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Guava : A promising memory enhancer in rodents

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SUMMARY

Psidium guajava commonly known as Guava in English and *Amrood* in Hindi is widely used as a fruit throughout the world. The present study was undertaken to investigate the effects of *Psidium guajava* fruit on memory of mice and rats. A total of 408 Swiss mice and 120 Wistar rats were used in the present study. The exteroceptive behavioral models employed in the present study to test memory were passive avoidance apparatus, Hebbs William's maze and elevated plus maze. *Psidium guajava* fruit (5, 10 and 15% w/w of diet) produced significant improvement in memory of young and aged rodents and reversed successfully the amnesia induced by scopolamine (0.4mg/kg, i.p.) and diazepam (1mg/kg, i.p.). Interestingly, brain cholinesterase activity, malondialdehyde levels and peripheral cholesterol levels were all reduced by *Psidium guajava* fruit. The memory-enhancing effect of *Psidium guajava* fruit may be attributed to its (i) acetyl cholinesterase inhibitory activity (ii) reduced malondialdehyde levels (iii) cholesterol lowering property and/or anti-oxidant activity. Thus, *Psidium guajava* fruit appears to be a promising memory enhancer.

Key words : *Psidium guajava*, Amnesia, Learning, Memory

Dementia is a collective name for progressive degenerative brain syndromes, which affect memory, judgment, thinking, behavior, emotions and communication skills. The most common cause of dementia is Alzheimer's disease, which is a progressive neurodegenerative disorder associated with loss of cholinergic neurons in distinct brain areas. The central cholinergic pathways play a prominent role in learning and memory processes (Nabeshima, 1993). Centrally acting antimuscarinic drugs (e.g. scopolamine) impair learning and memory both in animals (Higashida and Ogawa, 1987) and human beings (Sitaram *et al.*, 1978). Epidemiological studies of Indian population reveal that dementia is largely a hidden problem and the number of patients suffering from dementia is increasing especially in rapidly developing and heavily populated regions such as India, China and Latin America (Parle *et al*., 2005). Prevalence rates for dementia increase with advancing age (Kawas *et al.*, 2000). Management of neurodegenerative disorders is one of the thrust areas of research in the present scenario. Although, few medicines are available for the treatment of Alzheimer's diseases, the outcomes are often unsatisfactory, therefore, neurobiologists all over the world are looking for new directions and alternative strategies for managing cognitive disorders. In the light of above, we were interested to investigate the potential of guava fruit in the management of dementia.

The present study was undertaken to investigate the effects of *Psidium guajava* fruit on memory of rodents using elevated plus maze, passive avoidance apparatus and Hebbs–William's maze. Furthermore, the effects of guava fruit on brain acetylcholinesterase activity, total cholesterol and malondialdehyde levels were investigated in mice.

MATERIALS AND METHODS

Preparation of Amrood paste:

The fresh fruits of *Psidium guajava* (*Amrood*) were purchased from local market in Hisar, Haryana. The fresh fruits of *Amrood* were separately ground into a fine paste using an electric grinder. Different concentrations of *Amrood* paste (C_5, C_{10} and C_{15} *viz*. 5, 10 and 15% w/w) were fed to separate groups of rodents through a specially prepared diet. This special diet comprised of a mixture of *Amrood* Paste, wheat flour kneaded with water and a pinch of salt (sodium chloride), to impart taste. Each animal consumed around 3 g/day of this specially prepared diet. Control animals received the normal diet consisting of wheat flour, kneaded with water and a pinch of salt but was without *Amrood* paste.

Animals:

A total of 408 Swiss mice divided in 68 groups and 120 Wistar rats divided into 20 different groups were

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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 disease free small animal house, CCS Haryana Agriculture University, Hisar. The experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC) and care of laboratory animals was taken as per CPCSEA guidelines (Reg. No. 0436).

Statistical analysis:

All results were expressed as mean ± standard error of mean (S.E.M.). The data were analyzed using one-way ANOVA followed by Dunnett's 't' test.

