International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2020.375.381

Research Article
Pharmacokinetics Effect of Diclofenac or Ketorolac-methyl Eugenol and Their Implication in the Gastroprotection

[1]Leticia Cruz-Antonio, [2]María Elena Sánchez-Mendoza, [2]Yaraset López-Lorenzo, [2]Héctor Isaac Rocha-González, [1]Aida Robles-Sánchez and [2]Jesús Arrieta

[1]Área de Farmacéutica, Facultad de Estudios Superiores Zaragoza, Universidad Nacional Autónoma de México, Ciudad de México, México
[2]Sección de Estudios de Posgrado e Investigación, Escuela Superior de Medicina, Instituto Politécnico Nacional, Plan de San Luis y Díaz Mirón, Colonia Casco de Santo Tomás, Miguel Hidalgo, Ciudad de México, 11340, México

Abstract

Background and Objective: Gastric ulcers are a global health problem, in part caused by the frequent administration of NSAIDs. To reduce gastrointestinal damage, these drugs are often co-administered with gastroprotective agents, which also have severe adverse effects. The objective of this study was to evaluate the protective activity of methyl eugenol against diclofenac- and ketorolac-induced gastric damage and explore the pharmacokinetics of a possible drug interaction of the combined treatments. **Materials and Methods:** Rats were orally administered methyl eugenol at different doses and 1 h later given diclofenac (80 mg kg^{-1}) or ketorolac (35 mg kg^{-1}). The control groups were treated with diclofenac or ketorolac only. After 4 or 6 h, respectively, the animals were sacrificed and the stomachs removed. Gastric damage was quantified to calculate the percentage of gastroprotection. Additionally, a pylorus ligation was performed to test for an anti-secretory effect of methyl eugenol. Other animals received 10 mg kg^{-1} of diclofenac or ketorolac with or without methyl eugenol co-administration (100 mg kg^{-1}) to analyze possible drug interactions. The plasma concentrations of diclofenac and ketorolac were determined by HPLC and pharmacokinetics parameters were assessed. **Results:** Methyl eugenol decreased gastric lesions induced by diclofenac and ketorolac, achieving the maximum protection of 74.4 and 49.0%, respectively, at 100 mg kg^{-1}. No anti-secretory activity was detected. With the co-administration of methyl eugenol, the bioavailability of diclofenac was unchanged but that of ketorolac declined. **Conclusion:** Methyl eugenol provided greater protection against diclofenac- than ketorolac-induced gastric lesions. It affected the bioavailability of ketorolac (due to alterations in absorption) but not that of diclofenac.

Key words: Methyl eugenol , gastric damage, pharmacokinetics, drug interactions, ketorolac, diclofenac, gastroprotection

Citation: Leticia Cruz-Antonio, María Elena Sánchez-Mendoza, Yaraset López-Lorenzo, Héctor Isaac Rocha-González, Aida Robles-Sánchez and Jesús Arrieta, 2020. Pharmacokinetics effect of diclofenac or ketorolac-methyl eugenol and their implication in the gastroprotection. Int. J. Pharmacol., 16: 375-381.

Corresponding Author: Jesús Arrieta, Sección de Estudios de Posgrado e Investigación, Escuela Superior de Medicina, Instituto Politécnico Nacional, Plan de San Luis y Díaz Mirón, Colonia Casco de Santo Tomás, Miguel Hidalgo, Ciudad de México, 11340, México

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are frequently prescribed as an analgesic, anti-inflammatory and antipyretic agents. Their chronic use, however, is associated with adverse effects on the cardiovascular, neurological and renal systems and especially increased risk of gastrointestinal ulceration and bleeding[1,2]. It has been proposed that NSAIDs provoke gastrointestinal lesions by inhibiting cyclooxygenase-1 (COX-1), leading to decreased prostaglandin synthesis and reduced gastrointestinal protection[3].

The reduction of gastrointestinal damage in patients taking NSAIDs is primarily accomplished by co-administering gastroprotective agents such as H_2 receptor antagonists, misoprostol or proton pump inhibitors[4]. Nevertheless, there is evidence of adverse side effects produced by the prolonged use of these agents[5]. Proton pumps can be taken as an example. Although the profile of proton pump inhibitors appears to be the most favorable among such protective agents, offering the best efficacy and quality of life[6], their consumption is associated with a deficit in the absorption of vitamin B12 and calcium[7], cardiovascular problems[8] and renal failure[9].

It is necessary to find pharmacological alternatives that reduce these adverse effects. One plausible strategy is the combination of NSAIDs with an extract or compounds isolated from plants[10,11]. Methyl eugenol , alkenyl benzene found in a wide variety of plant species, is known to have gastroprotective activity on ethanol-induced gastric lesions[12] as well as an antinociceptive effect in the mouse formalin model[13]. Hence, it may also protect against diclofenac- or ketorolac-induced gastric damage. A possible interaction of the elements of a combined treatment must be considered, based on pharmacodynamics and/or pharmacokinetics[14].

