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Exploring the Effect of Caffeine on Decelerating the Lethargic Action of Diclofenac in the Treatment of Pain

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Authors' contributions

This work was carried out in collaboration between all authors. Author RK designed and supervised the work. Authors RH, AT and RS conducted laboratory experiment. Author MMB analyzed the data and wrote the manuscript with authors MSU, MSR and MMR. All authors read and approved the final manuscript.

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ABSTRACT

Background: Diclofenac is used as a potential analgesic for mild to moderately severe pain including migraine pain. However, the common side effect of this drug (drowsiness) becomes bothersome for patients taking diclofenac on regular basis in order to get rid of migraine pain. This hinders a person's daily routine and eventually causes loss of motivation for work. The central nervous system (CNS) stimulant activity of caffeine is thought to decelerate the undesirable effect of diclofenac.

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Objectives: The main aim was to investigate the efficiency of caffeine in decelerating the lethargic action of diclofenac at different dose regimen in pain induced Swiss Albino mice model.
Method: Different doses of caffeine (5 mg, 25 mg and 50 mg/kg body weight) were administered with diclofenac potassium tablet (50 mg/kg body weight) orally in acetic acid-induced pain in Swiss Albino male mice. The efficacy of caffeine (with and without diclofenac potassium 50 mg) in reducing pain was evaluated by writhing test. The CNS stimulating activity of caffeine to minimize lethargic properties of diclofenac was determined by measuring reflex action and locomotion of mice via conducting righting reflex test and open field test. Mean of the total duration of various locomotor activities of mice of different test groups was compared with normal and control groups by using ANOVA and Dunnet's test.
Results: The results of all tests demonstrated the reduced lethargic behaviours in mice with the maximum dose of caffeine (50 mg) in combination with diclofenac potassium (50 mg) at the same time the study revealed the overall effect of caffeine in enhancing total locomotion of mice. The statistical data of test results showed that higher dose of caffeine (50 mg) in combination with diclofenac potassium (50 mg) significantly increased ($P<0.01$) locomotor activities of mice as compared to the normal group. Moreover, for writhing counts significant result ($P<0.01$) was found in the combination of caffeine 50 mg with diclofenac potassium 50 mg when compared to the control group.
Conclusion: The present investigation suggests that the combination of diclofenac potassium (50 mg) with caffeine (50 mg) possess potent analgesic activity and successfully minimize the side effects (drowsiness and dizziness) of diclofenac.

Keywords: Analgesic; adjuvant; caffeine; diclofenac; locomotor activity; drowsiness; dizziness.

1. INTRODUCTION

Inadequate treatment of pain is widespread in various cases like post-operative period, trauma, accidents, during labour, migraine, cancer etc. According to the International Association for the Study of Pain, chronic pain should be considered as disease and relief of pain must be recognized as a human right [1]. Acute pain is often managed with medications like analgesics and anaesthetics. For such pain, caffeine may provide additional benefit when combined with analgesics like ibuprofen [2]. Caffeine is frequently used as a constituent in pain-relieving medicines accessible as an over-the-counter drug. An adjuvant is a substance that is added to a medicine to enhance its efficacy. The addition of caffeine as an adjuvant, at a specified dose, was found to relief pain up to 10% [3]. Although the mechanism by which caffeine contributes its role in enhancing analgesic efficacy is not well understood it was reported that caffeine induces mild analgesic effect and was found to be an effective analgesic adjuvant when combined with many traditional analgesics [3-5]. Use of chronic caffeine results is in the withdrawal of a headache [6,7]. Studies suggested that caffeine is mostly useful for enhancing relief of a headache rather than postsurgical pain where caffeine adds little as an adjuvant to the analgesics like aspirin and acetaminophen [8,9].

On the other hand, caffeine is also extensively consumed for its CNS stimulant effects such as increased attentiveness and reduced fatigue [10]. Caffeine mobilizes intracellular calcium and inhibits specific phosphodiesterases only at high non-physiological concentrations. The methylxanthine blocks adenosine receptors producing a stimulating effect [11]. Caffeine surges generation of energy throughout the brain but reduces cerebral blood flow, inducing a state of hypo-perfusion. Many of the effects concerning alertness caused by caffeine can be associated with its action on serotonin neurons. Caffeine also induces dose-dependent development in locomotor activity in animals [12].

