

Original Research Article

Synergistic analgesic and anti-inflammatory effect of alpha-lipoic acid co-administration with non-steroidal anti-inflammatory drugs in mice model

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Abstract

Purpose: To investigate the synergistic analgesic and anti-inflammatory effect of alpha-lipoic acid (α -ALA) co-administered with non-steroidal anti-inflammatory drugs (NSAIDs).

Methods: A total of 48 Albino mice were randomly and equally assigned to 8 groups. Group 1 was the control and received the vehicle (0.5 mL carboxymethyl cellulose (CMC) 1 % in water), group 2 received α -ALA (α -ALA; 100 mg/kg), group 3 received diclofenac sodium (25 mg/kg), group 4 received diclofenac sodium (25 mg/kg) + α -ALA (100 mg/kg), group 5 received Nimesulide (25 mg/kg), group 6 received Nimesulide (25 mg/kg) + α -ALA (100 mg/kg), group 7 received Celecoxib (25 mg/kg), and group 8 received Celecoxib + α -ALA (25+100 mg/kg) all in 0.5 mL 1 % CMC. The anti-inflammatory effect was assessed using carrageenan and formalin models to measure mouse paw edema, while the analgesic effect was assessed using acetic acid-induced writhing and hot plate tests.

Results: Co-administration of α -ALA with diclofenac significantly inhibited paw oedema compared to NSAID alone in the carrageenan and formalin models ($p < 0.001$). Furthermore, co-administration of α -ALA with diclofenac significantly reduced writhes, enhanced anti-nociceptive activity, and prolonged latency time compared to NSAID alone ($p < 0.001$).

Conclusion: Combination of alpha-lipoic acid (α -ALA) with NSAIDS (diclofenac) significantly reduced paw oedema and prolonged latency time compared to NSAIDs alone.

Keywords: Anti-inflammatory, Analgesic effect, Alpha-lipoic acid, NSAIDs, Synergism

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INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used for the treatment of pain and inflammatory conditions. Though widely prescribed to treat various diseases, there is the potential for serious adverse effects, particularly on the gastrointestinal tract and kidneys. Reactive oxygen species (ROS) have been implicated in the pathogenesis of NSAID-induced gastric and renal injuries, justifying the essential

role of ROS in oxidative stress-mediated tissue damage [1]. Pharmacological management of pain relies primarily on two main drug classes. These include non-steroidal anti-inflammatory drugs (NSAIDs), which exert their effect by inhibiting cyclooxygenase (COX) enzymes, and opioid analgesics [2]. Non-steroidal anti-inflammatory drugs (NSAIDs) are particularly preferred due to their potent efficacy, relatively low potential for abuse, and long-established safe clinical record [3,4]. These NSAIDs vary

structurally, but share a common mechanism of action. They are classified into non-selective COX inhibitors such as Aspirin, Ibuprofen, Diclofenac, Ketorolac, Ketoprofen, and Mefenamic acid; and Selective COX-2 inhibitors such as Celecoxib and Etoricoxib [5]. Under normal conditions, body tissues possess endogenous antioxidant defenses, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, which neutralize reactive oxygen species (ROS). Other antioxidants may include tryptophan, histidine, ascorbic acid, and alpha-tocopherol [6]. In cases of ischemia or hypoxia, these defenses may be depleted, leading to oxidative cellular damage following reperfusion. Reactive oxygen species (ROS) play a significant role in both gastric and renal toxicity of NSAIDs. Excess ROS results in lipid peroxidation, protein oxidation and DNA damage, worsening tissue injury induced by COX blockade [7]. Alpha-lipoic acid (α -ALA) is a naturally occurring organosulfur compound found in plants, animals, and humans. It possesses potent antioxidant properties, enhances endogenous antioxidant defenses and facilitates removal of heavy metals responsible for oxidative stress. Within the Krebs cycle, alpha-lipoic acid (α -ALA) functions as an essential cofactor for enzymes involved in cellular energy metabolism and forms covalent bonds with proteins. Owing to the presence of a chiral centre, α -ALA exists as two optical isomers, namely R-lipoic acid and S-lipoic acid [8]. Also, α -ALA is both lipid- and water-soluble, allowing broad tissue distribution and efficacy.

