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THE EFFECT OF CINNAMALDEHYDE ON STRESS-INDUCED DEFICITS IN ACQUISITION BEHAVIOR WITH BARNES MAZE IN RAT

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Abstract

Stress can disrupt the physiological homeostasis of biological organisms which follow by behavioral impairment; especially it can induce deficits in learning and memory process. Studies showed that Cinnamaldehyde (CA) have many pharmacological activities such as anti-diabetic, antioxidant, cognitive enhancer, anti-inflammatory and anti-cancer activity. To examine the effects of Cinnamaldehyde on stress-induced cognitive deficits, we assessed and compared the Electric foot Shock Stress (EShS) and Immobility stress (ImmS) on Barnes maze acquisition. In this experiment 60 Male Wistar rats were randomly divided into 5 groups (n=12): vehicle, two groups with stress and two groups with stress plus CA treatment. The learning and memory process were measured by Barnes maze. One week stress induction was begun two days before starting acquisition protocol and during it. Cinnamaldehyde was administered orally via gavage (20mg/kg/day) during experiment. This study demonstrated that both EShS and ImmS could disrupt the acquisition and memory with Barnes maze. The time of latency and distance pursuit to find goal box and number of errors increased in stress induced groups. Treatment with Cinnamaldehyde improves learning and memory process both in control and stress induced groups. Furthermore, the improvement effect of Cinnamaldehyde was better in electric foot shock stressed group than restraint group. The present study suggest that Cinnamaldehyde can improve learning and memory process both in control and stress-induced groups.The improvement against electric foot shock stress is better than immobilization.

Keywords: *Cinnamaldehyde, Stress, learning, memory, Barnes maze.*

Introduction

Stress seriously perturbs the physiological and psychological homeostasis and also induced alteration in the highly-conserved set of biological pathways to maintain physiologic integrity [1]. Hypothalamic– pituitary–adrenal (HPA) axis and the sympathetic Adrenal Medullary` (SAM) system are the main systems for the maintenance of physiological homeostasis. Activation of HPA leads to various alterations in the circulating hormones such as glucocorticoids. Furthermore, coordinate alteration of the SAM system following stress can increase heart rate, respiratory rate and brain function [2]. A body of research has shown that stress responses and hormones which primarily were regulated by HPA axis converge on learning and memory systems and can changes learning and memory process. Glucocorticoids, the main stress hormones, have an important contribution in the stress responses. Moreover, increased secretion of GC leads to memory impairment via an alteration of brain circuits that are responsible for memory consolidation and storage [3]. Although all of these alterations participate in memory storage, however their precise role in stress-induced responses is far from clear.

Cinnamaldehyde (CA) is the flavouring constituents derived from Cinnamomum verum it is used as an anti-inflammatory, antioxidant and anti-septic in the body. The CA uses in Chinese traditional medicine for curing patients. The recent studies have proved that CA can regulate gene expression and so may has an efficient role as an anti-cancer drugs [4]. However, few studies have focused on learning and memory. Jawale et al. showed that CA can improve behavioural deficits in diabetic induced cognitive deficits rats [5]. Donald grave and et al have shown the effect of CA in Alzheimer disease [6]. So, in the current study we have studied about the effects of application of CA in learning and memory of rats under stress conditions. There are many kinds of stress which the investigators use in animal studies such as immobilization, shaking, electrical foot shock and forced swimming. Chronic immobilization stress has many physiological effects including body weight loss, changes in neuro-architecture and function of brain and increases anxiety and fear of rats [7]. Electric shock stress as acute stress can stimulate behavioural responses similar to stress-enhanced fear learning [8]. In the current study, we have investigated the effect of one-week immobilization and electrical foot shock-induced stress on learning and memory process, also the effect of treatment with CA on learning and memory process which were changed by different kind of stress were examined. Barnes maze is a laboratory instrument that first described in 1979 by Barnes et al., and testing the navigational abilities of rodents [9, 10]. This maze is a type of non-invasive mazes that can assess function of hippocampal-dependent spatial learning and memory. We used Barnes maze for measurement of learning process because of less inducing stress than Morris water maze

Methods

Animals and groups

In the present study, 60 male Wistar rats (5 groups, n=12), weighing 200-250 gr were used. The rats were kept in standard cages with free access to food and water in temperature of 22± 2°C, humidity of 40% - 60%, and 12 hours light/dark cycle condition. The current study was approved in the ethics committee of Baqiyatallah University of medical science. The rats were divided into 5 groups randomly. The vehicle group which received solvent (corn oil) without any stress. A group was exposed to Electric foot Shock Stress (EShS) and in the other group the rats were in Immobilization Stress condition (ImmS) they did not have treatment with cinnamaldehyde (CA). The other two stress received groups were taken CA (20 mg/kg) via gavage during experiment.