RESULTS AND DISCUSSION

The results obtained from the present investigation are presented below:

Effect of Psidium guajava fruit on transfer latency:

Elevated plus-maze served as the exteroceptive behavior model to evaluate learning and memory in mice. Transfer Latency (TL) of first day (on 15th day of drug treatment) reflected learning behaviour of animals, whereas TL of the next day reflected retention of information/memory. *Psidium guajava* fruit (10% w/w, p.o.) administered for 15 days produced significant ($p<0.01$) reduction in TL of 16th day indicating improvement in memory of both young (22.33 ± 1.1) and aged (19.6+_0.8) mice as compared to respective control groups (27.05 ± 0.67 and 27.08 ± 0.63). Scopolamine (0.4mg/kg, i.p) and diazepam (1mg/kg, i.p.) produced significant ($p<0.01$) impairment in memory of mice. *Psidium guajava* fruit (5, 10 and 15% w/w) administered orally for 15 successive days successfully protected the animals ($p<0.01$) from developing memory deficits produced by the injection of scopolamine or diazepam (Fig. 1). Piracetam (400mg/kg, i.p.) was used as the positive control in the present study.

***Effect of Psidium guajava* fruit on step-down latency:**

Passive avoidance apparatus based on negative reinforcement was used to examine the long-term memory. *Psidium guajava* fruit (10 and 15 % w/w of diet, p.o.) administered for 15 days significantly ($P<0.01$) increased the step down latency of young and aged mice as compared to the respective control groups (Fig. 2). Furthermore, *Psidium guajava* fruit (5, 10 and 15% w/w, p.o.) reversed the amnesia produced by scopolamine (0.4mg/kg, i.p.) and diazepam (1mg/kg, i.p.) (Fig. 3).

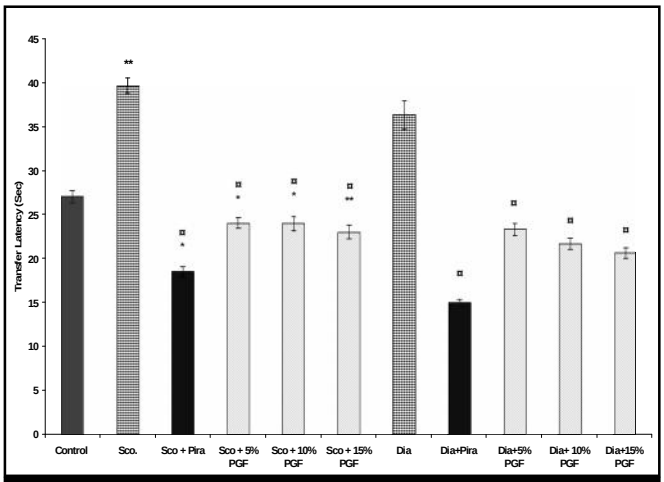

Fig.1 : Effect of *Psidium guajava* fruit on scopolamine and diazepam induced amnesia in young mice using elevated plus maze. Values are in mean ± SEM. (n=6). * denotes p< 0.05 and ** denotes p< 0.01 when compared to respective control group. ¤ denotes p< 0.01 when compared to scopolamine or diazepam alone group. (One-way ANOVA followed by Dunnett's t-test). Sco = Scopolamine, Dia = Diazepam, Pira = Piracetam, PGF = *Psidium guajava* fruit

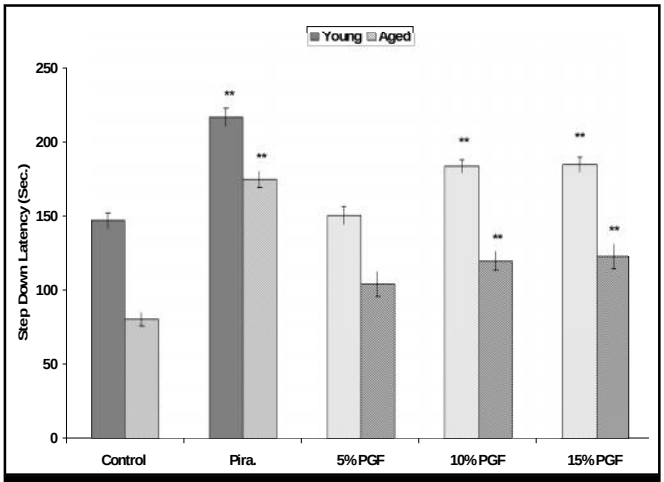

Fig.2 : Effect of *Psidium guajava* fruit on step down latency of young and aged mice using passive avoidance apparatus.Values are in mean ± SEM. (n=6). ** denotes p<0.01 when compared to respective control group of young or aged mice. (One-way ANOVA followed by Dunnett's t-test). PGF = *Psidium guajava*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

