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In-Vivo Study on Anxiolytic Effects of Hydroalcoholic Extract of *Agaricus blazei* Murill

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ABSTRACT

Background: *Anxiety involved panic attacks either having or not having social fear, social anxiety disorder, generalized anxiety disorder as well as separation anxiety disorder is known to be marked mental diseases. It is related to high medical cost and a significant load of disease. Agaricus blazei Murill (AbM) is a mushroom and possesses immune-modulating and antimicrobial effects both in-vivo and in-vitro and as well as it has been used to treat cancer, hepatitis, dermatitis, and hyperlipidemia traditionally.* ***Method:*** *In this experiment evaluation of anxiolytic effect of AbM on mice has been done by using Elevated Plus Maze test, open field test and motor co-ordination test by rotarod. Mice (Mus musculus) weighing 22-25 grams, were divided into 4 groups (n=6). Oral administration of hydro-alcoholic extract of AbM was utilized in 2 doses i.e. 136.5 mg/kg and 273 mg/kg. Group, I received vehicle (distilled water 10 ml/kg), p.o. Group II received standard (diazepam 1 mg/kg), i.p. Group III and IV orally received hydro-alcoholic extract of AbM (136.5 mg/kg and 273 mg/kg, respectively).* ***Result:*** *In Elevated Plus Maze test, oral administration of hydro-alcoholic extract of AbM (136.5 mg/kg and 273 mg/kg, respectively) exhibited significant ($p<0.01$) elevation in the percentage of number of open arm entries (48.0 ± 1.1% and 48.93 ± 2.1% respectively) and time spent in open arm (14.92 ± 1.9% and 84.17 ± 2.4%).* ***Conclusion:*** *Hence it is concluded that hydro-alcoholic extract of AbM can be a new therapeutic agent to treat anxiety.*

Keywords: Anxiety, *Agaricus blazei* Murill, Open field test, Elevated plus maze-test, Motor co-ordination test by rotarod

INTRODUCTION

Anxiety

The anxiety involved panic attacks either having or not having social fear, social anxiety disorder, generalized anxiety disorder as well as separation anxiety disorder is known to be marked mental diseases. It is related to high medical cost and a significant load of disease. The surveys related to immense societies revealed that globally up to 33.7% of the entire population is affected by anxiety disorders. There is no conforming data that shows the variation in prevalence rates of anxiety disorders in previous decades. According to cross-cultural studies, prevalence rates are markedly changeable. Most probably this divergence is because of methodological variations instead of cultural effects. Anxiety disorders have been related with long term medical problems although by increasing in age the prevalence rates have found to be getting lower. Anxiety disorders have been associated with additional anxiety problems as well as further mental problems. The common view related anxiety states that it is a marked factor of a recent era as some financial, constitutional, societal also natural modifications has caused to raise its prevalence rates [1]. Various drugs have been included in the pharmacologic treatment of anxiety at different times. Barbiturates were the first class of drugs that were greatly effective but unsuccessfully it was found to be narrow therapeutic index as well as its adverse effects include respiratory arrest [2]. For this reason, the benzodiazepines were established as a safer substitute for barbiturates. However, the beneficial effects of benzodiazepine are vitiated by physical and psychological dependence caused by

these agents [3]. Other drugs that are utilized to treat anxiety such as beta-blockers and buspirone antidepressants also produce considerable unwanted effects [4].

***Agaricus blazei* Murill**

*Ab*M is a mushroom, Basidiomycetes brown fungus, origin to Brazil. It is extensively utilized for medicinal functions together with non-prescript as a nutritious mushroom as well as in extract form [5]. *Agaricus blazei* is a nutritious mushroom referring to the family of Agaricaceae, has been frequently utilized as a health nutriment additive to prevent arteriosclerosis, cancer, hyperlipidemia, chronic hepatitis together with diabetes [6]. In Japan *Ab*M is familiar as Himematsutake, Agarikusutake or Kawarihiratake, in Brazil, it is known as Cogumelo do sol and in China as Ji Song Rong [7]. *Agaricus blazei* is thought to be a nutritious mushroom having nutritional value; also the secondary metabolites of this mushroom are varied chemically and possess an extensive range of biological functions. Including the bioactive substances are minerals, glucan, peptides, vitamins, polyphenols, polysaccharides, glycoproteins, polyunsaturated fatty acids and triterpenoids [8]. According to the culture of Brazil it would be effective against several conditions, for example, hepatitis, atherosclerosis, increased blood sugar, heart disease, dyslipidemia, etc. [9]. This mushroom possesses immune-modulating and antimicrobial effects both *in-vivo* and *in-vitro* and as well as it has been used to treat cancer, hepatitis, dermatitis, and hyperlipidemia traditionally [10].

