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Protective impact of Luteolin on Scopolamine induced-Amnesia in Wistar rats: an involvement of Acetylcholinesterase, dopamine and lipid peroxidation

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Abstract: ***Background:*** The alteration in cholinergic transmission is one of the hallmarks of amnesia which is frequently observed in Alzheimer's disease. ***Aim:*** The present study is focused to evaluate the possible protective effect of Luteolin on cognitive functions in scopolamine-induced amnesia in rats. Methods: The rats were divided into five groups containing six animals in each group; Group I the control group, Group II received Scopolamine (0.5 mg/ kg), Group III received Donepezil (10 mg/ kg) along with Scopolamine, and Group IV and V animals received Luteolin (10 mg/ kg and 20 mg/ kg) respectively along with Scopolamine. ***Results:*** Luteolin administration resulted in enhancement in learning tendency as observed by remarkable prolongation in time to reconnoiter novel objects, intensification in the retention trial, an increase of the time spent in open arms and diminution in level of AchE as well as lipid peroxidation, while up regulation of brain dopamine levels. ***Conclusion:*** Luteolin could be regarded as a beneficial agent proved to be effective against experimental induced-amnesia. Though, the molecular mechanism beyond this effect needs to be explored.
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1. Introduction

A rising number of people in elderly communities worldwide suffer from memory loss and amnesia as many countries are transitioning into ageing societies. Over the years, the number of patients with amnesia and memory impairment have been gradually grown, with more than 46 million people now living with the condition globally, and expected to increase to 131.5 million by 2050 (Mat Nuri et al., 2017). In 2010 the A cost of treatment and amnesia medication was estimated to be $604 billion and has risen annually (Wimo et al., 2013). This cost is estimated to increase two folds in the coming decades.

The hippocampus and cortex are actively involved in memory maintenance and regulation. The deposition of neurofibrillary tangles and senile plaques is correlated with dementia, which enhances oxidative stress and lowers cholinergic activity in the brain (Hampel et al., 2018). The alterations in the levels of the acetylcholine have been documented to influence cognitive function and been involved in memory loss (Decker and Duncan, 2020; Mineur and Picciotto, 2019). The deterioration in cognitive abilities contributes to amnesia which is one of the manifestations of some neurodegenerative diseases such as Alzheimer's disease (Matej et al., 2019). This phenomenon may occur perhaps due to brain damage or injury probably by the use of certain specific medications viz. sedatives. It may also occur as a result to the use of the muscarinic cholinergic receptor antagonists that worsen learning and cognition (Blackburn, 2017). It has been proposed that the enhancement of cholinergic activity in the brain is a standard strategy for delaying progression of the disease. In order to manage the symptoms of memory loss, several acetyl-cholinesterase blockers have been licensed and are associated with various adverse effects, including nausea, diarrhea, vomiting, anorexia and hepato-toxicity (Abeysinghe et al., 2020). In addition, no medication for treating the patient with memory deficits has been approved (Capouch et al., 2018).

The traditional medicine seems to be a vital choice to treat memory deficit. Flavonoids are widely acknowledged for antioxidant effect on biological system (Airoldi et al., 2018; Karak, 2019). Luteolin is a naturally-occurring flavonoid and well acknowledged for anti-inflammatory (Aziz et al., 2018), anti-oxidant, apoptosis-inducing and chemo preventive (Imran et al., 2019) activities. Luteolin is known to scavenge free radicals and hence protects

cells from reactive oxygen species associated damage. Luteolin is present in Artichoke extract and reported to be Phosphodiesterase-4 (PDE4) inhibitor. For neural signaling within brain cells, c-AMP is essential. The excess of PDE4 degrades c-AMP. Thus, Luteolin may prove to be beneficial in enhancing the process of learning and memory acquisition (Röhrig et al., 2017). Luteolin also demonstrated anti-amnesic effect against amyloid beta (25-35) peptide-induced toxicity in mice model (Liu et al., 2009). Therefore, our aim was to evaluate the anti-amnesic effects of Luteolin against Scopolamine induced amnesia in rats.

