ATTENUATION OF CADMIUM INDUCED OXIDATIVE STRESS AND COGNITIVE IMPAIRMENT BY NUTMEG EXTRACT IN RATS

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ABSTRACT

Cadmium compounds are considered toxic and can accumulate in the body as well as the environment. Cadmium has been shown to induce neurotoxicity and hepatocellular damage and adversely affects the biologic function of the liver. In the brain, Cd-induced neuronal damage occurs through amplifying the formation of ROS, which leads to oxidative stress and reducing the antioxidant defense mechanism, thus resulting in neurodegeneration and defects in memory. Natural products and their traditional uses can play an influential role in potential drug development at low cost by researchers. Role of herbs, spices and their constituents to maintain health and to cure diseases is a hot topic in food related research. Various herbs and spices have been shown to affect central nervous system, enhance learning, memory and intelligence. Nutmeg, an extremely high valued commodity not only because of its use as an exotic spice but also with growing evidence for many of its traditional uses as a natural remedy. In present study twenty-four animals were randomly divided into two equal groups control and cadmium treated rats. Animals of both groups were further divided into four groups (1) control-Water (2) nutmeg Extract (3) Cadmium treated rats-Water (4) Cadmium Nutmeg extract (300mg/ml). Weekly Behavioral alteration was monitored. The current study revealed that administration of cadmium exhibited anxiogenic effect by in light and dark box activity. The present study also revealed that cadmium administration significantly impaired short -term and long -term memory in MWM and also impaired recognition memory in NOR.

Keywords: Cadmium, Oxidative stress, Cognitive behavior, Nutmeg, Behavioral assessments

1.1 INTRODUCTION

The environmental exposure to the heavy metal, cadmium (Cd), has increased during the last decades due to its wide distribution in foods, vegetables, soil, smoking, and daily consumable products such as batteries. (Rafati *et al*., 2017). Environmental heavy metals are known to adversely affect mental health.1 Cadmium (Cd) is one of the most common heavy metals on Earth. (Forcella *et al.,* 2020, Almenara *et al.,* 2013). It is associated with neurotoxicity, cognitive decline, and neurodegeneration and is a true risk factor for the development of depression, psychological disorders, and neurodegenerative disorders such as dementia, Parkinson disease, and Alzheimer disease (AD) (Forcella *et al*., 2020).

Cadmium ions can increase the permeability of blood–brain barrier (BBB) and pass through to cause neural damage, primarily through the generation of reactive oxygen species (ROS) and induction of oxidative stress. (Goncalves *et al.,* 2011, El-Kott AF *et al.,* 2020). In past, a large number of chemical agents have been discovered or synthesized in order to treat and cure these infections but widespread and indiscriminate use of these drugs has led to the development of many drug resistant strains which constitutes a major problem worldwide as the existing drugs are becoming ineffective to control them. Consequently, there is an urgent need to look for alternatives of synthetic antibiotics and other drugs. (Hinneburg *et al*., 2006).

There has been a growing interest in natural antioxidants found abundantly in plants. Apart from green tea, numerous herbs and spices are the most important targets in the search for natural antioxidants from the point of view of safety. Spices that are normal ingredients of our diet are now known to exert health beneficial antioxidant effects in the mammalian system and in several model systems through their bioactive compounds A wide variety of phenolic compounds and flavonoids present in spices are now experimentally documented to possess potent antioxidant, anti-inflammatory, anti mutagenic, and anti carcinogenic activities.

The antioxidative and anti-inflammatory properties of these bioactive compounds from spices appear to contribute to their chemo preventive or chemo protective activity. (Srinivasan, 2014, Serafini *et al.,* 2016). The nutmeg is the seed of Myristica fragrans, which is well-known as a spice and functions as a natural antioxidant by preventing the peroxidation of lipids and quenching single oxygen (Piaru *et al.,* 2012).

