ABSTRACT
Mitochondria perform many pivotal functions essential for homeostasis, and metabolism. The dysfunction of the mitochondria has been proposed to be the main reason behind many diseases including diabetes mellitus, heart disease and neurodegeneration, as well as in the aging process. Tissues with high metabolism seem to be particularly vulnerable to mitochondrial dysfunction. Therefore, protection of mitochondria from oxidative stress is of prime importance to all organisms. This can be done by enrichment of the mitochondria with antioxidants which has been employed successfully in recent in vitro and in vivo experiments.

_Ganoderma lucidum_ popularly known medicinal mushroom and has been traditionally used as folk medicine for the promotion of health in the Orient and to prevent many diseases. But till now there are no reports on the mitochondrial protective effects of this mushroom. Thus, in the current study, the aqueous ethanol (70%) extract, protein bound polysaccharides and total triterpenes isolated from the _G. lucidum_ occurring in South India have been evaluated for their effects on the mitochondrial dehydrogenases, electron transport chain complex enzymes, mitochondrial antioxidant status, reactive oxygen species (ROS) levels, and mitochondrial membrane potential (Δψmt) in the various organs of rats against oxidative stress in aging as well as in cardiac and hepatic damages induced by chemicals. Again, the _in vitro_ antioxidant activities, toxicity studies, and phytochemical analysis of the aqueous ethanol extract, protein bound polysaccharides and total triterpenes isolated from the _G. lucidum_ were also evaluated.

The 70% ethanol extract was prepared using a water bath, the protein bound polysaccharides by the ethanol precipitation method and the total triterpenes from the ethanol extract by column chromatography using silica gel by gradient elution with n-hexane and chloroform. The _in vitro_ antioxidant activity was evaluated by different methods such as FRAP assay, DPPH assay, lipid peroxidation inhibition assay, hydroxyl radical scavenging assay, superoxide radical scavenging assay and ABTS radical scavenging assay. The results showed that the aqueous ethanol extract, protein bound polysaccharides and total triterpenes from _G. lucidum_ possess significant and dose dependant antioxidant activity and radical scavenging ability. The extract of _G. lucidum_ and the protein bound polysaccharides possess higher antioxidant activities than that of
the total triterpenes, except in the case of lipid peroxidation in which the extract showed lesser activity than that of protein bound polysaccharides and the total triterpenes. The water insoluble nature of total triterpenes made them unable to perform the hydroxyl radical scavenging activity and SOD activity. BHA, the synthetic antioxidant was used as the positive standard.

The anti aging study clearly showed that the activities of major innate mitochondrial antioxidant enzymes such as manganese superoxide dismutase (MnSOD), glutathione peroxidase (GPx) and the levels of mitochondrial glutathione (GSH) were decreased significantly \( (p<0.001) \) and the level of lipid peroxidation (MDA) was found to be enhanced significantly \( (p<0.001) \) during aging. Similarly, the activities of mitochondrial dehydrogenases such as isocitrate dehydrogenases (ICDH), \( \alpha \)-ketoglutarate dehydrogenases (\( \alpha \)-KGDH), succinate dehydrogenases (SDH) and malate dehydrogenases (MDH) and electron transport chain (ETC) enzymes such as NADH:ubiquinone oxidoreductase (complex I), succinate-coenzyme Q reductase (complex II), Ubiquinol-cytochrome c oxidoreductase (complex III) and Cytochrome c oxidase (complex IV) and the levels of ROS and \( \Delta \psi_{mt} \) were found to be declined significantly \( (p<0.001) \) during aging in all the organs studied. The lowest activities were found in old aged rats, than the middle aged or young aged rats. Thus, the current study supports the age related decline in the antioxidant status and mitochondrial dehydrogenases and ETC enzymes. The administration of aqueous ethanol extract of \textit{G. lucidum} (50 and 250 mg/kg b.w) had significantly \( (p<0.05) \) enhanced the antioxidant status, activities of mitochondrial dehydrogenases and ETC enzymes and the levels of \( \Delta \psi_{mt} \) and reduced the ROS levels in the middle and old aged rats, but the effect was non-significant \( (p>0.05) \) in the young aged rats. When the effects were compared, \textit{G. lucidum} showed more effects in old aged rats than that of middle aged rats.

The protein bound polysaccharides and total triterpenes were isolated from the \textit{G. lucidum} and evaluated for their effects in old aged rats. The doses used for the study, 25 and 50 mg/kg for protein bound polysaccharides and 2.5 and 5 mg/kg for total triterpenes, were found to enhance the antioxidant status, activities of mitochondrial dehydrogenases and ETC enzymes and the levels of \( \Delta \psi_{mt} \) and reduced the ROS levels significantly
(p<0.05) in the old aged rats. The protein bound polysaccharides found to be more effective than that of total triterpenes in its effects.