A modification in one of the stages of pharmacokinetics (absorption, distribution, metabolism or elimination) will probably cause an alteration of the blood concentration of a drug. Pharmacokinetic interactions that give rise to a blood concentration of the drug outside the therapeutic range are the most harmful. The more rapidly a change in blood concentration occurs, the more problematic the interaction, which could lead to an undesirable increase or decrease in the activity and/or bioavailability of either substance[14,15].

The present study aimed to evaluate the protective effect of methyl eugenol against diclofenac- and ketorolac-induced gastric damage in rats and to explore the possible pharmacokinetics of interaction between methyl eugenol and the respective NSAID.

MATERIALS AND METHODS

Study area: The study was carried out at the Multidisciplinary Unit for Experimental Research of the Facultad de Estudios Superiores (FES) Zaragoza, UNAM, from June, 2019 to March, 2020.

Chemicals: Methyl eugenol, diclofenac sodium salt, naproxen sodium and ketorolac tromethamine were purchased from Sigma Aldrich. Methanol and HPLC-grade acetonitrile were acquired from JT Baker. The other reagents were of analytical grade. Methyl eugenol was suspended in 0.5% carboxymethylcellulose, while diclofenac sodium, ketorolac tromethamine, omeprazole and carbenoxolone were dissolved in water.

Animals: Applicable international and national guidelines for the care and use of lab animals were followed. The current protocol was approved by the Internal Committee of the Escuela Superior de Medicina, Instituto Politécnico Nacional, (CICUAL-01/19-06 2014). Wistar rats (200-250 g) were provided by the FES Zaragoza, UNAM. The animals were individually housed in cages with wire-net floors and deprived of food for 18 h before experimentation, having free access to water. All assays involved 6-8 animals per group.

Diclofenac-induced gastric lesions: Methyl eugenol (10, 56 and 100 mg kg^{-1}), the reference drug carbenoxolone (100 mg kg^{-1}) or the vehicle were administered orally (0.1 mL/100 g of body weight). After 1 h, gastric lesions were induced with diclofenac (80 mg kg^{-1}, p.o.)[16]. Rats were sacrificed in a CO_2 chamber 4 h later. The stomach of each animal was removed, filled with formalin (2%) and Subsequently opened along the greater curvature. Henceforth, the methodology described by our workgroup was followed to determine the ulcer index and the percentage of gastroprotection[5].

Ketorolac-induced gastric lesions: Methyl eugenol (10, 30 and 100 mg kg^{-1}), the reference drug carbenoxolone (100 mg kg^{-1}) or the vehicle were administered orally. After 1 h, gastric lesions were induced with ketorolac (35 mg kg^{-1}, p.o.)[17]. The animals were sacrificed in a CO_2 chamber 6 h later and the percentage of gastroprotection was calculated.

Anti-secretory effect (pylorus ligation): Rats previously fasted for 18 h were anesthetized and immediately afterward the pylorus was ligated. The animals were then treated with methyl eugenol (100 mg kg^{-1}, p.o.) and sacrificed 4 h later to

dissect the stomachs and collect the gastric content. This was centrifuged at 3000 rpm for 5 min to determine the pH of the supernatant with a pH meter. Vehicle control and positive control (omeprazole) groups were included in the evaluation.

Pharmacokinetics study: Blood was sampled (200 µL) through a PE-10 catheter previously inserted in the caudal artery. The catheter was rinsed with heparin in saline solution (1 mg mL^{-1}) after each sampling[18]. Either the vehicle or methyl eugenol (100 mg kg^{-1}) was given orally and 30 min later diclofenac (10 mg kg^{-1}, p.o.)[19] or ketorolac (10 mg kg^{-1}, p.o.) was administered. Subsequently, blood samples were obtained at 0, 5, 10, 15, 20, 45, 60, 120, 240 and 360 min for diclofenac and at 0, 5, 15, 30, 45, 60, 90, 120, 180, 240 and 360 min for ketorolac. For blood sampling, the extracted volume was immediately replaced with physiological saline to avoid changes in the circulating volume. Plasma samples, obtained by centrifuging the blood samples at 12000 rpm for 10 min, were stored at -20°C to wait for processing.