A variety of chemical components and active phytochemicals, including vitamins, alkaloids, phenolic, flavonoids and lignan, improve their antioxidant effects against oxidative stress (Gupta *et al.*, 2013). M. fragrans is a plant with antithrombotic, antifungal, antibacterial, and anti-inflammatory properties (Dewi *et al*., 2015, Zhang W *et al*., 2016). Current study was design to evaluate the pharmacological effects of nutmeg extract in cadmium induced oxidative stress and cognitive impairment in rats.

2.1METHODS AND CHEMICALS

Animals Study was designed on 24 male Albino wistar rats (120-180 gm), purchased from the Dow university of Health and Sciences, Karachi, Pakistan and housed in individual cages and allowed to acclimatize to their surrounding with temperature (25±2ºC) for one week. Animals had free access to a normal standard diet during acclimatizing time before starting experiment. All experimental animals were approved by Institutional Ethics and Animal Care Committee, and the procedure was carried out in accordance with the National Institute of Health Guide for care and Use of Laboratory.

2.2. Collection and extract preparation.

Nutmeg was collected from the local market and seeds were grounded to powder and then soaked in ethanol for 8 days at room temperature. The soaked material was then filtered with the help of Whatman's No. 1 filter paper and supernatant was collected. Extraction procedure was repeated twice and filtrates were eventually combined earlier than subjecting to evaporation. To take out extract of nutmeg rotary apparatus was used at reduced pressure. Finally extract of nutmeg was obtained and stored until required.

2.3. Experimental protocol

Twenty-four animals were randomly divided into two equal groups control and cadmium treated rats. Animals of both groups were further divided into four groups (1) control-Water (2) nutmeg Extract (3) Cadmium treated rats-Water (4) Cadmium Nutmeg extract (300mg/ml). Weekly Behavioral alteration was monitored.

2.4. BEHAVIORAL ASESSMENT

2.4.1. Light and Dark Transition Box

Anxiolytic effects were determined by light/dark transition box. Procedure was same as described previously (Haleem and Mahmood, 2019).

2.4.2. Morris Water Maze

Brie fly the test comprised of training and test sessions during which the escape latency, that is the time taken by each rat to reach the hidden platform, was noted. The test phase was performed 24 h after a training session. The cutoff time was 2 min. A decrease in escape latency during test sessions was taken as an index of memory improvement.

2.3.3. Novel objects recognition (NOR) test

On the brief account NOR test was conducted to determine recognition ability in rats to distinguish between new and old object. Rats were trained to familiarize with two same objects during training session. During test session one old object was then replaced by a new object and time to sniff both objects was recorded. Recognition index was calculated by using the formula [time to sniff new object / (time to sniff old object + time to sniff new object)].

3.1. STATISTICAL ANALYSIS

Results are expressed as means ± SD. Effect of nutmeg extract administration on water or cadmium administrated rats were analyzed by three-way ANOVA (repeated measures design). The software used for the analysis was SPSS (version 16). Post-hoc comparison was done by Tukey HSD test. Values of $p<0.05$ were considered as significant.

4.1. RESULTS

4.1.1. Effect of Nutmeg Extract on anxiogenic or anxiolytic behavior (Time spent in light box) in cadmium induced oxidative stress and cognitive impairment in rats.

Figure 1 shows the effect of nutmeg extract administration on anxiogenic or anxiolytic behavior (no of entries in light box) in cadmium induced oxidative stress and cognitive impairment in rats. Anxiogenic or anxiolytic behavior was monitored by light dark transition box. Analysis of the data three-way ANOVA (repeated measures design), the effect of days ($F=0.118$, $df=18, 2$) were found to be non-significant. The effect of cadmium ($F=8.248$,

df=2,18, p<0.01).The effect of nutmeg extract (F=89.773, df=18,2,p<0.01) and the interaction between the days, cadmium and nutmeg (F=0.118,df=72,11,p<0.01)were found to be significant. Post hoc analysis by Tukey HSD test showed cadmium induced oxidative stress and cognitive impairment significantly showed anxiogenic behavior at 7^{th} (p<0.01) and 14^{th} (p<0.01) days of administration as compared to control group. This anxiogenic behavior is reversed by the administration of nutmeg extract at 7^{th} (p<0.01) and 14^{th} (p<0.01) of administration in cadmium treated rats groups.

