



## Original Paper

# Hepatotoxic Effects of Sub-chronic Paracetamol Administration on Serum ALT and AST Levels in Albino Rats in Kirkuk City, Iraq

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## ABSTRACT

The liver is the principal site of drug metabolism and biotransformation and is therefore vulnerable to toxic injury by xenobiotics. Paracetamol (PCM) is one of the most frequently used analgesic and antipyretic agents; however, excessive or prolonged use is associated with severe hepatic damage caused by the formation of reactive metabolites. This study aimed to assess the sub-chronic effect of PCM on serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in albino rats and to identify possible sex-related differences. Thirty albino rats of both sexes, aged 53–60 days and weighing 300–390 g, were randomly allocated to a control group ( $n = 10$ ) and a PCM-treated group ( $n = 20$ ). The treated group received PCM orally at 500 mg/kg body weight once daily for 30 days. Serum ALT and AST activities were determined by standard biochemical assays, and the data were analysed by Student's t-test. The study protocol was approved by the Animal Ethics Committee, College of Medicine, University of Kirkuk, Iraq (Approval No. 29 B, 27/3/2023). Serum ALT and AST were significantly higher in the treated group than in the control group ( $P \leq 0.05$ ), with very large effect sizes (Cohen's  $d = 8.53$  and  $7.75$ , respectively). In both groups, male rats showed higher enzyme activities than females. Sub-chronic PCM administration therefore produces marked hepatocellular damage, reflected by increased ALT and AST activities, and the injury is more pronounced in males than in females.

**KEYWORDS:** ALT, AST, Hepatotoxicity, Paracetamol (PCM), Sex differences, Albino rats.

## 1-INTRODUCTION

In addition to being the principal organ of drug metabolism and detoxification, the liver regulates numerous physiological processes within the body. It is therefore highly susceptible to injury caused by exogenous chemicals and medications [1]. Changes in serum biochemical markers, particularly alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are widely used to assess the degree of hepatic damage [2].

Paracetamol (PCM; acetaminophen) is among the most frequently used analgesic and antipyretic agents worldwide [3], owing to its high efficacy and relative safety at therapeutic doses. Nevertheless, overdose and prolonged use of this compound have repeatedly been identified as the main cause of severe hepatotoxicity and acute liver injury [4]. The hepatotoxicity of PCM arises from its bioactivation in hepatocytes to the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) [5]. Under normal conditions NAPQI is detoxified by conjugation with hepatic glutathione; however, depletion of glutathione during excessive exposure allows NAPQI to bind covalently to cellular proteins, leading to oxidative stress, mitochondrial dysfunction and hepatocellular necrosis [6].

Previous studies have shown that serum ALT and AST are sensitive indicators of hepatotoxicity induced by a variety of compounds, including PCM [7,8]. In addition, a growing body of evidence indicates that biological sex can influence susceptibility to drug-induced liver injury through differences in hormonal regulation and drug metabolism [9].

The aim of the present study was therefore to examine the sub-chronic hepatotoxic effect of PCM on serum ALT and AST activities in male and female albino rats.

## 2-MATERIALS AND METHODS

### 2.1. Experimental animals

The study was conducted between January and February 2026. Thirty adult albino rats of both sexes, aged 53–60 days and weighing 300–390 g, were obtained from a certified animal house. The animals were housed under standard laboratory conditions at 22–25 °C with a 12 h light/dark cycle and free access to standard feed and water.

### 2.2. Experimental design

The rats were randomly allocated to two groups. The control group (n = 10; 5 males and 5 females) received distilled water orally by gastric gavage once daily. The PCM-treated group (n = 20; 10 males and 10 females) received PCM orally by gastric gavage at a dose of 500 mg/kg body weight once daily for 30 consecutive days. The dose of 500 mg/kg was selected on the basis of previous experimental studies demonstrating its ability to induce hepatotoxicity in rats; it was chosen to evaluate the toxic effects of repeated PCM administration on serum ALT and AST rather than to simulate therapeutic exposure [10].

