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Alteration in thiols homeostasis, protein and lipid peroxidation in renal tissue following subacute oral exposure of imidacloprid and arsenic in Wistar rats

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ABSTRACT

The aim of present study was to assess whether No Observed Effect Level (NOEL) of imidacloprid (IMI) potentiates the arsenic induced renal toxicity at its maximum contaminant level in drinking water in Wistar rats. Significant elevation of lipid and protein oxidation with reduced level of total thiols and antioxidant enzymes (catalase, superoxide dismutase, glutathione reductase, glutathione peroxidase and glutathione-s-transferase) in renal tissue may have contributed to increased renal plasma biomarkers (creatinine and blood urea nitrogen) following repeated exposure of IMI and arsenic alone and in-combination. The altered renal biomarkers in co-exposed groups corroborated with histopathological alterations in renal tissue. The observations indicated that altered thiol homeostasis in renal tissue may be associated with increased lipid and protein oxidation in IMI and arsenic administered rats. It is concluded that administration of IMI potentiate the arsenic induced renal damage in Wistar rats.

1. Introduction

Kidney is the main organ responsible to maintain composition and volume of body fluids, acid-base balance and the redox status. In recent years, renal disorders have been on the rise and constitute a major health problem. The significant increase in the prevalence of renal disorders not only entails enormous cost of treatment but also has a role as a risk factor for cardiovascular disorders [1,2]. Therefore, the renal disorders are attracting greater attention of the health practitioners. Renal abnormalities are usually associated with chemically induced oxidative damage; however it is difficult to determine if this relationship contributes to the disease or is a consequence of disease [3]. Inhibition of protein synthesis and depletion of glutathione/thiols (-SH group) have been recognized as common pathophysiological mechanism(s) of renal tissue damage. Increased free radicals production with reduced scavenging capacity of renal tissue leads to the functional and structural alterations in kidney [4–6]. It has been reported that the exposure to pesticides, metals and metalloids which constitutes the major environmental toxicants induces oxidative damage to various tissues including kidneys [2,7–11].

Imidacloprid (IMI), a newer neonicotinoid insecticide, is used worldwide for insect and pest management in agriculture and flea control in dogs and cats [12]. It is one of the most popular insecticides across the world because of its highly selective toxicity to insects and being safe to non-target species. However, injudicious and continuous use of IMI has increased its levels in food chain and adversely affects the mammals and aquatic organisms [13–15]. High arsenic (As) levels in air and water is the major contaminant across the world [16]. Humans and animals usually get exposed to arsenic via air and food but it has been reported that in many countries including India the major route of arsenic exposure is through contaminated drinking water [17]. Therefore, arsenic exposure remains a major public health problem as millions of people are exposed to water levels above the recommended toxic limits. Although the mechanism for arsenic toxicity can be multi-

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factorial, oxidative stress is one of the most widely studied and accepted mechanisms [2,17–19].

In current scenario, dramatic and tremendous rise in the applications of IMI and higher complaints of arsenic contamination in ground water is not only common in India but in other industrial countries too. Therefore, present study was undertaken to evaluate the toxic renal effect of arsenic at maximum contaminant level (MCL) and NOEL dose of IMI alone and in-combination in wistar rats.

2. Materials and methods

2.1. Experimental animals and chemicals used

The study was conducted on healthy male Wistar rats (180–200 g m) procured from Indian Institute of Integrative Medicine (IIIM), Jammu. The animals were provided standard pellet ration and tap water for drinking *ad-libitum*. All the animals were maintained under standard managemental conditions (22 ± 3 °C, 50–60 % relative humidity and 12 h light-dark cycles). Prior to start of experiment, the rats were acclimatized in the laboratory conditions for a period of 15 days. The experimental protocol was dully approved by Institutional Animal Ethics Committee (IAEC) (Permission no. AU/ANN/13–14/IAEC/143-54). Imidacloprid (17.8%) used in the present study was commercially obtained from Mahindra and Mahindra Ltd. Agribusiness, Mumbai, India. Analytical grade Sodium Arsenate procured from SD Fine-Chemical Limited, Mumbai was used as a source of arsenic. All other chemicals used in the study were of analytical grade and purchased from different standard manufacturers.

2.2. Experimental protocol

The dose of imidacloprid used was 16.9 mg/kg body weight which is equivalent to NOEL (No Observed Effect Level) [20]. To determine its subtle effect on renal tissue alone and in-conjunction with the different doses of arsenic in drinking water. As per the WHO guidelines the MCL (maximum contaminant level) of arsenic in drinking water is 50 ppb [21] and in the present study, three dose levels viz. MCL (50 ppb), double than MCL (100 ppb) and triple than MCL (150 ppb) in drinking water were used. (4.165 mg of Sodium arsenate providing 1 mg arsenic).

