

# A Behavioral Study of Promethazine Interaction with Analgesic Effect of Diclofenac: Pain Combination Therapy

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## Key Words

antinociceptive, diclofenac, pain, promethazine

## Abstract

**Objectives:** Pain is considered as a cause of sickness and the most prevalent symptom which makes people visit a physician. Nowadays, combination therapy is becoming useful to relieve chronic and postsurgical pain. The aim of this study was to study the promethazine (as an antihistamine) interactions with antinociceptive effect of diclofenac (as a non-steroidal anti-inflammatory drugs).

**Methods:** In initial part of the study, we investigate the analgesic effect of diclofenac. Using writhing test, we demonstrate that diclofenac significantly reduces writhing response induced by acetic acid in a dose-dependent manner. In this study, we evaluate the combination effect of promethazine on diclofenac analgesic effect.

**Results:** We observed that diclofenac inhibited pain in the dose dependent manner which means that by increasing dose of diclofenac a significant decrease in pain was observed. This experimental setup allowed calculation of the dose that caused 50% antinocicep-

tion (ED50) for diclofenac. The ED50 for diclofenac in this study was determined to be 9.1 mg/kg according our previous study. Additionally, promethazine was showed a dose-dependent inhibition of writhes. The combination of different doses of promethazine (2, 4, 6 mg / kg) with diclofenac ED50 (9.1 mg / kg) was injected to mice. Promethazine 4 and 6 mg / kg in combination with diclofenac had significantly led to increase analgesic effect of diclofenac.

**Conclusion:** In conclusion, these results add important information to the existing knowledge on combination of diclofenac and antihistamine in pain therapies to be used in clinical practice and maybe helpful in designing the future guidelines.

## 1. Introduction

Pain defined by International Association for the Study of Pain as unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Pain is considered as a cause of sickness and the most prevalent symptom which makes people visit a physician. Pharmacotherapy is one of the most important ways of treatment; however, available drugs have limitations in terms of effectiveness and side effects. Nowadays, combination therapy is becoming useful to relieve chronic and postsurgical pain [1-5]. The usage of an adjuvant drug to reduce the dosage and side effects of main drugs is noticeable. Opioid and non-opi-

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oid drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) are used to control pain and inflammation which have significant side effects [6, 7].

Diclofenac is a kind of phenyl acetic acid derivate which inhibits cyclooxygenase enzymes. This drug has analgesic, antipyretic and anti-inflammatory effects [8]. Its analgesic effects are related to inhibitory effects of prostaglandin synthesis. Besides main mechanism, there are some secondary mechanisms through which diclofenac does operate [9]. The side effects of diclofenac include gastrointestinal effects, kidney dysfunction and CNS effects such as headache and dizziness [8].

Today, the beneficial effect of combination therapy to relieve chronic and postsurgical pain has been proven. Adjuvant analgesics represent a various group of drugs. These medications were originally developed for a primary indication other than pain, although they may enhance analgesia under specific circumstances when administered simultaneously [10]. The usage of an adjuvant drug to reduce the dosage and side effects of main drug is significant [5].

Antihistamines has been used as an adjuvant analgesic in controlling pain [11]. The effect of antihistamines on pain depends on their dosage, injection sites and evaluation tests [12]. In different studies antagonists of H1 have shown analgesic effects. Furthermore, a clinical study has shown that diphenhydramine is effective to reduce the cancer pains as an adjuvant to opioid [13]. Additionally, it is suggested that different opioid and non-opioid mechanisms could be active by antihistamines [14]. Promethazine is a Histamine H1 receptor antagonist that is derived from phenothiazine. It is known as an anti-allergic drug in clinical situations. Additionally, promethazine reacts with different kinds of receptors. It has antimuscarinic activity and leads to sedation, anesthesia, neuroprotective and antiemetic effects. Moreover, promethazine have local analgesic effect similar to those of local anesthetic [12]. Combination therapy of promethazine and sumatriptan showed to be more tolerable and proper for migraine therapy than monotherapy [15].

