Toxicology International, *30(4) : 675-680; 2023. DOI: 10.18311/ti/2023/v30i4/33071*

Online ISSN : 0976-5131; Print ISSN : 0971-6580

Toxicology International

Contents available at: www.informaticsjournals.com/index.php/toxi



Mitigating Effect of Cow Urine against Chlorpyrifos Induced Oxidative Stress

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Abstract

The present study was planned to evaluate the mitigating effect of cow urine against the detrimental effects induced by Chlorpyrifos (CPF), an organophosphate pesticide, in different tissues of rat. The LD_{50} was determined and $1/4^{th}$ of LD_{50} i.e., 38mg/ kg b.wt was selected for treatment. Sexually mature rats weighing 120±10 g was orally administered with selected dose and cow urine. After 24, 48 and 72 hours biochemical analysis was done using different biomarkers in different tissues. In light of available data, it was deduced that CPF has propensity to cause marked perturbations in the antioxidant enzymes. Further, cow urine mitigated the oxidative stress induced by CPF in different tissues. Thus, this study emphasizes the importance of use of cow urine as a natural source of antioxidants which could be beneficial in the therapeutic world for the treatment of various disorders implicating oxidative stress.

Keywords: Cow Urine, Chlorpyrifos, Oxidative Stress

1. Introduction

Widespread application of agrochemicals in agriculture to augment crop yield and to protect crops from a variety of pests has led to serious implication to boost productivity and sustainability of agriculture. Only 1% of the applied pesticide reaches the target and rest of it accumulates in the soil, spreads in the air, reaches water bodies or enters the food crops. Organophosphate Pesticides (OP) are among the most popular pesticides being used worldwide[1] and are among the most broadly used pesticides in diverse regions of the world such as USA[2], Africa[3,4] China[5]. Till 2020, Chlorpyrifos (CPF) has become the major commonly used organophosphate pesticide in agriculture for seed dressing and standing crop treatment throughout the world. Unfortunately, this compound is still being applied in India as well[6]. Recent studies by authors suggested presence of high amount of CPF in the soil which is approximately 3.58 mg/kg soil[7,8]. Consequently, it is highly desirable to search for antitoxic alternatives to minimize the toxic effects. The modern research world is taking interest in conventional medicines along with the modern therapies. In ancient Vedas cow urine has been considered holy and compared to the nectar (Rigveda 10.15). Due to its innumerable therapeutic potential traditional healers has been using it for treatment of various types of diseases such as diarrhoea, gastric infection, renal colic, jaundice, anaemia, skin diseases, abdominal pain, and arthiritis etc.[7,8]. It is also considered as an appetizer, a diuretic as well as a neuroprotective agent[7]. It is also known to generate bioenergy at cellular level[9]. Cow urine has been granted US Patent (No. 6896907 and 6410059.) for its various

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Article Received on: 20.02.2023 Revised on: 30.06.2023 Accepted on: 07.07.2023

medicinal properties including its antibiotic, antifungal and anticancerous potential[7,8] Though there are numerous examples stating the efficiency of cow urine yet scientific reports are few[7-9]. Therefore, the present study was undertaken to fill the lacuna in this regard and observe the antioxidative potential of cow urine against CPF.

2. Materials and Methods

2.1 Chemicals

CPF (99% pure solid) was purchased from Sigma Aldrich, St Louis, U.S. Cow Urine was purchased from Patanjali Ayurveda outlet, Amritsar, India.

2.2 Experimental Design

The experimental animals were divided into three groups having six rats each. First group was control and was treated with corn oil only, second group was treated with 38mg/kg b.wt. CPF i.e. 1/4th of LD_{50} (152mg/kg b.wt.) dissolved in corn oil and the third group was treated with cow urine and CPF. (CPF is 99% soluble in cow urine). Rats were pretreated with 0.5 ml cow urine for consecutive 10 days and then orally administered with CPF (38mg/kg of b.wt.).

