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LENTINULA EDODES: A PRODIGIOUS MUSHROOM POSSESSES ANXIOLYTIC POTENTIAL IN RODENTS

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Article Info

Abstract

Background: Mushrooms have been used from ancient times as traditional Chinese and Japanese medicines in number of diseases. Among them, *Lentinula edodes*, a prodigious mushroom contained flavonoids which are known to possess anxiolytic activities. In this study, we assessed the anxiolytic potential of *Lentinula edodes* extract.

Material and method: In this study light-dark box test, elevated plus maze test and open-field test were utilized to investigate the anxiolytic potential of L. edodes extract (LEE). The extract was given orally by gavage at the two doses of 250 mg/kg and 500 mg/kg. Diazepam (1 mg/kg) was utilized as the standard drug.

Result: It was observed that in light dark box the extract at both the doses produced significant and dose dependent increase in time remained in light chambers ($p<0.001$), transfer latency ($p<0.001$) and the sum of adaptations between two chambers ($p<0.001$) were significantly raised as compared the control group. However, in elevated plus maze animals in treated group spent more time in the open arm to that of closed as compared to the control group. *Lentinula edodes* also demonstrated significant dose dependent increase in the number of squares crossed ($p<0.001$) and rearings ($p<0.001$). However, fecal droppings were significantly decreased ($p<0.001$) in open field test as compared to that of animals in the control group.

Conclusion: The findings of this research concluded that *Lentinula edodes* extract possesses anxiolytic activity in animal models of anxiety.

Keywords:
Anxiety, light-dark box test, elevated plus test, open field test, Lentinula edodes extract.

Introduction

Anxiety disorders are the most common disorders faced by the humans throughout the world that produces significant impact on individual itself as well as on the society. According to GDP data of 2019, anxiety disorder affecting 301 million peoples with an estimation of around 4% population globally [1]. In Pakistan the prevalence rate of anxiety in young population is estimated around 30-50% [2]. Symptoms associated with anxiety are: avoidance behavior, irritability, severe anxiety attacks and apprehension [3]. GABA, Serotonin, Norepinephrine and Dopamine are the neurotransmitters that mediates significantly in anxiety and its related disorders [4].

In Allopathy, there are many standard drugs available to combat anxiety disorders such as: GABAA receptor agonist like Benzodiazepines, Buspirone, and other 5-HT1A receptor agonist balances the level of serotonin and dopamine in the brain [5]. However, these drugs may cause various undesirable reactions such as: muscle relaxation, dizziness, sedation, headache, nervousness, paresthesia, diarrhea, excitation and sweating [6]. Medicines obtained from the natural sources gain significant interest of public globally due to their less side effects and natural availability [7].

Mushrooms have been used from ancient times as a Traditional Chinese and Japanese medicines in the number of diseases [8]. There are many mushrooms available that possess significant medicinal activity such as *Ganoderma Lucidum* (Lingzi), *Lentinula edodes* (Xianggu or shitake), Grifola (Maitake) etc. [9]. Among them, *Lentinula edodes* (Xianggu or shitake) belongs to white rot species and is used as edible and as therapeutic mushroom cultivated in Japan and China. Polysaccharide from *Lentinula edodes* called lentinan showed anticancer properties in colon cancer cells, antifungal properties, prevents proliferation of leukemic cells, antioxidant anti-hepatotoxic, immune-stimulatory, anti-mutagenic and antitumor and anti-carcinogenic potential [9-12].

Lentinula edodes possess rich source of flavonoids and polyphenols [13]. Literature reveals that flavonoids are well known to possess anxiolytic activity [14]. To the best of our knowledge, there has been no investigation study to assess the anxiolytic potential of *Lentinula edodes*. In view of the idea that flavonoids present in *Lentinula edodes* could be useful in anxiety, we evaluated anxiolytic activity of *Lentinula edodes* using light dark box test, elevated plus maze test and open field test.

Figure 1. *Lentinula edodes*

Materials and methods:

The collection of plant material:

Lentinula edodes extract was purchased in powder form from Gluckspilze Austria. Diazepam was purchased from Hilton Pharma.

