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Research Article
Betahistine Protects Doxorubicin-Induced Memory Deficits via Cholinergic and Anti-Inflammatory Pathways in Mouse Brain

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Abstract

Background and Objective: Cognitive deficits are the most challenging complications with cancer-treated patients by doxorubicin chemotherapy. An anti-vertigo drug, betahistine acts as a strong antagonist at histamine H_3 receptors and a weak agonist at histamine H_1 receptors. The present study aimed to investigate the potential of betahistine on doxorubicin-induced cognitive impairment, neuronal cholinergic deficits and neuronal inflammation in mice. **Materials and Methods:** To induce cognitive impairment, four doses of doxorubicin (2 mg kg^{-1}, i.p.) were injected once a week in groups of mice. Betahistine (5 and 10 mg kg^{-1}) was administrated orally for 28 days and Elevated Plus Maze (EPM), Novel Object Recognition (NOR) and Y-Maze were used to measure cognitive behaviours. Acetylcholine (ACh) and pro-inflammatory cytokines (IL-6 and TNF-α) activity were estimated in brain tissues. **Results:** Betahistine reversed the behavioural deficits induced by doxorubicin. In EPM, it reduced the transfer latency on both acquisition and retention trails in doxorubicin-induced mice. A reversal in exploration time of both novel and familiar objects, higher exploration time with a novel object and improvement of discrimination index were observed in the betahistine administered group as compared with the doxorubicin-challenged group in the NOR test. Similarly, using the Y-Maze test, significant improvement in the number of entries in both known as well as novel arms, time spent in the novel arm and the total number of entries in the trail as well as in the test sessions by betahistine in doxorubicin-challenged mice. Mechanistically, it reversed the doxorubicin-induced cholinergic deficits in the brain by elevating the ACh levels. Additionally, betahistine attenuates the neuroinflammation by diminishing the levels of pro-inflammatory cytokines (TNF-α and IL-6) in the mouse brain. **Conclusion:** Betahistine highlights to induce neuroprotection against doxorubicin-induced cognitive impairments through facilitating cholinergic activity and ameliorating neuroinflammation in mice models.

Key words: Acetylcholine, betahistine, chemobrain, doxorubicin, memory, neuroinflammation, chemotherapy drugs

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Due to advancements in diagnostic technologies and treatment methods, the lifetime of cancer patients has increased significantly. Systemic chemotherapy with anticancer drugs plays a key role among other treatment methods. However, multiple organs toxicity including neurotoxicity, cardiotoxicity, nephrotoxicity and hepatotoxicity were commonly reported with chemotherapy drugs. Regarding neurotoxicity, around 70% of the chemotherapy survival patients affects by types of cognitive dysfunctions[1]. Recently, the National Cancer Institute (NCI), USA recognized chemobrain or Chemotherapy-Induced Cognitive Impairment (CICI) as one of the most troublesome morbidity to cancer servicers[2]. Presently, some drugs from cholinesterase inhibitors and anti-inflammatory agents were explored clinically for chemobrain but not yet approved.

Doxorubicin, a cytotoxic antitumor antibiotic and clinically it is more effective against types of major cancers including breast, lung, liver, stomach, ovary, thyroid, endometrium and bladder. Unfortunately, treatment with doxorubicin results in several adverse effects by toxic to healthy tissue including the brain that impacts the quality of life of the cancer patients. Besides, the number of clinical and preclinical reports have been underlined the Doxorubicin-Induced Cognitive Impairments (DICI) by highlighting the various mechanism of action[2-4]. Understandings of the major pathogenesis of DICI are still unclear and shreds of evidence have proposed several mechanisms including neuronal inflammation and decreasing cholinergic function[4].

In CNS, the neurotransmitter histamine plays a significant role in the regulation of several physiological and behavioural functions in animals and humans including locomotor activity, appetite, sleeping and wakefulness, learning and memory and neuroendocrine regulation[5]. Histamine produces its action in CNS through the activation of four G-protein-coupled receptors of histamine receptor subtypes such as H_1, H_2, H_3 and H_4. Among them, H_3 receptors are mainly located on presynaptic histaminergic and other neurons. They regulate the release of histamine and other neurotransmitters such ACh, norepinephrine and dopamine[6]. Due to the significant roles in the alteration of major neurotransmitters in CNS, histamine H_3 receptor has greater attention as a potential therapeutic target for several CNS-related disorders including schizophrenia, Alzheimer's Disease (AD), sleep-wake disorders, epilepsy, attention-deficit hyperactivity disorder and cognitive impairments[7]. Structurally, betahistine is an analogue of histamine and it acts as a strong antagonist on histamine H_3 receptors and a weak agonist on histamine H_1 receptors[8]. Therapeutically, betahistine is a drug of choice for the treatment of vertigo and vestibular disorders, particularly for symptoms of Meniere's disease[9]. Recently, groups of mice that received the betahistine injection promoted the recall of forgotten object memories in a NOR task and also it showed a significant increase of discrimination ratio in the same task. Additionally, the administration of betahistine to human volunteers improved their overall correct ratio in the object recognition task considerably[10]. During the past few years, the role of histamine antagonists has been studied extensively in several animal models to evidence their potential in the improvement of cognitive performance. However, there is lacking evidence related to the effect of betahistine on memory deficits.

Therefore, the present study was aimed to evaluate the effect of betahistine administration on memory deficit, cholinergic activity and proinflammatory cytokines in the doxorubicin-induced experimental model.

MATERIALS AND METHODS

Study area: The study was carried out at Pharmacology Research Laboratory, Department of Pharmacology and Toxicology, Qassim University, Saudi Arabia from November, 2020-March, 2021.

Animals: In this experiment, a total number of 24 adult male ICR mice (8-12 weeks old, 25-35 g b.wt.) were procured from the Animal Facility, Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, Saudi Arabia. Mice were divided into four groups at random, with six mice in each group. Maximum three mice were housed in each polypropylene cage and allowed free access to food and water throughout the acclimation and testing. All the animals were acclimatized for one week in standard laboratory conditions before starting the experiments. Institutional Animal Ethical Committee, College of Pharmacy, Qassim University, Saudi Arabia, authorized the current study's experimental protocols (Approval ID 2020-CP-8) and the animals have cared for according to the methods outlined in the National Research Council's (USA) Guide for the Care and Use of Laboratory Animals.

