ADAPTOGENIC (ANTISTRESS) ACTIVITY OF METHANOLIC EXTRACT OF *GANODERMA LUCIDUM* AGAINST PHYSICAL AND HYPOXIC STRESS IN MICE

Pawar Vinod S.[1*], Hugar Shivakumar[2]

[1] Dept of Pharmacology, S.V.P.M's College of Pharmacy, Malegaon (Bk), Baramati 413115, Maharashtra, India

[2] P.G. Dept of Pharmacology, BLDEA's. College of Pharmacy, Bijapur 586103, Karnataka, India

Summary

In the present study an adaptogenic effect of a methanolic extract of fruiting bodies of *Ganoderma lucidum* was evaluated against swimming endurance followed post swimming antifatigue and motor coordination and hypoxic stress tolerance test in mice. In swimming endurance, mice were treated for 7 days whereas in hypoxic stress animals were treated for 3 weeks. At the end of treatment all animals were individually subjected to respective stress stimuli. Oral administration (100, 300 and 500 mg/kg/day) of test extract showed dose dependant significant enhancement in swimming endurance time and antifatigue effect in post swimming antifatigue and motor coordination test. Concomitant treatment with test extract at dose of 300 and 500 mg/kg showed significant increase in hypoxic stress tolerance time. But the lower dose (100 mg/kg) of test extract produced considerable increase in hypoxia tolerance time. The findings from the present investigation indicate that test extract is known to possess significant antistress property as shown by its mitigating effects on both experimentally induced stress models.

Keywords: *Ganoderma lucidum,* swimming endurance, hypoxia test

Corresponding author
Pawar Vinod S.
Dept of Pharmacology,
SVPM's College of Pharmacy,
Malegaon (Bk), Tal Baramati,
Dist Pune, 413115 Maharashtra, India.
Tel.: +919420181501
Fax: 02112254447
E-mail: vinod_pharmacology@yahoo.co.in

Introduction

The adaptogen is a substance which can develop a state of raised resistance enabling an organism to cope with stressful situations[1]. The general aims of adaptogens therapy lie in its ability to reduce stress reactions during the alarm phase of stress response, prevent or at least delay the state of exhaustion and hence provide a certain level of protection against long term stress. A large variety of herbals have been studied for their Adaptogenic and rejuvenating properties. These plants are believed to promote positive health and maintain organic resistance against infections by re-establishing body equilibrium and conditioning the body tissues. Therapeutic approach for stress from ancient times has involved utilization of substances from natural origin, rather than synthesis of new chemical compounds. Since the introduction of adaptogens, several plants have been investigated, which were once used as tonics due to rejuvenating properties in traditional medicine[2].

Ganoderma lucidum, which belongs to the family of Ganodermataceae, is called *reishi* in Japanese and *lingzhi* in Chinese, is highly ranked in Oriental folkore medicine, considered as panacea because it is reported to have broad spectrum of medicinal properties for both health maintenance and treatment of diseases. *G. lucidum* is also called "marvelous herb" or "mashroom of immortality" emphasizing its function in enhancing longevity[3,4]. *G. lucidum* occurring in southern part of India possessed significant antioxidant, antitumor and anti-inflammatory activities[5].

With this view, present study was undertaken to evaluate adaptogenic activity of methanolic extract of fruiting bodies of *G. lucidumin* involving diverse stressors viz. swimming endurance, posts swimming muscular function test and hypoxia test.

Materials and methods

Plant material

Gift sample for research of fruiting bodies were obtained from Indian Council of Agriculture Research, Chambaghat, Solan (HP), India. (Ref. No. F. Corp Protection/Gano/09-10/6772). The specimen was identified by Prof. R. B. Deshmukh Department of Botany, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar, Baramati, Pune District, India. Plant specimen no. is PASR 122.