Stress methods

In this study two stress methods were used: Electric foot Shock Stress(EShS) and restraint Immobility Stress (ImmS). In the electric foot shock method, a box consisted of 4 glassy chambers, transferring electrical shock to feet of animals. The rats were put in the chambers for 30 minutes for adaptation and then the 2 mA electrical shock was applied to foot for 10 second the animals were also remained in chambers for 30 minutes after shock application this kind of stress was applied for 8 consecutive days (2 days before training protocol and 5 days during learning and memory procedure). In the restraint group immobilization stress was

applied 30 minute every day in 7 consecutive days (2 days before training and 5 days during learning and memory procedure), cognitive learning and memory were evaluated by the Barnes maze**.**

Barnes maze protocols

Barnes maze (Barnes, 1979) was a white circular surface with 120 cm diameter. It contained 16 holes that one of them was escape or goal hole with black box. Each hole has 4 centimeter distance from edge. The height of maze was 70 centimeters. The maze was easily cleanable with ethanol 70%. The maze was in the center of the room and three lights were placed in three lab roof corner around the maze with 120 degrees angles from the center of above maze. Simple colored-paper shapes (squares, triangles, circles) were mounted around the room as visual cues. All sessions were recorded using COP Monochrome CCD Camera (Model 15-CC20) and MyTV/x software. The day before starting the protocol animals were put on the maze for adaptation (30 min). In the first session of training the rats were put in a dark starting cylinder (15 cm height and 15 cm diameter) on the center of maze for 15 seconds, by removing the starting box the trajectory of the rats were recorded via the camera. Each session (day) consist of 5 trials each trial took 5 min until finding goal box, if did not, the animal was picked up manually and put in goal box, it was allowed to be in goal box for 60 secs before returning to stating box. The day 1-4 were training and the 5 day was the probe day, 24 hours after last training day. The items: time of latency in finding the goal box, total length survey and the number of errors to finding the goal box were computed. Maximum time a rat had the opportunity to find the goal box was 5 minutes.

Statistical analysis: Data were analyzed using IBM SPSS Statistics for Windows (version 20. The quantitative data were presented as Mean ± SEM and analyzed by using one way ANOVA followed by tukey post- hoc. The values with $P<0.05$ were considered as significant difference

Results

The effects of immobilization and electric foot shock stress on the learning process: Our results indicated that EShS increased the latency to finding the target during acquisition phase ($P<0.01$, $P<0.05$). Also, we identified that application of ImmS leads to an increase in the latency which were significant in first day ($P<0.001$) (Figure 1a). Furthermore, the EShS animals did not show improvement in the acquisition during 4days (20 trials). The ImmS also induced longer latency time in finding goal box which in the first day was significant in comparison with control group ($P<0.001$) but it improved during fallowing further three day of acquisition, however it could not reach to control group (Figure 1a). The distance parameter which was the long of the way which animals pursued toward the target hole was in consistence with time of latency during 4 consecutive training days (20 trials) in control group. The increase of distance parameter in EshS and ImmS groups were significant in the fourth day of acquisition ($P<0.0001$) (Figure 1b). Moreover, our results identified that numbers of errors reduced in the control and ImmS groups during four consecutive training days (20 trials). However, in spite of significant low errors in EshS group but it increased during later trials (Figure 1c). Both kinds of stress, ImmS and EShS, disrupted the progressive learning.

The effects of CA on the learning process in control group: Using CA in the vehicle group demonstrated that this component could help the animal to do the better performance in acquisition by decreasing in latency time and pursued distance to finding target hole, but it had not influence on number of errors ($P<0.05$) (Figure2 a, b, c).

