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Evaluation of prophylactic and therapeutic effects of silymarin and *N*-acetylcysteine in acetaminophen-induced hepatotoxicity in cats

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Avizeh, R., Najafzadeh, H., Razijalali, M., Shirali, S. Evaluation of prophylactic and therapeutic effects of silymarin and *N*-acetylcysteine in acetaminophen-induced hepatotoxicity in cats. *J. vet. Pharmacol. Therap.* **33**, 95–99.

Cats most commonly receive toxic amounts of acetaminophen (APAP) because owners medicate them without consulting a veterinarian. The aim of this study was to compare the hepatoprotective action of silymarin and *N*-acetylcysteine (NAC) against APAP poisoning. Twenty healthy cats were randomly allotted to five equal groups. Animals in group A were given APAP (single dose 150 mg/kg, p.o.); groups B and C consisted of cats that received NAC (100 mg/kg, p.o.) or silymarin (30 mg/kg, p.o.) concurrent with APAP administration respectively; groups D and E were treated like groups B and C, respectively, but 4 h after APAP administration. The serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), methemoglobin, and total and direct bilirubin were measured before APAP administration and 4, 24, and 72 h later. A single oral administration of APAP significantly elevated serum concentrations of ALT, AST, ALP, LDH, methemoglobin, and total and direct bilirubin. In both the groups receiving APAP plus NAC or silymarin, levels of serum enzyme activities, methemoglobin, and total and direct bilirubin remained within the normal values. It was concluded that silymarin as well as NAC can protect liver tissue against oxidative stress in cats with an APAP intoxication.

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INTRODUCTION

Acetaminophen (APAP) is one of the most common ingredients in most household medicine cabinets. Intake of a large dose of APAP may result in severe hepatic necrosis. For several reasons, cats are extremely sensitive to the toxic effects of acetaminophen, because of hepatic enzyme deficiency. Cats form glucuronides with many compounds slowly, or not at all, because they possess fewer isoforms of the enzymes that mediate the conjugation, that is, glucuronyl transferases (Allen, 2003). Oxidative stress mediated by oxidative capacities of the APAP metabolite, particularly the *N*-acetyl-*p*-benzoquinoneimine, is considered to be the main cause of hepatotoxicity of APAP (Olaleye and Rocha, 2008). Cats have a relative absence of a specific high-affinity acetaminophen glucuronyl transferase that conjugates acetaminophen with glucuronic acid. This relative deficiency of the glucuronide conjugation pathway results in more drug being conjugated to sulfates; however, the sulfation pathway has a finite capacity, which is also lower in cats than in other species. Once the sulfation pathway reaches its capacity, acetaminophen is allowed to persist in the blood and more is metabolized by cytochrome P-450 enzymes to NAPQI. Glutathione synthesis is suppressed by high concentrations of acetaminophen and the presence of NAPQI rapidly depletes glutathione stores (Allen, 2003). When glutathione is depleted, the reactive metabolite causes necrosis of hepatic and other tissues. Thus, treatment of APAP toxicity involves supplying alternate sulfhydryl donors or inhibiting oxidative formation of the reactive metabolite (Savides *et al.*, 1985).

Silymarin, an antioxidant flavonoid complex derived from the herb milk thistle (*Silybum marianum*), has long been used in the treatment of liver diseases (Reinhard *et al.*, 2001; Wellington and Jarvis, 2001). This property seems to be due to its ability to scavenge free radicals and to chelate metal ions (Das and Vasudevan, 2006).

N-acetylcysteine (NAC) is clinically used to decrease the toxicity of acetaminophen. NAC may protect against oxidative injury by providing cysteine for glutathione biosynthesis or by

direct reactions with electrophiles (Shattuck, *et al*. 1998; Ozkilic, *et al.* 2006).

The present study was conducted to compare the hepatoprotective action of silymarin and *N*-acetylcysteine in cases of APAP poisoning in cats.

MATERIALS AND METHODS

Animals

Twenty adult domestic short-haired cats of both sexes weighing between 2.82 and 4.57 kg were used. All cats appeared healthy, as determined by clinical examination, normal hematogram and clinical biochemical profiles. The cats were housed separately in a controlled environment and fed a home-made diet containing chicken and fish. Water was provided *ad libitum*. The animal care was provided under the supervision of a qualified veterinarian. This study was performed under control of Iranian Society for the Prevention of Cruelty to Animals.

