



Anti-Alzheimer Potential of Green Moong Bean

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Accepted on: 08-03-2016; Finalized on: 31-03-2016.

ABSTRACT

Green moong bean (*Vigna radiate*) has been cultivated for its therapeutic and traditional benefits all over the world. Anti-Alzheimer potential of green moong bean sprouts was investigated in the present study by employing interoceptive and exteroceptive behavioural models. A total of 138 Swiss mice divided into 23 groups were employed for the present research work. Three different concentrations (2, 4 & 8%w/w) of Moong bean sprouts (MBS) were administered with daily diet, for 15 successive days to mice. It was observed that MBS significantly reduced the transfer latency of mice in Elevated Plus Maze model and augmented the Step Down Latency in Passive Avoidance Paradigm thereby, indicating improvement in both short term and long term memory. Furthermore, ethanol and diazepam induced memory deficits were reversed by MBS in the present study. In addition, MBS produced marked increase in brain Glutathione levels, decrease in brain Acetylcholinesterase and diminished brain Malondialdehyde levels. The underlying mechanism of action of MBS appears to be a consequence of inhibition of acetylcholinesterase enzyme, scavenging of free radicals and neuroprotective effect mediated through its phytoestrogens. These findings, when taken together reflect that green moong bean sprouts possess promising anti-Alzheimer potential.

Keywords: Acetylcholinesterase, Anti-Alzheimer, Free radicals, Neuroprotection, Phytoestrogen, *Vigna radiate*.

INTRODUCTION

Sprouts or germinated seeds in addition to being excellent source of bioactive compounds are natural healthy dietary supplements.¹ They have become indispensable ingredients for major systems of medicines such as allopathy, ayurveda, naturopathy etc.

Botanically, green moong bean is known as *Vigna radiate* (Fabaceae).

It is commonly grown in India, Burma, Thailand and Indonesia. It is rich in phytoestrogens (isoflavones and coumestrol)², polyphenols (flavonoids), vitamins, proteins, phospholipids, protease inhibitors, γ aminobutyric acid³, minerals, fibres⁴, and tannins⁵.

Green moong bean relieves constipation and hot flushes. It regulates blood cholesterol levels, blood sugar levels, protects the heart³ and prevents osteoporosis. In addition to this, it is a natural immuno-booster, anti-inflammatory, anti-obesity, anti-oxidant³, anti-microbial agent³ and anti-cancerous activity⁶. However, there are no concrete reports about psychopharmacological actions of green moong bean sprouts.

Therefore, the present study was undertaken to provide scientific basis for the use of green moong bean sprouts in the management of Alzheimer's disease.

OBJECTIVE

The present research work was aimed to explore the Anti-Alzheimer Potential of Green moong bean sprouts using various exteroceptive and interoceptive behavioral models.

MATERIALS AND METHODS

Plant material

Moong bean seeds (*Vigna radiate*) were procured from Department of Pulses, College of Agriculture, Haryana Agriculture University (HAU), Hisar, Haryana, India.

Animals

A total of 138 Swiss mice were divided into 23 groups. Each group comprised of a minimum of 6 animals. Adult (6 months old) mice, of either sex, weighing around 20-25g were procured from the Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary Sciences, Hisar (Haryana). The experimental protocol was approved by the Institutional Animals Ethical Committee (IAEC) and the care of animals was taken as per guidelines of CPCSEA, Ministry of Forests and Environment, Government of India (Registration number 0436).

Drug protocol

Normal saline (vehicle), Piracetam (400mg/kg), Donepezil (1mg/kg), Ethanol (2.5g/kg) and Moong bean sprouts {(MBS) 2, 4 & 8% w/w} were administered for 15 successive days to mice. Diazepam (1mg/kg) was administered on 14th day. Biochemical studies were carried on 15th day 90 min after drugs/vehicle/MBS administration.

