International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

"Kamdhenu Ark" as a Genoprotective Agent Against Chlorpyrifos Induced Genotoxicity

Shelly Sharma[1], Pooja Chadha[2], Madhu Sharma[3]

[1, 2,3]Cytogenetics Laboratory, Department of Zoology, Guru Nanak Dev University, Amritsar-143005, Punjab, India

Abstract: ***Pesticides are being purposely added to the environment to control domestic and agricultural pests for increasing the yield of crops to satisfy the growing demands of population. The present study was aimed to evaluate the ameliorative effect of cow urine on chlorpyrifos-induced genotoxcity in vivo. Male healthy rats, 8–10 weeks old, weighing 120 ± 10 g were randomly selected and divided into eight groups, namely, corn oil (C); cow urine (CU),Group P-1/8 of LD50 i.e 19mg/kg b.wt CPF, Group Q-1/4 of LD_{50} i.e 38mg/kg b.wt CPF, Group R-1/2 of LD_{50} i.e 76mg/kg b.wt CPF, Group X-1/8 of LD_{50} i.e 76mg/kg b.wt + cow urine, Group Y-1/4 of LD_{50} i.e 38mg/kg b.wt + cow urine, Group Z-1/2 of LD_{50} i.e 19mg/kg b.wt + cow urine. All treatments were administered orally for 24, 48, 72 hours. Chlorpyrifos treated group showed increased chromosomal aberrations,as compared to controls. The groups pretreated with cow urine exhibited a significant decrease in frequency of aberrant cells as compare to the rats treated with chlorpyrifos alone. The present findings clearly show that oral administration of cow urine protects the rats from chlorpyrifos induced DNA damage and suggests that this treatment alleviates the genotoxicity of chlorpyrifos to a greater extent.***

Keywords: Chlorpyrifos, chromosomal aberrations, cow urine, genotoxicity

1. Introduction

Emergence of pesticides as genotoxicants is being a source of concern in the recent studies. The long- term genetic hazard of pesticides cannot be ignored and, it is, therefore highly desirable to search for naturally occurring genoprotective alternatives to minimize their toxic effects. Traditional products are being increasingly screened for their role in modulating the activity of environmental genotoxicants. Chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate) is a wide-ranging organophosphate insecticide used to control the agricultural and household pests[1]. It is an active ingredient of various preparations used against ectoparasites of dogs, cats and cattle [2]. Number of studies have reported the occurrence of chlorpyrifos in several media including air, dust, food, and hand wipe samples at preschool children's homes or day care centers (3,4,5,6,7,8). In India, CPF is classified as an extremely hazardous pesticide, its residue has been found in scented roses and their products [9]. Surprisingly, the soft drinks are also found to contain CPF in a concentration of 4.8 ppb, which is 47 times higher than permissible limit [10]. CPF, like other organophosphate compounds is known to produce toxic effects through the inhibition of acetyl cholinesterase (AChE) activity which leads to accumulation of acetylcholine in the cholinergic receptors. It also induces oxidative stress leading to generation of free radicals which play an important role in DNA damage, lipid per oxidation and protein oxidation. [11,12,13]. OP's are easily metabolized in mammals, as a result of their chemical structure containing ester bond which is hydrolyzed by animal estererases. In Veda, Kamdhenu ark (cow urine distillate) was compared to the nectar (Rigveda 10.15). From the ancient period in India, cow's urine has been used as a medicine. Cow urine is known to possess antioxidant and antimicrobial activities. [14]. It is considered useful in treating gastric infections, anemia, jaundice, piles, skin diseases and arthritis. It is also taken as an appetizer and a diuretic. Cow urine has volatile fatty acids and antioxidants which prevent formation of reactive oxygen species responsible for DNA damage. In a study, cow urine and combination of antioxidants (vitamin C, E) has shown protective effect against organochlorine pesticide [15]. As no study has been done to investigate the genoprotective potential of cow urine against organophosphate compounds, the aim of the present study was to investigate the ameliorative effect of cow urine "kamdhenu ark" against chlorpyrifos induced genotoxicity. Chromosomal aberration analysis was done as it is one of the reliable biomarkers for genotoxicity evaluation and good predictor in cancer risk assessment.

