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Hepatoprotective Potential of Aqueous Extract of *Ganoderma lucidum* Against Carbon Tetrachloride Intoxication in Rats

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ABSTRACT

The present study was undertaken to investigate the hepatoprotective effect and antioxidant property of aqueous extract from Ganoderma lucidum (GLE) on carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats. Treatment with 200 mg/kg body weight of GLE for six consecutive weeks prior to the administration of CCl_4 (1.5 ml/kg in olive oil, 20%) significantly prevented the increased serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, gamma glutamyltranspeptidase (γ-GT) and serum molodialdehyde (MDA) levels. The extract also induced the up-regulation of hepatic antioxidant enzymes namely superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GSSG-Rx) activities. In addition, pretreatment with GLE prior to CCl_4 intoxication drastically reduced incidences of ballooning degeneration, necrosis and portal traditis which are direct consequence of CCl_4-induced damage. These results attest to the hepatoprotective and antioxidant potentials of aqueous extract of G. lucidum in rats.

Key words: *Ganoderma lucidum*, toxicant, hepatoprotection, antioxidants, oxidative stress

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INTRODUCTION

Carbon tetrachloride is an extensively used xenobiotic to induced lipid peroxidation and hepatotoxicity. Carbon tetrachloride is metabolized by cytochrome P4502E1 (CYP2E1) to trichloromethyl radical ($CCl_3^{\cdot}$), which would initiate free radical-mediated lipid peroxidation

leading to accumulation of lipid –derived oxidative products that cause liver injury [1,2]. The polyunsaturated fatty acids (PUFAs) components of the membrane are particularly susceptible to free radical – initiated peroxidation [3]. The PUFAs in phospholipids of the endoplasmic reticulum were reported to decrease following in vivo CCl_4 administration [4].

Previous reports from a number of researchers have demonstrated that antioxidants would prevent CCl_4 toxicity in particular hepatotoxicity, by inhibiting lipid peroxidatio [5], suppressing serum alanine aminotransferase (ALT) and aspartate aminotransferase activities [6], and increasing the activities of antioxidant enzymes [7].

Mushrooms represent a major and as yet, largely untapped source of potent pharmaceutical products. Out of the approximately 10,000 known species of mushrooms, 2,000 are safe for people's health and about 300 of them possess medicinal properties [8]. *Ganoderma lucidum* (Fr.) P. Karst., which is commonly known as Reishi or Ling Zhi has been used in folk-medicine in China and Japan for 2000 years for a wide range of ailments. In Chinese folklore, fruiting bodies of *G. lucidum* have been regarded as a panacea for all types of diseases. This is probably due to the demonstrated efficacy of it as a popular remedy to treat several disease conditions, namely chronic hepatitis, arthritis, hypertension, hyperlipidemia, insomia, bronchitis, neoplasia, asthma, gastric ulcer, athreslerosis, dibetes, debility due to prolonged illness, etc [9]. These extensive range of traditional medical treatment have been scientifically substantiated by modern scientific standards. *Ganoderma* species were classified into several types, including black, red, purple, light black, yellow and white. Each type of *Ganoderma* has its own characteristic biological properties. The commonly used medicinal *Ganoderma* include, *G. lucidum*, *G. tsugae Murrill*, *G. capense Junhua* and *Ronglan* and *G. applanatum* (Pers.) Pat. Some of the physiological effects and distinctive properties of *Ganoderma* are strain dependent [10]. Polysaccharides and triterpenes of *Ganoderma* are the major source of its pharmacological active constituents.
Species of the genus, *Ganoderma* have been reported to occur throughout the world. *Ganoderma lucidum* has been found to occur widely in Nigeria, particularly in the rain forest zone. No attempt has been made to evaluate the medicinal properties of *Ganoderma* mushrooms occurring in Nigeria. Investigations carried out in our laboratory revealed that *Ganoderma lucidum* possessed antidiabetic, antilipidemic and cardioprotective activities. The present study was undertaken to investigate the hepatoprotective and antioxidant properties of wild species of *G. lucidum* found in Nigeria.

MATERIALS AND METHODS

Chemicals: All chemicals used were of analytical grade and were products of BDH Chemicals Ltd, Poole, England unless otherwise stated. Biochemical assay kits were obtained from Randox Laboratory Ltd, UK.

Plant material: Fruiting bodies of *G. lucidum* were collected from around Iwaro Oka Akoko in the month of June 2009. The plant material was identified and authenticated by Dr. Obembe of Plant Science Department, Adekunle Ajasin University, Akungba.

