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Effect of *Ganoderma lucidum* on MPTP Induced Behavioral Alterations in Swiss Albino Mice

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ABSTRACT

Objectives: To investigate the behavioral effect of *Ganoderma lucidum* polysaccharides (GLPS) and triterpenoid fractions (GLTT) in Parkinson’s induced mice. Methods: Parkinson’s disease (PD) was induced by intraperitoneal administration of 0.3 mg/kg 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP). *G. lucidum* was collected, authenticated and extracted with water and methanol to obtain GLPS and GLTT extracts respectively. A dose of 50 mg/kg of GLPS and GLTT were administered orally to PD mice for five consecutive days. Behavioral studies such as forced swim test, rota-rod test, grip strength test and locomotor activity were conducted in all the animals before and after toxicant, GLPS and GLTT treatment. Results: The mice injected with MPTP induced reasonable extent of Parkinsonism. In forced swim test, after treatment with GLPS and GLTT, the time for the mice to stop swimming decreased. In grip strength test, the motor coordination of PD induced mice seemed to be higher when compared to the normal mice, GLPS and GLTT treated groups. In rota-rod test, treatment with GLPS and GLTT showed mild decrease in the time for the mice to fall from a rotating rod. The results of the locomotor test showed that the mice had an increase in its locomotor activity after being induced with MPTP and decreased after being treated with GLPS and GLTT. Conclusion: This preliminary study results indicate that both GLPS and GLTT extract has therapeutic benefit on the PD mice.

Key words: 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP), Anti-parkinsonism activity, *Ganoderma lucidum*, Polysaccharide, Triterpenoid.

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INTRODUCTION

Parkinson’s Disease (PD) is the most common progressive neurodegenerative movement disorder which affects around 6 million people globally and the prevalence will rise with an aging population.¹,² Majority of the cases of PD are still considered idiopathic. There are some hypotheses of neuronal loss which involves genetically linked factors and environmental interaction factors. Ingestion or inhalation of neurotoxins (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [MPTP]), endogenous toxin releasing radical oxygen species (ROS) in brain and isoquinoline derivatives substances may cause PD.³

Although various therapeutic agents are available to cure PD symptomatically, there are many adverse effects associated with those medications. Taken levodopa as an example, even though it remains the pharmacologic mainstay for the treatment of PD, after several years of favorable responses, disabling motor complications frequently developed.³ Other than that, numerous side effects and complications of other dopaminergic medications and surgery had been tested too.⁴ So, these have reached our consensus that, till date, none of the drug or therapies has been convincingly shown to halt or cure the degenerative process in PD.

In light of these challenges, researches into alternative neuro-protective therapies area are rising at a feverish pace. Increasing number of patients are seeking for alternative treatments such as herbal therapies.⁴ Many researchers had discovered the broad therapeutic potential of a mushroom species *Ganoderma lucidum* (Family: Ganodermataceae). Among the bioactive elements in *G. lucidum*, polysaccharides, triterpenes, and peptidoglycans are three major physiologically active constituents in *G. lucidum*.⁵,¹⁰ *G. lucidum* polysaccharides (GLPS) obtained by aqueous extraction are reported to exhibit a wide-ranging bioactivities, including anti-inflammatory, hypoglycemic, anti-ulcer, anti-tumorigenic, and immunostimulating effects.¹¹,¹² On the other hand, the triterpenoids extract of *G. lucidum* (GLTT) by methanolic extraction demonstrated antipruritic effects¹³ and anti-cancer properties¹⁴,¹⁵ Previous studies which stated that the extract of *G. lucidum* is effective to improve the function of mitochondria in aged rat brain, suggested it as a possible therapeutic application against ageing-associated neurodegenerative diseases.¹⁶ Of this interest, we hypothesize that *G. lucidum* extract has its therapeutic efficacy on PD. By using MPTP, PD was induced to Swiss albino mice, after which the effects of *G. lucidum* polysaccharide (GLPS) and triterpenoid (GLTT) fractions were investigated.

