ORIGINAL ARTICLE

Antinociception and less gastric injury with the dexketoprofen-tapentadol combination in mice

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ABSTRACT

The purpose of this study was to evaluate the antinociceptive interaction between dexketoprofen and tapentadol in three different dose ratios, as well as the ulcerogenic activity of this combination. Dose–response curves were carried out for dexketoprofen, tapentadol, and dexketoprofen–tapentadol combinations in the acetic acid-induced writhing test in mice. On the other hand, the gastric damage of all treatments was assessed after the surgical extraction of the stomachs. Intraperitoneal administration of dexketoprofen and tapentadol induced a dose-dependent antinociceptive effect, reaching a maximal effect of about 58% and 99%, respectively. Isobolographic analysis and the interaction index showed that the three proportions produced an analgesic potentiation (synergistic interaction). Interestingly, the 1:1 and 1:3 ratios of the drugs combination produced minor gastric injury in comparison with the 3:1 proportion. Our data suggest that all proportions of the dexketoprofen–tapentadol combination produced a synergistic interaction in the acetic acid-induced visceral pain model in mice with a low incidence of gastric injury.

INTRODUCTION

Visceral pain is one of the most frequent reasons why patients seek medical attention [1]. The visceral pain may result from direct inflammation of a visceral organ (pancreatitis and appendicitis), occlusion of bile or urine flow (kidney stones), or from functional visceral disorders (irritable bowel syndrome) [2], among others. Among all types of stimuli, the mechanical ones can cause luminal distension, being harmful, and causing visceral pain in the bowel [3]. Drugs for the treatment of visceral pain have limited clinical effectiveness [1,4]. For this reason, it is necessary to carry out preclinical research to develop new therapeutic options to relieve the visceral pain states in the clinical setting [5].

Tapentadol is an analgesic with a dual mechanism of action that combines μ-opioid receptor activation and norepinephrine reuptake inhibition [6,7]. On the

other hand, dexketoprofen (S(+)-ketoprofen) is a non-steroidal anti-inflammatory drug (NSAID) with analgesic, anti-inflammatory, and antipyretic effects at peripheral and central sites. This drug produces its effects by inhibition of prostaglandin synthesis [8,9]. Hence, a combination of dexketoprofen, an NSAID, and a strong opioid, such as tapentadol (with a double mechanism of action at the spinal cord), is expected to induce a synergistic interaction [10,11]. However, so far there is no study about the possible synergistic interaction between these drugs in models of visceral pain.

Co-administration of NSAIDs—such as ketorolac, diclofenac, and acetaminophen—and tapentadol has previously been shown to produce synergistic analgesic effects and a better safety profile of the drugs combination when compared with each individual drug in some pain mouse models [12–15]. Thus, the purpose of this study was to evaluate the possible synergistic interaction between dexketoprofen and tapentadol in the writhing test in mice. We also evaluated the effects of treatments on gastric damage in mice.

MATERIALS AND METHODS

Animals
Experiments were carried out in adult male inbred balb/c mice (weighing 25–35 g) obtained from the Vivarium of the Centro Universitario de Ciencias de la Salud of the University of Guadalajara. Mice were housed in acrylic cages with metal caps in groups of 6 animals, with free access to water and food (LabDiet, St. Louis, MO). Animals were kept in a temperature-regulated room under a light-dark cycle of 12:12 h.

All experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals, Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals [16], and Norma Oficial Mexicana (NOM 062-ZOO-1999). The Ethics Committee of the Centro de Investigación y de Estudios Avanzados (Cinvestav) approved all the experimental procedures (Protocol 042/13). Efforts were made to minimize the number of animals used.

Drugs
Dexketoprofen tromethamine (Cat. 1179118) and acetic acid (Cat. 71251) were purchased from Sigma-Aldrich (St. Louis, MO). Tapentadol tablets of 10 mg were obtained from Grünenthal Laboratories (Mexico).

