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Antiparkinsonian Activity of Aqueous Extract of *Agaricus Blazei* Murill in Rotenone-induced Parkinson's Disease

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Authors' contributions

This work was carried out in collaboration among all authors. Author MA designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author HA wrote rest of the manuscript and managed the analyses of the study. Author TM and GH managed the final review of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Parkinsons disease is a chronic neurological disorder which may be due to reduction in the dopaminergic neurons in the brain**.** However, *Agaricus blazei* is a rich source of natural antioxidants.
Aim: In this study, antiparkinsonian activity of *Agaricus blazei* Murill was evaluated using different animal models.
Method: Antiparkinsonian activity was evaluated using two different doses (273 mg/kg and 819 mg/kg) of *Agaricus blazei* Murill. Rotenone and sunflower oil were used as positive and negative control, respectively. Catalepsy test, rotarod test, exploratory behavior test (rearing) and locomotor activity test were conducted to observe antiparkinsonian activity of the drug in rats.
Result: The results of the animal models were confirmed by determining the levels of reduced glutathione, total protein, thiobarbituric acid reactive substances (TBARS) and nitric oxide in the animal brain. Pretreatment with *Agaricus blazei* Murill, showed marked reduction in rotenone-

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induced catalepsy and a significant increase in exploratory behavior, muscular activity, and locomotor activity in rats. *Agaricus blazei* Murill has also shown extremely significant effect in decreasing the oxidative stress in the animal brain by increasing the brain levels of reduced GSH and total proteins and decreasing the levels of nitrite and TBARS.

Conclusion: The results of rotenone-induced catalepsy, exploratory behavior, rotarod test and locomotor activity showed that *Agaricus blazei* Murill exerts a significant ameliorative effect on Parkinson's disease in rats.

Keywords: Agaricus blazei; antiparkinsonian; antioxidants; oxidative stress and Parkinson's disease.

1. INTRODUCTION

Parkinson's disease (PD) may be due to the selective damage of dopaminergic neurons in substantia nigra pars compacta. It has been observed that oxidative stress plays a major role in the pathophysiology of Parkinson's Disease. Reactive oxygen species (ROS) are formed due to oxidative stress which result in neuronal death of the neurons This can be detected by decreased levels of endogenous antioxidants. Therefore, the use of antioxidants, along with other protective agents could be a better therapeutic intervention in PD. The current therapeutic agents, because of various side effects, have failed to prove to be a cure-all therapy for the PD patients [1]. Hence, the search for safer alternative/complementary medicines for the management of PD is an unfinished task for the researchers in this area of study.

Fungi, like primary basidiomycetes and mushrooms, are one of the significant edible foods. Numerous of these mushrooms are important because of their ability to produce some ingredients of high medicinal importance. Such mushrooms are called medicinal mushrooms [2]. A medicinal mushroom called *Agaricus blazei* Murill (ABM) commonly known as *Cogumelo do sol,* family Agariaceae, has a rich medicinal history. Traditionally, ABM has been well known for the management of physical and emotional stress, improvement and stimulation of immunity, and improvement in diabetic conditions. The herb is also potentially effective to fight against hypercholesterinemia, osteoporosis, peptic ulcer and digestive problems and different kinds of cancers Orivasios and Apuleius used the herb for the treatment of ulcers of the larynx in malignant conditions [2,3]. Scientifically, ABM is known for its pharmacological activities including, anticancer activity [4], immunostimulant and immunomodulatory activities [5,6], antiviral activity [7], antibacterial activity [8], cardioprotective effect [9], weight controlling and hypolipidemic effect [10] and hepatoprotective effect [11]. Antioxidants are famous for their neuroprotective potential [12]. *Agaricus blazei* Murill is an abundant source of naturally occuring antioxidants and aqueous extract of ABM has shown significant *in vitro* antioxidant activity [13]. This study was designed to investigate if *Agaricus blazei* possesses antiparkinsonian activity due to its well-established antioxidant potential.

2. MATERIALS AND METHODS

2.1 Preparation of the extract

Dehydrated basidiomes of *Agaricus blazei* Murill (ABM) were bought from Ibema, Brazil. The Fine powder was obtained by milling the basidiomes. The powder was then subjected to extraction by adding distilled water (100 ml) to 10 g each of powdered basidiome and was kept under agitation for 3 hours at a temperature of 28° C. The remaining solids were removed through filtration using Whatman filter paper (Size-1). This process of extraction was repeated three times whereas the remnants were lyophilized and stored at -20°C.

