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RESEARCH ARTICLE

Oxidative stress responses in Wistar rats on subacute exposure to pharmaceutical wastewater

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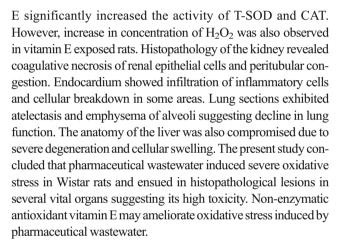Muhammad Furqan Akhtar[1] · Bushra Akhtar[3] · Ammara Saleem[4]

Abstract Water pollution has been a major concern for agrarian societies like Pakistan. Pharmaceutical industries are amongst the foremost contributor to industrial waste. Present study addresses the generation of oxidative stress caused by 2 months exposure to pharmaceutical wastewater in rats and their response to oral treatment with vitamin E, a potent antioxidant. The rats were randomized into five groups ($n = 5$) named as negative control, pharmaceutical wastewater (PEW) 100 %, PEW 10 %, PEW 1 %, and PEW 100 % + vitamin E. Oxidative damage in rats was evaluated by estimation of the activities of total superoxide dismutase (T-SOD), catalase (CAT), and the concentration of hydrogen peroxide (H_2O_2) in the liver, kidney, and blood/plasma. Exposure to pharmaceutical wastewater significantly decreased the activities of T-SOD and CAT and concentration of H_2O_2 in the liver and kidney and blood/plasma. Exposure to 100 % pharmaceutical wastewater exhibited a maximum decline in T-SOD activity, and activity was reduced to only 63.57 U/mL, 32.65, and 43.57 U/mg of protein in the plasma, kidney, and liver, respectively. Exposure to wastewater minimized activity CAT to 89.25 U/g of hemoglobin, 54.36, and 62.95 U/mg of protein in the blood, kidney, and liver, respectively. Treatment with vitamin E significantly increased the activity of T-SOD and CAT. However, increase in concentration of H_2O_2 was also observed in vitamin E exposed rats. Histopathology of the kidney revealed coagulative necrosis of renal epithelial cells and peritubular congestion. Endocardium showed infiltration of inflammatory cells and cellular breakdown in some areas. Lung sections exhibited atelectasis and emphysema of alveoli suggesting decline in lung function. The anatomy of the liver was also compromised due to severe degeneration and cellular swelling. The present study concluded that pharmaceutical wastewater induced severe oxidative stress in Wistar rats and ensued in histopathological lesions in several vital organs suggesting its high toxicity. Non-enzymatic antioxidant vitamin E may ameliorate oxidative stress induced by pharmaceutical wastewater.

Keywords Oxidative stress · Pharmaceutical wastewater · Total superoxide dismutase · Catalase

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Introduction

Hundreds of tons of pharmaceuticals are released into the environment either as such or as their metabolites. Most of pharmaceuticals have been reported at trace levels (hundreds of nanograms per liter) in the rivers and lake water. It indirectly substantiates capability to persist in surface waters. The xenobiotic nature of pharmaceuticals would suggest to impede their release to the environment by confining the sources of pollution (Andreozzi et al. 2004) (Andreozzi et al. 2002).

Toxic heavy metals such as cadmium, arsenic, mercury, and lead have been reported to occur in environment. Humans are exposed to these metals through different sources such as contaminated air, food, water, and soil. Transition metals play a role of catalyst in oxidative reactions of biological macromolecules so toxicity induced by heavy metals

might be due to oxidative damage. Iron, copper, and chromium undergoes redox recycling known as redox active metals whereas lead, cadmium, and mercury depletes major antioxidants especially thiol-containing antioxidants and enzymes (Ercal et al. 2001). It has been found that redox metals like iron (Fe), copper (Cu), and chromium (Cr) including some other metals have the property to produce reactive radicals such as super oxide anion radical and nitric oxide in biological systems (Jomova and Valko 2011).