Diclofenac is a broadly used non-steroidal anti-inflammatory drugs (NSAIDs). It is used for the treatment of mild to moderately severe pain involving conditions like arthritis, rheumatoid arthritis, osteoarthritis, dental pain, spondylitis, gout attack, normal headache, migraine and sometimes mild to moderate post-operative or post-traumatic pain. It is particularly useful for osteoarthritis [13] and acute migraine pain [14]. Drowsiness is one of its common side-effects [15]. This makes the drug very inconvenient for patients taking it for minor pains; they opt to continue to carry on their regular work. Caffeine as discussed earlier can ward off drowsiness due to its CNS stimulant activity and is also used in many analgesic formulations due to the

increased analgesic effect of the primary constituent, and so these make caffeine an ideal hypothetical candidate to try in combination with diclofenac. There are no previous works on this combination to measure the link with drowsiness. The only such work available was conducted to investigate the augmentation of the efficacy of diclofenac sodium soft gel (100 mg) with the caffeine (100 mg) as an adjuvant [16]. Therefore, the contemporary study was designed to ravel out the influence of different doses of caffeine (5 mg, 25 mg and 50 mg) when combined with diclofenac potassium tablet (50 mg), administered orally in mice with acetic acid-induced pain, on lowering the common side effect (drowsiness) of diclofenac. In this study, the CNS stimulating effects of caffeine was evaluated by analyzing locomotion of experimental animals via open field test. This method is widely used the tool in behavioural research of animals [17]. This involves an enclosed open area where an animal is placed and some form of activities or behaviours are measured [18,19]. The objectiveof this study was to examine the effectiveness of caffeine in reducing the lethargic action of diclofenac at various doses in pain induced mice model.

2. MATERIALS AND METHODS

2.1 Chemicals and Drugs

All chemicals used in this study were analytical grade. Diclofenac potassium (50 mg) tablets were purchased from the local registered medical store. The caffeine was collected from Eskayf Bangladesh Lid., Dhaka, Bangladesh.

2.2 Experimental Animals

Colony bred adult male healthy Swiss Albino mice weighing between 20-26 g were used for the present research. All the animals were obtained from the Jahangirnagar University. They were held in a standard light (14 h light: 10 h dark cycle), controlled room temperature (25 °C), with the delivery of laboratory feed and water. The animals were acclimatized for a week before commencing the experiment. Animal care was based on principles and guidelines approved by the Guide for the Care and Use of Laboratory Animals (NIH publication No: 85-23, revised in 1985). The experimental procedures were approved by the Biomedical Research Center, University of Dhaka, Bangladesh. The experiment was conducted from the month of July 2016 to July 2017 in the Department of Pharmacy, Primeasia University, Dhaka, Bangladesh.

2.3 Drug and Dose Regime

A comparative study of analgesic activity amid single dose of diclofenac potassium (50 mg/kg body weight, p.o.) and combined dose of diclofenac potassium (50 mg of b.w., p.o.) and caffeine (the applied doses of caffeine were 5 mg, 25 mg and 50 mg/kg body weight, p.o. respectively) was conducted. The trial was made on the same group of animals in order to determine the effect of caffeine in reducing the adverse effect (dizziness/weakness/fatigue) of diclofenac by administering orally different doses of caffeine (5 mg, 25 mg and 50 mg/kg body weight, p.o. respectively) with 50 mg of diclofenac potassium tablet. Conversion of human to animal dose for respective doses of diclofenac potassium and caffeine has been done by using previous method [20]. The experimental animals were divided into the following groups and received subsequent treatments accordingly:

Groups	Treatment
Group I (Normal)	No pain was induced and no medicine was administered
Group II (Control)	The pain was induced but no medicine was administered
Group III (Standard)	The pain was induced and diclofenac potassium 50 mg/kg body weight, p.o. was administered
Group IV (Test group 1)	The pain was induced and diclofenac potassium 50 mg + Caffeine 5 mg/kg of body weight, p.o. were administered
Group V (Test group 2)	The pain was induced and diclofenac potassium 50 mg + Caffeine 25 mg/kg body weight, p.o. were administered
Group VI (Test group 3)	The pain was induced and diclofenac potassium 50 mg + Caffeine 50 mg/kg body weight, p.o. were administered

2.4 Writhing Test

Acetic acid induced writhing method was implemented for evaluation of the analgesic activity. Writhing is characterized as a stretch, pressure to the other side, expansion of rear legs, constriction of the midriff so that the belly of mice touches the floor, followed by the turning of the trunk. Any writhing is recorded as a positive reaction. 30 male Swiss Albino mice weighing between 20-26 g were used for evaluation of analgesic activity in six groups, each group contained five mice. The animals were weighed and numbered appropriately. A solution of acetic acid (1% v/v) in distilled water was prepared in a volumetric flask and injected (0.01 ml/g) to the mice intraperitoneally [21]. The protocol for writhing test was carried out according to the previous report [22]. Writhing episodes were recorded for 10 minutes and the average writhes in each group was calculated in terms of percentage. Percentage inhibition of writhing is calculated using the formula:

Percentage of inhibition = [(Wc – Wt)/Wc] × 100

Where,Wc is the average writhing of the control group and Wt is the average writhing of the treated group.

The time period with the highest percentage of inhibition is thought-out as the peak time. A dose range is kept for those compounds which inhibit writhing more than 70%. Compounds with an inhibition percentage lower than 70% are considered to have the least activity [23].

