Hepatoprotective effect of cimetidine and ciprofloxacin on paracetamol–induced hepatotoxicity in male albino rats

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Abstract

Paracetamol induced hepatotoxicity results from hepatic enzymatic oxidation of paracetamol to a toxic, electrophilic intermediate. Cimetidine and ciprofloxacin has been shown to inhibit the hepatic oxidation of a number of drugs.The present study was designed to investigate the hepatoprotective effect of cimetidine and ciprofloxacin against paracetamol induced hepatotoxicity in male albino rats. The hepatotoxicity was induced by the intraperitoneal(i.p) administration of paracetamol at a dose of 500 mg/kg in male albino rats. Biochemical analysis of plasma levels of ALT, AST, ALP, LDH and bilirubin were determined as measures of liver function. Our results showed that levels of liver enzymes ALT, AST, ALP, LDH and bilirubin level were significantly enhanced by administration of paracetamol indicating a considerable hepatocellular injury. However, the co-treatment with 150 mg/kg cimetidine i.p 2 hours prior to paracetamol injection revealed a significant attenuation of serum ALT, AST, ALP, LDH and bilirubin levels.

On the other hand, the co-treatment with 25 mg/kg ciprofloxacin i.p 2 hours before paracetamol treatment didn't show any protective effect on liver function. The present results clearly demonstrate the marked protective effect of cimetidine against paracetamol induced hepatotoxicity in rats, effect which could have been mediated via its inhibition of the CYP450 system responsible for the metabolism of paracetamol. Therefore, the production of the toxic metabolite (NAPQI) will be decreased. Further studies are required, by measuring the CYP450 isoforms activities in human liver microsomes to confirm this protective effect.

Key words: hepatoprotective, ALT, AST, ALP, LDH, bilirubin, paracetamol, Cimitidine and ciprofloxacin.

Introduction

Acetaminophen (paracetamol) is a widely used analgesic and antipyretic drug,(1) known to cause hepatotoxicity in experimental animals and humans at high doses.(2) At therapeutic doses, it is mostly converted to nontoxic metabolites via Phase II metabolism by conjugation with sulfate and glucuronide, Furthermore, the mixed function oxidase system cytochrome P450 (CYP450) participates in metabolizing a small proportion of paracetamol, leading to the formation of N-acetyl-p-benzoquinoneimine (NAPQI),(3) a highly reactive intermediate metabolite which is normally detoxified by conjugation with reduced glutathione (GSH) and excreted in the

urine.(4) In cases of paracetamol overdose, the sulfate and glucuronide pathways become saturated, and more paracetamol is shunted to the CYP450 system to produce NAPQI. As a result, glutathione stores are depleted faster than they can be regenerated and the toxic metabolite is left to accumulate.(5) NAPQI therefore remains in its toxic form in the liver and reacts with cellular macromolecules, resulting in fatal hepatic cell death.(6) Moreover, NAPQI may generate reactive oxygen species (ROS) resulting in lipid peroxidation, mitochondrial damage, and ATP depletion.(7)

CYP2E1, CYP2D6, CYP1A2 and CYP3A4 have all been implicated in the metabolism of paracetamol to form NAPQI.(8-10) CYP2E1 has been considered to be a major isoform responsible for the bioactivation of paracetamol in humans.(11) Inhibition of CYP450 enzymes responsible for NAPQI formation might be useful besides N-acetylcysteine treatment in managing paracetamol overdose.(12,13) Experimental evidence indicates the important role of CYP450 inhibitors in the prevention of paracetamol-induced hepatotoxicity. This evidence is in line with the observation that the pretreatment of adult human volunteers by disulfiram, a potent inhibitor of CYP2E1, decreased the formation of NAPQI.(14) Moreover, intraperitoneal administration of a mixture of ketoconazole, isoniazid and caffeine (inhibitor solution), known inhibitors of CYP3A4, CYP2E1 and CYP1A2 ameliorated the development

of paracetamol-induced hepatotoxicity in rats .(15) Therefore, CYP450 inhibitors may exhibit a protective effect against paracetamol-induced hepatotoxicity and these calls for further researches regarding enzyme inhibitors that may be of therapeutic value.

Cimetidine is a H2 blocker that inhibits gastric acid secretion and is largely used in the treatment of peptic ulcers,(16) as well as it is a known inhibitor for CYP3A4 and CYTP2D6 isoenzymes of the CYP450 enzyme system.(17,18) Whereas, ciprofloxacin is a second-generation fluoroquinolone that can treat a number of bacterial infections including bone and joint infections, gastroenteritis, respiratory tract infections, skin infections and genatourinary infections.(19) It is also a potent inhibitor of CYP1A2.(20) The insufficient information regarding the protective role of both cimetidine and ciprofloxacin in paracetamol-induced hepatotoxicity was investigated in this study.

