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Bioactive extract of mycelia biomass of *Ganoderma lucidum* protects doxorubicin induced cardiomyopathy

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Cardiotoxicity induced by anticancer drug; doxorubicin (DOX) is a limiting factor for its prolonged use in chemotherapy. No effective drug is currently available to prevent DOX induced cardiomyopathy. *Ganoderma lucidum* is highly valued medicinal mushroom used in traditional medicine. Mycelia biomasses are considered as alternate sources of mushroom bioactive compounds. We examined the effect of bioactive extract of *G. lucidum* mycelia biomass (GLME) to prevent cardiotoxicity induced by DOX in rats using a cumulative dose 18 mg/kg body wt. GLME was administered to animals at doses of 250 and 500 mg/kg body wt. once daily for five days prior to DOX administration and continued for three more days. Animals were sacrificed 24 h after the last dose of drug. Activities of creatine kinase (CK), lactate dehydrogenase (LDH), endogenous antioxidant status, oxidative stress markers, electrocardiograph (ECG) and haematological parameters were evaluated. DOX administration drastically elevated CK, LDH, myocardial peroxidation and oxidative stress and significantly lowered endogenous antioxidant activity. GLME administration attenuated elevated levels of CK, LDH and oxidative stress and also ameliorated alterations in haematological and ECG parameters. Results revealed that bioactive extract of *G. lucidum* mycelia imparted significant protection against DOX induced cardiomyopathy suggesting the potential therapeutic significance of *G. lucidum* mycelia bioactives to alleviate DOX induced cardiomyopathy.

Keywords: Anticancer drug toxicity, Chemotherapy, Cardiac injury markers, Lingzhi, Mushroom, Oxidative stress, Reishi

Anthracycline antibiotic, doxorubicin (DOX) is one of the most extensively used drugs for cancer treatment. However, chronic use of this drug is delimited by its undesirable cardiotoxicity. The cardiotoxicity leading to heart failure (HF) is observed both in experimental animals and in humans[1]. DOX caused irreversible dilated cardiomyopathy and cognitive heart failure. The risk for delayed cardiac events such as cardiomyopathy and HF may develop at any time after DOX treatment, even after decade. DOX induced cardio toxicity arises in different ways; deleterious free radical generation by complexes with iron, redox cycling, mitochondrial dysfunction, DNA damage, alterations in protein and lipid, calcium overlord, etc[2].

Ganoderma lucidum (Fr.) P. Karst is a basidiomycetes belonging to Polyporaceae (Ganodermaceae) of Aphyllophorales, popularly known as Lingzhi in China and Reishi in Japan. Over millennia, Lingzhi considered as 'The mushroom of immortality' has been used in traditional Chinese medicine to treat several disease conditions and a tonic in promoting health and longevity, debility from prolonged illness, insomnia, anorexia and dizziness. More than 400 bioactive compounds have been reported from the fruiting body, mycelia and spores of *G. lucidum*[3]. The major bioactive components present are polysaccharides, and protein bound polysaccharides and triterpenes. In addition to these, the mushroom contains steroids, nucleic acids, glycoproteins, essential minerals, vitamins, amino acids, etc. The low-fat content and high proportion of polyunsaturated fatty acids accounts for its pharmacological and nutraceutical properties[4,5].

A number of *G. lucidum* products in the form of powders, dietary supplements and health drinks are produced from fruiting body, mycelia and spores. Modern pharmacological and clinical trials reveal that *G. lucidum* possessed significant protection against many disease conditions. For example, *G. lucidum* is the most effective antineoplastic agent among 58 basidiomycetes species tested[6]. The direct cytotoxic, antiangiogenesis properties and immunomodulatory

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activity by enhancing the cytokinine production by *G. lucidum* polysaccharides were well documented[7]. Investigations in our laboratory revealed that *G. lucidum* occurring in south India showed significant antioxidant, anti-inflammatory, anti-nociceptive, antimutagenic, anticarcinogenic, anticancer, hepatoprotective and nephroprotective activities[8-10]. Clinical trials have also been carried out using *G. lucidum* products[11].

Majority of mushroom products available today are developed from their fruiting bodies. Due to seasonal occurrence of mushroom fruiting bodies in the natural state and long duration of cultivation process submerged cultivation of mushroom mycelia biomass is considered as an alternative source to obtain consistent and safe mushroom components[3]. Recent investigations reveal that mushroom mycelia biomasses are excellent sources of bioactive metabolites[12]. Studies undertaken in our laboratory showed that *G. lucidum* mycelia biomass possessed significant antioxidant, antitumor and antimutagenic activities[8,9]. Although medicinal effects of Reishi – *G. lucidum* are well documented, studies on the therapeutic potential of its mycelia are inadequate. Here, we have made an attempt to evaluate the effect of the bioactive extract of mycelia biomass of *Ganoderma lucidum* (GLME) to ameliorate DOX induced myocardial complications.

