

38mg/kg b.wt and 76mg/kg b.wt of LD₅₀. Rats were divided into eight groups. 0.5ml of cow urine was given for seven consecutive days prior to administration of chlorpyrifos.

Group C- control (corn oil only)

Group CU- cow urine only

Group P-1/8 of LD50 i.e 19mg/kg b.wt CPF

Group Q-1/4 of LD50 i.e 38mg/kg b.wt CPF

Group R-1/2 of LD50 i.e 76mg/kg b.wt CPF

Group X-1/8 of LD50 i.e 19mg/kg b.wt + cow urine

Group Y-1/4 of LD50 i.e 38mg/kg b.wt + cow urine

Group Z-1/2 of LD50 i.e 76mg/kg b.wt + cow urine

CPF was administered orally in corn oil. Rats were orally administered with cow urine for consecutive seven days prior to the oral administration of different doses of CPF. All animals were humanly killed 24, 48 and 72 hrs after the last treatment and bone marrow were collected for the chromosomal aberrations.

2.4 Chromosomal aberrations

Chromosomal aberrations in bone-marrow metaphase cells was performed according to the technique described by Preston et al.[16]. with some modifications. Animals were intraperitoneally injected with 1.0 ml colchicines (1mg/kg b.w) 1 hr prior to the scheduled time of sacrifice, in order to accumulate metaphase cells. After 24 hours of given dose, the animals were sacrificed by cervical dislocations and bone marrow cells were harvested. Briefly, femur bones were excised and cleaned of any adhering muscle, the bone marrow was extracted in 0.56% KCL. The harvested cells were incubated at 37°C for 20-30 minutes and then centrifuged for 10 minutes at 1000-1200 rpm. Cells were fixed in Carnoy's fixative (methanol: acetic acid 3:1). Centrifugation and fixation were repeated three times at 10 minutes intervals. The material was re-suspended in a small volume of fixative, dropped on to clean slides, flame-dried, and stained with 5% Giemsa solution for 15 minutes and then put in xylene and mounted with DPX. A total of 200 well spread metaphase plates containing 42 chromosomes were scored for chromosomal aberrations at a magnification of 100X for each group. Different types of aberrations such

as clumping, gaps, chromatid breaks, dicentric, rings etc were scored and expressed as % chromosomal aberrations (%CA).

2.5 Statistical analysis

Results are expressed as means±SE. The statistical analysis was performed using one-way analysis of variance (ANOVA) and post hoc-tukey test.

3. Results

For the investigation of chromosomal aberrations (CA %), 200 metaphase cells for each treatment were scored. The mean value of %CA(chromosomal aberrations) observed for control(corn oil) was 10.42±0.32,10.26±0.15,9.66±0.24 and controls(cow urine) was 11.39±0.41,11.56±0.24,11.39±0.47 at 24hrs, 48hrs and 72hrs respectively. Tables 1-3 represent the various types of chromosomal aberrations observed in the rat bone marrow cells which include gap, break, rings, stickiness etc. The frequency of percent chromosomal aberrations induced by the different concentrations and the ameliorative effect of cow urine at different time intervals are given in Figures 1-3. After the treatment of 19 mg/kg b.wt of CPF ,the values of mean %CA(Chromosomal aberrations) were found to be 30.17±0.27, 26.82±0.36, 18.22±0.12 respectively at 24hrs, 48hrs and 72 hrs for animals treated with only chlorpyrifos. This increase was found to be significant (p<0.001) as compared to both the control groups i.e C and CU. Thus, there was a significant increase (P<0.001) in the frequency of aberrant cells in bone marrow of rats. However, in the group pretreated with cow urine before the administration of chlorpyrifos a significant (p<0.001) decrease in the frequency of chromosomal aberrations is observed (Fig. 1). Similar trend was observed in the rats with 38mg/kg b.wt and 76mg/kg b.wt CPF and CPF+U (Figs. 2-3). Perusal of Figure 1-3 also reveal that the highest number of chromosomal aberrations was observed in 24 hours of exposure.

Table 1: Frequency of chromosomal aberrations in rats treated with 19mg/kg b.wt(1/8 OF LD₅₀)CPF and CPF+U at 24hrs, 48hrs and 72 hours.