2. Material and Methods

2.1Chemicals

Chlorpyrifos was purchased from Sigma Chemicals, St Louis, MO. Metahnol, glacial acetic acid, Colchicines, potassium chloride, xylene was purchased from Merck, Germany and Giemsa was obtained from Fischer chemicals

2.2 Experimental Animals

Male albino rats, weighing 120 ± 10 g and 8–10 weeks old were procured. Animals were maintained on sterilized rice husk bedding in polypropylene cages and kept at a temperature of about 23 ± 3C with 12 ± 1 h light and day cycle. Animals were fed on standard pellet diet. Food and water were provided *ad libitum*. Rats were acclimatized for one week prior to the start of the experiments. Experimental protocol was approved by the Institutional Animal Ethics Committee. Handling of animals was according to the guidelines of Committee for Purpose of Control and Supervision of Experiments on Animals (C.P.C.S.E.A).

2.3 Experimental design

After range finding, LD_{50} was determined which was found out to be 152mg/kg body weight using probit analysis software. Three doses were selected i.e 19mg/kg b.wt,

38mg/kg b.wt and 76mg/kg b.wt of LD_{50}. 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 obsereved (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 LD_{50})CPF and CPF+U at 24hrs, 48hrs and 72 hours.

DOSE	TREATMENT	TIME	NO. OF METAPHASE	STICKINESS	GAP	BREAK	DICENTRIC	STRETCHED	All Dot Like	RINGS	TOTAL	%CA
One	C	24	208	18	2		2				23	10.54
Eigth of	CU	24	204	20	1	1	1				23	11.22
LD50	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

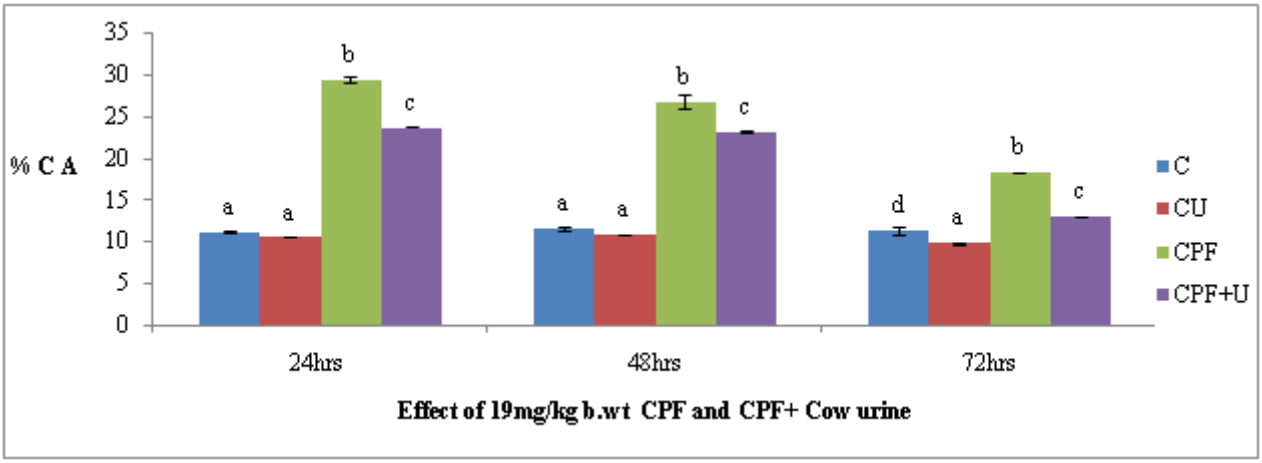

Figure 1: Effect of 19mg/kg b.wt CPF and CPF+U on rat bone marrow cells at different time intervals. Means that do not share a common letter are significantly different.

