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In vivo anxiolytic and locomotor activity of water extract of mung beans seeds in swiss albino mice

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Abstract
Mung beans seeds have been recognized as health-promoting pulses to alleviate anxiety and other related disorders. Though, being an important functional food, the antianxiety activity of these seeds is not explored yet. Thus, this research finalizes the anxiolytic action of various extracts of the Mung Beans seeds. However, extractions of Mung bean seeds have been carried by the triple maceration method and different extracts of solvents like petroleum ether, chloroform, ethanol, and water were prepared and dried. Besides, different doses of extracts *viz.* 50, 100, and 150 mg/kg. (p.o.) were given to female Swiss Albino mice as compared to standard drug diazepam 2mg/kg. (i.p) and their behavior was monitored on EPM and light & dark test model while the motor movement was assessed using a photoactometer; Although, each isolate was found to be safe with no deaths in female Swiss Albino mice when giving out orally with a dose of 1000 mg/kg(p.o.). Among all fraction, water extract (150mg/kg, p.o.) of the Mung bean seeds significantly boosted the entries and average spent time in the open arms in the EPM test along with progress in time spent as well significantly rise in duration of immobility and total of grade crossing inside the light and dark chamber but no expressive changes had been revealed in locomotor activity. These outcomes suggested that the aqueous extract at the dose of 150 mg/kg had shown anxiolytic effects and justify the traditional utilization of Mung bean seeds in the management of neurological syndromes.

Keywords: mung beans seeds; elevated plus-maze model; light and dark test, locomotor activity, anxiolytic

Introduction
Herbal remedies play a vital character in health programs around the world [1]. But anxiety can also lead to depression (or vice versa). Thus, new research into medicinal therapies of remedial plants for the treatment of mental illnesses has proceeded over the earlier period and their curative capacity has been evaluated[2]. In India, medicinal plants and their derivatives are an essential resource of drugs for the treatment of several mental illnesses [3].
Mung bean *V. radiata* is considered as Legumes that belong to Family-Leguminosae or Fabaceae. This plant family is broadly spread all over the globe and exists for the third position for the major family of blossoming plants. It has almost 650 genus and 20,000 species [4]. The familiar name of the Mung bean plant is mash, golden gram, green gram, and mung bean. The species ranges from small herbs to large tropical canopy trees. These plant species have located in the humid tropics, temperate zones, high land, arid zones, and low land [5]. Mostly, Dry seeds consume by human beings. Immature green pods are used as a source of vegetables in rural areas and cooked as oriental dishes. The stalks, husks, and leaves of the mung bean plant are their main parts. Seeds of Mung bean hold plenteous health aids and prevent human diseases [6]. The seeds are plentiful in protein and an amino acid such as lysine, minerals, and vitamins. Accordingly, it is widely used as a supplement to cereal-based human diets [7].
Mung seeds have numerous pharmacological activities such as anticancer [8], antihyperlipidemic [9], antidiabetic [10], antifungal and antibacterial [11], antiviral [12], antioxidant [13], hypertension [6], in liver diseases [14] and as memory enhancer [15]. Clinical evidence recommended that these seeds increase muscular strength and beneficial in rheumatism [15], useful to reduce obesity16and also act as anti-alzheimer [16].
However, there is no detailed study on the anxiolytic outcomes of Mung bean seeds. In this enact study, the antianxiety activity of different extracts obtained from Mung Bean seeds and was examined by the elevated plus-maze model (EPM), light, and dark test. However, motor activity was determined by the photoactometer model.

Materials and Methods
Herb material
Mung bean seeds were planted in June at Gurugram, India, and seeds of Mung beans were collected in August. The mung beans seed was identified by the National Institute of Science Communication and Information Resources (NISCAIR), detected at New Delhi, India, and voucher specimen of the plant (No. NISCAIR/RHMD/Consult/2018/3245-46) has been dropped in NISCAIR to furthest citation.
Solvents and instruments
Solvents like Pet. ether, chloroform, ethanol, and water were utilized for the extraction of the plant material.
Provision of plant extract Air-dried ground Mung Beans seed (5 kg) has been consecutively extracted independently with petroleum ether, chloroform, ethanol, and water by triple maceration process and their filtrate had been collected. However, petroleum ether, chloroform extract, ethanol extract was dried using a rotary vacuum evaporator. Water extract was prepared and dried. All the dried extracts

were deliberate and their fraction yields were estimated. The isolates had maintained at a refrigerator for auxiliary procedure.

