



**A COMPARATIVE STUDY OF THE EFFECT OF *SYZYGIVM CUMINI* AND WHEAT
GRAIN ON THE ANTI-ANXIETY ACTIVITY OF BLONANSERIN**

Gautam Nishu and Das Sanjita*

Pharmacy Institute, Pharmacy Institute, Noida Institute of Engineering and Technology, Greater Noida, Uttar Pradesh
(India).

***Corresponding Author: Das Sanjita**

Pharmacy Institute, Pharmacy Institute, Noida Institute of Engineering and Technology, Greater Noida, Uttar Pradesh (India).

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ABSTRACT

Objectives: Blonanserin is frequently used for the treatment of anxiety disease. This study includes the effect of *Syzygium cumini* and wheat grains on the drug (blonanserin) because the patients mostly take the wheat in their diet in India and other countries and *Syzygium cumini* is one seasonal fruit with anti-anxiety activity. **Methods:** This is a noble approach to assess the comparative study of the effect of *Syzygium cumini* and wheat grains on the anti-anxiety activity of blonanserin. The plant extract (200 mg/kg and 400 mg/kg) and the drug (2mg) were used for the evaluation of anti-anxiety activity using actophotometer, elevated plus maze and Y-maze. **Results:** Actophotometer, Elevated plus maze, Y-maze apparatus that wheat grain has the better anti-anxiety activity from then the *Syzygium cumini* but is comparatively less than the blonanserin grain showed, Whereas blonanserin with wheat grain showed significantly higher anti-anxiety activity than blonanserin alone. **Conclusions:** The present investigation shows that wheat grain can be advised to add to the diet of anxiety patient to improve the efficacy of blonanserin and the patients can also take *Syzygium cumini* in their diet for better effect.

KEYWORDS: Wheat grain, *Syzygium cumini*, blonanserin, anti-anxiety activity.

INTRODUCTION

An optimal health improves or maintains by the healthy person. In case of anxiety, healthy diet really makes a difference. Diet may help stay calm. Some diet to keep in mind for the diet of anxiety patient like Asparagus, Avocado, Blueberries, Milk, Almonds, Oranges, Salmon, Spinach, Turkey etc.^[1] Depression, stress, anxiety are of prevalent and highly psychiatric conditions in the world, which are defined as a negative emotional experience and consider with biochemical, behavioral and psychological changes. Herbal medicines have been widely used among sufferers of mood and anxiety disorders since antiquity.^[2] *Syzygium cumini* is an evergreen tropical tree belonging to the family Myrtaceae. *Syzygium cumini*, commonly known as jambolana, java plum, black plum or jamun.^[3] It is found in Bangladesh, India, Nepal, Pakistan, Sri Lanka, Philippines and Indonesia.^[4] *Syzygium cumini* was scientifically investigated to have prominent effect on CNS.^[5] Wheat grains is used for controls obesity, increases energy, reduces chronic inflammation, prevents metabolic disorders, prevents gallstones, improves metabolism, prevents breast cancer, prevents colon cancer, prevents childhood asthma etc.^[6] In the present study blonanserin is considered to be the marketed anti-anxiety agent, with which the effect of the extracts are to be compared.^[7]

MATERIALS AND METHODS

Collection of plant material and preparation of methanol extract of *Syzygium cumini* and wheat grains

The *Syzygium cumini* and Wheat Grains was collected from the local area of the Greater Noida. The plant was authenticated as *Syzygium cumini* (Myrtaceae) and Wheat grain (Poaceae) at National Bureau of Plant Genetic Resources (NBPGR), Pusa Institute of Agriculture, and New Delhi (Specimen No: NHCP/NBPGR/2017-6 and NHCP\NBPGR\2018-16). The dried crushed powder of *Syzygium cumini* and wheat grains was treated with methanol solvent (1000 ml) till 7 days at room temperature undergone maceration process. The extracts were separated with the help of vacuum filtration and collected into a container. The methanol extract of *Syzygium cumini* and wheat grains were subjected to the various phytochemical tests and then subjected to the pharmacological investigations.^[8]

Drugs and chemicals

Blonanserin (Intas Phramaceuticals Ltd., South Sikkim, India) was used as the standard anti-anxiety drugs. Methanol (Sigma Aldrich chemical Pvt. Ltd Bangalore, India) was purchased and used as analytical grade. Distilled water was used as vehicle.