Disturbance of metal ion homeostasis can lead to oxidative stress, which is characterized by generation of reactive oxygen species (ROS) which can overcome body antioxidant potential and results in damage to DNA, lipid peroxidation, protein modifications, and other effects which are indicative to certain diseases which include cardiovascular disease, diabetes, atherosclerosis, neurological disorders (Alzheimer's disease, Parkinson's disease), and chronic inflammation. The mechanism behind all these diseases is formation of superoxide radical, hydroxyl radical, and other ROS leading to mutagenicity and carcinogenicity due to malondialdehyde (MDA), 4-hydroxynoneal (HNE) and exocyclic DNA adducts (Jomova and Valko 2011). It is also found that metals owing to their toxicity interact with DNA and proteins leading to oxidative worsening of biological macromolecules. This process of breakdown of metal ions leads to a number of diseases (Mates 2000; Valko et al. 2006). A number of low molecular weight antioxidants, ascorbic acid (vitamin C), alpha tocopherol (vitamin E), glutathione (GSH), carotenoids, and flavonoids have the capacity of metal ions chelation reducing their catalytic activity to form ROS (Jomova and Valko 2011). It was suggested that biochemical markers used for hazard identification is the most suitable procedure. These biochemical markers have the potential of characterizing the hazardous substance under study and are considered a flexible approach in terms of using other tools (Jemec et al. 2010).

It has been determined earlier in our study that pharmaceutical wastewater (PEW) is a complex mixture of heavy metals and environmental contaminants (Sharif et al. 2016). In continuation of the study, oxidative stress and histopathological studies were conducted to validate that DNA damage caused by heavy metals, and environmental contaminants had also been associated with oxidative stress induction and toxicity to various organs. Furthermore, ameliorating effect of vitamin E on oxidative stress induction has also been investigated in the present study.

Material and method

Water sampling and characterization

PEW samples were collected from different pharmaceutical industries of Lahore in sterile glass bottles. Metal contents of the samples were determined by atomic absorption spectrophotometer (AAS), and gas chromatogram mass spectrophotometer (GC-MS) was used for screening of the organic environmental pollutants (Sharif et al. 2016).

Animals grouping

Experimental animals, 90-day-old male Wistar rats (130–150 g), were purchased from Department of Theriogenology, University of Veterinary and animal sciences (UVAS) Lahore. The rats were housed independently in stainless steel wire netting cages, air-conditioned room (temperature 21–25 °C and relative humidity was 50–60 %). All the animals were acclimatized for 10 days before the experiment start and were kept in 12 h day/light cycle.

Vitamin E (D-α-Tocopherol-polyethylene glycol 1000 succinate, water-soluble form) was obtained from Sigma-Aldrich. It was given orally as 100 mg/kg of body weight daily for 60 days. (El-Demerdash et al. 2004). The rats were randomized into five groups with five rats in each group and named as negative control, PEW 100 %, PEW 10 %, PEW 1 %, and PEW 100 % + vitamin E, respectively. The negative control group was provided with tap water, PEW 100 % group received pure pharmaceutical wastewater, PEW 10 % received 10 % pharmaceutical water, and PEW 1 % group was given 1 % of pharmaceutical wastewater. All the dilutions were made with the same water used for negative control. PEW 100 % + vitamin E group received pure pharmaceutical wastewater and vitamin E as an antioxidant. All the rats received treatment for 60 days. Rats were provided with same rat chow ad libtium (Banos et al. 2005).

Ethical consideration

All the experimental protocols were performed in compliance with Institutional Guidelines for the Care and Use of Laboratory Animals, UVAS, Lahore, Pakistan.

In vivo collection of samples

Wistar rats were fasted for 12 h, anesthetized (with diethyl ether), and were fixed on the experimental desk. Two to four milliliter blood was collected with the help of a catheter which was inserted into a celiac artery. The blood was centrifuged at 1000 rpm for 10 min at 4 °C. The plasma as top yellow layer was carefully removed and store on an ice pack for further analysis.

