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Gastroprotection and effect of the simultaneous administration of Cuachalalate (*Amphipterygium adstringens*) on the pharmacokinetics and anti-inflammatory activity of diclofenac in rats

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Abstract

This work aimed to study the effect of Cuachalalate methanol extract (CME) on the anti-inflammatory activity and pharmacokinetics of diclofenac sodium, a frequently prescribed non-steroidal anti-inflammatory drug (NSAID). The gastroprotective effect of CME on the gastric injury induced by diclofenac was studied in rats. CME showed a gastroprotective effect of 15.7% at 1 mg kg^{-1} and 72.5% at dose of 300 mg kg^{-1}. Omeprazole, used as anti-ulcer reference drug, showed gastroprotective effects of 50–89.7% at doses tested (1–30 mg kg^{-1}). The value of the 50% effective dose for the anti-inflammatory effect of diclofenac sodium (ED50 = 1.14 ± 0.23 mg kg^{-1}) using carrageenan-induced rat paw oedema model, was not modified by the concomitant administration of 30 or 100 mg kg^{-1} of CME. The effect of CME (30, 100 and 300 mg kg^{-1}, p.o.) on the pharmacokinetics of diclofenac sodium was studied. It was observed that the simultaneous administration of diclofenac sodium and 300 mg kg^{-1} of CME decreased significantly the values of C_{max} (7.08 ± 1.42 μg mL^{-1}) and AUC (12.67 ± 2.97 μg h mL^{-1}), but not the value of t_{max} (0.13 (0.1–0.25) h) obtained with the administration of diclofenac alone. The simultaneous administration of 30 or 100 mg kg^{-1} of CME did not modify the pharmacokinetic parameters of diclofenac. The experimental findings in rats suggest that CME at doses lower than 100 mg kg^{-1} protects the gastric mucosa from the damage induced by diclofenac sodium without altering either the anti-inflammatory activity or the pharmacokinetics of this NSAID.

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Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of several acute and chronic disorders, including arthritis and pain (Roth 1996). These drugs are classified into two groups: the conventional NSAIDs, also known as the nonselective NSAIDs; and the newer selective NSAIDs, known also as the cyclo-oxygenase (COX)-2 inhibitors (Rahme et al 2004). Nonselective NSAID use is frequently accompanied by upper gastrointestinal adverse effects. These effects can be minor, such as dyspepsia, abdominal pain and nausea, or very serious, such as perforation, ulcer or bleeding, which often require hospitalization (Rahme et al 2004). A number of approaches have been adopted to reduce the risk of NSAID-induced upper gastrointestinal complications, including reducing the NSAID dose, switching to NSAIDs perceived to be less toxic and using concomitant gastroprotective agents (Rahme et al 2004). The gastroprotective agents that have been used to heal or prevent an ulcer or to suppress abdominal pain and dyspepsia are misoprostol, histamine H_2-receptor antagonists, sucralfate and proton pump inhibitors (Carvajal et al 2004). However, the clinical experience with these agents is still limited and further surveillance to resolve this issue is needed. For this reason, considerable effort has focused on the identification of new gastroprotective agents. Some synthetic studies have been aimed at the preparation of new prostaglandins, prostacyclin mimetics and thromboxane antagonists (Ares & Outt 1998). New histamine H_2-receptor antagonists

have also been developed, which, unlike cimetidine or ranitidine, now appear to couple true gastroprotective activity with antisecretory properties (Ares & Outt 1998). Many other types of structures (flavonoids, peptides, terpenoids, xanthines, others), as well as compounds displaying certain pharmacological actions (5-hydroxytryptamine receptor binding, adrenergic receptor binding, mast cell stabilization, others) have been linked in some way to gastroprotection (Ares & Outt 1998). Several plants containing triterpenoids have been shown to possess anti-ulcer activity in several experimental ulcer models (Lewis & Hanson 1991; Borelli & Izzo 2000; Navarrete et al 2002). Gastroprotection, as an anti-ulcer mechanism of several natural products and crude extracts obtained from medicinal plants, plays an important role as a viable alternative in preventing NSAID-induced gastropathy (Arrieta et al 2003).

