Print ISSN: 2319-2003 | Online ISSN: 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

DOI: http://dx.doi.org/10.18203/2319-2003.ijbcp20175212

Original Research Article

Evaluation of the protective effect of *Prunus amagdylus* against aluminium chloride induced neurochemical alterations and spatial memory deficits in rats

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Received: 01 October 2017
Accepted: 27 October 2017

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ABSTRACT

Background: The present study was designed to evaluate the protective effect of *Prunus amagdylus* nut kernels against aluminium chloride induced spatial memory deficits in rats.
Methods: Plant material was extracted, and extracts were evaluated for anti-oxidants by DPPH method. Animals were divided into four groups of five animals each. Group 1 was normal group and was kept undisturbed. Group 2 was administered with Aluminium Chloride (4.2mg/kg i.p) for 21 successive days. Group 3 and 4 were pre-administered with *Prunus amygdalus* methanolic extract at dose 0.5 and 1mg/kg/ p.o) one hour prior to aluminium chloride administration. The memory parameters (both acquisition and retrieval) were evaluated using Morris water maze. After behavioural studies, the animals were sacrificed by decapitation and braintissue thiobarbituric acid reactive substances (TBARS), glutathione (GSH) and catalase activity were measured. Brain tissues from all the groups were histopathologically evaluated using Haematoxylin-eosin staining.
Results: Administration of Aluminium chloride resulted in severe memory deficits and neurochemical alterations as was indicated by significant increase in Transfer Latency (TL) time on Morris water maze and increase in the brain tissue TBARS levels in the control group animals. There was significant reduction in the GSH and catalase levels indicating decreased anti-oxidant defence. Histopathologically, control group animal brain tissue showed signs of neuroinflammation. All behavioural and neurochemical and histopathological changes were prevented to a significant extent in the animal groups pre-treated with *Prunus amygdalus* extract.
Conclusions: Methanolic extract of *Prunus amaygdalus* possesses protective activity against aluminium chloride induced neurotoxicity and associated memory deficits.

Keywords: Aluminium, Memory deficits, Neurotoxicity, *Prunus amygdalus*

INTRODUCTION

The human brain with nearly 1,000 trillion synaptic connections is susceptible to many types of damage and disease. It regulates and controls all bodily functions and is centre of higher-order thinking, learning and memory.[1] Learning and memory functions confer us the ability to accumulate knowledge from our experiences.[2] Learning refers to acquisition of any new information about the event in particular surroundings. Subsequent retrieval of this learned/acquired information is referred to as memory.[3] Information transmission within the brain, during the processes of memory encoding and retrieval, is achieved using a combination of chemicals (neurotransmitters) and neural current.[4] Memory one of the most complex functions of the brain, comprises of multiple components.[5,6] Memory may be sensory

(registration), short-term memory (STM) and long-term memory (LTM).[7] Consolidation refers to the process of conversion of STM to LTM.[8] Aluminium (Al) is abundantly distributed in our environment and has widespread and important uses in industrial applications and consumer products. Despite its environmental abundance, Al is not an essential element for living organisms and no enzymatic reaction requires Al.[9] However, Al directly enters human body because of its usage in pharmaceutical and cosmetic agents like antacids and antiperspirants.[10-12]

Aluminium (Al) is a neurotoxic metal and has been documented to be involved in the progression of neurodegenerative processes linked with various neurodegenerative diseases including Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and Parkinsonism dementia and the Gulf War syndrome.[13-17] Chronic administration of aluminium has been implicated in the apoptotic death of neurons and glial cells by impairing long term potentiation (LTP), which is a form of synaptic information storage leading to memory formation.[18] Therefore, Al causes spatial memory deficit, influences emotional reactivity, and impairs various brain functions related to learning and memory.[19,20]