2.3 Animals and Care

The male wistar rats weighing 120±20 g were used in the present experiment. The animals were housed in cages at room temperature with a relative humidity of 50% to 60% and on a 12hrs light–darkness cycle. The animals had free access to commercial pellet diet and water *ad libitum*. In the present study all the experiments were performed according to guidelines for use and care of laboratory animals. The experiments were approved by Committee for Purpose of Control and Supervision of Experiments on Animals (C.P.C.S.E.A) having no. 226/CPCSEA2013/20. All the animals were acclimated 15 days prior to any experiment.

2.4 Tissue Preparation

Three rats from each group were sacrificed after 24hrs, 48hrs and 72hrs of treatment. After decapitation, blood was obtained by heart puncturing and collected in heparin tubes to prevent clotting. Liver and kidney tissues were immediately removed; blotted, weighed and washed using chilled saline solution. Further, the tissues were 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. Furthermore, the homogenate was centrifuged at 16,000g for 30 min at 4°C. The resultant supernatant was used for the determination of different biochemical assays such as MDA (Malondialdehyde), CAT (catalase), SOD (Superoxide Dismutase), GST (Glutathione-S-Transferase) and AChE (Acetylcholinestrase) etc.

2.5 Biochemical Analysis

2.5.1 Determination of Malondialdehyde (MDA) in Rat Tissues (Liver and Kidney)

According to the method of Draper and Hadley[10], the extent of lipid peroxidation formation was measured. Blood and tissue supernatant was mixed with 1.25ml TCA, 2ml TBA (0.67%) and heated for 1hr 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 co-efficient of MDA, which is 1.56×10^5 M^{-1} cm^{-1}.

2.5.2 Determination of Glutathione S-transferase(GST) Activity in Rat Tissues (Liver and Kidney)

The activity of GST was measured according to the method of Chein and Dauterman[11]. Glutathione S-transferase catalyzes the conjugation reaction with glutathione in the first step of mercapturic acid synthesis. P-nitrobenzylchloride was used as substrate. The absorbance was measured spectrophotometrically at 310 nm using UV-Double Beam spectrophotometer. One unit of GST activity is defined as 1 mol product formation per minute.

2.5.3 Determination of Catalase (CAT) Activity in Rat Tissues (Liver And Kidney)

The enzyme catalase converts H_2O_2 into water. The CAT activity in blood, kidney and liver supernatant was measured spectrophotometrically at 240nm by calculating the rate of degradation of H_2O_2, the substrate of the enzyme[12]. Activity was monitored at 240nm for 60s. Data is expressed as U/mg protein.

2.5.4 Determination of Superoxide Dismutase (SOD) Activity in Rat Tissues (Liver and Kidney)

Superoxide dismutase was measured according to Kono[13]. The SOD activity in tissues was determined by measuring the inhibition of auto-oxidation of pyrogallol using some modification. 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.

2.5.5 AChE Estimation

The AChE content of the tissue homogenates mentioned earlier was determined by following the method described by Ellman *et al*[14].

2.5.6 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 test for multiple comparisons.

