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Neuroprotective activity of ethanolic extract of *Tamarindus indica* seeds against aluminium induced neurotoxicity

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ABSTRACT

The major aim of the study is to evaluate neuroprotective activity of ethanolic extracts of *Tamarindus indica* seeds against aluminium induced neurotoxicity in rats. Chronic administration of aluminium chloride significantly raised nitric oxide concentration, depleted reduced GSH, and decreased catalase activities in the whole brain compared to native rats($p<0.05$). However, chronic TSEE (250 and 500mg/kg) administration to the rats significantly attenuated oxidative damage (as indicated by reductions in nitric concentration and reduced GSH, and reduced catalase activities) as compared to control rats. Treatment with test drug that is ethanolic extract of *Tamarindus indica* seed extract and standard memantine (5mg/kg) significantly improved this memory performance (i.e., shortened mean acquisition latency) on day 19 and 20 ($p<0.05$) in the aluminium treated group. But comparatively test group of 500mg/kg showed more significance than 250mg/kg drug. This result indicating evidence for *Tamarindus indica* seeds had a neuroprotective effect on aluminium induced neurotoxicity. Chronic *tamarindus indica* seed treatment (250 and 500mg/kg) significantly improved memory performance (increased memory retention) for the 1st and 2nd RL on days 21 and 42 as compared to the aluminium chloride treated rats.

INTRODUCTION

Tamarindus indica Linn. (Family: Caesalpiniaceae) is known as tamarind, a well known plant of the Indian medicinal system. This plant possesses antidiabetic, hypolipidemic, hepatoprotective, antioxidant, antifungal, anti-inflammatory, antimalarial and antibacterial activities and antifluoride toxicity [1, 2]. The fruit pulp has been reported to contain ascorbic acid, β-carotene, tartaric acid, lactic acid, citric acid, and maleic acid which are responsible for hepatoprotective activity. Pulp of fruits has antidiabetic, hypolipidemic, antioxidant and hepatoregenerative activities [3]. Tamarind seed is a by-product of the commercial utilization of the fruit, the seed comprises the seed coat or testa (20-30%) and the kernel or endosperm (70-75%). However, it has several uses. It is commercially available as a food additive for improving the viscosity and texture of processed foods. The name "jellose" has been suggested for the seed polysaccharide as it describes both its jelly forming properties and the carbohydrate character [4]. Nature has provided an excellent store house of remedies to cure all aliments of Mankind. In ancient days, almost all medicines used were from natural sources, particularly from plants and even now plants continue to be an important source of new drugs. The importance of biological, chemical and pharmacological evaluation of plant derived agents used in the treatment of human aliments has been increasingly recognized in the last decade. Ayurveda is recorded in ancient scripture, handed down through generations and developed over 6000 years. This time-tested holistic medicinal system maintains that good health exists when the body, mind, spirit and environment are in perfect harmony. Good health is a phenomenon rare in today's fast moving world, where people live in a stressful environment and follow an unplanned diet and unbalanced life style. It has become the need of the hour that a new vibrant medical system evolves which is devoid of side effects and which leads to resurgence of Ayurvedic traditions. Herbal and herbal-based molecules are expected to form the basis for such development. Even in the era of genetic engineering, plants account for forty (40%) of all the medicinal formulation prescribed in the United States. In china about (40%) of the total medicinal consumption is attributed to traditional tribal medicines. About 1400 herbal preparation are widely, according to a recent survey in member states of European Union. This global awareness for everything natural is the biggest challenge for Indian pharmacist to come out with newer