Drugs and experimental design: Betahistine hydrochloride tablets were obtained from Qassim University Medical City (QUMC), Saudi Arabia. DOX (ADRIM®) injection was obtained from Fresenius Kabi Oncology Ltd. (India). The 0.5% w/v carboxymethylcellulose sodium (0.5% w/v CMC) was used to

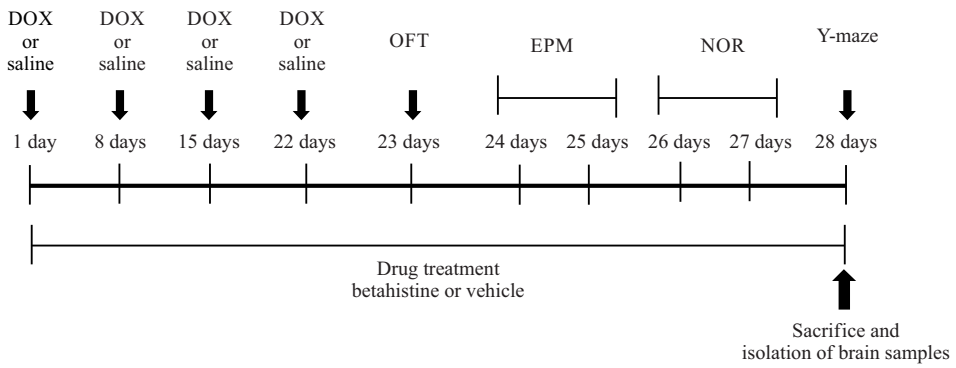

Fig. 1: Timeline administration of drug, behavioural assessments and isolation of brain samples

prepare betahistine (5 and 10 mg kg^{-1}) suspension and given to each group of mice orally. The dilution of doxorubicin (2 mg kg^{-1}) was prepared using normal saline and injected intraperitoneally (i.p.).

A total of 24 mice were divided into four groups (n = 6) and given either vehicle or betahistine treatment. The first group was considered as a control and treated with vehicle (0.5% w/v CMC) for twenty-eight days and injected four doses of normal saline (10 mL kg^{-1}, i.p.,) once per week (1, 8, 15 and 22 days) of treatment schedule (Fig. 1). The second group (DOX) was considered negative control that was treated with vehicle (0.5% w/v CMC) for 28 days and injected four doses of doxorubicin (2 mg kg^{-1}, i.p.) once per week (1, 8, 15 and 22 days) for four consecutive weeks. The dose of doxorubicin for inducing chemobrain in the rodent model was selected according to the earlier reports[11,12]. The third (DOX+BH5) and fourth (DOX+BH10) groups were treatment group those administered orally with betahistine (5 or 10 mg/kg/day, respectively) for 28 days and injected four doses of doxorubicin (2 mg kg^{-1}, i.p.) on once per week for four consecutive weeks. The body weight of each mouse was measured every week during the therapy and until the completion of the experiment.

During the treatment of the animals, the locomotor performance of each mouse was assessed using an open field test on 23 days of drug treatment. Continuously, the spatial memory assessments were performed using various behavioural tests including elevated plus maze (24 and 25 days of treatment), novel object recognition test (26 and 27 days of treatment) and Y-maze test (28 days of treatment) (Fig. 1). On 28 days, all of the animals were sacrificed at the end of the behavioural test and brain tissues were obtained for additional ELISA investigation.

Behavioural tests

Open field test (OFT): The OFT is a typical behavioural experimental model used to assess mouse motor activity[13]. It consists of a wooden open box (50×50×38 cm) and the bottom of the box is equally divided into 25 squares (2×2 cm). The entire experiment was maintained with minimum light in a calm environment. The experiment was performed on day 23 of the drug treatment. During the experiment, each mouse was placed in the centre of the open field and given 5 min to explore freely. This experiment recorded the total number of crossings (the animal crossing the total number of squires with all four paws) by each mouse.

Elevated plus maze (EPM) test: The EPM was made of wood and stood 25 cm above the ground with four equal-sized arms [16×5 cm (length×width)]. Two arms were surrounded by 12 cm high walls and arranged perpendicularly to two open arms on the opposite side. On training day (24th day of treatment), each of the mice was placed at the end of the open arm, away from the central platform. Transfer Latency (TL) was recorded for each animal as finding learning capability. The TL is defined as the time taken (in seconds) by the mice to move from the open arm to either one closed arm with all its four feet[14]. If the animal fails to enter into closed-arm within 90 sec, the mouse was gently pushed into the closed arm and allowed to explore the maze for another 2 min. After 24 hrs (on the 25th day of treatment), the again TL was recorded as retention of learned-task memory. During the experiment, the apparatus was cleaned with cotton soaked in water (90%) and ethanol (10%) after each trial to clear all odours.

Novel object recognition (NOR) test: The NOR was carried out in an open wooden box (80×60×40 cm) apparatus. The

test method was followed as described early with minor modifications[15]. The discriminating objects were selected with two dissimilar shapes (rectangle box as a familiar object and cylindrical box as a novel object, all at about the same tall and firm, so that they cannot be moved by the animals during the test). The experiments were performed in three phases such as habituation, training and test phases (the 26th and 27th days of drug treatment). In the habituation process, each animal was allowed free to explore the box without any objects for 5 min. The training session (T_1) was performed after 24 hrs (the 27th day of drug treatment). In this session, each mouse was allowed to explore with two similar rectangle samples [Familiar Object 1 (FO_1) and Familiar Object 2 (FO_2)] for 5 min during the familiarization process and the exploration times of FO_1 and FO_2 were noted. The exploration time was referred to as the total time spent by an animal directing its nose to an object at a distance $\leq$2 cm and touch it with the nose. Following the T_1 session, the test session (T_2) was performed after a 4 hrs inter-trial interval. During the familiarization and test phases, the experimental context was not significantly different. During T_2, the animal was allowed to explore with two objects, one similar to the sample (FO_1, rectangle box) and the other was novel (NO, cylindrical box) and the period of this phase was followed 5 min. The time of exploring the familiar object as well as the novel object was recorded. The discrimination index (D) was calculated to find the discrimination between the familiar and novel objects during the T_2 phase:

$$D = \frac{N - F}{N + E}$$

Where:

