

A significant hepatotoxicity associated with paracetamol overdose in the absence of kidney injury in rabbits

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ABSTRACT

Background: Overdose of paracetamol (PCM) is reported to cause hepatotoxicity and nephrotoxicity, or nephrotoxicity in absence of hepatotoxicity. This study was planned to investigate hepatotoxicity or nephrotoxicity induced by PCM.

Methods: Two groups of rabbits, six rabbits in each were used; control group were treated with normal saline, the second group was treated with PCM 1 g/kg/day orally for 9 days.

Results: PCM lead to a significant rise in serum liver enzymes, aspartate aminotransferase, alanine transaminase, alkaline phosphatase and total bilirubin with an increase in serum level of malondialdehyde (MDA) and reduction in serum glutathione (GSH). MDA level in liver homogenate was also significantly increased. These findings were further confirmed by histopathological changes suggestive of severe liver damage. On the contrary, PCM slightly increased serum creatinine, without changing MDA and GSH in kidney homogenate. Lack of PCM nephrotoxicity was further confirmed by histopathological examination.

Conclusion: PCM overdose produced severe hepatotoxicity without affecting the kidneys of the rabbits

Keywords: Paracetamol, Hepatotoxicity, Nephrotoxicity

INTRODUCTION

Paracetamol (PCM) is a widely prescribed over the counter analgesic and antipyretic medication. In general, PCM is accepted as effective and safe drug in practice, however, at overdose, hepatotoxicity is a commonly reported side-effect.^{1,2} Hepatic injury induced by PCM overdose is well-studied and found related to metabolism of PCM by hepatic cytochrome P-450 to a highly reactive toxic metabolite N-acetyl-p-benzo-quinone-imine (NAPQI).³ In excess of NAPQI, depletion of hepatic glutathione concentration takes place.⁴ In the setting of depleted glutathione (GSH), the parent drug PCM is shifted to cytochrome P-450 resulting in more NAPQI production and more liver injury. PCM induced renal injury and related mechanism, on the other hand, is not well-defined and data describing this effect is limited. Most of these data are inconclusive and based on case reports or small size studies.^{1,5-6} As cytochrome P-450 and reactive metabolite, NAPQI is equally described for PCM induced liver and renal injury, there are substantial

differences between the two organs in the way PCM is metabolized. One of these differences is related to an enzyme found in the medulla of the kidney, prostaglandin endoperoxidase synthetase, which also metabolizes PCM to NAPQI.^{7,8} The cytochrome P-450, which is present in the kidney is slightly different from that in the liver, but the formed metabolite (NAPQI) and its toxic effect on the liver and the kidney are the same.⁹ Based on these differences, the toxicity of PCM on the liver or the kidney may be discrete and may affect one organ more than the other¹⁰ and therefore, the aim of this study was to investigate toxicity produced by high doses of PCM on the liver and the kidney of rabbits.

METHODS

The study protocol was prepared and submitted for approval by the University Ethical Committee. The study was carried out between November 2012 and April 2013.

Animal handling

Sixteen healthy, locally bred, sexually mature male rabbits were obtained from a local market in Basrah, their body weights ranged between 1.5 and 2 kg, and ages between 4 and 6 months. The animals were kept in the animal house of the College of Medicine for acclimatization in an atmosphere controlled for light and constant room temperature. They were allowed for free movement; food and water were supplied ad libitum. Food was restricted to trefoil and lettuce. Carrots were discarded for its antioxidant effect.

Four animals were lost at various stages of the experiment, 2 rabbits suffocated due to wrong administration of PCM into the trachea and 2 rabbits were found dead few days after PCM dosing. 12 rabbits had completed the study.

Preparation of PCM suspension

PCM tablets (Panadol, 500 mg, GSK Company, UK) were purchased from a local market in Basrah city. 30 tablets of PCM were grinded by porcelain mortar, dissolved in 60 ml of distilled water to obtain a suspension of (250 mg/ml). The suspension was then placed in a closed container, shaken manually to obtain a homogenous suspension. A dose of 1 g/kg (4 ml/kg) from the suspension was taken into a syringe and given orally to the rabbits through a nasogastric tube (size 6). A wood tongue depressor with a hole in the middle placed between the teeth of the rabbit was used to facilitate passage of the tube and prevent chewing the tube by the animal.