technologies. World health organization, (WHO) currently encourages, recommends and promotes traditional herbal medicines in National theatre programs due their case of availability, low cost, safety, and people's faith in such remedies and has also made an attempt to identify all medicinal plants used golbally and listed more than 20,000 species. NAPALERT database documents ethnomedicinal uses alone for 9,200 of 33,000 species of monocots, dicots, gymnosperms, bryophytes and lichens, which would suggest that 28% of plants on earth have been used ethnomedicinally [5]. Herbal medicines are now being developed in dosage forms using modern manufacturing and processing techniques. Modern herbal research is focused mainly on activity-guided isolation (AGI) of phytoconstituents from the crude drug. Many of the plants used in herbal medicines contain principles whose effects can be demonstrated pharmacologically and the action of whole plant extract can usually be related to that of the isolated constituents, accurate methods of assays for herbal medicines are often lacking when the active constituents are unknown and there is no means of assessing the therapeutic potency. In this situation the use of a biological assay will reflect the true activity of the drug most clearly. Free radical reactions have been implicated in the pathology of many human diseases including atherosclerosis, ischemic heart disease, the aging process, inflammation, diabetes, immunodrepression, the neurodengenerative condition and other disease conditions [6]. Controlling the level of free radicals in the body is the role of antioxidants. Without balancing the level of free radicals to antioxidants we get deterioration of the system. In recent reviews in top nutritional journals it is underlined that the existing studies on human demonstrate a convincing effect of polyphenols on some some aspects of health. It is also a fact that bioavailabilty studies are accumulating, while new databases are created for various classes of polyphenols and estimates of intakes in many countries are discussed more and more seriously, the importance of antioxidant plant phenols is also seen in the efforts of researchers. In the last decade, much work has been presented by the scientific community, which focuses on [7]. The levels and chemical structure of antioxidant phenols in different plant foods, aromatic plants and various plant materials. The probable role of plant phenols in the prevention of various diseases associated with oxidative stress such as cardiovascular and neurodegenerative diseases and cancer. The ability of plant polyphenols to modulate the activity of enzymes, a biological action not yet understood. The ability of certain classes of plant phenols such as flavonoids (also called polyphenols) to bind to proteins. Flavonol-protein binding, such as binding to cellular receptors and transporters, involves mechanisms of polyphenols which are not related only to their direct activity as antioxidants. Phenolic antioxidants have been shown to play important roles in delaying of chronic diseases such as cardiovascular diseases (CVD), cancer, inflammatory bowel syndrome and Alzheimer's disease. A number of plants and plant isolates have been reported to protect free radical-induced damage and phenolic antioxidants are products of secondary metabolism in plants and are good sources of natural antioxidanys in human diets. Natural antioxidants from plant sources are potent and safe due to their harmless nature. Over the past 10 years, researchers and food manufacturers have become increasingly interested in polyphenols. Two aims of research are to establish evidence for the effects of polyphenols consumption on health and to identify which of the hundreds of existing polyphenols are likely to provide the greatest protection in the content of preventive nutrition. If these objectives are to be attained, it is essential to determine the nature and distribution if these compounds in our diet [8]. It is important to determine the amounts and species of polyphenols in plants, fruits and teas. The number of polyphenols has been estimated to be over one million, because they generally occurs as glycosides, and sugar species and binding forms show great variety. However, the bioactivity is attributed to aglycon structures, not to sugar moieties. The antioxidant potential is due mainly to the orthodiol (catechol) structures in aglycons.

MATERIALS AND METHODS

Plant collection

Seeds of *Tamarindus indica* ar collected in Guntur Dist, of Andra Pradesh, India, in April, 2013. The sample was identified and Authenticated by Mr. M.R. Paul Satyakeerthi, Head of the development, Department of Botany of Andra Christian College, Guntur.

Animals

Healthy male rats, *Rattunorvegicu* of Wister strain weighing 200-300 g were obtained from the Mahaveera enterprises, Hyderabad. Before starting experiment, animal were allowed to acclimatize for, at least 1 week. The animal were housed in polypropylene cages under pathogen free, uniform conditions of light (12 h) and dark (12h) cycle and temperature was maintained at 22±3°C. They were fed with standard pellet diet (Hindustan Lever Limited, India). Rats were kept on a 12 h light: 12-h dark cycle and their health status were checked frequently. All the experiment was performed in the morning and in accordance with the guidelines provided by the Institutional Animal Ethics Committee. Rats will be housed in solid bottom autoclaved polypropylene cages (Size: approximately L 425 x B 266 x H 185 mm), three rats per cage with stainless steel top grill having facilities for pellet feed and drinking water in polycarbonate bottle during acclimatization and throughout the study duration. Corn cob will be used as bedding material and will be changed twice a week. Animals were habituated/adapted to the experimenter and to the experimental conditions for measurement of Alzheimer's in the Lab (water maze (Model no: 306), Elevated plus Maze, Instruments Inc.) for 3-5 days prior to the initiation of experiment. A circular platform (4.5 cm diameter) was placed 1cm above the water level in one quadrant during acquisition phase. Each animal was subjected to four consecutive

