

Original Article

Effect of *Agaricus blazei* Murill on exploratory behavior of mice-model

Efeito de *Agaricus blazei* Murill no comportamento exploratório de modelo de camundongo

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Abstract

Increased anxiety and depressive symptoms have reported to be its association with long term illness. Because of having unwanted effects of newly available drugs, patients administering anxiolytic drugs usually discontinue the treatment before they are completely recovered. Therefore, there is a serious need to develop new anxiolytic drugs. The anxiolytic effect of hydro-alcoholic extract of *Agaricus blazei* in animal models was assessed. 24 male mice (*Mus musculus* genus) were included in the study. Four groups were prepared and each group contained six animals. The groups were vehicle control, positive control (diazepam 1.0 mg/kg, i.p.) as well as two treatment groups receiving *Agaricus blazei* hydro-alcoholic extract at a dose of 136.50 mg/kg and 273.0 mg/kg orally. The Marble burying test, Nestlet shredding test and Light and Dark box test used to assess anxiolytic activity. Mice administered with diazepam 1.0 mg/kg, i.p. while hydro-alcoholic extract of *AbM* (136.50 and 273.0 mg/kg, respectively) was administered via oral route which exhibited marked reduction in number of marbles-burying as compared to vehicle control group. Mice administered with diazepam 1.0 mg/kg, i.p. and Oral administration of hydro-alcoholic extract of *AbM* (136.50 and 273.0 mg/kg, respectively) exhibited significant decrease in nestlet shredding in comparison to vehicle control group. The oral administration of hydro-alcoholic extract at a dose of 136.5mg/kg and 273mg/kg showed elevation in time spent in light box and was comparable to standard treated group while time spent by mice following oral administration of hydro-alcoholic extract of *Agaricus blazei* at a dose of 273.0 mg/kg also showed elevation and was found to be more near to standard treated group (diazepam 1 mg/kg, i.p.).

Keyword: *Agaricus blazei*, therapeutic mushroom, anxiety, marble-burying, nestlet shredding.

Resumo

O aumento da ansiedade e dos sintomas depressivos têm relatado sua associação com doenças de longa duração. Por causa dos efeitos indesejáveis dos novos medicamentos disponíveis, os pacientes que administram medicamentos ansiolíticos geralmente interrompem o tratamento antes de estarem completamente recuperados. Portanto, há uma necessidade séria de desenvolver novos medicamentos ansiolíticos. Foi avaliado o efeito ansiolítico do extrato hidroalcoólico de *Agaricus blazei* em modelos animais. Vinte e quatro camundongos machos (gênero *Mus musculus*) foram incluídos no estudo. Quatro grupos foram preparados, e cada grupo continha seis animais. Os grupos foram controle de veículo, controle positivo (diazepam 1,0 mg/kg, i.p.), bem como dois grupos de tratamento recebendo extrato hidroalcoólico de *Agaricus blazei* na dose de 136,50 mg/kg e 273,0 mg/kg por via oral. O teste de enterrar Marble, o teste de retalhamento Nestlet e o teste de caixa clara e escura são usados para avaliar a atividade ansiolítica. Camundongos foram administrados com diazepam 1,0 mg/kg, i.p., enquanto o extrato hidroalcoólico de *AbM* (136,50 e 273,0 mg/kg, respectivamente) foi administrado por via oral, que exibiu redução acentuada no número de mármore enterrados em comparação com o grupo de controle de veículo. Camundongos administrados com diazepam 1,0 mg/kg, i.p. e a administração oral de extrato hidroalcoólico de *AbM* (136,50 e 273,0 mg/kg, respectivamente) exibiu diminuição significativa na trituração de ninhos em comparação ao grupo de controle de veículo. A administração oral de extrato hidroalcoólico na dose de 136,5mg/kg e 273mg/kg mostrou elevação no tempo gasto na caixa de luz e foi comparável ao grupo tratado padrão, enquanto o tempo gasto por camundongos após a administração oral de extrato hidroalcoólico de *Agaricus blazei* na dose de 273,0 mg/kg também mostrou elevação e foi mais próximo do grupo tratado padrão (diazepam 1 mg/kg, ip).

Palavra-chave: *Agaricus blazei*, cogumelo terapêutico, ansiedade, enterramento de mármore, trituração de nestlet.