Previous studies have indicated the synergistic effects of α -ALA in various treatment alternatives. Alpha-lipoic acid (α -ALA) has demonstrated beneficial effects in glycaemic control, prevention of diabetes-associated complications, and reduction of peripheral neuropathy symptoms, while also attenuating oxidative damage induced by heavy metals. Furthermore, combination of α -ALA with metformin exerted synergistic neuroprotective effects in diabetic encephalopathy by promoting coordinated activation of Nrf2 and AMPK signalling pathways [9]. In anticancer therapy, it reduced damage to healthy tissues by lowering levels of ROS produced by radiation [10]. When combined with clozapine, it reversed schizophrenia-like symptoms induced by ketamine in mice [11]. Also, α -ALA may serve as a valuable add-on to NSAID therapy, helping to boost anti-inflammatory and analgesic properties while protecting the stomach from potential damage [12]. This study therefore investigated the synergistic analgesic and anti-inflammatory effect of alpha-lipoic acid (α -ALA) co-

administered with non-steroidal anti-inflammatory drugs (NSAIDs).

EXPERIMENTAL

Animals

A total of 48 Albino mice (25 – 30 g) were randomly and equally assigned to 8 groups. The animals were sourced from Veterinary College at Basrah University and acclimatized in the animal house at the University of Basrah, College of Pharmacy, for 1 week under controlled conditions (20 – 25 °C, humidity of 30 $\pm$ 15 %, and 12 h dark/light cycle), with unrestricted access to feed and water *ad libitum*. The animals were randomized equally into 8 groups (n = 6).

Design

A total of 48 Albino mice were randomly and equally assigned to 8 groups. Group 1 was the control and received the vehicle (0.5 mL carboxymethyl cellulose (CMC) 1 % in water), group 2 received α -ALA (α -ALA; 100 mg/kg), group 3 received diclofenac sodium (25 mg/kg), group 4 received diclofenac sodium (25 mg/kg) + α -ALA (100 mg/kg), group 5 received Nimesulide (25 mg/kg), group 6 received Nimesulide (25 mg/kg) + α -ALA (100 mg/kg), group 7 received Celecoxib (25 mg/kg), and group 8 received Celecoxib + α -ALA (25+100 mg/kg) all in 0.5 mL 1 % CMC. All treatments were administered orally to overnight-fasted animals via a gavage tube. The carrageenan or formalin was administered 1 h after treatment, and inflammation and nociceptive responses were monitored over 4 h. Ethical approval was obtained from the Ethics Committee of the University of Basrah / College of Pharmacy (approval no: EC119), and complied with the guidelines for use and care of laboratory animals [13].

Anti-inflammatory activity

Carrageenan test

Acute inflammation was induced by palm injection of 1 % w/v carrageenan [14]. The animals were injected with stock solution (0.1 mL) into the right hind paw and paw volume was measured at baseline (pre-injection), 1, 2, and 4 h after injection.

Formalin test

Concentrated formalin solution (0.1 mL) was injected into the right hind foot. Thereafter, foot size and pain behaviors were recorded at

baseline, 1, 2, and 4 h following formalin injection [15].

Evaluation of parameters/indices

Writhing test

The acetic acid-induced abdominal writhing test was used to evaluate the analgesic and anti-inflammatory activity. Acetic acid solution was prepared by diluting 0.7 mL acetic acid in 100 mL distilled water and administered intraperitoneally to induce pain sensation [16].

Hot plate test

The hot-plate test was used to evaluate thermal nociception. The animals were placed on a heated surface maintained at 48 to 56 °C, although values as low as 44.5 °C and as high as 80 °C have been reported [17]. Latency of nociceptive reactions, such as paw licking or jumping, was recorded.

Statistical analysis

The statistical analysis was conducted using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). Data were presented as mean $\pm$ standard deviation (SD) and compared using one-way analysis of variance (ANOVA) and Dunnett's test. $P < 0.05$ was considered statistically significant.

RESULT

Carrageenan test

Alpha lipoic acid (α -ALA) combined with NSAIDs (diclofenac) significantly inhibited paw oedema

compared with diclofenac alone ($p < 0.01$), indicating a synergistic interaction (Table 1).