Removal of the liver and kidney

One gram of the kidney and liver tissues were taken, washed with cold normal saline, and dried with filter paper. These tissues were further minced and ground to prepare 10 % tissue homogenates according to the previous method (Akhtar et al.

2016a). One milliliter of supernatant was diluted with 9 mL normal saline to prepare 1 % tissue homogenate.

Measurement of protein content

Concentration of proteins in tissue was measured by Lowry's method of protein determination (Lowry et al. 1951). Bovine serum albumin was used as standard.

Measurement of SOD activity

Total SOD (T-SOD) activity was determined using SOD assay kit A001 (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China) (Ji et al. 1991). Superoxide anion free radical was generated in reaction system which comprised of xanthine and xanthine oxidase, and the scavenging activity of superoxide anion free radical was measured at 550 nm with glass cuvette. T-SOD activity of the plasma and tissues (liver and kidney) were expressed in units per milliliter and units per milligram protein, respectively (Luo et al. 2014).

Estimation of catalase activity

The activity of CAT was measured using a commercial kit A007–2 (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China). CAT activity was estimated by directly measuring the substrate H_2O_2 which decreased directly as the CAT level decreases in solution mixture. It was expressed as units per milliliter of blood and units per milligram of protein for the blood and tissue, respectively (Zhang et al. 2008).

Concentration of H_2O_2

The concentration of H_2O_2 was determined using a commercial kit A064 (Institute of Biological Engineering of Nanjing Jincheng, Nanjing, China). H_2O_2 forms a complex with molybdate, and OD value at 405 nm is directly associated with hydrogen peroxide in test sample. It was expressed as millimole per liter.

Histopathology

The rats were starved for 12 h and then sacrificed by decapitation by following the experimental protocol in compliance with the Institutional Guidelines for Care and Use of Laboratory Animals of University of Veterinary and Animal Sciences, Lahore, Pakistan DR/ 459. The liver and kidney were carefully removed and weighed. Any gross lesions observed were recorded. The tissues were fixed in 10 % PFA (formaldehyde solution) and processed for histological investigation. The tissues were embedded in paraffin wax, sectioned for 2 μm thickness, mounted on slides, and were stained with hematoxylin and eosin (H&E) for routine

light microscopy. The slides were investigated for any deleterious effects or appearance of lesions appeared after treatment with PEW (Ejaz et al. 2009).

Results

Chemical analysis

The results of chemical analysis of pharmaceutical wastewater revealed higher levels of Fe 4.96 mg L^{-1}. Similarly, Cr concentration was found to be 0.43 mg L^{-1}. Lead (Pb) was also present in higher than the normal permissible limits 0.21 mg L^{-1}. Arsenic (As) concentration of wastewater was found to be 0.83 mg L^{-1}. Cadmium (Cd) was also present in the sample, and its concentration was 0.02 mg L^{-1}. All these metals were present in concentrations higher than normal permissible limits of Environmental Protection Agency. Organic pollutants were also detected in the sample pharmaceutical wastewater using GC-MS. It was found that the wastewater was polluted with a number of organic pollutants including prednisolone, phenolic compounds, trimethoprim, and tolune (Sharif et al. 2016).

Estimation of T-SOD activity

T-SOD activity in plasma of control group was 92.54 ± 0.19 U/mL. The activity of T-SOD in PEW 100 % group was 63.7 ± 0.19 U/mL. It was found that T-SOD activity decreased significantly when rats were exposed to PEW 100 % orally (p 0.05). Administration of vitamin E helped in alleviating the decreased activity of T-SOD. It was found that the group PEW 100 % + vitamin E exhibited marked improvement in T-SOD activity (70.65 ± 2.44 U/mL) when compared to group PEW 100 % group (Table 1).

