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Research Article

Hepatoprotective Properties of Piperine in Experimentally Hepatotoxic Male Rats Exposed to Carbon Tetrachloride (CCl_4)

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Abstract | The aim of the current study was to evaluate the hepatoprotective effects of piperine against carbon tetrachloride (CCl_4)-induced liver injury in male rats. To assess the impact of piperine therapy on liver damage caused by CCl_4, 24 male rats were divided into four groups, with six rats in each group. Group 1 served as the control and received oral administration of distilled water. Group 2 underwent hepatotoxicity induction by administering CCl_4 at a dose of 0.8 ml/kg intraperitoneally. Group 3 received an oral dose of piperine at a concentration of 30 mg/kg body weight (bw) for 3 weeks. Finally, Group 4 received both piperine and CCl_4 for 3 weeks. The results showed a significant reduction ($p < 0.05$) in liver enzyme levels following piperine treatment in CCl_4-exposed rats. Additionally, piperine treatment resulted in significant improvements ($p < 0.05$) in lipid profiles, including reductions in total triglycerides, total cholesterol, low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL), while also increasing high-density lipoprotein (HDL) levels. Furthermore, piperine significantly increased ($p < 0.05$) antioxidant enzyme activities, including superoxide dismutase (SOD) and glutathione peroxidase (GPx), compared to the CCl_4-only treated rats. Overall, the findings of this study suggest that piperine is a potent hepatoprotective agent.

Keywords | Piperine, Liver, Rat, CCL_4, Oxidative damage

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INTRODUCTION

Various potent phytomedicines are available on the market in the form of entire plant extracts (Al-ameedi *et al*., 2023). Practitioners have long relied on the synergistic effects of the multiple components in these herbal preparations, whether derived from single herbs or blends, to significantly enhance their medicinal efficacy (Ayad *et al*., 2021). The exact mechanisms by which many herbal preparations, including botanical extracts, exert their effects are not yet fully understood (Paul and Kumar, 2024). However, several studies have indicated that the synergistic effect of combining various herbal extracts can lead to more potent and effective outcomes compared to using a single isolated component at the same dose (Harwansh *et al*., 2024).

Piperine, scientifically known as 1-peperoylpiperidine, is an alkaloid compound found in the fruit of plants from the *Piperaceae* family. Notable examples include black pepper (*Piper nigrum*) and long pepper (*Piper longum*) (Rajput and Panwar, 2024). Plants in this family have been widely used

in traditional medicine and as culinary spices across various cultures. Research has demonstrated that piperine possesses several pharmacological properties, such as antioxidant effects (Haq *et al*., 2021; Joshi *et al*., 2023), anti-inflammatory properties (Tiwari *et al*., 2020; Dhargawe *et al*., 2021), and vasodilatory effects (Rehman *et al*., 2020; Siddiqui *et al*., 2023). Additionally, piperine has shown potential neuroprotective effects against ischemia-reperfusion (Haq *et al*., 2021) and may also offer protection against lead acetate-induced nephrotoxicity (Sudjarwo *et al*., 2017).

Piperine naturally occurs in four isomeric forms (Vaibhav *et al*., 2012). However, only two isomers of piperine exhibit both pungency and biological activity, distinguishing them from the other two isomers. Carbon tetrachloride (CCl_4) is commonly used in liver studies due to its well-established role in inducing liver fibrosis and damage (Siddiqui *et al*., 2023). Liver damage induced by CCl_4 occurs through the generation of reactive oxygen species (ROS), triggered by CYP2E1 enzymes (Al-ameedi *et al*., 2016). Piperine helps mitigate liver damage by inhibiting the function of NF-κB and stimulating Nrf2, which in turn inhibits the synthesis of pro-inflammatory cytokines and mediators. Additionally, piperine activates the antioxidant response system, aiding in the removal of ROS and reducing lipid peroxidation (Rehman *et al*., 2020).

Beyond its hepatoprotective properties, piperine has a diverse array of biological activities, including antidepressant, anxiolytic, antithyroid, immune-stimulating properties against malignancies, anti-inflammatory, and anti-metastatic effects (Sudjarwo *et al*., 2017). Moreover, this alkaloid enhances the bioavailability of several drugs and provides hepato-protection against toxicity (Arora *et al*., 2023). The present investigation aimed to assess the efficacy of piperine in mitigating oxidative damage associated with hepatotoxicity and liver fibrosis induced by CCl_4.

MATERIALS AND METHODS

Chemicals

Piperine and CCl_4 were purchased from highly qualified supplier companies.

Animals

A total of 32 male Wistar rats, with weights ranging from 200 to 250 g, were used in the study. Each cage housed four animals and maintained controlled circumstances, including a steady ambient temperature of 22 ± 2 °C and a reverse light-dark cycle of 12 hours. Complimentary food and beverages were available.