Effect of Psidium guajava fruit on time taken to reach reward chamber:

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

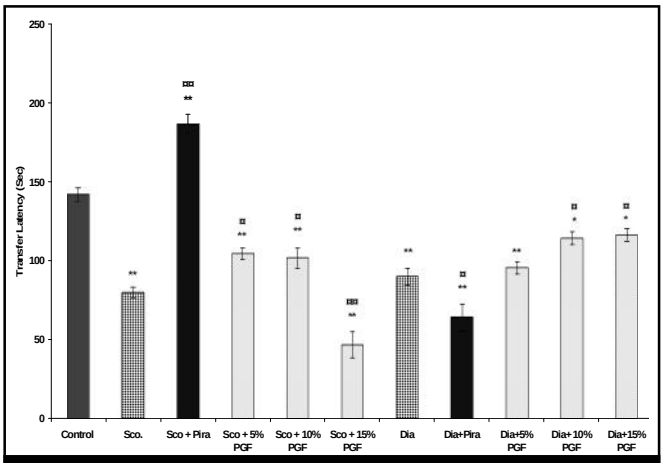

Fig.3 : Effect of *Psidium guajava* fruit on scopolamine and diazepam induced amnesia of young mice.Values are in mean ± SEM. (n = 6). ** denotes p<0.01 and * denotes p<0.05 as compared to respective control group. ¤¤ denotes p< 0.01 and ¤ denotes p< 0.05 as compared to scopolamine or diazepam alone groups.(One-way ANOVA followed by Dunnett's t-test). Sco = Scopolamine, Dia = Diazepam, PGF = *Psidium guajava* fruit

exposures. Thus, lower TRC value indicates improved memory, whereas high TRC value indicated poor memory. *Psidium guajava* fruit (5,10 and 15% w/w, p.o.) administered for 15 days along with diet produced significant (p<0.05) reduction in TRC at a dose of (5 and 15 % w/w) in young as well as aged rats whereas, *Psidium guajava* fruit (10% w/w, p.o.) showed remarkable (p<0.01) improvement in memory of young and aged rats as reflected by highly diminished TRC value

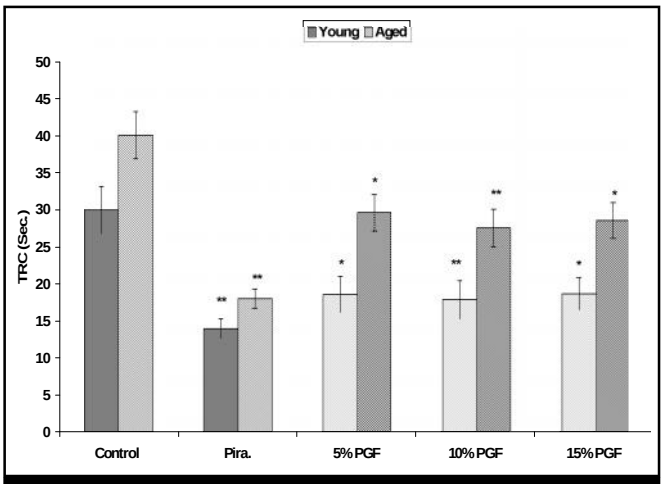

Fig.4 : Effect of *Psidium guajava* fruit on TRC of young and aged rats using Hebbs-William's maze. Values are in mean ± SEM. (n=6). * denotes p<0.05 and * * denotes p<0.01 as compared to control group of young and aged mice.(One-way ANOVA followed by Dunnett's t-test). PGF = *Psidium guajava* fruit, Pira = Piracetam

(Fig. 4). Scopolamine (0.4mg/kg, i.p.) and diazepam (1mg/kg, i.p.) produced significant (p< 0.01) impairment in memory of rats. *Psidium guajava* fruit (5,10 and 15%w/w) administered orally for 15 successive days successfully reversed (p<0.01) the memory deficits induced by scopolamine and diazepam. Piracetam (400mg/kg, i.p.) an established nootropic agent served as the positive control in this model.