The stem bark of *Amphipterygium adstringens* (Schltdl.) Schiede ex Standl. (Julianaceae), local name Cuachalalate, is the most important anti-ulcer remedy in Mexican traditional medicine (Navarrete et al 1990). Extensive studies published on the chemistry of this medicinal plant (Domínguez et al 1983; Soriano-García et al 1987; Watson et al 1987; Navarrete et al 1989; Mata et al 1991; Mata 1993; Pérez et al 1993; Olivera et al 1999; Makino et al 2004) have demonstrated two types of major components – triterpenoids and long-chain phenols. The gastroprotective effect of this medicinal plant has been very well demonstrated in animal models (Navarrete et al 1990, 1998). The triterpenes 3α-hydroxymasticadienonic acid and 3-epioleanolic acid, and β-sitosterol have been identified as the gastroprotective active principles in the stem bark of Cuachalalate (Arrieta et al 2003). The anti-inflammatory activity for this medicinal plant has also been described (Oviedo-Chávez et al 2004). Apart from indigenous uses, Cuachalalate has also gained importance in modern medicine due to its gastroprotective properties in attenuating the gastric-damage side effects of NSAIDs. In Mexico, elderly people, who have a tendency to take various kind of medications, are particularly found to use Cuachalalate.

Among the various NSAIDs, diclofenac sodium was selected for this study because it is frequently used in clinical medicine to treat inflammatory and postoperative conditions, and in chronic cancer pain (Minotti et al 1998). It also exhibits adverse effects such as gastritis and peptic ulceration, which result primarily from prostaglandin inhibition (Ares & Outt 1998). Diclofenac has been also classified as a NSAID with high probability of being prescribed with a gastroprotective agent (Carvajal et al 2004). However, there are no studies on the effects of the simultaneous administration of a gastroprotective agent on the pharmacokinetics and anti-inflammatory activity of diclofenac.

This work had three objectives: firstly, to investigate whether Cuachalalate methanol extract (CME) is able to protect against diclofenac-induced gastric lesions in rats; secondly, to study the effect of the simultaneous administration of CME on the anti-inflammatory activity and on the pharmacokinetics of diclofenac sodium; and, finally, to provide experimental support for the popular use of this medicinal plant in modern medicine to counteract the adverse effects of diclofenac on the gastric mucosa.

Materials and Methods

Materials

The stems bark of *A. adstringens* was collected in Costa Grande de Guerrero. Botanists from Herbario de Plantas Utiles Efraim Hernández X, Universidad Autónoma Chapingo, confirmed the botanical identity of the plant; a voucher specimen is XOLO 199021. The air-dried plant material was pulverized through a 2-mm screen using a Wiley Mill. A sample of plant material (200 g) was extracted with methanol (1.1 LX3) by maceration at room temperature ($22 \pm 2^{\circ}$C) for 3-day periods; evaporation of the solvents in vacuum gave 77.3 g of syrupy residues (CME). The content of the major triterpenes: 3α-hydroxymasticadienonic acid ($5.14 \pm 0.21\%$) and masticadienonic acid ($2.07 \pm 0.07\%$) in this extract was determined by an HPLC analytical method developed in our laboratory (Navarrete et al 2005). Briefly, the liquid chromatography system consisted of Waters (Waters Corp., Milford, MA, USA) model 600 pumps, a Waters model 717 Plus injector, a Waters model 600E automated gradient controller, a Waters model 2996 photodiode array detector and a computerized data station equipped with Waters Empower software. Separation was achieved on a Synergi MAX-RP 80A column (Phenomenex, 150×4.6 mm i.d.; 4 μm particle size) and operated at 40°C. Mobile phase: water (0.1% acetic acid) (A), acetonitrile (0.1% acetic acid) (B) and reagent alcohol (C) (A–B–C 18:52:30%) were used isocratically for 20 min; the flow rate was 1.0 mL min^{-1} and the detection wavelength was 215 nm (Navarrete et al 2005).

Diclofenac sodium, naproxen sodium, omeprazole, carragenin and Tween 80 were purchased from Sigma Co. (Sigma, St Louis, MO, USA). Methanol, glacial acetic acid, sodium acetate and ethyl acetate were of HPLC grade purchased from Fisher Scientific (Fair Lawn, NJ, USA). Water for the HPLC mobile phase was purified in a Milli-Q system (Millipore, Bedford, MA, USA).

Animals

All the experiments were performed with male Wistar rats, 55–60 days old, 180–220 g, obtained from Centro UNAM-Harlan (Harlan México, S.A. de C.V.). Procedures involving rats and their care were conduced conform the Mexican Official Norm for Animal Care and Handing (NOM-062-ZOO-1999) and in compliance with international rules on care and use of laboratory animals. The rats were placed in single cages with wire-net floors and deprived of food for 12, 24 or 48 h before experimentation according to related study, but allowed free access to tap water throughout.

All compounds were dissolved in saline solution (0.9%), with the exception of CME, which was suspended

in 0.5% Tween 80 in saline solution. The drugs were freshly prepared each time. Control rats received the same volume of vehicle (0.5% Tween 80 in saline or saline solution only) by the same route.

