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Effect of silybon 140® on pentobarbitone anaesthesia in dogs exposed to acetaminophen

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Onset and duration of anaesthesia as well as the changes in vital parameters associated with pentobarbitone-induced anaesthesia in normal dogs administered acetaminophen with and without Silybon 140® were studied. The rectal temperature, heart rates and respiratory rates of dogs before anaesthesia were higher than those after pentobarbitone (35 mg/kg) treatment alone or its combination with acetaminophen or acetaminophen plus Silybon 140®. The acetaminophen and Silybon 140® combination produced the least changes in vital parameters following pentobarbitone treatment. The duration of anaesthesia produced in dogs with pentobarbitone anaesthetic was significantly lower in dogs pre-treated with acetaminophen and Silybon 140® combination compared to those given pentobarbitone alone or its combination with acetaminophen. The dogs pre-treated with acetaminophen (300 mg/kg) had the highest duration of anaesthesia of 437± 64 min. These results suggest that pentobarbitone anaesthesia in dogs may be associated with decreased vital parameters. The decrease appears to be more in dogs treated with acetaminophen. The higher duration of anaesthesia and decreased vital parameters in dogs pre-treated with acetaminophen (300 mg/kg) could be associated with hepatotoxic effect of acetaminophen. Silybon 140® given in combination with acetaminophen ameliorated the enhanced vital parameters and duration of pentobarbitone anaesthesia.

Key words: Dog, acetaminophen, pentobarbitone, anaesthesia, silybon 140®.

INTRODUCTION

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Pentobarbitone (pentobarbital) sodium is a short acting barbiturate used occasionally as sedative, hypnotic and general anaesthetic in animals (Hanning, 1998). At a time, it was the most widely used anaesthetic agent in small animals, but with the introduction of halogenated inhalant anaesthetic agents, its use has declined (Harpal and Satyavan, 2006).

Pentobarbitone is known to provide good surgical anaesthesia after intravenous injection. It is a general body depressant, with respiratory and cardiovascular

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depression marked (Katzung, 1998). The drug is widely distributed to all organs and tissues of the body with highest concentration present in the brain and liver (Booth, 1988). Many anaesthetic agents demonstrate direct activity in modulating haemodynamic, myocardial energy supply and utilization (Muir, 1977), all of which influence heart and respiratory rates and temperature of the subject. Emergence from pentobarbitone anaesthesia depends mainly on metabolism of the drug, and since the metabolism of this agent is slow in dogs (about 15% per hour) the duration of anaesthetic action may be prolonged (Stoelting, 1990). The drug is used as an anaesthetic and for control of seizures, muscle rigidity and convulsion due to poisoning in dogs (Gray et al., 1987).

Acetaminophen is one of the most commonly used over the counter antipyretic and analgesic agent worldwide (Beltran-Olazabal et al., 2019). It is known to have produced acute liver failure in humans in many Western countries (Budnitz et al., 2011; Manthripogoda et al., 2011). The toxicity could be due to both intentional and non-intentional over dose (Dimitropoulos and Ambizas, 2014). Acetaminophen toxicity is known to have affected man and animals for decades and is as a result of interference with the metabolic pathway that involves the microsomes within the hepatocytes (Eric et al., 2016). The toxicity occurs with saturation of the major metabolic pathways: - the glucuronide and sulphate conjugation systems in large acute or with chronic use of acetaminophen, with more of the agent being metabolized by CYP450 system. The result is increased N-acetyl-P-Benzoquinoneimine (NAPQI) production. When glutathione is approximately 70% depleted, NAPQI begin to accumulate in the hepatocytes resulting in hepatic injury (Lee, 2003; Larson, 2007). N-acetyl-P-Benzoquinoneimine (NAPQ1) a metabolite of acetaminophen is highly toxic (Pathan et al., 2014). Acetaminophen was reported to prolong pentobarbital anaesthesia in rats (Nwachujor et al., 2012).

Silybon 140® (Silymarin) is obtained from an ancient medicinal plant Silybummarianum (L.) Gaertn., which has been in use for centuries for treatment of liver and gall bladder disorders. The active constituents of the plant are flavonoligans (Vogal, 1977; Kshirsagar et al., 2009). The agents induce hepatoprotective effects by various mechanisms including, regulatory action on cellular and mitochondrial membrane permeability (Muriter et al., 1986), prevention of toxin movement into the hepatocytes (Faulstich et al., 1980), inhibition of cytochrome P450 enzymes (Baer-Dubowska et al., 1998) and antioxidant and free radicals scavenging property (Miguez et al., 1994; Pradhan and Girish, 2013).

