Indian J. Anim. Res., 50 (2) 2016:168-174
Print ISSN:0367-6722 / Online ISSN:0976-0555

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
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Evaluation of oxidative stress in rats (*Rattus rattus*) inhabiting Bathinda region of Punjab, India

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Received: 29-02-2014 Accepted: 23-10-2014 DOI:10.18805/ijar.6704

ABSTRACT

Exposure to various toxic chemicals has become an increasingly recognized source of illness in human and animals, worldwide. The present study was designed to evaluate the oxidative stress in *Rattus rattus* inhabiting Bathinda region of Punjab. Punjab Agricultural University, Ludhiana campus rats served as control rats. *Rattus rattus* were brought to laboratory and separated according to sex. The animals were dissected, vital organ excised and weighed and blood and tissues were processed for histological and biochemical studies. Relative weights of liver, heart and kidney were observed to be higher in rats collected from Bathinda as compared to the control rats. Light microscopic observations of liver and kidney sections of Bathinda rats showed many histological changes. Levels of total proteins were found to be lower in blood, liver, kidney and brain of rats collected from Bathinda region. Activities of antioxidant enzymes namely catalase, glutathione peroxidase and glutathione transferase were reduced ($p<0.05$) and superoxide dismutase and glutathione reductase showed significant increase and the products of oxidation namely, malondialdehyde were significantly increased ($p<0.05$) in liver, kidney, brain and blood of rats collected from Bathinda district as compared to control rats. The results obtained indicate that environmental contaminants might be responsible for altering antioxidant defense system and inducing oxidative stress in rats inhabiting south- west region of Punjab.

Key words: Catalase, Glutathione peroxidase, Glutathione reductase, Lipid peroxidation, Proteins, Superoxide dismutase.

INTRODUCTION

In recent years, there has been growing concern regarding the adverse effects of various environmental contaminants on human health. With the advent of industrialisation, economic development and urbanisation, drastic changes have occurred in the lifestyle and surroundings of humans that have resulted in the extensive production and use of beneficial substances (Mathur and Cynthia 2011). As a result, many potentially hazardous chemicals have been released into the environment at an alarming rate and their exposure to both humans and wildlife has become inevitable. These chemicals that have been released into the environment are a leading causative factor in the high incidence of various pathological conditions, including cancers (Mathur and Cynthia 2011).

Pesticides have become integral part of agriculture in Punjab. Bathinda district in Punjab, an important belt of the country, irrigated by canal water, grows largely cotton and rice crops, the two crops known for excessive use of pesticides (Puri *et al.* 1999). Agrochemical processes in the waterlogged agricultural area with calcareous soil and use of phosphate fertilizers are favoured sources for deterioration of ground water quality in Bathinda districts (Bhalla *et al.* 2011). The consequences of high use of pesticides on the crops and indirectly in the environment has a wide spectrum of toxicological effects on the nervous, immune, cardiovascular, respiratory and reproductive systems in the humans and animals and even cancers that are mainly related to pesticides in creating oxidative stress (Mostafalou and Abdollahi 2012).

Oxidative stress induce diverse pathological conditions varying from aging to Parkinson's disease due to the surplus release of reactive oxygen species (Raina *et al.* 2009). The mammalian body has endogenous enzymatic defenses to fight oxidative stress such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-transferase (GST) and non-enzymatic components like reduced glutathione (GSH) (Raina *et al.* 2009). Therefore, the present study was undertaken to evaluate the oxidative stress in house rats inhabiting Bathinda region of Punjab, India.

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MATERIALS AND METHODS

House rats (*Rattus rattus)* were collected from Bathinda district using multicatch traps and those collected from Punjab Agricultural University campus, Ludhiana served as control rats. They were brought to laboratory and separated according to sex. The rats were mildly anaesthetized using chloroform and blood samples were collected directly from the heart in heparinised vials. The blood samples were centrifuged at 2300 rpm for 15 minutes to obtain erythrocytic pellets which settle as sediment. The pellets were washed with normal saline solution thrice and 10% haemolysate was prepared for biochemical studies.