4.1.2. Effect of Nutmeg Extract on Recognition memory (Time spent with novel object) in cadmium induced oxidative stress and cognitive impairment in rats

Figure 2 shows the effect of nutmeg extract administration on recognition memory (time spent with novel object) in cadmium induced oxidative stress and cognitive impairment. Analysis of the data on recognition memory by three-way ANOVA (repeated measures design), the effect of days ((F=0.749, df=2,18), the effect of cadmium (F=0.200, df=2,18) were found to be non-significant. the effect of nutmeg extract (F=15.690, df=2,18,p<0.01), and the interaction of cadmium, drug and days (F=4.023, df=72,11,p<0.01) were found to be significant. Post hoc analysis by Tukey HSD test showed that recognition memory was significantly increased at 7^{th} day (p<0.01), 14th day (p<0.01) of administration in nutmeg healthy rats group. Similarly in cadmium treated rats group, recognition memory was increased on 14^{th} (p<0.05) of administration of nutmeg extract.

4.1.3. Effect of Nutmeg Extract on Short term memory (Time to reached at platform) in cadmium induced oxidative stress and cognitive impairment in rats

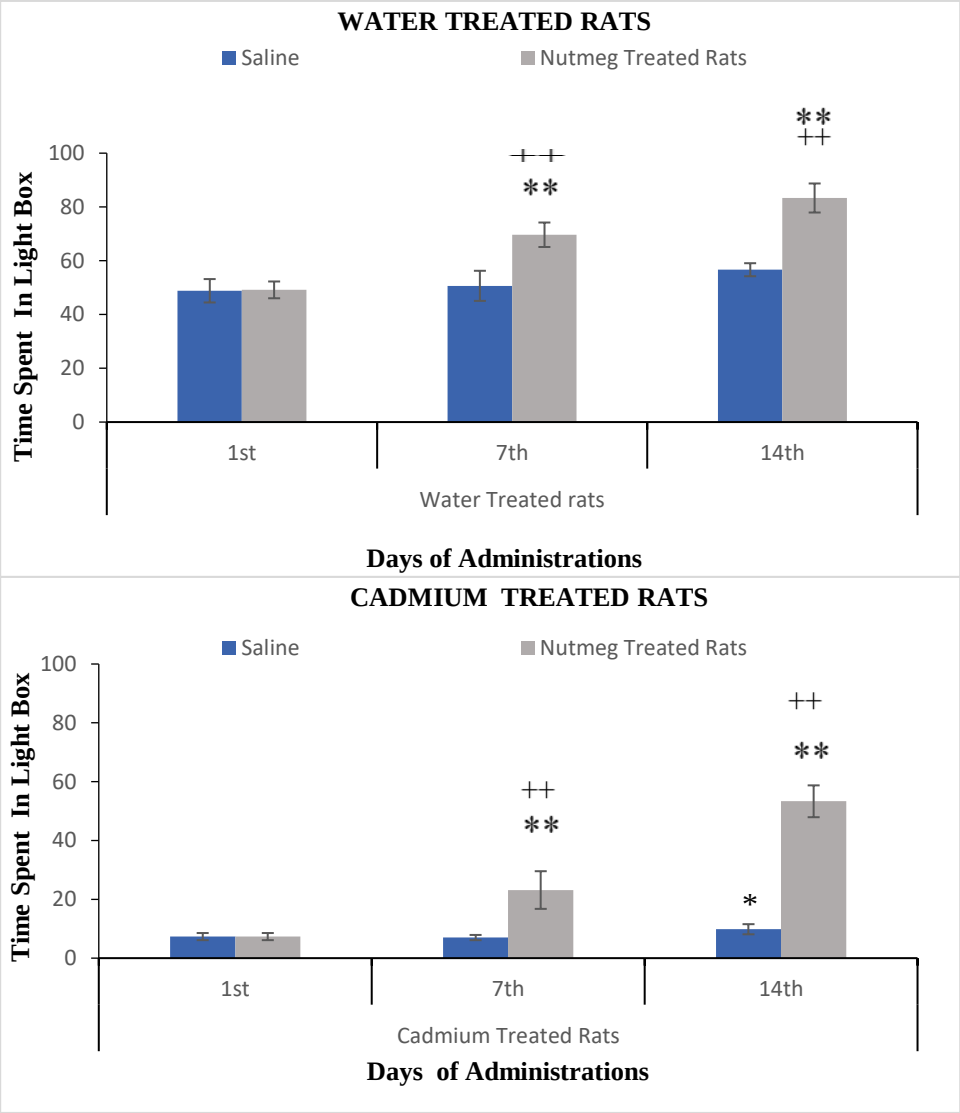
Figure 3 shows the effect of nutmeg extract administration on short term memory (time to reached at platform) in cadmium induced oxidative stress and cognitive impairment. Analysis of the data on by three-way ANOVA (repeated measures design), the effect of days (F=5.679, df=2, 18). the effect of cadmium (F=3.629, df=2, 18) and the effect of nutmeg extract (F=3.000, df=2, 18), were found to be non-significant. and the interaction of cadmium, drug and days (F=19.628, df=72, 11, p<0.01) were found to be significant. Post hoc analysis by Tukey HSD test showed that short term memory was significantly increased at 7^{th}