Preparation of extract

Fruiting bodies of *G. lucidum* were cut into small pieces, dried at 45-50^{o} C for 48 h, and powdered. 200 gms of the powdered material was defatted with petroleum ether. The marc obtained was then subjected to extraction with methanol (64 – 65.5^{0}C). The extract was concentrated using rotary flash evaporator

Animals

The pharmacological studies were conducted on Swiss albino mice (20-30g) of either sex, colony bred in the Institute's animal house. After procurement, all animals were left for one week for acclimatization and maintained in standard conditions (27 ± 3^{o} room temperature, 60-70 % relative humidity and 12h, photo period). The animals were fed with standard rodents pellet diet and were provided water *ad libitum.*

Acute toxicity study

An acute toxicity of METFGS was carried out in female albino mice (20-30 g). The animals were fasted over night prior to the experiment. Fixed dose (OECD Guideline No. 420) method of CPCSEA was adopted for toxicity study[6].

Swimming endurance and post–swimming muscle coordination test *(physical stress)*

Swiss albino mice of either sex having weight 20 – 30 g were divided into five groups (n=6) in which one group served as normal control (received only vehicle 1 ml/kg) and the animals treated with MEGL (100, 300, 500 mg/kg b.w.), WS (100 mg/kg b.w.) served as drug treated groups. Test and standard drugs were administered orally once a day for 7 days. On day 7, one hour after drug administration, the each animal was allowed to swim inside a perplex glass container (30 cm height with 20 cm diameter, containing water up to 25 cm height) maintained at 26 ± 1^{o}C. The end point was considered when animals were got exhausted[7,8]. The animals were removed and allowed to recover and dry for about 5 min. the animals were subsequently tested for muscle coordination on rota rod rotating at 15 rpm and the duration of stay on the rod was recorded[9].

Hypoxia test

Swiss albino mice of either sex weighing 20 – 30 g were selected and divided into five groups (n=6) in which one group served as normal control (received only vehicle 1 ml/kg p.o.) and the animals treated with MEGL (100, 300, 500 mg/kg b.w. p.o.), WS (100 mg/kg b.w. p.o.) served as drug treated groups. Animals were treated as shown above for 21 days. On day 7, 14 and 21, one hour after the treatment hypoxia time was recorded by placing each animal individually in the perplex jar of 1 lit capacity. The jars were made air-tight with greased stoppers and time until onset of convulsion was recorded as the end point[10]. Appearance of convulsion was very sharp end point, as delay by minute of removal of the animal from the vessel may lead to death of the same.

Statistical Analysis

All the values are expressed as mean ± SEM and data was analyzed by one–way ANOVA using Graph pad Prism. The post hoc analysis was carried out by Tukey's multiple comparison tests for expressing the significance and $p < 0.05$ was considered as significant.

Results

Acute toxicity study

Following the administration of single dose of 2000mg/kg body weight of MEGL to mice, no death was registered after 48h post treatment.

Swimming endurance and post swimming muscle coordination function

It was observed that MEGL (100,300, 500 mg/kg) treatment for 7 day showed striking increase in the swimming endurance time in mice which was significant *($p < 0.05$, $p < 0.01$, $p < 0.001$)* and dose dependant compared to control. In the post swimming muscle coordination (antifatigue) test in mice MEGL significantly increased the duration (sec) of stay on rota rod which was also dose dependant. (Fig 1 and 2)

