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ORIGINAL ARTICLE

Evaluation of oxidative stress and reproductive potential in male rats inhabiting South West region of Punjab.

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ABSTRACT
The present study was envisaged to investigate the possible effect of environmental contaminants on the reproductive outcome in male *T. indica* and *B. bengalensis* inhabiting South-West region of Punjab in North India. A significant difference in organ weights between Bathinda rats and control rats was observed. Levels of total proteins were found to be decrease in all the rats collected from Bathinda region. Activity levels of OS parameters viz. CAT, SOD, GST, GR and GPx were differentially altered and the products of oxidation namely, malondialdehyde increased significantly in rats collected from Bathinda district. The decrease in sperm motility and concentration was observed in Bathinda rats of both the species. Abnormal sperms were significantly increased in Bathinda rats. The results obtained indicate that environmental contaminants for prolonged period are responsible for altering antioxidant defence system and inducing oxidative stress, a cause for pathophysiological changes in rats inhabiting south- west region of Punjab.
Keywords: Antioxidants, Environmental pollutants, Oxidative stress, Sperm concentration, Sperm motility

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INTRODUCTION

With the advent of industrialization, economic development and urbanization, 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).

Contamination of natural resources by indiscriminate and hysterical use of pesticides is potential threat to animal and human health. Pesticides are linked to various chronic diseases like cancers, infertility, kidney failure, reproductive problems and nervous disorders in male and female (Agarwal and Sharma 2010). South-Western region of Punjab is known for its high pesticide use and deteriorated ground water quality due to Agrochemical processes and extensive use of phosphate fertilizers (Bhalla *et al* 2011). Acute occupational exposure for pesticides among sprayers was also high as they occasionally use protective devices while spraying (Thakur *et al* 2008, Singh and Kaur 2012).

Pesticides have become integral part of agriculture in Punjab, India. Bathinda district in Punjab, an important belt of the country, irrigated by canal water, grows largely cotton and rice crop, 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 also favoured sources for deterioration of ground water quality in Bathinda districts (Bhalla *et al* 2011).

Exposure of pesticides lowers the sperms count levels below the limit leading to male infertility (Mathur *et al* 2010, Rani *et al* 2013). Several research studies have indicated that sperm counts have been in decline for decades and scientists say modern lifestyles and contacts with chemicals are a contributing factor (Thakur *et al* 2008, Singh and Kaur 2012). Pesticides have the potential to interfere with androgen action and affect the development and maturation of the reproductive tract in males and cause declination in semen quality (Jurewicz *et al* 2009). The increase in insecticide levels in the blood of vertebrates has been reported to cause reproductive dysfunction (Singh *et al* 2008).

Earlier studies give some indication of increased reproductive risks of exposure to pesticide/heavy metals, but the epidemiological evidences do not allow any clear inference to be drawn (Thakur *et al* 2008, Singh and Kaur 2012, Singh and Sangha 2014). The present study was designed to examine the possible effect of environmental contaminants on oxidative parameters and male fertility indices in *Bandicota bengalensis* and *Tatera indica* inhabiting South-West region of Punjab.

MATERIAL AND METHODS

During the study, the field rats i.e., *Bandicota bengalensis and Tatera indica* were trapped from fields of Bathinda district of South West region of Punjab. Same species of rats were also collected from PAU, Ludhiana and adjoining areas and they served as control rats. Approval of Institutional Animal Ethical Committee, Guru Angad Dev Veterinary and Animal Science University (GADVASU), Ludhiana was obtained for the usage of animals vides letter no. 3901-35 dated 06-08-2012. Rats were brought to laboratory, separated according to species and observed for morphological symptoms. They were mildly anaesthetized using chloroform and blood samples were collected directly from the heart in heparinised vials. The blood samples were centrifuged at 2300g for 15 minutes to obtain erythrocytic pellets which settles as sediment. The pellet was washed with normal saline solution thrice and 10% haemolysate was prepared for biochemical studies.