Materials and Methods

Production of mycelia biomass and preparation of extract

Culture of *G. lucidum* was isolated and developed in our laboratory from fruiting bodies collected from *Caesalpinia coriaria*wild trees growing in Thrissur District, Kerala, India by standard microbiological methods. The isolate was maintained on potato–dextrose agar medium (Fig. 1) at 25-26°C with frequent transfers at every 21 days. For the production of mycelia, the isolate was grown in potato broth liquid medium containing (g/L) dextrose (20), yeast extract (1), KH_2PO_4 (5), $MgSO_4 .7 H_2O$ (2.5). Potato broth was prepared by boiling 200 g peeled potato in one litre distilled water for half an hour, filtered through several layers of cheese cloth and the final volume was made up to one litre. One hundred ml medium was poured to a number of 500 mL Erlenmeyer flasks, inoculated with actively growing cultures of the isolate and grown at 25-26°C on a shaker (Scigenics-Orbitech) at 180 rpm for 20 days. After the incubation period, mycelia biomass was recovered from the media by filtering through Whatman No. 1 filter paper, washed with distilled water several times and dried at 45-50°C for 48 h.

One hundred gram sample of powered mycelia biomass was defatted with petroleum ether using Soxhlet apparatus. The defatted material was then extracted with 70% aqueous ethanol for 12 h. The extract was filtered through Whatman No. 1 filter paper. The solvent was evaporated at 40°C using a rotary vacuum evaporator and finally lyophilized. The residue thus obtained was named alcoholic extract (GLME). The yield of GLME was 9.92%.

Experiment design

Doxorubicin hydrochloride (Doxowin), Wintac Pvt. Ltd. Bengaluru, India was procured from the local market. All other chemicals and reagents used in the experiment were of analytical grade.

Male Wistar rats weighing 220±20 g were purchased from Small Animal Breeding Centre, Agricultural University, Mannuthy, Thrissur, Kerala, India. Animals were kept for a week under standard environmental conditions with free access to feed and water *ad libitum*. Animal experiments were carried out following the guidelines of CPCEA (Government of India) and with approval of the Institutional Animal Ethics Committee (IAEC), Amala Cancer Research Centre, Thrissur, Kerala, India. (No. 149/1999/CPCSEA). The animals were divided into 5 groups. Six animals in each group were maintained except for DOX alone treated control group where number of animals used were higher in number (n = 12). High mortality rate (50%) in DOX alone treated groups was observed in the pilot study. Group I treated with distilled water was kept as

Fig 1. — Culture of *Ganoderma lucidum* in potato–dextrose agar medium.

normal. Group II treated with DOX (ip) was kept as control. Groups III and IV were administered with GLME at concentrations of 250 and 500 mg/kg body wt., respectively. Group V administered with DL-α-Lipoic acid (100 mg/kg body wt.) dissolved in 0.5% NaOH) was taken as standard. The DOX was administrated once daily (6 mg/kg body wt., i.p. 0.75 mL for 3 alternative days), (cumulative dose of 18 mg/kg body wt.) to groups II, III, IV and V. The extracts were given once daily for five days prior to DOX injection to groups III and IV and continued along with DOX injection for three more days .The animals were sacrificed 24 h after last dose of treatments. Heart was immediately excised, washed in saline weighed and kept at −70°C for biochemical analyses. Serum samples were used for the analysis of cardiac injury marker enzymes such as creatine kinase (CK) and lactate dehydrogenase (LDH).

Assays for *in vitro* and *in vivo* antioxidant activity

Antioxidant capacity of GLME was evaluated by commercially available and stable free radical DPPH (2,2-diphenyl-1-picryl-hydrazyl), soluble in methanol[13]. DPPH (a stable free radical commonly used to measure the antioxidant activity of compounds for their electron or hydrogen donating efficiency) radical reducing capacity of GLME at different concentrations was read by a spectro-photometer at 515 nm. Ferric reducing antioxidant activity of GLME was assayed by the method of Benzie & Strain[14], ABTS (2,2'-azino-bis(3-ethyl-benzthiazoline-6-sulphonic acid) radical scavenging activity was assayed by the method described by Long & Halliwell[15] and Superoxide anion scavenging activity was assayed by the method of Mc Cord & Fridovich[16]. Superoxide radical generated from the photo reduction of riboflavin was detected by NBT (Nitro blue tetrazolium) reduction.