The Selection of animals:

Swiss albino male mice having weight of 20–25 g and 2 months older were obtained from Karachi University were used for this study. The animals were deal with as per the specification given in NIH guidelines for the look after and usage of laboratory animals 8th edition.

Groupings of Animals

Animals were divided as 6 mice per group followed by 30 days treatment as follows:

Group 1: Distilled water 10 mL/kg, PO.

Group 2: *Lentinula edodes* 250 mg/kg, PO.

Group 3: *Lentinula edodes* 500 mg/kg, PO.

Group 4: Diazepam 1 mg/kg, PO.

Assessment of anxiolytic activity:

Light dark box test (LDBT)

The Light and dark test is the most common test use to check the anxiolytic potential of test compound in rodents. The apparatus consisted of two compartments one is light and other one is dark. A black chamber (25 cm long × 35 cm wide × 35 cm deep) covered with black Acrylic lid, and a bright chamber (25 cm long × 35 cm wide × 35 cm deep) is white and illuminated by a 40-W white light source or can also be transparent. A 7.5 cm x 5 cm open doorway in the center of the partition joined the two compartments. The mice were placed in the center of these two compartments after oral treatment to observe transfer latency, time remained in the light chamber and number of transition between these two compartments were observed for 5 minutes [15].

Elevated plus maze test (EPZT)

Elevated Plus Maze (EPM) test is extensively used to assess the anxiolytic potential of any pharmacological substance in rodents. The apparatus takes the shape of a plus symbol and is elevated off of the floor so as to create a conflict between the animals inherent fear of open, elevated areas and its inherent exploratory instinct. It comprises four arms, two open (16 x 5 cm) and two closed (16 x 5 cm) with vertical walls all rising out of a central platform (5 x 5 cm). The whole maze is raised to a 25 cm height off the ground. After 60 minutes of an oral (PO) administration of the test compound, every animal is put in the center of the maze with an open arm facing it. The parameters of the behavior such as the duration of staying in open and closed arms and the frequency of entries into each arm are measured during 5 minutes of observation. increments in duration of stay and entries into open arms reflect a low level of anxiety [16].

Open field test (OFT)

The Open Field Test (OFT) is widely used to evaluate locomotor activity, exploratory behavior and anxiety-like responses of rodents. testing is carried out in a square open field apparatus commonly of size 60 x 60 x 30 cm 3 made of wood or acrylic. Movement is quantified by marking the floor of the arena into 16 equal squares (15 x 15 cm 2). The animal is put alone in the center of the apparatus after administration of the test compound and left to explore freely during 10 minutes. A video camera is mounted on the top and behavior is recorded and the data is analyzed with the aid of a computerized tracking system.

Parameters deemed important are the number of squares crossed (ambulation), the frequency of rearing (standing on hind legs), time spent in the center or periphery, and the number of fecal boli which all give an indication of the locomotor functioning of the subject and its anxiety state. Anxiety is presumed to be lessened by the increased ambulation and central zone activity accompanied by decreased defecation, the latter being generally considered indicators of increased exploratory behavior [17].

Statistical analysis:

The data are shown as the mean ± standard error of the mean (SEM) with confidence intervals (CI) of 95%. The data are interpreted by using one-way ANOVA following Tukey's post hoc test. A probability level of 0.05 or less is accepted as significant.

Result:

The light and dark box test (LDBT)

In this test animals in the treated groups (LE 250mg/kg and LE 500mg/kg) shows significant dose dependent increased in the time at which animals remained in the bright chamber ($P < 0.001$), number of transition ($P < 0.001$) and transfer latency amongst both chambers ($P < 0.001$) as compared to the control group on 7th, 15th and 30th day of study (Figure 2,3 and 4). Whereas, diazepam (1 mg/kg), standard drug group shows significant increased in all the parameters assessed in light and dark box.