Experimental Design

Exteroceptive behavioral models

Elevated Plus Maze: The apparatus comprised of two open (16x5 cm²) and two covered arms (16x5x12 cm³),

which extended from a central platform (5x5 cm²). This apparatus was elevated to a height of 25cm from the floor. The procedure followed in the study was as described earlier.⁷

Passive avoidance paradigm: Passive Avoidance Paradigm consisted of a box (27x27x27 cm³) having three walls of wood and one wall of Plexiglas, featuring a grid floor (3mm stainless steel rods set 8mm apart), with a wooden platform (10x7x1.7 cm³) in the center of the grid floor. The box was illuminated with a 15W bulb during the experimental period. Electric shock (20V AC) was delivered to the grid floor. The procedure followed in the study was as described earlier.⁷

Interoceptive behavioral models

Diazepam induced amnesia⁸ and ethanol induced amnesia⁹ were used as interoceptive behavioral models.

Collection of Brain Samples

The animals were sacrificed by cervical decapitation under light anesthesia on the 15th day, 90min after administration of the last doses of Moong bean sprouts/standard drugs/vehicle. Then, the whole brain was carefully removed. It was weighed and transferred to a glass homogenizer and homogenized in an ice bath after adding 10 volumes of sterile normal saline. The homogenate was centrifuged at 3000 rpm for 10min and the resultant cloudy supernatant liquid was used for biochemical estimation.¹⁰

Biochemical estimations

Estimation of Brain Acetylcholinesterase: Brain acetylcholinesterase was estimated using Ellman method.¹¹

Estimation of Brain Glutathione (GSH) levels: Brain GSH was measured spectrophotometrically.¹²

Estimation of Malondialdehyde (MDA) levels: MDA, a measure of lipid peroxidation, was estimated spectrophotometrically.¹³

Statistical analysis: All the results were expressed as mean standard error (S.E.M). Data was analyzed using one-way ANOVA followed by Dunnett's *t*-test. *P* < 0.05 was considered as statistically significant.

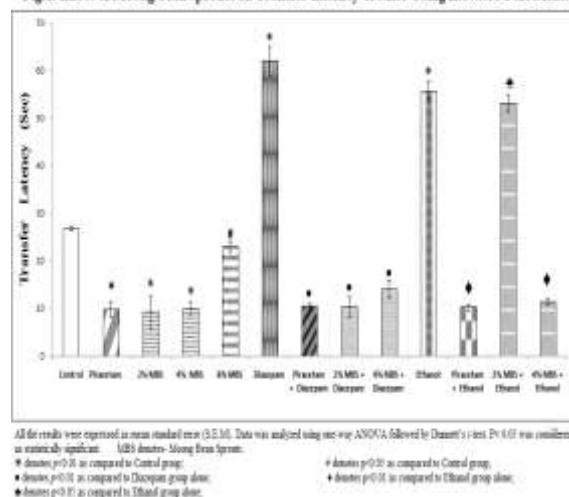
RESULTS

Elevated Plus Maze model

In it TL of first day represented learning ability of animals, whereas the TL of second day indicated memory. Moong bean sprouts (MBS) reduced the TL of mice remarkably (*p* < 0.01) in comparison to control group, when administered at 2 & 4% w/w concentrations (Fig. 1) along with diet for 15 successive days. Reduced TL indicates better memory performance of mice. Ethanol (2.5g/kg, i.p) and Diazepam (1mg/kg, i.p) increased the TL significantly, showing memory impairment. But, MBS at 2 and 4%w/w concentrations reversed these memory

deficits in Diazepam induced amnesia (Fig. 1). In ethanol induced amnesia significant reversal of memory impairment (*p* < 0.01) was only observed at 4%w/w concentration (Fig. 1). Further, animals treated with standard drugs, Piracetam showed remarkable improvement (*p* < 0.01) in memory and also reversed the amnesic effect of Ethanol and Diazepam. The influence of MBS was found to be comparable to that of Piracetam (nootropic agent).

