Evaluation of prophylactic and therapeutic effects of silymarin on acute toxicity due to tetracycline severe overdose in cats: a preliminary study

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Summary

The aim of the present study was to determine the protective action of silymarin on acute toxicity due to tetracycline severe overdose in cats. Thirty healthy cats were randomly allotted into five equal groups. Cats in group A were given tetracycline (single dose 120 mg⁄kg, p.o.); group B consisted of cats that received silymarin (single dose 30 mg⁄kg, p.o.) concurrent with tetracycline administration; groups C, D and E were treated as group B, but silymarin was administered 4, 12 and 24 h after tetracycline administration, respectively. The serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), BUN, serum creatinine and total and direct bilirubin were measured before tetracycline administration and 4, 12, 24 and 72 h later. A single oral administration of tetracycline increased, significantly, serum concentrations of ALT, AST, ALP, LDH in all cats of group A, after 24 h ($P<0.001$). In groups B and C, levels of serum enzyme activities remained within normal values. In group D, there were changes in levels of serum enzyme activities, but the difference was not significant ($P>0.05$). In group E, levels of serum enzyme activities were significantly higher than normal values ($P<0.05$). The difference was significant between groups A and E with groups B and C for the serum enzymes ($P<0.05$). In conclusion, silymarin can protect liver tissue against hepatotoxicity in cats with tetracycline severe overdose, particularly in the first 4 h after exposure.

Key words: Silymarin, Hepatotoxicity, Tetracycline, Cat

Introduction

Tetracyclines are broad-spectrum antibiotics and, as a class, inhibit the growth of a wide variety of bacteria, protozoa, and many intracellular organisms such as Mycoplasma, Haemofelis, Chlamydia (particularly in cats) and Rickettsia. Tetracyclines can be administered intravenously or intramuscularly, but the oral route is the preferred route in most animals. The most commonly reported side effect of the tetracyclines is gastrointestinal upset (Boothe, 2001; Lappin, 2001; Kayne and Jepson, 2004; Tilley and Smith, 2005). The mechanism of induction of hepatotoxicity is unknown, but it may result from accumulation of tetracyclines when they are not eliminated quickly enough or by administration of frequent and/or large doses above recommended therapeutic dosages (Kayne and Jepson, 2004). Moreover, although tetracycline has limited use in small animal medicine, its accidental ingestion may occur by cats. Mitochondrial damage, oxidative stress, and intracellular glutathione depletion, are the three most important features contributing to the hepatotoxicity prediction (Xu *et al*., 2008). The toxic responses of hepatocytes have caused cell death in steatohepatitis after 96 h of tetracycline treatment (Shen *et al*., 2009). Glutathione peroxidase (GPX), peroxiredoxin 1 (PRX1) and peroxiredoxin 2

(PRX2) are known to serve as antioxidative enzymes in cells. They can be utilized as biomarkers of hepatotoxicity (Yamamoto *et al*., 2005). Little information is available concerning the intoxication of tetracycline in cats. Tetracycline has been reported to induce a marked increase in alanine transaminase activity in the cat (Kaufman and Greene, 1993). One of the species variations in cats is a relative deficiency of glucuronyl transferase, which mediates conjugation of various drugs to glucuronide for elimination (Lappin, 2001; Maddison *et al*., 2002). During prolonged treatment, measurement of serum enzyme concentrations is recommended to allow dosage adjustment. The true incidence of drug-induced hepatic disease is unknown (Lappin, 2001; Maddison *et al*., 2002). Elevations in ALT and AST activity are the most consistent findings. Serum ALP and LDH activity may also be increased. Bilirubinuria and hyperbilirubinemia occur more commonly in cats than dogs (Lappin, 2001; Hsu, 2008). 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). Silymarin was chosen for this investigation because of it antioxidant properties. This property seems to be due to its ability to scavenge free radicals and to chelate metal ions (Asghar and Masood, 2008). The present study was conducted to evaluate the hepatoprotective action of silymarin as the standard drug for evaluation of its prophylactic and therapeutic effects on acute toxicity due to tetracycline severe overdose in cats.