trails with gap of 5 minutes. The animal was gently placed in water of the pool, with the drop location changed for each trail. The animal was then allowed 120 sec to locate the platform. Next the animal was allowed to stay on the platform for 20 sec, If the animal failed to reach the platform within 120 sec, it was guided to the platform and allowed to remain there for 20 sec. This was done before each testing, until exploration activities ceases. Animals which reach the platform within 60 sec are taken for further study. Animals were kept for one week, before enrollment into the study. Food, housing and water to the animals are also taken care. The following study protocol has been reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of SIMS College of pharmacy. The recommendations as per the animal welfare guidelines regarding animal care and handling will be strictly adhered to. The approval has been documented in the committee for the purpose of control and supervision of experiments on animals (CPCSEA) specified 'Form B' protocol *Approval Number (IAEC/SIMS 2013/004*). The procedures used in this protocol will be designed to conform to the accepted practices and to minimize/avoid risk of causing pain, distress or discomfort to the animals. The number of animals selected for use in this study will be considered to be the minimum requirement to meet rationale scientific endpoints [9]

Chemical and reagents

Tamarindus indica, memantine hydrochloride, aluminium hydrochloride and sodium carboxy methly cellulose.

EXPERIMENTAL DESIGN

Randomization and Grouping

A group of animals were examined for health and healthy *wistar* rats were selected for the study and were randomly assigned to different groups based on the body weight. The selected rats were divided into normal control, disease control, standard, test group 1 and 2. Disease induction is done. Administration of the disease, extract and standard drugs were done at time. Route and frequency of administration of formulations must be planned prior to the experiment [10].

Preparation of ethanolic extract of seed of T. indica

Ethanol extract of seed of *T. indica* was performed according to the method of National Institute of Health and Family Welfare (NIHFW), New Delhi.15. Fresh seeds of *T. indica* were dried in an incubator for 2 days at 400˚C, crushed in an electrical grinder to have coarse powdered. Then 100 g seed powders of *T. indica* was suspended in 500 mL of Ethanol then extracted for 18 h in a Soxhlet apparatus and a deep red ethanolic extract was obtained. The suspension was then filtered by coarse sieve filter paper. The filtrate was evaporated to dryness under reduced pressure in a rotary evaporator. A deep brown material was obtained (4 g/100 g of the dried seeds powder). It was stored at 0-40˚C until used. Photochemical tests have been done [11].

Table 1. : The initial acquisition latencies (IAL) on day 20 and retension latencies on day 20 and retension latencies on days 21 (1st RL) and (42nd RL) following Aluminum hydrochloride (100) co-current treatment were observed. Values are the means ± SEM. [a] P< 0.05 as compared to native group; [b] p<0.05 as compared to Aluminum hydrochloride (100) treated group; [c]p<0.05 as compared to TSEE (500mg) + Aluminum hydrochloride (100) group (repeated measures two-way ANOVA followed by Turkey's test for multiple comparisons).

Treatment (mg/kg)	Day 20 (IAL)	Day 21 (1st RL)	Day 42 (2nd RL)
Normal	78 ± 2.6	16 ± 2.6	10 ± 2.5
Aluminum hydrochloride (100)	79.6 ± 2.4	83.5±2.5	80.3±2.3[a]
Memantine (5mg/kg)	68.2 ± 3.5	15.5 ± 2.2	10.5 ± 2.5
TSEE (250mg/kg) + $AlCl_3$(100)	69.3 ± 2.5	45.6 ± 1.5[b]	46.2 ± 2.0[b]
TSEE (500mg/kg)+$AlCl_3$(100)	70.5 ± 2.7	47.33± 2.4[b,c]	45.2 ± 1.7[b,c]

