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ANTI-INFLAMMATORY, ANALGESIC, AND GASTRIC TISSUE EFFECTS OF LACIDIPINE, ASPIRIN, AND THEIR COMBINATION

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Abstract: The increase in cyclooxygenase-2 (COX-2) activity and the decrease in COX-1 activity were associated with increased intracellular calcium. Lacidipine, a calcium channel blocker, can protect gastric tissue from aspirin-induced damage while potentiating the analgesic and anti-inflammatory properties of aspirin. The aim of this study was to investigate the anti-inflammatory, analgesic, and gastric tissue effects of using lacidipine and aspirin in combination and separately. (LA). Twenty-four rats were randomly divided into CG (carrageenan control), LCG (carrageenan + lacidipine), ACG (carrageenan + aspirin), and LACG (carrageenan + LA) groups. Lacidipine 4 mg/kg, aspirin 100 mg/kg were given orally to the animals on an empty stomach. Carrageenan was injected into the hind paws of rats to induce inflammation and pain. Paw volumes were measured at 0, 1, and 4 hours after carrageenan administration, and paw pain thresholds were measured at 1 and 4 hours. After euthanasia (thiopental sodium, 50 mg/kg), paw and gastric tissues were excised and biochemically analyzed. Gastric tissues were also examined macroscopically. In the paw tissues of animals receiving lacidipine, malondialdehyde (MDA) was lower than in the CG and AG, while total glutathione (tGSH) was higher ($p<0.05$). Lacidipine inhibited COX-2, but not COX-1 isoenzyme. LA—lacidipine, and aspirin—inhibited inflammation and hyperalgesia at 1 and 4 hours, respectively. Lacidipine prevented aspirin-induced COX-1 inhibition and gastric ulcer formation. Lacidipine combined with aspirin may be beneficial for initiating its anti-inflammatory activity early, achieving a potent analgesic effect, and preventing aspirin-induced gastric injury.

Keywords: lacidipine, aspirin, inflammation, paw edema, analgesic, gastric damage

Abbreviations: CG: carrageenan control, LCG: carrageenan + lacidipine, ACG: carrageenan + aspirin, LACG: carrageenan + lacidipine + aspirin, MDA: malondialdehyde, tGSH: total glutathione, COX: cyclooxygenase

Lacidipine, like other dihydropyridine derivatives, is a voltage-dependent L-type calcium (Ca^{2+}) channel blocker drug (1). Dihydropyridine derivatives are more vasoselective than phenylethylamine and benzothiazepine derivative calcium channel blockers (2). Lacidipine exhibits stronger vasoselectivity than many dihydropyridine derivatives (1). Lacidipine is also known to have an antioxidant effect, which is attributed to inhibiting lipid peroxidation (LPO) reaction and glutathione (GSH) decrease in organs and tissues (3). It has been reported that lacidipine has a gastroprotective effect by suppressing the indomethacin-induced an increase in malondialdehyde (MDA), the toxic product of LPO, and a decrease in GSH and superoxide dismutase (SOD) in gastric tissue

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(4). Lacidipine has also been shown to inhibit the acute and chronic phases of carrageenan-induced inflammation (5). Lacidipine protects cardiomyocytes against the proinflammatory action of tumor necrosis factor-alpha (TNF-α) (6). It has been reported that oxidative stress and inflammation are closely related to the increase in intracellular Ca^{2+} (7). Thus, inhibition of calcium entry into the cells can explain the protective action of lacidipine on organs and tissues.

Aspirin (acetylsalicylic acid) is one of the most widely used drugs in the world as an analgesic, anti-inflammatory, antipyretic, and antiplatelet agent (8). It has been proven that the mechanism of anti-inflammatory, antipyretic, and analgesic action of aspirin is due to the inactivation of the cyclooxygenase (COX) enzyme, which participates in the synthesis of prostaglandins (PGs) responsible for inflammation, such as other nonsteroidal anti-inflammatory drugs (9). On the other hand, PGs help maintain the homeostasis of different organs and tissues and form a warning system against normal and pathological processes that cause inflammatory symptoms and pain (10). COX is known to have two distinct isoforms—COX-1 and COX-2. COX-1 is an enzyme constitutively synthesized in many tissues responsible for gastrointestinal system (GIS) protection, platelet aggregation, and maintenance of vascular homeostasis. COX-2, on the other hand, is an inducible enzyme responsible for inflammatory events; it is structurally synthesized in a few tissues, such as the brain and kidneys, in the absence of inflammation (9, 11). COX-1 enzyme inhibition is responsible for the side effects of aspirin and other NSAIDs, and COX-2 enzyme inhibition for its therapeutic effects (9). However, COX-2 is also known as cardioprotective. It has been shown experimentally shown that COX-2 inhibition causes increased thrombosis, decreased bleeding time, decreased kidney function, and increased blood pressure (11). On the other hand, there is evidence that aspirin increases gastric MDA production, decreases GSH levels, which is an endogenous antioxidant parameter, as well as COX-1 levels, and causes gastric damage (12, 13).

