Minireview Article

GANODERMA LUCIDUM MITIGATES ROTENONE-INDUCED PARKINSON'S DISEASE IN MALE WISTAR RATS

ABSTRACT

Background: Parkinson's disease (PD) has been declared as the second most common neurodegenerative disorder in the world. *Ganoderma lucidum*, Lingzhi or Reishi, is considered as a real therapeutic factory.

Aim: The current study was conducted to evaluate the antiparkinsonian activity of *Ganoderma lucidum* in rotenone-induced Parkinson's disease in male Wistar rats.

Method: The effects of *Ganoderma lucidum* (150 and 300 mg/kg) were studied on catalepsy, muscle rigidity and locomotor activity.

Result: The increased duration of catalepsy (induced by rotenone) was significantly reduced by *Ganoderma lucidum*. Rotenone significantly induced motor dysfunction as indicated by muscle rigidity and hypolocomotion. *Ganoderma lucidum* mitigated the motor dysfunction by improving rotarod performance and locomotor activity of the animals. Neurochemically, the activities of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), and the level of the antioxidant tripeptide glutathione (GSH) were significantly reduced by rotenone administration. Furthermore, rotenone increased the levels of the lipid peroxidation product malondialdehyde (MDA). However, *Ganoderma lucidum* supplementation to rotenone-injected mice significantly increased the levels of SOD, CAT, and GSH, and decreased the level of MDA.

Conclusion: This study strongly supports the neuroprotective and antiparkinsonian activity of *Ganoderma lucidum.*

Keywords: *Ganoderma lucidum,* Parkinson's disease, oxidative stress, rotenone

1. INTRODUCTION

Parkinson's disease (PD) has been declared as the second most common neurodegenerative disorder in the world. The disease is thought to be caused by reduced levels of dopamine and loss of dopaminergic neurons in substantia nigra and striatum [1]. It is important to know that 7-10 million people are globally affected by PD [2]. The etiology of PD is unclear and nearly all cases are sporadic in nature. The possible causes may include inflammation, free radicals, head trauma and exposure to environmental toxins [3, 4]. The role of oxidative stress has gotten the great importance in the pathology of PD [5]. It has been found that in Parkinson's disease cell death occurs due to the degeneration of dopaminergic neurons by oxidative stress because oxidative stress produces hindrance in physiological maintenance of redox potential in neurons by disrupting different biological processes. Aging, mitochondrial dysfunction, neuroinflammatory cells, dopamine metabolism, iron and calcium are being the mechanisms and sources for reactive oxygen species generation. [6].

The drugs available for Parkinson's disease are symptomatic in nature and unable to halt the progression of the disease. Moreover, long term therapy confronts big issues such as adverse effects related to available drugs, nonmotor symptoms and complexity of motor symptoms in advanced stages of Parkinson's disease [7].

A common herbicide/pesticide rotenone induces similar pathological features in animals as human Parkinson's disease thus making it a promising animal model for PD with construct validity [8, 9]. In rodents, rotenone induces the DJ-1 translocation and acidification, nigral iron accumulation, α-synuclein cytoplasmic inclusions formation in dopaminergic neurons and proteasomal dysfunction [10]. All these features make rotenone model a suitable one for the investigation of those therapeutic agents that target neuroinflammation and oxidative stress in PD.

Mushrooms have gained worldwide recognition for their therapeutic and nutritional values. Health-conscious people and those suffering from serious diseases need immunity boosters and cellular protection which increases the interest in mushrooms [11]. One such mushroom is *Ganoderma lucidum. Ganoderma lucidum* is a basidiomycete rot fungus. Lingzhi or Reishi are

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the common names of *Ganoderma lucidum*, which has a long history of use for overall health improvement and longevity. *Ganoderma lucidum*, is used only in medicine, and is not consumed as food as it has a bitter taste and hard texture. This fungus is considered as a real therapeutic factory [12].