Acute toxicity studies

In screening drugs, determination of LD 50 (the dose which proved to be lethal to 50% of the tested group of animals) is usually an initial step in the assessment of toxic manifestations (provides information on health hazards likely to arise from short term exposure to drugs) and is one of initial screening experiments performed with all compounds. Acute toxicity of *Tamarindus indica* Linn seeds have been already reported by Maiti et al and Chatterjee et al. The acute oral toxicity studies of extracts were carried out as per the organization for Economic co-operation and Development (OCED) guide lines, draft guidelines 423 adopted on 17 December 2001 received from committee for purpose of control and supervision of Experiment, Government of India. Administration of stepwise doses of dried extracts of *Tamarindus indica* seeds from the dose of 100mg/kg upto 2000mg/kg to young male albino mice and observed the signs of toxicity in the tested animals. The albino mice were divided into different groups of six animals each. The control group received 5ml/kg of distilled water orally. The other groups received the hydroalcoholic extracts of *Tamarindus indica* Linn at dose levels of 100, 500, 1000, 1500, 2000mg/kg through oral route. After administration of dose the animals were observed continuously for the first 4 hours and occasionally up to 24 hour and at the end of 72 hour for recording.

Table 2. : Values are the means ± SEM, P<0.05 as compared to native group. p< 0.05 as compared to aluminum hydrochloride (100) treated group; p<0.05 as compared to memantine(5.0)group+ Aluminum hydrochloride (100) group (repeated measures one-way ANOVA followed by turkey's test for multiple comparison.

	Nitrite µmol/mg of protein (% of control)	Reduced glutathione nmol/mg of protein(GSH)	Catalase µmol of hydrogen peroxide decomposed/min/mg of protein (% of control)	Superoxide dismutase units/mg of protein(% of control)	Glutathione S-transferase nmol CCDNB conjugated/mg/min of protein(% of control)
Normal	225.7±30.08 (100)	0.57± 0.08 (100)	5.137±1.02 (100)	1.41±0.28 (100)	95.9± 4 (100)
Aluminum hydrochloride (100)	652.81±27.32[a] (80.40)	0.05± 0.01[a] (39.200)	2.62±0.32[a] (51.0)	0.07±0.02[a] (4.96)	37.8±5.2[a] (38.6)
Memantine (5.0) + Aluminum hydrochloride (100)	220±35.2 (100.52)	0.57±0.028 (68.22)	5.25±0.06 (101.54)	0.80±0.06 (56.7)	99.5±5.1 (102.02)
TSEE(250) + Aluminum hydrochloride (100)	346.3±27.64[b] (163.2)	0.28±0.03[b] (43.58)	4.88±0.22[b] (94.3)	0.51±0.07[b] (36.17)	47.67±2.1[b] (48.69)
TSEE(500)+ Aluminum hydrochloride (100)	475±30.2[b,c] (210.30)	0.16±0.07[b,c] (99.24)	5.08±0.29[b,c] (98.25)	0.167±0.09[b,c] (1.18)	64.7±1.94[b,c] (65.98)

i. Behavioral profile: alertness, restlessness, irritability, Fearfulness.

ii. Neurological profile: spontaneous activity, reactivity, touch response, tremors.

iii. Autonomic profile: defecation and urination.

One tenth of upper limit dose and its half and double dose were selected as the levels for examination of therapeutic activity [10, 12].

Justification for test system

Wister rat is the species recommended for the assessment of neuroprotective activity as they are easily prone to Alzheimer's and they are well reported in the literature.

Personnel safety precautions

Gloves, head cap, face mask and goggles were used in addition to protective body garments and shoes to ensure adequate personal health and safety. Appropriate measures were taken to avoid inhalation and skin contact with the test item and although the risk of bites and scratches is species dependent, there are a few simple guidelines, which, if followed will significantly reduce the potential risks of such incidents. These include [13].

Always wear appropriate personal protective equipment, especially hand and face/eye protection;

If moving large contaminated items (e.g. non-human primate cages), wear heavy gloves;

When available and appropriate, use mechanical restrainers when performing procedures on unanesthesized animals;

All bites and scratches that result in bleeding should be immediately and thoroughly scrubbed and cleansed with soap and running water for at least 15 minutes;

Do not discard or disinfect any object which caused the injury; hold for analysis;

Notify supervisor and seek medical attention immediately.