day($p<0.05$), 14th day ($p<0.05$) of administration in nutmeg healthy rats group. Similarly in cadmium treated rats group, short term memory was increased on 7^{th} day ($p<0.05$), and 14^{th} ($p<0.01$) of administration of nutmeg extract.

4.1.4. Effect of Nutmeg Extract on Long term memory (Time to reached at platform) in cadmium induced oxidative stress and cognitive impairment in rats

Figure 4 shows the effect of nutmeg extract administration on long term memory (time to reached at platform) in cadmium induced oxidative stress and cognitive impairment. Analysis of the data on by three-way ANOVA (repeated measures design), the effect of days ($F=1.012$, $df=2, 18$), the effect of cadmium ($F=0.020$, $df=2, 18$) were found to be non-significant. the effect of nutmeg extract ($F=5.3000$, $df=2, 18$, $p<0.05$) and the interaction of cadmium, drug and days ($F=21.489$, $df=72, 11$, $p<0.01$,) were found to be significant. Post hoc analysis by Tukey HSD test showed that there was no effect on long term memory was in nutmeg healthy treated rats groups. Similarly in cadmium treated rats group, long term memory was increased on 7th day ($p<0.05$), and 14^{th} ($p<0.01$) of administration of nutmeg extract.

Figure 1. Effect of Nutmeg Extract on anxiogenic or anxiolytic behavior (Time spent in light box) in cadmium induced oxidative stress and cognitive impairment in rats.