Evaluation of the diclofenac and ketorolac plasma concentration: The plasma concentration of the drugs was determined by high-performance liquid chromatography (HPLC). For diclofenac, this was carried out according to the previously described method[20] and for ketorolac by a method formerly developed in our laboratory. Briefly, plasma samples (100 µL) were spiked with 200 µL of naproxen (30 µg mL^{-1}) as an internal standard and then 700 µL of methanol was added as the precipitant solvent. The mixture was shaken in a vortex for 3 min before centrifuging at 12500 rpm for 35 min. Supernatants were analyzed by HPLC at 40°C on a C18 (Zorbax Eclipse Plus®) column: 3.5 µm and 4.6×150 mm. The mobile phase: 0.04 mol L^{-1} potassium phosphate monohydrate buffer (pH 3.3) and methanol (57:43) v/v, at a flow rate of 1.5 mL min^{-1} monitored at 320 nm. Retention times for ketorolac and the IS were 3.3 and 6.4 min, respectively.

Statistics: Data are expressed as the mean±standard error of the mean. The values of gastroprotection were examined by the Kruskal-Wallis test followed by Dunn's multiple comparisons. The pharmacokinetics studies were analyzed by the Student's t-test. A value of $p \leq 0.05$ was considered statistically significant for all experiments.

RESULTS

Gastroprotection and anti-secretory activity: Methyl eugenol decreased diclofenac-induced gastric lesions in a

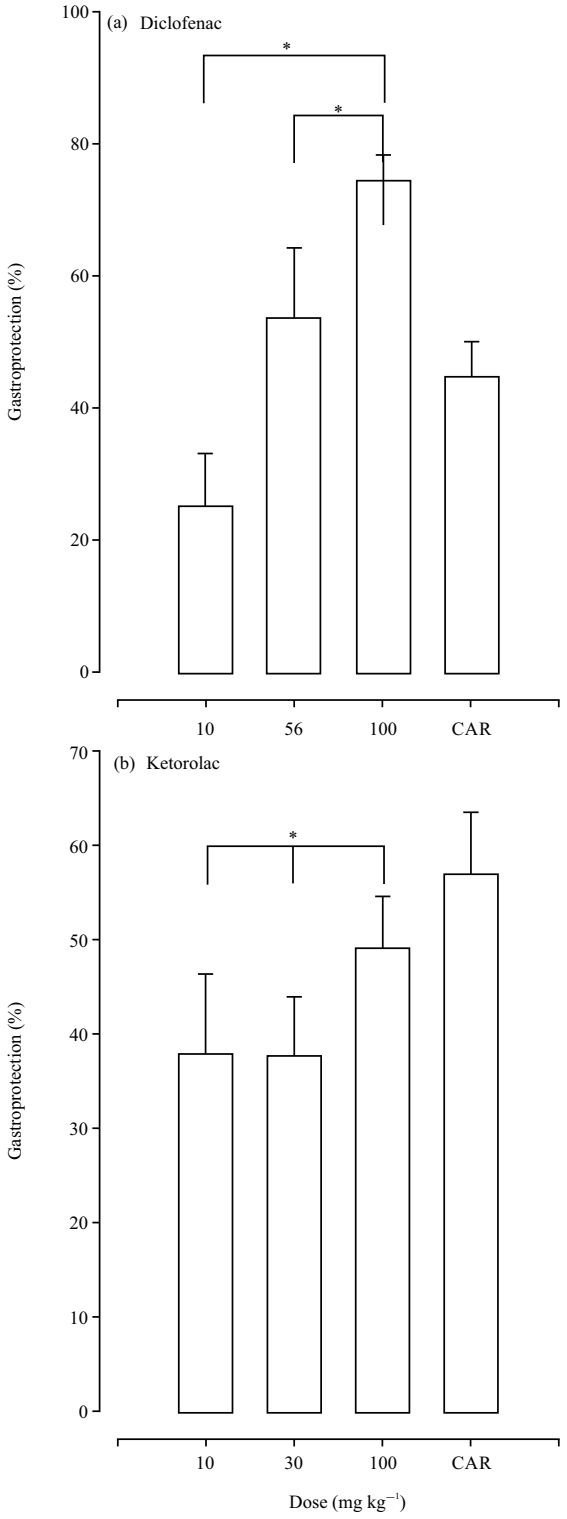

Fig. 1(a-b): Protection afforded by methyl eugenol against the gastric damage induced by (a) Diclofenac and (b) Ketorolac *$p \leq 0.05$, based on the Kruskal-Wallis test followed by Dunn's multiple comparisons
CAR: Carbenoxolone (100 mg kg^{-1})