Almonds are prunes that belong to the rose family, the Rosaceae. They were traditionally placed in a sub-family, the Prunoideae (or Amygdaloideae), but sometimes, they are placed in their own family, the Prunaceae (or Amygdalaceae). Almonds are a good source of nutrients such as mono unsaturated fatty acids, poly-unsaturated fatty acids (PUFA), arginine, and potassium.[20] Almonds are among the richest food sources of vitamin E, as RRR-α-tocopherol. Almonds also rich in phenolic compounds, which are localized principally in their skin, including flavonols (isorhamnetin, kaempferol, quercetin, catechin and epicatechin), flavanones (naringenin), anthocyanins (cyanidins and delohinidin), procyanidins, and phenolic acids (caffeic acid, ferulic acid, P-coumaric acid and Vanillic acid).[21] There are reports that the methanolic extracts of almonds possessed anti-oxidant activities and that their phenolic extract may be helpful in preventing or slowing the processes of various oxidative stress related diseases.[23] Due to well documented reports implicating the role of oxygen and nitrogen based free radicals in stress induced memory deficits and presence of a large array of anti-oxidant phytoconstituents in *Prunus amagdylus* nuts, the present study was designed to evaluate the effect of *Prunus amagdylus* nut extract on Chronic Unpredictable Stress induced memory deficits and associated biochemical and histopathological changes in rats.

METHODS

Animals

Wistar albino Rats (either sex), weighing between 200-250g, were used for the experimental protocol. They were fed with standard rodent pellet diet and water *ad libitum*. The care of laboratory animals was done as per the guidelines of CPCSEA, Ministry of Forests & Environment, and Government of India.

Drugs and chemicals

Crude drug

Seed nut kernels of the plant *Prunus amygdalus* family Rosaceae were collected from medicinal plant dealer Herbheal® Pvt. Limited of Amritsar (Punjab). The plant material was identified and authenticated by taxonomist of Herbheal®. All the reagents and chemicals employed in the study were purchased from either Qualikems, Fine Chem. Pvt. Ltd Mumbai or from Merck Specialities Pvt. Ltd., Worli, Mumbai and were of analytical grade.

Experimental procedure

Preparation of plant extracts

The nuts of *Prunus amygdalus* were coarsely powdered using an electrical grinder. Various extracts of the powdered drug were prepared using different solvents in the increasing order of their polarity. The chemical tests for the screening and identification of phyto-constituents were carried out for all extracts as per the standard methods.

Preliminary evaluation of antioxidant activity

Free radical scavenging activity of plant extracts against standard 2, 2 diphenyl 2 picryl hydrazyl hydrate (DPPH) was determined by the slightly modified method of Brand-Williams.[24]

Experimental protocol

The animals were divided into 4 different groups (n=5). Aluminium Chloride (4.2mg/kg) was administered through Intrapretoneial route daily to separate groups of Rats for 21 successive days. The doses of Aluminium chloride were selected on the basis of literature reports. *Prunus amygdalus* methanolic extract was administered through oral route daily to separate groups of Rats for 21 successive days at two doses i.e. 0.5 and 1mg/kg. During 21 days of Aluiminum chloride and *Prunus amygdalus* extract administration; the memory was evaluated using Morris water maze. From day 1st to 9th day rats were kept undisturbed except for the administration of Aluminium Chloride. From day 10th to 12th day after 30 minutes of administration of Aluminium, the rats were exposed to acquisition trials on Morris water maze as devised by method of Hoveida.[25] On 14th day retention of the short term memory was recorded. The rats were kept undisturbed except for cage cleaning and for the administration of Aluminium Chloride to all groups except normal group. On 22nd day (10 days after the last training trial) retention of the long term memory was recorded. After behavioural studies, the animals were sacrificed by

decapitation and brain catalase activity, Thiobarbituric acid reactive substances (TBARS) and GSH were measured.

Biochemical estimations

Brain Tissue Thiobarbituric Acid Reactive Substances (TBARS)

Brain tissue TBARS was measured as per the method of Will.[26]

Brain Tissue Reduced Glutathione (GSH)

The assay of GSH with DTNB was performed by following a standard Ellman's method.[27]

Barin tissue catalase

Barin Tissue Catalase activity was measured by method of Sinha.[28]

Statistical analysis

For evaluation of effect on spatial memory (acquisition phase)

We utilized two-way ANOVA with multiple comparison test followed by Tukey's post-hoc analysis with $p \leq 0.05$ considered significant for all values.

For retrieval trials and Biochemical parameters

The results were analyzed using one-way ANOVA followed by Tukey's post-hoc analysis with $p \leq 0.05$ considered significant for all values.

