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A novel medicine for alzheimer's disease

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Pomegranate, which contains very high levels of anti-oxidant polyphenolic substances, beta- secretase inhibitors and several other bioactive compounds is consumed all over the world without much knowledge of its medicinal properties. Polyphenols have been shown to be neuroprotective in different animal models. There are no proven ways to delay the onset or slow down the progression of Alzheimer's disease. This study was conducted to investigate the effects of pomegranate juice on memory deficits in mice and rats. A total of 216 Swiss mice and 72 Wistar rats were used in the present study. The exteroceptive behavioral models employed in the present study were elevated plus maze, passive avoidance apparatus and Hebb-William's maze. Pomegranate juice (10% v/v) produced significant improvement in the memory of young and aged rodents and reversed the amnesia induced by diazepam (1 mg/kg, i.p.) and scopolamine (0.4 mg/kg, i.p.). Furthermore, pomegranate juice inhibited significantly the brain acetylcholinesterase activity, diminished peripheral cholesterol and brain malondialdehyde levels. Pomegranate juice appears to be a useful anti-Alzheimer medicine on account of its multifarious beneficial effects such as memory improving effect, cholesterol lowering property, anti-cholinesterase and anti-oxidant activity. Therefore, pomegranate juice may be exploited clinically for the management of Alzheimer's disease.

Key words : Pomegranate, Memory, Amnesia, Alzheimer's disease

INTRODUCTION

When pregnant mice were fed with pomegranate juice in their diet, neuroprotective effect was observed in neonatal mice born to these mothers (West *et al.*, 2007). The pomegranate, *Punica granatum* L. (Family: Punicaceae), is consumed all over the world because of its delicious and pleasant taste. Pomegranate contains very high levels of antioxidant polyphenolic substances, beta- secretase inhibitors (Kwak *et al.*, 2005), mitogen activated protein kinase inhibitors and several other bioactive compounds.

Alzheimer's disease (AD) is a crippling, progressive, neurodegenerative heterogeneous brain disorder observed predominantly in senior citizens. Around 30 million patients are afflicted by AD all over the world. Presently, management of AD relies on nootropic agents, such as piracetam, nefiracetam, aniracetam, etc., anticholinesterases, such as donepezil, galantamine, rivastigmine and noncompetitive NMDA-receptor antagonists like memantine. However, it is worthwhile to explore new strategies for treating patients suffering from AD. In the light of above, the anti-Alzheimer potential of pomegranate juice was investigated in the present study.

The present study was undertaken to explore the anti-Alzheimer potential of pomegranate juice using elevated plus maze, passive avoidance apparatus and Hebb-William's maze in rodents. Furthermore, the effects of pomegranate juice on brain acetylcholinesterase activity, total cholesterol and brain malondialdehyde levels were investigated in mice.

MATERIALS AND METHODS

Preparation of pomegranate juice:

The fresh fruits of pomegranate (Mridula variety) were purchased from local market of Hisar and got authenticated from Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources, New Delhi (Ref.NISCAIR/RHMD/Consult/2008-09/1159/191). Fruits were washed with cold tap water and the outer leathery skin, which encloses hundreds of fleshy sacs was removed manually. The deep red coloured fruit juice was obtained using food grinder (Remi Anupam Mixie Ltd., Mumbai). Pilot study was conducted to determine the optimum dose of pomegranate juice (PJ) and duration of administration. Pomegranate juice was administered orally in mice and rats at the dose rate of 1 ml/100g body weight for a duration of 12 days.

Animals:

A total of 216 Swiss mice and 72 Wistar rats divided in 48 different groups were employed in the present study. Each group comprised of a minimum of 6 animals. Young (3-4 months old) and aged (12-15 months old) rodents

were procured from the Disease-Free Small Animal House of C.C.S. Haryana Agricultural University, Hisar. The experimental protocol was approved by the Institutional Animal Ethics Committee and the care of laboratory animals was taken as per the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India (registration number 0436).