The activity of T-SOD in the control group of kidney homogenate was 63.75 ± 0.15 U/mg of protein. T-SOD exhibited a decreased activity in PEW 100 % group (32.65 ± 0.77 U/ mg) of protein. The results were significantly different from control group (p 0.05). The group receiving PEW 100 % + vitamin E exhibited a significant increase in activity (43.07 ± 0.29 U/mg) of protein. These results were significantly difference (p 0.0.5) when compared with 100 %PEW group (Table 2).

Liver homogenates of group PEW 100 % exhibited T-SOD activity (43.57 ± 0.23 U/mg) of protein which is less than control group (57.91 ± 0.29 U/mg) of protein. The results were significantly different (p 0.05). However, treatment with vitamin E moderately improved the activity of T-SOD

Table 1 Effect of vitamin E on different oxidative changes in rat plasma/blood caused by chronic exposure to PEW at different levels of concentration

Treatments	T-SOD activity (U/mL)	CAT activity (U/g of Hb)	H$_2$O$_2$ concentration (mmol/L)
Control	92.54 ± 0.19	146.5 ± 1.32	220.6 ± 2.67
PEW 100 %	63.57 ± 0.19[a]	89.25 ± 2.03[a]	68.80 ± 2.67[a]
PEW 10 %	80.17 ± 0.38[a]	128.2 ± 0.78[a]	107.3 ± 2.67[a]
PEW1%	91.78 ± 0.38	141.9 ± 0.79	182.1 ± 3.5[a]
PEW 100 % + vitamin E	70.65 ± 2.44[a, b]	100.1 ± 0.77[a, b]	129.5 ± 2.67[a, b]

SOD, CAT, and H$_2$O$_2$ were measured after 60 days of oral exposure of PEW, followed by administration of vitamin E. Values are mean ± standard error ($n = 5$)

PEW pharmaceutical effluent wastewater, *SOD* superoxide dismutase, *CAT* catalase *H$_2$O$_2$* hydrogen peroxide

[a] p 0.05 versus control

[b] p 0.05100 % PEW versus 100 % PEW + vitamin E (analysis of variance followed by Tukey's test)

(47.36 ± 0.29 U/mg) of protein (p 0.05) as compared to PEW 100 % group (Table 3).

Estimation of catalase activity

The activity of CAT in blood of control group was 146.5 ± 1.32 U/g of hemoglobin. PEW 100 % group exhibited activity 89.25 ± 2.03 U/mg of hemoglobin which is statically different from control group (p 0.05). PEW 100 % + vitamin E increased the activity of catalase in blood (100.1 ± 0.77) which was found to be statically significant (p 0.05) when compared with PEW 100 % (Table 1).

Activity of catalase in control and PEW 100 % groups of the kidney were 130.7 ± 1.75 and 54.36 ± 1.71 U/mg of protein, respectively. Treatment with vitamin E increased the activity of catalase in the kidney (68.11 ± 4.55 U/mg) of protein. The results were found to be significantly different (p 0.05) when compared with PEW 100 % group (Table 2).

Liver homogenate of control and PEW 100 % group showed catalase activity of 135 ± 2.95 and 62.95 ± 2.82 U/mg of protein respectively. Catalase activity in PEW 100 % + vitamin E group was significantly different 72.75 ± 3.26 U/mg of protein (p 0.05) when compared with both groups mentioned above (Table 3).

Concentration of H$_2$O$_2$

It was found that the plasma concentration of H$_2$O$_2$ in control group rats was found to be 220.6 ± 2.67 mmol/L, in group PEW 100 % was 68.80 ± 2.67 mmol/L, and in group PEW 100 % + vitamin E was 129.5 ± 2.67 mmol/L. H$_2$O$_2$ concentration in PEW 100 % + vitamin E group differed significantly (p 0.05) when compared with all other groups (Table 1).

Results of kidney homogenate showed that group which received PEW 100 % + vitamin E exhibited higher concentration of H$_2$O$_2$ (279.3 ± 1.75 mmol/L) when compared with group which received PEW 100 %. Results also exhibited that concentration of H$_2$O$_2$ differed significantly (p 0.05) when compared with all other groups (Table 2).

