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Sub-chronic exposure to arsenic and dichlorvos on erythrocyte antioxidant defense systems and lipid peroxidation in rats

Nidhi Dwivedi and S.J.S. Flora*

Division of Regulatory Toxicology, Defence Research and Development Establishment, Gwalior-474 002, India

*Corresponding Author E-mail: sjsflora@drde.drdo.in

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Abstract

The effect of combined exposure to arsenic (25ppm in drinking water) and dichlorvos (2.5 mg kg^{-1}, orally) for 56 days on biochemical variables, indicative of lipid peroxidation, antioxidant enzyme system and AChE activity in erythrocytes of rats, were examined. While arsenic caused a significant increase in AChE, DDVP produced marked depletion. Combined exposure to arsenic and DDVP produced no additional decrease in AChE activity, which was comparable to DDVP. Arsenic and DDVP also increased the levels of reactive oxygen species (ROS) and thiobarbituric acid reactive substances (TBARS), suggesting free radical generation. Interestingly, glutathione linked enzymes (GSH, GPx, GST and GR) significantly increased on arsenic and DDVP exposure. SOD activity also increased significantly in the individually exposed groups, while catalase activity remained unchanged. Blood arsenic level increased significantly on co-exposure to arsenic alone and with DDVP exposed group. However, arsenic content in co-exposed group depleted marginally as compared to arsenic alone group, indicating possible arsenic redistribution. It might be concluded from the study that the combined exposure to arsenic and DDVP may lead to synergistic effects on certain biochemical indicators of oxidative stress like ROS, GSH and SOD, suggesting a more pronounced induction of lipid peroxidation in erythrocytes.

Key words

Arsenic, Dichlorvos, Erythrocyte, Interaction, Oxidative stress

Introduction

Humans are exposed to various kinds of environmental toxicants. These environmental stressors include toxic metals/metalloids, pesticides, chemical warfare agents etc. Of these, metals and pesticides were given much emphasis due to their persistence in environment, leading to their continual exposure and toxic effects.

Arsenic is among the most toxic pollutants, found in soil, water and air. It ranks first in the list of 20 hazardous substances by the Agency for Toxic Substances and Disease Registry (ATSDR 2005). Arsenic poisoning, as a result of natural occurrence in ground water remains a problem in many parts of the world, particularly in India and Bangladesh. Arsenic contamination in West Bengal, India has reported to be the biggest arsenic calamity (Mandal *et al.,* 1996). An estimated 30 million people in the Ganges delta are drinking well water, contaminated with arsenic. Besides India and Bangladesh, elevated levels of arsenic have been reported in other countries like China, Japan, Mexico, Taiwan, Thailand and the United States. As per the World Health Organization guidelines, population is currently exposed to arsenic level above 10 µg l^{-1} in drinking water (WHO, 2004; IPCS, 2001).

Pesticides are widely used in agricultural, household and public health settings resulting in continuing exposure to humans and animals. Efforts to increase the quality and quantity of crop yields in many countries have led to the significant rise in the usage of pesticides (Donaldson *et al.,* 1997). Pesticide poisoning is frequent particularly in developing countries and carries high mortality and morbidity (Jeyaratnam, 1990; Eddleston and Phillips 2004). Dichlorvos (2, 2-dichlorovinyl dimethyl phosphate, DDVP) is one of the broad-spectrum organophosphate compounds used throughout the world in agricultural, horticultural and public health programs (IARC, 1991).

Humans and animals are generally exposed to a variety of chemical mixtures prevailing in the environmental matrices. Although most of the studies have focused on studying the individual effects of toxicants, but considering that in actual scenario humans may get exposed to different chemicals, there is a need to study the effects of their concomitant exposure. There is every possibility that due to concurrent exposure, one toxicant may alter the biochemical activities of another, resulting in the response which may be additive or reductive in nature (Naraharisetti *et al.,* 2009, Flora *et al.,* 2013). Arsenic is a common global ground water contaminant while dichlorvos is one of the most commonly and widely employed organophosphate based insecticide for agro-production and protection of crops. There is thus a real situation where human may get exposed to these toxicants while working in a field (Aggarwal *et al.,* 2007; Flora *et al.,* 2013). There are several reports on the removal of arsenic, DDVP and other pesticides from ground/surface water which clearly indicates the presence of these toxicants in surface water (Liu *et al.,* 2009; Kosutic *et al.,* 2005; Bruggen and Vandecasteele, 2003). Recent reports have demonstrated the effects of combined exposure to arsenic with monocrotophos (MCP) and DDVP on biochemical variables, suggestive of hepatic and brain oxidative stress. These observations suggested that co-exposure to arsenic, DDVP and MCP may not necessarily lead to synergistic effects (Dwivedi and Flora, 2011). Flora *et al* (2013) in recent study suggested that individual exposure to arsenic or DDVP induces neuronal damage, mediated by alterations in biogenic amines and hepatotoxicity by excessive generation of ROS. Concomitant exposure to arsenic and DDVP also resulted in synergistic effects accompanied with some antagonistic effects.