Ethical Approval

The experimental design and methodologies used in this work were carefully assessed and approved to meet the animal welfare ethical criteria set by the Scientific Committee of the Faculty of Veterinary Medicine at Al-Qasim Green University.

Experimental Design

Group I: Six rats were administered 0.8 ml/kg.bw of distilled water daily by intragastric intubation and were designated as the control negative control.

Group II: 6 rats injected CCL_4 0.8 ml/kg intrapretonially, for 4 weeks (2 injection in each week) to induce hepatotoxicity (Wali *et al*., 2021).

Group III: 6 rats were administered CCL_4 (as in the preceding group) together with piperine by intragastric intubation at a daily dosage of 30mg/kg for a duration of 4 weeks.

Group IV: 6 rats were given 30 mg/kg.bw of piperine every day for four weeks through an intragastric tube.

Evaluation of Liver Enzyme

The levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) in the serum were measured using commercially available kits (Elab Science, China).

Evaluation of Lipid Profile

Serum samples were collected and analyzed for lipid profiles. Using a kit provided by Elabscience (China), the concentrations of triglycerides, high-density lipoproteins (HDL), and total cholesterol were measured. The low-density lipoprotein cholesterol (LDL-C) was determined by subtracting the concentration of high-density lipoprotein cholesterol (HDL-C) and very-low-density lipoprotein cholesterol (VLDL-C) from the total cholesterol.

Assessment of Antioxidant Biomarkers

The activity of superoxide dismutase (SOD) was assessed using the methodology by Marklund and Marklund (1974). The measurement of glutathione peroxidase (GPx) activity was conducted using the method outlined by Mohandas *et al*. (1984).

Statistical Analysis

The statistical approach used to analyze differences between groups was one-way analysis of variance (ANOVA). Comparisons were considered statistically significant at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Liver Enzymes

The animals exposed to CCl_4 exhibited a statistically sig-

nificant increase ($p < 0.05$) in the levels of liver enzymes compared to the control group. CCl_4 induces hepatotoxicity by promoting the release of liver enzymes into the bloodstream. However, administration of 30 mg/kg body weight (bw) of piperine resulted in a significant decrease ($p<0.05$) in the activity of liver enzymes, as shown in Figure 1.

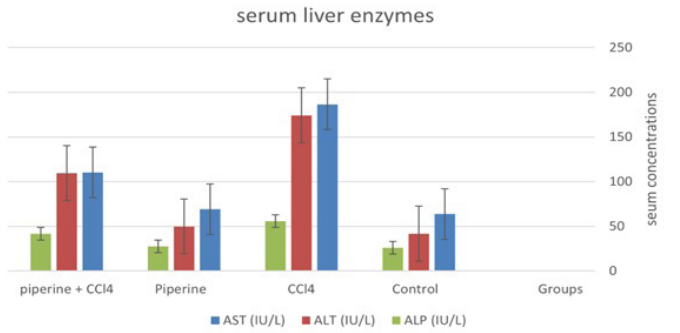

Figure 1: Levels of liver enzymes in different groups of the study.

Parameters of Lipid Profiles

The lipid profile showed a significant improvement ($p < 0.05$), except for HDL, which decreased following CCl_4 treatment compared to the control group, as shown in Figure 2. Administration of piperine at a dosage of 30 mg/kg significantly reduced ($p < 0.05$) the lipid profile, except for HDL, which exhibited an increasing trend.

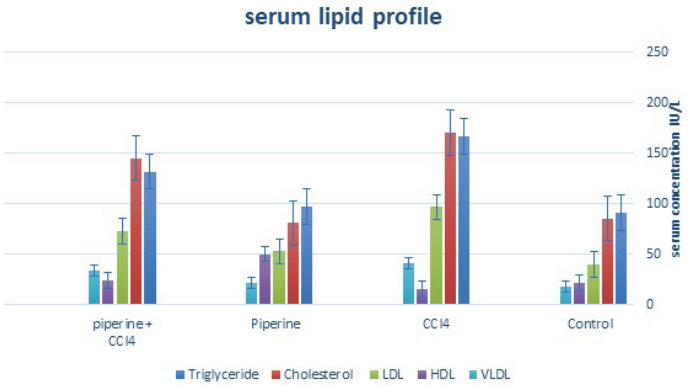

Figure 2: Levels of lipid profiles in different groups of the study.

Antioxidant biomarkers

The study investigated the protective effect of piperine on antioxidant enzyme activity. As shown in Figure 3, mice treated with piperine exhibited a statistically significant increase ($p < 0.05$) in antioxidant enzymatic activity compared to those exposed to CCl_4. The administration of piperine restored antioxidant enzyme activity to baseline levels.