Materials and Methods

Chemicals:

The drugs used in the experiment include paracetamol powder (purchased from Sigma Aldrich), cimetidine injectable solution (STEROP-Belgium), ciprofloxacin injectable solution (ciprofloxacin normon, Laboratorios Normon, S.A, Spain) and liver function tests were carried out in Al- saleem laboratory.

Animals:

Forty-eight male albino Sprague Dawley rats weighing 180-240g were obtained from Animal House at Benghazi University. The animal were housed in plastic cages and kept in controlled room temperature (24 ± 2 °C), with free access to standard rat diet and tap water for survival in a room under 12:12hr light/dark cycle. The cages were cleaned regularly to avoid any chance of infection. Experiments were carried out between 9:00a.m and 2p.m.

Experimental design:

The rats were adapted to laboratory condition for seven days before commencement of the experiment. Animals were divided into six groups, each consist of eight rats and treated as below:

Group 1: animals in this group received the vehicle (distilled water).

Group 2: animals in this group received paracetamol in a dose of (500 mg/kg, i.p).

Group 3: animals in this group received cimetidine in a dose of (150mg/kg, i.p).

Group 4: animals in this group received ciprofloxacin in a dose of (25 mg/kg, i.p).

Group 5: animals in this group received cimetidine in a dose of (150 mg/kg, i.p) 2 hours prior to paracetamol injection (500mg/kg, i.p).

Group 6: animals in this group received ciprofloxacin in a dose of (25 mg/kg, i.p) 2 hours prior to paracetamol injection (500mg/kg, i.p).

Sample collection and biochemical assays

At the end of the experiment, rats were sacrificed by cervical decapitation, and blood samples were collected into plastic test tubes 24h after administration of paracetamol and centrifuged at 10000 rev/min for 2 minutes to separate serum. Serum was preserved in eppendrof tubes and sent to Al-saleem medical laboratory for the determination of the biochemical parameters.

Assessment of serum marker enzymes

Serum alanine aminotrasferase (ALT), aspartate aminotranserase (AST), alkaline phosphates (ALP), lactate dehydrogenase (LDH) and bilirubin were all assayed using Roche diagnostic kits (USA). The absorbance of the reaction was determined at 690 nm by COBAS INTEGRA 400 PLUS.

Statistical analysis:

All data were expressed as mean±S.E.M. for eight animals per group. Statistical data was analyzed by one-way ANOVA. when one-way ANOVA showed significant differences among groups, Tukey's multiple comparisons test was used to determine the specific pairs of groups that were statistically different. P values of less than 0.05 were considered significant. Statistical analysis was

performed using statistical software GraphPad Prism (Version 6.01) for Windows.

Results

Biochemical analysis of plasma levels of ALT, AST, ALP, LDH and bilirubin were determined as measures of liver function. After the exposure of rats to paracetamol only a significant increase in the activities of liver enzymes ALT, AST, ALP, LDH and bilirubin level in comparison with the control group ($P<0.001$) see figures 1,2,3,4 and 5.Whereas, both cimetidine and ciprofloxacin alone didn't have any effect on liver function. They didn't cause increase in the levels of liver enzymes and bilirubin. Moreover, the pretreatment of rats with cimetidine demonstrated a significant decrease in the plasma levels of ALT, AST, ALP, LDH and bilirubin when compared to those of paracetamol treated group ($P< 0.001$). Although the levels of liver enzymes and bilirubin were significantly lower in the cimetidine+paracetamol-treated group compared with the paracetamol group, these levels were still higher than that of the control group see figures 1,2,3,4 and 5.

On the other hand, co-treatment of paracetamol with ciprofloxacin didn't decrease the plasma levels of liver enzymes and bilirubin. However, cimetidine induced marked hepatoprotective effect as shown in table-1.

Table 1: Effects of different treatments of paracetamol, cimetidine and ciprofloxacin alone and in combination on liver enzymes level.

Liver enzyme	Control	Paracetamol	Cimetidine	Ciprofloxacin	Paracetamol + Cimetidine	Paracetamol + Ciprofloxacin
ALT	56.67±5.48	172.5±5.98***	59.67±4.36	57.5±3.46	79.5±3.30###	171.17±6.06
AST	179.83±6.24	568.67±35.98***	184.5±4.74	176.83±6.87	260.67±26.18###	570.83±22.89
LDH	696±44.16	1410±79.13***	660.67±52.27	711.5±44.36	824±32.80###	1398.5±76.42
ALP	12.33±1.84	48.67±4.91***	16±2.86	14.67±1.84	22.33±2.50###	45.5±1.54
Bilirubin	0.46±0.04	2.06±0.05***	0.52±0.05	0.51±0.06	0.85±0.06###	2.02±0.04

Values are expressed as mean±SEM.

