



## Research Article



# Modulation of chlorpyrifos-induced genotoxicity and oxidative stress by cow urine supplementation in male wistar rats

Shelly Sharma, Sukanya Mehra and Pooja Chadha\*

*Cytogenetics Laboratory, Department of Zoology, Guru Nanak Dev University, Punjab, Amritsar-143005, India*

*Corresponding author e-mail: poojachadha77@yahoo.co.in*

**(Received: 08/01/2022; Revised: 13/04/2022; Accepted: 20/05/2022)**

### ABSTRACT

Due to extensive use of chemical fertilisers, pesticides and hormone injections our food is becoming poisonous and to eliminate such poisons from our body natural products can prove to be very effective. The therapeutic use of cow urine has a long history in India. And recently being considered as potent bio pesticide, the cow urine has been considered in the present study to evaluate its ameliorative potential against the oxidative stress and genotoxicity induced in the brain tissue of rat by intoxication of chlorpyrifos (CPF). Chlorpyrifos is a broad spectrum conventional organophosphate agrochemical used to control a variety of pests in agriculture and for domestic purposes. For the experiment the antioxidant potential of cow urine was measured by DPPH assay. Further in present inquisition rats were divided into two groups, one exposed to 1/8<sup>th</sup>, 1/4<sup>th</sup> and 1/2 of LD<sub>50</sub> of CPF and other group of rats were treated with cow urine consecutively for ten days prior to exposure of CPF. Brain tissue was collected after 24hrs, 48hrs and 72hrs of treatment. Symbolic elevation in %tail DNA, tail moment and MDA levels while reduction in SOD, CAT and GST activities was noted in chlorpyrifos treated groups as compared to control groups. The effect of dose was also ascertained for all parameters. On the other hand, pre-treatment with cow urine significantly abridged the effect of CPF intoxication. Thus, it appears from the study that cow urine ameliorates pesticide induced cancer insurgence in rat because of its high antioxidant levels and can be used as a viable potential substitute for anti-cancer therapy.

**Keywords:** Genotoxicity, Oxidative stress, Cow Urine, Chlorpyrifos, Brain.

### INTRODUCTION

During recent years agrochemicals being extensively and indiscriminately applied globally as plant protection agents and for boosting food production leading to discharge of large amounts of these chemicals into the environment. Among them many of them cause detrimental effects to non-target organisms. Organophosphate pesticides being pervasive in both developed as well as developing countries leads to the concerns regarding the relative safety of these chemicals on the environment as well as human health (Saulsbury et al., 2009). Chlorpyrifos (CPF) being highly applied organophosphorus pesticide against several pests. The advert effects of chlorpyrifos mainly attribute to the irreversible inhibition of enzyme acetylcholinesterase (AChE) which leads to accumulation of acetylcholine, its decreased activity and thus preventing smooth transmission of nerve impulse (Colovic et al., 2013; Singh et al., 2010) Thus due to extensive use and accessibility, exposure to CPF has become a major global health concern. Consequently, there is a dire need to search for natural antitoxic alternatives to alleviate the toxic effects.

Natural products include large group of chemical compounds produced by living organisms viz., animals,

bacteria, fungi and plants that have distinctive therapeutic effects. In the broadest sense, natural products include any substance produced by life (Strobel and Daisy, 2003). The bioactive compounds present inside the natural resources have persistently been of great interest to researchers working on several disorders (Hemraj et al., 2012). Most of the cancer therapeutic drugs have been derived from natural resources (Aggarwal et al., 2006). Researchers have generated number of new anti-cancer drugs but resistance to these drugs imposes great challenge to introduction of new drugs. Thus, the modern research world is taking huge interest in traditional medicines along with the modern therapies.

In ancient Vedas cow urine has been considered holy and compared to the nectar (Rigveda 10.15). According to Ayurveda Sangraha, the ancient scriptures, Sushrut samhita (45/221) and Charak (sloka-100), cow urine possesses medicinal values and is considered to be active in treating various types of disorders. Due to its several pharmaceutical values cow urine has been considered for the treatment of various diseases including diarrhoea, gastric infection, renal colic, jaundice, anaemia, skin diseases, fever, epilepsy, anaemia, abdominal pain, constipation and arthritis etc. (Nagda and Bhatt, 2014)

It is also considered neuroprotective agent and known to generate bioenergy at sub cellular level (Kelly, 1997; Randhawa, 2010). The analysis of cow urine also shows that it carries minerals, enzymes as well as vitamin A, B, C, D and E. Also, it is found to contain volatile fatty acids along with antioxidants which quench the formed reactive oxygen species responsible for DNA damage (Pathak ML & Kumar A., 2010). Also, cow urine has been found to carry antimicrobial and lipase potential which can be the important factor for its usage as a medicine (Kumar et al., 2004). The study permed by Jarald et al., (2008) revealed cow urine carries antioxidant and antimicrobial activities.

Though there are numerous examples stating the efficiency of cow urine yet scientific reports are few. However, its efficacy against toxicants such as pesticides induced toxic effects is scarcely explored. Therefore, the present study was undertaken to fill the lacuna in this regard. Consequently, in the present study the potential of cow urine in alleviating the oxidative stress and genotoxicity induced by chlorpyrifos in brain of rat has been investigated.

## MATERIALS AND METHODS

### Chemicals

CPF (99%) was purchased from Sigma Aldrich, St Louis, U.S. Cow urine was obtained from Divya Pharmacy, Haridwar, India, while all other chemicals used were of analytical grade and were obtained from Sisco Research Laboratory, Mumbai, India.

### Determination of Antioxidant Activity using DPPH free radical scavenging activity of cow urine

The free radical scavenging capacity of the cow urine was determined by DPPH method described by Brand-Williams et al. (1995) with slight modifications. The DPPH radical solution (200 $\mu$ M) was prepared in methanol. The cow urine sample (10 $\mu$ l) was added to the tubes followed by addition of 3 ml DPPH solution and was incubated for 30 min at room temperature. The absorbance was recorded at 517 nm using Shimadzu UV-1700 Pharma spec. Ascorbic acid was used as positive control. The results were expressed as % radical scavenging activity. The percentage radical (%) scavenging activity was calculated by the following formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

### Experimental Design

The experiment was divided into two parts. In the first part three groups of rats were treated with the three concentrations of chlorpyrifos i.e 1/8<sup>th</sup>, 1/4<sup>th</sup> and 1/2 of LD<sub>50</sub> (152 mg/kg b.wt) dissolved in corn oil along with one control group treated with corn oil. In the second part, again four groups were considered. All the groups were pre-treated with 0.5ml of cow urine for ten consecutive days and then administered with all three concentrations of chlorpyrifos in corn oil. Control group

is also treated with cow urine. Each group contains ten rats.

### Animals and care

Mature male Wistar rats having weight 120 $\pm$ 20 g was used for the experiment. Animals were caged at room temperature with a relative humidity and on a 12 h light–darkness cycle. The animals had free access to commercial pellet diet and water *ad libitum*. All the experiments were performed according to guidelines for use and care of laboratory animals and were approved by Committee for Purpose of Control and Supervision of Experiments on Animals (C.P.C.S.E. A). Animals were acclimated 15 days prior to any experiment.