Drugs in common use can induce toxic effects on the liver (Pradhan and Girish, 2013). The injury produced interferes with hepatic microsomal enzymes and therefore the metabolism of compounds bio-transformed in the liver (Adams and Dixit, 1970). This study investigates the effect of silybon 140® on pentobarbitone induced anaesthesia in dogs administered a hepatotixin, acetaminophen.

MATERIALS AND METHODS

Drugs

Silybon 140® (silymarin, Micro. Labs, Ltd, H.P. India), Acetaminophen (Paracetamol®, Pharmetex Pharmaceutical Industry LTD, Lagos, Nigeria) and Pentobarbitone sodium 6% (KyronPresriptions, P. O. Box 27329, Benrose, South African), were obtained from the Veterinary Teaching Hospital, Federal University of Agriculture, Makurdi.

Animals and treatments

The study was carried out in 12 male Nigerian dogs (Mongrel) of 12 months old and mean weight of 10±0.8 kg. The dogs were maintained in standard dog kennels with a temperature (31±3°C) and 40% humidity. The dogs were fed on standard dog feed with free access to clean drinking water. The animals were acclimatized for 2 weeks before the commencement of the experiment. The study was conducted on approval of the College of Veterinary Medicine, Federal University of Agriculture Ethics Committee.

Dogs were separated into four groups of three animals each. Baseline vital parameters including rectal temperature, respiratory and heart rates were obtained for the four groups prior to drug treatments. Acetaminophen induced hepatotoxicity model (Parmar et al., 2010) was used with slight modifications. Dogs were fasted overnight. Group 1 was administered distilled water only while acetaminophen in distilled water was administered orally to Group 2, 3 and 4 at the dose of 100, 200 and 300 mg/kg respectively. The animals in Group 4 further received orally silybon 140® at 100 mg/kg 2 h after acetaminophen administration. Eighteen hours later all the groups received pentobarbitone sodium at the rate of 35 mg/kg body weight. The onset of sleep and duration of sleep for each of the four groups were observed and measured according to the method of Fleknell (2006). The rectal temperature, respiratory and heart rates were also obtained during anaesthesia. The respiratory and heart rates were measured with stethoscope and rectal temperature obtained with rectal thermometer.

Statistical analysis

Data obtained from this study were expressed as mean value ± standard error of mean (S.E.M.). Vital parameter data were compared by student t-test. Analysis of variance was performed on the duration of anaesthesia by using Graph Pad Prism software. A probability of less than 5% ($P<0.05$) was considered significant.

RESULTS

Effect of silybon 140® on the mean rectal temperature of dogs administered acetaminophen and treated with pentobarbitone anaesthetic

The administration of pentobarbitone anaesthesia alone and its combination with 100 or 300 mg/kg of

Table 1. Effect of silybon 140® on the mean rectal temperature of dogs administered acetaminophen and treated with pentobarbitone anaesthetic.

Experimental groups	Mean rectal temperature (°C) ±SEM		Change (%)
	BAP	AAP	
Pentobarbitone	38.73 ± 0.35	35.07 ± 0.55	-9.45
Pentobarbitone + Acetaminophen (100 mg/kg)	39.87 ± 1.81	36.73 ± 0.23	-7.88
Pentobarbitone + Acetaminophen (200 mg/kg)	38.0 ± 0.49	34.15 ± 1.34	-10.50
Pentobarbitone + Acetaminophen (300 mg/kg) + silybon 140® (100 mg/kg)	38.30 ± 1.12	37.13 ± 0.19	-3.05

SEM = Standard error of mean; BAP = Before the administration of pentobarbitone; AAP = After the administration of pentobarbitone; -= Decreased compared to the value prior to administration of pentobarbitone.

Table 2. Effect of silybon 140® on the mean heart rate of dogs administered acetaminophen and treated with pentobarbitone anaesthetic.