3. Results

The biochemical analysis of different organs i.e., liver, kidney and blood of treated rats was done to evaluate the toxic potential of CPF and mitigating potential of cow urine. In each tissue different biochemical markers like MDA (Malondialdehyde), GST (Glutathione-S-Transferase), CAT (Catalase), SOD (Superoxide Dismutase) and AChE (Acetylcholinestrase) were studied. Acute exposure for 72h to 38mg/kg body weight of CPF resulted in significant alterations in all the oxidative stress analysis parameters studied in all the tissues (Figures 1-3) showing highest effect in liver. In liver tissue, MDA levels were found to be raised after 24h of exposure as compared to control. Maximum exposure was observed at 72h. GST, CAT, SOD activities which were found to be reduced symbolically. Similar trend was observed in all the other tissues. Cow urine pre-treated groups showed remarkable decrease in MDA levels and increase in GST, CAT, SOD and AChE activities for all doses and all durations revealing that cow urine modulated the genotoxic and oxidative stress effect induced by CPF. Pre-treatment with cow urine led to improvement in the levels of MDA and SOD, CAT, GST and AChE activities. In liver tissue, after 72h of exposure the GST activity increases from 39.02±0.37 to 58.18±1.39 after treatment of cow urine indicating 49.12% recovery. Likewise, CAT, SOD and AChE showed 45.19%, 108.20% and 95.96% recovery respectively in liver tissue after 72h of exposure. Likewise significant recovery noted in all the tissues reveals the mitigating effect of cow urine. Therefore, it is apparent that the cow urine counteracts toxic effects induced by CPF by observing decrease in detoxifying enzyme activities and hike in MDA levels. The pre-treatment with cow urine was found to be efficacious for all the organs considered for the experiment.

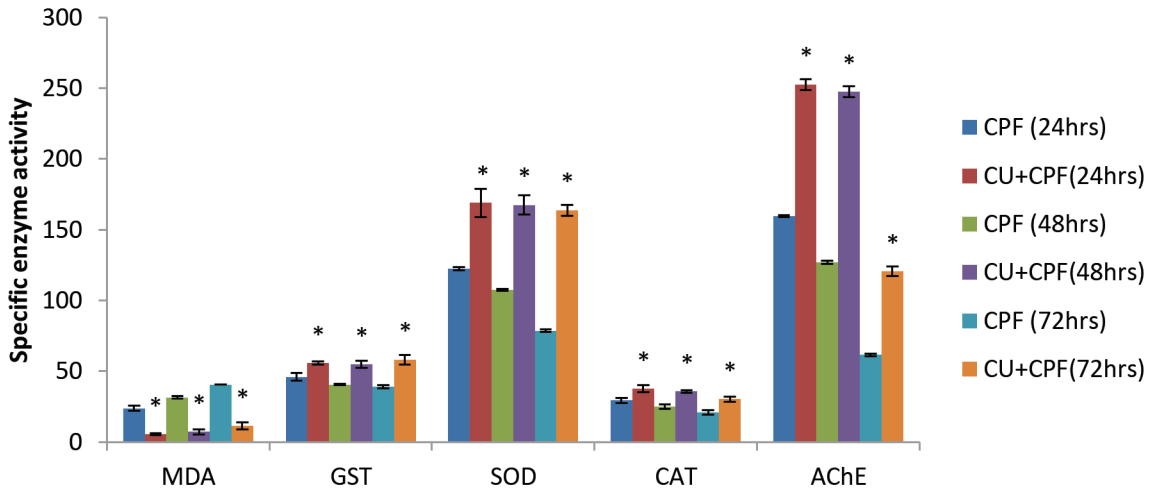


Figure 1. Effect of 38 mg/kg b.wt mg of CPF and CU+CPF on different enzyme levels in rat liver after 24, 48 and 72 hrs of exposure. Results are shown as means± S.E.
* significantly different ($p \leq 0.05$).