Experimental design

The animals were divided randomly into two groups (six rabbits in the group).

At the night of the experiment, to ensure fasting condition, the rabbit was transferred into a special constraint cage, which prevented it from eating its dropping.

Group 1 (control group)

The rabbits were treated with 2 ml distilled water orally for 9 days and were sacrificed at day 10 of the experiment.

Group 2 (PCM toxicity group)

The animals in this group were given a single dose of PCM (1 g/kg/day, orally) for 9 consecutive days, then were sacrificed at day 10 of the experiment.

Collection of blood and tissue samples

The rabbits were sacrificed by cervical dislocation at day 10 of the experiment after a short anesthesia with ether. 5-10 ml

of blood was collected directly from the heart transferred into a non-heparinized tube and allowed to clot for 10 mins. The serum was collected after centrifugation at 3000 rpm for 20 mins, divided into two portions, one for immediate measurement of malondialdehyde (MDA), and the other was frozen at -20°C for later measurements of liver enzymes, creatinine and GSH levels. The abdomen of the rabbit was opened; liver and kidneys were removed. Specimens from the liver and kidney were taken for immediate measurements of MDA and GSH. A piece from the liver and the kidney was taken, transferred into 10% formalin for histopathological examination.

Measurement of MDA in the serum, kidney and in liver homogenates

Estimation of serum MDA

A volume of 1 ml of fresh serum was used for measurement of serum MDA using thiobarbituric acid assay of Beuge and Aust (1978).¹¹

Estimation of MDA in kidney and liver homogenates

One g of fresh tissue from the kidney was taken, transferred into a small glass container, homogenized at 6000 rpm for 20 mins in cold phosphate buffer saline (pH=7.4) (using Hiedolph Electrical Homogenizer, Korea) to obtain 10% homogenate. Liver tissue homogenate was prepared by the same procedure. MDA levels in kidney and liver homogenates were estimated as described by Ohkawa et al., (1979).¹²

Measurement of GSH in serum, kidney and in liver homogenate

Homogenates obtained from kidney or liver were prepared from fresh specimens, stored at -20°C until analysis. GSH measurements of all specimens were performed in one run by ELIZA (Huma Reader HS, Germany) using a kit specific for rabbits (Cusabio reagents, Cusabio Laboratories, Daxueyuan Road, Donghu Hi-Tech Development Area, Wuhan, China).

Measurement of liver enzymes

Serum aspartate aminotransferase (S. AST), serum alanine aminotransferase (S. ALT) in serum were estimated using a commercial kit (Randox Diagnostic Reagents, Randox Laboratories, UK). Serum alkaline phosphatase (S. ALP) and serum total bilirubin were estimated by a commercial kit (Biolabo Reagents, Biolabo SA, France).

Measurement of serum creatinine (S. creatinine) and serum urea (S. urea) levels

S. creatinine level was measured spectrophotometrically

using a kit (H54, Human, Germany), and S. urea was measured by a standard method.

Histopathological examination

The specimens were examined at the Department of Pathology and Forensic Medicine, Basrah College of Medicine by an independent histopathologist. The slides were labeled with codes to ensure blindness of the examiner.

Statistical analysis

SPSS version 19 (Statistical Package for the Social Sciences, IBM, NY, USA) was used for statistical analysis. Data were fed into an excel spreadsheet, examined, and then transferred to SPSS data sheet for analysis. A non-parametric Mann–Whitney U-test for independent samples was used for comparing means. The data were presented as mean±standard deviation, $p < 0.05$ was considered significant.

RESULTS

Effect of PCM on liver enzymes

S. AST was significantly increased by 127% in PCM treated rabbits compared with the control group, $p=0.03$. S. ALT, S. ALP were also significantly increased by 140% and 69% respectively (in both $p=0.01$). In spite of the marked increase (115%) in S. total bilirubin from 0.33 ± 0.10 mg/dl in the control group to 0.72 ± 0.44 mg/dl, it did not achieve statistical significance (Table 1).

Effect of PCM on S. urea and S. creatinine

The level of S. urea was not affected and S. creatinine was slightly increased (27%) in PCM treated rabbits, however, the difference was small and obtained marginal statistical significance ($p=0.07$) (Table 1).