Preliminary phytochemical studies [17]

Preliminary phytochemical screening of active mung bean seed extracts was carried out using the standard procedure.

Animals

Adult female albino mice (18-25 g) were obtained from the University of Maliba University, Surat, Gujarat, and raised at the Animal Shelter of Maliba University. However, they were kept in groups in polypropylene cages (11 cm × 17 cm × 28 cm) together with wood shavings as bedding in controlled conditions of light (12 h light-dark cycle) and temperature (25 ± 1 ° C). However, mice were offered a free approach to diet and water. Besides, etiquettes for laboratory animals have been adopted by the Animal Ethics Institutional Committee (Reg. No. MPC/IAEC/05/2019).

Treatments Mung bean seed extract was independently dissolved in a medium containing 0.2% Tween 80 in normal saline (0.9% NaCl). Also, standard drug diazepam 2 mg/kg was dissolved in a medium. Although, the medium was employed as a control. Also, the prescribed amount of different test isolate/medium/diazepam had been used in concentrations to be administered orally to mice in volumes varying from 0.25 to 0.30 ml.

Experiments

Acute toxicity Study [18]

In these studies, Female swiss albino mice were selected by the random sampling method. Animals are not eaten for 4 hours and have free access to water.

All extracts i.e., Pet. ether extract, chloroform, ethanol and water extract from Mung bean seeds had been employed for mice for 3 days and their mortality rate was evaluated at doses of 50,200,500,1000 mg/kg. No mortality was found using these doses. Based on these findings, extracts were further evaluated for antianxiety studies.

Elevated plus-maze test [19]

Every mouse is positioned in the middle of the Elevated plus-maze (EPM) test model as well as its head facing of mice adjusted at open arms. However, through a 5-minute trial, the performance of each mice was documented as (a) average number of entries and (b) average time with open arms (mean time = total time in open arms/number of entries in open arms). However, direct test material is administered orally employing a syringe (tuberculin) equipped with an oral cannula. Besides, the dosing schedule was adjusted so that each mice should be on the EPM test model for 45 minutes after dosing. The animals were allocated to socialize during the experiment. However, every precaution has been taken to ensure that no external stimulus other than the height of the model. Comparable studies were documented to all groups as compared to the control group and standard drug diazepam at 2.0 mg/kg.

Light and dark test [20]

The Light and dark test models had made up of a wooden box. In this field are the two closest separate chambers i.e. a black section is prepared dark by being concealing with black plywood material, and the bright section is also coated with white and lights up brightly with a 40W lamp. Besides, the two chambers had been joined across a tiny free doorway that was located on the floor at the middle ground of the barrier. However, the albino mice had been positioned singly in the middle ground part of the light chambers. Oral therapy with water extract was given in different doses and also the behavior was checked for 5 minutes.

Locomotor Activity [21]

The outcome of Mung bean seeds extracts for the motor movement was assessed using a photoactometer. However, six albino mice had been selected for each dose (50 mg,100mg,150mg). Besides, the control group, standard diazepam (2 mg/kg i.p.), and water isolate extract had been given out orally to all mice before 30 minutes to start the experimentation. However, each albino mice were positioned and motor activity determination for each group was carried out within 5 minutes. Besides, therapy was randomized between 8:00 a.m. and 1:00 p.m. throughout the day for the determination of changes in behavior.

Statistical analysis

Values are stated as average ± SEM for all animal groups. However, a one-way analysis test of variance (ANOVA) was used. Although, data has been tracked by Studentized Tukey's trial for numerous analogies. The outcome was considered significant at $p \leq 0.05$ versus to control

Result

The fraction yields (w/w) of various extract like petroleum ether, chloroform, ethanol, and also water extracts were obtained as 0.48%,0.031%,0.62%, and 7%, respectively. Preliminary phytochemical screening of active extract carried out using the standard procedure and results are reported in table no 1.

Table 1: Phytochemical analysis of different extracts of seeds of Mung beans.