N = Exploration time of the novel object

F = Exploration time of the familiar object

Y-maze test: The Y-maze was made of wood and had three arms at a 120° angle (35×5×10 cm). To make it easier to see, the arms were painted brown. Each arm end was pasted with a picture contains a different pattern. The apparatus was set down on the ground. To ensure even lighting distribution, the light was given from above. The protocol of the test was modified from Tripathi *et al.*[16]. On the 28th day of treatment, during the training session (first trial) the novel arm was closed and each of the animals was allowed to explore another 5 min to freely another two arms. The number of entries in two arms at the trial session was recorded. The test session (second trial) was conducted after 4 hrs of the training session. In the test session, each mouse was allowed to explore the entire maze, including the novel arm for 5 min. In the test session, the number of entries in known and novel arms and time spent in the known and novel arm were recorded. An animal was classified to have entered an arm if it entered with 85% of its body. The percentage of time spent in the novel arm was calculated as the total time spent in the novel arm divided by the time spent in all the arms during the test session.

Biochemical assays using brain homogenate: At the end of the behavioural experiments (the 28th day of treatment), all the animals were sacrificed by cervical dislocation. The whole fresh brain was collected from the skull of each animal and the brain samples were homogenized with ice-cold phosphate-buffered saline (4°C, pH 7.4) using a homogenizer. The homogenate was then centrifuged for 10 min at 4000 rpm. The cloudy supernatant aliquot was transferred into 4 mL vials and stored at -80°C. The total protein content of the samples was quantified using the biuret colourimetric method (Crescent Diagnostics, Saudi Arabia). The samples were tested using Enzyme-Linked Immunosorbent Assay (ELISA) kits for Acetylcholine (ACh), Interleukin-6 (IL-6) and Tumour Necrosis Factor-α (TNF-α) antibodies as described in the manufacturer's (Cloud-Clone Corp., USA) protocol. Measurements were performed at 450 nm by using an EL×800 Absorbance Microplate Reader (BioTek Instruments, Inc.).

Statistical analysis: The results were indicated as Mean±Standard Error (SEM). The comparisons between the groups were analyzed using one-way ANOVA and followed Tukey-Kramer *post hoc* test for calculating significance levels between the two groups. For comparison of corresponding groups between two different objects in NOR were analyzed using unpaired Student's 't'-test. Graph Pad version 9 (GraphPad Software Inc., United States) was employed for statistical analysis. The $p<0.05$ was considered statistically significant.

RESULTS

Administration of betahistine and doxorubicin did not change the body weight of mice: The result in Fig. 2 shows the effect of betahistine on the weekly body weight of the doxorubicin-induced experiment model. From all the groups, each of the mouse body weights was measured every 7 days interval on 0, 7th, 14th, 21st and 28th days. There were no significant differences between control and doxorubicin-induced groups. In the same line, consider treatment groups, there were no significant variations in the body weight as compared to control as well as doxorubicin-induced groups.

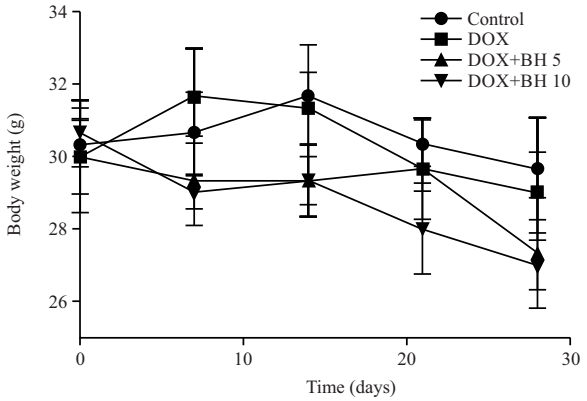

Fig. 2: Effect of betahistine on weekly body weight of doxorubicin-induced mouse model

Values are Mean±SEM (n = 6). One-way ANOVA [F (3,20): 0.095, p>0.05 for 0 day, F (3,20): 1.302, p>0.05 for 7 days, F (3,20): 1.447, p>0.05 for 14 days, F (3,20): 0.915, p>0.05 for 21 days, F (3,20): 1.101, p>0.05 for 28 days] followed by Tukey-Kramer multiple comparisons test. There were no statistically significant differences found between the groups in body weight

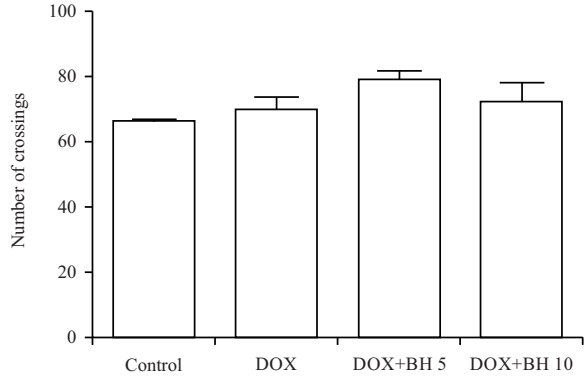

Fig. 3: Effect of betahistine on the total number of crossing in a doxorubicin-induced mouse model using the open-field test

Results are expressed by Mean±SEM (n = 6). One-way ANOVA [F (3,20): 2.100, p>0.05] followed by Tukey-Kramer multiple comparisons test. There were no statistically significant differences found between the groups in the total number of crossing

Administration of betahistine and doxorubicin did not alter the locomotion of mice in the open-field test: The result in Fig. 3 shows the effect of betahistine on the total number of the crossing of the doxorubicin-induced experiment model in the Open Field Test (OFT). Reference from results, there were no significant changes [F (3,20) = 2.100, p>0.05] noted between the groups concerning OFT parameter as a total number of the crossing during 5 min observation on 23 days of drug treatment.