The effects of CA on the learning process in EshS group: Administration of CA could improve acquisition performance in spite of electric foot shock stress induction.CA treatment reduce the latency time to finding goal box which was significant in fourth day($P<0.001$) (Figure3 a). Although the distance persuaded and the number of errors were significantly greater in the first day of training in EshS group but they increased during acquisition and the distance showed greater value ($P<0.001$) in EshS group in compare with treated group with CA (Figure3 a, b, c)

The effects of CA on the learning process in the ImmS group: Our results have shown a decreasing pattern in latency time through 4day training in immobilized animals. However, there were no significant different in the distance parameter

through 3 sessions just a bit increase in day 4. In this group. Treatment with CA had no effect on learning process (Figure4 a, b).

Probe trial data

The 24 hours memory indicated significant increase in latency, distance and number of errors in animals which exposed to EshS in compare with control group ($p<0.05$, $p<0.01$, $p<0.0001$). The immobilization stress also showed significant increase in latency time and distance persuaded in probe trial test but there was no difference in number of errors (Figure 1 a-c). Treatment with CA showed better memory performance in comparison with vehicle group (Figure2 a-c). The administration of AC in EshS group also reveal significant better memory by decreasing time of latency and distance against EshS group with no treatment. (Figure3 a-c). In the animals which were exposed to immobility stress the treatment with CA had not effect on memory test Figure4 a, b).

Discussion

The results of current study showed that one-week electric foot shock stress could significantly impair learning and memory process in animals. These impairments include time of latency and traversed distance and also number errors to finding goal target in Barnes maze. The one-week immobilization stress also could prolong the latency time in acquisition process. In the present investigation also we found that administration of the Cinnamaldehyde as an antioxidant and anti-inflammatory agent could improve learning and memory in both control and impaired groups with foot shock stress. In restraint immobilized stress group, significant changes in the time of latency and total surveyed way in acquisition periods and memory test were seen. In this group treatment with cinnamaldehyde could not improve the effect of stress. Many of studies demonstrate mild acute stress facilitate learning and memory formation, which represents an inverted U curve (Danielle et al, 2015) (Miracle et al, 2006). However, chronic stress can impair acquisition and memory consolidation (Holscher et al, 1999) (Miracle et al, 2006). The electric foot shock stress impairs performance in a water-maze spatial task (Oitzl and Kloet, 1992) (Chid et al, 2004) Some evidences indicate that electric foot shock stress result in apoptosis in mouse liver cells (Chid et al, 2004). Chronic restraint stress impairs rat spatial memory on the Y maze trough retraction of apical dendrites in pyramidal neurons (Conrad et al, 1996). A body of studies reveals that exposure to different kinds of stressor cause a variety of neurochemical changes in the brain especially in the medial prefrontal cortex and hippocampus, including increases in the glutamate and acetylcholine release (Bagley and Moghaddam, 1996). Various types of stress such as electric foot shock and restraint can increase dopamine metabolism (Dunn .1988). Reactive oxygen species (ROS) can be a major component which contribute inability of cell function even cell death (Uysaet al, 2005). Both physical and psychological types of stress promote adrenals to glucocorticoids secretion which in turn during long times and hours they increase glutamate receptors this may potentiate the response to prolonged stress, potentially including excitotoxic and cellular damage and also downregulation of hippocampal activity (Halliwell, 1992). On the other hands glucocorticoids increase the toxicity of ROS and increase the basal level of it in the cells (Mcintosh et al, 1988). Our results suggest that CA may improve learning and memory process. However, CA couldn't change the number of errors which we may contribute to more searching activity in these animals. Cinnamon has been used traditionally in food preparations and as an herbal medicine to treat a variety of ailments and their symptoms (Rafie et al, 2015). Cinnamon is known to have antioxidant effects (Eunice et al,2013). Oxidative stress, including protein oxidation, lipid peroxidation and protein nitration, are histological hallmarks in the experimental models of stress (Eunice et al, 2013). In our study, the improvement effects of cinnamaldehyd on the deficits induced by electrical foot shock stress are better than immobility. Some evidences identified that repeated restraint stress induces cognitive impairments that are dependent on the task and on stress intensity (Eunice et al, 2012). Furthermore, Chronic immobilization stress by restraint has been found to alter the pro-oxidant-antioxidant balance, leading to the development of various pathological states such as mitochondrial dysfunction, disruption of energy pathways, neuronal damage, impaired neurogenesis