RESULTS

Results of phytochemical screening

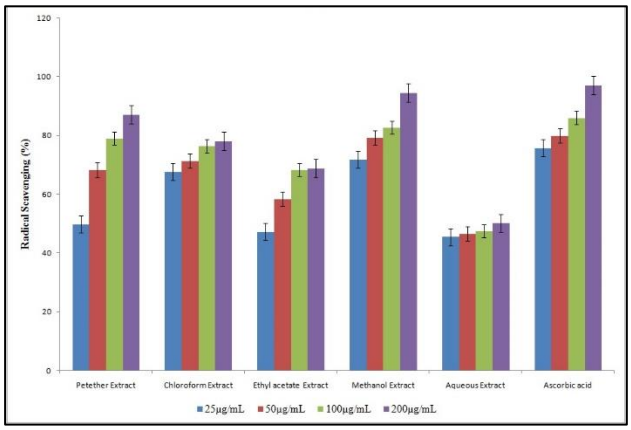
In the present study, alkaloids were found to be present in chloroform, methanolic and aqueous extracts. Presence of tannins and flavanoids was indicated in almost all of the extracts, however high amount was indicated in methanolic and aqueous extracts. Proteins and amino acids were detected in methanolic and aqueous extracts (Table 1).

Table 1: Result of various phytochemical screening tests of various extracts of *Prunus amygdalus* nut kernel.

Test	Pet ether	Chloroform	Ethyl acetate	Methanol	Aqueous
Alkaloids					
a) Dragondorff test	+	+	-	+	-
b) Wagner's test	+	+	+	-	-
Phenolic compounds and tannins and flavonoids					
a) Lead acetate	-	+	+	+	-
b) Bromine water	-	+	+	+	-
c) Potassium permanganate test	-	-	_	-	-
Proteins and Amino acids					
a) Millon's test	-	-	-	-	-
b) Ninhydrin test	-	-	-	-	-
Carbohydrates					
Tests for Hexose Sugars					
a) Tollen's pholorogucinol test for galactose	-	-	-	-	+
Tests for Pentose sugars					
a) Pentose sugars	-	-	-	-	-
Tests for Non-Reducing Polysaccharides					
a) Iodine test	-	-	-	-	-
Tests for Reducing Sugars					
a) Fehling Solution Test	-	+	-	+	+
Glycosides					
Cardiac Glycosides					
a) Keller Kiliani Test	+	+	-	+	-
b) Legal Test	+	+	+	-	-
c) Baljet Test	+	+	+	+	+
Anthraquinone Glycosides					
a) Borntrager's test	-	-	-	-	-
b) Modified borntrager's test	+	+	+	+	+
Phytosterol					
a) Salkowski test	-	-	-	-	-

Results of in-vitro anti-oxidant analysis

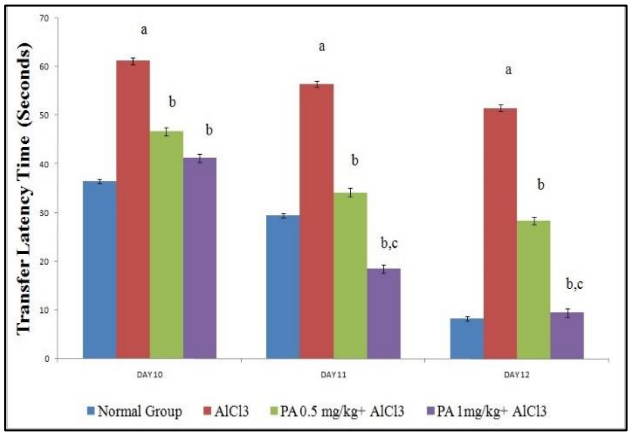
In-Vitro antioxidant analysis using DPPH method revealed that most potent scavenging was obtained using methanolic extract. Free radical scavenging was observed with all extracts, but significantly high results were obtained using methanolic extract (Figure 1).



mean±S.E.M with n = 5 in each group

Figure 1: Comparison of in-vitro free radical scavenging potential of different extracts of *Prunus amygdalus*.

