Research Article

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.

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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 serotonergic 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 *Paeonia lactiflora* [7], *Acorns calamus*[8], *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 inexpensively rich in beneficial phytochemicals such glutathione, sterols, flavonoids, and octacosanols. 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 diet[14]. 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

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

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

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

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. [22]

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.

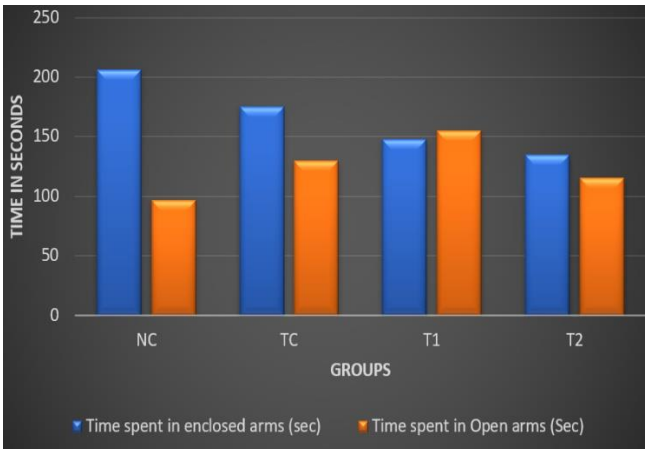

Figure 1: Anti-Anxiolytic Effects of Wheat Germ Oil in the Elevated Plus Maze Test in Albino Rats.

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 is difference between open arm visit into enclosed arm into 4 groups (n=5 in each group). 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.

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

Groups	Time spent in (sec)		Entries on (N)	
	Enclosed arms	Open arms	Enclosed arms	Open arms
NC	254.18 ±20.45	38.60 ± 5.14	11.67± 2.09	4.32 ± 1.23
TC	186.11 ± 16.73	88.11 ± 7.87	3.11± 1.21	9.57±1.62
T1	203.87 ± 17.71	66.23 ± 8.65	5.32± .69	9.83 ± 1.37
T2	208.11 ± 19.85	68.61 ± 7.98	8.21± 2.37	10.56 ± 1.21

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 and 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

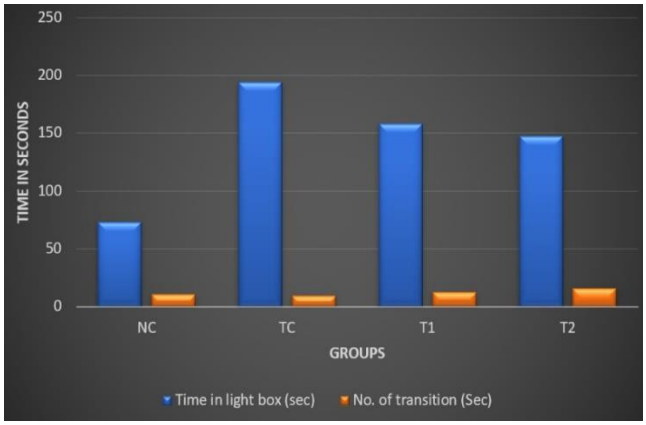

Figure 2: Evaluation of anxiolytic activity by light/dark method of wheat germ oil.

NC= Normal control; TC=Treatment control; T1=Treatment group (I) 200 mg/kg (orally administrated); T2= Treatment group (II) 400 mg/kg (orally administrated). 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.

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

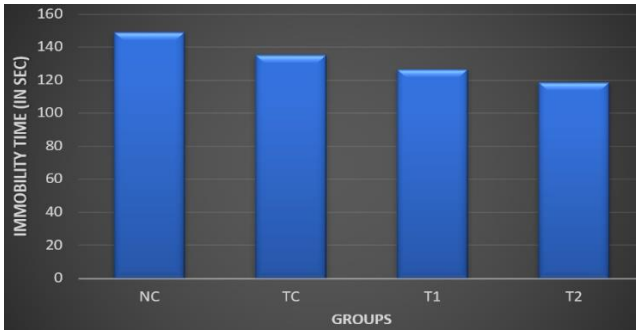

Figure 3: Effect of Wheat germ oil on immobility time in Forced swimming test.

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 wheat germ oil on FST in Wister albino rats. Imipramine=10mg/kg, T1= 200 mg/kg, T2= 400 mg/kg. Values are given as mean ±SEM (N=5 in each group), ***P<0.001, as compared to control.

