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Influence of Phenobarbital Pretreatment on Toxicity of *Calotropis procera* Latex in Nubian Goats

¹S.M.A. El-Badwi, ²A.O. Bakhiet, ¹A.B. Medani and ³Z.Y. Shamseldin

¹Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine University of Khartoum, P.O. Box 32, Khartoum North, Sudan

²Department of Pathology, Parasitology and Microbiology, College of Veterinary Medicine, Sudan University of Science and Technology, P.O. Box 204, Khartoum North, Sudan

³National Medicines and Poisons Board, Khartoum, Sudan

Corresponding Author: S.M.A. El-Badwi, Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine University of Khartoum, P.O. Box 32, Khartoum North, Sudan

ABSTRACT

The effect of phenobarbital pretreatment on the toxicity of *Calotropis procera* latex to Nubian goats was examined. The goats receiving phenobarbital sodium at daily oral doses of 20 mg/kg/day for 14 days plus an oral dose of *C. procera* latex on day 15, exhibited marked depression, lateral deviation of the head and neck, dyspnea and frequent urination and histo-pathologically, they showed centrilobular hepatocellular necrosis and fatty cytoplasmic vacuolation of the hepatocytes. The activities of GGT, GDH, LDH, AST and ALP and the concentration of creatinine were higher in this group than the group receiving latex alone. The study showed that phenobarbital pretreatment was found to potentiate the effect of *C. procera* latex in Nubian goat's kids.

Key words: Phenobarbital, *Calotropis procera*, toxicity, goats

INTRODUCTION

Phenobarbital is a known inducer of microsomal enzymes (Cytochrome P-450 (CYP), NADPH-cytochrome P-450 reductase, NADPH oxidase, glutathione -S-transferase) which are responsible for the metabolic breakdown of a large number of endogenous and exogenous chemical compounds (Demir *et al.*, 2001; Aniya *et al.*, 1993; Videla *et al.*, 2000).

It is reported that phenobarbital decrease the toxicity of some organo-phosphate as a result of an increase in paraoxonase activity. In addition, a number of xenobiotics increase serum AST and ALT activities and are used as markers of liver toxicity and it is reported that phenobarbital treatment increased these activities and protein levels in the liver (Clement, 1983).

Dandge and Govindwar (2011) reported that sulfamerazine treatment of phenobarbital pretreated rats caused marginal decrease in cytochrome b5 and significant decrease in the level of cytochrome P-450 activities of aminopyrine N-demethylase and aniline hydroxylase when compared with Phenobarbital treatment. Phenobarbital is a hepatotoxin and also has been used to promote carcinogenesis (hepatocarcinogenesis) induced by Diethyl Nitrosamine (DEN) in Wistar rats (Yoshiji *et al.*, 1991; Shahjahan *et al.*, 2004; Jahan *et al.*, 2007, 2011).

Calotropis procera latex is being known for its toxic and medicinal properties. It has been reported to cause congestion of eyes, iridocyclitis and dermatitis following accidental exposure (Tomar *et al.*, 1970; Biedner *et al.*, 1977; Handa *et al.*, 1984; Arya and Kumar, 2005) and it has

an inflammatory response that is mediated through histamine and prostaglandins (Singh *et al.*, 2000; Shivkar and Kumar, 2003, 2004; Arya and Kumar, 2005). It is used for the treatment of skin diseases, rheumatism and aches CSIR (1992) and is used as analgesic and weak antipyretic activities when administered orally (Dewan *et al.*, 2000a, b). De Lima *et al.* (2011) reported the toxic properties of *C. procera* latex to rats and *C. procera* leaves to sheep. El-Badwi, *et al.* (1998) studied the toxic effects on goats of *C. procera* latex given by different routes of administration. El-Badwi and Bakhiet (2010) examined the toxic effects of *Calotropis procera* latex on pregnant and nonpregnant Nubian goats. El-Sheikh *et al.* (1991) studies the activities of drug-metabolizing enzymes in goats treated orally with the latex of *Calotropis procera* and the influence of dieldrin pretreatment. They reported that Dieldrin pretreatment resulted in the induction of the activities of drug-metabolizing enzymes in the liver, kidneys and duodenal mucosa and it may have protected goats from the lethal effects of the latex.