Phytochemical	Chemical test	Pet.ether extract	Chloroform extract	Ethanol extract	Water extract
Alkaloids	Dragendorffs Test	-	-	-	-
	Mayer Test	-	-	-	-
Phenol	Ferric chloride test			+	+
Saponin	Foam test	-	-	+	+
Carbohydrates	Molisch test	-	-	-	+
	Fehling's test	-	-	-	+
Flavanoids	Shinoda test	-	-	+	+
	Lead acetate test	-	-	+	+
Terpenoids	Salkowski test	-	+	+	+
Tannin	Acetic acid test	-	-	+	+
Protein	Biuret test	-	-	-	+
Lipids	Sudan IV test	+	-	-	-
	Copper acetate test	+	+	-	-

(Positive sign specify the presence of several phytoconstituents with respective to concentration like +ve and –ve sign specify the absence of phytoconstituents).

Results revealed that ethanol extract of Mung bean seeds unveiled the existence of phenol, saponin, flavonoids, and tannins. Water extract specified the incidence of phenol, saponin, flavonoids, carbohydrates, and protein. The approximate LD50 of water extractive of Mung beans seeds was found to be 1000 mg/kg. Besides, the anxiolytic-like activity of seeds was also detected in EPM and light/dark exploration tests. Also, different doses of extracts *viz*. 50, 100, and 150 mg/kg had been processed to female albino mice, and behavior was perceived. Diazepam (2mg/kg,) was used as a standard drug. As usual, diazepam represented a significant boost in open arm entries and also raise mean time spent in open arms as reported in table no 2 and fig.1-2. Water extract of Mung Beans seeds at a dose of 150 mg/kg was realized to be exhibited a substantial boost of entries in open arms and average time expended in the open arms as compared to control. Besides, Mung bean seeds water extract had provoked behavioral changes in the elevated plus maze (EPM) test as similar to diazepam. Since, no significant anxiolytic activity was found in other extracts like Pet. ether, chloroform, and ethanol of mung bean seed in EPM studies. So, only water extractive was studied for light and dark model and locomotor activity. Like EPM, a similar anxiolytic effect had been found in the light and dark test model by water extract of seeds. However, water extract of mung bean seed had shown a significant rise of time spent in a light chamber and dark chamber as well as the significant progress in a number of crossings and also amplify in the duration of immobility between the light and dark chamber at dose 150mg/kg relative to control as clearly illustrated in table no.3 and fig 3. Water extractive (150 mg/kg) of Mung bean sees had not shown any expressive changes in locomotor activity related to diazepam as clearly depicted in table no. 4 and fig.4.

Table 2: Anxiolytic effects of different extracts from Mung bean seeds in albino mice by using EPM (elevated plus-maze model)

Group Treatment	Dose (mg/kg)	Entries of mice in open arms	Percentage of time spent open arms (%)
Control	0.25	1±0.36	1.9±0.26
Diazepam	2	12.5±0.92**	16.2±0.87**
Petroleum Ether extract	50	1.2±0.16	3.1±0.66
	100	2.7±0.42	5.7±0.62**
	150	1.7±0.21	5.4±0.36**
Chloroform extract	50	1.3±0.21	3.2±0.07
	100	3.5±0.42	5.0±0.45
	150	2.5±0.22*	4.0±0.45**
Ethanol extract	50	2.2±0.3	3.9±0.48
	100	3.2±0.79	4.2±0.38
	150	3.5±0.62	4.5±0.56
Water extract	50	3.7±0.49	6.3±0.34
	100	7.3±0.84	10.1±0.46*
	150	**7.8±0.6***	**13.3±0.73***

Data are stated as average ± SEM. ANOVA test was ensued by Studentized Tukey's Test, ** p <0.01, * p <0.05 versus control, Number of animals (n) = 6