The FST model is the more widely used animal model for screening antidepressant activity . Imipramine (10mglkg), Wheat germ oil (200, 400mg/kg) significantly decreases the duration of immobility by 37.43%, 57.44% and 67.32% respectively. Activity of wheat germ oil, in the doses studied is identical to that of im1pranune.

Biochemical Estimation

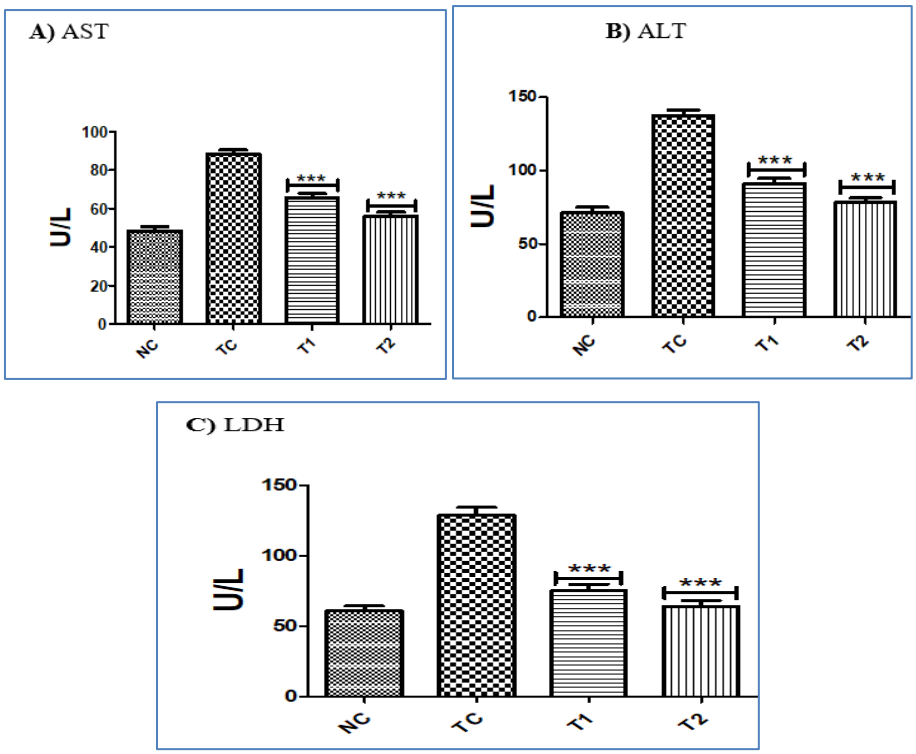

Figure 4: A) AST, B) ALT and C) LDH. Data are represented as mean ± SD (n=8). Statistically significant differences were observed between carcinogen control and test groups (one-way ANOVA followed by Bonferroni multiple comparison test; ***p<0.001, **p<0.01 and *p<0.05)

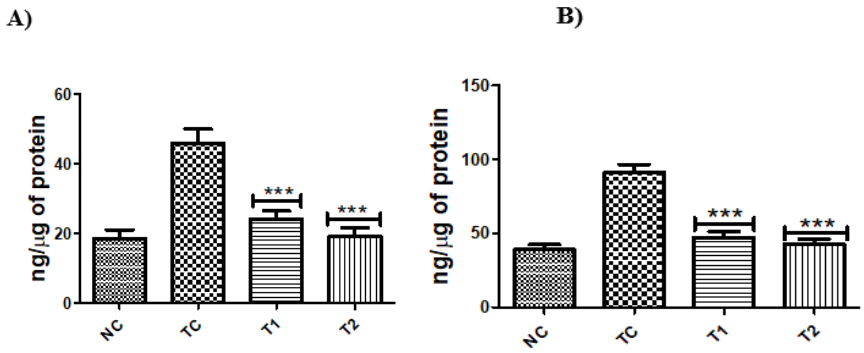

Figure 5: A) Bilirubin, **B)** Biliverdin. Data are represented as mean ± SD (n=8). Statistically significant differences were observed between carcinogen control and test groups (one-way ANOVA followed by Bonferroni multiple comparison test; ***p<0.001, **p<0.01 and *p<0.05)

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 up new avenues for future studies on this herbal medicine at various benefits, as an adjuvant to and in contrast with other anti-depressants and anxiolytic medications. Future integrated care of certain mental diseases might benefit from this.

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