### Tissue preparation

The animals were sacrificed after 24, 48 and 72hrs of treatment. After decapitation, brain was immediately removed; blotted, weighed and washed using chilled saline solution. One part of the brain tissues was minced, cut into small pieces and then dried on a filter paper and homogenized (10% w/v) separately, in ice- cold 1.15% KCl, 0.01 M sodium, potassium phosphate buffer (pH 7.4) in a Potter–Elvehjem type homogenizer. Further, the homogenate was centrifuged at 18,000g for 30 min at 4°C, and the resultant supernatant was used for the determination of different enzyme assays and TBARS and glutathione content. The other region of the brain was used for single cell gel electrophoresis for genotoxicity assessment. For this, homogenate (25% w/v) of fresh tissues was prepared in homogenizing buffer (0.075 M NaCl containing 0.024 M EDTA, pH 7.2) in a Potter Elvehjem homogenizer. To obtain nuclei, the homogenate was centrifuged at 900g for 10 min and the pellet was gently suspended in 4.0 ml of chilled homogenizing buffer.

### Determination of Genotoxicity

The potential genotoxicity of CPF was determined by following the basic protocol for the Comet Assay, with some modifications (Singh et al., 1988).

### Slide Preparation

Clean and dried slides were layered with 75 $\mu$ l of normal melting agarose (1% prepared in 0.1 M sodium phosphate buffer, pH 7.2 containing 0.9% NaCl) and allowed to solidify. The precoated slide was once again coated with second layer of 75 $\mu$ l of a mixture containing equal volumes of sample homogenate and low melting agarose (2% in phosphate buffer saline). Slides were then immersed in the lysing buffer (containing 2.5 M NaCl, 100 mM EDTA, 10 mM Trizma base, pH 10.0 adjusted with 10 N NaOH and with 5% DMSO and 1% Triton X-100) for 3 hrs in the dark. After lysis, electrophoresis was performed for 20 min for unwinding of DNA and left undisturbed. The slides were then placed and immersed in fresh electrophoresis buffer in order to remove all lysing solution. Electrophoresis was performed for 20 min at constant voltage (1 V/cm and 300 mA). After electrophoresis, the slides were washed thrice with freshly prepared neutralizing buffer (0.4 M Tris-HCl, pH 7.4) for 10 min each at room temperature. Slides were dehydrated in absolute methanol for 10 min

and left at room temperature to dry. The whole procedure was performed in dim light to minimize artifactual DNA damage. After drying, each slide was stained with 20µl of ethidium bromide rinsed with water, and covered with a coverslip. Slides were scored under fluorescence microscope and analyzed using casplab software. A total of 100 cells were scanned per three slides per tissue per animal randomly (with a total of 300 cells scored per tissue per animal) and analyzed using an image analysis system (casplab). The % tail DNA and tail moment (TM) were considered as the measure of DNA damage.

#### **Biochemical analysis**

##### **Determination of thiobarbituric acid reactive substances in rat brain**

According to the method of Draper and Hardley, 1990, the extent of lipid peroxidation in terms of thiobarbituric acid reactive substances (TBARS) formation was measured. Brain supernatant was mixed with 1.25 ml TCA, 2 ml TBA (0.67%) and heated for 1 hr at 80°C. After cooling, the precipitate was removed by centrifugation. The absorbance of the sample was measured at 532 nm using a blank containing all the reagents except the sample. As 99% TBARS are malondialdehyde (MDA), so TBARS concentrations of the samples were calculated using the extinction coefficient of MDA, which is  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ .

##### **Determination of glutathione S-transferase (GST) activity in rat brain**

Glutathione S-transferase catalyzes the conjugation reaction with glutathione in the first step of mercapturic acid synthesis. The activity of GST was measured according to the method of Habig, 1974. One unit of GST activity is defined as 1 mol product formation per minute.

##### **Determination of superoxide dismutase (SOD) activity in rat brain**

Superoxide dismutase was assayed according to Kono, 1978. One unit of enzyme activity was defined as the amount of the enzyme exhibiting 50% inhibition of auto-oxidation rate of pyrogallol. Data is expressed as U/mg protein.

##### **Determination of catalase (CAT) activity in rat brain**

The enzyme catalase converts  $\text{H}_2\text{O}_2$  into water. The CAT activity in brain supernatant was measured spectrophotometrically at 240 nm by calculating the rate of degradation of  $\text{H}_2\text{O}_2$ , the substrate of the enzyme (Aebi, 1984). Activity was monitored at 240 nm for 60 s. Data is expressed as U/mg protein.

##### **Protein estimation**

The protein content of the tissue homogenates mentioned earlier was determined by following the method described by Lowry, 1951 using bovine serum albumin as a standard.

##### **Statistical analysis**

The data were analyzed using SPSS 11.0 for Windows. The significance of differences was calculated using one-way analysis of variance (ANOVA) followed by Tukey's procedure for multiple comparisons.

## **RESULTS AND DISCUSSION**

### **DPPH Assay**

The amount of DPPH reduced was quantified by measuring a decrease in absorbance at 517nm. The cow urine sample exhibited a higher DPPH radical scavenging activity of  $80.93 \pm 0.32 \%$ .

The genotoxicity and oxidative stress results of the study have been given in the Tables 1-2 and Figures 1-4. Chlorpyrifos administration increased DNA damage, MDA production and decreased SOD, CAT and GST levels in brain tissues when compared to the control group. Pre-treatment with cow urine alleviates the effects of CPF treatment. The MDA level decreased while there was remarkable increase in the SOD, CAT and GST levels. The details of results have been described in the following sub sections.

### **Comet assay**

The DNA damage was expressed as % tail DNA and tail moment, as presented in Tables 1-2 respectively. The rat groups exposed to different concentrations of CPF exhibited significantly higher values of % tail DNA as well as tail moment ( $p \leq 0.01$ ) as compared to control groups at all-time intervals. Comparisons of the treatment groups within different doses and time intervals indicated significant differences as shown in Tables 1-2. Highest damage was observed at 24 hrs after treatment with all doses. It was found that increase in DNA damage was concentration dependent. Further, cow urine treatment caused a depression in the DNA damage as revealed by significant lowered values observed for % tail DNA and tail moment in all groups pretreated with cow urine as compared to only CPF treated group. No significant change was recorded in the control groups compared to cow urine groups respectively.

### **MDA levels**

The level of MDA was significantly increased in the chlorpyrifos treated groups as compared to the control (Figure 1.1). The effect of concentration and duration was found to be significant ( $p \leq 0.01$ ). The MDA level significantly decreased in brain tissues at all time durations in CPF plus cow urine treated groups as compared with the groups treated with chlorpyrifos only ( $p \leq 0.01$ , Fig. 1.1- 1.3).

### **GST activity**

Similar to the above results the GST activity decreased in the chlorpyrifos treated groups as compared to control groups. Effect of concentration and duration was found to be significant. A significant increase in GST levels was observed in groups pretreated with cow urine and then administrated CPF as compared to only CPF treated groups ( $p \leq 0.01$ , Fig. 2.1- 2.3).

### **CAT activity**

The activities of CAT were significantly inhibited to varying extents; however, pre-treatment with cow urine induced changes and significantly increased its activity ( $p \leq 0.01$ , Fig. 3.1- 3.3).

### **SOD activity**



A significant decrease in SOD activity was observed at the end of the all doses and time intervals in the group treated with CPF only as compared with the control group, while statistically significant changes were observed in the cow urine-plus-chlorpyrifos groups. SOD activity increased significantly in the cow urine plus chlorpyrifos treated groups as compared with the only chlorpyrifos treated group at all time intervals ( $p \leq 0.01$ , Fig. 4.1- 4.3).