The purpose of this study was to evaluate the effect of phenobarbital sodium on the toxicity of *Calotropis procera* latex in goats.

MATERIALS AND METHODS

Animals: Twelve 5-7 month-old male Nubian goat kids were purchased from a local market in Khartoum North and kept in pens within the premises of the Veterinary Teaching Hospital, University of Khartoum May 2010. The goats were given within the 2 week adaptation period oxytetracycline and sulphamethazine for the control of bacterial infections and coccidiosis respectively, fed on lucerne and allowed free access to drinking water.

Phenobarbital and latex administration: At the end of the adaptation period the goat kids were divided into 4 groups. Goats 1, 2 and 3 were the un-dosed controls (group 1) and goats 4, 5 and 6 (group 2) were given by the oral route phenobarbital only at 20 mg/kg/day for 14 days. Goats 7,8 and 9 were given an oral dose of *Calotropis* latex at 0.5 mL kg⁻¹ (group 3) and goats 10, 11 and 12 were given phenobarbital sodium at 20 mg/kg/day for 14 days plus a single oral dose of *Calotropis* latex at 0.5 mL kg⁻¹ (group 4). All groups were left for one week after the last dose then either died or slaughtered.

Parameters: Clinical signs and mortality rates were recorded. Blood samples were obtained from the jugular vein before the experiment started and thereafter for haematological investigation and serum analysis. Haemoglobin concentration (Hb), Packed Cell Volume (PCV), Red Blood Cell (RBC) and White Blood Cell (WBC) counts, Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) were estimated by standard methods (Schalm *et al.*, 1975). Sera were analyzed for the activities of GGT, GDH, LDH, AST and Alkaline Phosphatase (ALP) and concentrations of cholesterol, creatinine, bilirubin, urea, calcium, phosphorous, total protein, albumin and globulin using commercial kits (Stanbio Laboratory, Inc., San Antonio, TX, USA).

Statistical methods: The differences between mean values of data were analyzed by the unpaired student-t-test (Snedecor and Cochran, 1967).

RESULTS

The dosing schedule and fate of animals are given in Table 1.

Clinical findings: In non treated control goats in group 1 and the phenobarbital-dosed goats (group 2) there were no clinical abnormalities. The goats in group 4 receiving phenobarbital sodium

Table 1: The effect of phenobarbital pretreatment on the toxicity of *C. procera* latex to Nubian goats

Group No.	Goat No.	Phenobarbital (mg kg day ⁻¹ for 14 days)	Dose of latex 0.5 (mL kg ⁻¹ on day 15)	Survival times (days)	Fate of animals
1 (non-treated controls)	1	Nil	Nil	21	Slaughtered
	2	Nil	Nil	21	Slaughtered
	3	Nil	Nil	21	Slaughtered
2 (phenobarbital alone)	4	20	Nil	21	Slaughtered
	5	20	Nil	21	Slaughtered
	6	20	Nil	21	Slaughtered
3 (latex alone)	7	Nil	0.5	21**	Slaughtered
	8	Nil	0.5	21**	Slaughtered
	9	Nil	0.5	21**	Slaughtered
4 (phenobarbitale+latex)	10	20	0.5	17*	Died
	11	20	0.5	21**	Slaughtered in extremis
	12	20	0.5	21**	Slaughtered in extremis

*: 3 days post-latex administration. **: 7 days post-latex administration

at oral doses of 20 mg/kg/day for 14 days plus an oral dose of *C. procera* latex on day 15, exhibited marked depression, lateral deviation of the head and neck, dyspnoea and frequent urination within 10 h of the dosing. Signs lasted for 2 days and goat 10 died on day 3 and goats 11 and 12 were slaughtered in morbid condition on day 7 post-latex administrations. In goats treated with latex alone (group 3), the clinical sings were less marked than goats in group 4. Group 4 animals were slaughtered 7 days after latex administration.