Erythrocytes act as vehicles and help in rapid diffusion of O_2 and CO_2 between the lungs and tissues (Hamidi and Tajerzadeh, 2003). Dichlorvos has been selected for the present study in view of its widespread use and concern regarding its toxicity. Erythrocytes could be a possible target for both arsenic and DDVP induced oxidative damage leading to life threatening clinical manifestations such as anemia, hypoxia and several other disorders (Celik and Suzek 2009; Biswas *et al.,* 2008; Hinz *et al.,* 1996; Winski and Carter 1995). The present study was thus planned specifically to study the effects on erythrocytes as they may act as first target for both toxicants after systemic absorption (Celik and Suzek 2009; Saha *et al.,* 1999). In blood, erythrocytes function also depends on the intactness of cell membrane, which is the target for many toxic agents. Hence, it was considered interesting to study the effects of combined exposure to arsenic and DDVP on biochemical variables, indicative of oxidative stress in erythrocyte. Biswas *et al.* (2008) also reported the development of anemia during chronic arsenic exposure. Nemeti *et al.* (2003) extensively investigated the effect of arsenate on erythrocytes in humans and rats. Recent study was carried out to demonstrate that human erythrocytes efflux GSH in response to arsenic exposure (Yildiz and Cakir 2012). Celik and Suzek (2009) reported that individual exposure to DDVP led to lipid peroxidation and caused changes in the antioxidant enzyme system in erythrocytes and tissues. Reports are available on arsenic and DDVP individual exposure that can inhibit blood acetylcholinesterase activity (Bocquene *et al.,* 1995). The aim of the current study was to examine the sub-chronic individual and combined toxicity of arsenic and DDVP on lipid peroxidation, reactive oxygen species (ROS) and the role of antioxidant enzymes such as reduced glutathione (GSH), glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase and glutathione-S transferase (GST) in rat erythrocytes.

Materials and Methods

Chemicals and reagents : The organophosphorus compound Dichlorvos (DDVP; Nuvan 76%) was obtained from Syngenta Chemicals (India) and sodium arsenite ($NaAsO_2$, molecular weight 129.9) was procured from Sigma Chemical (USA). All other analytical laboratory chemicals and reagents were purchased from Merck (Germany), Sigma (USA) or BDH chemicals (Mumbai, India). Ultra pure water prepared by Millipore (New Delhi, India) was used throughout the experiment to avoid metal contamination and for the preparation of reagents and buffers used for various biochemical assays in the present study.

Animals and treatments : Male Wistar rats (110-120g) were obtained from Defence Research and Development Establishment (DRDE) animal facility and prior to use, were acclimatized for 7 days 12hr light/dark cycle. The Animal Ethical Committee of DRDE, Gwalior, India, approved the protocols for the experiments. The animals were housed in stainless steel cages in an air-conditioned room with temperature maintained at 25±2ºC. Rats were allowed standard chow diet (Ashirwad Feeds, Chandigarh, India) throughout the experiment and water *ad libitum*. Twenty-four animals were randomized into four groups of six rats each and treated for 56 days. A 56 day of exposure was chosen to determine the effects of combined sub-chronic exposure to these two toxicants using low doses. Animals of group I were control while, group II animals were treated with DDVP at a dose of 2.5 mg kg^{-1}, orally, group III animals were treated with sodium arsenite (25 ppm in drinking water). The animals of group IV were given combined treatment of DDVP (2.5 mg kg^{-1}) and sodium arsenite (25 ppm).

The selected dose of DDVP was 1/ 10th of their reported LD_{50} (Oral LD_{50} of sodium arsenite and DDVP in rat is 41 mg kg^{-1} and 25 mg kg^{-1}). After 56 days, blood was drawn from retro-orbital plexus and collected in heparinized treated vials for various biochemical, hematological parameters and metal analysis.

Preparation of erythrocyte: To prepare erythrocytes, blood was centrifuged at 1500 x g for 10 min at 4°C. After centrifugation, plasma and buffy coat were removed by aspiration. The obtained pellet was washed three times in phosphate buffer saline (0.1 M)

by centrifuging at 3000 rpm for 10 min. The erythrocytes obtained after centrifugation was divided into two parts to prepare hemolysate. One part diluted with chilled distilled water (100 µl of erythrocytes +1.9 ml of distilled water) and kept for the analysis of reactive oxygen species (ROS), thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) and acetylcholinesterase (AChE). The other part of hemolysate was used for the estimation of other antioxidant enzymes *viz* glutathione peroxidase (GPx), glutathione-S-transferase (GST), glutathione reductase (GR), SOD (Superoxide radicals) and catalase. For this, erythrocytes were precipitated by means of chloroform and ethanol. After centrifugation at 3000 x g for 10 min at 4°C, the supernatant obtained was used for estimating all the above mentioned enzymatic assays.