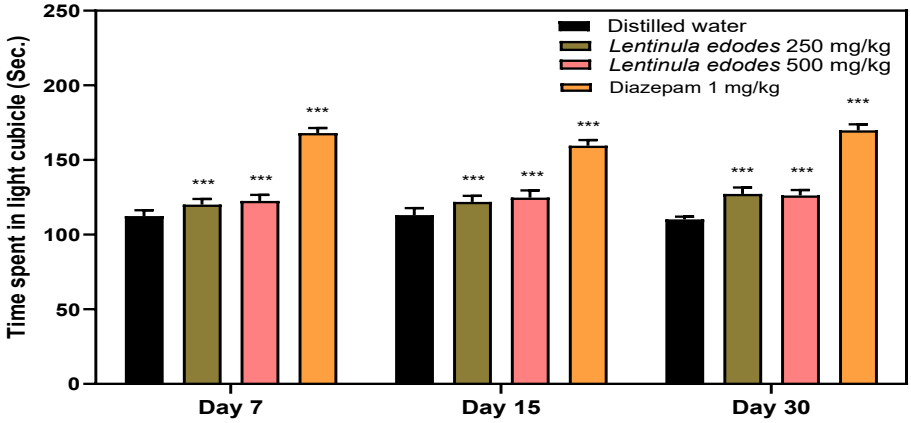

Figure 2. Effect of *Lentinula edodes* in time spent in light chamber

Animals per group (n) = 6. The values are mean ± S.E.M.; *< 0.05; **< 0.01; ***< 0.001 when compared with control group. (One-way ANOVA followed by Tukey's post hoc test)

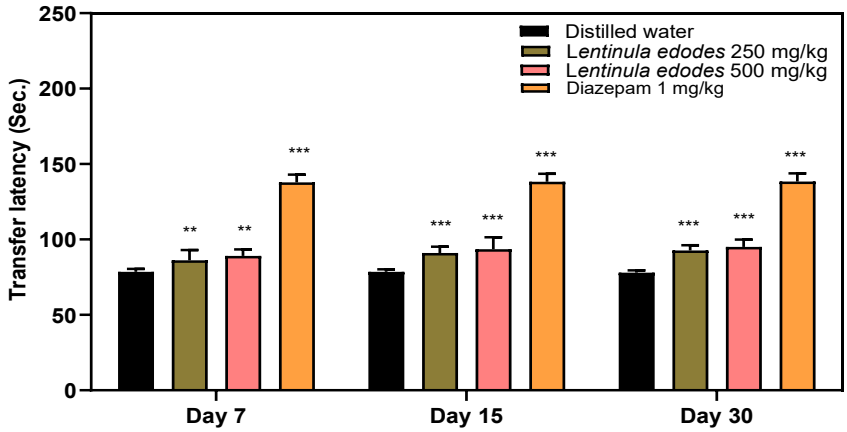

Figure 3. Effect of *Lentinula edodes* on transfer latency in light and dark box

Animals per group (n) = 6. The values are mean ± S.E.M.; *< 0.05; **< 0.01; ***< 0.001 when compared with control group. (One-way ANOVA followed by Tukey's post hoc test)

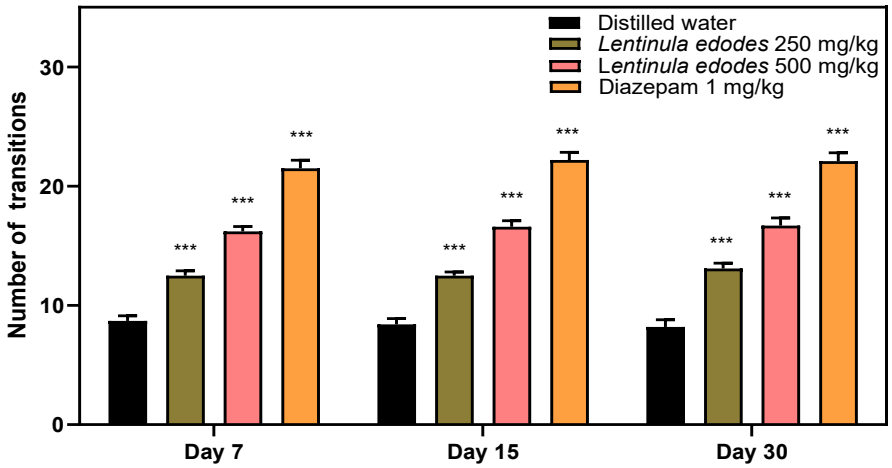

Figure 4. Effect of *Lentinula edodes* on number of transitions in light and dark box