H$_2$O$_2$ concentration was also measured in liver homogenates of all groups. PEW 100 % group exhibited 162.9 ± 2.67 mmol/L which was increased to 267.1 ± 1.75 mmol/L in group PEW 100 % which also received vitamin E. The results were significantly different when compared with control group (Table 3).

Elevated concentrations of H$_2$O$_2$ significantly tapered by vitamin E in the plasma, liver, and kidney. Changes induced by PEW were ameliorated by vitamin E (p 0.05).

Table 2 Effect of vitamin E on different oxidative changes in rat kidney caused by chronic exposure to PEW at different levels of concentration

Treatments	SOD activity (U/mg of protein)	CAT activity (U/mg of protein)	H$_2$O$_2$ concentration (mmol/L)
Control	65.75 ± 0.15	130.7 ± 1.75	402.7 ± 2.67
PEW 100 %	32.65 ± 0.77[a]	54.36 ± 1.71[a]	243.3 ± 5.11[a]
PEW 10 %	57.54 ± 2.53[a]	73.28 ± 4.56[a]	330.9 ± 3.5[a]
PEW 1 %	64.95 ± 0.6	116.7 ± 3.49	397.6 ± 1.75
PEW 100 % + vitamin E	43.07 ± 0.29[a, b]	68.11 ± 4.55[a]	279.3 ± 1.75[a, b]

SOD, CAT, and H$_2$O$_2$ were measured after 60 days of oral exposure of PEW, followed by administration of vitamin E. Values are mean ± standard error ($n = 5$)

PEW pharmaceutical effluent wastewater, *SOD* superoxide dismutase, *CAT* catalase, *H$_2$O$_2$* hydrogen peroxide
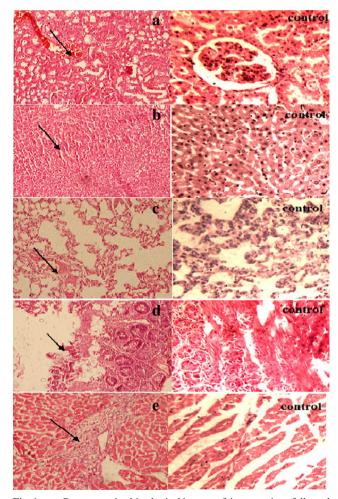
[a] p 0.05 versus control

[b] p 0.05100 % PEW versus 100 % PEW+ vitamin E (analysis of variance followed by Tukey's test)

Table 3 Effect of vitamin E on different oxidative changes in rat liver caused by chronic exposure to PEW at different levels of concentration

Treatments	SOD activity (U/mg of protein)	CAT activity (U/mg of protein)	H_2O_2 concentration (mmol/L)
Control	57.91 ± 0.29	135 ± 2.95	294.3 ± 2.72
PEW 100 %	43.57 ± 0.23^a	62.95 ± 2.82^a	162.9 ± 2.67^a
PEW 10 %	51.70 ± 1.14^a	87.51 ± 2.84^a	210.5 ± 2.67^a
PEW 1 %	57.80 ± 0.29	89.72 ± 1.65^a	236.8 ± 8.76^a
PEW 100 % + vitamin E	$47.36 \pm 0.29^{a, b}$	72.75 ± 3.26^a	267.1 ± 1.75^a

SOD, CAT, and H_2O_2 were measured after 60 days of oral exposure of PEW, followed by administration of vitamin E. Values are mean $\pm$ standard error ($n = 5$)

PEW pharmaceutical effluent wastewater, *SOD* superoxide dismutase, *CAT* catalase, H_2O_2 hydrogen peroxide

[a] p 0.05 versus control

[b] p 0.05100 % PEW versus 100 % PEW+ vitamin E (analysis of variance followed by Tukey's test)