The phenomenal health benefits of *Ganoderma lucidum* "the mushroom of immortality" have been reported and it has more than 400 bioactive components such as polysaccharides, triterpenoids, steroids, proteins/peptides, fatty acids, sterols, and nucleotides. These bioactive compounds have numerous medicinal effects [13]. *Ganoderma lucidum* polysaccharides and triterpenoids have many therapeutic effects such as anti-hepatotoxin, anti-inflammatory, anticancer, antimicrobial, anti-hypercholesterolaemic and antioxidant activity [12]. Antioxidant remedies for the treatment and prevention of Parkinson's disease are crucial. Therefore, the present study was undertaken to evaluate the antiparkinsonian activity of *Ganoderma lucidum* extract in rotenone induced neurodegeneration in mice.

2. MATERIALS AND METHODS

2.1 Experimental animals

For experiment 30 male Wistar rats (weight 200–220 g) were procured from the animal house of Liaquat National College, Karachi. Animals were kept in propylene cages under standard laboratory conditions including 12/12 light dark cycle, relative humidity 50 ± 5% and temperature 25 ± 2°C. There were 6 animals in each cage. Before starting the test, the animals were acclimatized to laboratory conditions for one week. The animals were handled according to the requirements in "Guidelines for care and use of laboratory animals, 8th edition" [14]. The study was approved by the Ethical Review Committee of Ziauddin University, Karachi.

2.2 Drugs & chemicals

Ganoderma lucidum extract comprised of cracked spores and fruiting bodies was purchased from Pharmanex Inc. (United States of America). Rotenone (ROT) and the assay kits for

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malondialdehyde (MDA), reduced glutathione (GSH), Catalase (CAT), Superoxide dismutase (SOD) were purchased from Sigma-Aldrich, St. Louis, MO, USA. Levodopa was purchased from Searle Pakistan Pvt Ltd.

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2.3 Experimental design

Rotenone (ROT) was administered at a dose of 2.5 mg/kg intraperitoneally (i.p.) once daily for four weeks for the induction of Parkinson's disease [15]. The animals were divided in five groups, six animals in each group. Group I, served as vehicle-injected control group, Group II served as the rotenone-injected (2.5 mg/kg i.p.) and vehicle-treated group, Group III served as levodopa (6 mg/kg p.o) and rotenone-injected (2.5 mg/kg i.p.) group, Group IV served as *Ganoderma lucidum* extract (150 mg/kg p.o.) and rotenone-injected (2.5 mg/kg i.p.) group, Group V served as *Ganoderma lucidum* extract (300 mg/kg p.o.) and rotenone-injected (2.5 mg/kg i.p.) group.

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2.4 Behavioral Procedures:

2.4.1 Catalepsy test

Catalepsy is a behavioural state in which the animal is unable to correct an externally imposed posture, A method given by Costall and Naylor for the measurement of catalepsy was used [16]. A bar of height 9 cm was used, and each animal was placed on the horizontal bar with both front paws in half rearing position. The duration of catalepsy was noted from the time when animal was positioned on the bar till the time the animal detached both front paws from the bar or climbed over the bar. Time was noted with the help of a stopwatch. Catalepsy was induced by rotenone (2.5 mg/kg, i.p.) and examined at every 30 min interval for 210 min. The cutoff time was five minutes.

2.4.2 Rotarod (Grip strength) test

Rotarod instrument was used to check the grip strength of all animals. The instrument is commonly used to assess the "minimal neurological deficit" such as coordination and motor function in rodents. Before starting the test, each animal was trained to acclimatize to the apparatus.

A rotating rod of diameter 7 cm and speed 25 rpm was used, and each animal was placed on it. The cutoff time was 300 sec. The average results were recorded as fall of time [17].

2.5 Dissection and homogenization

Animals after treatment period were scarified by decapitation under the influence of mild anesthesia. Brains were dissected out at once; the cerebellum was discarded while the forebrain was separated for the examination. Ice-cold isotonic saline was used for the removal of blood from the brain. 0.1M phosphate buffer (pH 7.4) was used to prepare 10% w/v tissue homogenate. The homogenate was then centrifuged at 10,000 rpm for 15 minutes to obtain aliquots of supernatant which was used for biochemical estimation [15].