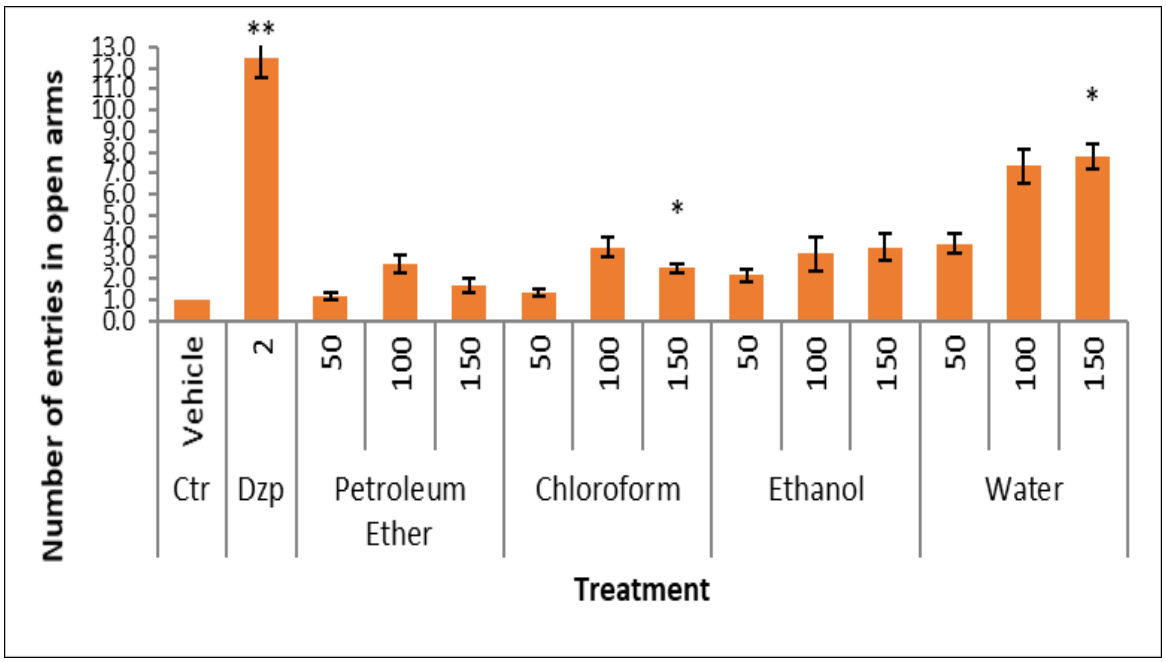


Fig 1: A comparative profile of a mean number of entries in open arms of EPM by mice treated with various extracts of seeds of Mung beans. Values are expressed as mean ± SEM. Number of animal (n) = 6. ** p <0.01, * p <0.05 relative to control.