Effect of Psidium guajava fruit on brain acetylcholinesterase activity:

The *Psidium guajava* fruit (5, 10 and 15% w/w, p.o.) produced significant reduction in brain acetylcholinesterase activity in young and aged mice (p<0.01) as compared to respective control groups. Similarly, donepezil (0.1mg/kg, i.p.), used as a standard drug evoked significant (p<0.01) reduction of brain AChE activity in young and aged mice, respectively (Fig.6).

Effect of Psidium guajava fruit on total cholesterol level:

Psidium guajava fruit (10 and 15% w/w) administered orally for 15 successive days significantly (p<0.01) decreased the total cholesterol level in both young and aged mice . The extent of reductions in total cholesterol levels with standard cholesterol lowering agent *viz*. simvastatin were 49.60% (p<0.01) in young and 41.0% (p<0.01) in aged mice, respectively (Fig.5).

Effect of Psidium guajava fruit on brain malondialdehyde (MDA) level:

Oral administration of (5, 10 and 15% w/w, p.o.)

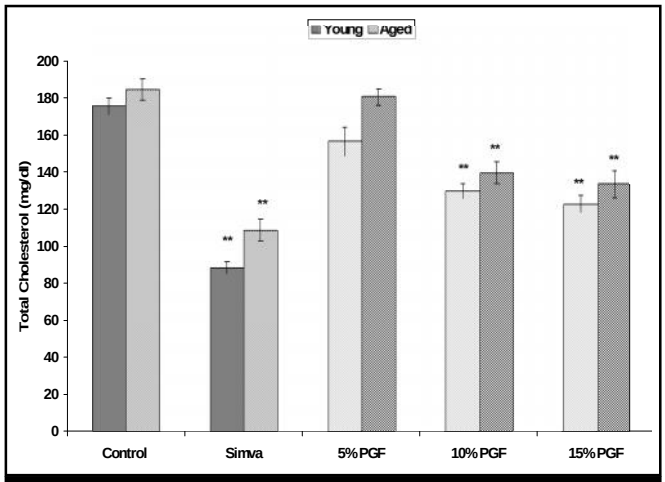

Fig.5 : Effect of *Psidium guajava* on total cholesterol levels of young and aged mice. Values are in mean ± SEM. (n=6). * * denotes p<0.01 as compared to respective control group of young and aged mice.(One-way ANOVA followed by Dunnett's t-test). PGF = *Psidium guajava* fruit, Simva = Simvastatin

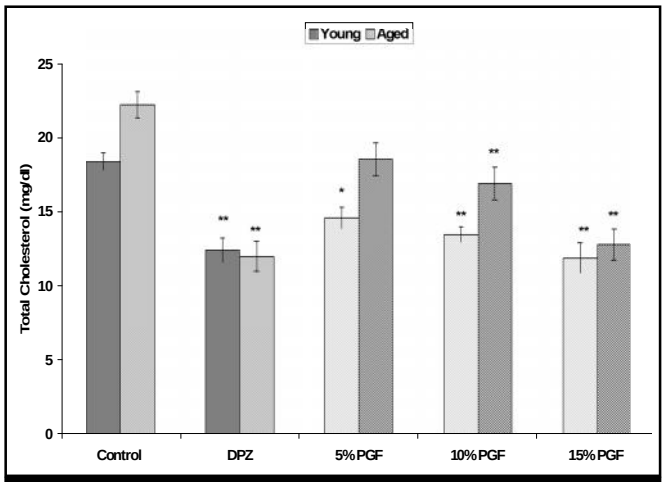

Fig. 6 : Effect of *Psidium guajava* on brain acetylcholinesterase activities of young and aged mice. Values are in mean ± SEM. (n=6). * denotes p<0.05 and ** denotes p<0.01 as compared to respective control group of young and aged mice.(One-way ANOVA followed by Dunnett's t-test). PGF = *Psidium guajava* fruit, DPZ = Donepezil

Psidium guajava fruit for 15 days produced significant ($p<0.05$) decrease in brain MDA levels of young and aged mice (Fig.7).

