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EVALUATION OF ANTIDEPRESSANT ACTIVITY OF SPROUTS OF VIGNA RADIATA IN RATS

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Abstract:

This study was carried out in order to know that the Methanolic Extract of Vigna radiata (MEVR) possess antidepressant activity or not. This study was done in acute models such as Forced Swim Test (FST), Tail Suspension Test (TST), Reserpine Induced Hypothermia (RIH) and Locomotor activity. Albino wistar rats of either sex weighing around 150-200 g were considered for the study. Imipramine (20 mg/kg, p.o.) was given for standard group, MEVR (400 mg/kg, p.o.) was given for test group and 0.9% NS, p.o. for Vehicle Group. Immobility time was calculated in FST and TST methods, rectal temperature and ptosis score were observed in RIH and in locomotor activity effect of MEVR on locomotion was observed by using Open Field Test in which parameters like total distance, time spent in outer zones and time spent in inner zones were observed.Finally MEVR showed antidepressant activity by significantly reducing the immobility time in FST and also in TST it reduced the immobility time when compare to vehicle group, in RIH it significantly reduced rectal temperature and ptosis when compared to vehicle group and in Locomotor activity no alteration in locomotion was observed.

Key words: *Antidepressant activity, Vigna radiata, Forced Swim Test (FST), Tail Suspension test (TST), Reserpine Induced Hypothermia (RIH)*

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INTRODUCTION:

Depression is said to be as a "state of reduced mood and aversion to activity that can affect a person's ideas, behaviour, feelings and sense of well-being[1]. According to World Health Organization (WHO), Depression is a common mental disorder. Around the world, more than 300 million people of all ages suffer with depression. At severe condition, depression may leads to suicide. Mostly,8,00,000 people may die due to suicide every year. In 15-25 years people the second leading cause of their death was suicide[2]. People with depression may show symptoms such as low interest, sad mood, less concentration, insomnia, weight gain/loss, decreased libido, hopelessness & suicidal tendency[3]. At present depression is treating with drugs such as Tri Cyclic Antidepressants (TCA's), Selective Serotonin Reuptake Inhibitors (SSRI's), Serotonin & Noradrenaline Reuptake Inhibitors (SNRI's), Mono Amine Oxidase Inhibitors (MAOI's). Other treatments include Psychotherapy[4] and Brain stimulation therapies[5]. Brain stimulation therapy includes Electro Convulsive Therapy (ECT), Vagus Nerve Stimulation (VNS), repetitive Transcranial Magnetic Stimulation (rTMS), Magnetic Seizure Therapy (MST) and Deep Brain Stimulation (DBS). This drugs and therapies are proved that they can reduce the depression condition[6]. But the symptoms cannot be reduced completely by this treatment. Even after 12 weeks of treatment for chronic depressive patient the remission rates was around 22-30%[7,8]. The side effects of these treatments include CNS (tremors, dizziness, agitation, edema, headache, increased/decreased sleep, and blurred vision), GI (abdominal pain, dry mouth, weight gain/loss, constipation, diarrhea, and nausea) and sexual dysfunctioning[9]. To overcome these side effects natural products can be preferred as they do not possess significant side effects. So, in this study *Vigna radiata* is considered. Also the constituents present in the *Vigna radiata* are flavonoids (quercetin, Kampferitin, rutin, apigenin), alkaloids, phenolic acids, glycosides etc. may be useful to possess antidepressant activity[10]. Sprouts of *Vigna radiata* consists of high flavonoid contents than the normal dried beans[11]. *Vigna radiata* had other pharmacological activities like antioxidant[11], antidiabetic[12], hepatoprotective[13], antihypertensive[14], antihyperlipidemic[15], anticancer & immunomodulatory[16], anti-inflammatory & antiarthritic[17], antifungal[18] and antiviral activity[19]. Until now no literature showed that *Vigna radiata* had antidepressant activity, so this study was planned to evaluate whether *Vigna radiata* possess antidepressant activity or not.

MATERIALS & METHODS:

Plant material and Authentication:

Dried beans of *Vigna radiata* were purchased from local distributor at Vijayawada. Those beans were identified and authenticated by P. Prasanna kumari, Head of the department of Botany, DNR College, Bhimavaram.

Animals:

Albino Wistar rats of either sex weighing around 150-250 g were considered for this study. Animals were purchased from Mahaveer enterprises, Hyderabad and were housed in the animal house at Shri Vishnu College of Pharmacy with register number 439/PO/S/01/CPCSEA, in which the room temperature was maintained at 23 ± 2^0C with 12 hrs light and dark cycles and free access to food and water. All the experimental protocols were approved by Institutional Animal Ethical Committee (IAEC): 439/PO/01/a/CPCSEA.

Chemicals:

Reserpine was purchased from LOBA CHEMIE Pvt. Ltd, Mumbai with Cas no: 50-55-5, Methanol was purchased from LOBA CHEMIE Pvt. Ltd, Mumbai with Cas no: 67-56-1.

