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Behavioral Alterations Caused by Pain and Inflammation in Rodent Models

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Abstract

The aim of the current study was to examine the impact of pain and inflammation on CNS behavior in rodent models. Test mice were segmented into a control group and two test groups, group 1 and group 2. 1 ml acetic acid (0.7%) per mouse was used for pain induction whereas inflammation was induced using 0.2 ml 1% carrageenan per mouse. The results demonstrated after pain induction, the test group writhes an average of 18.5 and 16 times/15 min while no writhing in control group, indicating successful pain induction in test group. A 38.88% and 52.94% increased paw volume was observed after 30 min of carrageenan induction in test group 1 and 2, respectively. Both pain induced group 1 and 2 showed 79% and 78% lowered head dipping while 62% and 57% lowered head dipping was observed among test group 1 and 2 with inflammation in Hole board test. Reduced mobility time was observed from tail suspension test. Pain induced and inflammation induced test groups 1 and 2 showed 29% and 14% as well as 28.19% and 15.14% reduction in mobility time, respectively. Similarly test group 1 and 2 enduring pains demonstrated 50% and 35% less exploration to the open arm whereas 75% and 89% lower exploration to the open arm was exhibited by the test group 1 and 2 with persuaded inflammation, respectively in elevated plus maze test. All these results of behavioral tests suggested that both test groups had reduced CNS activity which possibly due to the induced inflammation and pain.

Key word: Pain, inflammation, depression, behavioral test.

Introduction

A plethora of factors have been identified to impact the normal state of the central nervous system. Central nervous system depression has been associated with several factors. CNS depression has been found to impact day to day life of individuals. Inflammation and pain have been associated with numerous health outcomes including effects on central nervous system, cardiopulmonary system, causing rheumatoid disease etc (Epsley *et al*., 2021; Walsh *et al*., 2005). Again, a number of illnesses, including neurological, metabolic, and mental disorders, cause localized or systemic inflammation. Extensive research has demonstrated a robust association between inflammatory conditions and metabolic disorders (Esposito and Giugliano, 2004; Hotamisligil, 2006). Inflammation is a defense mechanism that is used by the human body in order to fight any infection, unwanted damage, changes or invading microorganisms. It acts as a symptom for a wide range of maladies. Pericarditis, gingivitis, arthritis, pleuritis etc. are commonly known inflammatory diseases. Diseases like osteoarthritis, rheumatoid arthritis, fibromyalgia, gout, lyme diseases are associated with inflammation as well as pain and it in turn affects the overall quality of life (Fernihough *et al*., 2004; Kluivers-Poodt *et al*., 2013). Chronic pain is associated with emotional instability, altered cognition anxiety and mood fluctuation all of which can lead to depressive behavior. In contrast, depression can cause localized as well as systemic inflammation and pain. Several

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studies have identified impact of pain on nervous system (Fine, 2011; Jensen *et al*., 2007; Mitchell and Boss, 2002). Neither pain nor inflammation is part of normal physiology thus occurrence of any of these two conditions can impact normal physiologic processes. The multifactorial nature of pain and inflammation contributes to depressive tendencies in individuals. A complex bidirectional relationship exists between inflammation, pain, and depression. Diversified research on pain focuses on altered behavioral responses of mice (Savage and Ma, 2015). Several CNS tests are available to elucidate the behavioral responses of animals under study. Hole board test, elevated plus maze test, tail suspension test, forced swim test etc. the hole board test studies the explorative behavior of mice whereas forced swim test and tail suspension test evaluate depressive behavior of rodents (Brown and Nemes, 2008; Can *et al*., 2012). Anxiety like behavior in mice model is studied by using elevated plus maze test (Komada *et al*., 2008). To conclude the occurrence of altered CNS behavior at least three different tests should be performed. The principal focus of the current study is to identify the occurrence and extent of local pain and inflammation on the CNS behavior of rodent models by employing hole board test, elevated plus maze test and tail suspension test.

Materials and Method

Test animals: Test animals were collected from International Center for Diarrheal Disease Research, Bangladesh (ICDDR, B). Swiss albino (*Mus musculus*) mice of both sexes were used for the study. Housing was done at the animal house of Department of Pharmacy, East West University.

Induction of pain: Pain induction in test animals was done using acetic acid which is a common irritant used for evaluation of peripheral analgesic activity of test substances (Koster *et al*., 1959). For this purpose, each mouse was injected with 0.7 ml 0.7% acetic acid solution intraperitoneally. The presence of writhing ensured successful induction of pain in test animals.