There might be some secondary mechanism(s), in addition to the main mechanism, through which analgesic drugs exert their analgesic effect. The probable mechanisms can be recognized by experimental studies. Different studies have shown the role of cholinergic, dopaminergic, opioidergic, adrenergic and serotonergic mechanisms in the analgesic effects of drugs [16-19]. The antagonists of these systems are useful to determine the mechanisms of analgesic drugs.

Adjuvant analgesics are used to decrease the dosage of non-steroidal anti-inflammatory drugs and their side effects [20]. It seems that antihistamines in combination with diclofenac can be effective to change the anti-nociceptive effect of diclofenac [21]. For this reason we aimed to study the promethazine interactions with antinociceptive effect of diclofenac.

## 2. Material and Methods

### 2.1. Chemicals

The materials include: Acetic acid (Merck, Germany), Diclofenac (Darou Pakhsh, Iran), Promethazine (Tehran Shimi, Iran), ondansetron (Tehran Shimi, Iran), metoclopramide (Zahravi, Iran), Atropine (Darou Pakhsh, Iran), Naloxene (Darou Pakhsh, Iran).

### 2.2. Animals

Male Naval Medical Research Institute (NMRI) mice (20 – 30 g) were purchased from animal house of Hamadan University of Medical Sciences (Hamadan, Iran) and were maintained in 12-/12-h day/night standard conditions with water and pellets ad libitum. The mice were randomly selected. All experiments were approved by the Ethical Committee of the Hamadan University of Medical Sciences (IR.UMSHA.REC.1394.6843) and were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication #85-23, revised in 1985).

### 2.3. Writhing test

The analgesic activity was assessed by the acetic acid abdominal constriction test (writhing test). Acetic acid (0.7%) was injected intraperitoneally (i.p.) in a dose volume of 10 ml/kg body-weight. The injection of main drug or adjuvant was operated 30 minutes before the injection of acetic acid and the injection of inhibitor was operated 30 minutes before the injection of analgesic drug. The total number of writhing was recorded over a period of 10 min, starting 5 min after acetic acid injection. Each mouse was received one injection and just joined the test in one turn. Antinociceptive activity was expressed as the inhibition percentage of the usual number of writhing in a drug-treated animal compared to the mean number of writhing obtained in control [22]. The percentage of analgesic activity was calculated as following:

$$\text{Percentage analgesic activity} = \frac{(N_c - N_t)}{N_c} \times 100$$

where  $N_c$  is the average number of stretches in the control group, and  $N_t$  is the average number of stretches in the test drug group [23].

### 2.4. Study the analgesic effect of Diclofenac

In this test, animals were divided into five groups ( $n = 6$ ) [24] as following: the control group (saline 0.9%, 10 ml / kg, i.p.); four groups of mice received different doses of diclofenac (5, 10, 20, 30 mg / kg) [25]. Mice were placed into transparent plastic box and the doses of diclofenac were administrated 30 minutes prior to i.p. injection of acetic acid 0.7% (in a dose volume of 10 ml/kg body-weight) for ability to suppress abdominal constriction responses. Afterward, their responses counted throughout continuous observation for 10 min. It has to be mentioned that each animal was used only once.

## 2.5. Study the analgesic effect of Promethazine

Herein, animals were divided into four groups ( $n = 6$ ) as following: the control group (saline 0.9%, 10 ml / kg, i.p.); three groups of mice received different doses of promethazine (2, 4, 6 mg / kg) [26]. Mice were placed into transparent plastic box and the doses of promethazine were administrated 30 minutes prior to i.p. injection of acetic acid 0.7% (in a dose volume of 10 ml/kg body-weight) for ability to suppress abdominal constriction responses. Afterward, their responses counted throughout continuous observation for 10 min. It has to be mentioned that each animal was used only once.

