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EVALUATION OF NOOTROPIC ACTIVITY OF DIFFERENT EXTRACTS OF *VIGNA MUNGO* (LINN) IN RATS

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ABSTRACT

The ethanol and aqueous seed extracts of *Vigna mungo* were evaluated for nootropic activity in mice. Acute oral toxicity study was performed on mice as per OECD guidelines. Scopalamine and Diazepam induced amnesia in mice were used to study the effects of extracts on learning and memory in the experimental animals using elevated plus maze. Both ethanol and aqueous extracts effectively reversed Sopalamine and Diazepam induced amnesia at a dose of 200 to 400 mg/ml by reducing transfer latency. This suggests that the extracts are capable of increasing memory and learning behavior in animals.

KEYWORDS: *Vigna mungo*, Nootropic activity, Amnesia, Transfer latency, Dementia.

INTRODUCTION

The learning and memory as well as its utilization in behavioral adaptation by an animal is a mystery which is not yet solved completely.[1] Learning and memory encompass the fields of neurophysiology, behavior, computational neurobiology, cognitive psychology and molecular biology. Dementia is an acquired syndrome of decline in memory and at least one other cognitive domain, such as language, visio-spatial, or executive function that is sufficient to interfere with social or occupational function in an alert person.[2] It can also be defined as the progressive decline in cognitive function due to damage or disease in the brain beyond what might be expected from normal aging. Particularly affected may be memory, attention, language, and problem solving. Especially in the later stages of the condition, affected persons may be disoriented in time, in place, and in person.

Various drugs have been used for the treatment of dementia, they are anticholinesterases, antipsychotics, antidepressants, anti-anxiety drugs, anti-oxidants. Drugs like Rivastigmine, tacrine, mimatamine, piracetam, thiamine can be used depending on cause of the disease.[3]

Many plant species such as A*corus calmus, Cymbopogot, Ttribulus terrestris, Clitoria ternatea* have been used in traditional system of medicine for treating disorders of nervous system, mental debility, insomnia, amnesia, and various mental disorders. Irrespective of tremendous advancements in the medicinal field, there is no truly satisfactory drug available for the treatment of dementia. Hence there is a need for nootropic drugs which can be used effectively to treat dementia and improve the mental condition.

Vigna mungo (Fabaceae) is an important pulse crop found throughout India. It is commonly known as black gram. Chemically it is rich in flavonoids. It has been used in traditional systems for various medicinal purposes, in Ayurvedic and Unani systems of medicine it is used as laxative, aphrodisiac, tonic, appetizer, diuretic, galactagogue and styptic; useful in piles, asthma, scabies, leucoderma, gonorrhoea, pains, epistaxis, paralysis, rheumatism and affections of the nervous system, liver and cough. It is also prescribed for dropsy and cephalalgia.[4] However there is no scientific data available about this drug for the treatment of memory enhancement. Hence, the present work was aimed to generate a scientific data and find the efficiency of extracts for their activity.

MATERIALS AND METHODS

Plant material

The seeds of *Vigna mungo* purchased from the local market. The berries were, cleaned, washed, dried and stored for further use.

Preparation of extracts

The seeds of *Vigna mungo* were powdered and sieved through No. 22 mesh. About 300 g of coarse powder was defatted using petroleum ether. The marc was subjected to extraction in soxhlet's apparatus for 48 hrs using ethanol as solvent. The marc left over after ethanol extraction was extracted using 4% chloroform water and aqueous extract was collected.

Preliminary Phytochemical Investigation

Phytochemical screening for Pet. ether, ethanol and aqueous extracts of *Vigna mungo* were performed for alkaloids, carbohydrates, glycosides, Phytosterols, Tannins, Saponins, Phenols,

Proteins by the standard procedure.[5]

Animals

Before selecting the animals, ethical clearance was obtained from animal ethical clearance committee, Mysore, India. Healthy male albino wistar rats (180-220g) were housed under standard conditions of temperature (22 ± 1.0C), relative humidity (55 ± 10%), 12 hr light/dark cycles and fed with standard pellet diet. After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under above said environmental conditions.

Acute Oral Toxicity Studies

The acute oral toxicity study was performed for ethanol extract (VMEE) and aqueous extract (VMAE) of *Vigna mungo* according to the OECD guidelines 423 (Acute Toxic Method). A starting dose used was 2000 mg/kg body weight p.o. of ethanol and aqueous extracts. The ethanol and aqueous extracts of *Vigna mungo* were administered to 3 male rats, observed for 14 days. The experiments were repeated again with the same dose level, 2000 mg/kg body weight p.o. of extracts for 3 days more, and observed for 14 days.[6] The doses of extracts for the study were selected as 100 mg/kg, 200 mg/kg and 400 mg/kg based on the ratio 1/20, 1/10 and 1/5 of safest dose.

