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Successful Treatment of Stanozolol Induced-hepatotoxicity with Silymarin in a Bitch

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ABSTRACT

The aim of this study was to detect of the protective action of silymarin on stanozolol-induced-hepatotoxicity in a bitch. A female dog with age 2.5 years-old, Doberman pinscher breed and weighing 24.15 kg was presented to Veterinary Hospital of Shahid Chamran University, in August 2010, with a history of depression, salivation, vomiting and seizures. Conjunctival hyperemia and dilated retinal vessels were main clinical signs. Stanozolol had been administered 24 h ago with high dose (2.5 times maximum dose) by owner. Blood sample was collected from the cephalic vein. Serum enzyme concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total and direct bilirubin, Blood Urea Nitrogen (BUN) and creatinine levels were measured immediately after referring, as indices of liver and kidney injuries. Administration of stanozolol was caused elevation of serum enzyme concentrations of ALT, AST, ALP, LDH, total and direct bilirubin in the affected dog. Silymarin (Sigma-Aldrich Co., St Louis, MO, USA) at a single dose of 30 mg/kg with other supportive treatments (fluid therapy and vitamin B. complex) were administered immediately. Application of silymarin improved clinical status and serum value enzyme activity was within normal, 24 h after treatment. Oral silymarin could prevent hepatotoxicity due to stanozolol in a dog.

Key words: Stanozolol, hepatotoxicity, silymarin, serum enzymes

INTRODUCTION

Stanozolol is an anabolic steroid that is commonly used in veterinary medicine to improve appetite, promote weight gain, to increase muscle strength and treat certain types of anemia in dogs, cats and horses. This medication should be used with caution in dogs and cats, since it may cause liver toxicity (Boothe, 2001; Kayne and Jepson, 2004; Tilley and Smith, 2005). Harkin *et al.* (2000) suggested that stanozolol is hepatotoxic in cats. Serum alanine transaminase (ALT) activity was significantly increased in 14 of 18 cats after stanozolol administration, but serum alkaline phosphatase (ALP) activity was mildly increased in only 3 cats (Harkin *et al.*, 2000). Little information is available concerning the intoxication of stanozolol in dogs. The mechanism of action of the hepatotoxicity is unknown but it has been postulated to be due to an immunologic response to reactive metabolites or to a difference in drug metabolism. Concurrent administration with other drugs that compete with the cytochrome P450 enzyme system may decrease the rate of metabolism of stanozolol (Lappin, 2001; Maddison *et al.*, 2002). The true incidence of drug-induced hepatic

disease is unknown in dogs. Clinical signs and laboratory test results are nonspecific and do not differentiate drug-induced from other causes of hepatic diseases. During prolonged treatment, measurement of serum enzyme concentrations is recommended, to allow dosage adjustment if necessary (Lappin, 2001; Maddison *et al.*, 2002). An elevation in ALT and aspartate aminotransferase (AST) activities is the most consistent findings. Serum ALP and lactate dehydrogenase (LDH) activities may be increased also (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 (Saller *et al.*, 2001; Wellington and Jarvis, 2001). Silymarin was chosen for this investigation because of antioxidant properties. This property seems to be due to its ability to scavenge free radicals. Primary antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) that remove superoxide radicals and hydrogen peroxide (Das and Vasudevan, 2006). To the best of our knowledge, this case is the first report of successful treatment of stanozolol-induced hepatotoxicity in a Doberman pinscher dog. In the present report, the hepatoprotective action of silymarin was evaluated as a standard drug, by measurement of serum enzyme concentrations.

MATERIALS AND METHODS

A female dog with age 2.5 years-old, Doberman pinscher breed and weighing 24.15 kg was presented to Veterinary Hospital of Shahid Chamran University, in August 2010, with a history of depression, salivation, vomiting and seizures. Conjunctival hyperemia and dilated retinal vessels were main clinical signs in examination of eyes with ophthalmoscope. Stanozolol had been administered 24 h ago with high dose (2.5 times maximum dose) by owner.