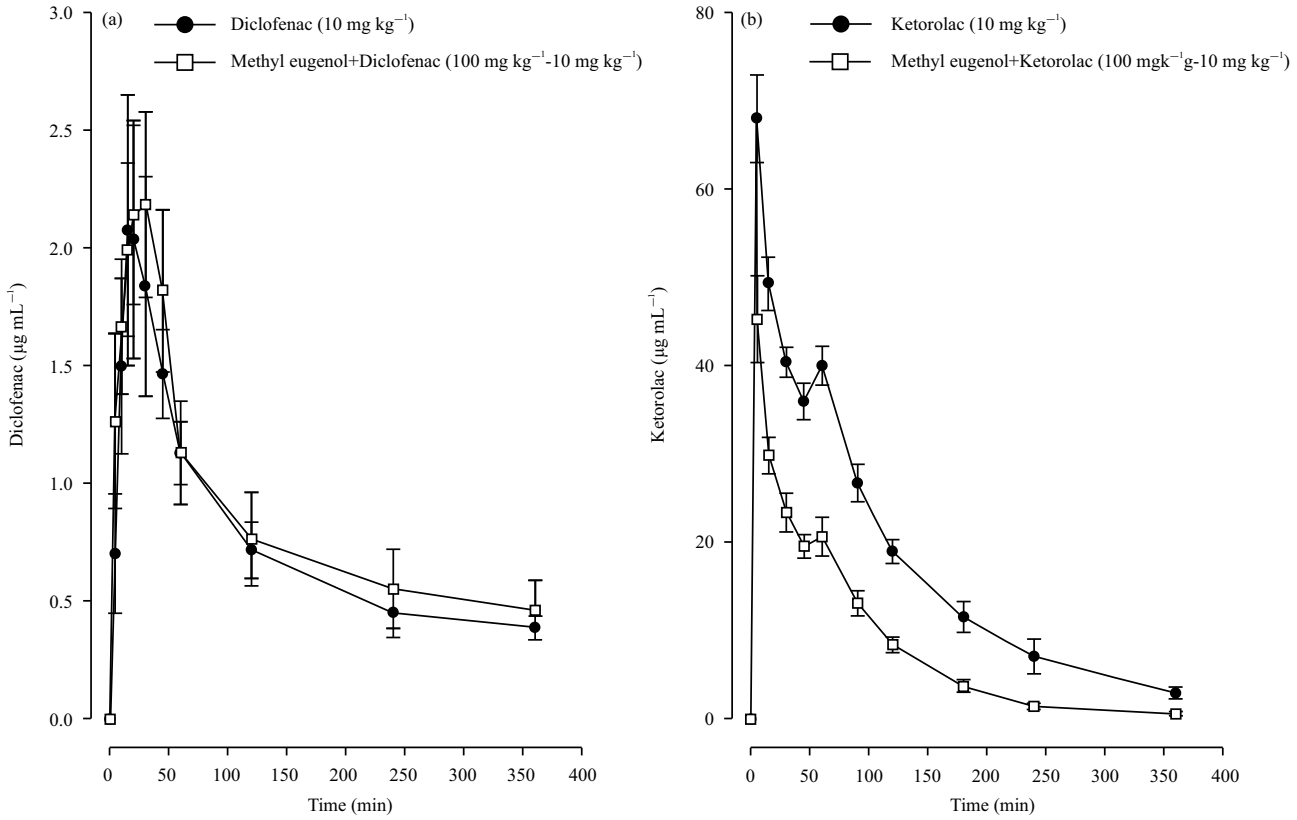

Fig. 2(a-b): Concentration-time profiles of (a) Diclofenac and (b) Ketorolac in plasma, in each case with and without methyl eugenol, *p≤0.05, based on the Student's t-test

Table 1: Anti-secretory activity of methyl eugenol

Treatments	Dose (mg kg^{-1})	N	Volume (mL)	pH
Control	--	7	1.68±0.19	1.66±0.12*
Methyl eugenol	100	7	1.48±0.07	1.50±0.07*
Omeprazole	30	7	1.37±0.07	5.85±0.36

*p≤0.05, based on the Kruskal-Wallis test followed by Dunn's multiple comparison

dose-dependent manner. The maximum protective effect was 74.42±3.86% for methyl eugenol (at 100 mg kg^{-1}) and 44.82±5.28% for carbenoxolone (the reference drug, at the same dose). Based on this substantially lower value for carbenoxolone, methyl eugenol is more potent (Fig. 1a).

Regarding the gastric damage caused by ketorolac, the protective effect of methyl eugenol was not dose-dependent (Fig. 1b). With a dose of 100 mg kg^{-1}, 49.00±5.62% gastroprotection was found for methyl eugenol and 56.95±6.50% for carbenoxolone, indicating a similar effect for both drugs.

In relation to anti-secretory activity (Table 1), there was a similar volume (1.48±0.07 mL vs. 1.68±0.19 mL) and pH (1.50±0.07 vs. 1.66±0.12) for methyl eugenol and the control group. However, the comparison of the pH of methyl eugenol and omeprazole (1.50±0.07 vs. 5.85±0.36) showed a significant difference. Overall, the results indicate that methyl eugenol has no anti-secretory activity.