The cardiac tissue was homogenized in 50 mmol phosphate buffer (pH7.0) to give 10% tissue homogenate (w/v) and used for the determination of reduced glutathione (GSH) by the method of Moron *et al.*[17]. Lipid peroxidation activity was determined by the assay of thiobarbituric acid reacting substance (TBARS) by the method of Ohkawa *et al.*[18]. The concentration of malondialdehyde (MDA) formed in the sample was calculated from the graph plotted using 1'1'3'3'-tetramethoxypropane as standard and expressed as n mol of MDA formed/mg protein. Tissue supernatant was used for the determination of the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) by the method of McCord & Fridovich[16], Hafeman *et al.*[19] and Beers & Sizer[20], respectively.

Assay for oxidative stress markers in experimental animals

Serum protein carbonyls (PCO) were determined by spectrophotometric assay method of Levine *et al.*[21] and the carbonyl content was calculated using the molar absorption coefficient of 22,000 $M^{-1}cm^{-1}$. The advanced oxidation protein products (AOPP) were assayed by a modified method of Kayali *et al.*[22]. Serum (0.2 mL) was mixed with 1 mL of Phosphate buffer and kept for 2 min at 27-28°C. Potassium iodide 0.1 mL (1.6 M) was added to the reaction mixture. The yellow coloured tri iodide formed after the addition of 0.2 mL of glacial acetic acid was read immediately using a double beam UV Visible spectrophotometer (Systronic India) at 340 nm against reagent blank. Concentration of AOPP was calculated using the extinction coefficient of 26 $mM^{-1}cm^{-1}$.

Assays for cardiac injury marker enzymes in experimental animals

Cardiac injury marker enzymes such as creatine kinase (CK) and lactate dehydrogenase (LDH) in the serum samples were analyzed by following the procedure provided by the manufacturer of the diagnostic kit (Agappe Diagnostic Pvt. Ltd, India).

Electrocardiography (ECG) analysis

ECG of experimental animals was recorded at the end of the treatment after the last dosing. All animals were anesthetized lightly with ether and needle electrodes were inserted under the skin for the limb lead at position II and ECG parameters ST, QT interval, QRS complex and heart rate were measured using the electrocardiograph (MAC 400, GE Medical System Information Technologies, Inc, USA).

Analysis of haematological parameter

Haematological components, such as HB, RBC, Platelet and WBC were analysed using automated counting chamber in order to evaluate the changes in the profile.

Examination of Histopathology of cardiac tissue

A portion of cardiac tissue of all treatments was fixed in 10% formalin and they were embedded in paraffin and 6µ microtone sections were prepared from each piece. The sections were stained with hematoxylin-eosin and examined under a light microscope.

Change in Body and organ weight of experimental animals

The weight of each experimental animal was recorded on first day and the last day of the experiment. The mean body weights were calculated. The weight of the heart excised from the animals was recorded and expressed in relation to final body weight of the animal

Chemical analysis of GLME for major chemical components

The extract (GLME) was treated with anthrone reagent and also with phenol-sulphuric acid reagent to find out the presence of polysaccharide component[23]. The protein component of GLME was determined by Bradford reagent[24].Chemical constituent profile of GLME was identified by high performance thin chromatography (HPTLC). The sample was dissolved in a mixture of methanol-water (98:2 and 4 µL was applied on 6×10 cm silica gel 60F254 thin layer chromatography plate (E. Merck, Germany) by a Linomat 5 sample applicator fitted with Camag microsyringe in nitrogen flow. The plate was developed in a twin trough glass chamber using chloroform-methanol-water (30:4:1) mobile solvent phase. The plate after solvent run was derivatized with anisaldehyde sulphuric acid reagent and heated at 110°C for 10 min and then scanned at 580 nm by a TLC scanner 3 densitometer equipped with WinCats software.

Statistical analysis

All data are presented as mean ± standard deviation. The mean values were analyzed using one way analysis of variance (ANOVA) (using the Graph Pad Instat software package). The significant differences between the groups were further analyzed by Bonferroni's t-test and *P* values less than 0.05 were considered as significant.

Results

There was marked changes in general appearance of DOX treated and GLME treated animals. The administration of DOX alone treated experimental animals caused marked weight loss and developed reddishness around the eyes and nose. They had alopecia, their fur became scruffy, back bone became sessile and brittle and had soft watery faces. These conditions were more severe at the end of study period indicating the severity of DOX toxicity compared to normal animals.

DOX alone treated animals showed high mortality before termination of the experiment. However, treatment with GLME was able to impart marked relief from the toxic manifestations. Body weight changes showed that all the treated groups compared to DOX (control) prevented loss of body (Fig. 2A) and heart weight (Fig. 2B).