2. Material and Methods

2.1 Animals

Wistar rats (180–200 g) of matched sex were housed in polypropylene cages under standard condition (12 h light/dark cycles at 28 ± 2°c). Lab animals were provided with standard pellet food and had free access to drinking water. Our animal study protocol was in a compliance with the guidelines of Institutional Animal Care and Use Committee (IACUC), Supervision of Experiments on Animals (CPCSEA) and ARRIVE (Animal Research: Reporting of In Vivo Experiments) and were duly approved by the Animal Care and Use Committee of Medical Research Institute, Alexandria University under the No. of 1015–11-MRI, May 2020.

2.2 Chemicals

Luteolin and Scopolamine were obtained from Sigma–Aldrich (MO, USA). Scopolamine was dissolved in normal saline (0.9% NaCl). While, Donepezil hydrochloride was purchased from Pfizer (Giza, Egypt) and was freshly prepared in 1% tween 80 in water. All other chemicals used in the study were of analytical grade.

2.3 Selection of Dose

As per the Wang et al. studies (Wang et al., 2016). Luteolin in the dose of 10 and 20 mg/kg per oral was performed in the study. Donepezil was used in the dose of (10 mg/kg) (Schreiber et al., 2007).

2.4 Grouping of Animals

Animal grouping and Dosing (n=6)

Group 1: Normal Control (2ml/ kg saline)

Group 2: Scopolamine (0.5 mg/ kg)

Group 3: Scopolamine (0.5 mg/ kg) + Donepezil (10 mg/kg)

Group 4: Scopolamine (0.5 mg/ kg) + Luteolin (10 mg/ kg)

Group 5: Scopolamine (0.5 mg/ kg) + Luteolin (20 mg/ kg)

2.5 Passive avoidance paradigm

The passive avoidance test was performed according to Yadav et al. method (Yadav et al., 2011) with a slight modification. Rats were placed in light compartment of a shuttle box as prerequisite for a passive avoidance test. The light compartment was isolated from the dark one by a guillotine door. After 30 s of acclimatization, the door was opened and closed after their entry into the dark compartment to receive a low-intensity foot shock (0.5 mA) for 10 s where, the transfer latency time (TLT) is recorded as well. The overall trial duration was 270 s divided into two partitions; The first one was for acquisition and the second one for retention testing after 1 day of the first one.

2.6 Novel object recognition test

Animals were tested using a (50 × 25 × 50 × 25) cm black box for couple days for object exploration (T1) which defined as sniffing or touching the object at <2 cm from the nose or exploring both the objects for ≥10 s. After identification, animals were reverted to its original cage (T2) for 24 h later to freely explore a familiar and novel object for 4 min. The time spent to explore objects was rerecorded on videotape (Mathiasen and DiCamillo, 2010).

2.7 Rotarod performance test

The ability to maintain balance and motor resistance was investigated using rotarod (Shiotsuki et al. 2010). First, the animal was placed on the rotating rod of the apparatus and allowed to walk at (7 rpm and 10 rpm acceleration). Thirty minutes later, rats were placed again and the time they took for balance and resist rod movement was recorded. The maximum time for each animal was 300 s.

2.8 Elevated plus maze test

This test is used to measure anxiety (Treit et al. 1993) by apparatus with two opposite open arms, two opposite closed arms, and a central sheath rise 50 cm above the floor. This test was done in a dark, silent chamber as each animal faced the open arm for 5 min. The number of entries and time spent in each arm were recorded.

2.9 Biochemical Estimation

2.9.1 Assay of Acetylcholinesterase (AChE) activity

The AChE activity was analyzed in hippocampus using acetylthiocholine iodide as substrate following the colorimetric method. Briefly, the reaction mixture in a final volume of 1.0 ml contained phosphate buffer (0.1 M, pH 7.4), post mitochondrial fraction of hippocampus containing around 15-20 µg protein, acetylthiocholine iodide and 5'dithionitrobenzoic acid (DTNB) (5 mM). The degradation of acetylthiocholine iodide was measured at 412 nm and results are expressed as µmol/mg protein (Ellman et al., 1961).