Preparation of dose formulation

Aluminium hydrochloride

The required quantity of aluminium hydrochloride was weighed using analytical balance and transferred into motar and pestle. Desired quantity of normal water added and triturated well to get final concentration of 150mg/ml solution [14].

Memantine

The required quantity of memantine were used in analytical balance and transferred into motar and pestle. Desired quantity of normal water added and triturated well to get final concentration of 5mg/ml solution.

Tamarindus indica ethanolic extract (TSEE)

The required quantity of TSEE was weighed using an analytical balance and transferred to a mortar and pestle. The desired volume of 0.5% *w/v* carboxymethyl cellulose sodium medium viscosity (CMC-Na) in water will be added and triturated to get the final concentration of 250 and 500 mg/mL suspensions. The suspension formulations will be transferred to a centrifuge tube and subjected to vortexing for 2 minutes to obtain homogeneous suspensions [15].

RESULTS

Effect of *Tamarindus indica* (250 and 500 mg/kg) on memory performance in the Morris water maze paradigm for aluminium hydochloride treated rats.

Aluminium treated rats significantly delayed acquisition latency to reach the visual platform compared to the naive group,

Table 3. : Values are the means±SEM, P<0.05 as compared to native group. p< 0.05 as compared to aluminum treated group; p<0.05 as compared to meantime(5.0)group+ Aluminum hydrochloride (100) group(repeated measures one-way ANOVA followed by turkey's test for multiple comparison.

Treatment(mg/kg)	Lipid peroxidation (mg of protein)	Acetylcholinestrase
Normal	1.54±0.13 (100)	0.1633±0.01 (100)
Aluminum hydrochloride (100)	3.96±0.39 (253.2)	0.31±0.018 (189.8)
TSEE (250) + Aluminum hydrochloride (100)	2.04±0.14 (132.46)	0.21±0.015 (128.5)
TSEE (500)+ Aluminum hydrochloride (100)	2.15±0.098 (139.6)	0.17±0.008 (104.1)
Memantine (5.0) + Aluminum hydrochloride (100)	2.58±0.35 (167.5)	0.17±0.012 (104.1)

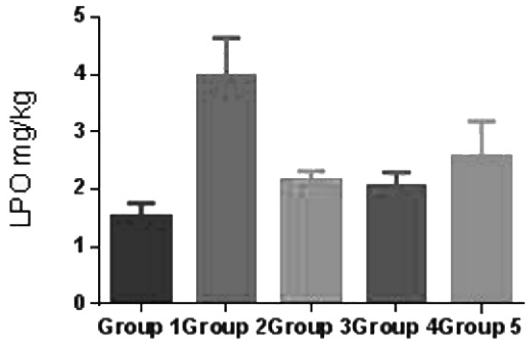

Figure 1. Effect of TSEE (250 and 500 mg/kg) on lipid peroxidation (LPO) levels in aluminium hydrochloride treated rats whole brain.

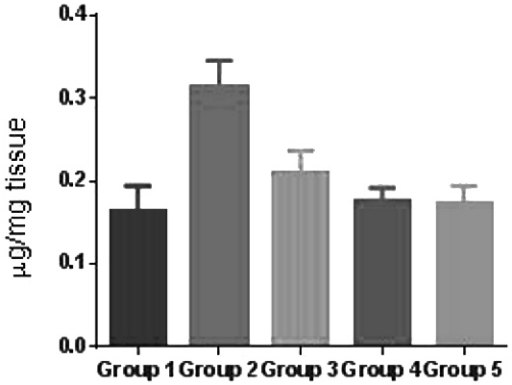

Figure 2. Effect of TSEE (250 and 500 mg/kg) on acetylcholinesterase levels in aluminium hydrochloride treated rats whole brain.