Organ weight and Organosomatic Index

Following humane sacrifice, the vital organs viz; brain, liver, spleen, stomach, heart, lungs and kidney; endocrine glands viz; adrenal, thyroid, parathyroid and reproductive organs viz. testis, epididymis (caput, corpus and cauda), vas deferns and other accessory organs like prostate and seminal vesicles were excised, cleaned off the adhering tissue and weighed separately. The organo-somatic index (OSI) was calculated by using the following formula as per Chattopadhyay *et al* (2011).

$$\textbf{Organo} - \textbf{somatic Index}(\textbf{OSI}) = \frac{\textbf{Organ weight}(\textbf{g})}{\textbf{Live body weight}(\textbf{g})} \textbf{X 100}$$

Biochemical studies

For biochemical studies, 0.5 g of liver, one whole kidney and testis was homogenized in 2 ml of phosphate buffer saline (PBS 0.1M, pH 7.4) and the homogenate was centrifuged at 3000 for r.p.m. for 10 min. 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 was assayed by the method of Stocks and Dormandy (1971).

Histological Studies

For histomorphological studies, testis of the rats collected from Bathinda region and PAU, Ludhiana were fixed in alcoholic bouin's solution 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 5µm thick sections were cut serially 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- eosin, cleared in xylene and mounted in DPX for microscopic examination.

Evaluation of male fertility

Cauda epididymis were separated from caput and corpus regions, weighed and suspended in 0.5 ml of phosphate buffer saline (PBS) at 37 °C. Epididymal fluid was collected in PBS by giving cuts to the cauda epididymis and sperm enriched epididymal fluid was collected in a tube. The spermatozoa were analysed for their motility, concentration and abnormalities.

Sperm motility: The motility of sperm was evaluated directly after mincing in drop of sperm suspension, microscopically. A drop of sperm suspension under the cover slip was examined for motility at X400 using Olympus microscope (CH-21). Sperm motility was expressed as percent of motile sperm of the total sperm counted, according to Linder *et al* (1986).

Sperm concentration: The spermatozoa concentration was carried out by diluting the sperm suspension with PBS (1:20), then mixed together, after that a drop of them delivered in to the Neubaure haemocytometer in each side of the counting chamber. The haemocytometer is allowed to stand for 5 min. for sedimentation, then sperms were counted in the large five square and expressed as sperm concentration in million, according to Feustan *et al* (1989).

Sperm abnormalities: About 100 spermatozoa were observed for normal morphology, headless, abnormal head, middle piece swelling, middle piece coiling, tail bending, tail coiling and multiple deformities were counted at x 1,000 using Olympus microscope (CH-21) and percent of each was calculated.

Statistical analysis

All statistical comparisons for organ weight and biochemical analysis were presented as the mean ±

standard error of mean (S.E.M). Comparisons were made between control and Bathinda rats belonging to different species on computer using t-test. A "P" value of 0.05 was selected as a criterion for statistically significant differences.

RESULT AND DISCUSSION

Organ weight

Bandicota bengalensis males collected from Bathinda region showed marked increase in the weight of liver and kidney and decrease in thyroid weight as compared to the PAU rats that served as control (Table 1). However, liver and kidney weight of *Tatera indica* showed significant decrease in the rats collected from Bathinda district. Other organs like spleen, heart, lungs, stomach and brain showed no significant difference in their weights in both the species of rats as compared to control rats (Table 1).

Significant increase in weight of testis of *Bandicota bengalensis* collected from Bathinda region was observed when compared with the respective control rats. However, weight of vas deferns and prostate of Bathinda *Bandicota bengalensis* rats showed a slight increase as compared to control rats (Table 2). The weight of epididymis (caput and corpus), seminal vesicles of *Bandicota bengalensis* and testis and vas deferns of *Tatera indica* collected from Bathinda region showed slight decrease as compared to control rats.

Biochemical observations

The total protein contents were found to be lower in the liver, kidney, brain and testis of male *Bandicota bengalensis* collected from Bathinda region as compared to control rats. Blood protein content of *Bandicota bengalensis* was found to be comparable in all the rats. *Tatera indica* rats also showed a non-significant decrease in protein content in all the organs as compared to control rats (Table 3-7).