Statistical analyses:

All the results were expressed as Mean ± Standard Error (SEM). Data were analyzed using one-way ANOVA followed by Dunnett's t-test.

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below:

Locomotor activity:

Pomegranate juice (10% v/v) administered orally for 12 successive days, was found to have no significant effect on the spontaneous locomotor activity of young (220.6 ± 7.99) or aged (255.5 ± 8.74) mice as compared to respective control group (216.83 ± 8.79 and 252.33 ± 9.13). Similarly, the spontaneous locomotor activity did not differ significantly between the control (294.3 ± 9.03, 320.34 ± 9.17) and PJ treated young (296.67 ± 8.52) and aged rats (326.66 ± 8.61) on day 12.

Effect of pomegranate juice on transfer latency:

Elevated plus maze served as the exteroceptive behavioral model to evaluate learning and memory in mice. Transfer latency (TL) of first day (on 12th day of drug treatment) reflected learning behavior of animals, whereas TL of next day reflected retention of information or memory. Pomegranate juice (10 % v/v) administered orally for 12 days produced significant ($p<0.01$) reduction in TL indicating improvement in learning and memory of both young and aged mice. Scopolamine (0.4 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) produced significant ($p<0.01$) impairment in learning and memory. Pomegranate juice (10% v/v) administered orally for 12 successive days successfully reversed ($p<0.01$) the memory deficits induced by scopolamine and diazepam (Fig. 1). Piracetam (400 mg/kg, i.p.) served as positive control in the present study.

Effect of pomegranate juice on step down latency:

Passive avoidance apparatus based on negative reinforcement was used to examine the long-term memory. Pomegranate juice (10 % v/v) administered orally for 12 days significantly ($p<0.01$) increased step down latency in young and aged mice as compared to respective control groups (Fig. 2) and reversed ($p<0.01$) amnesia induced by both scopolamine and diazepam (Fig. 3).

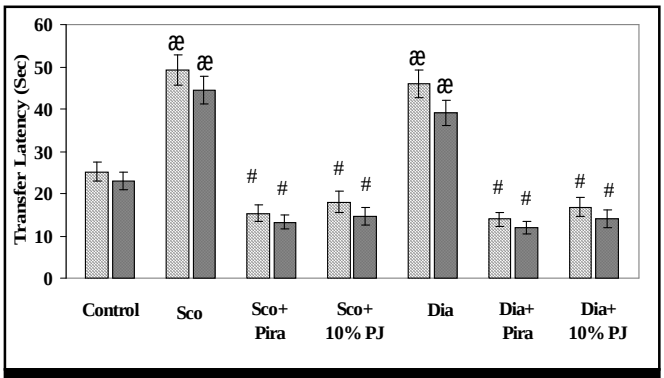

Fig. 1.: Effect of pomegranate juice on scopolamine and diazepam induced amnesia in young mice using elevated plus maze. Values are in Mean ± SEM. (n=6). æ denotes $p<0.01$ when compared to respective control group. # denotes $p<0.01$ when compared to scopolamine or diazepam alone group. (One-way ANOVA followed by Dunnett's t-test). Dia= Diazepam, Pira = Piracetam, PJ = Pomegranate juice, Sco = Scopolamine

Effect of pomegranate juice on time taken to reach reward chamber:

Hebb-William's maze is an incentive based exteroceptive behavioral model useful for measuring spatial and working memory of rats. Time taken to reach reward chamber (TRC) reflects the learning behavior of rats on first exposure and memory on subsequent

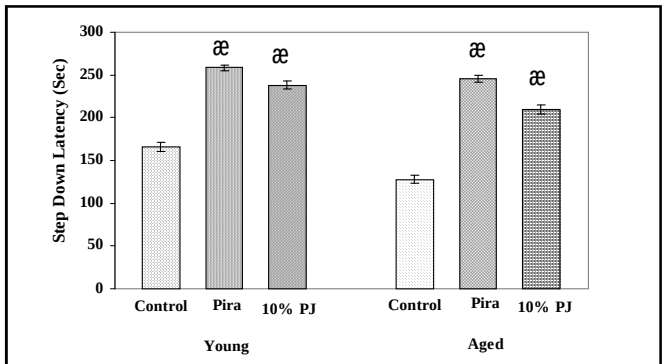

Fig. 2. : Effect of pomegranate juice on step down latency of young and aged mice using passive avoidance apparatus. Values are in Mean ± SEM. (n=6). æ denotes $p<0.01$ when compared to respective control group of young or aged mice. (One-way ANOVA followed by Dunnett's t-test). Pira = Piracetam, PJ = Pomegranate juice