Experimental protocol

The cats were allocated to five groups consisting of four cats each:

- Group A: Acetaminophen (Jalinous Pharmaceutical Co., Tehran, Iran) was administrated orally at a single dose of 150 mg/kg in gelatin capsules.
- Group B: NAC (Zambon Co., Cadem Pino, Switzerland) was administered orally at a single dose of 100 mg/kg, as a 14% solution in distilled water by gavages, concurrent with APAP administration.
- Group C: Silymarin (Sigma-Aldrich Co., St Louis, MO, USA) was administered orally at a single dose of 30 mg/kg, in gelatin capsules, concurrent with APAP administration.
- Group D: NAC was administered orally at a single dose of 100 mg/kg, 4 h after APAP administration.
- Group E: Silymarin was administered orally at a single dose of 30 mg/kg, 4 h after APAP administration.

Blood sampling and blood chemistry

Blood samples were collected from the jugular veins under ketamine anesthesia. Serum concentrations of ALT, AST, ALP, LDH, and total and conjugated bilirubin were measured in an automated chemical analyzer (BT 3000 Plus, Biotechnica, Milan, Italy) using diagnostic kits (Pars Azmoon Co., Tehran, Iran) before APAP administration and 4, 24 and 72 h thereafter.

Data analysis

The arithmetic mean of ALT, AST, ALP, LDH, methemoglobin, and bilirubin was compared between groups using one-way analysis of variance (ANOVA) and *post hoc* Fisher Least Significant Difference tests (SPSS, version 10, SPSS Inc., Chicago, IL, USA). The level of significance was set at 0.05.

RESULTS

Clinical findings

After APAP administration, all cats of group A, given acetaminophen only, showed signs of intoxication within 4–72 h. Clinical findings included cyanosis, facial edema, hemoglobinuria, anorexia, and depression. Concurrent or therapeutic application of NAC and silymarin improved clinical status of cats and abolished clinical signs. So that only one of the cats from each other groups developed a few mild signs of intoxication including anorexia, discolored urine and or facial oedema within hours after APAP ingeastion that resolved after 1 day. None of the cats in all groups died during the experiment.

Enzyme concentrations

In group A cats, after APAP administration, ALT concentration increased within 24–72 h. The mean serum ALT concentration increased from 46.75 IU/L before APAP administration to 92.5 and 162.75 IU/L, 24 and 72 h after APAP administration respectively. Concurrent or therapeutic (4 h later) application of NAC and silymarin significantly prevented an increase in serum ALT concentration in other groups ($P < 0.05$) (Fig. 1). ALT concentration was not different between groups B–E statistically.

Acetaminophen significantly increased the AST concentration in group A from 50.25 IU/L before to 88.25 IU/L 72 h after APAP administration. This effect was prevented by NAC and silymarin in other groups (Fig. 2).

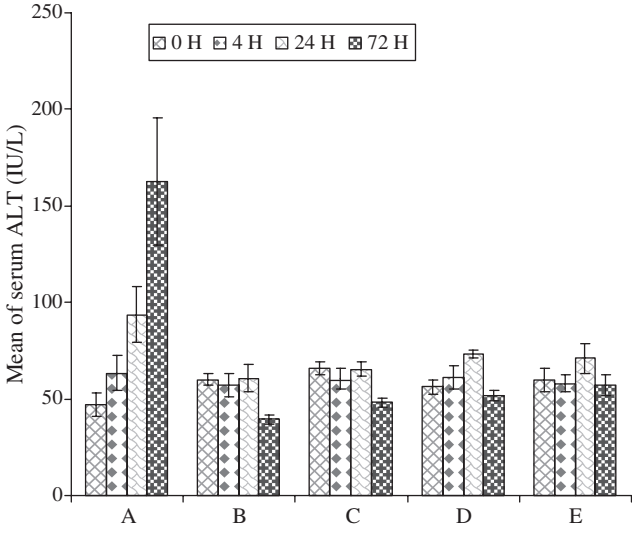

Fig. 1. The mean ± SE of serum ALT concentration of cats: A: APAP treated; B: concomitant application of *N*-acetylcysteine; C: concomitant application of silymarin; D: *N*-acetylcysteine application 4 h after APAP administration; E: silymarin application 4 h after APAP administration.