Materials and Methods

Animals

Thirty adult domestic short-haired cats of both sexes weighing between 2.65 and 4.20 kg were used. All cats appeared healthy as determined by clinical examination, normal haematogram and biochemical profiles. The studied cats were between 11 months and 2.5 years and had been vaccinated against panleucopenia, calicivirus, herpesvirus and rabies. Antiparasite drugs were administered two weeks before starting the project. They were fed a home-made diet containing chicken and fish. Water was provided *ad libitum*. This study was performed under the control of the Iranian Society for the Prevention of Cruelty to Animals.

Experimental protocol

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

Group A: Tetracycline hydrochloride was administered orally at a single dose of 120 mg⁄kg, in gelatin capsules.

Group B: Silymarin (Sigma-Aldrich Co., St Louis, MO, USA) was administered orally at a single dose of 30 mg⁄kg, in gelatin capsules, concurrent with tetracycline hydrochloride administration.

Group C: Silymarin was administered orally at a single dose of 30 mg⁄kg, 4 h after tetracycline hydrochloride administration.

Group D: Silymarin was administered orally at a single dose of 30 mg⁄kg, 12 h after tetracycline hydrochloride administration.

Group E: Silymarin was administered orally at a single dose of 30 mg⁄kg, 24 h after tetracycline hydrochloride administration.

Powder of tetracycline hydrochloride was pooled from the available drug dosage form (250 mg capsules, Daroopakhsh, Tehran, Iran). Administration of tetracycline hydrochloride above 120 mg/kg caused severe vomiting and emesis in cats (obtained data from a pilot study), so this dose was selected for this study.

Blood sampling and blood chemistry

Blood samples were collected from the jugular or femoral veins. Serum concentrations of ALT, AST, ALP, LDH, BUN, creatinine and total and direct bilirubin were measured in an automated chemical analyser (BT 3000 Plus, Biotechnica, Milan, Italy) using diagnostic kits (Pars Azmoon Co., Tehran, Iran) before tetracycline hydrochloride administration and 4, 12, 24 and 72 h after. Normal values were referred to Tilley and Smith (2005).

Statistical analysis

The arithmetic mean of serum ALT, AST, ALP, LDH, BUN, serum creatinine

and total and direct bilirubin were compared between groups using repeated measures analysis of variance and Bonferroni test (SPSS, version 10, SPSS Inc., Chicago, IL, USA). Differences were considered significant when $P<0.05$.

Results

Oral administration of tetracycline hydrochloride significantly increased serum concentrations of ALT, AST, ALP and LDH in all cats of group A, 24 h after treatment ($P<0.05$). Clinical findings mainly included depression, anorexia, ptyalism, diarrhoea, emesis and abdominal pain. There were differences in enzyme activity among the different therapeutic groups. Concurrent or therapeutic (4 h later) application of silymarin prevented a significant increase in serum enzyme concentrations in most cats of groups B and C ($P<0.05$). Concurrent or therapeutic application of silymarin in the first 4 h improved the clinical status of cats and abolished clinical signs in most cats of groups B and C also. In group D, there were changes in levels of serum enzyme activities, but the difference was not significant ($P>0.05$). In group E, levels of serum enzyme activities were significantly higher than normal values ($P<0.05$). The difference was significant between groups B and C with groups A and E for the following serum enzymes: ALT, AST, ALP, and LDH ($P<0.05$). None of the cats died during the study. The course of the biochemical profile activities is summarized in Tables 1-6 (mean±SD). The concurrent or therapeutic application of silymarin had a beneficial effect and prevented a significant increase in serum enzyme concentrations. Changes in levels of BUN and creatinine were not significant in all groups ($P>0.05$). Total and direct bilirubins were normal in all of the studied cats also.