Blood samples were taken from the cephalic vein. A biochemical analysis was performed after centrifugation at 1200 g for ALT, AST, ALP, LDH, total and direct bilirubin, Blood Urea Nitrogen (BUN) and creatinine levels in an automated chemical analyzer (BT 3000 Plus, Biotechnica, Milan, Italy) using diagnostic kits (Pars Azmoon Co., Tehran, Iran), as indices of liver and kidney injuries. Blood glucose concentration was determined by a glucometer (Cleverchek, Taiwan) using strips also. Normal values for serum enzymes were referred to Tilley and Smith (2005). Silymarin (Sigma-Aldrich Co., St Louis, MO, USA), in gelatin capsules, at a single dose of 30 mg kg⁻¹ with other supportive treatments (fluid therapy and vitamin B complex) were administered immediately.

RESULTS

There was a significant difference in the enzyme activities, before and after treatment. A single injection administration of stanozolol elevated serum enzyme concentrations of ALT, AST, ALP, LDH and total and direct bilirubin in the affected dog (265, 186, 325, 485 IU L⁻¹ and 0.8 and 0.3 mg dL⁻¹, respectively) but BUN and creatinine were normal (22 and 1.2 mg dL⁻¹). Blood glucose level was normal (95 mg dL⁻¹) also. The studied animal was presented with a history of seizures (several times after some physical activity) which at the time of arrival it was stopped. Clinical signs (conjunctival hyperemia and dilated retinal vessels) were probably due to hypertension. Vital signs were normal. Based on that data, hepatotoxicity due to stanozolol was diagnosed. The owner was advised to avoid injection of the drug. Application of silymarin with other supportive treatments improved clinical status and symptoms of the disease (particular seizures) until 72 h later. Serum enzyme of ALT, AST, ALP, LDH and total and direct bilirubin in the affected dog activities were within normal value (55, 43, 78, 294 IU L⁻¹ and 0.3 and 0.1 mg dL⁻¹, respectively), 24 h after treatment (Fig. 1).

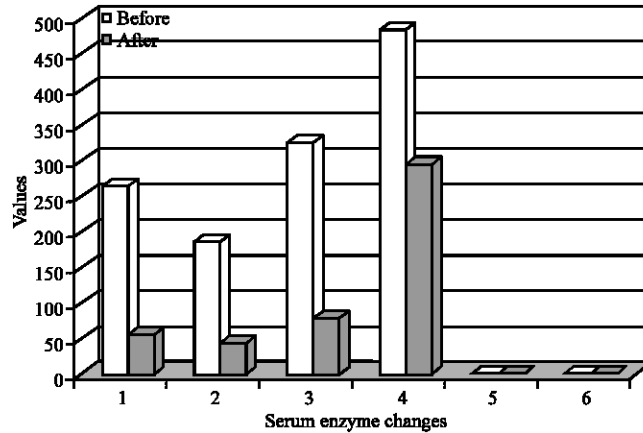


Fig. 1: Enzyme changes before and 24 h after treatment with silymarin: 1= ALT (IU L⁻¹), 2= AST (IU L⁻¹), 3 = ALP (IU L⁻¹), 4= LDH (IU L⁻¹), 5= Total bilirubin (mg dL⁻¹) and 6= Direct bilirubin (mg dL⁻¹)