Animals per group (n) = 6. The values are mean ± S.E.M.; *< 0.05; **< 0.01; ***< 0.001 when compared with control group. (One-way ANOVA followed by Tukey's post hoc test)

Elevated plus maze test (EPZT)

In this test animals both the treated groups (LE 250 mg/kg and LE 500 mg/kg) deduce significant raise ($P < 0.001$) in the time spent in open arm and entries in open arm as compared to that of control group on 7th, 15th and 30th day of the study (Fig 5 and 6).

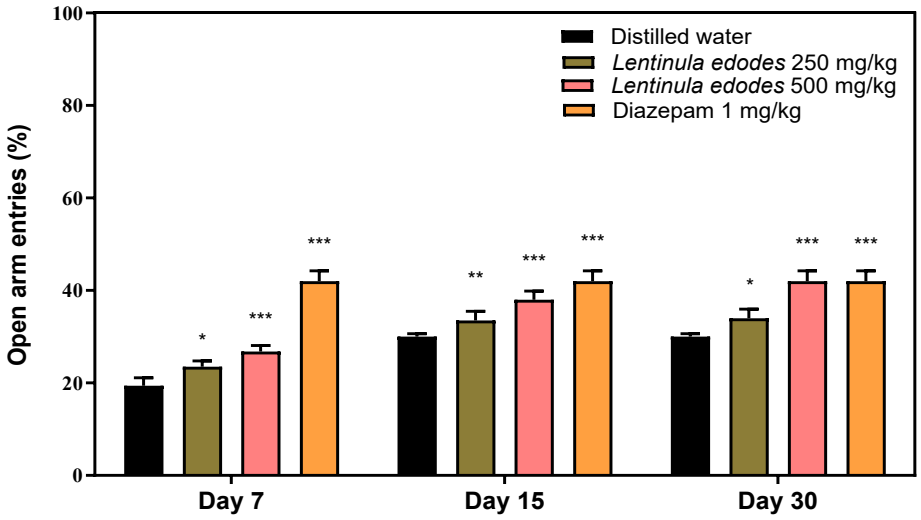

Figure 5. Effect of *Lentinula edodes* on open arm entries in Elevated plus maze

Animals per group (n) = 6. The values are mean ± S.E.M.; *< 0.05; **< 0.01; ***< 0.001 when compared with control group. (One-way ANOVA followed by Tukey's post hoc test

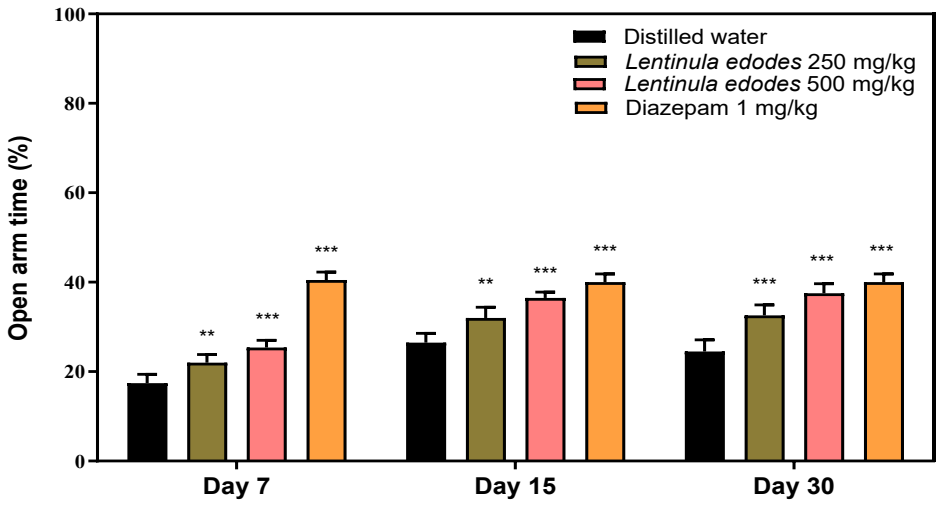

Figure 6. Effect of *Lentinula edodes* on number of transitions in light and dark box

