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Amelioration of neurodegeneration and cognitive impairment by Lemon oil in experimental model of Stressed mice

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ABSTRACT

Citrous lemon (Rutaceae) an Indian folk medicine has been used for the treatment of various pathological diseases viz., diabetes, cardiovascular, inflammation, hepatobiliary dysfunction and neurodegenerative disorder. Can lemon oil altered the memory of unstressed and stressed mice, a basic question for which the present work was put on trial. The present investigation was intended to assess the impact of Lemon oil on memory of unstressed and Stressed Swiss young Albino mice. Lemon oil (50 and 100 mg/kg o.r.) and donepezil (10 mg/kg) were guided for three weeks to different groups of stressed and unstressed mice. The nootropic movement was assessed utilizing elevated plus maze and Hebbs Williams Maze. Cerebrum acetylcholinesterase (AChE), plasma-corticosterone, decreased glutathione, lipid per oxidation alongside superoxide dismutase and catalase was surveyed as marker for disease. Histopathology was performed for estimation of drug effects. Acute immobilized stress was induce, lemon oil (100 mg/kg) and donepezil together indicated memory enhancing movement both in stressed and unstressed mice. Lemon oil significantly ($p < 0.001$) altered and lowered brain AChE activity both in stressed and unstressed mice. Scopolamine induced amnesia was also significantly altered and reversed both in stressed and unstressed mice by lemon oil at a dose of 50 and 100 mg/kg. Lemon oil (50 and 100 mg/kg) was further able to control the corticosterone level in plasma for stressed mice. Lemon oil significantly ($p < 0.001$) elevated the level of catalase, superoxide dismutase and reduced glutathione levels both in stressed and unstressed animals with respect to controlled group along with TBARS both in stressed and unstressed compared with control group. Hence it can be concluded that memory enhancing activity might be related to reduction in AChE and TBARS activity and by elevated GSH, SOD and catalase through decrease in raised plasma corticosterone levels

1. Introduction

Dementia a brain and social challenge characterized by corrosion of memory through Acetylcholine a pertinent neurotransmitter [1]. There are some areas where the frailty of acetylcholine is discipline to rebound the levels of acetylcholinesterase (AChE) in the brain. AChE degrades acetylcholine and from this point forward, costing an arm and a leg. AChE life leads to frailty of acetylcholine and flash from the past impairment am within one area that occur to desolate [2]. Thus, facilitation of cholinergic pathways commits one of the targets for the service of memory impairment. AChE inhibitors are clinically capable for review of recollection in patients of Alzheimer's disease. Stress plays an important role in neurodegeneration. Stress details the excess of preserve corticosterone in the body. Stress-induced restore in corticosterone levels has been hang in suspense to impair flash from the past retrieval [3]. The inherent pathogenesis is a exodus of neurons in the hippocampus, cortex, and subcortical structures [4]. The onset symptoms may range from forgetting names, inability to recall the taste, loss

Abbrevations: AD, Alzheimer disease; AChE, acetylcholinesterase; EO, Essential oil; IAEC, Institutional Animals Ethics Committee; EPM, Elevated plus Maze; TL, Transfer latency; HWM, Hebbs William Maze; TRC, Time taken by the animal to reach reward chamber; MDA, Malondialdehyde; GSH, reduced glutathione; AIS, Acute Immobilized Stress; ANOVA, Analysis of variance; TBARS, Thiobarbituric acid reactive substances; SOD, Super Oxide Dismutase; WHO, World Health organization; ROS, Reactive Oxygen Species; NFT, Neurofibrillary tangles; HNE, 4-hydroxy-2,3-nonenal

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of collection may be short term, mood swing, losing the things and items etc, along with frustration and stiffness of heart. In severe condition cognitive impairment patient is totally dependent on others ranging from absolutely inconsistent with frequent loss of path.

Learning and memory are contending forms. Learning is the understanding of new information whiles the maintenance of learned information is known as memory. Learning requires adaptability though memory requires steadiness within the sight of outside jolts [5]. Memory function is helpless against an assortment of pathologic procedures including neurodegenerative diseases, depression, head injury, hypoxia, cardiovascular surgery, depression, anxiety, strokes the reactions of prescription, tumors and normal aging [6]. Different neuromodulators associated with the way toward learning and memory is acetyl choline, platelets activating factor, dopamine, serotonin, GABA (c-amino butyric acid), neuropeptides, angiotensin converting enzymes, histamine, insulin, estrogen, nitric oxide, calcium, oxygen free radical, epinephrine and nor-epinephrine and neurotrophic factors [7]. Dementia is a psychological issue described by impedance of memory. Acetylcholine is a critical neurotransmitter administering learning and memory [1]. There perhaps insufficiency of acetylcholine because of increment in the levels of acetylcholinesterase (AChE) in the brain. AChE degrades acetylcholine and henceforth, over the top AChE movement prompts lack of acetylcholine and memory debilitation may happen in the individual [8]. In this manner, assistance of cholinergic pathways might be one of the objectives for the treatment of memory weakness. There are many neurodegenerative diseases and cognitive impairment is one of them, that develops unrelenting cease and arrest brain functions in the hippocampal angle of the sage, involving senile plaques. Now days the trend for cognitive impairment is more prevalent in middle age and first born adult, affecting more than 5 millions. Americans and by 2030 the number may raise to 7.7 millions. This figure reflects the intensity of problem in America only at global level the condition is much worse. Its symptoms are reflected in early 60's with some defects. The biggest problem is that it has no fixed etiology with genetic factors only responsible up to 10–15%. There is no clear cut antidote for this and most of the treatment is limited to symptoms only that make both physician and patient helpless.

