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Research Article

AMELIORATION OF ANXIOLYTIC BEHAVIOR IN INTRACEREBROVENTRICULAR COLCHICINE INJECTED RATS BY NAPROXEN

SUSMITA SIL, TUSHARKANTI GHOSH*

Neurophysiology Laboratory, Department of Physiology, University College of Science and Technology, University of Calcutta, Kolkata - 700 009, West Bengal, India. Email: tusharkantighosh53@yahoo.in

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ABSTRACT

Objective: Anxiety behavior in experimental model of Alzheimer's disease (AD) in rats by intracerebroventricular (ICV) injection of colchicine is important to characterize this animal model, but it has not been sufficiently investigated in this animal model. The different attributes of anxiety behavior in ICV colchicine injected rats (ICIR) was studied, and the effects of naproxen, a non-steroidal anti-inflammatory drug on the anxiety status of these AD animals were assessed since in earlier studies naproxen protected cognitive impairments and neurodegeneration in ICIR.

Methods: The anxiety status was assessed in an elevated open field with a novel object in two study durations (14-day and 21-day study). After measuring the anxiety behavior in two study durations, rats were sacrificed, and blood was collected for measuring the serum corticosterone (CORT) level.

Results: Anxiolytic behavior along with lower CORT level was observed in ICIR in both the 14- and 21-day studies. After p.o. administration of different doses of naproxen (5, 10, 20 mg/kg body wt.) in ICIR, this anxiolytic behavior along with low serum CORT level showed gradual recovery and eventually both the parameters attained normal level at the dose of 20 mg/kg body weight in 21-day study.

Conclusion: The present study showed an anxiolytic behavior in ICIR, and which may result from the colchicine induced neurodegeneration along with the impaired activity of the hypothalamo-pituitary-adrenal axis. Some parameters appeared to be sensitive for determination of anxiety status in this model.

Keywords: Colchicine, Anxiolytic, Naproxen, Corticosterone, Alzheimer's disease.

INTRODUCTION

Alzheimer disease (AD) is a form of dementia in which loss of memory is the first and the most characteristic symptom. There are around 35 million patients suffering from AD worldwide [1]. Although amnesia is the first and foremost clinical symptom of AD, other manifestations such as excitation, aggressiveness, apathy, disinterest, anxiety, depression, and disinhibition are also observed in this disease and these symptoms contribute significantly to the clinical profile in mild cognitive impairments, including AD [2]. Anxiety symptoms are common in AD patients and the prevalence of anxiety in AD patient was found to be in excess of 30% of the total AD population [3]. However, the anxiety and other symptoms are not consistently found in all AD patients [4].

Animal models are considered valuable tools for studying the pathophysiological mechanisms involved in the disease processes as well as for evaluating new therapeutic strategies for treatment of human diseases [5]. Memory impairment is the first and foremost characteristic feature in animal models of AD and the histopathological features are found in some animal models of AD. Emotional characteristics like anxiety in animal models of AD have not been studied sufficiently. However, some transgenic and chemical induced animal models of AD were used to evaluate anxiety-like behavior in rats and mice. Anxiogenic, anxiolytic or no change in anxiety behavior were observed in transgenic mice model of AD [6-8]. This inconsistency in the anxiety-like behavior in transgenic mice may originate from the different neuropathological changes, and different methods used in these models. Anxiogenic behavior has been indicated from chemically induced AD animal model such as streptozotocin and ibotenic acid-induced AD models in elevated plus maze (EPM) [9]. The method of assessing the anxiety-like behavior in EPM has been questioned by some investigators [10] because the animal takes shelter in the enclosed arm in the anxious state which resembles passive avoidance from open arms to reduce risk of threat [10]. Therefore, anxiety is the most persistent in a situation where uncertainty is predominant. An alternative method has been used by Ennaceur *et al*., [10] to measure anxiety by the response of rats to novelty in an elevated open field, which involves uncertainty with no safer alternative. The anxiety level may be truly assessed by this procedure in the animal model of AD, but no such experiments have been carried out as evident from the literature.

Intracerebroventricular (ICV) injection of colchicine, to rodents resulted in progressive impairment of memory and neurodegeneration which were characterized as a sporadic model of AD [11-18]. However, very little is known about non-cognitive behavioral effects of ICV injection of colchicine in rodents. Recently, Raghavendra *et al*., [19] have demonstrated that the anxiety-like behavior in colchicine induced model of AD in EPM was changed. It was reported that the administration of naproxen, a non-steroidal anti-inflammatory drug (NSAID) in ICV colchicine induced rat model resulted in the inhibition of neurodegeneration, with the concomitant recovery of cognitive impairments in this model [14,17]. The inhibition of neurodegeneration by naproxen in colchicine induced rats may have effects on the anxiety behavior similar to the memory impairments, as the neuronal network for anxiety may be protected in this experimental condition, and such study on the anxiety behavior may help to functionally characterize the ICV colchicine induced neurodegeneration. However, the naproxen mediated protection of colchicine induced neurodegeneration on anxiety behavior of ICIR has not been investigated.

Therefore, the present study was designed to investigate the different attributes of anxiety in ICV colchicine injected rats (ICIR) by the response to novelty in an elevated open field space and to study the effects of naproxen on the different attributes of anxiety in these rats.

METHODS

Animals

One hundred sixty-eight healthy, adult male albino rats (Charles–Foster strain) weighing 200-250 g were used in this study. Animals were housed individually in polypropylene animal cages with food pellet and water ad libitum. The animal room was maintained at the temperature of 25±1°C with a 12 hrs light-dark cycle (light 7 a.m. to 7 p.m.). The experimental protocol was approved by an Institutional Review Committee for the use animals. According to the regulations set by Institutional Animal Ethical Committee, all adequate measures were taken to minimize the pain and discomfort of the rats used for the study.

