

*Full Length Research Paper*

# Toxicity of *Calotropis procera* latex in pregnant and non-pregnant goats

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Experiments were carried out to examine the effect of *Calotropis procera* latex on pregnant and non-pregnant Nubian goats. In pregnant goats during 2 - 3 months of pregnancy, the latex was given at 0.3 ml/kg/day intra vaginally, the animals showed small amounts of mucus at the vulva and plugs, cried in pain and there was maternal and fetotoxicity on day 3 of the experiment. The toxicity was correlated with pathological changes which include higher activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP) and higher concentrations of creatinine, globulin, urea and progesterone while in non-pregnant goats given *C. procera* latex at 0.3 ml/kg/day or at 0.005 ml/kg/day intravaginally, showed vaginitis, anorexia and loss of condition..

**Key words:** *Calotropis procera*, latex, maternal and fetotoxicity, goats.

## INTRODUCTION

*Calotropis procera* belonging to the family Asclepiadaceae, known in Sudan as Ushar. Parts and products of *C. procera* is used for the treatment of variety of ailments such as; fever, joint pain, muscular spasm, constipation, and gastric disorders (Diaziel, 1937; Derasari, 1965; Iwu, 1993). It has been found that latex can be used to treat leprosy, ulcers, tumors and piles and to act as anti-inflammatory agents in laboratory animals affected with carragenan or formalin-induced paw oedema (Kumar and Basu, 1994; Jangde et al., 1994).

The latex, flowers and root bark of this plant are used in Indian folk medicine mainly for digestive system disorders but also for the treatment of asthma, cough, catarrhal inflammation of the upper respiratory tract and skin diseases (Khan and Abdul-Malik, 1989). Diarrhea has been reported as one of the toxic signs of the latex of *C. procera* in animals (Mahmound et al., 1979; Dada et al., 2002). Eghianruwa et al. (2006) reported that the use of

*C. procera* latex as a purgative may not produce required effects in all cases. The effects of *C. procera* on several smooth muscles have been reported. It has been observed to stimulate the uterus (Saha et al., 1961; Chopra et al., 1965). The plant has also been described as abortifacient (Saha et al., 1961; Al-Robai et al., 1993).

The ethanol extract of *C. procera* latex yielded two compounds which were identified as tetraxasteryl acetate and a triterpene, taraxcast (Radha-Pant et al., 1989).

Experiments were carried out to examine the effects of *C. procera* on Nubian goats during 2 - 3 months of pregnancy and consequences on developing fetuses of *C. procera* latex.

## MATERIALS AND METHODS

### Animals

Fifteen, 24 - 36 month-old female Nubian goats were purchased from Kuku Livestock Market, Khartoum North and kept within the premises of the Veterinary Teaching Hospital, University of Khartoum. Six out of these animals were pregnant at 2 - 3 months. All animals were given the 2-weeks adaptation period Penicillin and Sulphamethazine for bacterial infections and coccidiosis,

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**Table 1.** Dosing schedule and time of death of pregnant and non-pregnant goats given *C. procera* latex via intra vaginal route.

Group no.	Goat no.	Dose of latex (ml/kg/day)	Fate of animals	Mortality (%)
1 (Pregnant controls)*	19	Nil	3 days (Slaughtered)	0
	20	Nil	3 days (Slaughtered)	
	21	Nil	3 days (Slaughtered)	
2 (Pregnant goats)*	22	0.3	3 days (Died)	100
	23	0.3	3 days (Died)	
	24	0.3	3 days (Died)	
3 (Non-pregnant controls)	25	Nil	30 days (Slaughtered)	0
	26	Nil	30 days (Slaughtered)	
	27	Nil	30 days (Slaughtered)	
4 (Non-pregnant goats)	28	0.005	30 days (Slaughtered)	0
	29	0.005	30 days (Slaughtered)	
	30	0.005	30 days (Slaughtered)	
5 (Non-pregnant goats)	31	0.3	21 days (Slaughtered in extremis)	0
	32	0.3	21 days (Slaughtered in extremis)	
	33	0.3	21 days (Slaughtered in extremis)	

\* = At 2-3 months of pregnancy.

respectively, fed on Lucerne and were given free access to drinking water.