2.5 Righting Reflex Test

The righting reflex test is a basic, fast test to judge locomotor capacities in mice. It assesses overall body strength by gaging the ability of mice to return to their four paws after having been placed on their side. Because of its minimalism, and like other, non-terminal checks, it permits the longitudinal study of the development of a locomotorimpairment or its enhancement by therapeutic compounds [24]. The test was run on a smooth flat surface with even texture. This was necessary to ensure a just surface that neither facilitated nor hindered righting. The time was recorded manually using a stopwatch [25]. Scoring of the mice was evaluated based on standard protocol [26].

2.6 Open Field Test

The open field test provides instantaneous measures of locomotion, exploration and anxiety. The total line crosses and the occurrence of rearing are typically used as actions of locomotoractivity but are also events of exploration and anxiety. A high frequency of these behaviours specifies amplified locomotion and exploration or a minor level of anxiety. The quantity of central square entries and the period of time spent in the central square are measures of exploratory behaviour and anxiety. A high occurrence of these behaviours directs high exploratory behaviour and low anxiety levels [27].

The open field apparatus was assembled of plywood and measuring 72 ×72 cm with 36 cm thick walls in which different areas have been marked where various behaviours of mice were recorded. Dark lines were drawn on the floor with a marker that separated the floor into sixteen 18 × 18 cm squares. A central square (18 cm × 18 cm) was drawn in the middle of the open field [28]. The central square is used as some mouse strains have high locomotor activity and cross the lines of the test chamber numerous times during a test session. Moreover, this has an adequate space neighbouring it to give meaning to the central location as being different from the outer locations [29]. The maze was positioned in a 1.8 × 4.6 m test room and lit by a 60-watt red lamp for contextual lighting. The open field maze was cleaned between each mouse by means of 70 % ethyl alcohol. Records of line crosses were obtained with a fixed ceiling camera programmed (Samsung, Malaysia) to track through a computer system for visual monitoring.

Mice were employed at the centre or one of the four bends of the open field and permitted to explore the apparatus for 5 minutes. After the 5 minutes, the mice were returned to their cages and the open field was cleaned with 70 % ethyl alcohol and allowed to dry between tests. To evaluate the course of habituation to the uniqueness of the arena, mice were exposed to the apparatus for 5 minutes on two successive days [17].

2.7 Measurement of Locomotor Activity

The animals' behaviours scored according to previously established procedure [28]. The total locomotor activity of each animal was considered as the sum of line crosses and a number of rears [28]. The frequency of central square entry and

duration of residing within central squares are measures of animal's exploratory behaviour. Several dependent variables measured in the open field correlate significantly with one another, such as: line crosses and rearing, as well as line crosses and central square activity [28]. A high frequency of all these behaviours indicates increased locomotion and exploration by the animal.

2.8 Factor analysis for Locomotor Activity

Factors for open field behaviour of animals were analyzed according to Jahkel et al., identifying three significant factors including activity (line crosses, time active), exploration (centre squares crossed, time in centre, sniffing), and irritation (time passive) [26].

2.9 Statistical Analysis

The values are represented as mean ± SEM, and statistical significance between treated and control groups was analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's test where $P<0.05$ was considered statistically significant.

3. RESULTS

The results of statistical analyses of all tests have been presented in bar diagrams (Fig. 1, 2, 3, 4 and 5) where the effects of diclofenac (50 mg/kgb.w., p.o.) alone and in combination with different doses of caffeine (5 mg, 25 mg and50 mg/kgb.w., p.o.) were compared. The analgesic activity of single dose of diclofenac (50 mg/kgb.w., p.o.) and its combination with caffeine (5 mg, 25 mg and 50 mg/kg b.w., p.o.) was determined and compared with the control group (animals with pain only) via acetic acid-induced pain in mice. The duration and frequency of different locomotor activities have been presented in bar graphs showing the fundamental effects of treatment of animals with a combination of diclofenac and caffeine on different behaviours regarding locomotion of the open field test (Fig. 1). The righting reflex test was done to further assess the locomotor abilities in mice. This test is more rapid and simple as compared to open field test.