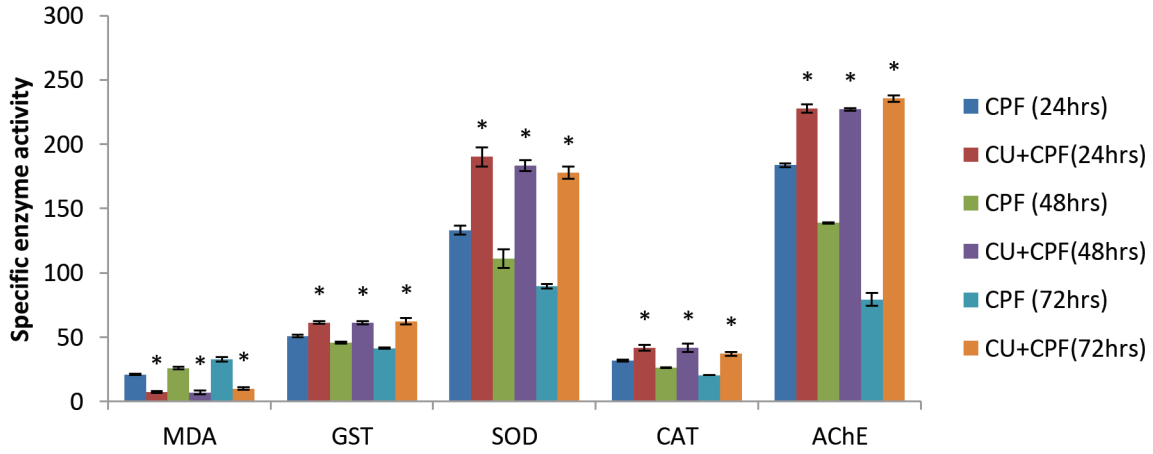


Figure 2. Effect of 38 mg/kg b.wt mg of CPF and CU+CPF on different enzyme levels in rat kidney after 24, 48 and 72 hrs of exposure. Results are shown as means± S.E.
* significantly different (p≤0.05).

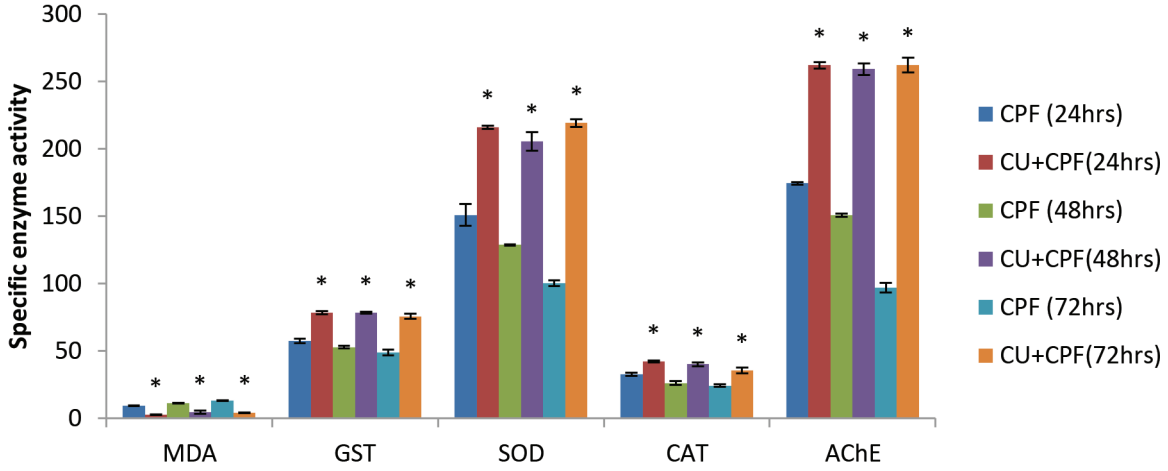


Figure 3. Effect of 38 mg/kg b.wt mg of CPF and CU+CPF on different enzyme levels in rat blood after 24, 48 and 72 hrs of exposure. Results are shown as means± S.E.
* significantly different (p≤0.05).

