

Short Communication

Preventive Effect of *Ganoderma lucidum* on Paracetamol-induced Acute Hepatotoxicity in Rats

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Abstract

The preventive effect of *Ganoderma lucidum* on paracetamol-induced acute hepatotoxicity was investigated in Wistar rats in the present study. Hepatotoxicity was induced by oral administration of paracetamol (500 mg/kg of body weight) for the last 7 consecutive days of the dietary regimen *G. lucidum*. The extent of liver damage was determined by assessing the plasma levels of alanine aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total bilirubin (TB) and total plasma total protein (TP). Oral administration of paracetamol significantly increased the plasma levels of AST, ALT, ALP and LDH, suggesting a clear cut hepatotoxicity in these rats. However, the pretreatment of *G. lucidum* (1% powder with basal food) significantly decreased these hepatotoxic indices of ALT, AST, ALP, LDH in the *G. lucidum* plus paracetamol-administered rats nearly to those of the control rats. Plasma total protein levels also ameliorated by the pre-treatment of *G. lucidum*. Thus, the results of the present investigation demonstrate that the *G. lucidum* provides significant hepatoprotective activity against paracetamol-induced acute hepatotoxicity in rats.

Keywords: *Ganoderma lucidum*; Paracetamol; Hepatotoxicity; Liver enzymes.

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1. Introduction

Liver is the largest and functionally crucial organ of human body. It plays an important role in the regulation of the physiological processes, metabolism, synthesis, secretion and storage [1]. Detoxification of a variety of drugs, xenobiotics, environmental pollutants and chemotherapeutic agents occurs in liver [2]. Toxic chemicals (e.g. peroxidized oil, carbontetrachloride, chlorinated hydrocarbons etc.), several drugs (paracetamol,

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isoniazide, certain antibiotics, chemotherapeutics etc.), viral infections, autoimmune challenges and excess alcohol consumption are known to cause liver diseases [3]. Liver disease is still a serious health problem affecting more people worldwide at an alarming rate. About 35 million people are somehow suffering from the liver disease in Bangladesh [4], while liver disease deaths account for 1.6% of total deaths in 2011 [5].

Side effects of conventional synthetic drugs urge scientific community to evaluate complimentary herbal medicines with hepatoprotective activity [6]. *Ganoderma lucidum* (family- Ganodermataceae), a saprophytic edible fungus, is new in Bangladesh. It is long being used in China, Japan and Korea as a traditional or folk medicine for the treatment of a wide variety of diseases [7]. Therefore, the present study was conducted to evaluate whether *G. lucidum* protects against paracetamol-induced acute hepatotoxicity in rats.

2. Materials and Methods

2.1. Collection of mushroom

G. lucidum mushroom was obtained as gift from the Bangladesh Herbal and Nutrition Research Limited (BAHANUR), Savar, Dhaka, Bangladesh. Fresh mushroom was washed in tap water and cut into small pieces. The mushroom was then dried in the Sun and powdered using mechanical grinder. The fine powdered mushroom was then stored in air-proof plastic packets until further use.

2.2. Experimental animals

Twenty-four Wistar adult rats (~46 weeks) weighing about 160~170 g were used in the present investigation. The rats were housed in plastic cages under hygienic conditions at $25\pm 2^{\circ}\text{C}$ and 12 h light-darkness cycles (light 8.00-20.00 h; dark 20.00- 8.00 h). Animals had free access to feed and tap water throughout the experiment. Experimental rats were randomly divided into four groups: Untreated Control Group (UCG), orally received sterile water; Paracetamol Treated Group (PTG) orally received paracetamol at a dose of 500 mg/kg body weight; *G. lucidum* Treated Group (GTG) received 1% *G. lucidum* powder mixed with basal food; Co-treatment Group (CTG) orally received 1% *G. lucidum* powder (mixed with basal food) plus paracetamol at a dose of 500 mg/kg body weight. Feeding of *G. lucidum* was continued for 30 days, while acute hepatotoxicity was induced by oral administration of rats with paracetamol during the last 7-consecutive days of the 30-days *G. lucidum* feeding regimen. The animals were cared and handled according to ethical norms approved by Bangladesh Association for Laboratory Animal Science.