Table2: Frequency of chromosomal aberrations in rats treated with 38mg/kg b.wt(1/4 of LD_{50}) 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	C	24	208	18	2		2				23	10.54
of LD50	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	13	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

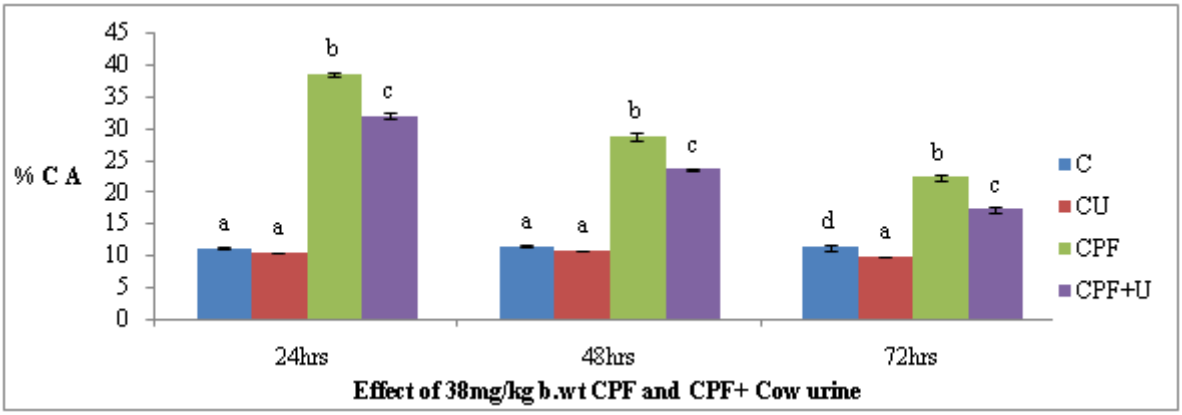

Figure 2: Effect of 38mg/kg b.wtCPF and CPF+U on rat bone marrow cells at different time intervals. Means that do not share a common letter are significantly different.

Table 3: Frequency of chromosomal aberrations in rats treated with 76 mg/kg b.wt(1/2 of LD_{50})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
Half of	C	24	208	18	2		2				23	10.54
LD50	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

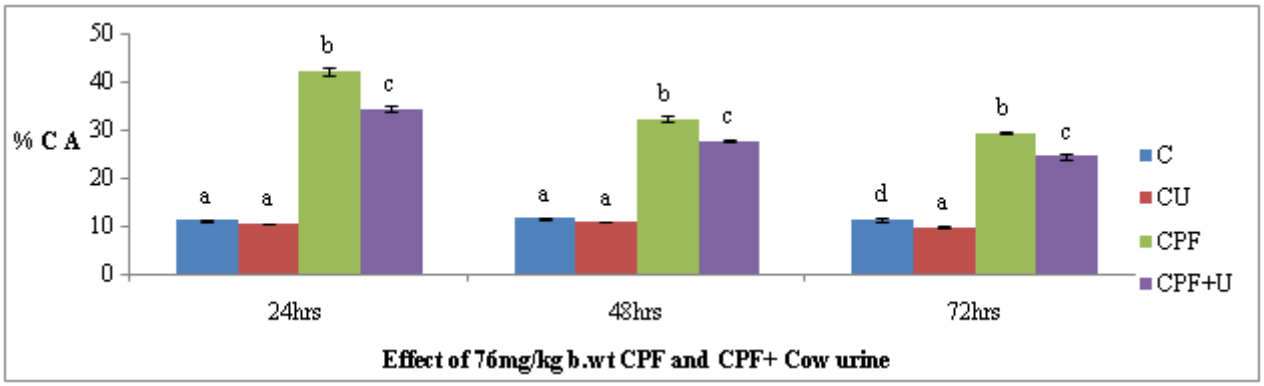

Figure 3: Effect of 76mg/kg b.wt CPF and CPF+U on rat bone marrow cells at different time intervals. Means that do not share a common letter are significantly different.

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 cytoxicity induced by commonly used organophosphate pesticides individually and in mixture was also earlier done on rat tissues[20].Other OP's such as alachor, atrazine ,maleic hydrazine, paraquat and trifuralin 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 incuded 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.

References

[1] J.E. Casida, G.B. Quinstad, "Organophosphate toxicology: safety aspects of non-acetylcholine cholinesterase secondary targets," Chemical Research in Toxicology, (17), pp. 983-98, 2004.

[2] B.L. Blangburn, D.S. Lindsay, "Ectoparasiticides: In: Adams HR (ed) Veterinary Pharmacology and Therapeutics," 8th edition, The Iowa State University Press, Ames, IA, USA, pp. 1008, 2001.