and induction of signaling events in apoptotic cell death (Hyo et al, 2014). Several studies show that Trans-cinnamaldehyde improves memory impairment in neuro inflammation conditions. The microglia cells were used to assess the potential anti-neuro inflammatory effects of CA by examining the production of nitric oxide, pro-inflammatory cytokines and activation of MAPKs (Zhang et al, 2016). In addition, Modi et al., showed that cinnamon and its metabolite protect memory and learning in an animal model of Alzheimer disease. It suppresses neuronal apoptosis, glial activation, and Aβ burden in the hippocampus and protects memory and learning in the transgenic mice (Khushbu et al, 2015). Thus, cinnamon may be a promising natural supplement in halting or delaying the progression of AD. Chronic stress has long been implicated in the pathogenesis of many disorders and diseases. The scientists discovered that Cinnamon compounds have potential ability to prevent Alzheimer disease by inhibition of tau aggregation (Dylan et al, 2009). Some studies showed that cinnamon increased the activity of PKA, the level of phospho-CREB and neurotrophic factors e.g., brain-derived neurotrophic factor (BDNF). It is possible CA improve stress induced memory deficits by up-regulation neurotrophic factors. It seems that cinnamon and its metabolites may be useful for the various neurodegenerative disorders (Jana et al, 2013). In conclusion our results indicate that cinnamaldehyde could improve learning and memory process in stressful condition, specially its effect in electric foot shock is better than immobilized condition.

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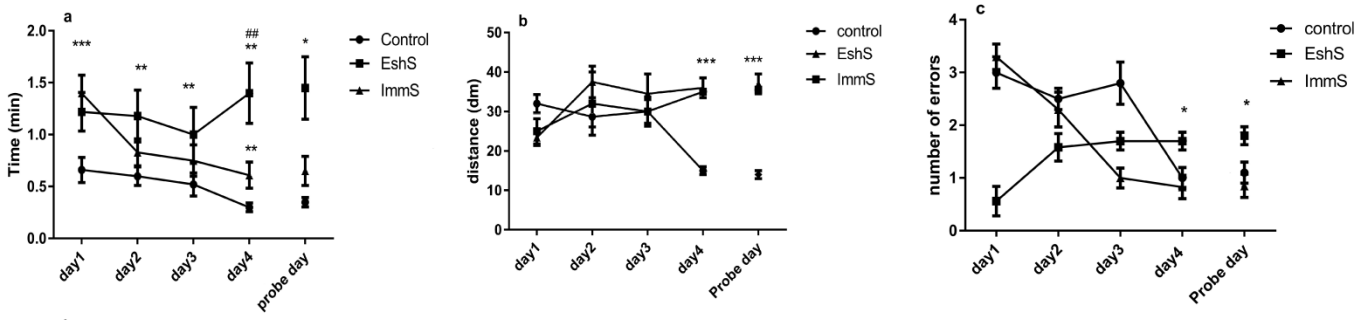

Figure 1. Acquisition and memory probe test curves in control group and stress induced groups (Electric Foot Shock Stress (EshS) and Immobilization Stress (ImmS). Values expressed as Mean ± SEM, n = 12. a) latency time b) the distance surveyed c) errors. * Demonstrated difference with control group and # indicated difference between EShS and ImmS groups. * =p<0.05, **=p<0.01 and ***=p<0.001.

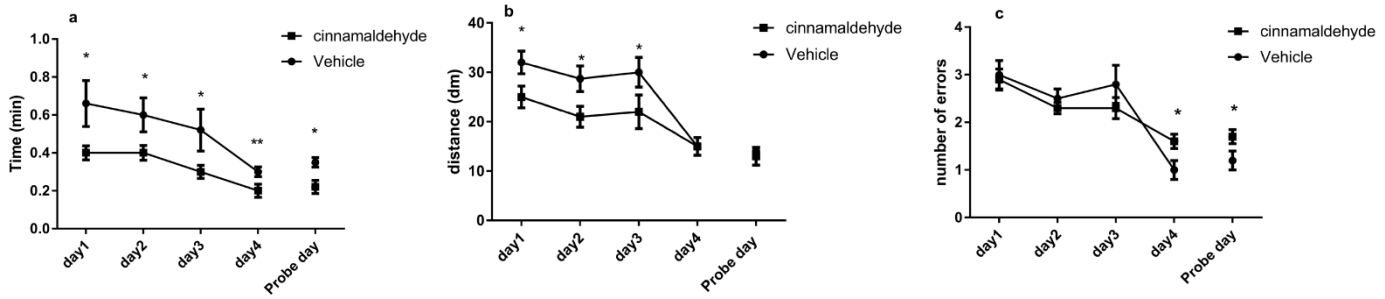

Figure 2. Acquisition and memory probe test curves in control and Cinnamaldehyde (CA) treated groups. Values expressed as Mean ± SEM, n = 12. a) the latency time is decreased in treated group, b) the total path length show decreasing manner, c) the number of errors are the same in both groups. *= p<0.05, **=p<0.01

