Available online at **www.ijmrhs.com**

ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2016, 5, 6:57-60

Anxiolytic-like activity of ethanol extract of *Ganoderma Lucidum* (Reishi) in mice

Nasir Ali[1], *Aslam Muhammad[1] and Sial Ali Akbar[2]

[1]*Department of Pharmacology, Faculty of Pharmacy, Ziauddin University, Karachi 75600, Pakistan*
[2]*Department of Pharmacy Practice, Faculty of Pharmacy, Ziauddin University, Karachi 75600, Pakistan*
Corresponding Email:Pharmacologist1@yahoo.com

ABSTRACT

Ganoderma lucidum is used for the treatment and prevention of many ailments including arthritis, hepatitis, hypercholesterolemia, bronchitis, gastric cancer and hypertension. In this study we have evaluated the potential anxiolytic-like activity of the mushroom in mice models of anxiety. Light/dark box and elevated plus maze test were used to evaluate the anxiolytic-like activity of ethanol extract of fruiting bodies and cracked spores of Ganoderma lucidum. The extract was given orally by gavage at the dose of 20 mg/kg, 75 mg/kg and 130 mg/kg body weight. Diazepam (1 mg/kg i.p.) was used as the standard drug. The results reveal that Ganoderma lucidum demonstrated significant increase in the time spent in light cubicle when compared with the control group in light/dark box test and the results of elevated plus maze test reveal that Ganoderma lucidum, showed significant increase in the time spent in open arms and number of open arm entries when compared with the control group. Overall evidences propose that ethanol extract of Ganoderma lucidum has anxiolytic activity which is comparable to 1 mg/Kg diazepam.

Key words: *Ganoderma lucidum*, Anxiety, Light/dark box test, Elevated plus maze test

INTRODUCTION

Anxiety is a sensation of uneasiness, uncertainty, and tension arising from hypothetical or hallucinatory threat together with increase in sympathetic activity and alteration of psycho physiological signs including increased skin conductance, heart rate and muscle tone. Patients of anxiety have feeling of fear, dispersed and greatly unpleasant feelings [1]. Anxiety is a psychiatric condition which disrupts routine life activity and affects 4-6% of the population [2]. Phobias, panic disorders and generalized anxiety are clinical symptoms of anxiety [3]. In generalized anxiety disorders, benzodiazepines are commonly used although these agents have many side effects including memory disturbance, muscle relaxation, physical dependence, sedation and interaction with many drugs. Drugs from traditional herbs as well as newly synthesized compounds can have relevance in the therapy of anxiety. Notably for this purpose, utilization of natural, tolerable and mild phytopharmaceuticals are in public interest [4].

Ganoderma lucidum, known as "Lingzhi" in China, is one among greatly regarded fungi around the world. Its production is mainly seen in Southwest, East China and provinces of Guangxi and Hebei[5]. Reishi, Lingzhi, Sachitake, Munnertake and Youngzhi are the common names of *Ganoderma lucidum*[6]. *Ganoderma lucidum* contains various types of active substances that are inorganic ions, organic germanium, triterpenoids, sterols, polysaccharides, oils and fats [7].Primary medicinal constituent of *Ganoderma lucidum* are triterpenoids and polysaccharides [8,9]. *Ganoderma lucidum* for centuries particularly in China, Japan, and Korea, was frequently related to health, long life, healing and happiness [10]. In Asian countries, *Ganoderma lucidum* has been utilized widely as a traditional medicine for thousands of years[11]. This miraculous mushroom is used for the treatment and

prevention of many ailments including arthritis, hepatitis, hypercholesterolemia, bronchitis, gastric cancer and hypertension [8].In this study we have evaluated the potential anxiolytic-like activity of the mushroom in mice models of anxiety.

MATERIALS AND METHODS

Animals

In this study we have used male Swiss albino mice. These mice weighed in between 25g 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 hours light and dark cycle. The mice were provided with water and food *ad libitum*. All experiments were carried according to the guidelines set forth by the National Institutes of Health (Bethesda, MD, USA) and prior approval was taken from the ethical review committee, Ziauddin University.

Different groups of mice

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

Group I: Normal control, given normal saline 2ml/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 diazepam 1 mg/kg, *i.p.*

Dosing

Normal saline and *Ganoderma lucidum* were given orally by oral feeding tube/gavage. Intraperitoneal route was used for the administration of diazepam. The dosing was done once daily (OD) at 9 a.m. continuously for 28 days.

The light/dark box test

The two compartments or light/dark box method titrates the natural habit of mice to analyze a new environment, contrary to the callous properties of blazingly light open field. The steadiest parameter for analyzing anxiolytic activity is exploratory behavior and the time spent in light region. The light/dark box consists of two compartments: one light region or white region of size (25L×25W×27H cm) brightened by desk lamp and the other dark region or black region of size (25L×25W×27H cm), both the compartments were separated via tunnel of size (7.5×7.5 cm) for allowing mice to move from one compartment to other. The parameter being observed was time spent in the light region or white region of cage[12,13].