Animals per group (n) = 6. The values are mean ± S.E.M.; *< 0.05; **< 0.01; ***< 0.001 when compared with control group. (One-way ANOVA followed by Tukey's post hoc test).

Open field test (OFT)

The result shows significant increase (P < 0.001) in the number of boxes crossed by the animals in both the treated groups (LE 250 mg/kg and LE 500 mg/kg) and standard drug group (diazepam 1mg/kg) as compared with the control group (Fig 7). Number of rearing are also increase significantly in the aforementioned groups (P < 0.001) in dose dependent fashion (Fig 8). However, droppings of faeces are significantly (P < 0.001) reduced in treated and standard drug group whereas increase in the control group (Fig 9).

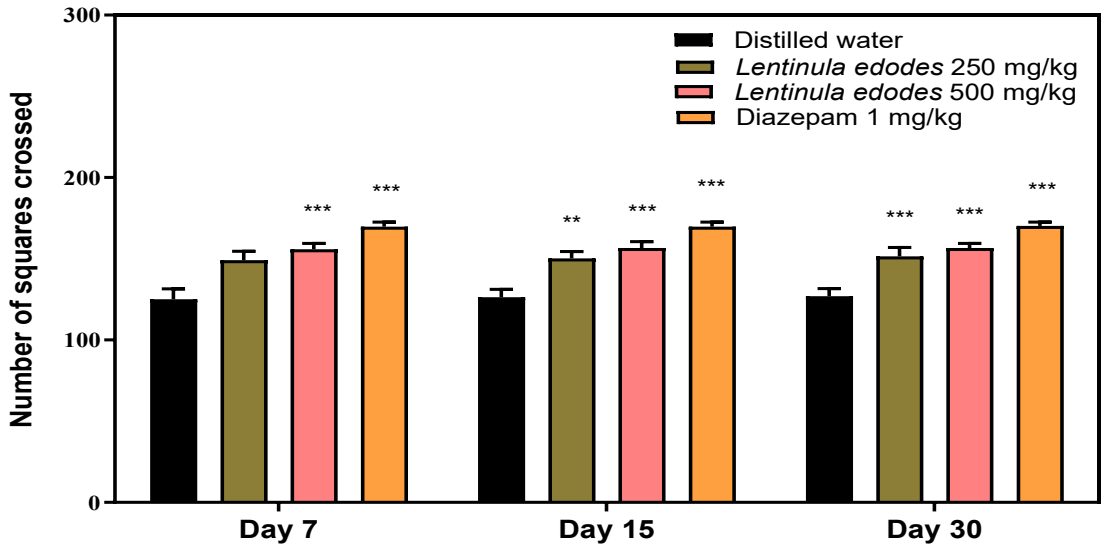

Figure 7. Effect of *Lentinula edodes* on number of squares crossed in open field

Animals per group (n) = 6. The values are mean ± S.E.M.; *< 0.05; **< 0.01; ***< 0.001 when compared with control group. (One-way ANOVA followed by Tukey's post hoc test).