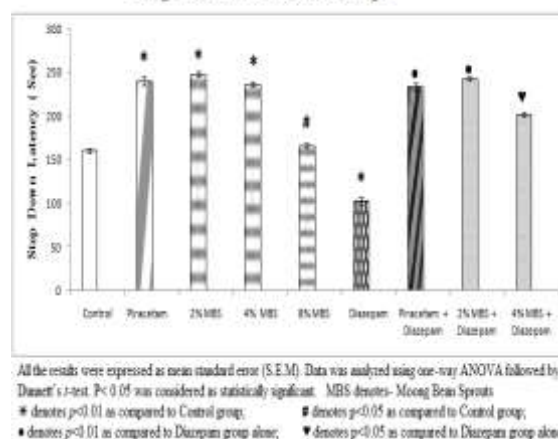
Fig.1. Effect of Moong bean sprouts on Transfer Latency of Mice Using Elevated Plus Maze.



Passive Avoidance Paradigm

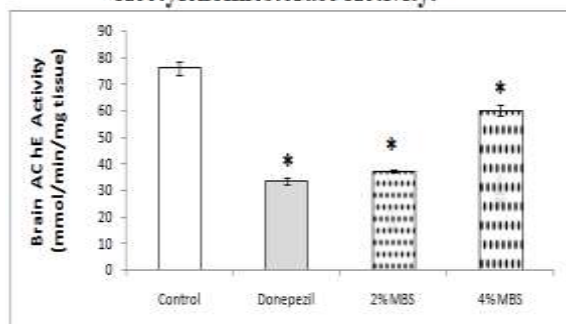
Administration of MBS for 15 successive days (2 & 4 % w/w concentration) augmented the SDL (*p* < 0.01) in comparison to control group (Fig. 2). Significant increase in SDL indicates improved long term memory. Diazepam decreased the SDL significantly showing memory impairment. MBS administered at 2 and 4% w/w concentration reversed the memory deficits induced by Diazepam (Fig. 2). This memory improving effect of MBS was similar to standard drugs Piracetam.

Fig.2. Influence of Moong bean sprouts on Step Down Latency of mice using Passive Avoidance Paradigm.



Brain AChE activity

In the present study, inclusion of both 2% and 4% w/w MBS (Fig. 3) in the daily diet produced remarkable inhibition (*p* < 0.01) of AChE activity, when measured after 15 days of MBS administration.

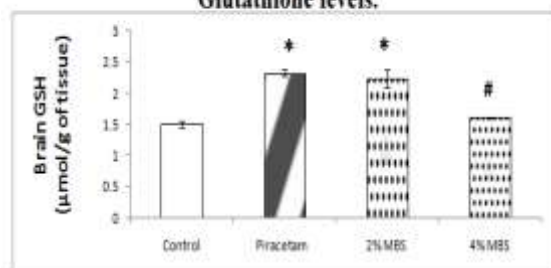
Fig.3. Influence of Moong bean sprouts on Brain Acetylcholinesterase Activity.

All the results were expressed as mean standard error (S.E.M). Data was analyzed using one-way ANOVA followed by Dunnett's *t*-test. $P < 0.05$ was considered as statistically significant. MBS denotes- Moong Bean Sprouts

* denotes $p < 0.01$ as compared to Control group.

Brain GSH levels

Administration of MBS at 2% w/w concentration for 15 successive days significantly ($p < 0.01$) rise the levels of GSH in the brains of mice. At 4% w/w concentration little rise ($p < 0.05$) was seen in GSH level (Fig.4).

Fig.4. Influence of Moong bean sprouts on Brain Glutathione levels.

