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GANODERMA LUCIDUM ATTENUATES AND PREVENTS CCL4-INDUCED HEPATIC AND RENAL DAMAGE IN SPRAGUE – DAWLEY RATS

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Abstract
Ganoderma lucidum (G. lucidum) is considered to be a medicinal mushroom and it is widely used as anti-oxidants to prevent or treat of different types of diseases including cancer, cardiovascular disease and renal dysfunction. This study aimed to indicate whether G. lucidum could attenuate oxidative stress and prevent Hepatorenal damage. CCl_4 was used to induce oxidative stress in adult male of Sprague–Dawley rats (about 8-weeks old, 200–220g weight). Adult rats were randomly divided into four equal groups A, B, C and D. Group A was determined as a control one, group B received daily oral dose of Ganoderma Extract, (600 mg/kg/bw) for 12 weeks, while groups C and D received 0.1ml\100g b.w. of CCl4 (50% in olive oil) via the intraperitoneal route twice a week for 12 weeks, followed by daily oral dose of GAN extract, (600 mg/kg/bw). Blood samples were later collected for biochemical analysis. Liver and Renal Function Tests, such as ALT (alanine transaminase), ALP (alkaline phosphatase) and AST (aspartate aminotransferase), uric acid, creatinine and urea were determined. The current study is also determined the antioxidant enzyme activity, such as malondialdehyde (MDA), catalase (CAT), glutathione (GSH), superoxide dismutase (SOD) and H_2O_2 induced by CCl4. The results demonstrated that G. lucidum can significantly prevent the CCl4 induced liver and kidney damage.

Keywords: Ganoderma lucidum; CCl_4; Hepatoprotective; Oxidative stress; kidney damage; medicinal mushroom; Sprague–Dawley

INTRODUCTION

Several studies have reported that mushrooms extract and other herbal medicines such as *Asparagus racemosus*, *Panax Notoginseng* and *Rosmarinus officinalis* play novel functions as antidepressant (Matsuzaki et al., 2013). Mushrooms have a dozen types of bioactive substances including vitamins, proteins carbohydrates, minerals, fibers triterpenoids, polysaccharides, sterols, and fatty acids (Babu and Subhasree, 2008). They are also rich in antibacterial and other medicinal compounds such as antiviral, antioxidant and anticancerousn. *Ganoderma lucidum* (*G. lucidum*), also terms the reishi mushroom, is a popular medicinal mushroom used for promoting health and longevity in India, China, and Japan (Kao et al., 2013). This type of mushroom has been widely utilized as therapeutic to treat or prevent insomnia, neurasthenia, carcinoma, deficiency fatigue (Deng et al., 2020) as well as treatment gastric ulcer, nephritis, hypertension, Melanoma and Breast Cancer (Barbieri et al., 2017). *G. lucidum* is also produced phyto-constituents such as adenosine, polysaccharides, ergosterols, coumarin, ganoderic acids, , lactones, minerals, organic germanium and mannitol (Bao et al., 2002). They are probably improve energy, strengthens, enhance blood circulation and attenuate various diseases including diabetes, atherosclerosis, liver disease, heart disease, kidney diseases and microbial infection (Geng et al., 2019; Wihastuti and Heriansyah, 2017; Zhao et al., 2018). It has been reported that cultured mycelia of *G. lucidum* possesses immunomodulatory (Bao et al., 2002), antitumor (Lin and Zhang, 2004), antibacterial, antiviral activities (Eo et al., 1999), anti- inflammatory hepatoprotect role and also prevent peroxidation and free radicals from causing damage of healthy cells in the body (Batra et al., 2013; Rai et al., 2015). In addition, water extracts and ethanol extracts of *G. lucidum* give protection against inflammation of the liver, the biggest gland of the body and the main detoxifying organ that regulates drugs metabolism and chemical toxicity (Lu et al., 2013). Triterpenoid extracted from *G. lucidum* is considered a protective factor against Acute viral hepatitis and chronic liver induced by the carbon tetrachloride (CCl4) (Wang et al., 2000). Results from animal experiments have indicated that CCl4 causes fibrosis in the liver tissue, damage of the hepatic parenchyma and increases liver enzymes particularly Alanine aminotransferase (ALT) and Aspartate transaminase (AST) (Gao et al., 2019). This is probably because production of free radicals during the activation of CCl4 by drug-metabolizing enzymes placed on the endoplasmic reticulum (Slater and Sawyer, 1971). In vivo and in vitro experiments have showed a potent action of *G. lucidum* as antioxidant and its radical-scavenging impacts for protection of the liver (Wang et al., 2000). Ganoderma extract could also protect the kidney from superoxide induced renal damages (Shieh et al., 2001). *G. lucidum* has been approved to exhibit pathophysiological mechanisms in attenuating and treating various renal diseases, such as chronic kidney disease (CKD) and acute kidney injury (AKI) (Geng et al., 2019). However, it is still unclear the impacts of *G. lucidum* on hepatorenal damage induced by CCl4. Consequently, the present study proposed that *G. lucidum* can effectively counteracting oxidative stress and could act as therapy against liver and