Formalin test

Alpha-lipoic acid (α -ALA) alone produced a moderate but significant inhibition compared to control ($p < 0.01$). Furthermore, combining α -ALA with NSAIDs significantly enhanced the anti-inflammatory effect ($p < 0.001$; Figure 1).

Hot plate model

Alpha lipoic acid (α -ALA) alone significantly increased latency time in a time-dependent manner, reaching 44 % MPE at 90 min ($p < 0.01$). Co-administration of α -ALA with diclofenac significantly enhanced the anti-nociceptive effect, resulting in longer latency time and highest analgesic activity (73.1 % MPE) at 90 min ($p < 0.01$).

DISCUSSION

Carrageenan-induced inflammation is a well-established experimental model characterized by the release of inflammatory mediators such as histamine, serotonin, bradykinin, and prostaglandins, which contribute to edema formation [14]. As a result, inhibition of paw swelling reflects suppression of these inflammatory pathways. This study revealed that α -ALA showed moderate anti-inflammatory activity and significantly enhanced the anti-inflammatory effects of different NSAIDs. This is attributed to the potent antioxidant properties of α -lipoic acid (reduced oxidative stress and inhibited production of pro-inflammatory cytokines and mediators).

Table 1: Anti-inflammatory effects of α -ALA in combination with NSAIDs (mean $\pm$ SD, %)

Group	Paw thickness (mm) (% Inhibition)			
	0 h	1 h	2 h	4 h
Control	1.88 $\pm$ 0.19	2.84 $\pm$ 0.28	2.96 $\pm$ 0.18	2.86 $\pm$ 0.22
α -lipoic acid	2.08 $\pm$ 0.18	2.72 $\pm$ 0.24** (33.33)	2.70 $\pm$ 2.22** (42.59)	2.58 $\pm$ 0.20*** (48.97)
Diclofenac	2.14 $\pm$ 0.23	2.45 $\pm$ 0.18*** (67.7)	2.40 $\pm$ 0.23*** (75.92)	2.30 $\pm$ 0.16*** (83.67)
Diclofenac + α -lipoic acid	1.95 $\pm$ 0.17	2.24 $\pm$ 0.22*** (69.79)	2.18 $\pm$ 0.24*** (78.70)	2.02 $\pm$ 0.21*** (92.85)
Nimesulide	1.96 $\pm$ 0.21	2.40 $\pm$ 0.24*** (54.16)	2.32 $\pm$ 0.18*** (66.66)	2.20 $\pm$ 0.24*** (75.51)
Nimesulide + α -lipoic acid	1.89 $\pm$ 0.16	2.28 $\pm$ 0.2 *** (59.37)	2.20 $\pm$ 0.16*** (71.29)	2.0 $\pm$ 0.22*** (88.78)
Celecoxib	2.1 $\pm$ 0.22	2.58 $\pm$ 0.14** (50.0)	2.50 $\pm$ 0.17*** (62.96)	2.40 $\pm$ 0.22*** (69.38)
Celecoxib + α -lipoic acid	1.90 $\pm$ 0.16	2.3 $\pm$ 0.21*** (58.33)	2.24 $\pm$ 0.14*** (68.51)	2.10 $\pm$ 0.2*** (79.6)

Data expressed as mean $\pm$ SD. * $P < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs control