Preparation of extract:

Mung beans were thoroughly washed with water in order to remove the dirt and then soaked for 10 hrs. Then the soaked seeds were tied in a muslin cloth for 2 days and for every 6 hrs water had sprinkled on the cloth in order to keep the cloth moist. Seeds were germinated and sprouts had produced. These sprouts were then shade dried for 15-20 days. Dried sprouts were then pulverized to obtain fine powder. Extraction was carried out for 3 days using soxhlet and the obtained extract was concentrated with the help of rotary vacuum evaporator, dried and stored in a desiccator.

Phytochemical Screening:

The dried Methanolic Extract of *Vigna radiata* (MEVR) was subjected to various Phytochemical tests[20] in order to identify the type of chemical constituents present in it, such as alkaloids, flavonoids, proteins, amino acids, carbohydrates, phenolic acids, steroids.

IRWIN Test:

This is mainly done in rodents for assessing acute toxicity of a test agent and its effects on behaviour and physiological function. The International Committee

for Harmonization (ICH S7A) certifies that the Irwin test and Functional Observation Battery (FOB) are equivalent for the purpose of assessing safety.

In this study the rats were administered with two doses of Methanolic Extract of *Vigna radiata* such as 200 mg/kg as low dose and 400 mg/kg as high dose and observed the effects on behaviour and physiological changes by comparing with the control rats where n=3 for each group. The animals were assessed for behaviours specifically related to Neurotoxicity:convulsions and tremor, Central Nervous System (CNS) stimulation: excitation, Straubtail, jumping, stereotypy and aggressive behaviour, CNS depression: sedation, rolling gait, loss of balance, loss of traction, decreased muscle tone, analgesia, corneal reflex, catalepsy, Autonomic functions: temperature, salivation and defecation.All of these parameters were observed at 0 h, ½ h, 1 h, 3 h, 4 h, 24 h after dosing by comparing with the Control (0.9 % NS, p.o.) group[21].

EXPERIMENTAL DESIGN:

Forced Swim Test (FST):

Animals were divided into 3 groups (n=8). Filled the transparent cylinder (60 cm height & 25 cm diameter) with water and placed each rat for 15 mins which is considered as pre-test. After completion of pre-test at 1, 5 & 23 hrs, animals were treated with their respective compounds. At 24 hrs the actual test was started, where the cylinder was filled, video was started to record and finally placed each animal for 6 mins. Immobility within 6 mins was noted with the help of a stopwatch. Immobility is the time during which the animals were float on the surface with front paws together, and its head above the surface & made only those movements which were necessary to keep afloat[22].

Tail Suspension Test (TST):

Animals were divided into 3 groups (n=8). Before starting, animals were treated with their respective compounds at 24, 20 & 1 hr before the test. Then the animals were hung to a suspension bar which is 50 cm above the ground with the help of a tape for 6 mins. Before starting the test process, video was started in order to record the test process. Immobility within the 6 mins was noted with the help of stopwatch. Immobility is the time during which the animals were hung passively without any movement. In this study pendulum like movements due to vigorous mobility of previous seconds were also considered as immobility[23].

Reserpine Induced Hypothermia (RIH):

Animals were divided into 3 groups (n=6). Initial rectal temperature & ptosis score was measured and considered as normal values (-18 h). Then all the animals were treated with Reserpine (2 mg/kg, s.c.). After 18 hrs of Reserpine treatment rectal temperature was measured and ptosis score was observed and considered as 0 h. Then the animals were treated with their respective compounds and again temperature and ptosis score was measured at 1 h, 2 h, 3h & 4 h after treatment. Temperature was measured with the help of digital thermometer. Ptosis score was given as: Normal eye – 0, 1/4th closed eye – 1, 1/2 closed eye – 2, 3/4th closed eye – 3, fully closed eye – 4[24,1].

Locomotor Activity:

Locomotor activity was done by using Open Field Test (OFT). Animals were divided into 3 groups (n=5). Then the animals were treated with their respective compounds. After an hour of dosing, the animals were individually placed in the centre of the OFT apparatus, which is a simple circular dark wooden box with the floor divided into 19 sections to make up the central and peripheral sections. The OFT apparatus was illuminated by neon bulbs placed perpendicularly above it. Time spent in outer zones, time spent in inner zones and total distance were recorded within 5 min[25].

RESULTS:

Preliminary Phytochemical Screening:

The Methanolic Extract of sprouts of *Vigna radiata* (MEVR) was subjected to preliminary phytochemical screening and the results were tabulated in Table No. 1. The results showed the presence of Alkaloids, Amino acids, Carbohydrates, Flavonoids, Glycosides, Proteins, Steroids & Terpenoids.