Grouping of animals: Healthy albino rats were selected and the animals were divided into 9 groups consisting of 6 animals each which are as follows

Group1: Normal control treated with 2% tween 20.

Group 2: Disease control treated with Diazepam (5 mg/kg).

Group 3: Standard treated with Diazepam (5 mg/kg) and Piracetam 100mg/kg.

Group 4: VMEE 100 treated with Diazepam (5 mg/kg) and ethanolic extract of *Vigna mungo* (100mg/kg i.p).

Group 5*:* VMEE 200 treated with Diazepam (5 mg/kg) and ethanolic extract of *Vigna mungo* (200mg/kg i.p).

Group 6*:* VMEE 400 treated with Diazepam (5 mg/kg) and ethanolic extract of *Vigna mungo* (400mg/kg i.p).

Group 7: VMAE 100 treated with Diazepam (5 mg/kg) and aqueous extract of *Vigna mungo* (100mg/kg i.p).

Group 8*:* VMAE 200 treated with Diazepam (5 mg/kg) and aqueous extract of *Vigna mungo* (200mg/kg i.p).

Group 9*:* VMAE 400 treated with Diazepam (5 mg/kg) and aqueous extract of *Vigna mungo* (400mg/kg i.p).

Evaluation of Nootropic Activity

The ethanol and aqueous extracts of *Vigna mungo* berries were evaluated for their *in vivo* nootropic potentials by the following methods.

Elevated plus-maze (Exteroceptive Behavior model)[7]

Elevated plus-maze served as the exteroceptive behavior model to evaluate learning and memory in rats. The apparatus consisted of two open arms (16 cm × 5 cm) and two closed arms (16 cm × 5 cm × 12 cm). The arms extended from a central platform (5 cm × 5 cm) and the maze is elevated to a height of 25 cm from the floor. Each rat was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) i.e. the time taken by mouse with all its four legs to move into one of the closed arm was recorded on the 1st day. If the animal did not enter into one of the closed arms within 90 sec, it was gently pushed into one of the two closed arms and the TL was assigned as 90 sec. The mouse was allowed to explore the maze for another 10 sec, and then returned to its home cage. Retention of this learned-task was examined 24 hrs and 9th day after the first day's trial.

Scopolamine-induced amnesia (Interoceptive Behavior Model)[8]

Scopolamine (0.4 mg/kg) was injected i.p 90 min after administration of extracts of *vigna mungu or* standard on 7th day. TL (Transfer latency) was recorded after 45 min of injection and retention (memory) of learned task was examined after 24 hrs. Scopolamine when administered through i.p route to the animals will induce the impairment of memory by acting through muscarinic system.

Diazepam-induced amnesia (Interoceptive Behavior Model)[9]

Diazepam (5 mg/kg) was injected i.p after 90 min of administration of extracts of *Vigna mungu* or standard on 7th day. TL (Transfer latency) was recorded after 45 min of injection and retention (memory) of learned task was examined after 24 hrs. Diazepam when administered through i.p. route to the animals will induce the impairment of memory by acting through GABAergic system.

Statistical Analysis

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean±standard error of mean (S.E.M.) and analyzed for ANOVA and post hoc Dunnet's t-test using Graphpad prism5 software.

RESULTS

Preliminary Phytochemical Investigation

The Pet.ether, ethanolic and aqueous extracts of seeds of *Vigna mungo* were subjected to different preliminary chemical tests to determine the presence of phyochemical constituents. The results revealed the presence of secondary metabolites such as steroids, triterpenoids, anthraquinnes, alkaloids, flavonoids and tannins.

Acute Oral Toxicity Studies

The acute oral toxicity study was performed for ethanol extract (VMEE) and aqueous extract (VMAE) of *Vigna mungo.* The doses of extracts for the study were selected as 100 mg/kg, 200 mg/kg and 400 mg/kg based on the ratio 1/20, 1/10 and 1/5 of safest dose.

Evaluation of Nootropic Activity

Elevated plus-maze (Exteroceptive Behavior model)

The transfer Latency (TL) of first day reflected learning behavior of animals whereas, TL of second day reflected retention of information or memory. *Vigna mungo* extract at low dose (100mg/kg) administered for 7 days orally did not have any significant ($p>0.5$) effect on TL of first day of training, on second day and ninth day of the study as compared to control. The moderate (200 mg/kg) and higher dose (400 mg/kg) of the ethanol and aqueous extracts of *Vigna mungo* significantly decreased TL on 1st, 2nd and 9th day indicating significant improvement of learning and memory.

Table No 1: Effect of Extracts Vigna mungo on transfer latency in elevated plus maze model.