The experimental work was divided into three sections: gastroprotective studies, anti-inflammatory studies and pharmacokinetics studies.

Gastroprotective studies

Ulceration was induced according to the method described by Rabin et al (2000). Briefly, intragastric administration of diclofenac sodium ($80\,mg\,kg^{-1}$) to male rats was carried out after a 48-h fasting period with free access to water. For these experiments, rats in groups of ten were treated orally either with CME (1, 3, 10, 30, 100 or $300\,mg\,kg^{-1}$), omeprazole (1, 3, 10 or $30\,mg\,kg^{-1}$, used as anti-ulcer reference drug) or vehicle (0.5% Tween 80 in saline) 30 min before diclofenac sodium administration. Eighteen hours after diclofenac administration, the rats were killed by ether inhalation. The stomach and duodenum were dissected out, inflated with 10 mL of 2% formalin and placed in 2% formalin for 15 min to fix both the inner and outer layers. The duodenum was opened along its anti-mesenteric side and the stomach along the greater curvature. An observer, unaware of the drug treatment, measured the damage area (mm^2) under a dissection microscope ($\times 10$) with an ocular micrometer. The sum of the area of all lesions in the corpus for each rat was calculated and served as the ulcer index. Gastroprotection (%) was calculated according to: % Gastroprotection $= (UIC - UIT) \times 100/UIC$, where UIC is ulcer index in control and UIT is ulcer index in test (Navarrete et al 1998).

Anti-inflammatory studies

To evaluate the anti-inflammatory activity of diclofenac sodium with and without CME it was used the carrageenan-induced rat hind paw oedema model (Winter et al 1962).

The right hind paw of the rats was marked to a point on the skin over the lateral malleolus and the initial paw volume was recorded (Vo, basal volume). Fasted rats (24 h) were grouped randomly into groups of five rats each, to determinate the dose–response curve for anti-inflammatory effect of diclofenac sodium (0.1, 0.3, 1.0, 2.5, 5.0 or $10\,mg\,kg^{-1}$, p.o.), CME (1.0, 3.0, 10, 30, 100 or $300\,mg\,kg^{-1}$, p.o.) or the combination diclofenac sodium (0.1, 1.0, 3.0, $10\,mg\,kg^{-1}$, p.o.) + CME (30 or $100\,mg\,kg^{-1}$, p.o.). A control group treated with vehicle was included in each experiment. After a period of 30 min, 0.1 mL of carrageenan solution (3% w/v) was injected subcutaneously in the plantar region of the right hind paw of each rat. Following injection of carrageenan, the paw volumes of each rat were measured at different time intervals (1, 2, 3, 4 and 5 h) with a plethysmometer (Plethysmometer 7150; Ugo Basile), by the volume displacement method; this volume was denominated Vt. The variation of the oedema volume at each time was calculated as delta volume ($\Delta Vt = Vt - Vo$) in mL. A delta volume versus time curve was constructed for each treatment and its area under the curve (AUC) was calculated for the period of observation ($AUC_{0-5\,h}$) using the trapezoidal method (Cruz et al 1999). Inhibition of oedema (%EI) was calculated by the equation: $\%EI = (AUCcontrol - AUCtreat) \times 100/AUCcontrol$.

Data were expressed as the mean ± s.e.m. of 5 rats. The 50% effective doses (ED50) and their associated 95% confidence limits (CL_{95}) were estimated according to standard linear regression analysis (Tallarida 2000).