Extract preparation: The fruiting bodies were dried under shade and ground to powder using mechanical grinder. About 500 g of the powder was macerated in 2.5 L distilled water at room temperature for 24 h. It was then filtered using Whatmann filter paper (number 1) and filtrate evaporated to dryness in water bath at 60^0 C. A brownish residue about 46.3 g (9.26% yields) was obtained and kept in airtight container at 40^0 C until used.

Animal treatment: 40 adult male Wistar rats weighing 63 – 78 g obtained from the animal laboratory of the Department of Biochemistry, University of Ilorin, Ilorin were used for the study. The animals were housed individually in stainless steel cages with raised wire floor at 25 - 30^0 C and 12 h light/darkness condition. Maintenance and treatment of animals were in accordance with the principles of the "Guide for care and use of laboratory animals in research and teaching" prepared by the National Academy of Sciences and published by the National Institute of Health (NIH) publication 86 – 23 revised in 1985. They were fed commercial rat's feed (Bendel Flour Mill Ltd, Ewu) and water *ad libitum* for the initial period of two weeks of acclimatization and throughout the experiment. Rats were randomly distributed into four groups of ten (10) animals each and treated as follows: (1) Group I, Normal control (NC): rats i.p. injected with 10 ml/kg body weight sterile saline solution serving as normal controls; (2) Group II, GLE: rats i.p. treated with 200 mg/kg aqueous extract of *G. lucidum* once daily for five weeks; (3) Group III, CTC: rats intoxicated with 1.5 ml CCl_4 (CCl_4/olive oil 1:5v/v) 3 times in a week for five weeks; (4) Group IV, GLE + CTC: rats i.p. treated with 200 mg/kg aqueous extract of *G. lucidum* once daily for five weeks (1 h before each CCl_4 injection).

Collection of samples: At the end of the treatment period, the animals were fasted overnight but allowed access to water *ad libitum*, weighed, and sacrificed by cervical dislocation while under mild anesthesia, blood was collected by cardiac puncture and centrifuged at 3000 rpm (Beckman GS – 6R, Germany) for 5 min at 4^0C. Serum was obtained at the supernatant for measuring enzyme level. Liver, heart, kidneys and spleen were quickly dissected out, rinsed in isotonic sterile saline, blotted dry on a filter paper and weighed. The organ weight to body weight ratio was calculated as a useful index of toxicity [11]. Each tissue was placed in separate plastic vial containing ice-cold sterile saline and stored at -8^0C for further analysis is carried out.

Preparation of liver homogenate: Whole liver homogenate (5%) in buffer solution (50 mM Tris – HCl, 0.25 M sucrose, pH 7.4) was prepared for determination of malondialdehyde (MDA) and total protein concentrations. Antioxidant enzymes activity were determined in the cytosolic fraction of the liver homogenate obtained by centrifugation at 10,000 rpm for 15 min at 40C, and the enzymes activity were expressed relative to protein concentration in the same fraction.

Biochemical assays: Serum total protein concentration was colorimetrically determined using commercial kits (Randox Laboratory Ltd, UK). Lipid peroxides levels were assessed as thiobarbituric acid reactive substances (TBARS) using MDA as a standard with an extinction coefficient of 1.53×10^5 $M^{-1}cm^{-1}$. Total protein concentration was determined in the whole tissue homogenate and their cytosolic fractions by the dye binding method of Bradford [12]. Serum ALT, AST and γ-GT activities were determined using commercial kits (Randox laboratory Ltd, Uk)

Histopathological examination: Hepatic tissues of rats from each group were collected from the same lobe of the livers and trimmed to an approximately 2 mm thickness. The tissues were then placed in 10% of neutral buffered formalin for histopathological studies. For histopathological examination, the formalin fixed liver was embedded in paraffin, cut into 4 – 5 µm thick sections, stained with hematoxylin-eosin, and observed under a photomicroscope.

Statistical analysis: Data were expressed as mean ± SEM. Statistical analysis was performed using ANOVA. Inter-group comparism was achieved by Duncan Multiple Range Test using SPSS 11.0. Differences were judged to be statistically significant when p was less the 0.05.