Table No-1: Results of phytochemical screening of *Vigna radiata*

Phytochemical	**Phytochemical Test**	**Results**
Alkaloids	Dragendorff's Test	+
Glycosides	Keller-Killiani Test, Baljet's Test	+
Flavonoids	Zinc Hydrochloride Test	+
Proteins	Xanthoproteic Test	+
Aminoacids	Millon's&Ninhydrine Test	+
Phytosterols	Salkowski Test	+
Carbohydrates	Molisch's Test	+

+ = Presence of the compound

IRWIN Test:

Both 200 mg/kg and 400 mg/kg of MEVR does not showed any toxic effects when compared with the Control group at ½ h, 1 h, 3 h, 4 h and 24 h after treatment schedules. So, 400 mg/kg was considered for further antidepressant studies.

Forced Swim Test:

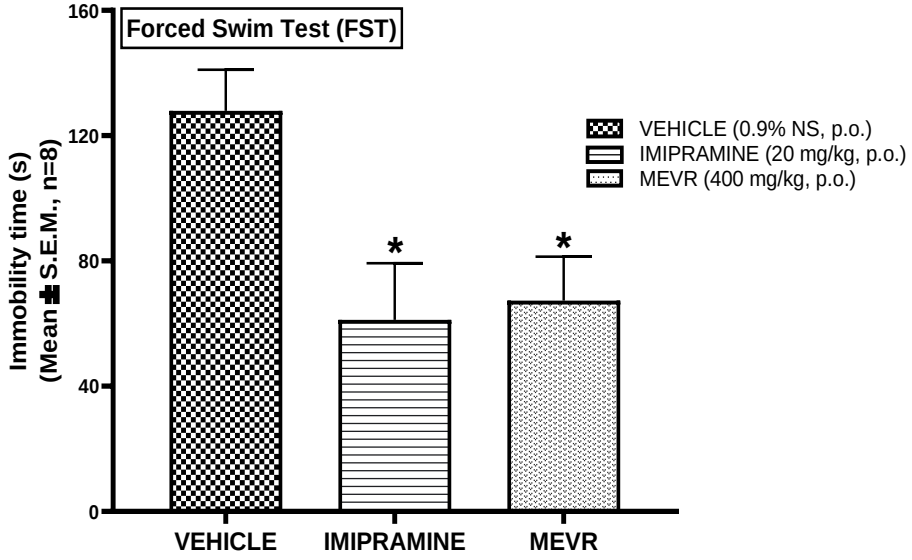

Fig-1: Effect of MEVR on Immobility Time (s) in Forced Swim Test

Results were represented as the Mean ± S.E.M., n= 8/ group; data was analysed using Graph pad prism 8.2 by one-way ANOVA using Dunnett's multiple comparison test; Significance at *P < 0.05 vs VEHICLE group. Imipramine (20 mg/kg, p.o.) and MEVR (400 mg/kg, p.o.) were significantly reduced the immobility time (s) when compare with the Vehicle (0.9% NS, p.o.) group.

Tail Suspension Test:

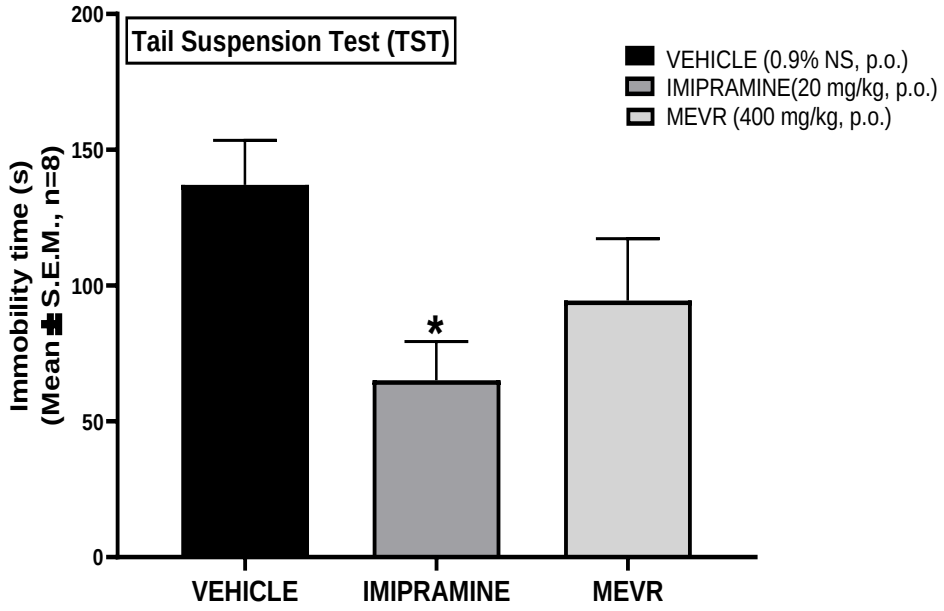

Fig-2: Effect of MEVR on Immobility Time (s) in Tail Suspension Test

Results were represented as the Mean ± S.E.M., n= 8/ group; data was analysed using Graph pad prism8.2 by one-way ANOVA using Dunnett's multiple comparison test; significance at *P < 0.05 vs VEHICLE group. Imipramine (20 mg/kg, p.o.) significantly reduced the immobility time (s) whereas MEVR (400 mg/kg, p.o.) reduced the immobility time (s) but not significant when compared with the Vehicle (0.9% NS, p.o.) group.