Male Wistar rats were randomly allocated into eight groups of six rats each. Group I served as the control received distilled water (1 ml/day), whereas group II received oral gavage of IMI at the dose rate of 16.9 mg/kg body weight. Group III, IV and V were provided access exclusively to drinking water containing arsenic in the concentrations of 50, 100 and 150 ppb, respectively while group VI, VII and VIII received combined administration of IMI and arsenic at the dose rate of 16.9 mg/kg + 50 ppb, 16.9 mg/kg + 100 ppb and 16.9 mg/kg + 150 ppb, respectively. The animals received daily dosing of IMI between 9.00–10.00 A M daily for a period of 28 days. All animals were weighed weekly for readjustment of the total quantity of IMI administered. Animals were also monitored for any clinical sign(s) during entire period of study.

2.3. Sample collection and analysis

At the end of 28 days of daily treatments animals were sacrificed by cervical dislocation and 3–4 ml blood samples were collected directly from heart in sterilized heparinised tubes. A part of kidney tissue (1 *g*) was collected in 10 ml ice cold phosphate buffer solution (0.5 M, pH-7.4) and rest of renal tissue in formal saline (10%) for antioxidant parameters and histopathological studies, respectively. The blood samples were centrifuged at 4000 rpm for 10 min and plasma was separated and was used for the determination of renal biomarkers using Chemistry Analyzer (CHEM-7, ERBA, Mannheim) by diagnostic kits (Transasia Bio-Medicals Ltd, India). Tissue samples were homogenized using teflon coated homogenizer at 1000 rpm for 5–7 min at refrigerated temperature and 10% tissue homogenate was prepared for determining various antioxidant parameters and cellular damage indicators.

2.4. Determination of cellular damage indicators in kidney

Tissue levels of malondialdehyde (MDA) and Advanced Oxidation Protein Product (AOPP) were determined in the renal tissue. MDA level of renal tissue was analyzed at 535 nm by thiobarbituric acid reactive substance estimation using standard method [22] and values were expressed as nmol MDA produced/g of tissue/h. AOPP of renal tissue was analyzed at 340 nm according to method described earlier [23] and values were expressed as μM using Chloramine T as standard.

2.5. Determination of antioxidant parameters in kidney

Total thiols (TTH) level was determined in renal tissue and expressed as mM reduced glutathione as a standard [24]. Antioxidant enzymes in renal tissue viz. catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-s-transferase (GST) were determined spectrophotometrically (UV-1601, Shimadzu) to assess the alterations in antioxidant system. GST and GR activities were determined according to standard methods and activities expressed as μmol GSH-CDNB conjugate formed/min/g of tissue and nmol NADPH/min, respectively [25,26]. Similarly activities of GPx, SOD and CAT were determined in renal tissue homogenate using standard methods [27–29].

2.6. Histopathological studies

The histopathological studies were carried out according to standard method. Formalin fixed renal tissue of different groups were embedded in paraffin, sectioned, stained with hematoxylin and eosin and examined under a light microscope for histopathological studies.

2.7. Statistical analysis

The biochemical and oxidative stress parameters were analyzed for analysis of variance at 5% level of significance using the Duncan Multiple Range test.

3. Result and discussion

The environment is complex fabric of chemical agents and physical factors which vary both in time and space. It is becoming evident that environmental exposures in humans are not just limited to single chemical rather they are exposed concurrently to a large number of chemicals. At the present time, issues related to assessing, managing and communicating the health risk associated with exposures to chemical mixtures are becoming steadily more critical from a public health perspective [30–32]. Continuous exposures to cocktail of chemicals may induce subtle health effects which may later get manifested into serious health disorders in humans. Kidney is the principal organ responsible for elimination of most of these chemicals and maintaining the cellular redox status. Nowadays incidences of chronic renal disorders are ever increasing due to increased levels of environmental contaminants.

Repeated administrations of IMI significantly ($P < 0.05$) increased plasma levels of blood urea nitrogen (91.3%) and creatinine (54.7%) while as exposure to arsenic alone at 50, 100 and 150 ppb significantly increased BUN (56.1–136.2%) and CR (49.1–83.0%) compared to control (Table 1). The increased levels of BUN and CR may be either due to increased breakdown of tissue or dietary protein and/or impaired renal excretion of nitrogenous waste [33]. Increased CR levels are associated with degenerative changes of cardiac and skeletal

Table 1
Effect of repeated oral administrations of imidacloprid (IMI) and arsenic (As) alone and in-combination on plasma renal biomarkers, total thiols and renal tissue damage indicators in Wistar rats.