MATERIALS AND METHODS

Animals

Mice (*Mus musculus*) weighing 22-25 grams, 24 in number, were purchased from Karachi University's animal house, Pakistan. When animals had completed the habituate period of 1-week in the laboratory, the drug was given. Propylene cages (4 in number) were arranged for animals; in each cage a group of 6 animals was settled, 12/12-hour light-dark cycle was provided to animals. Animals were settled at room temperature at 25-30°C. Standard diet and water ad libitum had been given to mice.

Extract

Agaricus blazei proprietary extract was purchased from ORIVeDA, Amsterdam, Netherlands. *Agaricus blazei* extract was utilized in 2 doses i.e. 136.5 mg/kg and 273 mg/kg, p.o[7].

Drug and Chemicals

Diazepam was bought from Roche Pakistan (PVT.) LTD., while all other chemicals were purchased from Sigma-Aldrich (USA). Based on the literature, the doses of different drugs were selected [7]. Diazepam was utilized in a dose of 1 mg/kg (0.001 mg/g) (i.p.) [11].

Sample Size

Animals: 24 mice were divided into the following 4 groups:

- **Group I (n=6):** Vehicle control: The animals in this group were provided with distilled water 10 ml/kg, p.o.
- **Group II (n=6):** Positive control: The animals in this group were administered with diazepam 1 mg/kg, i.p.
- **Group III (n=6):** Treatment group: The animals in this group were provided with extract at a dose of 136.5 mg/kg, p.o.
- **Group IV (n=6):** Treatment group: The animals in this group were provided with extract at a dose of 273 mg/kg, p.o.

Open Field Test

To assess anxiolytic behavior under similar situations, the open field test was selected. To test the mice, distinct types of open field apparatus have been utilized. The apparatus was composed of a PVC chamber (60 cm × 60 cm × 60 cm). The platform of the open field was divided into 25 squares (15 cm × 15 cm), the centers having the 9 inner squares and the periphery contains 16 squares by the side of the wall. The laboratory area was kept dim as well as constrict. The lamp (40 W) was used to lighten the open field arena that focuses on the field from a height of about 75-100 cm. For open field test vehicle and hydro-alcoholic extract of *Agaricus blazei* Murill was administered orally and after 1 hour of administration, mice settled separately in a single square of the corner. For the 5 minutes number of pass over squares, number of rearings and assisted rearings were observed [12].

Elevated Plus-Maze Test

This test used to analyze anxiolytic behavior of animals. Vehicle and hydro-alcoholic extract of *Agaricus blazei* Murill was administered to animals orally and the activity was assessed after 1 hour of administration. The equipment contains 2 contrary open arms (40 cm × 11 cm) pass over with 2 closed arms having equal dimension by 65 cm height. The central square having a dimension (11 cm × 11 cm) connects arms. The apparatus consists of walls and floor which was painted with black color and 200 lux light illuminated, sound proof room was used to place the apparatus. To evaluate, the animal settled separately on the center area directing open arm. For Elevated Plus-Maze test the time spent (duration) in open arm which was taken as the percentage as well as frequency of entries of open arm estimated for 5 minutes. The precautions were made to make sure that except the height of plus-maze; no external stimuli could be the cause of anxiety in mice. Entry in arm explained as all 4 paws traverse the line that separates the central area and an arm. The formula [100 × open/(open+enclosed)] was used to estimate the percentage of time spent in open arms and the formula (100 × open/total entries) was used to calculate number of open arm entries [12].

