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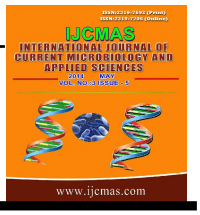

Original Research Article

Rejuvenating Consequence of *Ganoderma lucidium* on Cellular Integrity and Function of CCl_4 Induced Liver of Mice

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A B S T R A C T

To investigate the effects of Reishi mushroom, *Ganoderma lucidum* extract (GLE), on Cellular Integrity and Function of CCl_4 Induced Liver of Mice. Thirty mice were used in the study and were grouped into three with 10 mice in each group. Group A: untreated mice kept as control and served with equal volume of distilled water by gavage method. Group B: mice treated with 2.8 ml/kg b. w. of (CCl_4) twice a week for 5 weeks to make chronic liver injury model. Group C: mice treated with *Ganoderma lucidium* followed by CCl_4 by gavage method @ 75 mg/kg b.w. (body weight) for eight weeks. After making several trials of dose of *Ganoderma lucidium*, oral dose of 75 mg/kg b.w. (body weight) was used for amelioration. The treated and control group of mice were sacrificed on targeted day for estimation of cellular integrity like SGPT/ALT, SGOT/AST, ALP and for functionality bilirubin, albumin and globulin were determined. Oral administration of GLE (75 mg/kg b.w.) significantly ($p < 0.00$) reduced the CCl4-induced increase in AST, ALT and ALP activities. A marked significant ($p < 0.001$) increase in bilirubin level as compared to the control group, while significant ($p < 0.001$) decreased level were observed in GLE treated group. The CCl4-induced decrease in plasma albumin concentration and globulin were significantly ($p < 0.001$) increased following the administration of *Ganoderma lucidium* extract. Oral administration of GLE is effective in the reduction of chronic liver injury, probably via a protective effect by its free-radical scavenging ability and normalizing the physiology of the body and maintaining the cellular integrity.

Keywords

Ganoderma lucidium, CCl_4; Swiss albino mice; Liver Function.

Introduction

Ganoderma lucidum, an oriental fungus has a long history of use for promoting health and longevity in China, Japan, and other Asian countries. In China, *G. lucidum* is called lingzhi, whereas in Japan the name for the Ganodermataceae family is reishi.

Ganoderma has a unique double walled basidiospore with a shining skin. Some of the active compounds identified in the cell wall of the mushrooms include protein bound polysaccharides or long chain glucose (Hsu et al., 2009; La Clair et al., 2011; Zhou et al., 2012). Most mushrooms

are composed of around 90% water by weight. The remaining 10% consists of 10–40% protein, 2–8% fat, 3–28% carbohydrate, 3–32% fiber, 8–10% ash, and some vitamins and minerals, with potassium, calcium, phosphorus, magnesium, selenium, iron, zinc, and copper accounting for most of the mineral content (Borchers et al., 1999). These compounds along with probably others have been found useful in the treatment of malignancies such as leukemia as well as immunodeficiency states. Similarly extracts of *Ganoderma lucidum* specifically has been found useful in the treatment of viral, bacterial as well as some parasitic infections and infestations (Karaman et al., 2010; Kim et al., 2008; Sadava et al., 2009).

Polysaccharides, peptidoglycans, and triterpenes are three major physiologically active constituents in *G. lucidum* (Boh et al., 2007).

A number of animal studies have indicated that water or ethanol extracts of *G. lucidum* showed protective actions against acute hepatitis in rats or mice (Lin et al., 1995, 1993., Liu et al., 1979). Other reports had previously indicated that triterpenoids isolated from *G. lucidum* possessed the protective effect against acute hepatitis caused by CCl4 (Kim et al., 1999; Wang et al., 2000). Furthermore, Park et al. (1997) demonstrated that, in rats, polysaccharides extracted from *G. lucidum* could antagonize liver fibrosis caused by biliary obstruction. These results demonstrate that *G. lucidum* possesses a protective effect in the liver.

The main aim of this study to investigate the effects of Reishi mushroom, *Ganoderma lucidum* extract (GLE), on Cellular Integrity and Function of CCl_4 Induced Liver of Mice. Since no reports have recorded the effect of *G. lucidium* on Cellular Integrity and Function of CCl_4 Induced Liver. In the present study, we therefore investigated the probable effect, if any, of extracts of *G. lucidum* on chronic CCl_4-induced liver injury.