Design of experiments

168 rats were used in this study and were divided in the following way:

14-day study

72 rats were divided into three division: control, sham (ICV artificial CSF), and AD (ICV colchicine injection). Each one was divided into 4 groups which contain 6 animals each: Group I (without any naproxen), Group II (5 mg/kg body wt. of naproxen), Group III (10 mg/kg body wt. of naproxen), and Group IV (20 mg/kg body wt. of naproxen). Therefore, 12 groups were used in this study. On 14th day after ICV injection of colchicine/artificial CSF, all the rats in each group were subjected to a 10 minutes anxiety test in an elevated open field in response to a novel object. After the end of the anxiety test rats were sacrificed (under ether anesthesia) between 11 and 11.30 am and 1.5 ml blood was collected from the heart by a syringe and was kept for serum collection without any anticoagulant. With this serum corticosterone (CORT) level was measured in different groups of rats.

21-day study

72 rats were divided similar to the 14-day study and thus 12 groups were used in this study. All the rats in the 21-day study were subjected to anxiety test after 21-days of ICV colchicine/artificial CSF injection and then serum CORT levels were measured in these rats in the same manner like the 14-day study.

Study with alprazolam (positive control)

24 rats were divided into 4 groups: Group I (without any alprazolam), Group II (0.02 mg/kg body wt. of alprazolam), Group III (0.04 mg/kg body wt. of alprazolam), and Group IV (0.08 mg/kg body wt. of alprazolam). All the groups of alprazolam treated rats were subjected to anxiety test 1 hr after administration of alprazolam in the said doses.

Preparation of experimental rat model of AD by colchicine

In the lateral ventricle of rat, 7.5 µg of colchicine (SRL, India) dissolved in 2.5 µl artificial CSF [15] was injected slowly for 5 minutes. The lateral ventricle of both sides of the brain was approached stereotaxically [20] (AP-0.6 mm from bregma, L ±1.5 mm and V +2.8 mm below cortical surface) through a steel cannula (0.45 mm diameter) connected to a Hamilton syringe in anesthetized rats (Na-thiopentone, 50 mg/kg body wt. i.p.) and the method has been briefly described in Sil *et al*. [17].

Drug treatment

Treatment of naproxen

Naproxen (RPG Life Science Ltd., India) was dissolved in 10% alcohol and it was administered orally through a gastric cannula attached to a 1-ml syringe. The daily dose of naproxen was divided equally into two parts given at 6 hrs intervals each. Three doses of naproxen (5, 10, and 20 mg/kg body wt.) were given p.o. to different groups of rats for 14 and 21 days each starting from 4 days prior to colchicine (ICV) injection (for AD rats) and 4 days prior to vehicle (ICV) injection (for sham-operated rats). Control rats were also treated with naproxen for the same time period.

Treatment of alprazolam

Alprazolam (Sun Pharma Drugs Pvt. Ltd., India) was dissolved in distilled water and it was administered orally through a gastric cannula attached to a 1-ml syringe.

Measurement of anxiety behavior

The anxiety behavior was measured by the method described by Ennaceur *et al*. [10] in open field space. This method use response of rats to novelty in an open space field. The adopted method is briefly described below:

Apparatus

The apparatus consists of a wooden platform (width 80 cm × length 80 cm × height 50 cm) elevated by 120 cm from the ground. The objects (width 8 cm × length 8 cm × height 13 cm), which were to be explored were in triplicate and were alternated between animals. They were made of white ceramic, a biologically neutral material. The objects were of heavy weight so that the animals could not move them around in the arena. These objects had never been associated with a reinforcer and are not known to have any ethological significance for the rats. The field of the open space was divided in the outer area, inner area, and object area. The outer area was 15 cm wide from the edge of the field. The inner area was 10 cm × 10 cm wide located in the middle of the field. The object area is defined by a circle drawn in the center, which is 2 cm larger than the bottom surface of the objects that was used in the experiment.

Testing

The experiments were carried out in open space with an object in the center. These required testing animals in a single session on 14th and 21st day after colchicine or vehicle injection along with control rats for all the groups. Alprazolam treated rats were also tested in a single session after 1 hr of drug administration. In each session, all the rats in each group were tested for 10 minutes. The surface of the platform of the apparatus was cleaned with 90% ethanol after testing of each rat and left to dry before the introduction of the next rat to minimize the effects of lingering olfactory cues. Rats were released into an open space from any part of the outer area (randomly pre-defined) facing away from the inner area. They were released for 10 minutes to explore the area of the open space. Rats were observed on a screen monitor connected to a video camera suspended above the test arena. The latency of first entry to the inner area, frequency of entry to the inner area, total time spent in the inner area, total time spent in the outer area, latency of first approach to the object area and frequency of approach to the object area were noted in each session.

Blood collection

Blood was collected (1.5 ml, between 11:00 a.m. and 11:30 a.m.) from the heart of anesthetized rat (Diethyl Ether, Kabra Drugs, India) by a syringe and was kept for serum collection without any anticoagulant. With this serum, CORT level was measured in different groups of rats in 14- and 21-day study.

CORT level

Serum CORT concentration was determined by radioimmunoassay using a commercially available kit (^{125}I Rat CORT [MP Biomedicals, LIC, Diagnostics Division, Ohio]) and gamma counter. The antisera used for the assay was highly specific for the rat CORT and it had 1.58% cross reactivity with 11-deoxy CORT. The assay sensitivity is approximately 10 ng/ml and intra inter-assay coefficient variation was <10%. Quality control serum was used for the assay, and all the experimental samples were run in duplicate.

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). One-way ANOVA was employed to compare the data of the control, sham-operated, and AD experimental groups, followed by Tukey's multiple comparison test using the Statistical Package for Social science Software (SPSS software: 20.0.0, USA).

RESULTS

Latency of first entry to the inner area

The latency of first entry to the inner area was significantly increased (F [23, 120] = 2.773, [p<0.001]] in AD rats in both the 14-day and 21-day study compared to that of respective control (C) and sham-operated (S) rats. After administration of naproxen in AD rats at the doses of 5 mg, 10 mg, and 20 mg/kg body weight, the latency of first entry to the inner area was significantly lower (p<0.001) compared to AD rats in 21-day study but not in the 14-day study. The latency of first entry to the inner area was not significantly changed among the eight groups of control and sham rats in both the study durations (Fig. 1).

Positive control

The latency of first entry to the inner area was significantly increased (F [3, 20] = 6.936, [p<0.01]) in rats treated with 0.04 and 0.08 mg/kg body weight of alprazolam compared to control rats, but not with alprazolam at the dose of 0.02 mg/kg body wt. The increase of latency of first entry to the inner area in rats after administration of different doses of alprazolam showed a graded effect and there was significant difference (p<0.05) between 0.02 mg and 0.04 mg/kg body weight dose in this study (Fig. 2).