Reserpine Induced Method:

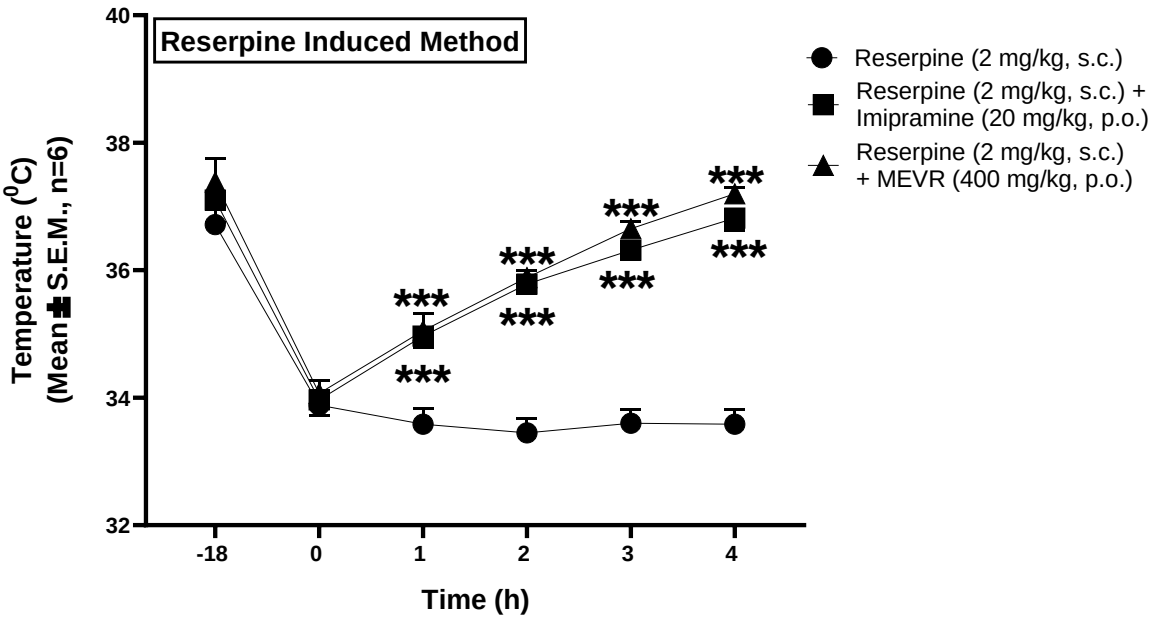

Fig-3: Effect of MEVR on Hypothermia (^{0}C) in Reserpine Induced Method

Results were represented as the Mean ± S.E.M., n= 6/ group; data was analysed using Graph pad prism 8.2 by two-way ANOVA using Dunnett's multiple comparison test; significance at ***P < 0.001 vs RESERPINE group. Both Imipramine (20 mg/kg, p.o.) and MEVR (400 mg/kg, p.o.) were significantly reduced the hypothermic condition when compare with the Vehicle (0.9% NS, p.o.) group at all time points.