2.9.2 Assay of lipid peroxidation

The extent of lipid peroxidation was performed by a reported method (Ohkawa et al., 1979). Briefly, homogenate of hippocampus in phosphate buffer (0.1

M, pH 7.4) was incubated with sodium dodecyl sulfate (10%, w/v) for 10 min followed by the addition of 20% acetic acid. The reaction mixture was incubated with thiobarbituric acid (0.8%) for 1 h in boiling water bath. The pink color formed was measured at 532 nm and the amount of Thiobarbituric acid reactive substances (TBARS) was calculated using a molar extinction coefficient of 1.56 × 10 5 M/cm.

2.9.3 Assay of Dopamine levels

About 1 ml of brain sample was homogenized with 1.00 ml of 1.5 × 10–2 M ferric chloride, and 1.00 ml of 1.5 × 10–2 M potassium ferricyanide then transferred into a tube and diluted up to 25 ml with distilled water. Then, shaking well and incubate for 35 min at room temperature. Finally, the absorbance of the solution was measured at 735 nm against blank (Guo et al., 2009).

2.10 Statistical analysis

Data were expressed as mean ± SEM. Statistics was applied using Graph Pad Prism version 8.0 for Windows, Graph Pad Software, San Diego, California, USA. One-way ANOVA test with Dunnett's comparison test were performed to analyze statistical significance. Differences were considered significant if $p < 0.05$.

3. Results

3.1 Passive avoidance paradigm

It is an affiliative model of learning in which the subject tends to equate the occurrence of an aversive stimulus with a specific circumstance. The passive avoidance activity in rats is the repression of the inherent affinity for the test apparatus's dark compartment. Figure 1 shows the effects of the latencies in the passive avoidance task. There was no notable differentiation between the groups in the initial latency to reach the dark chamber, according to the findings. In the scopolamine-administered group (Group II), the secondary latency period was substantially decreased than in the control group (Group I). The administration of Luteolin resulted in a significant increased the time to explore novel objects (Group IV and V). The treatment with Donepezil (Group II) significantly rise in the secondary latency time ($p < 0.001$).

3.2 Novel object recognition test

The novel object recognition test is used to assess the memory of recognition. In contrast to the acquisition studies, exposure of rats to scopolamine induced a substantial decrease in the transmission latency time of the retention trials, suggesting a decline in the learning and memory performance of rats relative to controls. In the retention trial, the transition latency period in rats in the control and concurrent experimental groups with Donepezil and scopolamine was increased when compared to the acquisition trial. In addition, simultaneous treatment with scopolamine in rats with Luteolin (20 mg/kg and 40 mg/kg) resulted in a substantial improvement in the retention trial relative to the acquisition trial. The treatment with Luteolin in rats treated with scopolamine resulted in a substantial improvement in the retention trial relative to the acquisition trial, suggesting better memory and learning in rats (Figure 2).

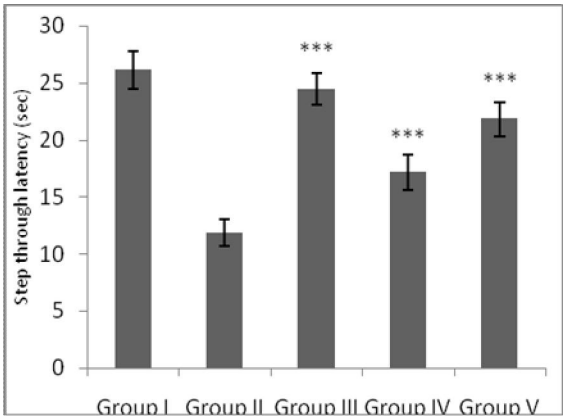

Figure 1. Effect of administration of Luteolin on step through latency in scopolamine induced dementia in rats. The data were expressed as mean ± standard deviation (SD) and *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ was considered significance level.