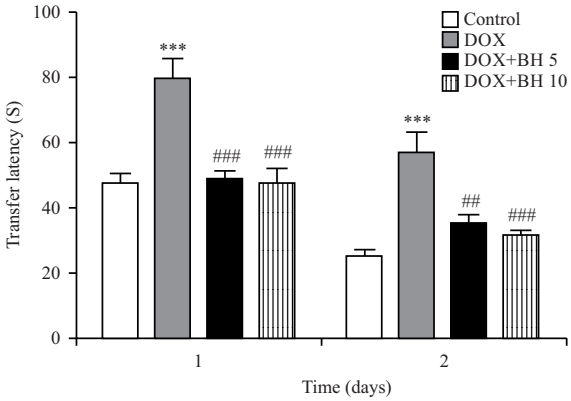

Fig. 4: Effect of betahistine on doxorubicin-induced cognitive impairment in mice using elevated plus-maze

Results are expressed by Mean±SEM (n = 6). One-way ANOVA [F (3,20): 13.88, p<0.001 for 1 day and F (3,20): 13.87, p<0.001 for 2 days of EPM test] followed by Tukey-Kramer multiple comparisons test. ***p<0.001 as compared to the control group, ##p<0.01 and ###p<0.001 as compared to the doxorubicin-induced group

Treatment of betahistine shortens the transfer latency of doxorubicin-challenged mice in the elevated plus-maze test: Using Elevated Plus Maze (EPM), the reduction in Transfer Latency (TL) on acquisition (training day/day) and retention (after 24 hrs, 2 days) were considered as an improvement in animals learning capability and memory capacity. Here, the effect of betahistine continuous 28 days administration on doxorubicin-induced spatial memory impairment of mice in the EPM test is depicted in Fig. 4. Analyzing one-way ANOVA showed that there were significant differences in TL values amongst the groups on day [F (3,20) = 13.88, p<0.001) and 2 days (F (3,20) = 13.87, p<0.001] of the EPM test. From *post hoc* analysis, it was found that the doxorubicin-induced group showed significant elevation (p<0.001) in day and 2 days TL values as compared to the control group on respective days. The obtained results confirmed the doxorubicin-induced cognitive impairment in the mice model. However, the oral administration of betahistine at 5 mg kg^{-1} significantly reduced the TL values on day (p<0.001) and 2 days (p<0.01) of the EPM test, when compared to the doxorubicin-induced group. Similarly, a higher dose of betahistine (10 mg kg^{-1}, p.o.) also significantly reduced (p<0.001) the TL values both on day (acquisition) and 2 days (retention) of the EPM test as compared to the doxorubicin-induced group.

Treatment of betahistine improved cognitive functions of doxorubicin-challenged mice in the novel object recognition (NOR) test: The result in Fig. 5 indicates the effect of betahistine on various behavioural parameters of

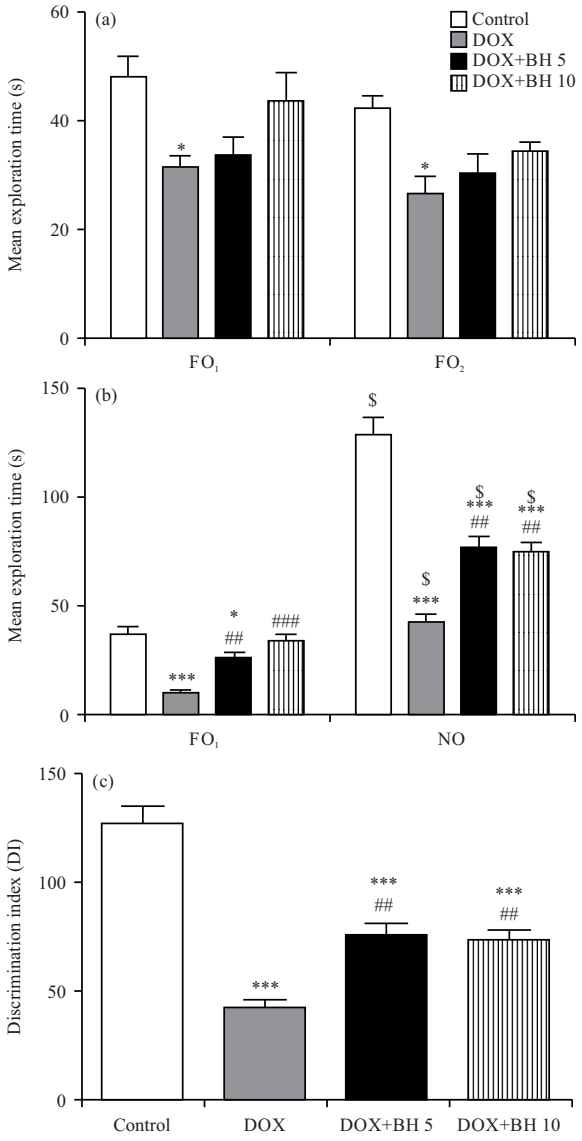

Fig. 5(a-c): Effect of betahistine on doxorubicin-induced cognitive impairment in mice using novel object recognition test, (a) Exploration time of two familiar objects (FO_1 and FO_2) during the training session (T_1), (b) Exploration time of familiar (FO_1) and novel (NO) objects during the test session (T_2), and (c) Discrimination index