Frequency of entry to the inner area

Frequency of entry to the inner area significantly decreased (F [23, 120] = 5.221) in AD rats in both the 14-day (p<0.05) and 21-day (p<0.001) study compared to that of respective control (C) and sham-operated (S) rats. After administration of naproxen in AD rats at the dose of 20 mg/kg body weight, frequency of entry to the inner area was significantly higher (p<0.001) compared to AD rats in 21-day study, but not in the 14-day study. The frequency of entry to the inner area was not significantly changed among the eight groups of control and sham rats in both the study durations (Fig. 3).

Positive control

The frequency of entry to the inner area was significantly decreased (F [3, 20] = 12.603) in rats treated with 0.04 (p<0.01) and 0.08 (p<0.001) mg/kg body weight of alprazolam compared to control rats, but not with alprazolam at the dose of 0.02 mg/kg body wt. The decrease of frequency of entry to the inner area in rats after administration of different doses of alprazolam showed a graded effect and there was significant difference between 0.02 mg and 0.04 mg (p<0.05), 0.02 and 0.08 (p<0.001) mg/kg body weight doses in the present study (Fig. 4).

Total time spent in the inner area

The total time spent in the inner area significantly decreased (F [23, 120] = 8.323, [p<0.001]) in AD rats in both the 14-day and 21-day study compared to that of respective control (C) and sham-operated (S) rats. After administration of naproxen in AD rats at the dose of 20 mg/kg body weight, total time spent in the inner area was significantly higher (p<0.05) compared to AD rats in both the study durations. The increase in total time spent in the inner area of AD animals after administration of different doses of naproxen showed a graded effect and there was significant difference (p<0.001) between AD rats treated with 5 mg and 10 mg with 20 mg/kg body weight of naproxen in 14-day study. The total time spent in the inner area was not significantly changed among the eight groups of control and sham rats in both the study durations (Fig. 5).

Positive control

The total time spent in the inner area was significantly decreased (F [3, 20] = 37.7, [p<0.001]) in rats treated with 0.02, 0.04, and 0.08mg/kg body weight of alprazolam compared to control rats (Fig. 6).

Total time spent in the outer area

The total time spent in the outer area significantly increased (F [23, 120] = 61.581, [p<0.001]) in AD rats in both the 14-day and 21-day study compared to that of respective control (C) and sham-operated (S) rats. After administration of naproxen in AD rats at the dose of 20 mg/kg body weight, total time spent in the outer area was significantly lower (p<0.001) compared to AD rats in14-day study. Similarly, after administration of naproxen in AD rats at the doses of 10 and 20 mg/kg body weight, total time spent in the outer area did not increase and it was significantly lower (p<0.001) compared to AD rats in 21-day study. The decrease in total time spent in the outer area of AD animals after administration of different doses of naproxen showed a graded effect and there was significant difference between AD rats treated with 5 mg and 10 mg with 20 mg/kg body weight of naproxen

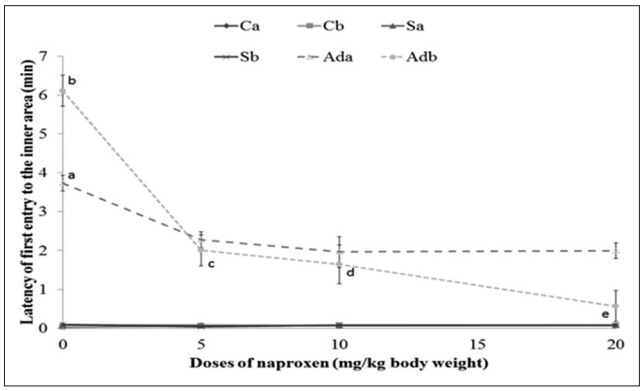

Fig. 1: Latency of first entry to the inner area of different groups of rats. [a]Significant at p<0.001 (Alzheimer's disease [AD] vs. control/sham-operated rats in 14-day study), [b]Significant at p<0.001 (AD vs. control/sham-operated rats in 21-day study), [c]Significant at p<0.05 (AD vs. naproxen treated AD rats at the dose of 5 mg/kg body weight in 21-day study), [d]Significant at p<0.05 (AD vs. naproxen treated AD rats at the dose of 10 mg/kg body weight in 21-day study), [e]Significant at p<0.001 (AD vs. naproxen treated AD rats at the dose of 20 mg/kg body weight in 21-day study). Abbreviations: Ca (Control rats of 14-day study), Cb (Control rats of 21-day study), Sa (Sham-operated rats of 14-day study), Sb (Sham-operated rats of 21-day study), Ada (Alzheimer's disease rats of 14-day study), Adb (Alzheimer's disease rats of 21-day study). Values are expressed in mean ± standard error of mean (n=6 in each group)

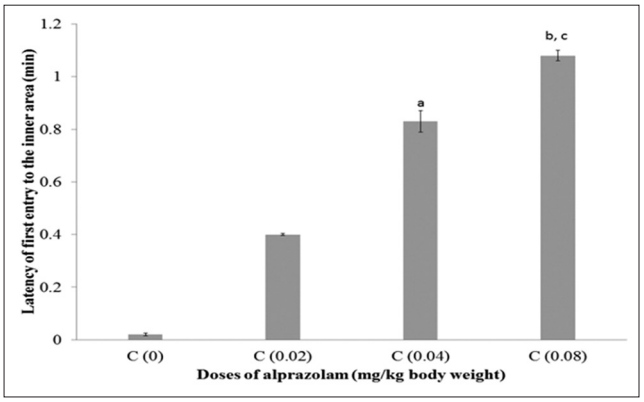

Fig. 2: Latency of first entry to the inner area of the positive control group. [a]Significant at p<0.01 (control vs. rats treated with 0.04 mg/kg body weight of alprazolam), [b]Significant at p<0.01 (control vs. rats treated with 0.08 mg/kg body weight of alprazolam), [c]Significant at p<0.05 (rats treated with 0.02 mg/kg body weight of alprazolam vs. rats treated with 0.08 mg/kg body weight of alprazolam). Abbreviations: C (0) (control rats without any drug treatment), C (0.02) (rats treated with 0.02 mg/kg body weight of alprazolam), C (0.04) (rats treated with 0.04 mg/kg body weight of alprazolam), C (0.08) (rats treated with 0.08 mg/kg body weight of alprazolam). Values are expressed in mean ± standard error of mean (n=6 in each group)