Discussion

The results of the present study show that a single dose of oral tetracycline hydrochloride (120 mg/kg) can induce acute hepatotoxicity in cats of group A, as verified

Table 1: The mean ± SEM of serum ALP concentration (IU/L) in experimental groups of cats based on group and time

Group	Time				
	Before	After 4 h	After 12 h	After 24 h	After 72 h
A	^{B} 43.50±7.16^{a}	^{B} 40.00±3.72^{a}	^{B} 44.00±3.64^{a}	^{A} 178.33±28.25^{a}	^{A} 182.50±27.55^{a}
B	^{A} 46.50±4.12^{a}	^{A} 36.33±8.94^{a}	^{A} 42.83±5.97^{a}	^{A} 60.50±9.6^{b}	^{A} 55.83±13.22^{b}
C	^{A} 49.83±7.99^{a}	^{A} 46.00±5.19^{a}	^{A} 37.33±10.01^{a}	^{A} 59.00±8.31^{b}	^{A} 42.33±14.08^{b}
D	^{A} 45.67±8.47^{a}	^{A} 43.83±4.88^{a}	^{A} 35.67±8.02^{a}	^{A} 109.50±38.58^{ab}	^{A} 119.00±37.29^{ab}
E	^{B} 37.50±9.4^{a}	^{B} 38.33±9.02^{a}	^{B} 44.33±4.97^{a}	^{A} 178.50±26.53^{a}	^{A} 186.33±33.68^{a}

Significant differences are presented by capital letters in each row and lowercase letters in each column ($P<0.05$). Group A: Tetracycline hydrochloride was administered orally at a single dose of 120 mg⁄kg. Group B: Silymarin was administered orally at a single dose of 30 mg⁄kg, concurrent with tetracycline hydrochloride. Group C: Silymarin was administered orally at a single dose of 30 mg⁄kg, 4 h after tetracycline hydrochloride. Group D: Silymarin was administered orally at a single dose of 30 mg⁄kg, 12 h after tetracycline hydrochloride. Group E: Silymarin was administered orally at a single dose of 30 mg⁄kg, 24 h after tetracycline hydrochloride

Table 2: The mean ± SEM of serum AST concentration (IU/L) in experimental groups of cats based on group and time

Group	Time				
	Before	After 4 h	After 12 h	After 24 h	After 72 h
A	^{B} 43.17±4.75^{a}	^{B} 42.17±4.86^{a}	^{B} 35.50±4.37^{a}	^{A} 152.00±32.78^{a}	^{A} 137.83±16.14^{a}
B	^{A} 38.00±3.18^{a}	^{A} 42.33±3.19^{a}	^{A} 40.00±2.93^{a}	^{A} 43.00±9.42^{b}	^{A} 47.17±7.83^{b}
C	^{A} 44.50±6.35^{a}	^{A} 38.83±2.63^{a}	^{A} 42.50±3.22^{a}	^{A} 50.00±9.76^{b}	^{A} 55.00±15.47^{ab}
D	^{A} 43.17±3.42^{a}	^{A} 38.33±3.06^{a}	^{A} 41.33±6.58^{a}	^{A} 89.00±26.73^{ab}	^{A} 104.33±29.12^{ab}
E	^{B} 45.83±2.64^{a}	^{B} 47.67±5.17^{a}	^{B} 39.67±3.15^{a}	^{A} 139.17±26.94^{a}	^{A} 131.83±26.88^{ab}

Significant differences are presented by capital letters in each row and lowercase letters in each column ($P<0.05$)

Table 3: The mean ± SEM of serum ALT concentration (IU/L) in experimental groups of cats based on group and time

Group	Time				
	Before	After 4 h	After 12 h	After 24 h	After 72 h
A	^{B} 54.17±4.73^{a}	^{B} 55.67±4.03^{a}	^{B} 52.33±4.17^{a}	^{A} 164.83±18.74^{a}	^{A} 220.50±25.69^{a}
B	^{A} 56.67±3.84^{a}	^{A} 48.67±5.28^{a}	^{A} 44.50±6.49^{a}	^{A} 56.17±15.13^{b}	^{A} 67.17±13.31^{c}
C	^{A} 62.17±5.04^{a}	^{A} 48.83±6.85^{a}	^{A} 50.33±7.23^{a}	^{A} 67.00±11.4^{b}	^{A} 66.83±10.07^{c}
D	^{A} 45.33±6.16^{a}	^{A} 49.33±6.62^{a}	^{A} 52.67±3.96^{a}	^{A} 112.83±26.85^{ab}	^{A} 124.33±29.23^{bc}
E	^{B} 43.67±6.15^{a}	^{B} 52.17±3.97^{a}	^{B} 54.83±7.17^{a}	^{A} 169.83±24.16^{a}	^{A} 166.33±24.15^{ab}