Biochemical assays

Clinical hematological variables: Level of mean cell volume (MCV), hematocrit (HCT), hemoglobin (HGB), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelets (PLT), red blood cell (RBCs) count and white blood cell (WBCs) count were measured using a Sysmex hematology analyzer (model K4500).

Reactive oxygen species (ROS) : Amount of ROS was measured using 2, 7-dichlorofluorescin diacetate (DCFDA) that gets converted into highly fluorescent DCF by cellular peroxides (including hydrogen peroxide). The assay was performed as described in the literature (Socci *et al.,* 1999). Briefly, 5% RBC hemolysate was prepared in ice-cold 40 mM Tris–HCl buffer (pH 7.4), and this was further diluted to 0.25% with the same buffer (40 mM Tris–HCl, pH 7.4) and placed on ice. The samples were divided into two equal fractions (2 ml each). In one fraction, 40 ml of 1.25 mM DCFDA, in methanol, was added for ROS estimation. Another fraction, in which 40 ml of methanol was added served as control for hemolysate auto fluorescence. All the samples were incubated for 15 min in water bath at 37ºC. Fluorescence was determined at 488 nm excitation and 525 nm emission wavelength, using a fluorescence plate reader (Perkin-Elmer, LS-55, United Kingdom).

Blood reduced glutathione (GSH): Determination of GSH concentration was carried out by the modified method of Jollow *et al.* (1974) 1.8 ml of distilled water was added to 0.2 ml of blood and incubated for 10 min at 37ºC for complete hemolysis. After adding 3 ml of sulphosalicylic acid (4%), tubes were centrifuged at 1200g for 15 min .To the supernatant, 200µl of DTNB (10 mM) was added in presence of phosphate buffer (0.1 M pH 7.4). Absorbance need at 412 nm was used for calculation of GSH concentration.

Thiobarbituric reactive substances (TBARS) : Lipid peroxidation was estimated following the method of Ohkawa *et al.* (1979) 0.1 ml of 5% RBC hemolysate was added to 0.2 ml of 8.1% SDS (w/v) and incubated for 10 min. Then 1.5ml of 20% acetic acid (pH 3.5) was added followed by addition of 1.5 ml of 0.8% thiobarbituric acid (w/v) and 0.7 ml distilled water and incubating for 1 hr in boiling water bath. One millilitre of distilled water was added to the solution after cooling and centrifuged at 6000 rpm for 15 min. The malondialdehyde formation was determined by reading the absorbance at 535 nm and the values were expressed as nmoles of MDA ml^{-1} blood.

Glutathione peroxidase (GPx) and glutathione S-transferase (GST) activity: Glutathione peroxidase in erythrocyte was determined by the method of Flohe and Gunzler (1984) at 37ºC. One milliliter of reaction mixture was prepared, which contained 0.3 ml of phosphate buffer (0.1 M, pH 7.4), 0.2 ml of GSH (2 mM), 0.1 ml of sodium azide (10 mM), 0.1 ml of H_2O_2 (1 mM) and 0.3 ml of hemolysate. After incubation at 37ºC for 15 min, reaction was terminated by addition of 0.5 ml of 5% TCA. Tubes were centrifuged at 1500xg for 5 min and supernatant was collected. About 0.2 ml phosphate buffer (0.1 M pH 7.4) and 0.7 ml of DTNB (0.4 mg ml^{-1}) was added to 0.1 ml of reaction supernatant. After mixing, absorbance was read at 420 nm. A molar extinction coefficient of 6.22 x 103 M cm^{-1} was used to determine the activity.

GST activity was determined following the protocol of Habig *et al.* (1974) The reaction mixture contained 0.02 ml of 1-chloro 2, 4-dinitro benzene (1 mM) and 2.9 ml of GSH (0.3 mg GSH ml^{-1} in 0.2 M phosphate buffer, pH 7.4) and 30 µl of hemolysate and change in color was monitored by reading the absorbance (340 nm) at 30 s intervals for 3 min. The enzyme activity was expressed in nano mole conjugate min^{-1} mg^{-1} protein.

Erythrocytes glutathione reductase activity : Glutathione reductase activity was determined in erythrocytes by the method of Worthington *et al.* (1974) To measure GR activity, 60mM EDTA was mixed with 0.1 M phosphate buffer (pH 7.4), 2 mM NADPH and 60 mM glutathione disulfide (GSSG). To 270 µl of above mixture, 30 µl of each hemolysate was added and mixed in the well plate reader to start the reaction. The assay was run at 340 nm for 5 min with absorbance readings taken every 60 sec.