Pharmacokinetics studies: When comparing diclofenac administered alone or with methyl eugenol (Fig. 2a), there are no significant differences in the pharmacokinetic parameters. This can be appreciated concerning the pattern of the plasma concentration of diclofenac, the maximum concentration (C_{max}, 2.46±1.04 vs. 2.54±0.79 μg mL^{-1}), the area under the curve (AUC_{0-t}, 317±119.31 vs. 344.94±101.66 μg×min mL^{-1}); $AUC_{0-\infty}$, (384.17±162.39 vs. 417.57±149.20 μg×min mL^{-1}) and the T_{max} (45±16.43 vs. 62.5±24.03) (Table 2).

The pharmacokinetics of ketorolac followed a different pattern. Compared to ketorolac given alone, its co-administration with methyl eugenol significantly decreased the C_{max} (68.04±12.25 μg mL^{-1} vs. 45.29±12.06 μg mL^{-1}; Fig. 2b). The same pattern was observed with the AUC_{0-t}

Table 2: Pharmacokinetics parameters related to the oral administration of diclofenac with or without methyl eugenol

Parameters	Diclofenac	Methyl eugenol+Diclofenac
C_{max} (μg mL^{-1})	2.46±1.04	2.54±0.79
T_{max} (min)	45.00±16.43	62.50±24.03
AUC_{0-t} (μg×min mL^{-1})	317.00±119.31	344.94±101.66
$AUC_{0-\infty}$ (μg×min mL^{-1})	384.17±162.39	417.57±149.20

Data are expressed as the mean±SEM of six replicates for each treatment

Table 3: Parameters of the pharmacokinetics involved in the oral administration of ketorolac with or without methyl eugenol

Parameters	Ketorolac	Methyl eugenol+Ketorolac
C_{max} (μg mL^{-1})	68.042±12.25	45.29±12.06*
T_{max} (min)	5.00±0	5.00±0
AUC_{0-t} (μg×min mL^{-1})	6329.90±1164.65	2931.55±597.96*
$AUC_{0-\infty}$ (μg×min mL^{-1})	6721.04±1427.09	2989.11±634.61*

Data are expressed as the mean±SEM of six replicates for each treatment, *$p \leq 0.05$ vs ketorolac administered alone

(6329.9±1164.65 vs. 2931.55±597.96 μg×min mL^{-1}) and $AUC_{0-\infty}$ (6721.04±1427.09 vs. 2989.11±634.61 μg×min mL^{-1}) (Table 3). The T_{max} values (5±0 vs. 5±0), however, were not significantly different (Table 3). Therefore, the administration of methyl eugenol reduces the bioavailability of ketorolac.

DISCUSSION

Methyl eugenol was herein evaluated as a possible candidate for co-administration with NSAIDs to help counteract the gastrointestinal lesions generated by these drugs. Accordingly, the gastroprotective activity of methyl eugenol against diclofenac- and ketorolac-induced damage was quantified in a rat model. The pharmacokinetics of drug interaction was examined for methyl eugenol with diclofenac and ketorolac.

The capacity of non-selective NSAIDs to provoke gastric ulcers is based on a multifactorial process encompassing the production of reactive oxygen species, leukotrienes, inflammatory molecules from COX-2 and other molecules, as well as the activation of phospholipase A2, the process of lipidic peroxidation and the inhibition of antioxidant enzymes[21-23]. Another mechanism that contributes to the development of gastric ulcers with the inhibition of prostaglandin synthesis is increased gastric motility, which promotes the restriction of blood flow[24].

In the current study, the gastroprotection afforded by methyl eugenol was greater for diclofenac- than ketorolac-induced damage (Fig. 1a, b). Methyl eugenol is known to inhibit COX-2[25] but not COX-1 and diclofenac preferentially inhibits COX-2[26]. The overall effect is the inhibition of the prostaglandin E_2 (PGE_2) synthesis resulting from COX-2 but not from COX-1 and therefore the continuing existence of gastroprotection furnished by PGE_2 synthesized from the latter enzyme. Ketorolac, however, inhibits both COX-2 and COX-1[27], thus decreasing the synthesis of PGE_2 from each of these enzymes and avoiding gastric protection by methyl eugenol. The integrity of the gastric mucosa depends mostly on the synthesis of PGE_2 from COX-1. Although prostaglandins from COX-2 are also involved in gastroprotection, their participation is minimal[21].

According to previous studies, nitric oxide, sulfhydryl groups and prostaglandins do not participate in the gastroprotective mechanisms of action of methyl eugenol[12]. The present study demonstrated that methyl eugenol does not act as an anti-secretory agent (Table 1). The gastric volume and gastric pH detected after treatment with methyl eugenol at the dose of 100 mg kg^{-1} differed significantly from the values found with the reference drug omeprazole, but not from those of the vehicle control group. Further research is needed on the possible mechanism of action of the gastroprotective activity of methyl eugenol, which may be related to its antioxidant capacity.