DOSE	TREATMENT	TIME	NO. OF METAPHASE	STICKINESS	GAP	BREAK	DICENTRIC	STRETCHED	AllDot Like	RINGS	TOTAL	%CA
One	C	24	208	18	2		2				23	10.54
Eighth of LD50	CU	24	204	20	1	1	1				23	11.22
	P	24	200	32	6	5	4	3	6	3	59	29.45*
	X	24	202	18	5	6	4	1	5	9	48	23.76**
	C	48	201	10	4	4	4				22	10.89
	CU	48	200	10	2	3	2	2	2	1	23	11.56
	P	48	205	30	8	3	7		3	4	55	26.83*
	X	48	202	10	4	3	7	3	6	4	37	18.31**
	C	72	204	10	4	4			2		20	9.87
	CU	72	202	12	2	5	3	1			23	11.39
	P	72	206	22	6	1	5	4	4	6	48	23.29*
	X	72	200	9	2	1	1	3	4	6	26	13**

*p<0.001(statistical difference between control and CPF);**p<0.001(statistical difference between CPF and CPF+U), CA: Chromosomal aberrations

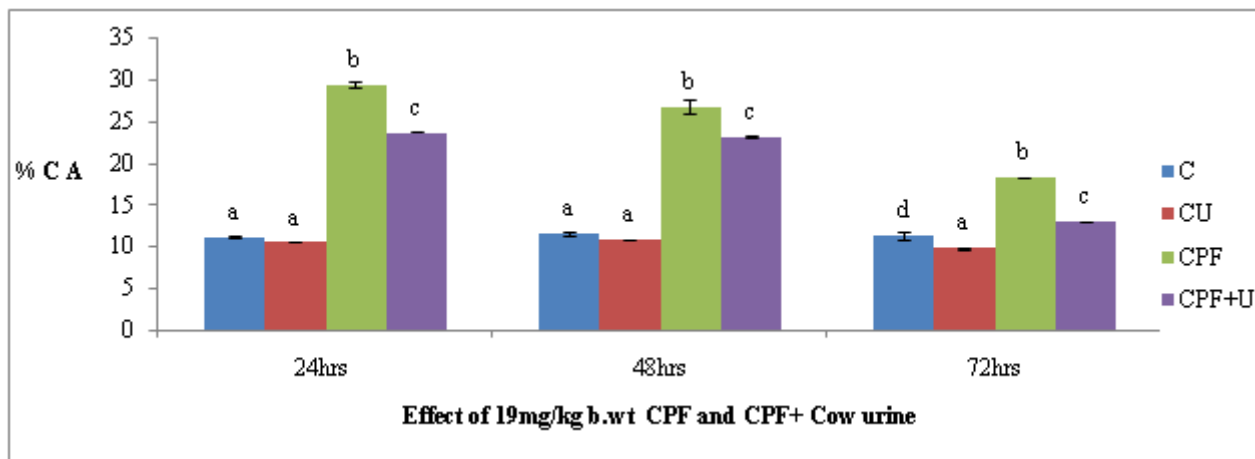


Figure 1: Effect of 19mg/kg b.wt CPF and CPF+U on rat bone marrow cells at different time intervals. Means that do not share a common letter are significantly different.

Table2: Frequency of chromosomal aberrations in rats treated with 38mg/kg b.wt(1/4 of LD₅₀) CPF and CPF+U at 24hrs, 48hrs and 72 hrs

DOSE	TREATMENT	TIME	NO. OF METAPHASE	STICKINESS	GAP	BREAK	DICENTRIC	STRETCHED	All Dot Like	RINGS	TOTAL	%CA
One Fourth of LD ₅₀	C	24	208	18	2		2				23	10.54
	CU	24	204	20	1	1	1				23	11.22
	Q	24	205	44	9	9	3	4	4	6	79	38.53*
	Y	24	200	32	6	6	5	4	4	3	64	32**
	C	48	201	10	4	4	4				22	10.89
	CU	48	200	10	2	3	2	2	2	1	23	11.56
	Q	48	201	36	4	4	3	3	5	3	58	28.86*
	Y	48	203	18	4	3	6	3	4	8	48	23.64**
	C	72	204	10	4	4			2		20	9.87
	CU	72	202	12	2	5	3	1			23	11.39
	Q	72	201	31	4	2	3	1	3	1	45	22.38*
	Y	72	202	15	2	1	7	2	3	7	35	17.32**