2.3. Assessment of liver function

Blood was collected from the inferior vena cava into heparinized syringe under anesthesia at the end of the experimental session. The blood was then centrifuged at 1600 rpm for 10 min at room temperature to separate plasma. The plasma was used to estimate liver marker enzymes such as alanine aminotransaminase (ALT), aspartate aminotransaminase

(AST), γ -glutamyl transferase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and also plasma total protein (TP), total bilirubin (TB) using available commercial diagnostic kits. These estimations were done according to the standard procedures given along with the kits purchased.

2.4. Statistical analyses

The results are expressed as mean \pm SEM (Standard error of mean). The analyses of all parameters for inter-group differences were performed by one-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Tests. The statistical program used was GRAPHPAD PRISM® (version 4.00; GraphPad Software Inc., San Diego, CA, USA). A level of $P < 0.05$ was considered statistically significant.

3. Results and Discussion

The results of hepatoprotective effect of *G. lucidum* against paracetamol-induced acute hepatotoxicity have been summarized in Table 1. Oral administration of paracetamol significantly increased the plasma levels of structural integrity-related hepatic enzyme markers including ALT, AST, ALP and LDH. Feeding of *G. lucidum* alone showed no significant alterations in the plasma levels of these hepatic marker enzymes. Co-treatment of *G. lucidum* with paracetamol significantly decreased the plasma levels of ALT, AST, ALP, and LDH in the *G. lucidum*+paracetamol rats nearly to those of the normal control rats.

Table 1. Effect of *Ganoderma lucidum* on the hepatic enzymatic markers.

Parameters	UCG	PTG	GTG	CTG
ALT (U/L)	38.8 \pm 1.5 ^a	49 \pm 1.46 ^b	33.7 \pm 0.9 ^a	40.3 \pm 1.5 ^a
AST (U/L)	99.8 \pm 2.6 ^a	120.7 \pm 2.87 ^b	90 \pm 1.5 ^a	101.3 \pm 2.7 ^a
ALP (U/L)	179.3 \pm 2.9 ^a	198.8 \pm 2.71 ^b	177.7 \pm 3.5 ^a	185.8 \pm 2.5 ^a
LDH (U/L)	556.7 \pm 11.3 ^a	830.3 \pm 11.3 ^b	539.3 \pm 15.6 ^a	650.2 \pm 17.9 ^c

Results are expressed as mean \pm SEM. UCG (untreated control group), orally received sterile water; PTG (paracetamol treated group), orally received paracetamol; GTG (*G. lucidum* treated group), received 1% *G. lucidum* powder; CTG (Co-treatment group), orally received 1% *G. lucidum* powder plus paracetamol. Data are analyzed by ANOVA followed by Tukey's Multiple Comparison Tests as post hoc comparison. Values in the same row that do not share common superscripts are significantly different at $P < 0.05$.

In the present study, the hepatoprotective effect of *G. lucidum* was investigated by using paracetamol for the induction of liver damage. Normally 95% of paracetamol is metabolized in liver by glucuronidation and sulfation. Only 5% of paracetamol is metabolized by several of the cytochrome P₄₅₀ enzymes system into the reactive intermediate N-acetyl- p-benzoquinone imine (NAPQI) [8]. NAPQI is detoxified at the

expense of reduced glutathione (GSH). Thus, the overdose of acetaminophen depletes glutathione stores, leading to accumulation of NAPQI, mitochondrial dysfunction and the development of acute hepatic necrosis [9].

