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Research Article

AN EXPERIMENTAL STUDY OF ETHANOLIC SEED EXTRACT OF VIGNA MUNGO LINN. FOR NOOTROPIC ACTIVITY ON ALBINO RATS

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ABSTRACT :

Purpose: The present study was designed to evaluate the Nootropic potential of Ethanolic seed extracts of Vigna mungo.Linn.(ESVM).

Design/methodology/approach: The objective of the present study was achieved using following methods such as Clonidine induced hypothermia and Haloperidol induced catalepsy. This study was carried out to evaluate the effectiveness of the ethanolic seed extracts of Vigna mungo.Linn.(ESVM) as Nootropic drug.

Findings: In Clonidine induced hypothermia model, ESVM show significantly increased rectal temperature in animals, showing antagonistic effect of extract. The extract also shows significantly decreased Lithium induced head twitching indicating anti seritonergic properties

Conclusion: The results concluded that Vigna mungo Linn plant has nootropic activity which is most useful for patients suffering from dementia.

Key words : *Ethanolic seed extracts of Vigna mungo Linn.(ESVM), Nootropic activity, amnesia, transfer latency, Haloperidol, Clonidine, hypothermia, catalepsy.*

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Introduction

The learning and memory as well as its utilization in behavioural adaptation by an animal is a mystery which is not yet solved completely.[1] 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] In traditional system of medicine (Ayurveda) Vigna mungo Linn. was used for the treatment of mental disorders as reported. But there was no scientific data available for the treatment for the memory enhancement. So the present work was aimed to generate a scientific data and find the efficiency of extract of title plant for their activity.[3,4]

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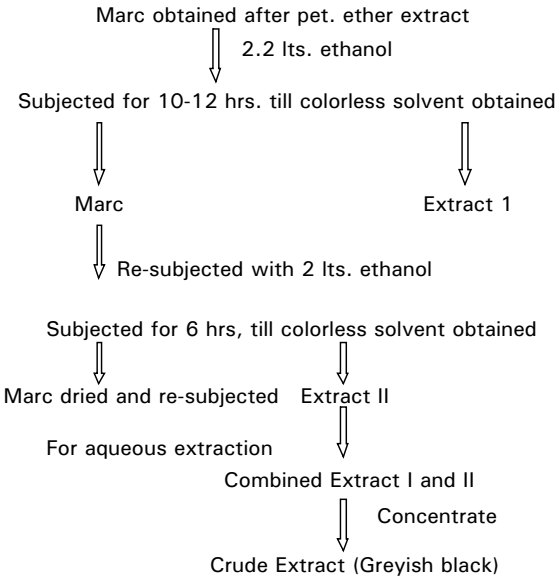
Material and Methods:

Plant material: The seeds of Vigna mungo Linn. were purchased from the local market. The seeds were cleaned, washed and dried for further use.

Preparation of extracts: The shade dried seeds of Vigna mungo Linn was powdered and sieved through No. 22 mesh. About 500 g (appx.) of coarse powder was defatted using petroleum ether. The marc left over was subjected to ethanol extract in Soxhlet apparatus for 48 hrs.

Preparation of ethanol extract Vigna mungo Linn:

Marc obtained after petroleum ether extract Solvent used : Ethanol 2.2 lts.



Preliminary phytochemical investigation :

Phytochemical screening for Ethanolic seed extract of Vigna mungo Linn.(ESVM) were subjected for alkaloids, carbohydrate, glycoside, Phytosterol, Tannins, saponins, Phenols, Proteins tests using standard procedure.[5]

Animals

Healthy male albino wistar rats (180-220g) were procured from Sri Venkateswara Enterprises, Bangalore. The animals were housed under standard conditions of temperature (22 ± 1^0), relative humidity (55 ± 10%), 12h light/dark cycles and fed with standard pellet diet (Amrut, Pranav Agro Industries Ltd., Sangli, India) and water ad libitum. 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. The experimental protocol has been approved by the Institutional Animals Ethics Committee (60/CP/2014-15, Dt 17/4/2015).

Acute Oral Toxicity Studies

The acute oral toxicity study was performed for Ethanolic seed extract of Vigna mungo Linn.(ESVM) according to the OECD guidelines 423 (Acute Toxic Method). A starting dose used was 2000 mg/kg body weight p.o. of ESVM. The ESVM was administered to 3 male rats and observed for 14 days. The experiments was 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 ESVM 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

The Ethanolic seed extracts of Vigna mungo. Linn. (ESVM) were evaluated for their in vivo nootropic potentials for the following methods.

Behavioral study:

Clonidine-induced hypothermia [7]

Clonidine induced hypothermia is used to assess the effect of drug influencing NA (nor-adrenaline) mediated behaviour. Albino rats will be used in groups of 6 in each. The rectal temperature will be recorded every 30 min after clonidine (0.1 mg/kg i.p) till 180 min. Piracetam , ESVM & vehicle will be administered 30 min before clonidine treatment and reversal effect (inhibition of hypothermia due to clonidine) has to be recorded.