Group	1st DAY	2nd DAY	3rd DAY
Normal	54.14 ± 1.064	50.62 ± 1.040	23.67 ± 1.022
Piracetam	49.74 ±1.055	33.31±1.060**	4.500 ± 0.4282***
VMEE 100	52.48 ± 0.9984	48.84 ± 0.8314	23.33 ± 0.4944
VMEE 200	50.28 ± 0.2699	39.97 ± 0.6924***	15.67 ± 0.5578***
VMEE 400	50.18 ± 0.4730	32.13 ± 1.306***	7.667 ± 0.4216***
VMAE 100	52.77 ± 0.7567	49.61 ± 1.117	24.67 ± 0.8028
VMAE 200	51.50 ± 0.4872	41.88 ± 0.7410***	16.67 ± 0.6667***
VMAE 400	48.48 ± 0.3944	31.89 ± 0.5591***	7.833 ± 0.5426***

Note: VMEE= *Vigna mungo* ethanol extract, VMAE=*Vigna mungo* aqueous extract

Values are mean ± S.E.M, n=6 symbols represent statistical significance.

+ p<0.05, ++ p<0.01, +++p<0.001 Disease vs Normal control

* p<0.05, ** p<0.01, ***p<0.001 Therapeutic vs disease control

Scopolamine-induced amnesia (Interoceptive Behavior Model)

This study was conducted to test the ability of the extracts to counteract the amnesia produced by the scopolamine. The administration of scopolamine in control animals exhibited significant increase in the TL compare to normal animals. The pretreatment for 7 days with standard drug piracetam, *Vigna mungo* ethanol extract (VMEE) and *Vigna mungo* aqueous extract (VMAE) at medium (200mg/kg) and higher (400mg/kg) significantly reduced (p<0.01) TL compared to control animals. But the effect of low doses of extracts on TL was not significant (p>0.5).

Table No 2: Effect of extracts of *Vigna mungo* on scopolamine induced amnesia.

Group	Transfer Latency
Normal	55.09± 1.155
Scopolamine	71.91+++± 1.350
Piracetam	34.05***± 0.4889
VMEE 100	62.65± 1.261
VMEE 200	54.43***± 0.6316
VMEE 400	33.94***± 0.6323
VMAE 100	66.13± 1.668
VMAE 200	52.71***± 0.4467
VMAE 400	35.26***± 0.3107

Note: VMEE= *Vigna mungo* ethanol extract, VMAE=*Vigna mungo* aqueous extract

Values are mean ± S.E.M, n=6 symbols represent statistical significance.

+ p<0.05, ++ p<0.01, +++p<0.001 Disease vs Normal control

* p<0.05, ** p<0.01, ***p<0.001 Therapeutic vs disease control

Diazepam induced amnesia (Interoceptive Behavior Model)

The diazepam induced amnesia in rats was performed to evaluate the ability of the extracts to increase memory and learning in animals. In the present study, significant reduction of Transfer Latency (TL) was observed in control animals treated with diazepam alone compared to normal animals due to amnesia. In the therapeutic animals treated with piracetam, medium (200mg/kg) and higher doses (400mg/kg) of VMEE and VMAE, significant reduction in TL (p<0.01) was observed, but low doses of extracts did not have significant reduction compared to control animals.

Table No 3: Effect of extracts of *Vigna mungo* on diazepam induced amnesia

Group	Transfer Latency
Normal	53.28±0.5295
Diazepam	77.11+++±0.8143
Piracetam	36.68±0.2930
VMEE 100	61.03±0.4103
VMEE 200	54.72***±0.4309
VMEE 400	37.71***±0.3161
VMAE 100	66.46±1.150
VMAE 200	57.10***±0.2605
VMAE 400	37.88***±0.3178

Note: VMEE= *Vigna mungo* ethanol extract, VMAE=*Vigna mungo* aqueous extract

Values are mean ± S.E.M, n=6 symbols represent statistical significance.

+ $p<0.05$, ++ $p<0.01$, +++$p<0.001$ Disease vs Normal control

* $p<0.05$, ** $p<0.01$, ***$p<0.001$ Therapeutic vs disease control

DISCUSSION AND CONCLUSION

The ethanol and aqueous extracts of *Vigna mungo* have been tested for facilitatory effect on learning behavior in rats using elevated plus maze model (EPM). The present study has attempted to correlate the effect on monoamine mediated behavior and the brain levels of 5-HT, DA and GABA with the nootropic activity.

The reduction in the transfer latency by extracts of VMEE (200 and 400 mg/kg) and VMAE (200 and 400 mg/kg) has proved that they can enhance learning behavior in mice. This observation has been strengthened by the finding that extracts have shortened the transfer latency in the elevated plus maze model indicating improvement in memory, which is in accordance with the hypothesis.[10, 11]

The results of the present study suggesting that, ethanol and aqueous extracts of *Vigna mungo* posses significant memory enhancing activity and also suggested several possible mechanisms for its beneficial effects. The extracts have significantly reversed diazepam and scopolamine induced amnesia models which explore the GABA antagonist and cholinomimetic potentials of these extracts.

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