Neuroprotective Effects of Pomegranate Juice in Mice

MILIND PARLE AND MANAS KUMAR SAMAL

In the food industry, pomegranate is listed under a novel category of exotic fruits called ‘super fruits’. Pomegranate juice is reported to possess several useful medicinal properties such as anti-carcinogenic, anti-inflammatory, hypolipidemic and anti-hypertensive (Jurinca, 2008). Alzheimer’s disease (AD) is said to be the leading cause of dementia in elderly individuals. Alzheimer patients exhibit deterioration in cognitive functions rendering them incapacitated to perform normal daily activities (Parle and Kadian, 2009).

A keen interest in therapeutic properties of pomegranate has prompted numerous in vitro and in vivo animal and clinical studies. However, not much work has been done on the psychopharmacological actions of pomegranate juice. Therefore, this study explore the neuroprotective potential of pomegranate juice in mice. A total of 204 Swiss male mice divided into 34 groups were employed in the present investigation. Pomegranate juice (10% v/v) showed significant improvement in the memory of young and aged mice, when tested in object recognition task model. The pomegranate juice also reversed the memory deficits induced by diazepam (1 mg/kg, i.p.), scopolamine (0.4 mg/kg, i.p.) and sodium nitrite (75 mg/kg, s.c.). Furthermore, pomegranate juice showed hypoglycemic effect in mice and increased brain reduced glutathione levels. The underlying mechanism of action for the observed memory enhancing effect of pomegranate juice could be attributed to its anti-oxidant and glucose lowering property. This study highlights the neuroprotective potential of pomegranate juice in various experimental models.

Key words: Pomegranate juice, Memory, Alzheimer’s disease, Neuroprotective

ABSTRACT

A keen interest in therapeutic properties of pomegranate has prompted numerous in vitro and in vivo animal and clinical studies. However, not much work has been done on the psychopharmacological actions of pomegranate juice. Therefore, this study explores the neuroprotective potential of pomegranate juice in mice. A total of 204 Swiss male mice divided into 34 groups were employed in the present investigation. Pomegranate juice (10% v/v) showed significant improvement in the memory of young and aged mice, when tested in object recognition task model. The pomegranate juice also reversed the memory deficits induced by diazepam (1 mg/kg, i.p.), scopolamine (0.4 mg/kg, i.p.) and sodium nitrite (75 mg/kg, s.c.). Furthermore, pomegranate juice showed hypoglycemic effect in mice and increased brain reduced glutathione levels. The underlying mechanism of action for the observed memory enhancing effect of pomegranate juice could be attributed to its anti-oxidant and glucose lowering property. This study highlights the neuroprotective potential of pomegranate juice in various experimental models.

Materials and Methods

Study was conducted to determine the optimum dose of pomegranate juice (PJ) and duration of administration. Pomegranate juice (10% v/v) was administered orally in mice for a duration of 12 days.

Animals:

All the experiments were performed using male Swiss mice procured from the Disease-Free Small Animal House of C.C.S. Haryana Agricultural University, Hisar. Young (3-4 months old) and aged (12-15 months old) animals were used in the present study. The experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC) 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).

Memory deficits were produced in mice using drugs such as diazepam, scopolamine (Parle and Kadian, 2009) and sodium nitrite. Aged animals also served as memory compromised animals.

Sodium nitrite induced hypoxia model:

Sodium nitrite induced hypoxia model was
used to test the memory of mice (Schindler et al., 1984). Under normal circumstances, thirsty mice spend more time in small chamber, wherein a water bottle had been placed, as compared to time spent in large chamber. This behavior of animals was attenuated by pretreatment with sodium nitrite. Sodium nitrite is reported to induce brain hypoxia leading to amnesia in animals. In this model, the duration of exploration of small chamber (DESC) while searching for the water bottle is evaluated over a period of 3 minutes and an increase in duration signifies improved memory.

Object recognition task:
Object recognition task is a convenient model used to evaluate the object recognition memory in mice. (Sik et al., 2003; Dere et al., 2007).