4. Discussion

When an organism is exposed to any toxic substance, body's detoxification mechanisms are activated. Pesticides act as pro-oxidants and elicit effects in multiple organs[15]. Some pesticides are metabolized into different chemicals and excreted while some are stored in fatty tissues of the body. As oxidative stress results when the balance between antioxidant system and ROS is lost therefore intoxication by CPF may hamper balance of antioxidant system[16]. There was increase in MDA levels and decrease in the SOD, CAT and AChE activity for all the organs. Decrease in the activity of GST indicates suppression of detoxifying system of body against CPF-induced toxicity in all organs. GST is a detoxifying enzyme and detoxifies a variety of electrophilic compounds to less toxic forms by conjugation with –SH groups such as GSH[18]. The antioxidant enzymes can alleviate the toxic effects of ROS[17,18,32]. For instance, SOD protects cells against oxidative damage and catalyzes the conversion of superoxide radical to Hydrogen Peroxide (H_2O_2) while CAT is an effective protective enzyme against lipid peroxidation and converts hydrogen peroxide to water[19]. CPF-induced toxicity resulted in decreased levels of SOD and CAT and produced adverse effects in liver, kidney and

blood tissue as revealed by Mansour *et al.*,[20]. Induction of tissue damaging effect by altering levels of enzymatic and non-enzymatic enzymes in the target organs viz., blood, liver and kidney in the present finding are in agreement with the findings of Sharma and Chadha[6], Ambali *et al.*,[21], Demir *et al.*,[22], Ahmed *et al.*,[23], Mansour and Mossa[20].

However, pretreatment with cow urine led to improvement in the levels of MDA and SOD, CAT, GST, AChE activity. Results revealed the efficacy of cow urine against CPF-induced oxidative stress. Earlier reports have also shown that supplementation with cow urine attenuated the increased MDA levels due to cisplatin in mice[24]. In another study, cow urine was found to be useful in mitigating and modulating lindane induced nephrotoxicity and oxidative stress with decreased levels of MDA, SOD and CAT activities[8]. The lipid lowering, antidiabetic and antioxidant properties of cow urine have also been reported by Sachdev *et al.*,[25]. Gosavi *et al.*,[26] emphasized presence of high amounts of vitamin C in cow urine which is responsible for its immunomodulatory potential and helps in restoring antioxidant status by lowering the increased MDA levels. Further, vitamin E and C play important role in defence against oxidative stress generated in the body due to xenobiotics[27]. Copper supplementation of vitamin C and E has known to reduce gentamicin induced hepatotoxicity in rats[28]. The analysis of cow urine has shown the presence of manganese, carbolic acid, iron, silicon, succinic, calcium salts, minerals, lactose, chlorine, nitrogen, sulphur, phosphate, sodium, magnesium, citric, titric, enzymes, creatinine, hormones, gold acids and water soluble vitamins A, B, C, D and E in it[29]. Natural occurrence of Vitamin C and E in cow urine further supports the antioxidant potential of cow urine and increases its modulating potential. Furthermore, another possible mechanism for protective effect of cow urine may be due to its antioxidant property contributed mainly due to the presence of volatile fatty acid and its free radical scavenging property[25]. The volatile fatty acids were reported to activate the third compliment component in serum. The product of this activation has some histamine releasing effects which leads to acute fall in monocyte and neutrophil count in blood within 4hrs[30] of treatment. In another recent study by Streepada *et al.*,[31] it was found that cow urine possessed remarkable antioxidant and anti-inflammatory activities which further supports the present study.

5. Conclusion

It is concluded that CPF intoxication caused oxidative stress in blood, kidney and liver in rat. However, the cow urine act synergistically in reducing chlorpyrifos induced toxic effects as it was found to be helpful in mitigating and modulating the oxidative stress induced in the different organs due to CPF. Thus, the highlight of the study is efficiency of cow urine against the pesticide toxicity which can open new insights in the field of medicine.

6. Acknowledgement

The authors wish to thank the CSIR-UGC, Centre with Potential for Excellence in Particular Area (C.P.E.P.A), University Grants Commission, New Delhi, India, and Special Assistance Programme (SAP), UGC, New Delhi, for providing financial assistance.