Effect of Prunus amygdalus nut kernel methanolic extract on aluminium chloride induced changes in spatial memory deficits

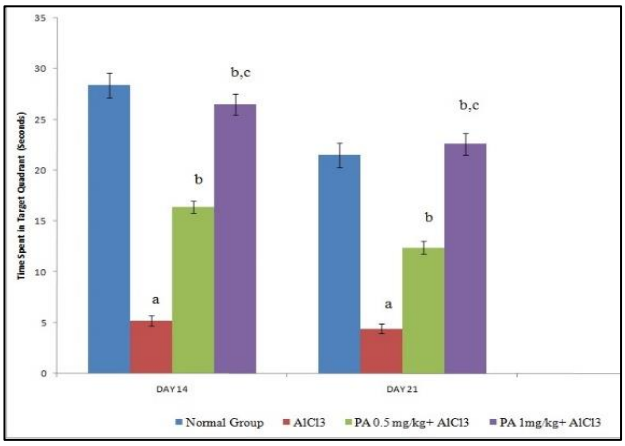


mean±S.E.M with n = 5 in each group;
[a]denotes p <0.05 as compared to the Normal group; [b]denotes p <0.05, as compared to $AlCl_3$ group; [c]denotes p <0.05, as compared to $AlCl_3$ + PA 0.5mg/Kg group

Figure 2: Changes in average transfer latency time on day 10, day11 and day 12 of the normal, aluminium chloride and extract treated groups.

In the present study, there was a significant increase in the Transfer Latency (TL) time on day 10, day11 as well as on day 12 in the acquisition trails in $AlCl_3$ treated group as compared to normal group indicating a significant lack in the learning ability and memory development in $AlCl_3$ treated group as compared to normal group. Administration of methanolic extract of *Prunus amygdalus* nuts at doses of 0.5 mg/Kg and 1 mg/Kg *p.o* in rats resulted in a significant decrease in the TL time value as compared to $AlCl_3$ treated group (Figure 2).

In the memory retrieval trials, there was a significant decrease in the average time spent in target quadrant both on day 14 and on day 21 in $AlCl_3$ treated group as compared to normal group indicating a significant lack in the ability to retrieve learned information both on short term basis as well as on long term basis. Administration of methanolic extract of *Prunus amygdalus* nuts at doses of 0.5mg/Kg and 1mg/Kg p.o in rats resulted in a significant dose dependent increase in the time spent in target quadrant both on day 14 and on day 21 as compared to $AlCl_3$ treated group (Figure 3).



mean±S.E.M with n = 5 in each group;
[a]denotes p <0.05 as compared to the Normal group; [b]denotes p <0.05, as compared to $AlCl_3$ group; [c]denotes p <0.05, as compared to $AlCl_3$ + PA 0.5 mg/Kg group

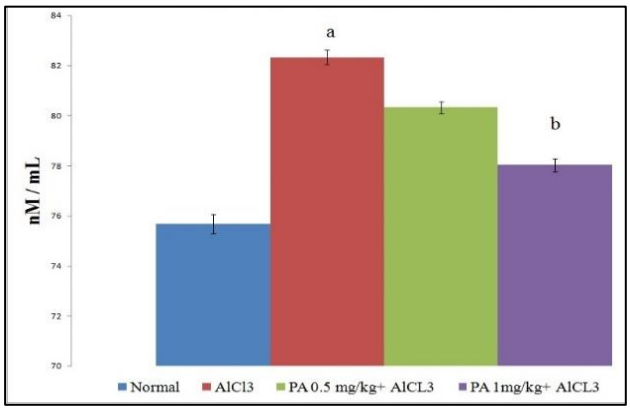
Figure 3: Changes in average time spent in target quadrant on day 13 and day 23 of the normal, aluminium chloride and extract treated groups.

Effect of Prunus amygdalus nut kernel methanolic extract on aluminium chloride induced changes in brain tissue biochemical levels

In the present study, there was a significant increase brain tissue Thiobarbituric Acid Reactive Substances (TBARS) levels in $AlCl_3$ treated group as compared to normal group indicating a significant increase in the generation of free radicals in the brain tissue of $AlCl_3$ treated group as compared to normal group. Administration of methanolic extract of *Prunus amygdalus* nuts at doses of 0.5mg/Kg and 1mg/Kg p.o in rats resulted in a significant decrease in brain tissue TBARS levels as compared to $AlCl_3$ treated group (Figure 4).