The activity of catalase decreased non-significantly in liver, blood and testis of rats collected from Bathinda region as compared to the control rats. Slight increase in CAT activity was observed in kidney of *Bandicota bengalensis* and brain of *Tatera indica* rats collected from Bathinda district (Table 3-7). The level of SOD activity increased significantly in liver and kidney of *Bandicota bengalensis* collected from Bathinda region as compared to the control rats while non-significant increase was observed in liver, kidney, brain, blood and testis of *Tatera indica* and *Bandicota bengalensis* of Bathinda district as compared to control rats (Table 3-7). GST activity showed a slight increase in liver, kidney, brain, blood and testis of *Bandicota bengalensis* and *Tatera indica* and brain of female *Tatera indica* collected from Bathinda region as compared to control (Table 3-7).

The activity of GR increased non-significantly in kidney, blood and testis of *Bandicota bengalensis* and liver, brain and blood of *Tatera indica* collected from Bathinda district when compared to the control rats .GR activity was comparable in liver and brain of *Bandicota bengalensis* and in kidney and testis of *Tatera indica* (Table 3-7). Reduced activity of glutathione peroxidase was observed in liver, kidney and blood of *Bandicota bengalensis* and in liver, brain and blood of *Tatera indica* rats collected from Bathinda region as compared to control. Activity of glutathione peroxidase was comparable in liver of *Bandicota bengalensis* and in kidney and testis of *Tatera indica* (Table 3-7).

LPO showed non-significant increase in liver, kidney, brain and testis and significant increase in blood of male *Bandicota bengalensis* and *Tatera indica* collected from Bathinda region (Table 3-7).

Histological Studies in Testis

The use of histopathological evaluations, while evaluating animal tissue is of prominent role in male reproductive risk assessment. Light microscopic examination of sections of the testes of control *Bandicota bengalensis* and *Tatera indica* revealed that the parenchyma of testis was formed of rounded seminiferous tubules (Fig.1 A, C). In transverse sections testis contain numerous seminiferous tubules which are different in their shape and size. The primary spermatogonial cells, primary and secondary spermatocytes, spermatids and sperm bundles are visible in the seminiferous tubules (Fig.1A, C). Each seminiferous tubule is lined by a thin basement membrane and the interstitial cells and connective tissues are present in between the tubules. The lumen of the seminiferous tubule contains numerous sperms (Fig.1A, C).

Light microscope examination of testis section of the Bathinda rats revealed some distorted seminiferous tubules in *Bandicota bengalensis* (Fig. 1B) and *Tatera indica* (Fig. 1D), clumped spermatozoa which was found to be more prominent in *Tatera indica* (Fig.1D) as compared to *Bandicota bengalensis* (Fig.1B). Non-significant change was observed in the tubules of all the rats while slight larger spaces were seen in the interstitial tissue between the tubules in all the rats collected from Bathinda region as compared to control rats (Fig.1B, D).

Sperm parameters

There was a decrease in the percent sperm motility in *T. indica* and *B. bengalensis* Bathinda rats compared to Ludhiana rats (Table 8). Sperm concentration was significantly reduced in *T. indica* and *B. bengalensis*

Bathinda rats as compared to Ludhiana rats (Table 8). The percent morphological abnormalities (head, tail and multiple) in spermatozoa of rats of South Western Punjab were much higher as compared to control rats (Table 9). Non significant increase in percent tail and multiple sperm abnormalities was observed in these rats as compared to the rats of Ludhiana district.

DISCUSSION

Organ weights were recorded as potential indicator of a dietary effect on the organism. (Piao *et al* 2013). Liver and kidneys are central metabolic organs and 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). Reduced weight of liver and kidney may be considered as indicators of toxins induced damage (Grunhage *et al* 2003). The environmental contaminants/pesticides might have induced injury that could have induced impairment in metabolic activity of liver and kidney and caused a disturbance in metabolic activity require for maintainace of tissue (Benjamin *et al* 2006). Shekhar *et al* (2011) also reported the decrease in total body, testis and epididymis weights in cypermethrin treated mice. The reduction in weight of accessory organs indicates atrophy of glandular tissue and also a reduction in secretionary ability. The decline in testis weight may also be a result of a decrease in sperm count as well as reduction in weight of seminal vesicles and ventral prostate, which may reflect an interference with androgen output. Unpublished data of our laboratory have also revealed pesticide chlorpyrifos and malathion residues in liver and blood of male *T. indica and B. bengalensis* rats inhabiting Bathinda district of Punjab.