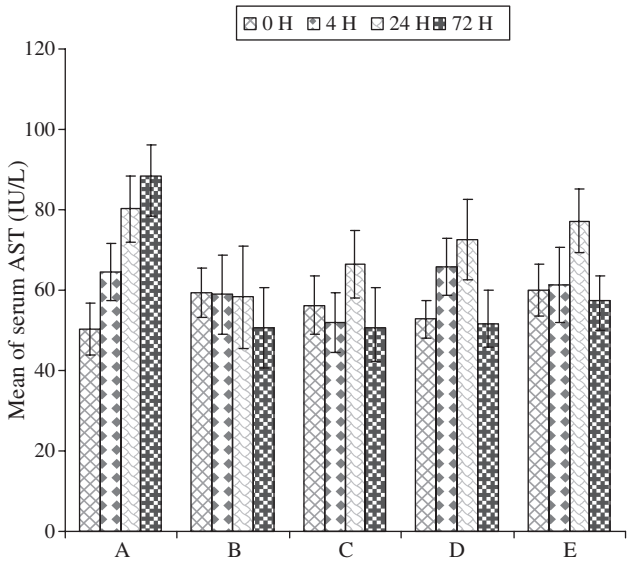

Fig. 2. The mean ± SE of serum AST concentration of cats: A: APAP treated; B: concomitant application of *N*-acetylcysteine; C: concomitant application of silymarin; D: *N*-acetylcysteine application 4 h after APAP administration; E: silymarin application 4 h after APAP administration.

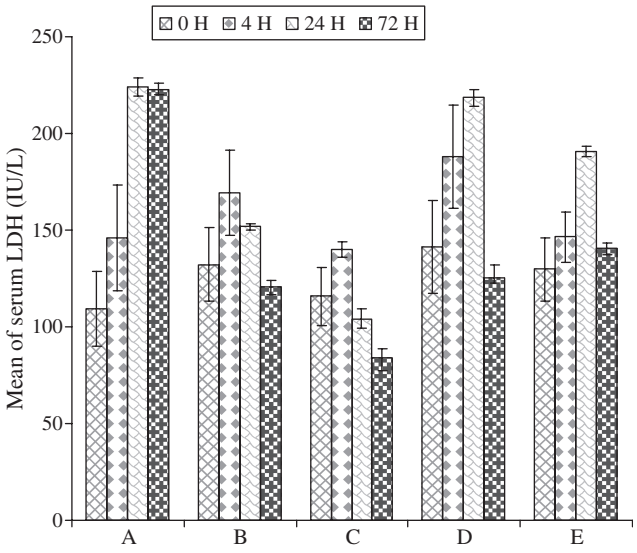

Fig. 4. The mean ± SE of serum LDH concentration of cats: A: APAP treated; B: concomitant application of *N*-acetylcysteine; C: concomitant application of silymarin; D: *N*-acetylcysteine application 4 h after APAP administration; E: silymarin application 4 h after APAP administration.

The serum concentrations of ALP and LDH were also increased in the APAP-treated group, and this effect was significantly reduced by NAC and silymarin in other groups except B and D. In group B, ALP and in group D, LDH changes were similar to group A through 24 h, but thereafter, significant reduction (improvement) was noted. (Figs 3 & 4).

Bilirubin concentration

After APAP administration, serum total bilirubin concentration increased in group A cats within 24–72 h, and the concentrations at 72 h differed significantly from pretreatment values. Concurrent or therapeutic application of NAC did not decrease serum total bilirubin concentration toward normal values. The concurrent or therapeutic application of silymarin had a beneficial effect and returned serum total bilirubin concentration toward normal values (Fig. 5). The results for serum conjugated bilirubin levels were similar to that of total bilirubin, and silymarin could also revert these concentrations toward normal values (Fig. 6).