*** (P<0.001) denotes significant difference vs. control values.

(P<0.001) denotes significant difference vs. paracetamol values.

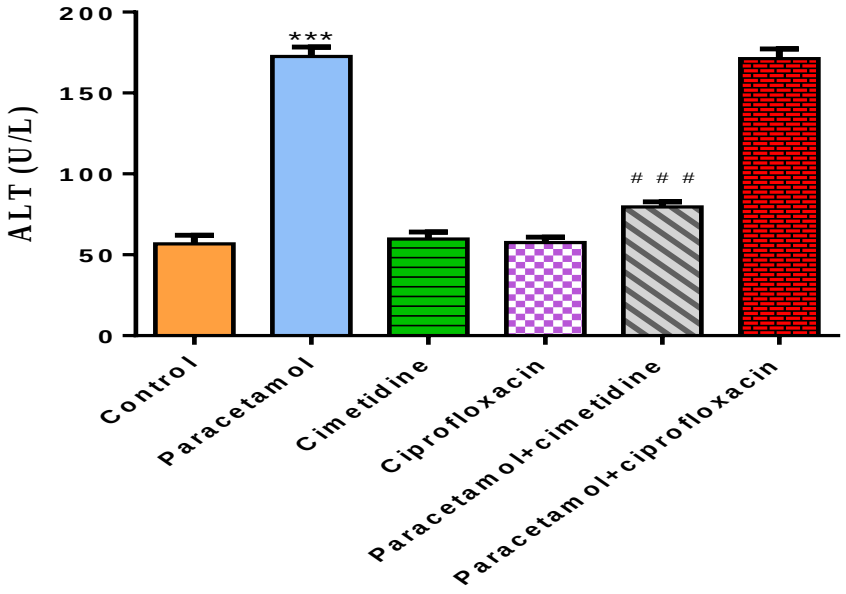

Figure 1. Showed the effect of paracetamol, cimetidine and ciprofloxacin on the liver ALT level. Values are expressed as mean±SEM. *** ($P<0.001$) denotes significant difference vs. control values. # # # ($P<0.001$) denotes significant difference vs. paracetamol values.

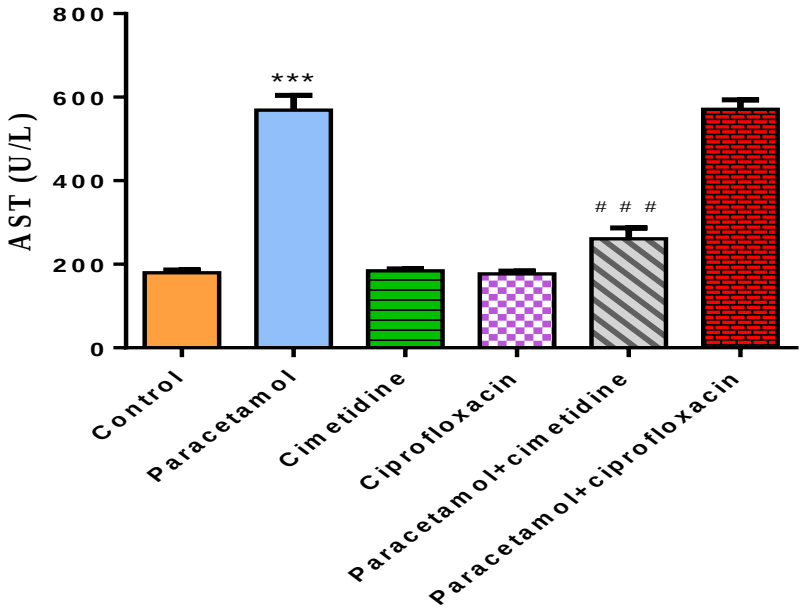

Figure 2. Showed the effect of paracetamol, cimetidine and ciprofloxacin on the liver AST level. Values are expressed as mean±SEM. *** ($P<0.001$) denotes significant difference vs. control values. # # # ($P<0.001$) denotes significant difference vs. paracetamol values.