Significant differences are presented by capital letters in each row and lowercase letters in each column ($P<0.05$)

Table 4: The mean ± SEM of serum LDH concentration (IU/L) in experimental groups of cats based on group and time

Group	Time				
	Before	After 4 h	After 12 h	After 24 h	After 72 h
A	^{B} 210.50±33.78^{a}	^{B} 207.33±22.4^{a}	^{B} 178.33±22.98^{a}	^{A} 437.17±31.33^{a}	^{A} 405.33±14^{a}
B	^{A} 209.67±15.66^{a}	^{A} 237.67±25.29^{a}	^{A} 213.00±35.2^{a}	^{A} 232.33±36.54^{b}	^{A} 261.50±33.67^{b}
C	^{A} 223.17±22.34^{a}	^{A} 237.33±23.89^{a}	^{A} 217.50±21.7^{a}	^{A} 271.83±31.96^{ab}	^{A} 255.67±36.7^{b}
D	^{A} 210.83±24.37^{a}	^{A} 223.83±26.68^{a}	^{A} 225.00±19.57^{a}	^{A} 349.83±68.76^{ab}	^{A} 353.17±60.7^{ab}
E	^{B} 229.67±18.83^{a}	^{B} 207.00±20.29^{a}	^{B} 221.17±26.32^{a}	^{A} 416.50±45.79^{ab}	^{A} 474.17±44.66^{a}

Significant differences are presented by capital letters in each row and lowercase letters in each column ($P<0.05$)

Table 5: The mean ± SEM of serum BUN concentration (mg/dl) in experimental groups in cats based on group and time

Group	Time				
	Before	After 4 h	After 12 h	After 24 h	After 72 h
A	27.00±2.39	23.67±1.94	23.83±1.85	30.33±5.09	34.33±4.96
B	24.83±1.74	26.17±1.74	25.17±2.37	23.83±2.21	24.67±1.54
C	26.00±2.33	27.00±2.78	25.83±1.54	26.50±1.98	25.33±1.54
D	24.00±1.64	25.83±3.31	24.00±2.27	24.00±1.83	31.33±4.81
E	25.17±2.01	23.50±2.36	26.83±3.35	33.67±6.11	32.00±5.64

No significant difference was observed in statistical analysis ($P>0.05$) in all comparisons

Table 6: The mean ± SEM of serum creatinine concentration (mg/dl) in experimental groups in cats based on group and time

Group	Time				
	Before	After 4 h	After 12 h	After 24 h	After 72 h
A	1.48±0.18	1.40±0.2	1.33±0.22	1.83±0.27	2.02±0.4
B	1.57±0.15	1.65±0.2	1.68±0.14	1.57±0.21	1.30±0.12
C	1.57±0.14	1.68±0.24	1.63±0.24	1.72±0.17	1.38±0.12
D	1.63±0.24	1.57±0.22	1.33±0.13	1.60±0.23	2.13±0.33
E	1.70±0.26	1.45±0.14	1.67±0.25	2.13±0.33	2.05±0.35

No significant difference was observed in statistical analysis ($P>0.05$) in all comparisons

by clinical and biochemical findings. This dosage is almost six-times higher than the therapeutic dosage (20 mg/kg) which is normally used in feline medicine. Administration of the drug increased serum concentrations of liver enzymes in all cats of group A.

The result of therapeutic dose of silymarin (4 h after administration of tetracycline hydrochloride) was similar to prophylactic dose (co-administration of both the drugs) in the prevention of hepatotoxicity in the present study. Silymarin had high inhibitory effects on tetracycline-induced hepatotoxicity in most cats in groups B and C, so parameters

remained within the normal range. In group E, similar to group A, the variables increased according to the time that the drug was administered. On the basis of the presumption that tetracycline hydrochloride is a hepatotoxic drug, an increase in serum enzyme activities within 24 h after treatment, indicates a need to suspend the drug administration and to provide supportive care.

Most cases of drug-induced hepatopathy are mild and present with vague signs of lethargy and anorexia with or without vomiting or jaundice. In the present study, the most common clinical signs were depression, anorexia, ptyalism, diarrhoea, emesis and abdominal pain. There were no clinical signs such as hypersensitivity reactions as observed in humans (Bircher, 2007). Kaufman and Greene (1993) reported that administration of tetracycline was associated with an adverse drug reaction in a cat. Clinical signs consisted of anorexia, ptyalism, and signs of depression. The most noticeable biochemical abnormality was a markedly high serum ALT activity.