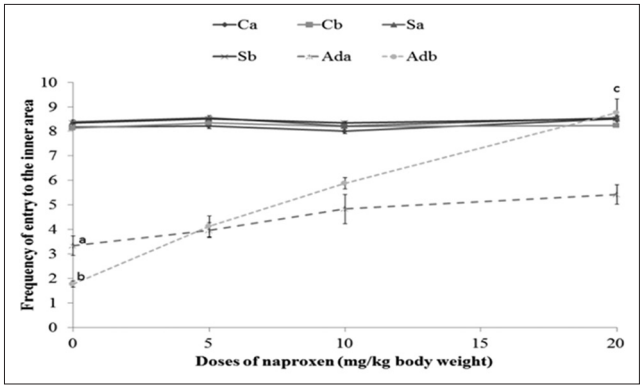

Fig. 3: Frequency of entry to the inner area of different groups of rats. [a]Significant at p<0.01 (Alzheimer's disease [AD] vs. control/ sham-operated rats in 14-day study), [b]Significant at p<0.001 (AD vs. control/sham-operated rats in 21-day study), [c]Significant at p<0.001 (AD vs. naproxen treated AD rats at the dose 20 mg/kg body weight in 21-day study). Abbreviations are same as Fig. 1. Values are expressed in mean ± standard error of the mean (n=6 in each group)

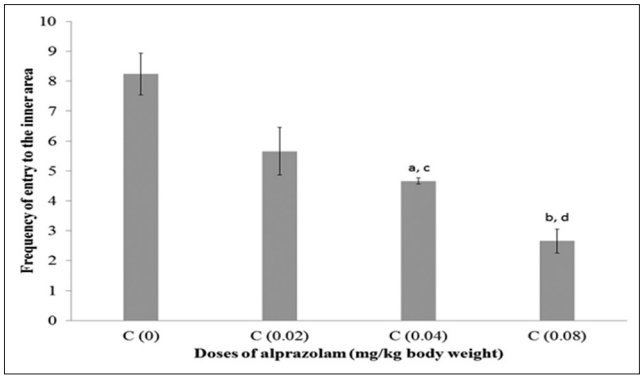

Fig. 4: Frequency of entry to the inner area of the positive control group. [a]Significant at p<0.01 (Control vs. rats treated with 0.04 mg/kg body weight of alprazolam), [b]Significant at p<0.001 (Control vs. rats treated with 0.08 mg/kg body weight of alprazolam), [c]Significant at p<0.05 (Rats treated with 0.02 mg/kg body weight of alprazolam vs. rats treated with 0.04 mg/kg body weight of alprazolam), [d]Significant at p<0.001(Rats treated with 0.02 mg/kg body weight of alprazolam vs. rats treated with 0.08 mg/kg body weight of alprazolam). Abbreviations are same as Fig. 2. Values are expressed in mean ± standard error of the mean (n=6 in each group)

in both the study durations (p<0.001). The total time spent in the outer area was not significantly changed among the eight groups of control and sham rats in both the study durations (Fig. 7).

Positive control

The total time spent in the outer area was significantly increased (F [3, 20] = 155.561, [p<0.001]) in rats treated with 0.02, 0.04 and 0.08 mg/kg body weight of alprazolam compared to control rats (Fig. 8).

Latency of first approach to the object area

The latency of first approach to the object area significantly increased (F [23, 120] = 15.024, [p<0.001]) in AD rats in both the 14-day and 21-day study compared to that of respective control (C) and sham-operated (S) rats. After administration of naproxen in AD rats at the doses of 10 and 20 mg/kg body weight, latency of the first approach to the object area was significantly lower (p<0.001) compared to AD rats in both the study durations. Latency of the first approach to the object area was not significantly changed among the eight groups of control and sham rats in both the study durations (Fig. 9).

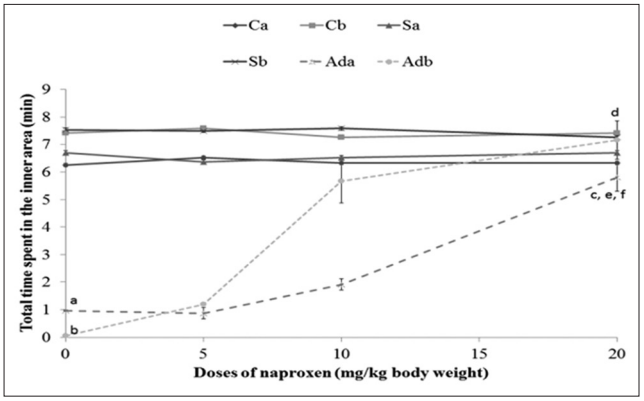

Fig. 5: Total time spent in the inner area of different groups of rats. [a]Significant at p<0.001 (Alzheimer's disease [AD] vs. control/sham-operated rats in 14-day study), [b]Significant at p<0.001 (AD vs. control/sham-operated rats in 21-day study), [c]Significant at p<0.05 (AD vs. naproxen treated AD rats at the dose 20 mg/kg body weight in 14-day study), [d]Significant at p<0.05 (AD vs. naproxen treated AD rats at the dose 20 mg/kg body weight in 21-day study), [e]Significant at p<0.01(naproxen treated AD rats at the dose 5 mg/kg body weight vs. 20 mg/kg body weight in 14-day study), [f]Significant at p<0.01 (naproxen treated AD rats at the dose 10 mg/kg body weight vs. 20 mg/kg body weight in 14-day study). Abbreviations are same as Fig. 1. Values are expressed in mean ± standard error of the mean (n=6 in each group)