For both drugs, stock solutions were prepared. All drugs were dissolved in saline.

Nociception test
The acetic acid-induced visceral pain test was carried out as previously reported [12]. Mice were placed in individual acrylic observation chambers for a 60-min acclimatization period. Then, animals were intraperitoneally (ip) injected with 10 mL/kg of a 1% acetic acid solution in order to induce the characteristic writhing (a contraction of the abdominal muscle together with a stretching of the hind limbs). The animals were immediately returned to the observation chambers, and the number of writhes in a 5 min period was counted during the next 30 min. The reduction of the number of writhes in the 30-min period was considered as antinociception.

Experimental design
Dexketoprofen (56.2, 100, 177.8, and 316 mg/kg, ip) or tapentadol (3.16, 5.62, 10, and 17.78 mg/kg, ip) were administered 15 min before the painful stimulus (acetic acid injection). The antinociceptive effect percentage was obtained as follows: [(vehicle-post compound)/vehicle] X 100. The ED_{50} of each drug was calculated using their dose-response curves by linear regression. The ED_{50} of both drugs was employed in combinations in three different proportions. Each combination was evaluated in 4 subsequent doses. Calculation of the total dose in each combination was as follows: for the proportion 0.5:0.5 (1:1), total dose $1 = ED_{50Dexketoprofen}/1 + ED_{50Tapentadol}/1$; total dose $2 = ED_{50Dexketoprofen}/2 + ED_{50Tapentadol}/2$; total dose $3 = ED_{50Dexketoprofen}/4 + ED_{50Tapentadol}/4$ and total dose $4 = ED_{50Dexketopreofen}/8 + ED_{50Tapentadol}/8$. The same equation was used for the 0.75:0.25 (3:1) and 0.25:0.75 (1:3) proportions. These doses were tested in independent groups for the combination dose-response relationship. Euthanasia was performed with an overdose of pentobarbital at the end of each experiment.

Gastric injury
Male balb/c mice were used ($n = 6$ per group). Animals had free access to water but without food for 18 h prior to the experiments. Then, the greatest doses of each individual drug and the highest doses of each proportion of the combinations were evaluated to determine the possible gastric damage. The experimental groups were as follows: Group 1 = vehicle (saline), Group 2 = ethanol, Group 3 = dexketoprofen 316 mg/