Motor Co-ordination Test by Rotarod

The rotarod equipment revolving at a velocity of 32 rotations per minute was used to examine the effect on motor coordination test. The equipment was composed of a base raised area, also an iron rod of 42 cm long in addition having 6 cm diameter with the non-polished plane. There are 6 disks which divide the rod into 3 same sections; hence 6 mice can walk on the rod at the same velocity and time. For Motor co-ordination test by rotarod, performance time was recorded by the periods between the rising of the mice on the rod and falling off. The practicing of animal was provided 20 times at 5-15 minutes period. After that 6 animals were picked on random basis to detect the holding of the walking technique. Animals were selected for the study that performed exceeding 10 seconds. The performance time was monitored at 15 minutes time period for 90 minutes following administration of the standard or test drug, for each dose with 6 trained mice [12].

RESULTS

In the Open Field Test (OFT), diazepam-treated mice showed a significant increase ($p<0.05$) in the number of rearings, number of squares crossed and assisted rearings during 5-min interval of test as compared to vehicle-treated groups. Oral administration of mice treated with hydroalcoholic extract of *Agaricus blazei* Murill (136.5 mg/kg and 273 mg/kg, respectively) exhibited significant increase in the number of rearings ($p<0.05$), assisted rearings and squares crossed ($p<0.01$) (Table 1).

Table 1 Comparison of Diazepam and hydro-alcoholic extract of *Ab*M on the behavior of mice in open field test (n=6)

Treatment	No. of rearing	No. of assisted rearing	No. of squares crossed		
			Peripheral	Central	Total
Vehicle (10 ml/kg, p.o)	9.2 ± 3.3	13.3 ± 2.5	39 ± 3.0	14.8 ± 3.5	53.8 ± 1.2
Diazepam (1.0 mg/kg, i.p)	32.3 ± 23	68.3 ± 4.3	84 ± 1.7	15.7 ± 1.2	99.7 ± 3.8
Hydroalcoholic ext. (136.5 mg/kg, p.o)	22.8 ± 1.2	57.2 ± 1.3	78.2 ± 1.4	13.7 ± 4.1	91.9 ± 1.5
Hydroalcoholic ext. (273 mg/kg, p.o)	24.7 ± 2.7	63.5 ± 3.1	81.8 ± 2.1	14.8 ± 3.2	96.6 ± 2.2

The results showed that the mice administered with vehicle (10 ml/kg, p. o. normal saline) expended maximum period in the closed arm (196.3 ± 33 seconds) and showed fewer entries (5.2 ± 33 seconds) in open arm in comparison to closed arm at 5 minutes. Mice administered with diazepam (1 mg/kg, p.o.) presented marked ($p<0.001$) elevation in the percentage of entries of open arms (44.0 ± 3.6%) and period expended in open arm (16.78 ± 1.8%) whereas, in closed arm number of entries and time spent were significantly ($p<0.001$) decreased. Oral administration of hydro-alcoholic extract of *Ab*M (136.5 mg/kg and 273 mg/kg, respectively) exhibited significant ($p<0.01$) increase in the percentage of number of open arm entries (48.0 ± 1.1% and 48.93 ± 2.1% respectively) and time spent in open arm (14.92 ± 1.9% and 84.17 ± 2.4%) however, in the closed arm number of entrance, also period expended was markedly ($p<0.01$) decreased in comparison to vehicle-administered group (Table 2).

Table 2 Comparison of Diazepam and hydro-alcoholic extract of *Ab*M on the behavior of mice in Elevated Plus-Maze test (n=6)

Treatment	No. of entries		Time spent (s)		Open arm entries (%)	Time spent (%)
	Open arm	Closed arm	Open arm	Closed arm		

Vehicle (10 ml/kg, p.o)	5.2 ± 2.3	15.5 ± 5.3	27.8 ± 3.4	196.3 ± 1.3	25.1 ± 2.3	12.4 ± 1.6
Diazepam (1.0 mg/kg, i.p)	11.7 ± 2.1	13.3 ± 1.4	41.7 ± 1.7	206.7 ± 2.5	44.0 ± 3.6	16.78 ± 1.8
Hydroalcoholic ext. (136.5 mg/kg, p.o)	10.8 ± 3.5	11.7 ± 3.9	35.3 ± 1.1	201.2 ± 1.1	48.0 ± 1.1	14.92 ± 1.9
Hydroalcoholic ext. (273 mg/kg, p.o)	11.5 ± 4.1	12.0 ± 4.1	39.0 ± 2.9	207.5 ± 1.4	48.9 ± 2.1	84.17 ± 2.6