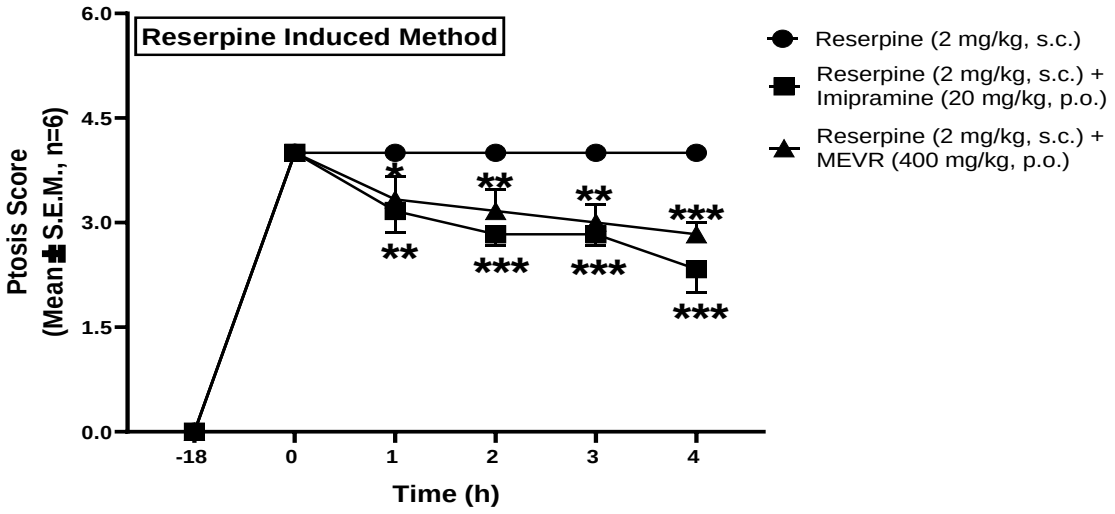

Fig-4: Effect of MEVR on Ptosis in Reserpine Induced Method

Results were represented as the Mean ± S.E.M., n= 6/ group; data was analysed using Graph pad prism 8.2 by two-way ANOVA using Dunnett's multiple comparison test; significance at ***P < 0.001, **P <0.01, *P < 0.05 vs RESERPINE group. At 4 h MEVR (400 mg/kg, p.o.) and Imipramine (20 mg/kg, p.o.) had shown equally significant effect when compared with the Vehicle (0.9% NS, p.o.) group in reducing ptosis.

Locomotor Activity:

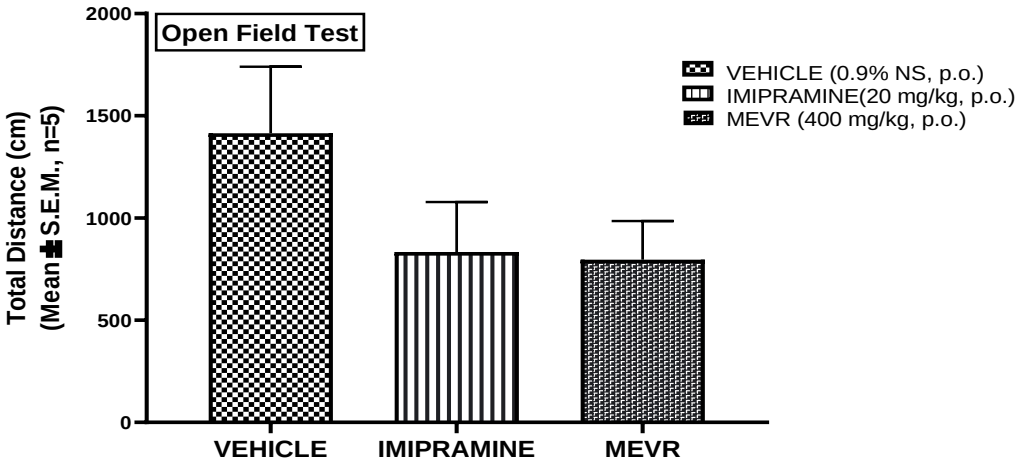

Fig-5: Effect of MEVR on Total Distance in Open Field Test

Results were represented as the Mean ± S.E.M., n= 5/ group; data was analysed using Graph pad prism 8.2 by One way ANOVA using Dunnett's multiple comparison test. Both Imipramine (20 mg/kg, p.o.) and MEVR (400 mg/kg, p.o.) reduced the total distance in locomotor activity when compared with the Vehicle group (0.9% NS, p.o.) group.

