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Effect of the Chloroform Extract of *Tanacetum vulgare* and one of its Active Principles, Parthenolide, on Experimental Gastric Ulcer in Rats

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Abstract

This study examines the anti-ulcerogenic activity of a chloroform extract of *Tanacetum vulgare* and purified parthenolide, the major sesquiterpene lactone found in the extract.

Gastric ulcers induced by oral administration of absolute ethanol to rats were reduced dose-dependently by oral pretreatment of animals with the chloroform extract (2·5–80 mg kg^{-1}) or parthenolide (5–40 mg kg^{-1}). When administered 30 min before challenge with the alcohol the protection ranged between 34 and 100% for the extract and 27 and 100% for parthenolide. When the products were administered orally 24 h before treatment with ethanol, 40 mg kg^{-1} of the extract and of the lactone reduced the mean ulcer index from $4{\cdot}8 \pm 0{\cdot}3$ for control animals to $1{\cdot}4 \pm 0{\cdot}2$ and $0{\cdot}5 \pm 0{\cdot}1$, respectively. The products also prevented alcohol-induced reduction of the number of sulphydryl groups within the gastric mucosa ($50{\cdot}6 \pm 2{\cdot}3$ μg (mg protein)$^{-1}$ for normal animals compared with $17{\cdot}7 \pm 3{\cdot}0$ μg (mg protein)$^{-1}$ for alcohol-treated animals). Administration of the extract (80 mg kg^{-1}) or parthenolide (40 mg kg^{-1}) 24 h before ethanol treatment restored the numbers of mucosal -SH groups to values near those found for normal animals.

These results suggest that the products assayed, in particular parthenolide, might find therapeutic application, although further work is required to establish their profit/risk ratio.

Plants belonging to the genus *Tanacetum* L. (Compositae) have been used medically since ancient times. Crude extract from *T. parthenium* (feverfew) has become popular for the treatment of arthritis, migraine and psoriasis (Berry 1984; Johnson et al 1985; Murphy et al 1988). Organic extracts of *T. microphyllum* D. C. have been shown to contain principles with anti-inflammatory activity (Abad et al 1991). *Tanacetum vulgare* L., known as tansy, and the subject of this work, is a common herb in Europe, Asia and America which has been used in folk medicine as a stimulant, emmenagoge and antihelminthic (Evans 1996). A chloroform extract of this plant has anti-inflammatory activity in acute and subchronic models (Mordujovich-Buschiazzo et al 1996). Assays have shown the presence of sesquiterpene lactones in a chloroform extract of *T. vulgare* (Nano et al 1980). The major sesquiterpene lactone found in chloroform extracts of Argentine-grown *T. vulgare* is parthenolide (Schinella et al 1998), a germacranolide isolated from *T. parthenium* and other plants (Bohlmann & Zdero 1982). Sesquiterpene lactones have recently received considerable attention because of their complex pharmacological actions (Robles et al 1995).

Plants containing sesquiterpene lactones have also been used in popular medicine as a cytoprotective agent against gastric ulcers. It has been speculated that this anti-ulcerogenic activity could be related to interactions between the α-methylene-γ-lactone group and thiol constituents in the gastric mucosa (Giordano et al 1990).

The aim of this study was to explore the gastroprotective action of the chloroform extract from *T. vulgare* and of purified parthenolide on ethanol-induced injury to the gastric mucosa of rats.

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Materials and Methods

Plant material

Aerial parts of *Tanacetum vulgare* grown for ornament in the province of Buenos Aires, Argentina, were collected during flowering and authenticated by Dr Marta Nájera (Department of Botany, National University of La Plata). A voucher sample (LPE no. 968) was deposited in the Carlos Spegazzini Museum of Botany and Pharmacognosy, National University of La Plata, Argentina.