Effect of PCM on parameters of oxidative stress in the serum

Effect on S. MDA and serum GSH (S. GSH)

Treatment with PCM has resulted in a significant increase in S. MDA by 200% compared with the control group, $p=0.01$, and a significant reduction in S. GSH by 40% compared with the control group, $p=0.02$ (Table 1).

Effect of PCM on parameters of oxidative stress in kidney and liver homogenates

The level of kidney MDA was slightly and insignificantly increased by 9% in PCM treated group. While in liver homogenate, PCM treatment markedly increased MDA level by 84%, $p=0.002$ (Table 2).

There were small changes in GSH after treatment with PCM both in kidney and liver homogenates. In the kidney, there was a slight increase in GSH (4.7%) in PCM treated group compared with the control group, which did not achieve statistical significance. While, in liver homogenate, the level of GSH was decreased by 22%, the change was so small to obtain statistical significance.

Histopathological examination

There were no histopathological changes in the kidneys of rabbits in the control group. The glomeruli and renal tubules appeared normal (Figure 1a). Similarly, treatment with an overdose of PCM 1 g/kg for 9 days revealed no histopathological changes in the glomeruli and in renal tubules. These findings were seen in all treated rabbits (Figure 1b).

Histopathological examination of all liver specimens of the control group revealed normal liver cells, bile ducts and portal veins (Figure 1c), while marked histopathological changes were seen in all liver specimens of rabbits treated with an overdose of PCM such as infiltration with lymphocytes, bile duct proliferation and sinusoidal dilatation (Figure 1d).

Table 1: Effect of PCM (1 g/kg orally) on liver and renal functions and parameters of oxidative stress in rabbits.

Laboratory measurements	Control	PCM	% change from control	Significance
S. AST (U/I)	11±2.45	25±10.29	+127	$p=0.03$
S. ALT (U/I)	6.66±1.63	16.66±7.20	+140	$p=0.01$
S. ALP (IU/L)	39.3±18.96	66.23±4.81	+69	$p=0.01$
S. total bilirubin (mg/dl)	0.33±0.10	0.72±0.44	+115	$p=0.2$ (NS)
S. urea (mg/dl)	24±0.10	23.7±5	-1.25%	$p=0.9$ (NS)
S. creatinine (mg/dl)	0.67±0.08	0.85±0.19	+27	$p=0.07$ (NS)
S. MDA (μ mol/l)	0.18±0.01	0.55±0.22	+200	$p=0.01$
S. GSH (nmol/ml)	16.5±4.11	9.83±3.00	-40	$p=0.02$

All values are mean±SD, S. AST: Serum aspartate aminotransferase, S. ALT: Serum alanine aminotransferase, S. ALP: Serum alkaline phosphatase, S. MDA: Serum malondialdehyde, S. GSH: Serum glutathione, NS: Not significant, PCM: Paracetamol, NS: Not significant, SD: Standard deviation

Table 2: The effect of PCM (1 g/kg orally) on parameters of oxidative stress in kidney and liver homogenate in rabbits.

Laboratory measurements	Kidney tissue homogenate				Liver tissue homogenate			
	Control	PCM	% change from control	Level of significance	Control	PCM	% change from control	Level of significance
MDA (nmol/mg)	5.22±1.19	5.70±1.50	+9	p=0.6 (NS)	2.14±0.22	3.96±0.82	+84	p=0.002
GSH (nmol/ml)	9.85±1.83	10.31±1.06	+4.7	p=0.6 (NS)	10.5±3	8.14±2.10	-22	p=0.1 (NS)

All values are mean±SD, MDA: Malondialdehyde, GSH: Glutathione, NS: Not significant, PCM: Paracetamol, SD: Standard deviation