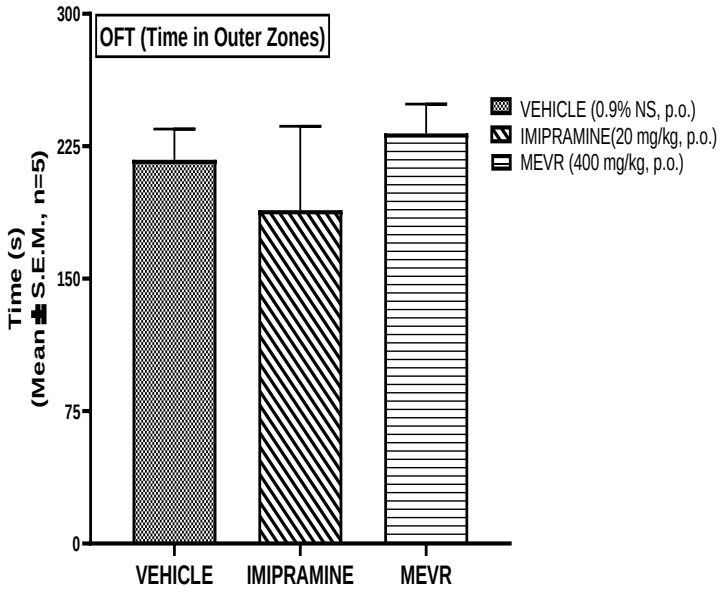

Fig-6: Effect of MEVR on Time (s) spent in Outer zones in Open Field Test

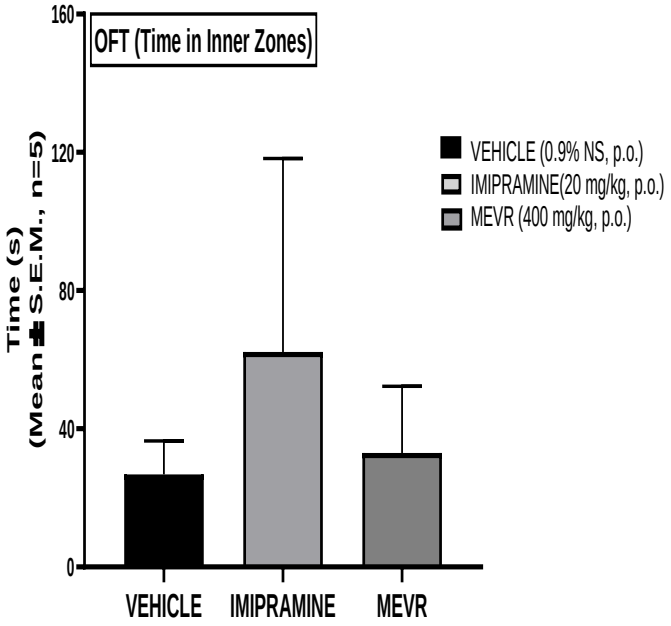

Fig-7: Effect of MEVR on Time (s) spent in Inner Zones in OFT

Results were represented as the Mean ± S.E.M., n= 5/ group; data was analysed using Graph pad prism 8.2 by One way ANOVA using Dunnett's multiple comparison test. Both Imipramine (20 mg/kg, p.o.) and MEVR (400 mg/kg, p.o.) does not showed much alteration in time spent in outer zones when compared with the Vehicle (0.9% NS, p.o.) group.

DISCUSSION:

The present study was done on Methanolic Extract of *Vigna radiata* to know whether it may possess antidepressant activity using albino wistar rats.

Vigna radiata commonly called as green gram in English. *Vigna radiata* is found abundantly in India and can be taken frequently by most of the people in their food. It consists of various phytochemicals such as flavonoids, alkaloids, glycosides, carbohydrates, aminoacids, proteins & steroid as per the phytochemicals screening. Flavonoids such as quercetin[26], kampferetin[27], rutin[28], apigenin[29] and phenolic acids such as gallic acid[30] and ferulic acid[31] showed antidepressant activity as per the literature. There is no literature showing that *Vigna radiata* had antidepressant activity. So, this study was carried to identify whether it had antidepressant activity or not. Also germinated seeds are considered good for health when compared to normal beans. Recently people are becoming more health conscious and are taking sprouts in their diet. From the literature we found that sprouts had high flavonoid content than the dried beans[11]. So, this study was carried by using sprouts of *Vigna radiata*.

Then Primary Observation (IRWIN) test was done to know the safety of the extract. The Irwin test is used to estimate the minimum lethal dose of a test substance, the dose range for CNS responses and the primary effects on behaviour and physiological functions. The International Committee for Harmonization (ICH S7A) certifies that the Irwin test and FOB are equivalent for the purpose of assessing safety[21]. As per the literature *Vigna radiata* does not showed any toxic effects at 2 g/kg[17]. So 200 and 400

mg/kg was considered here to do the IRWIN test. MEVR did not show any lethal effect in both 200 mg/kg and 400 mg/kg which were administered through per oral route. As 400 mg/kg does not showed any lethal effect this dose was selected for investigating the antidepressant activity.

Further Antidepressant activity of MEVR was estimated by using different methods such as Forced Swim Test, Tail Suspension Test and Reserpine Induced Hypothermia and Locomotor activity.FST and TST are the preliminary screening methods to assess the antidepressant activity of the compounds. Mainly few things have to be considered in this method which includes, after 6 mins the rats were taken out from the water and wipe them neatly with dried towel and place them in separate cages, this is mainly to prevent the behaviour alteration of the rats which will further undergone for test process. The water in the tank has to be replaced with fresh water for each rat. Here in FST rats which were treated with Imipramine (61.175±18.08286*) and MEVR (67.3125±14.0804*) had shown equally significant decrease in immobility time than the Vehicle group (127.9125±13.13287).

Tail Suspension Test (TST) is also widely used method to determine the antidepressant activity. Here Imipramine (65.1375±14.21973*) significantly reduced immobility time when compared to the Vehicle group (137.0375±16.45736), whereas MEVR (94.4875±22.81062) reduced the immobility time but not significant when compared to the Vehicle group (137.0375± 16.45736).

Reserpine Induced Hypothermia (RIH) is one of the acute models to determine antidepressant activity. Reserpine is mainly involved in inhibiting the reuptake of monoamines from the storage vesicles which leads to cause hypothermia by increasing in the metabolism of monoamines[32]. Here hypothermia was decreased by the MEVR at each hour equally significant to Imipramine. Initially at 0 h Vehicle (33.5±0.288675), Imipramine (34.05±0.144338) and MEVR (33.95±0.317543) had shown. At 4 h MEVR (36.9±0.057735***) and Imipramine (37.2±0.23094***) were showed equally significant decrease in hypothermia than Vehicle group (33.45±0.202073). In case of ptosis score initially at 0 h Vehicle group (4), Imipramine group (4) and MEVR (4) were observed. At 4 h MEVR (2.5±0.288675***) and Imipramine (2±0.57735***) were showed equally significant effect than Vehicle group (4).

