Biochemical Studies on Adverse Health Effects of Arsenic and Chromium in Buffaloes

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A B S T R A C T

The present study was undertaken to assess the damage caused by heavy metals, arsenic and chromium in slaughtered buffaloes. The assessment of adverse effects of these heavy metals in tissues (Liver, kidney, pancreas, and ovary) and blood have been determined by the expression of metallothionein, antioxidants and biochemical parameters. Tissue and blood samples were collected from buffaloes (n=50) from local abattoir to assess the level of arsenic and chromium. The concentration of these heavy metals was found to be significantly (p<0.05) higher in exposed group as compared to the control group. Malonldialdehyde (MDA) level in tissues and blood of each arsenic and chromium exposed groups were significantly (p<0.05) higher than the non-exposed control group. The activity of superoxide dismutase (SOD) and catalase (CAT) was found to be significantly increased (p<0.05) in tissues and blood of exposed group. The exposed buffaloes were found to have significantly (p<0.05) increased glucose, cholesterol, AST, LDH, CK, ALP, urea, and creatinine level as compared to control group. The fold change expression level of metal binding protein metallothionein (MT-2) in the heavy metal exposed tissues and blood was found to be variable expressed. liver and pancreas had maximum and minimum fold change MT-2 expression respectively. The study concluded that arsenic and chromium exposure in buffaloes could result in oxidative damage and alterations in the expression of metallothionein.

Introduction

Few of the metals are important elements required in maintaining the homeostasis in animal body.When ingested in large quantity via food chains; they cause deleterious effect on animal and human health leading to toxicity due to their potential accumulation in biological ecosystem. Heavy metals are referred to as metals with density (specific gravity) greater than five g/cm3). Their sources can be natural (rocks) and anthropogenic (agriculture fertilizers, industrial waste, mining) (Vanderlinden *et al.,* 2006; Conesa *et al.,* 2007). To fulfill increased demand of food grains and

livestock products, modernization of industries and agriculture continues at an accelerated pace causing increase in environmental concentration of heavy metals in water, feed, air and soil. Arsenic is one of the most lethal heavy metals to ruminants, their permissible limit for organs and blood is 0-0.05 ppm and 0.04-0.5 ppm, respectively (ANZFA, 2001).

Chromium (III), on the other hand, is an essential nutritional supplement for animals and humans and has an important role in glucose metabolism. The uptake of hexavalent chromium compounds through the airways and digestive tract is faster than that of trivalent chromium compounds (Zhitkovich, 2005). The permissible limit in body for Chromium in tissues and blood are 0-0.5 ppm, 0.5-2.5 PPB respectively (ANZFA, 2001) exceeding this limit leads to the toxicity and lethal effects on animal body.

Arsenic and Chromium are mainly accumulated in soft tissues like liver, kidney and muscle causing damage and dysfunction of the organs, increase in liver enzyme and kidney parameters in plasma.

A metal binding protein Metallothionein (MT), is a ubiquitous, low molecular weight cysteine rich protein which has high affinity for essential (Zn and Cu) micro minerals and provide innate defense mechanism (Margoshes *et al.,* 1957). These proteins bind tightly to heavy metals to decrease toxicity (Klaassen *et al.,* 2009). For mammals, MTs bind zinc (Kagi, 1991), but with excess copper or cadmium, zinc can be easily be replaced by these metals (Shaw *et al.,* 1991). MT has four isoforms in which mainly MT-1 and MT-2 isoform are present almost in all soft tissues (Masters *et al.,* 1997; Searle *et al.,* 1984). MT-3 isoform was expressed mainly in brain and MT-4 was detected in different epithelial cells (Uchida *et al.,* 1991; Quaife *et al.,* 1994).

Thus, due paucity of data related to MT2 expression in buffaloes affected with heavy metal toxicity, this approach was made to study the level of MTs in blood as marker, which could determine the exposure of heavy metals in buffaloes. The corresponding level of MTs in tissues was also studied to find out the extent of damage caused by heavy metals in various tissues.

Materials and Methods

Sample collection

The liver, kidney, pancreas and ovary tissue samples were collected on ice pack in tissue collection vials and blood samples were collected in heparinized vial from local buffalo abattoir near Ludhiana, Punjab, India. For RNA isolation buffaloes tissue samples were collected in RNALater® and blood sample in Tri-Reagent BD respectively from freshly slaughtered apparently healthy animals and store at -80°C till processing.

Processing of samples

Separation of plasma

Blood samples from slaughtered buffaloes were collected in sterile tube containing anticoagulant (heparin). The samples were centrifuged at 3000rpm, for 10 min at room temperature to separate plasma. The plasma samples were stored in aliquots in vial free from any mineral at -20°C.