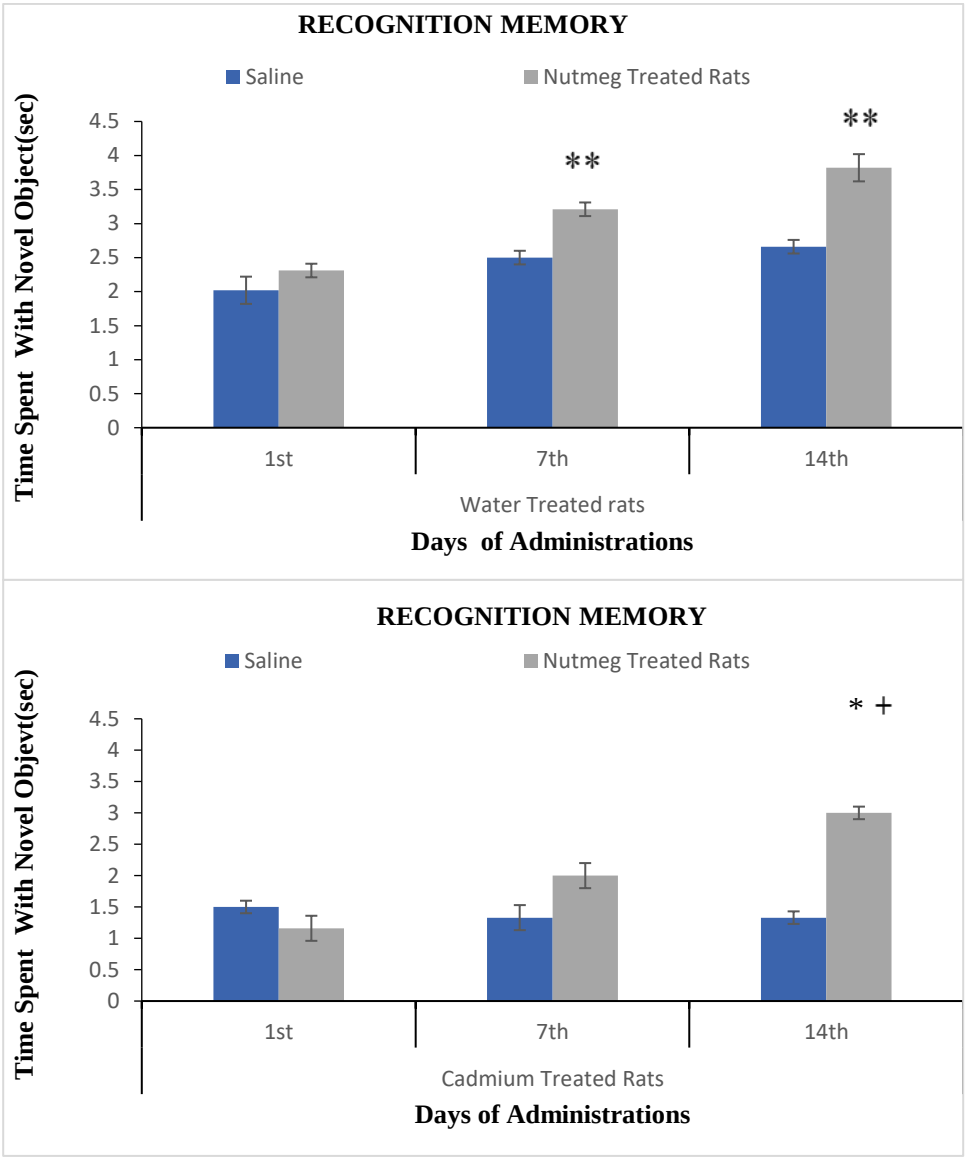


Values are means ± SD (n=6). Significant differences by Tukey HSD test:

* $p < 0.05$, $p < 0.01$ when compared among saline inject or nutmeg extract from 1st day administration;

+ $p < 0.05$, ++$p < 0.01$ when compared saline inject cadmium induced animals or cadmium induced animals with nutmeg extract samples; following three-way ANOVA (repeated measure design).