Figure 1 Time courses of the antinociceptive activity of dexketoprofen (a), tapentadol (b), and the different proportions of dexketoprofen (DXK) and tapentadol (TAP), 0.5:0.5 (c), 0.75:0.25 (d), and 0.25:0.75 (e), in the mice writhing test. Data are mean ± SEM of at least six animals.
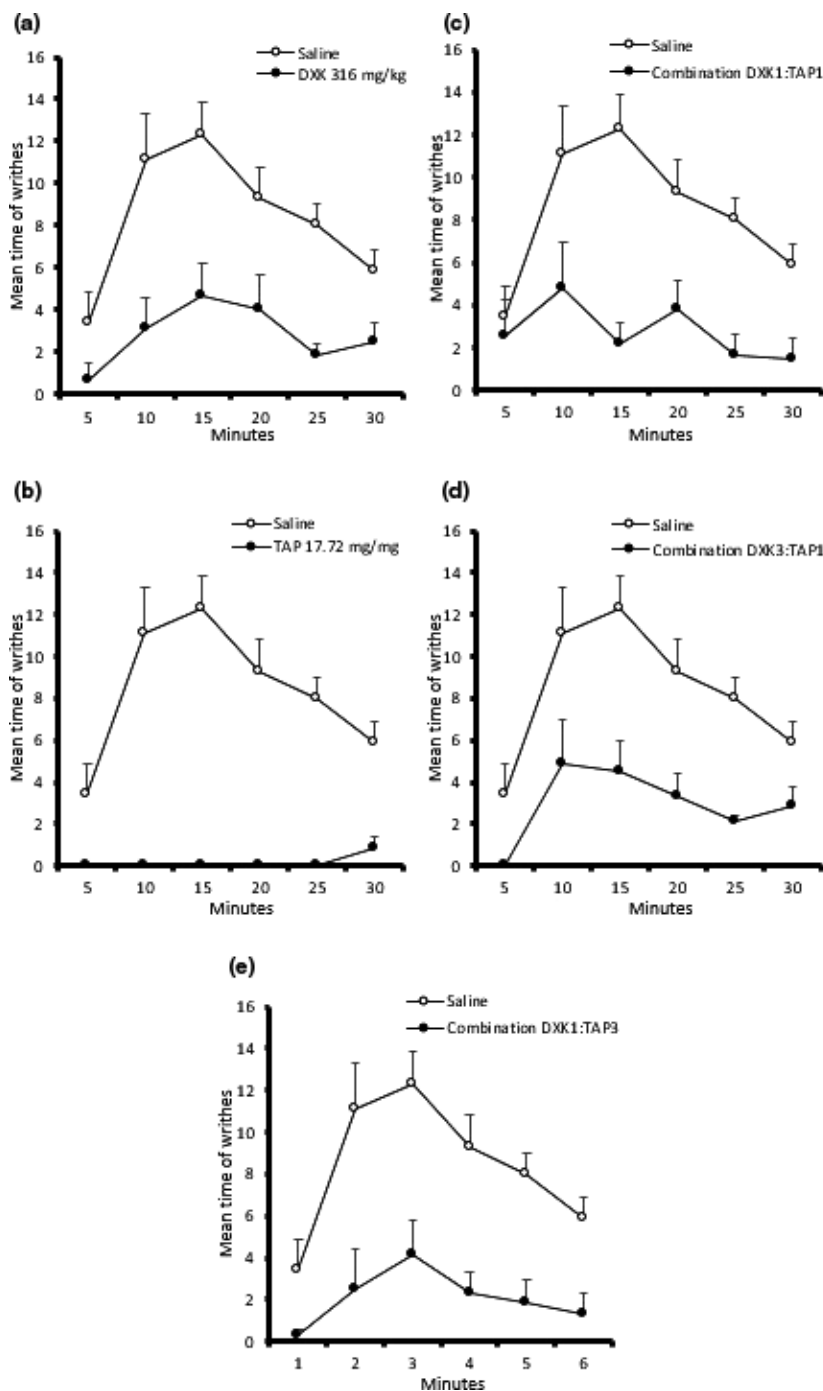
kg, Group 4 = tapentadol 17.78 mg/kg, Group 5 = dexketoprofen 169.1 mg/kg and tapentadol 2.2 mg/kg (1:1 ratio of the combination), Group 6 = dexketoprofen 84.5 mg/kg and tapentadol 3.3 mg/kg (1:3 ratio of the drug mixture), and Group 7 = dexketoprofen 253.6 mg/kg and tapentadol 1.1 mg/kg (3:1 ratio of the drugs combination). Animals