#### Extraction of the latex

The latex of *C. procera* was obtained from several plants located at the Faculty of Veterinary Medicine University of Khartoum in February 2009. The plants were first identified and authenticated by the Medicinal and Aromatic Plant Research Institute, National center for Research, Khartoum Sudan. Latex was obtained by breaking the leaf stock and allowing the latex to flow into a glass beaker. Fresh latex was obtained in the morning on the day of experiment.

#### Administration of the plant latex

At the end of the adaptation period, the animal were weighed and allotted to five groups, each of three goats. Goats no. 19, 20 and 21 were the un-dosed pregnant controls (group 1). *C. procera* latex was given at 0.3 ml/kg/day to pregnant goats no. 22, 33 and 24 (group 2) by intra vaginal route.

Goats no. 25, 26 and 27 (group 3) were the un-dosed non-pregnant controls. Goats no. 28, 29 and 30 (group 4) were given the *C. procera* latex 0.005 ml/kg/day. *C. procera* latex was given at 0.3 ml/kg/day to non-pregnant goats no. 31, 32 and 33 (group 5) by intra vaginal route.

#### Parameters

Clinical signs and mortality rates were recorded. Blood samples were obtained from the jugular vein before the experiment started and thereafter for heamatological 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 heamoglobin concentration (MCHC) were estimated by standard methods (Shalm et al., 1975).

Sera were analyzed for the activities of GGT, GDH, LDH, AST, ALP and concentration of cholesterol, creatinine, bilirubin, urea, calcium, phosphorus, total protein, albumin and globulin using commercial kits (Stanbio Laboratory Inc., San Antonio, TX, USA).

Concentration of progesterone in serum was determined by radioimmunoassay after extraction from serum (0.1 ml) with *n*-hexane as described by Behrens et al. (1993).

## RESULTS

### Clinical findings and mortality

The dosing schedule and time of death of pregnant and non-pregnant goats' dosed intra vaginally with *C. procera* latex are given in Table 1. In pregnant goats given the latex of *C. procera* intra vaginally at 0.3 ml/kg/day (group 2) showed one hour after dosing, excessive urination, abduction of the hind limbs, obvious abdominal movements, uneasiness and tendency to lie down. Five hours post dosing, small amount of mucus were seen at the vulva and plugs were observed 19 h later with signs of uneasiness. Vaginoscopic examination revealed hyperemia of the vaginal mucosa and increased mucus containing blood around the urethra and cervix. These signs were exaggerated on day 2 and 3 and goats 22, 23 and 24 moaned loudly, cried in pain for approximately 20 min and regurgitated ruminal contents prior to death on day 3.

**Table 2.** Necropsy findings in pregnant and non-pregnant goats given *C. procera* latex by intra vaginal route.

Site	Findings	3 (Pregnant)	Groups 5 (Non-pregnant)	4 (Non-pregnant)
Vagina	Vaginitis	+++	+	+
Cervix	Cervicitis	++	+	(-)
	Mucous plug	++	(-)	(-)
Uterus	Endometritis	+++	++	+
Ovaries	Haemorrhage	++	+	(-)
	Congestion	++	+	(-)
Liver	Enlargement	+++	++	+
	Fatty change	+++	++	++
Gall bladder	Distension with bile	+++	(-)	(-)
	Cholecystitis	+++	(-)	(-)
Spleen	Enlargement	+++	++	(-)
Heart	Haemorrhage	++	+	(-)
	Congestion	++	+	(-)
Kidneys	Haemorrhage	++	+	+
	Congestion	++	+	+
Urinary bladder	Distention with urine	++	(-)	(-)
	Cystitis	++	(-)	(-)
Abomasum	Abomasitis	+	(-)	(-)
Intestine	Enteritis	+++	(-)	(-)

+ ... +++ = degree of change; = no change.

In non-pregnant goats 31, 32 and 33 given the latex of *C. procera* at 0.3 ml/kg/day through intra vaginal routes (group 5), there was vaginitis abduction of the hind limbs and excessive urination following daily administration of the latex. These signs were observed daily in addition to anorexia, loss of condition and depression which developed between days 7 and 20. These goats were slaughtered in extremis on day 21.

In non-pregnant goats 28, 29 and 30 dosed intra vaginally with 0.005 ml/kg/day of latex of *C. procera* (group 4), the signs were similar but less pronounced than those seen in previous groups. These goats were slaughtered on day 30.