Group Classification : The healthy albino rats were selected and the animals will be divided into 6 groups consisting of 6 animals each as follows.

- Group1: Normal control treated with 2% tween 20.
- Group 2: Disease control treated with clonidine (0.1 mg/kg i.p) alone.
- Group 3: Standard treated(Piracetam 100mg/kg) with clonidine (0.1 mg/kg i.p)
- Group 4: ESVM100mg/kg p.o treated with clonidine (0.1 mg/kg i.p)
- Group 5: ESVM 200 mg/kg p.o treated with clonidine (0.1 mg/kg i.p
- Group 6: ESVM 400 mg/kg p.o treated with clonidine (0.1 mg/kg i.p)

Lithium-induced head twitch[8]

Lithium-induced head twitching were used to assess the effect of drugs influencing second messenger system. Rats were treated with vehicle or Ethanolic seed extract of VignamungoLinn.(ESVM) 30 min before lithium sulphate(190 mg/kg i.p) and the number of head twitch were counted for 60 min and the prevention of head twitch by the extract were recorded

Group Classification: The healthy albino rats were selected and the animals will be divided into 6 groups consisting of 6 animals each as follows.

- Group1: Normal control treated with 2% tween 20.
- Group 2: Disease control treated with lithium sulphate (190 mg/kg, ip).
- Group 3: Standard treated (Piracetam 100mg/kg) with lithium sulphate(190 mg/kg, ip)
- Group 4: : ESVM 100 mg/kg p.o treated with lithium sulphate (190 mg/kg, ip)
- Group 5: ESVM 200 mg/kg p.o treated with lithium sulphate (190 mg/kg, ip)
- Group 6: ESVM 400 mg/kg p.o treated with lithium sulphate (190 mg/kg, ip)

Statistical Analysis

All the result were expressed as mean±standard error of mean (S.E.M.), analysed for ANOVA and post Dunnet's t-test using Graph pad prism-5 software.

Results & Discussion:

Preparation of extracts

About 500g of seeds of Vigna mungo was powdered and extracted with petroleum ether and ethanol.

Preliminary phytochemical investigation:

The ethanolic seed extract of Vigna mungo Linn. (ESVM) was subjected to different preliminary phytochemical tests to determine the chemical constituents present in the extracts, the results of which are tabulated as below (Table 1).

Table 1: Results of preliminary phytochemical tests

Sl. No.	Chemical Test	Ethanolic extract
1.	**Test for Carbohydrates**	
	Molish's Test (General test)	++
	A. Test for reducing sugars	
	a. Fehling's test	++
	b. Benedicts test	++
	B. Test for Monosaccharides	
	Barfoed's test	+
	C. Test for Non-reducing Polysaccharides	
	Tannic acid test for starch	+
2.	**Test for Proteins**	
	a. Millon's test	+
	b. Test for proteins containing sulphur	+
	c. Precipitation test	+
3.	**Test for Amino acids**	
	a. Test for tyrosine	+
	b. Test for cystein	+
4.	**Test for Steroids**	
	Salkowski test	+
5.	**Test for Triterpenoids**	
	a. Salkowski reaction	+
	b. Liebermann – Butchard reaction	+
6.	**Test for Flavonoids**	
	a. Shinoda test	+
	b. Ferric chloride test	+
	c. Alkaline reagent test	+
	d. Lead acetate test	+
7.	**Test for Tannins and phenolic compounds**	
	a. Ferric chloride test	+
	b. Dilute iodine solution	+
	c. Potassium dichromate test	+

Acute oral toxicity :

The results of acute oral toxicity study suggested that the Ethanolic seed extract of Vignamungo.Linn. (ESVM) were safe up to 2000mg/kg. As per the above study, dose fixation was calculated. The doses for the further study were selected as low dose (100mg/kg), medium dose (200mg/kg) and high dose (400 mg/kg) on the ratio $1/20^{th}$, $1/10^{th}$ and $1/5^{th}$ of safe dose.

Behavioural studies

Clonidine-induced hypothermia

Administration of Clonidine produced a fall in rectal temperature in control animals. The fall in temperature was maximum at 150 min after administration of clonidine i.e. from initial temperature of 35.10^{0} C to 31.65^{0}C at 120 min. In the animals pre-treated with Piracetam, ESVM (200mg/kg and 400mg/kg) reduction in the rectal temperature was not significant. But significant reduction in rectal temperature was observed in animals treated with low doses of ESVM 100mg P.O. The observations are given in Table 2.