2.2 Drugs and Chemicals

Rotenone (R8875 Sigma) and the other chemicals which were used in this study were procured from Sigma-Aldrich.

2.3 Phytochemical Screening

Freshly prepared *Agaricus blazei* Murill (ABM) extract was put to different qualitative tests as per procedures defined elsewhere [14] to evaluate the existence of phytochemical constituents such as carbohydrates, protein, saponins, flavonoids, alkaloids, cardiac glycosides, and anthraquinones, through virtue of colour changes.

2.4 Selection of doses

The selection of doses of different drugs was based on literature i.e., 273 mg/kg and 819 mg/kg for *Agaricus blazei* Murill [15] whereas 2.5 mg/kg for rotenone were used [16].

2.5 Animals

In this study, male wistar rats bearing the weight of 200-220 g, were bought from the animal house of University of Karachi. However, all the animals were placed in the environment having optimum temperature of 25-30 ˚C under 12\12-hour light-dark cycle. Water *ad libitum* and free excess of standard diet were provided to the animals.

2.5.1 Grouping of animals

Animals used in this study were divided in four groups (n =6) as follows.

I: This group received sunflower oil 4 ml/kg, *i.p*.
II: This group received rotenone 2.5 mg/kg, *i.p*. dissolved in sunflower oil 1 mg/2 ml
III: This group received *Agaricus blazei* Murill 273 mg/kg, *p.o*. and rotenone 2.5 mg/kg, *i.p*.
IV: *Agaricus blazei* Murill 819 mg/kg, *p.o*. and rotenone 2.5 mg/kg, *i.p*.

All the groups of the animals received the doses of above agents on daily basis for a period of 28 days.

2.6 Acute Toxicity of the Extract

Toxicity was assessed as per OECD-423 guidelines. Briefly, female Wistar rats weighing between 200 to 220 g fasted overnight and a single dose of 2000 mg/kg of the extract was administered. For the detection of any signs of toxicity the animals were observed for the next 24 hours. However, animals were carefully monitored during the first 4 hours after the administration of the extract [17].

2.7 Behavioral Assessment

Behavioral analyses were performed on 7^{th}, 14^{th} and 28^{th} day of the study. All groups of the rats were subjected to the following behavioral tests:

2.7.1 Catalepsy test

Catalepsy is a behavioral state in rodents in which the animals become unable to correct the externally imposed postures. In this study, rotenone (2.5 mg/kg, i.p.) was used to induce the catalepsy in rats. The period of catalepsy was calculated in seconds. Catalepsy was measured using a standard bar test. The bar was 9 cm above the base. The front paws of the rat were placed onto the 9 cm wooden bar and the duration of retaining the forepaws on the elevated bar until they touch the floor was noted as the cataleptic score. The cutoff time of 180 s was applied. The test was performed on 7^{th}, 14^{th} and 28^{th} day of the study [18].

2.7.2 Exploratory behavior (rearing)

Rodents show exploratory behavior, including rearing, whenever they are placed in a new container. During rearing behaviour, the forelimbs will touch the container wall. In this study, small plexiglass cages size (30× 20× 30 cm) were used individually for each animal. The rats were given 5 min habituation period before the commencement of the test. After completion of the habituation period, the number of rearing was noted for the next 5 min. [19].

2.7.3 Rotarod motor coordination test

Rotarod was used to check the grip strength and muscle rigidity of all animals. Rotarod test is commonly used model for the assessment of muscle coordination and motor function. Before starting the therapy, to adjust rats on the rotarod apparatus, each rat was trained. During the test, each rat was placed on a rotating rod at a speed of 25 rpm. A cutoff time of 180 seconds was maintained during the experiment. Fall of time for each animal was noted in seconds. The test was performed on 7^{th}, 14^{th} and 28^{th} day of the study [18].

2.7.4 Locomotor activity

In this test, locomotor activity of animals was evaluated on 7^{th}, 14^{th} and 28^{th} day of the study by using digital actophotometer having infrared photocells for a period of 5 min. The values for locomotor activity of animals were noted as counts per 5 min [20].

