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Diuretic Activity and Cytotoxic Study of Various Extracts of *Ganoderma lucidum*

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Abstract: *Ganoderma lucidum* has been in practice traditionally for a multitude number of human diseases. In vivo study in BALC/c mice was conducted for the investigation of diuretic potential of this mushroom. The diuretic property was studied against Furosemide 40mg/kg, a standard drug. The results of aqueous extract of *Ganoderma lucidum* at doses 300 mg/kg and 500 mg/kg were 1.8 ±0.45ml and 2.1 ± 0.11ml respectively i.e slight increase; while the results of methanolic extract at doses 300 mg/kg and 500 mg/kg were 3.4± 0.27 ml and 5.1±0.11mli.e the most promising result compared to control which resulted as 1.48 ± 2.44ml, while for positive control was recorded as 5.32± 0.44ml. It was also observed that the aqueous extract killed the brine shrimp with percentage lethality/mortality of 60%, 50% and 43.33% at a concentration of 1000, 100 and 10ppm respectively and the methanol extract percentage mortality/lethality was 70%, 60% and 50% at concentrations of 1000, 100 and 10ppm respectively. It concludes that methanolic extract has more diuretic capability and not having any significant cytotoxicity.

Key words: *Ganoderma lucidum* · Diuretic · Cytotoxicity · Mushroom

INTRODUCTION

Ganoderma lucidum is a Basidiomycetes fungus, a mushroom belonging to the family Polyporaceae and is one of the most famous traditional Chinese medicinal herb [1,2]. The extracts of mushroom has immense biological activities like antibacterial, antifungal, antitumor, immunomodulatory, cardiovascular, anti hepatotoxic and analgesic anti hypertensive and anti cancerous [3-7]. Diuretics are drugs that increase the flow rate of urine and sodium excretion [8]. These drugs are prescribed to adjust the volume and composition of body fluids in several clinical medical conditions [9]. Drug induced diuresis is beneficial in a number of medical complications such as congestive heart failure, nephritic syndrome, cirrhosis, renal failure, hypertension etc [10]. For the sake of cytotoxicity studies, traditional brine shrimp assay is used [11]. The*Artemia*nauplii have been in practice for the last 3 decades for testing test general toxicity [12]. Despite of having wonderful therapeutic potential that have been explored extensively, present study is an attempt to search for diuretic potential of aqueous and various organic extracts of *Ganoderma lucidum*.

MATERIALS AND METHODS

Collection of Mushroom: *Ganoderma lucidum* mushroom was collected and identified by Pakistan Museum of Natural History-Islamabad. The sample was carefully transported to the Research Laboratory-Federal Urdu

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University of Arts, Science and Technology-Karachi for exploring its biological and pharmacological potential.

Animals: BALB/c mice were used in this study. Mice (20-25 g) of either sex were generously gifted by Faculty of Pharmacy, Federal Urdu University of Arts, Science and Technology-Karachi-Pakistan. Animals were kept in animal house following the standard conditions and maintained at 23-25°C and was given standard diet and tap water *ad libitum*.

Aqueous Extract Preparation: The aqueous extract was prepared by boiling method i.e 5 grams of fine grounded mushroom powder in 100 ml of distilled autoclaved water and then move gently the flask for 15 minutes [13]. After extract preparation, coarse suspended particles of tea were removed by passing through strainer and later by passing via 0.22um filter. The extract was stored in refrigerator in small vials as aliquots for further use [14].

Methanol Extraction: The methanol extraction of *Ganoderma lucidum* was prepared by using Harbone method in which 25g of mushroom powder were Soxhelt extracted using 250ml of 95% methanol. These extracts were lasted from half an hour. The volatile substances were concentrated by evaporation using water bath 100°C [15].

Acute Toxicity Study: The acute toxicity of ethanolic and methanolic extracts was determined by using BALB/C mice. The animals were divided into 3 different groups. The group1 received normal saline (25 mg/kg), served as control. The groups 2 and 3 received 3000 mg/kg body weight of both types of organic extracts. After oral administration of these extracts via gavage needle, the animals were observed continuously for the behavioral changes for the first 24 hrs and then observed for mortality if any, after 24 hrs.

Diuretic Activity: In order to find out the diuretic potential of aqueous and various organic extracts modified method of was used [16]. Animal groups were divided into four groups and marked them alphabetically. Group A control, Group B: 300 mg/kg of *Ganoderma lucidum* (aqueous),Group C: 500 mg/kg (Aqueous) and Group D: Furosemide 20mg/kg (reference drug) were kept separately into the metabolic cages and 24 hours urine (ml) was collected into tubes fitted to the bottom of the metabolic cage. Same procedure was adopted by changing Group B formethanolicextract. During this period of study, no food and water was made available to animals.

