EFFECT OF HOLSTEIN FRIESIAN CROSS BRED COW URINE ON BIOCHEMICAL PROFILE OF RATS IN SUB ACUTE SAFETY STUDY

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ABSTRACT

An acute and subacute oral toxicity study of Holstein Friesian cross bred cow urine was conducted to determine the median lethal dose for both male and female rats separately. Five groups of male rats and five groups of female rats, each consisting of six rats were used for estimating LD50 value. The study was conducted as per the OECD guidelines. In the acute toxicity study in 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 subacute oral toxicity study the serum samples were analyzed on day 0, 14 and 28 for different biochemical parameters such as AST, ALT, ALP, BUN, Creatinine, Bilirubin and STP concentration.

There were significant ($P<0.01$) changes in biochemical values as well as concentration of various biochemical parameters, but the significant values were under normal ranges. Study concludes that Holstein Friesian cross bred cow urine has no effect on biochemical parameters in rats.

KEY WORDS : cow urine, biochemical, acute, subacute toxicity, rats

INTRODUCTION

Cows were regarded as wealth and were the backbone of the economy of ancient India. Cattle were one of the most frequently used animals described in Vedas. Cows were regarded as mother ("Gau-mata") and referred to as Aghnya.From the ancient period, cow urine has been used as a medicine. In Veda, cow's urine was compared to the nectar. In Sushrut, several medicinal properties of cow's urine have been mentioned and cow urine was known to cause weight loss and to cure leprosy, cardiac and kidney problems, indigestion, stomach ache, edema, etc. (Kaviratna and Sharma., 1996).

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 the observed protective effects (Krishnamurthi et al., 2004). Cow 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).The detailed studies on pharmacological activities and safety evaluation of cow urine employing controlled studies in experimental animals are scanty. Hence the present work was undertaken to study the effect of cow urine on biochemical profile of rats.

MATERIALS AND METHODS

Wistar albino rats aged between six to seven weeks and weighed 130-150g were used in the study. They were acclimatized to the experimental conditions for one week. After acclimatization, animals were grouped and housed in standard polypropylene rat cages during the experiment. They were maintained under standard laboratory hygienic conditions, providing standard laboratory animal feed and water *ad libitum*. The approval of the Institutional Animal Ethics Committee was obtained prior to start of the experiment vide No.VPT.1.CPCSEA.2008

Holstein-Friesion (HF) cross- bred, milking cows aged about 2-3 years were selected for urine collection. Animals were hygienically maintained in the Department of Livestock Production and Management, Veterinary College, Hebbal, Bangalore.

Natural voiding mid- stream urine was collected in a sterile glass container and stored in refrigerator at 4^0C and utilized for the experiment within two hours of collection. Precaution was taken to avoid any external contamination. Immediately after collection physical, chemical and microscopic examination of urine was done.

Safety evaluation of cow urine

Acute toxicity study

Acute toxicity study was conducted to determine the median lethal dose for both male and female rats separately. Five groups of male and female rats, each consisting of six rats were used for estimating LD50 value.

The rats were fasted overnight prior to administration of the urine. A graded dose (0.05, 0.1, 0.2 and 0.3 ml) of urine was administered in a single dose to rats by gavage using a stomach tube. The rats were observed for a period of 14 days for clinical signs. An attempt was made to arrive at the median lethal dose using the method of Finney (1971).

Serum biochemical parameters were estimated from the serum samples of the experimental rats on day 0, 14 and 28 in sub-acute toxicity study to investigate the toxic effect on organs and tissues specifically on liver and kidney using ARTOS Semi-Automatic Biochemical Analyzer. The commercially available diagnostic kits from M/s. Swemed diagnostics, Bangalore, were employed in the estimation of the following parameters:

1. Aspartate aminotransferase (AST) activity
2. Alanine aminotransferase (ALT) activity
3. Alkaline phosphatase (ALP) activity
4. Blood urea nitrogen (BUN) concentration
5. Creatinine concentration
6. Bilirubin concentration
7. Total serum protein (TSP) concentration

Histopathology

At the end of the test period i.e., on day 14 and 28 the rats were sacrificed under diethyl ether anesthesia for histopathological study.

Organs namely liver, kidney, heart, lung and spleen were collected for histopathology. The organs were cleared off from the adnexal 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).

The data were analyzed by One-way ANOVA with Dunnett's post test, using Graph Pad Prism statistical software, version 4.01. The results were presented as mean ± S.E.M.

RESULTS AND DISCUSSION

Acute toxicity study

There were no observable clinical signs. Cow urine did not result in mortality of either male or female rats. Further LD50 value was also not determined.