2.8 Biochemical Estimation

2.8.1 Decapitation and homogenization

After the completion of behavioral tests, the animals were anaesthetized and sacrificed by decapitation for biochemical estimation. Immediately after decapitation, the brains of the animals were extract out, washed carefully with

ice-cold normal saline, and homogenized in Tris HCL. The supernatant fluid formed due to homogenization was centrifuged for the period of 10 min at 10,000 ×g. The neuroprotective activity of ABM extract was measured by estimating the concentrations of brain antioxidant enzymes [12].

2.8.2 Lipid peroxidation assay (TBARS)

By measuring the byproduct of lipid peroxide i.e. thiobarbituric acid reactive substances (TBARS), lipid peroxide level can easily be measured. For this purpose, aqueous solution of thiobarbituric acid, sodium dodecyl sulphate and acetate buffer (pH 3.5) were blended with (10 % w/v) tissue homogenate. Red pigment was obtained after heading the mixture at 95 °C for 1 hour, which was then extracted with *n*-butanolpyridine mixture. The absorbance for this mixture was noted at 532nm. Lipid peroxide level was expressed as nmol malondialdehyde, whereas tetramethoxypropane was used as an external standard [21].

2.8.3 Reduced glutathione assay (GSH)

An assay of reduced glutathione was done by precipitating 1 ml of both 10% trichloroacetic acid (TCA) and the tissue homogenate. In addition, 5,5-dithio-bis-(2-nitrobenzoicacid)(DTNB)reagent 0.5 ml and 4 ml of phosphate solution were added to the homogenized supernatant liquid. The absorbance was noted at 412 nm [21].

2.8.4 Estimation of nitrite

Nitrite can be detected spectrophotometrically by using Griess reagent. A mixture of Griess reagent and brain homogenate was formed by mixing them in equal proportions and incubated. The absorbance of mixture was noted at 546 nm [22].

2.8.5 Determination of protein

For the determination of protein, Lowry method was used. Whereas bovine serum albumin was used for standard curve determination [23].

2.9 Statistical Analysis

Data were expressed as ± standard error of mean (SEM). One-way ANOVA followed by Tukey's post hoc test was applied for the analysis of data.

3. RESULTS

3.1 Phytochemical Screening

The aqueous extract of ABM showed the presence of pharmacologically important phytoconstituents, which are summarized in Table 1.

3.2 The Effect of ABM Extract on Rotenone-induced Catalepsy in Rats

The result of one-way ANOVA showed extremely significant difference between the four groups of rats in duration of catalepsy ($F_{3, 8}$=15.16, P = 0.001). The means of treatment groups viz. Group III (ABME 273 mg/kg) and Group IV (ABME 819 mg/kg) were compared with the means of Group II (rotenone 2.5 mg/kg) using Tukey's *post hoc* test. However, it has been observed that the activity of animals was significantly reduced in the treatment groups when compared with the rotenone group (Fig. 1).

3.3 The Effect of ABM Extract on Exploratory Behavior (rearing) in Rats

In this behavioral test, there were significant differences among four groups of the rats as indicated by one-way ANOVA ($F_{3, 8}$=6.15, P = 0.014). The means of the treatment groups viz. Group III (ABME 273 mg/kg) and Group IV (ABME 819 mg/kg) were compared with the means of Group II (rotenone 2.5 mg/kg) using Tukey's *post hoc* test. However, it has been observed that the activity of animals was significantly increased in the treatment groups when compared with the rotenone group (Fig. 2).

3.4 The Effect of ABM Extract on RotarodMotorCoordinationTest in Rats

It this test, there were extremely significant difference among the four groups of the rats as indicated by one-way ANOVA ($F_{3, 8}$=43.60, P = 0.001). The means of the treatment groups viz. Group III (ABME 273 mg/kg) and Group IV (ABME 819 mg/kg) were compared with the means of Group II (rotenone 2.5 mg/kg) using Tukey's *post hoc* test. It has been observed the fall off time in rotarod test was significantly increased in the treatment groups when compared with the rotenone group (Fig. 3).