Experimental groups	Mean heart rate (beats/min) ±SEM		Change (%)
	BAP	AAP	
Pentobarbitone	90.67 ± 7.06	86.6 ± 8.74	-4.41
Pentobarbitone + Acetaminophen (100 mg/kg)	94.67 ± 18.42	85.41 ± 6.33	-9.26
Pentobarbitone + Acetaminophen (200 mg/kg)	102.67 ± 11.39	92.34 ± 6.93	-12.73
Pentobarbitone + Acetaminophen (300 mg/kg) + silybon 140® (100 mg/kg)	99.67 ± 6.06	96.10 ± 3.31	-3.25

SEM = Standard error of mean; BAP = Before the administration of pentobarbitone; AAP = After the administration of pentobarbitone
-= Decreased compared to the value prior to administration of pentobarbitone.

acetaminophen to dogs decreased the temperature by 9.45, 7.88 and 10.5% respectively when compared to the values before pentobarbitone administration. However, when pentobarbitone was combined with 300 mg/kg acetaminophen and silybon 140®, the decrease in body temperature was only 3.05% which was less than those of pentobarbitone alone, and its combination with acetaminophen (Table 1).

Effect of silybon 140® on the mean heart rate of dogs administered acetaminophen and treated with pentobarbitone anaesthetic

The heart rates of dogs were not significantly (P>0.05) altered by the administration of pentobarbitone alone. Pentobarbitone in combination with 100 or 200 mg/kg of acetaminophen, respectively decreased the heart rate by 9.26 and 12.73% compared to values prior to pentobarbitone treatments. The combination of pentobarbitone, acetaminophen (300 mg/kg) and silybon 140® (100 mg/kg) only decreased the heart rate by 3.25% (Table 2).

Effect of silybon 140® on the mean respiratory rate of dogs administered acetaminophen and treated with Pentobarbitone anaesthetic

The respiratory rate of dogs was depressed after administration of pentobarbitone alone from 25.67±3.28 to 11.33±1.20 cycle per min, a 55.86% decrease (Table 3). The administration of pentobarbitone plus 100 mg/kg acetaminophen decreased the respiratory rate by 64.5% (from 31.0±4.04 to 11.0±1.00 cycles/min). Pentobarbitone combined with 300 mg/kg acetaminophen decreased respiratory rate from 34.67±3.11 to 17.34±1.26 cycles/min (that is, 50.01% decrease). Pentobarbitone, acetaminophen (300 mg/kg) and Silybon 140® (100 mg/kg) combination resulted in the least respiratory rate decrease of 21.0% (from 33.33±1.76 to 26.33±10.26 cycle/min). The pentobarbitone post treatment respiratory rates in all the treated dogs were decreased compared to the pre-treatment values.

Effect of silybon 140® on the mean sleeping time of dogs administered acetaminophen and treated with pentobarbitone anaesthetic

The longest duration of anaesthesia of 421.9±10.5 min was recorded when acetaminophen (200 mg/kg) was administered prior to pentobarbitone administration. The least anaesthetic duration (153.8±13.2 min) was obtained with the combination of pentobarbitone, acetaminophen (300 mg/kg) and silybon 140® (100 mg/kg) (Table 4). Pentobarbitone alone and its combination with 100 mg/kg acetaminophen produced anaesthetic durations of 194.4±21.4 and 166.2±11.6 min respectively. The anaesthetic duration of 421.9±10.5 min produced by

Table 3. Effect of silybon 140® on the mean respiratory rate of dogs administered acetaminophen and treated with pentobarbitone anaesthetic.

Experimental groups	Mean respiratory rate (cycles/min) ±SEM		Change (%)
	BAP	AAP	
Pentobarbitone	25.67 ± 3.28	11.33 ± 1.20	-55.86
Pentobarbitone + Acetaminophen (100 mg/kg)	31.0 ± 4.04	11.0 ± 1.00	-64.50
Pentobarbitone + Acetaminophen (200 mg/kg)	34.67 ± 3.11	17.34 ± 10.26	-50.01
Pentobarbitone + Acetaminophen (300 mg/kg) + silybon 140® (100 mg/kg)	33.33 ± 1.76	26.33 ± 2.33	-21.00

SEM = Standard error of mean; BAP = Before the administration of pentobarbitone; AAP = After the administration of pentobarbitone
-= Decreased compared to the value prior to administration of pentobarbitone.

Table 4. Effect of silybon 140® on the mean sleeping time of dogs administered acetaminophen and treated with pentobarbitone anaesthetic.