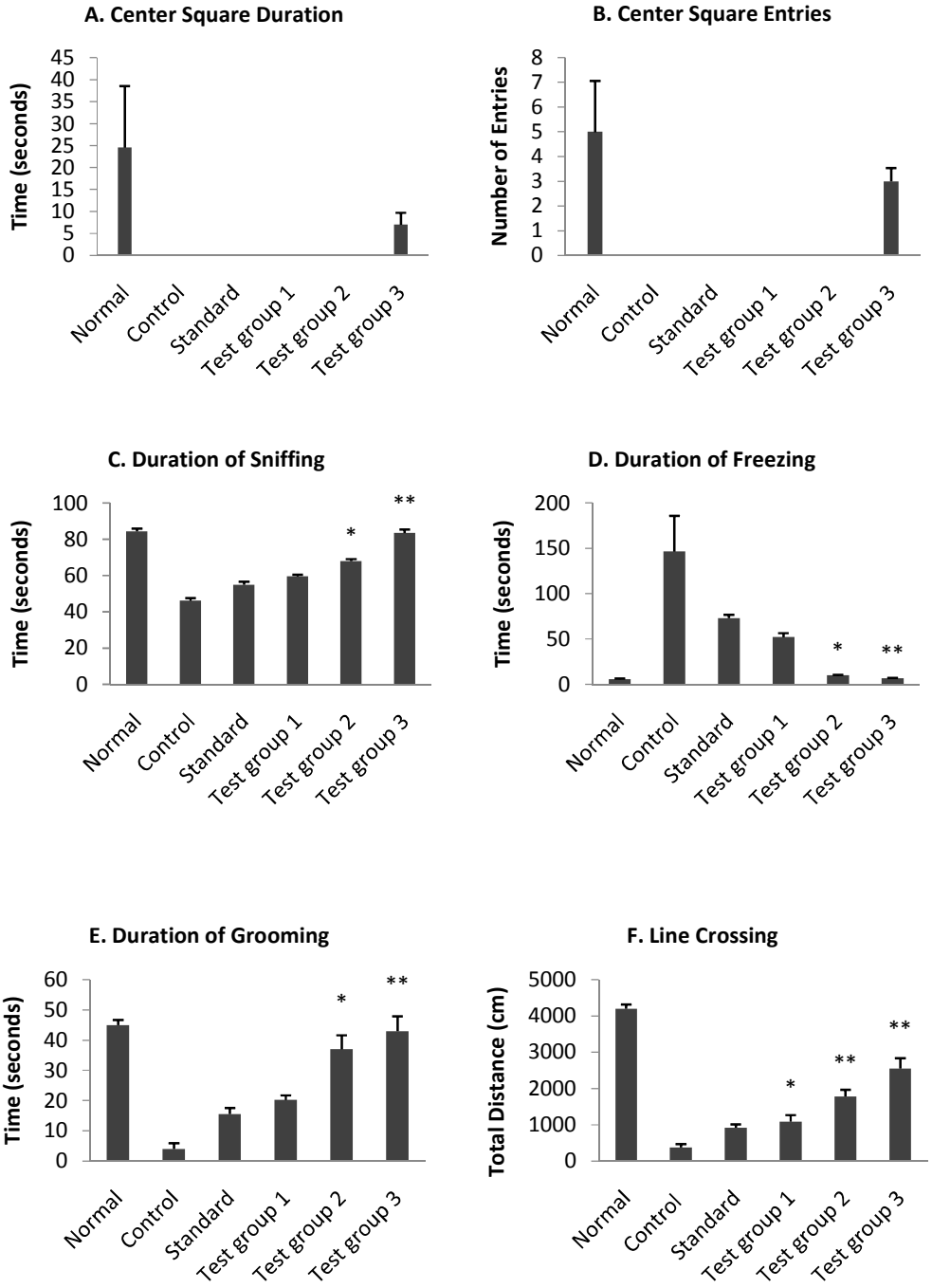
Generally, a good relationship exists between the analgesic potency of drugs in writhing assays and their clinical potencies. In this study, the analgesic activity of diclofenac (50 mg/kg b.w. p.o.) alone and in combination with different doses of caffeine (5 mg, 25 mg and 50 mg/kg b.w., p.o.) have been evaluated as percentage of pain inhibition (Fig. 2) and the average number of writhes as SEM of writhing test (Fig. 3). The test result shows pronounced analgesic activity of diclofenac (50 mg/kg b.w., p.o.) single dose as compared to the control group. However, a combination of caffeine with diclofenac exhibited the more potent analgesic effect. The efficacy in reducing pain significantly increased ($P<0.01$) with an increased dose of caffeine (50 mg/kg b.w., p.o.) in combination with diclofenac potassium (50 mg/kg b.w., p.o.) when compared to the control group. At this dose of caffeine (50 mg b.w., p.o.) with diclofenac inhibited pain by 88.41% while diclofenac potassium (50 mg/kg b.w., p.o.) alone inhibited 63.98%. Thus 50 mg caffeine with diclofenac potassium (50 mg/kg b.w., p.o.) showed around 24.43% more analgesic efficacy than diclofenac potassium (50 mg/kg b.w., p.o.) alone.

The time change sin righting reflex test of different test groups and control group have been graphically represented in the Fig.4. The scores of righting reflex test of different groups of experimental animals based on the time taken to turn over were represented in the bar graph (Fig.5). In most of the cases, the time changes in score result from muscle weakness. The results revealed that the righting latency reduced in mice received various treatments with diclofenac potassium 50 mg + caffeine 5 mg/ 25 mg/50 mg/kg b.w. (p.o.) as compared to the control group. The highest score was found in test Group VI (pain + diclofenac potassium 50 mg + caffeine 50 mg/kg b.w. (p.o.) showing the most significant result ($P<0.01$) as compared to the control group (pain induced group).