3.5 The Effect of ABM Extract on Rotenone-induced locomotor Activity in Rats

The results of one-way ANOVA ($F_{3, 8}$=53.92, P = 0.001) indicated a significant difference among four groups of rats in actophotometer test. The means of treatment groups viz. Group III (ABME

273 mg/kg) and Group IV (ABME 819 mg/kg) were compared with the means of Group II (rotenone 2.5 mg/kg) using Tukey's *post hoc* test. The results showed that the locomotor activity was significantly increased in treatment groups when compared with rotenone group (Fig. 4).

3.6 The Effect of ABM Extract on Brain Nitrite, TBARS, GSH and Total Proteins in Rats

In biochemical assay, a one-way ANOVA indicated a significant difference among four groups of rats in brain levels of nitrite ($F_{3, 20}$ = 39.59, P = 0.0001), TBARS ($F_{3, 20}$ = 14.17, P = 0.0001), GSH ($F_{3, 20}$ = 77.21, P = 0.0001) and total proteins ($F_{3, 20}$ = 57.57, P = 0.0001). The means of treatment groups viz. Group III (ABME 273 mg/kg) and Group IV (ABME 819 mg/kg) were compared with the means of Group II (rotenone 2.5 mg/kg) using Tukey's *post hoc* test. The results of the analysis reveal that the levels of nitrite and TBARS were significantly decreased while the levels of reduced GSH and total proteins were significantly increased in treatment groups when compared with rotenone group (Figs. 5-8).

Table 1. Phytochemical analysis of the aqueous extract of *Agaricus blazei* Murill

Phytochemical	Test	Observation
Carbohydrates	Barfoed's and Molisch's test	+
		+
Protein	Biuret test	+
Flavonoids	Pew's test	+
	Shibita's reaction test	+
Saponins	Frothing test	+
Alkaloids	Mayer's and Dragendorff's test	+
		+
Anthraquinones	Borntrager's test	--
Cardiac glycosides	General test	--

(+ present; -- absent)

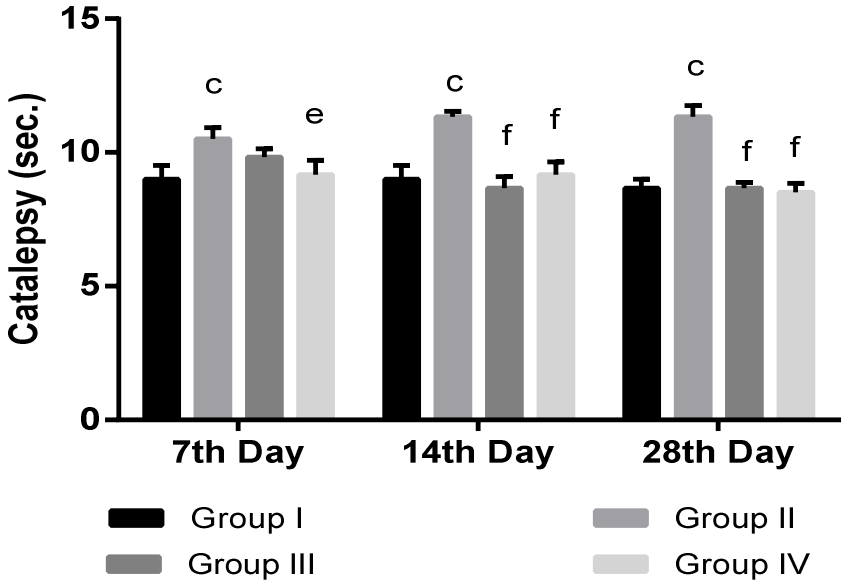

Fig. 1. Effect of ABM extract on rotenone-induced catalepsy in rats.

The values are mean ± SEM

[a]p < 0.05, [b]p < 0.01, [c]p < 0.001 when compared with the control group, [d]p < 0.05, [e]p < 0.01, [f]p < 0.001 when compared with the rotenone group;

(ANOVA followed by Tukey's post hoc test)