Effect of *G. lucidum* feeding on structural integrity of liver was evaluated by the assessments of the levels of ALT, AST, ALP and LDH on serum. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the most specific indicators of hepatic injury and represent markers of hepatocellular necrosis [10]. Oral administration of paracetamol significantly increased the plasma levels of aminotransaminases (ALT and AST), while pretreatment of *G. lucidum* significantly decreased the levels of these enzymes in the *G. lucidum*+paracetamol rats to those of the control rats. Rises in these enzymes correspond to hepatocyte necrosis or abnormal membrane permeability, while depletion indicates healing of hepatic parenchyma [11]. Therefore, we speculate that the decreases of the elevated plasma levels of these enzymes might occur by the prevention of the leakages of intracellular enzymes in the *G. lucidum*+paracetamol rats. Liver function associated hepatic enzymatic markers, including GTT, TB and TP also were measured in plasma to evaluate whether *G. lucidum* feeding has any effect on liver function under acute hepatotoxicity (Fig. 1). Plasma levels of these enzymes were not significantly altered as a result of feeding of *G. lucidum* alone. Oral administration of paracetamol increased the levels of GTT ($P>0.05$) while decreased the plasma level of TB ($P>0.05$) and TP ($P<0.05$). *G. lucidum* and paracetamol co-treatment decreased plasma levels of GGT ($P>0.05$) concomitantly with increases in TB ($P>0.05$) and TP ($P<0.05$) levels.

Plasma GGT, bilirubin and total protein levels are related to the functions of hepatic cells [1]. The rise in the bilirubin is one of the most useful clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte [12]. Plasma protein provides information about the severity of the parenchyma liver disease or necrosis as well as synthetic capacity [13]. Activity of the microsomal enzyme GGT reflects the extent of intracellular oxidative stress in drug and xenobiotics detoxification function [14, 15]. Oral administration of paracetamol significantly depressed the synthetic capacity of liver, as indicated by the decreased level of TP (Fig 1.B). The secretory and detoxification function was also reduced as a result of paracetamol administration, as reflected by the decrease in the level of TB (Fig 1.A) and increase in the level of GTT (Fig. 1.C), respectively. But, co-treatment of *G. lucidum* significantly reversed the levels of GTT, TB and TP nearly to those of the control rats. Therefore, these results suggest that *G. lucidum* probably ameliorates the hepatocellular functions, including haepatobiliary and synthetic function, concomitantly with the improvement of structural integrity, at least partially, though not prominently.

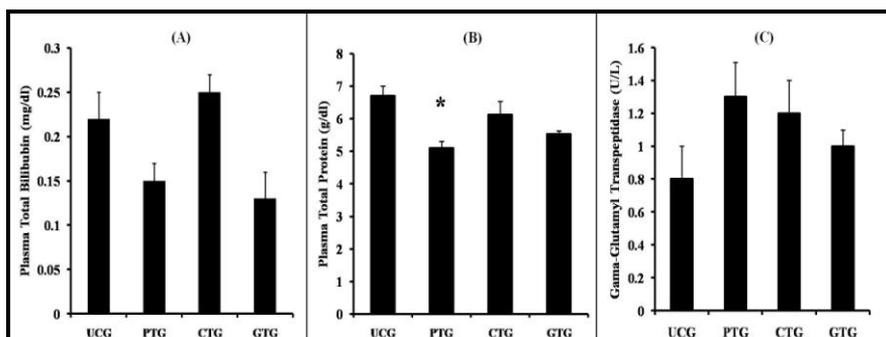


Fig. 1. Effect of *G. lucidum* on liver function associated enzymatic markers. Results are expressed as mean \pm SEM. UCG (Untreated Control Group), orally received sterile water; PTG (Paracetamol Treated Group), orally received paracetamol; GTG (*G. lucidum* Treated Group) received 1% *G. lucidum* powder; CTG (Co-treatment Group) orally received 1% *G. lucidum* powder plus paracetamol. Data are analyzed by ANOVA followed by Tukey's Multiple Comparison Tests as post hoc comparison. '*' indicates statistically significant ($P < 0.05$) difference with respect to UCG (Untreated Control Group).

4. Conclusion

To our knowledge, this is the first study to describe the hepatoprotective effect of *G. lucidum* as raw powder form, while several other studies showed the hepatoprotective effect of *G. lucidum* by using its extract. The extraction solvent property is known to have significant impact on the therapeutic property while the intake of raw powder is cheap, simple and easy [16]. Thus, the results of the present study again demonstrate that the *G. lucidum* has significant hepatoprotective activity against paracetamol-induced acute hepatotoxicity in rats. Further investigations are suggested to explore the elaborated mechanism of hepatoprotection with disease related therapeutic intervention.

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