## 2.6. Study the analgesic effect of Diclofenac and Promethazine combination

In this experimental setup, animals were divided into four groups ( $n = 6$ ) as following: the control group (diclofenac effective dose for 50% of the population (ED50), 9.1 mg/kg, i.p.); three groups of mice received diclofenac (9.1 mg / kg) and different doses of promethazine (2, 4, 6 mg/kg). Mice were placed into transparent plastic box and the effective dose (ED50) of diclofenac (9.1 mg/kg) and different doses of promethazine (2, 4, 6 mg / kg) were administrated 30 minutes prior to i.p. injection of acetic acid 0.7% (in a dose volume of 10 ml/kg body-weight) for ability to suppress abdominal constriction responses. Then, their responses counted during continuous observation for 10 min. It has to be mentioned that each animal was used only once.

## 2.7. Study the possible mechanism of Diclofenac analgesic activity through different pathways

The possible contribution of opioid, serotonin, dopamine, and cholinergic receptor in antinociceptive effect of diclofenac was assessed by antagonist studies. Four groups ( $n = 6$ ) of mice were received diclofenac in combination with inhibitors of different pain pathways (naloxone, ondansetron, atropine and metoclopramide). In this part, the antagonists of different pain pathways were injected 30 minutes prior administration of diclofenac 30 mg / kg and subsequently 30 minutes later the acetic acid (0.7%) was injected to mice. Afterward, their responses counted throughout continuous observation for 10 min. It has to be mentioned that each animal was used only once.

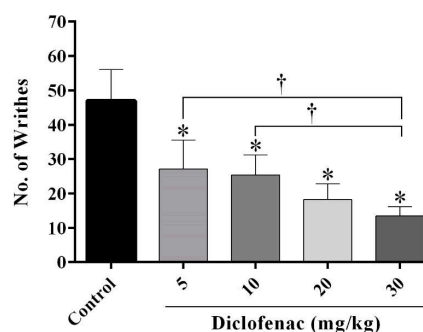
## 2.8. Statistical analysis

In this study statistical analysis operates by software Spss20. For each group of experiments, the number of animals was  $n \geq 6$ . Data are presented as mean  $\pm$  SD. Statistical significance was examined by one-way ANOVA followed by Turkey's test. The figures were draw using Graphpad Prism 6 software (Graphpad Software, Inc., San Diego, CA). A difference of  $p < 0.05$  was considered significant.

## 3. Results

### 3.1. Analgesic effect of Diclofenac

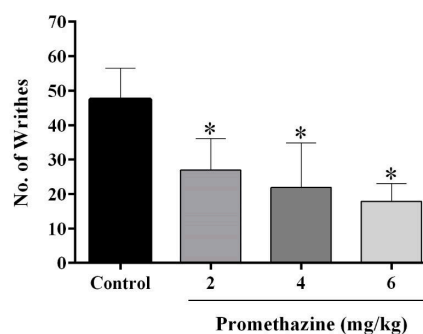
In this test, application doses of 5, 10, 20 and 30 mg/kg ( $p < 0.001$ ) were effective in decreasing writhing compared to the control group (Fig. 1). Diclofenac at dose of 5 mg / kg had inhibited pain 42.9% compared to control group which was a significant difference. Diclofenac 10 mg / kg had inhibited pain 46.6%, diclofenac 20 mg / kg had inhibited pain 61.6%, and diclofenac 30 mg/kg had inhibited pain 71.5%. Additionally, injection of the 30 mg / kg dose of the diclofenac were significantly effective in decreasing writhing compared to the group which were received diclofenac 5 and 10 mg / kg ( $P < 0.001$ ). We observed that diclofenac inhibited pain in the dose dependent manner which means that by increasing dose of diclofenac a significant decrease in pain was observed (Fig. 1). This experimental setup allowed calculation of the dose that caused 50% antinociception (ED50) for diclofenac. The ED50 for diclofenac in this study was determined to be 9.1 mg/kg according our previous study [22].



**Figure 1** The dose-related effect of diclofenac in the writhing test. Each column represents the writhing response of 6 animals per group ( $N = 6$ ) as mean  $\pm$  SD. \* $p < 0.05$  as significant difference to respect control treated with vehicle. † $p < 0.05$  as significant difference to diclofenac 30 mg / kg.