Results are expressed by Mean±SEM (n = 6), One-way ANOVA [F (3,20): 4.020, $p<0.05$ for FO_1 and F (3,20): 5.427, $p<0.01$ for FO_2 during (T_1), F (3,20): 21.92, $p<0.001$ for FO_1 and F (3,20): 39.68, $p<0.001$ for NO during (T_2), F (3,20): 39.49, $p<0.001$ for discrimination index] followed by Tukey-Kramer multiple comparisons test for comparisons of within the groups. The student's unpaired 't' test was used to comparisons of corresponding each group of exploration time. $^{\$}p<0.001$ as compared to the corresponding group, $^{*}p<0.05$ and $^{***}p<0.001$ as compared to the control group, $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ as compared to the doxorubicin-induced group

doxorubicin-induced cognitive deficiencies in mice tested with the NOR test. When both of the objects were similar during the training session (T_1), there were significant differences between the groups in the mean exploration time of familiar objects such as FO_1 [F (3,20) = 4.020, $p<0.05$] and FO_2 [F (3,20) = 5.427, $p<0.01$] using one-way ANOVA analysis (Fig. 5a). Further *post hoc* test explained that there was a significant decrease ($p<0.05$) in exploration time of both objects FO_1 and FO_2 in the doxorubicin-induced group when compared with the control group. The exploration times (FO_1 and FO_2) of both betahistine treatment groups (5 and 10 mg kg^{-1}, p.o.) did not show any significant differences as compared to the control group. Also, the comparisons between corresponding groups of FO_1 and FO_2 showed similarities in exploration time.

During the test session (T_2), when one of the Familiar Objects (FO_2) was replaced with a novel object (NO), each group of mice was spent significantly higher exploration time ($p<0.001$) with NO as compared to the corresponding similar group of a familiar object (FO_1) (Fig. 5b). When compared the exploration time within the groups, there were significant differences in exploration time of NO [F (3,20) = 39.68, $p<0.001$] and FO_1 [F (3,20) = 21.92, $p<0.001$] using one-way ANOVA analysis. Extension of *post hoc* comparison between the NO groups indicated that a significant reduction ($p<0.001$) in exploration time of the doxorubicin-induced group when compared to the control group. However, 28 days of consecutive betahistine (5 and 10 mg kg^{-1}, p.o.) administration to groups of mice significantly increased the exploration time ($p<0.01$) of NO as compared to the doxorubicin-induced group.

Effect of betahistine treatment in the ability of the discrimination between the familiar object (FO_1) and Novel Object (NO) during session T_2 on doxorubicin-induced mice was calculated as a Discrimination Index (DI) and displayed in Fig. 5c. Analysis by one-way ANOVA indicated that there were significant differences [F (3,20) = 39.49, $p<0.001$] in the DI as compared between the groups. Administration of doxorubicin (2 mg kg^{-1}, i.p.) four doses caused a significant decrease ($p<0.001$) in DI value when compared to the control animals. Continuous twenty-eight days of treatment with betahistine, however, ameliorated the effect of doxorubicin-induced cognitive deficits. Both doses (5 and 10 mg kg^{-1}, p.o.) of betahistine were significantly improved ($p<0.01$) the DI values as compared to doxorubicin-challenged mice. Unfortunately, the reversal of the DI values of both doses of betahistine was not comparable with control animals.

