cyclo-oxygenase in leukocytes by feverfew. Biochem. Pharmacol. 43: 2313–2320

Williams, C. A., Hoult, J. R. S., Harborne, J. B., Greenham, J., Eagles, J. (1995) Biologically active lipophilic flavonol from *Tanacetum parthenium*. Phytochem. 38: 267–270