Organ weights: After collection of blood, the animals were dissected and vital organs viz; brain, liver, spleen, stomach, heart, lungs and kidney and endocrine glands viz; adrenal, thyroid, parathyroid were excised, cleaned off the adhering tissue and weighed separately and observed for morphological data.

Histological studies: For histopathological studies, liver and kidney of *Rattus rattus* were fixed in bouin's fixative for 24 hours. After complete fixation, the tissue was dehydrated in graded series of ethanol, cleared in xylene and embedded in paraffin wax (Melting point between 58-60°C). The 5-7µm thick sections were cut with the help of microtome and after usual de-waxing and rehydration in descending series of ethanol to water, the sections were stained in haematoxylin, counterstained with eosin, dehydrated in ascending ethanol series, cleared in xylene and mounted in DPX.

Biochemical studies: For biochemical studies, 0.5 g of liver, brain and kidney were homogenised in 2 ml of phosphate buffer saline (PBS) 0.1M, pH 7.4 and centrifuged. Supernatant was used for estimation of proteins by method of Lowry *et al.* (1951). The activity of glutathione peroxidase, superoxide dismutase, catalase, glutathione reductase and glutathione-S-transferase in erythrocyte lysate/tissue homogenate was assayed by the method of Hafeman et al. (1984), Marklund and Marklund (1974), Aebi (1983), Carlberg and Mannervik (1985) and Habig *et al.* (1974) respectively. Lipid peroxidation in erythrocytes was assayed by the method of Stocks and Dormandy (1971).

Statistical analyses: All statistical comparisons for organ weight and biochemical analyses were presented as the mean ± standard error of mean (S.E.M). Comparisons were made between control and Bathinda rats belonging to different sex on computer using t-test. A "P" value of 0.05 was selected as a criterion for statistically significant differences.

RESULTS AND DISCUSSION

A slight increase in weight of all the vital organs of male house rats collected from the Bathinda region was observed when compared with their control rats (Table 1). A significant increase in weights of liver and lungs in male rats and brain in female rats were observed in rats collected from Bathinda district as compared to control rats. Weight of all other vital organs *viz;* liver, spleen, heart, lungs, brain, stomach and kidneys in females was comparable with their control counterparts (Table 1).

No significant difference was observed in weight of thyroid and parathyroid glands in male and female rats when compared with their respective control rats. Significant increase in weight of adrenals was observed in rats collected from Bathinda district as compared to control rats (Table 1).

Liver and kidneys are important for metabolic and excretory processes and are therefore often regarded as indicator organs for toxic effects. The differences in liver and kidney weights are considered as sensitive risk parameters (Piao *et al.* 2013). Relative weights of liver, heart and kidney in male and female was observed to be higher in *Rattus rattus* collected from Bathinda as compared to control rats. Spleen and stomach showed decrease in weight of Bathinda rats as compared to control group. Organ weight and histological

Table 1: Relative weight of vital organs and endocrine glands (g/100 g bw) in male and female *Rattus rattus.*

	Male		Female	
Organs	**Control**	**Bathinda**	**Control**	**Bathinda**
Liver	3.25±0.29	3.92±0.50*	4.43±0.30	4.47±0.72
Kidney	0.44±0.02	0.48±0.03	0.41±0.01	0.47±0.02
Spleen	0.23±0.03	0.29±0.04	0.27±0.03	0.25±0.02
Heart	0.39±0.10	0.43±0.03	0.38±0.02	0.42±0.03
Lungs	0.38±0.05	0.48±0.04*	0.48±0.03	0.50±0.03
Stomach	2.38±0.17	2.55±0.68	2.05±0.10	2.03±0.75
Brain	1.38±0.10	1.43±0.11	1.45+0.09	1.63+0.07*
Thyroid	0.210±0.02	0.220±0.02	0.200±0.01	0.200±0.01
Parathyroid	0.024±0.00	0.021±0.00	0.018±0.00	0.017±0.01
Adrenal	0.023±0.01	0.029±0.01*	0.023±0.01	0.028±0.01*

Values are Mean ± SE,

*Significant difference at ($p \leq 0.05$) as compared to control

investigations were performed to detect the changes at the organ and cellular level between control and rats from Bathinda district. Organ weight can also be the most sensitive indicator of an effect of drug toxicity, as significant differences in organ weight between treated and control animals may occur in the absence of any morphological changes (Piao *et al*. 2013). Increased adrenal weight might reflect a state of physiological stress in the body of rats. Glucocorticoids released from adrenals might play an important role in mediation of depressed immune response (Gatterman *et al*. 2002).