[3] C.L. Curl, R.A. Fenske., J.C. Kissel, J.H. Shirai, T.F. Moate, W. Griffith, G. Coronado, B. Thompson, "Evaluation of take-home organophosporus pesticide exposure among agricultural workers and their children," Environmental Health Perspectives, 110(12), pp. A787–A792, 2002.

[4] R.A. Fenske, C.Lu , D. Barr, L. Needham, "Children's exposure to chlorpyrifos and parathion in an agricultural community in central Washington state," Environmental Health Perspectives , 110(5), pp.549–553, 2002.

[5] A.C. Clayton, E.D. Pellizzari, R.W. Whitemore, J.J. Quackenboss, J. Adgate, K. Sexton, "Distributions, associations, and partial aggregate exposure of pesticides and polynuclear aromatic hydrocarbons in the Minnesota Children's Pesticide Exposure Study (MNCPES)," Journal of Exposure Analysis and Environmental Epidemiology, 13(2), pp. 100–111, 2003.

[6] P.J. Lioy, R.D Edwards, N. Freeman, S. Gurunathan, E. Pellizzari, J.L Adgate, J. Quackenboss, K. Sexton, "House dust levels of selected pesticides and a herbicide measured by the EL and LWW samplers and comparisons to hand rinses and urine metabolites," , Journal of Exposure Analysis and Environmental Epidemiology(10), pp. 327–340, 2000.

[7] K. Sexton, J.L. Adgate, L.E. Eberly, C.A. Clayton, R.W. Whitmore, E.D. Pellizzari, P.J. Lioy, J.J. Quackenboss, "Predicting children's short-tem exposure to pesticides: results of a questionnaire screening approach," Environmental Health Perspectives, (111), pp. 549–553, 2003.

[8] N.K. Wilson, J.C. Chuang, R. Iachan, C. Lyu, S.M. Gordon ,M.K. Morgan, H. Ozkaynak , L.S. Sheldon, "Design and sampling methodology for a large study of preschool children's aggregate exposures to persistent organic pollutants in their everyday environments." Journal of Exposure Analysis and Environmental Epidemiology, (14), pp. 260–274, 2004.

[9] A. Kumar , G. Nandda, A. Shanker, "Determination Of Chlorpyrifos 20%Ec(Dursban 20% Ec) in scented rose and its product," Journal Chromatography, (1050), pp. 193-502, 2014.

[10] CSE (2006)-Centre For Science And Environment,Issue In Depth http://www.cse.org/misc/cola-indepth/cola 2006/cola-press 2006.htm,2006

[11] F. Canadas, D. Cardona, E. Davila, F. Sanchez-Santed, "Long-term neurotoxicity of chlorpyriphos; spatial learning impairment on repeated acquisition in a water maze," Toxicological Sciences (85), pp.944-51, 2005.

[12] C.N. Pope, T.K. Chakraborti, M.L. Chapman, J.D. Farrar, "Long-term neurochemical and behavioural effects induced by acute chlorpyriphos treatment," Pharmacology Biochemistry Behaviour, (42), pp. 251-56, 1992.

[13] H. Shi, Y. Sui, X. Wang, Y. Luo, L. Ji, "Hydroxyl radical production and oxidative damage induced by cadmium and naphthalene in liver of Carassius auratus," Comparative Biochemistry and Physiology, (C 140) , pp. 115–121, 2005.

[14] E. Jarald, S. Edwin, V. Tiwari, R. Garg and E. Toppo, **"**Antioxidant and antimicrobial activities of cow urine," Global Journal of Pharmacology, 2 (2), pp.20-22, 2008.

[15] G. Nagda, D. Kumar Bhatt, "Effect of treatment of cow's urine ''Gomutra'' and antioxidants in alleviating the lindane-induced oxidative stress in kidney of Swiss mice (Mus musculus)," DOI 10.1007/s11033-014-3044-6, Molecular Biology, 2014.

[16] R.J. Preston, B.J. Dean, A.F. Galloway, S. Mcfree, "Mammalian *in vivo* cytogenic assay-analysis of chromosomal aberration in bone marrow cells mutation," Mutation Research, (189), pp. 157-165, 1987.

[17] D**.** Primentel, A. Greiner, T. Bashore, "Economic and environmental costs of pesticide use In: Rose" J (ed) Environmental Toxicology: Current Developments. Gordon and Breach Science Publishers, New Delhi, India, pp 121-151, 1998.