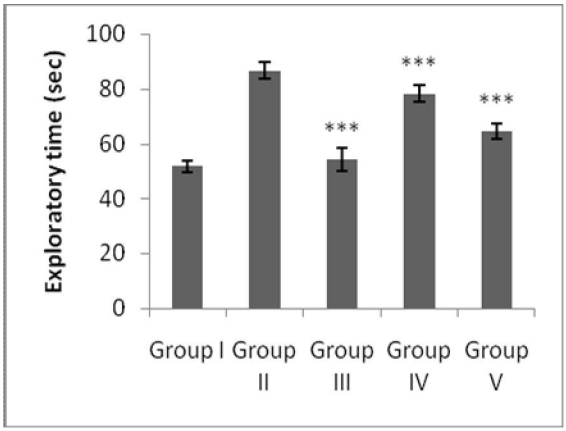

Figure 2. Effect of administration of Luteolin on exploratory time in scopolamine induced dementia in rats. The data were expressed as mean ± standard deviation (SD) and *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ was considered significance level.

3.3 Plus maze test

The effect of Scopolamine, Donepezil and Luteolin was evaluated on plus maze apparatus (Figure 3; Figure 4). The administration of Scopolamine resulted in the notable increase in transfer latencies on acquisition along with retention

days in comparison with normal control group. Luteolin treatment (20 mg/ kg and 40 mg/ kg) resulted in notable changes in transfer latency. The treatment with Scopolamine caused a substantial increase in the time duration consumed in the closed arms and a attenuation in the time resided in the open arms. Treatment of rats with Luteolin substantially decreased the residence time in the closed arms and increased the time spent in the open arms ($p < 0.001$).

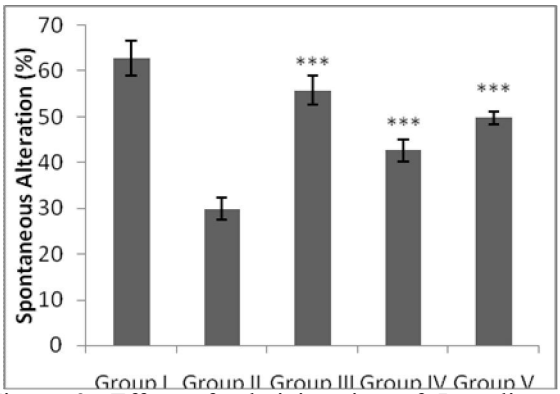

Figure 3: Effect of administration of Luteolin on spontaneous alteration in scopolamine induced dementia in rats. The data were expressed as mean ± standard deviation (SD) and *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ was considered significance level.

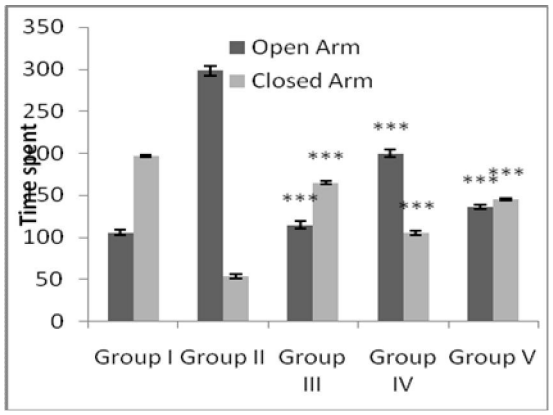

Figure 4: Effect of administration of Luteolin on open and closed arm in plus maze in scopolamine induced dementia in rats. The data were expressed as mean ± standard deviation (SD) and *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ was considered significance level.

3.4 Rotarod test

The figure 3 showed the outcomes for the period of the rotarod test equilibrium in various groups. The duration of balance was notably less in the scopolamine group as compared to the control group. No notable change in the equilibrium period was caused by the treatment with Luteolin. The administration of Luteolin (20 mg/ kg and 40 mg/ kg) resulted in increased residence time on rotarod (Figure 5).