Table 2: Effect of extracts of Vignamungo Linn. on Clonidine-induced hypothermia

Group	30 min	60min	90 min	120 min	150 min	180 min
Normal	35.87± 0.1282	35.68± 0.2197	35.70 ± 0.2236	35.67± 0.2108	36.08± l0.1352	36.13± 0.1333
Clonidine	35.10 ± 0.06325	34.75± 0.1310	33.33 ± 0.2108	33.78+± 0.1558	33.15+± 0.1708	32.22+± 0.1641
Piracetam	35.75 ± 0.1727	35.88± 0.06540	35.90± 0.1966	35.50 ± 0.2236	36.12*± 0.1167	36.12*± 0.1167
ESVM 100	35.82± 0.2182	34.68± 0.1376	34.75± 0.1586	34.83± 0.3073	34.20± 0.2620	34.45± 0.2872
ESVM 200	35.93 ± 0.1054	35.33± 0.2108	35.32± 0.2638	35.32± 0.2167	35.07*± 0.2028	35.28*± 0.1833
ESVM 400	35.90± 0.1528	35.90± 0.06831	36.08± 0.05426	35.65 ± 0.2062	35.75*± 0.1708	36.20* ± 0.1291

Values are mean ± SEM, 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 Treatment vs disease control

Lithium-induced head twitch

In this study, administration of lithium induces head twitches on animals. Treatment with standard drugpiracetam, extract of ESVM (200mg/kg and 400mg/kg) have shown significant reduction inthe number of head twitches ($p<0.01$), while effects of low dose 100mg/kg of extract was not significant in reducing number of head twitches(See Table 3.)

Table 3 : Effect of extracts of VignamungoLinn. on lithium-induced head twitching in rats

Group	No of Head Twitching
Normal	1.167± 0.3073
Lithium	23.67+++± 1.022
Piracetam	4.500***± 0.4282
ESVM 100	23.33± 0.4944
ESVM 200	15.67***± 0.5578
ESVM 400	7.667***± 0.4216

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 Treatment vs disease control

DISCUSSION :

Dementia is a mental disorder characterized by loss of intellectual ability sufficiently severe as to interfere with ones occupational or social activities. Dementia is of several types and it invariably involves impairment of memory. The most common cause of dementia is Alzheimer's disease, which is a progressive neurodegenerative disorder associated with loss of neurons in distinct brain areas. The present study performed to evaluate Ethanolic seed extracts of Vignamungo Linn.(ESVM) which contain several phyto-constituents for nootropic activity.

The clonidine is a- agonist that enhances secretion of NA in the brain and increased transmission of NA in the hypothalamus activates cold sensitive neurons results in hypothermia. In clonidine induced hypothermia model, there was significant reduction in the rectal temperature was observed in control animals. But piracetam and ESVM have inhibited clonidine induced hypothermia shows the ability to decrease the transmission of NA in the brain which is responsible for nootropic activity.

It has been indicated that an increase in serotonergic transmission can interfere with learning acquisition and memory consolidation.[9] The lithium was administered to experimental animals to induce head twitches which are one of the 5-HT mediated behaviours. There was significant number of head twitches were observed in the disease control animals of the present study. In animals treated with Piracetam & ESVM (200mg/kg and 400mg/kg), we had observed the reduction in lithium-induced head twitches indicating the ability of the ESVM, extracts to counteract serotonergic action. This may be due to the stimulation of somato-dendritic 5-HT 1A receptors which inhibits the postsynaptic receptors.[10] The results of the present study suggesting that, ethanolic seed extracts of Vignamungo Linn. (ESVM) possess significant memory enhancing activity and also suggested several possible mechanisms for its beneficial effects. In behavioural studies, the extracts have shown for the antagonism of NA mediated behaviour in clonidine induced hypothermia model in rats. The extracts also antagonized lithium induced head twitching in rats and shows that it possesses anti-serotonergic activity. According to results of study, several mechanisms can be assumed for the Nootropic activity but further study is necessary to explore exact mechanism of action for the Nootropic activity of the plant and also for the isolation and evaluation of specific phytochemical-constituents for Nootropic activity.

CONCLUSIONS :

The Ethanolic seed extract of Vignamungo Linn.(ESVM) has reversed the dementia induced by various agents, many mechanisms can be expected for its activity due to the presence of large number of chemical constituents. The ESVM also inhibited lithium induced head twitches indicating that it can antagonize the serotonergic action which is also one of the possible mechanisms for Nootropic activity. The plant extracts also significantly inhibited clonidine induced hypothermia in rats.

Although a great deal of evidence supports that Immunomodulators affect learning and memory, the exact mechanism that underlies this effect is yet to be determined. It also possesses antioxidant, anti-inflammatory, Immunomodulatory which makes very difficult to judge the mechanism of action. All these mechanisms may act in conjunction facilitating the acquisition and retention of learned activity. Further study is needed to determine the mechanism of action and to find out the active component responsible for its activity.

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