***In vitro* and *in vivo* antioxidant activities of GLME**

GLME showed significant (69%) free radical scavenging activity at 100 µg/mL concentration. This was evident from DPPH radical scavenging activity. DPPH scavenged the free radicals by suppressing the chain initiation or by inhibiting the chain propagation reactions. An inhibition of 76% free radical scavenging activity was observed in both ABTS and superoxide assays of GLME. Ferric reducing assay of GLME showed significantly high activity (84% reduction of ferric ions). Free radical scavenging activity summarizes the potential role of GLME in preventing oxidative stress (Fig. 3).

DOX treatment caused drastic depletion of cardiac antioxidant enzymes (Table 1). The activities of the antioxidants such as SOD, GPx and CAT were significantly increased in GLME treated group of animals compared to DOX alone treated control. Treatment with 250 mg/kg body wt. of GLME did not alter the drop in the enzyme activity caused by DOX treatment. While the activities were restored to normal level by GLME (500 mg/kg body wt.) and α-lipoic acid treated groups.

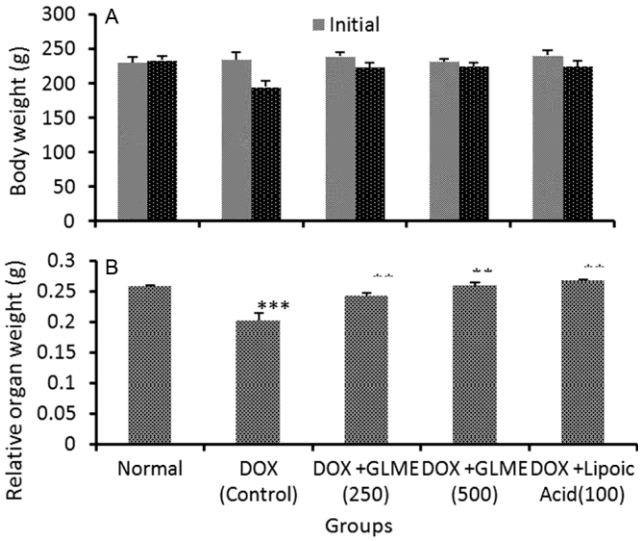

Fig 2. — Effect of GLME on (A) body weight; and (B) relative organ weight of animals treated with DOX. [Body weight changes during the experiment in DOX challenged group of rats. Initial and final body weight is recorded. Values are the mean ± S.D; n=6 animals. ****P* <0.001 significantly different from DOX control (Bonferroni test). DOX, Doxorubicin; and GLME, *Ganoderma lucidum* mycelia biomass extract]

Table 1 — Effect of GLME on DOX induced alteration in the activity of antioxidant enzymes in the myocardium

Groups	Treatments (mg/kg body wt.)	SOD (U/mg protein)	GP_X (U/mg protein)	Catalase (U/mg protein)
Normal	-	4.91±0.47	36.21±6.39	2.89±0.62
Doxorubicin (Control)	Cumulative dose of 18 mg/kg body wt. (3 doses of 6 mg/kg body wt.)	1.84±0.52***	13.38±4.78***	1.09±0.36[c]
G. *lucidum*	250+DOX	1.23±0.19[ns]	18.53±1.89[ns]	1.6±0.16*
	500+DOX	3.6±0.51***	27.91±4.1*	1.72±0.27**
DL-α-Lipoic acid	100+DOX	5.34±0.85***	28.99±6.8[ns]	2.2±0.42***

[Values are the mean±SD; n=6 animals. ***P <0.001,**P <0.01, *P <0.05[ns] and P >0.05 significantly different from Doxorubicin control (Bonferroni test). SOD, Superoxide dismutase; GPx, Glutathione peroxidise]

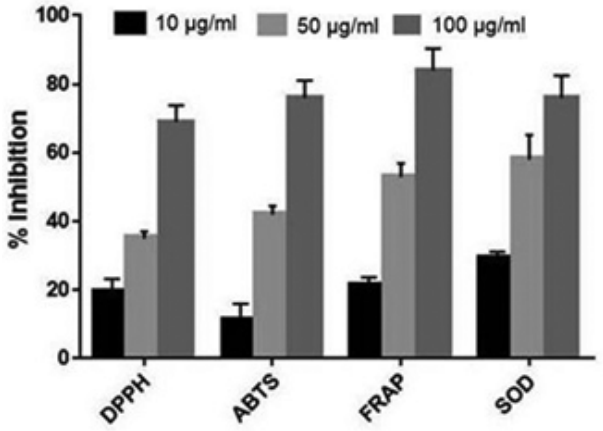

Fig 3. — *In vitro* antioxidant activities of GLME. [Data represent the percentage inhibition by different concentrations of GLME. Values are the mean ± SD; n=3]