Superoxide dismutase (SOD) : Erythrocyte SOD activity was assayed by the method of Kakkar *et al.* (1984) SOD was extracted from purified RBCs by ethanol chloroform extraction as described by Steck and Kant (1974). Reaction mixture was prepared which contained 1.2 ml of sodium pyrophosphate, 0.3 ml of PMS, 0.3 ml of NBT, 0.2 ml of hemolysate, 0.8 ml of distilled water and 0.2 ml of NADH. The control reaction mixture was prepared and contained 1.2 ml of sodium pyrophosphate, 0.3 ml of PMS, 0.3 ml of NBT, 1ml of distilled water and 0.2 ml of NADH. Both mixtures were incubated at 37ºC for 90 sec and then 1 ml of acetic acid was added and the mixture was allowed to stand for 10 min. The absorbance was read at 560nm.

Catalase : Catalase activity, in purified erythrocytes, was

assayed following the procedure of Sinha *et al.* (1972). Reaction mixture containing 1 ml of phosphate buffer, 0.1ml of hemolysate, 0.4 ml of distilled water and 0.2 ml of H_2O_2 was prepared. The control mixture was prepared; containing 1 ml of phosphate buffer, 0.5ml of distilled water and 0.2 ml of H_2O_2. Both the mixtures were incubated at 37°C for 15 min and reaction was stopped by additing 2 ml of acetic acid with dichromate (1:3 ratio of 5% potassium dichromate in distilled water and glacial acetic acid respectively). Above mixture was boiled for 15 min and the mixture was cooled and absorbance was read at 570 nm

Acetylcholinesterase (AChE) activity: Activity of acetyl cholinesterase (AChE) in erythrocyte was determined according to the method of Ellman *et al.* (1961) using acetylthiocholine as substrate. A reaction mixture was prepared which contained 2.6 ml of phosphate buffer [0.1 M, pH 8.0], 0.1 ml of DTNB and 0.02 ml of hemolysate. The above mixture was incubated at 37°C for 5 min and then 0.02 ml of acetylcholine iodide was added. AChE activity was measured at 412 nm and its unit was expressed as nmol min^{-1} mg^{-1} protein.

Arsenic estimation: Arsenic concentration in blood was measured after wet acid digestion, using a Microwave Digestion System (CEM, USA, model MDS-2100). Arsenic was estimated using a Hydride Vapor Generation System (Perkin Elmer model MHS-10), fitted with an atomic absorption spectrophotometer (AAS, Perkin Elmer model Analyst 100).

Statistical analysis : Data was presented as mean+SE. Data was also analyzed for statistical comparison using ANOVA followed by Bonferroni test. A significance of P< 0.001 and P< 0.05 was considered significant.

Table 1 : Effects of individual and combined exposure to DDVP and arsenic for 56 days on body weight of rats

Groups	Initial body weight (g)	Final body weight (g)
Control	118.3±3.10	185.2 ± 5.96*
DDVP	115.6±5.24	165.8±11.67†
Arsenic	113.6±2.12	171.0±15.03‡.
DDVP+As	112.5±1.62	161.5 ±9.62†

Values are mean of b replicates ± SE. *,†,‡. Differences between values with matching symbol notations within each row are not statistically significant at 5% probability level

Results and Discussion

It is known that pesticide toxicity and persistence of heavy metals in the environment may lead to various adverse effects. Arsenic contamination in groundwater is a major public health concern and DDVP is among the most widely used pesticides by the farmers. Interestingly, toxic effects of arsenic and DDVP are reported to be mediated through oxidative stress leading to the generation of ROS, peroxidation of lipids. These changes alter the permeability and the functional status of cell membranes, disturbing the anti-oxidative defence mechanisms and ultimately leading to possible cell death. Exposure to arsenic and DDVP also generates superoxide anion, hydroxyl radical and hydrogen peroxide during their metabolism in the body (Flora 2011, Binukumar and Gill 2010, Shi *et al.,* 2004, Celik *et al.,* 2009; Bhadauria and Flora 2007).

Body weight gain was significantly less pronounced in animals exposed to DDVP (43%) and arsenic (50%), while animals co-exposed to DDVP and arsenic (43%) showed weight