2.6 Biochemical estimation

2.6.1 Malondialdehyde (MDA) level:

The detection kit of malondialdehyde (MDA) was used to find out the lipid peroxidation amount following manufacturer's instructions. In the presence of thiobarbituric acid (250 µL) and acid reagent, samples (250 µL) were incubated and vortexed strenuously. Samples were put for incubation at 60°C for 60 minutes then the samples were centrifuged at 10,000× g for 3 minutes. The spectrum of reaction mixture was recorded at 532 nm. The results were expressed as µm MDA/mg protein [15].

2.6.2 GSH level (reduced glutathione):

Reduced glutathione GSH assay kit was used for the evaluation of reduced GSH level following manufacturer's instructions. For the deproteinization of samples 5% 5-sulfosalicylic acid solution was used and then centrifuged for the removal of precipitated protein the resultant supernatant was used to estimate GSH level. The incubation period for samples (10 µL) along with working mixture (150 µL) (assay buffer + GSH reductase + 5,5′-dithiobis (2-nitrobenzoic acid)) in 96-well plates was 5 minutes. 50 µL NADPH solutions (dil) were properly mixed in each sample. Using a microplate reader with the kinetics for 5 minutes sample's absorbance was measured at 412 nm. The results were expressed as µm GSH/mg protein [15].

2.6.3 Superoxide dismutase (SOD) level:

The antioxidant enzyme superoxide dismutase (SOD) activity was determined by Cayman assay kit as per manufacturer's instructions [15].

2.6.4 Catalase (CAT) level:

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The antioxidant enzyme Catalase (CAT) activity was determined by Cayman assay kit as per manufacturer's instructions [15].

2.7 Statistical Analyses:

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A one-way analysis of variance (ANOVA) and Tukey's *post hoc* test were used to determine the statistical significance between different groups. The criterion for any statistically significant difference was set as $p < 0.05$.

3 RESULTS

3.1 The effect of *Ganoderma lucidum* on rotenone-induced catalepsy:

The results showed that vehicle + rotenone treated group has increased duration of catalepsy as compared to vehicle treated group. Contrary, *Ganoderma lucidum* extract given at doses (150 and 300 mg/kg) prior to rotenone injection significantly reduced the duration of catalepsy when compared with the vehicle + rotenone treated group.

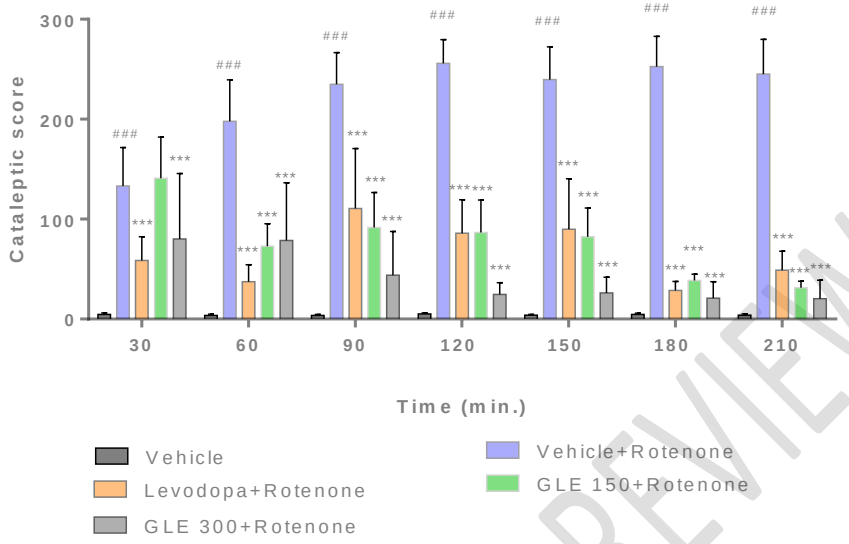

Figure 1: Effect of *Ganoderma lucidum* on rotenone-induced catalepsy

n = 6 (Animals in every group). The given values are mean ± SEM, One-way ANOVA and Tukey's test were used for data analysis. Rotenone exposure induced a significant increase (###p < 0.001) in cataleptic time of rotenone treated mice when compared to vehicle treated control mice. Supplementation with *Ganoderma lucidum* in rotenone treated mice significantly decrease the cataleptic score at dose150 mg/kg (**p < 0.01) and at dose 300 mg/kg (***p < 0.001).