7. References

1. Abhilash PC, Singh N. Pesticide use and application: An Indian scenario. J Hazard Mater. 2009; 165:1–12. https://doi.org/10.1016/j.jhazmat.2008.10.061
2. Grube A, Donaldson D, Kiely T, Wu L. Pesticides industry sales and usage 2006 and 2007 market estimates. Washington DC: United States environmental protection agency, office of pesticide programs, biological and economic analysis division; 2011.
3. Umosen AJ, Ambali SF, Ayo JO, Mohammed B, Uchendu C. Alleviating effects of melatonin on oxidative changes in the testes and pituitary glands evoked by subacute chlorpyrifos administration in Wistar rats. Asian Pacific J Tropical Biomed. 2012; 8:645-50. https://doi.org/10.1016/S2221-1691(12)60113-0
4. Copaja S, Vergara R, Bravo H. Bioavailability of chlorpyrifos in wheat plants (*Triticum aestivun*). Agricul Sci. 2014; 5:660-7. https://doi.org/10.4236/as.2014.58069
5. Zhang Q, Zhu L, Han C, Wang J, Xie H, Wang J, Sun S. Analysis of chlorpyrifos and TCP residues in agricultural soil and apples by HPLC. J Food Agricul Environ. 2011; 9:659-62.
6. Wolejko E, Lozowicka B, Jablonska-Trypuc A, Pietruszynska M, Wydro U. Chlorpyrifos occurrence and toxicological risk assessment: A review. Int J Environ Res Public Health. 2022; 19(19):12209. https://doi.org/10.3390/ijerph191912209
7. Sharma S, Chadha P. Induction of neurotoxicity by organophosphate pesticide chlorpyrifos and modulating role of cow urine. Springerplus. 2016; 5:1344. https://doi.org/10.1186/s40064-016-3004-9