It has been shown in the literature that an increase in COX-2 activity (14) and a decrease in COX-1 activity are directly proportional to the increase in intracellular Ca^{2+} amount (15). This information obtained from the literature suggests that lacidipine may be effective in protecting gastric tissue from aspirin damage and may potentiate the analgesic and anti-inflammatory activity of aspirin. Therefore, this study aimed to investigate the anti-inflammatory and analgesic effects of using lacidipine and aspirin together (LA) and separately in rats in carrageenan-induced inflammation and to investigate how gastric tissue is affected by these treatments.

MATERIALS AND METHODS

Animals

For the experiment, 24 albino Wistar-type male rats, 6 months old, weighing between 280-290 grams, were purchased from the Erzincan Binali Yildirim University Experimental Animals Application and Research Center. To ensure adaptation to the environment, the animals were housed and fed in groups for one week in a controlled environment that had a suitable temperature (22°C) and light exchange (12 hours of darkness and 12 hours of light). All experiments were performed after approval from the Local Ethics Committee for Animal Experiments (meeting date: 02/23/2023, decision no. 05).

Drugs

The thiopental sodium used in the experiment was procured from IE Ulagay in Turkey, the carrageenan from Sigma (Germany), the lacidipine (4 mg tablet) from GlaxoSmithKline Drugs in Turkey, and aspirin (100 mg tablet) from Abdi Ibrahim Pharmaceutical Industry in Turkey.

Experimental Groups

The rats were randomly divided into 4 groups treated as follows: carrageenan administered control (CG), carrageenan + lacidipine (LCG), carrageenan + aspirin (ACG), and carrageenan + LA (LACG).

Experimental Procedure

Animal weights were determined for drug administrations and the amount was calculated for each animal separately. To conduct this experiment, lacidipine (4 mg/kg, 1 mg/1cc solution) (16), aspirin (100 mg/kg, 2 mg/1cc solution) (17), and lacidipine (4 mg/kg) + aspirin (100 mg/kg) were administered once orally to animals in the LCG (n = 6), ACG (n = 6), and LACG (n = 6) groups after 12 hours of fasting. A similar volume of 0.9% NaCl was given orally to the CG group (n = 6). For oral treatments, the drug drawn into the syringe was applied to the stomachs of the animals with blunt, curved gavage tips made of steel material. Inflammation was induced by injecting 0.1 mL of 1% carrageenan into the plantar surface of the hind paws of all rats one hour after drug application (17). The anti-inflammatory activity was evaluated by measuring the

animal paw volumes up to the knee joint with a plethysmometer (Cat. No. 37140, Ugo Basile, ITALY) before the carrageenan injection and at the first and fourth hours following the induction of inflammation after the carrageenan injection.

Anti-inflammatory activity was calculated according to the formula: Anti-inflammatory activity = 100 - (volume difference before and after carrageenan administration for the CG group X100/ volume difference before and after carrageenan administration for the treated group) (18). In addition to measuring paw volume, Basile Algesimeter (Cat no: Cat. No. 37215, Ugo Basile, ITALY) was also used to measure the hind paw pain thresholds of the animals at the first and fourth hours (19). To determine paw pain thresholds, the threshold stimulus at which paw retraction occurred was measured using a weight (maximum limit of 300 g) applied to the pads of the hind paws of the rats (20).

Analgesic activity was calculated according to the formula: Analgesic activity = 100 - (paw pain threshold measurement value of the CG group X100/paw pain threshold value of the treated group) (21). Immediately after completing the measurements, all animals were euthanized with high-dose thiopental sodium (50 mg/kg) anesthesia. After the animals were euthanized, all paw and stomach tissues of all animals were removed and biochemically analyzed. Before investigating the gastric tissue biochemically, macroscopic examinations were performed. In the macroscopic examination, the stomach tissues were evaluated for ulcerative areas (black bleeding areas) and erosion. Millimetric paper was used to calculate the ulcerated areas in the mucosa.

