Evaluation of Antidepressant and Anxiolytic Activity of Wheat Germ Oil in Experimental Animals

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ABSTRACT

Traditional medicine has long employed wheat germ oil for its antioxidant, antifungal, and antipsychotic properties. Studies on the antidepressant and anxiolytic properties of wheat germ oil do not exist, though. In this work, rats were used to test the SCF method's antidepressant and anxiolytic effects on wheat germ oil extract. We discovered that giving 200mg/kg WGO for 7 days dramatically decreased the amount of time that FST spent immobile. The percentage of time spent and the number of entry into the open arms of the EPMT considerably increased after oral administration of 200 mg/kg WGO or 4 mg/kg diazepam for 7 days. Rats spent more time in the LDBT's lit side, which extended their stay there. These findings corroborate wheat germ oil's possible anxiolytic and antidepressant properties and support the historic usage of wheat germ oil in the management of emotional problems.

Keywords: Wheat germ oil, anxiolytic, antidepressant, elevated plus maze, Forced swim test.

INTRODUCTION

Mental illness and behavioural problems affect around 45 million individuals globally, making up 12.3 percent of the global disease burden ¹. By 2020, it is predicted that this number would increase to 15% ². The two most common psychiatric diseases among the various mental illnesses and behavioural disorders are depression and anxiety ³. The treatment of these disorders involves the use of several traditional anxiolytic and antidepressant medications, including monoamine oxidase inhibitors, benzodiazepines, selective serotonin reuptake inhibitors, tricyclic antidepressants, noradrenergic, serotonin-norepinephrine reuptake inhibitors, and specific serotonin antidepressants. However, using the aforementioned medications to treat a condition can also result in unfavourable side effects, such as weight gain, sexual dysfunction, cardiovascular toxicity, and drug interactions ⁴-⁶. Therefore, the creation of potent anxiolytic and antidepressant medicines with no or minimal side effects is urgently needed.

Many traditional Chinese medicinal herbs have been used effectively in recent years to prevent or cure anxiety and depression, including Paonia lactiflora ⁷, Acorns calamus⁸, Ginkgo biloba ⁹, Acanthopanax senticosus ¹⁰, Albizia julibrissin ¹¹ and Hypericum perforatum ¹². To treat mental problems, traditional Chinese medicines may be a viable alternative.

The Poaceae family includes annual plants like wheat (Triticum L.). It is a good source of minerals, unsaturated fatty acids, vitamin E, vitamin B group, proteins, and dietary fibre. Additionally, it is said to be relatively inexpensive rich in beneficial phytochemicals such glutathione, sterols, flavonoids, and octacosanol. Consequently, WG is regarded as a nutritious meal that can aid in the prevention of several cancers and other illnesses. Raw wheat germ (RWG), which contains up to 10% to 15% oil, is primarily employed as an oil source in the culinary, pharmaceutical, and cosmetic sectors ¹³. Recently, a lot of study has been concentrated on the possible use of wheat as a material. Wheat germ oil is a laxative, lowers fats, and protects and nourishes the skin. Given the abundance of polyunsaturated fatty acids and vitamin E, it is a beneficial diet¹⁴. Tocopherols and tocotrienols, which are components of vitamin E, are powerful inhibitors of lipid oxidation in dietary and biological systems. Because only plants can produce tocopherols, they are an essential nutritional component for both humans and animals ¹⁵, ¹⁶.

The present study investigated the anxiolytic and antidepressant activities of WGO in rats, at doses of 200, and 400mg/kg/day, using the light-dark box test (LDBT) and the elevated plus-maze test (EPMT), and investigated the antidepressant activities of WGO by the forced swimming test (FST).

MATERIALS AND METHODS

Chemicals

The chemicals used in the experiment include diazepam and Imipramine (Intlas Pharmaceuticals, India), tween 80
(Research-lab fine Chem Industries, India), and ethyl alcohol (Changshu Yangyuan Chemical, China).

**Plant Material**
The fresh parts of Wheat were purchased from local market in Local Market.

**Experimental animals**
Albino Wistar rats weighting 140-180 g were obtained from rodent breeding unit of the Aryakul College of Pharmacy and Research, Lucknow, India. The animals were housed under standard environmental conditions and were allowed free access to tap water and standard laboratory pellet ad libitum. The ethical handling of rats used in our study and the experimental protocols used were approved by Institutional Animal Ethics Committee.

