



Antidepressant-Like Activity of Ethanol Extract of *Ganoderma lucidum* (Reishi) in Mice

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ABSTRACT

Ganoderma lucidum, known as “Lingzhi” in China, is one among greatly regarded fungi around the world. In old Chinese encyclopedias of medical “Shen Nong’s Ben Cao Jing” and “Ben Cao Gang Mu”, it is rated as extraordinarily precious fungus. In this study, antidepressant activity of ethanol extract of *Ganoderma lucidum* has been assessed. The extract was given orally by gavage at the dose of 20 mg/kg, 75 mg/kg, and 130 mg/kg body weight. Fluoxetine (20 mg/kg p.o.) was used as the standard drug. The results of our study show that *Ganoderma lucidum* significantly decreased immobility time in forced swim test and tail suspension test. Open field test was used to assess locomotor activity of the mice to exclude the false positive results. In open field test, *Ganoderma lucidum* didn’t affect the total movement and ambulatory movement at the same doses that significantly reduced immobility time in the forced swim test and tail suspension test. Thus, it is concluded that ethanol extract of *Ganoderma lucidum* has antidepressant activity in mice.

Keywords: *Ganoderma lucidum*, depression, forced swim test, tail suspension test

INTRODUCTION

Depression is a mental/mood disorder that portrays persistent and profound perception of hopelessness, sadness, or failure of attraction in things those were previously delightful. Risk of depression varies in lifetime of both men and women. In men, the risk of depression is around 5% to 12% and in women it is bit higher i.e. 10% to 25% [1]. Depression is culpable for the biggest fraction of disease load that are reasoned to non-fatal health consequences. Depression is responsible for 12% of disability in the world [2]. Depressive patients are often disturbed by increase pain and physical illness as compare to general population. Moreover, depressive patient has decreased educational, occupational, and social functioning and also have high medical morbidity. Precise diagnosis with effective treatment can improve the outcome of suicide in severe depressive episodes. Around 15% patients commit suicide with episodes of severe depression. Tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs) are commonly used amongst the various pharmacological drugs for treatment of depression, but they have many distressful adverse effects because they are frequently used for a long time. For that reason, hunt for antidepressants with wide action and lesser adverse actions continue [3].

Ganoderma lucidum, known as “Lingzhi” in China, is one among greatly regarded fungi around the world. In old Chinese encyclopaedias of medical “Shen Nong’s Ben Cao Jing” and “Ben Cao Gang Mu”, it is rated as extraordinarily precious fungus. Its production is mainly seen in Southwest, East China and provinces of Guangxi and Hebei [4-7]. In this study, antidepressant activity of *Ganoderma lucidum* has been assessed.

MATERIALS AND METHODS

Animal selection

In this study, we have used male Swiss albino mice. These mice weighed in between 25 g to 30 g. The animals were bought from the animal house of Aga Khan University. The temperature in the housing area was adjusted to 23°C ± 0.5°C, with 12 h light and dark cycle. The mice were provided with water and food ad libitum.

Different groups of mice

Mice were divided into different groups in the following manner for the assessment of antidepressant activity.

Group I: Normal control, given normal saline 2 ml/kg, p.o.

Group II: Treatment group, given extract 20 mg/kg, p.o.

Group III: Treatment group, given extract 75 mg/kg, p.o.

Group IV: Treatment group, given extract 130 mg/kg, p.o.

Group V: Positive control, given fluoxetine 20 mg/kg, p.o.

Dosing

Ganoderma lucidum, fluoxetine and normal saline were given orally by oral feeding tube/gavage. The dosing was done once daily (OD) at 9 a.m. continuously for 28 days.