Groups (n = 6)	Treatment	BUN	CR	TTH	MDA	AOPP
I	Control	$21.99^{a} \pm 2.50$	$0.53^{a} \pm 0.06$	$3.65^{d} \pm 0.141$	$21.65^{a} \pm 2.45$	$1.60^{a} \pm 0.046$
II	Imidacloprid (IMI)	$42.06^{bc} \pm 3.33$	$0.82^{b} \pm 0.05$	$2.45^{c} \pm 0.340$	$43.83^{b} \pm 2.14$	$1.99^{cd} \pm 0.036$
III	Arsenic (50 ppb)	$34.33^{b} \pm 2.31$	$0.79^{b} \pm 0.08$	$3.16^{d} \pm 0.290$	$37.82^{b} \pm 1.85$	$1.84^{b} \pm 0.040$
IV	Arsenic (100 ppb)	$43.61^{c} \pm 2.39$	$0.91^{b} \pm 0.04$	$2.55^{c} \pm 0.026$	$59.63^{c} \pm 1.89$	$1.93^{bc} \pm 0.031$
V	Arsenic (150 ppb)	$51.94^{d} \pm 3.02$	$0.97^{bc} \pm 0.06$	$2.30^{bc} \pm 0.052$	$67.69^{d} \pm 2.24$	$2.02^{cd} \pm 0.050$
VI	IMI + As (50 ppb)	$62.11^{e} \pm 3.02$	$1.03^{bc} \pm 0.09$	$1.94^{bc} \pm 0.120$	$74.38^{d} \pm 2.36$	$2.08^{d} \pm 0.041$
VII	IMI + As (100 ppb)	$75.01^{f} \pm 2.81$	$1.19^{c} \pm 0.09$	$1.72^{ab} \pm 0.214$	$85.46^{e} \pm 2.61$	$2.20^{e} \pm 0.037$
VIII	IMI + As (150 ppb)	$85.37^{g} \pm 3.04$	$1.53^{d} \pm 0.13$	$1.32^{a} \pm 0.177$	$108.52^{f} \pm 3.72$	$2.24^{e} \pm 0.031$

Values are given as mean ± SE of 6 animals unless otherwise stated.
Values having different superscript (a,b,c,d,e,f) in a column are statistically different from one another at 5% level of significance.
Values of Blood urea nitrogen (BUN) and plasma creatinine (CR) are expressed in mg/dl.
Values of TTH (total thiols) are expressed in μM.
Lipid peroxidation is expressed in nmol MDA produced/g of tissue/hr whereas AOPP in μM of Chloramine T.

Table 2
Effect of repeated oral administrations of imidacloprid (IMI) and arsenic (As) alone and in- combination on activities of antioxidant enzymes of renal tissue in Wistar rats.

Groups (n = 6)	Treatment	GST	GR	GPx	SOD	CAT
I	Control	$92.55^{f} \pm 4.58$	$56.11^{e} \pm 3.34$	$45.19^{f} \pm 3.67$	$378.08^{e} \pm 14.9$	$3352.99^{g} \pm 84.3$
II	Imidacloprid (IMI)	$42.85^{cd} \pm 4.51$	$39.26^{cd} \pm 2.89$	$22.51^{cd} \pm 2.26$	$241.92^{d} \pm 12.8$	$2145.15^{e} \pm 59.2$
III	Arsenic (50 ppb)	$61.73^{e} \pm 4.34$	$44.71^{d} \pm 2.17$	$32.67^{e} \pm 2.09$	$266.99^{d} \pm 18.2$	$2840.30^{f} \pm 79.3$
IV	Arsenic (100 ppb)	$49.45^{d} \pm 3.71$	$42.10^{d} \pm 3.36$	$25.52^{d} \pm 2.25$	$232.47^{cd} \pm 13.6$	$2141.05^{e} \pm 77.9$
V	Arsenic (150 ppb)	$44.15^{d} \pm 2.95$	$37.03^{cd} \pm 2.38$	$18.30^{bc} \pm 1.58$	$194.52^{bc} \pm 13.9$	$1700.15^{d} \pm 73.2$
VI	IMI + As (50 ppb)	$33.43^{bc} \pm 2.78$	$32.90^{bc} \pm 2.31$	$16.46^{bc} \pm 1.50$	$171.64^{ab} \pm 14.4$	$1488.44^{c} \pm 67.5$
VII	IMI + As (100 ppb)	$27.95^{ab} \pm 1.57$	$25.87^{ab} \pm 1.03$	$13.95^{ab} \pm 1.20$	$147.95^{a} \pm 12.7$	$1294.78^{b} \pm 44.5$
VIII	IMI + As (150 ppb)	$21.05^{a} \pm 1.60$	$23.98^{a} \pm 2.00$	$9.56^{a} \pm 0.692$	$133.70^{a} \pm 14.7$	$1077.24^{a} \pm 34.8$