*p<0.001(statistical difference between control and CPF);**p<0.001(statistical difference between CPF and CPF+U),CA: Chromosomal aberrations

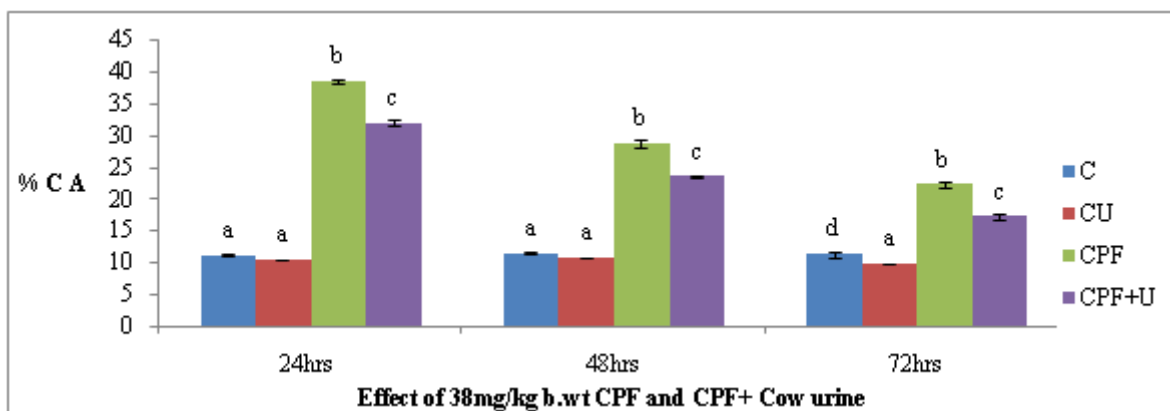


Figure 2: Effect of 38mg/kg b.wt CPF and CPF+U on rat bone marrow cells at different time intervals. Means that do not share a common letter are significantly different.

Table 3: Frequency of chromosomal aberrations in rats treated with 76 mg/kg b.wt(1/2 of LD₅₀) CPF and CPF+U at 24hrs, 48hrs and 72 hrs

DOSE	TREATMENT	TIME	NO. OF METAPHASE	STICKINESS	GAP	BREAK	DICENTRIC	STRETCHED	All Det Lib e	RINGS	TOTAL	%CA
Half of LD50	C	24	208	18	2		2				23	10.54
	CU	24	204	20	1	1	1				23	11.22
	R	24	201	53	7	9	7	2	6	1	85	42.28*
	Z	24	200	35	7	7	4	5	7	4	69	34.5**
	C	48	201	10	4	4	4				22	10.89
	CU	48	200	10	2	3	2	2	2	1	23	11.56
	R	48	203	41	7	10	2	1	3	3	66	32.51*
	B	48	201	4	5	7	6	7	4	10	56	27.86**
	Z	72	204	10	4	4			2		20	9.87
	CU	72	202	12	2	5	3	1			23	11.39
	R	72	200	24	7	5	7	4	6	6	59	29.5*
	Z	72	207	16	8	7	5	5	4	8	51	24.63**

* $p < 0.001$ (statistical difference between control and CPF); ** $p < 0.001$ (statistical difference between CPF and CPF+U), CA: Chromosomal aberrations