### 2.3. Sample collection and biochemical analysis

At the end of the experimental period, blood samples were collected by retro-orbital puncture under light anaesthesia. Serum was separated by centrifugation and used for biochemical analysis. Serum ALT and AST activities were measured using standard commercial kits according to the manufacturer's instructions.

### 2.4. Statistical analysis

Data are expressed as mean  $\pm$  standard deviation (SD). Differences between the treated and control groups, and between males and females within each group, were analysed by Student's t-test using SPSS software. Cohen's d effect size and the 95% confidence interval (CI) of the mean difference were calculated for the principal comparisons. Differences were considered statistically significant at  $P \leq 0.05$ .

### 2.5. Ethical approval

All experimental procedures involving animals were conducted in accordance with the institutional guidelines for the care and use of laboratory animals. The study protocol was approved by the Animal Ethics Committee, College of Medicine, University of Kirkuk, Iraq (Approval No. 29 B, 27/3/2023).

## 3-RESULTS

### 3.1. Effect of PCM on serum ALT and AST

Sub-chronic PCM administration produced a highly significant increase in serum ALT compared with the control group (Table 1). Mean ALT activity rose from  $39.1 \pm 4.3$  U/L in the control group to  $202.0 \pm 23.0$  U/L in the treated group ( $P < 0.001$ ). The magnitude of this difference was very large (Cohen's d = 8.53), with a mean difference of 162.9 U/L (95% CI 151.85–173.95 U/L).

Similarly, serum AST was significantly elevated in the treated group compared with the control group (Table 1). Mean AST activity increased from  $101.2 \pm 10.0$  U/L to  $329.0 \pm 35.0$  U/L ( $P < 0.001$ ), again with a very large effect size (Cohen's d = 7.75) and a mean difference of 227.8 U/L (95% CI 210.39–245.21 U/L).

**Table (1) Serum ALT and AST activities (U/L) in control and PCM-treated rats**

| Parameter | Control (n = 10) | PCM-treated (n = 20) | Mean difference (95% CI) | Cohen's d | P-value |
|-----------|------------------|----------------------|--------------------------|-----------|---------|
| ALT (U/L) | $39.1 \pm 4.3$   | $202.0 \pm 23.0$     | 162.9 (151.85–173.95)    | 8.53      | < 0.001 |
| AST (U/L) | $101.2 \pm 10.0$ | $329.0 \pm 35.0$     | 227.8 (210.39–245.21)    | 7.75      | < 0.001 |

Data are presented as mean  $\pm$  SD. ALT: alanine aminotransferase; AST: aspartate aminotransferase; CI: confidence interval; PCM: paracetamol. P-values refer to the comparison between the control and treated groups (Student's t-test).

### 3.2. Sex-related differences

Serum ALT was higher in males than in females in both groups (Table 2). In the control group, males showed higher ALT values ( $43.2 \pm 2.6$  U/L) than females ( $35.0 \pm 2.0$  U/L;  $P = 0.01$ ). In the treated group, ALT remained higher in males ( $222.5 \pm 13.5$  U/L) than in females ( $181.5 \pm 8.5$  U/L;  $P < 0.001$ ).

A comparable pattern was observed for AST (Table 2). In the control group, AST was higher in males ( $111.6 \pm 5.0$  U/L) than in females ( $94.8 \pm 3.6$  U/L;  $P = 0.02$ ), and in the treated group male AST activity ( $358.5 \pm 18.0$  U/L) exceeded that of females ( $299.5 \pm 12.5$  U/L;  $P < 0.001$ ). Thus, the PCM-induced elevation of both enzymes was more pronounced in male rats.