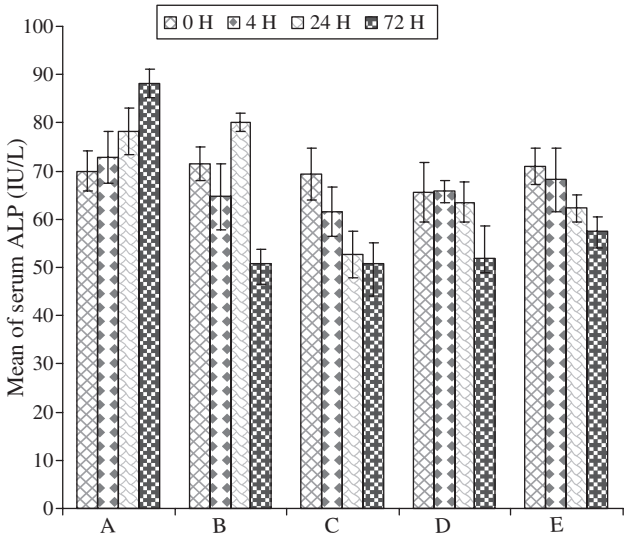

Fig. 3. The mean ± SE of serum ALP concentration of cats: A: APAP treated; B: concomitant application of *N*-acetylcysteine; C: concomitant application of silymarin; D: *N*-acetylcysteine application 4 h after APAP administration; E: silymarin application 4 h after APAP administration.

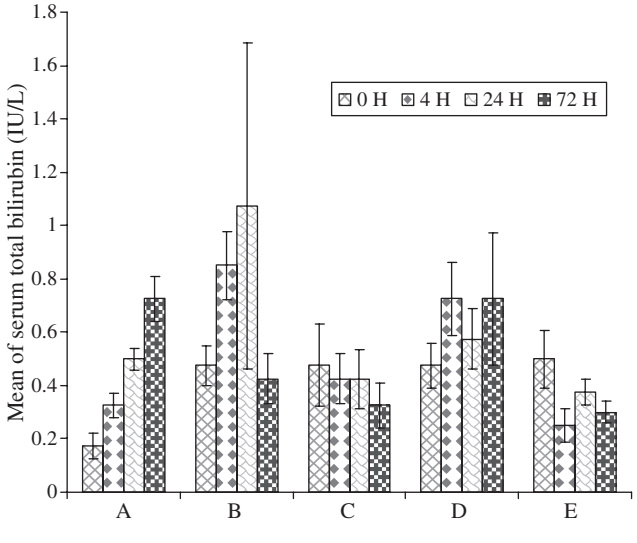

Fig. 5. The mean ± SE of serum total bilirubin concentration of cats: A: APAP treated; B: concomitant application of *N*-acetylcysteine; C: concomitant application of silymarin; D: *N*-acetylcysteine application 4 h after APAP administration; E: silymarin application 4 h after APAP administration.

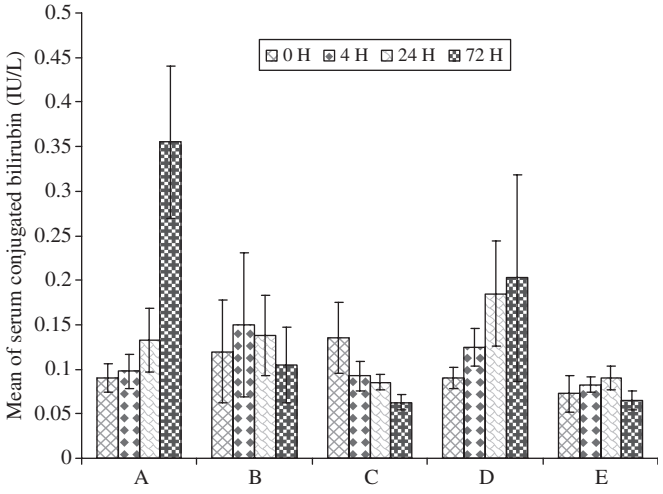

Fig. 6. The mean ± SE of serum conjugated bilirubin concentration of cats: A: APAP treated; B: concomitant application of *N*-acetylcysteine; C: concomitant application of silymarin; D: *N*-acetylcysteine application 4 h after APAP administration; E: silymarin application 4 h after APAP administration.

Methemoglobin level

Acetaminophen induced methemoglobinemia to a significant level in group A cats. Methemoglobin values increased from 0.205 to 0.76 g/L 72 h after APAP administration. This effect of APAP on serum methemoglobin concentration was significantly reversed by NAC and silymarin in all treatment groups (Fig. 7).

DISCUSSION

A single dose of acetaminophen (150 mg/kg) was toxic for cats in the present study as indicated by the occurrence of methemoglobinemia and an increase in serum concentrations of diagnostic liver enzymes and total and conjugated bilirubin. Various dosages of acetaminophen were used in other studies for APAP toxicity. For examples Gaunt *et al.* (1981) used 143 mg/kg; Rumbeiha *et al.* (1995) used 120 mg/kg; and St Omer and McKnight (1980) used a 325-mg tablet of APAP/cat. However, the result of the present study shows a single dose of 150 mg/kg b.w. acetaminophen can induce acute hepatotoxicity as verified by clinical and biochemical investigations.