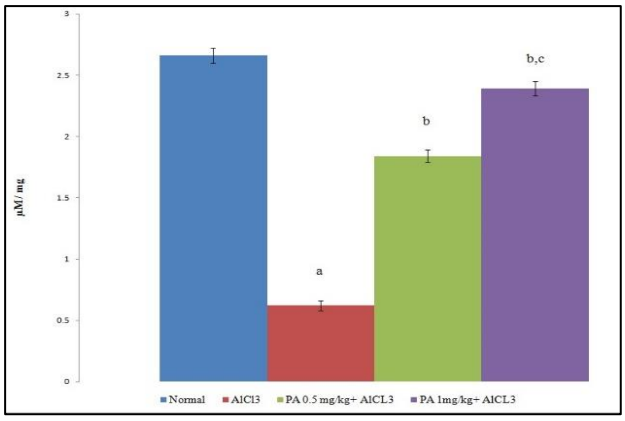
Furthermore, there was a significant decrease in the brain tissue reduced glutathione (GSH) and catalase levels in $AlCl_3$ treated group as compared to normal group indicating an overload of the free radical scavenging

mechanisms in the brain as compared to brains of animals of normal group. Administration of methanolic extract of *Prunus amygdalus* nuts at doses of 0.5mg/Kg p.o and 1mg/Kg p.o in rats resulted in a significant dose dependent decrease in brain tissue GSH and catalase levels as compared to $AlCl_3$ treated group (Figure 5 and Figure 6).



mean±S.E.M with n = 5 in each group;
[a]denotes p <0.05as compared to the Normal group; [b] denotes p <0.05, as compared to $AlCl_3$ group.

Figure 4: Changes in brain tissue TBARS levels of the normal, aluminium chloride and extract treated groups.



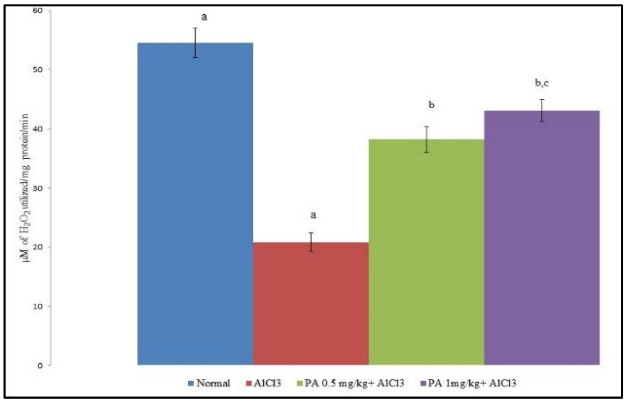
mean±S.E.M with n = 5 in each group;
[a]denotes p <0.05 as compared to the Normal group; [b]denotes p <0.05, as compared to $AlCl_3$ group; [c]denotes p <0.05, as compared to $AlCl_3$ + PA 0.5 mg/Kg group

Figure 5: Changes in brain tissue GSH levels of the normal, aluminium chloride and extract treated groups.

Effect of Prunus amygdalus nut kernel methanolic extract on aluminium chloride induced changes in histopathology of the brain tissue

Histological evaluation was performed on brain samples on the last day of the experimental protocol. Hematoxylin and eosin stained sections of tissues were evaluated for signs of neuro-inflammation. Comparison of brain tissue section from the extracts-treated rats with control group showed significant improvement in the neuro-inflammation in the extracts-treated groups. The microscopic photographs are shown in (Figure 7). Slides of Normal group showed well defined neural cell morphology with no signed of inflammation or glial cell margination (Figure 7A).

Aluminium chloride treated group showed significant inflammatory changes with widespread glial cell margination and multifocal vacuolization (a hallmark of imminent neuronal damage), indicating severe neuro-inflammation (Figure 7B). In *Prunus amygdalus* nut kernel methanolic extract (0.5mg/Kg p.o) treated groups, the neuro-inflammation process had been dampened relatively (Figure 7C). In *Prunus amygdalus* nut kernel methanolic extract (1mg/Kg p.o) treated groups, the hippocampal region in the medial ipsilateral part of the whole brain slide showed less regions of glial cell margination and diminished vacuolization indicating restoration of neuro-inflammation cascade (Figure 7D).



mean±S.E.M with n = 5 in each group;
[a]denotes p <0.05as compared to the Normal group; [b]denotes p <0.05, as compared to $AlCl_3$ group; [c]denotes p <0.05, as compared to $AlCl_3$ + PA 0.5 mg/Kg group

Figure 6: Changes in brain tissue catalase levels of the normal, aluminium chloride and extract treated groups.