4. DISCUSSION

Various ethological and conventional parameters have been noted during the open field test session [26]. However, in the present experiment, there were no significant effects of treatment on defecation and urination responses of mice. Results for the locomotor activity of animals and the effect of three different dose regimens of caffeine (5, 25 and 50 mg/kg b.w.) on locomotion are shown in Fig. 6. Changes in each behaviour based on time duration and number of centre square entries have been provided by repeated measures in two successive days and the arithmetic mean was determined for each group of animals using ANOVA followed by Dunnett's test. The statistical

results show direct comparison between the normal group (no pain + no treatment) and the treatment groups (pain + diclofenac potassium 50 mg + caffeine caffeine 5 mg/25 mg/50 mg/kg b.w., p.o.) as well as control group (pain + diclofenac potassium 50 mg/kg b.w., p.o.) and treatment groups.



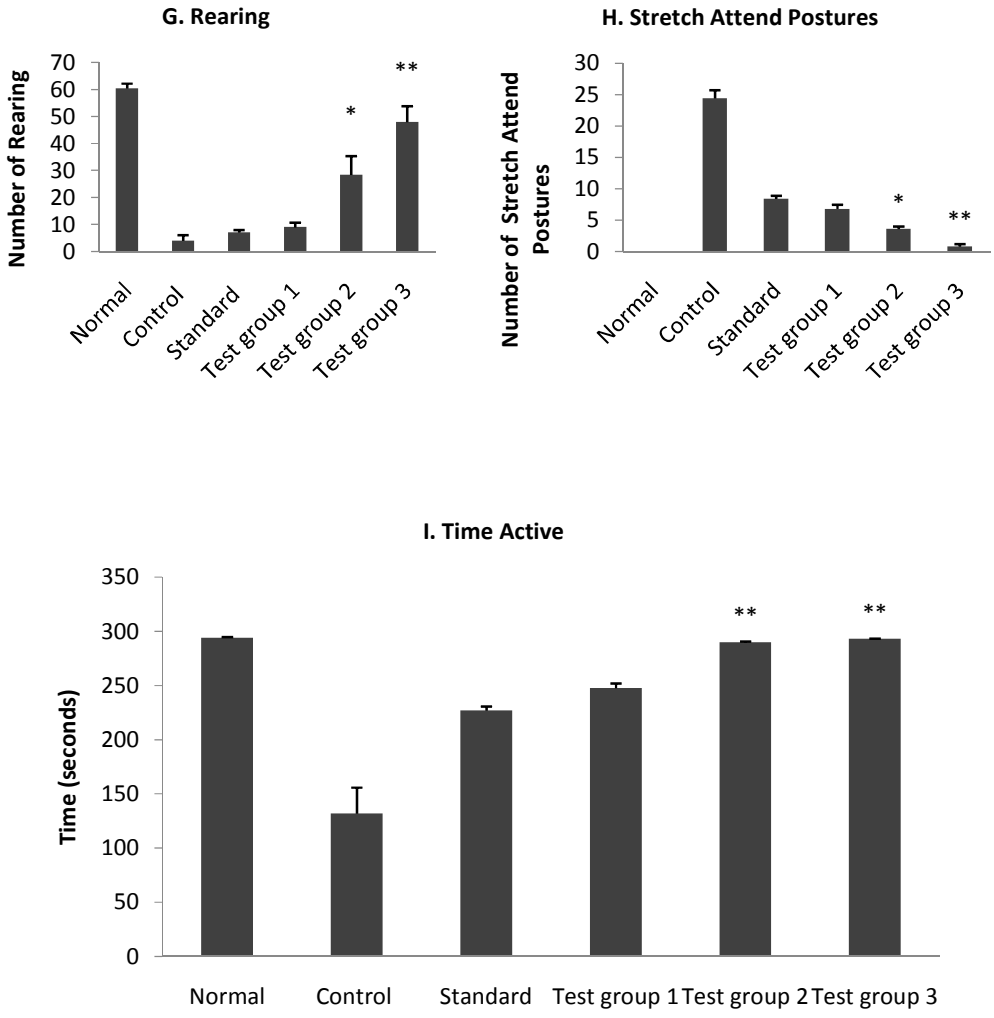


Fig. 1. Behavioral profile in five minutes time of different groups of animals.