Extraction and identification procedures

The chloroform extract of *Tanacetum vulgare* was obtained as reported elsewhere (Nano et al 1980). In brief, air-dried powdered material was extracted with dichloromethane in a Soxhlet apparatus. After vacuum concentration, the dichloromethane extract (7% of plant dry weight) was dissolved with warm ethanol, treated with 4% aqueous lead acetate and left to stand overnight. A chloroform extract (1·9% of plant dry weight) was obtained from the filtrate. The extract was filtered through a Sephadex LH-20 (Pharmacia) 40 cm × 3 cm gel column which was eluted with methanol to yield 57 10-mL fractions. Fractions 25–30 were combined and separated on a 40 cm × 2·5 cm silica gel column eluted with chloroform (100 mL) and a 9 : 1 mixture of chloroform–ethyl acetate (300 mL) to give 30 10-mL fractions. Those numbered 13–20 contained parthenolide (21% w/w of the chloroform extract). The purity of the compound was checked by high-performance liquid chromatography (HPLC) with diode-array detection (DAD) on a Merck-Hitachi system fitted with an RP-18 column (5 μm) eluted with a water–methanol–trifluoroacetic acid gradient (99 : 1 : 0·05 → 1 : 99 : 0·05) for 30 min. The UV detector was set at 210 nm.

The ^{1}H and ^{13}C NMR spectra of parthenolide (4,5-epoxygermacra-1(10),11(13)-dien-6β,7αH-12,8-olide) were recorded on a Varian 400 MHz spectrometer. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm, TMS = 0) 6·33 d (J = 3 Hz), H-13a; 5·62 d (J = 3 Hz), H-13b; 5·20 br d (J = 7·7 Hz), H-1; 3·85 t (J = 4·9 Hz), H-6; 2·78 br d (J = 4·9 Hz), H-5; 2·4 m, H-2 and H-9′; 2·5 m, H-2′, H-3′, H-8′ and H-9; 1·70 s, methyl H-14 (3); 1·29 s methyl H-15 (3). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm, TMS = 0) 169·23 (C-12), 139·19 (C-11), 134·56 (C-10), 125·23 (C-1), 121·22 (C-13), 82·42 (C-6), 66·36 (C-5), 61·50 (C-4), 47·64 (C-7), 41·18 (C-9), 36·32 (C-3), 30·62 (C-8), 24·12 (C-2), 17·96 (C-15), 16·93 (C-14).

Ethanol-induced gastric ulcer

Experiments were performed on male Wistar rats, 200–250 g. Food was withdrawn 14–16 h before the experiments. Acute gastric ulceration was induced by ethanol (Robert et al 1979). Briefly, absolute ethanol (1 mL/animal) was administered intragastrically to control rats and to rats pretreated 30 min or 24 h before with the chloroform extract of *Tanacetun vulgare* (2·5–80 mg kg^{-1}, p.o.) or parthenolide (5–40 mg kg^{-1}, p.o.) as a single dose. The control group received the vehicle (poly(-vinylpyrrolidone) in saline). Animals were killed by decapitation 1 h after administration of ethanol and the stomach was removed, opened along the greater curvature and washed with 150 mM NaCl. The extent of erosion of the stomachs was measured according to the method of Merazzi-Uberti and Turba as described previously (Giordano et al 1994). The results were expressed in terms of an ulcer index (UI) which is the average severity of erosions/rat for each group on the scale from 0 to 5. The sum of these values was divided by the number of animals.

Analytical methods

The mucosa of the glandular stomach was removed by scraping with a blunt knife, and homogenized with 40 vols 0·2 M EDTA. The non-protein sulphydryl content of the homogenate was determined by use of Ellman's reagent (Sedlak & Lindsay 1968). Reduced glutathione was used as a standard. The protein content of the gastric mucosa was quantified by the method of Lowry et al (1951) with bovine serum albumin as the standard.

Data analysis

All values are expressed as means ± standard deviations (s.d.). Statistical analysis was performed by use of Student's *t*-test, with $P < 0{\cdot}05$ being regarded as indicative of significance.