Slides of Normal group showed well defined neural cell morphology with no signs of inflammation or glial cell margination (Figure 7A). Aluminium chloride treated group showed significant inflammatory changes with widespread glial cell margination (Figure 7B) and multifocal vacuolization (Arrows), indicating severe neuro-inflammation (a hallmark of imminent neuronal damage). In *Prunus amygdalus* nut kernel methanolic extract (0.5mg/Kg p.o) treated groups, the neuro-inflammation process had been dampened relatively (Figure 7C). In *Prunus amygdalus* nut kernel methanolic extract (1mg/Kg p.o) treated groups, the hippocampal region in the medial ipsilateral part of the whole brain slide showed less regions of glial cell margination and diminished vacuolization indicating restoration of neuro-inflammation cascade (Figure 7D).

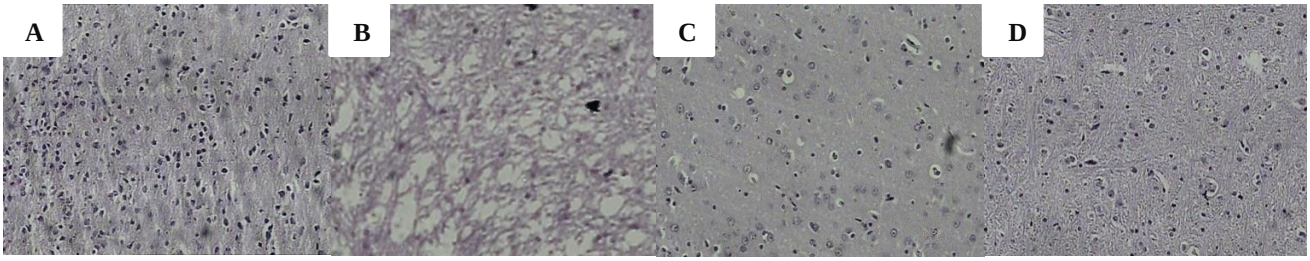


Figure 7: The pictophotographs of samples of brain tissues of normal, aluminium chloride and aluminium chloride + extract treated groups.

DISCUSSION

We explored the role of *Prunus amygdalus* nut kernel methanolic extract administration in $AlCl_3$ induced neurotoxicity. On Chronic exposure, Aluminium accumulates in brain tissues; maximum being in hippocampus, which is the site of memory and learning which results in learning impairment.[29] Al toxicity affects ionic, cholinergic and dopaminergic neurotransmission which exacerbate brain oxidative damage. Therefore, Al is considered as an etiological factor in a range of neurodegenerative disorders.[30]

In the present study, exposure to aluminium altered the performance of rats in Morris water maze, a test commonly used to assess learning ability and memory development. The $AlCl_3$ (4.2 mg/kg i.p) exposure significantly increased the transfer latency time (time taken by the rat to reach the hidden platform in water maze) as compared to the normal (untreated) group indicating impairment of learning ability over a period of three days (day 10 - day 12) of training.

Moreover, the retrieval ability of the learned information also showed significant downfall as evaluated by time spent in target quadrant in short term retrieval trials on day 14 as well as on long term retrieval trials on day 22, in contrast to Normal group (untreated) rats, clearly reflecting $AlCl_3$ induced impairment in learning and memory. These findings are in concordance with previously documented effects of aluminium on neuro-inflammation and associated spatial memory impairment.[31,32]

Administration of *Prunus amygdalus* nut kernel methanolic extract (0.5mg/kg p.o and 1mg/kg p.o) significantly improved the learning and memory ability as evident by a significant decrease in the transfer latency time in the extract treated groups over a training period of three days (day 10 - day 12). Moreover, there was significant improvement in the retrieval ability of the learned information as evident by a significant increase in the time spent in target quadrant in short term retrieval trials on day 14 as well as on long term retrieval trials on day 22, in contrast to $AlCl_3$ treated group rats.