[18] C.R. Alavanja, M.K. Ross , M.R. Bonner, "Increased cancer burden among pesticide applicators and others due to pesticide exposure," CA Cancer Journal of Clinicians, (63), pp. 120-142, 2013.

[19] F.M. El-Demerdesh, "Lipid peroxidation, oxidative stress and acetylcholinesterase in rat brain exposed to organophosphate and pyrethroid insecticides," Food and Chemical Toxicology, (49), pp.1346-1352, 2011.

[20] A. Ojha, S. Yaduvanshi, S. Pant, V. Lomash, N. Srivastava, " Evaluation of DNA damage and cytotoxicity induced by three commonly used organophosphate pesticides individually and in mixture, in rat tissues," Wiley Periodicals, Inc. Environmental Toxicology , (28), pp.543-552, 2011.

[21] G. Ribas, G. Frenzilli, R. Barale, R. Marcos, "Herbicide-induced DNA damage in human lymphocytes evaluated by the single-cell gel electrophoresis (SCGE) assay," Mutation Research , (344), pp.41-54, 1995.

[22] A. Mehta, R.S. Verma, N. Srivastava, " Chlorpyrifos induced DNA damage in rat liver and brain cells," Environ Mol Mutagen, (49), pp. 426-33, 2008.

[23] S.M. Amer, F.A.E. Aly, "Cytogenetic effects of pesticides. IV. Cytogenetic effects of the insecticides Gardona and Dursban," Mutation Research, (279), pp. 165-170, 1992.

[24] R.S. Verma, A.Mehta, N.Srivastava, "in vivo chlorpyrifos induced oxidative stress: Attenuation by antioxidant vitamins", Pesticide Biochemistry and Physiology, (88), pp. 191-196, 2007.

[25] M.F. Rahman, M. Mahboob, K. Danadevi, B.B. Saleha, P. Grover, " Assessment of genotoxic effects of chlorpyrifos and acephate by comet assay in mice leukocytes," Mutatation Research, (516), pp.139-47, 2002.
[26] N.S. Ahmed , A.S. Mohamed , M.A. Abdel-Wahhab , "Chlorpyrifos-induced oxidative stress and histological changes in retinas and kidney in rats: protective role of ascorbic acid and alpha tocopherol," Pesticide Biochemistry and Physiology , (98), pp.33-38, 2010.
[27] S.F. Ambali, D.O. Akanbi, A.G. Giwa, O.O. Oladipo, J.O. Ayu, "Chlorpyrifos- induced clinical, haemotological and biochemical changes in Swiss albino mice-mitigating effect by co-administration of vitamins C and E," Life Science Journal,(3),7, pp. 37-44, 2010.
[28] R.S. Chauhan, B.P. Singh, L.K. Singhal, "Immunomodulation with kamdhenu Ark in mice," J Immunol Immunopathol , (71), pp. 89-92, 2001.
[29] D. Dutta ,S.S. Devi , K. Krishnamurthi et al, " Anticlastogenic effect of redistilled cow's urine distillate in human peripheral lynphocytes challenged with manganese dioxide and hexavalent chromium," Biomedical and Environmental Sciences, 19, pp. 487-494, 2006.
[30] W. Raja, R.C. Agarwal, "Chemoprotective potential of cow urine against 7, 12- dimethylbenz(a) antracene-induced skin papillomagenesis in mice," Academic Jornal of Cancer Research, (3), pp. 7-10, 2010.
[31] K. Krishnamurthi, D. Dutta, S.S. Devi, T. Chakrabarti, "Protective effect of diatillate and redistillate of cow,s urine in human polymorphonuclear leukocytes challenged with established genotoxic chemicals," Biomedical and Environmental Sciences, (17), pp. 57-66, 2004.

Author Profile

Shelly Sharma is doing Ph.D as Research Fellow in Cytogenetics Laboratory, Department of Zoology, Guru Nanak Dev University, Amritsar, Punjab, India.

Dr Pooja Chadha is working as assistant professor in. Department of Zoology, Guru Nanak Dev University, Amritsar, Punjab, India

Madhu Sharma is doing Ph.D as Research Fellow in Cytogenetics Laboratory, Department of Zoology, Guru Nanak Dev University, Amritsar, Punjab, India