CHAPTER 5: Effect of protein bound polysaccharides isolated from the *Ganoderma lucidum* on the mitochondrial oxidative stress and dysfunction in old aged rats
TABLE OF CONTENTS

5. INTRODUCTION

5.2. MATERIALS AND METHODS

5.2.1. Animals

5.2.2. Isolation of the protein bound polysaccharides from *G. lucidum*

5.2.3. Effect of protein bound polysaccharides from *G. lucidum* on the mitochondrial antioxidant status and mitochondrial enzymes of aged rats

5.2.4. Isolation of mitochondria

5.2.5. Determination of enzymatic antioxidant activity

5.2.6. Determination of reduced glutathione

5.2.7. Determination of lipid peroxidation

5.2.8. Determination of the activities of Krebs cycle dehydrogenases

5.2.8.1. Determination of activity of the isocitrate dehydrogenase (ICDH) activity

5.2.8.2. Determination of α- ketoglutarate dehydrogenase (α-KGDH) activity

5.2.8.3. Determination of succinate dehydrogenase (SDH) activity

5.2.8.4. Determination of malate dehydrogenase (MDH) activity

5.2.9. Determination of activities of the respiratory chain complexes

5.2.9.1. Determination of complex I activity

5.2.9.2. Determination of complex II activity

5.2.9.3. Determination of complex III activity

5.2.9.4. Determination of complex IV activity

5.2.10. Determination of reactive oxygen species (ROS) level

5.2.11. Determination of mitochondrial membrane potential (ΔΨmt)

5.2.12. Determination of protein content
5.3. RESULTS

5.3.1. Effect of *G. lucidum* protein bound polysaccharides on the innate mitochondrial antioxidant activities in the old aged rats

5.3.2. Effect of *G. lucidum* protein bound polysaccharides on the levels of mitochondrial GSH and lipid peroxidation in the old aged rats

5.3.3. Effect of *G. lucidum* protein bound polysaccharides on the activities of mitochondrial dehydrogenases in the old aged rats

5.3.4. Effect of *G. lucidum* protein bound polysaccharides on the activities of mitochondrial respiratory chain complexes in the old aged rats

5.3.5. Effect of *G. lucidum* protein bound polysaccharides on the levels of mitochondrial reactive oxygen species (ROS) in the old aged rats

5.3.6. Effect of *G. lucidum* protein bound polysaccharides on the levels of mitochondrial membrane potential (ΔΨmt) in old aged rats

5.4. DISCUSSION
5.1. INTRODUCTION

During aging, the decay of mitochondria and related mitochondrial dysfunction are playing important roles. Studies related to mitochondria and aging showed that mitochondria ultimately cause their own decay during aging, although the factors involved in mitochondrial dysfunction and heterogeneity remain to be clarified (Cadenas and Davies, 2000). The increased oxidant production and lipid peroxidation in the mitochondrial membrane and associated decline of cellular antioxidant levels during aging clearly supports the enhanced mitochondrial susceptibility to oxidative damage (Arivazhagan et al., 2001). The resultant mitochondrial decay may eventually cause inadequate energy production and/or the loss of calcium homeostasis. Such changes could result in unwarranted cellular apoptosis and lead to the general metabolic decline evident in aging. Increasing evidence suggests that defects in mitochondrial energy production are involved in the neuronal damage observed in aging and several neurodegenerative diseases (Beal, 1998). Since the first implication of mitochondria in cell aging (Harman, 1972), many reports have demonstrated a key role by mitochondrial oxidative damage in aging and age associated diseases.

Supplementation with dietary antioxidants has been one of the approaches utilized to test the free radical theory of aging, and, at least, to try to reduce the impact of age related dysfunctions (Beckman and Ames, 1998; Miquel, 2002). Several attempts have been made to slow down the age associated mitochondrial decay by means of dietary interventions with antioxidants (Miquel, 2002) and some have succeeded in partially restoring mitochondrial structure and function by reducing the oxidative damage to lipids, proteins and nucleic acids. However, issues of dosing and timing of antioxidant treatments remain still quite unexplored. Thus, it is essential to explore new natural antioxidants to protect human from free radicals and the progression of many chronic diseases. Polysaccharides, which widely distributed in animals, plants and microorganisms, have been demonstrated to play an important role as free radical scavengers in the prevention of oxidative damage in living organism (Tsiapali et al., 2001).

Since polysaccharides are one of the major bioactive components in *G. lucidum*, researches direct their focus on *G. lucidum* polysaccharides (Rui-fang and Rong-chun, 2004). In recent years, polysaccharides extracted from *G. lucidum* have been regarded as an important class of anticoagulant, immunomodulating and
antitumour with antioxidant activities (Bao et al., 2002; Zhou et al., 2007; Xu et al., 2011). In an earlier study from our lab, we obtained a *G. lucidum* polysaccharide preparation (GLPP) with potential radioprotective activity (Pillai et al., 2008). It protects against radiation induced damages including induction of micronucleus in reticulocytes of mice, strand breaks in plasmid pBR322 DNA and inhibits lipid peroxidation. Another recent study from our lab also showed that *Ganoderma* mushroom polysaccharide also prevents radiation induced DNA strand breaks in human cells (Pillai et al., 2010). As observed in the earlier chapter, the aqueous ethanol extract of *G. lucidum* had more efficiency in old aged rats, the protein bound polysaccharides isolated from *G. lucidum* were evaluated for their role in age related oxidative stress and cellular energy status. The effects of the protein bound polysaccharides isolated from *G. lucidum* on the antioxidant status, on the activities of mitochondrial dehydrogenases as well as on respiratory chain complexes in the aged rats were evaluated and the findings are reported in this chapter.

5.2. MATERIALS AND METHODS

5.2.1. Animals

Male albino rats of Wistar strain approximately 3-4 months old (young aged; weighing approximately 130-160 g), and above 24 months old (old aged; weighing approximately 350- 400 g) were used in this study.