Values are given as mean ± SE of 6 animals unless otherwise stated.
Values having different superscript (a,b,c,d,e,f) in a column are statistically different from one another at 5% level of significance.
Values of GST (glutathione S transferase) are expressed in μmol of CDNB conjugate formed/ min/ g of tissue.
Values of GR (glutathione reductase) are expressed nmol of NADPH/min.
Values of SOD (Superoxide dismutase) and GPx (glutathione peroxidase) are expressed in Unit/ g of tissue.
Values of CAT (Catalase) are expressed in μmol H_2O_2 decomposed/ min/ g of tissue.

muscles. Impaired renal function in present investigation may be as a resultant of oxidative insults induced by either IMI [14,34–36] or arsenic in mammals [37,38]. Co-administrations of the toxicants produced more pronounced increase in BUN (288.2%) and CR (188.7%) levels indicative of more severe renal dysfunction as compared to individual administration of either toxicant (Table 1). Similar observations have also been reported with co-exposure of different metals with different insecticides [39–41].

Oxidative stress may be induced due to excessive free radicals like reactive oxygen/nitrogen species (ROS/RNS) generated during metabolism of toxicants which may directly interact with cellular macromolecules including DNA, lipids and proteins resulting into altered structural and functional integrity of cells. Total thiols (-SH group) plays a major role in the detoxification of extra- and intra-cellular RNS/ROS, and therefore, is an indispensible component of the cellular antioxidant defenses. Its concentration is also responsible for maintaining redox state of protein thiols involved in DNA synthesis and repair. Repeated oral administration of IMI produced significant reduction in the TTH level (32.9%) in the renal tissue of rats [14,42,43]. Similarly, exclusive access to drinking water containing arsenic in drinking water in different concentrations (50, 100 and 150 ppb) resulted to reduction in TTH level (13.4–36.9%) of renal tissues of rats [37,44,45]. Co-exposure to IMI and arsenic produced more pronounced fall in TTH level (63.8%) in renal tissue as compared to control (Table 1). Similar decline in TTH level have also been reported in different visceral organs in arsenic exposed rats [46–48].

The increased free radicals generated during IMI and arsenic metabolism with reduced cellular free radicals scavenging capacity (decreased TTH) might be responsible for free radicals induced membrane lipid (MDA) and protein oxidation (AOPP) in renal tissue. A significant increase in the MDA (102.4%) and AOPP (24.4%) following repeated oral administrations of IMI alone was observed compared to control. Similar observations have also been reported in deltamethrin [42] and dichlorodiphenyltrichloroethane [43] exposure in rats. Similarly