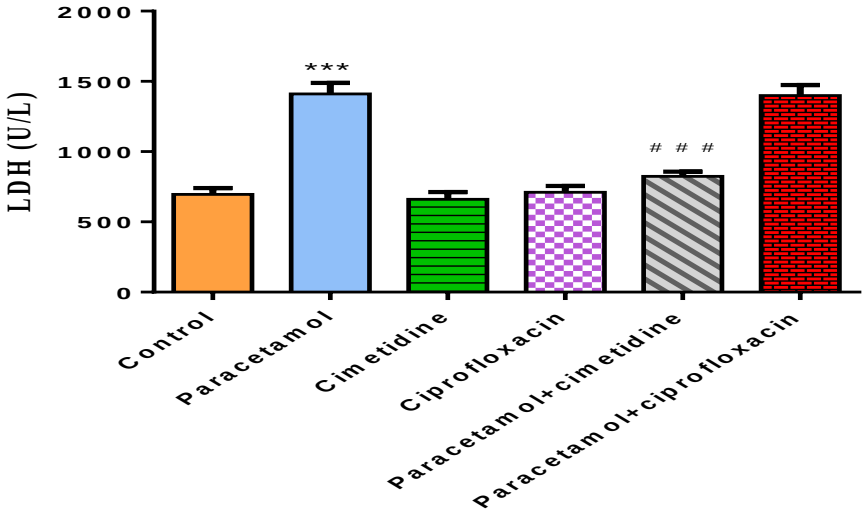

Figure 3. Showed the effect of paracetamol, cimetidine and ciprofloxacin on the liver LDH level. Values are expressed as mean±SEM. *** ($P<0.001$) denotes significant difference vs. control values. # # # ($P<0.001$) denotes significant difference vs. paracetamol values.

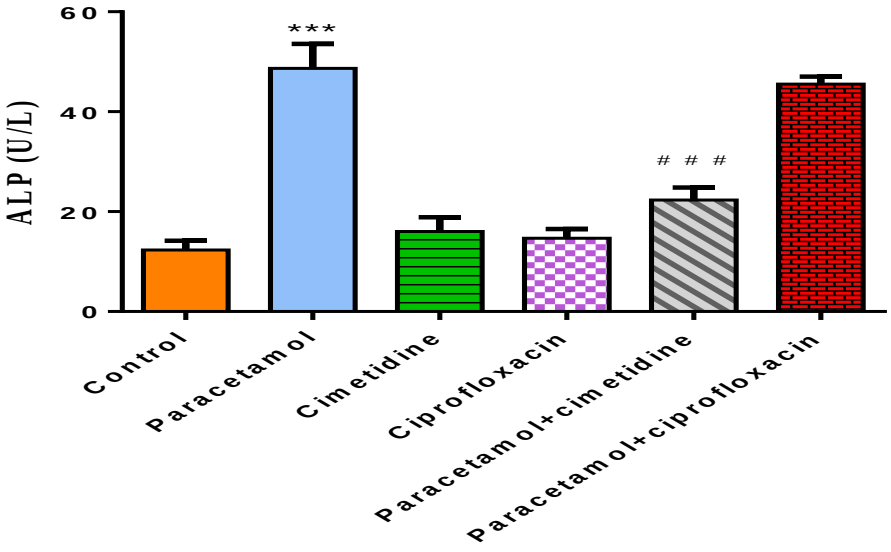

Figure 4. Showed the effect of paracetamol, cimetidine and ciprofloxacin on the liver ALP level. Values are expressed as mean±SEM. *** ($P<0.001$) denotes significant difference vs. control values. # # # ($P<0.001$) denotes significant difference vs. paracetamol values.