Elevated plus maze test

Exploratory behavior of mice was determined by using the apparatus named elevated plus maze following the method described elsewhere [14,15]. The apparatus was elevated 50 cm over the floor and consisted of two closed arms that were opposite to each other and two open arms that were also opposite to each other. Both the arms (open and closed) were of same dimensions: 50cm long x 10cm wide, closed arms were surrounded by side walls of size 40cm in height that extended from central platform of size 10cm x 10cm. At the beginning of the test, mice were placed one by one over the central area or platform, and the behavior was analyzed for 10 mins using a video camera mounted over the apparatus. The total time spent by mouse in each of the arms as well as the total number of entries towards each arm were analyzed and recorded.

Statistical analysis

One-way ANOVA and Tukey's *post hoc* test were used to calculate the statistical significance. The data is expressed as mean ± SEM. Statistically significant difference was accepted at $P < 0.05$.

RESULTS

The results reveal that *Ganoderma lucidum*, at the dose of 75 mg/kg and 130 mg/kg, demonstrated significant and dose dependent increase in the time spent in light cubicle when compared with the control (saline-treated) group. Diazepam, as a standard drug, also exhibited significant increase in the time spent in light (Table 1).

Table 1. Effect of *Ganoderma lucidum* on the time spent in the light cubicle in light/dark box test

Treatment groups	Time spent in light compartment		
	7th day	14th day	28th day
Normal saline	62.232 ± 2.264	5.509 ± 0.884	2.449 ± 0.801
*Ganoderma lucidum*20 mg/kg	64.062 ± 1.691	62.628 ± 1.656	60.882 ± 1.575
Ganoderma lucidum 75 mg/kg	121.212 ± 1.277	65.928 ± 2.143*	120.93 ± 2.119**
Ganoderma lucidum 130 mg/kg	125.01 ± 0.963**	120.312 ± 0.963*	122.76 ± 2.173***
Diazepam	123.84± 1.571*	123.84± 1.571***	123.84± 1.571***

*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'spost hoc test).*

The results of elevated plus maze test reveal that *Ganoderma lucidum*, at the dose of 75 mg/kg and 130 mg/kg, demonstrated significant and dose dependent increase in the time spent in open arms and number of open arm entries when compared with the control (saline-treated) group. Diazepam, as a standard drug, also exhibited significant increase in the time spent (Table 2 & 3).

Table 2. Effect of *Ganoderma lucidum* on the time spent in the open arms in elevated plus maze

Treatment groups	Time spent in open arm		
	7thday	14thday	28thday
Normal saline	1.570 ± 1.209	2.352 ± 1.56	1.05 ± 0.991
*Ganoderma lucidum*20 mg/kg	2.19 ±1.086	1.999 ± 1.315	63.828 ± 1.390
Ganoderma lucidum 75 mg/kg	63.702 ±1.447	61.638 ± 1.238	123.42 ± 2.689*
Ganoderma lucidum 130 mg/kg	62.67 ± 1.408	2.569 ± 0.732	124.368 ± 4.851*
Diazepam	123.6± 5.258*	123.24± 5.389*	124.242± 4.913*

*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'spost hoc test).*

Table 3: Effect of *Ganoderma lucidum* on number of entriesin the open arms in elevated plus maze

Treatment groups	Entries in open arm		
	7thday	14thday	28thday
Normal saline	1.333 ± 0.989	2.167 ± 1.014	0.5000 ± 0.224
*Ganoderma lucidum*20 mg/kg	2.500 ± 0.992	2.333 ± 1.282	1.667 ± 0.558
Ganoderma lucidum 75 mg/kg	5.500 ± 1.384	6.667 ± 1.085*	6.167 ± 1.352**
Ganoderma lucidum 130 mg/kg	6.167 ± 1.195*	3.667 ± 0.843	5.167 ± 1.400*
Diazepam	3.833 ± 1.046	1.000± 0.365	5.833 ± 0.401**

*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

A number of scientific studies have been carried out on *Ganoderma lucidum* for various pharmacological activities. However, no scientific study has been carried out to explore anxiolytic effect of this miraculous mushroom. This research study was designed in an attempt to assess the anxiolytic potential of ethanol extract of *Ganoderma lucidum* in mice.

Anxiolytic activity was assessed using two different animal models for anxiety named as elevated plus maze and light and dark box test. The conventional plus maze is greatly sensitive to the effect of anxiolytic as well as anxiogenic medications interacting at $GABA_A$-BZD (benzodiazepine) complex [16]. Elevated plus maze is thought-out amongst widely validated models for the assessment of anxiolytic and sedative substances such as benzodiazepines. On elevated plus maze or EPM, mice commonly spend more time in the closed arms when compared to open arms[17]. This choice reflects rodents' disliking of the open arms due to anxiety of open arena. Anxiolytics drugs are those that increase the number of entries and time spent in open arms and the inverse remains true for anxiogenics [16, 18]. Results of this study demonstrated that *Ganoderma lucidum* treated mice showed statistically significant increase, compared to control group, in time spent in open arms and the number of entries in open arms and revealed anxiolytic potential in elevated plus maze (EPM) model.