**Table 1.** Effect of different concentrations of CPF and CPF+ CU on % tail DNA of rat brain at different time intervals.

	24 hrs	48hrs	72hrs
CO	6.07±0.05 <sup>a,p</sup>	6.26±0.20 <sup>a,p</sup>	5.57±0.38 <sup>a,p</sup>
CU	5.20±0.01 <sup>a</sup>	6.52±0.46 <sup>a</sup>	5.63±0.32 <sup>a</sup>
38mg CPF	48.08±0.15 <sup>b,r</sup>	46.35±0.22 <sup>c,r</sup>	41.17±0.13 <sup>d,r</sup>
38mg CPF +CU	43.99±0.10 <sup>*</sup>	40.19±0.10 <sup>*</sup>	33.70±0.31 <sup>*</sup>
76mg CPF	61.73±0.21 <sup>b,s</sup>	56.39±0.02 <sup>c,s</sup>	50.47±0.36 <sup>d,s</sup>
76mg CPF+CU	54.77±0.12 <sup>*</sup>	48.48±0.05 <sup>*</sup>	45.57±0.39 <sup>*</sup>

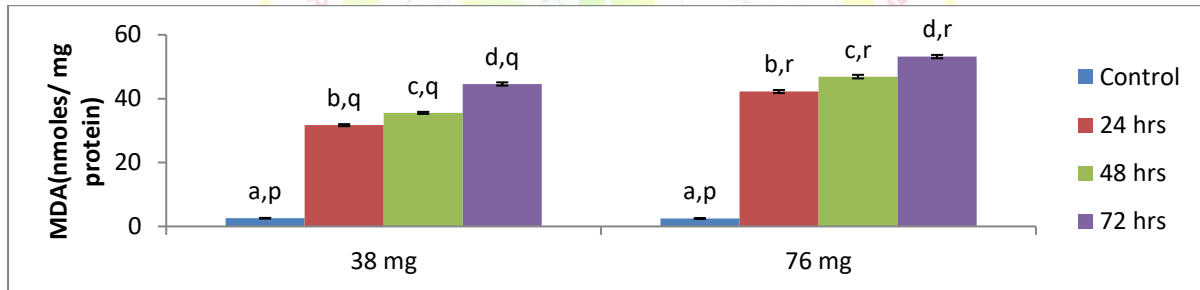
Results are shown as means± S.E. Means that do not share a common letter are significantly different ( $p \leq 0.01$ ). Different letters a, b, c, d signify the effect of duration and p, r, and s signify the effect of

concentration. \*means significantly lower as compared to CPF treated group ( $p \leq 0.01$ ). CO: Corn oil, CU: Cow urine CPF: Chlorpyrifos, CPF+CU: Chlorpyrifos and cow urine.

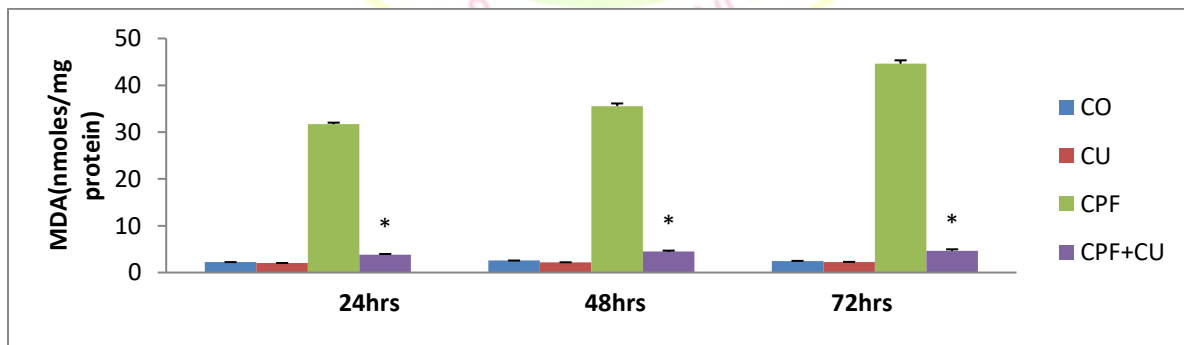
**Table 2.** Effect of different concentrations of CPF and CPF+ CU on tail moment of rat brain at different time intervals

	24 hrs	48hrs	72hrs
CO	0.67±0.01 <sup>a,p</sup>	0.69±0.03 <sup>a,p</sup>	0.59±0.04 <sup>a,p</sup>
CU	0.54±0.01 <sup>a</sup>	0.72±0.06 <sup>a</sup>	0.58±0.05 <sup>a</sup>
38mg CPF	33.15±0.17 <sup>b,r</sup>	30.19±0.80 <sup>c,r</sup>	26.09±0.09 <sup>d,r</sup>
38mg CPF+CU	27.79±0.09 <sup>*</sup>	25.37±0.23 <sup>*</sup>	20.18±0.21 <sup>*</sup>
76mg CPF	46.57±0.39 <sup>b,s</sup>	40.87±0.22 <sup>c,s</sup>	35.60±0.41 <sup>d,s</sup>
76mg CPF+CU	38.65±0.11 <sup>*</sup>	33.12±0.05 <sup>*</sup>	30.80±0.38 <sup>*</sup>

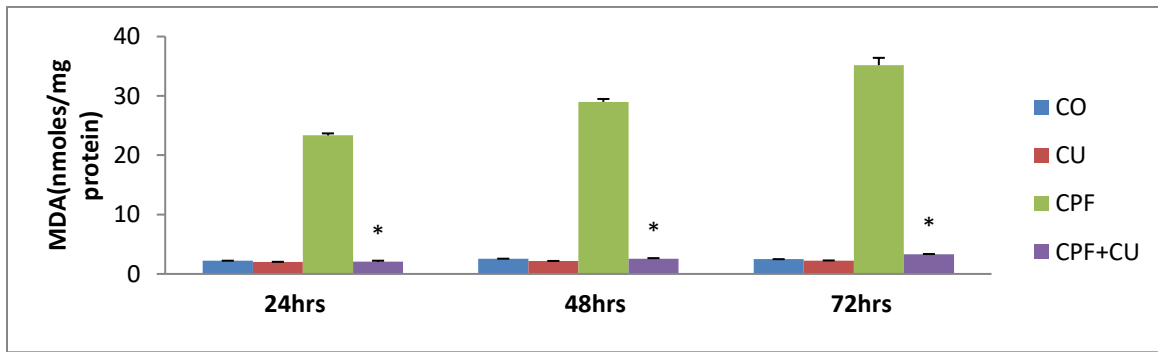
Results are shown as means± S.E. Means that do not share a common letter are significantly different ( $p \leq 0.01$ ). Different letters a, b, c, d signify the effect of duration and p, r, and s signify the effect of concentration. \*means significantly lower as compared to CPF treated group ( $p \leq 0.01$ ). CO: Corn oil, CU: Cow urine CPF: Chlorpyrifos, CPF+CU: Chlorpyrifos and cow urine.