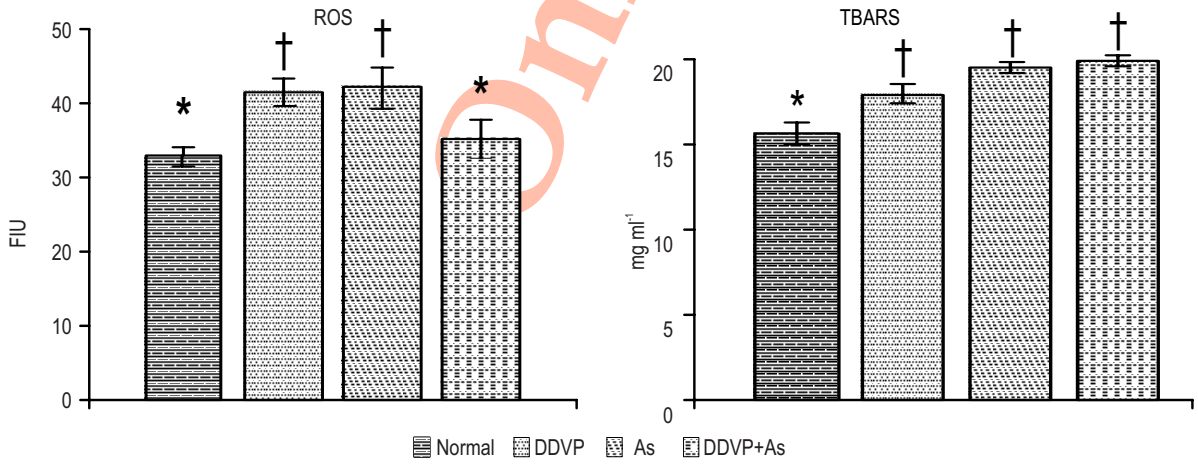

Fig. 1 : Effect of individual and combined exposure to DDVP and arsenic on reactive oxygen species (ROS) and thiobarbituric reactive substance (TBARS) levels in rat erythrocytes. Values are mean of 6 replicates ± SE; n = 6. *,† Differences between values with matching symbol notations within each bar is not statistically significant at 5% probability level

Table 2 : Effects of individual and combined exposure of DDVP and arsenic for 56 days on different hematological variables in rats

	Control	DDVP	As	DDVP+As
WBC	14 ± 0.63*	16.1 ± 0.90†	22.7 ± 2.07‡	20.6 ± 2.54‡
RBC	10.6 ± 0.56*	10.7 ± 0.34*	12.2 ± 0.25*	10.88 ± 0.18*
HGB	15.1 ± 0.52*	13.9 ± 0.33*	15.2 ± 0.33*	14.9 ± 0.21*
HCT	63.4 ± 1.87*	59.6 ± 1.75*	64.5 ± 1.20*	65.5 ± 0.61*
MCV	57.8 ± 0.46*	56.1 ± 0.59*	56.0 ± 0.22*	58.9 ± 0.58*
MCH	14.4 ± 0.94*	13.3 ± 0.19*	12.7 ± 0.26*	13.9 ± 0.30*
MCHC	25.05 ± 1.45*	23.8 ± 0.22*	23.7 ± 0.40*	23.6 ± 0.34*
PLT	1052.2 ± 73.5*	1242.3 ± 96.7†	1217.3 ± 89.4‡	1225.2 ± 75.5‡

RBC-Red blood cells as $\times 10^6$ μl^{-1}; WBC-White blood cells as $\times 10^3$ μl^{-1}; HGB-Hemoglobin as g dl^{-1}; HCT-Hematocrit as %; MCV-Mean cell volume as fl; MCH-Mean cell haemoglobin as pg; MCHC-Mean cell haemoglobin concentration as g dl^{-1}; PLT-Platelet as $\times 10^6$ μl^{-1}. Values are mean of 6 replicates ± SE. *,†,‡ Differences between values with matching symbol notations within each row are not statistically significant at 5% probability level

loss which was comparable to DDVP (43%) (Table 1). A significant increase in WBC counts (16.1 ± 0.90) in arsenic, DDVP (22.7 ± 2.07) and in animals co-exposed to arsenic and DDVP (20.6 ± 2.54) was noted as compared to untreated animals (14 ± 0.63). However, PLT counts were significantly increased in all the exposed groups (1242.3 ± 96.7 in arsenic, 1217.3±89.4 in DDVP, 1225.2 ± 75.5 in arsenic + DDVP) as compared to control (1052.2±73.5) with no synergistic effects on co-exposure to these toxicants. Most of the other hematological variables remained significantly unchanged (Table 2). A significant change in the body weight is considered as a classical indicator for the development of toxicity in animals co-exposed to arsenic and DDVP, while changes in haematological variables like WBC and PLT counts can be attributed to the normal pathological manifestation in response to toxicity as a possible defence mechanism (Celik *et al.,* 2009).

ROS and TBARS increased significantly on individual exposure to DDVP (41.37± 1.89) and arsenic (42.09±2.66), while during co-exposure (35.23±2.61) it remained unchanged as compared to control (32.82±1.35) (Fig. 1). A significant increase in TBARS was observed too on individual exposure to the two toxicants (17.9±0.58 DDVP; 19.5±0.30) while no synergistic effects were noted in DDVP+ As co-exposed animals (19.7±0.17) as compared to control (15.65±0.66). Lipid peroxidation is a biomarker of oxidative stress indicating damage to biological cell membranes present interior to the cell. Both arsenic and DDVP are known to induce lipid peroxidation (Flora, 2011; Nain and Smits 2012). Co-exposure to arsenic and DDVP produced no marked change in TBARS level as compared to the effects in individual groups suggesting possible formation of insoluble complex. Lipophilic characteristic of organophosphates allows them to interact with lipid-rich bio-membranes present within the cell and damages as a result of oxidative polyunsaturated fatty acids, leading to lipid peroxidation (Yarsan and Cakir 2006). Erythrocytes function depends on the intactness of cell membrane and presence of high concentrations of polyunsaturated fatty acids, molecular oxygen and numerous antioxidant enzymes (GSH, GPx, GR and GST) making erythrocytes as a major target for many toxicants. Thus the