Preparation Methanolic extract of the plant

The fruit of the plant *Syzygium cumini* and wheat grains were dried and crushed by the mechanical grinder. The crushed powder was treated with methanol solvent (1000ml) till 7 days at room temperature by maceration process after 7 days the extract was filtered and the filtrate was concentrated over vacuumed filter and the solvent was evaporated to get the extract for investigations.

The extracts obtained were subjected for the primarily investigation for the presence of various chemical constituents such as alkaloids, glycosides, carbohydrates, gums and mucilaginous substances, flavonoids, phenols, starch, fibers, saponins, tannins, steroids, sterols, and proteins.^[9]

Animals

Swiss albino mice weighing between 20-25 grams of either sex were utilized for the present study to induce hemorrhoids and irritable bowel syndrome. They were obtained from Central Animal House of NIET Pharmacy Institute, Greater Noida. The animals were housed in standard polypropylene confines at $25 \pm 2^{\circ}\text{C}$ with 12 hrs light and day cycle. Every one of the animals was free access to standard rodent feed, and palatable water. The animals were given a period of seven days for acclimatization to laboratory conditions. The protocol of the present study using experimental animals with protocol number IAEC/NIET/2017/01/02 was approved by Institutional Animals Ethics Committee (1845/PO/Re/S/16/CPCSEA).

EVALUATION OF ANTIANXIETY ACTIVITY

Gross Behavioral Study

The behavioral studies of the methanol extract of *Syzygium cumini*, wheat grains and blonanserin were performed on mice at the dose of 200 mg/kg, 400 and 8 mg/kg. The following behavioral studies were performed to get maximum information about the effect of *Syzygium cumini*, wheat grains and blonanserin on the CNS.

Righting reflex, pinna reflex, corneal reflex, muscle tone, motor activity, cataleptogenic activity, traction test, stereotyped behavior, awareness, somersault test, muscle relaxant activity using rotaroad test and locomotor activity using actophotometer.^[10]

Elevated Plus Maze Test

The elevated plus maze is mostly used as a behavioral assay for rodents and it had been validated to assess the anti-anxiety effect. The mice were placed at the junction of the four arms of the maze, facing an open arm, and number of entries/duration in each arm was observed for 5 minutes. An increase in open arm activity (duration and/or entries) reflected the anti-anxiety behavior. The evaluation of the anti-anxiety activity of the *Syzygium cumini*, wheat grains and blonanserin at 200, 400 and 8

mg/kg p.o. respectively were performed using Elevated plus maze.^[11-12]

Locomotor activity

By using an actophotometer the locomotor activity was measured. The locomotor activity can be defined as an index of wakefulness (alertness) of mental activity. Actophotometer operates on photoelectric cells which are connected in circuit. Six lights and six photocells are placed in the outer periphery of the bottom in such a way that a single mouse can block only one beam. The mice were tested for antianxiety activity of the methanol extract of the *Syzygium cumini*, wheat grains and blonanserin at 200, 400 and 8 mg/kg p.o. respectively in the actophotometer by observing their effect on locomotion for 10 min.^[12-13]

Y-Maze

The Y-Maze is a widely used behavioral task in neuroscience for studying spatial learning and memory. This test is based on that the rodents are motivated to explore their environment and locate food quickly and efficiently. Mice were treated with the methanol extract of the *Syzygium cumini*, wheat grains and blonanserin (200, 400 and 8 mg/kg p.o.) was given 30 min prior to the experiment and kept individually in one arm of the apparatus. Each mouse was placed in one of the arm compartments and was allowed to move freely until its tail completely enters another arm. The sequence of arm entries is manually recorded, the arms being labeled A, B, or C. An alternation is defined as entry into all three arms consecutively, for instance if the animal makes the following arm entries; ACB,CA,B,C,A,CAB,C,A, in this example, the animal made 13 arm entries 8 of which are correct alternations. The number of maximum spontaneous alternations is then the total number of arms entered minus two, and the percentage alternation is calculated as $\{(\text{actual alternations} / \text{maximum alternations}) \times 100\}$. The total numbers of visits in different arm were observed for 10 min.^[14]

RESULTS

It is evident from the results of a phytochemical investigation that the common phytoconstituents of the methanol extract of *Syzygium cumini* are flavonoids, tannins, saponin, carbohydrates and glycosides. Starch, fibers, proteins, carbohydrates and flavonoids were found to be present in the methanolic extract of wheat grains.

