

**EFFECT OF CROSS BRED COW URINE IN RAT DURING SUB ACUTE SAFETY STUDY**

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Received 16-6-2012 Accepted 18-8-2012

**ABSTRACT**

Acute and sub acute oral toxicity studies of Holstein Friesian cross bred cow urine was conducted to determine the median lethal dose for both male and female rats. There was no mortality even at the highest dose tested (5 ml/kg body weight) indicating that cow urine was practically non-toxic. In the repeated dose 28 day sub acute oral toxicity study the blood and serum samples were analyzed on day 0, 14 and 28 for estimating different hematological parameters (TEC, TLC, DLC, Hb, Hct, MCV, MCH and MCHC). There were significant ( $P < 0.01$ ) changes in hematological values as well as concentration of various hematological parameters, but the values were under normal ranges.

**KEYWORDS :** Cow urine, Hematological, Acute, Subacute, Rats

**INTRODUCTION**

Cows were regarded the backbone of the economy of ancient Indians. From the ancient period, cow's urine has been used as a medicine. In Veda, cow's urine was compared to the nectar. The redistillate of cow's urine was found to possess total antioxidant status of around 2.6 mmol, contributed mainly by volatile fatty acids (1500 mg/L) as revealed by the GC-MS studies. These fatty acids and other antioxidants might cause protective effects (Krishnamurthi et al., 2004). Cow's urine concoction is a traditional preparation commonly administered to convulsing children by the Yoruba-speaking people of Nigeria. Its use is, however, often complicated by severe poisoning (Ayorinde et al., 1982). Further, the toxicity was also experimentally confirmed in mice (Oyebola and Elegbe, 1975). In our previous study no significant alteration in biochemical profile were observed during administration of Holstein Friesian cross bred cow urine (Jagadeesh et al., 2011).

The detailed studies on safety evaluation of cow urine employing controlled studies in experimental animals are scanty. Hence the present work was undertaken to study the effect of cross bred cow urine on hematological profile in rats.

**MATERIALS AND METHODS****Safety evaluation of cow urine**

Acute toxicity study was conducted as per method of Jagadeesh et al. (2011). Hematological parameters were estimated using blood samples collected on day 0, 14 and 28 in sub-acute toxicity study by retro-orbital plexus puncture method using microhaematocrit capillary tubes containing disodium EDTA as an anticoagulant. The hematological parameters, Haemoglobin concentration (Hb), Haematocrit (Hct), Total erythrocyte count (TEC), Total leukocyte count (TLC), Differential leukocyte count (DLC), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were estimated following the standard method of Jain (1990).

**Histopathology:** At the end of the test period i.e., on day 14 in acute toxicity and day 28 in subacute toxicity study the rats were sacrificed under diethyl ether anesthesia and organs namely liver, kidney, heart, lung and spleen were collected for both gross and histopathology study.

Table 1. Effect of cow age on haematological parameters of male Friesian day calving cows

Group	Days	Hb (g/dl)	Hct (%)	WBC (10 <sup>9</sup> /l)	MCV (fl)	MPV (fl)	MCHC (g/fl)
Group I (2-3)	0 DAY	7.99 ± 0.17	39.65 ± 0.53	13.56 ± 0.51	52.97 ± 0.27	78.27 ± 0.79	39.83 ± 0.25
	7 DAY	7.37 ± 0.23	44.28 ± 0.59	16.37 ± 0.57	55.23 ± 0.83	78.69 ± 0.77	41.67 ± 0.18
	28 DAY	7.57 ± 0.28	37.93 ± 0.72	17.97 ± 0.72	57.07 ± 0.31	78.96 ± 0.73	39.06 ± 0.33
Group II (4-5)	0 DAY	7.71 ± 0.23	39.38 ± 0.37	17.70 ± 0.15	50.30 ± 0.67	18.37 ± 0.77	39.13 ± 0.38
	7 DAY	7.97 ± 0.39	39.99 ± 0.13	17.36 ± 0.19	53.77 ± 0.37	18.57 ± 0.30	39.33 ± 0.99
	28 DAY	7.87 ± 0.27	40.73 ± 0.23	17.68 ± 0.21	52.29 ± 0.28	19.20 ± 0.76	37.60 ± 0.83
Group III (6-7)	0 DAY	7.89 ± 0.77	38.60 ± 0.73	17.70 ± 0.70	50.92 ± 0.58	19.20 ± 0.38	39.13 ± 0.21
	7 DAY	7.22 ± 0.39	38.16 ± 0.63	13.81 ± 0.23***	76.77 ± 1.78***	19.33 ± 0.57	36.66 ± 0.35**
	28 DAY	6.97 ± 0.27*	35.92 ± 0.93	13.33 ± 0.21***	78.92 ± 1.73***	19.37 ± 0.73	38.67 ± 0.23
Group IV (8-9)	0 DAY	7.18 ± 0.72	38.68 ± 0.00	17.50 ± 0.73	75.69 ± 0.26	19.38 ± 0.23	40.02 ± 0.39
	7 DAY	6.30 ± 0.19*	36.70 ± 0.09*	13.37 ± 0.13***	78.73 ± 0.13***	19.16 ± 0.39	36.80 ± 0.79**
	28 DAY	5.86 ± 0.23**	34.20 ± 0.05*	13.29 ± 0.16***	77.97 ± 0.13***	18.87 ± 0.27**	36.33 ± 0.71
Group V (10-11)	0 DAY	7.18 ± 0.71	36.97 ± 0.22	17.10 ± 0.60	52.29 ± 0.77	19.33 ± 0.33	39.37 ± 0.97
	7 DAY	6.57 ± 0.77	35.97 ± 0.52**	13.79 ± 0.19***	76.99 ± 0.65***	18.87 ± 0.32	35.67 ± 0.3***
	28 DAY	5.73 ± 0.22***	33.38 ± 0.12***	13.26 ± 0.18***	78.93 ± 0.13***	18.77 ± 0.18**	37.86 ± 0.96***