At the 45-min interval, diazepam and hydro-alcoholic extract of *Ab*M (273 mg/kg) significantly ($p<0.01$) decreased motor movement of the animal however hydro-alcoholic extract of *Ab*M (136.5 mg/kg) did not succeed to produce result on motor coordination. At 60 minute time period the doses of hydro-alcoholic extract of *Ab*M (136.5 mg/kg and 273 mg/kg) and diazepam significantly ($p<0.01$) decreased motor movement of animal in comparison to vehicle-administered group, however at 75 minutes and 90 minutes time period, both the doses of hydro-alcoholic extract of *Ab*M (136.5 and 273 mg/kg) induced significantly ($p<0.001$) comparable results with diazepam (Table 3).

Table 3 Comparison of Diazepam and hydro-alcoholic extract of *Ab*M on rotarod performance in mice (n=6)

Treatment	Time of animals remained without falling from revolving rod (sec)					
	15	**30**	**45**	**60**	**75**	**90 min**
Vehicle (10 ml/kg, p.o)	253.7 ± 4.3	242.2 ± 1.1	225.3 ± 2.6	211.5 ± 5.2	195.2 ± 4.1	178.8 ± 2.3
Diazepam (1.0 mg/kg, i.p)	271.0 ± 3.7	218.7 ± 2.7	176.8 ± 5.6	137.0 ± 4.7	110.3 ± 3.4	96.5 ± 3.9
Hydroalcoholic ext. (136.5 mg/kg, p.o)	258.0 ± 1.9	233.8 ± 5.1	213.5 ± 4.7	158.2 ± 1.3	123.0 ± 2.7	102.7 ± 4.1
Hydroalcoholic ext. (273 mg/kg, p.o)	263.8 ± 2.5	216.3 ± 4.8	160.2 ± 7.1	95.5 ± 7.8	77.8 ± 5.2	75.7 ± 3.8

DISCUSSION

The anxiety involved panic attacks either having or not having social fear, social anxiety disorders, generalized anxiety disorder as well as separation anxiety disorder are known to be marked as mental diseases [1]. It is an unfavorable emotion which is a result of receiving danger that can cause from the inner or outer resource, also can be actual or predictable [13]. Because of having unwanted effects of newly available drugs, patients administering anxiolytic drugs usually discontinue the treatment before they are completely recovered. Moreover, among the controlled studies one-third of patients are insensitive to any one of the available medications. Therefore, there is a serious need to develop new anxiolytic drugs [14]. To find new therapeutic agents to treat neurological illness, medicinal plant research, globally, has developed continually by describing the pharmacological uses of various species of plants in different animal models [15].

Numerous plants that are utilized in cultural medicine for anxiolytic action are found to do rise in traveling around in the open arms of Elevated Plus Maze test. In elevated plus maze test mice usually, consume their assigned time in closed arms. This shows dislike towards open arms that are caused by fright of open spaces [16]. Results collected after administering hydro-alcoholic extract of *Ab*M on the Elevated Plus-Maze showed anxiolytic action because rise in open arm entry is the parameter that represents anxiolytic action. In the open field test anxiety is the reason for the marked reduction of passage in peripheral also central area as well as decreased rearing showing stress and fear [17]. Results collected after administering hydro-alcoholic extract of *Ab*M on the open field indicated improved number of rearing, assisted rearing and number of squares crossed as compared to vehicle-treated group. The observed data showed anxiolytic effect. Rotarod test is utilized to assess motor coordination as well as neuromuscular resistance peripherally. A decrease in motor coordination is probable to influence performance in behavioral tests [18]. According to our findings hydro-alcoholic extract of *Ab* M (136.5 mg/kg and 273 mg/kg), unlike diazepam (1 mg/kg), had no marked action on motor coordination. Moreover the extract did not influence motor coordination nor found to cause neuromuscular blockade.

CONCLUSION

Hence it is concluded that hydro-alcoholic extract of *Ab*M can be a new therapeutic agent to treat anxiety.