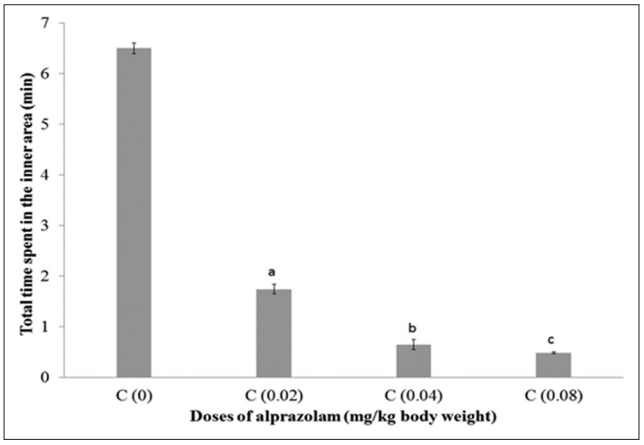

Fig. 6: Total time spent in the inner area of the positive control group. [a]Significant at p<0.001 (Control vs. rats treated with 0.02 mg/kg body weight of alprazolam), [b]Significant at p<0.001 (Control vs. rats treated with 0.04 mg/kg body weight of alprazolam), [c]Significant at p<0.001 (Control vs. rats treated with 0.08 mg/kg body weight of alprazolam). Abbreviations are same as Fig. 2. Values are expressed in mean ± standard error of the mean (n=6 in each group)

Positive control

The latency of first approach to the object area was significantly increased (F [3, 20] = 34.237, [p<0.001]) in rats treated with 0.04 and 0.08 mg/kg body weight of alprazolam compared to control rats, but not with alprazolam at the dose of 0.02 mg/kg body wt. The increase of latency of first approach to the object area in rats after administration of different doses of alprazolam showed a graded effect and there was significant difference (p<0.001) between 0.02 mg and 0.04 mg, 0.02 and 0.08 mg/kg body weight doses in the present study (Fig. 10).

Frequency of approach to the object area

The frequency of approach to the object area significantly decreased (F [23, 120] = 38.031, [p<0.001]) in AD rats in both the 14-day and 21-day study compared to that of respective control (C) and sham-

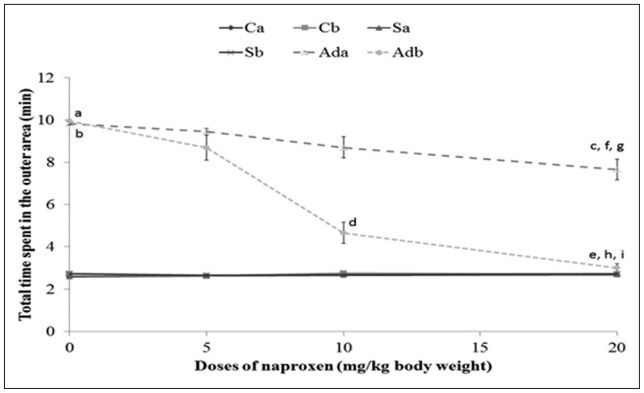

Fig. 7: Total time spent in the outer area of different groups of rats. [a]Significant at p<0.001 (Alzheimer's disease [AD] vs. control/ sham-operated rats in 14-day study), [b]Significant at p<0.001 (AD vs. control/sham-operated rats in 21-day study), [c]Significant at p<0.001 (AD vs. naproxen treated AD rats at the dose of 20 mg/kg body weight in 14-day study), [d]Significant at p<0.01 (AD vs. naproxen treated AD rats at the dose of 10 mg/kg body weight in 21-day study), [e]Significant at p<0.001 (AD vs. naproxen treated AD rats at the dose of 20 mg/kg body weight in 21-day study), [f]Significant at p<0.001 (naproxen treated AD rats at the dose 5 mg/kg body weight vs. 20 mg/kg body weight in 14-day study), [g]Significant at p<0.001 (naproxen treated AD rats at the dose 10 mg/kg body weight vs. 20 mg/kg body weight in 14-day study), [h]Significant at p<0.001 (naproxen treated AD rats at the dose 5 mg/kg body weight vs. 20 mg/kg body weight in 21-day study), [i]Significant at p<0.001 (naproxen treated AD rats at the dose 10 mg/kg body weight vs. 20 mg/kg body weight in 21-day study). Abbreviations are same as Fig. 1. Values are expressed in mean ± standard error of the mean (n=6 in each group)

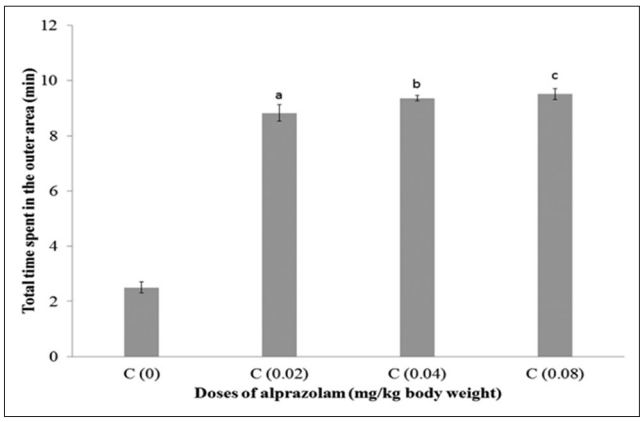

Fig. 8: Total time spent in the outer area of positive control group. [a]Significant at p<0.001 (Control vs. rats treated with 0.02 mg/kg body weight of alprazolam), [b]Significant at p<0.001 (Control vs. rats treated with 0.04 mg/kg body weight of alprazolam), [c]Significant at p<0.001 (Control vs. rats treated with 0.08 mg/kg body weight of alprazolam). Abbreviations are same as Fig. 2. Values are expressed in mean ± standard error of the mean (n=6 in each group)

operated (S) rats. After administration of naproxen in AD rats at the dose of 20 mg/kg body weight, frequency of approach to the object area did not decrease and it was significantly higher (p<0.001) compared to AD rats in 21-day study. The increase in frequency of approach to the object area of AD animals after administration of different doses of naproxen showed a graded effect and there was significant difference between AD rats treated with 5 mg and 20 mg/kg body weight of naproxen (p<0.001) and again between AD rats treated with 10 mg and