Here, Normal = No pain and no medicine, Control = Pain only, Standard = Pain + Diclofenac potassium 50 mg/kgb.w. (p.o.), Test group 1 = Pain + Diclofenac potassium 50mg + Caffeine 5 mg/kgb.w. (p.o.), Test group 2 = Pain + Diclofenac potassium 50 mg + Caffeine 25 mg/kgb.w. (p.o.), Test group 3 = Pain + Diclofenac potassium 50 mg + Caffeine 50 mg/kgb.w. (p.o.). The total time spent in the center square, the number of center square entries followed by total duration of locomotion and other activities (eg., sniffing, grooming, rearing, stretch attend postures) were recorded within various locations of the open field (center, inner ring, outer ring, four corners marked in red and walls)

Data were represented as mean ± SEM, n = 5 in each group

**Indicates significance where P<0.05*

***Indicates very significance where P<0.01)*

Expected differences in animal behaviour and movement distortion among the groups (normal versus treatment and control versus treatment) were found. The normal group showed maximum entries in the centre square with the longest duration of stay. Among the test groups, Group VI (pain + diclofenac potassium 50 mg + caffeine 50 mg/kg b.w., p.o.) represented most of the entries in the centre square while other groups did not attempt for entries in five minutes of the experiment. The significant result (P<0.01) was found in Group VI when compared to the normal group for the behaviours involving sniffing, freezing, grooming, line crossing, rearing and

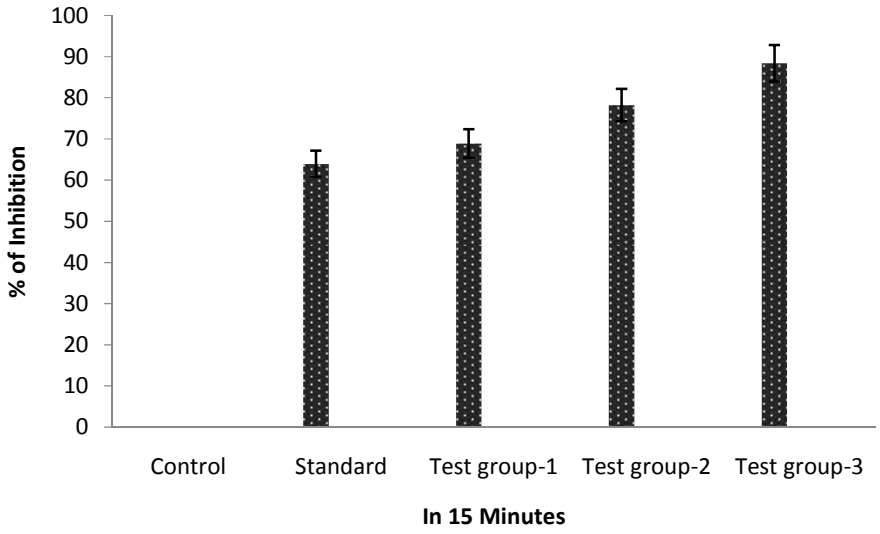


Fig. 2. Graphical representation of the percentage of pain inhibition in acetic acid (0.01 ml/g) induced writhing test in mice of different groups.

Here, Control = Pain + No drug, Standard = Pain + Diclofenac potassium 50 mg/kg b.w. (p.o.), Test groups 1/2/3 = Pain + Diclofenac potassium 50 mg + Caffeine 5 mg/25 mg/ 50 mg/kg b.w. (p.o.)
Data were represented as mean ± SEM, n = 5 in each group

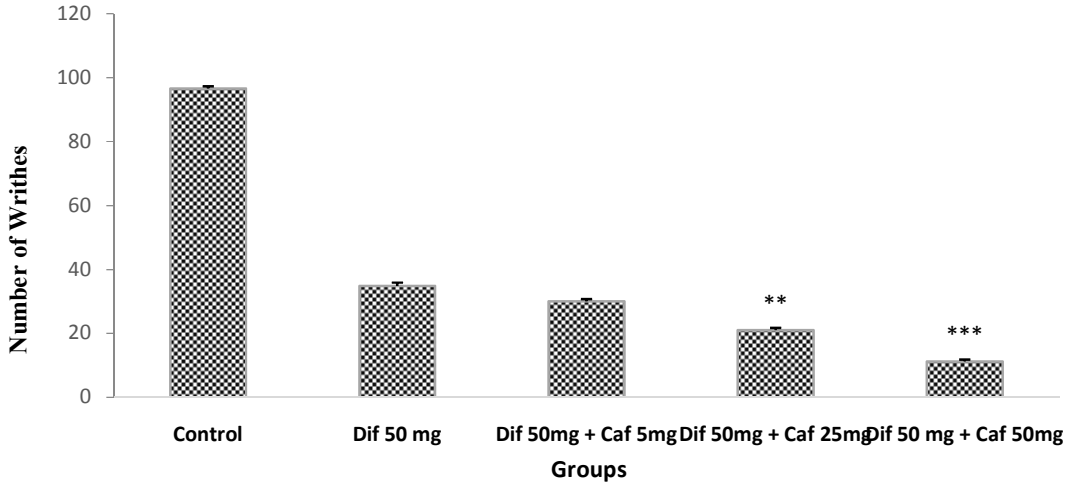


Fig. 3. Graphical representation of a number of writhes in acetic acid-induced writhing test in mice.