Histopathology

Histopathological lesions were observed in Wistar rats after 60 days exposure to PEW 100 %. Coagulative necrosis of renal epithelial cells and widespread disintegration of tubular cells were observed. Some tubular cells exhibited cellular swelling, and peritubular congestion was also observed. The anatomy of the liver was also compromised due to severe degeneration and cellular swelling. The hepatocytes of hepatic cord also indicated coagulative necrosis. The histopathological evaluation of lung sections exhibited atelectasis and emphysema of alveoli suggesting decline in lung function. Pharmaceutical wastewater adversely affected the intestinal epithelial cells, leading to their degeneration and sloughing off. Exposure to pharmaceutical wastewater also adversely affected histology of heart. Endocardium showed infiltration of inflammatory cells and cellular breakdown in some areas (Fig. 1). No significant changes were found in the brain (cerebellum and cerebrum). There were no changes in cell bodies. Microglial cells were intact, and no obvious pathological changes were observed.

Discussion

In the present study, role of pharmaceutical wastewater was investigated for induction of oxidative stress in Wistar rats. Role of vitamin E role in ameliorating oxidative stress in the kidney, liver, and blood/plasma was investigated after an exposure for 60 days. The liver and kidneys were selected because these are considered to be the major target of toxicity. The liver-containing metabolizing enzymes have the ability to biotransform the xenobiotics into less toxic or active metabolite. The liver has been a site for lipid peroxidation so considered a hall mark for the oxidative stress tests. The kidneys do perform same activities along with filtration (Patlolla et al. 2009).

Fig. 1 a–e Representative histological images of tissue sections followed oral exposure of PEW for 60 days. **a** Kidney section of rat highlighting necrosis of cells along with control group showing intact glomerulus. **b** Liver section of rat highlighting degeneration and swelling of hepatocytes along with control **c** Lungs section of rat representing atekation and emphysema of alveoli **d** Intestine section of rat highlighting mild degeneration and sloughing of intestinal epithelial cells **e** Heart section of rat highlighting break down of cells in endocardium and presence of some inflammatory cells

Heavy metals whether redox active or redox inactive might have the potential of increasing the production of reactive oxygen species (ROS) e.g., hydroxyl (OH$^{\cdot}$), superoxide (O$_2^{-}$) radicals, or H$_2$O$_2$. Generation of ROS in a large quantity overwhelms the intrinsic antioxidant defenses resulting in condition known as oxidative stress. Cells under oxidative stress display various anomalies which might be caused by interaction of ROS with lipids, proteins, and DNA (Ercal et al. 2001).

It was reported that K$_2$Cr$_2$O$_7$ depressed the activities of SOD, CAT, and GSH-Px in rat's epithelial intestinal cell (Sengupta et al. 1990). It was later confirmed that chromium (VI) decreased the enzymatic antioxidant levels which included SOD and CAT (Susa et al. 1996). Our study is in accordance with a previous investigation which reported that a 60 days exposure of textile wastewater ensued induction of oxidative stress in Wistar rats. However, treatment with vitamin C exhibited a decline in wastewater induced oxidative damage (Akhtar et al. 2016a). Normally enzymatic and non-enzymatic antioxidant systems exist in body. Overproduction of free radicals or diminished scavenging capacity of enzymes or both mechanisms has been involved in lipid peroxidation which is a chain process, initial oxidation of few molecules leads to massive tissue destruction (Zaidi and Banu 2004). Several diseases of the central nervous system and cardiovascular disorders have been linked to reactive oxygen species and the inhibition of antioxidant defense mechanism (Smythies 1999).