Post-mortem findings: In goats in group 4 which received *C. procera* latex there was haemorrhagic enteritis and congestion of spleen, heart, liver and kidneys. In goats in group 3 receiving the plant latex alone, there were less severe lesions than those seen in group 4. Phenobarbital group (group 2) showed slight hepatic fatty change and congestion of the kidneys.

Histopathological findings: In goats of group 4, there was centrilobular hepatocellular necrosis and fatty cytoplasmic vacuolation of the hepatocytes (Fig. 1), degeneration of the somniferous tabulates and some of the cardiac muscle fibres and renal tubular cells, lymphocytic infiltration in the glomeruli and haemosiderosis of the spleen. In goats in group 3, the liver cells were vacuolated or neurotic, the renal tubular cells appeared degenerated and the splenic pulp was congested and contained hemosidrin deposits. In group 2, fatty vacuolation of some of the contribular hepatocytes was detected. No lesions were seen on the non-treated controls in group 1.

Changes in serum constituents: The effect of *C. procera* latex on the activities of GGT, GDH, LDH, AST and ALP and the concentration cholesterol, creatinine, bilirubin, urea, calcium, phosphorus, total protein, albumin and globulin is given in Table 2 and 3. In groups 3 and 4 the activities of GGT, GDH, LDH, AST and ALP and the concentrations of cholesterol, globulin and urea were higher ($p < 0.05$ - 0.001) than those in groups 1 and 2. The concentration of creatine was higher ($p < 0.05$) in group 4 than other groups and no changes in calcium or bilirubin concentration was detected in the test groups. In group 2, the activities of AST and ALP and the concentration of globulin and cholesterol were higher ($p < 0.05$ - 0.01) than group 1.

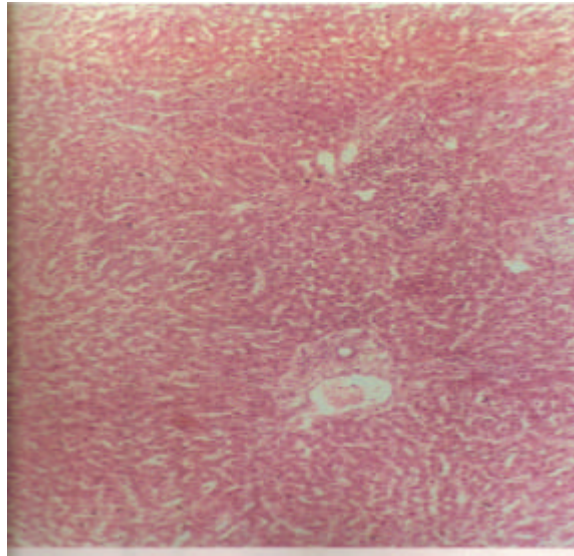


Fig. 1: Centrilobular hepatocellular necrosis and fatty cytoplasmic vacuolation of the hepatocytes in a goat given phenobarbitone and *C. procera* latex H and E x 40

Table 2: The effect of phenobarbitone and *C. procera* latex on the concentration of serum constituents of Nubian goat kids