Biochemical parameters are sensitive index of the changes due to pesticide toxicity and can constitute important diagnostic tool in toxicological studies (Singh and Saxena 2001). Significant decrease in total protein content was reported in liver and kidney tissues of rats inhabiting Bhatinda region. Otitoju and Onwurah (2007) also reported a reduction in rat plasma protein exposed to pesticides. The decrease in testicular proteins from Bathinda rats may indicate the induced degenerative changes in the testis of rats or general disturbance of the protein metabolism which may be due to androgen/estrogen deficiency leading to impaired gametogenesis. Depressed level of proteins was also found in males and females treated with 2400 ppm imidacloprid (Solecki 2001).

Reduced activity of catalase, glutathione reductase and glutathione peroxidase indicates increased production of free radicals under the influence of environmental intoxicants. Khan *et al* (2010) suggested that genetic polymorphisms in glutathione peroxidise enzymes and their altered expressions and activities are associated with oxidative DNA damage and subsequently the individual risk of cancer susceptibility. Reduced activity of glutathione peroxidase was in accordance with previous studies where Heikal *et al* (2013) observed a significant decrease in levels of glutathione peroxidase in liver of rats administered with Cyromazine, Chlorpyrifos and both as compared to control rats.Increase in the activity of superoxide dismutase was also observed by Tunçmen and Tüzmen (2007) in blood, brain, kidney and liver of rats fed with drinking water of Buyukkabaca. The GR activity was significantly increased after exposure to the concentrations of 0.4 μg/L and 0.8 μg/L of deltamethrin in liver in common carp (Ensibi *et al* 2013).

Malondialdehyde (MDA) is an end product of lipid peroxidation including phospholipids in the cell membrane and the enhanced levels of MDA is an indicator of oxidative stress. The increased levels of MDA may pose the survival threat to live cells, which may have the potential to affect various organs and their normal physiology leading to severe pathophysiological conditions (Glutekin 2001). 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.

The microscopic examination of testis section of the Bathinda rats revealed some distorted and degenerative changes in seminiferous tubules. Histological evaluations are especially useful in providing a relatively sensitive indicator of damage; and with short-term dosing, providing information on target cells, extent of toxicity, and, indicating the potential for recovery (Akinloye *et al* 2002). In the lindane treated rats, the cells were irregularly shaped and there was marked intercellular space between the spermatogenic cells and cell disorganization was found (Simic *et al* 2012). Sakr and Azab (2001) reported abnormal seminiferous tubules with many vacuoles, marked reduction in spermatogenic cells, and degenerated Leydig cells in albino rats inhaling pyrethroids. Manna *et al* (2004) found edema between seminiferous tubules, vacuolization and hyalinization in the tubules of the testes of rats exposed to α-cypermethrin. A decrease in luminal sperm and apparent dilation of tubules, together with oxidative stress, has been associated with testicular damage from sodium fluoride in rats (Ghosh *et al* 2002).

The assessment of sperm motility is one of the basic elements of semen analysis and is especially important in samples where many sperm are immotile, to distinguish between immotile dead sperm and immotile live sperm (Björndahl *et al* 2003). Sperm count is liable to decrease either due to decreased

sperm production in seminiferous tubules or decreased sperm maturation in epididymis under effects of some chemical (Rani *et al* 2013, Taib *et al* 2013). Decline in sperm motility and concentration can be attributed to environmental contaminants leading to androgen insufficiency (Chitra *et al* 2001, Mathur *et al* 2010). Sperm motility evaluation done by Perobelli *et al* (2010) also revealed a statistically significant reduction in the number of sperms in rats exposed to single or mixed pesticides as compared to control group. Treatment with either the higher or lower lindane dose also induced a significant decrease in epididymal sperm numbers and sperm motility (Simic *et al* 2012).

Table 1: Relative weight of various organs (g/100 g bw) in different species of male rats.