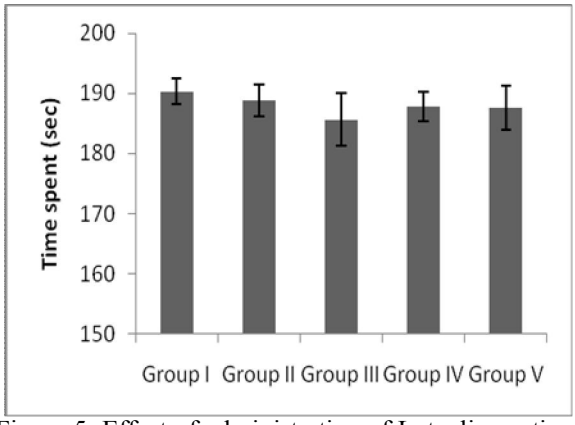

Figure 5: Effect of administration of Luteolin on time spent in Rotarod apparatus in scopolamine induced dementia in rats. The data were expressed as mean ± standard deviation (SD) and *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ was considered significance level.

3.5 Assay of AChE activity

The exposure of rats to scopolamine triggered a substantial increase in the activity of AChE, an enzyme that is associated with the metabolism of acetylcholine in rats. Treatment with donepezil in rats treated with scopolamine produced a substantial decrease in AChE activity in the brain of rats relative to those treated with scopolamine alone. In addition, treatment with Luteolin in rats, relative to those treated with scopolamine alone, caused a significant increase in AChE activity in the brain in rats (Table 1).

Table 1. Effect of administration of Luteolin on biochemical markers in scopolamine induced dementia in rats.

Treatment	AChE activity (ng/g wet tissue)	TBRAS formation inhibition (%)	Dopamine levels (ng/g wet tissue)
Group I	0.0085±0.00067	369.24±21.74	347.58±11.23
Group II	0.0222±0.00056	137.67±17.23	417.24±13.71
Group III	0.0121±0.00049***	296.52±17 ***	384.03±14.54***
Group IV	0.0193±0.00075***	196.95±19.82***	408.52±12.82***
Group V	0.0152±0.00067***	254.24±18.57***	364.28±14.35***

‡The data were expressed as mean ± standard deviation (SD) and *p < 0.05, **p < 0.01, ***p < 0.001 was considered significance level.

3.6 Assay of lipid peroxidation

The exposure of Scopolamine in rats resulted in a notable increment in lipid peroxidation in rat brain in comparison with normal control. TBRAS levels are the remarkable indicator of lipid peroxidation. TBARS are generated as a by-product of lipid peroxidation that can be identified by the TBARS assay utilizing thiobarbituric acid. The treatment with Donepezil in rats caused in a notable decrement in the extent of lipid peroxidation in rat brain as compared to those treated with scopolamine. However, the treatment with Luteolin in rats caused a notable decrease in the level of lipid peroxidation in rat brain as compared to those treated with scopolamine (Table 1).

3.7 Assay of Dopamine

The administration of scopolamine in animals resulted in increase in dopamine levels in the rat brain. Administrations of Luteolin tend to decrease dopamine levels in animals. Donepezil administration also resulted in decrement in the level of brain dopamine.

4. Discussions

Scopolamine, a non-selective muscarinic receptor blocker prevents cholinergic signaling and induces memory and learning afflictions, resulting in long-term and short-term memory impairment, involving learning and memory deficits. It also produces amnesia in rodents. Scopolamine treatment results in increment in oxidative stress that alters antioxidant defense system (San Tang, 2019). There are numerous studies that document the association of oxidative stress and its connotation in progression of amnesia and further Alzheimer's disease (Birla et al., 2020). The free radicals (oxygen) cause age associated decrement in memory functioning that leads to pathogenesis of dementia and Alzheimer's disease (Hachinski et al., 2019).

Passive Avoidance task is the fear-aggravated measure used for testing memory and learning in rat model of CNS disorders. Subjects learn to escape from the environment in which an undesired stimulus that has previously been given (Zameer et al., 2019). Animals with healthy memory and learning can stop accessing the area where even the shock was previously exposed to them. These are determined by measuring the duration of crossing between the compartments through the door. The Passive Avoidance task is important for measuring the effects on memory and learning of novel therapeutic agents, and also examining the processes involved in cognition (Leblanc and Ramirez, 2020). The protection offered by Luteolin in present study justifies its utility in enhancing memory.