Values are mean ± S.E., \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, n=6

Table 2. Effect of cross bred cow urine on the growth of rats in the presence of 28 days of rat body weight

Group	Days	No. of rats (P <sub>0</sub> )	Initial weight (g)	Final weight (g)	Survival (%)	Mortality (%)	Survival (%)
Control	0 DAY	17.35 ± 0.91	3.77 ± 0.38	78.65 ± 1.36	100	0.00	100 ± 0.15
	17 DAY	17.72 ± 0.91	3.77 ± 0.37	78.58 ± 0.76	100	0.00	100 ± 0.11
	28 DAY	17.35 ± 0.91	3.57 ± 0.25	79.97 ± 0.76	100	0.00	100 ± 0.07
Cross bred	0 DAY	18.73 ± 1.11	3.73 ± 0.76	78.37 ± 0.97	100	0.00	100 ± 0.13
	17 DAY	22.88 ± 0.75**	3.77 ± 0.75*	77.88 ± 1.81	100	0.00	100 ± 0.13
	28 DAY	23.55 ± 0.92**	3.38 ± 0.32***	77.58 ± 2.37***	100	0.00	100 ± 0.15***
Cross bred + urine	0 DAY	17.77 ± 0.69	3.76 ± 0.71	78.87 ± 2.85	100	0.00	100 ± 0.12
	17 DAY	17.87 ± 0.72	3.72 ± 0.55	75.31 ± 1.61	100	0.00	100 ± 0.11
	28 DAY	17.70 ± 0.69	3.78 ± 0.27	77.97 ± 1.17**	100	0.00	100 ± 0.15**
Cross bred + urine	0 DAY	17.60 ± 0.56	3.78 ± 0.25	75.90 ± 1.98	100	0.00	100 ± 0.02
	17 DAY	17.61 ± 0.99	3.75 ± 0.29	75.92 ± 1.89	100	0.00	100 ± 0.01
	28 DAY	25.80 ± 0.53***	7.85 ± 0.13	73.67 ± 1.19*	100	0.00	100 ± 0.01*
Cross bred + urine	0 DAY	18.00 ± 0.53	3.18 ± 0.77	75.99 ± 1.17	100	0.00	100 ± 0.03
	17 DAY	18.25 ± 0.83	3.15 ± 0.26	77.68 ± 1.23	100	0.00	100 ± 0.01
	28 DAY	25.71 ± 0.33***	5.79 ± 0.17	73.72 ± 2.29***	100	0.00	100 ± 0.01***

\* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001, \*\*\*\* P < 0.0001

Table 3. Effect of day of treatment on the response of some of the parameters in the study.