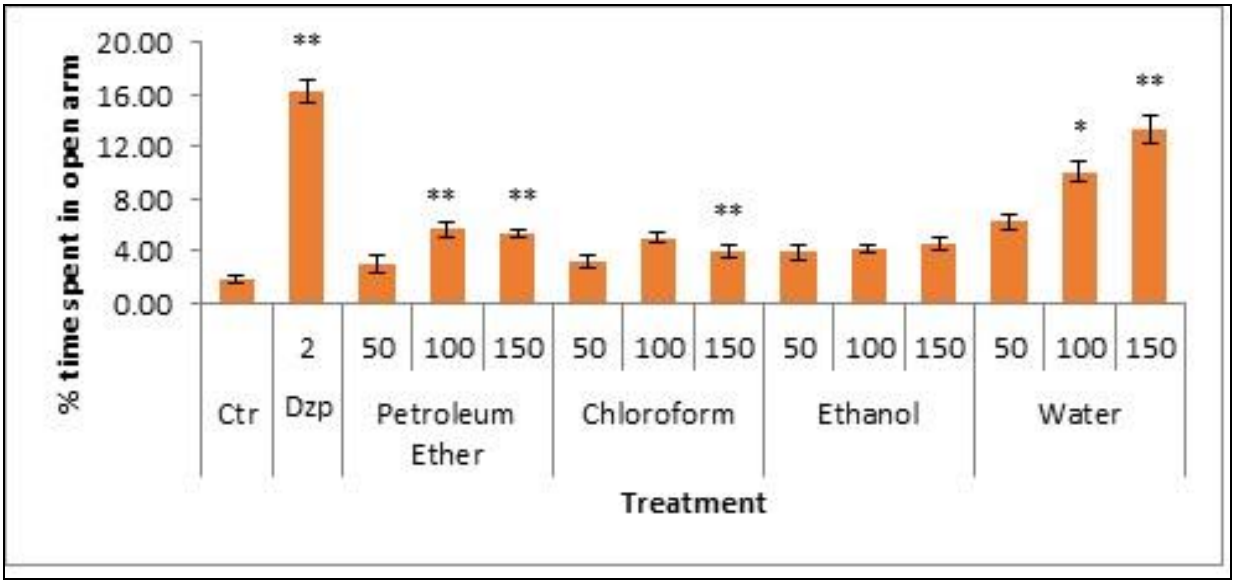
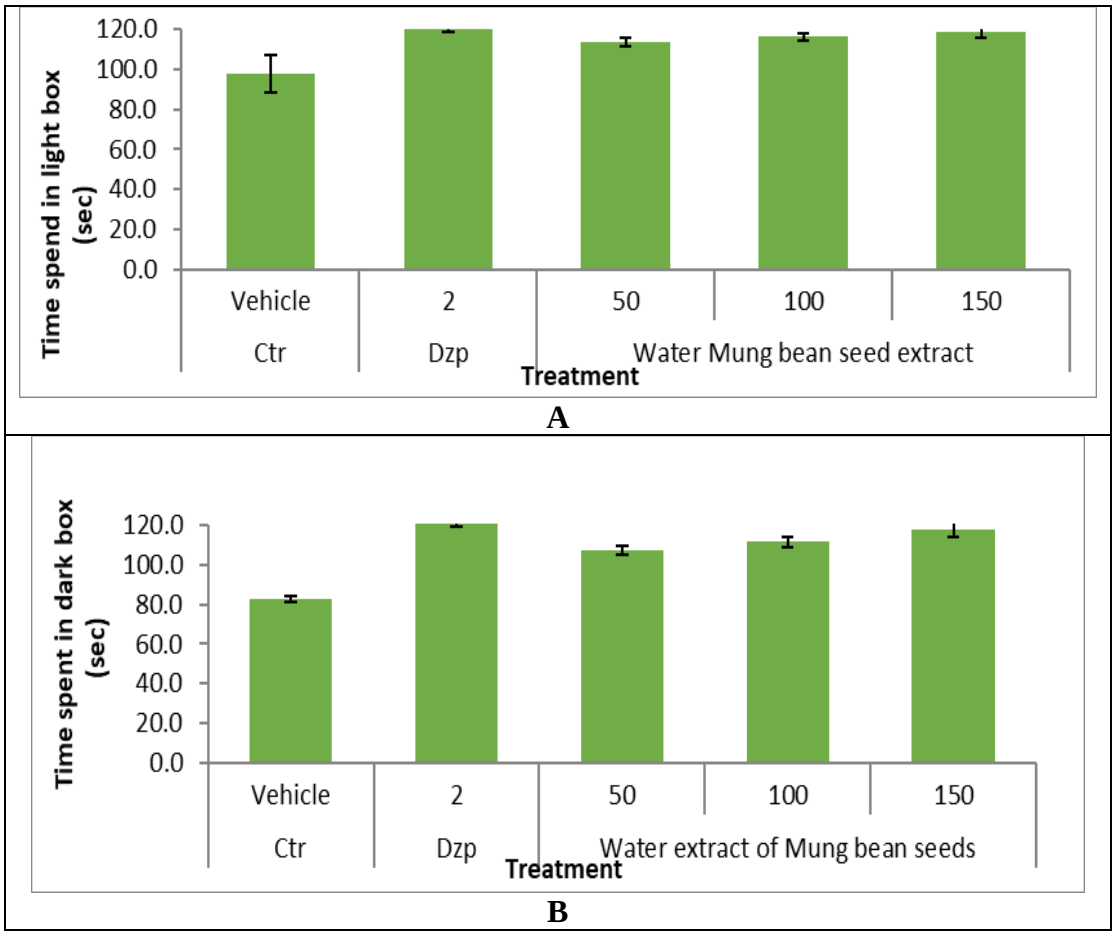


Fig 2: A comparative profile of a mean number of entries in open arms of EPM by mice treated with various extracts of seeds of Mung beans. Values are expressed as mean ± SEM. Number of animal (n) = 6. ** p <0.01, * p <0.05 relative to control.

Table 3: The effect of giving water extract of mung bean seeds on the conduct of female albino mice in the light and dark test model.