Parameters groups	Cholesterol	Creatinine	Bilirubin	Urea	Calcium	Total protein	Albumin	Globulin
	----- (mg dL ⁻¹) -----					----- (g dL ⁻¹) -----		
1 (controls)	45.84±6.26	0.71±0.18	0.19±0.05	21.09±2.78	7.12±0.34	6.88±0.38	4.12±0.13	71.00±0.35
2 (20 mg/kg/day phenobarbitone)	123.23±2.37*	1.71±0.18 ^{NS}	0.36±0.04 ^{NS}	28.82±4.29 ^{NS}	6.08±0.85 ^{NS}	8.90±0.18 ^{NS}	4.08±0.29 ^{NS}	4.82±0.22*
3 (0.5 mg kg ⁻¹ <i>C. procera</i> latex)	120.00±4.32*	1.45±0.09 ^{NS}	0.23±3.21 ^{NS}	53.34±3.21*	7.63±0.83 ^{NS}	7.66±0.43 ^{NS}	2.94±0.21*	4.72±0.17*

NS: Not significant. *: p<0.05, **: p<0.01

Table 3: The combined effect of phenobarbital and *C. procera* latex on activities of serum enzymes in Nubian goats

Parameters groups	GGT IU	GDH IU	LDH IU	AST IU	ALP IU
1 (controls)	13.17±1.34	7.51±0.45	90.10±3.12	31.51±2.02	44.32±1.98
2 (20 mg/kg/day phenobarbital)	16.23±1.01 ^{NS}	12.35±2.11*	92.30±2.56 ^{NS}	66.11±2.31*	96.01±3.33*
3 (0.5 mg kg ⁻¹ <i>C. procera</i> latex)	18.11±1.54*	17.51±1.35**	117.50±3.09*	95.20±4.08**	163.22±3.47**
4 (20 mg/kg/day Phenobarbital +0.5 mL kg ⁻¹ <i>C. procera</i> latex)	23.53±2.01**	22.01±1.78**	148.33±3.52**	198.31±3.41**	172.21±4.04**

NS: Not significant. *: p<0.05, ** p<0.01

Haematological findings: In group 4, the values of PCV and RBC were lower (p<0.05) and those of WBC and MCHC were higher (<0.05) than the controls. In group 3, the values of MCHC and RBC were lower (p<0.05) and those of MCV and PCV were higher (p<0.05) than the controls. MCV values were higher (p<0.05) in group 2 than group 1.

DISCUSSION

There is complete lack of information on the response of ruminants to treatment with phenobarbital sodium prior to administration of laticiferous plants. However, the toxicity of

Calotropis procera latex to goat kids and to pregnant and non-pregnant goats was reported by El-Badwi *et al.* (1998). In the present study, phenobarbital sodium at daily oral doses of 20 mg/kg/day for 14 days was found to potentiate the effect of *C. procera* latex in Nubian goats kids. Potentiation by microsomal enzymes of the toxicity of drugs such as halothane and carbon tetrachloride (Ford *et al.*, 1972) and chloroform (Abdelsalam *et al.*, 1982) is well documented. For example, Gopinath and Ford (1975) found that carbon disulphide, an inhibitor of microsomal enzymes, protects the liver of rats against necrosis produced by chloroform. Dieldrin is an equally effective inducer of the activity of hepatic microsomal enzymes in Nubian goats (Abdelsalam *et al.*, 1982).

Atessahin *et al.* (2004) reported that liver paraoxanase and arylerase activities were increase in rats orally treated with Phenobarbital and in study by Demir *et al.* (2001) found that Phenobarbital caused decrease in brain tissue superoxide dismutase activity.

However, Chladek *et al.* (2001) reported that, pretreatment of rat with Phenobarbital did not change significantly the steady-state hepatic, biliary and partial metabolic decreases of 50 $\mu\text{mol L}^{-1}$ methotrexate.

Since, induction of microsomal enzymes stimulate drug metabolism, the metabolites may be toxic or less toxic than the parent compound. However, it can be assume there will be no changes in the intensity or the duration of action of a drug if the later is not metabolized by the induced enzymes. On the other hand, if the metabolite has little effect, enzyme induction increases the rate of drug inactivation and may prevent deleterious effects of compounds (Conney *et al.*, 1956). This process is useful in detoxification of powerful poisons and carcinogens encountered in the environment of man or animals.

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