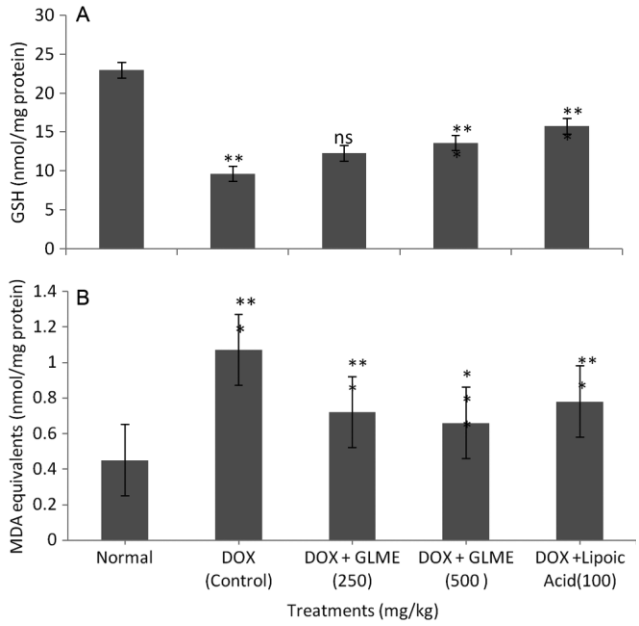

Fig 4. — Effect of GLME on the levels of (A) GSH activity; and (B) Lipid peroxidation. [Values are the mean ± SD, n=6 animals. ***P <0.001, *P <0.05[ns] significantly different from DOX control (Bonferroni test). DOX, Doxorubicin; and GLME, *G. lucidum* mycelia biomass extract]

Table 2 — Effect of GLME on the myocardial injury marker Enzymes: Creatine kinase (CK) and Lactate dehydrogenase (LDH)

Groups	Treatments (mg/kg body wt.)	CK (U/L)	LDH (U/L)
Normal	-	98.59±14.89	280.0±16.67
Doxorubicin (Control)	Cumulative dose of 18 mg, and 3 doses of 6 mg/kg body wt.)	598.9±72.46***	1683.3±198.82[c]
G. *lucidum*	250+DOX	450.47±20.67**	598.45±155.69***
	500+DOX	319.59±16.86***	412.77±16.99***
DL-α-Lipoic acid	100+DOX	270.12±54.53***	423.84±63.70***

[Values are the mean±SD, (n=6); ***P <0.001 and **P <0.01 significantly different from Doxorubicin control Group (Bonferroni test). CK, Creatine Kinase; and LDH, Lactate Dehydrogenase]

DOX administration caused marked decline in GSH level in the cardiac tissue. The level of GSH was enhanced by the administration of GLME at a concentration of (500 mg/kg body wt.). Administration of lower concentration of GLME did not show any significant increase in GSH level (Fig. 4A). DOX treatment up regulated lipid peroxidation in cardiac tissue. This was evident from increased levels of MDA in the DOX treated group of animals. Administration of GLME consistently reduced the levels of lipid peroxidation and thereby protecting the myocardium from membrane damage (Fig. 4B).

Effect of GLME on myocardial injury markers

Myocardial injury marker enzymes, such as creatine kinase (CK) and lactate dehydrogenase (LDH) activities were drastically elevated by DOX treatment compared to the normal group of animals. A significant elevation in the activities of CK and LDH was observed (Table 2). There was 4.27 and 5.11 fold decline in the activities of CK and LDH in animals treated with GLME (500 mg /kg body wt.), respectively. However, treatment with 250 mg/kg GLME was not effective to lower activity of myocardial injury markers.

Effect of GLME on Oxidative stress markers

Administration of DOX drastically elevated oxidative stress in myocardium. Advanced oxidation protein products (AOPP) and Protein carbonyl (PCO) levels were significantly increased in the DOX treated group of animals. Administration of GLME imparted a notable decrease in AOPP (Fig. 5A) and PCO (Fig. 5B).

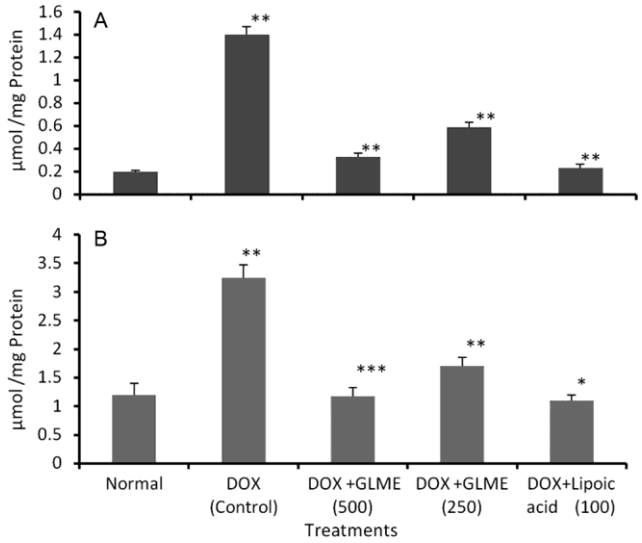

Fig 5 — Effect of GLME on protein oxidation markers. (A) Advanced oxidation protein product (AOPP); and (B) Protein Carbonyls (PCO). [Values are the mean ± S.D n=6 animals. ****P*<0.001 significantly different from DOX control (Bonferroni test). DOX, Doxorubicin; and GLME, *Ganoderma lucidum* mycelia biomass extract]

