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EFFECT OF *PHASEOLUS VULGARIS* SEEDS ON LEARNING, BEHAVIOUR AND MEMORY ENHANCEMENT IN RATS

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ABSTRACT

Phaseolus vulgaris belongs to family fabaceae is naturally available plant. Seeds of this plant are highly nutritious and contains medicinal properties. It is indigenous to central and south America. *Phaseolus vulgaris* have wide number of uses but mainly taken for weight loss and diabetes. The current study explains the memory enhancement activity of ethanolic extract of *Phaseolus vulgaris* seeds (EEPV). The extract was administered orally for 7 days in two doses (100mg/kg and 200mg/kg). The extract reduced the amnesia brought on by diazepam (1mg/kg) and improved the levels of memory. Memory improvement was described using behavioural models like Hebbs William maze and labyrinth maze. Study shows that memory improvement was occurred due to the presence of flavonoids.

Keywords: *Phaseolus vulgaris*, fabaceae, Hebbs William maze, labyrinth maze, flavonoids

INTRODUCTION

Dementia is often referred as a clinical syndrome characterized by continuous rise in impairments of cognitive skills and ability to work independently. The syndrome is a result of many diseases, with Alzheimer's disease,

vascular dementia and dementia with Lewy bodies together involving most cases [1].

There are two types of dementia young onset dementia (early called as "pre -senile dementia") and late onset dementia (early

called as “senile” dementia) young onset dementia includes people who acquires dementia before reaching to the age of 65 and late onset dementia includes people who acquire dementia after reaching the age of 65. Alzheimer’s disease is one of the major cause of dementia [2].

Natural remedies have gained the forefront in treatment of human ailments in the current herbal age which have turned the focus back on nature’s bounty of plant, animal and mineral wealth the world over. The quality of human life has improved and the lifespan of the species has grown because to this return to nature.

One of the most crucial functions of the brain is memory. Because memory is the mechanism through which organisms are able to record their experiences and use this information to change their reactions to the environment, memory is essential for survival. Memory loss and cognitive impaired functions are the major features of Alzheimer’s disease (AD).

Phaseolus vulgaris is commonly known as bean or French bean, widely used plant source for treating various diseases. It belongs to family fabaceae [3].

In Folk medicine, Beans are mainly used for burns, itch, bladder, cardiac, hiccups, depurative, diabetes, diarrhoea, diuretic,

acne, dysentery, eczema, emollient, dropsy, kidney stones, rheumatism, sciatica, and tenesmus [4].

Phaseolus vulgaris L. is indigenous to Tropical America and expanded its cultivation throughout the temperate and tropical regions of the world [5].

Phaseolus vulgaris is commonly used in the treatment of obesity and diabetes.

MATERIALS AND METHODS

Plant material

Fresh seeds of *Phaseolus vulgaris* seeds have been purchased from local market in Guntur in the state of Andhra Pradesh. The plant material was taxonomically identified and authenticated by Dr. P.Satyanarayana Raju garu M.Sc., M.Phil., Ph.D from Department of Botany and Microbiology, Acharya Nagarjuna University, Nagurjuna Nagar, AP on 15th Oct. 2020.

The freshly collected seeds were broke into small pieces and dried under shade. The fully dried material was pulverized into coarse powder by using mechanical grinder and then passed through 40 mesh sieve to get uniform powder and preserved in an air tight container.

Preparation of the extract [6]

The ethanolic extract of *Phaseolus vulgaris* linn (EEPV) was prepared by using soxhlation, at room temperature. The

powdered dried seeds of *Phaseolus vulgaris* Linn. (250g) were extracted with 50% ethanol (EtOH). The extract was filtered, dried and concentrated by using rotary evaporator. The resultant extract was used for further studies.

Animals

Male wistar rats were used for this experiment as estrogens (female sex hormones) have been discovered to have effect on memory. Animals were obtained from disease free small animal house. Wistar rats weighing 150-250g were taken for this study. The animals have free access to food and water, and they were housed in a natural (12 h each) light dark cycle. Food feeded to animals consisted of wheat flour kneaded with small amount of water and followed by drops of refined vegetable oil. The animals were habituated for at least 5 days to the laboratory conditions in advance of behavioral experiments. Experiments were carried out between 0900 h and 1800 h. The experimental design was approved by the institutional animal ethics committee, and the laboratory animals were monitored and cared as per the guidance of CPCSEA, Ministry of Forests and Environment, Government of India (registration number 1048).