Brine Shrimp Lethality Assay: The assay was performed by slight modification [17]. In this method, first for hatching purpose, 70 mg of shrimp eggs was gently sprinkled into container having 250 ml distilled sea water. The container was placed beside a light ray source like window and keeps the widow open slightly for proper ventilation. After 48 hours, brine shrimp larvae were collected carefully with the help of dropping pipette. Then add about 4.5ml of brine solution (sea water) into each test tube. Prepare the concentration in an order of 1000 ppm, 100 ppm and 10 ppm. The 0.5ml diluted test solution of the extracts was added into the test tubes making 5ml, the final volume. Then add 10 active brine shrimp (nauplii) into each of these vials. Replicates of each of the dose levels were prepared, using seawater as control number of survivors, deaths and nauplii and noting down the lethargic movement after 24 hours [11]. The percentage mortality was calculated from the following formula:

% mortality = No. of dead *nauplii* / Initial no. of live *nauplii*x 100

RESULTS AND DISCUSSION

Mushrooms contribute greatly as a wonderful source for manufacturing powerful and effective pharmaceutical drugs. Mycologists have identified over 10,000 mushrooms and among them 2,000 are good for human health and 300 have medicinal nature [18]. For centuries, it has been observed in East Asia, the fruiting body of the fungus *Ganoderma lucidum*has been in practice for curative purpose [19]. In this study, *Ganoderma lucidum* both methanolic and ethanolic extracts were found to be safe orally at the dose of 3000 mg/kg body weight. No mortality and any other signs were observed in any of the treated animals after 24 hrs. Based upon these results, finally 300mg/kg and 500mg/kg doses were selected for the sake of investigation. The diuretic property of *Ganoderma lucidum* was evaluated against Furosemide 40mg/kg which is loop diuretic, result of aqueous dose of *Ganoderma lucidum* at doses 300 mg/kg and 500 mg/kg result were 1.8 ± 0.45ml and 2.1 ± 0.11ml respectively i.e slight increase as mentioned in Table 1; while the results of methanolic extract at doses 300 mg/kg and 500 mg/kg were 3.4± 0.27 ml and 5.1±0.11ml i.e the most promising result as mentioned in Table 2 with respect to control which resulted as 1.48 ± 2.44ml, while for positive control

Table 1: Assessment of diuretic activity of aqueous extract of *Ganoderma lucidum*

		Diuretic activity	
Treatment	Dose mg/kg orally	Volume (ml) Mean ±SEM	Diuretic action
Control	0.5ml saline	1.48± 2.44	0
Crude extract			
Water extract of *Ganoderma lucidum*	300 mg/kg	1.8 ±0.45	1.21
	500 mg/kg	2.1± 0.11	1.41
Furosemide	40 mg/kg	5.32±0.44	3.63

Table 2: Assessment of diuretic activity of methanolic extract of *Ganoderma lucidum*

		Diuretic activity	
Treatment	Dose mg/kg orally	Volume (ml) Mean ±SEM	Diuretic action
Control	0.5ml saline	1.48± 2.44	0
Crude extract			
Of *Ganoderma lucidum*	300 mg/kg	3.4±0.27	2.29
	500 mg/kg	5.1± 0.11	3.44
Furesamide	40 mg/kg	5.32±0.44	3.63

Table 3: Brine shrimp lethality of aqueous extract of *Ganoderma lucidum*

Dose level ppm	Initial napaulii	Number survived after 24hrs	Number died after 24 hrs	% mortality/lethality
1000	30	12	18	60%
100	30	15	15	50%
10	30	17	13	43.33%
Control	30	24	6	20%

Fig. 1: *Ganoderma lucidum* mushroom at the point of collection near tree in Pakistan.

Fig. 2: Principal author is engaged in the collection of urine sample from metabolic cage at FUUAST-University-Pakistan

reading was noted as 5.32± 0.44ml, as indicated in the results. The concentrations of the extracts was prepared in parts per million (ppm). It was observed that the aqueous extract killed the brine shrimp with percentage lethality/mortality of 60%, 50% and 43.33% at a concentration of 1000, 100 and 10ppm respectively as indicated in Table 3.On the other hand, the methanol extract percentage mortality/lethality was 70%, 60% and 50% at concentrations of 1000, 100 and 10ppm respectively as mentioned in Table4. Both the extracts showed more or less same level of mortality and reflected that not having any significant toxicity even at higher doses. Scientists believed that the bioactivity is probably due to certain chemical compounds like polysaccharides or triterpenes, proteins and some other bioactive substances [20]. Another study indicated that *Ganoderma lucidum* extract possesses 120 different triterpenes [21]. The brine shrimp lethality bioassay was performed for testing cytotoxicity of methanolic and aqueous extracts in this study. Brine shrimp assay is nodoubt a rapid, simple and cost effective bioassay for the determination of toxicity of natural products [22].

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