Liver fibrosis is the common end-stage of most chronic liver disease, regardless of etiology, and its progression leads to cirrhosis and liver cancer (Alcolado et al., 1997). Although the exact mechanisms of pathogenesis in liver cirrhosis are still obscure, the role of free radicals and lipid peroxides has attracted considerable attention (Gebhardt , 2002). It has been found that metabolism of CCl4 involves the production of free radicals through its activation by drug-metabolizing enzymes located in the endoplasmic reticulum (De Pomerai et al., 1977). CCl_4 is capable of causing liver lipid peroxidation, resulting in liver fibrosis (Kim et al., 1999).

Materials and Methods

Animals

In the present investigation, experiments were performed on 10-12 weeks old healthy Swiss albino mice, *Mus musculus*. For the optimal growth and development, the mice were kept in ideal condition under a well regulated light and dark (12h:12h) schedule at 23±1°C in the animal house, Mahavir cancer Institute & Research centre, patna, India (CPCSEA Regd. No. 1129/bc/07/CPCSEA, dated 13/02/2008) and the experiment was duly approved by the IAEC. Animals were given food and water *ad libitum*. Carbon tetrachloride : 2.8 ml/kg b. w. of (CCl_4) was used to make liver injury model of mice & it was procured from Sigma-Aldrich Chemicals Pvt Limited, Bangalore (India).

***Ganoderma lucidium*:** *G. lucidum*, was obtained from the Daxen Agritech India Pvt. Ltd.

Methodology

Study Design

Thirty mice were used in the study and were grouped into three with 10 mice in each group. Group A: untreated mice kept as control and served with equal volume of distilled water by gavage method. Group B: mice treated with 2.8 ml/kg b. w. of (CCl_4) twice a week for 5 weeks to make chronic liver injury model model. Group C: mice treated with *Ganoderma lucidium* followed by CCl_4 by gavage method @ 75 mg/kg b.w. (body weight) for eight weeks.

After making several trials of dose of *Ganoderma lucidium*, oral dose of 75 mg/kg b.w. (body weight) was used for amelioration. The treated and control group of mice were sacrificed on targeted day for estimation of cellular integrity like SGPT/ALT, SGOT/AST (Liver transaminases). These are useful biomarkers of liver injury. Albumin test and some with conditions linked to the biliary tract (bilirubin and alkaline phosphatase) have done for functionality.

Collection of Blood

The blood from the control and treated mice has been taken out as a sample to test and collect the data. Blood samples were obtained from mice by orbital sinus puncture. Mice were anaesthetized for this purpose. Collection of blood from orbital sinus with a Hematocrit tube is one of the most effective methods, which causes least stress to the animal.The blood was collected in EDTA vaccutainer tube for biochemical study.

Assessment of liver functions

Whole blood was centrifuged at 3500 r/min at 4 ºC for 15 min to separate the plasma. Aspartate aminotransferase (AST)/SGOT and alanine aminotransferase (ALT)/SGPT, ALP albumin and globulin were determined spectrophometrically with an automatic analyzer using commercially available kits (Roche Diagnostics).

Statistical analysis

Data were analyzed with statistical software (Graphpad Prism 5) and values were expressed as Mean ± SEM. And differences between the groups were statistically analyzed by one-way analysis of variance (ANOVA) using the Dunnett's test.

Results and Discussion

Analysis of liver functions

The results for the liver function parameters are presented in Table.1. A day's dependent study of *Ganoderma lucidium* was done against CCL4 induced mice. Effect of *G. lucidium* showed restored value of liver function parameters in Group III & IV. As shown in (Table), CCl_4 treatment resulted in a significant ($p < 0.001$) increase in plasma AST ALT and ALP activities as compared to the control group. Oral administration of GLE (75 mg/kg b.w.) significantly ($p < 0.00$) reduced the CCl4-induced increase in AST, ALT and ALP activities. A marked significant ($p < 0.001$) increase in bilirubin level as compared to the control group, while significant ($p < 0.001$) decreased level were observed in GLE treated group.

The plasma albumin content and globulin in CCl4-treated groups were significantly ($p < 0.001$) lower than that in the control group. The CCl4-induced decrease in plasma albumin concentration and globulin were significantly ($p < 0.001$) increased following the administration of *Ganoderma lucidium* extract.

The liver filters and processes blood as it circulates through the body. It metabolizes nutrients, detoxifies harmful substances, makes blood clotting proteins, and performs many other vital functions. The cells in the liver contain proteins called enzymes that drive these chemical reactions. When liver cells are damaged or destroyed, the enzymes in the cells leak out into the blood, where they can be measured by blood tests. Liver tests check the blood for two main liver enzymes: Aspartate aminotransferase (AST), formerly called SGOT. The AST enzyme is also found in muscles and many other tissues besides the liver. Alanine aminotransferase (ALT), formerly called SGPT. ALT is almost exclusively found in the liver. If ALT and AST are found together in elevated amounts in the blood, liver damage is most likely present.