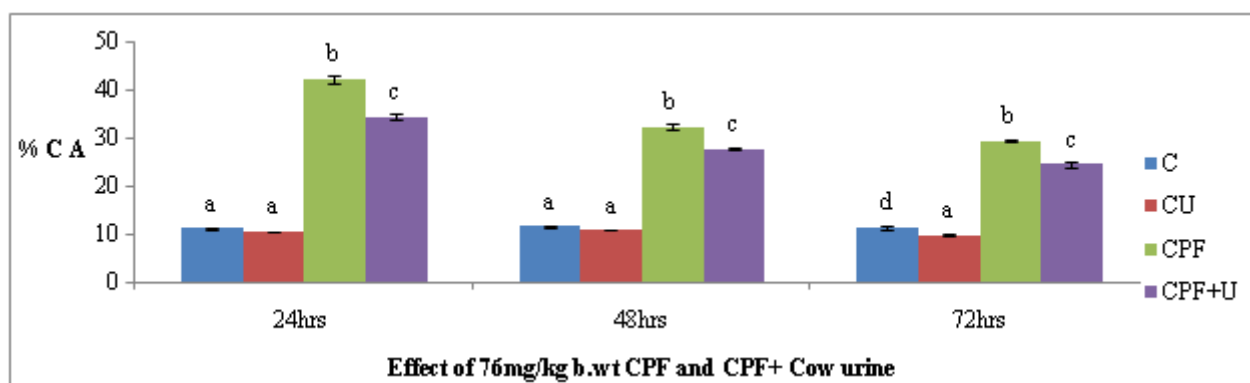


Figure 3: Effect of 76mg/kg b.wt CPF and CPF+U on rat bone marrow cells at different time intervals. Means that do not share a common letter are significantly different.

4. Discussion

Pesticides are widely used in agricultural areas to improve the crop yield, but the indiscriminate use of these chemicals in the environment causes toxicity to the non-target organisms. Organophosphates (OP's) belong to the most commonly used groups of insecticides. It is estimated that about 2 million tons of organophosphorus (OP) pesticides are used in a year throughout the world [17]. Use of organophosphate pesticides has been and remains pervasive in both developed and developing nations; as a result concerns are increasing regarding the relative safety of these chemicals to the environment and human health. Earlier organophosphate pesticides were considered safer alternative to organochlorines [18] but it was revealed in a study that organophosphate insecticides had the propensity to cause significant oxidative damage in rat brain, which was found to be associated with marked perturbations in antioxidant defense system [19]. Evaluation of DNA damage and cytotoxicity induced by commonly used organophosphate pesticides individually and in mixture was also earlier done on rat tissues [20]. Other OP's such as alachlor, atrazine, maleic hydrazine, paraquat and trifluralin were found to have positive results for genotoxicity by increasing comet tail length [21]. During the present investigation CPF was found to possess genotoxic potential as revealed by significant increase in the chromosomal aberrations in the rats treated with CPF as compared to controls. Similarly the assessment of chlorpyrifos induced DNA damage in rat liver and brain cells was observed through comet assay [22]. The significant

increase in chromosomal aberrations in mouse spleen cells after treatment with CPF was observed by Amer et al. [23]. The formation of oxygen free radicals seems to be a major factor in the toxicity of pesticides and both organochlorines and organophosphates have been reported to produce oxidative stress [24]. The increased ROS due to high oxidative stress may attack the biomolecules like DNA which may result in the increased chromosomal aberrations as observed in the present study. The DNA damaging effect of CPF was found to be alleviated in the present study when the group given CPF was pretreated with the cow urine. Cow urine is well known to possess antioxidant and antimicrobial properties [25]. It is rich in vitamin A, B, C, D, E and volatile fatty acids. The ameliorative effect of these vitamins single or in combination, in protecting DNA damage have been revealed from the number of studies [15, 23, 26]. The immunomodulatory [27], anticlastogenic [28] and chemoprotective [29] effects of distillate and redistillate of cow urine have also been reported. Protective effect of cow's urine has also been established in human polymorphonuclear leukocytes challenged with genotoxic chemicals [30]. In a study it was observed that cow urine alone or in combination with other antioxidants resulted in alleviation of lindane-induced oxidative stress in kidney of Swiss mice [15].

5. Conclusion

So our present study highlighted the importance of "Kamdhenu Ark" (cow urine) to possess the genoprotective effect against CPF induced genotoxicity. Further, there is

need to develop strategies for promoting the vital medicinal potential of cow urine for the benefit of mankind.

6. Acknowledgement

Authors are thankful to C.P.E.P.A (Centre with Potential for Excellence In Particular Field) for providing financial assistance and the Head, department of Zoology for providing necessary laboratory facilities.

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