RESULTS

Interperitoneal administration of aqueous extract of *G. lucidum* (GLE) to normal rats for six weeks caused non-significant changes in the relative weights of liver, heart, kidneys and spleen. However, absolute and relative weights of the liver, heart, kidneys and spleen were significantly ($p<0.05$) elevated following CCl_4 intoxication (Table 1). The results shown in Table 1 revealed that pretreatment with GLE before CCl_4 intoxication prevented the negative effect of the toxicant on organ weights and organ to body weight ration by keeping liver, heart, kidney and spleen weights and absolute weights close to normal values.

Table 1: Absolute weights (g) and relative weights (x 10^{-2}) of some tissue in rats after GLE and/or CTC treatments.

Groups	Liver		Heart		Kidneys		Spleen	
	Wet weight	Weight/body weight	Wet weight	Weight/body weight	Wet weight	Weight/body weight	Wet weight	Weight/body weight
NC	2.30±0.2^{a}	2.40±0.2^{a}	0.32±0.1^{a}	0.33±0.01^{a}	0.55±0..01^{a}	0.57±0.03^{a}	0.28±0.02^{a}	0.29±0.01^{a}
GLE	2.43±0.01^{a}	2.55±0.01^{a}	0.33±0.01^{a}	0.35±0.02^{a}	0.58±0.01^{a}	0.61±0.05^{a}	0.28±0.02^{a}	0.29±0.01^{a}
Change (%)	5.65	6.25	3.13	6.06	5.45	7.02	0.00	0.00
CCT	3.85±0.05^{b}	2.71±0.2^{a}	0.56±0.05^{b}	0.54±0.01^{b}	0.88±0.02^{b}	0.69±0.01^{ab}	0.42±0.01^{b}	0.50±0.01^{b}
Change (%)	67.4	12.9	75.0	63.6	60.0	21.1	50	74.4
GLE + CCT	2.65±0.02^{ab}	2.44±0.1	0.38±0.01^{a}	0.35±0.01^{a}	0.68±0.02^{ab}	0.63±0.01^{a}	0.35±0.02^{a}	0.32±0.01^{a}
Change (%)	15.20	1.67	18.75	2.00	23.64	6.00	7.00	3.00

NC = Normal control. GLE = *Ganoderma lucidum* extract. CTC = Carbon tetrachloride. Values in the same row carrying different superscripts are significantly different at $p<0.05$.

As shown in Table 2, serum total protein concentration was significantly ($p<0.05$) increased in normal rats following GLE i.p. administration. Intoxication with CCl_4 however brought about a pronounced reduction in serum total protein concentration. Results obtained (Table 2) also revealed that pretreatment with GLE before toxicant challenge ameliorated the effect of the toxicant on serum protein level. GLE administration produced a non-significant change on serum ALT, AST and γ-GT activities in normal rats, the extract however prevented leakages of these enzymes from the tissues into the serum following CCl_4 treatment.

Table 2: Serum total protein, ALT, AST and γ-GT activities in rats after GLE and/or CTC treatments.

Groups	Total protein ($mgdl^{-1}$)	ALT (UL^{-1})	AST (UL^{-1})	γ-GT (UL^{-1})
NC	78.5 ± 9.5^{a}	4.38 ± 0.2^{a}	5.20 ± 0.5^{a}	7.52 ± 0.3^{a}
GLE	96.9 ± 6.9^{b}	4.41 ± 0.3^{a}	5.15 ± 0.2^{a}	8.10 ± 0.2^{a}
Change (%)	23.4	0.68	-1.54	7.71
CTC	43.7 ± 10.2^{c}	12.53 ± 2.5^{b}	17.80 ± 3.7^{b}	33.50 ± 2.5^{b}
Change (%)	-44.3	186.1	242.3	345.5
GLE + CCT	82.3 ± 8.5^{ab}	7.80 ± 2.2^{c}	9.65 ± 2.2^{c}	13.33 ± 1.5^{c}
Change (%)	4.8	78.1	85.6	77.3

NC = Normal control. GLE = Ganoderma lucidum extract. CTC = Carbon tetrachloride. ALT = Alanine aminotransferase. AST = Aspartate aminotransferase. γ-GT = Gamma glutamyl transpeptidase. Values in the same row carrying different superscripts are significantly different at $p<0.05$.

As observed in Table 3, hepatic total protein concentration was not significantly altered when GLE was administered to normal rats. Liver total protein concentration was significantly reduced by 42.5% following CCl_4 challenge. Pretreatment with GLE before CCl_4 intoxication significantly ($p<0.05$) elevated hepatic total protein concentration above normal level by 16.7%. Lipid peroxidation product (MDA) concentration was significantly increased (72.8%) in rats following treatment with CCl_4. GLE administration to rats before treatment with CCl_4 significantly reduced liver concentration of MDA by 56.7%.