	Bandicota bengalensis		***Tatera indica***	
Organs	**Control**	**Bathinda**	**Control**	**Bathinda**
Liver	4.05±0.39	4.19±0.60*	4.00±0.20	3.11±0.33*
Kidney	0.45±0.02	0.50±0.04*	0.35±0.01	0.25±0.03*
Spleen	0.39±0.16	0.30±0.06	0.34±0.02	0.32±0.03
Heart	0.49±0.06	0.48±0.03	0.40±0.05	0.40±0.04
Lungs	0.48±0.23	0.41±0.08	0.51±0.05	0.50±0.04
Stomach	3.35±0.31	3.22±0.16	2.90±0.08	2.93±0.27
Brain	2.00±0.72	1.55±0.09	1.26±0.07	1.23±0.11
Thyroid	0.260±0.15	0.210±0.04*	0.230±0.012	0.220±0.02
Parathyroid	0.023±0.01	0.024±0.01	0.024±0.00	0.024±0.00
Adrenal	0.028±0.00	0.028±0.00	0.036±0.00	0.035±0.01

Values are Mean ± SE,
*Significant difference at ($p \leq 0.05$) as compared to control

Table 2: Relative mass of reproductive organs (g/100 g bw) in different species of male rats

	Bandicota bengalensis		***Tatera indica***	
Organs	**Control**	**Bathinda**	**Control**	**Bathinda**
Epididymis	0.28±0.15	0.27±0.18	0.30±0.06	0.29±0.06
Cauda	0.19±0.01	0.19±0.12	0.14±0.02	0.13±0.03
Caput	0.17±0.01	0.16±0.10	0.12±0.02	0.11±0.03
Corpus	0.032±0.01	0.029±0.01	0.034±0.01	0.032±0.03
Testis	0.54±0.19	0.65±0.04*	0.68±0.11	0.63±0.13
Seminal vesicles	0.39±0.11	0.33±0.06	0.38±0.003	0.38±0.07
Vas deferns	0.04±0.01	0.05±0.01	0.05±0.01	0.04±0.05
Prostate	0.21±0.01	0.23±0.01	0.24±0.01	0.24±0.03

Values are Mean ± SE,
*Significant difference at ($p \leq 0.05$) as compared to control

Table 3: Liver enzymatic antioxidant parameters (mg g^{-1} wet weight of tissue) of *Bandicota bengalensis and Tatera indica* male rats.

	Bandicota bengalensis		***Tatera indica***	
Parameters	**Control**	**Bathinda**	**Control**	**Bathinda**
Protein	4.92±0.76	4.50±0.55	4.23±0.02	3.75±0.31
CAT	45.06±1.80	44.27±0.57	40.34±4.45	39.10±1.22
SOD	12.62±3.18	15.83±2.36*	14.87±0.02	16.49±1.63
GST	0.44±0.05	0.45±0.07	0.42±0.01	0.43±0.06
GR	0.08±0.02	0.08±0.02	0.06±0.00	0.08±0.01
GPx	0.42±0.05	0.33±0.01	0.29±0.00	0.27±0.09
LPO	10.61±0.94	11.92±1.10	9.11±0.75	9.40±1.33

All the values are Mean ± SE of 5 animals in each group,
*Statistically significant different ($p \leq 0.05$) 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: Kidney enzymatic antioxidant parameters (mg g^{-1} wet weight of tissue) of *Bandicota bengalensis and Tatera indica* male rats.

	Bandicota bengalensis		*Tatera indica*	
Parameters	**Control**	**Bathinda**	**Control**	**Bathinda**
Protein	4.08±0.88	3.73±0.19	3.51±0.12	3.05±0.02
CAT	42.29±1.65	42.55±4.75	45.66±2.85	43.29±2.02
SOD	15.30±1.91	20.53±0.08*	11.12±3.23	12.24±0.46
GST	0.39±0.01	0.41±0.06	0.40±0.03	0.41±0.03
GR	0.06±0.01	0.10±0.01	0.09±0.01	0.09±0.01
GPx	0.24±0.12	0.23±0.04	0.33±0.05	0.33±0.07
LPO	9.72±1.03	10.69±1.20	9.42±0.62	9.66±0.12

All the values are Mean ± SE of 5 animals in each group,
*Statistically significant different (p≤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 5: Brain enzymatic antioxidant parameters (mg g^{-1} wet weight of tissue) of *Bandicota bengalensis and Tatera indica* male rats.