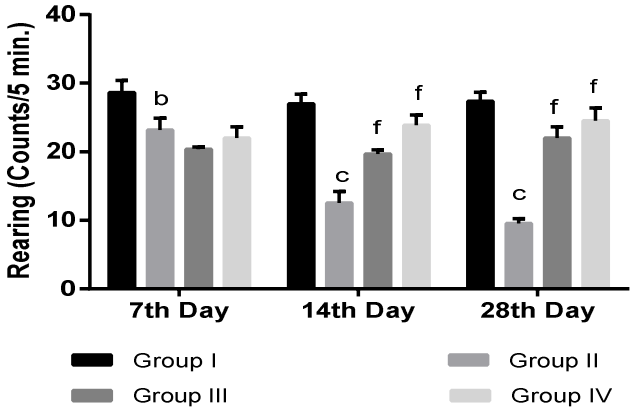

Fig. 2. Effect of ABM extract on exploratory behavior (rearing) in rats

The values are mean ± SEM; [a]p < 0.05, [b]p < 0.01, [c]p < 0.001 when compared with the control group, [d]p < 0.05, [e]p < 0.01, [f]p < 0.001 when compared with the rotenone group; (ANOVA followed by Tukey's post hoc test)

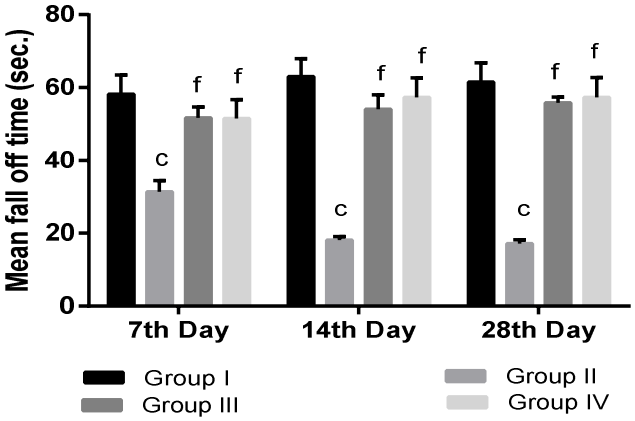

Fig. 3. Effect of ABM extract on rotarod motor coordination test in rats

The values are mean ± SEM.; [a]p < 0.05, [b]p < 0.01, [c]p < 0.001 when compared with the control group., [d]p < 0.05, [e]p < 0.01, [f]p < 0.001 when compared with the rotenone group; (ANOVA followed by Tukey's post hoc test).

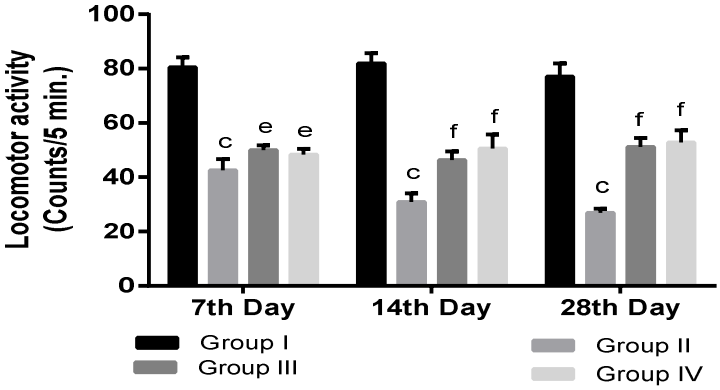

Fig. 4. Effect of ABM extract on locomotor activity in rats

The values are mean ± SEM; [a]p < 0.05, [b]p < 0.01, [c]p < 0.001 when compared with the control group, [d]p < 0.05, [e]p < 0.01, [f]p < 0.001 when compared with the rotenone group; (ANOVA followed by Tukey's post hoc test)

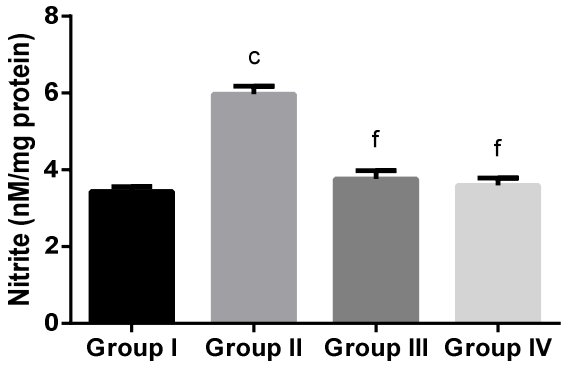

Fig. 5. Effect of ABM extract on nitrite levels in the rat brain

The values are mean ± SEM

[a]p<0.05, [b]p<0.01, [c]p<0.001 when compared with the control group, [d]p<0.05, [e]p<0.01, [f]p<0.001 when compared with the rotenone group; (ANOVA followed by Tukey's post hoc test)