Here, Control = Pain + No drug, Standard = Pain + Diclofenac potassium 50 mg/kg b.w. (p.o.), Test groups = Pain +Diclofenac potassium 50 mg + Caffeine 5 mg/25 mg/50 mg/kg b.w. (p.o.)
Data were represented as mean ± SEM, n = 5 in each group
***Indicates significance where P<0.05*
****Indicates significance where P<0.01*

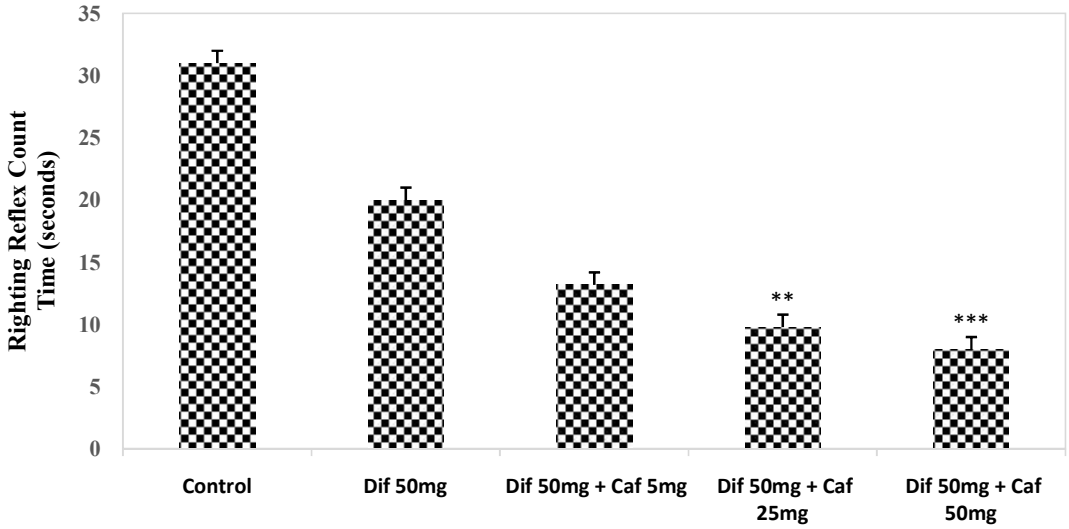


Fig. 4. Graphical representation of changes in righting reflex time.

Here, Control = No drug, Standard = Diclofenac potassium 50 mg/kg b.w. (p.o.), Test groups = Diclofenac potassium 50 mg/kg b.w. (p.o.) + Caffeine 5 mg/25 mg/50 mg/kg b.w. (p.o.)
Data were represented as mean ± SEM, n = 5 in each group
***Indicates significance where P<0.05*
****Indicates significance where P<0.01*

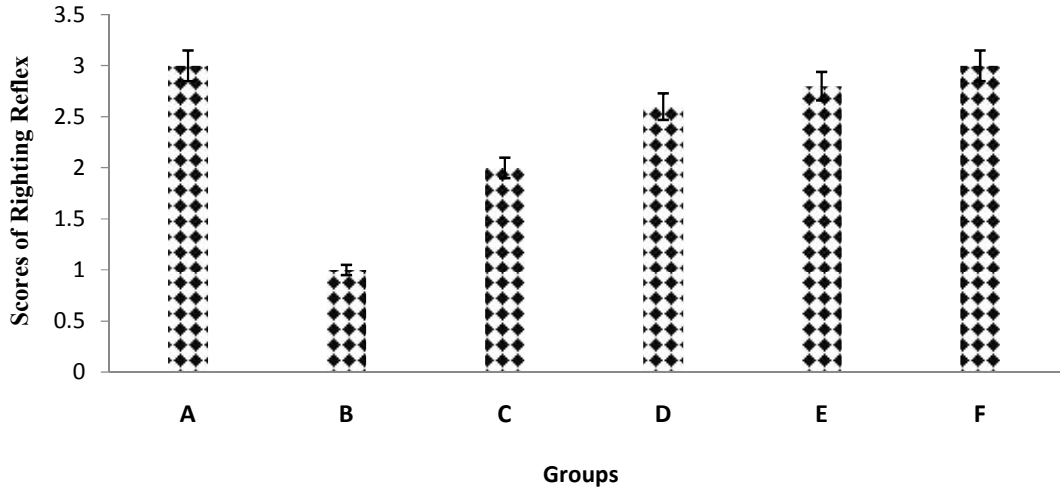


Fig. 5. Graphical representation of scores of righting reflex test among different groups of animals.