Biochemical Analyses

The excised tissues were weighed and placed in Petri dishes after washing with physiological saline. Liquid nitrogen was used to grind all tissues into powder. The tissue samples were homogenized to determine MDA and reduced GSH levels.

Determination of tissue MDA and tGSH

MDA (μmol/g protein) and tGSH (nmol/g protein) levels in paw and gastric tissues were analyzed separately for each animal. MDA and tGSH concentrations were determined using enzyme-linked immunosorbent assays (ELISAs) according to the manufacturer's manual. MDA was analyzed using the analysis kit with product number 10009055, and tGSH was analyzed using the analysis kit with product number 703002 (Cayman Chemical Company, Ann Arbor, MI, USA).

Measurement of COX activity

The paw and gastric tissues' COX activity was measured using a COX activity assay kit (Item No. 760151, Cayman, Ann Arbor, MI, USA). Until analysis, the tissues were washed thoroughly with Tris buffer, pH 7.4, containing 0.16 mg/mL heparin and stored at -80°C. The tissues were homogenized at 5 mL per gram in a cold buffer (0.1 M Tris-HCl, pH 7.8, containing 1 mM EDTA) and centrifuged at 10000 xg for 15 minutes at 4°C. In the next step, the protein concentration in the supernatant was measured with the Bradford method (22). Peroxidase activity of COX was measured using the COX activity analysis kit (Item No. 760151, Cayman, Ann Arbor, MI, USA). This activity was determined colorimetrically by monitoring the appearance of oxidized N, N, N', N'-tetramethyl-p-phenylenediamine at 590 nm. A COX-1-specific inhibitor was used to determine COX-2 activity (23). The results for COX-1 and COX-2 activity are presented in units per milligram of protein. COX activity in tissue is expressed as nmol/min/mg protein (U/mg protein) (24).

Statistical Analysis

Statistical analysis was performed using IBM SPSS 22 (IBM Corp. Released 2013, IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The results are presented as the mean ± standard error of the mean (SEM). The normality assumption was confirmed with the Shapiro-Wilk test. The groups were compared using one-way ANOVA because the data were normally distributed. Based on the homogeneity of variances, LSD or Games-Howell were used as post hoc tests after performing ANOVA. The statistical level of significance for all tests was considered to be 0.05.

RESULTS

Carrageenan-Induced Paw Inflammation Measurement

As seen in Table 1, carrageenan injection induced inflammatory edema in animal paw tissue. Lacidipine significantly reduced carrageenan-induced inflammation at the first ($p = 0.036$) and fourth ($p < 0.001$) hours, whereas aspirin reduced it insignificantly ($p = 0.719$) at the first hour and significantly ($p < 0.001$) at the fourth hour. LA suppressed carrageenan-induced inflammation significantly ($p = 0.024$) in the first hour and more significantly ($p < 0.001$) in the fourth hour. As can be observed in Table 1, the anti-inflammatory effect of lacidipine was 62% in the first hour and 54.4% in the fourth hour. The anti-inflammatory effect of aspirin was

Table 1. Changes in paw volumes, anti-inflammatory activity, mechanical paw pain threshold, and analgesic activity in the experimental groups at 1 and 4 hours after carrageenan injection into the paw pad.

		CG	LCG	ACG	LACG	F	p values
Paw volume (mL)	Before the carrageenan	0.89 ± 0.01	0.91 ± 0.02	0.88 ± 0.02	0.93 ± 0.02		
	1st hour	0.99 ± 0.03	0.95 ± 0.01	0.97 ± 0.01	0.96 ± 0.01		
	4th hour	1.57 ± 0.02	1.22 ± 0.01	1.24 ± 0.02	0.99 ± 0.02		
Difference between inflamed and baseline paw volumes (mL)	1st hour	0.100 ± 0.02	0.038 ± 0.01*	0.090 ± 0.02**	0.033 ± 0.02*	3.166	0.047
	4th hour	0.680 ± 0.01	0.310 ± 0.03*	0.360 ± 0.01*	0.060 ± 0.03*,***	132.884	<0.001
Anti-inflammatory activity (%)	1st hour	-	62.0	10.0	67.0		
	4th hour	-	54.4	47.1	91.2		
Paw pain threshold (gram)	1st hour	11.00 ± 0.58	14.83 ± 1.48*	21.50 ± 1.23*	52.33 ± 1.36*,***	359.896	<0.001
	4th hour	7.83 ± 0.60	13.50 ± 0.76*	16.33 ± 1.41*	72.83 ± 1.66*,***	648.099	<0.001
Analgesic activity (%)	1st hour	-	25.7	48.8	79.0		
	4th hour	-	42.2	52.1	89.3		

