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Central nervous system activity of the methanol extracts of *Helianthus annuus* seeds in mice model

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ABSTRACT

Helianthus annuus seeds contain various chemical components and evaluate different biological activities. The present study was carried out to investigate the central nervous system (CNS) activity of methanolic extract of *Helianthus annuus* seeds in mice model. General behaviour, antidepressant activity and anxiolytic activity was observed. The results revealed that the methanol extract of *Helianthus annuus* seeds at 100 and 200 mg/kg caused a significant increase in the spontaneous activity (general behavioural profile), moderate increase in anxiolytic activity (light-dark box and elevated plus maze test) and remarkable increase in antidepressant activity (tail suspension test). The results suggest that methanol extract of *Helianthus annuus* exhibit significant antidepressant and moderate anxiolytic activity in tested animal models.

***Key Words*:** Sunflower seeds, asteraceae, general behaviour, antidepressant activity, anxiolytic activity.

INTRODUCTION

The sunflower seed is the fruit of the sunflower (*Helianthus annuus*). The term "sunflower seed" is actually a misnomer when applied to the seed in its pericarp (hull). These seeds are usually pressed to extract their oil. It is a potential protein supplement for human diet. However, the primary use of sunflower seed is not for edible protein, it is for oil because certain attributes of sunflower seed oil have particularly attractive to the food industry. It is the rich source of vitamins especially vitamin E. Sterols, saponins, flavonoids, and unsaturated terpenoids are the main chemicals revealed in the phytochemical test of *H. annuus*. Flavonoids have antidepressant as well as mild anxiolytic properties. It has been reported that flavonoids and their synthetic derivatives selectively bind to the central benzodiazepine receptors, and shows anxiolytic and other benzodiazepine-like effects in animal model (Onasanwo *et al.*, 2010). Flavonoids, saponin and terpinoids modulate the level of neurotransmitters such as serotonin, noradrenaline and dopamine (Patel *et al.*, 2014). As there are limited numbers of research work has been carried out on *H. annuus* seed extract this study was conducted to investigate the central nervous system effects (general behaviours, antidepressant and anxiolytic effects) of methanolic extract of *H. annuus* by using mice model.

MATERIALS AND METHODS

Animal

Albino male Swiss mice (22-25g) obtained from BCSIR (Bangladesh Council of Scientific and Industrial Research) Chittagong, were used in the study. The mice were kept at constant temperature (22±2°C) and 12-h light/12-h dark. Mice were fed standard laboratory food (Hind Lever diet pellets) and water was given *ad libitum*. Each animal was used once in the behaviour tests. The experimental protocols for this study were approved by the Institutional Ethical Committee of University of Science and Technology Chittagong (*Reference: USTC/2015/1822/02*) following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

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Plant material and extraction

300gm dried powder of seed was weighed and taken in an aspirator (2.5L). Before placing powders into the aspirator, the jar was washed properly with acetone and then dried. 800ml of solvent i.e. methanol was added gradually. The container with its content was sealed & kept for 20 days with occasional shaking & stirring. The major portion of the extractable compounds of the plant materials were dissolved in the solvent. Then whole mixture was filtered through cotton wool and the filtrate was concentrated by evaporation in dry and clean air. It was kept for 15 days to get the final extract of the seed.

Phytochemical screening

Preliminary Phytochemical screening of the powdered seed was performed for the presence of alkaloids, carbohydrates, flavonoids, steroid and triterpenoids (Trease and Evans, 1995) **(Table 1)**.

Drugs and chemicals

The following drugs were used: Diazepam, Imipramine, Propylene glycol and methanolic extract of *Helianthus annuus* seed.

General behavioural profiles

To determine the general behavioural profiles the test was performed by the method of Dixit and Varma (1976). Sixteen adult albino mice were divided in to four groups (n = 4). Methanol extract of *H. annuus* was administered at the dose of 100 and 200 mg/kg (i.p.) for the first two groups of animals respectively. The last two groups were administered propylene glycol (5 ml/kg) as a vehicle control and diazepam (5 mg/kg) as a drug control. For their behavioural changes the animals were kept under

Table 1: Chemical group test of *Helianthus annuus* seeds.

Phytochemicals	Test	Inference
Carbohydrates	Fehling's test	+
Alkaloids	Wagner's test	+
Flavonoids	Alcoholic test	+
Tannins	Ferric chloride test	-
Glycosides	Keller-Killiani test	-
Saponins	Foam test	-
Steroids	Libermann-Burchard test	+
Triterpenoids	Libermann-Burchard test	+
Gum	Molisch's test	-

observation, if any, at 30 min intervals in the first one hour and at the hourly intervals for the next 4 hour for the following parameters.