indicating memory deficits. Treatment with test drug *Tamarindus indica* seed extract and standard memantine (5mg/kg) significantly improved this memory performance (i.e., shortened mean acquisition latency) on day 19 and 20 (p<0.05) in the aluminium hydrochloride treated group. But comparatively test group of 500mg/kg showed more significance than 250mg/kg drug. Following training, the visual platform was hidden. These results suggested that aluminium chloride caused significant cognitive impairment. Aluminium hydrochloride treatment was then found to significantly delay mean acquisition latency (on day 20) and retention latencies (1st and 2nd RL on day and 42, respectively) to escape on to the hidden platform compared to the normal group. These results suggested that aluminium hydrochloride caused significant cognitive impairment. Further, chronic *tamarindus indica* seed treatment (250 and 500mg/kg) significantly improved memory performance (increased memory retension) for the 1st and 2nd RL on days 21 and 42, respectively, compared to the aluminium hydrochloride treated rats (Table 1). However, chronic TSEE (250 and 500mg/kg) administration to the rats significantly attenuated oxidative damage (as indicated by reductions in nitric concentration and reduced GSH, and reduced catalase activities) as compared to control rats (Table 2 & 3).

Effect of ethanolic extraction of *Tamarindus indica* seed extract on nitrite, reduced glutathione and catalase activity in whole brain of rats treated with aluminium hydrochloride

Chronic administration of aluminium hydrochloride significantly raised nitric oxide concentration, depleted reduced GSH, and decreased catalase activities in the whole brain compared to native rats (p<0.05) [16]. However, *Tamarindus indica* (250 and 500 mg/kg) administration to the rats significantly attenuated oxidative damage (as indicated by reductions in nitrite oxide concentration and reduced GSH, and decreased glutathione S-transferase, superoxide dismutase, and catalase activities) as compared to control rats (Table-2). Furthermore, *Tamarindus indica* (250 and 500 mg/kg) treatment alone did not significantly influence these parameters compared to naive rats.

Effect of *Tamarindus indica* on aluminium concentration in aluminium chloride treated rats