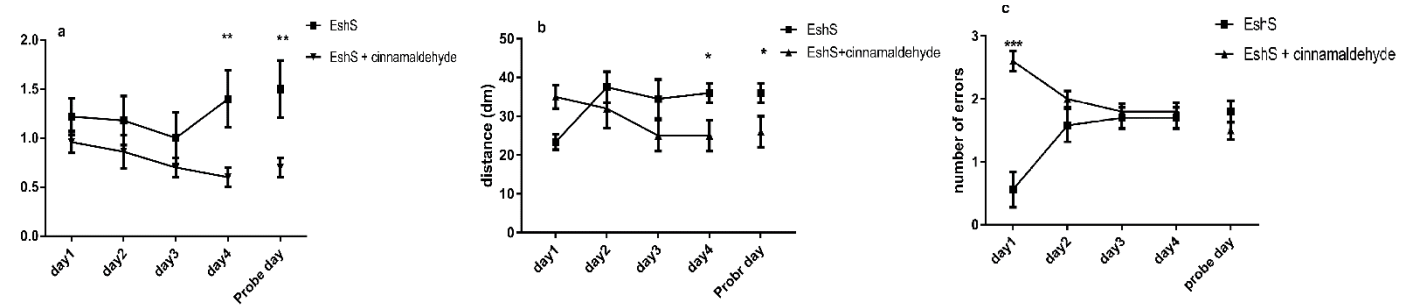

Figure 3. Acquisition and memory probe test curves in EShS group and treated group with CA. Values expressed as Mean ± SEM, n = 12. a) the CA improve the time of latency induced by foot shock stress, especially in 4th session, b) CA repair the total path length which was increased stressed group, c) indication of different acquisition manner in EShS and treated group with CA. * =p<0.05, **=p<0.01 and ***=p<0.001.

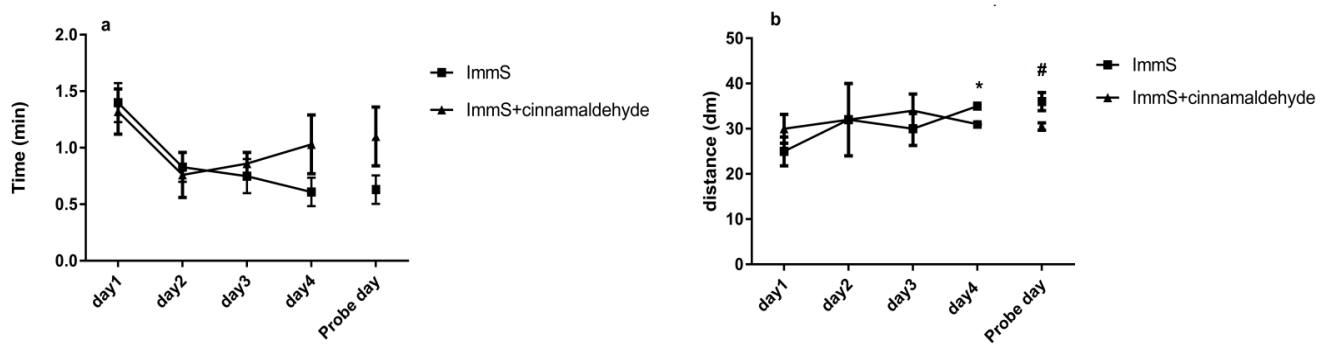

Figure 4. Acquisition and memory probe test curves in ImmS and treated group with CA. Values expressed as Mean ± SEM, n = 12. a,b) Demonstrate that neither time of latency nor total path length (distance) are affected by CA treatment of ImmS group, just in day four there is a bit reduction in total length path in treated group. # = $p<0.05$