Pharmacokinetic studies

Fasted rats (12 h) were grouped randomly into four groups of six rats each and orally administered as follows: the first group with diclofenac sodium ($10\,mg\,kg^{-1}$) + vehicle (0.5% Tween 80 in saline solution, 0.5 mL/100 g body weight, control group); second group with diclofenac sodium ($10\,mg\,kg^{-1}$) + $30\,mg\,kg^{-1}$ CME (30 CME group); third group with diclofenac sodium ($10\,mg\,kg^{-1}$) + $100\,mg\,kg^{-1}$ CME (100 CME group); and the fourth group with diclofenac sodium ($10\,mg\,kg^{-1}$) + $300\,mg\,kg^{-1}$ CME (300 CME group). For the determination of plasma diclofenac before (t = 0 min) and after (3, 6, 10, 15, 20, 30, 45, 60, 90, 120, 240, 480 and 600 min) the administration, whole blood samples ($100\,\mu L$) were taken from the cannula inserted and fixed in the caudal artery (Cruz et al 1999). The same volume of blood extracted at each time was replaced immediately after sampling with physiological isotonic saline solution to avoid any reduction in circulation volume. The concentration of diclofenac sodium was determined in whole blood by HPLC using a procedure of internal standard developed in our laboratory. Whole blood samples were added with $20\,\mu L$ of 1% acetic acid, $40\,\mu L$ of naproxen sodium ($10\,\mu g\,mL^{-1}$) used as internal standard (IS) and $500\,\mu L$ of ethyl acetate (in that order). The samples were shaken in a vortex mixer for 3 min and centrifuged for 10 min at $12\,000\,rev\,min^{-1}$. The supernatant was separated and a second extraction with $500\,\mu L$ of ethyl acetate was performed. The supernatants were combined and dried through a nitrogen current at $50 \pm 2°C$. The residue was reconstituted with $100\,\mu L$ of mobile phase (acetate buffer pH 5.5–methanol, 3:7). A sample of $20\,\mu L$ of this mixture was subjected to liquid chromatography (LC) analysis.

The LC system consisted of Waters (Waters Corp., Milford, MA, USA) model 600 pumps, a Waters model 717 Plus injector, a Waters model 600E automated gradient controller, a Waters model 2996 photodiode array detector, and a computerized data station equipped with Waters Empower software. Separation was achieved on a Symmetry C_{18} column (Waters, 150×4.6 mm i.d.; $5\,\mu m$ particle size) and operated at 25°C. The column was equipped with a 2-cm LC-18 guard column (Supelco, Bellefonte, PA, USA). The mobile phase consisted of 75 mM sodium acetate buffer adjusted pH to 5.5 with glacial acetic acid (A) and methanol (B) (A–B, 30:70%), was used isocratically for 15 min; the flow rate was $1.0\,mL\,min^{-1}$ and detection wavelength 275 nm. Each run was followed by a 5-min wash with 100% methanol

and an equilibration period of 15 min. The retention time for IS (naproxen sodium) and diclofenac sodium were 3.3 and 5.8 min, respectively (Figure 1). To determine the accuracy of the method, rat blood samples were spiked with 0.5, 1.5 and 4.5 $\mu g\,mL^{-1}$ of the standard diclofenac sodium and recovery rates were 92.8%, 101.1% and 99.3%, respectively. An indicator for precision is the standard deviation (δ). All samples were injected in triplicate and the standard deviation of standard compound was below 15.0%. Calibration standards ranging from 0.03 to 7.0 $\mu g\,mL^{-1}$, a relatively broad concentration range, were analysed in triplicate, and the resulting peak–height ratio (height diclofenac sodium/height IS) responses were regressed on the concentration with the weighting factors: $1/x^0$, $1/x$, $1/x^2$, $1/x^{1/2}$, $1/y$, $1/y^2$ and $1/y^{1/2}$ (Almeida et al 2002). The weighting factor $1/x^2$ produced the least sum of percentage relative error ($\Sigma\ \%RE = 1.5 \times 10^{-12}$). The calibration curve obtained with this weighting factor was: $Y = 0.257x + 0.02$, with $r^2 = 0.991$. With this calibration curve the accuracy of the data, expressed by bias value ($\%bias = (C_{mean} - C_{nominal})/C_{nominal} \times 100$) was evaluated across the whole concentration range. Percentage bias values were in the range −2.4% to −12.5% and all of them were lower than acceptable limits of ±20% (Almeida et al 2002). The limit of detection of diclofenac (obtained from the relation: $LOD = 3.3 \times Sb/b$; where Sb is the standard deviation for the blank and b is the slope of the calibration curve) was 0.02 $\mu g\,mL^{-1}$. The components of blood and CME did not interfere with the quantification of plasma diclofenac sodium (Figure 1). The lectures of diclofenac concentration in analysed samples were higher than 0.3 $\mu g\,mL^{-1}$.