Behavioral Study

From the behavioral study it has been observed that the methanol extract of the *Syzygium cumini*, wheat grains and blonanserin were found to have less depressant effect as reflected by pinna reflex. These are found to have significant muscle relaxant property as well as have depressant effect, which were well comparable to that of the standard drug. From the overall study of the methanol extract of the *Syzygium cumini*, wheat grains and blonanserin possess significant CNS depressant

activity that were well comparable to that of the standard drug.

Elevated Plus Maze

On the administration of the extract of *Syzygium cumini* and wheat grains (200mg/kg and 400mg/kg) and blonanserin (8mg/kg), it was observed that the time spent in open arm was increased significantly ($P < 0.01$) as

compared to the control group (Table-1). The number entries into open arm by the animals treated with blonanserin with *Syzygium cumini* and wheat grain was found to be significantly increased as compared to that of the control group. The ratio of entries to the time spent in open arm was observed to be significantly increased on treatment with blonanserin with *Syzygium cumini* and wheat grain.

Table 1: Observation of open arm in Elevated plus maze using *Syzygium cumini*, wheat grain and blonanserin.

Groups	Response after			
	30min	60min	90min	120min
Normal Saline	0.452 ± 0.30	0.552 ± 0.31	0.304 ± 0.58	0.494 ± 0.36
Wheat Grains (400 mg/kg)	0.429 ± 0.99	0.752 ± 0.30	0.758 ± 0.30	0.794 ± 0.36
<i>Syzygium cumini</i> (200 mg/kg)	0.429 ± 0.99	0.647 ± 0.22	0.516 ± 0.21	0.747 ± 0.23
Blonanserin (8 mg/kg)	0.771 ± 1.62	1.469 ± 0.48	1.987 ± 0.41	2.049 ± 0.428*
Drug+ wheat grains (8 mg/kg +400 mg/kg)	0.877 ± 0.22	1.849 ± 0.42	2.449 ± 0.48*	3.522 ± 0.70*
Drug+ <i>Syzygium cumini</i> (8 mg/kg +200 mg/kg)	0.877 ± 0.22	1.694 ± 0.36	1.993 ± 0.40	2.149 ± 0.42*

Results are represented as mean ± S.E.M; n=6. * $p \leq 0.01$ are considered significance versus positive control.

It had been observed from the observation of Elevated plus maze apparatus in open arm that wheat grain has the better anti-anxiety activity then the *Syzygium cumini* but is comparatively less than the blonanserin, whereas blonanserin with wheat grain showed significantly higher anti-anxiety activity then blonanserin alone.

Locomotor activity

Locomotor activity is considered as an index of alertness, and the spontaneous decrease in basal activity score implicates the reduction of anxiety. Such types of effect can be found in the case of sedatives. The basal activity score (mean ± standard error) in four groups recorded for 10 minutes are represented in Table 2. There is a significant ($P < 0.01$) decrease in the locomotor score in case of animal treated with the extract of

Syzygium cumini and wheat grains (200mg/kg and 400mg/kg) and blonanserin (8mg/kg) as compared to the control group. It was also observed that the locomotor score by the animals treated with blonanserin with *Syzygium cumini* and wheat grain was found to be significantly decreased ($P < 0.001$) as compared to that of the control group. But the animals treated with wheat grains (400 mg/kg) and *Syzygium cumini* (200 mg/kg) alone showed minimal changes in locomotor score. It had been observed from the observation of Actophotometer apparatus that *Syzygium cumini* has the better anti-anxiety activity then the wheat grain but is comparatively less than the blonanserin, whereas blonanserin with wheat grain showed significantly higher anti-anxiety activity then blonanserin alone.

Table 2: Observation of antianxiety activity of *Syzygium cumini*, wheat grain, and blonanserin using actophotometer.