Awareness, alertness and spontaneous activity
The awareness and alertness was recorded by visual measure of the animal's response when placed in a different position and its ability to orient itself without bumps or falls The normal behaviour at resting position was scored as mild movement(+), moderate movement (++), strong movement (+++) and extreme movement as (++++). The spontaneous activity of the mice was recorded by placing the animal in a bell jar. It usually shows a significant degree of inquisitive behaviour. Moderate activity was scored as (++) and strong activity as (+++). If there is little motion, the score was (+), while if the animal sleeps, the score was (–). Excessive or very strong inquisitive activity like constant walking or running was scores as (++++). A similar test was performed with the same scoring, when the animals are removed from the jar and placed on a table (Ramanathan *et al.* 2008).

Pinna Reflex
It was measured by touching the centre of pinna with a hair or other fine instrument. The unaffected mouse withdraws from the irritating hair.

Grip Strength
It was measured by allowing the animal to grasp a pencil in the horizontal position and noting the time taken by the animal to drop the pencil on the table.

Touch response
The touch response was recorded by touching the mice with a pencil or forceps at the various part of the body (*i.e.*, on the side of the neck, abdomen and groin).

Pain response
The pain response was graded when a small artery clamp was attached to the base of the tail, and response was noted.

Anxiety model
Light-Dark Box Test
Crawley and Goodwin procedure (1980) was done to assess the anxiolytic activity of the compounds (light-dark box test). The apparatus consisted of a light compartment and a dark compartment. Light dark box is a rectangular box of 46 X 27 X 30 cm (l X b X h), which is divided into 2 compartments. A central opening (7 X 7 cm) on the floor level is placed for the joining of the two compartment. For this experiment, albino mice were divided into four groups, each group comprising of four animals. Vehicle (distilled water 10 ml/kg), standard (diazepam 1 mg/kg), and extract (100 and 200mg/kg) were administered *p.o.* One hour after administration, each mouse was placed individually in the illuminated part of the light/dark box. During the test session of 5 min., latency (the time it takes for the animal to move into the dark compartment for the first time), number of entries into the light and dark compartments, total time spent in the light compartment were recorded.

Elevated plus maze
The Elevated plus maze is structured by two open arms and two closed arms (50X10 X40 cm each) elevated to a height of 50 cm. Distilled water (10 ml/kg), *H. annuus* (100 and 200mg/kg) and diazepam (1 mg/kg) were administered p.o to 4 groups of 4 mice each. One hour post-treatment, each mouse was placed in turn in the centre of the maze facing one of the closed arms. The total time spent by each mouse in the open and closed arms of the maze and the number of entries was recorded for 5 minutes (Abidemi *et al.*, 2012).

Depression model
Tail suspension test
Tail suspension test was done by the method described by Steru *et al.*, (1985). The mice were suspended 60 cm above the surface of table with an adhesive tape placed 1 cm away from the tip of the tail. Immobility duration was recorded for the last 5 minutes. Mice were considered to be immobile when it did not show any movement of body and hanged passively. One hour prior to test, single administrations (p.o.) of *H. annuus* extract (100 and 200mg/kg) and Imipramine (60mg/kg) was given.

Statistical analysis
The results were expressed as mean±S.E.M. Statistical software SPSS® version 16 was used and ANOVA test was done for statistical analysis of difference between groups. The p values less than 0.05 were considered significant.

RESULTS

Response in general behavioural profile
H. annuus showed significant increase (++++) in grip strength and pain responses at dose of 200 mg/kg. It also showed strong movement (+++) response in spontenuous activity, pinna reflex and touch response at dose 200mg/kg. There was also moderate increase (++) in awareness and alertness at dose 200mg/kg. However, the standard drug diazepam caused mild increase in all these responses compared with the methanol extract of *H. annuus* (**Table 2)**.

Response in Light-Dark box test
H. annuus showed moderate increase in the latency of entry into the light box with peak effect produced at the dose of 200 mg/kg (72±0.85 seconds) compared to control (34±5.63 seconds). The effect at this dose was almost similar to that of diazepam (84.00±16.29 seconds). In respect of latency of entry into the light box and number of entries, the values for *H. annuus* showed moderately significant anxiolytic effect at the dose of both 100mg/kg (63±0.62 seconds) and 200 mg/kg (72±0.85 seconds) **(Table 3)**.

Response in Elevated plus maze
H. annuus produced a significant increase in the time spent in the open arms with peak effect produced at the dose of 100 mg/kg (51±0.62 seconds) relative to control (30.23±0.62 seconds). In respect of entry into open arms,

Table 2: Response in general behaviour profile with *H. annuus* seeds.

Behaviour	Extract (mg/kg)		Diazepam 5mg/kg	Propylene glycol
	100	200		
Awareness	+	++	+	-
Alertness	+	++	+	-
Spontaneous activity	+	+++	+	-
Pinna reflex	++	+++	+	-
Grip strength	++	++++	+	-
Touch response	++	+++	+	-
Pain response	++	++++	+	-

+ = mild movement; ++ = moderate movement; +++ =strong movement; ++++ =extreme movement

Table 3: Response in light-dark box test with *H. annuus* seeds.