Biochemical estimations:
Collection of blood and brain samples:
The animals were sacrificed by cervical decapitation under light anesthesia using ether on the 12th day. Immediately after decapitation, the trunk blood was collected. Then whole brain was carefully removed from the skull and kept at 20°C. The collected blood was centrifuged at 3000 rpm for 15 min so as to separate the plasma for estimation of glucose level.

Estimation of brain reduced glutathione level:
Reduced glutathione was measured spectrophotometrically by using method of Ellman (1959).

Estimation of plasma glucose level:
GOD-POD (Miksch and Wiedemann, 1973) method was used for the estimation of blood glucose level using semi autoanalyzer.

Experimental design:
A total of 204 Swiss mice divided in 34 different groups (n=6) were employed in the present study.

Statistical analysis:
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

Effect of pomegranate juice on duration of exploration of small chamber:
The duration of exploration of small chamber (DESC) is defined as the total time spent inside the small chamber over 3 minute period. An increase in duration signifies improved memory. Pomegranate juice (10 % v/v) administered orally for 12 days significantly (p<0.01) increased the DESC of young and aged mice and reversed (p<0.01) the amnesia induced by sodium nitrite (75 mg/kg, s.c.). Piracetam (400 mg/kg, i.p.) an established nootropic agent served as the positive control in the present study (Fig. 1).

Effect of pomegranate juice on discrimination index:
Discrimination index (d1) is the difference between the exploration time of novel object and familiar object. Increase in d1 indicates enhanced recognition memory. Pomegranate juice (10 % v/v) administered orally for 12 days produced significant (p<0.01) increase in discrimination index indicating improvement in memory of both young and aged mice (Fig. 2). 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) the memory deficits induced by scopolamine and diazepam (Fig.3).

Effect of pomegranate juice on brain reduced glutathione level:
Oral administration of 10% (v/v) PJ for 12 successive days produced significant increase in brain reduced glutathione level in both young (33.06 %, p<0.01) and aged (29.54 %, p<0.05) mice (Fig. 4).
functions (Gauthier, 2002). Hypoxia induced with sodium nitrite reduces incorporation of choline into acetylcholine and thereby decreases the synthesis of acetylcholine (Gibson et al., 1976). Pomegranate juice reversed the memory impairing effects of scopolamine, diazepam and sodium nitrite probably through facilitated cholinergic transmission.

Immunohistochemical studies suggested the existence of chronic inflammation in certain regions of the brain in Alzheimer patients. Since inflammation can be damaging to host tissue, it was hypothesized that anti-inflammatory drugs might be inhibiting both the onset and progression of AD. This hypothesis is supported by the observation that indomethacin, a non-steroidal anti-inflammatory drug exhibited a memory protective effect against electroconvulsive shock-induced retrograde amnesia (Rao et al., 2002). Anti-inflammatory action (Schubert et al., 1999) exhibited by PJ might be contributing favorably to the memory enhancing activity observed in the present study.

In the present investigation, PJ administered orally for 12 successive days significantly decreased plasma glucose level in both young and aged mice. As compared to respective control groups (124.46 ± 4.89, 142.05 ± 5.08) the per cent reduction in blood glucose levels were 14.12% in young (106.9 ± 4.03, p<0.01) and 11.82% in aged mice (125.26 ± 4.253, p<0.05).

In the present investigation, PJ administered orally for 12 successive days improved the memory of mice, when tested using object recognition task model. Furthermore, pretreatment with PJ for 12 days protected the mice from developing memory deficits after the administration of scopolamine, diazepam and sodium nitrite. Acetylcholine is considered as the most crucial neurotransmitter involved in the regulation of cognitive
by increasing the endogenous defensive capacity of brain.

**Conclusion:**

In the present study, it was observed that pomegranate juice (i) enhanced reduced glutathione levels (ii) reduced glucose levels (iii) and ultimately improved memory of both young and aged mice. These findings reveal the neuroprotective potential of pomegranate juice in different animal models.

**Authors’ affiliations:**

MANAS KUMAR SAMAL, Pharmacology Division, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, HISAR (HARYANA) INDIA

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