Forced swim test

Forced swimming test was performed in accordance with the method described elsewhere with little alteration. Concisely, on pre-test session that was first day, mice were put one at a time in a clean cylinder (45 cm in height and 28 cm in diameter) that was filled with water to a depth of 15 cm and temperature of water inside cylinder should be $25 \pm 1^\circ\text{C}$. The mice were then compelled to swim in water for 15 minutes. Afterward, mice were taken out from the cylinder. Before transferring to their home cages mice were completely dried by the help of towels and warmed by a drier or heater. For each mouse, the water filled in the tank was changed. On the second day that was test session day, mice were put back into the swimming tank (cylinder) for 6 minutes. Immobility and mobility time was scored during the last 4 minutes of aggregate 6-minute test time. Immobility time was calculated if the mice continued floating without hustle and only made movements for keeping its nose or head above the water [8,9].

Tail suspension test

The method described by Steru, et al. [10] was used to measure the total immobility time in mice caused by suspending it from tail. Each mouse was suspended from tail by adhesive tape on suspension bar which is elevated 50 cm over the floor. The tape should be placed on tail in a way that it is 1 cm away from the tail tip. Each and every mouse should be isolated visually and acoustically while suspended. The immobility was measured during a test period of 6 minutes. Immobility in mice was considered if they hung completely motionless.

Open field test

To rule out the stimulant effects of the extract on immobility period, locomotor activity of mice was determined by using the apparatus named open field by following the method described elsewhere [11]. The apparatus is composed of white box of dimension (70 L \times 70 W \times 40 H cm) and the base of the box is divided into 25 squares of equal area of 10 cm \times 10 cm. At beginning of the test, mice were placed individually in the central box of open field and left to acclimatize in the open environment. Behaviour was assessed for 5-minute test period using a video camera mounted over the open field. The total number of boxes crossed centrally and peripherally, time spent in central and peripheral boxes, and number of rearing was analyzed. The apparatus was cleaned after each test by using isopropanol.

Statistical analysis

One-way ANOVA and Turkey's hoc test was used to calculate the statistical significance. The data is expressed as mean \pm SEM. Statistically significant difference was accepted at $p < 0.05$.

RESULTS

The results reveal that all three doses of *Ganoderma lucidum*, used in this study, demonstrated significant decrease in immobility time when compared with the normal control (saline-treated) group. Fluoxetine was used as a positive control drug and it also exhibited significant decrease in immobility time (Table 1).

Table 1 Effect of *Ganoderma lucidum* on the immobility time in forced swim test

Treatment groups	Immobility time		
	7th day	14th day	28th day
Normal saline	193.98 \pm 9.942	175.02 \pm 12.096	150.9 \pm 26.79
<i>Ganoderma lucidum</i> 20 mg/kg	139.8 \pm 16.53	99.3 \pm 23.55	51.798 \pm 28.662*
<i>Ganoderma lucidum</i> 75 mg/kg	145.38 \pm 30.36	98.22 \pm 33.306	46.302 \pm 23.862*
<i>Ganoderma lucidum</i> 130 mg/kg	96.78 \pm 27.192*	83.4 \pm 6.21*	36.3 \pm 15.876*
Fluoxetine	76.5 \pm 18.39**	70.32 \pm 2.081**	26.298 \pm 11.616**

Number of animals n=10. The values are mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when compared with the control group (One-way ANOVA followed by Tukey's post hoc test).

The results of tail suspension test reveal that *Ganoderma lucidum*, at the dose of 130 mg/kg, demonstrated significant decrease in immobility time when compared with the normal control (saline-treated) group. Fluoxetine was used as a positive control drug and it also exhibited significant decrease in immobility time (Table 2).

Table 2 Effect of *Ganoderma lucidum* on the immobility time in tail suspension test

Treatment groups	Immobility time TST		
	7th day	14th day	28th day
Normal saline	202.92 ± 14.634	220.5 ± 13.002	192 ± 33.282
<i>Ganoderma lucidum</i> 20 mg/kg	158.4 ± 10.416	181.32 ± 21.468	158.7 ± 25.122
<i>Ganoderma lucidum</i> 75 mg/kg	153.48 ± 20.694	156.72 ± 16.02	129.6 ± 4.246
<i>Ganoderma lucidum</i> 130 mg/kg	123.9 ± 11.04**	94.38 ± 25.296***	91.32 ± 15.318*
Fluoxetine	89.4 ± 13.614***	54.798 ± 8.742***	84.48 ± 11.13**

Number of animals n=10. The values are mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 when compared with the control group (One-way ANOVA followed by Tukey's post hoc test).