### Post-mortem findings

These are summarized in Table 2. In group 2 goats, lesions were more severe than in goats of groups 4 and 5. Vaginitis, slight cervicitis, endometritis, enteritis, hepatitis, abomasitis, haemorrhage on kidneys and heart and pulmonary cyanosis were more marked than those in non-pregnant goats in groups 4 and 5. The cervical plug was disrupted but was not completely removed in group 2 goats. In non-pregnant goats in both group 4 and 5, the cervix was open and inflamed.

### Histopathological findings

In group 2, the liver showed diffuse fatty cytoplasmic vacuolation of the hepatocytes and centrilobular

hepatocellular necrosis, aggregates of lymphocytes and congestion of sinusoids.

The heart muscle fibers were focally vacuolated or necrotic with lymphocytic infiltration, the intestine revealed haemorrhagic enteritis with marked lymphocytic infiltration in lamina propria. Placentitis, polemic haemosiderosis and necrosis of renal tubular cells were detected. In non-pregnant goats in group 5, hepatic congestion and fatty changes, degeneration of the renal tubular cells, spleen haemosiderosis and enteritis were less marked. In non-pregnant goats in group 4, lesions were almost similar but less pronounced than group 5.

### Changes in serum constituents

These are presented in Table 3. The activities of serum AST and ALP and the concentration of creatinine, globulin and urea were higher ( $p < 0.05 - 0.001$ ) in the test group than the control groups. The concentration of bilirubin, cholesterol and total protein in groups 3 and 5 were higher ( $p < 0.05 - 0.01$ ) than the other groups. The concentration of calcium tended to decrease ( $p < 0.05$ ) in groups 4 and 5.

### Changes in progesterone concentration

The concentration of progesterone in pregnant goats given the latex of *C. procera* at 0.3 ml/kg/day decreased to low levels but not so dramatic and that in non-pregnant

**Table 3.** Change in the concentration of serum constituents in pregnant and non-pregnant goats dosed intra vaginally with *C. procera* latex.

Groups/Parameters	GGT i.u	GDH i.u	LDH i.u	AST i.u	ALP i.u	Cholestrol (mg/dl)	Creatinine (mg/dl)
1 (Pregnant controls)	20.95 ± 1.12	17.16 ± 0.75	75.98 ± 2.11	45.43 ± 3.49	166.34 ± 3.27	50.35 ± 4.50	0.94 ± 0.52
2 (Pregnant 0..3 ml/kg/day)	45.55 ± 1.31**	67.89 ± 0.93***	234.03 ± 4.01***	128.80 ± 5.30**	307.74 ± 4.02**	165.80 ± 7.50**	4.23 ± 0.35*
3 (Non-pregnant controls)	19.84 ± 0.93	10.22 ± 0.70	48.63 ± 1.76	33.31 ± 2.90	157.82 ± 4.52	68.39 ± 3.89	0.84 ± 0.31
4 (Non-pregnant 0..005 ml/kg/day)	25.67 ± 0.83*	67.61 ± 1.35***	236.31 ± 2.19***	130.83 ± 5.00**	363.92 ± 5.10***	128.17 ± 4.03**	3.42 ± 0.32*
5 (Non-pregnant 0..3 ml/kg/day)	28.56 ± 1.05*	56.09 ± 1.46**	349.52 ± 3.53***	185.33 ± 6.17**	497.96 ± 3.12***	78.16 ± 12 <sup>NS</sup>	3.10 ± 0.37*

  

Groups/Parameters	Bilirubin (mg/dl)	Urea (mg/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
1 (Pregnant controls)	0.37 ± 0.01	31.81 ± 2.10	6.71 ± 1.30	2.80 ± 0.21	3.54 ± 0.26	3.54 ± 0.12	4.30 ± 0.25
2 (Pregnant 0..3 ml/kg/day)	0.75 ± 0.05*	46.34 ± 2.35**	6.62 ± 0.29 <sup>NS</sup>	3.80 ± 0.30 <sup>NS</sup>	2.61 ± 0.12*	2.61 ± 0.16*	7.10 ± 0.14**
3 (Non-pregnant controls)	0.35 ± 0.07	28.29 ± 1.54	7.34 ± 0.50	2.73 ± 0.11	3.25 ± 0.37	3.25 ± 0.12	3.93 ± 0.35
4 (Non-pregnant 0..005 ml/kg/day)	1.17 ± 0.02**	48.31 ± 2.97*	10.87 ± 0.37*	3.40 ± 0.18 <sup>NS</sup>	2.97 ± 0.57*	2.97 ± 0.12 <sup>NS</sup>	7.86 ± 0.74*
5 (Non-pregnant 0..3 ml/kg/day)	0.13 ± 0.07 <sup>NS</sup>	56.92 ± 4.19*	13.29 ± 1.12*	5.90 ± 0.54*	2.87 ± 0.56 <sup>NS</sup>	2.87 ± 0.25 <sup>NS</sup>	6.45 ± 0.23*

N.S = Not significant. \* = p < 0.05; \*\* p < 0.01.