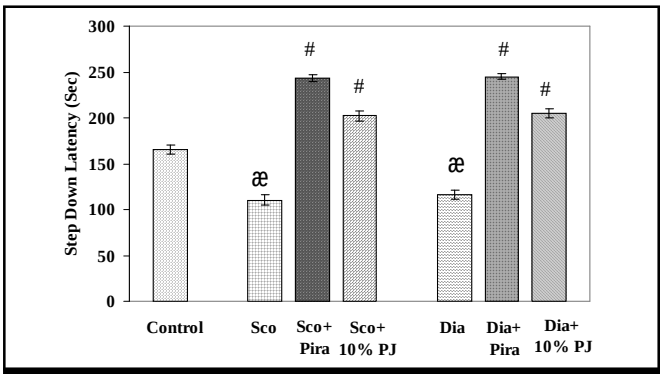

Fig 3.: Effect of pomegranate juice on scopolamine and diazepam induced amnesia of young mice. Values are in Mean ± SEM. (n=6). æ denotes p<0.01 when compared to respective control group. # denotes p <0.01 when compared to scopolamine or diazepam alone group. (One-way ANOVA followed by Dunnett's t-test). Dia = Diazepam, Pira = Piracetam, PJ = Pomegranate juice, Sco = Scopolamine

exposures. Thus lower TRC value indicates improved memory, whereas high TRC value indicated poor memory. Pomegranate juice (10 % v/v) administered orally for 12 days produced significant ($p<0.01$) reduction in TRC indicating improvement in memory of both young and aged rats. (Fig. 4). Scopolamine (0.4 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) produced significant ($p<0.01$) impairment in memory. Pomegranate juice (10% v/v) administered orally for 12 successive days successfully reversed ($p<0.01$) memory deficits induced by scopolamine and diazepam. Piracetam (400 mg/kg, i.p.) an established nootropic agent served as the positive control in this model.

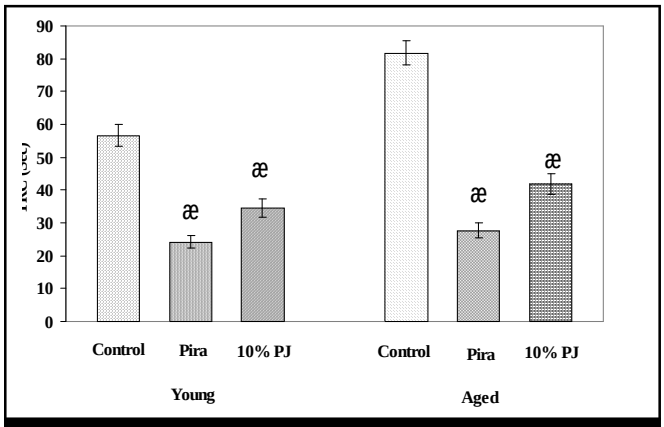

Fig. 4.: Effect of pomegranate juice on TRC of young and aged mice using Hebb-William's maze. Values are in Mean ± SEM. (n=6). æ denotes p<0.01 when compared to respective control group of young or aged mice. (One-way ANOVA followed by Dunnett's t-test). Pira =Piracetam, PJ = Pomegranate juice, TRC = Time taken to reach the reward chamber

Effect of pomegranate juice on brain acetylcholinesterase activity:

The PJ (10% v/v) produced significant reduction in brain acetylcholinesterase activity in young (17.61 %, $p<0.05$) and aged mice (21.463 %, $p<0.01$) as compared to respective control groups. Similarly, donepezil (0.1 mg/kg, i.p.) used as a standard drug evoked significant ($p<0.01$) reduction of brain AChE activity in young (31.34 %) and aged (33.82 %) mice, respectively (Fig. 5).