Group	Days	WBC (x10 <sup>9</sup> /mm <sup>3</sup> )	Haematocrit (%)	ESR (mm/hr)	MCV (fl)	MCH (pg)	MCHC (g/l)
Control	0 DAY	8.07 ± 0.16	38.90 ± 0.32	15.90 ± 0.71	93.06 ± 0.77	77.32 ± 0.61	39.23 ± 0.25
	1 DAY	7.57 ± 0.13	38.92 ± 0.28	15.72 ± 0.78	97.33 ± 0.37	78.37 ± 0.73	77.71 ± 0.37
	2 DAY	7.77 ± 0.25	38.97 ± 0.37	15.10 ± 0.92	97.99 ± 0.75	78.52 ± 0.92	39.52 ± 0.76
	0 DAY	8.73 ± 0.25	38.75 ± 0.26	15.00 ± 0.12	97.87 ± 0.72	77.67 ± 0.31	78.96 ± 0.37
	1 DAY	7.82 ± 0.27	38.62 ± 0.32	17.72 ± 0.73	97.71 ± 0.82	79.75 ± 0.71	39.72 ± 0.25
	2 DAY	7.75 ± 0.16	38.33 ± 0.77	17.93 ± 0.73	96.92 ± 0.99**	79.20 ± 0.57	38.19 ± 0.75
Group 1	0 DAY	8.02 ± 0.77	38.25 ± 0.22	15.00 ± 0.72	98.98 ± 0.71	77.72 ± 0.22	39.72 ± 0.73
	1 DAY	7.72 ± 0.22	38.52 ± 0.12	17.92 ± 0.23*	92.57 ± 0.77	79.25 ± 0.56	37.71 ± 0.92**
	2 DAY	7.27 ± 0.16	37.65 ± 0.22**	13.77 ± 0.27**	95.07 ± 0.62	72.77 ± 0.76	35.22 ± 0.35*
	0 DAY	7.57 ± 0.77	38.92 ± 0.76	15.20 ± 0.37	92.77 ± 0.95	73.12 ± 0.22	77.69 ± 0.63
	1 DAY	7.63 ± 0.15	38.10 ± 0.22**	13.92 ± 0.19**	97.05 ± 0.19	79.79 ± 0.25	35.93 ± 0.27***
	2 DAY	6.52 ± 0.34**	37.90 ± 0.55***	13.53 ± 0.13**	97.63 ± 0.25	72.36 ± 0.21*	37.70 ± 0.66**
Group 2	0 DAY	7.23 ± 0.30	37.55 ± 0.75	15.20 ± 0.63	61.03 ± 0.77	77.27 ± 0.57	77.37 ± 0.22
	1 DAY	7.13 ± 0.37	38.53 ± 0.06	13.72 ± 0.30***	95.20 ± 0.33	79.52 ± 0.22	36.21 ± 0.36**
	2 DAY	5.52 ± 0.23***	37.67 ± 0.25***	13.22 ± 0.27***	96.76 ± 0.67**	77.22 ± 0.27**	37.39 ± 0.37***

\* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001, \*\*\*\* p < 0.0001, \*\*\*\*\* p < 0.00001.

Table 1. Effect of cross bred cow urine on the growth, survival, and productivity of rats.

Group	Day	Survival (%)	FCR (g/g)	FCR (g/g)	FCR (g/g)	FCR (g/g)	FCR (g/g)	FCR (g/g)
Control	0 Day	100	1.00	1.00	1.00	1.00	1.00	1.00
	15 Day	100	1.00	1.00	1.00	1.00	1.00	1.00
	30 Day	100	1.00	1.00	1.00	1.00	1.00	1.00
U1	0 Day	100	1.00	1.00	1.00	1.00	1.00	1.00
	15 Day	100	1.00	1.00	1.00	1.00	1.00	1.00
	30 Day	100	1.00	1.00	1.00	1.00	1.00	1.00
U2	0 Day	100	1.00	1.00	1.00	1.00	1.00	1.00
	15 Day	100	1.00	1.00	1.00	1.00	1.00	1.00
	30 Day	100	1.00	1.00	1.00	1.00	1.00	1.00
U3	0 Day	100	1.00	1.00	1.00	1.00	1.00	1.00
	15 Day	100	1.00	1.00	1.00	1.00	1.00	1.00
	30 Day	100	1.00	1.00	1.00	1.00	1.00	1.00
U4	0 Day	100	1.00	1.00	1.00	1.00	1.00	1.00
	15 Day	100	1.00	1.00	1.00	1.00	1.00	1.00
	30 Day	100	1.00	1.00	1.00	1.00	1.00	1.00
U5	0 Day	100	1.00	1.00	1.00	1.00	1.00	1.00
	15 Day	100	1.00	1.00	1.00	1.00	1.00	1.00
	30 Day	100	1.00	1.00	1.00	1.00	1.00	1.00

FCR = Feed Conversion Ratio; FCR = Feed Conversion Ratio; FCR = Feed Conversion Ratio; FCR = Feed Conversion Ratio; FCR = Feed Conversion Ratio.

The organs were cleared off from the adrenal tissues using saline and placed on a blotting paper and gently pressed to remove excess saline adhering to the organ. The representative tissue samples were collected in neutral buffered formalin and processed for histopathology by cutting sections of 5 micron thickness and staining with haematoxylin and eosin (Luna, 1968).

## RESULTS AND DISCUSSION

### Acute toxicity and Sub acute toxicity study

Cow urine even at the highest dose (5ml/kg) tested did not result in mortality indicating that, cow urine was practically non toxic and no observable clinical signs were noted , further LD50 value for cow urine could not be established.

### Hematology

The hematological values for male and female rats are presented in Table 1, 2, 3 and 4.

From the table 1, 2, 3 and 4 it is revealed that among all hematological parameters, a significant ( $P<0.01$ ) decrease was observed in MCHC and erythrocyte count in both male and female rats, at the same time a significant ( $P<0.01$ ) increase was noted in neutrophil counts as well as MCV in both male and female rats after administration of cow urine at 28 days .

There were no gross lesions noticed at the end of 28 day repeated oral safety study. All organs were having normal architecture. No gross lesions were found in any of the treated groups.

No histopathologic lesions noticed in rats on 28 day repeated oral safety study. Microscopically all organs showed normal architecture.

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