Aluminium chloride treatment significantly increased the

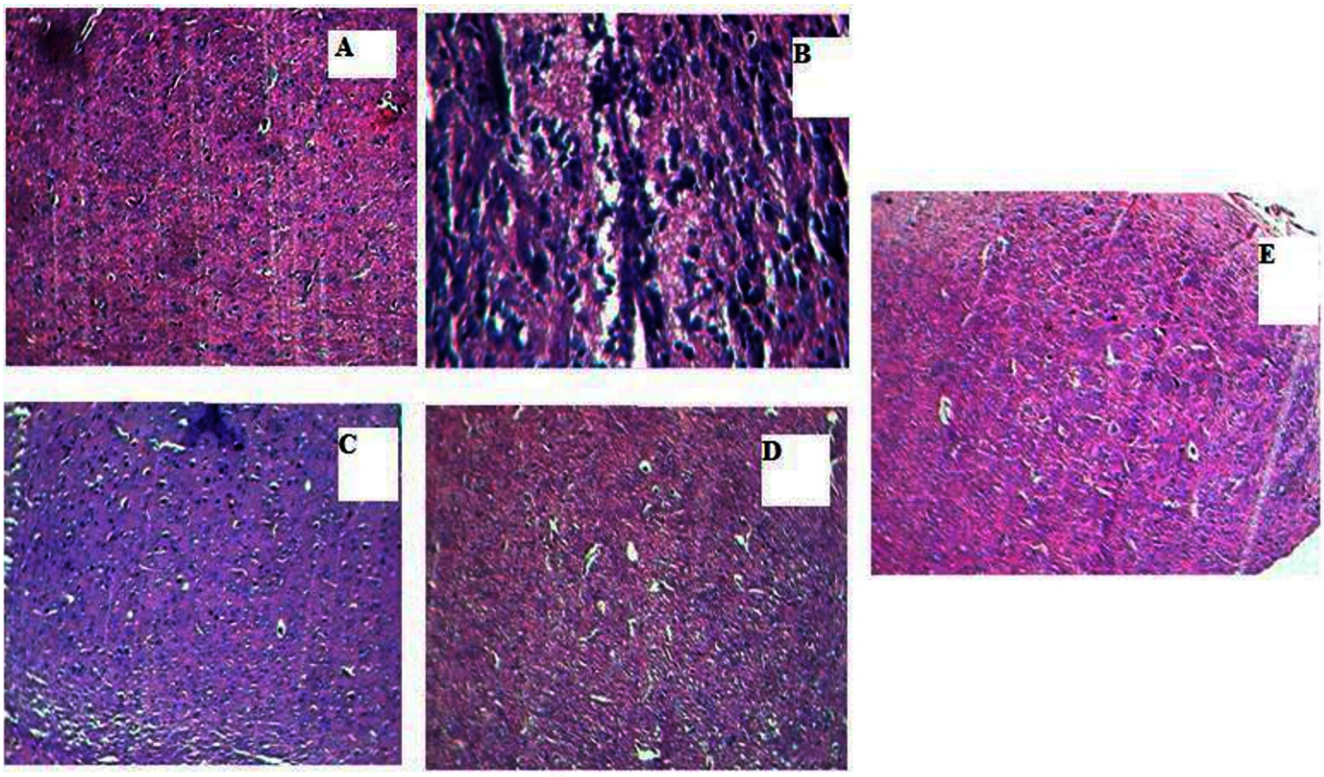

Figure 3. Microscopic study of lateral sections of mice brain (400 magnifications). Histological sections of brain were stained with haematoxylin & eosin (H&E). (A) Control rat brain showing intact neurons, without any spongiosis. (B) rat exposed to 100 mg/kg/day AlCl3 for 6 weeks clearly showing perinuclear spaces, lipofuscin deposits, congestion inthe blood vessels and prominently degenerated neurons and disrupted nuclei and oedema. (C) rats administered memantine(5mg/kg+100mg/kg/day Alcl3) for 6 weeks showing existence of theperinuclear spaces. (D-E) administered TSEE (250 mg/kg, 500 mg/kg,) for 6 weeks showing normalisation of the brain microstructural elements, with normal neurons and intact nucleus and astrocytes. No spongiosis/vacuolization and degenerated neurons could be observed.

aluminium concentration in the hippocampus and cortex of rats compared to control. However, *Tamarindus indica* (250 and 500 mg/kg) treatment significantly attenuated the aluminium concentration in the hippocampus and cortex compared to control ($p < 0.05$).

Effect of *Tamarindus indica* on acetylcholinesterase (AChE) activity in aluminium chloride treated rats

Chronic aluminium chloride treatment significantly increased the whole brain AChE activity compared tonaive rats. However, chronic *Tamarindus indica* (250 and 500 mg/kg) treatment significantly attenuated AChE activity, as compared to the control rats ($p < 0.05$).

DISCUSSION

The results of the present study indicated that chronic administration of aluminium chloride resulted in progressive deterioration of spatial memory as determined by Morris water maze task paradigms. Experimentally, it was demonstrated that intracerebral administration of aluminium chloride caused learning deficits in the Morris water maze task in rabbits [16], which was in agreement with our findings. This phenomenon could be attributed to the ability of aluminium to interfere with downstream effector molecules, such as cyclic GMP, involved in long-term potentiation [17]. This disruption could then explain the memory impairment and neurobehavioral deficits observed. In present study, chronic exposure of aluminium increased aluminium concentration in hippocampus and cerebral cortex as compared to the control animals. It has been observed that high aluminium level in brain is associated with decline in visual memory and attention concentration in hemodialysis patients. The results of our study indicate that chronic administration of aluminium chloride results in progressive deterioration of spatial memory in both Morris water maze paradigm. In the present study, chronic aluminium chloride treatment caused a significant decrease in the acetyl cholinesterase activity leading to memory deficits, but later was significantly restored by chronic co-administration with test drug *Tamarindus indica* seed extract and standard memantine(5mg/kg). However chronic co-administration of *Tamarindus indica* seed extract and standard memantine (5mg/kg) attenuated acetylcholine esterase enzyme activity which was not significantly different ($P < 0.05$) potentiated their protective effects (decreased MDA levels and restored SOD and catalase levels) as compared to their effects alone and control group [18]. Thus the results strongly support our hypothesis that the memory deficits observed after chronic aluminium chloride treatment might have arisen as a result of mitochondria dysfunction, which is the key factor for the production of ROS generation and ultimately causing oxidative injury to neurons, which could therefore be prevented by antioxidant treatment. The use of memantine is expected to compensate cholinergic deficits indirectly by inhibiting the destruction of acetylcholine and directly by increasing the expression of choline acetyltransferase. Memantine reversed

histopathological and biochemical impairments (oxidative stress parameters) caused by aluminium [19]. The corrected biochemical parameters, in addition to enhancement of cholinergic activity, might explain the ability of memantine to improve the behavioural performance in aluminium exposed animals. But some side effects reported in connection with chronic use of memantine include irritability, nervousness, agitation, anxiety and sleep disturbances. Hence coadministration of *Tamarindus indica* seed extract and standard memantine (5mg/kg) study plan was designed to decrease the toxic profile of memantine and help to achieve better therapeutic efficiency. However, results also clearly indicates that chronic co administration of memantine combination have potent therapeutic activity, which was indicated through behavioural and oxidative stress parameters [20].