Several studies have described pharmacological interactions between natural products and drugs (e.g., NSAIDs)[28-30]. Most of the reported interactions affect the absorption or metabolic process of drugs[31]. Regarding drugs that dissolve in the stomach, any pharmacological, physiological or pathological circumstance capable of modifying gastric emptying can significantly alter absorption and, consequently, the pattern of pharmacokinetics. Since drugs must dissolve to access the bloodstream, absorption depends on their lipophilicity as well as the pH of the absorption site[32].

Methyl eugenol is a natural compound with rapid absorption (due to its lipophilic properties), enabling it to cross gastric membranes rapidly[33]. At a dose of 100 mg kg^{-1}, it does not modify gastric pH (Table 1). Moreover, the co-administration of methyl eugenol does not substantially change the degree of ionization of diclofenac or ketorolac. As a result, methyl eugenol should not alter the rate or magnitude of absorption of either of these NSAIDs.

The data presently obtained in relation to the pharmacokinetics of diclofenac agree with reports in the literature[19,34]. The co-administration of diclofenac with methyl eugenol did not affect the pharmacokinetics of the former (Fig. 2a). Hence, the bioavailability of diclofenac would not be modified by its co-administration with a single 100 mg kg^{-1} dose of methyl eugenol.

The inhibition of COX-2 by methyl eugenol[25] impedes the synthesis of PGE_2 and this in turn is involved in hypermotility and reduced blood flow in the gastric mucosa[21,24]. However, such physiological processes did not change the

bioavailability of diclofenac when used in a combined treatment with methyl eugenol. Contrarily, the co-administration of methyl eugenol with ketorolac diminished the values of Cmax (65%), AUC at time t and AUC at infinity by about 45% compared to the treatment with ketorolac alone (Fig. 2b, Table 3).

As aforementioned, ketorolac inhibits both COX-2 and COX-1 and methyl eugenol inhibits COX-2, thus eliminating the synthesis of PGE_2 by both enzymes. The gastric hypermotility reportedly caused by ketorolac is somehow associated with a prostaglandin deficiency generated by COX-1 inhibition,[21] resulting in the modification of physiological processes and a decrease in absorption. Since these alterations lead to increased gastric motility and reduced blood flow, they should impede the bioavailability of ketorolac.

Concerning the pharmacokinetic interactions between two drugs, when the metabolism of a drug is promoted or inhibited by another drug concomitantly administered, the cytochrome P450 (CYP) system of enzymes is necessarily involved[28]. Accordingly, diclofenac is known to be metabolized mainly by CYP2C9 and CYP3A4[14,19] and ketorolac by CYP2C8 and CYP2C9[35,36]. Meanwhile, CYP2E1, CYP2B7 and CYP2A2 participate in the metabolic pathway of methyl eugenol in rodents[37,38]. Therefore, methyl eugenol does not promote the metabolism of diclofenac or ketorolac, which reinforces the idea that the pharmacokinetic interaction is due solely to the absorption process.

CONCLUSION

The current results demonstrate the good gastroprotective activity by methyl eugenol against diclofenac-induced damage and moderate effectiveness against ketorolac-induced lesions. Methyl eugenol did not modify the pharmacokinetic behavior of diclofenac. Furthermore, it was found that methyl eugenol does not have anti-secretory activity, thus ruling out this route as its gastroprotection mechanism.

SIGNIFICANCE STATEMENT

In the clinical setting, the treatment of pain with NSAIDs is associated with various adverse effects, including gastric damage. The latter condition negatively affects the quality of life of patients and can lead to serious systemic complications. Overall, the present findings suggest the advantage of co-administering methyl eugenol with diclofenac to reduce gastric injury. The results are congruent with the fact that methyl eugenol by itself exerts analgesic and gastroprotective activity and that gastric damage is one of the adverse effects of NSAIDs.

ACKNOWLEDGMENT

This study was supported by PAPIIT grant IN213918 from the DGAPA-UNAM.

We thank Ana Laura Rodríguez Durán and Lourdes González Flores for their technical assistance.