Effect of GLME on haematology profile

Administration of DOX at a cumulative dose of 18 mg/kg body wt. caused significant undesirable changes in haematology profile. Treatment with GLME (500 mg/kg body wt.) was able to alleviate these changes. This was evident in group of animals treated with GLME (Table 3). However, GLME at its lower concentrations did not show any marked change in RBC count compared to DOX treated group. Haemoglobin count was higher in DOX alone treated group of animals than control.

Effect GLME on histopathology of cardiac tissue

Histopathology observation of cardiac tissue of normal animals and animals that were administered with α-lipoic acid (100 mg/kg body wt.) showed normal structure and morphology. Cardiac tissue of animals that were treated with DOX showed myocardial hypertrophy coupled with eosinophlic cytoplasm and haemorrhage lesions. Heart tissue of animals that were administered with GLME largely ameliorated hypertrophy and haemorrhage lesions (Fig. 6).

Effect of GLME on electrocardiography (ECG) parameters

Doxorubicin treatment showed significant changes in electrocardiogram (ECG). DOX treatment caused significant prolongation (P <0.05) of QT interval, QRS complex and heart rate (HR) while no significant effect on ST segment and PR interval was

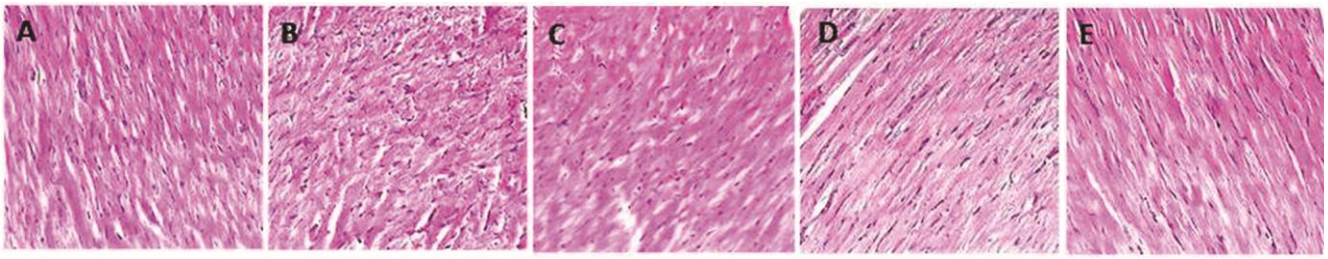

Fig 6 — Effect of GLME against DOX induced cardiotoxicity: Histopathological observation of cardiac tissue sections stained with H&E. (A) Gr. I, Normal; (B) Gr. II, DOX (3 doses of 6 mg/kg, cumulative dose of 18 mg/kg); (C & D) Gr. III & IV, GLME 250 and 500 mg/kg+DOX, respectively; and (E) Gr. V, DL-α-Lipoic acid 100 mg/kg + DOX. [Magnification 10X. DOX, Doxorubicin; and GLME, *Ganoderma lucidum* mycelia biomass extract]

Table 3 — Effect of GLME on haematological parameters of DOX treated animals

Groups	Treatments (mg/kg body wt.)	Hb (g/dL)	RBC ($\times10^6$)	Platelets ($\times10^6$)	WBC (mm^3)
Normal	-	12.50±0.95	5.43±0.99	5.29±1.03	6050±22
Doxorubicin (Control)	Cumulative dose of 18 mg/kg body wt. (3 doses of 6 mg/kg body wt.)	17.54±1.21***	3.87 ±0.80***	4.55 ±0.87***	2369±274^c
G. *lucidum*	250+DOX	14.17±0.73***	3.9±0.46^{ns}	4.55±0.24*	3742±461***
	500+DOX	13.04±0.28***	4.31±0.29**	5.6±0.35***	5759±297***
DL-α-Lipoic acid	100+DOX	14.17±1.86***	3.69 ±1.78**	4.9±0.99***	4782±377***

[Values are the mean±SD; n=6 animals. ****P* <0.001, ***P* <0.01, **P* <0.05^{ns} and *P* >0.05 significantly different from Doxorubicin control Group (Bonferroni test). <*P* 0.001 significantly different from normal. Hb, Haemoglobin; RBC, Red blood cells; and WBC, White blood cells]