Results

The results of our experiments show that rat mucosa gastric injury induced by ethanol is significantly and dose-dependently reduced both by chloroform extract of tanacetum vulgare and by parthenolide (Table 1). The chloroform extract (2·5–10 mg kg^{-1}, p.o.) administered 30 min before treatment with ethanol, exerted a significant protective effect against alcohol-induced ulceration—inhibition of damage ranged between 34 and 100%. Pretreatment of animals with parthenolide (5–40 mg kg^{-1}, p.o.) also reduced the gastric damage but total protection was obtained with higher doses.

Table 1. Effect of chloroform extract and of parthenolide from *Tanacetum vulgare* on gastric ulcer induced by ethanol.

Treatment	Dose ($mg\,kg^{-1}$)	Ulcer index	Inhibition (%)
Ethanol + vehicle (n = 8)	0	4·8 ± 0·3	0
Ethanol + chloroform extract (n = 6)	2·5	3·2 ± 0·5*	34
	5·0	2·2 ± 0·6**	53
	7·5	1·0 ± 0·3**	79
	10·0	0†	100
Ethanol + parthenolide (n = 6)	5	3·5 ± 0·4*	27
	10	0·8 ± 0·2**	83
	40	0†	100

Rats were given absolute ethanol (1 mL) 30 min after receiving the chloroform extract or parthenolide. They were killed 1 h later. Results are expressed as means ± standard deviation and were analysed by use of Student's *t*-test: $*P < 0{\cdot}05$, $**P < 0{\cdot}01$ compared with result from ethanol + vehicle group.

Table 2. Relationship between the severity of ethanol-induced gastric lesions and the concentration of non-protein sulphydryl groups in the mucosa of treated rats.

Treatment	Dose ($mg\,kg^{-1}$)	Ulcer index	Inhibition (%)	Non-protein -SH groups ($\mu g\,GSH\,(mg\,protein)^{-1}$)
No treatment (n = 4)	0	0	–	50·6 ± 2·3
Ethanol + vehicle (n = 8)	0	4·9 ± 0·3	0	17·7 ± 3·0
Ethanol + chloroform extract (n = 6)	10	3·0 ± 0·7*	39	25·7 ± 2·0*
	40	1·4 ± 0·2**	71	33·1 ± 1·8**
	80	1·0 ± 0·2**	80	38·8 ± 2·1**
Ethanol + parthenolide (n = 6)	10	2·6 ± 0·2*	47	26·2 ± 0·8*
	20	1·8 ± 0·2**	63	36·7 ± 5·0**
	40	0·5 ± 0·1**	91	51·3 ± 4·2**

Rats were given absolute ethanol (1 mL) 24 h after receiving the chloroform extract or parthenolide. They were killed 1 h later. Results are expressed as means ± standard deviation and were analysed by use of Student's *t*-test: $*P < 0{\cdot}05$, $\dagger P < 0{\cdot}01$ compared with result from ethanol + vehicle group.

In another assay, chloroform extract or parthenolide was administered to animals 24 h before treatment with ethanol. The results from this assay show a profile of gastrointestinal effects similar to that observed when the extract was administered 30 min before the alcohol (Table 2). When the dose of the chloroform extract or of parthenolide was $40\,mg\,kg^{-1}$ the mean ulcer index was reduced from 4·9 ± 0·3 in controls to 1·4 ± 0·2 ($P < 0{\cdot}01$) and 0·5 ± 0·1 ($P < 0{\cdot}01$), respectively.