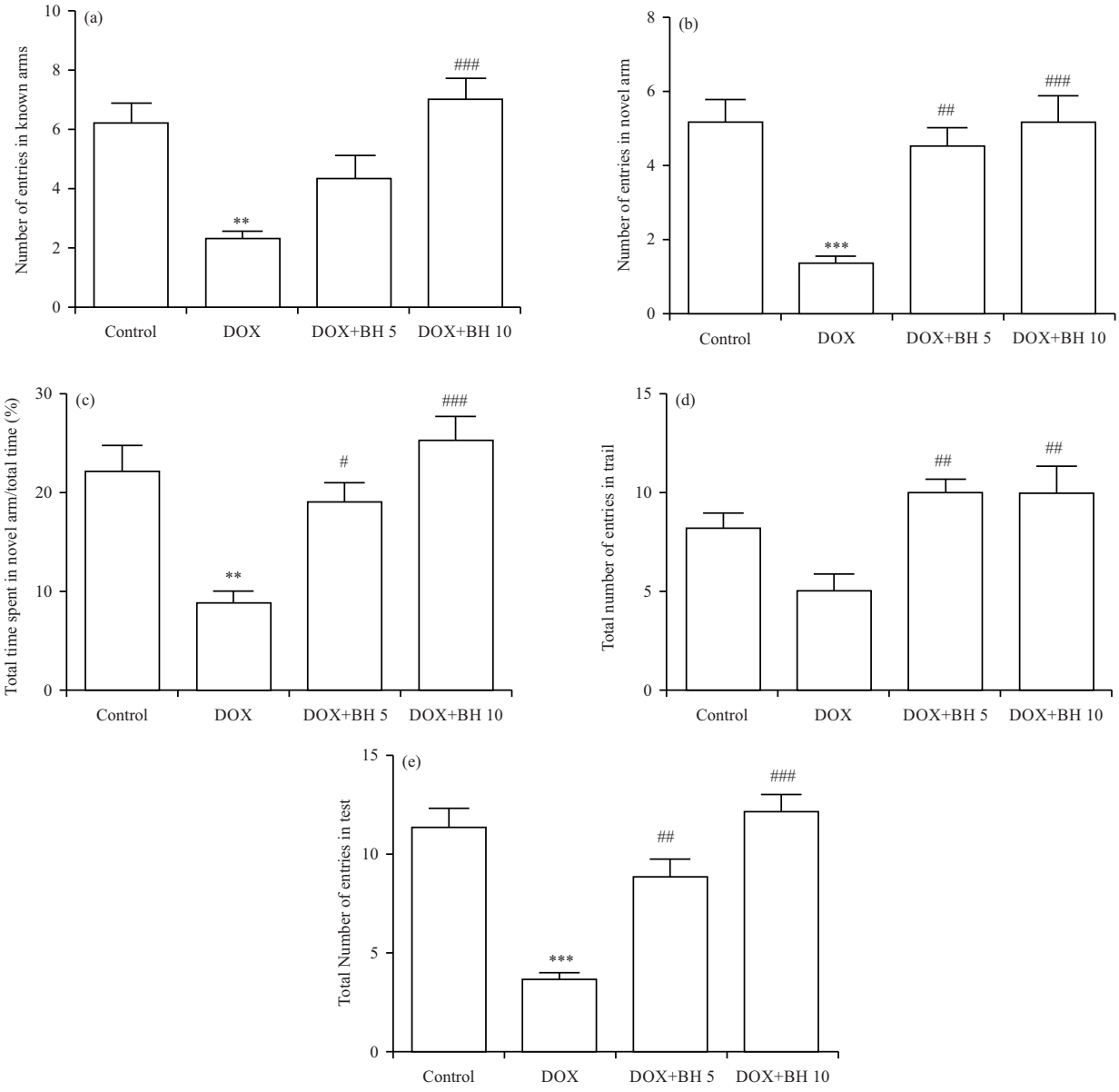

Fig. 6(a-e): Effect of betahistine on doxorubicin-induced cognitive impairment in mice using Y-maze test, (a) Number of entries in known arms in test, (b) Number of entries in novel arm in test, (c) Time spent (%) in the novel arm in test, (d) Total number of entries in the trail and (e) Total number of entries in the test

Results are expressed by Mean±SEM (n = 6). One-way ANOVA [F (3,20): 10.870, p<0.001 for the number of entries in known arm, F (3,20): 11.680, p<0.001 for the number of entries in novel arms, F (3,20): 11.100, p<0.001 for the percentage of time spend in novel arm, F (3,20): 6.133, p<0.01 for the total number of entries in the trail, F (3,20): 21.350, p<0.001 for the total number of entries in test] followed by Tukey-Kramer multiple comparisons test. **p<0.01 and ***p<0.001 as compared to the control group, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p<0.001 as compared to the doxorubicin-induced group

Treatment of betahistine improved cognitive functions of doxorubicin-challenged mice in the Y-maze test: The result in Fig. 6 highlights the effect of betahistine on various behavioural parameters of the Y-maze test in the doxorubicin-induced mouse experimental model. The number of entries in the known arm and novel arm results are displayed in Fig. 6(a-b). The comparison among the groups exhibited that there were significant differences in the number of entries in known [F (3,20) = 10.870, p<0.001] and novel [F (3,20) = 11.680, p<0.001] arms during the test session. Results showed that four doses of doxorubicin (2 mg kg^{-1}, i.p. per week) significantly reduced the number of know arm entries (p<0.01) and novel arm entries (p<0.001) when compared to respective control animals. The oral

administration of betahistine at the dose level of 10 mg kg^{-1} showed a significant increase ($p<0.001$) in the number of entries in the know arm when compared to the doxorubicin-induced group. Moreover, treatment of both doses (5 and 10 mg kg^{-1}, p.o.) of betahistine treatment significantly improved ($p<0.01$ and $p<0.001$, respectively) the number of novel arms entries as compared to the doxorubicin-induced group.

The result in Fig. 6c represents the percentage of time spent by mice in the novel arm of the Y-maze apparatus during the test session. Statistically, there were significant differences [$F(3,20) = 11.100$, $p<0.001$] between the groups in the percentage of time spent in the novel arm. The group of mice only administrated with doxorubicin (2 mg kg^{-1} per week) was highlighted a significant reduction ($p<0.01$) in the percentage of time spent in the novel arm, when compared to control mice. However, the group of mice treated with betahistine significantly enhanced ($p<0.05$ for 5 mg kg^{-1}, $p<0.001$ for 10 mg kg^{-1}) the performance of mice in more time spent at the targeted novel arm as compared to the doxorubicin-induced group.

The total number of entries to the arms in the Y-maze test in trial and test sessions find in Fig. 6(d-e), respectively. Statistical analysis results that there were significant differences in the total number of entries by animals during the trial [$F(3,20) = 6.133$, $p<0.01$] and test [$F(3,20) = 21.350$, $p<0.001$] sessions as compared among the groups. Further comparison between selective groups showed there was no significant difference between control and doxorubicin-induced groups in the total number of entries during the trial session (Fig. 6d). Nevertheless, both doses of betahistine (5 and 10 mg kg^{-1}, p.o.) still improved ($p<0.01$) the number of arm entries in the trial session as compared to doxorubicin-induced animals. On the other hand, in the test session, doxorubicin administration significantly declined ($p<0.001$) the total number of arm entries as compared to normal animals (Fig. 6e). Remarkably, there were significant improvements in the number of arm entries by oral treatment of betahistine at the dose levels at 5 mg kg^{-1} ($p<0.01$) and 10 mg kg^{-1} ($p<0.001$), when compared to doxorubicin-induced mice.

Treatment of betahistine elevated acetylcholine (ACh) levels in brain homogenate of doxorubicin-challenged mice: Figure 7 illustrates the effect of concurrent 28 days of administration of betahistine on ACh levels in different groups of doxorubicin (2 mg kg^{-1} per week, i.p.) treated mice brain homogenate. Statistical analysis performed among the groups showed significant differences [$F(3,20) = 12.53$, $p<0.001$] in

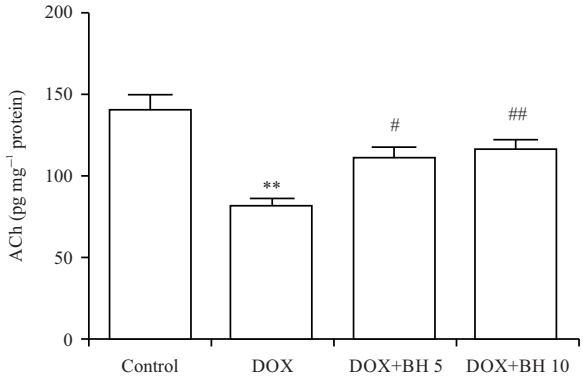

Fig. 7: Effect of betahistine on doxorubicin-induced declined acetylcholine (ACh) levels in mouse brain

Results are expressed by Mean±SEM (n = 6). One-way ANOVA [F (3,20): 12.53, p<0.001] followed by Tukey-Kramer multiple comparisons test, **p<0.01 as compared to the control group, #p<0.05 and ##p<0.01 as compared to the doxorubicin-induced group