*p < 0.05 vs. CG, **p > 0.05 vs. CG, ***p < 0.05 vs. LG and AG. One-way ANOVA, LSD or Games Howell test. Statistical significance level was accepted as 0.05.

calculated to be 10% in the first hour and 47.1% in the fourth hour. Whereas, LA had an anti-inflammatory effect of 67% in the first hour, its anti-inflammatory effect increased to 91.2% in the fourth hour.

Mechanical Pain Threshold Measurement

As seen in Table 1, mechanical paw pain thresholds of LCG, ACG, and LACG were higher than CG at the first and fourth hours ($p < 0.05$). The mechanical pain threshold measurement values at the first and fourth hours in the LACG group were higher than those of LCG and ACG ($p < 0.001$). The LG and AG groups were similar at the fourth hour ($p = 0.354$). According to these data, lacidipine produced 25.7% analgesia in the first hour and 42.2% in the fourth hour. The analgesic effect of aspirin was 48.8% in the first hour, it increased to 52.1% in the fourth hour. Whereas, the analgesic effect of LA was 79.0% in the first hour, it increased to 89.3% in the fourth hour.

Biochemical Findings

MDA and tGSH analysis in the paw tissue

When the MDA and tGSH data in the paw tissue were evaluated, at least one group was found to be statistically different (F/p = 43.353/<0.001; 51.915/<0.001, respectively). As indicated in Figure 1A, there was no difference between the MDA values of CG and ACG ($p = 0.999$). MDA data of LACG were found to be similar ($p = 0.889$) to LCG, but lower than ACG and CG ($p < 0.001$). CG and ACG were similar ($p = 0.732$) in terms of tGSH levels. LACG's tGSH levels were similar ($p = 0.985$) to LCG, but higher than CG and ACG (Figure 1B, $p < 0.001$).

COX-1 and COX-2 analysis in the paw tissue

When the COX-1 and COX-2 data in the paw tissue were evaluated, at least one group was found to be statistically different (F/p = 297.777/<0.001; 1099.250/<0.001, respectively). As seen in Figure 1C, COX-1 activity in the hind paw tissue of LCG and LACG was significantly ($p < 0.001$) higher than that of the carrageenan and aspirin group. In addition, it was observed that COX-1 activity in the aspirin group was lower than in the carrageenan group ($p < 0.001$). Whereas, lacidipine and aspirin suppressed the increase in COX-2 activity in the paw tissue injected with carrageenan at almost the same levels ($p = 0.207$), LA suppressed it more significantly ($p < 0.001$) and more strongly than lacidipine and aspirin separately (Figure 1D).