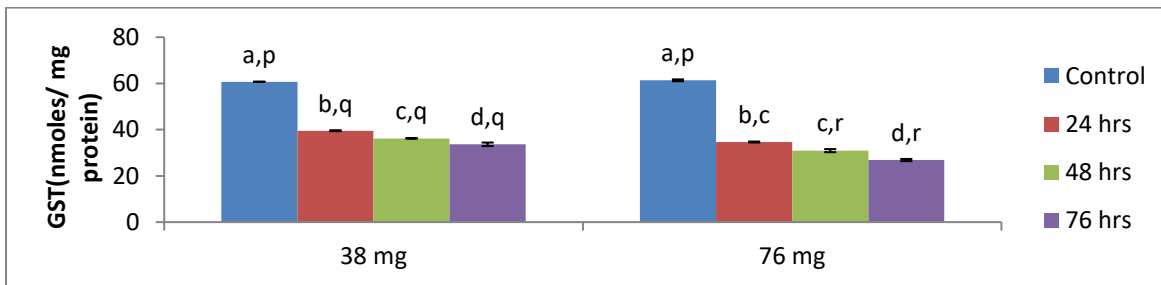
**Figure 1.1:** Effect of different concentrations of CPF on MDA levels in rat brain at different time intervals. Results are shown as means± S.E. Means that do not share a common letter are significantly different ( $p \leq 0.01$ ). Different letters a, b, c, d signifies the effect of duration and p, q, r, and s signifies the effect of concentration.



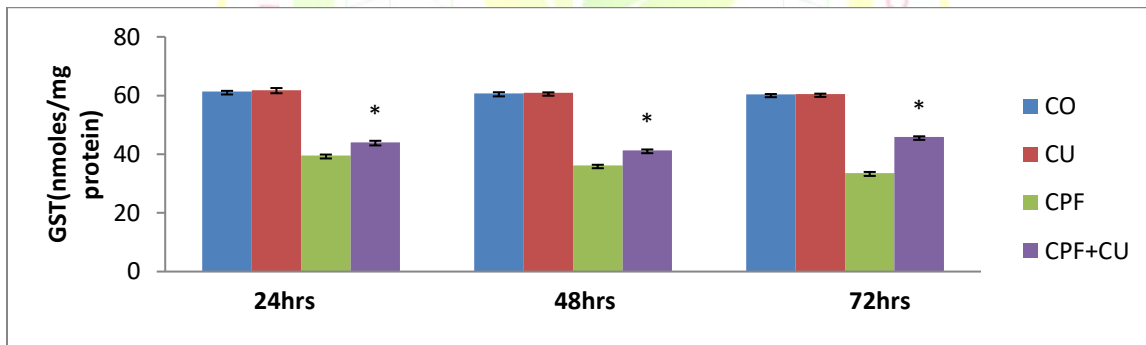
**Figure 1.2:** Effect of 38mg CPF and CPF+CU on MDA levels of rat brain at different time intervals. Results are shown as means± S.E \*means significantly lower as compared to CPF treated group ( $p \leq 0.01$ ). CO: Corn oil, CU: Cow urine CPF: Chlorpyrifos, CPF+CU: Chlorpyrifos and cow urine.



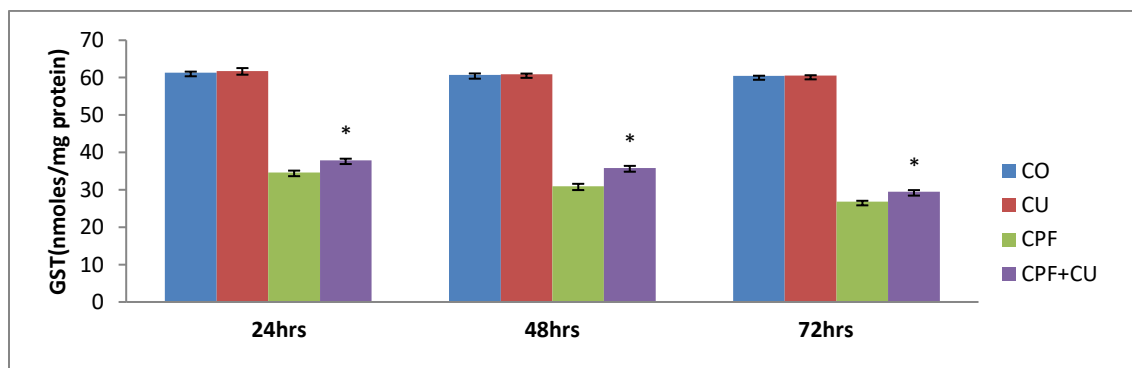
**Figure 1.3:** Effect of 76mg CPF and CPF+CU on MDA levels of rat brain at different time intervals. Results are shown as means± S.E. \*means significantly lower as compared to CPF treated group ( $p \leq 0.01$ ). CO: Corn oil, CU: Cow urine CPF: Chlorpyrifos, CPF+CU: Chlorpyrifos and cow urine.



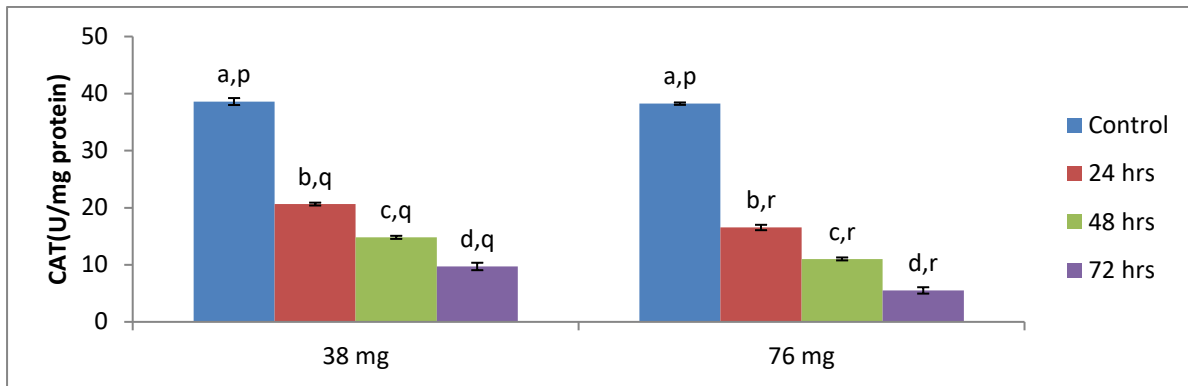
**Figure 2.1:** Effect of different concentrations of CPF on GST levels in rat brain at different time intervals. Results are shown as means± S.E. Means that do not share a common letter are significantly different ( $p \leq 0.01$ ). Different letters a, b, c, d signifies the effect of duration and p, q, r and s signifies the effect of concentration.