	Bandicota bengalensis		*Tatera indica*	
Parameters	**Control**	**Bathinda**	**Control**	**Bathinda**
Protein	4.57±0.01	3.71±0.22*	4.25±0.06	4.24±0.08
CAT	45.88±6.12	43.87±3.10	49.03±3.44	50.24±6.12
SOD	16.85±3.32	17.64±0.59	15.42±1.11	15.46±2.77
GST	0.48±0.00	0.49±0.03	0.43±0.05	0.52±0.04*
GR	0.07±0.00	0.07±0.01	0.06±0.00	0.08±0.02
GPx	0.21±0.16	0.21±0.07	0.21±0.05	0.16±0.03
LPO	11.18±2.31	11.36±0.59	8.34±0.04	8.29±1.48

All the values are Mean ± SE of 5 animals in each group,
*Statistically significant different (p≤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 6: Blood enzymatic antioxidant parameters of *Bandicota bengalensis and Tatera indica* male rats.

	Bandicota bengalensis		*Tatera indica*	
Parameters	**Control**	**Bathinda**	**Control**	**Bathinda**
Protein	34.0±0.52	34.0±0.11	29.3±0.01	29.0±0.08
CAT	11.21±6.12	9.04±3.10	11.98±3.44	11.34±6.12
SOD	3.31±0.18	3.15±0.74	4.12±0.19	4.22±0.27
GST	0.007±0.00	0.009±0.00	0.007±0.00	0.008±0.00
GR	0.008±0.00	0.009±0.00	0.009±0.00	0.010±0.00
GPx	0.04±0.01	0.03±0.01	0.04±0.01	0.03±0.02
LPO	160.3±3.21	192.8±2.11*	170.8±0.03	202.4±4.32*

All the values are Mean ± SE of 5 animals in each group,
*Statistically significant different (p≤0.05) as compared to control
Units: Proteins (mg/ml 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 sample).

Table 7: Effect of environmental contaminants on biochemical parameters of male rats of different species

	Bandicota bengalensis		*Tatera indica*	
Parameters	**Control**	**Bathinda**	**Control**	**Bathinda**
Protein	4.78±0.50	3.60±0.21*	4.12±0.03	4.09±0.10
CAT	32.26±3.68	31.72±5.59	31.89±2.05	29.31±0.16
SOD	19.09±2.20	20.49±0.65	17.09±2.22	18.05±1.55
GST	0.47±0.02	0.48±0.02	0.46±0.01	0.47±0.02
GR	0.07±0.02	0.08±0.01	0.06±0.01	0.06±0.01
GPx	0.20±0.13	0.21±0.07	0.19±0.04	0.19±0.09
LPO	11.64±1.79	12.00±0.41	8.34±1.48	8.52±0.79

Values expressed as Mean ± SE (n=5).
*Significant difference (P ≤ 0.05) as compared to control
Units: Proteins (g/dL sample), CAT (µmole of H_2O_2 decomposed/min/mg protein), SOD (U/mg protein), GST (µmoles of GSH-CDNB conjugate formed/ min/mg protein), GR (µmoles of NADPH oxidized/ min/mg protein), GPx (U/mg protein), LPO (nM MDA/ml sample).

Table 8: Effect of environmental contaminants on Sperm mortality and Sperm concentration

Parameters	*Bandicota bengalensis*		*Tatera indica*	
	Control	**Bathinda**	**Control**	**Bathinda**
Sperm motility(%)	91.00±0.03	89.56±1.01	91.56±6.87	88.45±1.93
Sperm concentration (10^7)	21.00±0.01	20.76±1.38	22.79±0.36	17.98±1.78*

Values are Mean ± SE
*Significant difference at (p≤0.05) as compared to control

Table 9: Effect of contaminants on percent sperm abnormalities in male rats

Type of sperm abnormality	*Bandicota bengalensis*		*Tatera Indica*	
	Control	**Bathinda**	**Control**	**Bathinda**
Normal	69.00 ± 0.02	62.67 ± 1.01	71.22 ± 1.04	60.67 ± 0.51
Headless	16.00 ± 1.20	15.02 ± 1.72	15.00 ± 0.04	14.00 ± 1.72
Abnormal Head	5.90 ± 1.54	6.00 ± 0.04	5.67 ± 1.54	3.67 ± 0.50
Middle piece bending	1.55 ± 0.40	3.01 ± 0.19	4.10 ± 0.01	5.66 ± 1.09
Middle piece coiling	4.00± 0.01	2.66 ± 1.27	3.20± 0.40	2.00 ± 0.20
Tail bending	13 ± 0.07	12 ± 1.72	11.00 ± 0.40	9.00 ± 0.02
Tail coiling	2.66 ± 0.02	3.33 ± 0.94	2.00 ± 0.70	2.50 ± 0.90
Multiple deformities	1.66 ± 0.01	3.00 ± 0.21	3.6 ± 0.20	3.60 ± 1.27