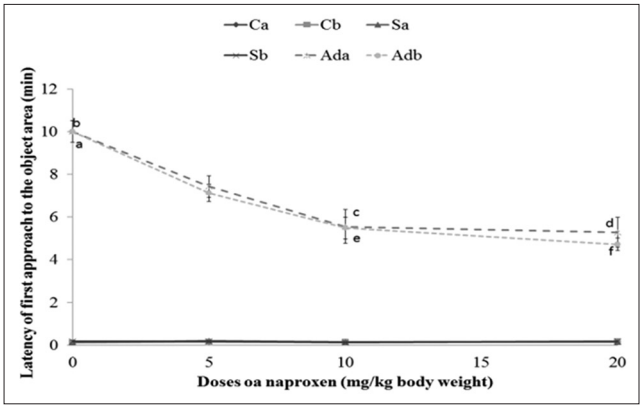

Fig. 9: Latency of first approach to the object area of different groups of rats. [a]Significant at p<0.001 (Alzheimer's disease [AD] vs. control/sham-operated rats in 14-day study), [b]Significant at p<0.001 (AD vs. control/sham-operated rats in 21-day study), [c]Significant at p<0.001 (AD vs. naproxen treated AD rats at the dose of 10 mg/kg body weight in 14-day study). [d]Significant at p<0.001 (AD vs. naproxen treated AD rats at the dose of 20 mg/kg body weight in 14-day study). [e]Significant at p<0.001 (AD vs. naproxen treated AD rats at the dose of 10 mg/kg body weight in 21-day study). [f]Significant at p<0.001 (AD vs. naproxen treated AD rats at the dose of 10 mg/kg body weight in 21-day study). Abbreviations are same as Fig. 1. Values are expressed in mean ± standard error of the mean (n=6 in each group)

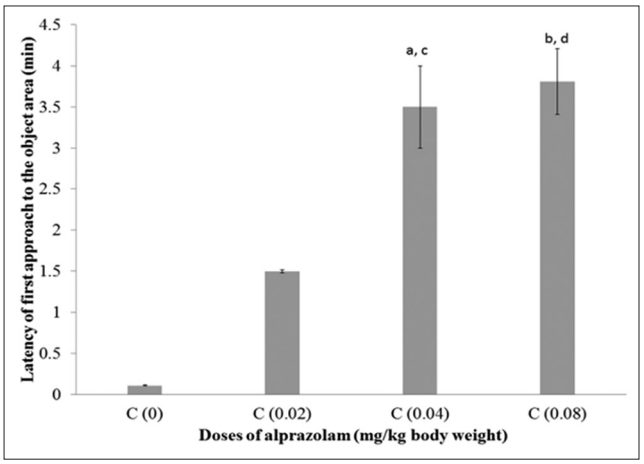

Fig. 10: Latency of first approach to the object area of the positive control group. [a]Significant at p<0.001 (Control vs. rats treated with 0.04 mg/kg body weight of alprazolam), [b]Significant at p<0.001 (Control vs. rats treated with 0.08 mg/kg body weight of alprazolam), [c]Significant at p<0.001 (Rats treated with 0.02 mg/kg body weight of alprazolam vs. rats treated with 0.04 mg/kg body weight of alprazolam), [d]Significant at p<0.001 (Rats treated with 0.02 mg/kg body weight of alprazolam vs. rats treated with 0.08 mg/kg body weight of alprazolam). Abbreviations are same as Fig. 2. Values are expressed in mean ± standard error of the mean (n=6 in each group)

20 mg/kg body weight of naproxen (p<0.01) in 21-day study. There was a significant difference between the 14-day and 21-day study of 20 mg treated AD rats (p<0.05). The frequency of approach to the object area was not significantly changed among the eight groups of control and sham rats in both the study durations (Fig. 11).

Positive control

The frequency of approach to the object area was significantly decreased (F [3, 20] = 41.848, [p<0.001]) in rats treated with 0.02, 0.04

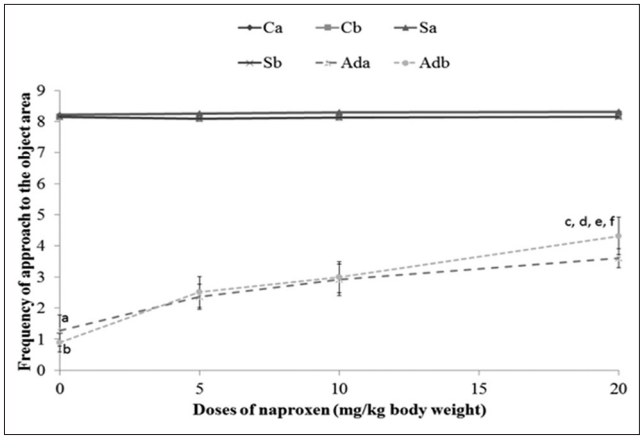

Fig. 11: Frequency of approach to the object area of different groups of rats. [a]Significant at P<0.001 (Alzheimer's disease [AD] vs. control/sham-operated rats in 14-day study). [b]Significant at p<0.001 (AD vs. control/sham-operated rats in 21-day study). [c]Significant at p<0.001 (AD vs. naproxen treated AD rats at the dose of 20 mg/kg body weight in 21-day study).[d]Significant at p<0.001(naproxen treated AD rats at the dose 5 mg/kg body weight vs. 20 mg/kg body weight in 21-day study), [e]Significant at p<0.01 (naproxen treated AD rats at the dose 10 mg/kg body weight vs. 20 mg/kg body weight in 21-day study), [f]Significant at p<0.05 (naproxen treated AD rats at the dose of 20 mg/kg body weight between 14-day and 21-day study). Abbreviations are same as Fig 1. Values are expressed in mean ± standard error of the mean (n=6 in each group)

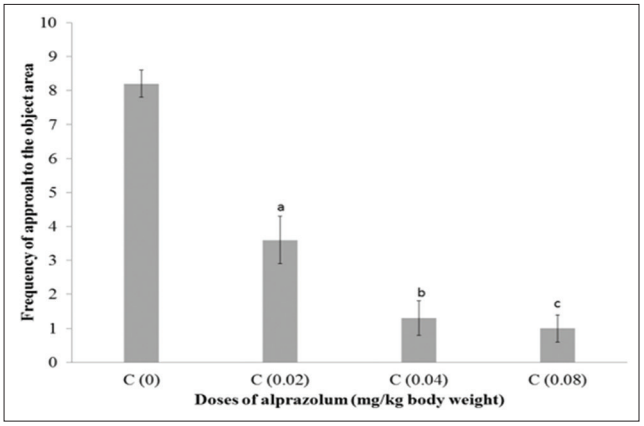

Fig. 12: Frequency of approach to the object area of the positive control group. [a]Significant at p<0.001 (Control vs. rats treated with 0.02 mg/kg body weight of alprazolam), [b]Significant at p<0.001 (Control vs. rats treated with 0.04 mg/kg body weight of alprazolam), [c]Significant at p<0.001 (Control vs. rats treated with 0.08 mg/kg body weight of alprazolam). Abbreviations are same as Fig. 2. Values are expressed in mean ± standard error of the mean (n=6 in each group)

and 0.08 mg/kg body weight of alprazolam compared to control rats (Fig. 12).