Natural herbs are the best solution to fix all the symptoms associated with cognitive impairment. Plant base drugs are highly standardise with perfect demonstration of treatment. Talking about lemon from Rutaceae family called *Citrous lemon* (LO) is full of ascorbic acid, citric acid and flavnoids along with minerals. The health related benefit is not only related to Vitamin C but also associated with flavanoids with antiallergic, antioxidative, antimutagenic, antiviral, anti-inflammatory, and anticarcinogenic activities. This fruit is a bank of variety of chemical including phenolic, vitamins, dietary fibres, minerals and essential oils along with carotenoids [9].

By inhibiting the cellular oxidative damage neurodegenerative diseases is prevented by essential oil of lemon. The botanical aspect of Rutaceae family state that 150 genera with 2000 species and the biggest among all these are Citrus with 70 species of *Terminalia* [10]. Anti-inflammatory and analgesic, antifungal, antioxidant, antiviral and antibacterial are the common activities exhibited by citrus fruits. Brazil is a birth place of *citrus limon* (L.) Burma mainly the north east and north part of the country commonly known as "limoeiro" is second to it [11]. Its infusion of aerial parts of leaves mainly find its use in treatment of various cancer, brain disorder, diabetes, obesity, cardiovascular disorder along with blood lipid lowering potential [12].

C.limon is well known for potential that assist in neuroprotective activities but its nootropic activity till date was untouched. Present research was designed to explore the effect of EO on memory of stressed and unstressed mice with estimation of, corticosterone and brain AChE levels, super oxide dismutase, lipid peroxidation, reduced glutathione of mice. The dose of *C.limon* EO (50 and 100 mg/kg) was selected through the erstwhile studies to what place lemon have been declared publicly to assuage cognitive degeneration, mitochondrial dysfunction and oxidative stress in mice.

2. Materials and methods

2.1. Drugs and chemicals

Metyrapone from Sigma-Aldrich, St Louis, USA, Scopolamine hydrobromide, essential oil lemon, sulphanilamide, m-phosphoric acid, acetylcholine iodide, dithiobisnitrobenzoate, p-nitroso-*N,N*-dimethylaniline were locally purchased from Hi- Media labs Pvt. Ltd. India; Donepezil from Ranbaxy, India. Normal saline was used for dissolving the drugs, except Donepezil where gentle heat was used. Essential lemon oil was separately dissolved in DMSO.

2.2. Experimental animals

20–25 g of Swiss albino mice (2–3 month old, male) acclimatize for 5 days were used in the study caged under standard laboratory condition (6 animals per cage) with alternating dark and light cycle of 12/12 h. The protocol was approved by IAEC of Sam Higginbottom University of Agriculture, Technology & Sciences Allahabad, Uttar Pradesh. India.

2.3. Experimental design

The animals were categorized in 32 groups. Each group have 6 mice.

3. Groups for elevated plus maze

The effect of drugs on memory was widely understood by plus maze EPM model. This method is described as the parameters for testing memory [13]. Fig. 1 represented the different group of mice and treatment received during the entire experimental study. On the stage before unassailable testing (i.e., administration of abused drug on 20th day), each mouse is arranged toward the finish of an unmask arm, confronting thus from the central platform. As the time taken by the animal to charge from the unmask arm into an outstanding of the cover arm by the greater part of all its four legs. TL is recorded for exclusively for each animal. Retention about (memory) is examined 24 h a short time later the trial.

3.1. Groups for Hebbs Williams Maze (HWM)

For measuring spatial working memory, HWM model is used. It consists of three chamber (1) (start box), which is fitted to (2) the central chamber (or exploratory area) and (3) reward chamber in which (food) is kept. All the three segments are given guillotine removable entryways. Fig. 2 showed the different group of mice and received the treatment received during the end of the experimental study. The mice were set in the start box keeping the entryway opened to enhance the animal passage into the following later chamber on the primary day of the drug administration i.e., (drug administration on 20th day). The passageway of the begin box is shut effortlessly a short time later the creature oblige into the following parliament in order to prevent back section. The learning index reflects (TRC) from start box on 1 st day. Every animal was permitted to disentangle the maze for 3 min with every one of the entryways opened once coming back to the cage. Retention (memory score) about the learned task was contemplated 24 h after the principal day trial. Noteworthy loss of significant value in TRC shows enhancement in memory

3.2. Stress mice with acute immobilization

Mice were stressed by immobilizing them for 150 min by placing them on board on their back and tying the four limbs. Behavioural parameters were noted after the test on individual mice. The