The amount of proteins did not vary significantly in liver of male and female rats from Bathinda district and those from PAU which served as control rats. CAT activity was significantly reduced and the activity of GR and LPO levels were significantly increased in the liver of male rats inhabiting Bathinda district than the control rats (Table 2). GPx activity was also significantly reduced in female house rats collected from Bathinda district as compared to PAU rats. No significant difference in activity of enzymes, SOD, CAT, GR, GST and LPO levels observed in the liver of female rats (Table 2).

Increase in concentration of proteins and SOD levels in the kidney was observed in male rats collected from Bathinda district as compared to the control rats while the female rats showed a slight decrease in their levels. Non-significant decrease in concentration of GPx and CAT and increase in GR, GST, LPO levels was observed in rats trapped from Bathinda region when compared with the control male and female rats (Table 3).

Increase in concentration of proteins was observed in brain of male rats collected from Bathinda district as compared to control PAU rats while non-significant decrease in concentration of proteins and catalase activity was observed in brain of female rats trapped from Bathinda region when compared with the control rats. Glutathione peroxidase levels were also non-significantly lower in brain of all rats collected from Bathinda as compared to control male and female rats. SOD, GR, GST and LPO levels increased non-significantly in brain of male and female house rats that belong to Bathinda region as compared to control (Table 4).

Non-significant decrease in concentration of proteins and catalase activity was observed in haemolysate

Table 2: Liver enzymatic antioxidant parameters in male and female house rats.

	Male		Female	
	Control	**Bathinda**	**Control**	**Bathinda**
Protein	4.74±0.16	4.67±0.63	4.08+0.15	4.08+0.07
GPx	0.37±0.05	0.30±0.03	0.35±0.04	0.25±0.18*
SOD	11.32±0.21	11.24±3.37	13.59±0.21	11.89±3.43
CAT	40.59±0.13	37.11±3.06*	46.23±6.12	45.83±3.91
GR	0.06±0.01	0.14±0.01*	0.06±0.01	0.07±0.01
GST	0.32±0.01	0.36±0.04	0.42±0.02	0.42±0.07
LPO	11.02±0.60	16.06±0.63*	10.17±0.62	11.41±0.95

Values are Mean ± SE (n=10)

*Significant difference at ($p \leq 0.05$) as compared to control

Units: Proteins (mg/g tissue), GPx (U/mg protein), SOD (U/mg protein), CAT (µmole of H_2O_2 decomposed/min/mg protein), GR (µmoles of NADPH conjugate/ min/mg protein), GST (µmoles of GSH-CDNB conjugate formed/ min/mg protein), LPO (nM MDA/100 mg tissue).

Table 3: Kidney enzymatic antioxidant parameters of male and female house rats.

	Male		Female	
	Control	**Bathinda**	**Control**	**Bathinda**
Protein	4.16±0.60	4.97±0.81*	3.27±0.13	3.18±0.27
GPx	0.22±0.23	0.21±0.05	0.40±0.08	0.38±0.13
SOD	16.09±1.26	18.18±1.77	13.46±0.10	11.90±1.90
CAT	48.69±2.62	47.73±6.78	40.78±0.66	40.15±0.36
GR	0.07±0.01	0.08±0.01	0.08±0.02	0.09±0.02
GST	0.47±0.01	0.50±0.04	0.49±0.07	0.48±0.06
LPO	10.18±0.58	10.85±0.99	8.85±0.32	9.31±0.02

Values are Mean ± SE (n=10)

*Significant difference at ($p \leq 0.05$) as compared to control

Units: Proteins (mg/g tissue), GPx (U/mg protein), SOD (U/mg protein), CAT (µmole of H_2O_2 decomposed/min/mg protein), GR (µmoles of NADPH conjugate/ min/mg protein), GST (µmoles of GSH-CDNB conjugate formed/ min/mg protein), LPO (nM MDA/100 mg tissue).