Figure.2.Effect of Nutmeg Extract on Recognition memory (Time spent with novel object) in cadmium induced oxidative stress and cognitive impairment in rats

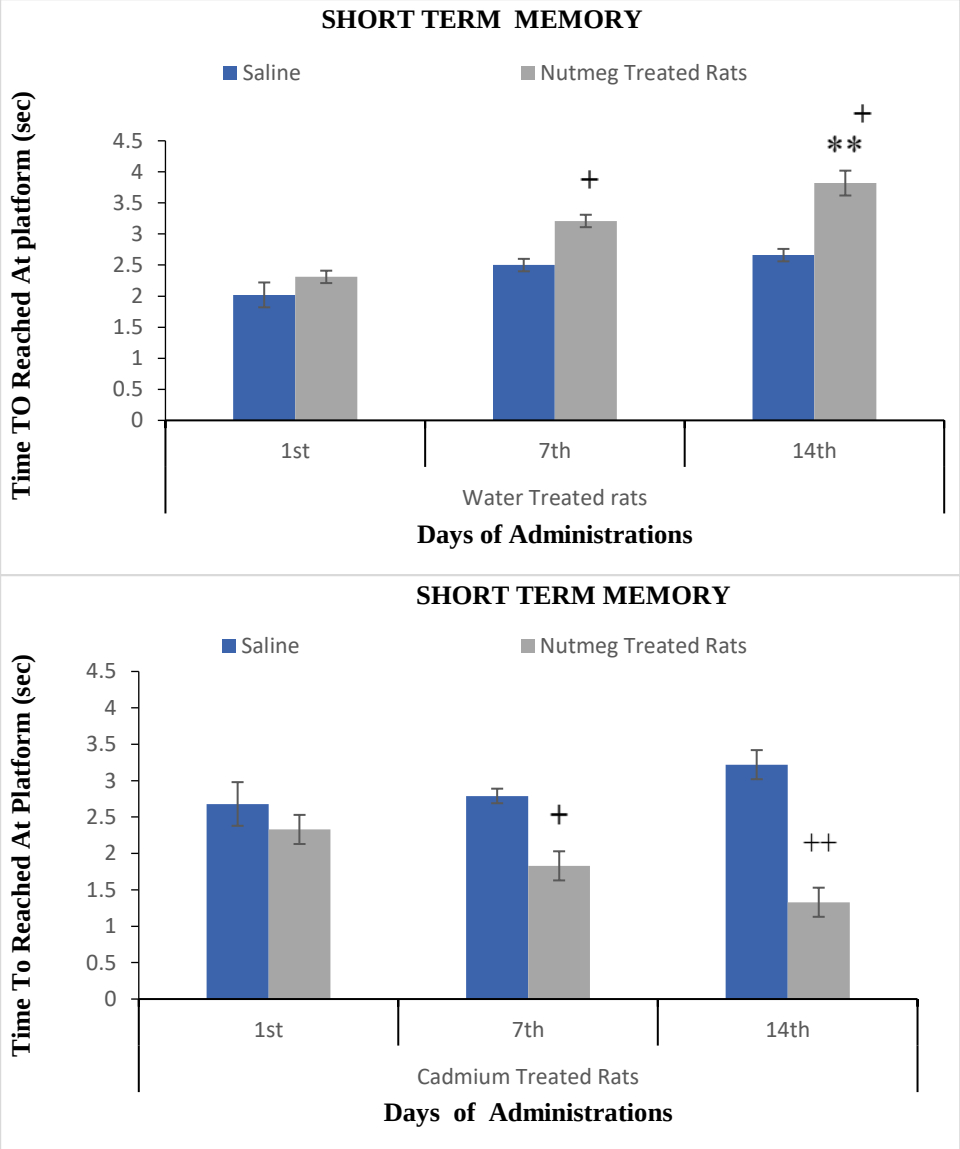


Values are means ± SD (n=6). Significant differences by Tukey HSD test:

* $p < 0.05$, $p < 0.01$ when compared among saline inject or nutmeg extract from 1st day administration;

+ $p < 0.05$, ++$p < 0.01$ when compared saline inject cadmium induced animals or cadmium induced animals with nutmeg extract samples; following three-way ANOVA (repeated measure design).