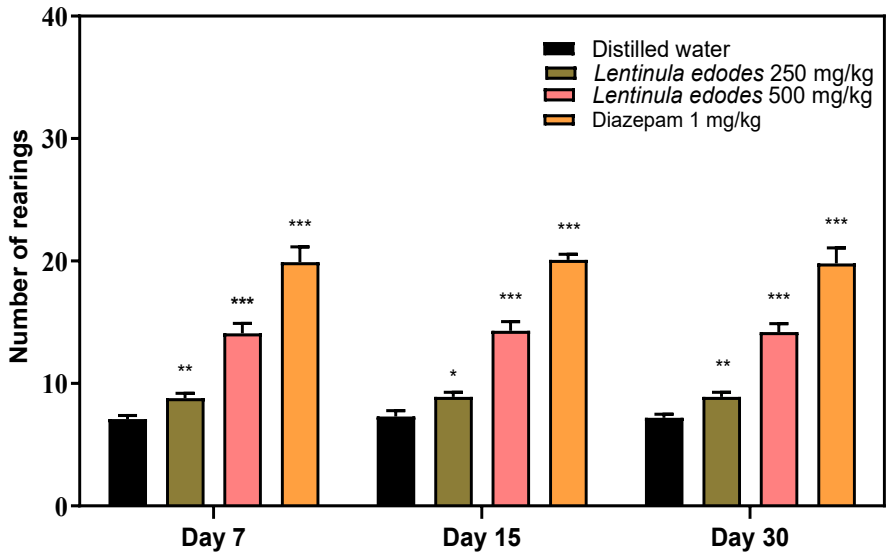

Figure 8. Effect of *Lentinula edodes* on number of rearinbgs crossed in open field

Animals per group (n) = 6. The values are mean ± S.E.M.; *< 0.05; **< 0.01; ***< 0.001 when compared with control group. (One-way ANOVA followed by Tukey's post hoc test).

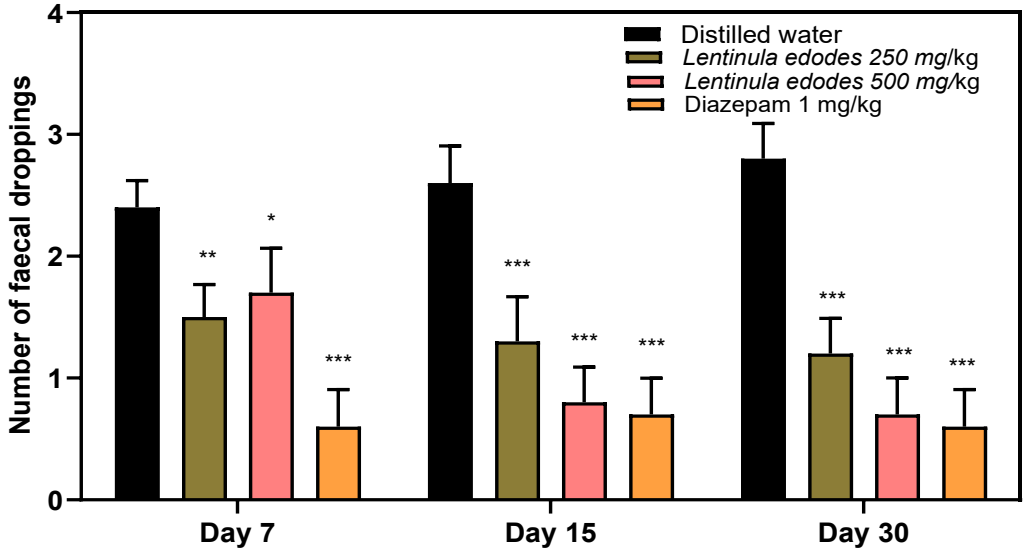

Figure 9: Effect of *Lentinula edodes* on faecal droppings in open field test

ANOVA following that Tukey's post-hoc analysis. Number of animals per group (n) = 06. The conclusions are given like mean ± S.E.M. $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$;

Discussion:

This study the anxiolytic effect of *Lentinula edodes* (shiitake mushroom) in rodents with the help of the commonly accepted behavioral tests: Elevated Plus Maze (EPM), Light and Dark Box (LDB), and Open Field Test (OFT). Two doses of the mushroom extract, 250mg/kg and 500mg/kg, administered orally were tested and compared with a reference anxiolytic drug, diazepam (1mg/kg). The results obtained suggest that

L. edodes has dose-dependent anxiolytic effect, and that the high dose has a more significant and uniform anxiolytic effect in all the models.