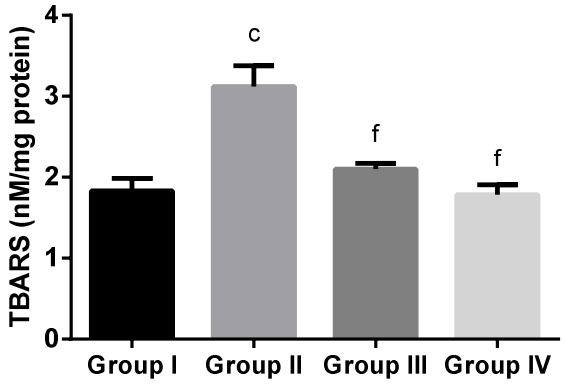

Fig. 6. Effect of ABM extract on lipid peroxidation in the rat brain

The values are mean ± SEM; [a]p<0.05, [b]p<0.01, [c]p<0.001 when compared with the control group, [d]p<0.05, [e]p<0.01, [f]p<0.001 when compared with the rotenone group; (ANOVA followed by Tukey's post hoc test)

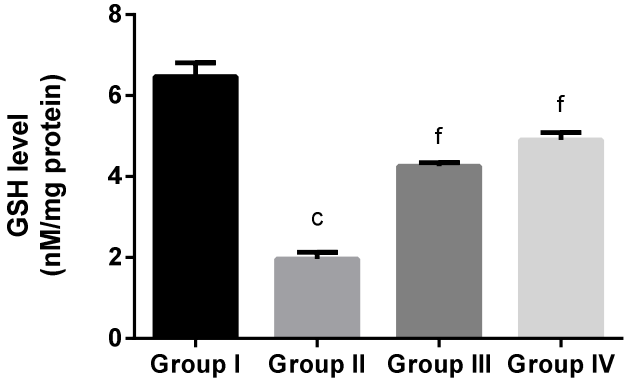

Fig. 7. Effect of ABM extract on reduced glutathione levels in the rat brain.

The values are mean ± SEM; [a]p<0.05, [b]p<0.01, [c]p<0.001 when compared with the control group, [d]p<0.05, [e]p<0.01, [f]p<0.001 when compared with the rotenone group; (ANOVA followed by Tukey's post hoc test)

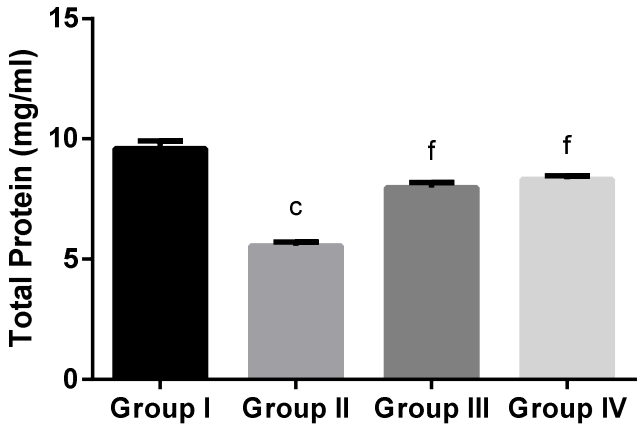

Fig. 8. Effect of ABM extract on total protein levels in the rat brain.
The values are mean ± SEM; [a]p<0.05, [b]p<0.01, [c]p<0.001 when compared with the control group, [d]p<0.05, [e]p<0.01, [f]p<0.001 when compared with the rotenone group; (ANOVA followed by Tukey's post hoc test)

4. DISCUSSION

Parkinson's Disease, a chronic neurodegenerative disorder which may be due to the reduction in dopaminergic neurons present in the region of substantia nigra pars compacta. Whereas, several pathologies such as mitochondrial dysfunction, oxidative stress, protein accumulation like a-synuclein and apoptosis are involved in this disease. However, the most important pathology of PD has been oxidative stress [24].