Since the effect of extract in old aged rats was found to be better than that of the isolated components, its effect was evaluated against chemical induced oxidative stress and associated mitochondrial damage. The hepatic mitochondrial damage induced by acetaminophen (APAP) and carbon tetrachloride (CCl₄) and cardiac mitochondrial damage induced by isoproterenol (ISO) were used. The study clearly showed that after 3 hours of the single dose of APAP (3g/kg b.w, p.o) there was potent hepatic damage as evident by the significantly (p<0.001) enhanced activities of liver marker enzymes such as serum glutamic pyruvic transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT) and alkaline phosphatase (ALP). Again, the APAP challenge significantly (p<0.001) declined the mitochondrial antioxidant status, activities of mitochondrial dehydrogenases and ETC enzymes and the levels of Δψmt and enhanced the ROS levels. Similarly, after 24 hrs of the single dose of CCl₄ /paraffin oil (1:5, v/v, 1.5 ml/kg, i.p), the activities of SGPT, SGOT and ALP were significantly (p<0.001) enhanced suggesting the hepatic damage and it also resulted in the declined mitochondrial antioxidant status, activities of mitochondrial enzymes and the levels of Δψmt and enhanced the ROS levels than that of the normal rats. In the case of ISO, two subcutaneous injection of ISO (85 mg/kg) at an interval of 24 h for 2 days resulted in cardiac damage as evidenced by the significantly (p<0.001) enhanced activities of creatine kinase (CK) and lactate dehydrogenases (LDH) than that of normal group. In this model also cardiac damage was associated with mitochondrial dysfunction supported by declined the mitochondrial antioxidant status, activities of mitochondrial enzymes and levels of Δψmt and enhanced ROS levels than the normal rats.

In the three models aqueous ethanol extract of G. lucidum (100 and 250 mg/kg) was employed keeping α-tocopherol (100 mg/kg) as the standard. The observations clearly showed that G. lucidum could significantly protect against hepatic damage induced by APAP and CCl₄ as evidenced by the significantly decreased activities of SGPT, SGOT and ALP and significantly increased antioxidant status, activities of mitochondrial enzymes and the levels of Δψmt and reduced the ROS levels compared to
the APAP and CCl₄ control groups. Similarly, the ISO model clearly showed that *G. lucidum* significantly protected against ISO induced cardiac damage and mitochondrial dysfunction as there was significantly decreased activities of CK and LDH than that of ISO control group. There was significantly enhanced mitochondrial antioxidant status, activities of mitochondrial enzymes and the levels of Δψₘ and reduced ROS levels in the *G. lucidum* treated groups compared to the ISO control group. There was no dose dependant nature among the two doses of *G. lucidum* used. Similarly, α-tocopherol significantly protected against the APAP and CCl₄ induced hepatic and ISO induced cardiac mitochondrial damage.

The acute and sub acute toxicity studies of aqueous ethanol extract, the protein bound polysaccharides and total triterpenes of *G. lucidum* have been performed. The extract upto a dose of 2500 mg/kg body weight, total polysaccharide upto the dose of 500 mg/kg body weight and the total triterpene fraction upto the dose of 50 mg/kg body weight orally did not produce any external symptoms of toxicity or mortality. In subacute toxicity studies, treatment with the different concentration of the extract (50 and 250 mg/kg), protein bound polysaccharides (25 and 50 mg/kg) and total triterpenes (2.5 and 5 mg/kg) were not able to produce any statistically significant changes in the hematological or biochemical parameters compared to the normal group of animals. Results of these toxicity studies clearly indicated the non toxic nature of these extract and the isolated fractions/compounds. The histopathological examination of the liver and kidney tissues of the treated animals also supports this.

Further, the phytochemical evaluation of the aqueous ethanol extract of *G. lucidum* showed the presence of polysaccharides, triterpenes and proteins. The extract contains 8-9% carbohydrate and 40% protein contents. The HPTLC analysis showed that the extract contains 19 compounds corresponding to the 19 peaks. Similarly, the isolated polysaccharide from *G. lucidum* showed as a protein-bound polysaccharide with 60% carbohydrate and 18% protein. Similarly, the paper chromatography, TLC and HPLC analysis of the isolated polysaccharide also showed that the monosaccharide present is D-glucose. Similarly, the analysis of amino acid composition of protein bound polysaccharide by the TLC method showed that it contains aspartic acid, glutamic acid,
alanine and threonine. The HPTLC analysis of the total triterpenes fraction showed that there are 14 peaks.

The study concluded that aqueous ethanol extract of *G. lucidum* is capable of protecting the mitochondria against oxidative stress under different conditions including, aging, liver diseases and MI by maintaining the mitochondrial antioxidant status, activities of mitochondrial enzymes such as TCA and ETC complexes. The major chemical components of *G. lucidum* such as the protein bound polysaccharides and total triterpenes were also capable of protecting mitochondria against oxidative stress. There was no dose dependant effect observed and in comparison the protein bound polysaccharides showed more effects than that of total triterpenes. *G. lucidum* attenuate ROS by maintaining the mitochondrial antioxidant status and membrane potential thus enhances TCA cycle and ETC during oxidative stress. Thus, the present study revealed that this mushroom can be used as a safe and non toxic drug or food supplement which is effective to protect the mitochondria against oxidative stress and hence can be effective against various mitochondria mediated diseases.

**Key Words:** *Ganoderma lucidum*, Mushroom, Mitochondria, Antioxidants, Free radicals, Aging, TCA enzymes, respiratory chain complexes, Hepatoprotection, Carbon tetrachloride, Acetaminophen, Isoproterenol, Myocardial infarction, Triterpenes, Protein bound polysaccharide