Light/dark box test is another broadly utilized animal model for the assessment of anxiolytic and anxiogenic drugs[17]. This model is dependent on the innate reluctance of rodents to lighted areas as well as abrupt natural behavior of rodents due to mild stressors that is light [12]. Drug-induced behavioral increment towards the enlighted

region of light/dark box, is proposed as an index of anxiolytic potential [19,20]. In light/dark box model, the results were similar to (EPM or elevated plus maze) model. Compared to control group, the groups that were treated with the *Ganoderma lucidum* extract at the dose of 75 mg/kg and 130 mg/Kg revealed statistically significant rise in total time spent in the light arena, identical to the standard anxiolytic drug, suggesting anxiolytic potential of *Ganoderma lucidum.*

CONCLUSION

Overall evidences propose that ethanol extract of *Ganoderma lucidum*has anxiolytic activity which is comparable to 1 mg/Kg diazepam.

REFERENCES

[1] Talbott J. Prevalence, Severity, and Comorbidity of 12-Month DSM-IV Disorders in the National Comorbidity Survey Replication. Yearbook of Psychiatry and Applied Mental Health. 2006;2006:131-132.
[2] Kasper S. Social phobia: the nature of the disorder. Journal of Affective Disorders. 1998;50:S3-S9.
[3] Morgan Kclarke D. Longitudinal trends in late-life insomnia: implications for prescribing. Age and Ageing. 1997;26(3):179-184.
[4] Vitale G, Arletti R, Ruggieri V, Cifani C, Massi M. Anxiolytic-like effects of nociceptin/orphanin FQ in the elevated plus maze and in the conditioned defensive burying test in rats. Peptides. 2006;27(9):2193-2200.
[5] Xia Q, Zhang H, Sun X, Zhao H, Wu L, Zhu D et al. A Comprehensive Review of the Structure Elucidation and Biological Activity of Triterpenoids from Ganoderma spp. Molecules. 2014;19(11):17478-17535.
[6] Paterson R. Ganoderma — A Therapeutic Fungal Biofactory. ChemInform. 2006;37(50).
[7] El-Mekkawy S, Meselhy M, Nakamura N, Tezuka Y, Hattori M, Kakiuchi N et al. Anti-HIV-1 and anti-HIV-1-protease substances from Ganoderma Lucidum. Phytochemistry. 1998;49(6):1651-1657.
[8] Zhang Q, Xiang J, Zhang L, Zhu X, Evers J, van der Werf W et al. Optimizing soaking and germination conditions to improve gamma-aminobutyric acid content in japonica and indica germinated brown rice. Journal of Functional Foods. 2014;10:283-291.
[9] Shiao M, Lin L, Yeh S. Triterpenes from Ganoderma lucidum. Phytochemistry. 1988;27(9):2911-2914.
[10] Pawlik A, Janusz G, Dębska I, Siwulski M, Frąc M, Rogalski J. Genetic and Metabolic Intraspecific Biodiversity ofGanoderma lucidum. BioMed Research International. 2015;2015:1-13.
[11] Ji Z, Tang Q, Zhang J, Yang Y, Jia W, Pan Y. Immunomodulation of RAW264.7 macrophages by GLIS, a proteopolysaccharide from Ganoderma lucidum. Journal of Ethnopharmacology. 2007;112(3):445-450.
[12] BourinMHascoët M. The mouse light/dark box test. European Journal of Pharmacology. 2003;463(1-3):55-65.
[13] Chen S, Wang W, Li W, Wang R, Li Y, Huang Y et al. Anxiolytic-like effect of asiaticoside in mice. Pharmacology Biochemistry and Behavior. 2006;85(2):339-344.
[14] Bertoglio L, Joca S, Guimarães F. Further evidence that anxiety and memory are regionally dissociated within the hippocampus. Behavioural Brain Research. 2006;175(1):183-188.
[15] Matsuzaki H, Shimizu Y, Iwata N, Kamiuchi S, Suzuki F, Iizuka H et al. Antidepressant-like effects of a water-soluble extract from the culture medium of Ganoderma lucidum mycelia in rats. BMC Complementary and Alternative Medicine. 2013;13(1):370.
[16] Bhattacharya S, Satyan K. Experimental methods for evaluation of psychotropic agents. Indian Journal of Experimental Biology.35:565-575.
[17] Aslam M., Hussain S. Anxiolytic-like Activity of Methanol Extract of Leaves of *Cydonia oblonga*Miller (Quince) in Mice. Inventi Impact: Ethnopharmacology. 2015;3:96-101.
[18] Jaiswal A, Bhattacharya S, Acharya S. Anxiolytic activity of *Azadirachtaindica* leaf extract in rats. Indian Journal of Experimental Biology.1994; 32:489-491.
[19] Aslam M, Sultana N. Evaluation of anxiolytic-like activity of *Vitis vinifera* juice in mice. Avicenna Journal ofPhytomedicine. 2016; 6:344-350.
[20] Ihne J, Fitzgerald P, Hefner K., Holmes, A. Pharmacological modulation of stress-induced behavioral changes in the light/dark exploration test in male C57BL/6J mice. Neuropharmacology. 2012; 62:464-473.