Table 4: Brain enzymatic antioxidant parameters in male and female house rats.

	Male		Female	
	Control	**Bathinda**	**Control**	**Bathinda**
Protein	4.41±0.58	4.86±0.33*	4.34±0.24	4.12±0.02
GPx	0.20±0.10	0.18±0.03	0.29±0.01	0.26±0.04
SOD	14.93±1.92	15.58±2.57	11.80±0.85	14.97±3.58
CAT	49.00±3.39	49.20±6.13	40.65±2.49	40.59±2.31
GR	0.07±0.00	0.09±0.00	0.06±0.01	0.09±0.02
GST	0.41±0.08	0.43±0.03	0.49±0.02	0.49±0.05
LPO	9.82±0.49	10.20±0.68	8.06±0.12	8.22±0.21

Values are Mean ± SE (n=10)

*Significant difference at ($p \leq 0.05$) as compared to control

Units: Proteins (mg/g tissue), GPx (U/mg protein), SOD (U/mg protein), CAT (µmole of H_2O_2 decomposed/min/mg protein), GR (µmoles of NADPH conjugate/ min/mg protein), GST (µmoles of GSH-CDNB conjugate formed/ min/mg protein), LPO (nM MDA/100 mg tissue).

of male and female rats collected from Bathinda district as compared to control rats. GPx, GR and GST was found to be comparable while SOD activity showed a slight increase in both the male and female rats collected from Bathinda district as compared to PAU. LPO levels increased significantly in blood of male and female rats that belong to Bathinda region as compared to control (Table 5).

The amount of proteins decreased in liver, kidney, brain and blood of all the rats. Significant decrease in total protein content was reported in liver and kidney tissues of malathion treated rats compared with the control group by Ozden *et al.* (2009) and Othman (2011). Various oxidative stress parameters *viz.* GPx, SODs, catalase, GR and indicator of oxidative damage that is, lipid peroxidation were evaluated in all the rats. Significant increase in the value of superoxide dismutase, glutathione-S-transferase and lipid peroxidation was observed in liver and kidney, of Bathinda rats. However, reduction in the activity of glutathione peroxidase, catalase and glutathione reductase was observed in Bathinda rats as compared to control rats. Sodhi *et al.* (2008) also found decrease in the mean activity of glutathione peroxidase in

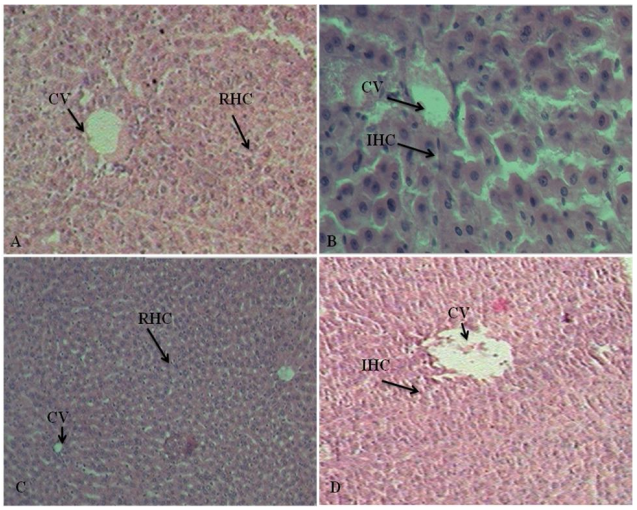

Fig 1: A and C: T.S. of liver of a control male and female *Rattus rattus* respectively showing radially arranged hepatic cords (RHC) with normal outlines of central vein (CV). Hepatocytes nuclei are round with dispersed chromatin and prominent nucleoli (X100). B and D: T.S. of liver of a male and female *Rattus rattus* respectively collected from Bathinda district showing slight loosening in arrangement of hepatic cords, irregularly arranged hepatic chords (IHC), infiltration of leucocytes and dilation in central vein (CV) (X400).