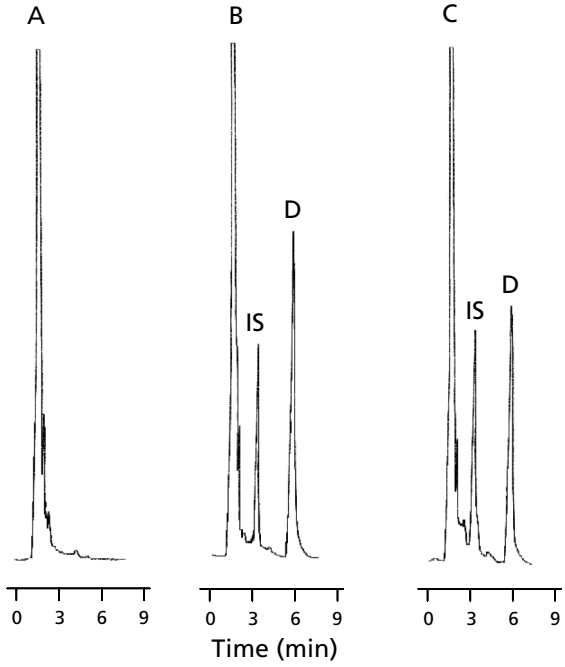

Figure 1 Chromatograms illustrating blank of rat blood (A), blank rat blood containing naproxen sodium as internal standard and diclofenac sodium (B) and a sample after 10 min of the administration of CME (100 mg kg^{-1}, p.o.) and diclofenac sodium (10 mg kg^{-1}, p.o.) (C). Mobile phase: 0.075 M sodium acetate buffer adjusted pH to 5.5 with glacial acetic acid–methanol (30:70%) used isocratically; column Symmetry C_{18} (Waters, 150 × 4.6 mm i.d.; 5 μm particle size); flow rate 1.0 mL min^{-1}; injection volume 20 μL; detection wavelength 275 nm. Peaks: D = diclofenac, IS = internal standard.

The maximum plasma concentration (C_{max}) and the time to reach the C_{max} (t_{max}) after oral administration of diclofenac were determined directly from the measurement values. The area under the plasma concentration–time curve ($AUC_{0-10\,h}$) was calculated by the trapezoidal method using blood concentration data (Cruz et al 1999).

Statistics

The values obtained in the gastroprotective and anti-inflammatory experiments are reported as the mean ± s.e.m., statistically significant differences between the treatments for these experiments were tested by one-way analysis of variance followed by Dunnett's t-test. Statistically significant differences between treated and control rats were determined by Student's t- test for unpaired data for ED50. The values for C_{max} and $AUC_{0\text{-}10\,h}$ are reported as the mean ± s.d. in the text and tables and as mean ± s.e.m. in the figures. The median, minimum and maximum were given as summary statistics for t_{max}. Statistically significant differences between treated and control rats were determined by Student's t-test for unpaired data for C_{max} and AUC, and the Mann–Whitney rank sum test was used to determine the significance in t_{max}. Probability (P) values less than 0.05 were considered significant.

Results

The CME showed a dose-dependent gastroprotective effect (Figure 2A) against diclofenac sodium-induced gastric lesions, reaching a gastroprotection of 15.7% at 1 mg kg^{-1} and 72.5% at dose of 300 mg kg^{-1}. This effect was less potent than that of omeprazole (Figure 2B), which at doses tested (1–30 mg kg^{-1}) showed gastroprotective effects of 50–89.7%.

Diclofenac sodium showed a dose-dependent anti-inflammatory effect in the carrageenan-induced rat hind paw oedema model (Figure 3A), whereas CME did not (Figure 3B). At 0.3 mg kg^{-1} diclofenac sodium inhibited the inflammation by 36% and its effect increased with increasing dose until reaching 70% at 10 mg kg^{-1}. Table 1 summarized the anti-inflammatory activity calculated as percent oedema inhibition when diclofenac and CME were administered simultaneously. The values of the 50% effective dose (ED50) for the anti-inflammatory effect of diclofenac sodium (1.14 ± 0.23 mg kg^{-1}) were slightly decreased by the simultaneous administration of 30 mg kg^{-1} (0.85 ± 0.16 mg kg^{-1}) or 100 mg kg^{-1} (0.79 ± 0.09 mg kg^{-1}) of CME, although these values were not statistically different from those of the control group ($P = 0.07$).