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1. Introduction

Anxiety is the highest frequently occurring disorder found among people with fifteen percent prevalence (Boyle et al., 2017). According to surveys of immense societies it has been found that overall 33.7 percent of entire population has faced anxiety problems (Ali et al., 2019). Increased anxiety and depressive symptoms have reported to be its association with long term illness (Lerman et al., 2018). Reduced sleep aspect is markedly related with higher symptoms of anxiety as well as depression (Okun et al., 2018). 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 (Goldstein and Levitt, 2008). *Agaricus blazei* Murill (*AbM*) is a nutritive mushroom origin to Brazil. It is cultivated in countries like Indonesia, Taiwan, China, Japan and Korea (Yeh et al., 2015). It is basidiomycete generally familiar as sun mushroom. It is marketed as natural form for utilization and to use in capsules, solutions and syrups in Brazil. According to culture of Brazil it would be effective against several conditions, for example hepatitis, atherosclerosis, increased blood sugar, heart disease, dyslipidemia etc. (Liu et al., 2015). A number of researches have been revealed that the mushroom *Agaricus blazei* Murill contain polysaccharides which are produced by mycelial fermentation and is reason for its anti-tumor together with immune-modulating effects and possess biological functions (Wang et al., 2018). *Agaricus blazei* Murill contains compounds like active hemi-cellulose compounds, 1,6-beta-glucan, anti-tumor organic substance and 1,3-betaglucan. This mushroom enhances the number and functions of natural killer (NK) cells (Verçosa Júnior et al., 2015). *Agaricus blazei* is thought to be a nutritious mushroom due to 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 (Fanhani et al., 2016). A number of researches have been revealed that the mushroom *Agaricus blazei* Murill containing polysaccharides are reason for its anti-tumor together with immunomodulating effects. The structure also the activities of xyloglucan-protein complexes together with glucan have been investigated, also glucans with α -(1,4; 1,6) linkages, β -(1,6) linkages together with α -(1,4) linkages with drawn from fruiting body of this mushroom have been investigated. Moreover, this mushroom contains polysaccharides which are produced by mycelial fermentation for example β -(1,3) glucan, glucomannan also β -(1,6) glucan have revealed to possess biological functions (Wang et al., 2018).

2. Materials and Methods

2.1. Animals and grouping

24 male mice weighing 22-25 grams, 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 were arranged for animals; in each cage a group of 6 animals was settled, 12/12-hour light-dark cycle was provided to animals at room temperature ranging 25-30°C. Standard diet and water ad libitum had been administered to mice.

- Group I (n=6): Vehicle control: The animals in this group were provided with distilled water 10.0 ml/kg, p.o.
- Group II (n=6): Positive control: The animals in this group were administered with diazepam 1.0 mg/kg, i.p.
- Group III (n=6): Treatment group: The animals in this group were provided with extract at a dose of 136.50 mg/ kg, p.o.
- Group IV (n=6): Treatment group: The animals in this group were provided with extract at a dose of 273.0 mg/kg, p.o.

2.2. Extract

Agaricus blazei Murill (*AbM*) proprietary extract was purchased from ORIVeDA, Amsterdam, Netherlands. *AbM* extract was utilized in two doses i.e. 136.50 mg/kg and 273.0 mg/kg, p.o (Ni et al., 2013).

2.3. Drug and chemicals

Diazepam was bought from Roche Pakistan (PVT.) LTD., while all other chemicals were bought from Sigma Aldrich (USA). Based on the literature, the doses of different drugs were selected (Ni et al., 2013). Diazepam was utilized in a dose of 1.0 mg/kg (0.001 mg/g, i.p.) (Foyet et al., 2012).

2.4. Marble burying test

The marble-burying test was used to assess the Occlusive Compulsive Disorder (OCD) in mice. Plastic cage of 40cm in length, 27cm in breadth and 15.5cm in height was bedded with 5 cm layer of sawdust and 20 neat glass marbles having diameter of 10 mm with levelly spaced on sawdust. Each of the male mice was separately kept in the cage for ten minutes. Later animal were taken out and the burying action was calculated by counting the number of marbles that were more than two-third enclosed with saw dust. Reduction of the burying action indicates an anxiolytic action (Kalariya et al., 2015; Angoa-Pérez et al., 2013; Nirwane et al., 2015).