DISCUSSION

The result of the present study showed that administration of stanozolol with high dose (2.5 times maximum dose) could induce hepatotoxicity in dog as verified by clinical and biochemical investigations. Administration of stanozolol increased serum enzyme concentrations (ALT, AST, ALP, LDH) and total and direct bilirubin. Silymarin had inhibitory effects on stanozolol-induced hepatotoxicity in the Doberman pinscher dog, so the parameters remained within the normal value. On the basis of the presumption that stanozolol is a hepatotoxic drug, its administration with high dose can increase serum enzyme activities within 24 h after onset, indicates a need to suspend drug administration and to provide supportive care. Stanozolol at high doses should not be administered for dogs (Figure not shown). Many highly effective anabolic steroids are available but they must be used correctly to obtain favorable clinical response. The ideal anabolic steroids should have a wide margin of safety, considerable activity, easy to administer and compatibility with other compound (Kahn and Line, 2007). 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 conjunctival hyperemia and dilated retinal vessels. Depression, salivation, vomiting and seizures were reported in history. Harkin *et al.* (2000) suggested that most healthy cats and cats with renal failure developed marked inappetence, groomed less and were less active within 7 to 10 days after initiation of stanozolol administration.

Cowan *et al.* (1997) showed that stanozolol had positive effects on nitrogen balance and lean body mass in dogs with mild-to-moderate, non-uremic, experimentally induced, chronic renal failure. Delgado *et al.* (2010) reported that stanozolol treatment increased antioxidant capacity in selected skeletal muscles from sedentary rats. While we concluded that the stanozolol decreases antioxidant capacity and this was the cause of use of silymarin as an antioxidant agent. Effect of initial high-dose of stanozolol is possible reason of different results. 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 diseases (Webster and Cooper, 2009). Silymarin is a scavenger of radicals, such as hydroxyl, superoxide and hydrogen peroxide, increases sorbitol dehydrogenase and decreases lipid peroxidation (Lappin, 2001; Oliveira *et al.*, 2001). All the provided reviews are not in support to our results.

We used silymarin for treatment of hepatotoxicity due to stanozolol in a Doberman pinscher dog. It protects liver cells directly by stabilizing the membrane permeability through inhibiting lipid peroxidation and prevents liver glutathione depletion (Paulova *et al.*, 1990; Mira *et al.*, 1994). Silymarin can protect liver tissue against oxidative stress with different intoxications in dogs (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; Kalender *et al.*, 2005). The major activity of silymarin is its antioxidant property, which makes it useful in the prevention of other organ-specific toxicities related to the induction of oxidative stress (Varzi *et al.*, 2007). Treatment effects of silymarin and role of serum enzyme activities in stanozolol-induced hepatotoxicity in this study was similar to results of Avizeh *et al.* (2010). In their research, a single oral administration of acetaminophen significantly elevated serum concentrations of ALT, AST, ALP, LDH, methemoglobin and total and direct bilirubin in cats. In both groups received acetaminophen plus N-acetylcysteine or silymarin, levels of serum enzyme activities, methemoglobin 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 methemoglobinemia in dogs and deficiency of N-acetylcysteine activity contributes to this species-dependent toxicity. In another research, serum creatinine and BUN concentrations were increased in the dogs under administration of gentamicin-induced nephrotoxicity. Silymarin and vitamin E decreased nephrotoxicity in dogs (Varzi *et al.*, 2007). Stanozolol had no nephrotoxicity effects in the present study, because BUN and creatinine levels were normal in the affected dog.

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 decrease in younger dogs because of lower hepatic enzyme activities. Older dogs are more likely to have preexisting disease and alterations in hepatic blood flow that affect rates of drug metabolism (Lappin, 2001; Kahn and Line, 2007). Hepatotoxicity of individual drugs varies significantly among different species. Agents known to be hepatotoxic in other species can not be assumed to be hepatotoxic in dogs. Likewise, agents may cause hepatic injury in dogs but not in other species (Lappin, 2001). Further clinical and biochemical investigations are needed to detect various aspects of stanozolol-induced-hepatotoxicity in dogs.

CONCLUSION

The results of this study showed that silymarin had protective effect in treatment of stanozolol-induced hepatotoxicity and provided a useful therapy, at least in the first 24 h of intoxication. Results were verified by clinical and biochemical investigations. Clinician education and follow-up is recommended to avoid stanozolol induced- hepatotoxicity in dogs.

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