5.2.2. Isolation of the protein bound polysaccharides from *G. lucidum*

The protein bound polysaccharides were isolated from *G. lucidum* as described previously (2.2.2). The protein bound polysaccharide fraction was suspended in distilled water and employed for the experiment.

5.2.3. Effect of protein bound polysaccharides from *G. lucidum* on the mitochondrial antioxidant status and mitochondrial enzymes of aged rats

Animals were divided into 5 groups of 6 animals each.

1. Group I: old aged rats administered with distilled water kept as old aged control group,
2. Group II: young aged rats administered with distilled water kept as young aged control group,
3. Group III: old aged rats administered with protein bound polysaccharides 25 mg/kg body weight,
4. Group IV: old aged rats administered with protein bound polysaccharides 50 mg/kg body weight,

5. Group V: old aged rats administered with DL-α-Lipoic acid 100 mg/kg body weight.

The DL-α-Lipoic acid was dissolved in alkaline saline (0.5% NaOH, w/v). The DL α-lipoic acid, protein bound polysaccharides or distilled water (to aged control and young control) were administered orally once daily for 15 days. Twenty-four hours after the completion of drug administration, the animals were sacrificed by cervical decapitation. The heart, brain, skeletal muscle, liver and kidneys were excised out immediately and kept in -70ºC for the determination of enzymatic and non-enzymatic mitochondrial antioxidant status, activities of mitochondrial dehydrogenases, mitochondrial respiratory chain complexes, level of ROS, and membrane potential.

5.2.4.Isolation of mitochondria

Mitochondria were isolated from the heart, brain, skeletal muscle (soleus), liver and kidney homogenates by differential centrifugation according to the method described in section 2.2.4. Mitochondrial fraction was frozen and thawed 3 to 5 times centrifuged at 3000g and the supernatant was used for the enzyme assays.

5.2.5.Determination of enzymatic antioxidant activity

The supernatant (approximately 3 mg/ml protein) was used for the determination of activities of manganese-superoxide dismutase (Mn SOD) (section: 2.2.5) and glutathione peroxidase (GPx) (section: 2.2.6) using a double beam spectrophotometer (2202-Systronics India Ltd, Hyderabad, India)

5.2.6.Determination of reduced glutathione

The level of reduced glutathione in the mitochondrial fraction was determined by the method described in the section 2.2.7.

5.2.7.Determination of lipid peroxidation

The level of lipid peroxidation measured as malondialdehyde equivalents formed in the mitochondrial fraction was determined by the method described in the section 2.2.8.
5.2.8. Determination of the activities of Krebs cycle dehydrogenases

5.2.8.1. Determination of activity of the isocitrate dehydrogenase (ICDH) activity

ICDH activity was estimated according to the method of Fatania et al. (1993) as described in the section 2.2.10.1. The activity was expressed as micromoles of NAD reduced/min/mg protein using extinction coefficient 6.3 mM⁻¹ cm⁻¹.

5.2.8.2. Determination of α- ketoglutarate dehydrogenase (α-KGDH) activity

α-KGDH activity was estimated by the method of Reed and Mukherjee (1969) as described in the section 2.2.10.2. The activity was expressed as μmoles of NAD (reduced/ min/mg protein) using extinction coefficient 6.3 mM⁻¹ cm⁻¹.

5.2.8.3. Determination of succinate dehydrogenase (SDH) activity

SDH activity was estimated by the method of Nulton-Persson and Szweda (2001) as described in the section 2.2.10.3. The activity was calculated using the extinction coefficient of DCPIP (19.1 mM⁻¹ cm⁻¹) and expressed as μmoles of DCPIP reduced/min/mg protein.

5.2.8.4. Determination of malate dehydrogenase (MDH) activity

MDH activity was estimated by the method of Mehler et al. (1948) as described in the section 2.2.10.4. The activity was expressed as μmoles of NADH oxidized/min/mg protein using the extinction coefficient of NADH 6.3 mM⁻¹ cm⁻¹.

5.2.9. Determination of activities of the respiratory chain complexes

5.2.9.1. Determination of complex I activity

Estimated by the method of Janssen et al. (2007) described in the section 2.2.11.1. The activity was expressed as μmoles of DCPIP reduced/ min/mg protein (extinction coefficient of DCPIP is 19.1mM⁻¹ cm⁻¹)

5.2.9.2. Determination of complex II activity

Complex II activity was estimated by the method of Janssen et al. (2007) as described in the section 2.2.11.2. The activity was expressed as micromoles of DCPIP reduced/min/mg protein (extinction coefficient of DCPIP is 19.1mM⁻¹ cm⁻¹)
5.2.9.3. Determination of complex III activity

Complex III activity was estimated by the method of Krahenbuhl et al. (1991) by preparing Decyl ubiquinol as described in the section 2.2.11.3. Activity of complex III was expressed as micromoles of ferricytochrome-C reduced/min/mg protein.

5.2.9.4. Determination of complex IV activity

Complex IV activity was determined by the method of Capaldi et al. (1995) by preparing ferrocytochrome C as described in the section 2.2.11.4.

5.2.10. Determination of reactive oxygen species (ROS) level

The level of ROS in the mitochondrial fraction was determined by the method described in the section 2.2.12 by using a Nanodrop flurospectrometer, Wilmington, USA and represented as relative fluorescence units (RFU).

5.2.11. Determination of mitochondrial membrane potential (ΔΨmt)

The ΔΨmt was quantified in the mitochondrial fraction by the method described in the section 2.2.13 by using a Nanodrop flurospectrometer and represented as relative fluorescence units (RFU).

5.2.12. Determination of protein content

The amount of protein present in the mitochondrial fraction was determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard as described in the section 2.2.9.