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3.2 The effect of *Ganoderma lucidum* on rotarod test:

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The result showed that vehicle + rotenone treated group significantly decreased the time of fall as compared to vehicle group. However, the chronic oral administration of *Ganoderma lucidum* extract at doses (150 and 300 mg/kg) prior to rotenone injection significantly increased the time of stay on the apparatus when compared with the vehicle + rotenone treated group.

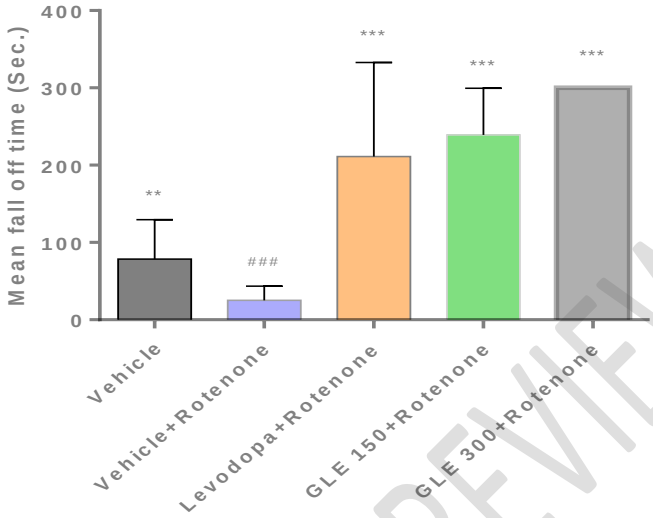

Figure 2: Effect of *Ganoderma lucidum* on rotarod test

n = 6 (Animals in every group). The given values are mean ± SEM, One-way ANOVA and Tukey's test were used for data analysis. Rotenone exposure induced a significant decrease (###$p < 0.001$) in time of falloff of rotenone treated mice when compared to vehicle treated control mice. Supplementation with *Ganoderma lucidum* in rotenone treated mice significantly increase the time of stay (***$p < 0.001$).

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3.3 Effect of *Ganoderma lucidum* on malondialdehyde (MDA) level

Next, we examined whether the neuroprotective effect (observed in our models) of *Ganoderma lucidum* is due to its antioxidant activity or not. The effect of *Ganoderma lucidum* against neurodegeneration induced by rotenone was investigated by testing the level of malondialdehyde (MDA). MDA is a marker of lipid peroxidation in the midbrain. Immensely increased level of MDA was observed in vehicle + rotenone treated groups of animals when compared with vehicle group. However, *Ganoderma lucidum* extract given at doses (150 and 300 mg/kg) prior to rotenone injection significantly suppressed MDA level when compared with vehicle + rotenone treated groups.

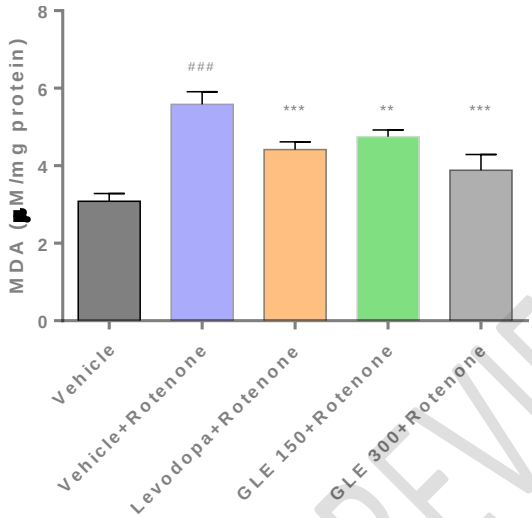

Figure 3: Effect of *Ganoderma lucidum* on malondialdehyde (MDA) level

n = 6 (Animals in every group). The given values are mean ± SEM, One-way ANOVA and Tukey's test were used for data analysis. Rotenone exposure induced a significant increase (###p < 0.001) in MDA level in the midbrain of rotenone treated mice when compared to vehicle treated control mice. Supplementation with *Ganoderma lucidum* in rotenone treated mice significantly decrease the MDA level at dose150 mg/kg (**p < 0.01) and at dose 300 mg/kg (***p < 0.001).