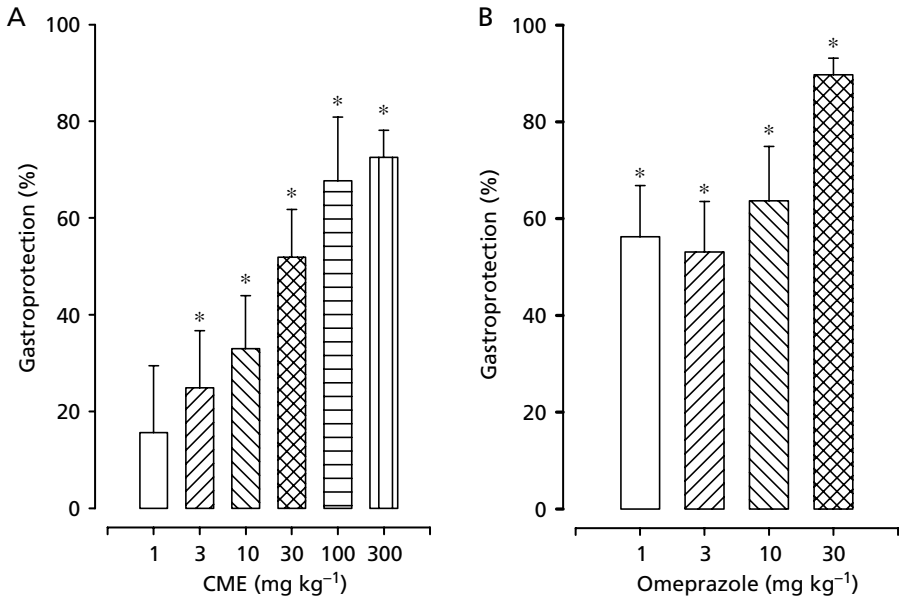

Figure 2 Doses–response curves of the gastroprotective effect of CME (A) and omeprazole (B) against diclofenac-sodium-induced gastric lesions in rats. Bars represent the mean ± s.e.m. (n = 10) of %gastroprotection calculated from the mean ulcer index obtained in the control group ($16.23 \pm 4.2\,mm^2$). *$P < 0.05$ compared with the control group.

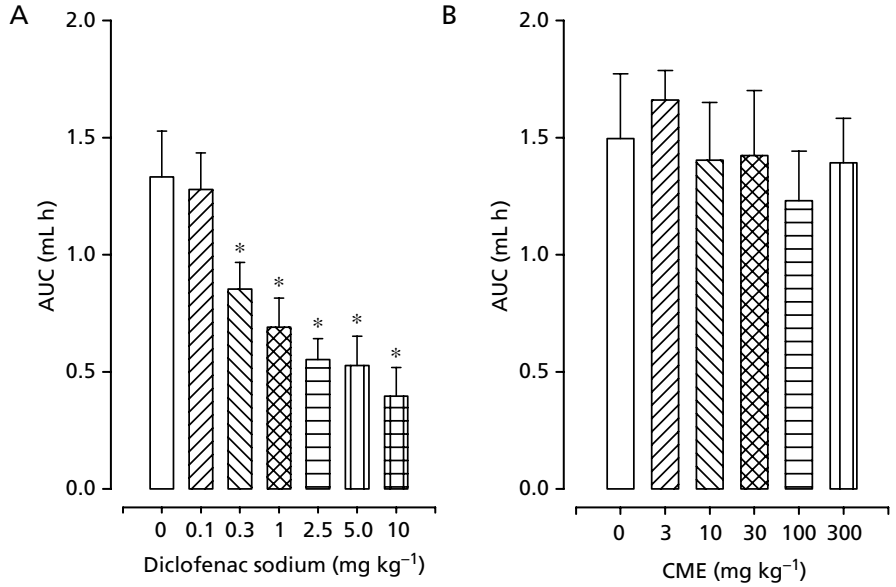

Figure 3 Anti-inflammatory effect of diclofenac sodium (A) and CME (B), expressed as area under the curve obtained from the delta volume vs time graph for each dose tested in the carrageenan-induced rat hind paw oedema model. Bars represent the mean ± s.e.m. (n = 5). *$P < 0.05$ compared with the control group.

The findings of the pharmacokinetic studies are shown in Table 2 and the plasma concentration profile at different time intervals is depicted in Figure 4. It was observed that concomitant administration of diclofenac sodium and CME at $300\,mg\,kg^{-1}$ decreased significantly ($P = 0.031$) the value of C_{max} ($4.90 \pm 1.63\,\mu g\,mL^{-1}$) and AUC ($8.66 \pm 2.54\,\mu g\,h\,mL^{-1}$) versus the values of C_{max} ($7.08 \pm 1.42\,\mu g\,mL^{-1}$) and AUC ($12.67 \pm 2.97\,\mu g\,h\,mL^{-1}$) obtained with the administration of diclofenac alone. However, there were no significant differences ($P = 0.86$) between the values obtained for these pharmacokinetic parameters when diclofenac ($10\,mg\,kg^{-1}$) was simultaneously administered with CME at 30 or $100\,mg\,kg^{-1}$ versus diclofenac alone (Table 2). The values in Table 2 clearly suggest that there was no alteration in t_{max} ($P = 0.24$). The range of time to reach C_{max} in the different treatments was found to be 0.05–0.5 h.