kidney damage.

MATERIALS AND METHODS

Mushroom water extraction (aqueous extract)

300 grams of *G. lucidum* was weighed and measured by a Top Loader Balances. 600 ml of distilled water (D.W.) was added onto the material, which then immediately placed in a water bath for about 2h at 70^{oC}. Liquid filtration was carried out using Whatman filter paper size 1, and the extract placed into a plastic tube and then stored at -18° ^{C} to avoid decomposition and prevent contamination. Later, the extract was liquefied at 70^{oC} using a hot water bath then transferred into rotary evaporator flask. The extract was then swirled using Silica Crucible contains acetone and solid carbon dioxide to adsorb the extracts on to the flask. Next step was freeze-drying the flask at -40^{oC} with a vacuum pressure for two days. The extract was then filtered (Harborne, 1998).

Animal exposure

Sprague–Dawley rats (male/ age 8 weeks weighing between 200–220g) were ordered from the Laboratory Animal Center, Veterinary Medicine school - Baghdad University - Iraq. The animals were kept in standard laboratory conditions. All the experiments and protocols were carried out in accordance with international guidelines in laboratory animal care and use legislation, approved by a research ethics committee. After two weeks of acclimatization, the rats were divided into four groups A, B, C and D. A normal control group (n=7), received just normal saline solution. Group B (n=7) received daily dose of GAN extract, (600 mg/kg/bw) for 12 weeks orally using. Group C (n=7) received 0.1ml\100g b.w. of CCl4 (50% in olive oil) via the intraperitoneal route twice a week for 12 weeks. The last group D (n=7) received 0.1ml\100g b.w. of CCl4 through the intraperitoneal route twice weekly for 12 weeks, followed by dally oral dose of GAN extract, (600 mg/kg/bw) for 12 weeks.

Samples of Blood

After 12 weeks treatment, rats were anaesthetized with (50 mg/kg i.p.) sodium pentobarbital. The blood then was collected using cardiac puncture, followed by centrifugation (500×g) for 5 min, to obtain serum for biochemical analysis.

Biochemical investigations

The left side of rat liver was homogenized in ice-cold (20 mM) phosphate-buffered saline (PBS; pH 7.4). The thiobarbituric acid (TBA) assay was performed to mesure Malondialdehyde (MDA), due to the reaction of MDA with TBA to produce thiobarbituric acid reactive substances (TBARS), red species that absorbs at 535 nm (Ohkawa et al., 1979). Glutathione (GSH) level was detected at 412 nm (Sedlak and Lindsay, 1968). The catalase (CAT) activity was spectrophotometrically determined according to (Johansson and Borg, 1988) by measuring the decomposition of (H_2O_2) at 240 nm. The activity of SOD was also measured spectrophotometrically using phenazine methosulfate in order to generating superoxide radicals that react with nitroblue tetrazolium (Nishikimi et al., 1972).

Serum Biochemistry

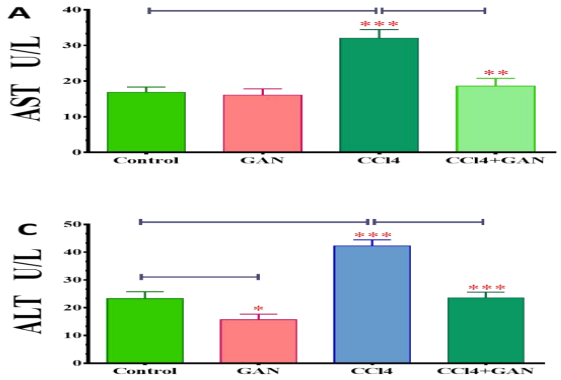
Serum Aspartate Aminotransferase (AST), serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined using Reflation plus Analyzer and Roche kits (Roche Diagnostics GmbH, Mannheim, Germany). While urea and creatinine levels were estimated using standard test kits (Randox Laboratories, Crumlin in County Antrim, Northern Ireland, UK).