were treated during 6 h and then sacrificed to surgically remove the stomachs. These were placed in 50 mM phosphate buffer (pH 7.4) at 48 °C for 5 min, inflated using 1 mL of 2% formalin, and immersed in 2% formalin to fix both inner and outer layers.
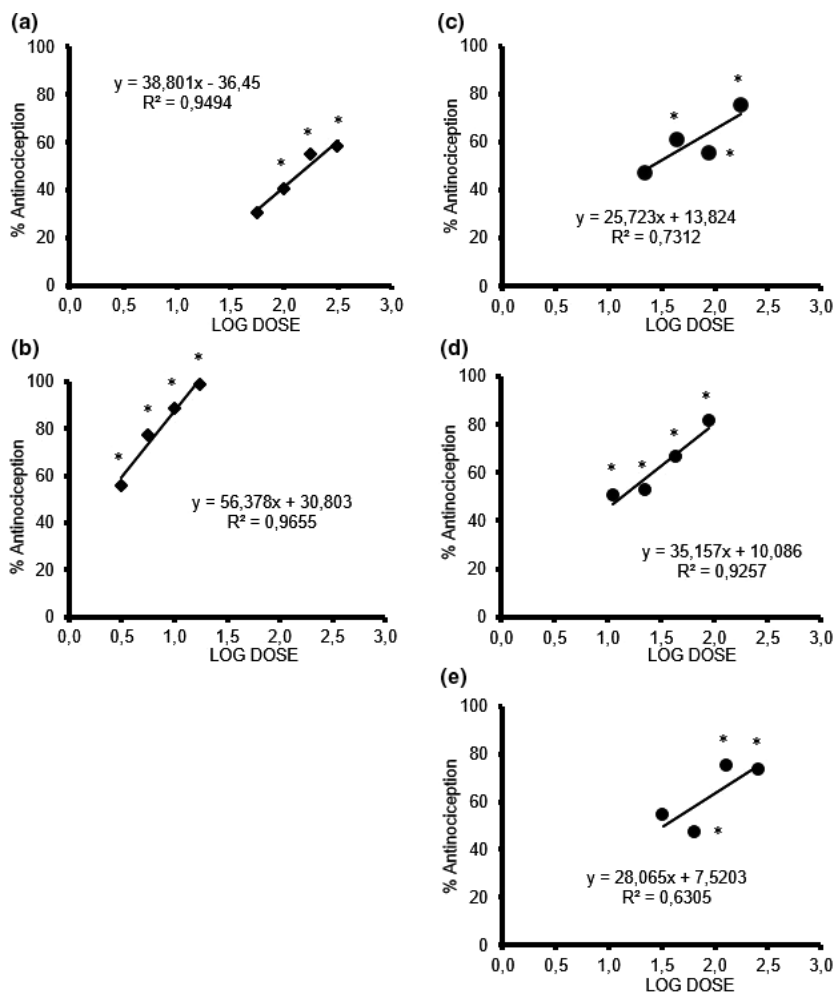
Figure 2 Dose–response curve for dexketoprofen (a), tapentadol (b), and the combination of dexketoprofen (DXK) and tapentadol (TAP) at different proportions, 0.5:0.5 (c), 0.75:0.25 (d), and 0.25:0.75 (e). Data are means ± SEM of at least six animals. *Statistical difference when compared to control group, by one-way ANOVA followed by the Tukey test ($P < 0.05$).