Preliminary phytochemical screening

An attempt was made to observe the presence and absence of diverse phytochemical constituents in the EEPV, viz., alkaloids (Wagner's test), flavonoids (Shinoda test), tannins (Ferric chloride test), steroids and triterpenes (Lieberman-Burchard's test), terpenoids (Salkowski test), glycoside (Baljet's test) and saponins (Foam test) according to standard methods

DRUGS

The drugs used in this study are diazepam injection, piracetam injection (Nootropil) (Dr. Reddy's Laboratories, Utaarakhand).

VEHICLE [7]

Plant extract (EEPV) was suspended in Carboxy methyl cellulose and administered orally to rats. Piracetam and Diazepam were dissolved individually in normal saline and injected i.p. Volume of oral administration and i.p. injection was 1ml/1000g of rat.

Drug treatment [7]

In the current study, the rats were split into 5 different groups for testing memory. Each group consists of 5 animals. Group I is represented as control and administered only saline. Group II is represented as inducing and administered only diazepam. Group III is represented as standard and administered diazepam along with standard drug piracetam .Group IV and V are represented as test and

administered diazepam and different doses of extract EEPV (100 and 200 mg/kg) .

All control group animals received vehicle (saline) for 7 consecutive days. Group III and IV received EEPV orally for 7 successive days by using water as solvent. Ninety minutes after administration of last dose (on the 7th day) rats were exposed to training session by using Hebb-william , labyrinth and rectangular mazes. Retention of memory was recorded after 24 days (In the 8th day). Amnesia was caused by diazepam (1mg/kg i.p.)

HEBB-WILLIAMS MAZE [7]

Hebb-williams maze is a behaviour model for measuring memory. It is partitioned into 3 components: animal chamber (or start box), which is connected to the middle chamber (or exploratory area), and a reward chamber at the other side of the maze in which the reward (food) is provided. All three

chambers are equipped with guillotine removable doors. Prior to the experiment animals are habituated to the maze for significant results. On the first day (i.e, seventh day of drug treatment), the rat was allowed in the animal chamber or start box and the door was opened to facilitate the entry of the animal into the next chamber to prevent back entry the door was closed immediately after the movement of animal. Here time is taken as the parameter to measure the activity.

Time it takes the animal to go from the start box to the prize chamber was noted on first day (training session) for each animal. Each animal was allowed to explore the maze for 3min with all the doors opened before returning to its home cage. Retention of this learned task(memory)was examined 24 hrs after the first day trail (i.e., eight day, 24 hrs after last dose).



LABYRINTH MAZE [8]

Labyrinth maze is one of the finest devices for studying spatial memory in animals. It also consists of 3 chambers. Chamber A in which animal was allowed to start its journey which is immediately connected to Chamber C which is exploratory area for animals is spread with Y shaped figures in a zig-zag position and the final Chamber B in which the food reward is placed. Prior to the experiment all the animals are allowed to habituate the equipment for good results. On the first day (i.e, seventh day of drug

treatment), the rat was allowed to enter in the Chamber A and the door was opened to facilitate the entry of the animal into the next chamber to prevent back entry the door was closed. Time required for the animal to reach the Chamber B from the Chamber A was noted on first day (training session)for each animal. Each animal was permitted to explore the maze for 3min with all the doors opened before returning to its home cage. Retention of this learned task(memory)was examined 24 hrs after the first day trail (i.e., eight day, 24 hrs after last dose).



RESULTS

Preliminary phytochemical screening

The result of phytochemical screening show the presence of glycosides, flavonoids, phenols, saponins, tannins, terpinoids by usind standard methods

Hebbs William maze

Effect on transfer latency (using hebbs William maze)

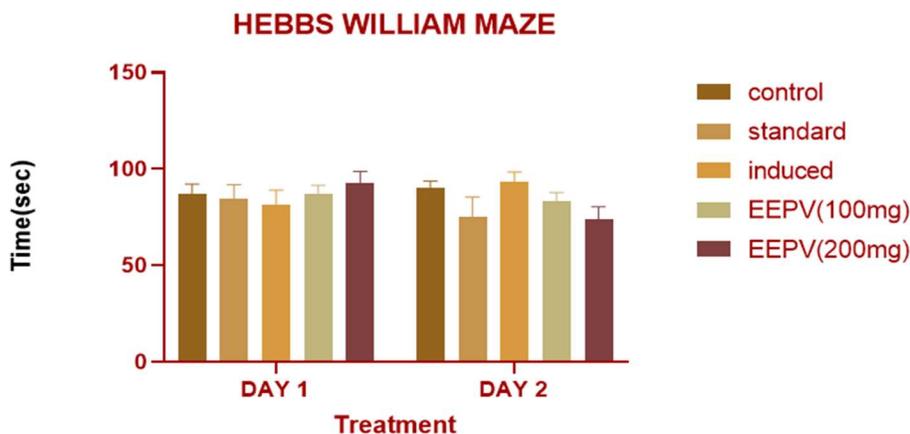
Time required to reach the reward chamber is described as latency and it explains about memory of animals on eighth day. EEPV

(100 mg/kg and 200mg/kg) administered orally for 7 days have shown significant effect on Transfer latency equal to standard piracetam (400 mg/kg). Amnesia was induced by diazepam (1mg/kg i.p.) have increased the latency scores of animals which were significantly reduced by standard

piracetam (400 mg/kg) and EEPV (200mg/kg p.o.). (p<0.0001) represent significant reduction in latency scores which in turn represent improvement in memory levels which were decreased by diazepam (1mg/kg i.p.)