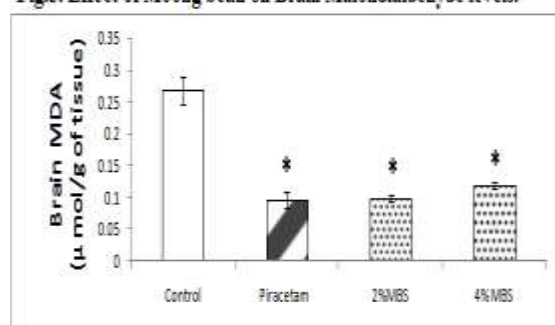
All the results were expressed as mean standard error (S.E.M). Data was analyzed using one-way ANOVA followed by Dunnett's *t*-test. $P < 0.05$ was considered as statistically significant. MBS denotes- Moong Bean Sprouts

* denotes $p < 0.01$ as compared to Control group;

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Brain MDA levels

Feeding mice with MBS for 15 successive days produced a significant ($p < 0.01$) fall in MDA levels at 2 and 4 % w/w concentrations (Fig.5).

Fig.5. Effect of Moong bean on Brain Malondialdehyde levels.

All the results were expressed as mean standard error (S.E.M). Data was analyzed using one-way ANOVA followed by Dunnett's *t*-test. $P < 0.05$ was considered as statistically significant.

MBS denotes- Moong Bean Sprouts

* denotes $p < 0.01$ as compared to Control group;

DISCUSSION

Cognition is an ability of an individual to perceive or record sensory events, retain this data over a short or long period of time and recall the same at a later date when needed¹¹. Learning and memory are two fundamental cognitive functions.¹⁴ Learning is an adaptive change in behavior caused by prior experience.¹⁵ Subsequent retrieval or retention of the acquired information is referred to as memory. The cognitive dysfunction includes delirium, behavioral disorders and dementia. It is also a lifestyle related disorder¹⁶, which significantly impairs the functions of certain regions of central nervous system and degrade the quality of life. Dementia is characterized by loss of neurons and atrophy of brain tissue.¹⁷ This leads to limitations in cognitive performance and poor concentration.¹⁸ Alzheimer's disease (AD) is the most common form of dementia.¹⁹ It is an age-related progressive neurodegenerative disease caused by severe neurodegeneration in the hippocampus and neocortical regions of the brain of the affected individuals.²⁰ It is an incurable and terminal disease.²¹ AD destroys brain cells thereby interfering with memory, thinking, and behavior which affect work, family, social relationships and the most basic activities of daily living. But, with the growing impact of dementia on today's society, new treatments need to be developed that could effectively improve the cognitive and physical performance of patients suffering with dementia. The main histological features of AD include extracellular protein deposits termed as amyloid beta ($A\beta$) plaques and intraneuronal neurofibrillary tangles. Abnormal accumulation of cholesterol levels increase $A\beta$ in cellular and most animal models of AD; and drugs that inhibit cholesterol synthesis lower $A\beta$ in these models. A number of epidemiological studies point out that high level of cholesterol contribute to the pathogenesis of AD.⁸ Since moong bean sprouts are rich in soluble dietary fibers which, lower LDL cholesterol³, might benefit Alzheimer patients.

In the present study, we measured the short term and long term memory of mice using Elevated Plus Maze and Passive Avoidance Paradigm respectively. Furthermore, ethanol and diazepam induced memory deficits were reversed by green moong bean sprouts. Piracetam (Nootropic agent) was employed as a standard memory enhancer and donepezil was used as standard acetylcholinesterase inhibitors. Administration of Moong bean sprouts (MBS) for 15 successive days in different concentrations showed memory enhancement in mice as reflected by reduced Transfer Latency using Elevated Plus Maze and enhanced in Step Down Latency in Passive Avoidance Paradigm. Furthermore, pretreatment of animals with MBS for 15 days, protected the animals from amnesic effect of Ethanol and Diazepam. These findings suggest the neuroprotective role of green moong bean sprouts in mice. Acetylcholine is the most important neurotransmitter involved in regulation of cognitive functions.²² Neuronal loss of acetylcholine particularly in