Table 5: Blood enzymatic antioxidant parameters of male and female house rats.

	Male		Female	
	Control	**Bathinda**	**Control**	**Bathinda**
Protein	31.09±0.01	30.76±0.06	31.9±2.15	30.28±2.68
GPx	0.03±0.00	0.02±0.01	0.02±0.001	0.02±0.01
SOD	4.03±0.19	4.20±0.97	3.05±0.19	4.80±0.97
CAT	12.12±1.20	12.11±3.21	11.54±0.95	10.73±3.55
GR	0.007±0.00	0.008±0.00	0.007±0.001	0.007±0.006
GST	0.009±0.00	0.01±0.00	0.009±0.001	0.008±0.002
LPO	120.8±0.44	210±0.43*	160.3±0.55	196.8±0.15*

Values are Mean ± SE (n=10)

*Significant difference at ($p \leq 0.05$) as compared to control

Units: Proteins (mg/g tissue), GPx (U/mg protein), SOD (U/mg protein), CAT (µmole of H_2O_2 decomposed/min/mg protein), GR (µmoles of NADPH conjugate/ min/mg protein), GST (µmoles of GSH-CDNB conjugate formed/ min/mg protein), LPO (nM MDA/100 ml tissue).

chicks after receiving malathion (10mg/kg bw) as compared to control. Superoxide dismutase (SODs) is class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide (Zelko *et al.* 2002).The increase in the activity of SOD indicates an increase in O_2Éproduction (Zhang *et al.* 2004, Tunçmen and Tüzmen 2007).This was in accordance with previous studies where Heikal *et al.* (2013) observed increase in the activity of SOD and a significant decrease in levels of glutathione peroxidase in liver of rats administered with Cyromazine, Chlorpyrifos and both as compared to control rats.

Catalase detoxifies the ROS/ H_2O_2, therefore it acts as an antioxidant enzyme (Mates 2000). Presence of catalase in erythrocytes may also help to protect somatic cells exposed to high levels of H_2O_2 during active inflammation (Agar *et al.* 1986).Glutathione reductase (GR) plays a vital role in cell's defense against reactive oxygen metabolites by sustaining the reduced status of an important antioxidant glutathione (Sung *et al.* 2007). This enzyme is essential for reduction of glutathione disulfide (GSSG) to the reduced form glutathione (GSH), necessary for protection of cells against oxidative stress (Tekman *et al.* 2008). Glutathione-S-transferase (GST) have been suggested to participate in a wide variety of bio-transformations, including xenobiotic detoxification, ligand binding, transport, as well as synthesis and modification of prostaglandins, leukotrienes and steroids. GST is a detoxifying enzyme that catalyzes the conjugation of a variety of electrophilic substrates to the thiol group of GSH, producing less toxic forms (El –Gendy *et al.* 2010).

Lipid peroxidation (LPO) is the process of oxidative degradation of polyunsaturated fatty acids (PUFA) and its occurrence in biological membranes causes impaired membrane function, structural integrity, decrease in membrane fluidity and inactivation of a several membrane bound enzymes. Increase in LPO levels was also observed by Tunçmen and Tüzmen (2007) in blood, brain, kidney and liver of rats fed with drinking water of Buyukkabaca. Free radicals like superoxide and peroxyl radicals formed as a result of excessive level of ethion in Buyukkabaca drinking water might be responsible for the increase in LPO and alteration in antioxidative defence mechanisms.

Light microscopic observation of the liver sections of the control rats showed a normal histological architecture with clearly outlined in the sections of anastomosing hepatocytes along with adjacent sinusoids radiating from the central veins towards the periphery of the liver lobules (Fig.1A). Normal outlines of the central vein (CV) were clearly visualized. Hepatocytes nuclei were round with dispersed chromatin and prominent nucleoli. Hepatocytes were organized into plates separated by vascular channels (sinusoids). No infiltration of leucocytes was observed in the sinusoids. Each lobule was bounded by scanty connective tissue (Fig 1A,C).