REFERENCES

1. Davis, J.S., H.Y. Lee, J. Kim, S.M. Advani and H.L. Peng *et al*., 2017. Use of non-steroidal anti-inflammatory drugs in US adults: changes over time and by demographic. Open Heart, 10.1136/openhrt-2016-000550.
2. Pok, L.S.L., F.H. Shabaruddin, M. Dahlui, S. Sockalingam and M.S.M. Said *et al*., 2018. Clinical and economic implications of upper gastrointestinal adverse events in Asian rheumatological patients on long term non steroidal anti inflammatory drugs. Int. J. Rheumat. Dis., 21: 943-951.
3. Melcarne, L., P. García-Iglesias and X. Calvet, 2016. Management of NSAID-associated peptic ulcer disease. Expert. Rev. Gastroenterol. Hepatol., 10: 723-733.
4. Sanalkumar, K.B., K.T. Shenoy, K. Arun, K.M.H. Ilavarasi, P.G. Venugopalan and K.B. Leena, 2016. Pattern of use of gastroprotective agents along with the anti – inflammatory and analgesics drugs. J. Evolution. Med. Dent. Sci., 5: 50-54.
5. Sánchez-Mendoza, M.E., Y. López-Lorenzo, A.S. Matus-Meza and J. Arrieta, 2018. Gastroprotective effect of calealactone B: Lack of involvement of prostaglandins, nitric oxide and sulfhydryls. Drug Dev. Res., 79: 11-15.
6. Yeomans, N., I. Wilson and G. Län, 2001. Quality of life in chronic NSAID users: a comparison of the effect of omeprazole and misoprostol. Scand. J. Rheumatol., 30: 328-334.
7. Heidelbaugh, J.J., 2013. Proton pump inhibitors and risk of vitamin and mineral deficiency: evidence and clinical implications. Ther. Adv. Drug. Saf., 4: 125-133.
8. Shah, N.H., P. LePendu, A. Bauer-Mehren, Y.T. Ghebremariam and S.V. Iyer *et al*., 2015. Proton pump inhibitor usage and the risk of myocardial infarction in the general population. PLoS ONE, Vol. 10. 10.1371/journal.pone.0124653.
9. Xie, Y., B. Bowe, T. Li, H. Xian, S. Balasubramanian and Z. Al-Aly, 2016. Proton pump inhibitors and risk of incident CKD and progression to ESRD. J. Am. Soc. Nephrol., 27: 3153-3163.
10. Figueredo, S.M., F.P. do Nascimento, C.S. Freitas, C.H. Baggio and C. Soldi *et al*., 2011. Antinociceptive and gastroprotective actions of ethanolic extract from *Pluchea sagittalis* (Lam.) Cabrera. J. Ethnopharmacol., 135: 603-609.