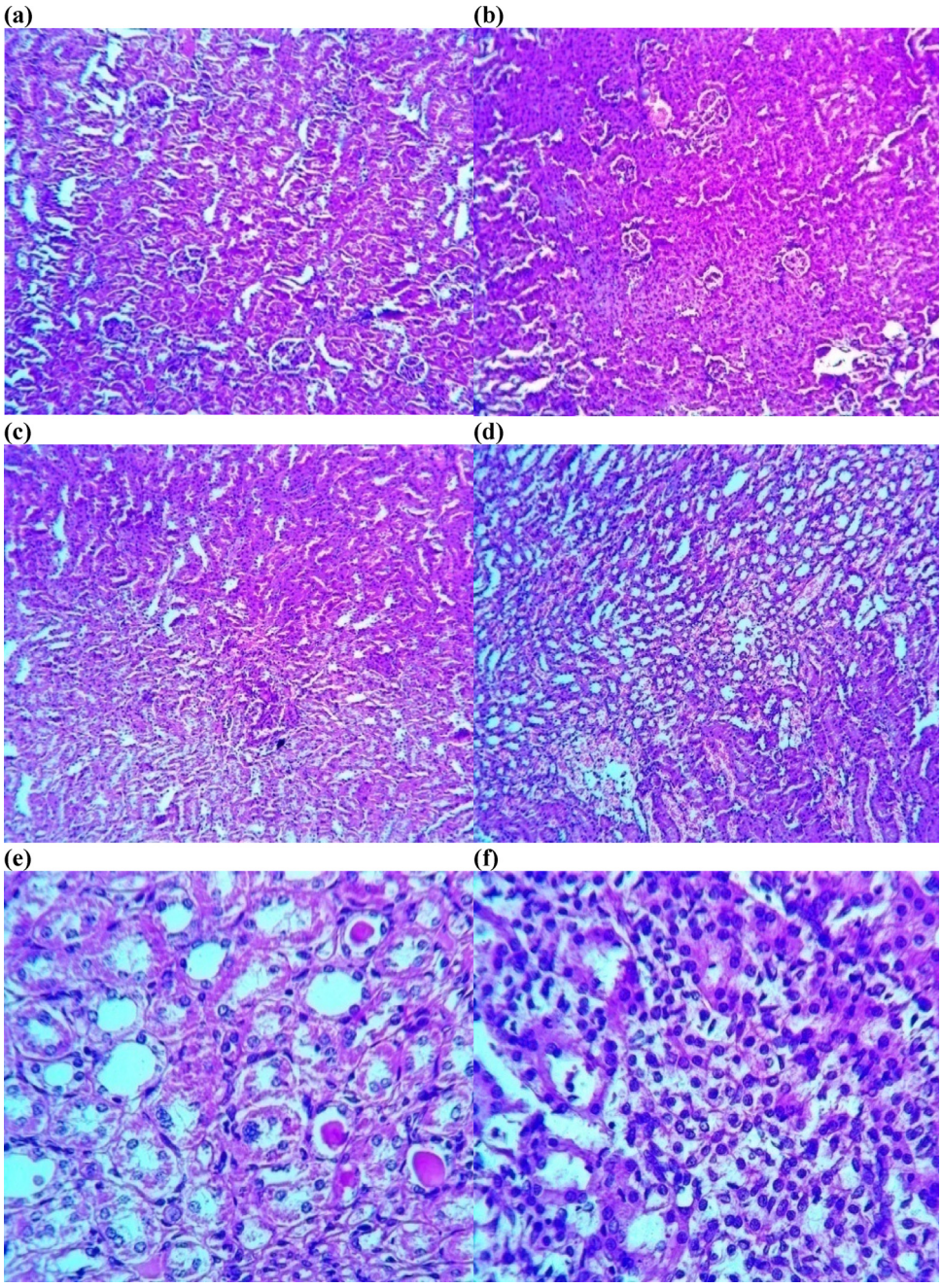

Fig. 1. Photomicrograph of Hematoxylin and Eosin (10X) stained sections of formalin fixed kidney (a) control animals showing normal renal parenchyma (b & c) group II and III showing alterations in histological appearance of renal parenchyma with slight degenerative changes (d) group VI: hemorrhages and congestion in renal interstitial tissues (e) group VII: vacuolar degeneration of tubular epithelium and presence of hyaline casts in tubular lumen of rats (f) and group VIII: necrosis of tubular epithelium in Wistar rats.

exposure of different levels of arsenic in drinking water produced significant rise in levels of MDA (74.7–212.7%) and AOPP (15.0–26.3%). The increased membrane lipid and protein peroxidation have been reported in renal [37,49,50], hepatic [47] and testicular tissue [48] of rats following exposure of arsenic. Simultaneous exposure of both the toxicants produced more pronounced increase in oxidation of membrane lipids (401.2%) and proteins (40.0%) of renal tissue compared to control as well as exposure of either toxicant (Table 1). These observations are in accordance with the observations reported with co-exposure of metals and insecticides to rats [39,41,46].

Reduced antioxidant enzymes (GST, GR, GPx, SOD and CAT) responsible for the scavenging of super oxides, peroxides and hydroxides generated during toxicant induced oxidative insults might have been responsible for increased MDA and AOPP levels in renal tissue. In the present study, IMI caused a significant decline in the activities of SOD (36.0%), GST (53.7%), GR (30.1%), GPx (50.2%) and CAT (36.0%) after repeated oral administrations in rats for 28 days (Table 2). Similar results have also been observed following subacute exposure of deltamethrin [42], cypermethrin [51] and acetamiprid [52].

Arsenic at 50, 100 and 150 ppb in drinking water also produced reduction in the activities of SOD (54.6–64.6%), GST (33.3–52.3%), GR (20.3–34.0%), GPx (27.7–59.5%) and CAT (15.3–49.5%) in renal tissue of Wistar rats (Figure 5a and b). Similar findings were also reported by Patel and Kalia (2010) and Lakshmi et al. (2015) following exposure of arsenic to rats. Co-exposure of IMI and arsenic at 150 ppb produced more pronounced decrease in activities of SOD (64.6%), GST (52.3%),

GR (57.3%), GPx (57.5%) and CAT (67.9%) in renal tissue of Wistar rats. The reduction in activities of these antioxidant enzymes might be either due to increased concentration of free radicals or decreased concentration of reduced glutathione a substrate for different antioxidant enzymes like GPx, GST and GR. Such pronounced alterations in different antioxidant parameters in different visceral organs of animals exposed concurrently to insecticides and metals have been reported [41,46–48].

3.1. Histopathological alterations

Microscopic examination of kidney sections of the IMI administered group revealed minor alterations from the normal renal structure of control rats with no appreciable histopathological abnormalities. There was slight shrinkage of glomeruli with a mild degeneration of the epithelial cells in the kidneys. Administration of IMI at dose higher than NOAEL has been reported to cause degeneration of renal tubules and glomeruli of female rats [20,34]. A dose of 80 mg/kg/day in IMI in chicken and rats has been reported to cause dilatation of tubules, shrunken glomeruli, vacuolation, inter tubular hemorrhages and hyaline casts [35,53].