Locomotor activity by Open Field Test was mainly done in order to overcome nonspecific locomotor activity the extract might posses. Compounds having psychostimulant activity show increase in locomotor activity whereas antidepressants shows decrease in locomotion[33]. Here MEVR reduced the locomotor activity compared to the normal group. In case of total distance Imipramine (833.304 ± 218.74) and MEVR (795.84 ± 168.50) showed decrease in locomotion when compared to Vehicle group (1413.792 ± 291.52). In case of time spent (sec) in outer zones Imipramine (188.8 ± 42.49) and MEVR (232.2 ± 14.95) does not show significant alteration when compared to Vehicle group (217.4 ± 15.55).
Also time spent (sec) in inner zones, Imipramine (62.2 ± 50.08) and MEVR (33 ± 17.22) not showed significant alteration than Vehicle group (26.8 ± 8.66). So this test was used to confirm that the observed antidepressant activity was not due to stimulation of general motor activity.

CONCLUSION:

By considering the above results obtained Methanolic Extract of *Vigna radiata* reduced the immobility time in Forced Swim Test and Tail Suspension Test and also in Reserpine induced method it significantly reduced the hypothermic condition and ptosis. So, *Vigna radiata* may possess antidepressant activity without any psychostimulant effect.

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REFERENCES:

1. Babu PN, Nagaraju B, Yamini K, Dhananjaneyulu M, Venkateswarlu K, Mubina M. Evaluation of antidepressant activity of ethanolic extract of Dacuscarota in mice. Journal of Pharmaceutical Sciences and Research. 2014 Feb 1;6(2):73.
2. 2018. Available at: https://www.who.int/news-room/fact-sheets/detail/depression. [Accessed May 2019].
3. 2018. Available at: https://www.nimh.nih.gov/health/topics/depression/index.shtml. [Accessed May 2019].
4. Available at: https://www.nimh.nih.gov/health/publications/depression-what-you-need-to-know/index.shtml. [Accessed May 2019].
5. 2016. Available at: https://www.nimh.nih.gov/health/topics/brain-

stimulation-therapies/brain-stimulation-therapies.shtml. [Accessed May 2019].
6. Rakofsky JJ, Holtzheimer PE, Nemeroff CB. Emerging targets for antidepressant therapies. Current Opinion in Chemical Biology. 2009 Jun 1;13(3):291-302.
7. Keller MB, McCullough JP, Klein DN, Arnow B, Dunner DL, Gelenberg AJ, Markowitz JC, Nemeroff CB, Russell JM, Thase ME, Trivedi MH. A comparison of nefazodone, the cognitive behavioral-analysis system of psychotherapy, and their combination for the treatment of chronic depression. New England Journal of Medicine. 2000 May 18;342(20):1462-70.
8. Kocsis JH, Gelenberg AJ, Rothbaum BO, Klein DN, Trivedi MH, Manber R, Keller MB, Leon AC, Wisniewski SR, Arnow BA, Markowitz JC. Cognitive behavioral analysis system of psychotherapy and brief supportive psychotherapy for augmentation of antidepressant nonresponse in chronic depression: the REVAMP Trial. Archives of general psychiatry. 2009 Nov 1;66(11):1178-88.
9. Vanderkooy JD, Ken nedy SN, Bagby RM. Antidepressant side effects in depression patients treated in a naturalistic setting: a study of bupropion, moclobemide, paroxetine, sertraline, and venlafaxine. The Canadian Journal of Psychiatry. 2002 Mar;47(2):174-80.
10. Silva LR, Pereira MJ, Azevedo J, Gonçalves RF, Valentão P, de Pinho PG, Andrade PB. Glycine max (L.) Merr., Vigna radiata L. and Medicago sativa L. sprouts: A natural source of bioactive compounds. Food research international. 2013 Jan 1;50(1):167-75.
11. Tiwari U, Servan A, Nigam D. Comparative study on antioxidant activity, phytochemical analysis and mineral composition of the Mung Bean (Vigna Radiata) and its sprouts. Journal of Pharmacognosy and Phytochemistry. 2017;6(1):336-40.
12. Yeap SK, Mohd Ali N, Mohd Yusof H, Alitheen NB, Beh BK, Ho WY, Koh SP, Long K. Antihyperglycemic effects of fermented and nonfermented mung bean extracts on alloxan-induced-diabetic mice. BioMedical Research International. 2012 Oct 3;2012.
13. Mohd Ali N, Mohd Yusof H, Long K, Yeap SK, Ho WY, Beh BK, Koh SP, Abdullah MP, Alitheen NB. Antioxidant and hepatoprotective effect of aqueous extract of germinated and fermented mung bean on ethanol-mediated liver damage. BioMedical research international. 2012 Dec 24;2013.
14. Nakamura K, Koyama M, Ishida R, Kitahara T, Nakajima T, Aoyama T. Characterization of bioactive agents in five types of marketed sprouts and comparison of their antihypertensive, antihyperlipidemic, and antidiabetic effects in fructose-loaded SHRs. Journal of food science and technology. 2016 Jan 1;53(1):581-90.
15. Solanki YB, Jain SM. Antihyperlipidemic activity of Clitoriaternatea and Vigna mungo in rats. Pharmaceutical biology. 2010 Aug 1;48(8):915-23.
16. Hafidh RR, Abdulamir AS, Bakar FA, Jalilian FA, Abas F, Sekawi Z. Novel molecular, cytotoxical, and immunological study on promising and selective anticancer activity of Mung bean sprouts. BMC complementary and alternative medicine. 2012 Dec;12(1):208.
17. Venkateshwarlu E, Reddy KP, Dilip D. Potential of Vigna radiata (L.) sprouts in the management of inflammation and arthritis in rats: Possible biochemical alterations. International Journal of Experimental Biology. 2016 Jan;54(01)37-43.
18. Ye XY, Ng TB. Mungin, a novel cyclophilin-like antifungal protein from the mung bean. Biochemical and biophysical research communications. 2000 Jul 14;273(3):1111-5.
19. Hafidh RR, Abdulamir AS, Bakar FA, Sekawi Z, Jahansheri F, Jalilian FA. Novel antiviral activity of mung bean sprouts against respiratory syncytial virus and herpes simplex virus− 1: an in vitro study on virally infected Vero and MRC-5 cell lines. BMC complementary and alternative medicine. 2015 Dec;15(1):179.
20. Banu KS, Cathrine L. General techniques involved in phytochemical analysis. International Journal of Advanced Research in Chemical Science. 2015;2(4):25-32.
21. Roux S, Sablé E, Porsolt RD. Primary observation (Irwin) test in rodents for assessing acute toxicity of a test agent and its effects on behavior and physiological function. Current protocols in pharmacology. 2004 Dec;27(1):10.
22. Slattery DA, Cryan JF. Using the rat forced swim test to assess antidepressant-like activity in rodents. Nature protocols. 2012 Jun;7(6):1009.
23. Aslam M. Tail suspension test to evaluate the antidepressant activity of experimental drugs. Bangladesh Journal of Pharmacology. 2016 Mar 13;11(2):292-4.
24. Rojas-Corrales MO, Berrocoso E, Gibert-Rahola J, Micó JA. Antidepressant-Like Effect of tramadol and its Enantiomers in Reserpinized Mice: Comparativestudy with Desipramine,