11. Kupeli, E., F.P. Sahin, I. Calıs, E. Yesilada and N. Ezer, 2007. Phenolic compounds of *Sideritis ozturkii* and their *in vivo* anti-inflammatory and antinociceptive activities. J. Ethnopharmacol., 112: 356-360.
12. Sánchez-Mendoza, M.E., L. Cruz-Antonio, D. Arrieta-Baez, I.M. Olivares-Corichi, R. Rojas-Martínez, D. Martínez-Cabrera and J. Arrieta, 2015. Gastroprotective activity of methyleugenol from *Peperomia hispidula* on ethanol-induced gastric lesions in rats. Int. J. Pharmacol., 11: 697-704.
13. Yano, S., Y. Suzuki, M. Yuzurihara, Y. Kase and S. Takeda *et al*., 2006. Antinociceptive effect of methyleugenol on formalin-induced hyperalgesia in mice. Eur. J. Pharmacol., 553: 99-103.
14. León-Reyes, M.R., G. Castañeda-Hernández and M.I. Ortiz, 2009. Pharmacokinetic of diclofenac in the presence and absence of g libenclamide in the rat. J. Pharm. Pharm. Sci., 12: 280-287.
15. Sandritter, T.L., M. McLaughlin, M. Artman and J. Lowry, 2017. The interplay between pharmacokinetics and pharmacodynamics. Pediatr. Rev., 38: 195-206.
16. Choi, Y.J., N. Kim, J.Y. Lee, R.H. Nam and H. Chang *et al*., 2014. Protective effects of garlic extract, PMK-s005, against nonsteroidal anti-inflammatory drugs–induced acute gastric damage in rats. Dig. Dis. Sci., 59: 2927-2934.
17. Nagarsenker, M.S., R.N. Meshram and G. Ramprakash, 2000. Solid dispersion of hydroxypropyl β-cyclodextrin and ketorolac: Enhancement of in-vitro dissolution rates, improvement in anti-inflammatory activity and reduction in ulcerogenicity in rats. J. Pharm. Pharmacol., 52: 949-956.
18. Rodríguez-Romero, V., K.I. González-Villalva, J.L. Reyes, R.E. Franco-Bourland, G. Guízar-Sahagún, G. Castañeda-Hernández and L. Cruz-Antonio, 2015. A novel, simple and inexpensive procedure for the simultaneous determination of iopamidol and p-aminohippuric acid for renal function assessment from plasma samples in awake rats. J. Pharm. Biomed. Anal., 107: 196-203.
19. De Paz-Campos, M.A., M.I. Ortiz, A.E.C. Pina, L. Zazueta-Beltran and G. Castaneda-Hernandez, 2014. Synergistic effect of the interaction between curcumin and diclofenac on the formalin test in rats. Phytomedicine, 21: 1543-1548.
20. Rodríguez-Cal y Mayor, A., G. Castañeda-Hernández, L. Favari, A. Martinez-Cruz, G. Guízar-Sahagún and L. Cruz-Antonio, 2020. Pharmacokinetics and anti-inflammatory effect of naproxen in rats with acute and subacute spinal cord injury. Naunyn-Schmiedeberg's Arch Pharmacol., 393: 395-404.
21. Tanaka, A., H. Araki, S. Hase, Y. Komoike and K. Takeuchi, 2002. Up-regulation of COX-2 by inhibition of COX-1 in the rat: a key to NSAID-induced gastric injury. Aliment. Pharmacol. Ther., 16: 90-101.
22. Heeba, G.H., M.K.A. Hassan and R.S. Amin, 2009. Gastroprotective effect of simvastatin against indomethacin-induced gastric ulcer in rats: Role of nitric oxide and prostaglandins. Eur. J. Pharmacol., 607: 188-193.
23. Lim, J.H., J.H. Kim, N. Kim, B.H. Lee and P.J. Seo *et al*., 2014. Gastroprotective effect of *Cochinchina momordica seed* extract in nonsteroidal anti-inflammatory drug-induced acute gastric damage in a rat model. Gut. Liver., 8: 49-57.
24. Takeuchi, K., 2012. Pathogenesis of NSAID-induced gastric damage: Importance of cyclooxygenase inhibition and gastric hypermotility. World. J. Gastroenterol., 18: 2147-2160.
25. Tang, F., F. Chen, X. Ling, Y. Huang, X. Zheng, Q. Tang and X. Tan, 2015. Inhibitory effect of methyleugenol on ige-mediated allergic inflammation in RBL-2H3 cells. Mediators Inflammation, 2015: 1-9.
26. Walker, C., 2018. Are All oral COX-2 selective inhibitors the same? A consideration of celecoxib, etoricoxib and diclofenac. Int. J. Rheumatol., 10.1155/2018/1302835.
27. Sinha, V.R., R.V. Kumar and G. Singh, 2009. Ketorolac tromethamine formulations: an overview. Expert. Opin. Drug. Deliv., 6: 961-975.
28. Butterweck, V., H. Derendorf, W. Gaus, A. Nahrstedt, V. Schulz and M. Unger, 2004. Pharmacokinetic herb-drug interactions: Are preventive screenings necessary and appropriate? Planta Med., 70: 784-791.
29. Izzo, A.A., 2012. Interactions between herbs and conventional drugs: overview of the clinical data. Med. Princ. Pract., 21: 404-428.
30. Abebe, W., 2002. Herbal medication: Potential for adverse interactions with analgesic drugs. J. Clin. Pharm. Ther., 27: 391-401.
31. Alissa, E.M., 2014. Medicinal herbs and therapeutic drugs interactions. Therapeut. Drug Monit., 36: 413-422.
32. Brake, K., A. Gumireddy, A. Tiwari, H. Chauhan and D. Kumari, 2017. In vivo studies for drug development via oral delivery: challenges, animal models and techniques. Pharm. Anal. Acta., 08: 560-571.
33. Committee on Herbal Medicinal Products (HMPC), 2005. Public statement on the use of herbal medicinal products containing methyleugenol. https://www.ema.europa.eu/en/documents/scientific-guideline/public-statement-use-herbal-medicinal- products-containing-methyleugenol_en.pdf
34. Zhong, Z.Y., B.B. Sun, N. Shu, Q.S. Xie and X.G. Tang *et al*., 2016. Ciprofloxacin blocked enterohepatic circulation of diclofenac and alleviated NSAID-induced enteropathy in rats partly by inhibiting intestinal β-glucuronidase activity. Acta. Pharmacol. Sin., 37: 1002-1012.

35. Välitalo, P.A., H. Kemppainen, A. Kulo, A. Smits, K.V. Calsteren *et al*., 2017. Body weight, gender and pregnancy affect enantiomer-specific ketorolac pharmacokinetics. Br. J. Clin. Pharmacol., 83: 1966-1975.
36. Kulo, A., K. van Calsteren., R. Verbesselt, A. Smits and R. Devlieger, *et al*., 2012. The impact of caesarean delivery on paracetamol and ketorolac pharmacokinetics: a paired analysis. J. Biomed. Biotechnol., 10.1155/2012/437639.
37. Gardner, I., H. Wakazono, P. Bergin, I. De Waziers, P. Beaune, J.G. Kenna and J. Caldwell, 1997. Cytochrome P450 mediated bioactivation of methyleugenol to 1- hydroxymethyleugenol in fischer 344 rat and human liver microsomes, Carcinogenesis, 18: 1775-1783.
38. Robison, S.H. and D.B. Barr, 2006. Use of biomonitoring data to evaluate methyl eugenol exposure. Environ. Health. Perspect., 114: 1797-1801.