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Bandelow, Borwin, and Sophie Michaelis. "Epidemiology of anxiety disorders in the 21st century." *Dialogues in Clinical Neuroscience*, Vol. 17, No. 3, 2015, p. 327.
2. Lafferty, KA. "*Barbiturate Toxicity*:" Medscape, 2017, https://emedicine.medscape.com/article/813155-clinical.
3. Bandelow, Borwin, et al. "The diagnosis of and treatment recommendations for anxiety disorders." *Deutsches Ärzteblatt International*, Vol. 111, No. 27-28, 2014, p. 473.
4. Bleakley, S. "Anxiety disorders: management." *The pharmaceutical Journal*, Vol. 12, 2017, p. 17.
5. Wang, Hang, et al. "The protective effect of *Agaricus blazei* Murrill, submerged culture using the optimized medium composition, on alcohol-induced liver injury." *BioMed Research International*, 2014, pp. 1-9.
6. Shimizu, Takamitsu, et al. "Agarol, an ergosterol derivative from Agaricus blazei, induces caspase-independent apoptosis in human cancer cells." *International Journal of Oncology*, Vol. 48, No. 4, 2016, pp. 1670-78.
7. Ni, Wei-Ya, et al. "Extract of medicinal mushroom *Agaricus blazei* Murill enhances the non-specific and adaptive immune activities in BALB/c mice." *In Vivo*, Vol. 27, No. 6, 2013, pp. 779-86.
8. Fanhani, Jamile Corina, et al. "Effect of *Agaricus blazei* in the diet of broiler chickens on immunity, serum parameters and antioxidant activity." *Semina: Ciências Agrárias*, Vol. 37, No. 4, 2016, pp. 2235-46.
9. Liu, Yi, et al. "Polysaccharide *Agaricus blazei* Murill stimulates myeloid-derived suppressor cell differentiation from M2 to M1 type, which mediates inhibition of tumor immune-evasion via the Toll-like receptor 2 pathway." *Immunology,* Vol. 146, No. 3, 2015, pp. 379-91.
10. Lima, Cristiane UJO, Eliana F. Gris, and Margô GO Karnikowski. "Antimicrobial properties of the mushroom *Agaricus blazei*-integrative review." *Revista Brasileira de Farmacognosia*, Vol. 26, No. 6, 2016, pp. 780-86.
11. Foyet, Harquin Simplice, et al. "Anxiolytic and antidepressant-like effects of the aqueous extract of Alafia multiflora stem barks in rodents." *Advances in Pharmacological Sciences,* 2012, pp. 1-8.
12. Nagaraja, Thandaga S., et al. "Evaluation of anxiolytic effect of *Erythrina myogenesis* Gamb. in mice." *Indian Journal of Pharmacology*, Vol. 44, No. 4, 2012, p. 489.
13. Moser, Debra K. "The rust of life": impact of anxiety on cardiac patients." *American Journal of Critical Care*, Vol. 16, No. 4, 2007, pp. 361-69.
14. Goldstein, Benjamin I., and Anthony J. Levitt. "The specific burden of comorbid anxiety disorders and of substance use disorders in bipolar I disorder." *Bipolar Disorders,* Vol. 10, No. 1, 2008, pp. 67-78.
15. Arora, Disha, and Anupam Sharma. "Isolation and characterization of the chemical constituents of *Stellaria media* Linn." *International Journal of Pharmaceutical Sciences and Research,* Vol. *5*, 2012, pp. 4629-32.
16. Harsha, Singapura Nagesh, and Kandangath Raghavan Anilakumar. "Anxiolytic property of Hydro-alcohol extract of *Lactuca sativa* and its effect on behavioral activities of mice." *Journal of Biomedical Research*, Vol. 27, No. 1, 2013, p. 37.
17. Sulakhiya, Kunjbihari, et al. "Effect of *Beta vulgaris* Linn. leaves extract on anxiety-and depressive-like behavior and oxidative stress in mice after acute restraint stress." *Pharmacognosy Research*, Vol. 8, No. 1, 2016, p. 1.
18. Doukkali, Zouhra, et al. "Evaluation of anxiolytic activity of methanolic extract of *Urtica urens* in a mice model." *Behavioral and Brain Functions*, Vol. 11, No. 1, 2015, p. 19.