5.3. RESULTS

The experimental results showed that the antioxidant status and activities of mitochondrial dehydrogenases and respiratory chain complexes were declined significantly with advancing age. There was significant decrease in the membrane potential and increase in the ROS level in the old aged rats with respect to that of young rats. However, treatment with *G. lucidum* protein bound polysaccharides effectively ameliorated the oxidative stress and cellular energy status in the old aged rats.
5.3.1 Effect of *G. lucidum* protein bound polysaccharides on the innate mitochondrial antioxidant activities in the old aged rats

In this study, the activities of antioxidant enzymes such as Mn SOD and GPx were highest in the young aged control than that of old aged controls which clearly showed that the antioxidant enzymes are declined during aging in all the major organs such as heart, brain, skeletal muscle, liver and kidneys (Table 5.1). In the current investigation, there was approximately 3.14 and 1.97 fold decreases in the case of heart MnSOD and heart GPx, 1.97 and 2.05 fold decreases for brain MnSOD and brain GPx, 3.41 and 1.79 fold decreases for skeletal muscle MnSOD and skeletal muscle GPx, 2.01 and 2.38 fold decreases for liver MnSOD and liver GPx, and 1.46 and 1.56 fold decreases for kidney MnSOD and kidney GPx respectively in the case of old aged control compared to that of young aged control.

In all the organs studied, such as heart, brain, skeletal muscle, liver and kidneys, the treatment of *G. lucidum* protein bound polysaccharides and the positive standard, DL-α-Lipoic acid (LA) showed significant changes in the activities of Mn SOD and GPx in the old rats (Table 5.1). Similarly, LA had significant effects in the old aged group.

There was approximately 3.80, 3.93 and 4.51 fold increases for heart MnSOD, 1.16, 1.06 and 0.88 fold increases for brain MnSOD, 1.35, 2.15 and 1.81 fold increases for skeletal muscle MnSOD, 1.37, 1.38 and 1.83 fold increases for liver MnSOD and 1.18, 1.20 and 1.20 fold increases for kidney MnSOD respectively in the case of *G. lucidum* protein bound polysaccharide treatment at 25, 50 mg/kg and LA 100 mg/kg groups more than that of aged control. There was approximately 1.94, 1.77 and 1.50 fold increases for heart GPx, 1.54, 1.97 and 2.53 fold increases for brain GPx, 1.23, 1.38 and 1.55 fold increases for skeletal muscle GPx 1.91, 2.01 and 1.93 fold increases for liver GPx and 1.21, 1.90 and 1.77 fold increases for kidney GPx respectively in the case of *G. lucidum* protein bound polysaccharide 25, 50 mg/kg and LA 100 mg/kg treated groups more than that of aged control.
Table 5.1: Effect of protein bound polysaccharides from *G. lucidum* on the innate antioxidants in the mitochondria of in the old age rats

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Organ</th>
<th>Old age control</th>
<th>Young control</th>
<th>Old age rats+ Protein bound polysaccharides (25 mg/kg)</th>
<th>Old age rats+ Protein bound polysaccharides (50 mg/kg)</th>
<th>Old age rats + DL-α-Lipoic acid (100 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn SOD (U/mg protein)</td>
<td>Heart</td>
<td>6.23 ± 1.21</td>
<td>32.25 ± 8.88***</td>
<td>36.18 ± 7.84***</td>
<td>41.52 ± 12.25***</td>
<td>39.44 ± 5.88***</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>10.25 ± 2.25</td>
<td>30.25 ± 5.58***</td>
<td>18.90 ± 4.44*</td>
<td>18.94 ± 5.58*</td>
<td>16.56 ± 5.54**</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>6.21 ± 1.25</td>
<td>33.25 ± 7.77***</td>
<td>15.72 ± 5.58*</td>
<td>19.28 ± 3.24**</td>
<td>18.07 ± 4.57**</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>32.75 ± 6.50</td>
<td>85.65 ± 6.75***</td>
<td>66.15 ± 12.25***</td>
<td>69.51 ± 15.58***</td>
<td>83.99 ± 12.25***</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>31.25 ± 5.56</td>
<td>60.25 ± 6.36***</td>
<td>48.89 ± 5.55**</td>
<td>52.25 ± 8.99***</td>
<td>54.42 ± 10.20***</td>
</tr>
<tr>
<td>GPx (U/mg protein)</td>
<td>Heart</td>
<td>16.09 ± 3.71</td>
<td>44.10 ± 5.01***</td>
<td>45.39 ± 6.90***</td>
<td>45.30 ± 8.10***</td>
<td>35.99 ± 6.17***</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>13.42 ± 3.52</td>
<td>41.75 ± 7.08***</td>
<td>30.09 ± 4.08***</td>
<td>38.06 ± 4.75***</td>
<td>36.75 ± 6.08***</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>16.75 ± 3.70</td>
<td>41.42 ± 4.75***</td>
<td>28.46 ± 3.34***</td>
<td>34.82 ± 6.66***</td>
<td>36.74 ± 5.12***</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>31.13 ± 7.13</td>
<td>96.13 ± 5.13***</td>
<td>81.35 ± 8.13***</td>
<td>86.98 ± 9.99***</td>
<td>83.68 ± 10.00***</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>26.13 ± 4.39</td>
<td>60.13 ± 12.63***</td>
<td>50.63 ± 13.63***</td>
<td>65.11 ± 7.13***</td>
<td>60.18 ± 6.28***</td>
</tr>
</tbody>
</table>

Values are the mean ± SD; n = 6.

***p<0.001, **p<0.01 and *p<0.05 significantly different from old aged control (Bonferroni test).
5.3.2. Effect of *G. lucidum* protein bound polysaccharides on the levels of mitochondrial GSH and lipid peroxidation in the old aged rats

In the study, the levels of mitochondrial GSH were decreased significantly (Fig. 5.1.A) and the level of lipid peroxidation as MDA formed were significantly enhanced (Fig.5.1.B) in the old aged control group than that of young aged control in all the organs studied.

There was approximately 1.28 fold decrease in the heart mitochondrial GSH, 1.36 decrease in the brain mitochondrial GSH, 2.28 fold decrease for skeletal muscle mitochondrial GSH, 1.19 fold decrease for liver mitochondrial GSH and 1.46 fold decrease for kidney mitochondrial GSH levels respectively in the old aged group than that of young aged group. There were approximately 1.70 fold increase in the heart MDA level, 2.34 fold increase in the brain MDA level, 1.12 fold increase in the skeletal muscle MDA level, 1.21 fold increase in the liver MDA level and 2.19 fold increase in the kidney MDA level respectively in the old aged group than that of young aged group.