Table 4 — Effect of GLME on ECG parameters of animals treated with Doxorubicin

Groups	Treatments (mg/kg body wt.)	HR (b/min)	QT (ms)	ST (ms)	QRS (sec)	PR (sec)
Normal	-	432±20.83	70±28	27±6	0.040±0.007	0.03±0.009
Doxorubicin (Control)	Cumulative dose of 18 mg/kg body wt. (3 doses of 6 mg/kg body wt.)	341±38^{b}	121±15.9^{c}	39±4^{a}	0.069±0.02^{b}	0.03±0.001^{ns}
G. lucidum	250+DOX	366±39.74^{ns}	100± 15**	33±5^{ns}	0.046±0.01**	0.024±0.002^{ns}
	500+DOX	396±15.16*	86±16***	27±8^{ns}	0.046±0.003**	0.024±0.005^{ns}
DL-α-Lipoic acid	100+DOX	390 ±30.10^{ns}	120±19^{ns}	28±8^{ns}	0.046±0.02***	0.022±0.003^{ns}

[Values are the mean ± SD; n=6 animals. ***P <0.001,**P <0.01, *P <0.05^{ns} and P >0.05 significantly different from Doxorubicin control Group (Bonferroni test). ^{c}P <0.001, ^{b}P <0.01 and ^{a}P <0.05 significantly different from normal, HR, Heart rate; QT,QT intervals; ST, ST segments; QRS, QRS complex; and PR, PR interval]

observed compared to other treated groups (Table 4).GLME treatment at 500 mg/kg body wt. significantly ameliorated the changes in QT interval and QRS complex.

Mycochemical profile of GLME

Anthrone reagent test showed the presence of polysaccharide component in GLME and phenol-sulphuric acid reaction confirmed this observation. The protein component in GLME was detected by Bradford reagent. The chemical analysis revealed that GLME contained 13% polysaccharides and 49% protein. Thus, GLME was found to be a protein bound polysaccharide. HPTLC analysis showed terpenes as the other major component of GLME. This was evident from the positive reaction of the spots with anisaldehyde-sulphuric spray reagent. HPTLC figure print of GLME is presented in Fig. 7.

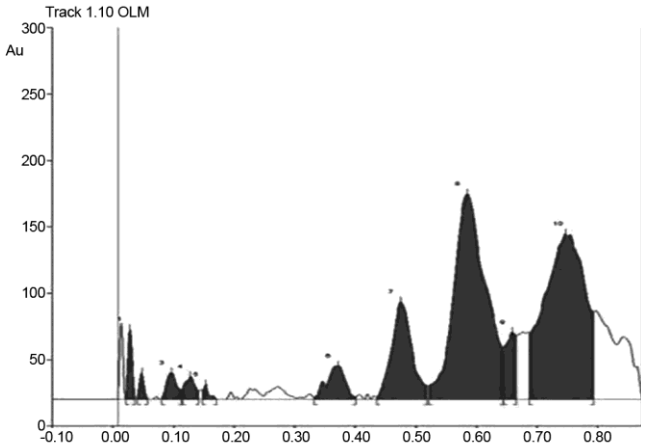

Fig 7. — HPTLC finger print of *Ganoderma lucidum* mycelia biomass extract (GLME).

Discussion

Chemotherapy is the most acclaimed treatment option for cancer. DOX is an extensievely used chemotherapy drug for a large number of cancer patients world wide. However, the cardiotoxicity caused by DOX in high doses delimit its therapeutic success. Several mechanisms are known to cause the DOX induced cardiotoxicity. Among these, oxidative stress based intra myocardial free radical generation gained wide acceptance. DOX causes the formation of ROS by electron exchange between quinine moiety of DOX with other oxygen molecules or with electron donors present in the cellular environment[25]. This noxious cycle generates excessive formation of free radicals and thereby causing oxidative damage to proteins and lipids. As in patients, DOX causes myelosupression and ECG variations in experimental animals[26-29].

The present experimental study reveals the significant protective effects of cultured mycelia biomasss extract of *G. lucidum* (GLME) on DOX induced complications of cardiomyopathy. Bioactive extracts of mushrooms are known to possess a large number of therapeutic effects[3].However, the mycelia biomass of most of therapeutically useful mushrooms is not exploited adequately for product development. The mushroom mycelia production by submerged fermentation provides a scalable method to obtain bioactive compounds. In the present study, we observed a marked elevation of serum as creatine kinase (CK) and lactate dehydrogenase (LDH) in DOX alone administered control group of animals. Treatment with mycelia extract (GLME) restored the activities of serum CK and LDH to normal level. This reveals the cardioprotective effect of GLME against the elevation of activity of cardiac injury marker enzymes.