Statistical analysis

ANOVA is the test used in Graph Prism Software V. 7.00 for analysis of the results, Statistical significance was considered as $p < 0.05$.

RESULTS

Treatment with CCl4 dramatically increased the activity of the AST, ALT and ALP serum in treatment rats compared to the control group (figure 1). As shown in the same figure, *G. lucidum* alone did not change the level of AST and ALP (Fig.1A and B respectively), but it caused lower decrease in ALT level as compared to that in the control group (Fig.1C).



However, CCl4 +GAN treatment resulted in a dramatic decrease in plasma AST, ALP and ALT activities when compared to the respective control group (Fig.1).

Figure 1. Effect of G. lucidum on hepatic enzymes, AST, ALP and ALT levels in CCl4-treated rats. Each group represents mean± S.D. of seven animals Mean values in each row having different superscript (***, **, and *) are significant at $p < 0.05$

In order to further explore of *G. lucidum* effects, the levels of creatinine, Uric acid and Urea were assayed. As shown in Fig. 2, CCl4 significantly increased the serum creatinine, Urea and Uric acid as compared to the control group. However, CCl4+ GAN obviously decreases the CCl4-induced elevation creatinine, Uric acid and Urea levels as compared to the control group (Fig. 2).

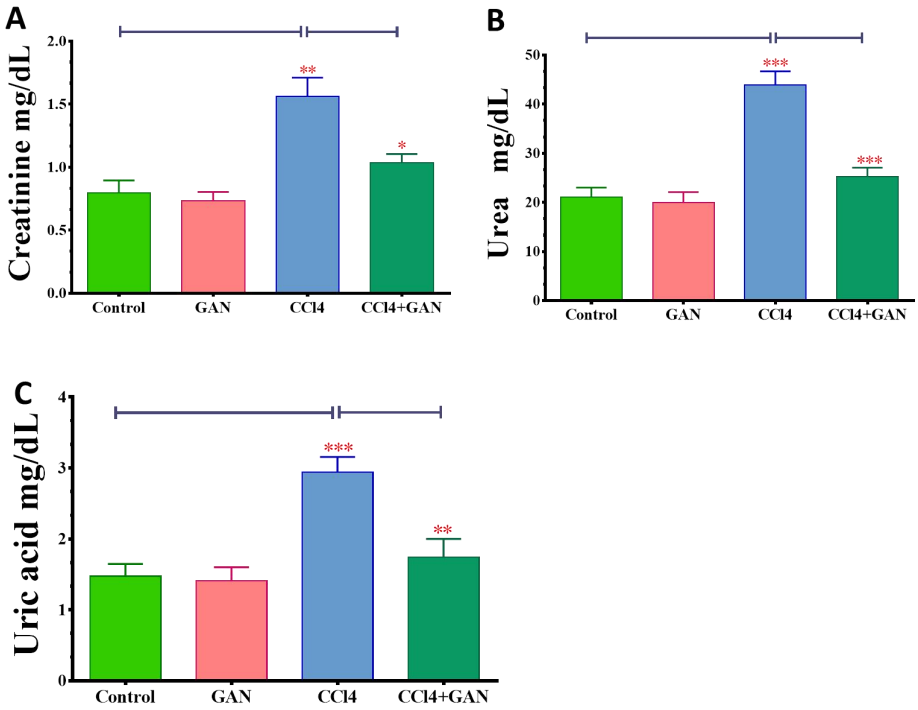


Figure 2. Effect of *G. lucidum* on the serum creatinine, Uric acid and Urea in CCl4-treated rats
Each group represents mean± S.D. of seven animals Mean values in each row having different superscript (***, **, and *) are significant at $p < 0.05$

We also examined the effect of *G. lucidum* on CCl4 as an index induced hepatic oxidative stress. For this step, we measured the activities of hepatic MDA, H_2O_2, GSH, CAT and SOD. As shown in table (1), injection of CCl4 significantly increased MDA and H_2O_2 in comparison with control. There was lower decrease in the treatment with GAN alone in both MDA and H_2O_2 compared with control. However, no differences were seen in MDA and H_2O_2 in the CCl4+ GAN groups. CCl4 dramatically decreased in SOD and CAT levels and lower decreased in GSH compared to the control (see Table 1). Treatment with GAN alone and CCL4+ GAN groups showed clear elevation in SOD and GSH but did not change CAT when compared to the control (see Table 1).