Treatment (mg/kg, p.o.)	Time spent in the lighted box (sec)	Time spent in the dark box (sec)	No. of crossings	Duration of immobility (sec)
Vehicle	97.8±6.4	82±1.1	19.0±1.12	198.2±12.0
Diazepam (1)	122±2.8	120.8±1.6	37.5±1.54	48.7±5.6**
Water extract of MB(50)	113.7±1.3	107.0±1.7	23.8±1.034	184.3±13.5
Water extract of MB (100)	116.5±1.2	111.5± 1.8	26.8±2.63	113.5±12.5**
Water extract of MB (150)	**119±2.2**	**118.0±2.8**	**30.8±2.22**	80.8±10.8**

Data are stated as average ± SEM. ANOVA test was ensued by Studentized Tukey's Test, ** p <0.01, * p <0.05 versus control, Number of animals (n) = 6



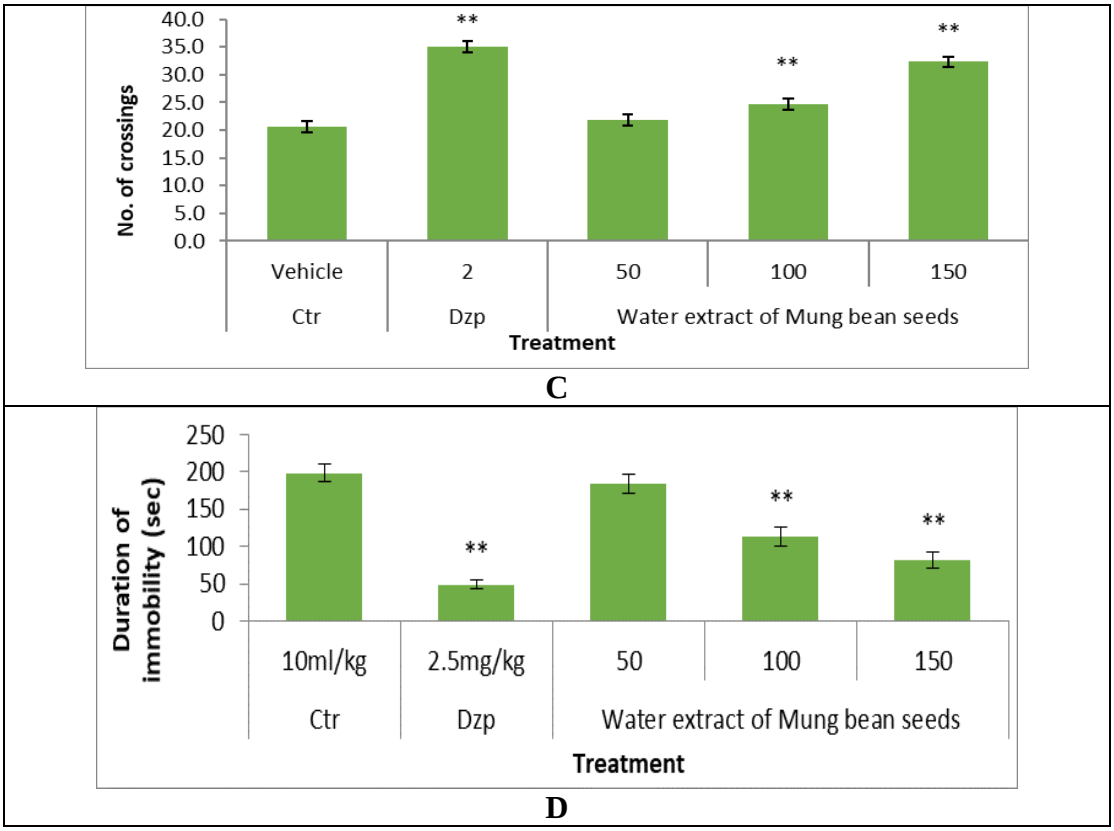


Fig 3: Graphical studies of water extracts of seeds of Mung beans in the light and dark test model in female albino mice as follows. Data have ensued as average ± SEM. ** p <0.01, * p <0.05 relative to control.