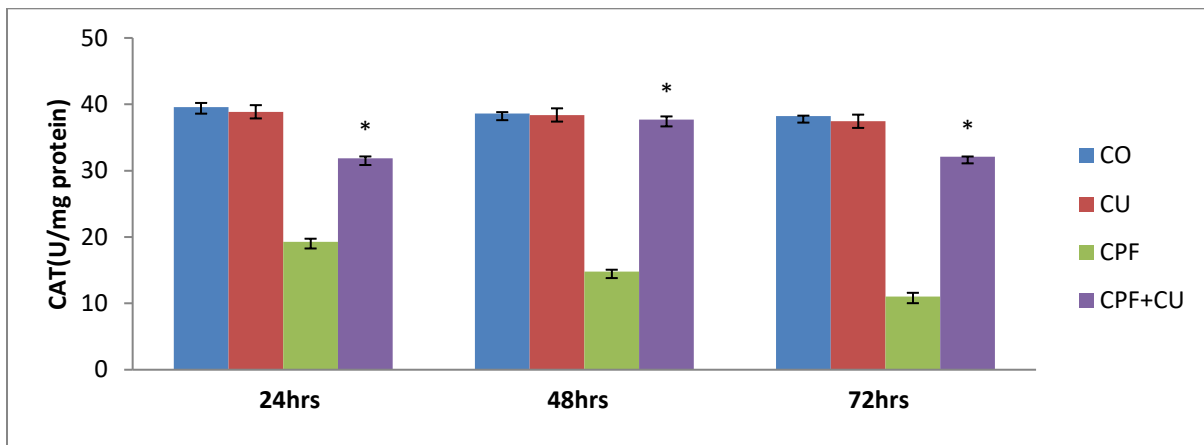
**Figure 2.2:** Effect of 38mg CPF and CPF+CU on GST activity of rat brain at different time intervals. Results are shown as means± S.E. \*means significantly higher as compared to CPF treated group ( $p \leq 0.01$ ). CO: Corn oil, CU: Cow urine CPF: Chlorpyrifos, CPF+CU: Chlorpyrifos and cow urine.



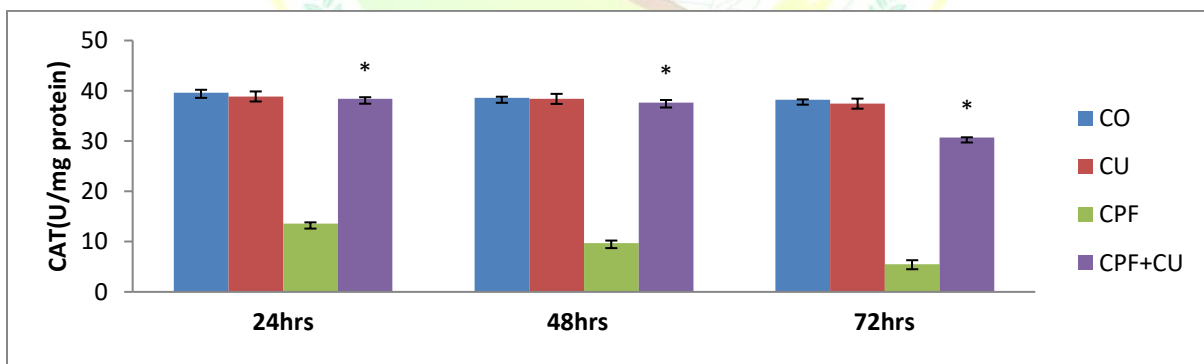
**Figure 2.3:** Effect of 76mg CPF and CPF+CU on GST activity of rat brain at different time intervals. Results are shown as means± S.E. \* means significantly higher as compared to CPF treated group ( $p \leq 0.01$ ). CO: Corn oil, CU: Cow urine CPF: Chlorpyrifos, CPF+CU: Chlorpyrifos and cow urine.



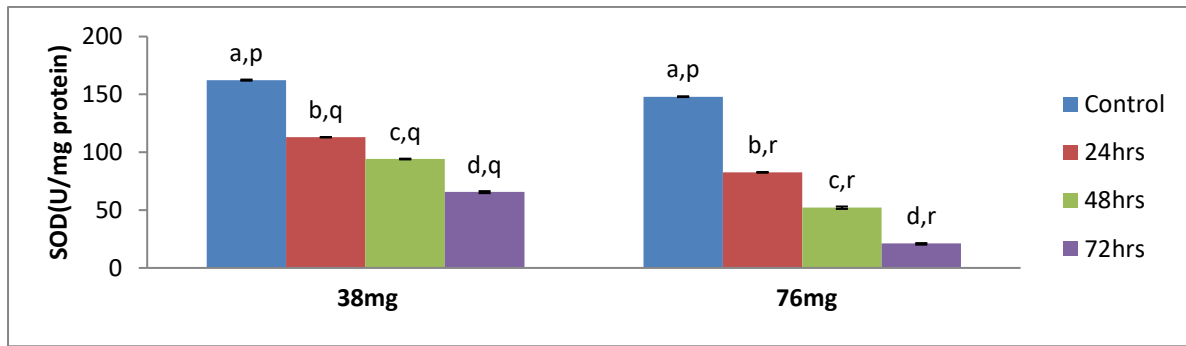
**Figure 3.1:** Effect of different concentrations of CPF on CAT levels in rat brain at different time intervals. Results are shown as means± S.E. Means that do not share a common letter are significantly different ( $p \leq 0.01$ ). Different letters a, b, c, d signifies the effect of duration and p, q, r and s signifies the effect of concentration.



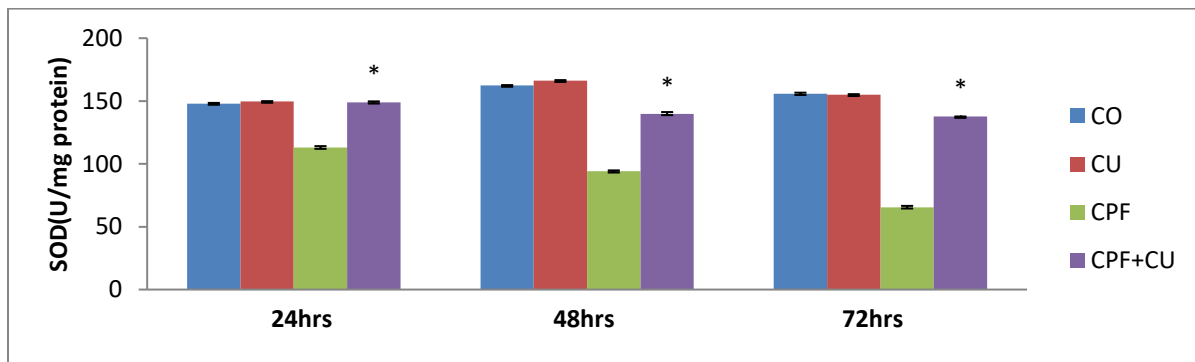
**Figure 3.2:** Effect of 38mg CPF and CPF+CU on CAT activity of rat brain at different time intervals. Results are shown as means± S.E. \* means significantly higher as compared to CPF treated group ( $p \leq 0.01$ ). CO: Corn oil, CU: Cow urine CPF: Chlorpyrifos, CPF+CU: Chlorpyrifos and cow urine.



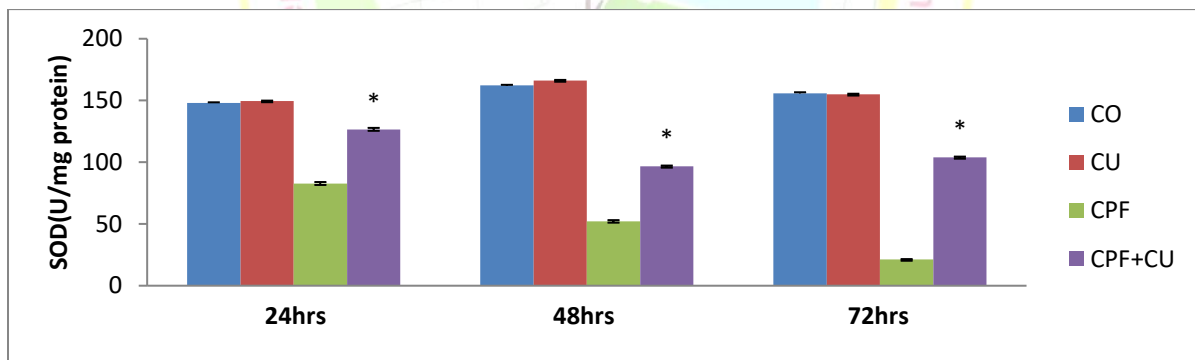
**Figure 3.3:** Effect of 76mg CPF and CPF+CU on CAT activity of rat brain at different time intervals. Results are shown as means± S.E. \*means significantly lower as compared to CPF treated group ( $p \leq 0.01$ ). CO: Corn oil, CU: Cow urine CPF: Chlorpyrifos, CPF+CU: Chlorpyrifos and cow urine.