In the current study, exposure to different concentrations of arsenic produced histopathological lesions which increase in severity with the increasing concentration of the arsenic in drinking water. At low dose of arsenic (50 ppb), slight alterations with no appreciable pathological lesions were observed but with the increasing concentration, mild to moderate vacuolar degeneration of tubular epithelium was seen. Such findings in renal tissue are in accordance with the findings of Chowdhury et al. [54]. Co-administration of IMI and arsenic produced histopathological lesions like degeneration and necrotic changes in tubular epithelial cells, necrosis and infiltration of phagocytic cells, disruption of tubular basement membrane along with oedema, hemorrhages and inter-tubular congestion. Such changes were very prominent and more severe with co-exposure of IMI with arsenic (Fig. 1). Similar administration of arsenic at 10 mg/kg, orally for 8 weeks in mice showed severe degenerative changes with mononuclear cell infiltration in kidney [55].

4. Conclusion

Elevated renal biomarkers, tissue damage indicators with reduced activities of antioxidant enzymes and total thiols along with histopathological alterations following repeated oral administrations of IMI or arsenic in drinking water indicate renal damage. Such cellular alterations in renal tissue due to reduced antioxidant defense were more pronounced in co-exposed toxicant groups indicating thereby the potentiation of arsenic induced renal damage by IMI in Wistar rats.

Conflict of interest

Authors declare there are no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Transparency document

The Transparency document associated with this article can be found in the online version.

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References

[1] A.S. Levey, A.C. Schoolwerth, N.R. Burrows, Comprehensive public health strategies for preventing the development, progression and complications of CKD: report of an expert panel convened by the Centres for Disease control and Prevention, Am. J. Kidney Dis. 53 (2009) 522–535.
[2] M.N. Rana, J. Tangpong, M.M. Rahman, Toxicodynamics of lead, cadmium, mercury and arsenic- induced kidney toxicity and treatment strategy: a mini review, Toxicol. Rep. 5 (2018) 704–713.
[3] M. Araujo, W.J. Welch, Oxidative stress and nitric oxide in kidney function, Curr. Opin. Nephrol. Hypertens. 15 (1) (2006) 72–77.
[4] R.S. Barsoum, Chronic kidney disease in the developing world, New Engl. J. Med. 354 (2006) 997–999.
[5] S. Uchino, The epidemiology of acute renal failure in the world, Curr. Opin. Crit. Care 12 (2006) 538–543.
[6] 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 Biochem. Physiol. 98 (1) (2010) 33–38.
[7] N. Ercal, H. Gurer-Orhan, N. Aykin-Burns, Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage, Curr. Top. Med. Chem. 1 (6) (2001) 529–539.
[8] P.D. Stivaktakis, M.P. Kavvalakis, M.N. Tzatzarakis, et al., Long-term exposure of rabbits to imidaclorpid as quantified in blood induces genotoxic effect, Chemosphere 149 (2016) 108–113.
[9] V. Kant, A.K. Srivastava, P.K. Verma, R. Raina, Alterations in biochemical parameters during subacute toxicity of fluoride alone and in conjunction with aluminum sulfate in goats, Biol. Trace Elem. Res. 130 (1) (2009) 20–30.
[10] M.A. Dar, A.M. Khan, R. Raina, P.K. Verma, M. Sultana, Effect of repeated oral administration of bifenthrin on lipid peroxidation and anti-oxidant parameters in wistar rats, Bull. Environ. Cont. Toxicol. 91 (1) (2013) 125–128.