Stomachs were opened lengthwise through the greater curvature and sandwiched between two microscope slides.

Analysis of the data

The % of antinociception was calculated using the 30-min full period. Isobolographic analysis and interaction index were employed to assess the kind of interaction, both using the Tallarida methods [17–20]. The isobolographic analysis considers that the combination of drugs uses equieffective doses of individual drugs. Using the dose–response curve of each drug, the effective dose 50 (ED_{50}) of each therapeutic agent was calculated. For this, we used a log-linear regression method to determine ED_{50} from the experimental dose-response curves. This method considers that the antinociceptive effect ranges from 0 to 100%. Then, we determined the ED_{50} the dexketoprofen-tapentadol combination

(experimental ED_{50}) using the equation described above [17–20]. Theoretical versus experimental ED_{50} values were compared using Student's *t*-test.

To analyze the data on gastric ulceration, the stomachs were scanned and the gastric damage area (mm^2) was quantified using the software Scion Image for Windows, Release Beta 4.0.2. [21]. The ulceration area was analyzed by one-way analysis of variance (ANOVA) followed by the Tukey test. A $P < 0.05$ was considered significant.

RESULTS

Antinociceptive effect

Acetic acid-induced nociception (measured as the mean number of writhes) is shown in *Figure 1*. The individual administration of dexketoprofen (316 mg/kg) and tapentadol (17.7 mg/kg), as well as the combinations

of dexketoprofen and tapentadol in proportions 1:1, 1:3, and 3:1 reduced acetic acid-induced writhing (*Figure 1*). All treatments reached the maximal effect in about 15 min and then declined in about 30 min. Both dexketoprofen and tapentadol produced dose-dependent antinociceptive effects in the acetic acid-induced writhing test (*Figure 2a* and *b*). The maximal antinociceptive effect of dexketoprofen was about 58% while that of tapentadol was about 99% (*Figure 2a* and *b*). Furthermore, dexketoprofen–tapentadol combinations at 0.5:0.5 (1:1), 0.25:0.75 (1:3) and 0.75:0.25 (3:1) proportions also induced a dose-dependent antinociceptive effect, reaching a maximal effect of about 75%, 80%, and 75%, respectively (*Figure 2c–e*).

Isobolographic analysis and interaction index

All proportions of the dexketoprofen–tapentadol combination produced similar effects of antinociceptive synergism according to the results obtained with the isobolographic analysis (*Figure 3a–c*). In the same manner, the interaction index of all ratios of the combination supported the antinociceptive synergistic interactions shown by the isobolographic analysis (*Table 1*).

Gastric injury

Ethanol, but not saline, produced several ulcerative lesions to the stomach of mice. Dexketoprofen and tapentadol induced some damage to the stomach, but much less than ethanol. The 0.5:0.5 (1:1) and 0.25:0.75 (1:3) dexketoprofen–tapentadol proportions of the drugs combination produced minor damage than 0.75:0.25 (3:1) proportion (*Figure 4*).

DISCUSSION

The results of our experimental assay showed that dexketoprofen induces a dose-dependent antinociceptive activity in the acetic acid-induced abdominal contortions test in mice. Dexketoprofen produced similar antinociceptive activity to that previously reported in the writhing test in mice [22,23], as well as in other animal pain models [24–26]. The antinociceptive potency of the individual drugs was performed by comparing the ED_{50} of our study with that of previous reports. Thus,
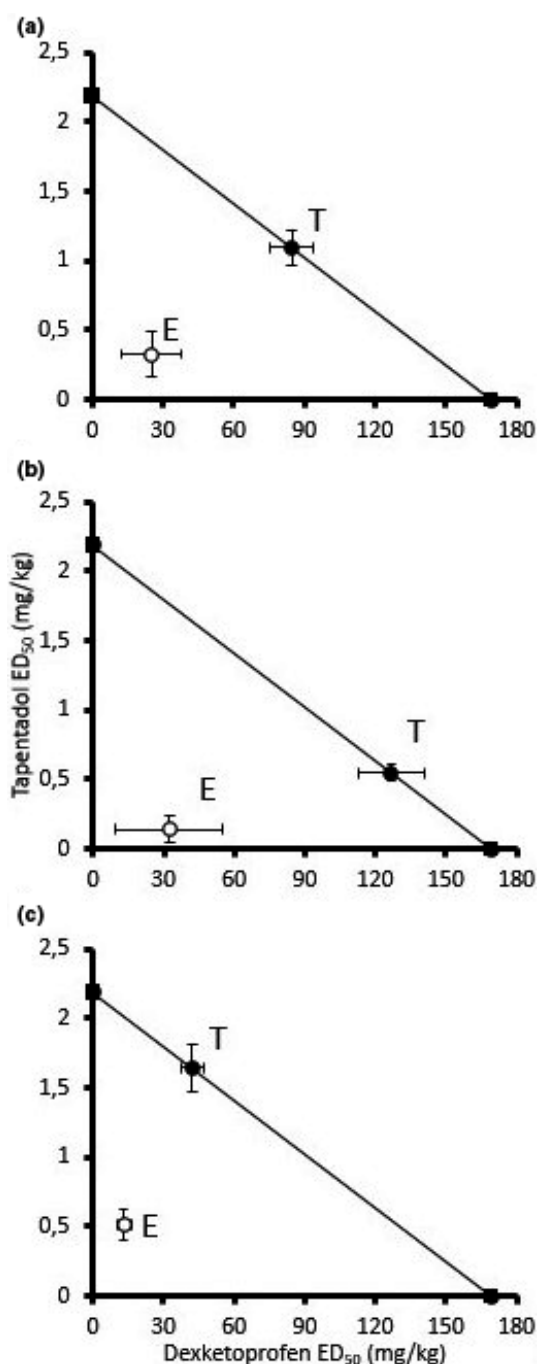