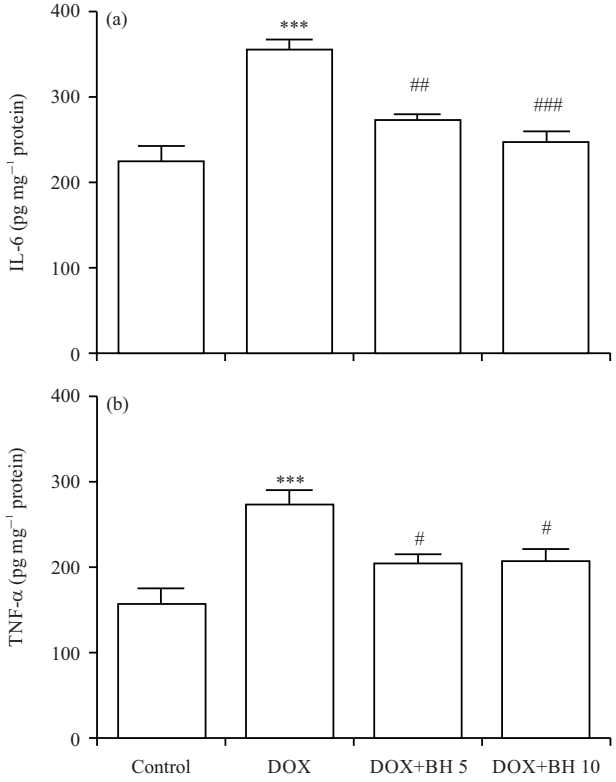

Fig. 8(a-b): Effect of betahistine on doxorubicin-induced elevated pro-inflammatory cytokines levels in mouse brain, (a) IL-6 level and (b) TNF-α level

Results are expressed by Mean±SEM (n = 6). One-way ANOVA [F (3,20): 17.620, p<0.001 for IL-6 level, F (3,20): 9.544, p<0.001 for TNF-α level] followed by Tukey-Kramer multiple comparisons test, ***p<0.001 as compared to control group, #p<0.05, ##p<0.01 and ###p<0.001 as compared to the doxorubicin-induced group

brain ACh levels. When compared to the control group, it was found that doxorubicin-induced mice exhibited significantly lower ACh levels ($p<0.01$) in brain homogenate. Further, the treatment of betahistine (5 and 10 mg kg^{-1}, p.o.) significantly restored the brain ACh levels in a dose-dependent manner ($p<0.05$ and $p<0.01$, respectively) as compared to doxorubicin-induced mice.

Treatment of betahistine reduced pro-inflammatory cytokines (IL-6 and TNF-α) in brain homogenate of doxorubicin-challenged mice: The result in Fig. 8 highlights the effect of two doses of betahistine treatment on interleukin-6 (IL-6) and Tumour Necrosis Factor-α (TNF-α) levels in the brain homogenate of doxorubicin-induced animals. There were significant differences among the treatment groups in IL-6 levels [$F(3,20) = 17.620$, $p<0.00$] as well as TNF-α levels [$F(3,20) = 9.544$, $p<0.001$] when using one-way ANOVA analysis. It was found that treatment of doxorubicin (2 mg kg^{-1} per week, i.p.) elicited significantly higher levels ($p<0.001$) of the two targeted pro-inflammatory cytokines such as IL-6 and TNF-α as compared to control animals. Simultaneous 28 days oral administration of betahistine (5 and 10 mg kg^{-1}) significantly reduced ($p<0.01$ and $p<0.001$, respectively) IL-6 levels in mice brains as compare to doxorubicin-induced animals (Fig. 8a). Additionally, the treatment of betahistine also significantly attenuated the increased brain TNF-α levels by $p<0.05$ at both dose levels (5 and 10 mg kg^{-1}, p.o.) of betahistine when compared to the doxorubicin-induced group (Fig. 8b).

DISCUSSION

The present study evidenced the neuroprotective potential of betahistine against doxorubicin-induced memory deficit. Our results found that continuous 28 days of oral betahistine treatment at doses 5 and 10 mg kg^{-1} markedly reversed the memory impairment, which was induced by doxorubicin using various maze models like an elevated plus maze, novel object recognition and Y-maze tests. Furthermore, the same treatment significantly increased neurotransmitter acetylcholine levels and also attenuated the pro-inflammatory cytokines IL-6 and TNF-α levels in the doxorubicin-challenged mouse brain. Principally, memory deficits associated with doxorubicin treatment have been reported in various animal models and cognitive studies in humans[2,17]. In another view, presently the member of histamine H_3 receptor antagonists have great attention as a potential target for the management of CNS disorders including cognitive deficiencies[18].

Regarding Elevated Plus Maze (EPM) assay, the present results showed that a longer TL of both sessions on 1 days (acquisition) and 2 days (retention) with doxorubicin-induced animals as compared with the control group explained the impairment of memory with doxorubicin treatment. Furthermore, using Novel Object Recognition (NOR) task to discriminate between a novel and a familiar object, the animals must first attend to two identical objects and keep the two objects in working memory[19]. During training session (T_1), when used both similar objects (FO_1 and FO_2) were, the results showed that the group of mice treated with doxorubicin significantly lowered the exploration time as compared to control. It is evidenced that doxorubicin treatment affects the ability of acquisition in animals. While the continuation of test session (T_2) in NOR test, when keeping one of the familiar objects (FO_1) from T_1 and a Novel Object (NO), the groups of animals showed a significant increase in exploration time of novel object as compared to the corresponding group familiar object exploration time. It is indicating that the animals were spent more time with the novel object as compared to the familiar object, which specifies the retention capacity and discrimination ability of both objects as well as remembering the familiar object from T_1. Furthermore, the treatment of doxorubicin resulted in significantly lower exploration time of novel as well as familiar objects explained the lower retention as well as the discrimination ability of animals. However, continuous treatment of betahistine (5 and 10 mg kg^{-1}, p.o.) improved the cognitive functions in the doxorubicin-challenged mice group. In the NOR task, more cognitive skills are required to recognition of novelty as exploring a single novel object or a task of a novel environment[19]. As reported early, when animals are allowed with exposing to a novel object and a familiar object, they approach frequently and like to spend more time exploring the novel object than previously familiar one[13,20]. Additionally, the Discrimination Index (DI) of the treatment groups offers further evidence of the discrimination ability of animals during T_2. Oral administration of betahistine demonstrated a better DI than that of doxorubicin-induced animals. Commonly, the DI explains to understand the discrimination between the familiar and novel objects by animals.