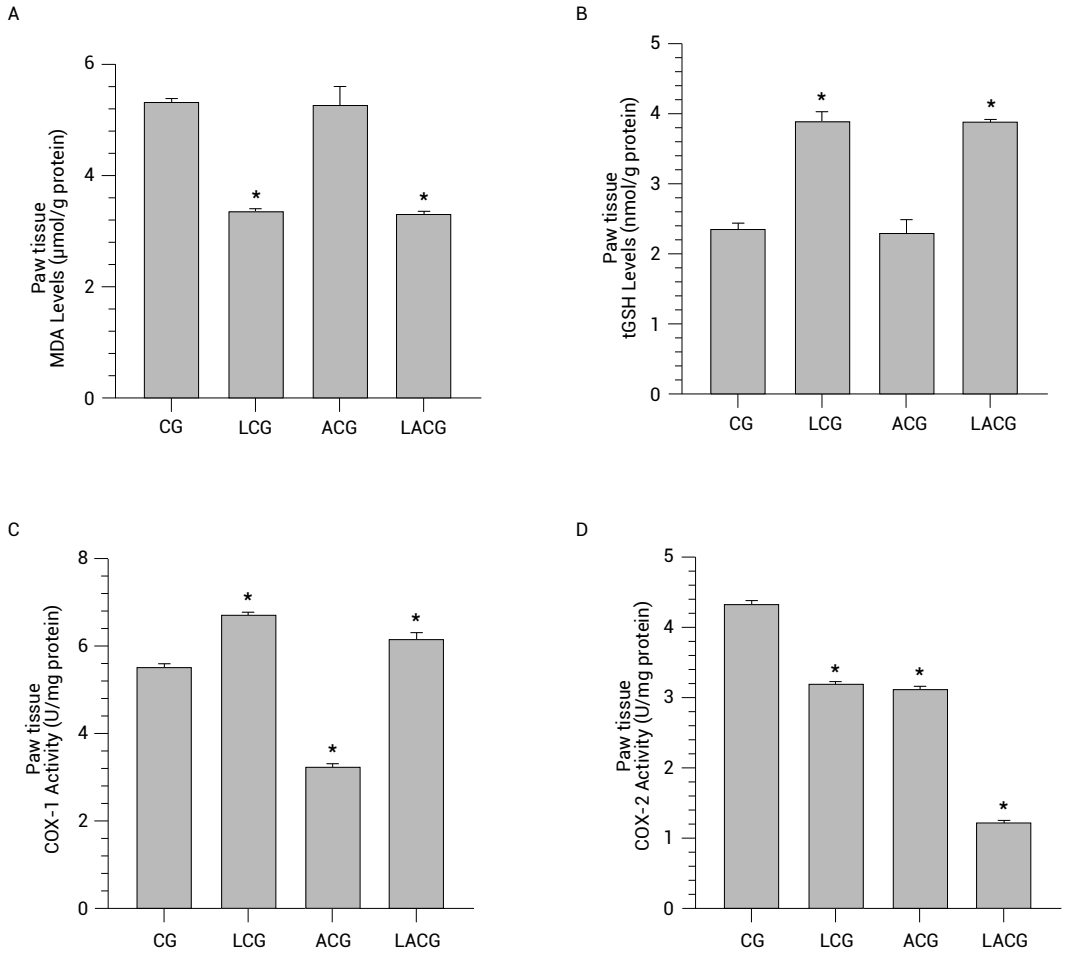


Figure 1. Analysis of MDA (A), tGSH (B), COX-1 (C), and COX-2 (D) activity obtained from paw tissues of animals. The bars show the mean ± SEM (n = 6). *: $p < 0.05$ vs CG.

MDA and tGSH analysis results of gastric tissue

When the MDA and tGSH data in the gastric tissue were evaluated, at least one group was found to be statistically different (F/p = 486.790/< 0.001; 619.711/< 0.001, respectively). As presented in Figure 2A, no significant (p = 0.521) difference was observed between the MDA amounts in the gastric tissues of the saline and lacidipine groups. However, MDA levels in the gastric tissue of the aspirin-treated group were found to be significantly ($p < 0.001$) higher than those of the CG, LCG, and LACG groups. Moreover, the tGSH amount in the gastric tissue of the aspirin group was lower than that of the CG, LCG, and LACG groups ($p < 0.001$; Figure 2B).

COX-1 and COX-2 analysis results of gastric tissue

Differences were found in at least one group for COX-1 data in paw tissue. (F/p = 706.383/<0.001). No significant (p = 1.000) difference was found between COX-1 activities in the gastric tissues of animals treated with saline and lacidipine. The results for the LCG and LACG groups didn't differ either (p = 0.186). COX-1 activities were calculated to be significantly lower in the gastric tissues of the animals treated with aspirin compared to the CG, LCG, and LACG groups ($p < 0.001$; Figure 2C). However, COX-2 activity was found to be similar in all groups (F/p = 0.404/0.752) (Figure 2D).

Macroscopic Evaluation of Gastric Tissue

As seen in Figures 3A 3B, and 3D, macroscopic ulceration was not observed in the gastric tissues of animals treated with carrageenan, lacidipine, and LA. There were, however, hyperemia and edema observed in some areas of the gastric lining in the LACG group. All gastric mucosal tissues from the animals treated with aspirin were hyperemic. A large