Values are Mean ± SE

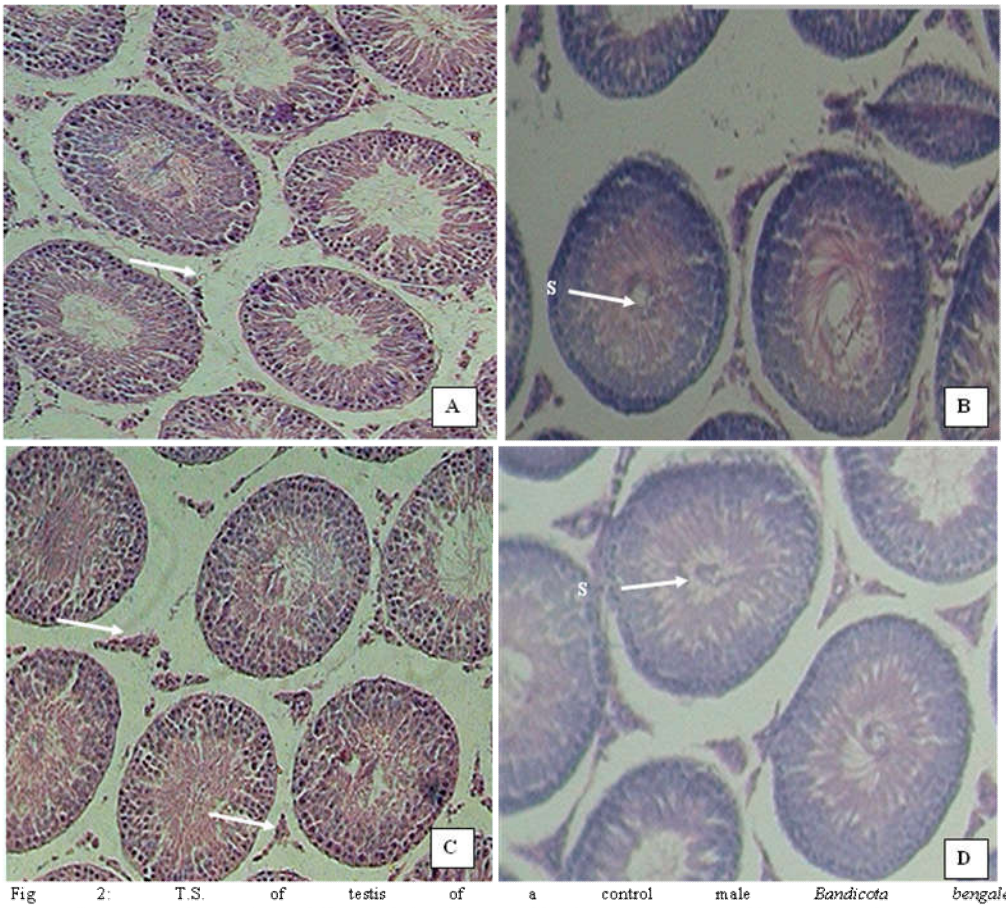

Fig 2: T.S. of testis of a control male *Bandicota bengalensis* (A) and *Tatera indica* (C) showing seminiferous tubules with spermatid and mature spermatozoa with an outer membrane, theca albuginea alongside Leydig cells lying between seminiferous tubules (arrow).T.S. of testis of a male *Bandicota bengalensis(B) and Tatera indica (D)* collected from Bathinda district showing clumped spermatozoa (S) and seminiferous tubules present with high propotion of spermatozoa (X100).

CONCLUSION

In conclusion, it can be inferred that environmental contaminants may produce adverse reproductive effects, by the altering levels or activities of antioxidants such as CAT, GR, GPx, SOD and GST enzymes and lipid peroxidation and histopathology of reproductive organs. Reduced epididymal weight, sperm concentration and motility in *Bandicota bengalensis* and *Tatera indica* inhabiting Bathinda district of South-west Punjab indicates the negative effects of environmental contaminants.

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