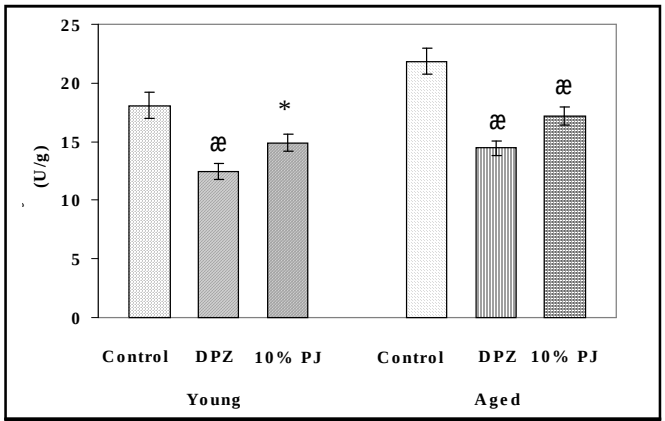

Fig. 5:Effect of pomegranate juice on brain acetylcholinesterase activities of young and aged mice. Values are in Mean ± SEM. (n=6).æ denotes p<0.01 when compared to respective control group of young or aged mice. * denotes p<0.05 when compared to control group of young mice. (One-way ANOVA followed by Dunnett's t-test). DPZ = Donepezil, PJ = Pomegranate juice

Effect of pomegranate juice on total cholesterol level:

Pomegranate juice (10% v/v) administered orally for 12 successive days significantly ($p<0.01$) decreased the total cholesterol level in both young (27.23 %) and aged (19.10 %) mice. The extent of reduction in total cholesterol levels with standard cholesterol lowering agent *viz*. simvastatin were 35.74 % ($p<0.01$) in young and 31.77 % ($p<0.01$) in aged mice, respectively (Fig. 6).

Effect of pomegranate juice on brain malondialdehyde level:

Oral administration of 10% (v/v) PJ for 12 successive days produced significant ($p<0.01$) decrease in brain MDA level in both young (18.39 %) and aged (15.51 %) mice (Fig. 7).

Alzheimer's disease is a genetically heterogeneous neurodegenerative disorder, which is slow in onset but relentless in progress. It is characterized by decline in cognitive abilities, manifested by loss of memory, impaired judgment, aphasia, apraxia, agnosia, disorientation,

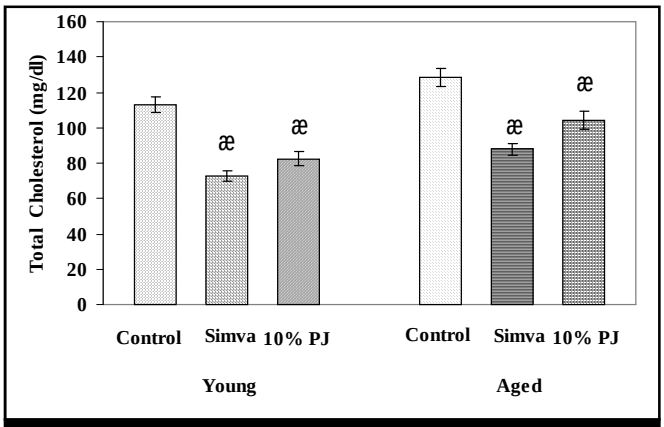

Fig. 6. : Effect of pomegranate juice on total cholesterol levels of young and aged mice.
Values are in Mean ± SEM. (n=6). æ denotes p <0.01 when compared to respective control group of young or aged mice. (One-way ANOVA followed by Dunnett's t-test). Simva = Simvastatin, PJ = Pomegranate juice