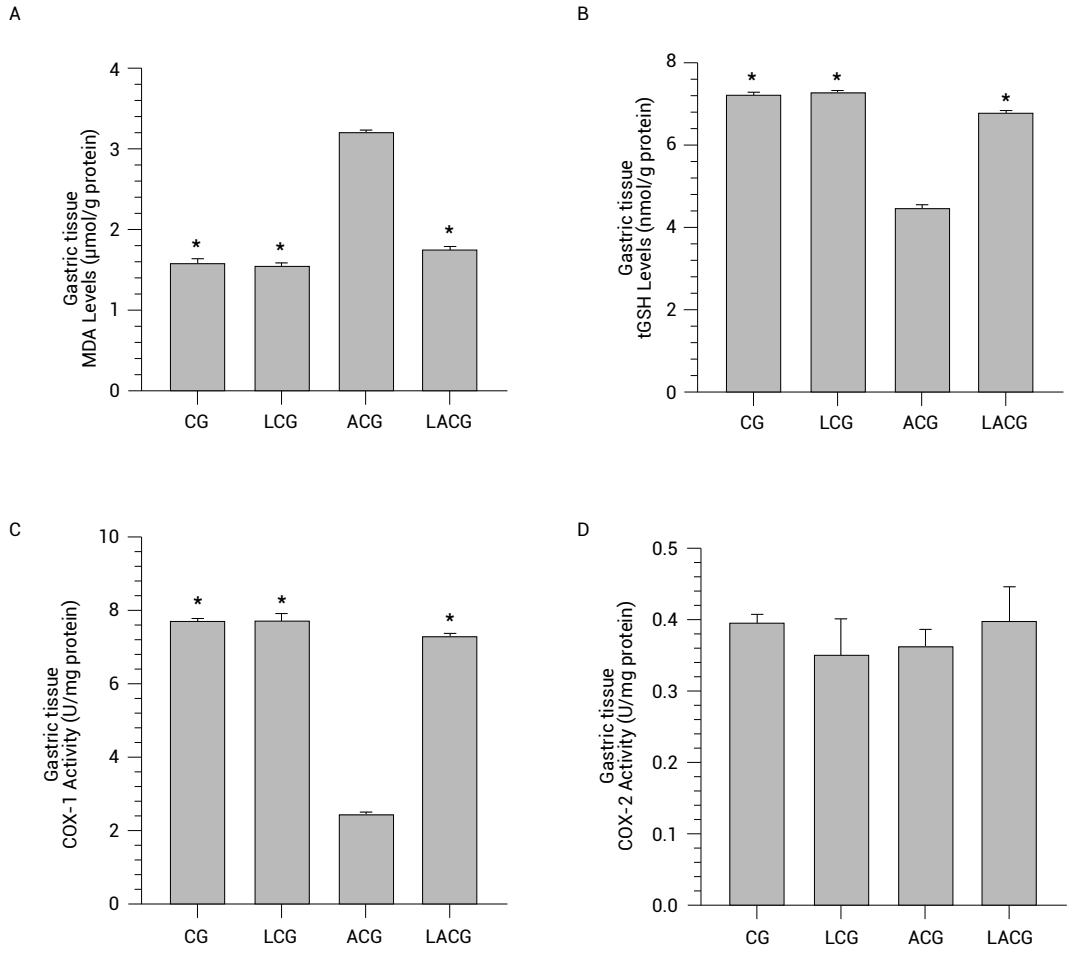


Figure 2. Analysis of MDA (A), tGSH (B), COX-1 (C), and COX-2 (D) activity obtained from gastric tissues of animals. The bars show the mean ± SEM (n = 6). *p < 0.05 vs ACG.

part of the gastric tissue had ulcer foci of different sizes and colors, some of which were black and some dark red. There were clear borders around the ulcer foci, which were characterized by round, oval, and irregular mucosal defects. Edema was observed in the ulcerated gastric tissue (Figure 3C). Moreover, the aspirin group had an average of 22 ulcers in their gastric tissue, and their average diameter was 28 mm^2.

DISCUSSION

In this study, the effects of lacidipine, aspirin, and LA on carrageenan-induced inflammation and pain in paw tissues of rats and how these treatments affect gastric tissues were investigated. In addition, MDA and tGSH levels and COX-1 and COX-2 activities in paw and stomach tissues were biochemically analyzed. The current study showed that lacidipine significantly reduced carrageenan-induced inflammation in the first and fourth hours, while aspirin was not effective in the first hour and provided a decrease in the fourth hour. Aspirin reduced carrageenan-induced pain more potently than lacidipine at the first and fourth hours. According to the results of both measurements, LA showed the best anti-inflammatory and analgesic activity. While no damage was observed in the stomach tissue of the animals administered lacidipine, we observed that aspirin caused serious damage to the stomach. LA was able to suppress aspirin-induced gastric injury.