Experimental groups	Mean anaesthetic time (min) ±SEM	
	Onset of anaesthetic	Duration of anaesthetic
Pentobarbitone	1.5 ± 0.3	194.4 ± 21.4[b]
Pentobarbitone + Acetaminophen (100 mg/kg)	1.2 ± 0.4	166.2 ± 11.6[b]
Pentobarbitone + Acetaminophen (200 mg/kg)	0.6 ± 0.40	421.9 ± 10.5[a]
Pentobarbitone + Acetaminophen (300 mg/kg) + silybon 140® (100 mg/kg)	1.4 ± 0.5	153.8 ± 13.2[c]

SEM = Standard error of mean; [a,b,c] = signifiacantly (P<0.05) different from each other.

combination of pentobarbitone plus 200 mg/kg acetaminophen was significantly (P<0.05) higher than those of the other treatment groups.

DISCUSSION

Acetaminophen administration prior to pentobarbitone administration altered the vital parameters as well as the duration of anaesthesia in the dogs. The decrease in body temperature when pentobarbitone was used either alone or in combination with acetaminophen with or without silybon 140® may have resulted from the central nervous system (CNS) depression. Hypothermia could result from CNS depression (Irwin et al., 1959). The dogs treated with a combination of pentobarbitone, 300 mg/kg acetaminophen and silybon 140® had the lowest body temperature decrease of all the treated groups.

Administration of acetaminophen prior to pentobarbitone treatment resulted in decreased heart rate apparently from increased concentration of pentobarbitone in the system due to the postulated inhibition of the biotransformation by hepatic microsomal enzymes. The animals treated with acetaminophen prior to pentobarbitone administration usually have longer anaesthetic duration, indicating reduced inactivation of pentobarbitone, an indirect evidence of inhibition of hepatic metabolizing enzymes. Pentobarbitone is a general depressant of the body systems (Muir, 1977; Cooper, 1989; Vater, 1998).

Earlier report (Hall and Clarke, 1991; Booth, 1988; Vater, 1998) indicated that pentobarbitone caused respiratory depression in man and animals. The lower rate respiratory depression noticed when 300 mg/kg acetaminophen and 100 mg/kg silybon 140® were used prior to pentobarbitone administration compared to the other groups probably reflected the anti-hepatotoxic activity of silybon 140®, a hepatoprotective drug. The depressant effects of pentobarbitoneanaesthetic on respiration has been known since the early days when the depth, character and rate of respiration were recognized as important clinical signs to the depth of anaesthesia (Hanning, 1998). The anaesthetic pentobarbitone is biotransformed by the hepatic microsomal enzymes (Booth, 1988). The interference with the biotransformation mechanism by acetaminophen (a hepatic toxin) without silybon140® may have prevented the detoxification of pentobarbitone.

The duration of anaesthesia obtained when pentobarbitone was used alone was lower than when acetaminophen was administered prior to pentobarbitone. The increase in anaesthetic period following acetaminophen administration prior to pentobarbitone administration may be associated with the hepatotoxic effect of acetaminophen resulting in the destruction of microsomal enzymes, thereby sustaining the effect of pentobarbitone on the systems. The administration of silybon 140® plus acetaminophen (300 mg/kg) prior to

pentobarbitone administration produced the least anaesthetic duration. This may be due to the protective effect of silybon 140® on the liver cells, resulting in reduced destruction of the hepatocytes which remained viable to carry on biotransformation of pentobarbitone. The hepatoprotective and antioxidant activity of silybon 140® is due to its ability to inhibit the free radicals that are produced from the metabolism of toxic substances such as acetaminophen (Vargas-Mendoza et al., 2014). The generation of free radicals is known to damage cellular membranes and induce lipoperoxidation. Silybon 140®enhances hepatic glutathione and may contribute to the antioxidant defence of the liver.

Conclusion

The administration of acetaminophen prior to pentobarbitone increased significantly the duration of pentobarbitone anaesthesia, however, silybon 140® was observed to ameliorate the increased anaesthetic duration produced when acetaminophen alone was administered with pentobarbitone. It is therefore suggested that pentobarbitoneanaesthesia in dogs with hepatic injury should be accompanied with silybon140® treatment to reduce the duration of anaesthesia.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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