Table 2 also shows the relationship between the severity of ethanol-induced gastric ulcer and the concentration of non-protein sulphydryl groups in the rat gastric mucosa. Intragastric administration of 1 mL ethanol to vehicle-treated rats produced gastric damage with an ulcer index of 4·9 ± 0·3 and a 65% reduction in the sulphydryl content of the mucosa. The concentration of these groups was $50{\cdot}6 \pm 2{\cdot}3\,\mu g\,(mg\,protein)^{-1}$ in control stomachs and $17{\cdot}7 \pm 3{\cdot}0\,\mu g\,(mg\,protein)^{-1}$ after ethanol treatment. Pretreatment of the animals with chloroform extract or parthenolide 24 h before administration of the alcohol not only protected the gastric mucosa against damage but also prevented the reduction in the number of -SH groups. Chloroform extract ($80\,mg\,kg^{-1}$, p.o.) restored the mucosal sulphydryl content to 77% of that found in the mucosa of control rats ($38{\cdot}8 \pm 2{\cdot}1$ compared with $50{\cdot}6 \pm 2{\cdot}3\,\mu g\,(mg\,protein)^{-1}$). The pattern of results for rats pretreated with parthenolide was similar to that for the chloroform extract. Parthenolide seems to be more effective than the chloroform extract—when the dose was $40\,mg\,kg^{-1}$ mucosal protection was almost complete (91%) and the sulphydryl content was completely restored to normal values ($51{\cdot}3 \pm 4{\cdot}2\,\mu g\,(mg\,protein)^{-1}$).

Discussion

The results of this study clearly show that the chloroform extract of the aerial parts of *Tanacetum vulgare*, and the sesquiterpene lactone parthenolide, when administered 30 min or 24 h before injury with ethanol, dose-dependently reduced the

mucosal lesions produced by the alcohol. It is known that ethanol reduces the concentration of non-protein sulphydryl groups in gastric mucosa and that administration of thiols induces gastric protection (Szabo et al 1981). The gastric mucosa contains a high concentration of reduced glutathione, and agents that markedly reduce it cause severe gastric ulceration (Boyd et al 1979). Our results indicate that ethanol-induced erosion of the gastric mucosa occurs in parallel with a substantial reduction in the number of sulphydryl groups. Oral administration of a chloroform extract of *Tanacetum vulgare* provided gastric cytoprotection and also prevented the reduction of the number of non-protein thiols.

Chromatographic purification of the chloroform extract yielded parthenolide, a sesquiterpene lactone of the germacranolide type containing α-methylene butyrolactone functionality. This function is known to have chemical reactivity toward biological nucleophiles, e.g. thiols and amines (Begley et al 1989). Assays with parthenolide were undertaken to determine whether this lactone contributed to the biological activity of the chloroform extract. Our data clearly indicate that parthenolide was as effective as the extract in the cytoprotection of gastric mucosa against ethanol injury. Pretreatment with the lactone also effectively prevented the alcohol-induced reduction in the number of gastric mucosa thiols.

It was reported recently that dehydroleucodine, a sesquiterpene lactone isolated from *Artemisia douglasiana* prevents the development of gastric lesions induced by ethanol (Guardia et al 1994). Involvement of the -SH compounds of the gastric mucosa in the cytoprotection was suggested, although the content of these groups was not measured.

Chloroform extracts of *Tanacetum vulgare* also contain different flavonoids (Ognyanov & Todorova 1983; Schinella et al 1998), compounds reported to inhibit gastric acid secretion and to protect against experimental ulcers (Motilva et al 1992; Vela et al 1997). The mixture of flavonoid methyl ethers isolated from the chloroform extract of tansy also had anti-ulcerogenic properties in our experiments, but the protection reached only 25% for a dose of 100 mg kg^{-1} administered 24 h before treatment with ethanol (data not shown). We cannot exclude the possibility of a topical effect of flavonoids being involved in the anti-ulcerogenic effect of the chloroform extract administered 30 min before ethanol injury.

The combination of the anti-inflammatory and anti-ulcerogenic activities of the chloroform extract and of parthenolide from *Tanacetum vulgare* as seen here is unusual but therapeutically desirable. The results of our experiments suggest that naturally-occurring sesquiterpene α-methylene butyrolactones like parthenolide might find therapeutic application. The chemical reactivity of the α-methylene function has been invoked as the explanation of certain toxic effects of such lactones (Hay et al 1994). Further work is required to assess the actual profit/risk ratio of this class of natural product widely used for self-medication.

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