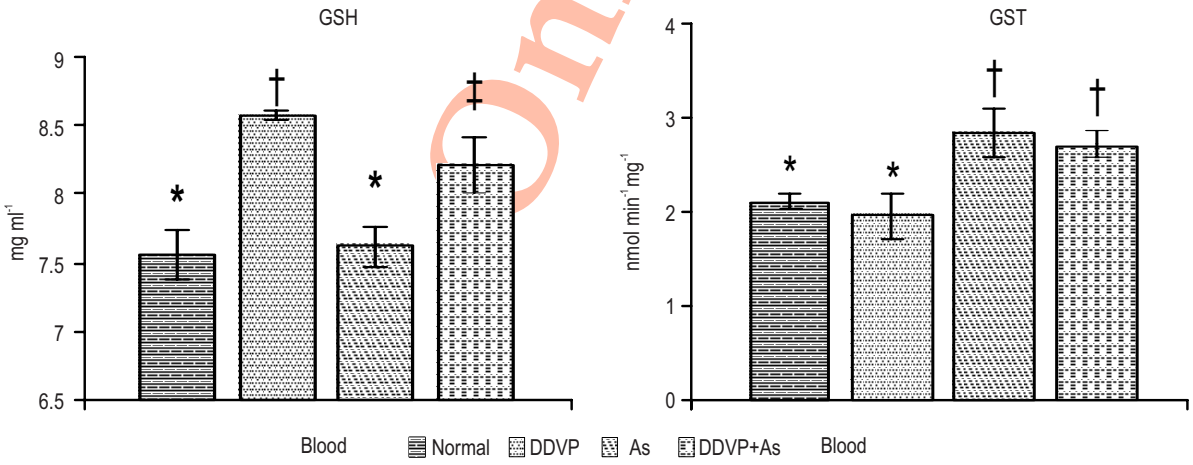

Fig. 2 : Effect of individual and combined exposure to DDVP and arsenic on reduced (GSH) and glutathione S-transferase (GST) activity in rat erythrocytes. Values are mean of 6 replicates ± SE; *,†,‡.Differences between values with matching symbol notations within each bar is not statistically significant at 5% level of probability

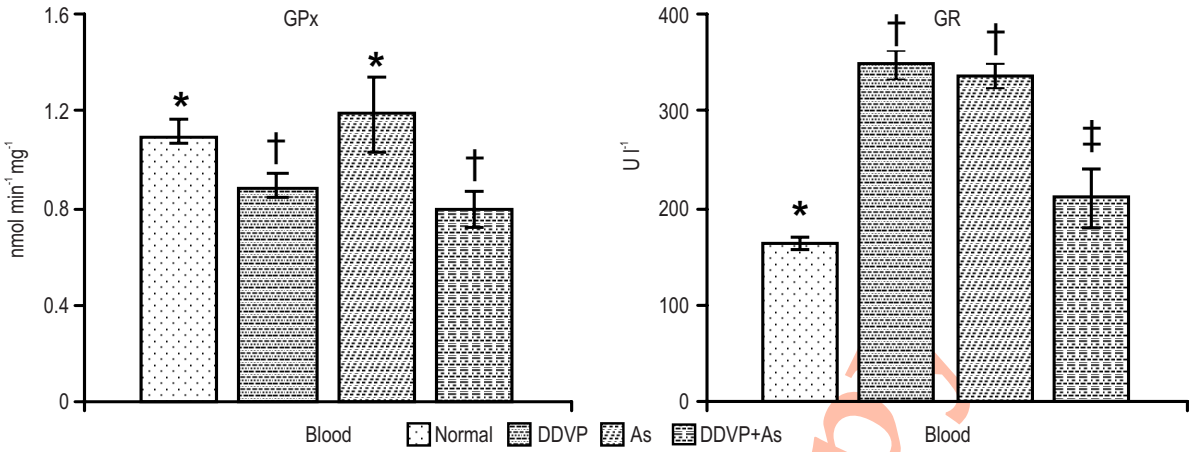

Fig. 3 : Effect of individual and combined exposure to DDVP and arsenic on glutathione peroxidase (GPx) and glutathione reductase (GR) activities in rat erythrocytes. Values are mean of 6 replicates ± SE. *, † Differences between values with matching symbol notations within each bar are not statistically significant at 5% probability level

measurement of lipid peroxidation plays a key role in investigating the toxic effects of arsenic and organophosphate pesticides towards erythrocytes in rat model (Nain and Smits 2012, Biswas *et al.,* 2008, Catagol *et al.,* 2007, Aggarwal *et al.,* 2009). The results further suggest that arsenic and DDVP alone increased ROS but concomitant exposure to these toxicants had no additional effects on ROS. These observations are in agreement with previous finding in liver and brain (Ajiboye, 2010; Flora *et al.,* 2008; Kitchin and Ahmad, 2003). Increased TBARS and ROS levels confirm generation of free radicals, resulting in oxidative stress conditions, leading to the structural and functional alterations in erythrocytes.