Pb has also been associated with oxidative stress. Lead nitrate decreased the activities of SOD, CAT, and GSH and resultant tissues (liver and kidneys) were exposed to per oxidative damage. Both of these enzymes are metalloproteins which perform their function by detoxifying peroxides ($^{-}$OOH), H$_2$O$_2$, and O$_2^{*}$ (Lakshmi et al. 2013). Pb toxicity has also been found to be multifactorial. Possible mechanisms associated with Pb induced oxidative stress include enzyme inhibition and a decline in mineral absorption, and it reduced binding with sulfhydryl groups. As has also been linked with oxidative damage in tissues through generation of ROS e.g., superoxide, hydroxyl, and peroxyl radicals. Decreased activity of SOD and CAT in the liver of rats and increased MDA levels were suggested to be the possible mechanisms of arsenic induced oxidative injury (Santra et al. 1999), (Xu et al. 2013). Present study suggests that the occurrence of excess amounts of metals such as Pb, As, and Cr in pharmaceutical wastewater contributed to the oxidative potential of this wastewater.

Oxidative stress has been associated with redox imbalance in various cancer cells when compared with normal cells. This redox imbalance might be attributed to oncogenic stimulation. Mutation of DNA is linked with lesions associated with DNA which have been appeared in different tumors showing the involvement of oxidative stress in etiology of cancer (Akhtar et al. 2016b).

Pharmaceutical residues, sex steroid hormones, and personal care products have been categorized as new emerging pollutants. Prednisolone was detected in the pharmaceutical wastewater. Steroids have been associated with deterioration of endocrine and reproductive function. Studies have proposed increased risk of breast, prostate, and testicular carcinomas on exposure to endocrine disrupting substances like prednisolone (Kudłak and Namieśnik 2008). Phenolic compounds have been identified as potent environmental pollutants. Pharmaceutical, petroleum, and chemical industries have contributed in the discharge of a large amount phenolic compounds. Phenolic compounds led to the generation of free radicals in organs like the liver leading to the generation of phenoxy radicals and intermediate metabolites resulting in generation of superoxide radicals and hydrogen peroxide (Michałowicz and Duda 2007). Toluene has been found associated with the increase in formation of reactive oxygen species, and decrease in oxidative stress markers like SOD, CAT, and glutathione S-transferase when different concentrations of toluene were exposed to *Drosophila melanogaster* was exposed to different concentrations of toluene (Singh et al. 2009).

Non-enzymatic antioxidants such as vitamin A, C, and E are effective antioxidants and had a potential to mitigate oxidative stress induced injuries (Olas and Wachowicz 2002) (Chaudiere and Ferrari-Iliou 1999). Several studies have reported that vitamin E therapy mitigated oxidative damage in the liver and kidney (Onyema et al. 2006), (Rao et al. 2006). This study is in agreement with previous investigations that vitamin E do play some role in relieving oxidative stress and improving the biomarkers related to stress. Although ameliorating effects of vitamin E on the activities of SOD and CAT were observed, yet H$_2$O$_2$ concentration in control group was higher than those of other groups. Although increase in SOD activity leads to an increase production of H$_2$O$_2$. However, some heavy metals like arsenic have an interesting pathway of increasing the production of H$_2$O$_2$ via mechanisms other than SOD induction which is explicated by oxidation of As (III) to As (V). High level of H$_2$O$_2$ causes induction of CAT activity. It is suggested that under physiological conditions arsenic can directly produce H$_2$O$_2$. These heavy metals also cause the production of free radicals usually through reactions independent of CAT activity such Fenton's reactions which further affects H$_2$O$_2$ concentration in the liver and kidney tissues (Jomova and Valko 2011; Jurczuk et al. 2004).

Environmental metals like lead, chromium, and cadmium are known hepatotoxic agents (Cui et al. 2004), (Mousa 2004), (Ramm 2005). They have a potential to cause chronic renal diseases (Shaikh et al. 1999), (Barbier et al. 2005). It was found that a mixture of heavy metals can induce oxidative stress in the liver, kidney, brain, and erythrocytes. The mechanism involved might be lipid peroxidation along with decreasing ability of antioxidative defense systems in male rat (Jadhav et al. 2007a), (Jadhav et al. 2006). The final

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