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3.4 Effect of *Ganoderma lucidum* on reduced glutathione (GSH) level

Glutathione (GSH) is a tripeptide antioxidant, whose bioavailability is correlated with increased MDA level due to oxidative stress. A significant decrease in GSH level was observed in vehicle + rotenone treated group when compared with vehicle group, whereas *Ganoderma lucidum* given at doses (150 and 300 mg/kg) prior to rotenone injection treated groups significantly increased GSH level when compared with vehicle + rotenone treated group.

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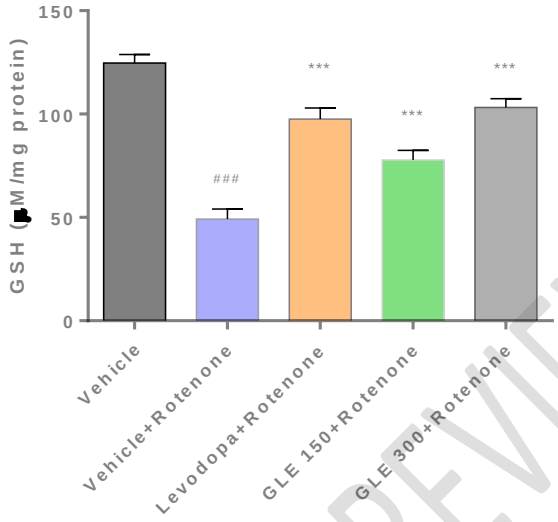

Figure 4: Effect of *Ganoderma lucidum* on reduced glutathione (GSH) level

n = 6 (Animals in every group). The given values are mean ± SEM, One-way ANOVA and Tukey's test were used for data analysis. Rotenone exposure induced a significant decrease (###p < 0.001) in GSH level in the midbrain of rotenone treated mice when compared to vehicle treated control mice. Supplementation with *Ganoderma lucidum* in rotenone treated mice significantly increase the GSH level at dose150 mg/kg (**p < 0.01) and at dose 300 mg/kg (***p < 0.001).

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3.5 Effect of *Ganoderma lucidum* on Superoxide dismutase (SOD) level

The activity of antioxidant enzyme superoxide dismutase (SOD) was also measured. The significant decrease in the activity of SOD was observed in vehicle + rotenone treated group when compared with vehicle control group. *Ganoderma lucidum* given at doses (150 and 300 mg/kg) prior to rotenone injection significantly increased the activity of SOD when compared with vehicle + rotenone treated group.

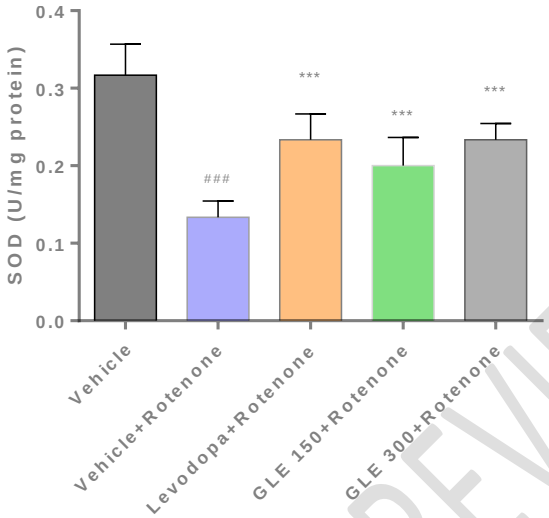

Figure 5: Effect of *Ganoderma lucidum* on superoxide dismutase (SOD) level

n = 6 (animals in every group). the given values are mean ± sem, one-way anova and tukey's test were used for data analysis. rotenone exposure induced a significant decrease (###p < 0.001) in sod level in the midbrain of rotenone treated mice when compared to vehicle treated control mice. supplementation with *Ganoderma lucidum* in rotenone treated mice significantly increase the sod level at dose150 mg/kg (**p < 0.01) and at dose 300 mg/kg (***p < 0.001).