A. A comparative profile of time consumed in the lighted chamber (sec)
B. A comparative profile for Time consumed in the darkness box (sec)
C. A comparative profile of no. of crossings
D. A comparative profile of Duration of immobility (sec)

Table 4: Effects of different extract on mice in Locomotor activity

Treatment	Dose(mg/kg)	Locomotor activity
Control	10ml/kg	182.16±2.5
Diazepam	2mg/kg	67.66±2.4**
Water	50	184.33±9.7
	100	124.33±4.7**
	150	120.66±4.7**

Data are stated as average ± SEM. ANOVA test was ensued by Studentized Tukey's Test. Locomotor activity was measured for 5 min ** p <0.01, * p <0.05 relative to control, Number of animals (n) = 6

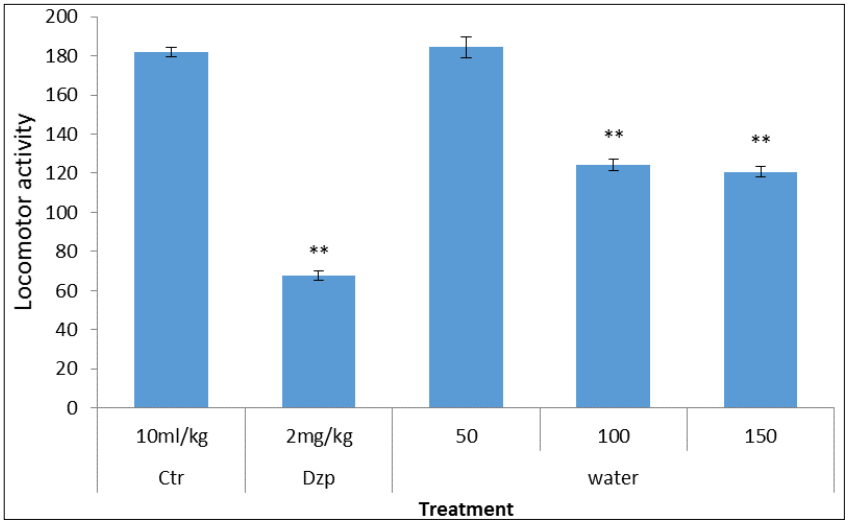


Fig 4: Comparative outline of mean locomotor activity by female Swiss albino mice treated with water extracts of seeds of Mung beans. These data are stated as average ± SEM. ** p <0.01, * p <0.05 relative to control.

Discussion

Benzodiazepine is an especially famous drug and used to cure numerous varieties of anxiety ailments, but due to its undesirable side effects, there is an urgent need to develop a marginal, exact, and possibly cost-free therapy. So, research has been focused to investigate to develop natural anxiolytic drugs. The water extract of seeds of Mung bean contains flavonoids, amino acids, carbohydrates, glycosides, tannins, saponins, and phenolic compounds. All extracts i.e., petroleum ether, chloroform, ethanol, and water extracts were recognized to be safe with no deaths in female Swiss albino mice when administered orally with 1000 mg/kg. Different doses of mung bean seeds extract *viz*. 50, 100, and 150 mg/kg were administered orally to mice. Further, water extract (150mg/kg) of the Mung bean seeds significantly improved the entries and mean time consumed in the wide-open arms in the EPM model. Also, time consumed in the light box and the dark box was found to be increased as well as increases duration of immobility (sec), and also a number of the crossing were found to be higher relative to the control group in the light and dark test. This outcome was comparable to the effect produced by diazepam at 2mg/kg. i.p. However, no changes in spontaneous locomotor activity were observed. The literature revealed that Mung bean seeds contain flavonoids and Gamma-aminobutyric acid and administration of GABA-enriched food is effective for the regulation of depression, sleeplessness [22]. As a result, Mung bean seeds can be used for the management of anxiety disorders.

Conclusion

This information suggests that the aqueous extract (150mg/kg) from the seeds of Mung Beans has anxiolytic effects without compromising motor activity. Water extract of Mung bean seeds had noticeable outcomes on the anxiety-related syndrome on exposure to the elevated plus-maze model and the light/dark test. Further studies are underway to finalize the anxiolytic compound from Mung bean seeds.

Acknowledgments

None

Conflict of Interest Statement

The author declares no conflict of interest, financial, or otherwise.

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