Table 3: Hepatic protein and malondialdehyde concentrations in rats after GLE and/or CTC treatment

Groups	Total protein (mg/g)	MDA (nmol/g)
NC	165.8 ± 12.2^{a}	33.5 ± 3.8^{a}
GLE	172.5 ± 9.6^{a}	26.1 ± 2.5^{b}
Change (%)	4.0	-22.1
CTC	95.3 ± 4.8^{b}	72.8 ± 5.2^{c}
Change (%)	-42.5	117.3
GLE + CTC	193.5 ± 10.2^{c}	38.9 ± 3.1^{d}
Change (%)	16.7	16.1

NC = Normal control. GLE = Ganoderma lucidum extract. CTC = Carbon tetrachloride.
MDA = Malondialdehyde. Values in the same row carrying different superscripts are significantly different at $p<0.05$.

The results shown in Table 4 showed that liver antioxidant enzymes (SOD, GSH-Px, GSSG-Rx, and CAT) activities are significantly increased when GLE is administered to normal rats. The results also showed that exposure to CCl_4 caused a pronounced decrease in liver activities of these enzymes. However, pretreatment with GLE before exposure to the toxicant (CCl_4) significantly raised the concentration of these enzymes in the liver above the levels observed in normal rats,

Table 4: Hepatic antioxidant enzymes activity in rats after GLE and/or CTC treatment

Groups	SOD (Umg-1 protein)	GSH-Px (Umg-1 protein)	GSSG-Rx (Umg-1protein)	CAT (kcatmg-1 protein)
NC	5.8 ± 1.2^{a}	27.3 ± 2.2^{a}	21.5 ± 2.1^{a}	3.6 ± 0.1^{a}
GLE	11.3 ± 2.5^{b}	42.5 ± 3.8^{b}	33.3 ± 5.3^{b}	5.4 ± 0.2^{b}
Change (%)	90.8	55.6	54.9	50
CTC	3.1 ± 0.2^{c}	15.0 ± 1.5^{c}	10.6 ± 1.2^{c}	2.2 ± 0.01^{c}
Change (%)	-46.6	-45.1	-68.2	-38.9
GLE + CTC	9.5 ± 1.2^{d}	38.7 ± 3.2^{d}	28.2 ± 2.3^{d}	4.7 ± 0.1^{d}
Change (%)	63.8	41.8	31.2	30.6

NC = Normal control. GLE = Ganoderma lucidum extract. CTC = Carbon tetrachloride. SOD = Superoxide dimutase. GSH-Px = Glutathione peroxidase. GSSG-Rx = Glutathione reductase. CAT = Catalase. Values in the same row carrying different superscripts are significantly different at $p<0.05$.

The results of the histopathological examinations of the livers of rats for each group is shown in Plates 1-4. The results showed that CCl_4 could induce histological changes in the liver, including increased degeneration, necrosis, and portal traditis. Rats in the various groups except those in control group exhibited ballooning degeneration in the centrolobular zone and hepatocyte necrosis. Animals in the CTC treated group suffered more severe damage than those pre-treated with GLE.