Dementia is a common age-related mental problem, and also a characteristic symptom of Alzhiemers disease. Nootropic agents like piracetam and cholinesterase inhibitors like donepezil or rivastigmine are clinically used in situations, where cognitive impairment is observed, but the resulting side-effects associated with these agents have made their utility limited. Ayurveda emphasizes use of herbs, nutraceuticals or life-style changes for controlling

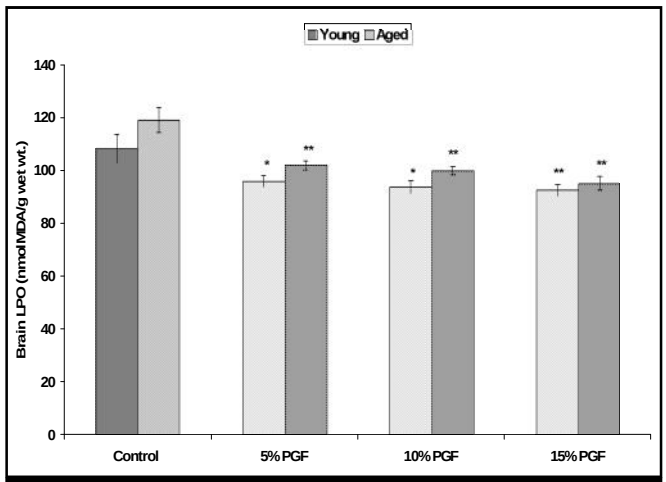

Fig. 7 : Effect of *Psidium guajava* on brain malondialdehyde levels of young and aged mice. Values are in mean ± SEM. (n=6). * denotes p<0.05 and ** denotes p<0.01 as compared to respective control group of young and aged mice.(One-way ANOVA followed by Dunnett's t-test). PGF = *Psidium guajava* fruit

age related neurodegenerative disorders. In the present study, *Psidiun guajava* fruit administered orally for 15 successive days improved the memory of rodents, when tested using elevated plus maze, passive avoidance apparatus and Hebbs-William's maze. Furthermore, pretreatment with *Psidium guajava* fruit for 15 days protected the animals from developing memory deficits by scopolamine or diazepam. A number of epidemiological studies point out that Alzheimer's disease is mainly characterized by extracellular protein deposits termed as beta-amyloid (AB) plaques, intraneuronal neurofibrillary tangles and loss of cholinergic neurons (Sayre *et al.*, 1997; Sparks *et al.*, 1994). Acetylcholinesterase enzyme (AChE) metabolizes the neurotransmitter Ach and thereby limits its action in healthy individuals. In the present study, the *Psidium guajava* fruit produced elevation of brain acetylcholine level by significant reduction of acetylcholinesterase activity. Oxygen free radicals are the chemical entities that can exist separately with one or more unpaired electrons. Free radicals are generated during normal metabolism of drugs, environmental chemicals as well as endogenous chemicals, especially stress hormones such as, adrenaline and nonadrenaline (Masuda *et al.*, 2003). These Oxygen free radicals are implicated in the process of ageing and may be responsible for the development of Alzheimer's disease in elderly (Berr, 2002, Floyd and Hensley, 2002, Rogers *et al.*, 2003). The significant decrease in MDA levels in the brains of mice treated with *Psidium guajava* fruit indicated attenuation of lipid peroxidation and inhibition of the generation of free radicals. This anti-oxidant effect of *Psidium guajava* fruit might be beneficial in protecting the brains of rodents against the oxidative stress. Abnormal accumulation of cholesterol increases the accumulation of â-amyloid plaques and drugs that inhibit cholesterol synthesis lower â-amyloid plaques deposition (Puglielli *et al.*, 2003; Mori *et al.*, 2001, Fassbender, *et al.*, 2001).). In the present study, young and aged mice treated with *Psidium guajava* fruit showed significant reduction in cholesterol levels. Thus, *Psidium guajava* fruit may be enhancing memory of rodents in multiple ways.