The results of locomotor activity, open field test, showed that the extract has insignificant effect on the locomotor activity of the animals (Table 3).

Table 3 Effect of *Ganoderma lucidum* on number of boxes crossed by mice in open field

Treatment groups	Total number of boxes crossed		
	7th day	14th day	28th day
Normal saline	58.333 ± 13.771	90.500 ± 14.322	53.500 ± 14.787
<i>Ganoderma lucidum</i> 20 mg/kg	117.33 ± 16.818	74.333 ± 21.235	65.167 ± 10.597
<i>Ganoderma lucidum</i> 75 mg/kg	111.67 ± 23.196	70.833 ± 16.782	64.000 ± 17.407
<i>Ganoderma lucidum</i> 130 mg/kg	105.00 ± 10.050	73.667 ± 22.896	42.500 ± 9.084
Fluoxetine	146.50 ± 45.541	26.000 ± 5.112	34.167 ± 12.046

Number of animals n=10. The values are mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 when compared with the control group (One-way ANOVA followed by Tukey's post hoc test).

DISCUSSION

The present study showed that *Ganoderma lucidum* also has antidepressant-like activity similar to that of fluoxetine. The findings that *Ganoderma lucidum* significantly reduces the time of immobility in FST and TST as compared to vehicle control proves that *Ganoderma lucidum*, has antidepressant potential. Furthermore, the present study also showed that antidepressant activity of *Ganoderma lucidum* is not greater than that of fluoxetine. Out of the three doses of *Ganoderma lucidum* used, the highest dose of 130 mg/kg, p.o showed the most significant antidepressant activity.

Antidepressants can be distinguished from stimulants, because antidepressant does not cause motor stimulation, in contrast to stimulants, which causes marked motor stimulation [12]. To determine whether *Ganoderma lucidum* actually possesses an antidepressant activity, we tested the locomotion counts to exclude the inhibitory or excitatory effects. *Ganoderma lucidum* didn't affect the total movement and ambulatory movement at the same doses that significantly reduced immobility time in the TST and FST.

The locomotor activity of animals was measured to differentiate between sedative and central nervous system stimulant activity of drugs. It was measured by using an instrument named open field. Locomotion of the animal was expressed in terms of total number of boxes crossed during a 5-min test for each mouse.

The vertical movement (rearing) is an index of the locomotor activity [13] while the horizontal movement (increased number of lines crossed) is an indication of the central nervous system stimulant properties. The results of open field showed that *Ganoderma lucidum* did not increase the locomotor activity as compared to control, thus showing that the antidepressant like effect was not because of central nervous stimulation. *Ganoderma lucidum* treated mice didn't show any significant change in locomotor activity of mice in comparison to the control, so it hasn't produce any overt motor effects. This supports the hypothesis that the antidepressant activity of the *Ganoderma lucidum* is not a false positive and is specific.

It has been proposed, in the amine hypothesis of depression, that low levels of noradrenaline (NA) and serotonin (5-

HT) in the brain triggers depression. Furthermore, the medicines that increase the levels of amine neurotransmitters lessen depression [14]. Nearby all the antidepressants produce their effects by acting on one or more of the following mechanisms: inhibition of monoamine oxidase, inhibition of 5-HT reuptake or NA (and DA), or antagonism of inhibitory preganglionic NA and/or 5-HT receptors. These mechanisms result in an increased level of NA and/or 5-HT. A wide evidence from the scientific studies propose that noradrenaline is a major neurotransmitter involved in the pathophysiology and management of depression and related disorders [15]. Antidepressant-like activity of *Ganoderma lucidum* might be due to its effect on noradrenergic or serotonergic neurotransmission.

CONCLUSION

The results of this study suggest that ethanol extract of *Ganoderma lucidum* possesses antidepressant-like activity in animal models of depression.

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