The treatment of *G. lucidum* protein bound polysaccharides and LA significantly enhanced the GSH level and reduced the MDA level in the aged rats (Fig.5.1.A and Fig.5.1.B). There was approximately 1.74, 2.03 and 1.25 fold increases for heart GSH, 1.09, 0.95 and 1.27 fold increases for brain GSH, 1.65, 1.98 and 2.19 fold increases for skeletal muscle GSH, 0.99, 1.08 and 1.06 fold increases for liver GSH, 1.08, 1.29 and 1.28 fold increases for kidney GSH respectively in the case of *G. lucidum* protein bound polysaccharide 25, 50 mg/kg and LA 100 mg/kg treated groups than that of aged control.

Similarly, there was approximately 2.23, 2.36 and 2.57 fold decreases in the level for heart MDA, 1.92, 2.52 and 2.08 fold decreases in the level for brain MDA, 1.63, 1.17 and 1.08 fold decreases in the level for skeletal muscle MDA, 1.39, 1.95 and 1.23 fold decreases in the level for liver MDA, and 2.53, 3.01 and 2.25 fold decreases in the level for kidney MDA respectively in the case of *G. lucidum* protein bound polysaccharide 25, 50 mg/kg and LA 100 mg/kg treated groups than that of aged control.
Fig. 5.1.A. Effect of the protein bound polysaccharides from *G. lucidum* on the mitochondrial GSH level in the old aged rats

Values are the mean ± SD; n = 6.

***p<0.001, **p<0.01, *p<0.05 significantly and ns p>0.05 non-significantly different from old aged control (Bonferroni test)

Fig. 5.1.B. Effect of the protein bound polysaccharides from *G. lucidum* on the mitochondrial MDA level in the old aged rats

Values are the mean ± SD; n = 6.

**p<0.01 significantly different from old aged control (Bonferroni test)
5.3.3. Effect of *G. lucidum* protein bound polysaccharides on the activities of mitochondrial dehydrogenases in the old aged rats

In the current study the activities of mitochondrial dehydrogenases such as ICDH, α-KGDH, SDH and MDH were significantly declined in the old aged control than that of young aged control (Table 5.2).

There was approximately 1.87, 1.91, 1.75, 1.10 and 1.49 fold decreases for heart, brain, skeletal muscle, liver and kidney ICDH activities, 5.10, 2.39, 4.08, 1.28 and 1.8 fold decreases for heart, brain, skeletal muscle, liver and kidney α-KGDH activities, 1.74, 2.12, 1.37, 1.22 and 1.42 fold decreases for heart, brain, skeletal muscle, liver and kidney SDH activities, and 1.85, 3.40, 1.63, 1.32 and 1.37 fold decreases for heart, brain, skeletal muscle, liver and kidney MDH activities respectively in the old aged group than that of young aged group.

The treatment of *G. lucidum* protein bound polysaccharides and LA significantly enhanced the activities of mitochondrial dehydrogenases in old aged rats (Table 5.2). There was approximately 2.99, 3.48 and 2.05 fold increases in the activity for heart ICDH, 1.48, 1.38 and 1.39 fold increases in the activity for brain ICDH, 1.07, 1.89 and 1.03 fold increase in the activity for skeletal muscle ICDH, 1.55, 1.85 and 1.47 fold increase in the activity for liver ICDH and 1.37, 1.51 and 1.21 fold increase in the activity for kidney ICDH respectively in the case of *G. lucidum* protein bound polysaccharide 25, 50 mg/kg and LA 100 mg/kg treated groups than that of aged control.

There was approximately 3.42, 3.30 and 3.21 fold increases in the activity for heart α-KGDH, 2.26, 2.35 and 1.71 fold increases in the activity for brain α-KGDH, 2.77, 2.93 and 3.52 fold increases in the activity for skeletal muscle α-KGDH, 1.0, 1.22 and 1.03 fold increases in the activity for liver α-KGDH, 1.08, 1.28 and 0.97 fold increases respectively in the activity for kidney α-KGDH respectively in the case of *G. lucidum* protein bound polysaccharide 25, 50 mg/kg and LA 100 mg/kg treated groups than that of aged control.

Similarly, there was approximately 1.26, 1.80 and 1.06 fold increases in the activity for heart SDH, 1.54, 1.92 and 1.75 fold increases in the activity for brain SDH, 1.10, 1.32 and 1.40 fold increases in the activity for skeletal muscle SDH, 1.32, 1.54 and 1.12 fold increases in the activity for liver SDH, 1.18, 1.25 and 1.35 fold increases in the activity for kidney SDH respectively in the case of *G. lucidum* protein bound polysaccharide 25, 50 mg/kg and LA 100 mg/kg treated groups than that of aged control.
Table 5.2: Effect of protein bound polysaccharides from *G. lucidum* on the activities of mitochondrial dehydrogenases in the old aged rats