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3.6 Effect of *Ganoderma lucidum* on Catalase (CAT) level

The activity of antioxidant enzyme catalase (CAT) was also measured. The significant decrease in the activity of CAT was observed in vehicle + rotenone treated group when compared with vehicle control group. *Ganoderma lucidum* given at doses (150 and 300 mg/kg) prior to rotenone injection significantly increased the activity of CAT when compared with vehicle + rotenone treated group.

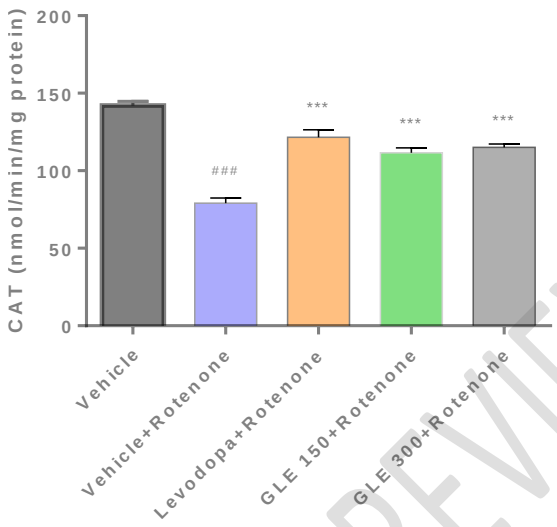

Figure 6: Effect of *Ganoderma lucidum* on catalase (CAT) level

n = 6 (Animals in every group). The given values are mean ± SEM, One-way ANOVA and Tukey's test were used for data analysis. Rotenone exposure induced a significant decrease (###p < 0.001) in CAT level in the midbrain of rotenone treated mice when compared to vehicle treated control mice. Supplementation with *Ganoderma lucidum* in rotenone treated mice significantly increase the CAT level at dose150 mg/kg (**p < 0.01) and at dose 300 mg/kg (***p < 0.001).

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4. DISCUSSION

One of the most common neurodegenerative disorders is Parkinson's disease. Parkinson's disease has progressive nature and complex pathogenesis. Development of Parkinson's disease involves neuroinflammation, oxidative stress and mitochondrial dysfunction [10]. The pathological and behavioral symptoms of Parkinson's disease have been produced, in rats, by the systemic administration of a pesticide called rotenone. Rotenone causes oxidative stress, neurodegeneration of dopamine, glial activation, α-synucleinopathy, inflammation and motor dysfunction [18]. The damage caused by free radicals can be alleviated by *Ganoderma lucidum*, an acclaimed antioxidant [12]. The present study was directed towards the antiparkinsonian effect of *Ganoderma lucidum* against rotenone induced neurotoxicity in rats.

Our results of behavioral assessment through rat models such as catalepsy and rotarod performance test proposed antiparkinsonian potential of *Ganoderma lucidum*. Our results also proposed the attenuation of oxidative stress, through determination of malondialdehyde and reduced glutathione levels and antioxidant enzymes such as catalase and superoxide dismutase activities in the brain tissues.

Rotenone induced catalepsy is commonly used animal model for Parkinson's disease. The neurotoxicity induced by rotenone in the substantia nigra of the rat is facilitated by a dopamenergic neuron transmitter i.e., oxidation of dopamine [19]. In the present study rotenone significantly induced catalepsy in rats when compared with the duration vehicle control group. However, chronic oral administration of *Ganoderma lucidum* at doses (150 and 300 mg/kg) prior to rotenone injection significantly reduced the duration of catalepsy in rats when compared to the vehicle control group. This may indicate the neuroprotective potential of *Ganoderma lucidum* in dopaminergic neurotransmission in striatum.