MATERIALS AND METHODS

*G. lucidum* was gifted by DXN Pharma Sdn Bhd., Malaysia. 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP) was obtained from Sigma Aldrich Co, St. Louis, USA. All chemicals, consumables and solvents used in this project were of analytical grade and purchased from Bendor森 laboratory chemicals, Malaysia. EDTA, diethyl ether and formaldehyde were procured from RM chemicals/HmbG chemicals, Malaysia.

Animals

Healthy, adult, both the gender of Swiss albino mice weighing 20-30 g were obtained from Central Animal house, AIMST University, Malaysia. The animals were housed in large, spacious poly acrylic cages at an ambient room temperature with 12 h light and dark cycle. The animals were fed with water and food *ad libitum*. The PD induced animal model study was approved by AIMST University Human and Animal Ethics Committee and the study was conducted according to Animal Research Review Panel guidelines.
Extraction of GLPS and GLTT

*G. lucidum* was extracted with distilled water by maceration in a dark place for 6 days. Few drops of 1% chloroform were used as preservative to prevent fungal growth. Later, the extract was filtered and dried at 60°C. The semisolid form of *G. lucidum* polysaccharide (GLPS) extract was preserved in cold conditions until further studies.

For the triterpenoid extraction, *G. lucidum* powder extracted with methanol by Soxhlet extraction for 24 h. The extract was evaporated to dryness on a rotary evaporator to obtain *G. lucidum* triterpenoid (GLTT). The extract was stored in cold conditions until further studies.

Antiparkinson activity of GLPS and GLTT

Healthy Swiss albino mice were divided into five groups each of five animals as follows.

- Group I: Control
- Group II: MPTP control
- Group III: MPTP+GLPS 50 mg/kg
- Group IV: MPTP+GLTT 50 mg/kg

A control group animal were administered with normal saline (1 ml/kg) and the animals in group II to IV were administered with 3 mg/kg MPTP (dissolved in saline), intraperitoneally for 5 consecutive days. After induction of PD, the animals in group III and IV were treated with GLPS (50 mg/kg) and GLTT (50 mg/kg). The drugs were suspended with 0.5% carboxymethyl cellulose and administered orally for 5 consecutive days. During the experiment, body weight variations, regular food and water intake was measured. Prior to the experiment and at the end of the experiment, the rats’ behavior was studied using forced swim test, grip strength test, rota-rod test and locomotor activity. At the end of the study the rats were sacrificed and brain samples were collected and preserved in 10% neutral formalin for histopathological studies.

**Forced swim test:** In this test, the tank with water of temperature 24°C-30°C with depth that the rodent's tails and feet not touching the bottom of the tank was prepared. The rodent was then put into the tank upon which the time between the rodent to stop swimming and start sinking was recorded. The rodent was removed immediately as soon as it started to sink. The procedure was repeated for all the rodents. The water tank was then cleaned as the accumulation of faeces and urine could cause bacterial contamination.

**Grip strength test:** The forelimb of the rodent was left to cling on a rope while its body and tail was suspended in the air above 50 cm from the ground. The time of the rodent clinging on the rope was recorded. The procedure was repeated for all 5 rodents of both control and treated group. The height between the rope and ground was ensured not be too high, to prevent the rodent hurt when falling.

**Rota-rod test:** The rodent was placed on a rota-rod with speed of 20 RPM. The time between rodent maintain on the rota-rod until it fall was recorded. The procedure was repeated for each rodent.

**Locomotor activity:** The locomotor activity of the rats was recorded in actophotometer provided with an acrylic cage and 8 beams of infrared light along both the x- and y-axis. The activity of each rat was monitored at room temperature over 10 min.

**Histopathological analysis**

The brain samples were preserved in 10% neutral formalin for histopathological analysis. The brain sample were embedded in paraffin after being dehydrated in alcohol and subsequently cleared with xylene. A 5-6 micrometer thickness of brain section were prepared from paraffin blocks and stained with hematoxylin and eosin and mounted in neutral DPX medium, and the sections were examined under light microscope.

**Statistical analysis**

All statistical data are represented as mean ± SEM of the mentioned number of testing in five animals. One way ANOVA (Graph Pad Prism 5.00, Instat software, San Diego, CA < USA) followed by Tukey’s post hoc test were used for the statistical analysis.