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Organ</th>
<th>Old age control</th>
<th>Young control</th>
<th>Old age rats+ Protein bound polysaccharides (25 mg/kg)</th>
<th>Old age rats+ Protein bound polysaccharides (50 mg/kg)</th>
<th>Old age rats+ DL-α-Lipoic acid (100 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart</td>
<td>1689.36 ± 400.25</td>
<td>4032.25 ± 1212.25***</td>
<td>5314.50 ± 789.28***</td>
<td>5944.50 ± 677.58***</td>
<td>4085.56 ± 258.58***</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>777.25 ± 124.25</td>
<td>1977.40 ± 254.54***</td>
<td>1514.42 ± 177.25**</td>
<td>1828.41 ± 588.58***</td>
<td>1588.55 ± 333.38**</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>977.63 ± 294.07</td>
<td>2581.13 ± 350.00***</td>
<td>1485.20 ± 122.78**</td>
<td>2483.28 ± 88.79***</td>
<td>1917.68 ± 611.28***</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>1125.52 ± 200.25</td>
<td>2005.55 ± 544.25**</td>
<td>2249.25 ± 188.88***</td>
<td>2703.22 ± 255.25***</td>
<td>2433.20 ± 488.10***</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>1425.25 ± 248.25</td>
<td>2689.25 ± 199.25***</td>
<td>2480.22 ± 189.25***</td>
<td>2689.25 ± 155.55***</td>
<td>2425.15 ± 400.22***</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>82.25 ± 14.25</td>
<td>549.69 ± 102.25***</td>
<td>444.73 ± 114.50***</td>
<td>495.53 ± 177.25***</td>
<td>410.55 ± 100.35***</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>32.25 ± 9.25</td>
<td>121.88 ± 22.55**</td>
<td>124.04 ± 30.25**</td>
<td>138.66 ± 41.25***</td>
<td>125.55 ± 54.48**</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>90.25 ± 11.25</td>
<td>539.32 ± 125.25***</td>
<td>351.47 ± 69.98**</td>
<td>408.39 ± 111.25***</td>
<td>545.22 ± 188.22***</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>388.25 ± 24.28</td>
<td>554.25 ± 27.25***</td>
<td>451.46 ± 47.25**</td>
<td>513.49 ± 11.25***</td>
<td>500.28 ± 75.25**</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>218.25 ± 17.25</td>
<td>346.11 ± 22.25***</td>
<td>284.78 ± 31.25**</td>
<td>320.66 ± 19.55**</td>
<td>305.25 ± 78.55**</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>16.05 ± 4.25</td>
<td>48.25 ± 5.28***</td>
<td>47.90 ± 16.69***</td>
<td>53.15 ± 14.25***</td>
<td>45.56 ± 9.99**</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>20.25 ± 5.58</td>
<td>46.72 ± 11.25**</td>
<td>42.51 ± 14.20*</td>
<td>47.52 ± 13.32*</td>
<td>45.10 ± 8.88**</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>22.25 ± 7.44</td>
<td>51.60 ± 15.25***</td>
<td>43.50 ± 4.25*</td>
<td>52.50 ± 6.69***</td>
<td>45.55 ± 12.15**</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>25.25 ± 4.77</td>
<td>45.84 ± 3.25***</td>
<td>42.76 ± 7.25**</td>
<td>47.25 ± 8.77***</td>
<td>48.86 ± 8.99***</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>199.25 ± 19.25</td>
<td>541.41 ± 136.25***</td>
<td>441.24 ± 120.25**</td>
<td>451.70 ± 144.25**</td>
<td>425.25 ± 75.75*</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>75.25 ± 12.25</td>
<td>430.90 ± 133.32***</td>
<td>352.70 ± 101.25**</td>
<td>429.46 ± 140.25***</td>
<td>410.55 ± 139.10***</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>175.25 ± 25.25</td>
<td>356.73 ± 29.32**</td>
<td>331.61 ± 120.25*</td>
<td>350.64 ± 102.25*</td>
<td>350.55 ± 88.99*</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>206.28 ± 44.28</td>
<td>385.26 ± 55.61***</td>
<td>310.18 ± 62.28*</td>
<td>339.85 ± 39.39**</td>
<td>313.46 ± 52.24*</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>174.28 ± 21.32</td>
<td>304.36 ± 37.32***</td>
<td>271.77 ± 23.08***</td>
<td>275.44 ± 30.12***</td>
<td>319.86 ± 44.82***</td>
</tr>
</tbody>
</table>

Units: isocitrate dehydrogenase (ICDH) – µmoles of NAD+ reduced/min/mg protein; α ketoglutarate dehydrogenase (α-KGDH) – µmoles of NAD+ reduced/min/mg protein; succinate dehydrogenase (SDH) – µmoles of DCPIP reduced/min/mg protein; malate dehydrogenase (MDH) – µmoles of NADH oxidized/min/mg protein.

Values are the mean ± SD; n = 6.

***p<0.001, **p<0.01, *p<0.05 significantly and "p>0.05 non-significantly different from old aged control (Bonferroni test)
bound polysaccharide 25, 50 mg/kg and LA 100 mg/kg treated groups than that of aged control.

Also, there was approximately 1.47, 1.41 and 1.60 fold increases in the activity for heart MDH, 2.87, 3.31 and 3.10 fold increases in the activity for brain MDH, 1.05, 1.24 and 1.30 fold increases in the activity for skeletal muscle MDH, 0.99, 1.20 and 1.04 fold increases in the activity for liver MDH, 1.27, 1.25 and 1.41 fold increases in the activity for kidney MDH respectively in the case of *G. lucidum* protein bound polysaccharide 25, 50 mg/kg and LA 100 mg/kg treated groups than that of aged control.

### 5.3.4. Effect of *G. lucidum* protein bound polysaccharides on the activities of mitochondrial respiratory chain complexes in the old aged rats

In the current study, the activities of respiratory chain complexes such complex I, II, III and IV were significantly declined in the old aged control than that of young aged control group (Table 5.3).

There was approximately 1.94, 1.83, 1.70, 1.28 and 1.81 fold decreases for heart, brain, skeletal muscle, liver and kidney complex I activities, 1.89, 2.25, 1.5, 1.13 and 1.74 fold decreases for heart, brain, skeletal muscle, liver and kidney complex II activities, 2.16, 1.64, 1.50, 1.68 and 1.11 fold decreases for heart, brain, skeletal muscle, liver and kidney complex III activities, and 1.98, 1.80, 1.24, 1.20 and 1.36 fold decreases for heart, brain, skeletal muscle, liver and kidney complex IV activities respectively in the old aged group than that of young aged group.