A novel rodent object recognition test is a non-reward approach focused on random observation of novel and similar objects. It is the task that reflects non-spatial working memory. The test for novel object recognition in the rat is the innate propensity to explore a novel as opposed to a familiar object (Neill et al., 2010). This task has exploratory behavior element and a memory preservation element, so that throughout the pre-test process, an animal should have adequately explored the familiar object to differentiate between it and a new object subsequent during the testing stage (Lueptow, 2017). In this study, animals treated with scopolamine displayed overall less exploration time than normal animals during pre-training. This is in accordance with previous outcomes in other behavioral arche-types that indicate that adult animals treated with scopolamine have diminished explorative activity and a significant decrease in the identification of novel object in the mission discrimination index (Lu et al., 2018).

The Rota rod test is the study based on ‘performance’ of experimental animals that focuses on the solicitation of a rotating rod with forced motor activity in rodent (Jamwal et al., 2017). The stability, strength training and motor coordination of the animal can be determined primarily following an injury or the impact of experimental drugs (Mishra et al., 2018). In the present study, treatment with Luteolin demonstrated no notable change in the muscle coordination. Thus, the phenomenon of muscle

coordination was not influenced by treatment with Luteolin.

The elevated plus maze is an anxiety test that typically uses rodents for screening of putative anxiolytic or anxiogenic compounds and as a specific research instrument in the research of neurobiological distress (Sotoudeh et al., 2020). The more time animals spends in open arm, the less is the anxiety in animals. Findings from Elevated plus maze test suggest Luteolin had a dose-dependent preventive role on transfer latency toward scopolamine-induced amnesia. In addition, the decline in transfer latency during the retention period implied a response from Luteolin to resolve the scopolamine-induced learning and memory deficit.

Acetylcholine is believed to be the most significant neurotransmitter associated with cognitive function control. Substantial evidence is available that connects the central cholinergic system to memory. It has been shown that cognitive impairment is linked to impaired cholinergic activity and facilitation of central cholinergic activity results improvement in learning memory (Verma et al., 2018). A characteristic feature of Alzheimer's type of senile dementia has been documented to be selective loss of cholinergic neurons and decreased AChE production (De Jaco et al., 2017). The study results showed that Luteolin possibly showed an increase in the amount of acetylcholine by significantly reducing the activity of AChE in the brain (Peña-Bautista et al., 2019a).

In the phase of 'age-associated' deterioration in learning and memory, oxygen free radicals are involved that are associated with the progression of Alzheimer's disease in geriatric (Peña-Bautista et al., 2019b). Luteolin is well documented for antioxidant effects. The 'neuroprotective' role of Luteolin can be associated with radical scavenging and antioxidant properties making vulnerable brain cells exposed to less oxidative stress. This results in decreased brain injury and enhanced neuronal function. Low levels of dopamine are associated with the risk towards the development of Alzheimer's disease. The increase in level of dopamine due to administration of Luteolin provides an insight about the retardation towards the progression of Alzheimer disease (Pan et al., 2019).

The previous studies cite the role of Luteolin as phosphodiesterase inhibitor. For proper neural signaling within neurons, c-AMP is crucial second messenger. The excess of PDE4 causes degradation c-AMP. Thus, Luteolin may prove to be beneficial in enhancing the process of learning and memory acquisition (Röhrig et al., 2017). This could be one of the necessary mechanisms along with inhibition of cholinesterase and lipid peroxidation that predisposes towards anti-amnesic effect of Luteolin.

5. Conclusion

The results of the study suggest that Luteolin pre-treatment prevents scopolamine-induced impairment of memory in scopolamine induced amnesia. The protective effect was observed by increased learning tendency as evident by significant increased the time to explore novel objects, improvement in the retention trial relative, prolonged the duration spent in the open arms and decreases AChE as well as lipid peroxidation. Luteolin upregulated dopamine levels in rat brain. Thus, Luteolin can be regarded as a novel therapeutic agent against amnesia. However, the molecular mechanism behind this effect needs to be explored.

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