Figure 3 Isobolographic analysis showing the kind of interaction between dexketoprofen (DXK) and tapentadol (TAP) at different proportions, 0.5:0.5 (a), 0.75:0.25 (b), and 0.25:0.75 (c), in the acetic acid-induced visceral pain test in mice. Data are means ± SEM for at least six animals.

Table 1 Comparison of the theoretical and experimental values of the three proportions of the drugs combination and interaction index

|	Combinations		
	1:1	DXK3:TAP1	DXK1:TAP3
Theoretical ED_{50} values (mg/kg)	85.63 ± 9.46	43.91 ± 4.74	127.35 ± 14.18
Experimental ED_{50} values (mg/kg)	25.49 ± 0.22*	13.66 ± 0.10*	32.63 ± 0.30*
Interaction Index	0.29 ± 0.15	0.31 ± 0.07	0.25 ± 0.18

Values are means $\pm$ SEM.

DXK, dexketoprofen; TAP, tapentadol.

*$P < 0.05$ indicates statistical difference between theoretical vs. experimental ED_{50} values.

Figure 4 Representative images of stomachs of mice used for the evaluation of gastric injury. Groups included saline, ethanol, dexketoprofen, tapentadol and the 0.5:0.5 (1:1), 0.75:0.25 (3:1), and 0.25:0.75 (1:3) proportions of the dexketoprofen–tapentadol combination in the mouse model of the visceral pain.
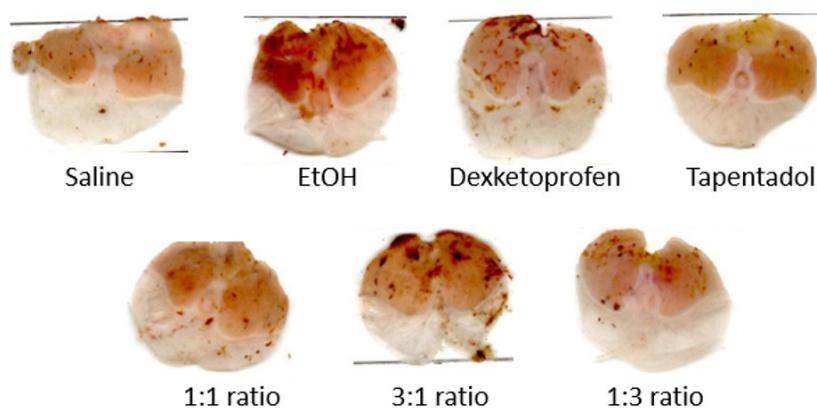
dexketoprofen ($ED_{50} = 168.07$ mg/kg) had a lower antinociceptive potency than other NSAIDs in the abdominal writhing model in mice, such as diclofenac ($ED_{50} = 7.2$ mg/kg) [27], ketoprofen ($ED_{50} = 30.3$ mg/kg) [27], metamizole ($ED_{50} = 28.5$ mg/kg) [27], meloxicam ($ED_{50} = 6.5$ mg/kg) [27], piroxicam ($ED_{50} = 8.5$ mg/kg) [27], and ketorolac ($ED_{50} = 8$ mg/kg) [13] when administered by the intraperitoneal route in acetic acid-induced contortions model. Likewise, tapentadol induced a dose-dependent antinociceptive action, which is similar to those reported previously in the writhing test [12,13]. Moreover, tapentadol showed better antinociceptive action in the acetic acid-induced abdominal stretch assay compared with its effects in the orofacial and hind paw formalin test [12–15]. Regarding to the antinociceptive potency of tapentadol ($ED_{50} = 2.19$ mg/kg), it showed minor antinociceptive potency than morphine ($ED_{50} = 0.12$ mg/kg) [22], methadone ($ED_{50} = 0.08$ mg/kg) [28], fentanyl ($ED_{50} = 0.02$ mg/kg) [28,29], and superior than tramadol ($ED50 = 3.9$ mg/kg) [28] in the acetic acid-induced writhing test.