Plate1: Liver section of normal rat

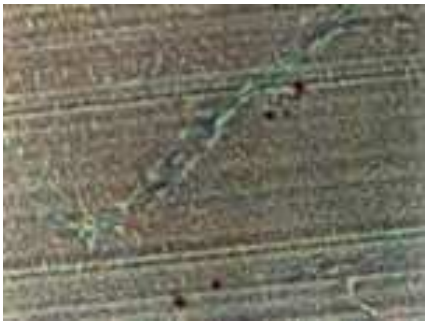

Plate 2: Liver section of rats treated with GLE

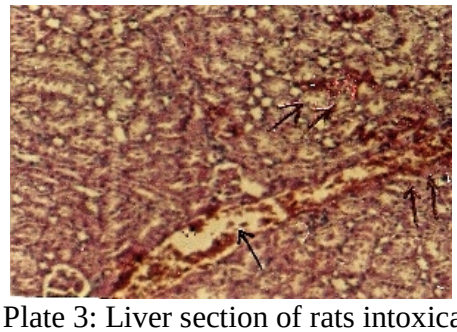

Plate 3: Liver section of rats intoxicated with CCl_4

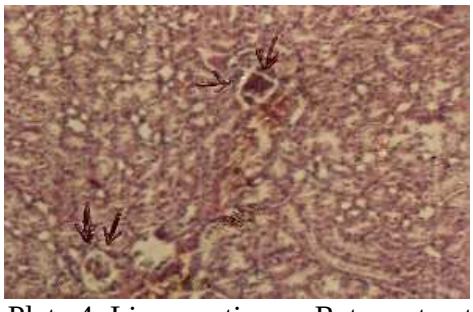

Plate 4: Liver section or Rats pretreated with GLE before CCl_4 intoxication

DISCUSSION

Data generated in this study showed that i.p. administration of GLE to normal rats produced no toxic effect on the tissues. This is revealed by non-significant difference in the absolute and relative weights of the liver, heart, kidneys and spleen in the GLE group compared to normal control group. The observed increased in both absolute and relative weights of these tissues in the CCl_4 group confirm its toxic effect on rats as reported by other researchers. CCl_4 induced tissue damage involves its biotransformation by cytochrome P4502E1 (CYP2E1) into trichloromethyl radical (CCl_3^*) which initiates lipid peroxidation, distrupt Ca^{2+} homeostasis, and eventually caused cell death [2,13]. Administration of CCl_4 markedly increases serum levels of ALT, AST and γ-GT, an observation indicative of tissue damage. Increased serum concentrations of these enzymes commonly reflect the severity of liver injury [14,15]. Reports from other study have shown that polysaccharide extract extract of *Ganoderma lucidum* could protect against chemically-induced hepatotoxicity [16]. In the present study, the increased levels of enzymes were considerably reduced by pre-treatment with aqueous extract of *G. lucidum*. This observation was consistent with the results of Lin *et al*. [6]. From the afore-going it could thus be suggested that *G. lucidum* extract tend to protect prevent liver damage and suppressed the leakage of ALT, AST and γ-GT through cellular membranes. In support of earlier reports from other researchers, histopathological examination of liver sections of rats challenged with CCl_4 revealed ballooning degeneration and inflammation of lymphocytes [16,17]. In contrast, liver sections of rats pre-treated with *G. lucidum* extract showed well-preserved musculature as also observed by Refaie *et al*. [17]. Treatment of CCl_4-intoxicated rats with GLE suppressed ALT, AST and γ-GT leakage into the serum, which indicates the attenuation of hepatocytes damage.
Many studies have shown that the hepatoprotective activities may be associated with antioxidant capacity to scavenge reactive oxygen species [18].

Interactions of these reactive oxygen species lead to the development of pathological situations often characterized by compromised membrane integrity and thus malfunctioned cellular transport system. Antioxidant enzymes SOD, catalase, GSH-Px and GSSG-Rx offer protection against oxidative tissue damage [19]. Wang *et al*. [20] had earlier demonstrated that biochemical evaluations of rats 24 h after subcutaneous CCl_4 administration (CCl_4/olive, 1:1, 3 ml/kg) led to increases in activities of antioxidant enzymes including SOD, catalase and GSH-Px in the liver. Halliwell [21] had earlier suggested that CCl_4 may induce oxidative stress and consequent down-regulation of antioxidant enzymes thus render cells more susceptible oxidative damage. The resultant up-regulation of antioxidant enzymes; SOD, catalase, GSH-Px and GSSG-Rx by *G. lucidum* extract could thus render the cell more resistant to CCl_4-induced damage. This conclusion is premised on the results of the histological examinations of liver sections of the different treatment groups which revealed minimal parenchymal spotty inflammation in CCl_4 challenged rats pre-treated with GLE in contrast to distorted architecture, ballooning with foci of necro-inflammation and spotty necrosis in CCl_4 challenged rats without GLE treatment.

Previous reports from our laboratory on phytochemical analysis of aqueous extract of *G. lucidum* (from south western Nigeria) have shown that it contains ample amount of triterpenes and polysaccharides. CCl_4-intoxication caused a pronounced elevation of both serum and hepatic MDA (a by-product of peroxidation of biological membrane polyunsaturated fatty acids); these observations demonstrated the inadequacy of the hepatic antioxidant defenses in combating the

reactive oxygen species produced as a result of CCl_4 intoxication. The ability of GLE to normalize the reduced level of serum and hepatic total protein level as a result of CCl4-induced damage also demonstrated its potent therapeutic ability in initiating cell repair.

In conclusion, the results of this study revealed that pretreatment with aqueous extract of G. lucidum was effective in alleviation CCl_4-induced liver damage in rats. One mechanism may be through its antioxidant activity.

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