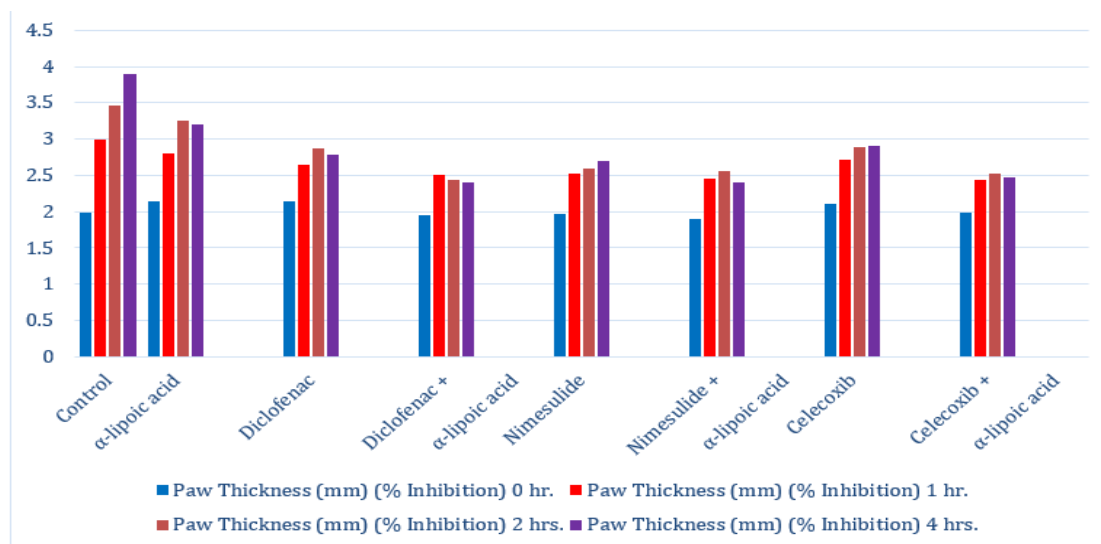


Figure 1: Anti-inflammatory effects of α-ALA and its combinations with NSAIDs on the formalin-induced paw edema in mice

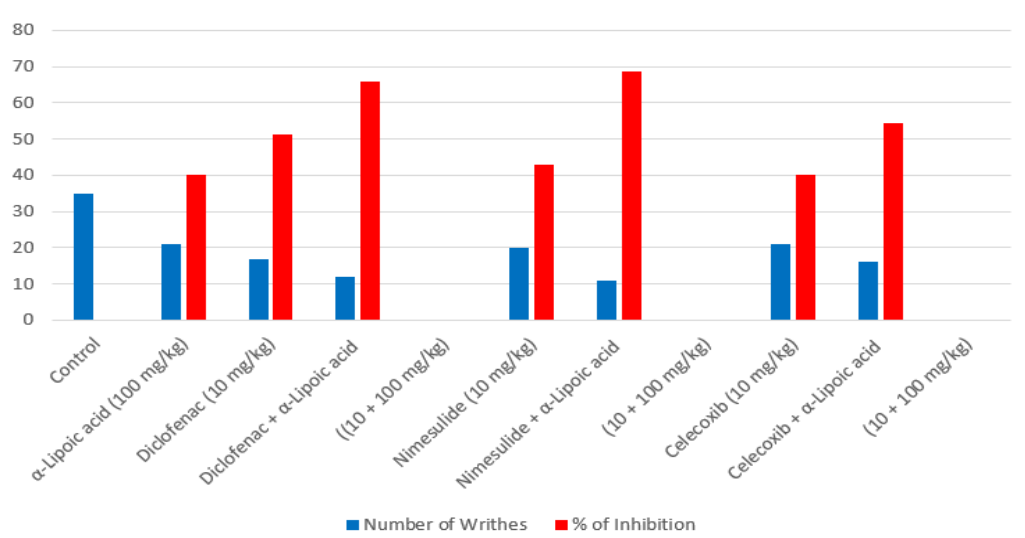


Figure 3: Anti-nociceptive effects of α-ALA and NSAID on an acetic acid-induced writhing test in mice

Table 2: Anti-nociceptive effects of α-lipoic acid and NSAIDs (mean ± SD, %)