Group	Dosage (mg/kg)	Number of entry in light box	Time in light box (seconds)
Control	-	4	34±1.65
Diazepam	1	8	84±1.65
H. annuus	100	5	63±0.62
	200	6	72±0.85

Table 4: Response in tail suspension test with *H. annuus* seeds.

Test Group	Dose (mg/kg)	Immobility duration (second)
Control	-	190.8 ± 0.75
Imipramine	60	30.2 ± 0.64
H. annuus	100	93 ± 0.47
	200	78 ± 1.3

the extract at the dose of 100 mg/kg significantly ($p<0.05$) increased the number of entries compared to control. The number of entries into the closed arms was reduced by *H. annuus* at doses of 100 and 200 mg/kg, and diazepam, with values of 90.7±0.64, 80±1.08, and 39.6±0.40 respectively, compared to control (110.7±0.70) which shows moderate anxiolytic activity **(Figure 1)**.

Response in tail suspension test

H. annuus showed significant antidepressant activity ($p<0.05$) by decreasing the immobility time (*H. annuus* 100mg/kg, 93±0.47; *H. annuus* 200mg/kg, 78±1.3) as compared with Imipamine (60mg/kg, 30.2±0.64) and control (190.8±0.75). There was significant difference between the effect of the various doses of *H. annuus* and that observed with control on the immobility time group when the mice were exposed to the tail suspension test **(Table 4)**.

DISCUSSION

The present study investigated the central nervous system effects of the methanolic extract from *Helianthus annuus seed* in mice. To the best of our knowledge and for the first time, this research work on *H. annuus* produced significant antidepressant-like effects and moderate anxiolytic effects. When assessed in Tail Suspension Test (Steru *et al.*, 1985), the fraction was able to induce antidepressant-like effects after oral administration of varying doses of *H. annuus* with the 100mg/kg and 200mg/kg dose showing the highest immobility. Based on these findings, it can be said that *H. annuus* which decreases the immobility time in tail suspension test is similar to the mechanism of fluoxetines via the serotonin system in depression.

H. annuus can also mediate its activity through the same mechanism as that of Imipramine. Imipramine belongs to the class of tricyclic antidepressant drugs

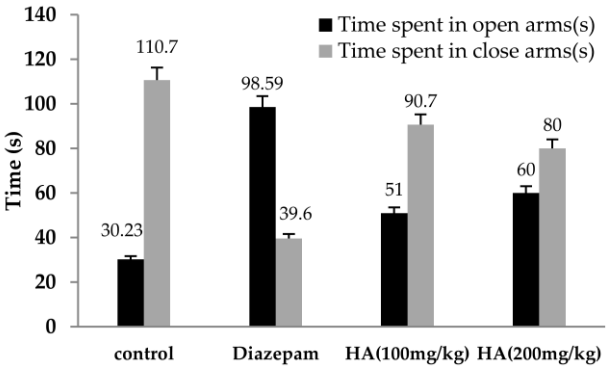

Figure 1: Response in Elevated plus maze test with *H. annuus* seeds. Data presented as average±SEM, n=4.

which blocks the reuptake of norepinephrine (NE) and 5-hydroxytryptamine (5-HT) into their respective neurons. The light-dark and elevated plus maze tests which were used to explore the anxiolytic potentials of *H. annuus*, where 200mg/kg dose showed moderate anxiolytic properties. Our study showed significant anxiolytic effect in light-dark box test (Young and Johnson, 1991) in compared to elevated plus maze test which showed moderate anxiolytic activity.

H. annuus was able to show a moderate anxiolytic-like properties in elevated plus maze test with the time the mice spent at the opened arm being more than the observed time with the mice that were given Diazepam. It is possible that each chemical constituents of the fraction exhibited the biological activity influencing on the neuro behaviours involving antidepressant activity.

However, the precise mechanism underlying *H. annuus* activity will still require further investigations. This might have been attained through its influence on the levels of monoamines. This research work has eliminated the involvement of neurotoxicity in the use of *H. annuus* for pharmacotherapy in anxiety and depression.

CONCLUSION

The results obtained in this study indicate that the methanol extract of the seed of *Helianthus annuus* have effect on central nervous system with significant antidepressant and moderate anxiolytic activity in different *in-vivo* animal model. The medicinal values of the plant seed may be related to their constituent phytochemicals. So, further detailed investigations are needed to isolate and identify the active compounds present in the plant extract and its various fractions and their efficacy need to be done. It will help in the development of novel and safe drugs for the treatment of different types of central nervous system disorders.

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