Induction of inflammation: Carrageenan, a polysaccharide derived from red seaweed, is used as an inflammatory agent in a variety of research / studies (Otterness and Moore, 1988; Winter *et al.*, 1962)**.** 0.2 ml 1% carrageenan solution was injected in the sub plantar region of the right hind paw of each mouse. Paw volume measurement was done using plethysmometer before and after injecting carrageenan solution. Increase in paw volume indicated edema formation which is one of the basic symptoms of inflammation development.

Evaluation of CNS depression: Three different tests were employed for the evaluation of CNS depression in mice.

Elevated plus maze test (EMT): Each mouse is placed at the center of a four-arm maze of which two arms are closed and two are open. The mice are allowed to move freely in the apparatus and are observed for five minutes in order to determine the number and duration of times each mouse goes to the open and close ends of the maze. This is a widely used method to evaluate CNS activity of test rodents (Komada *et al*., 2008).

Tail suspension test: The tail suspension test is a well-established method for evaluating antidepressant effects of chemicals (Can *et al*., 2012). In this study, the test animal was suspended for five minutes from a certain distance above the ground. The immobility time for each mouse of both control and treatment groups *i.e.* inflammation and pain induced groups was measured and compared.

Hole board test: The exploratory capacity as well as anxiogenic behavior of test animal are measured by hole board test (Brown and Nemes, 2008; Takeda *et al*., 1998). Here a hole board consisting of 16 holes was used. The test mice were allowed to freely explore the hole board. The number and frequencies of head dipping showed by each mouse of every group was recorded and compared for five minutes.

Result and Discussion

Results of pain induction: Writhing is a positive indicator of the onset of pain. As a measure of pain

creation, Table 1 shows the average number of writhing in both the test and control groups. The study's conclusion demonstrated that whereas the control group did not exhibit any writhing, the test groups, which received a 1 ml solution of 0.7% acetic acid, did exhibit an average of 18.5 and 16 writhing per 15 minutes. The test groups' writhing indicate that the pain induction process was effective.

Table 1. Number of writhing among control and test group of mice.

Groups (n=5)	Average number of writhing per 15 minutes
Control	Nil
Test group 1	18.5
Test group 2	16

Results of inflammation induction: In case of, research mice an elevated paw volume signals inflammation induction. As represented in figure 1, initially, at 0 Hour, the average paw volume (ml) was 0.18 and 0.17 ml for the test group 1 and test group 2 and 0.17 ml for the control group. Following a 0.5-hour treatment with 1% carrageenan, test groups exhibited an increased paw volume of 38.88% and 52.94%, respectively although no such alteration was presented by the control group. This observation points to a definite sign of inflammation development in the mice in the test group.

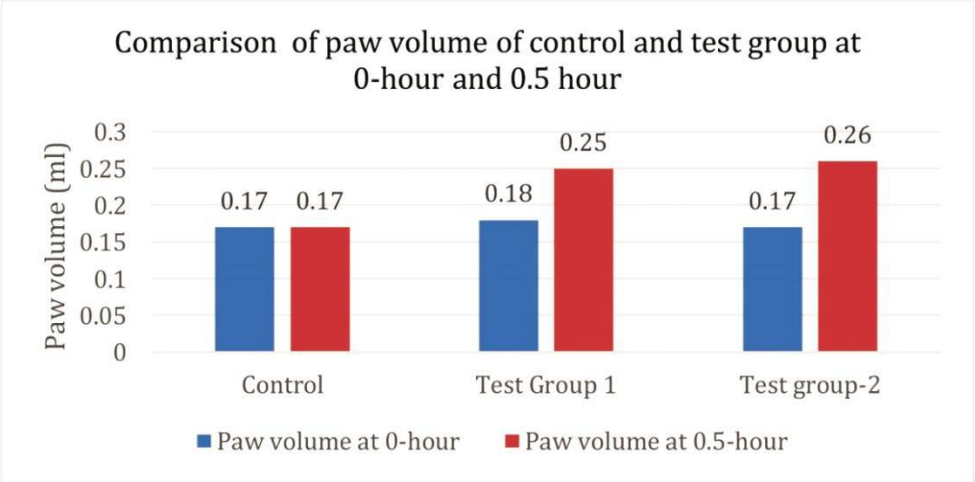

Figure 1. Comparison of paw volume at 0-hour and 0.5 hour between control and test group of mice.