Figure. 3. Effect of Nutmeg Extract on Short term memory (Time to reached at platform) in cadmium induced oxidative stress and cognitive impairment in rats

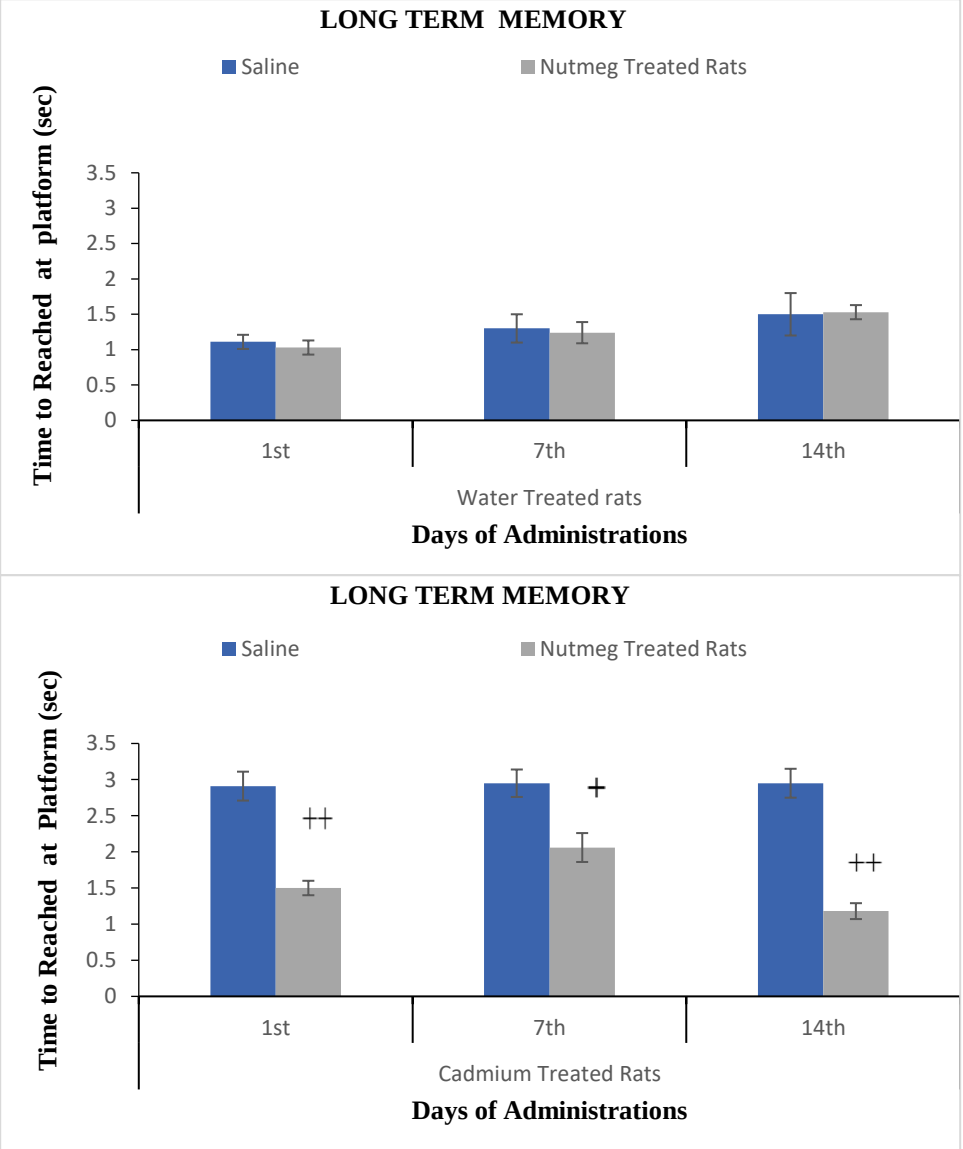


Values are means ± SD (n=6). Significant differences by Tukey HSD test:

* $p < 0.05$, $p < 0.01$ when compared among saline inject or nutmeg extract from 1st day administration;

+ $p < 0.05$, ++$p < 0.01$ when compared saline inject cadmium induced animals or cadmium induced animals with nutmeg extract samples; following three-way ANOVA (repeated measure design).