[11] F.G. Apaydın, H. Baş, S. Kalender, Y. Kalender, Subacute effects of low dose lead nitrate and mercury chloride exposure on kidney of rats, Envir. Toxicol. Pharmacol. 41 (2016) 219–224.
[12] M. Ihara, L.A. Brown, C. Ishida, H. Okuda, D.B. Sattelle, K. Matusda, Actions of imidacloprid, clothianidin and related neonicotinoids on inicotinic acetylcholine receptors of American cockroach neurons and their relationship with insecticidal potency, J. Pesticide Sci. 31 (2006) 35–40.
[13] M. Tomizawa, J.E. Casida, Neonicotinoid insecticide toxicology: mechanisms of selective action, Ann. Rev. Pharmacol. Toxicol. 45 (2005) 247–268.
[14] Y. Arfat, N. Mahmood, M.U. Tahir, et al., Effect of imidacloprid on hepatotoxicity and nephrotoxicity in male albino mice, Toxicol. Rep. 1 (2014) 554–561.
[15] S. Özdemir, S. Altun, H. Arslan, Imidacloprid exposure cause the histopathological changes, activation of TNF-α, iNOS, 8-OHdG biomarkers, and alteration of caspase 3, iNOS, CYP1A, MT1 gene expression levels in common carp (L.), Toxicol. Rep. 5 (2018) 125–133.
[16] M. Tuzen, K.O. Saygi, I. Karaman, M. Soylak, Selective speciation and determination of inorganic arsenic in water, food and biological samples, Food Chem. Toxicol. 48 (1) (2010) 41–46.
[17] C.H. Tseng, A review on environmental factors regulating arsenic methylation in humans, Toxicol. Appl. Pharmacol. 235 (3) (2009) 338–350.
[18] X. Wang, H. Zhao, Y. Shao, et al., Nephroprotective effect of astaxanthin against trivalent inorganic arsenic-induced renal injury in wistar rats, Nutr. Res. Pract. 8 (1) (2014) 46–53.
[19] Y. Hu, C. Yu, M. Yao, et al., The PKCδ-Nrf2-ARE signalling pathway may be involved in oxidative stress in arsenic-induced liver damage in rats, Environ. Toxicol. Pharmacol. 62 (2018) 79–87.
[20] U. Kapoor, M.K. Srivastava, S. Bhardwaj, L.P. Srivastava, Effect of imidacloprid on antioxidant enzymes and lipid peroxidation in female rats to derive its No Observed Effect Level (NOEL), J. Toxicol. Sci. 35 (4) (2010) 577–581.
[21] C.M. Steinmaus, Y. Yuan, A.H. Smith, The temporal stability of arsenic concentrations in well water in western Nevada, Envir. Res. 99 (2) (2005) 164–168.
[22] Shafiq-ur-rehman, Lead-induced regional lipid peroxidation in brain, Toxicol. Lett. 21 (1984) 333–337.
[23] V. Witko-sarsat, M. Friedlander, C. Capeillère-blandin, T. Nquyen-Khoa, A.T. Nquyen, J. Zingraff, P. Jungers, B. Descamps-Latscha, Advanced oxidation protein products as a novel marker of oxidative stress in uremia, Kidney Int. 49 (5) (1996) 1304–1313.
[24] A.P. Motchnik, B. Frei, N.B. Ames, Measurement of antioxidants in human blood plasma, protein thiols, in: L. Packer (Ed.), Oxygen Radicals in Biological Systems, Methods in Enzymology, Academic Press, California, 1994234(D):273-274.
[25] W.H. Habig, M.J. Pabst, W.B. Jakoby, Glutathione S-transferases. The first enzymatic step in mercapturic acid formation, J. Biol. Chem. 249 (22) (1974) 7130–7139.
[26] I. Carlberg, B. Mannervik, Purification and characterization of the flavoenzyme glutathione reductase from rat liver, J. Biol. Chem. 250 (14) (1975) 5475–5480.
[27] D.G. Hafeman, R.A. Sunde, W.G. Hoekstra, Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat, J. Nutr. 104 (5) (1974) 580–587.
[28] S. Marklund, G. Marklund, Involvement of superoxide anion radical in autoxidation of pyrogallol and a convenient assay for superoxide dismutase, Eur. J. Biochem. 47 (1974) 469–474.
[29] H.E. Aebi, Catalase, in methods of enzymatic analysis, in: H.U. Bergmeyer (Ed.), 276-286, Academic Press, New York, 1983.
[30] J.L. Mattsson, Mixtures in the real world: the importance of plant self-defense toxicants, mycotoxins, and the human diet, Toxicol. Appl. Pharmacol. 223 (2) (2007) 125–132.