Results of behavioral tests: Findings of diversified research works found to have positive correlation between depression, pain and inflammation. Different pathological conditions involve the presence of inflammation and pain which alter certain physiological processes that ultimately affect the behavioral patterns among individuals. CNS function can be quantified by employing a variety of behavioral tests including hole board test, tail suspension test and elevated plus maze test.

Outcomes of the performed hole board test, tail suspension test and elevated plus maze test indicated that test group had an increased depressive behavior although behavioral pattern among the control group remains invariable.

Results of hole board test: In the hole board study, the estimation of the number of head dips is utilized to investigate depressive behavior in the study object. As depicted in figure 2, the average head dipping (an average head dipping of 18.5 and 19.5 times per 5 minutes was portrayed by test group 1 and 2, respectively) of the mice in test groups 1 and 2 encountering pain was, 79% and 78% lower respectively, than that of the control group,

suggesting a higher depressive behavior among the test group of mice. The HBT results of test groups 1 and 2 with generated inflammation also showed depressive behavior in the test group of mice, with 62% and 57% less head drooping (test group 1 showed an average of 16 head dipping per 5 min, whereas test group 2 demonstrated an average of 18 head dipping per 5 min) than the control group. Even while both groups with pain and inflammation had depressive behavior, the findings of the hole board test showed that the test group in pain had a higher level of depression.

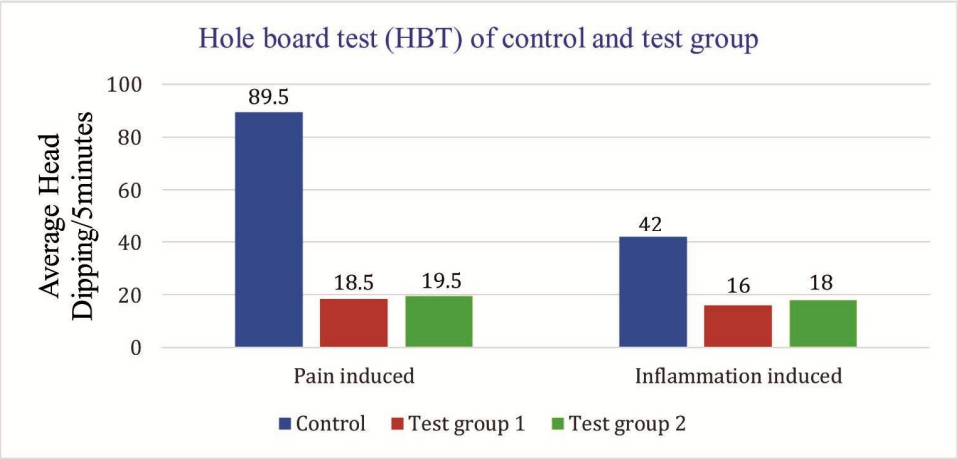

Figure 2. Average head dipping among control and study group of mice experiencing pain and inflammation.

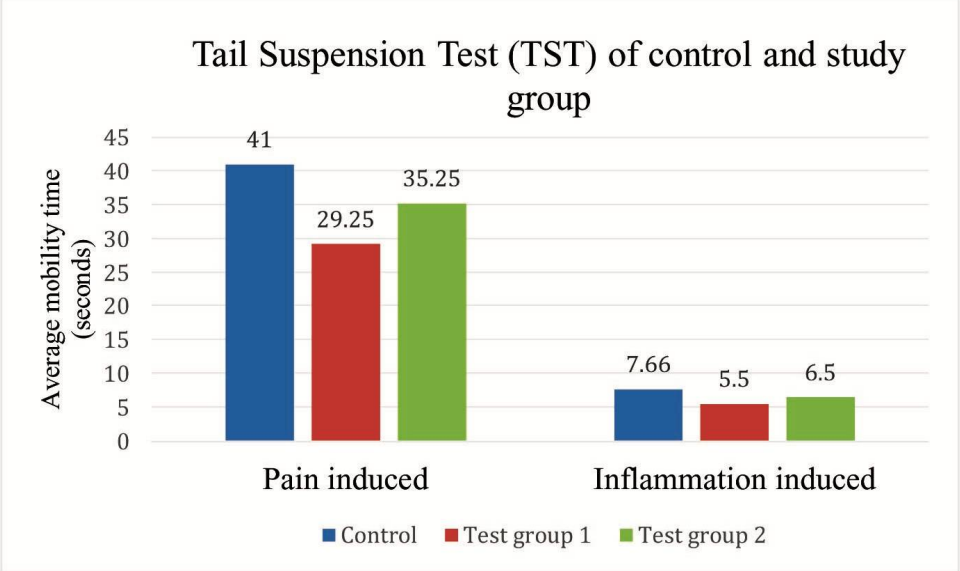

Figure 3. Average mobility time among control and test group with pain and inflammation.