The treatment of *G. lucidum* protein bound polysaccharides and LA significantly enhanced the activities of mitochondrial respiratory chain complexes in the old aged rats (Table 5.3). There was approximately 2.14, 1.57 and 1.67 fold increases in the activity for heart complex I, 1.51, 1.75 and 1.89 fold increases in the activity for brain complex I, 1.04, 1.01 and 1.11 fold increases in the activity for skeletal muscle complex I, 1.07, 1.24 and 1.10 fold increases in the activity for liver complex I and 1.43, 1.396 and 1.58 fold increases in the activity for kidney complex I respectively in the case of *G. lucidum* protein bound polysaccharide 25, 50 mg/kg and LA 100 mg/kg treated groups than that of aged control.

There was approximately 1.89, 2.25 and 1.98 fold increases in the activity for heart complex II, 1.42, 1.67 and 1.71 fold increases in the activity for brain complex
Table 5.3: Effect of protein bound polysaccharides from *G. lucidum* on the activities of respiratory chain complexes in the old aged rats

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Organ</th>
<th>Old age control</th>
<th>Young control</th>
<th>Old age rats+ Protein bound polysaccharides (25 mg/kg)</th>
<th>Old age rats+ Protein bound polysaccharides (50 mg/kg)</th>
<th>Old age rats + DL-α-Lipoic acid (100 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I</td>
<td>Heart</td>
<td>11.25 ± 5.25</td>
<td>43.18 ± 11.25**</td>
<td>32.98 ± 14.25*</td>
<td>41.21 ± 15.25**</td>
<td>40.10 ± 12.55**</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>17.25 ± 4.25</td>
<td>59.51 ± 20.22***</td>
<td>46.79 ± 14.25*</td>
<td>55.45 ± 17.25**</td>
<td>53.28 ± 12.55**</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>18.25 ± 2.22</td>
<td>31.25 ± 4.99**</td>
<td>24.71 ± 2.58**</td>
<td>26.54 ± 1.25**</td>
<td>27.75 ± 5.25***</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>19.25 ± 3.25</td>
<td>52.95 ± 12.25***</td>
<td>43.32 ± 11.25**</td>
<td>45.58 ± 14.25**</td>
<td>44.35 ± 8.88**</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>11.25 ± 1.22</td>
<td>35.25 ± 7.25***</td>
<td>23.01 ± 5.25**</td>
<td>27.13 ± 6.36**</td>
<td>31.25 ± 9.88***</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>10.25 ± 3.33</td>
<td>32.25 ± 11.25**</td>
<td>25.47 ± 5.25*</td>
<td>29.21 ± 9.21**</td>
<td>27.75 ± 8.55**</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>21.25 ± 5.25</td>
<td>38.25 ± 8.28***</td>
<td>36.44 ± 4.25**</td>
<td>44.14 ± 6.66***</td>
<td>42.25 ± 7.75***</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>14.25 ±1.25</td>
<td>32.25 ± 5.25***</td>
<td>25.89 ± 4.25**</td>
<td>34.91 ± 6.25***</td>
<td>31.75 ± 7.25***</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>6.99 ± 1.25</td>
<td>15.78 ± 2.25**</td>
<td>11.87 ± 3.32**</td>
<td>12.59 ± 4.25**</td>
<td>14.10 ± 5.56**</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>14.25 ± 4.25</td>
<td>39.25 ± 8.25***</td>
<td>30.89 ± 7.25***</td>
<td>34.11 ± 8.25**</td>
<td>37.10 ± 12.55**</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>24.25 ± 6.25</td>
<td>46.25 ± 12.44**</td>
<td>33.90 ± 6.25**</td>
<td>42.75 ± 7.25*</td>
<td>47.15 ± 12.10**</td>
</tr>
<tr>
<td>Complex IV</td>
<td>Heart</td>
<td>27.25 ± 8.25</td>
<td>85.97 ± 15.52***</td>
<td>56.00 ± 7.25**</td>
<td>71.19 ± 8.88***</td>
<td>74.10 ± 12.17***</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>48.25 ± 12.25</td>
<td>157.35 ± 48.25***</td>
<td>134.50 ± 44.25**</td>
<td>159.19 ± 22.21***</td>
<td>150.10 ± 28.88***</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>72.25 ± 11.25</td>
<td>136.08 ± 32.25**</td>
<td>132.16 ± 25.52**</td>
<td>143.83 ± 17.25***</td>
<td>140.15 ± 38.10**</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>110.25 ± 18.25</td>
<td>180.83 ± 27.25**</td>
<td>163.71 ± 29.25*</td>
<td>173.98 ± 19.25**</td>
<td>188.10 ± 35.25***</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>92.25 ± 13.25</td>
<td>130.07 ± 12.25***</td>
<td>160.99 ± 12.25**</td>
<td>190.10 ± 60.12***</td>
<td>190.10 ± 60.12***</td>
</tr>
</tbody>
</table>

Units: complex I – µmoles of DCPIP reduced/min/mg protein; complex II – µmoles of DCPIP reduced/min/mg protein; complex III – µmoles of ferricytochrome-C reduced/ min/mg protein; complex IV – µmoles of ferrocytochrome-C oxidized/min/mg protein.

Values are the mean ± SD; n = 6.

***p<0.001, **p<0.01, *p<0.05 significantly and ns>0.05 non-significantly different from old aged control (Bonferroni test)
II, 1.49, 1.47 and 1.41 fold increases in the activity for skeletal muscle complex II, 1.21, 1.41 and 1.30 fold increases in the activity for liver complex II, 1.40, 1.85 and 1.58 fold increases respectively in the activity for kidney complex II respectively in the case of *G. lucidum* protein bound polysaccharide 25, 50 mg/kg and LA 100 mg/kg treated groups than that of aged control.

Similarly, there was approximately 1.31, 1.26 and 3.30 fold increases in the activity for heart complex III, 1.04, 1.01 and 1.04 fold increases in the activity for brain complex III, 1.47, 1.72 and 1.34 fold increases in the activity for skeletal muscle complex III, 1.28, 1.40 and 1.33 fold increases in the activity for liver complex III, 0.91, 1.16 and 1.15 fold increases in the activity for kidney complex III respectively in the case of *G. lucidum* protein bound polysaccharide 25, 50 mg/kg and LA 100 mg/kg treated groups than that of aged control.