Latency scores of Hebbs William Maze

Groups	Day 1	Day 2
Control	87±4.60	90±3.34
Standard	81.6±6.62****	93.4±4.49****
Induced	84.4±6.71 ^{ns}	75.4±9.02 ^{ns}
EEPV (100mg)	87.2±3.86****	83.6±3.72****
EEPV (200 mg)	92.8±5.46****	74±5.76****



Data represent Mean ±SEM, (n=5). Two way Anova followed by Dunnett’s multiple comparison test. Significance was shown at p<0.0001 when compared with control.****denotes p<0.0001 which is compared with control group of rats

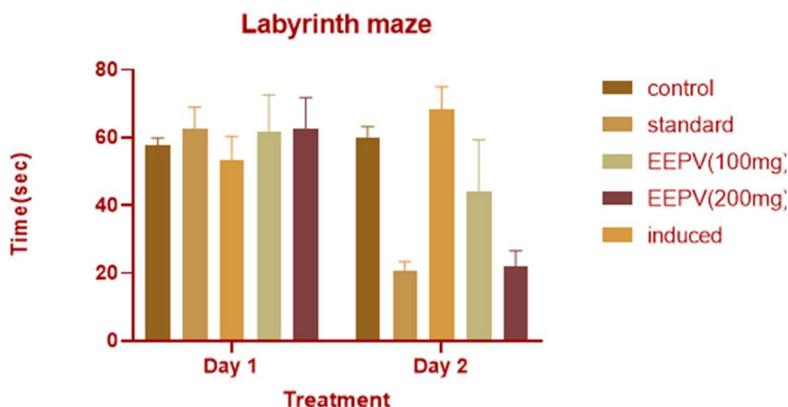
Labyrinth maze

Time required to reach the reward chamber is described as latency and it explains about memory of animals on eighth day. EEPV (100mg/kg) administered orally for 7 days did not shown any significant changes. EEPV (200 mg/kg) administered orally for 7 days have shown significant effect on Transfer latency equal to standard piracetam (400

mg/kg) . Amnesia was induced by diazepam (1mg/kg i.p.) have increased the latency scores of animal which were significantly reduced by standard piracetam (400 mg/kg) and EEPV (100 mg/kg and 200mg/kg p.o.). (p<0.0001) represent significant reduction in latency scores which in turn represent rise in memory levels which were decreased by diazepam (1mg/kg i.p.)

Latency scores of Labyrinth Maze

Groups	Day 1	Day 2
Control	57.6±2.05	59.8±3.05
Standard	53.4±6.15****	68.4±5.88****
Induced	62.4±5.88 ^{ns}	20.6±2.49 ^{ns}
EEPV (100mg)	61.6±9.76 ^{ns}	44±13.6 ^{ns}
EEPV (200 mg)	62.4±8.42****	21.8±4.308****



Data represent Mean ±SEM, (n=5). Two way Anova followed by Dunnett’s multiple comparison test. Significance was shown at p<0.0001 when compared with control. ****denotes p<0.0001 which is compared with control group of rats. (ns) denotes non significant figures when compared with control

DISCUSSION

The EEPV has shown significant effect at a dose of 200 mg/kg which is similar to standard piracetam 400 mg/kg due to presence of phytochemical constituents called flavonoids. The investigation reveals EEPV has memory enhancement activity at a standard level useful to mankind. Hebb’s William Maze and Labyrinth Maze are standard methods for evaluating memory.

STATISTICAL ANALYSIS

All the above results were denoted as mean standard error (SEM). Data was analyzed using Two-way ANOVA followed by Dunnett’s t-multiple comparison test. p values p<0.0001 were considered as statistically significant.

CONCLUSION

Behaviour models for studying drugs or conditions that affect cognitive processes was standardized and evaluated by using seed extracts of *Phaseolus vulgaris*. The ethanolic extract of *Phaseolus vulgaris* has shown significant memory enhancement activity by Labyrinth maze, Hebb’s William maze. Further investigation of EEPV may reveal the mechanism of memory enhancement activity.

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