Table 1 Effect of CCl4 and GAN on the levels of the lipid peroxidation in treated rats

	Control	GAN	CCl4	CCl4+ GAN
MDA	**6.537 ±0.635**	**4.343 ±0.391***	**14.71 ±0.721*****	**5.732± 0.642****
H_2O_2	**0.449 ±0.04**	**0.277 ±0.044***	**1.206 ±0.085*****	**0.409± 0.03*****
GSH	**0.089 ±0.004**	**0.11 ±0.009**	**0.029 ±0.004*****	**0.105±0.007****
CAT	**1.137 ±0.065**	**1.138 ±0.108**	**0.441 ±0.078*****	**1.132± 0.097*****
SOD	**59.12 ±3.313**	**62.37 ±3.361**	**39.1 ±2.26*****	**65.46±3.203*****

Effect of CCl4 and GAN on the levels of the lipid peroxidation product MDA (nmol/mg protein) , hydrogen peroxide ($H2O_2$)(mmol/g protein), glutathione (GSH; mg/g protein), catalase (CAT;_mol H2O2/Sec/g protein), and superoxide dismutase (SOD; U/mg protein) in hepatic protein of rats in different groups. The values are expressed as the means ± S.D. of seven animals Mean values in each row having different superscript (***, **, and *) are significant at $p < 0.05$

DISCUSSION

Herein, we revealed the beneficial impact of *G. lucidum* in prevention biochemical alterations in CCl4-induced liver fibrosis. The liver is considered a central organ in the metabolic process (Bechmann et al., 2012). After liver damage, there would probably be metabolic disorder glucose level (Bahr et al., 2006) and formed abnormal liver (Yin et al., 2013). The environmental pollutants, drugs and toxic chemicals cause cellular damage by activation of reactive oxygen species (Othman et al., 2016). CCl4, also called hepatotoxic substance, is one common environmental toxicant used in the experimental study for stimulation animal models of acute hepatic and renal damage (Ali and Rajab; Gao et al., 2019). The aim of this study, therefore, is to determine the role of *G. lucidum* in preventing liver and kidney injury caused by CCl4-intoxicated in rat.

The progression of the pathological changes in liver damage is a major contributor to the proliferation of connective tissue and fibrotic response (Kao et al., 2013). CCl4 inside the cell and could in the endoplasmic reticulum leads to the converting Cl_3COO and CCl_3 by cytochrome P-450 (Li et al., 2019). This process elevates permeability of the calcium in the plasma membrane and probably lead to calcium deficiency disease and then cell death (Strehler and Thayer, 2018). CCl4 can also mediate acute toxicity by changing the permeability of cellular membrane and also leakage the hepatocellular enzymes from the cytoplasm into the bloodstream, which in turn indicates liver damage and loss of functional integrity (El-Bakry et al., 2017).

In rats, CCl4 induces liver fibrosis and leads to reduction in plasma hepatic protein contents (Lin and Lin, 2006). The current study have detected that CCl4-induced increased

levels of APT, ALT, and AST. The serum level of these marker enzymes indicates liver physiological state. The alteration in APT, ALT and AST levels occurs as a result of the liver distortion, which in turn induces cellular injury of the internal organs, in response to the toxic metabolites and diseases (Patrick-Iwuanyanwu et al., 2007). AST, ALT, and ALP in serum are experimentally used to evaluate hepatic damage. In addition, damage the hepatocytes leads to easily leakage of liver enzymes into the blood vessels (Sokar et al., 2017).

The transaminases AST and ALT play a crucial role in Amino Acid biosynthesis and catabolism. They are mainly used as indicator for liver damage, promoted various types of detoxification processes, biosynthesis and metabolism of energetic macromolecules for many different functions (Gupta, 2019; Seven et al., 2004). AST, ALP and ALT serums are considered to be reliable markers of the liver (Gao et al., 2019) and the elevated serums mainly indicate liver necrosis and alterations in membrane permeability and probably cell morphology (Neuman, 2020). In agreement with (Gao et al., 2019), the recent study detected that CCl4-induced hepatotoxicity in rats as a result of the increase in AST ALP and ALT.