**Table 4.** Haematological changes in pregnant and non-pregnant goats dosed with *C. procera* latex via intra vaginal route.

Groups/Parameter	Hb (g/dl)	PCV (%)	RBC (x10 <sup>6</sup> mm <sup>3</sup> )	WBC(x10 <sup>3</sup> mm <sup>3</sup> )	MCV (m <sup>3</sup> )	MCHC (%)
1 (Pregnant controls)	8.72 ± 0.39	29.00 ± 0.81	9.19 ± 0.28	7.40 ± 0.65	31.55 ± 2.11	30.10 ± 0.90
2 Pregnant (0.3 ml/kg/day)	7.05 ± 0.28 <sup>N.S</sup>	42.05 ± 1.18**	6.58 ± 0.43	10.00 ± 0.43*	63.90 ± 2.69**	16.82 ± 0.33**
3 Non-pregnant (controls)	9.03 ± 0.30	27.67 ± 0.35	8.58 ± 0.29	7.40 ± 0.65	32.24 ± 1.19	32.63 ± 1.75
4 Non-pregnant (0.005 ml/kg/day)	5.67 ± 0.20**	23.00 ± 0.9*	4.35 ± 0.87*	14.80 ± 0.58*	52.87 ± 1.05**	24.65 ± 0.57**
5 Non-pregnant (0.3 ml/kg/day)	7.14 ± 0.08	33.51 ± 0.19	7.98 ± 0.34 <sup>N.S</sup>	16.10 ± 0.52**	41.49 ± 0.16*	21.32 ± 0.36*

N.S = Not significant. \* = p < 0.05; \*\* p < 0.01.

goats given the latex at 0.3 ml/kg/day or 0.005 ml/kg/day also tended to decrease.

### Changes in haematology

These are described in Table 4. In group 2, the values of PCV and MCV were higher (p < 0.01) and those of MCHC were lower (p < 0.05 - 0.01) than group 1. The values of Hb and WBC did

not change. The values of MCHC and Hb were lower (p < 0.05 - 0.01) in groups 4 and 5 than group 3. PCV was higher (p < 0.01) in group 5 and lower (p < 0.05) in group 4 than the non-pregnant control goats in group 3.

### DISCUSSION

The intra vaginal administration of *C. procera*

latex at 0.3 ml/kg/day; caused death of all pregnant goats at day 3 of the experiment. This might be attributed to rapid absorption of the active ingredients of the plant latex and of the consequent development of a general toxicity in pregnant goats. Saha et al. (1961); Al-Robai et al. (1993) described the plant as abortifacient; although abortion did not take place but foetotoxicity was an ultimate development with an obvious patchy endometritis. The decrease in

serum progesterone concentration in pregnant goats treated with *C. procera* latex was not observed in non-pregnant goats treated via the intravaginal route with *C. procera* latex at 0.3 ml/kg/day for 21 days or 0.005 ml/kg/day for 30 days.

A number of medicinal plants such as *Phoenix dactylifera* (Palmae); *Triticum aestivum* (Grammineae); *Phaseolus vulgaris* (Fabaceae) and *Cyperus esculentus* (Cyperaceae) contain estrone and 17  $\alpha$ -estradiol (Watt and Breyer-Brandwijk, 1962; Bakhiet and Adam, 1995).

Toxicity of plants to goats was observed by Panter et al. (1990), in their study, three piperidine alkaloid containing plants, *Conium maculatum*, *Nicotiana glauca* and *Lupinus formsus* included multiple congenital contractures and palatoschisis in goat kids when their dams were gavaged with the plant during gestation days 30 - 60. Also maternal and fetotoxicity were observed at 2000 ppm toluene via whole body exposure in pregnant rats from gestation day 6 - 15 inclusive 6 h/day.

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