**Figure 4.1:** Effect of different concentrations of CPF on SOD levels in rat brain at different time intervals. Results are shown as means± S.E. Means that do not share a common letter are significantly different ( $p \leq 0.01$ ). Different letters a, b, c, d signifies the effect of duration and p, q, r and s signifies the effect of concentration.



**Figure 4.2:** Effect of 38mg CPF and CPF+CU on SOD activity of rat brain at different time intervals. Results are shown as means± S.E. \*means significantly higher as compared to CPF treated group ( $p \leq 0.01$ ). CO: Corn oil, CU: Cow urine CPF: Chlorpyrifos, CPF+CU: Chlorpyrifos and cow urine.



**Figure 4.4:** Effect of 76mg CPF and CPF+CU on SOD activity of rat brain at different time intervals. Results are shown as means± S.E. \*means significantly higher as compared to CPF+CU treated group ( $p \leq 0.01$ ). CO: Corn oil, CU: Cow urine CPF: Chlorpyrifos, CPF+CU: Chlorpyrifos and cow urine.

Genotoxicity and toxicity of pesticides for non-target organisms and their influence on ecosystem are a worldwide concern specially when it possesses bio-accumulative properties and enter the food chain (Pimentel et al., 1998). The pesticide owing to its consistency, contaminate the environment even at sub-lethal doses and tends to accumulate in the tissue of the organisms. Pesticides are used in variety of combinations and researchers are actively involved in carrying out the studies to assess the risk involved in direct and indirect exposure. In the recent years, comet assay has been used as sensitive biomarker of

genotoxicity by different research groups using different animal models. viz drosophila (Carmona et al., 2011), fish (Ali et al., 2009) birds (Baos et al., 2006), reptiles (Bronikowski et al., 2008) and amphibians (Ismail et al., 2014; Yin et al., 2009). The results of the present study clearly demonstrate that there has been significant increase in % tail DNA and tail moment in chlorpyrifos treated groups as compared to control groups. Similarly, the genotoxic effects of CPF has also been reported by all these authors (Li et al., 2015; Ojha et al., 2015; Muller et al., 2015; Ojha et al., 2011; Mehta et al., 2008) in wistar rats and dose dependent increase was also

highlighted. Martinez et al. (2014) also reported the genotoxic effects of commercial formulations of chlorpyrifos.

Pesticides have been known to augment the production of reactive oxygen species, which in turn generate oxidative stress in different tissues (Mansour et al., 2009). Further, generation of free radicals cause lipid peroxidation, DNA damage and later promotion and progression of carcinogenesis. Though, the organisms are equipped with an antioxidant defence system to shield tissues against oxidative damage but if the rate of reactive oxygen species production exceeds the capacity of defence mechanisms, cellular and DNA damage can occur (Cadet et al., 2003). This may be one of the possible reasons for DNA damage in brain. Results of present study indicate that exposure to chlorpyrifos significantly increase MDA levels in brain tissue. MDA is one of the major oxidation products of peroxidized polyunsaturated fatty acids and increased MDA content is an important marker of lipid peroxidation (Celik & Suzek, 2009). Thus, results of the present study are in agreement with previous reports where increase in MDA was also observed *in vivo* and *in vitro* due to chlorpyrifos in different tissues (Demerdash, 2011). However, supplementation of cow urine along with the administration of chlorpyrifos led to significant decrease in DNA damage and MDA levels, indicating that it may have beneficial role in lowering the chlorpyrifos induced toxicity. Earlier reports have also shown that supplementation with cow urine attenuated the increased MDA levels due to cisplatin (Ajith et al., 2007). The results reveal that treatment with cow urine was effective in lowering the MDA levels. Thus, reduced levels of lipid peroxides indicate the effective antioxidant property of the cow urine in the moderation of tissue damage.

As oxidative stress results when the balance between antioxidant system and ROS is lost therefore intoxication by chlorpyrifos may hamper balance of antioxidant system (Rao and Chhunchha, 2010). This is further depicted by decrease in the activities of CAT, SOD and GST, which further deteriorates the situation. Under physiological conditions, antioxidant enzymes such as SOD and CAT eliminate ROS, thereby playing an integral role in the antioxidative stress defence of cell (Bukowski, 2004). Similar to earlier reports, results of the present study also show a decrease in SOD and CAT levels in the chlorpyrifos treated group and in all such cases the main culprit being superoxide radicals (Demir, 2011). Brain SOD and CAT levels were also found to be lowered due to treatment with methyl parathion (Gultekin et al., 2011; Verma et al., 2009; Eraslan et al., 2007). SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, while CAT converts hydrogen peroxide into water. Therefore, decrease in the activity of SOD may be attributed to the saturation of SOD during the process of converting O<sub>2</sub> to H<sub>2</sub>O while that of CAT may involve saturation during the breakdown of free radicals and H<sub>2</sub>O<sub>2</sub> or inhibition by

radicals. All the pretreatment groups showed improvement in the levels of SOD and CAT.

GST's are the family of detoxifying enzymes that catalyse the addition of tripeptide glutathione to xenobiotic substrates which have electrophilic functional groups viz conjugation of variety of electrophiles to thiol group of GSH producing less toxic forms. Thus, they play an important role in detoxification and metabolism of many toxic compounds (Ahmed et al., 2010; Carr et al., 1999; Mehra and Chadha, (2020). Our present study found a decreased GST activity in animals intoxicated with chlorpyrifos. The decreased GST activity might reflect cellular oxidative stress due to chlorpyrifos exposure.

So, in the present investigation, cow urine has been found to ameliorate toxic potential of CPF due to its high antioxidant activity as depicted by DPPH antioxidant activity test of cow urine. Similarly, the treatment with cow urine in combination with synthetic antioxidants was found to modulate the lindane induced oxidative stress in kidney of Swiss mice (Nagda and Bhatt, 2013). The protective effect of cow urine may have been due to the antioxidant properties of it as revealed by Jarald et al. (2008).

#### CONCLUSION

The highlight of the study is the competence of cow urine against the pesticide induced toxicity, which can open new insight in the therapeutic world for treatment of various disorders implicating oxidative stress and DNA damage. The pharmaceutical industries can further explore its exquisite properties and use it for preparation of various types of drugs against various diseases like stress, cancer etc. Thus, sufficient routine consumption of cow urine by the individuals, especially farmers, who are recurrently exposed to pesticides, is recommended as it can prove beneficial in combating adverse effects of pesticides.

#### ACKNOWLEDGEMENTS

The authors wish to thank Centre with Potential for Excellence in Particular Area, University Grants Commission, New Delhi, India for providing financial assistance.

#### Ethics approval

All the experiments were performed according to guidelines for use and care of laboratory animals and were approved by Committee for Purpose of Control and Supervision of Experiments on Animals (C.P.C.S.E.A, Reference number: 226/CPCSEA2 013/20).