**RESULT**

The semisolid form of *G. lucidum* polysaccharide (GLPS) and triterpenoid (GLTT) extracts were used in this study. The percentage yield of GLPS and GLTT were found to be 8.88% and 6.67% respectively.

The effect of GLPS and GLTT extract on MPTP induced behavioral alterations were summarized in Table 1. On day of termination MPTP treated animals showed increased swimming ability, muscular strength, and increased locomotor activity. Whereas GLPS and GLTT significantly inhibited the MPTP induced behavioral alterations. No significant alteration was observed in rota-rod test. Histopathological analysis did not indicate any abnormality in the brain tissue of GLPS and GLTT treated animals (Figure 1).

**DISCUSSION**

Parkinson's disease is characterized by the tetrad of tremor at rest, rigidity, decreased voluntary movements, and postural instability due to depletion of dopamine levels in nigrostriatal system and loss of neurons of nigrostriatal dopaminergic pathway. Loss of dopamine producing neurons in the nigrostriatal system. The mice were injected with MPTP to induce PD, because the pathogenesis of MPTP induced PD in mice is clearly linked to a form of human parkinsonism. Since GLPS and GLTT, has combination benefit without toxicity which represents the desired end results in the development of effective therapeutics interventions, it has been used for hundreds of years as a health promotion and treatment strategy.

In the present study, MPTP administered group showed an increased neuronal and muscular activities rather than a decrease. PD was char-

<table>
<thead>
<tr>
<th>Behavioral Tests</th>
<th>Baseline (seconds)</th>
<th>1st day after MPTP (seconds)</th>
<th>5th day after MPTP (seconds)</th>
<th>GLPS 50 mg/kg (seconds)</th>
<th>GLTT 50 mg/kg (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forced Swim Test</td>
<td>84.00 ± 1.44</td>
<td>118.00 ± 4.98</td>
<td>158.20 ± 5.51</td>
<td>57.20 ± 9.83</td>
<td>60.50 ± 6.10</td>
</tr>
<tr>
<td>Grip Strength Test</td>
<td>89.20 ± 1.48</td>
<td>120.20 ± 4.73</td>
<td>160.80 ± 5.20</td>
<td>81.60 ± 4.40</td>
<td>90.10 ± 5.40</td>
</tr>
<tr>
<td>Rota-rod Test</td>
<td>25.60 ± 3.65</td>
<td>25.20 ± 1.37</td>
<td>29.10 ± 2.57</td>
<td>17.70 ± 3.39</td>
<td>19.50 ± 2.84</td>
</tr>
<tr>
<td>Locomotor activity</td>
<td>79.00 ± 1.12</td>
<td>118.00 ± 2.00</td>
<td>136.00 ± 1.77</td>
<td>108.00 ± 4.12</td>
<td>142.00 ± 2.98</td>
</tr>
</tbody>
</table>

*The values are mean ± SEM of five animals.*
characterized by a decreased voluntary movement, but we observed an increased motor activity after MPTP administration and this may be due to its sympathomimetic over activation or frequency of MPTP which may not be sufficient to induce PD in experimental animals. Porras et al induced PD in monkey and mouse and they found that “MPTP mouse models for instance failed to replicate symptomatic manifestation of PD”. GLPS and GLTT were found to alter the MPTP induced increase in behavioral function and this indicates that both GLPS and GLTT has some degree of neuroprotective effect. Zhang et al., and Zhou et al., studied the neuroprotective effect of G. lucidum and the effect is due to reduction in the percentage of apoptotic neurons, suppressed overexpression of active caspases-3, -8 and -9, myeloperoxidase activity, malondialdehyde levels, nitric oxide levels and inhibition of the reduction of Bcl-2 expression.

CONCLUSION
The mice models injected with MPTP successfully induced reasonable extent of Parkinsonism. After employing GLPS and GLTT into the PD induced mice models, the behavioral studies of forced swim test and grip strength test reflected mean values which indicated that GLPS and GLTT held therapeutic benefit on the mice models, when compared with control.

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CONFLICT OF INTEREST
All authors declare no conflict of interest.

ABBREVIATIONS USED
PD: Parkinson’s disease. MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; GLPS: Ganoderma lucidum polysaccharides; GLTT: Ganoderma lucidum triterpenoid; ROS: Radical oxygen species.

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