Results of tail suspension test: The tail suspension test was also involved to evaluate depressive behavior in test and control mice. Each mouse was observed for 5 min. Test group 1 and 2 with induced pain exhibited 28.65% and 14.02% reduced mobility time (an average mobility time of 29.25 and 35.25 seconds per 5 min was depict by test group 1 and 2 respectively) compared to the control

group. Similarly, test group 1 and 2 which experienced inflammation depict 28.19% and 15.14% reduced mobility time than the control group respectively (5.5 seconds and 6.5 seconds of mobility time per 5 min was showed by test group 1 and 2). A reduction in mobility is indicative of a higher degree of depression. Reduced mobility time was seen in both the pain- and inflammation-induced groups indicating depressive behavior among the test groups. These results demonstrated that both inflammation and pain contributed to depression and thus affecting central nervous system function (Figure 3).

Result of elevated plus maze test: To assess how pain and inflammation affect CNS behavior, an elevated plus maze test was also performed. EMT used an assay of exploration to both open and closed ends where a lower exploration number indicates a higher depressive behavior. Compared to the control group, test groups 1 and 2 with pain demonstrated 50% and 35% less exploration (an average exploration of 5 times and 6.5 times per 5 min was demonstrated by test group 1 and 2 respectively) to the open end. Test groups with inflammation displayed a similar pattern, except they restricted their exploration to a greater extent *i.e.* 75% and 89%, respectively (an average exploration of 3.25 times and 1.33 times per 5 minutes was demonstrated by test group 1 and 2 respectively) (Figure-4).

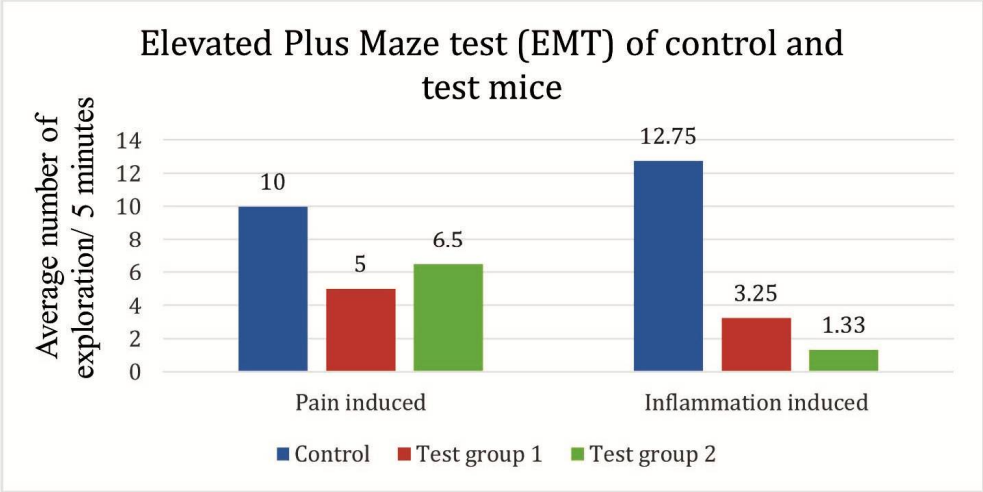

Figure 4. Number of explorations to open end by control group and test group.

Conclusions

The outcome of the study portrayed a considerable rise in depressive behavior in the test groups enduring pain or inflammation. The results of this study showed the need for more investigation into the processes underlying these phenomena as they show a direct correlation between pain, inflammation, and alterations in central nervous system function. The outcomes of this study also highlighted the significance of comprehensive studies to evaluate the intricate association among these physiological implications. To conclude, this study sheds light on potential directions for therapeutic interventions meant to lessen the undesirable impacts of pain, inflammation, and CNS behavior on general health and well-being. It also provides insightful information about the intricate interactions between these ailments. Further study is required to better comprehend the impact and develop therapeutic approaches to alleviate the detrimental impacts of these comorbidities.

Declaration by the authors

The current research is a modification of our previous work under same title which was published as an abstract for an oral presentation for the 5th

International Conference on Biotechnology in Health and Agriculture – 2023.

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