It has been shown that the pharmacokinetic of tetracycline is favourable for therapeutic use in the dog (Rajaian *et al*., 2008). Nevertheless, in a case report, two dogs that were given two doses of oxytetracycline 24 h apart at a dose of 130 mg/kg died, indicating that high doses of oxytetracycline can induce a nephrotoxicosis (Stevenson, 1980). In our study, changes of BUN and creatinine were not significant between different groups.

The pharmacokinetic of doxycycline was compared in cats given 5 mg/kg intravenously. The peak serum concentration was 22.89 µg/ml and fell to 0.89 µg/ml 20 h after injection, falling to nondetectable levels at 32 h and beyond. Doxycycline was more extensively bound to serum proteins in cats than dogs (Riond *et al*., 1990).

Many medicinal, nutraceutical, and botanic extracts such as S-adenosylmethionine, N-acetylcysteine, ursodeoxycholic acid, silymarin, and vitamin E have been used as cytoprotective agents in liver disease (Webster and Cooper, 2009). We used silymarin for the prevention of hepatotoxicity effects of tetracycline hydrochloride in cats. Silymarin is a scavenger of radicals, such as hydroxyl, superoxide, and hydrogen peroxide (H_2O_2), increases sorbitol dehydrogenase and decreases lipid peroxidation (Lappin, 2001; Oliveira *et al*., 2001). In the present study, the prophylactic and therapeutic effects of silymarin administration and the role of serum enzyme activities in tetracycline-induced hepatotoxicity were similar with results published by Avizeh *et al*. (2010). In their research, a single oral administration of acetaminophen significantly elevated serum concentrations of ALT, AST, ALP, LDH, methaemoglobin, and total and direct bilirubin. In both groups that received acetaminophen plus N-acetylcysteine or silymarin, levels of serum enzyme activities, methaemoglobin, and total and direct bilirubin remained within the normal values. McConkey *et al*. (2009) showed that para-aminophenol is the metabolite responsible for acetaminophen-induced methaemoglobinaemia, and deficiency of N-acetyle cysteine activity in cats contributes to this species-dependent toxicity. In another research, serum creatinine and BUN concentrations increased in dogs under administration of gentamicin-induced nephrotoxicity. Silymarin and vitamin E decreased nephrotoxicity in dogs (Varzi *et al*., 2007).

The major activity of silymarin is its antioxidant property, which makes it useful in the prevention of organ-specific toxicities related to the induction of oxidative stress (Varzi *et al*., 2007). It protects liver cells directly by stabilizing the membrane permeability through inhibiting lipid peroxidation and prevents liver glutathione depletion (Paulová *et al*., 1990; Mira *et al*., 1994). Silymarin can protect liver tissue against oxidative stress with different intoxications in cats (Valenzuela and Garrido, 1994). Silymarin alone or in supplement with other drugs, can influence the therapy of different diseases of liver in many animals (Oliveira *et al*., 2001; Sumathy *et al*., 2001). Host factors such as age, sex, individual genetic constitution, malnutrition, especially protein deficiency, disease status, and prior or concomitant use of other medications can affect the severity of drug-induced hepatic disease. The rate of metabolism of some drugs may be decreased

in younger cats because of lower hepatic enzyme activities. Older cats are more likely to have pre-existing disease and alterations in hepatic blood flow that affect rates of drug metabolism (Lappin, 2001). In this study the cats were young adults and clinically healthy. Many highly effective antibiotics are available, but the ideal antibiotics should have a wide margin of safety and considerable activity against immature stages, be easy to administer, inhibit re-infection and be compatible with other compounds (Kahn, 2007). In conclusion, the results of this study showed that silymarin had protective effects in prophylaxis and treatment of tetracycline-induced hepatotoxicity in cats and might provide a useful therapy in cats, at least in the first 4 h of intoxication. Owner education and follow-up is recommended to avoid hepatotoxicity or probably nephrotoxicity when prescribing tetracycline hydrochloride in cats.

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