The hepatotoxic effects of CCl_4 are primarily attributed to the generation of harmful free radicals, specifically the trichloromethyl radical (CCl_3) and the trichloromethyl peroxy radical (CCl_3O_2) (Al-ameedi *et al.*, 2016; Biswas *et al.*, 2021). Liver enzymes serve as reliable biomarkers for assessing both the rate and extent of liver damage. Elevated levels of these enzymes in the bloodstream are directly correlated with liver injury (Saha *et al.*, 2019). In our study, we observed clear evidence of hepatic damage in Wistar rats exposed to CCl_4. The significant increase in the levels of AST, ALP, and ALT ($p \leq 0.05$) indicates that CCl_4 acts as both an inflammatory and hepatotoxic agent (Bahceci-oglu *et al.*, 2008).

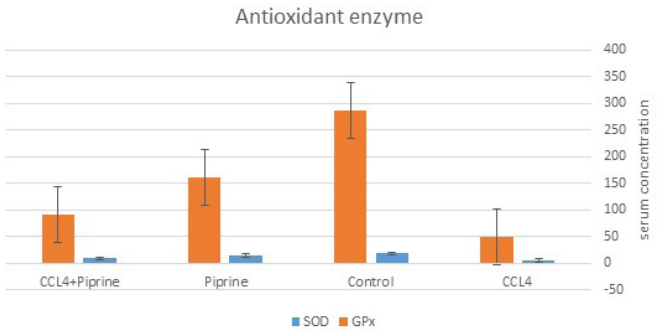

Figure 3: Levels of antioxidant enzymes in different groups of the study.

The administration of piperine effectively reduced the elevated liver enzyme levels, as shown in Figure 1. This suggests that piperine may protect hepatocytes from damage induced by CCl_4. The protective effect could involve maintaining the structural integrity of hepatocyte membranes and preventing the release of enzymes. Our findings are consistent with several studies that report the hepatoprotective effects of piperine (Desai *et al.*, 2008; Biswas *et al.*, 2021; Wali *et al.*, 2021).

Additionally, rats treated with CCl_4 exhibited a significant ($p \leq 0.05$) increase in their lipid profile, including total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL), along with a decrease in high-density lipoprotein (HDL) levels. However, oral administration of piperine resulted in the restoration of the lipid profile, as shown in Figure 2. This aligns with a study by Tu *et al.* (2014), which found that a high-cholesterol diet led to an increase in TC levels and the TC/HDL ratio, though methyl piperine effectively reduced this increase.

Superoxide dismutase (SOD) activity is a reliable biomarker for hepato-renal damage, as it neutralizes superoxide anions and produces hydrogen peroxide, which helps alleviate harmful effects. Hepatic cells rely on the combined actions of glutathione peroxidase (GPx) and catalase to eliminate peroxides during detoxification processes (Solanki and Jain, 2011; Wali *et al.*, 2021). Antioxidant enzymes, such as GPx and SOD, are essential components of the body's internal defense mechanisms against oxidative stress. Our

study found a significant decrease in SOD and GPx levels in rats treated with CCl_4. However, the administration of piperine led to a notable recovery of these enzymes, as depicted in Figure 3. This suggests that piperine plays a critical role in promoting the efficient removal of reactive oxygen species (ROS) produced by the P450 bio-activation induced by CCl_4.

Our findings are in line with research by Abdel-Daim *et al.* (2019), which demonstrated the antioxidant potential of piperine. Furthermore, piperine has been shown to enhance the bioavailability of both synthetic drugs and bioactive phytochemicals. For instance, Bi *et al.* (2019) used piperine to improve the absorption of silybin, a flavonoid known for its liver-protective properties, in a CCl_4-induced liver damage model. Additionally, research by Jin *et al.* (2018) found that the bioavailability of ginsenoside Rh_2, a compound known to modulate the immune system, was significantly enhanced when co-administered with piperine.

CONCLUSIONS AND RECOMMENDATIONS

The current study highlighted the hepatoprotective potential of piperine in preventing hepatotoxicity induced by CCl_4. Piperine exerts a protective effect by suppressing the excessive production of liver damage indicators and enhancing antioxidant biomarkers. It also prevents liver cell death, reduces free radical levels, and boosts antioxidant activity. Therefore, our study suggests that piperine could be a potent remedy for the prevention of liver damage.

NOVELTY STATEMENTS

The novelty of this research lies in the investigation of piperine for its hepatoprotective effects. The objective of this study was to elucidate the hepatoprotective potential of piperine by analyzing the levels of certain enzymes that are elevated during CCl_4-induced hepatotoxicity.

AUTHOR'S CONTRIBUTIONS

Mohammed A. Aboktifa conceptualized and designed the study Ali I. Al-ameedi and Zahraa M Ayad participated in the investigation and the drafting of the paper as well as, analyzed and interpreted the data. Ahmed Hamzah Mosa revised the paper critically for intellectual content and approved the final version of the paper to be published. All authors agree to be accountable for all aspects of the work.

Conflict of Interest

The authors have declared no conflict of interest.

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