Also, there was approximately 1.37, 1.78 and 1.74 fold increases in the activity for heart complex IV, 1.49, 2.26 and 2.00 fold increases in the activity for brain complex IV, 1.28, 1.52 and 1.22 fold increases in the activity for skeletal muscle complex IV, 1.05, 1.20 and 1.73 fold increases in the activity for liver complex IV, 1.13, 1.36 and 1.23 fold increases in the activity for kidney complex IV respectively in the case of *G. lucidum* protein bound polysaccharide 25, 50 mg/kg and LA 100 mg/kg treated groups than that of aged control.

5.3.5. Effect of *G. lucidum* protein bound polysaccharides on the levels of mitochondrial reactive oxygen species (ROS) in the old aged rats

In the current study, the levels of mitochondrial ROS were significantly increased in the old aged control than that of young aged control group (Fig.5.2.A).

There were approximately 3.90, 2.34, 6.04, 1.82 and 2.06 fold increases in the heart, brain, skeletal muscle, liver and kidney mitochondrial ROS levels respectively in the old aged control than that of young aged control group of animals. The treatment of *G. lucidum* protein bound polysaccharides and LA were significantly effective to reduce the ROS levels in old aged rats (Fig.5.2.A).

There was approximately 1.18, 1.18 and 2.47 decreases in the heart mitochondrial ROS level, 1.03, 1.82 and 2.48 fold decreases in the brain mitochondrial ROS level, 0.89, 1.14 and 1.56 fold decreases in the skeletal muscle mitochondrial ROS level, 1.29, 1.35 and 1.48 fold decreases in the liver mitochondrial
Fig. 5.2.A. Effect of the protein bound polysaccharides from *G. lucidum* on the mitochondrial ROS level in the old aged rats

Values are the mean ± SD; n = 6.

***p<0.001, **p<0.01 significantly different from old aged control (Bonferroni test)

Fig. 5.2.B. Effect of the protein bound polysaccharides from *G. lucidum* on the mitochondrial membrane potential level in the old aged rats

Values are the mean ± SD; n = 6.

***p<0.001, **p<0.01, *p<0.05 significantly and nsp>0.05 non-significantly different from old aged control (Bonferroni test).
ROS level and 2.84, 3.38 and 2.04 fold decreases in the kidney mitochondrial ROS level respectively in the case of *G. lucidum* protein bound polysaccharides 25 and 50 mg/kg and LA 100 mg/kg treated old aged rats.

5.3.6. **Effect of *G. lucidum* protein bound polysaccharides on the levels of mitochondrial membrane potential (ΔΨmt) in old aged rats**

In the current study, the levels of ΔΨmt were significantly declined in the old aged control than that of young aged control group (Fig.5.2.B). There were approximately 4.10, 2.37, 2.89, 2.03 and 1.85 fold decreases in the heart, brain, skeletal muscle, liver and kidney ΔΨmt levels in the old aged control than that of young aged control group of animals.

The treatment of *G. lucidum* protein bound polysaccharides and LA were significantly effective to enhance the ΔΨmt levels in middle aged and old aged rats (Fig.5.2.B). There was approximately 2.53, 2.73 and 3.09 increases in the heart ΔΨmt level, 1.34, 1.58 and 1.41 fold increases in the brain ΔΨmt level, 2.27, 2.49 and 2.49 fold increases in the skeletal muscle ΔΨmt level, 1.38, 1.52 and 1.23 fold increases in the liver ΔΨmt level and 1.06, 1.17 and 1.19 fold increases in the kidney ΔΨmt level respectively in the case of *G. lucidum* protein bound polysaccharides 25 and 50 mg/kg and LA 100 mg/kg treated middle aged rats.

These values were calculated using the maximum values (mean + SD value) for aged control group and minimum values (mean - SD value) for the three treated groups so that assessments will be modest and the expectations in the enhanced activities are a minimum.

5.4. **DISCUSSION**

The results of the present study showed that the activities of antioxidant enzymes such as Mn SOD and GPx and TCA cycle enzymes such as ICDH, α-KGDH, SDH and MDH as well as the respiratory chain complexes such as complex I, II, III and IV and the levels of glutathione (GSH) and mitochondrial membrane potential (ΔΨmt) were lowered significantly and the levels of lipid peroxidation as well as the ROS were increased significantly in the heart, brain, skeletal muscle, liver and kidneys mitochondria of the aged rats in comparison to that of young rats.

In the *G. lucidum* polysaccharide treated groups, the activities of antioxidant enzymes, TCA enzymes, and respiratory chain complexes as well as the levels of
GSH and ΔΨ(mt) were enhanced significantly and the levels of lipid peroxidation and ROS were lowered significantly than that of aged control group. In the two doses 25 and 50 mg/kg, there was no dose dependency seen in any of the activities. The standard used in the study DL-α-Lipoic acid (100 mg/kg) (LA) was effective. The dose and selection of LA as a reference standard was based on a recent study that reported the improved mitochondrial supported bioenergetics and the antioxidant activity of when LA (100 mg/kg orally) was treated once daily for 28 days (Savitha and Pannerselvam 2006).

Our findings as regards the declined MnSOD, and GPx activities in the mitochondria of aged animals also agree with the previously reported data (Kumaran et al., 2003). As enzymes are proteins, the reduced protein synthesis during aging due to decreased ATP production (Miquel et al., 1980), may be the cause for the reduction in the activities of these enzymes. The decreased GPx activity observed in this study in aged animals can be attributed to the decreased GSH level. Increased production of ROS with concomitant decreases in antioxidant status, DNA modifications and a progressive decline of overall protein synthesis has been reported to accompany aging (Shigenaga et al., 1994; Richter, 1995).