Blood GSH level increased on DDVP exposure (8.58±0.04) while remained statistically unchanged on arsenic exposure (7.62±0.15) as compared to control (7.56±0.19). During co-exposure to arsenic and DDVP, the effect (increase) was less pronounced (8.21±0.21) as compared to DDVP alone exposed group (8.58±0.04). GST activity, on the other hand, increased on arsenic exposure (2.85±0.26) while remained unchanged on DDVP (1.96±0.24) or arsenic + DDVP exposure (2.61±0.18) as compared to control (2.10±0.08) (Fig. 2).

Fig. 3 shows increased activities of GPx and GR in DDVP, arsenic and arsenic + DDVP co-exposed animals. GPx activity decreased following exposure to DDVP (0.89±0.05) while remained statistically unchanged on arsenic exposure (1.18±0.15) as compared to control (1.09±0.03). In animals co-exposed to arsenic and DDVP (0.79±0.07), the effects were predominantly of DDVP (0.89±0.05). Increase in GR activity following arsenic (336.6±17.7) or DDVP exposure (345.9±31.2) were comparatively less pronounced in arsenic + DDVP co-exposed animals (210.6±31.3) as compared to control (163.2± 6.71).

No change was noted in catalase activity in all the exposed group as compared to untreated animals. While, SOD activity increased significantly on DDVP (65.9±10.2) or arsenic exposure (130.6±15.0), co-exposure to these toxicants produced no additional effects (149.8±7.23) as compared to control (22.5±2.42) and the values were almost same as in case of arsenic. SOD activity increased more prominently in arsenic exposed animals than DDVP, while in animals co-exposed to two toxicants produced no additive effects. Catalase activity, on the other hand, remained unchanged on any of the above treatments as compared to control (60.2±2.56) (Fig. 4). Increased GSH level in DDVP exposed animals might be an indication of body's initial self protective mechanism against excessive generation of reactive oxygen species. Arsenic being more toxic than DDVP, and it's exposure may either produce a depletion or no change (as in the present case). In animals concomitantly exposed to arsenic and DDVP no -significant change in GSH level was noted. This may be attributed to its utilization to challenge the prevailing oxidative stress condition. SOD and catalase are antioxidant enzymes, and play a coordinated role in prevention of oxidative damage mediated by excessive generation of reactive oxygen species. Co-administration of arsenic and DDVP produced significant increase in SOD activity but the most prominent change was observed in arsenic alone group, suggesting protective responses to eliminate the reactive free radicals. Lipid peroxidation elevated despite increase in SOD, leading to overproduction of superoxide radicals ($^{\cdot}O^{-}$), which synergistically inhibited catalase activity. It was previously reported that diazinon, phosphomidon, dimethoate and malathion caused an increase in SOD activity (Altuntas *et al.,* 2004; Sharma *et al.,* 2005; Catalgol Karademir *et al.,* 2007). Glutathione related enzymes like GPx, GR and GST function in the detoxification process. GPx and GR enzymes play a key role in maintaining the redox cycle of GSH