The 500 mg/kg dose of L. edodes produced a significant increase of the time spent by rodents in the bright compartment and the frequency of transitions between the compartments in the Light and Dark Box test (Figure 2,3 and 4). This change in behavior indicates less anxiety-like behavior since the rodent innate behavior is to stay in dark, closed areas but when their anxiety is low, they will explore the lighted area [15]. These findings were similar to the effects achieved with diazepam, which was used as a positive control of anxiolytic activity.

Likewise, the Elevated Plus Maze, an anxiety test depending on the conflict between innate fear of open areas and exploratory drive, revealed that animals dosed with 500 mg/kg spent much time in open arms and had more entries than control group (Figure 5 and 6). These actions were in line with known anxiolytic behaviour [18] . Although the lower dose (250 mg/kg) elicited modest effects, the higher dose was as efficacious as diazepam, indicating that the active constituents in L. edodes might exert their effects by converging central nervous system pathways, which might involve GABAergic system, although it is still to be established further.

These findings were also confirmed by the Open Field Test. The 500 mg/kg dose group displayed elevated locomotor activity, diminished freezing, and elevated entries into the central region of the open field (Figure 7, 8 and 9), which is suggestive of lowered anxiety and an intensified exploratory motivation [19]. Notably, there were no reports of sedation at either dose of mushroom extract, which is especially promising compared to benzodiazepines such as diazepam which regularly produce sedation at anxiolytic doses.

A possible mechanism of anxiolytic-like activity of L. edodes could be ascribed to a high content of neuroactive phytochemicals, especially β-glucans (including lentinan), ergothioneine, and phenolic antioxidants. Lentinan is well-researched in terms of its immunomodulatory and neuroprotective properties, such as the reduction of neuroinflammation which is highly connected with the state of anxiety and depression [20]. Ergothioneine is a special sulfur-bearing amino acid that is naturally found in mushrooms and can penetrate the blood-brain barrier; it has been shown to have strong antioxidant properties, which may help neuronal cells avoid oxidative stress, which is known to cause anxiety disorders [21].

Other studies sustain the health promoting properties of L. edodes. It is deemed as a functional food owing to its antioxidant, anti-inflammatory, and adaptogenic effects [22]. These actions can have synergistic action in attenuating stress-related behaviors in rodent models, representing an holistic mechanism of action, as opposed to the specific receptor-based modulations produced by conventional anxiolytics. In addition to that, it has also been shown that dietary mushroom supplementation can regulate gut microbiota which is critical in the gut-brain axis, a novel segment in the practice of neuropsychiatry. The mechanism of action of polysaccharides found in mushrooms to stimulate positive intestinal bacteria can have an indirect effect on the brain and its emotional control [23].

L. edodes exhibited anxiolytic potential similar to diazepam at high doses with a possibly more favorable safety profile. Benzodiazepines have been known to have such adverse effects as sedation, dependency, tolerance and withdrawal symptoms. On the contrary, L. edodes as a functional food or dietary supplement could offer a natural and less-toxic option that could be employed in the management of mild to moderate anxiety.

Nevertheless, there are also limitations in the study. The molecular level of mechanism of action was not investigated. Neurotransmitter quantification (e.g., GABA, serotonin), receptor binding assays and chronic

exposure studies to assess long-term efficacy and safety should be left to future studies. Also, the bioavailability and pharmacokinetics of the active compounds in L. edodes are topics of future investigation.

Conclusion

The present study demonstrates encouraging findings that *Lentinula edodes* has anxiolytic-like effects on rodent models, especially at 500 mg/kg dose. The extract also enhanced anxiety-related behaviours in three distinct paradigms, whose results were similar to those of the conventional diazepam drug, but without the sedative actions. Such results form the basis of exploring the possibility of using L. edodes as a natural anxiolytic. Additional clinical and mechanistic investigations are justified to confirm its safety in human population and to completely clarify neuro pharmacological profile.

Conflict of interest:

The authors declare that there is no conflict of interests regarding the publication of this paper.

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