The most abundant intracellular non-protein thiol molecule with predominant defense against ROS in post mitotic tissues is GSH (Dringen et al., 2000). It reacts directly with ROS and electrophilic metabolites, protects essential thiol groups from oxidation and serves as a substrate for GSH-related enzymes such as GPx and glutathione S-transferases (Townsend et al., 2003). In this study, we found that levels of GSH in the heart, brain, skeletal muscle, liver and kidney mitochondria were lowered significantly in the aged rats with respect to that of young. The declined GSH level during aging is in accordance with the recent reports of depleted GSH content during aging in post-mitotic tissues (Kumaran et al., 2004). The enhanced oxidative damage due to free radicals is supposed to be the main reason behind the reduced GSH level during aging. One of the major consequences of aging is the depletion of the important cellular antioxidant GSH, which results in oxidation of protein thiols and loss of activities of critical enzymes with active thiol groups. Thus maintenance of thiol in the cell is most important for the normal functioning of it.

In our experiments, we found a marked increase in the levels of lipid peroxidation in the mitochondria of the aged rats. Lipids act as vital substrates for
lipid peroxidation and the enhancement of lipid profile during aging may be the cause for increased lipid peroxidation. Also, an enhanced level of lipid peroxides in hyperlipidaemia was reported (Celine, 1992), suggesting a casual relationship between lipids and lipid peroxidation. Antioxidants are essential for preventing the cellular damage caused by free radicals and free radical-mediated lipid peroxidation. Therefore, the increased LPO observed in this study results from the declined activities of SOD and GPx as well as decreased level of GSH. By enhancing these antioxidants status, by the treatment of \textit{G. lucidum} protein bound polysaccharides was able to could protect from the LPO.

In our study, the estimation of ROS production in the mitochondria of different tissues revealed that during aging the level of ROS had increased significantly (p<0.001) and treatment with \textit{G. lucidum} protein bound polysaccharides had significantly reduced the generation of ROS in the aged rats with respect to the aged control, which clearly shows the significant effect of \textit{G. lucidum} protein bound polysaccharides in scavenging the ROS during aging. The primary factor governing mitochondrial ROS generation is the redox state of the respiratory chain (Tompkins et al., 2006). Thus; the increases in the electron transport chain activity of aged rats by the administration of \textit{G. lucidum} protein bound polysaccharides may have decreased the formation of ROS in the mitochondria.

The determination of the $\Delta \Psi_{\text{mt}}$ in the mitochondria of major organs such as heart, brain, muscle, liver and kidneys in the current study clearly shows that during aging there is significant decline in the $\Delta \Psi_{\text{mt}}$. This is in accordance with many other recent studies of various organs (Navarro and Boveris, 2010; Niemann et al., 2010). These results suggest in old age, the oxidative damage in membranes takes place and this is supported by a rise in membrane polarization (Savitha and Panneerselvam, 2006). Studies shows that the increased ROS level and decreased antioxidant enzymes may damage the expression of mitochondrial components, and damage to mitochondrial calcium metabolism and there by leads to defective assembly of respiratory complexes (Calderón-Cortés et al., 2008). Recent studies showed that the defects in oxidative phosphorylation are likely the cause of the drop in $\Delta \Psi_{\text{mt}}$ and leads to the reduced ATP synthesis (Hiona et al., 2010). Hence, these findings may be the underlying causes behind the decreased $\Delta \Psi_{\text{mt}}$. Thus, decrease in the ETC enzymes and resultant enhanced ROS generation and decreased antioxidant enzymes
have been suggested to be the reason behind the declined $\Delta \Psi_{\text{mt}}$ in old aged rats. On the other hand $G. \ lucidum$ protein bound polysaccharides and LA administration enhanced the $\Delta \Psi_{\text{mt}}$ level in the old aged rats, which is supposed to be by the enhancement in the ETC, TCA and antioxidant enzymes and scavenging of the ROS in the mitochondria.

In our studies, we found that the activities of TCA enzymes such as ICDH, $\alpha$-KGDH, SDH and MDH as well as that of respiratory chain complexes such as Complex I, II, III and IV in the heart, brain, skeletal muscle, liver and kidney mitochondria were decreased in the aged rats compared to that of young rats. But, the treatment of $G. \ lucidum$ protein bound polysaccharides and LA significantly enhanced their activities. Numerous studies that have examined the effects of aging on enzyme function of ETC complexes in the mitochondria of both rodents and humans have reported conflicting results possibly due to use of various markers for normalization of mitochondrial content and/or failure to include enzyme activities as well as the Complex I– Complex III and Complex II– III coupled activities (Sandhu and Kaur, 2003; Choksi and Papaconstantinou, 2008).

The deletions in the mtDNA are tissue specific and that this interferes with the functionality of the complexes of the mitochondrial ETC (Van Remmen et al. 2003). Consequently, the activities of the TCA enzymes and mitochondrial ETC complexes have been reported to decrease with age (Kumaran et al., 2004; Kumaran et al., 2005). Some protein complexes of the ETC may be more prone to oxidative damage. Complex I has been shown to be particularly sensitive to oxidative damage because of its Fe-S clusters (Van Remmen and Richardson 2001), and its activity has been reported to decrease with age (Mansouri et al. 2006).

Furthermore, because the age-dependent decline of the glutamate-malate supported respiration was found to be more evident than that of the succinate-supported respiration, it has been suggested that mutation(s) in the seven genes of NADH dehydrogenase (complex I) encoded by mtDNA may be involved in this age associated decline of respiratory function (Wei and Lee 2002). Complex IV activity (COX) has also been shown to decrease with age, (Kumaran et al. 2004).

Thus this study demonstrates that the protein bound polysaccharides isolated from $G. \ lucidum$ is capable to enhance the cellular energy status during aging by enhancing the antioxidant level, activities of TCA enzymes and respiratory chain
complexes and $\Delta \Psi_{mt}$ and by reducing the ROS level. The results thus reveal that *G. lucidum* protein bound polysaccharides is capable of preventing age related decline of antioxidant status in the heart, brain, skeletal muscle, liver and kidneys mitochondria suggesting the potential role of this mushroom in anti-aging.