#### Competing interest

The authors declare that they have is no competing interests.

#### REFERENCES

- Aebi H, 1984. Catalase *in vitro*. *Methods Enzymol*, 8,105: 121.
- Aggarwal BB, Ichikawa H, Garodia P, Weerasinghe P, Sethi, G, Bhatt ID, Pandey MK, Shishodia S & Nair MG, 2006. From traditional Ayurvedic medicine to modern medicine: identification of



- therapeutic targets for suppression of inflammation and cancer. *Expert Opinions on Therapeutic Targets*, **10**: 87.
- Ahmed NS, Mohamed AS & Abdel-Wahhab MA, 2010. Chlorpyrifos-induced oxidative stress and histological changes in retinas and kidney in rats: protective role of ascorbic acid and alpha tocopherol. *Pestic Biochem Physiol*, **98**: 33.
- Ahuja YR & Saran, R, 1999. Alkaline single cell gel electrophoresis assay. *International Protocol J Cytol Genet*, **34**: 57.
- Ajith TA, Usha S & Nivith AV, 2007. Ascorbic acid and a-tocopherol protect anticancer drug cisplatin induced nephrotoxicity in mice: a comparative study. *Clinica Chimica Acta*, **375**: 82.
- Ali D, Nagpure NS, Kumar S, Kumar R, Kushwaha B & Lakra WS, 2009. Assessment of Genotoxic and mutagenic effects of chlorpyrifos in fresh water fish *Channa punctatus* (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. *Food Chem Toxicol*, **47**: 650.
- Aly N, EL-Gendy K, Mahmoud, F & El-Sebae AK, 2010. Protective effect of vitamin C against chlorpyrifos oxidative stress in male mice. *Pestic Biochem Physiol*, **97**: 7.
- Banudevi S, Krishnamoorthy G, Venkataraman P, Vignesh C, Aruldas MM & Arunakaran J, 2006. Role of  $\alpha$ -tocopherol on antioxidant status in liver, lung and kidney of PCB exposed male albino rats. *Food Chem Toxicol*, **44**: 2040.
- Baos R, Jovani R, Pastor N, Tella JL, Jimenez B, Gomez G, González MJ & Hiraldo F, 2006. Evaluation of genotoxic effects of heavy metals and arsenic in wild nestling white storks (*Ciconia ciconia*) and black kites (*Milvus migrans*) from Southwestern Spain after a mining accident. *Environ Toxicol Chem*, **25**: 2794.
- Bhatti, G. K., Kiran, R. & Sandhir, R, 2010. Modulation of ethion-induced hepatotoxicity stress by vitamin e supplementation in male wistar rats. *Pestic Biochem Physiol*, **98**: 26.
- Bindhumol V, Chitra KC & Mathur PP, 2003. Bisphenol A induces reactive oxygen species generation in the liver of male rats. *Toxicol*, **188**: 117.
- Brand-Williams W, Cuvelier ME & Berset C, 1995. use of a free radical method to evaluate antioxidant activity. *LebenWissen Technol*, **28**: 25.
- Bronikowski AM, 2008. The evolution of aging phenotypes in snakes, a review and synthesis with new data, *Age (Dordr)* **30**: 169.
- Bukowska B, 2004. 2, 4, 5-T and 2, 4, 5-TCP induces oxidative damage in human erythrocytes, the role of glutathione. *Cell Bio Interact*, **285**: 57.
- Cadet J, Douki T, Gasparutto D & Ravanat JL, 2003. Oxidative damage to DNA, formation, measurement and biochemical feature. *Mutat Res*, **531**: 5.
- Carmona ER, Guecheva TN, Creus A & Marcos R, 2011. Proposal of an in vivo comet assay using haemocytes of *Drosophila melanogaster*. *Environ Mol Mut*, **52**(2): 165.
- Carr AC & Frei B, 1999. toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *American J Clin Nutr*, **69**: 1086.
- Cavalcante DGSM, Martinez CBR & Sofia SH, 2008. Genotoxic effects of Roundup on the fish *Prochilodus lineatus*. *Mut Res*, **655**: 41.
- Celik, I & Suzek, H, 2009. Effects of subacute exposure of dichlorvos at sublethal dosages on erythrocyte and tissue antioxidant defense systems and lipid peroxidation in rats. *Ecotoxicol Environ Safe*, **729**: 905.
- Colovic MB, Krstic DZ, 2013. Lazarevic –Patsi TD, Bondizic AM & Vasic MV, Acetylcholinesterase Inhibitors, Pharmacology and Toxicology. *Curr Neuropharmacol*, **11**(3): 315.
- Demir F, Uzun FG, Durak D & Kalender Y, 2011. Subacute chlorpyrifos-induced oxidative stress in rat erythrocytes and the protective effects of catechin and quercetin. *Pestic Biochem Physiol*, **99**: 79.
- Draper HH & Hadley M, 1990. Malondialdehyde Determination as an index of lipid peroxidation. *Methods Enzymol*, **186**: 421.
- Durak S, Kalender FG, Uzun F, Demir Y & Kalender, 2010. Mercury chloride induced oxidative stress and the protective effect of vitamins C and E in human erythrocytes *in vitro*. *Afr J Biotechnol*, **94**: 88.
- El-Demerdash, FM, 2011. Lipid peroxidation, oxidative stress and acetylcholinesterase in rat brain exposed to organophosphate and pyrethroid insecticides. *Food Chem Toxicol*, **49**: 1346.
- Eraslan G, Saygi S, Essiz D, Aksoy A, Gul H & Macit E, 2007. Evaluation of aspect of some oxidative stress parameters using vitamin E, proanthocyanidin and N-acetylcysteine against exposure to cyfluthrin in mice. *Pestic Biochem Physiol*, **88**(1): 43.
- Gultekin F, Ozturk M & Akdogan M, 2000. The effect of organophosphate insecticide chlorpyrifos-ethyl on lipid peroxidation and antioxidant enzymes (*in vitro*). *Arch Toxicol*, **74**: 533.
- Habig WH, Pabst MJ & Jakoby WB, 1974. Glutathione-S-transferase. The first enzymatic step in mercapturic acid formation. *J Biol Chem*, **49**: 7130.
- Hemraj, Upmanyu N, Gupta A, Jindal A & Jalhan S, 2012. Pharmacological activities of *Stephania glabra*, *Woodfordia fruticosa* and *Cissempeles pareira* – A review. *Int J Pharm Pharmaceutic Sci* **4**(3): 16.
- Ismail M, Khan MQ, Ali R, Ali T & Mobeen A, 2014. Evaluation of the Genotoxicity of Chlorpyrifos in Common Indus Valley Toad, *Bufo stomaticus* using alkaline single-cell gel electrophoresis (Comet) Assay. *Agric Sci*, **5**: 376.