Heart tissues are highly susceptible to free radical attack due to suppressed activities of antioxidant enzymes compared to other tissues[30]. The results show that GLME imparted significant enhancement of both *in vitro* and *in vivo* antioxidant systems. The finding suggests the beneficial effect of GLME to ameliorate oxidative stress. GLME showed significant free radical scavenging activity in *in vitro* antioxidant assays. The metal iron chelating property of extract is evident from FRAP radical scavenging assay. Hydrogen donating ability of extract is obvious from

DPPH assay. Superoxide anion is a potent radical in cellular system which reduces molecular oxygen. Superoxide radical scavenging assays depict the role of GLME in eliminating superoxide radicals. The ability of GLME to scavenge ABT radical demonstrates its oxygen radical absorbance capacity.

In vivo assay findings provide strong correlation with *in vitro* antioxidant enzymes activities. Administration of DOX at a cumulative dose of 18 mg/kg depleted the enzymatic antioxidants, such as SOD, GPx and CAT present in the cardiomyocytes. The activities of these enzymes are augmented by the administration of GLME at a dose of 500 mg/kg body wt. This suggests the strong antioxidant property of cultured mycelia biomass extract.

GSH, a thiol containing tripeptide acts as a direct antioxidant in cellular systems. The mechanism was associated to decomposing superoxide radicals, hydrogen peroxide and singlet oxygen. The depletion of GSH is a direct indication of peroxidation of lipids[31]. Free radicals mainly attack the unsaturated fatty acids present in the membrane leading to deterioration of biological membrane. DOX treatment depleted the GSH stores and up regulated the peroxidation. Elevation of TBARS was observed in DOX treated group resulting in depletion of GSH level. GLME was able to scavenge peroxide radicals formed in the cardiomyocytes and thereby restoring the normal membrane fluidity. This effect is contributed by high polyunsaturated fatty acid (PUFA) content present in cultured mycelia biomass. This observation is consistent with the earlier report by investigators[32].

Protein oxidation is the covalent modification of a protein either directly by ROS or indirectly by the by-products of oxidative stress. Protein carbonyl derivatives are the most common protein oxidative products in biological systems. Free radicals may react with amino acids, sulfhydryl groups of protein and there by interfere electron transferring between amino acids. The damage caused by ROS upon proteins found mainly on several amino acid residues including cysteine, proline, histidine, methionine and arginine[33], which eventually lead to the generation of PCO and AOPP.The results of the present study show the occurrence of protein oxidation is very high in DOX treated group. GLME even at its low concentration of 250 mg/kg body wt. was capable to scavenge the oxidants and protect the proteins from oxidation. This indicates the significant beneficial effect of *G. lucidum* mycelia biomass extract against cardiomyopathy.

Previous experimental studies demonstrated that the administration of DOX resulted in myelosupressents and thereby affecting erythropoiesis[26]. The results of present study also show that DOX treatment caused severe thrombocytopenia and leukopenia. The haemoglobin levels are found very high in the DOX control group. However, mechanism of this effect is obscure. In clinical trials, DOX has been reported to show variations in ECG parameters in cumulative doses[27]. A significant increase in the heart rate, as well as significant increase in QT interval prolongation and widening of QRS complex in the DOX alone treated animals compared to GLME treated groups was observed in the present study. QT prolongation is an indication of doxorubicin induced abnormal ventricular depolarization and repolarisation. DOX treatment is reported to present marked bradycardia[34]. Our observation also supports this finding .The present study has shown that administration GLME imparts significant protection against DOX induced altered ECG parameters. The results, thus reveal the myocardial protection by the cultured mycelia biomass extract of *G. lucidum.*

DOX induced cardiotoxicity has attracted great attention in clinical practice because it is one of the impediments in the use of this versatile anticancer drug. However, amelioration of the cardiotoxicity of this drug might significantly enhance its therapeutic value in clinical settings. Hence, a therapeutic agent capable to prevent the DOX induced cardiotoxicity has great clinical importance.

Conclusion

In this study we investigated the protective effect of bioactive extract of *Ganoderma lucidum* mycelia biomass against doxorubicin (DOX) induced cardiotoxicity in rats. Results of current investigation revealed that aqueous alcoholic extract of the mycelia biomass showed profound protective effect. Mycelia of *Ganoderma* contain a large number of bioactive compounds such as polysaccharides, triterpenes and polyphenols. The observed bioactivity might be attributed to these secondary metabolites. The finding suggests the potential therapeutic use of bioactive extract of cultured mycelia biomass of *G. lucidum.*

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Conflict of interest

Authors declare no conflict of interests.

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