8. Nagda G, Bhatt DK. Effect of cow's urine "Gomutra" and antioxidants in alleviating the lindane-induced oxidative stress in kidney of Swiss mice (*Mus musculus*). Mol Biol Rep. 2014; 41(4):1967-76. https://doi.org/10.1007/s11033-014-3044-6
9. Randhawa GK. Cow urine as distillate as bioenhancer. J Ayur Inte Med. 2010; 4: 240-1. https://doi.org/10.4103/0975-9476.74089
10. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Meth Enzymol. 1990; 186:421–31. https://doi.org/10.1016/0076-6879(90)86135-I
11. Chien C, Dauterman WC. Studies on glutathione-S-transferase in *Helicoverpa* (*Heliothis*) zea. Insect Biochem. 1991; 21(8):857–64. https://doi.org/10.1016/0020-1790(91)90092-S
12. Aebi H. Catalase. In: Bergmeyer HU, editor. Methods in Enzymatic Analysis. New York: Academic Press; 1983. p. 276–86.
13. Kono Y. Generation of superoxide radical during autooxidation of hydroxylamine and an assay for superoxide dismutase. Arch Biochem Biophys. 1978; 186:189–95. https://doi.org/10.1016/0003-9861(78)90479-4
14. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961; 7:88-95. https://doi.org/10.1016/0006-2952(61)90145-9
15. Limon-Pacheco J, Gonsebatt ME. The role of antioxidants and antioxidant related enzymes in protective responses to environmentally induced oxidative stress. Mutat Res. 2009; 674:137–47. https://doi.org/10.1016/j.mrgentox.2008.09.015
16. Gultekin F, Delibas N, Yasar S, Kilinc I. *In vivo* changes in antioxidant systems and protective role of melatonin and a combination of vitamin C and vitamin E on oxidative damage in erythrocytes induced by chlorpyrifos-ethyl in rats. Arch Toxicol. 2001; 75:88–96. https://doi.org/10.1007/s002040100219
17. Mansour SA, Mossa HAT. Lipid peroxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and the protective effect of zinc. Pestic Biochem Phys. 2009; 93:34–39. https://doi.org/10.1016/j.pestbp.2008.09.004
18. Altuntas I, Delibas N, Demirci M, Kilinc I, Tamer N. The effects of methidathion on lipid peroxidation and some liver enzymes: Role of vitamins E and C. Arch Toxicol. 2002; 76:470–3. https://doi.org/10.1007/s00204-002-0359-1
19. Winston GW, Di Giulio RT. Prooxidant and antioxidant mechanisms in aquatic organisms. Aquat Toxicol. 1991; 19:137–61. https://doi.org/10.1016/0166-445X(91)90033-6
20. Mansour SA, Mossa AH. Oxidative damage, biochemical and histopathological alterations in rats exposed to chlorpyrifos and the antioxidant role of zinc. Pestic Biochem Physiol. 2010; 96:14–23. https://doi.org/10.1016/j.pestbp.2009.08.008
21. Ambali SF, Ayo JO, Ojo SA, Esievo KAN. Vitamin E protects Wistar rats from chlorpyrifos-induced increase in erythrocyte osmotic fragility. Food Chem Toxicol. 2010; 48:3477–80. https://doi.org/10.1016/j.fct.2010.09.026
22. Demir F, Gökce Uzun F, Durak D, Kalender Y. Subacute chlorpyrifos-induced oxidative stress in rat erythrocytes and the protective effects of catechin and quercetin. Pesti Biochem Physiol. 2011; 99:77–81. https://doi.org/10.1016/j.pestbp.2010.11.002
23. Ahmed NS, Mohamed AS, Abdel-Wahhab MA. Chlorpyrifos-induced oxidative stress and histological changes in retinas and kidney in rats: Protective role of ascorbic acid and alpha tocopherol. Pestic Biochem Physiol. 2010; 98:33-8. https://doi.org/10.1016/j.pestbp.2010.04.006
24. Ajith TA, Usha S, Nivith AV. Ascorbic acid and a-tocopherol protect anticancer drug cisplatin induced nephrotoxicity in mice: A comparative study. Clinica Chimica Acta. 2007; 375:82–6. https://doi.org/10.1016/j.cca.2006.06.011
25. Sachdev DO, Gosavi DD, Salwe KJ. Evaluation of antidiabetic, antioxidant effect and safety profile of gomutra ark in wistar albino rats. Anc Sci Life. 2012; 31(3):84-9. https://doi.org/10.4103/0257-7941.103180
26. Gosavi DD, Sachdev D, Salwe K. Immunomodulatory and antioxidant effect of gomutra ark in rats. J Maha Gan Inst Med Sci. 2011; 16(2):37-41.
27. Ray G, Hussain SA. Oxidants, antioxidants and carcinogenesis. Ind J Exp Bio. 2002; 42:1213–32.
28. Kadkhodaee M, Khastar H, Faghihi M, Ghaznavi R, Zahmatkesh M. Effects of co-supplementation of vitamins E and C on gentamicin-induced nephrotoxicity in rats. Exp Physiol. 2004; 90(4):571–6. https://doi.org/10.1113/expphysiol.2004.029728
29. Jain NK, Gupta VB, Garg R, Silawat N. Efficacy of cow urine therapy on various cancer patients in Mandsaur district, India: A survey. Int J Green Pharm. 2010; 4:29–35. https://doi.org/10.4103/0973-8258.62163
30. Olusi SO, Ojewole JA. Evidence for complement activation following the oral administration of cow's urine concoction in rats. Afr J Med Sci. 1978; 7:79-83.
31. Streepada K. Antioxidant and anti-inflammatory activities of cow urine from malnad gidda-an indigenous breed. Int J Pharma Sci Res. 2019; 10(2):612-8.
32. Mehra S, Sharma S, Chadha P. Assessment of antioxidant and ameliorative potential of aqueous extract of *Thuja orientalis* (*Platycladus orientalis* [L.]). Asian J Pharm Clin Res. 2018; 11:1-5. https://doi.org/10.22159/ajpcr.2018.v11i12.28205