It is known that serotonin, bradykinin, and histamine are secreted within the first hour after carrageenan injection, with proinflammatory PGs being secreted within the following hours (25). Lacidipine significantly reduced paw volume increases at the first and fourth hours, indicating that it inhibited the increase of serotonin, bradykinin, histamine, and proinflammatory PGs. Lacidipine

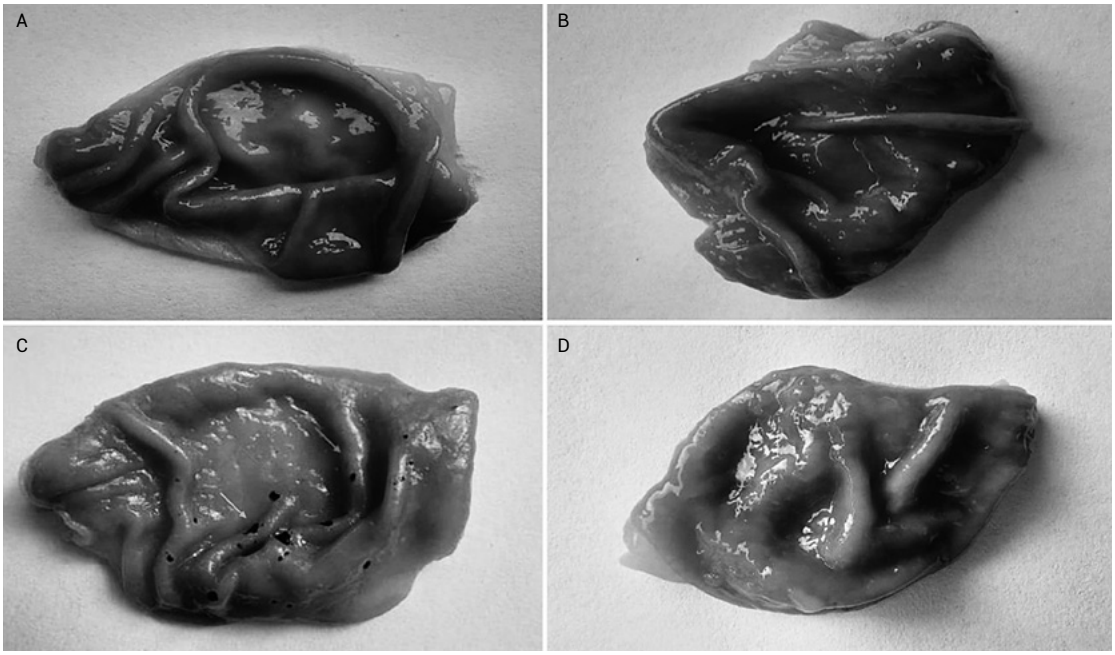


Figure 3. Macroscopic view of gastric tissues. A: Normal gastric tissue appearance of the CG group. B: Normal gastric tissue appearance of the LCG group. C: Gastric tissue after a single aspirin (100 mg/kg) dose. Arrows: ulcer areas. D: Normal gastric tissue appearance of the LACG group.

also prevented carrageenan-induced mechanical paw threshold reduction. It has been reported in the literature that the Ca2+ channel blockade has analgesic activity in experimental thermal and visceral pain models (26). Moreover, lacidipine was reported to alleviate experimentally induced hyperalgesia (27). The role of intracellular Ca^{2+} increase in the pathogenesis of carrageenan-induced inflammation was shown (28). The increase in intracellular Ca2+ levels may cause an increase in ROS production and oxidative stress (29), resulting in deterioration of the intracellular redox state and cell death. (30). By inducing LPO in the cell membrane, ROS produces toxic products such as MDA from lipids (31, 32). Lacidipine, which significantly inhibited the increase of MDA levels in paw tissue, also significantly suppressed carrageenan-induced inflammation in this study. This is consistent with the knowledge that a reduction in ROS leads to a reduction in the overall inflammatory environment (33).

In the case of oxidative stress, an increase in oxidants is accompanied by a decrease in antioxidants (34). Here we showed that lacidipine prevented the decrease in tGSH induced by carrageenin. GSH is a non-enzymatic endogenous antioxidant molecule that protects cells against ROS (35). Previous research has also reported a relationship between a GSH decrease and carrageenan-induced inflammation (25).