Serum CORT level (ng/ml)

The serum CORT level was significantly decreased (F [23, 120] = 7.136, [p<0.001]) in AD rats in both the 14-day and 21-day study compared to that of respective control (C) and sham-operated (S) rats. After administration of naproxen in AD rats at the dose of 20 mg/kg body weight, serum CORT level was significantly higher (p<0.001) compared to AD rats in 14-day study but in 21-day study serum CORT level was significantly increased after administration of naproxen in all doses. The serum CORT level in AD animals after administration of different doses of naproxen showed a graded effect and there was a significant difference in serum CORT between AD rats treated with 5 mg and 20 mg/kg body weight of naproxen (p<0.001) in 14-day study. The serum CORT level was not significantly changed among the eight groups of control and sham rats in both the study durations (Fig. 13).

DISCUSSION

The procedure of the measurement of anxiety status of rats in the present study is different from the EPM. In an open space animals face intermingled threat and fear from the entire surrounding and novelty [10], but the threat is more on the surroundings of the elevated open area compared to the inner area. On the other hand, the novel object in the inner area is another source of fear but the inner part is comparatively safe place than the outer area. According to Ennaceur *et al.* [10] the rats with high anxiety level would spend more time in the inner area in spite of the threat of novelty, but they would spend more time in outer area when anxiety status is reduced. In the present study, all the parameters probably indicate the anxiolytic behavior in ICIR. Though the molecular pathogenesis of transgenic AD rats and ICIR are not similar, some transgenic AD mice also showed reduced anxiety-like behavior [21]. However, the anxiety status in transgenic mice as reported by several investigators is inconsistent and contradictory. In EPM while anxiogenic behavior was observed in Tg 2576 mice [22], anxiolytic behavior was noted in other type of transgenic mice (mutant PS1 and PS2 genes) and still in transgenic mice overexpressing APP, anxiety status was not changed [6]. Arendash *et al.* [23] reported no change in anxiety status and Reiserer *et al*. [7] reported decreased anxiety in APP 1 PS1 mice. Similarly, Touma *et al*. [6] reported no change in anxiety status in TgCRND8 mice and Lee *et al*. [24] showed anxiogenic behavior in the same mice model in the EPM. Raghavendra *et al.,* [19] reported anxiogenic behavior in ICIR using EPM as indicated by the decrease in the number of entries and time spent in the open arms in comparison to the closed arms. The present study, however, indicates an opposite change in anxiety behavior in ICIR. The entry and staying in the enclosed space in EPM may not indicate the changes of anxiety as the animals prefer a safe alternative of the enclosed arm or dark space rather than taking a risk of entering in the open space and staying there [25]. Anxiety is expressed when neither escape nor avoidance is possible [10] unlike what is observed in EPM. Therefore, the design of behavioral test in EPM may not indicate true anxiety level [10]. Knyazev *et al*. [26] concluded that anxiety is generated by the concurrent and equivalent activation of fear and reward system, and therefore, anxiety should be the most evident in a situation of uncertainty when chances of gaining and losing are about equal. If uncertainty disappears anxiety should give way to the satisfaction or sadness. The anxiety assessment procedure that involves uncertainty is considered appropriate one to indicate its status. The method of response to novelty in an open space model has been developed by Ennaceur *et al*. [10] which can be considered an appropriate procedure to measure anxiety. The parameters for anxiety assessment such as total time spent in the inner area and outer area, latency of first approach to the inner/object area and frequency of approach to the inner/object area were altered significantly in ICIR compared to that of control in both the study durations. These parameters may be considered sensitive for determination of anxiety status in this model. ICIR has been used by several authors as a sporadic model of AD [13,15-18,27]. The current study provides, to our knowledge, the first demonstration of reduced anxiety-like behavior in colchicine induced AD rats.

The method used to measure the anxiety behavior in the present study has been validated by the positive control experiment, i.e. the parameters of anxiety were measured by this method in control animals after the administration of p.o. administration of alprazolam, an anxiolytic drug. Rats treated with alprazolam showed an increase in the latency to enter the inner area and object area, but the frequency of entries to these areas was decreased. Results also showed that time spent in the outer area was increased whereas time spent in the inner area was decreased

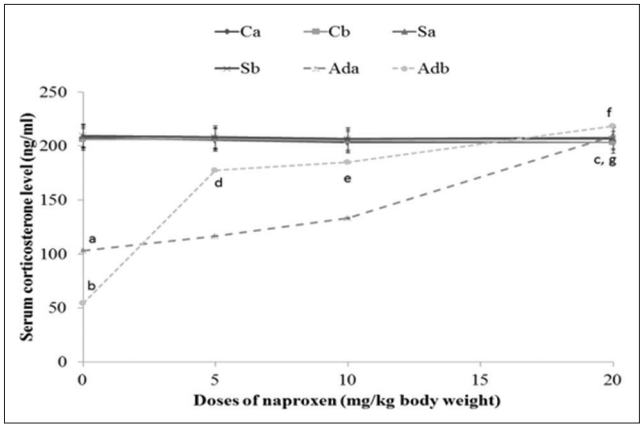

Fig. 13: Serum corticosterone level in different groups of rats. [a]Significant at p<0.001 (Alzheimer's disease [AD] vs. control/ sham-operated rats in 14-day study). [b]Significant at p<0.001 (AD vs. control/sham-operated rats in 21-day study). [c]Significant at p<0.001 (AD vs. naproxen treated AD rats at the dose of 20 mg/kg body weight in 14-day study). [d]Significant at p<0.001 (AD vs. naproxen treated AD rats at the dose of 5 mg/kg body weight in 21-day study). [e]Significant at p<0.001 (AD vs. naproxen treated AD rats at the dose of 10 mg/kg body weight in the 21-day study). [f]Significant at p<0.001 (AD vs. naproxen treated AD rats at the dose of 20 mg/kg body weight in 21-day study). [g]Significant at p<0.001 (naproxen treated AD rats at the dose 5 mg/kg body weight vs. 20 mg/kg body weight in14-day study). Abbreviations are same as Fig 1. Values are expressed in mean ± standard error of the mean (n=6 in each group)