Another of the liver's key functions is the production of bile, which helps digest fat. Bile flows through the liver in a system of small tubes (ducts), and is eventually stored in the gallbladder, under the liver. When bile flow is slow or blocked, blood levels of certain liver enzymes rise: Alkaline phosphatase, 5' nucleotidase, Gamma-glutamyl transpeptidase (GGT). Liver tests may check for any or all of these enzymes in the blood. Alkaline phosphatase is by far the most commonly tested of the three. If alkaline phosphatase and/or 5' nucleotidase and GGT are elevated, a problem with bile flow is most likely present. Bile flow problems can be due to a problem in the liver, the gallbladder, or the tubes connecting them.

A number of animal studies have indicated that water or ethanol extracts of *G. lucidum* showed protective actions against acute hepatitis in rats or mice (Lin et al., 1995, 1993, 2002, Liu et al., 1979). Other reports had previously indicated that triterpenoids isolated from *G. lucidum* possessed the protective effect against acute hepatitis caused by CCl_4 (Kim et al, 199, Wang et al, 2000). Furthermore, Park et al. (Park et al., 1997) demonstrated that, in rats, polysaccharides extracted from *G. lucidum* could antagonize liver fibrosis caused by biliary obstruction. These results demonstrate that *G. lucidum* possesses a protective effect in the liver.

The present study showed the rejuvenating effect of *Ganoderma lucidium* extract in prevention of liver injury induced by CCl4 treatment. An amelioration brought about by GLE was seen in plasma biochemical parameters. CCl_4 treatment caused hepatocellular damage in mice, as indicated by a drastic increase in both plasma ALT and AST levels after CCl4 administration. Mice treated with GLE showed a protection against CCl_4-induced hepatotoxicity, with the levels of both plasma AST and ALT being reduced.

The liver synthesizes not only the protein it needs, but also produces numerous

Table.1 The results for the liver function parameters

Parameter	Control I	CCL_4 Treated II	*G. lucidium* 6 wks Treated III	*G. lucidium* 8 wks Treated IV
ALT/SGPT (U/ml)	22.4 ± 0.672	86.6 ± 0.773	40.0 ± 0.741	32.2 ± 0.634
AST/SGOT (U/ml)	26.3 ± 0.088	58.2 ± 0.722	36.6 ± 0.407	29.5 ± 1.829
ALP (KA units)	7.0 ± 0.771	18.3 ± 1.880	12.2 ± 0.336	9.5 ± 0.327
Bilirubin (mg/dl)	0.64 ± 0.052	3.55 ± 0.120	1.4 ± 0.048	0.85 ± 0.063
Albumin (g/dL)	3.46 ± 0.065	2.47 ± 0.055	2.76 ± 0.025	3.02 ± 0.049
Globulin (g/dL)	2.19 ± 0.018	1.54 ± 0.034	1.68 ± 0.063	1.86 ± 0.012

ALT/SGPT = Alanine aminotransferase **,** (AST)/SGOT = Aspartate aminotransferase, ALP = Alkaline phosphatase

export proteins. Among the latter, plasma albumin is the most important (Podolsky and Isselbacher, 1998). Export proteins are synthesized on polyribosomes bound to the rough endoplasmic reticulum of the hepatocytes. In contrast, protein destined for intracellular use is synthesized on free polyribosomes rather than bound polyribosomes (Podolsky and Isselbacher, 1998). In this experiment, CCl_4 induced liver injury in mice and causing a decrease in plasma albumin and globulin contents as compared to control. GLE helped to increase in albumin and globulin content in the plasma; thus it was shown that GLE ameliorate the decline in liver synthesis function caused by CCl_4-induced liver injury

The entire study concludes that CCl4 induces hepatic injury leading to abnormal functioning of the liver. CCl4 causes biochemical changes in enzymes of Liver function test as increase in SGPT, SGOT, ALP and bilirubin levels in comparison to control. *Ganoderma lucidium* aqueous extract administration upon the CCl_4 treated group resulted in decreased level of SGPT, SGOT, ALP and bilirubin levels as to the control levels indicates the ameliorative properties. *Ganoderma lucidium* extract also helped to increase the concentration of albumin and globulin content in the plasma.

In conclusion, CCl_4 involves the production of free radicals through its activation by drug-metabolizing enzymes located in the endoplasmic reticulum and CCl4 is capable of causing liver lipid peroxidation, resulting in liver injury. Oral administration of GLE is effective in the reduction of chronic liver injury, probably via a protective effect against hepatocellular necrosis by its free-radical scavenging ability and normalizing the physiology of the body and maintaining the cellular integrity.

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