Another significant factor contributing to carrageenan-induced inflammation was the increase in COX-2 activity, which was responsible for inflammatory PG synthesis (25). PG synthesis is initiated by the stimulation of phospholipase A_2 in the cell membrane with Ca^{2+} (36). In this study, lacidipine significantly suppressed the increase in COX-2 activity in the paw tissue treated with carrageenan. This finding was consistent with studies indicating that an increase in COX-2 activity was directly associated with an increase in intracellular Ca^{2+} (14, 15).

In this study, aspirin had no significant effect on MDA and tGSH in paw tissue treated with carrageenan. Aspirin even decreased GSH levels in gastric tissue and increased MDA (12, 13). While aspirin significantly inhibited COX-1 and COX-2 activity, it did not significantly reduce carrageenan-induced inflammation at the first hour, but significantly at the fourth hour. In the literature, carrageenan was associated with the release of histamine, serotonin, and bradykinin during the initial stages of edema (37). The inhibition of carrageenan-induced inflammation by aspirin was due to the inactivation of COX-1 and COX-2 enzymes involved in PG synthesis. Studies have reported that aspirin has an anti-inflammatory effect by inactivating the COX enzyme, which is involved in the synthesis of PGs that cause inflammation (9, 11). Even though COX inhibition induced the

late-term anti-inflammatory and analgesic effects of aspirin, early-term analgesic effects were not attributed to COXs.

In this study, LA suppressed carrageenan-induced inflammation more potently when compared with lacidipine and aspirin alone. LA had a weaker anti-inflammatory effect at the first hour than at the fourth hour. The reason might be that aspirin could not inhibit histamine, serotonin, or bradykinin secretion in the early stages of inflammation in response to carrageenan (37). According to the literature, lacidipine inhibited carrageenan-induced inflammation more strongly in the first hour (5). Aspirin and lacidipine both have significant anti-inflammatory effects, which could explain LA's stronger anti-inflammatory effect at the fourth hour. These findings were consistent with the pathogenesis of late-phase carrageenan-induced inflammation (25).

It was also found that LA suppressed carrageenan-induced hyperalgesia more strongly than lacidipine or aspirin alone in the first hour. There is information in the literature that increased ROS and PG production increase pain sensitivity in inflammatory conditions (38). Therefore, the strongly analgesic activity of LA, which suppressed COX-2 increase in paw tissue the most, may be related to PG synthesis inhibition.

In this study, no macroscopic damage was observed in the stomach tissues of the animals in the saline, lacidipine, and LA groups. However, significant damage was observed in the gastric tissue of the aspirin group, with high MDA and low tGSH levels and COX-1 activity. This study confirmed that lacidipine had no damage to the gastric mucosa, which was consistent with the literature findings (4). Lacidipine was even known to have an antiulcer effect by inhibiting the increase of MDA and decrease of tGSH (4). It was also possible that lacidipine did not cause gastric damage because it did not reduce COX-1 activity. As known, COX-1 was a constitutive enzyme responsible for gastrointestinal system protection (9). The lack of damage caused by a Ca^{2+} channel blocker—lacidipine—in the gastric tissues was consistent with the findings reporting that decreases in COX-1 activities were directly related to intracellular Ca^{2+} levels (15). There was a relationship between the Ca^{2+} level and gastric ulcers according to a study by Fomina (39). These findings confirmed the role of these parameters in the pathogenesis of the ulcerogenic effect of aspirin. The experimental results obtained from the lacidipine and aspirin group revealed why ulcers did not develop in the gastric tissue of LACG. The doses of these drugs used in experimental animals are different from the doses administered to patients in the clinic. However, in the clinic, the combination of lacidipine and aspirin in therapeutic doses may also be beneficial in humans.

Strengths and Limitations

The current study is the first study to support the combination of aspirin with a Ca channel blocker to potentiate its analgesic anti-inflammatory power and at the same time prevent its adverse effects on gastric tissue. It would be helpful to measure the second and third hours of the carrageenan-induced inflammation and pain test and assess it biochemically to clarify the mechanism of action and duration of action of lacidipine and aspirin. In addition, histopathological examination of paw and stomach tissues is also important.

CONCLUSIONS

This study revealed that a faster and stronger analgesic effect and minimal gastrotoxic effect can be achieved with LA compared to aspirin therapy. This combination may be advantageous in clinical situations where aspirin therapy is preferred. In addition, this study may lead to studies on whether the maximum effect and minimum toxic effect can be achieved by using N and T-type Ca channel blockers together with NSAI drugs.

Conflicts of Interest

The authors declare there are no conflicts of interest to report.

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