**Extraction of Oil**
The supercritical fluid extract technique was used to extract 150 grammes of fresh wheat germ oil over the course of around 3 hours. Following extraction, the first separator was used to collect the oil, while the second was used to collect the water and volatile substances. Following collection, the amount of extracted oil was evaluated gravimetrically, and the yield of wheat germ oil is reported as a percentage of weight. 17

**Institutional Ethical Committee Approval**
The Institutional Animal Committee (IAEC) has approved the experimental protocols for the anxiolytic and anti-depressant activity and approval number is 1896/PO/Re/S/16/CPCSEA/2022/5.

**In Vivo Anxiolytic Activity**
- **Animal:** Albino Wistar rats
- **Age and Weight:** 8-11 weeks/ 140-180 gms
- **Gender:** Male/Female

**Table 1:** Experimental Design for Anxiolytics Activity

<table>
<thead>
<tr>
<th>Groups (N=5)</th>
<th>Treatment and route of Administration</th>
<th>Dose and duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>Normal Saline</td>
<td>10ml/kg</td>
</tr>
<tr>
<td>Positive control/ Standard</td>
<td>Diazepam</td>
<td>10mg/kg (1 day)</td>
</tr>
<tr>
<td>(subcutaneously)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment group (I)</td>
<td>Wheat germ oil</td>
<td>200 mg/kg (7 days)</td>
</tr>
<tr>
<td>Low dose (orally administrated)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment group (II)</td>
<td>Wheat germ oil</td>
<td>400 mg/kg (7 days)</td>
</tr>
<tr>
<td>High dose (orally administrated)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Elevated Plus Maze (EPM)**
The test was carried out using equipment that Lister had approved 18. The number of entries made into the open and closed arms, as well as the time spent in them, were recorded using a video camera for the following five minutes after each rat was put in the centre of the labyrinth facing one of the open arms. These statistics were used to compute the proportion of entries and the amount of time spent in each arm.

**Light-Dark Box Test (LDBT)**
The light-dark box test was conducted using the Costall et al. 19 approaches. The testing equipment was a plexiglass box (45 cm long, 27 cm wide, and 27 cm high) divided into two compartments, each having a white surface that was 60% brightly lighted and 40% dark. Rats were positioned individually facing one of the light sources in the centre of the lit enclosure. Five minutes of the time spent in the light box was recorded. Entry inside the light box was thought to be a sign of reduced anxiety. 20

**In Vivo Anti-Depressant Activity**
- **Animal:** Albino Wistar rats
- **Age and Weight:** 8-11 weeks/ 140-180 gms
- **Gender:** Male/Female

**Table 2:** Experimental Design for Anti-Depressant Activity

<table>
<thead>
<tr>
<th>Groups (N=5)</th>
<th>Treatment and route of Administration</th>
<th>Dose and duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>Normal Saline</td>
<td>10ml/kg</td>
</tr>
<tr>
<td>Positive control/ Standard (i.p)</td>
<td>Imipramine</td>
<td>10 mg/kg (twice a day for one weeks)</td>
</tr>
<tr>
<td>Treatment group (I)</td>
<td>Wheat germ oil</td>
<td>200 mg/kg (7 days)</td>
</tr>
<tr>
<td>Low dose (orally administrated)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment group (II)</td>
<td>Wheat germ oil</td>
<td>400 mg/kg (7 days)</td>
</tr>
<tr>
<td>High dose (orally administrated)</td>
<td></td>
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</tr>
</tbody>
</table>

**Forced Swimming Test**
Individual rats were made to swim for 15 minutes in a glass beaker with a 1 cm diameter and 15 cm height filled with fresh water to a 6 cm height, at a temperature of 27.2 °C. The "pre-test" session ended here. Each mouse was once more made to swim in the same setting for 6 minutes in a "test-session" twenty-four hours later. The test session was performed before and following the medication treatment (0 day apart) (on 8th day). When the rats float still or move barely enough to maintain their heads above the water's surface, they are deemed immobile. The duration of the immobility for the final four minutes of the six-minute test was timed. 21

**Collection of Serum**
After 24 hours after the last treatment, ketamine (60 mg/kg) and xylazine (5 mg/kg) were administered intraperitoneally to induce anaesthesia. Animal experimentation blood samples were collected using the retro orbital sinus puncture method. The blood was moved...
to a clean container once it had been collected. For the purpose of blood coagulation, the blood sample containers were maintained at 37 °C for 40 min. The remaining serum from the test animals was added to the centrifuge tube after the clot was removed from the container. Serum-filled centrifuge tubes underwent 10 minutes of 3000RPM centrifugation. The clear serum that resulted was poured into a clean container and refrigerated. Erba diagnostic kit and semi-auto analyzer are used for these estimations.