Rotenone-induced catalepsy, exploratory behavior, rotarod test and locomotor activity are most frequently used models for animal to evaluate neurodegenerative disorders. A specific complex I inhibitor i.e., rotenone which is a common herbicide, reproduces Parkinsonian signs and symptoms in rodents [18]. Many research studies have exhibited that systemic administration of rotenone can lead to the degradation of dopaminergic neurons in the nigrostriatal pathway that progresses the development of behavioural, neurochemical and pathological events of PD [25].

The results of rotenone-induced catalepsy showed that *Agaricus blazei* Murill provides significant ameliorative effect on Parkinson's disease in rats. The effect of *Agaricus blazei* Murill extract on rearing, muscle rigidity and locomotor activity of rats was also evaluated because earlier studies show that the patient suffering from PD reflects loss of brain motor coordination and becomes unable to maintain normal limb posture [26]. Efficient locomotor activity was observed among the animals treated with *Agaricus blazei* Murill extract at the doses of 273 and 819 mg/kg when compared to control group which provided more evidence of the ameliorative effect of *Agaricus blazei* Murill on PD.

Dysfunction of mitochondrial complex-1 generates oxidative stress and plays an imperative role in the pathogenesis of Parkinson's Disease [24]. *Agaricus blazei* Murill is a well-recognized antioxidant mushroom. [27]. Considering the potent antioxidant profile of *Agaricus blazei*, it is assumed that the beneficial effect of *Agaricus blazei* Murill in aforesaid models of PD could be due to its lessening effect on the oxidative stress in the brain. To confirm the assumption, the levels of reduced glutathione, total protein, nitric oxide and thiobarbituric acid reactive substances (TBARS) were estimated in the animal brain.

Glutathione peroxidase is one of the important neuroprotective enzymes in the brain. It acts as a scavenger of H_2O_2 produced by cellular metabolism besides balancing the composition and disintegration of H_2O_2 in normal conditions. The decreased level of glutathione is the limiting factor in the elimination of H_2O_2. However, in Parkinson's disease, glutathione is reduced extensively in the substantia nigra because of neuronal loss [28]. It has been reported that excess stimulation of neurons by the glutamic acid and significant activation of macrophages through nitric oxide can promote the toxicity of neuronal cells in the brain [29]. The production of nitric oxide in biological materials can be assessed by the determination of nitrite. In this

study, the level of nitric oxide in the brain was detected spectrophotometrically by using Griess reagent [30]. The biomarker of oxidative stress is lipid peroxidation which was determined by measuring the byproduct of lipid peroxide i.e., thiobarbituric acid reactive substances (TBARS). Lipid peroxidation ensues because of the attack on double bonds of arachidonic acid and unsaturated fatty acid. This generates the radicles of lipid peroxyl, which initiate a series of additional strikes on other unsaturated fatty acids that can leads to the oxidative degradation of polyunsaturated fatty acids and its incidence in bio membranes results in impaired structural integrity, inactivation of several membrane-bound enzymes, impaired membrane function and reduced fluidity. Earlier studies show that in the substantia nigra of PD patients the levels of lipid peroxidation product is significantly increased [31]. In the current study, analogous results have been found in the brain homogenate of rotenone treated rats. Rotenone animal group exhibited a steady decline in GSH and total protein levels and a substantial rise in the levels of nitrite and TBARS in the animal brain when compared to the control group. Two different doses of aqueous extract of *Agaricus blazei* Murill (273 and 819 mg/kg, p.o.) were used in the rotenone-induced PD models. Both doses were found to be significant in decreasing oxidative stress in the animal brain by increasing the brain levels of reduced GSH and total proteins and decreasing the levels of nitrite and TBARS.

5. CONCLUSION

The results of rotenone-induced catalepsy, exploratory behavior, rotarod test and locomotor activity showed that *Agaricus blazei* Murill exerts a significant ameliorative effect on Parkinson's disease in rats. Biochemically, both doses were found to be significant in decreasing oxidative stress in the animal brain by increasing the brain levels of reduced GSH and total proteins and decreasing the levels of nitrite and TBARS. Thus, we conclude that *Agaricus blazei* Murill possesses antiparkinsonian activity in rats. This activity of the mushroom could be due to its antioxidant potential. The mushroom can be used alone or in combination with other antiparkinsonian drugs. We believe that consumption of the mushroom will be highly fruitful for the PD patients.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was reviewed and approved by institutional ethical committee of Ziauddin University, Karachi.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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