Figure 4. Effect of Nutmeg Extract on Long term memory (Time to reached at platform) in cadmium induced oxidative stress and cognitive impairment in rats



Values are means ± SD (n=6). Significant differences by Tukey HSD test:

* $p < 0.05$, $p < 0.01$ when compared among saline inject or nutmeg extract from 1st day administration;

+ $p < 0.05$, ++$p < 0.01$ when compared saline inject cadmium induced animals or cadmium induced animals with nutmeg extract samples; following three-way ANOVA (repeated measure design).

DISCUSSION

The current study revealed that administration of cadmium exhibited anxiogenic effect by decrease the time spent in light box in light and dark box activity. This anxiogenic behavior was reverse by the administration of nutmeg extract at 14th day of administration. The present study also revealed that cadmium administration significantly impaired short -term and long -term memory by decreasing time to reach at platform in MWM and also impaired recognition memory neither in NOR By decline in time spent with novel object. The administration of nutmeg extract significantly enhanced short-term and long-term memory at 7th and 14th day administration and it also improved recognition memory at 14th day administration.

This study revealed that Cadmium exposure induced anxiogenic behavior by decreasing the time spent in light box. the present study also reveled that cadmium exposed rats treated nutmeg extract significantly showed anxiolytic behavior at 14th day of administration by increasing the time spent in light box in light and dark box activity. Previously study also reported the anxiolytic behavior of nutmeg. It may be the presences of Myristicin, a component of nutmeg showing evaluation of anxiolytic (Ingale. S. *et al*., 2012).

Hippocampus and frontal cortex regions of brain are highly implicated in learning and memory processes. These regions play a critical role in everyday memory formation for facts and habituations (Xu and Südhof, 2013). Different types of water maze models are available but Morris water maze test has been used in various experiments to assess the effect of drugs on cognitive impairment (Xu *et al*., 2019; Schröder *et al*., 2020) due to possess additional advantages (Bromley-Brits *et al*., 2011). Current study revealed that short term and long term memory Significantly impaired by increase in time to reach at platform induced by Cd was observed in rats at 7th and 14th day of administration. It also revealed that cadmium exposed rats significantly impaired recognition memory as compared to control. Previous literature also demonstrates that cadmium is a neurotoxin, its administration alters learning and memory functions (Lukawski *et al*. 2005). Present study revealed that cadmium treated nutmeg extract significantly enhanced short, long term and recognition memory by decreasing the time to reached at platform and increasing the time spent with novel object in MWM and NOR respectively.

Past study similar reported that M. fragrans significantly improved learning and memory functions. It may due to the decrease acetyl cholinesterase activity. Acetyl cholinesterase is an enzyme that inactivates acetylcholine and the central cholinergic pathways play a prominent role in the learning and memory process (Thouvarecq*et al*., 2001, Dhingra *et al*., 2006).

CONCLUSION

Present study shows cadmium administration revealed anxiogenic behavior and leading to impaired memory performance. Culinary herbs and spices are foods that are a rich source of bioactive molecules such as sulfur-containing compounds, tannins, alkaloids, phenolic diterpenes, and vitamins, especially flavonoids and polyphenols. Research over the past decade has reported that bioactive constituents of spices possess the diverse range of health benefits. Spices and herbs are excellent sources of antioxidants with their high content of phenolic compounds. Furthermore, antioxidant components of these spices may augment the antioxidant potential of neuron thereby reducing cadmium-induced oxidative stress and thus improve memory function. Our findings assume special significance for general population exposed to heavy metal through occupation or daily life routine. Daily consumption of nutmeg may provide beneficial effects against heavy metal-induced toxicity due to environmental pollution. These results may help to improve therapeutics in oxidative stress-induced memory related disorder

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