Conclusion:

In the present study, *Psidium guajava* fruit (i) inhibited brain acetyl cholinesterase enzyme, thereby elevating acetylcholine concentrations in brain, (ii) lowered serum cholesterol levels, (iii) reduced MDA levels and (iv) eventually improved memory of both young and aged rodents. These findings reveal the therapeutic potential of *Psidium guajava* fruit in the management of cognitive disorders.

REFERENCES

Berr, C. (2002). Oxidative stress and cognitive impairment in the elderly. *J. Nutr. Health Aging.*, **6**: 261-266.

Fassbender, F., Simons, M., Bergmann, C., Stroick, M., Lutjohann, D., Keller, P., Runz, H., Kuhl, S., Bertsch, T., Bergmann, K.V., Hennerici, M., Beyreuther, K. and Hartmann, T., (2001). Simvastatin strongly reduces Alzheimer's disease Ab 42 and Ab 40 levels *in vitro* and *in vivo*. *Proc. National Acad. Sci. USA*, **98** : 5856-5861.

Floyd, R. A. and Hensley, K. (2002). Oxidative stress in brain aging. Implications for therapeutics of neurodegenerative disease. *Neurobiol. Aging*., **23**: 795-807.

Higashida, A. and Ogawa, N. (1987). Differences in the acquisition process and the effect of scopolamine on radial maze performance in the strains of rats. *Pharmacol. Biochem. & Behavior,* **27** : 483–489

Masuda, T., Inaba, Y., Maekawa, T., Takeda, Y., Yamaguchi, H., Nakamoto, K., Kuninaga, H., Nishizato, S. and Nonaka, A. (2003). Simple detection method of powerful antirradical compounds in the raw extract of plants and its application for the identification of antiradical plants constituents. *J. agric. & Food Chem.,* **51** : 1831–1838

Mori, T., Parris, D., Town, T., Rojiani, A.M., Sparks, D.L., Delledonne, A., Crawford, F., Asdullah, L.I., Humphery, J.A., Diickson, D.W. and Mullan, M.J. (2001). Cholesterol accumulation in senile plaques of AD patient & in transgenic APP Swiss mice.Neuropath. *Exp. Neurol.,* **60** : 778-785.

Nabeshima, T. (1993). Behavioral aspects of cholinergic transmission: role of basal forebrain cholinergic system in learning and memory. *Progress in Brain Res.*, **98** : 405–411.

Parle, M., Vasudevan, M. and Singh, N. (2005). Swim everyday to keep dementia away. *J. Sport Sci. Med.*, **4** : 37-46.

Puglielli, L., Tanzi, R.E. and Kovacs, D.M. (2003). Alzheimer's disease: the cholesterol connection. *Nature Neuroscience,* **6** : 345-351.

Rogers, E.J., Milhalik, S. and Ortiz Shea, T.B. (2003). Apple juice prevents oxidative stress and impaired cognitive performance caused by genetic and dietary deficiencies in mice. *J. Nut. Health Aging,* **7** : 1-6

Sayre, L.M, Zagorski, M.G., Surewicz, W.K., Krafft, G.A. and Perry, G.. (1997). Mechenism of neurotoxity associated with Ab deposition and the role free radicals in the pathogenises of AD: a critical appraisal. *Chem. Res. Toxicol.,* **336** : 1216-1222.

Sitaram, N., Weingartner, H. and Gillin, J.C. (1978). Human serial learning. Enhancement with arecholine and choline and impairment with scopolamine. *Sci.*, **201** : 247–276.

Sparks, D.L., Liu, H., Gross, D.R. and Scheff, S.W.(1994). Induction of Alzhiemers-like beta amyloid immuno-reactivity in the brain of rabbits with dietary cholesterol. *Exp. Neurol.*, **126** : 88-94.

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