Aluminium was previously found to be a potent pro-oxidant known to enhance lipid peroxides in the cortex and hippocampus [21]. It also caused alterations in iron homeostasis, resulting in excessive free iron ions, which undergo the Fenton reaction and cause oxidative damage, finally culminating in neurodegeneration. Furthermore, it also targets mitochondria, causing the release of cytochrome c and the activation of proapoptotic proteins like bax and caspase-3, which trigger neuronal apoptotic death [22].

As oxidative damage is mediated by free radicals, it was necessary to investigate the status of endogenous antioxidant enzymes like catalase, superoxide dismutase and glutathione, which are the first line of defense against free radical damage under oxidative stress conditions. In our study, chronic administration of aluminium chloride resulted in marked oxidative stress as indicated by increases in lipid peroxidation and nitrite concentration, as well as decreases in reduced glutathione, catalase, superoxide dismutase and glutathione S-transferase activity. These changes could have been due to the reduced axonal mitochondria turnover, disruption of the golgi or reduction of synaptic vesicles induced by aluminium treatment, all of which result in the release of oxidative products like malondialdehyde, carbonyls, and peroxynitrites, and of enzymes like superoxide dismutase, within the neurons [23]. Under oxidative stress conditions, SOD presents the first line of defense against superoxide as it dismutates the superoxide anion to H_2O_2 and O_2. Catalase protects SOD by converting H_2O_2 to water and oxygen. Catalase is present in the peroxisomes of mammalian cells, and probably serves to destroy H_2O_2 generated by oxidase enzymes located within these subcellular organelles. Aluminium is a potent cholinotoxin [24]. It has a biphasic effect on acetylcholinesterase activity, with an initial increase in the activity of this enzyme during the first 414 days of exposure followed by a marked decrease. This biphasic effect has been attributed to the slow accumulation of aluminium in the brain. This would explain the increase in acetylcholinesterase activity observed in the aluminium chloride treated rats. Because oxidative stress and cognitive dysfunction are strongly correlated, agents that modulate reactive oxygen species may be potentially useful as antidementia agents. Administration of seed extracts of drug was found to improve not only the memory retention but also reduced oxidative damage induced by chronic aluminium administration. Seed extract of Tamarindus indica treatment also attenuated the rise in MDA and NO concentration of aluminium treated rats [25].

The results presented here showed that *Tamarindus indica* (250 and 500 mg/kg) was able to attenuate the increased concentration of aluminium in both of these regions of the brain in rats. Therefore, the present study highlights that *Tamarindus indica* improves behavioral and biochemical function in the aluminium-treated brain, an effect that could be partially correlated with its neuroprotective properties. However, further cellular studies are required to understand the effect of *Tamarindus indica* on oxidative stress in different experimental systems.

CONCLUSION

In conclusion the result of the present study indicates that ethanolic extract of *Tamarindus indica* seeds prevented aluminium hydrochloride induced neurotoxicity in the cerebral cortex of rat. Besides, inhibiting oxidative stress and histopathological alterations this study demonstrates that Tamarindus indica showed promising results in normalising the altered activity/levels of proteins at cholinergic synapse induced by aluminium hydrochloride. Thus our ethanolic extract is a potential formulation for treating aluminium hydrochloride induced neurotoxicity. Further studies are warranted to determine the active principle of these extracts and also to determine the exact mechanism by which the phytochemicals exert a neuroprotective effect. Overall results from the present study support the potential of ethanolic extract as a remedy to prevent memory loss in natural aging as well as an alternative remedy for neurodegenerative disorders associated with oxidative stress and aluminum-induced memory loss.

DECLARATIONS

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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