**Statistical Analysis**

Results from the pharmacological screening were expressed as Mean ± standard error of the mean (SEM). Differences between the control and treatment groups in the experiments were tested for significance using unpaired student’s ‘t’ test. values of P<0.05 were considered as statistically significant.

**RESULTS AND DISCUSSION**

**Elevated Plus maze method**

Diazepam treated rats showed significant increase (P < 0.05) in the number of open arm entries, time spent in open arms and the number of rears in the open arm. They showed a reduction in the time spent in closed arm. Wheat germ oil treated rats exhibited significant increase (P<0.05) in the number of open arm entries (200 and 400 mg/kg), time spent in open arm, percentile ratio of open arm to total arm entries, the number of total arm entries, and the number of rears in the open arm entries, but decrease in time spent in closed arm.

![Figure 1: Anti-Anxiolytic Effects of Wheat Germ Oil in the Elevated Plus Maze Test in Albino Rats.](image1)

**Table 3: Anti-Anxiolytic Effects of Wheat Germ Oil in The Elevated Plus Maze Test in Albino Rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time spent in (sec)</th>
<th>Entries on (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enclosed arms</td>
<td>Open arms</td>
</tr>
<tr>
<td>NC</td>
<td>254.18 ±20.45</td>
<td>38.60 ± 5.14</td>
</tr>
<tr>
<td>TC</td>
<td>186.11 ± 16.73</td>
<td>88.11 ± 7.87</td>
</tr>
<tr>
<td>T1</td>
<td>203.87 ± 17.71</td>
<td>66.23 ± 8.65</td>
</tr>
<tr>
<td>T2</td>
<td>208.11 ± 19.85</td>
<td>68.61 ± 7.98</td>
</tr>
</tbody>
</table>

NC= Normal control; TC=Treatment control; T1=Treatment group (I) 200 mg/kg (orally administrated); T2= Treatment group (II) 400 mg/kg (orally administrated). Effect of the Wheat germ oil on the number of times spend a number of entries in the open arm and enclosed arm in rat. Wheat germ oil (200 mg/kg, po), (400 mg/kg, po) and diazepam (1 mg/kg, po). Each values represents mean ± SEM of rats. The data was Analyzed ANOVA followed by Dunnett’s test. ***P.a <0.001 **P<0.01. Show significant different as compared to vehicle control group.

**Light/ Dark Model**

![Figure 2: Evaluation of anxiolytic activity by light/dark method of wheat germ oil.](image2)

In the light/dark test, anxiety is generated by the conflict between the tendency to explore and the initial tendency to avoid the unfamiliar and can be evaluated according to the number of transitions in to and the time spent in the light chamber where in increase in these parameters is considered to reflect anxiolytic-like properties. Our results showed that the extract (200 mg/kg) increased time spent in the light chamber, suggesting anxiolytic action.
Anti-Depressant Activity

Wheat germ oil on forced swimming test

Figure 3: Effect of Wheat germ oil on immobility time in Forced swimming test.

Biochemical Estimation

Figure 4: A) AST, B) ALT and C) LDH. Data are represented as mean ± SD (n=8). Statistically significant differences were observed between carcinogen control and test groups (one-way ANOVA followed by Bonferroni multiple comparison test; ***p<0.001, **p<0.01 and *p<0.05)

Figure 5: A) Bilirubin, B) Biliverdin. Data are represented as mean ± SD (n=8). Statistically significant differences were observed between carcinogen control and test groups (one-way ANOVA followed by Bonferroni multiple comparison test; ***p<0.001, **p<0.01 and *p<0.05)
CONCLUSION

It is clear from the current study that wheat germ oil has antidepressant and anxiolytic properties in experimental mice. As a result, depression and anxiety can be prevented and treated with this composition. The current study offers new avenues for future studies on this herbal medicine and treated with this composition. The current study provides evidence that wheat germ oil is effective in treating depression and anxiety in mice. As a result, depression and anxiety can be prevented and treated with this composition. The current study offers new avenues for future studies on this herbal medicine and treated with this composition.

REFERENCES


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