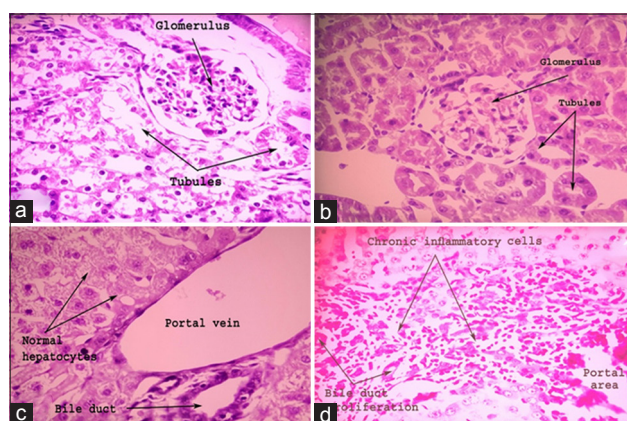


Figure 1: Representative histopathological findings of kidney and liver from a rabbit treated with paracetamol (PCM) 1 g/kg, (a) A kidney from a rabbit treated with normal saline (control) which shows normal glomerulus surrounded by normal renal tubules, (b) kidney after PCM treatment which revealed normal renal tissue, (c) liver (control) group, (d) liver, after PCM treatment which showed significant histopathological changes, chronic inflammatory cells (lymphocytes) and bile duct proliferation can be seen. (H and E, ×40).

DISCUSSION

Reports from clinical studies have shown that PCM commonly induces hepatotoxicity with minimal kidney damage.^{13,14} However, there are many studies reporting PCM nephrotoxicity in absence of hepatotoxicity.^{15,17}

In view of these discrepancies, the present study was undertaken to investigate the toxic effect of an overdose of PCM (1 g/kg) orally on the liver and kidney of rabbits. Among available animal models for PCM induced hepatotoxicity or nephrotoxicity, the rabbit was chosen for the present study since other animals like rats are not sensitive to PCM toxicity. Furthermore, the rabbit was described as a better model for studying PCM induced nephrotoxicity as there are some similarities with human kidney. Larsson et al., 1985⁸ has shown that the microsomes in the medulla of human kidney metabolize PCM through Prostaglandins pathways at rates similar to those present in rabbit kidneys.

In the present study, it was demonstrated that the administration of an overdose of PCM for 9 consecutive days produced

severe liver toxicity in the rabbits as manifested by significant increases in serum liver enzymes, S. MDA and significant reduction in S. GSH. In liver homogenate, PCM produced a significant increase in MDA level with a small reduction in GSH. This pattern of change in MDA and GSH in liver tissue may indicate PCM induced lipid peroxidation and compromised antioxidant defense mechanism.⁴

These changes were further confirmed by severe damage of liver tissue as shown by histopathological examination. Sinusoidal dilatation observed in histopathological examination is consistent with findings reported by McCuskey et al.¹⁸ which may indicate involvement of hepatic microvasculature (sinusoidal endothelial cells) injury which may play a role in the toxicity and event of changes in liver enzymes and parameters of oxidative stress associated with PCM toxicity.

The kidney was not affected by PCM overdose as manifested by little and insignificant effect on S. urea and S. creatinine with slight changes in MDA and GSH levels in kidney homogenate and further confirmed by normal histopathological examination. The results of the present study are in agreement with many clinical case reports in patients ingesting a suicidal overdose of PCM.^{14,19} The mechanism behind absence of PCM induced nephrotoxicity is not clearly understood. It should be noted that nephrotoxicity may appear late in PCM toxicity.¹⁴ However, important findings obtained from case reports revealed that most cases of nephrotoxicity were diagnosed between 2 and 5 days after PCM overdose ingestion,²⁰ with a peak S. creatinine to occur at the 1st week. In the present study, lack of nephrotoxicity, noted after 9 days of PCM treatment cannot definitely rule out late appearance of nephrotoxicity. PCM induced liver damage has been reported to occur within 24 hrs and reached maximum 3 days after ingestion,^{21,22} which indicates that liver damage occurs before renal damage. These findings have some clinical importance since, by the time the liver is severely damaged, renal function is preserved and ready to play a role in elimination of the formed water soluble phase II glucuronide or sulfate conjugate or even the toxic metabolite (NAPQI) which may help minimize the burden on the liver. Moreover, the study may also raise the possibility, though theoretical, of considering donating kidneys for transplantation of patients died from PCM induced liver failure. Such assumption needs further in-depth long-term studies in human to ensure that kidney injury does not appear as a late complication. It can be concluded that PCM induced

hepatotoxicity can occur in the absence of renal damage in the rabbit model.

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Conflict of interest: None declared

Ethical approval: The study protocol was approved by the University Ethical Committee

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