Table 1 Anti-inflammatory effect of diclofenac alone and in combination with Cuachalalate methanol extract at 30 and 100 mg kg^{-1} in the carrageenan-induced rat paw oedema model

Group	Diclofenac (mg kg^{-1})	$AUC_{0-5\,h}$ (mL h)	%EI observed	%EI predicted	Residuals	ED50 (CL_{95}) (mg kg^{-1})
Diclofenac + vehicle	Control	1.331 ± 0.197	—	—	—	
	0.1	0.994 ± 0.0652	25.29 ± 4.9	26.91	−1.62	1.141 (0.86–1.52)
	1.0	0.687 ± 0.151*	48.38 ± 11.3	48.75	−0.37	r = 0.996
	3.0	0.546 ± 0.110*	59.01 ± 8.3	59.17	−0.16	
	10.0	0.395 ± 0.122*	70.32 ± 9.2	70.59	−0.327	
Diclofenac + 30 mg kg^{-1} CME	Control	1.140 ± 0.160	—	—	—	
	0.1	0.728 ± 0.173	36.17 ± 15.14	36.15	0.02	0.849 (0.72–0.99)
	1.0	0.555 ± 0.106	51.34 ± 9.31	51.06	0.28	r = 0.995
	3.0	0.584 ± 0.120*	57.55 ± 10.51	58.17	−0.61	
	10.0	0.385 ± 0.116*	66.27 ± 10.21	65.96	0.31	
Diclofenac + 100 mg kg^{-1} CME	Control	1.431 ± 0.172	—	—	—	
	0.1	1.025 ± 0.181	28.32 ± 12.7	29.59	−1.27	0.789 (0.60–1.04)
	1.0	0.675 ± 0.127	52.80 ± 8.9	52.33	0.47	r = 0.997
	3.0	0.500 ± 0.226*	65.04 ± 15.8	63.19	1.85	
	10.0	0.382 ± 0.096*	73.31 ± 6.7	75.08	−1.77	

The $AUC_{0-5\,h}$ obtained from the curve delta volume versus time for each treatment is expressed as the mean ± s.e.m. of 5 rats. % Oedema Inhibition (%EI) was calculated for each rat treated with drug with reference to the respective control group and according the equation expressed in the Materials and Methods section. %EI predicted for the linear regression analysis (%EI observed vs log dose), the correlation coefficient (r) and residuals (%EI observed − %EI predicted) are shown as elements of goodness of fit of the model used to determine the 50% effective dose (ED50) and their associated 95% confidence limits (CL_{95}). *$P < 0.05$, vs respective control; Dunnett's *t*-test after one-way analysis of variance.

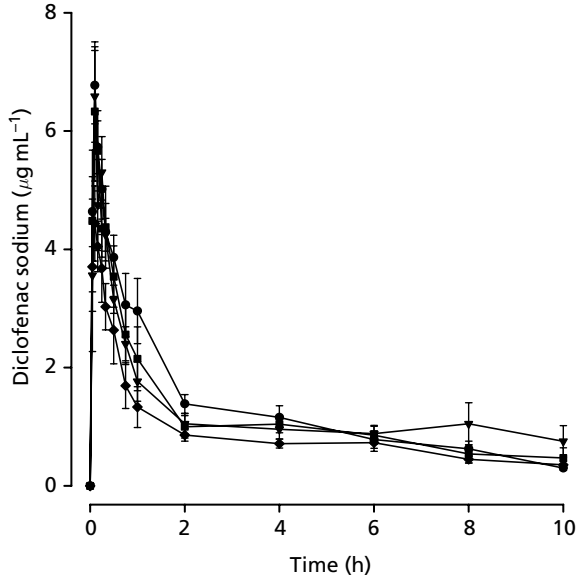

Figure 4 Mean plasma concentration–time curves in rat after single oral administration of 10 mg kg^{-1} diclofenac sodium, alone (circles) or with 30 mg kg^{-1} (triangles), 100 mg kg^{-1} (squares) or 300 mg kg^{-1} (diamonds) oral doses of CME. Data are the mean ± s.e.m. for 6 rats.

