IN VIVO ANTIDEPRESSANT AND ANXIOLYTIC ACTIVITY OF THE METHANOL EXTRACT OF HELIANTHUS ANNUUS SEEDS

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Abstract:

Objective: Helianthus annuus seeds contain various chemical components and evaluated for different biological activities. The present study was carried out to investigate the antidepressant and anxiolytic activity of methanolic extract of Helianthus annuus seeds in mice model.

Methods: The efficacy of extract (100-200mg/kg) was compared with standard antidepressant drug Imipramine 60 mg/kg and anxiolytic drug diazepam (1mg/kg). Antidepressant activity was done in mice model by tail suspension test and anxiolytic activity was determined both light-dark box test and elevated plus maze test.

Results: The results revealed that the methanol extract of Helianthus annuus seeds showed a significant result in tail suspension test at 100 mg/kg (93 ± 0.47 seconds) and 200 mg/kg (78 ± 1.3 seconds) and moderate result in light-dark box test at 100 mg/kg (63±0.62) and 200 mg/kg (72±0.85), in elevated plus maze test at 100 mg/kg (51±0.58 seconds) and 200 mg/kg (60±0.62 seconds).

Conclusion: The results suggest that methanol extract of Helianthus annuus exhibit significant antidepressant and moderate anxiolytic activity in tested animal models.

Key Words: Helianthus annuus, antidepressant activity, anxiolytic activity, mice model.

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 specially vitamin E. Sterols, saponins, flavonoids, and unsaturated terpenoids are the main chemicals revealed in the phyto chemical test of H. annuus. Satisfactory results from CNS-antidepressant activity tests reveal that the H. annuus display a behavioral profile that is consistent with an anxiolytic and CNS-antidepressant action. It is possible that the presence of several polyphenols in the extract, could account for its effect on CNS. 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 [1]. Flavonoids, saponin and terpinoids modulate the level of neurotransmitters such as serotonin, noradrenaline and dopamine [2].

As there are limited numbers of research work has been carried out on H. annuus seed extract and presence of flavonoids, terpenoids have effects on central nervous system, so this study was conducted to investigate the central nervous system effects (antidepressant and anxiolytic effects) of methanolic extract of H anuuus by using mice model.
Material 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 behavior tests. The experimental protocols for this study were approved by the Institutional Ethical Committee of University of Science and Technology Chittagong (USTC/2015/1850/02) following the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Collection of seeds:
The air dried seeds of *H. annuus* was collected at their matured form from Bangladesh Agriculture Research Institute, Gazipur, Bangladesh in June 2014 (Voucher No. 56). Undesired plant parts or additives were removed after the collection. Then it was dried through air but absences of sun light for several days. The seeds were ground into fine powder with the help of electric grinder. Then the powder was stored in air tight container and placed in a cool, dry and dark place.

Plant material and extraction
300gm dried powder of seed was weighed & taken in a 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 & clean air. And 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 [3].

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Test</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>Fehling’s test</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Wagner’s test</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Alcoholic test</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride test</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Keller-Killiani test</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>Libermann-Burchard test</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>Libermann-Burchard test</td>
<td>+</td>
</tr>
<tr>
<td>Gum</td>
<td>Molisch’s test</td>
<td>-</td>
</tr>
</tbody>
</table>

Drugs and chemicals
The following drugs were used: Diazepam (Square Pharmaceutical Ltd, Bangladesh), Imipramine( Novartis Bangladesh Ltd) and methanolic extract of *Helianthus annuus* seed.

Depression model
Tail suspension test
Tail suspension test was done by the method described by Steru [4]. 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.
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) [5]. 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 (50 x 10 x 40 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 [6].

Statistical analysis
The results were expressed as mean ± S.E.M. Statistical analysis of difference between groups was evaluated by ANOVA test. The p value less than 0.01 and 0.05 were considered high significant and moderate significant.

Results
Response in tail suspension test
H. annuus showed significant antidepressant activity (p<0.01) by decreasing the immobility time (H. annuus 100mg/kg, 93 ± 0.47; H. annuus 200mg/kg, 78 ± 1.3) as compared with Imipramine (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 1)

Table 1: Response in Tail Suspension test with H. annuus seeds.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Immobility duration (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>190.8 ± 0.75</td>
</tr>
<tr>
<td>Imipramine</td>
<td>60</td>
<td>30.2 ± 0.64</td>
</tr>
<tr>
<td>H. annuus</td>
<td>100</td>
<td>93 ± 0.47*</td>
</tr>
<tr>
<td>H. annuus</td>
<td>200</td>
<td>78 ± 1.38**</td>
</tr>
</tbody>
</table>

All values are mean ±SEM (n=4); **p< 0.01, *p< 0.05 when compared to control.

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 2)
Table 2: Response in Light-dark box test with *H. annuus* seeds

<table>
<thead>
<tr>
<th>Group</th>
<th>Dosage (mg/kg)</th>
<th>Number of entry in light box</th>
<th>Time in light box (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>4</td>
<td>34±1.65</td>
</tr>
<tr>
<td>Diazepam</td>
<td>1</td>
<td>8</td>
<td>84±1.65</td>
</tr>
<tr>
<td><em>Helianthus annuus</em></td>
<td>100</td>
<td>5</td>
<td>63±0.62</td>
</tr>
<tr>
<td><em>Helianthus annuus</em></td>
<td>200</td>
<td>6</td>
<td>72±0.85</td>
</tr>
</tbody>
</table>

All values are mean ±SEM (n=4); **p< 0.01, *p<0.05 when compared to control.

**Response in Elevated plus maze**

*H. annuus* produced a moderate increase in the time spent in open arms at the dose of 200 mg/kg (60±0.62 seconds) relative to control (30.23±0.62 seconds). In respect of entry into open arms, the extract at the dose of 200 mg/kg moderately 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 80.7±0.64, 90±1.08, and 39.6±0.40 respectively, compared to control (110.7±0.70) which shows moderate anxiolytic activity (P< 0.05) (Figure 1)

![Figure 1: Response in Elevated plus maze test with *H. annuus* seeds](image-url)
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 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 fluoxetine 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 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 [7] 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 behaviors involving antidepressive 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 significant effect on antidepressant and moderate effect on 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.

References