The dexketoprofen–tapentadol combination produced lower antinociceptive activity (Emax = 75.25%), when compared to the tapentadol–ketorolac (Emax = 82%) and tapentadol–diclofenac (Emax = 97%) combinations. Furthermore, the ED_{50} of the dexketoprofen–tapentadol ratios were higher than for tapentadol–diclofenac and tapentadol ketorolac mixtures [12,13].

In this study, the intraperitoneal administration of the individual drugs and the different proportions of the drugs combination 30 min before the acetic acid could have produced a local effect. Dexketoprofen has shown a peripheral analgesic effect when used in the formalin pain model in mice [30]. Tramadol, an opioid analgesic with similar mechanisms of action to tapentadol, has shown to produce local peripheral analgesic action in the hind paw formalin and thermal plantar tests [31,32]. Local peripheral and/or regional activity of opioid analgesics has also been reported [33,34]. However, this could not be ruled out until the corresponding studies have been carried out.

The antinociceptive activity of this drug combination could be explained by the mechanisms of action reported for each individual drug. Like other NSAIDs, dexketoprofen (S(+)-ketoprofen) inhibits in a

stereoselectivity manner cyclooxygenase (COX) *in vitro* and *in vivo* [35,36]. Moreover, some authors have reported the antinociceptive activity of this drug in rodents involves the participation of nitric oxide and serotonergic receptors [25]. On the other hand, tapentadol produces antinociception by binding to μ-opioid receptors and through the norepinephrine reuptake blockade. Thus, activation of μ-opioid receptors, as well as α_2 adrenergic receptors at several levels (peripheral, spinal, and supraspinal), could lead to the antinociceptive effects of this drug [6,7,10,11,37,38].

We found that dexketoprofen alone (316 kg/kg) produced a stomach injury lower than that induced by the positive control (ethanol). This result disagrees with that previously reported in which a lower dose of dexketoprofen (3–25 mg/kg) was used [39]. However, as expected, this drug-induced greater damage than tapentadol and saline. Regarding the combinations, we found that the 0.5:0.5 and 0.25:0.75 proportions induced a small gastric injury when compared with the 0.75:0.25 dexketoprofen–tapentadol combination. This could be due to the amount of dexketoprofen in the combination (dexketoprofen 253.6 mg/kg [0.75:0.25] vs. 169.1 [0.5:0.5] and 84.5 mg/kg [0.25:0.75]). To our knowledge, this is the first report about the gastric injury of the dexketoprofen–tapentadol combination. According to the results of this study and a previous report also conducted by our group, tapentadol produces damage to the gastric mucosa [12].

In conclusion, our data suggest that all proportions of the dexketoprofen–tapentadol combination produce an antinociceptive synergistic interaction in the acetic acid-induced visceral pain model in mice. Moreover, the 0.5:0.5 (1:1) and 0.25:0.75 (1:3) dexketoprofen–tapentadol proportions of the drugs combination produced minor damage than 0.75:0.25 (3:1) proportion and each individual drug. Thus, this combination could be useful to treat visceral pain in humans with good efficacy and an improved side effect profile.

CONFLICTS OF INTEREST

Authors do not have conflict of interest.

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