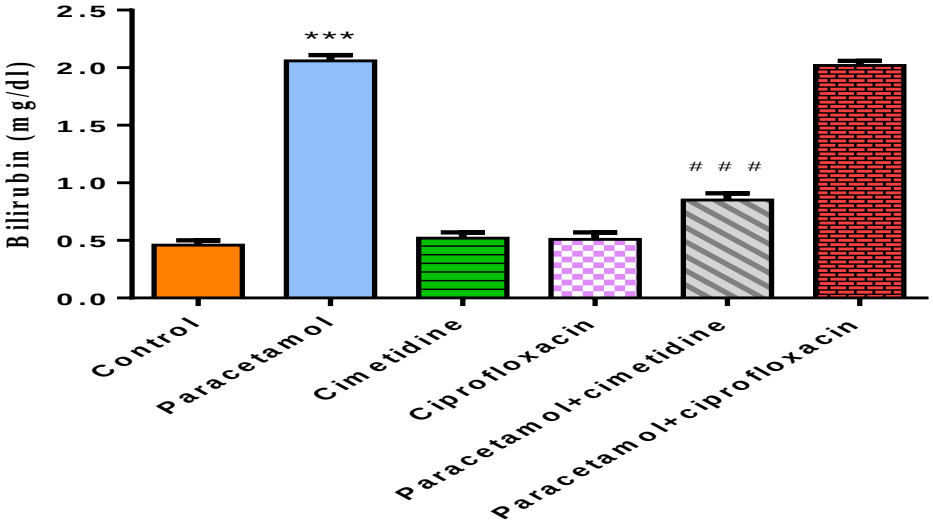

Figure 5. Showed the effect of paracetamol, cimetidine and ciprofloxacin on the liver bilirubin level. Values are expressed as mean±SEM. *** (P<0.001) denotes significant difference vs. control values. # # # (P<0.001) denotes significant difference vs. paracetamol values.

Discussion

Paracetamol is one of the safest over-the counter drugs when used in recommended doses, but is capable of producing massive hepatic necrosis on acute overdose or chronic low dose use.(21) Hepatotoxicity results not from paracetamol itself, but from its metabolism by the mixed function oxidase system CYP450, leading to the formation of N-acetyl-p-benzoquinoneimine (NAPQI). NAPQI is a toxic metabolite which depletes the liver's natural antioxidant glutathione and directly causes damage

to liver cells, and subsequent liver failure.(22) However, different treatment strategies have been proposed to prevent paracetamol-induced hepatotoxicity.(23) For this purpose, adult male albino rats were divided into six groups and were treated with a high dose of paracetamol in the absence or presence of cimetidine and ciprofloxacin. The liver damage was reflected by an increase in level of serum transaminases and bilirubin, these are located in the cytoplasm and leak into blood stream after hepatic injury.(24) The current study revealed a significant ($P< 0.001$) rise in the level of serum marker enzymes ALT, AST, ALP, LDH and serum bilirubin level on exposure to a toxic dose of paracetamol, indicating considerable hepatocellular injury. However, these elevations were significantly ($P<0.001$) attenuated by cimetidine pretreatment, this effect clearly indicated that cimetidine may offer protection in hepatic damage induced by paracetamol. Such observation is in accordance with previous experimental studies,(25,26) where they showed that cimetidine is useful in the treatment of paracetamol overdose because of its hepatic microsomal enzyme system inhibitory effect, (27-29) mainly CYP3A4 and CYP2D6 leading to decrease in NAPQI formation.

However, cimetidine is not only an enzyme inhibitor but is capable of decreasing covalent binding of paracetamol to liver protein and decreases the rate of glutathione depletion.(30) Furthermore, cimetidine has a strong radical-scavenging activity

and reduces the generation of superoxide anion. In addition, cimetidine is able to reduce the iron-induced rise in lipid peroxidation. (31,32)

On the face of this observation, another mechanism has been involved in the explanation of this hepatoprotection induced by cimetidine is its ability to reduce the rate and extent of paracetamol absorption via reductions in gastric emptying time, (33) as proposed by Garba et al had previously postulated an additional mechanism for cimetidine drug interactions, suggesting that cimetidine can also cause direct relaxation of gastrointestinal tract smooth muscle which can in turn reduce the rate of gastric emptying and consequently cause a decrease in absorption rate of co-administered drugs. (34)

Additionally, the pretreatment of paracetamol with ciprofloxacin didn't reduce the plasma level of liver enzymes and bilirubin. Although, there are several forms of cytochrome P450 in humans, includingCYP2E1, CYP2D6, CYP1A2 and CYP3A4 have been shown to catalyze the oxidation of paracetamol to NAPQI, our study demonstrated that ciprofloxacin, which is known for its inhibition of CYP2A1 didn't show any inhibitory effect toward NAPQI formation. The explanation for this came from studies using CYP1A2 null mice,(35) where they indicated that CYP1A2 didn't participate in NAPQI synthesis.(36,37)

Conclusion

Our results demonstrate that paracetamol is capable of inducing marked alterations in biochemical parameters of the liver in a rat model. The present study highlights the hepatoprotective role of cimetidine, administered after paracetamol exposure. The hepatoprotective activity of cimetidine may be due to its inhibition of the CYP3A4 and CYP2D6 responsible for the metabolism of paracetamol. Therefore, the production of the toxic metabolite (NAPQI) will be decreased. Whereas, the administration of ciprofloxacin didn't prevent the liver damage induced by paracetamol, this result suggests that CYP1A2 didn't contribute significantly to NAPQI formation. Clearly, These interesting findings await for the measurement of CYP450 isoforms activities in human liver microsomes. Furthermore, the measurement of oxidant and defensive antioxidant parameters are needed.

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