**Table (2) Serum ALT and AST activities (U/L) in male and female rats of the control and PCM-treated groups**

| Parameter | Group                    | Male             | Female           | P-value |
|-----------|--------------------------|------------------|------------------|---------|
| ALT (U/L) | Control (5 M, 5 F)       | $43.2 \pm 2.6$   | $35.0 \pm 2.0$   | 0.01    |
|           | PCM-treated (10 M, 10 F) | $222.5 \pm 13.5$ | $181.5 \pm 8.5$  | < 0.001 |
| AST (U/L) | Control (5 M, 5 F)       | $111.6 \pm 5.0$  | $94.8 \pm 3.6$   | 0.02    |
|           | PCM-treated (10 M, 10 F) | $358.5 \pm 18.0$ | $299.5 \pm 12.5$ | < 0.001 |

Data are presented as mean  $\pm$  SD. M: male; F: female; ALT: alanine aminotransferase; AST: aspartate aminotransferase; PCM: paracetamol. P-values refer to the comparison between males and females within each group (Student's t-test).

### 4-DISCUSSION

In the present study, 30 days of PCM administration produced a marked increase in serum ALT and AST activities, indicating hepatocellular injury in the treated animals compared with controls. Damage to hepatocytes leads to leakage of these intracellular enzymes into the bloodstream. The findings agree with previous reports in which PCM-induced liver toxicity in experimental animal models was accompanied by significantly elevated serum aminotransferases [11,12].

Elevated ALT is a reliable indicator of liver damage because the enzyme is located predominantly in hepatocytes [13], whereas elevated AST, which is present in large amounts in the liver as well as in other tissues, points to more severe hepatic injury [14]. Together, these findings indicate that prolonged administration of PCM at a high dose causes substantial hepatic damage [15].

The mechanism underlying PCM hepatotoxicity involves its bioactivation in the liver, mainly by cytochrome P450 2E1 (CYP2E1), to the reactive metabolite NAPQI [16]. NAPQI is normally neutralised by glutathione; with excessive exposure, glutathione stores become depleted, allowing NAPQI to induce oxidative stress and mitochondrial dysfunction that culminate in hepatocyte death [17].

A sex-related difference in ALT and AST activities was also observed: males in both the control and treated groups had significantly higher enzyme activities than females, in line with the male-biased susceptibility to acute liver injury described in experimental models [18]. This difference may be attributed to hormonal influences and to differences in antioxidant defence between the sexes, which could increase male susceptibility to PCM-induced liver injury [19,20]. Experimental evidence suggests that male rats exhibit greater CYP-mediated bioactivation of PCM, leading to increased NAPQI formation; combined with reduced glutathione availability during toxic exposure, this may contribute to the greater vulnerability of males to PCM-induced hepatocellular injury [21]. Oestrogen, in contrast, may enhance glutathione synthesis and antioxidant defence, which could partly explain the lower susceptibility of female rats [22].

Several limitations of this study should be acknowledged. Histopathological examination of liver tissue was not performed to confirm the biochemical findings, and oxidative stress biomarkers such as malondialdehyde (MDA), reduced glutathione (GSH) and superoxide dismutase (SOD) were not evaluated. Only a single dose of PCM (500 mg/kg) and a single treatment duration (30 days) were investigated. In addition, the unequal group allocation (10 control and 20 treated rats) and the absence of an *a priori* sample-size or power calculation may have affected the

statistical precision of the results and limit the generalisability of the findings. Future studies incorporating histopathological assessment, oxidative stress markers, balanced group allocation, *a priori* sample-size calculation and several dose levels and treatment durations are recommended to provide a more comprehensive evaluation of PCM-induced hepatotoxicity.

## CONCLUSION

Sub-chronic administration of PCM at 500 mg/kg for 30 days caused marked hepatic dysfunction in albino rats, as reflected by significantly increased serum ALT and AST activities, and male rats were more susceptible than females to this toxicity. These findings provide preliminary evidence of PCM-induced hepatotoxicity in this experimental model; further studies including histopathological evaluation and oxidative stress biomarkers are recommended to confirm and extend these results.

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