### 3.2. Analgesic effect of Promethazine

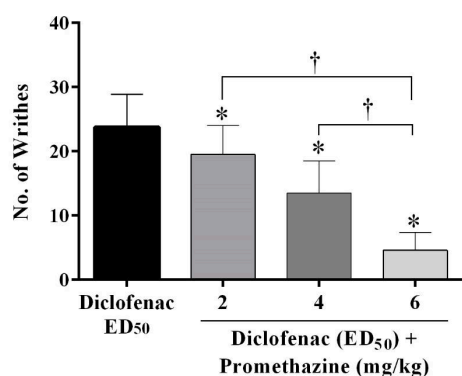
To investigate the possible antinociceptive effect of promethazine, different doses of promethazine was used. In this laboratory model, application doses of 2, 4, 6 mg / kg ( $p < 0.001$ ) were effective in decrease of writhing response compared to the control group (Fig. 2). It was observed that promethazine 2, 4, 6 mg/kg had inhibited writhes by 43.2%, 53.9% and 62.3% which were significantly different from control group (Fig. 2).



**Figure 2** The dose-related effect of promethazine in the writhing test. Each column represents the writhing response of 6 animals per group (N = 6) as mean  $\pm$  SD. \* $p < 0.05$  as significant difference to respect control treated with vehicle.

### 3.3. Analgesic effect of Diclofenac in combination with Promethazine

In order to consider the interaction of diclofenac with promethazine, diclofenac ED<sub>50</sub> and promethazine 2, 4, 6 mg / kg were used (Fig. 3). The results have shown that promethazine 2 mg/kg in combination with diclofenac had inhibited pain 59% which was not significantly different with diclofenac group. The combination of diclofenac and promethazine 4 mg / kg had inhibited writhes 71.6% which was significantly different from diclofenac ED<sub>50</sub> group ( $P < 0.001$ ). Using diclofenac ED<sub>50</sub> along with promethazine 6 mg / kg have resulted in pain inhibition by 90.2%, which was significantly different from diclofenac ED<sub>50</sub> ( $P < 0.001$ ). Additionally, the result of this experiment showed that combination of diclofenac and promethazine (2, 4 mg / kg) was significantly decreasing writhing compared to the combination of diclofenac and promethazine 6 mg / kg ( $P < 0.001$  and  $P < 0.01$ , respectively). The result of this experiment was shown that promethazine could increase analgesic effect of diclofenac (Fig. 3).



**Figure 3** The combination effect of diclofenac ED<sub>50</sub> and promethazine on the anti-nociceptive effect of the diclofenac in the writhing test. Each column represents the writhing response of 6 animals (n = 6) per group as mean  $\pm$  SD. \* $p < 0.05$  as significant difference to respect control treated with ED<sub>50</sub>. † $p < 0.05$  as significant difference to promethazine 6 mg / kg group.

### 3.4. The effect of inhibitors of different analgesic pathways on analgesic effect of Diclofenac

In this experimental setup, it was revealed that using naloxone (1 mg / kg; an opoidic receptor antagonist) [27] or ondansetron (0.5 mg / kg; serotonin receptor antagonist) [28] or atropine (1 mg/kg; cholinergic receptor) [27] plus diclofenac 30 mg / kg caused the reverse of the pain relief effects of the diclofenac ( $P < 0.05$ ). The results have shown that inhibition of writhes by naloxone, ondansetron and

atropine were 50.7%, 57.1% and 43.2%, respectively. However, the combination of metoclopramide (1 mg / kg; dopamine receptor antagonist) [29] plus diclofenac 30 mg / kg inhibited writhes 69.2% which was not significantly effective in reversing of the pain relief effect of diclofenac 30 mg / kg. Additionally, the combination of atropine or naloxone plus diclofenac were significantly different from combination of metoclopramide and diclofenac in terms of writhes inhibition ( $P < 0.05$ ).