Table 1. Effect of cow urine on biochemical parameters of male rats in 28 day oral safety study

Groups	Days	ALT	AST	BUN	Creatinine	ALP	Total protein	Bilirubin
GroupI Control	**0 DAY**	45.50±1.06	46.98±2.34	20.38±1.35	0.50±0.19	132.20±0.86	7.57±0.23	0.25±0.01
	14 DAY	50.88±0.87	49.21±1.47	21.89±1.10	0.47±0.59	132.70±1.21	7.68±0.16	0.34±0.02
	28 DAY	48.28±1.57	53.44±0.72	23.73±0.92	0.51±0.36	132.28±0.57	7.58±0.23	0.31±0.05
Group II 0.05 ml	**0 DAY**	51.92±1.38	46.87±1.22	21.21±0.69	0.42±0.35	132.47±1.17	7.73±0.23	0.26±0.02
	14 DAY	51.68±0.83	48.45±2.96	22.06±0.52	0.48±0.38	133.18±1.51	7.80±0.26	0.32±0.02
	28 DAY	49.20±1.83	45.19±1.39	23.10±0.90	0.48±0.62	141.25±3.93*	7.46±0.26	0.20±0.03
Group III 0.1 ml	**0 DAY**	51.97±1.00	48.08±2.64	21.06±0.65	0.53±0.39	130.25±2.22	7.42±0.33	0.27±0.01
	14 DAY	50.23±1.61	45.91±1.02	22.13±0.43	0.57±0.03	135.33±1.61	7.45±0.10	0.19±0.03
	28 DAY	45.66±1.33	45.83±1.35*	21.94±0.40	0.70±0.50 ***	140.66±1.92*	7.26±0.13	0.20±0.03
Group IV 0.2 ml	**0 DAY**	51.90±2.32	53.65±4.66	21.38±0.39	0.44±0.06	133.68±2.46	7.72±0.37	0.30±0.03
	14 DAY	52.93±1.49	50.21±1.33	20.72±0.16	0.64±0.03 ***	130.06±2.78	7.50±0.11	0.27±0.36
	28 DAY	50.23±2.08	48.75±2.34	21.30±0.23*	0.63±0.49 ***	133.80±1.22	7.16±0.19	0.21±0.02
Group V 0.3 ml	**0 DAY**	49.76±2.70	51.72±1.45	21.91±0.67	0.48±0.34	132.97±2.04	7.92±0.29	0.28±0.02
	14 DAY	49.83±1.75	53.91±2.26	22.36±0.31	0.64±0.05 ***	124.10±2.45	7.33±0.13	0.211±0.05
	28 DAY	53.34±0.99	54.20±1.29	23.21±0.55	0.80±0.11 ***	132.20±2.84*	7.13±0.19	0.24±0.03

Values are mean ± SE
***P<0.05 **P<0.01 ***P<0.001 n = 6**

Table 2. Effect of cow urine on biochemical parameters offemale rats in 28 day oral safety study

Groups	Days	ALT	AST	BUN	Creatinine	ALP	Total protein	Bilirubin
GroupVI Control	0 DAY	52.17±1.25	68.65±1.39	22.05±1.04	0.40±0.63	132.10±0.79	7.73±0.17	0.33±0.02
	14 DAY	50.87±0.87	52.78±1.35	20.96±0.84	0.38±0.05	132.70±1.21	7.51±0.20	0.26±0.04
	28 DAY	50.78±1.19	56.70±2.98	22.73±0.79	0.37±0.02	132.28±0.57	7.31±0.24	0.28±0.05
Group VII 0.05 ml	0 DAY	51.92±1.38	71.87±1.77	26.28±0.74	0.50±0.05	132.80±1.95	7.90±0.32	0.33±0.02
	14 DAY	51.68±0.83	53.11±2.93	24.90±0.77**	0.54±0.09**	134.25±1.45	7.63±0.22	0.31 ±0.05
	28 DAY	52.20±1.33	53.45±1.14	24.43±0.98	0.53±0.09	137.92±1.71*	7.30±0.05	0.29±0.05
GroupV III 0.1 ml	0 DAY	51.97±1.00	69.75±1.44	22.85±0.62	0.41±0.07	132.75±1.49	7.58±0.35	0.31±0.03
	14 DAY	51.90±0.80	61.53±2.62	26.36±0.00**	0.44±0.06	135.33±1.61	7.11±0.27	0.34±0.08
	28 DAY	51.85±0.97	61.00±2.35	25.16±0.78	0.48±0.06	138.00±1.03*	7.26±0.13	0.27±0.02
Group IX 0.2 ml	0 DAY	48.32±1.68	71.98±1.76	23.02±0.85	0.39±0.04	132.02±1.04	7.72±0.37	0.35±0.03
	14 DAY	54.48±3.05	56.38±1.40	23.28±0.86	0.46±0.04	140.06±1.82**	7.47±0.09	0.46±0.09
	28 DAY	56.06±1.99	58.75±2.34	23.13±0.80	0.53±0.04	140.36±2.51**	7.16±0.11	0.36±0.09
Group X 0.3 ml	0 DAY	47.09±2.31	80.05±2.55	23.85±0.80	0.45±0.02	132.97±1.28	8.08±0.38	0.35±0.02
	14 DAY	53.25±1.37	60.58±2.98	23.06±0.89	0.60±0.10**	139.10±2.35*	7.16±0.18	0.32±0.05
	28 DAY	61.68±3.19	62.53±2.48	20.21±0.05	0.69±0.08***	152.20±1.45***	7.63±0.22	0.36±0.06

Values are mean ± SE *P<0.05 **P<0.01 ***P<0.001 n = 6

There was no gross and no histopathological lessions were noticed on completion of 28 day repeated oral safety study.

Biochemical parameters

Serum obtained from blood samples collected on day 0, 14, 28 of experiment period were used to estimate ALT, AST, ALP, creatinine, BUN, bilirubin and TSP for both male and female rats separately. From Table 1 and 2 it is revealed that there is a significant change in biochemical profile after administration of cow urine. There was a significant increase ($P < 0.05$) in all biochemical parameters after administration of cow urine at intermediate and high dose in both male and female rats.

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