2.5. Nestlet shredding test

In this test each mice was settled into a cage composed of an individual, pre-weighted nestlet. After that the cage was covered with filter-top. Food and water were not allowed throughout this test duration. The mice was placed in the cage without any disturbance, accompanying the nestlet for thirty minutes. After 30 minutes, the mice was removed and the remaining unshrouded part of the nestlet material was taken out from the cage with the help of forceps and

let it dry, and weighed. Weight after the shredding was divided by the initial weight of the nestlet to estimate percentage of shredded nestlet (Angoa-Pérez et al., 2013).

2.6. The Light or Dark box test (LDBT):

The equipment is composed of wooden chamber open from top having 2 definite cubicles; a light and a dark cubicle. The dark cubicle is painted with a black color. The light cubicle is transparent. The experiment initiated by settling an animal into center of light compartment. The factors such as the transfer latency; the time consumed to enter firstly in dark compartment, the number of transitions between two compartments and the time spent in the dark box was monitored. The readings were taken for 10 minutes (Aslam and Najam, 2013).

3. Results

Mice administered with diazepam (1 mg/kg, i.p.) and Oral administration of hydro-alcoholic extract of

AbM (136.5 and 273 mg/kg, respectively) exhibited significant ($P < 0.05$) decrease in number of marbles-burying response as compared to vehicle control group (Table 1).

The results showed that Mice administered with diazepam (1 mg/kg, i.p.) and Oral administration of hydro-alcoholic extract of AbM (136.5 and 273 mg/kg, respectively) exhibited significant ($P < 0.05$) decrease in nestlet shredding as compared to vehicle control group (Table 2).

In control group, mice administered with vehicle (10 ml/kg, p. o. normal saline) spent average time in light box was 78.5 ± 5.25 seconds while in dark box they spent 184.75 ± 7.25 seconds. Mice administered with diazepam (1 mg/kg, i.p.) presented marked ($P < 0.001$) elevation in the average time spent in light box (102.5 ± 3.6) and decrease in time average spent in dark box (86 ± 13). Oral administration of hydro-alcoholic extract of AbM (136.5 and 273 mg/kg, respectively) exhibited significant ($P < 0.01$) raise in time spent by mice in lighted area as well as lowered the time spent in darkened area (Table 3).

Table 1. Comparison of Diazepam and hydro-alcoholic extract of AbM by the Assessment of Marble-burying Behavior in mice.

Treatment	No. of Marbles Buried
Vehicle (10 ml/kg, p.o)	15.16 ± 3.3
Diazepam (1.0 mg/kg, i.p)	3.7 ± 23**
Hydroalcoholic ext. (136.5 mg/kg, p.o)	5.83 ± 1.2*
Hydroalcoholic ext. (273 mg/kg, p.o)	4.5 ± 2.7***

n=6, all value are shown as mean ± SEM. Statistical analysis of data were carried out by one-way ANOVA * $P < 0.01$ *** $P < 0.05$ and **** $P < 0.001$ when compared with control. SEM=Standard error of the mean, AbM = *Agaricus blazei* Murill.

Table 2. Comparison of Diazepam and hydro-alcoholic extract of AbM by Nestlet Shredding Test in mice.

Treatment	Percentage of weights after Nestlet Shredded
Vehicle (10.0 ml/kg, p.o)	5.5 ± 1.3
Diazepam (1.0 mg/kg, i.p)	7.0 ± 3.15**
Hydroalcoholic ext. (136.5.0 mg/kg, p.o)	9.8 ± 1.4*
Hydroalcoholic ext. (273.0 mg/kg, p.o)	11.5 ± 4.9***

n=6, all value are shown as percent mean ± SEM. Statistical analysis of data were carried out by one-way ANOVA * $P < 0.01$ ** $P < 0.05$ and **** $P < 0.001$ when compared with control. SEM = Standard error of the mean; AbM = *Agaricus blazei* Murill.

Table 3. Effect of hydro-alcoholic extract of AbM on exploratory behavior in Light or Dark Box Test (LDBT).