- Jarald E, Edwin S, Tiwari V, Garg R & Toppo E, 2008. Antioxidant and antimicrobial activities of cow urine. *Global J Pharmacol*, **2**(2): 20.
- Kelly JF, 1997. The urine cure and other curious medical treatments. <https://wfm.u.org/LCD/19/urine.html>, accessed on 11 august 2016
- Kono Y, 1978. Generation of superoxide radicals during auto-oxidation of hydroxylamine and an assay for superoxide dismutase. *Arch Biochem Biophys*, **186**: 189.
- Kumar A, Kumar P, Singh LK & Agarwal DK, 2004. Pathogenic effect of free radicals and their prevention through Cowpathy. *Indian Cow*, **4**: 27.
- Li D, Huang Q, Zhang L, Yang Z, Zong, M & Tao L, 2015. The organophosphate insecticide chlorpyrifos confers its genotoxic effects by inducing DNA damage and cell apoptosis. *Chemosphere*, **135**: 387.
- Lowry OH, Rosebrough NJ, Farr AL & Randall RJ, 1951. Protein measurement with Folin phenol reagent. *J Biol Chem*, **193**: 265.
- Mansour SA & Mossa HAT., Lipid peroxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and the protective effect of zinc. *Pestic Biochem Physiol*, **93**: 34.
- Martinez RS, Di Marzio WD & Saenz M E, 2014. Genotoxic effects of commercial formulations of chlorpyrifos and tebuconazole on green algae. *Ecotoxicol*, **24**: 45.
- Mehra S, Chadha P. 2021. Genetic and biochemical changes in liver and kidney of *Channa punctatus* after treatment with 2-naphthalene sulfonate. *Toxicol Res (Camb)*. Mar **23**;10(2):292-298. doi: 10.1093/toxres/tfab010. PMID: 33884180; PMCID: PMC8045590.
- Mehra S, Chadha P. 2021. Naphthalene-2-sulfonate induced toxicity in blood cells of freshwater fish *Channa punctatus* using comet assay, micronucleus assay and ATIR-FTIR approach. *Chemosphere*. **265**: 129-147. doi: 10.1016/j.chemosphere.2020.129147. Epub 2020 Dec 1. PMID: 33302202.
- Mehra, S., & Chadha, P. 2020. Bioaccumulation and toxicity of 2-naphthalene sulfonate: an intermediate compound used in textile industry. *Toxicology research*, **9**(2): 127–136. <https://doi.org/10.1093/toxres/tfaa008>
- Mehra, S., Sharma, S., & Chadha, P. 2018. Assessment of antioxidative and ameliorative potential of aqueous extract of leaves of *Thuja Orientalis* (*Platyclusus orientalis* [L.]). *Asian Journal of Pharmaceutical and Clinical Research*, **11**(12), 325–329. <https://doi.org/10.22159/ajpcr.2018.v11i12.28205>
- Mehta A, Verma RS & Srivastava N, 2008. Chlorpyrifos-induced DNA damage in rat liver and brain. *Environ Mol Mut*, **49**(6): 426.
- Mittal S, Kaur G & Vishwakarma GS, 2013. Effect of environmental pesticides on the health of rural communities in the malwa region of Punjab, India, A Review, Human and Ecological Risk Assessment, *An Int J*, **20**(2): 366.
- Muller M, Hess L, Tardivo A, Lajmanovich R, Attademo A, Poletta G, Simoniello MF, Yodice A, Lavarello S, Chialvo D & Scremin O, 2014. Neurologic dysfunction and genotoxicity induced by low levels of chlorpyrifos. *Neurotoxicol*, **45**: 22.
- Nagda G & Bhatt DK, 2014. Effect of cow's urine "Gomutra" and antioxidants in alleviating the lindane-induced oxidative stress in kidney of Swiss mice (*Mus musculus*). *Mol Bio R*, **41**(4): 1967.
- Ojha A & Gupta YK, 2015. Evaluation of genotoxic potential of commonly used organophosphate pesticides in peripheral blood lymphocytes of rats. *Human Exp Toxicol*, **34**(4): 390.
- Ojha A, Yaduvanshi S, Pant S, Lomash V & Srivastava N, 2011. "Evaluation of DNA damage and cytotoxicity induced by three commonly used organophosphate pesticides individually and in mixture, in rat tissues". *Environ Toxicol*, **28**: 543.
- Pandey AK, Nagpure NS, Trivedi SP, Kumar R & Kushwaha B, 2011. Profenos induced DNA damage in freshwater fish *Channa punctatus*(bloch) using single cell gel electrophoresis. *Mut Res* **726**: 209.
- Pathak ML & Kumar A, 2003. Gomutra a descriptive study. *Sachitra Ayurveda*, **7**: 81.
- Pimentel, D., Greiner, A & Bashore, T, 1998. Economic and environmental costs of pesticides use *Environmental Toxicology, Current Developments*, (Gordon and Breach Science Publisher, London, UK), 121.
- Randhawa GK, 2010. Cow urine as distillate as bioenhancer. *J Ayur Integr Med*, **1**(4) 240.
- Rao MV & Chhunchha B 2010. Protective role of melatonin against the mercury induced oxidative stress in the rat thyroid. *Food Chem Toxicol*, **48**: 7.
- Saulsbury MD, Heyliger SO, Wang K & Johnson DJ, 2009. Chlorpyrifos induces oxidative stress in oligodendrocyte progenitor cells. *Toxicol*, **259**: 1.
- Shiva V, 1991. The green revolution in the Punjab. *The Ecologist* **21**: 2. <http://livingheritage.org/green-revolution.htm>, accessed on 10 august 2016.
- Singh NP, McCoy MT, Tice RR & Schneider EL, 1988. A simple technique for quantification of low levels of DNA damage in individual cells. *Exp Cell Res*, **175**: 84.
- Singh S, Kumar V, Thakur S, Banerjee BD, Chandna S, Rautela RS, Grover SS, Rawat DS, Pasha ST, Jain SK, Ichhpujani RL & Rai A, 2010. DNA damage and cholinesterase activity in occupational workers exposed to pesticides. *Environ Toxicol Pharm*, **31**: 278.

- Strobel GA & Daisy B, 2003. Bioprospecting for microbial endophytes and their natural products. *Microbiol Mol Biol Rev*, **67**: 491.
- Verma RS, Mehta A & Srivastava N, 2009. Comparative studies on chlorpyrifos and methyl parathion induced oxidative stress in different parts of rat brain, attenuation by antioxidant vitamins. *Pestic Biochem Physiol*, **951**: 52.
- Weichenthal S, Moase C & Chan P, 2012. A review of pesticide exposure and cancer incidence in the agricultural health study cohort. *Environ Health Persp*, **118**: 1117.
- Yadav, S, 2013. Green revolution's cancer train. <http://www.hardnewsmedia.com/2006/11/648>, accessed on 10 august 2016.
- Yin X, Zhu G, Li XB & Liu S, 2009. Genotoxicity evaluation of chlorpyrifos to amphibian Chinese toad (Amphibian: Anura) by Comet assay and Micronucleus test, *Mut Res* **680**: 2.
- Zama Z, Meraihi S, Tebibel W, Benayssa FS, Benayache AJ & Vlitinck, 2007. Chlorpyrifos-induced oxidative stress and tissue damage in the liver, kidney, brain and fetus in pregnant rats, the protective role of the butanolic extract of *Paronychie argentea* L. *Indian Pharmacol*, **391**: 145.

**Citation:** Sharma, S.; Mehra, S. and Chadha, P 2022. Modulation of chlorpyrifos-induced genotoxicity and oxidative stress by cow urine supplementation in male wistar rats. *International Journal of Agricultural and Applied Sciences*, 3(1):79-89. <https://doi.org/10.52804/ijaas2022.3113>

**Copyright:** © Sharma et al. 2022. Creative Commons Attribution 4.0 International License. IJAAS allows unrestricted use, reproduction, and distribution of this article in any medium by providing adequate credit to the author(s) and the source of publication.