It is well documented that *G. lucidum* polysaccharide peptide has no affects gross liver pathology and histology in rats and mice (Dewi et al., 2015). *G. lucidum* is attracting elevated attention in current years as because its ability in reducing oxidative stress. the current results have found that treatment with *G. lucidum* decreased the effect of CCl4-induced hepatotoxicity as obviously shown by the decrease of liver enzymes AST, ALP and ALT. These results generally agree with other earlier studies (Wang et al., 2019; Zhang et al., 2016).

For better understanding, the current study examined the toxicity of CCl4 in inducing kidney oxidant injury in rats. Results have found that CCl4 toxicity obviously increased in blood urea, uric acid, and creatinine levels compared to the control. However, pre-treatment of *G. lucidum* extracts significantly decreased urea, uric acid, and creatinine levels, proposing that it would counteract nephrotoxicity caused by CCl4. These results were in the same line with (Pillai et al., 2011, Abdullah et al., 2018) and demonstrated that Ganoderma can prevent elevate urea, creatinine levels (Essam El-Din and El-Mowafy, 2014). These results proposed that Ganoderma probably possesses a potential therapeutic for preventing nephrotoxicity caused by drugs or even chemical materials.

Elevation of uric acid is probably because the degradation of nitrogenous bases such as purines and pyrimidines, while the elevation of creatinine level in the blood determines impaired kidney function (Mossa and Abbassy, 2012; Rifai, 2019). The increased level of both urea and creatinine refers to the decline of glomerular filtration rate which then elevated by *G. lucidum*. Therefore, treatment of nephrotoxicity using *G. lucidum* supports its activity as antioxidant (Essam El-Din and El-Mowafy, 2014).

The hepatotoxicity of CCl4 is ultimately due to the active metabolite, trichloromethyl free radical (Stoyanovsky and Cederbaum, 1999). It binds to macromolecule of tissue and hence stimulates oxidative of membrane lipid degradation and ultimately membrane damage assumed that such development results in production of lipid peroxidation, which in turn yields different products among them is (MDA) (Lee et al., 2019; Patrick-Iwuanyanwu et al., 2007). In this work, injection of CCl4 increased hepatic MDA level. This result reflects the increased lipid peroxidation, leading to failure of antioxidant defence and then liver damage (Cemek et al., 2010).

It has been determined that treated liver tissues and cells with CCl4 induces a complex biological process for resistance to the toxicity (Dong et al., 2016). The metabolism of CCl4 in the liver leads to the formation of free radicals (Sancheti et al., 2013), and then triggers oxidative stress, which known as a conjoint pathological mechanism to contribute in launch and progression of liver injury (Souza et al., 2017). Oxidative stress, in turn, induces the inflammatory cytokines (Heeba and Morsy, 2015) and results in apoptosis and necrosis of hepatocytes, inflammation induction and further stimulation progression process of liver fibrogenesis and fibrosis (Heeba and Mahmoud, 2014; Unal-Cevik et al., 2004).

The current study found that oxidative stress parameters were remarkably altered in rats exposed to CCl4. In addition to MDA, the increase in H_2O_2 level and decrease of GSH, CAT and SOD were compared to the control group. These results support the conclusion that the depletion of GSH, CAT and SOD by CCl4 extract in treated animals leads to abolition antioxidant in oxidative stress (Li et al., 2019). GSH, CAT and SOD are considered endogenous antioxidants which play a vital role in deactivation the free radicals (Abdulhadi et al.; Jamor et al., 2019). However, treatment with *G. lucidum* extract restored MDA, H_2O_2 contents, SOD, CAT and GSH levels. These results were in line with (Gao et al., 2019). The restoration activities of oxidative stress parameters by *G. lucidum* can protect the enzymes (Cayir et al., 2009; Sheena et al., 2003). Inhibition the increased MDA content by *G. lucidum* is a powerful antioxidant and exhibits significant strong scavenging free radical activity (Gao et al., 2019).

GSH, the main non-protein thiol presents in many living organisms, plays an important role in coordinating innate defense mechanisms of antioxidant. It maintains cells structure and function by its detoxification reactions. Depletion of GSH results in the increase in CCl4 toxicity (Jayakumar et al., 2006). Glutathione depletion also leads to lipid peroxidation and this probably being the prime factor that permits the oxidative degradation of lipids and impairs the activities of antioxidant enzymes (Essam El-Din and El-Mowafy, 2014). In conclusion, the present work established that CCl4 is a potent hepatotoxic and nephrotoxic substance, which results in oxidative stress by depleting antioxidant enzymes activities. *G. lucidum* is probably a good therapeutic as anticancer and a good source for the immunomodulatory.

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