Treatment	Time Spent in Light Box	Time Spent in Dark Box	No. of Transitions between the two boxes
Vehicle (10 ml/kg, p.o)	178.5 ± 5.25	284.75 ± 7.25	12.25 ± 17
Diazepam (1.0 mg/kg, i.p)	356.5 ± 3.6**	186.0 ± 13**	22.0 ± 2.3**
Hydroalcoholic ext. (136.5 mg/kg, p.o)	301.0 ± 1.5*	192.0 ± 0.23*	19.5 ± 1.7*
Hydroalcoholic ext. (273 mg/kg, p.o)	349.35 ± 1.9*	208.28 ± 7.1*	24.15 ± 3.8*

n=6, all value are shown as mean ± SEM. Statistical analysis of data were carried out by one-way ANOVA. * $P < 0.01$ and ** $P < 0.001$ when compared with control. SEM = Standard error of the mean; AbM = *Agaricus blazei* Murill.

4. Discussion

Anxiety is remarkably prevalent psychological behavioral disorder all over. It involves disturbed social as well as occupational activities, personal discomfort, decreased quality of life and serious damage economically. Diazepam is most frequently prescribed anxiolytic drug having adverse effects like dependence, varied attentiveness and cognitive behavior. By discontinuation diazepam withdrawal symptoms are also observed (Srinivas et al., 2018). This study analyzed anxiolytic effects of mushroom *Agaricus blazei* Murill by the tests that are predictive models of anxious behavior.

The marble burying test gives analysis relating anxiety. Usually when animals distinguish materials as unsafe they are more likely to cover up those materials. As newer substance kept in cage they cover up them with dust. The anxiolytic agents weaken this action. The animal is said to have anxious state if they cover up more number of marbles (Choukairi et al., 2019). In this study it is found that there are lesser number of marbles buried in *AbM* treated group at both doses as compared to vehicle and found to be near to standard treated group. Nestlet Shredding Test is influential behavioral tests that evaluate repetitive together with compulsive-like activities. Elevated shredding habit indicated by nestlet shredding test shows repetitive as well as compulsive habit and indicates obsessive-compulsive disorder (Murphy et al., 2017). The anxiety causing agents tend to increase shredding habit while anxiolytic agents help to reduce shredding behavior (Eissa et al., 2018). Mice administered with diazepam (1 mg/kg, p. o.) and Oral administration of hydro-alcoholic extract of *AbM* at both doses (136.5 and 273 mg/kg, respectively) showed decrease in nestlet shredding as compared to vehicle control group. The light or dark box apparatus used to analyze values associated with anxiety in mice. Animal is allowed to place in light-dark box and they were having an option to explore light cubicle. This behavior seems to decrease in anxious behavior. Also in anxious state animal is more likely to attract dark cubicle. Elevation in time spent in light cubicle indicates anti-anxiety action (Holden et al., 2015). The results of the study confirmed that administration of hydro-alcoholic extract of *AbM* lowers levels of anxiety as there was an increase in time spent in light cubicle by mice and decrease the time spent in dark cubicle. The oral administration of hydro-alcoholic extract at a dose of 136.5mg/kg showed elevation in time spent in light box and was comparable to standard treated group while time spent by mice following oral administration of hydro-alcoholic extract of *AbM* at a dose of 273mg/kg also showed elevation and was found to be more near to standard treated group. The time spent in dark box decreased following oral administration of *Agaricus blazei* Murill extract at both doses. The number of transitions between two boxes also increased following oral administration of hydro-alcoholic extract of *AbM* at both doses and were near to standard treated group. The anxiolytic effect given by *Agaricus blazei* Murill may be due to its compound β -glucan. Some studies showed that mushrooms having β -glucan containing either β -1,6-linked glucan with β -1,3 branches or β -1,3-linked glucan branched

with β -1,6 glucosides as a marked polysaccharide is the bioactive compound of Maitake mushroom which proved to lower depression in animal study (Bao et al., 2016).

5. Conclusion

It was concluded that extract of *Agaricus blazei* Murill possesses anxiolytic-like activity in mice, proved by designed experiments. In this study evaluation of anxiolytic effect of *AbM* on mice has been done by using marble burying behavior, nestlet shredding test and Light and dark box test. Mice (*Mus musculus*) weighing 22–25 grams, were divided into four groups. Each group contains six mice. Oral administration of hydroalcoholic extract of *AbM* was utilized in two doses i.e. 136.5 mg/kg and 273 mg/kg. According to the results of the test it has been concluded that hydro-alcoholic extract of *AbM* can be new therapeutic agent to treat anxiety.

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