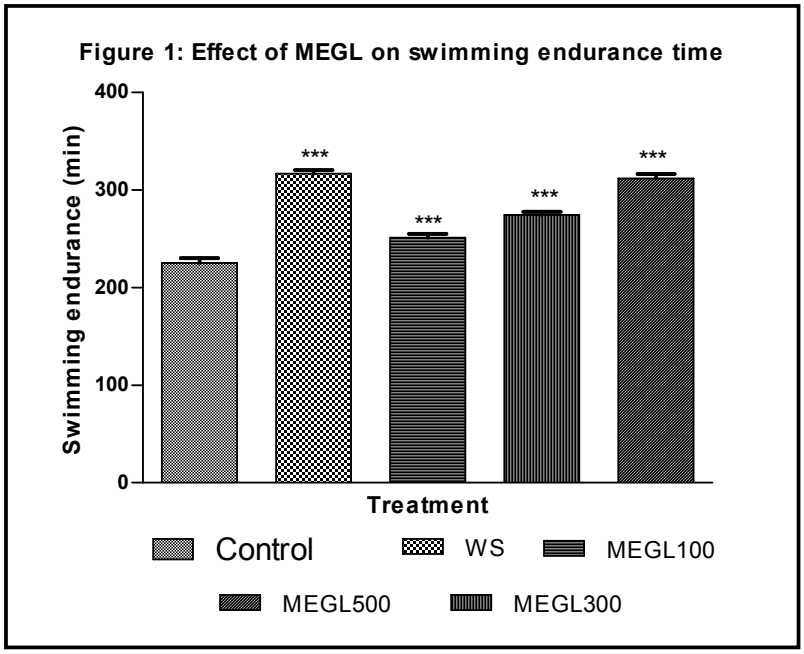
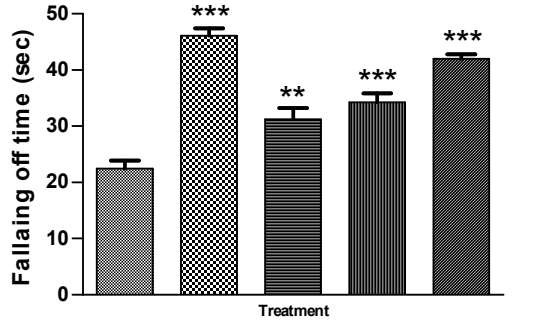


Figure 2: Effect of MEGL on post swimming antifatigue test



Hypoxia test

Anoxia stress tolerance time was determined by taking the appearance of convulsion as end point. On 7th day of MEGL (100, 300, 500 mg/kg) showed increase in stress tolerance time compared with normal control but results were not significant statistically. MEGl 300 and 500 mg/kg offered significant *($p < 0.05$ and $p < 0.01$),* dose dependant increase in hypoxia stress tolerance on 14th day. There was also significant rise in anoxia tolerance time seen on day 21 in both 300 and 500 mg/kg of MEGL *($p < 0.01$, $p < 0.001$).*

Table 1: Effect of MEGL on hypoxia time in mice

Treatment	Dose (mg/kg, p.o)	Hypoxia time (min) Mean ± SEM		
		7th Day	14th Day	21st Day
Normal Control	--	148.79 ± 4.32	149.80 ± 4.20	152.60 ± 3.86
WS	100	192.41 ± 3.62***	197.63 ± 4.16***	203.20 ± 4.32***
MEGL	100	148.20 ± 3.31	154.13 ± 4.30	164.91 ± 4.51
MEGL	300	156.40 ± 3.18	169.60 ± 3.78 *	181.80 ± 4.60**
MEGL	500	162.30 ± 3.34	175.10 ± 4.53**	189.78 ± 5.22***

Hypoxia time expressed as the mean ± SEM; (n = 6).
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared to control.
WS: *Withania somnifera standerdised extract*
MEGL: Methanolic extract of *Ganoderma lucidum*

Discussion

In the present study MEGL has been evaluated for the adaptogenic (antistress) activity against swimming endurance followed by antifatigue effect and hypoxia test in mice. The well known adaptogen *Withania somnifera* was taken as a reference standard in the present study.

The anti-hypoxia effect is related to improved or raised cerebral resistance to hypoxia and reduced cerebral consumption of oxygen in acute hypoxia. When mice are exposed to a hypobaric environment for a specified period, the mitochondria of heart and brain cells of are seriously damaged and brain neurotransmitters, i.e. norepinephrine, dopamine, serotonin and acetylcholine are significantly decreased. Our results demonstrated that MEGL at dose of 300 and 500 mg/kg exhibited significant and dose dependant antistress activity as indicated by increase in duration of anoxia stress tolerance time. This protective action of MEGL on hypoxia in mice may be due to action on the pituitary-adrenal gland axis[7].

Mice when forced to swim in a restricted space from which they cannot escape, become immobile after an initial period of vigorous activity. It has been suggested that the observed immobile immobility signifies behavioral despair which is now recognized as common consequence of stress[12]. A dose dependant significant antistress property was exhibited by MEGL in swimming endurance test as evident by striking augmentation in swimming endurance time and also offered significant post swimming antifatigue effect in mice.