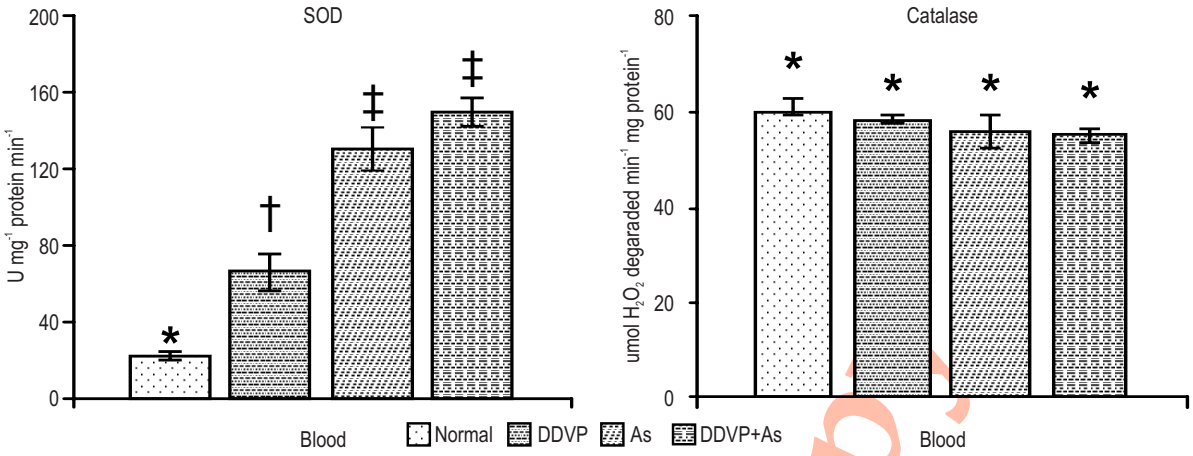

Fig. 4 : Effect of individual and combined exposure to DDVP and arsenic on superoxide dismutase (SOD) and Catalase activity in rat erythrocytes. Values are mean of 6 replicates ± SE; *, †, ‡ Differences between values with matching symbol notations within each bar are not statistically significant at 5% probability level

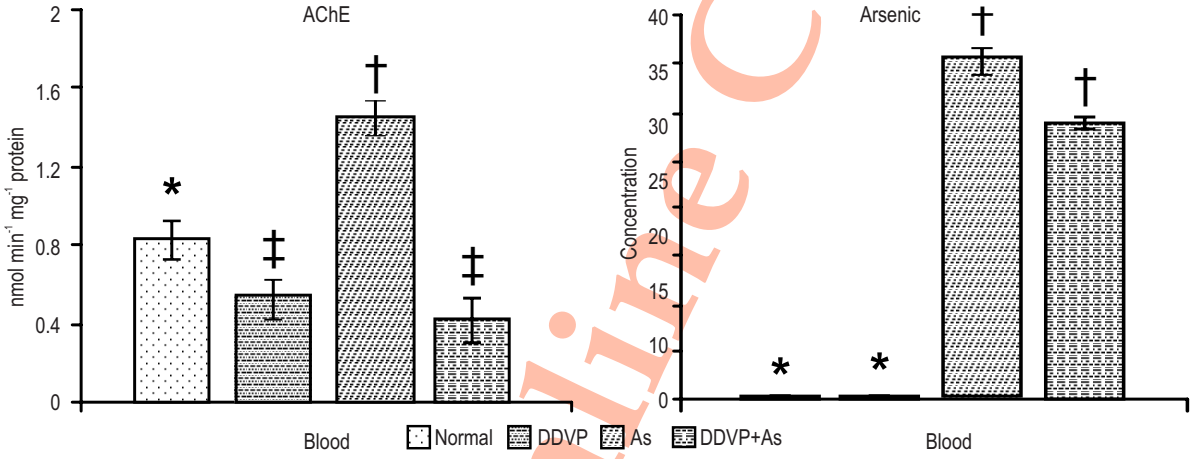

Fig. 5 : Effect of individual and combined exposure to DDVP and arsenic on Acetylcholinesterase activity (AChE) activity and arsenic content in erythrocytes. Values are mean of 6 replicates ± SE; *, †, ‡ Differences between values with matching symbol notations within each bar are not statistically significant at 5% probability level

and protects cell against oxidative damage. In the present study, GPx, GR and GST activities increased significantly during individual and co-exposure to arsenic and DDVP, indicating that GSH was efficiently converted to GSSG and vice-versa. However, GST activity also increased significantly in arsenic alone and in co-exposed group suggesting protection against reactive oxygen species (Mishra *et al.,* 2008, Celik and Isik 2009). AChE activity decreased on DDVP exposure while increased on arsenic exposure as compared to control group (Fig. 5). Interestingly, following co-exposure the effects were similar as observed in DDVP alone exposed rats. Acetylcholinesterase is a known biomarker of organophosphate toxicity and it phosphorylates serine residue present at the active site of AChE, thus inhibiting this enzyme and resulting in accumulation of Ach, that produces hyperstimulation of cholinergic effects (Bainy *et al.,* 2006). In the present study, AChE activity was significantly inhibited by DDVP alone (0.54±0.16) and during arsenic + DDVP co-exposure (0.43±0.12) compared to control (0.83±0.12). Interestingly, arsenic exposure led to a pronounced increase (1.45±0.20) in AChE activity which may be attributed to the fact that metals might interact with acetylcholine receptors affecting their binding efficiency leading to an increased AChE synthesis (Bainy *et al.,* 2006, Dwivedi *et al.,* 2010). This observation might be attributed to arsenic concentration which was less pronounced (not significant) in DDVP + As co-exposed animals (28.8±0.59) as compared to arsenic exposed (35.2±1.8) (Fig. 5).

In conclusion, the present study depicts interesting observation between arsenic and DDVP exposure on erythrocytes, suggesting increased lipid peroxidation mediated by excessive generation of reactive oxygen species (ROS) and simultaneous protection by antioxidant enzymes in response to free radicals generated during exposure of these toxicants. However, the study needs further exploration by exploring dose and duration related effects to evaluate the possible mechanism.

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