Groups	Response after			
	30min	60min	90min	120min
Normal Saline	74.13 ± 30.26	111.64 ± 45.75	113.78 ± 46.45	139.96 ± 57.14
Wheat Grains (400 mg/kg)	73.82 ± 87.29	90.50 ± 20.68	85.842 ± 6.46	64.64 ± 26.39
<i>Syzygium cumini</i> (200 mg/kg)	68.45 ± 7.53	37.57 ± 15.33	32.703 ± 13.35	28.30 ± 23.39
Blonanserin (8 mg/kg)	42.66 ± 37.83	6.97 ± 2.84*	5.010 ± 2.04*	4.97 ± 6.11**
Drug+ wheat grains (8 mg/kg +400 mg/kg)	19.82 ± 4.01	6.12 ± 2.50*	2.639 ± 1.07*	3.12 ± 12.29**
Drug+ <i>Syzygium cumini</i> (8 mg/kg +200 mg/kg)	14.43 ± 5.83	11.8 ± 82.38*	4.930 ± 2.01*	4.84 ± 57.90**

Results are represented as mean ± S.E.M.; n=6. * $p \leq 0.01$ and ** $p \leq 0.001$ are considered significance versus positive control.

Y-Maze Model

A significant decrease in the number of visits in the three arms of the Y-maze was observed in the wheat, *Syzygium cumini* and blonanserin ($p \leq 0.01$) treated animals as compared to the control animals. It was also observed

that the locomotor score by the animals treated with blonanserin with *Syzygium cumini* and wheat grain was found to be significantly decreased ($P \leq 0.01$) in the number of visits in the three arms of the Y-maze as compared to that of the control group. It had been

observed from the observation of Y-maze apparatus that wheat grain has the better anti-anxiety activity than the *Syzygium cumini* but is comparatively less than the

blonanserin, whereas blonanserin with wheat grain showed significantly higher anti-anxiety activity than blonanserin alone.

Table 3: Observation of antianxiety activity of *Syzygium cumini*, wheat grain, and blonanserin using Y-maze.

Groups	Response			
	30min	60min	90min	120min
Normal Saline	45.211 ± 0.45	46.983 ± 0.41	39.033 ± 0.42	42.304±0.58
Wheat Grains (400 mg/kg)	22.816 ± 0.34	21.816 ± 0.34	21.547 ± 0.22	20.894±0.36*
<i>Syzygium cumini</i> (200 mg/kg)	20.894 ± 0.37	19.752±0.31	18.049±0.42	18.156 ± 0.42*
Blonanserin (8 mg/kg)	16.983 ± 0.41	14.983 ± 0.41	12.816 ± 0.34*	10.752 ± 0.30*
Drug+ wheat grains (8 mg/kg +400 mg/kg)	14.983 ± 0.41	10.836 ± 0.31	8.816 ± 0.34*	8.516 ± 0.21*
Drug+ <i>Syzygium cumini</i> (8 mg/kg +200 mg/kg)	14.836± 0.34	12.752 ± 0.30	10.049 ± 0.42*	9.095 ± 0.44*

Results are represented as mean ± S.E.M.; n=6. *p≤0.01 are considered significance versus positive control.

CONCLUSION

The target of the project is to improve the activity of mostly prescribed antianxiety drug blonanserin by the normal diet having potential antianxiety effect. The patients mostly take wheat in their diet in India and other countries. The result of the present project suggests that wheat grains have better significant antianxiety activity and potentiate the antianxiety activity of blonanserin. On the other hand it has been observed that *Syzygium cumini* is having significant antianxiety activity and found to potentiate the antianxiety of blonanserin, but less than that of wheat grain. It was also observed that both the methanol extract of *Syzygium cumini* and wheat grains have the presence of phytoconstituents mainly flavonoids and carbohydrates. Their antianxiety activity may be due the presence of flavonoids.^[15-16] The result of the present project suggests that wheat grains potentiate the antianxiety activity of blonanserin than *Syzygium cumini*. The results clearly concluded that *Syzygium cumini* and mainly wheat grain should be included in the diet of anxiety patients prescribed blonanserin to improve its efficacy. Further studies are in progress to isolate and identify the components responsible for anxiolytic activity and the mechanism of action involved. Results will pave a way for the isolation of bioactive principles and new drug search for anxiety.

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