Fluvoxamine, Venlafaxine and Opiates. Journal of Psychopharmacology. 2004 Sep;18(3):404-11.
25. Fekadu N, Shibeshi W, Engidawork E. Evaluation of the antidepressant-like activity of the crude extract and solvent fractions of Rosa abyssinica Lindley (Rosaceae) using rodent models of depression. Clinical Experimental Pharmacology. 2016;6(208):2161-1459.
26. Rinwa P, Kumar A. Quercetin suppress microglial neuroinflammatory response and induce antidepressent-like effect in olfactory bulbectomized rats. Neuroscience. 2013 Dec 26;255:86-98.
27. Park SH, Sim YB, Han PL, Lee JK, Suh HW. Antidepressant-like Effect of Kaempferol and Quercitirin, Isolated from Opuntia ficus-indica var. saboten. Experimental neurobiology. 2010 Jun 1;19(1):30-8.
28. Machado DG, Bettio LE, Cunha MP, Santos AR, Pizzolatti MG, Brighente IM, Rodrigues AL. Antidepressant-like effect of rutin isolated from the ethanolic extract from Schinusmolle L. in mice: evidence for the involvement of the serotonergic and noradrenergic systems. European Journal of Pharmacology. 2008 Jun 10;587(1-3):163-8.
29. Nakazawa T, Yasuda T, Ohsawa K. Antidepressant-like effects of magnolol from Magnolia officinalis in the forced swimming test. Natural medicines= 生藥學雜誌. 2003;57(6):221-6.
30. Chhillar R, Dhingra D. Antidepressant-like activity of gallic acid in mice subjected to unpredictable chronic mild stress. Fundamental & clinical pharmacology. 2013 Aug;27(4):409-18.
31. Zeni AL, Zomkowski AD, Maraschin M, Rodrigues AL, Tasca CI. Involvement of PKA, CaMKII, PKC, MAPK/ERK and PI3K in the acute antidepressant-like effect of ferulic acid in the tail suspension test. Pharmacology Biochemistry and Behavior. 2012 Dec 1;103(2):181-6.
32. Yaffe D, Forrest LR, Schuldiner S. The ins and outs of vesicular monoamine transporters. The Journal of general physiology. 2018 May 7;150(5):671-82.
33. Saleem AM, Hidayat MT, Jais AM, Fakurazi S, Moklas MM, Sulaiman MR, Amom Z. Antidepressant-like effect of aqueous extract of Channastriatus fillet in mice models of depression. European Review for Medical and Pharmacological Sciences. 2011 Jul 1;15(7):795-802.