Increased generation of oxidative free radicals (OFR) or impaired antioxidant defense mechanism have been implicated in stress induced perturbed homeostasis including immunosuppression, inflammation, diabetes mellitus, peptic ulceration and other stress related disease[13]. Mushrooms have a notable place in the folklore throughout the world and in traditions of many cultures[14]. The fruiting body of *Ganoderma lucidum* has been regarded panacea for all types of diseases[15]. Literature reveals that *G. lucidum* possessed significant antioxidant properties[5]. Thus at least part of observed adaptogenic (antistress) effect of MEGL may be due to the antioxidant activity.

Conclusion

The findings that it increases resistance to stress against diverse aversive stimuli in a non-specific manner indicates that it could have adaptogenic activity but the study did not include the tests for elucidating the mechanism of action.

Acknowledgement

The authors are thankful to Director, Indian Council of Agriculture Research, Chambaghat, Solan (HP) India for providing the *Ganoderma lucidum.* Also financial support from BCUD, University of Pune, Maharashtra is gratefully acknowledged.

References

1. Lazarev NV. General and specific in action of pharmacological agents. Farmacol Toxicol 1958; 21: 81-6.
2. Rege NN, Thatte UM, Dahanukar SA. Adaptogenic properties of six rasayana herbs used in Ayurvedic medicine. Phytother Res 1999; 13: 275-291.
3. Yuen JWM, Gohel MDI. The dual rples of *Ganoderma* antioxidants on urothelial cell DNA under carcinogenic attack. J Ethnopharmacol 2008; 118:324-330.
4. Shiao MS, Lee KR, Lin LJ, Wang CT. Natural products and biological activities of the Chinese medical fungus, *Ganoderma lucidum.* In: Ho CT, Osawa T, Hung MT, Rosen RT, editors. Food phytochemicals for cancer prevention. II. Teas, species and herbs. Washington, DC: American Chemical Society; 1994: 342-354.
5. Jones S, Janardhanan KK. Antioxidant and antitumor activity of *Ganoderma lucidum* (Curt. Fr.) P. Karst- Reishi (Aphyllopharmocetideae) from South India. Int J Med Mushr 2000, 86: 195-200.
6. Mrs. Prema Veeraraghavan. (2000). Expert consultant, CPCSEA, OECD guide line No. 420.
7. Bani Sarang, Pandey Anjali, Kour Kiranjeet et al. Adaptogenic activity of *Labisia pumila* extract. PCT WO2010/147449 A1: 23 December 2010.
8. Pawar VS, Shivakumar H. Antistress activity of *Trigonella foenum graecum* (Linn) seeds using swimming endurance and cold stress in rodents. Indian Drugs 2011;48(2): 56-61.
9. Ghufran Ahmad, Yusuf Amin KM, Naeem A Khan, Tajuddin. The antistress activity of a gem-containing unani formulation against diverse stressors. J Ethnopharmacol 1998; 59: 187-193.
10. Shivakumar H, Talha Javed, Prakash T, Nagendra Rao, Jayakumar Swamy BHM & Veerana Goud A. Adaptogenic activity ethanolic extract of *Tribulus terrestris* Linn. J Nat Rem 2006; 6/1: 87-85
11. Vinod S Pawar, Shivakumar H. Screening methods for evaluation of adaptogenic agents: a review. J Pharmacy Research 2011; 4(3): 763-765.
12. Porsolt RD, Bertin A, Jlafre M. Behavioural despair in mice. A primary screening test for antidepressants. Arch Int Pharmacodyn Ther 1977, 229: 327-335.
13. Anand kumar Singh, Sunil S Dhamanigi, mohammad Asad. Antistress activity of hydroalcoholic extract of *Eugenia caryaphyllus buds.* Indian J Pharmacol 2009 41(1): 28-31.
14. Chang ST, Buswell JA. Mushroom nutriceuticals. World J Microbiol Biotechnol 1996, 12: 473-476.
15. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxide in animal tissue by thibarbituric acid reaction. Ann Biochem 1979, 95: 351-358.