Group	Latency time (s; % MPE)			
	Pretreatment	30 min	60 min	90 min
Control	7.8±0.7	7.6±0.5	8.1±0.6	7.4±0.8
α-Lipoic acid (100 mg/kg)	8.2±0.3	11.8±1.2** (30.5)	12.3±0.9*** (34.7)	13.4±1.5*** (44)
Diclofenac (10 mg/kg)	7.6±0.5	12.2±1.1*** (37.1)	14.1±1.7*** (52.4)	15.3±0.8*** (62.1)
Diclofenac + α-ALA (10 + 100 mg/kg)	8.1±0.6	13.7±1.4*** (47)	16.3±2.1*** (68.9)	16.8±2.0*** (73.1)
Nimesulide (10 mg/kg)	7.9±0.5	12.0±1.6*** (33.9)	13.6±1.7*** (47.1)	14.5±2.1*** (54.5)
Nimesulide + α-ALA (10 + 100 mg/kg)	8.3±0.7	13.4±1.5*** (43.6)	15.7±1.1*** (63.2)	16.2±1.6*** (67.5)
Celecoxib (10 mg/kg)	8.2±0.4	11.6±0.9*** (28.8)	12.5±1.3*** (36.4)	13.4±1.4*** (44)
Celecoxib + α-ALA (10 + 100 mg/kg)	7.8±0.8	12.5±1.1*** (38.5)	13.8±1.6*** (49.1)	14.6±1.6*** (55.7)

Also, α -ALA has been reported to modulate nuclear factor-kappa B (NF- κ B) signaling and reduce cyclooxygenase-mediated inflammatory responses, which is attributed to the anti-inflammatory activity observed in this study [8]. Similarly, NSAIDs are potent anti-inflammatory drugs that inhibit COX at the site of inflammation [3,4]. The significantly improved anti-inflammatory effect of Diclofenac observed in this study may be due to the nonselective inhibition of COX. The formalin test, commonly used to assess pain and inflammation in rodents, produces a biphasic pain response. There was an early phase representing direct activation of pain receptors and a later phase indicating peripheral and central sensitivity associated with inflammation. A gradual increase in paw thickness in the control group confirmed successful induction of inflammation, while treatment groups showed significant oedema suppression.

The findings of the present study revealed that α -ALA attenuated formalin-induced paw edema and enhanced the anti-inflammatory activity of NSAIDs when co-administered, potentially through mechanisms involving its potent antioxidant activity [8]. The results provided evidence for the utility of α -ALA as a novel adjuvant with NSAIDs, presumably to enhance the anti-inflammatory effects of NSAIDs while facilitating lower doses with minimal long-term treatment-related adverse effects [12]. This activity indicated a synergistic interaction rather than an additive effect, acting simultaneously on several pathogenic pathways at the same time. Pain was induced following injection of irritants into the peritoneal cavity, causing a characteristic stretching response, called writhing [18]. Writhing is defined as a stretch, tension to one side, extension of hind legs, or contraction of the abdomen so that it touches the floor or turning of the trunk (twist). Any form of writhing is considered a positive response [18]. Results of this study showed that α -ALA significantly reduced writhing response, demonstrating an intrinsic analgesic activity likely related to the attenuation of oxidative stress associated with pain and inflammation [19]. This reduction reflected a lesser degree of pain intensity in the animal under treatment. Treatment based solely on NSAIDs was also found to have relevant analgesic effect, in agreement with the proven mechanism of NSAIDs. These results suggest that both alpha-lipoic acid α -ALA and NSAIDs possessed potent anti-nociceptive effects [13]. The hot plate model was used to evaluate the central anti-nociceptive activity of α -ALA and NSAIDs by measuring latency time to a thermal stimulus in mice. An increase in reaction latency

reflects an elevation in pain threshold and indicates effective analgesic activity [17]. Results showed that α -ALA alone significantly and progressively prolonged latency time, reflecting a moderate but prolonged anti-nociceptive activity. However, in combination with NSAIDs, results showed improved analgesic efficacy and duration of action, indicating a synergistic effect. Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxygenase, reducing prostaglandin synthesis [3,4], while α -ALA acts as a potent antioxidant, scavenging ROS. This reduces oxidative stress, which is known to contribute to pain and inflammation. By targeting both inflammatory mediators and oxidative pathways, the combination therapy achieved significant pain relief compared to either treatment alone, consistent with findings from a previous study [13].

CONCLUSION

Combination of alpha-lipoic acid (α -ALA) with NSAIDS (diclofenac) significantly reduced paw oedema, showed better anti-nociceptive activity and prolonged latency time compared to either treatment alone. However, the potentiation mechanism should be investigated in future studies.

DECLARATIONS

Acknowledgement/Funding

None.

Ethical approval

It is stated in the methods

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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