Discussion

The gastroprotective effect shown by omeprazole in reducing the gastric damage induced by diclofenac sodium (80 mg kg^{-1}) is through the inhibition of gastric acid secretion. The data are consistent with the clinical use of omeprazole to protect against diclofenac gastric damage (Carvajal et al 2004) and with basic studies on the gastroprotective effect of omeprazole in models of gastric damage induced with acidified ethanol and indometacin (Chandranath et al 2002). Several plants containing high amounts of triterpenoids have shown anti-ulcer activity in experimental ulcer models (Borelli & Izzo 2000). The gastroprotective activity of these plants is not due to inhibition of gastric acid secretion but probably due to activation of mucous membrane protective factors (Borrelli & Izzo 2000). It has been reported that CME did not modify gastric secretion in Say pyloric-ligated rats (Navarrete et al 1990), suggesting that the gastroprotection shown here by CME is independent of the inhibition of the gastric acid secretion. Also, previously we reported that in the gastroprotection of CME on ethanol-induced gastric mucosal lesions, endogenous nitric oxide plays an important role and that there is partial participation by prostaglandins and endogenous sulfydryls (Arrieta et al 2003), indicating that the gastroprotective effect of CME may be due to an increase of the gastroprotective factors.

Our findings on the anti-inflammatory effect of CME contrast with those of Oviedo-Chavez et al (2004), who reported anti-inflammatory activity for Cuachalalate in the same animal model at 31 and 100 mg kg^{-1}. This discrepancy can be attributed to those authors using intraperitoneal administration and an aqueous extract of Cuachalalate. We did not observe any tendency to reduce the oedema even though we used a dose of 300 mg kg^{-1}, which represents 10 and 3 times the dose used by those authors (Oviedo-Chavez et al 2004). The range of doses tested in the anti-inflammatory studies was in the same range as the doses at which CME

Table 2 Pharmacokinetic parameters of diclofenac sodium after single oral doses of $10\,mg\,kg^{-1}$ alone or in the presence of Cuachalalate methanol extract (CME) at 30, 100 or $300\,mg\,kg^{-1}$ orally in rat

Treatment	C_{max} ($\mu g\,mL^{-1}$)	t_{max} (h)	AUC_{0-10} ($\mu g\,h\,mL^{-1}$)
Diclofenac ($10\,mg\,kg^{-1}$)	7.08 ± 1.42	0.13 (0.1–0.25)	12.67 ± 2.97
Diclofenac ($10\,mg\,kg^{-1}$) + CME ($30\,mg\,kg^{-1}$)	6.90 ± 2.26	0.10 (0.05–0.16)	12.35 ± 4.22
Diclofenac ($10\,mg\,kg^{-1}$) + CME ($100\,mg\,kg^{-1}$)	6.88 ± 2.47	0.10 (0.05–0.5)	11.48 ± 3.85
Diclofenac ($10\,mg\,kg^{-1}$) + CME ($300\,mg\,kg^{-1}$)	4.90 ± 1.63*	0.13 (0.05–0.25)	8.66 ± 2.54**

The results for AUC and C_{max} are given as mean ± s.d. of six repetitions for each treatment. The results for t_{max} are given as median with maximum and minimum value in parenthesis. $^{**}P = 0.033$ and $^{**}P = 0.031$, relative to the control group (Student's *t*-test unpaired data).

showed gastroprotection, although the concomitant administration of diclofenac sodium plus CME did not modify the anti-inflammatory ED50 of diclofenac sodium. The results obtained in this work give experimental support to the empirical use of Cuachalalate to decrease the gastropathy which develops following the use of diclofenac and may represent an alternative in the research into strategies to decrease the gastric damage due to chronic use of NSAIDs (Ares & Outt 1998).

The C_{max} and AUC values of diclofenac were significantly ($P = 0.03$) decreased by the administration of $300\,mg\,kg^{-1}$ of CME. This denotes that there is a possible kinetic interaction between diclofenac and the components of CME when it is administered at high doses. However, at 30 and $100\,mg\,kg^{-1}$ CME did not produce changes in the pharmacokinetics of diclofenac. While herb–drug interactions may involve a pharmacodynamic and pharmacokinetic mechanism, they may result in either beneficial or adverse effects (Abebe 2002). The results obtained here suggest that concomitant administration of diclofenac sodium and CME may represent a beneficial effect, because doses lower than $100\,mg\,kg^{-1}$ did not modify the anti-inflammatory activity and pharmacokinetics of diclofenac. However, further research is needed to assess the clinical significance of these results and to ensure effective use of Cuachalalate to counteract the gastropathy induced by drugs.

Conclusion

We found that Cuachalalate methanol extract (CME) at doses lower than $100\,mg\,kg^{-1}$ protected against the gastric lesions induced by high-dose diclofenac sodium without altering either the anti-inflammatory effect or the concentration versus time curve of diclofenac sodium in rats. The results provide important information on the beneficial effects of the concomitant administration of CME and diclofenac sodium. They also give experimental support to the empirical use of Cuachalalate to attenuate the gastric-damage side effects induced by a non-steroidal anti-inflammatory drug.

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