## 4. Discussion

In this study, we describe the impact of promethazine as an adjuvant on analgesic effect of diclofenac. Additionally, we show the possible mechanism of action which might diclofenac exert its analgesic effect. Available knowledge on combination therapy of pain shows that the combination of antihistamine and NSAIDs or opioids are commonly used in clinical practice with generally good results [13, 16]. We hypothesized that a similar scenario might occur when promethazine and diclofenac are combined and, therefore, we investigated the combination effect as well as the possible mechanism of action involved in the analgesic effect of diclofenac.

In initial part of the study, we investigate the analgesic effect of diclofenac. Using writhing test, we demonstrate that diclofenac significantly reduces writhes response induced by acetic acid (Fig. 1) which is in consistence with the results of other study [21].

According to our previous study, by drawing dose-response figure and linear regression analysis, ED<sub>50</sub> was determined 9.1 mg / kg [22]. However, in other studies ED<sub>50</sub> was determined to be 8.1 [21] and 9.8 mg / kg [26]. It seems that these differences are because of dissimilarities between concentrations of acetic acid and the time of writhes counting.

The role of histaminergic system in controlling pain has been represented in different studies [13, 30]. It has been postulated that histamine plays role in controlling pain by H<sub>2</sub> receptor [31]. For this reason, to investigate the role of promethazine as an adjuvant analgesic, a range of promethazine doses from 2 to 6 mg / kg was tested (Fig. 2). Promethazine has showed a dose-dependent inhibition of writhes, which is in consistence with our results [32].

Diphenhydramine has been studied as an analgesic adjuvant [13]. It has been shown that diphenhydramine was effective on relieving resistant pains of cancer when it was added to different analgesic regimen as an adjuvant. In patients who had cancer and they were resistant to oral, injection and epidural use of opioids, diphenhydramine had reduced the pain by being used continuously [13]. Additionally, different studies have proved that analgesic effect of diclofenac can be increased by some adjuvant [21, 33]. In this study, we evaluate the combination effect of promethazine on diclofenac analgesic effect. The combination of different doses of promethazine with diclofenac was injected to mice. In this experimental setup, we used diclofenac ED<sub>50</sub> (9.1 mg / kg), we observed a significant decrease in writhes number which consequently lead to increase analgesic effect of diclofenac (Fig. 3). Promethazine 4 and 6 mg/kg in combination with diclofenac had

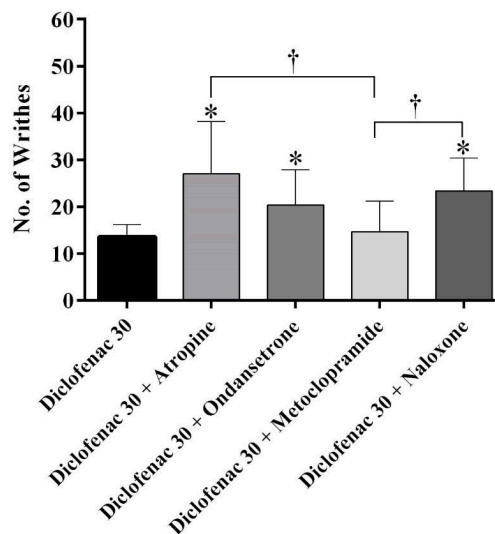


significantly led to increase analgesic effect of diclofenac (Fig. 3). Accordingly, the results suggest the efficacy of this combination which might lead to decrease the dose of diclofenac and consequently decrease its side effect. This finding might be helpful to create future guidelines for relieve of pains using diclofenac. The study of Paz-Compos et al. suggested that diclofenac-curcumin combination may have therapeutic advantages for the clinical treatment of inflammatory pain [33]. The synergism effect of diclofenac in combination with clonidine or diclofenac with phenylephrine have been demonstrated in other study as well [21].