Liver of Bathinda male and female rats showed slight loosening in arrangement of hepatic cords around central vein (Fig.1B, D). Large vacuolization or empty spaces in all the rats were observed that were more prominent in male house rats as compared to the female rats. The loss of radial arrangements of hepatocytes was also seen in male rats. The dilation of CV and sinusoids between hepatocytes were also increased (Fig.1B) and showed infiltration of large mass of leucocytes inflammatory cells in CV and sinusoids. The dilation of central vein (CV) in all the female rats collected from Bathinda district was found to be higher as compared to the male *Rattus rattus* of the same region (Fig.1D).

Histopathological examination photomicrographs of the kidney sections in the control male and female *Rattus rattus* showed a well demarcated medulla and cortex with intact capsule and well-formed glomerular tuft (Fig.2A,C). Kidney sections of rats collected from Bathinda district showed variable changes in some parts of the urinary tubules and distension of glomerular capillaries in *Rattus rattus*. Sections of kidney showed swelling appearances, increasing urinary space, and inflammatory cell infiltration degeneration of glomeruli and associated tubules structure (Fig.2B, D).

Histopathological changes in liver and kidney were observed in accordance with our biochemical results,

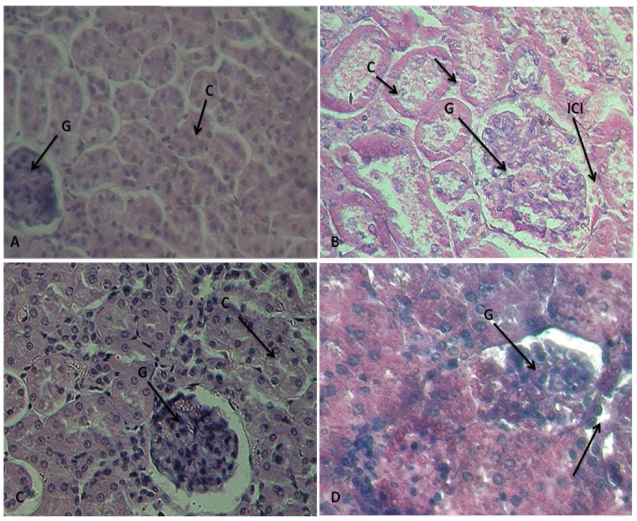

FIG 2: A and C: Photomicrograph of a T.S. of kidney of a control *Rattus rattus* male and female rat respectively showing intact capsule with well demarcated glomerular tuft (G) and normal arrangement of tubules in the cortical portions (C) (X400).B and D: Light micrograph of a T.S. of kidney of *Rattus rattus* male and female rat respectively collected from Bathinda region showing mild to moderate cellular swelling with mild inflammatory cell infiltration (ICI) and tubular degeneration (arrow) (X400).

showing minor damages which were not critical on animal health. Light microscopic observation of the liver sections of Bathinda region rats showed many histopathological changes as compared to control rats. The rats from Bathinda region had dilation of central veins and infiltration by large mass of leucocytic inflammatory cells in the blood vessels. Kidney sections of all the rats of Bathinda region showed variable pathological changes in glomeruli and some parts of the urinary tubules as compared to control rats. Heikal *et al.* (2013) reported histopathological alterations in liver of cyromazine and chlorpyrifos treated rats which include degeneration and coagulative necrosis in the hepatocytes, inflammatory cells infiltration and kupffer cells proliferation.

CONCLUSION

It is concluded that the biochemical evaluation resulted in the induction of lipid peroxidation (MDA), depletion in tissue protein and alteration in antioxidant enzymes, decreased glutathione peroxidase, catalase and glutathione reductase and increased superoxide dismutase and glutathione transferase). It is speculated that environmental contaminants may be the reason of inducing oxidative stress by increasing LPO levels and altering the antioxidant defense system in rats collected from Bathinda region.

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