Furthermore, for the assessment of grip strength, rotarod performance test was used. Rotenone treated group has a significant reduction in time of fall when compared with vehicle control group. In contrast chronic oral administration of *Ganoderma lucidum* at doses (150 and 300 mg/kg) prior to rotenone injection significantly increased the time of stay of rats on the apparatus when compared with vehicle control group. This may also a sign of neuroprotective potential of *Ganoderma lucidum* against these behavioral symptoms [20].

Oxidative stress is a key factor in Parkinson's disease pathogenesis. Cellular death takes place because of high free radical discharge that occurs when there are defects in electron transport of mitochondrial complex-I [21]. The generation of reactive oxygen species and loss of ATP production are due to the systemic administration of rotenone which is a mitochondrial complex-I electron chain transport inhibitor. The oxidation of polyunsaturated fatty acid induced by reactive oxygen species is called lipid peroxidation. Lipid peroxidation yields MDA which adducts with proteins and DNA bases to induce cellular damage. Parkinson's disease patient's brain reportedly has increased oxidative damage of lipids, proteins, and DNA [10]. Our study revealed that increased MDA level was observed in rotenone treated rats when compared with vehicle treated rats. However, pretreatment with *Ganoderma lucidum* at doses (150 and 300 mg/kg) in rotenone treated rats showed a significant decrease in MDA level when compared with

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vehicle treated rats. In the present study the inhibitory effect of *Ganoderma lucidum* against lipid peroxidation may be due to reactive oxygen species and peroxy radical's detoxification.

Parkinson's disease etiology encompasses an important factor known as glutathione (GSH), an enzyme. Neuronal loss is correlated with the depletion of reduced glutathione. The risk of lipid peroxidation and formation of free radical is increased when there is decreased availability of reduced glutathione, that ultimately disrupts neuronal capacity to detoxify hydrogen peroxide [22]. In our study, a decrease in GSH level was observed in rotenone treated animals when compared with vehicle treated control animals. However, pretreatment with *Ganoderma lucidum* at doses (150 and 300 mg/kg) in rotenone treated animals, significantly increased GSH level when compared with vehicle treated rats. This evidence suggests the antioxidant effect of *Ganoderma lucidum*.

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SOD an enzyme that catalyzes the dismutation of superoxide into hydrogen peroxide and nonreactive oxygen species. When cells are exposed to oxygen, SOD appears to be an antioxidant defense, which is available in almost all cells. SOD has a neutralizing effect on free radical toxicity [23]. In the brain tissues of rotenone treated rats, a reduced level of SOD was observed, which indicated oxidative stress. However, pretreatment with *Ganoderma lucidum* at doses (150 and 300 mg/kg) in rotenone treated group showed a significant increase in SOD level when compared with vehicle treated rats.

Another antioxidant enzyme that neutralizes the toxic effects of hydrogen peroxide is catalase (CAT). CAT converts hydrogen peroxide into nonreactive oxygen specie and water and stops those precursors accumulation that biosynthesizes free radicals. CAT level is decreased by oxidative stress [24]. In our study rotenone treated rats showed a decrease in CAT level when compared with vehicle treated control rats. However, pretreatment with *Ganoderma lucidum* at doses (150 and 300 mg/kg) in rotenone treated rats showed increased CAT levels when compared with vehicle treated rats.

It has been found that, in neurodegenerative disorders, such as Parkinson's disease, stroke and Alzheimer's disease, neuroinflammation and oxidative stress plays very important role. For neurodegenerative diseases, drugs with anti-inflammatory and antioxidant activities suit well [25, 26]. Therefore, our study suggests that *Ganoderma lucidum*, due to its strong antioxidant and

anti-inflammatory activity, may be liable for neuroprotection in neurodegenerative diseases including Parkinson's disease.

6. CONCLUSION

Based on above facts, we get into the conclusion that *Ganoderma lucidum* (Reishi) effectively ameliorates the neurodegeneration induced by rotenone due to its strong antioxidant and anti-inflammatory activity. This proves *Ganoderma lucidum* do have antiparkinsonian activity. However, further investigation is required for the mechanism through which *Ganoderma lucidum* exerts its neuroprotective effect in Parkinson's disease and to find the specific constituents of the mushroom responsible for these activities.

ETHICAL APPROVAL

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

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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