Free radicals have been well implicated in neurodegeneration and cognitive decline.[33] Reactive oxygen species (ROS) can accumulate excessively in the brain to severely attenuate the neuronal functions, which may be the basis of cognitive impairment.[34,35] Moreover, oxidative stress severely overburdens antioxidant defence system of the brain, which may form the basis for neuroinflammation induced memory impairment.[36] In the present investigation, chronic aluminium chloride treatment (4.2mg/kg i.p) resulted in significant oxidative damage as indicated by increased oxidative stress clearly evident from a significant increase in lipid peroxidation (TBARS) levels. Furthermore, there was a compromised antioxidant defence situation as evident by a significant decline in the level of brain tissue GSH and Catalase levels. However, administration of *Prunus amygdalus* nut kernel methanolic extract (0.5mg/kg p.o and 1mg/kg p.o) exhibited its significant ($P < 0.05$) protective effects (decreased TBARS and restored GSH and Catalase levels) as compared to aluminium chloride treated group.

One possible mechanism of $AlCl_3$ induced neurotoxicity is by oxidative stress. $AlCl_3$ is a non-redox active metal capable of increasing the cellular oxidative milieu by potentiating the pro-oxidant properties.[37] Chronic $AlCl_3$ exposure generates reactive oxygen species (ROS) that cause lipid peroxidation (LPO) and oxidative damage to proteins, DNA, and decreasing intracellular antioxidants. Our results strongly support the hypothesis that the memory deficits observed after chronic aluminium chloride treatment might have arisen as a result of ROS mediated mitochondrial dysfunction, ultimately causing oxidative injury to neurons, resulting in evident neuroinflammation which could therefore, be prevented by antioxidant treatment.

It is interesting to quote here that, Al compounds can reach systemic circulation in different ways and ultimately increasing its concentration in brain. Upon entering the brain, it affects the slow and fast axonal transports, induces inflammatory responses, inhibits long-term potentiation, causes synaptic structural abnormalities which results profound neurodegeneration. $AlCl_3$ cause degeneration of cholinergic terminals in the cortical areas and cell depletion in the brain regions to induce learning deficits.[38]

Chronic administration of Al is also documented to disturb the structure and function of blood brain barrier.[39] This effect of aluminium may possibly have played an important role in facilitating the entry of chemical constituents of *Prunus amygdalus* phytoconstituents to the brain.

Our hypothesis was strengthened by histopathological studies which indicated severe neuroinflammatory changes especially neuronal tissue vacuolization in the hippocampal region of Al treated brains which may be the basis of dementia. The effects were reversed to a significant extent by treatment with methanolic extract of *Prunus amygdalus*.

Several natural compounds such as curcumin, Lagenaria siceraria (bottle gourd), Clove (Syzygium aromaticum) and naringin have been reported to exhibit neuroprotective effect against the $AlCl_3$ induced cell damages using different experimental model.[40]

Antioxidant potential of *Prunus amygdalus* and its phytoconstitutents such as amygdalin is well documented. The present results are in concordance with observed antioxidant and neuroprotective claims of *Prunus amygdalus*. The present results highlight the beneficial potential of *Prunus amygdalus* in Aluminium induced neuroinflammation and associated neurochemical alterations. However, further research is still warranted to delineate the molecular mechanism of observed beneficial effects.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the contribution of Dr. Neeraj Bhatia, Central Registration Officer, Guru Nanak Dev Hospital, Amritsar and staff members of Khalsa Diagnostic Laboratory, Amritsar for their technical guidance in histopathology of brain tissue.

Funding: Funding sources from Khalsa College of Pharmacy, Amritsar, India
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Animal Ethics Committee of Khalsa College of Pharmacy, Amritsar, India

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Cite this article as: Bhatia N, Kaur G, Bhatia G, Kaur N, Rahar S, Lalit, et al. Evaluation of the protective effect of *Prunus amagdylus* against aluminium chloride induced neurochemical alterations and spatial memory deficits in rats. Int J Basic Clin Pharmacol 2017;6:2881-8.