In order to study probable mechanisms of diclofenac, other than inhibiting cyclooxygenase, the role of opioidergic, serotonergic, cholinergic and dopaminergic systems were studied. Naloxone is competitive antagonist of opioidergic receptors which can antagonize analgesic and other effects of morphine by  $\mu$ ,  $\sigma$  and K receptors [24]. Naloxone has proved dose dependent effects on controlling the pain of human and animals [34]. In experimental studies, very low doses of naloxone have shown analgesic effects [35]. The results of this study suggest that naloxone can lead to increase the number of writhes which were inhibited using diclofenac Fig. 4. This outcome indicates that opioidergic system may play role in analgesic effect of diclofenac. This finding is in consistence with other study which has showed that i.p. injection of naloxone has reversed the analgesic effect of diclofenac [36]. Pernia Andrade et al. have shown that naloxone 1 mg/kg has inhibited analgesic effect of Lysine acetylsalicylate (injection form of Aspirin) in hot plate and tail flick tests [37]. Additionally, it has been reported that naloxone can antagonize analgesic effect of some non-opioidergic drugs such as acetaminophen and can act as an antagonist of gabanergic receptors [38]. There are some reports which have been shown that 5-hydroxytryptamine 3 receptors (5HT3) play role in inducing and inhibiting pain [39, 40]. These effects depend on the location of analgesic receptors, the way of administration and evaluating pain test [40]. In order to study the possible role of serotonin on analgesic effect of diclofenac, the ondansetron as an antagonist of 5HT3 receptors was used. We observed significant increase in the number of writhes (Fig. 4) which means that ondansetron inhibited analgesic effect of diclofenac. This result indicates that serotonin receptors, especially 5HT3 might play role in analgesic effect of diclofenac. In accordance with our study, other investigation has shown that ondansetron had decreased analgesic effect of diphenyl diselenide [29]. Different studies have been shown the effect of 5HT1A, 5HT2A and 5HT3 receptors in the analgesic effect of aspirin and acetaminophen [41, 42].

Atropine, an antagonist of the muscarinic acetylcholine receptors, was use to investigate the effect of muscarinic receptors in analgesic activity of diclofenac [43]. In this study, atropine 1 mg/kg has significantly inhibited analgesic effect of diclofenac (Fig. 4). This result suggests the possible analgesic effect of diclofenac throughout cholinergic pathway. Intraperitoneally injection of atropine has significantly antagonized effect of NSAIDs in tail flick test [43]. Dopamine  $D_1$  and  $D_2$  receptor antagonists have been reported to play role in analgesic effect [27, 44]. In

this study, metoclopramide, as a dopaminergic antagonist, increase the number of writhes (Fig. 4); however, this increase was not statistically significant. This finding indicates the possible involvement of dopamine receptor in the analgesic effect of diclofenac. Different studies have shown that  $D_2$  agonists and indirect stimulant of dopamine receptors have shown analgesic effect in different models of pain in animals [25, 27, 44].



**Figure 4** The influence of the pre-treatment with naloxone (1 mg / kg), ondansetron (0.5 mg / kg), atropine (1 mg / kg) and metoclopramide (1 mg / kg) on the anti-nociceptive effect of the diclofenac (30 mg / kg) in the writhing test. Each column represents the writhing response of 6 animals per group as mean  $\pm$  SD. \*  $p < 0.05$  as significant difference compare to Diclofenac 30 mg / kg; †  $p < 0.05$  as significant difference compare to the combination of Diclofenac 30 mg / kg and metoclopramide.

## 5. Conclusion

This study demonstrates the combination effect of promethazine on diclofenac analgesic effect. Promethazine showed to be effective in increasing analgesic effect of diclofenac, which may suggest usage of lower diclofenac dose with consequently lower side effects. According to the results, we suggest that at least some parts of analgesic effect of diclofenac might be due to interaction with opioidergic, serotonergic, cholinergic and dopaminergic pathways. However, more studies should be performed to evaluate the precise pharmacokinetic and pharmacodynamics profile of such combination. In conclusion, these results add important information to the existing knowledge on combination of diclofenac and antihistamine in pain therapies to be used in clinical practice and maybe helpful in designing the future guidelines.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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