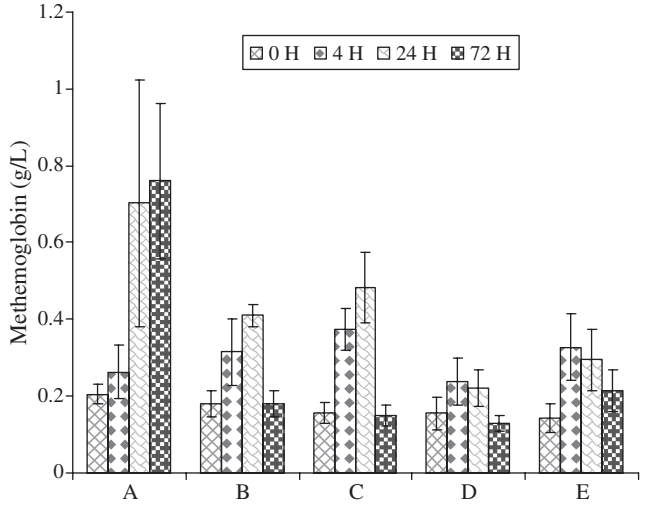

Fig. 7. The mean ± SE of serum methemoglobin concentration of cats: A: APAP treated; B: concomitant application of *N*-acetylcysteine; C: concomitant application of silymarin; D: *N*-acetylcysteine application 4 h after APAP administration; E: silymarin application 4 h after APAP administration.

The prophylactic effect of administration of NAC in this study was similar to that in the study of Gaunt *et al.* (1981). Their regimen consisted of four oral doses of NAC (200 mg/kg, given three times and 100 mg/kg, given once) with 2 h between doses. The result of therapeutic dose of NAC was similar to prophylactic dose in this study. Oral NAC has been used with effective results in treating cats poisoned with APAP. In those regimens, cats were treated several times with initial up to loading dosage levels of between 140 and 280 mg of NAC/kg of body weight and later dosage levels of between 70 and 100 mg of NAC/kg (St Omer and McKnight, 1980; Rumbeiha *et al.*, 1995). The ability of acetylcysteine to provide cysteine, which serves as a precursor for glutathione, has considerably extended as a major therapeutic agent for scavenging free radicals (Ozkilic, *et al.* 2006).

The co-administration or 4 h after administration of silymarin had similar effects on APAP-induced hepatotoxicity in the present study. This therapeutic regimen was even better than the NAC application. Silymarin is a scavenger of radicals, such as hydroxyl, superoxide, and hydrogen peroxide (H_2O_2); increases sorbitol dehydrogenase (SOD); and decreases lipid peroxidation (Oliveira *et al.*, 2001). It protects liver cells directly by stabilizing the membrane permeability through inhibiting lipid peroxidation (Paulova *et al.*, 1990; Mira *et al.*, 1994) and prevents liver glutathione depletion (Valenzuela and Garrido, 1994). The role of oxidative stress in acetaminophen-induced hepatotoxicity and preventive and therapeutic effects of natural antioxidants were evaluated in some other studies (Allison *et al.*, 2000; Balaji Raghavendran *et al.*, 2005). For example, the hepatoprotective effect of aqueous ethanol extract of *Zingiber officinale* against acetaminophen-induced acute toxicity is mediated either by preventing the decline of hepatic antioxidant status or due to its direct radical scavenging capacity (Ajith *et al.*, 2007). The ethanolic extract of *Cuscuta chinensis* can prevent hepatic injuries of acetaminophen-induced hepatotoxicity in rats and this is likely mediated through its antioxidant activities (Yen *et al.*, 2007). Similar results were seen in *Phyllanthus niruri* administration in mice (Bhattacharjee and Sil, 2006). Lipoic acid did not act as an antioxidant but appeared to enhance oxidant effects of acetaminophen, but vitamin E plus cysteine did have protective effects (Hill *et al.*, 2005).

In conclusion, the results of this study showed that silymarin has protective effect similar to *N*-acetylcysteine at least in prophylaxis and treatment of APAP-induced hepatotoxicity in cats and might provide a useful therapy for intoxication patients. The major activity of both the compounds is their antioxidant property, which makes them useful in the prevention of other

organ-specific toxicities related to the induction of oxidative stress (Varzi et al., 2007).

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