[31] S.J. Ryker, M.J. Small, Combining occurrence and toxicity information to identify priorities for drinking-water mixture research, Risk Anal. 28 (3) (2008) 653–666.
[32] S. Shukla, R.C. Jhamtani, M.S. Dahiya, R. Agarwal, Oxidative injury caused by individual and combined exposure of neonicotinoid, organophosphate and herbicide in zebrafish, Toxicol. Rep. 4 (2017) 240–244.
[33] B.M. Bush, Interpretation of Laboratory Results for Small Animals, Clinical Blackwell Scientific Publication, London, 1991.
[34] S. Bhardwaj, M.K. Srivastava, K. Upasana, L.P. Srivastava, A 90 days oral toxicity of imidacloprid in female rats: morphological, biochemical and histopathological evaluations, Food Chem. Toxicol. 48 (5) (2010) 1185–1190.
[35] S. Soujanya, M. Lakshman, Imidacloprid induced nephrotoxicity in male rats, Ind. J. Appl. Res. 6 (2013) 415.
[36] M. Hussein, V. Singh, R. Sethi, A.K. Singh, M.K. Hassan, Studies on embryonic effects of neonicotinoid insecticide on chick embryos, J. Anat. Soc. India 63 (2014) 125–129.
[37] H.V. Patel, K. Kalia, Sub-chronic arsenic exposure aggravates nephrotoxicity in experimental diabetic rats, Ind. J. Exper. Biol. 48 (2010) 762–768.
[38] B.V.S. Lakshmi, M. Sudhakar, F.J. Sudha, M.V. Gopal, Ameliorative effect of *Triticum aestivum* Linn against experimentally induced arsenic toxicity in male albino rats, Pharm. Lett. 7 (1) (2015) 202–211.
[39] A.M. Khan, R. Raina, N. Dubey, P.K. Verma, Effect of deltamethrin and fluoride co-exposure on the brain antioxidant status and acetylcholinesterase activity in wistar rats, Drug Chem. Toxicol. 41 (2) (2018) 123–127.
[40] R. Raina, N.A. Baba, P.K. Verma, M. Sultana, M. Singh, Hepatotoxicity induced by subchronic exposure of fluoride and chlorpyifos in wistar rats: mitigating effect of ascorbic acid, Biol. Trace Elem. Res. 166 (2) (2015) 157–162.
[41] N. Baba, R. Raina, P.K. Verma, M. Sultana, Free radical induced nephrotoxicity following repeated oral exposure to chlorpyrifos alone and in conjunction with fluoride in rats, Turk. J. Med. Sci. 46 (2016) 512–517.
[42] A.M.A. El-Saad, Lycopene protects against deltamethrin induced oxidative renal dysfunction in rats, Egypt J. Exper. Biol. (Zool.) 7 (2) (2011) 111–121.
[43] N. Marouani, D. Hallegue, M. Sakly, M. Benkhalifa, K.B. Rhouma, O. Tebourbi, Involvement of oxidative stress in the mechanism of p,p'-DDT induced nephrotoxicity in adult rats, Gen. Physiol. Biophys. 36 (3) (2017) 309–320.
[44] L. Wang, Z.R. Xu, X.Y. Jia, J.F. Jiang, X.Y. Han, Effect of arsenic (AsIII) on lipid peroxidation, Glutathione content and antioxidant enzymes in growing pigs, Asian-Aust. J. Anim. Sci. 19 (5) (2006) 727–733.
[45] F.M. El-Demerdash, M.I. Yousef, F.M. Radwan, Ameliorating effect of curcumin on sodium arsenite-induced oxidative damage and lipid peroxidation in different rat organs, Food Chem. Toxicol. 47 (1) (2009) 249–254.
[46] A. Jain, S. Agrawal, S.J.S. Flora, Arsenic and nicotine co-exposure lead to some synergistic effects on oxidative stress and apoptotic markers in young rat blood, liver, kidney and brain, Toxicol. Reprod. 2 (2015) 1334–1346.
[47] L. Mahajan, P.K. Verma, R. Raina, S. Sood, Toxic effects of imidacloprid combined with arsenic: Oxidative stress in rat liver, Toxicol. Ind. Health 34 (10) (2018) 726–735.
[48] L. Mahajan, P.K. Verma, R. Raina, S. Sood, Potentiating effect of imidacloprid on arsenic-induced testicular toxicity in Wistar rats, BMC Pharmacol. Toxicol. 19 (1) (2018) 48.
[49] J. Zhang, X. Pan, N. Li, X. Li, Y. Wang, X. Liu, X. Yin, Z. Yu, Grape seed extract attenuates arsenic induced nephrotoxicity in rats, Exp. Therap. Med. 7 (2014) 260–266.
[50] P. Reckziegel, V.T. Dias, D.M. Benvegnú, N. Boufleur, R.C.S. Barcelos, H.J. Segut, C.S. Pase, C.M.M. Dos Santos, E.M.M. Flores, M.E. Burger, Antioxidant protection of gallic acid against toxicity induced by Pb in blood, liver and kidney of rats, Toxicol. Reprod. 3 (2016) 351–356.
[51] S.A. Sakr, A.Y. Albarakai, Effect of cinnamon on cypermethrin induced nephrotoxicity in albino rats, Int. J. Adv. Res. 2 (7) (2014) 578–586.
[52] R.K.S. Devan, A. Mishra, P.C. Prabu, T.K. Mandal, S. Panchapakesan, Sub-chronic oral toxicity of acetamiprid in Wistar rats, Toxicol. Environ. Chem. Rev. 97 (9) (2015) 1236–1252.
[53] A.M. Kammon, R.S. Brar, H.S. Banga, S. Sodhi, Patho-biochemical studies on hepatotoxicity and nephrotoxicity on exposure to imidacloprid in layer chickens, Vet. Arhive 80 (5) (2010) 663–672.
[54] D.U.S. Chowdhury, S. Islam, R. Akter, L. Khaleda, Z. Rahman, M. Al-Forkan, A study on the effect of arsenic on tissue histology and its deposition pattern in various organs of wistar albino rats, Eur. J. Pharmacol. Med. Res. 3 (5) (2016) 580–587.
[55] A.S. Noman, S. Dilruba, N.C. Mohanto, et al., Arsenic-induced histological alterations in various organs of mice, J. Cytol. Histol. 6 (3) (2015).