Here, A = Normal (no pain, no medicine), B = Control (pain only), C = Diclofenac potassium 50 mg/kg b.w. (p.o.), D = Diclofenac potassium 50 mg + Caffeine 5 mg/kg b.w. (p.o.), E = Diclofenac potassium 50 mg+Caffeine 25 mg/kg b.w. (p.o.), F = Diclofenac potassium 50 mg+Caffeine 50 mg/kg b.w. (p.o.)
Data were represented as mean ± SEM, n = 5 in each group

stretchattend postures. The animals of the same group (Group VI) exhibited maximum active time and minimum freezing time as compared to the control group and the standard group (pain +

diclofenac potassium 50 mg/kg b.w., p.o.). This indicates that the effect of dizziness or drowsiness due to the administration of diclofenac potassium (50 mg/kg b.w., p.o.) in reducing pain was effectively minimized by the higher dose of caffeine (50 mg/kg b.w., p.o.). The key findings of the present study are that the combined effect of diclofenac and caffeine exhibited statistically significant benefit against pain and drowsiness as compared to diclofenac alone. Several studies have been conducted through years using animal and human models regarding the effect of caffeine in relieving pain [3]. Data from previous studies showed caffeine as an analgesic adjuvant against mild to severe pain (including migraine pain) although the mechanisms by which it contributes to enhancing the efficacy of other analgesics (NSAIDs) are yet to be understood [3]. On the other hand, caffeine is considered as a competitive antagonist of adenosine receptors (A_1 and A_2) as a result this blocks the peripheral pro-nociceptive adenosine signalling and hence activate the central nor adenosine pathway (a pain suppressing system) [28]. Early reports suggested caffeine as an analgesic adjuvant in combination with a number of NSAIDs like paracetamol, ibuprofen, aspirin, diclofenac sodium and tolfenamic acid where caffeine was incorporated in different dose regimen [30,31]. The previous study was conducted in the human model using diclofenac sodium soft gel (100 mg) + caffeine (100 mg) versus diclofenac sodium soft gel (100 mg) where caffeine has been marked as an analgesic adjuvant in reducing migraine pain [23]. NSAIDs like paracetamol, ibuprofen, and aspirin are marketed in combination with caffeine. However, no oral dosage forms of diclofenac with caffeine are available in the market.

In the present study it was incorporated diclofenac potassium (50 mg/kg b.w., p.o.), a drug promptly used to treat mild to moderately severe pain like migraine pain, in combination with different dose regime of caffeine involving low to higher dose (5 mg, 25 mg and 50 mg/kg b.w., p.o.) in pain induced mice model. The present work demonstrated not only the role of caffeine as an analgesic adjuvant with diclofenac but also as an agent that could help to reduce its (diclofenac) adverse effect. Caffeine is widely used central nervous system stimulant of methylxanthine class as it increases the energy metabolism throughout the brain [29]. It activates noradrenalin neurons and causes the release of dopamine. The methylxanthine reportedly induced dose-response increase in locomotor activity in animals [26]. The study revealed the expectedbehaviour of animals when caffeine was added along with the analgesic (diclofenac potassium 50 mg) to treat acetic acid-induced pain. Caffeine potently minimized the side effect (drowsiness) of diclofenac potassium (50 mg/kg b.w., p.o.) by inducing maximum locomotion in animals during the experiment. This was analyzed by comparing the test groups (pain + diclofenac potassium 50 mg + caffeine 5 mg, 25 mg, and 50 mg/kg b.w., p.o.) with the standard group (pain + diclofenac potassium 50 mg/kg b.w., p.o.). The higher dose of caffeine (50 mg/kg b.w., p.o.) showed a significant benefit against the lethargy caused by diclofenac potassium (50 mg/kg b.w., p.o.). Although caffeine has been widely recognized as analgesic adjuvant it may simultaneously bring about anti-lethargic/anti-drowsiness efficacy when administered with diclofenac in reducing mild to moderately severe pain.

5. CONCLUSION

Overall, the results of the present study suggested that the combination of diclofenac potassium (50 mg/kg b.w., p.o.) and caffeine (50 mg/kg b.w., p.o.) may exhibit a potential approach to the treatment of mild to moderately severe pain with minimum side effect (i.e. drowsiness/dizziness). As caffeine helped to reduce the lethargic effect of diclofenac, therefore, the present combination may also be a promising therapeutic in case of migraine patients who are often prescribed with diclofenac potassium (50 mg) tablets as daytime doses since this will not have any interference in patient's day time activities. With regards to this beneficial effect, further studies are planned to develop a combined oral formulation (tablet) of diclofenac potassium (50 mg) and caffeine (50 mg) along with pre-clinical and clinical trials to assess its clinical efficacy in the treatment of pain with least drowsiness.

CONSENT

Consent is not applicable.

ETHICAL APPROVAL

All experimental procedures were approved by the Biomedical Research Center, University of Dhaka, Bangladesh.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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