hippocampus area is the major cause for the development of Alzheimer's disease. Facilitation of central cholinergic activity by the use of anticholinesterases is presently the mainstay of the pharmacotherapy of dementia in AD.²³ In the present study, green moong bean sprouts produced significant inhibition of AChE activity. This might have led to the accumulation of Ach at the synapse and facilitation of cholinergic transmission. Polyphenols have been associated with a reduced risk of developing dementia, improved cognitive performance in elderly individuals and overall improved cognitive function. They exert their beneficial actions through their ability to suppress neuro-inflammation and protection of neurons against neurotoxins induced injury.²⁴ There is ample evidence regarding the presence of polyphenolic compounds in moong bean sprouts.³

Glutathione (GSH) is body's master antioxidant found in every cell. GSH is an endogenous tripeptide (3 amino acids bonded together) known as L-GlutamylCysteinylGlycine.²⁵ It protects the brain cells from the damaging effect of ROS (reactive oxygen species - free radicals and peroxides).²⁶ GSH also eliminates toxins from the body and boosts immune system. Thus, higher levels of glutathione concentrations are associated with good health. In the present research work, administration of moong bean sprouts for 15 successive days elevated the brain GSH levels indicating protective role in AD. Thiobarbituric Acid Reactive Substances are one of several products of damage produced by oxidative stress. TBARS measure Malondialdehyde (MDA) levels, which is an end product of lipid peroxidation. MDA further is a Reactive Oxygen Species (ROS), which serves as a biomarker of oxidative stress *in-vivo*. Oxidative stress occurs when the generation of ROS in a system exceeds system's ability to neutralize and eliminate these free radicals.²⁷ The human brain is responsible for approximately 20% of our body oxygen consumption and thus subjected to a high metabolically derived level of ROS.²⁸ The lipid bilayer of the brain is rich in polyunsaturated fatty acids and oxygen. Therefore, it is highly susceptible to lipid peroxidation²⁹, which plays an important role in the pathogenesis of neurodegenerative disorders like Alzheimer's disease. The polyunsaturated fatty acids present in the brain tissue cells are attacked by free radicals leading to oxidative lesions. Thus, increase in MDA levels correspond to increased oxidative stress leading to brain damage and decreased MDA levels reflects neuroprotection. We observed that, in the present study moong bean sprouts reduced the MDA levels, ultimately providing a neuroprotective role in AD.

Estrogen is an essential hormone for maintenance of feminine sex traits. It can exert effects on the brain, including cognitive function, co-ordination of movement, pain and neuroprotection. Recently evidence has accumulated signifying a protective role for estrogens against Alzheimer's disease, Parkinson's disease and schizophrenia, through their ability to prevent neuronal

cell apoptosis, reduction in beta amyloid accumulation, enhancing neurogenesis and cognitive function.³⁰ Estrogen levels can be restored naturally by consuming phytoestrogens. Moong bean sprouts possess phytoestrogens (isoflavones, coumestrol)² which, might have helped favourably in its anti-Alzheimer action.

Hence, consumption of moong bean sprouts on daily basis can prevent the development of Alzheimer's disease.

CONCLUSION

In the present study, moong bean sprouts (MBS) showed anti-Alzheimer activity as reflected by i) reduced Transfer Latency using Elevated Plus Maze, and ii) enhanced Step Down Latency in Passive Avoidance Paradigm. Furthermore, pretreatment of animals with MBS for 15 successive days, protected the animals from amnesic effect of Ethanol and Diazepam. The underlying mechanism of action of moong bean appears to be a consequence of inhibition of acetylcholinesterase enzyme, enhanced scavenging of free radicals and neuroprotective effect mediated through its phytoestrogens.

Acknowledgement: Ms. Sushila Kaura is an Inspire Research Fellow of DST, Government of India, New Delhi.

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Source of Support: Nil, Conflict of Interest: None.