The Y-maze test was used to measure spatial recognition memory, general exploratory behaviour and anxiety-like behavior[16]. The number of entries in known arms and a novel arm indicates the alterations in arm discrimination behaviour of animals. Present results showed that doxorubicin treatment reduced both known and novel arms but failure in alteration of arm discrimination. However, the lower the number of novel arm entries highlighting the loss of spatial memory of

the animals[16]. Interestingly, the treatment of betahistine dose-dependently reversed the number of novel arm entries in the Y-maze test indicating attenuation of doxorubicin-induced spatial memory impairment. Coping behaviour to the novel environment of animals during the test session was calculated by the percentage of radio between the time spent in the novel arm and total time including time spent in all the arms as well as the centre of the Y-maze. The decrease in the value of the coping behaviour of the animal was indicated by the increase in anxiety behavior[21]. As compared with the control group, doxorubicin treatment showed a lower value of coping behaviour indicated that minimum time spent in the novel environment by animals correlated to anxiety-like behaviour. Oral administration of betahistine, however, attenuates the doxorubicin-induced loss of coping strategy by increasing the time sent in a novel environment of animals. Furthermore, the total number of entries in arms during trial and test sessions in the Y-maze test was indicating the curiosity behaviour of the animals[22]. Both doses of betahistine also significantly increased the curiosity behaviour of doxorubicin-challenged mice.

To elucidate the molecular mechanisms of memory-enhancing effect by betahistine were explored with examining its effect on cholinergic transmission and neuroinflammatory mediators in the doxorubicin-induced mouse. Early preclinical studies have indicated that treatment of doxorubicin altered the biosynthesis and release of neurotransmitters in the animal brain[2]. Intraperitoneal injection of doxorubicin decreased the release of choline which undergoes the acetylation with enzyme choline acetyltransferase to synthesis ACh that resulted in depletion of ACh biosynthesis at mouse hippocampus[23,24]. The present results found that treatment of betahistine significantly enhanced the ACh levels in doxorubicin-induced animals. Early findings reported that the number of histamine H_3 receptor antagonists were enhanced the release of neurotransmitters including histamine and ACh by blocking presynaptic histamine H_3 receptors in various brain areas[18]. One of our research findings resulted that a histamine H_3 receptor antagonist ciproxifan improved the cholinergic transmission by increased the ACh levels and decreased the AChE activity in an AD transgenic mouse model of B6.129-Tg (APPSw)40BTLA/J mice[6].

It is evidenced that intraperitoneal treatment of doxorubicin showed increased levels of TNF-α in the hippocampus and cortex area in experimental mice[25]. Recent research supported that a weekly dose of 2.5 mg kg^{-1} (i.p.) of doxorubicin for 4 weeks significantly elevated the IL-6 levels of serum and brain in rats[4]. With these elevations of TNF-α and other proinflammatory cytokines levels, oxidative damage occurs in neuronal tissues and neuronal death can result in neuronal toxicity of doxorubicin treatment[26]. The present study found that doxorubicin triggered the generation of inflammatory cytokines TNF-α and IL-6 in brain tissues that indicate the inflammatory conditions of the brain. Nevertheless, the obtained results elaborated the effective inhibition of continuous 28 days oral administration of both doses of betahistine (5 or 10 mg kg^{-1}) significantly reduced the generation of pro-inflammatory mediators, TNF-α and IL-6 in doxorubicin-induced brain tissues. Besides reference from our previous laboratory study, the treatment of a histamine H_3 receptor antagonist ciproxifan showed its neuroprotective effects by attenuating the neuroinflammation by reducing the pro-inflammatory cytokines like IL-6, IL-1α and IL-1β in brain tissues of AD transgenic mouse B6.129-Tg (APPSw)40Btla/J[6].

The current results designate that betahistine promises as a therapeutic target for reducing doxorubicin-induced cognitive deficits by improving cognitive ability, enhancing cholinergic transmission and attenuating pro-inflammatory cytokine release in the mouse brain. Clinically, betahistine is commonly prescribed medication for the treatment of vertigo and vestibular disorders. The present study extended with further evaluations of its beneficial effects on chemotherapy-induced cognitive deficits in a mouse model. It is a preliminary pre-clinical evaluation using an animal model with focused mechanisms. However, these results will initiate the researchers to explore the additional supportive evidence against chemotherapy-induced cognitive deficits to support its further clinical use.

CONCLUSION

Collectively, the achieved results supported the neuroprotective effect of betahistine against doxorubicin-induced cognitive impairment in experimental mice. The 28 days of treatment of betahistine (5 and 10 mg kg^{-1}, p.o.) showed improvement of various cognitive behavioural parameters using maze models such as elevated plus-maze, novel object recognition and Y-maze tests. Additionally, the treatment of betahistine improved the CNS cholinergic activity by elevating the acetylcholine level and suppressed the neuroinflammation through control the production of pro-inflammatory cytokines, TNF-α and IL-6 in the doxorubicin-induced mouse brain.

SIGNIFICANCE STATEMENT

The goal of this study was to evaluate the potential of betahistine on doxorubicin-induced cognitive impairment, neuronal cholinergic deficiencies and neuronal inflammation using an experimental model. The finding of our study recommended that antagonizing histamine H_3 receptors using betahistine improved memory functions, cholinergic transmission and attenuating neuroinflammatory cytokines in doxorubicin-challenged mice. These results showed a therapeutic direction to explore the benefits of histamine H_3 antagonists for chemotherapy-induced cognitive deficiencies.

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