in the alprazolam treated rats compared to that of control rats. These results indicate that rats treated with alprazolam showed reduced anxiety-like behavior in the elevated open field with a novel object. Moreover, different doses of alprazolam showed the graded response on the anxiolytic behavior. This indicates the pharmacological effect of alprazolam on anxiolytic behavior. Other investigators have also reported anxiolytic behavior in alprazolam treated rats [28]. Therefore, it appears that the method used in this study is the appropriate one for measuring anxiety status and can identify the anxiolytic behavior. The anxiolytic behavior of colchicine induced AD rats observed in the present study probably depicts the true anxiety status of this animals.

Neural areas such as amygdala and hippocampus play important roles in the regulation of anxiety status [29,30]. The amygdala helps in the development of anxiogenic behavior by increasing secretion of hypothalamic corticotropin-releasing hormone (CRH) whereas hippocampus provides a negative feedback control in anxiogenic condition (by inhibiting hypothalamic CRH secretion). Neurodegeneration in the amygdala has been reported in ICV ICIR [13,17] and that may induce anxiolytic behavior. Hippocampal neurodegeneration was observed previously in ICIR [17,18], and such neurodegeneration would have increased the anxiety behavior had the negative control of this neural structure become non-existing. It has been reported previously that different brain areas including hippocampus and amygdala showed neurodegeneration in ICV ICIR [18]. The anxiolytic behavior in the ICIR rats of the present study may be the sum total effect of the impaired function of different neural areas which were affected by colchicine induced neurodegeneration.

Hypothalamo-pituitary-adrenal axis (HPA axis) has been reported to play an important role in the control of anxiety-like behavior [31]. Abnormality in HPA axis indicated by sustained increased level of glucocorticoids is associated with anxiety and depression [32]. The normal feedback mechanism in the HPA axis allow glucocorticoids to inhibit CRH production but in those patients feedback system is impaired and CRH level may be high. In rats, it has been found that central administration of CRH resulted in behavioral activities such as anxiety, and arousal. on the other hand lower CRH production in rats is indicated to be a reason behind anxiolytic behavior in them [33]. The sustained lower serum level of CORT in ICIR of the present study probably is the resultant effect of lower secretion of CRH from the hypothalamus. Therefore, the anxiolytic behavior in the ICIR may develop due to the impaired HPA axis. Moreover, in naproxen treated ICIR, CORT levels were gradually increased in a graded manner with increasing doses of naproxen at both the time points and at the highest dose CORT level reached the control value. This graded increase of CORT in naproxen treated ICIR can be corroborated with the recovery of anxiety-like behavior in these rats. The recovery of CORT level by naproxen a non-steroidal anti-inflammatory drug indicates that decrease of CORT level in ICIR may be mediated by inflammation.

The administration of naproxen (p.o.) at different doses in ICIR showed the gradual recovery of anxiety-like behavior during 2 and 3 weeks of postoperative study. Naproxen at the dose of 20 mg/kg body weight showed complete recovery of all the behavioral parameters in a 21-day study. Administration of lower dose of naproxen caused partial recovery of most of the parameters. The latency of first approach to the object area significantly decreased in both the study durations after administration of naproxen at the dose of 10 mg/kg body wt. However, the latency of first entry to the inner area was significantly decreased in 21-day study after administration of naproxen at the dose of 5 and 10 mg/kg body weight and the total time spent in the outer area was decreased after administration of 10 mg/kg body weight of naproxen in a 21-day study. Other parameters in ICIR remained unaltered at the dose of 5 and 10 mg/kg body weight of naproxen. These results indicate that the most potent effect of naproxen on the colchicine induced anxiolytic behavior in rats was observed at the dose of 20 mg/kg body weight in a 21-day study. The recovery from the anxiolytic behavior after the administration of naproxen in the AD rats of the present study may be due to the inhibitory effect of this NSAID on the colchicine induced neurodegeneration, neuroinflammation, and lower CORT level. Kumar *et al*., [14] also reported that the administration of naproxen, a cyclooxygenase inhibitor, causes recovery of cognitive impairments in ICIR by reducing the oxidative stress in the brain. It was observed in our previous study that naproxen had reduced the markers of neuroinflammation, chromatolysis and number of beta-amyloid plaques in the hippocampus in a dose-dependent manner in ICIR and most effective dose in this regard was found to be 20 mg/kg body weight [17]. It was also observed that the low level of serum CORT concentration in ICIR gradually increased in a dose-dependent manner after administration of naproxen and complete recovery was found at the dose of 20 mg. The anxiolytic behavior in ICIR was also recovered at this dose of naproxen (20 mg/kg body wt). It appears from these observations that the observed changes in the anxiety behavior in ICIR and its recovery by the administration of naproxen may be linked with the colchicine induced neurotoxicity, neuroinflammation, CORT level and its inhibition with naproxen respectively.

The present study showed for the first time that ICV colchicine induced neurodegeneration resulted in reduced anxiety-like behavior in rats in an elevated open space in response to novelty. This reduced anxiety-like behavior is inflammation induced neurodegeneration mediated as naproxen can inhibit the impairment of anxiety behavior. Moreover, lower serum CORT level in ICIR may also be linked with the reduced anxiety-like behavior. The parameters of anxiety like total time spent in the inner area and outer area, latency of first approach to the object area and frequency of approach to the object area appear to be sensitive for determination of anxiety status in this model.

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