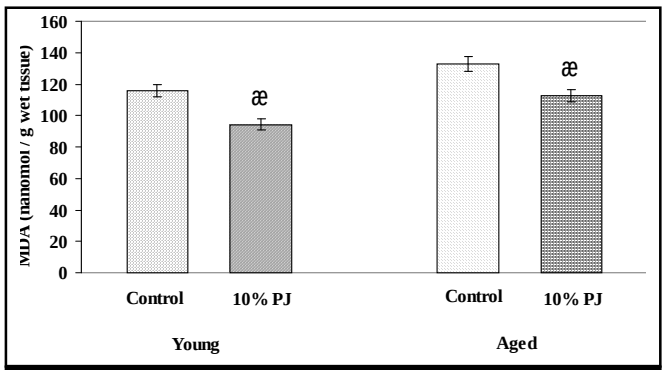

Fig. 7.: Effect of pomegranate juice on brain malondialdehyde levels of young and aged mice.
Values are in Mean ± SEM. (n=6). æ denotes p <0.01 when compared to respective control group of young or aged mice. (One-way ANOVA followed by Dunnett's t-test). PJ = Pomegranate juice, MDA = Malondialdehyde

confusion, impaired judgment, disturbed sleep and loss of interest in life (Parle and Parle, 2008). Despite the severity of this disease, allopathic system of medicine is yet to provide a satisfactory antidote, therefore, it becomes worthwhile to investigate the potential of pomegranate juice in the management of AD. In the present study, PJ administered orally for 12 successive days improved the memory of rodents, when tested using elevated plus maze, passive avoidance apparatus and Hebb-William's maze. Furthermore, pretreatment with PJ for 12 days protected the animals from memory deficits produced by scopolamine and diazepam. No significant difference was observed in locomotor activity of PJ treated and control group of animals. This rules out the possibility that the locomotor activity *per se* may have contributed to the changes in performance of treated animals in elevated plus maze, passive avoidance apparatus and Hebb-William's maze experiments.

The main characteristic features of AD include selective loss of cholinergic neurons, extracellular protein deposits termed as β-amyloid (Aβ) plaques and intraneuronal neurofibrillary tangles. In the present study, the pomegranate juice produced elevation of brain acetylcholine level by significant reduction of acetylcholinesterase activity. Beta-amyloid is produced by the action of β-secretase enzyme on amyloid precursor protein and neurofibrillary tangles are the result of hyperphosphorylation of tau protein. Thus, β-secretase inhibitors (Kwak *et al.*, 2005) and mitogen activated protein kinase inhibitors (Jurenka, 2008), which are abundantly present in pomegranate juice may be favorably reducing both, the hyperphoshorylation of tau proteins and production of Aβ. Abnormal accumulation of Aβ is associated with high cholesterol levels and drugs that inhibit cholesterol levels reduce the secretion and accumulation of Aβ (Zipp *et al.*, 2007). Interestingly the animals, which were treated with PJ showed significant reduction in cholesterol levels as compared to control group of animals. Therefore, it seems likely that PJ may prove to be a useful anti-Alzheimer agent because of its cholesterol lowering property. The malondialdehyde is an end product of lipid peroxidation, an index of free radical generation. Oxygen free radicals are implicated in the process of ageing and may be responsible for the development of AD (Rogers *et al.*, 2003). The significant decrease in MDA levels in the brains of mice treated with PJ indicated attenuation of lipid peroxidation. This anti-oxidant effect of PJ might be beneficial in protecting mice against the oxidative stress.

Conclusion :

An ideal anti-Alzheimer agent should be able to successfully arrest the deposition of Aâ plaques in brain, attentuate the formation of neurofibrillary tangles, increase cholinergic transmission, lower cholesterol levels, ameliorate oxidative stress and improve memory. In the present study, it was observed that pomegranate juice (i) increased cholinergic transmission (ii) decreased total cholesterol levels (iii) diminished lipid peroxidation (iv) and ultimately improved memory. Thus, present investigation indicates the potential of pomegranate juice in the management of neurodegenerative disorders like Alzheimer's disease.

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