Preparation of tissue homogenate

A 10% tissue homogenate was prepared in 0.1M Tris-HCl, pH - 7.5 by using all glass tissue homogenizer with Teflon pestle followed by sonication of the homogenized tissue by sonicator. The homogenate obtained was centrifuged at 5000 rpm for 10 min (10000×g for 15 min) at 4°C in a refrigerated

centrifuge. The supernatant was collected and immediately used for assaying of Arsenic and chromium determination.

Preparation of 10% RBC hemolysate

Freshly, collected heparinized blood samples after separation of plasma sediment cell was washed with 0.9% NaCl solution. This process was repeated thrice then these erythrocytes obtained after washing were hemolyzed with 9-fold volume of distilled water to prepare 10 % hemolysate.

Tissue homogenate and hemolysate was used to analyze reduced blood glutathione, superoxide dismutase (SOD), lipid peroxidation, catalase and glutathione peroxidase.

Determination of arsenic and lead in tissues

Glassware decontamination

All glassware used for arsenic and lead estimation were washed in detergent, soaked overnight in chromic acid and rinsed several time with triple distilled water and dried in hot air oven. Blood plasma and tissue homogenate were digested in a conical flask after adding 5 ml triple acid (HNO_3, 70% $HClO_4$ and H_2SO_4 in 10:3:1, v/v) (Ludmila, 1976). It was covered and kept overnight at room temperature. Then solution was heated on hot plate until it becomes clear and about 0.5-1 ml of solution is left. Then it was diluted to 10ml with triple distilled water and used for estimation of arsenic and chromium. The concentration of Arsenic and chromium were estimated by inductively coupled plasma optical emission spectrometer (PerkinElmer, Optima 2100DV) by using specific standard operating conditions meant for specific mineral. All the determinations were performed in duplicate.

Antioxidants and biochemical parameters

Estimation of lipid peroxidation in tissues and erythrocytes was done by the reaction of thiobarbituric acid (TBA) with malonyldialdehyde (MDA) (Placer *et al.,* 1966). The activities of superoxide dismutase (SOD), in tissue and blood was estimated by method based on principle that the nitrobluetetrazolium inhibits superoxide dismutase with reduced nicotinamide adenine dinucleotide (NADH) mediated by phenazonium methosulphate under aerobic conditions (Nishikimi *et al.,* 1972). The activity of erythrocytic as well as tissue glutathione peroxidase, based on the principle that glutathione peroxidase catalyzes the reaction between hydrogen peroxide (H_2O_2) and reduced glutathione (GSH) to form oxidized glutathione (GSSG) and water (H_2O) (Hafeman *et al.,* 1974). The level of antioxidants (catalase, SOD, MDA, glutathione peroxidase (GPx), glutathione (GSH), and Vitamin E and C was determined manually.

The blood profile (glucose, total protein [TP], albumin [ALB], and globulin), lipid profile (cholesterol and triglyceride), and liver and kidney function parameters (alanine aminotransferase [ALT], aspartate aminotransferase [AST], blood urea nitrogen [BUN], and creatinine) was determined by kits (ErbaManheim). Vitamin E and C were analyzed manually.

Isolation of RNA

0.1% DEPC treated and properly sterilized laboratory wares was used to minimize ribonuclease activity during collection of samples and all other RNA works. Isolation of RNA was done by standard protocol given in "TRI Reagents^TM RNA ISOLATION SYSTEM" for tissue and for blood TRI Reagent BD (Sigma-Aldrich, USA).The

optical density of nucleic acid (RNA) was measured in an ultraviolet light Nano drop spectrophotometer (Thermo, USA). For quantification of RNA concentration, the readings were taken at a wavelength of 260 nm and 280 nm. Pure preparations of RNA with OD260/OD280 ratio greater than 1.7 were selected for further studies (according to Sigma-Aldrich, USA guidelines).

After isolation of RNA, Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) was done for synthesis of cDNA using MMLV Reverse transcriptase 1st-Strand cDNA Synthesis kit (Epicenter®). The cDNA was used immediately for real-time PCR or stored at –20°C for future use. Integrated Dna Technologies (India) synthesized primers used for qPCR. Simple PCR was run at different gradient temperature (60°C to 62°C) using specific MT2 primers. The oligonucleotide sequences for MT2 forward and reverse primers are. 5'A AAGATTGCAAGTGCGCCTC3' and 5' CA CTTGTCCGAAGCCCCTTT 3' respectively. RPL4 was used as the reference gene (Nygard *et al.,* 2007) and forward and reverse primer sequence were 5'TT GGAAACATG TGTCGTGGG3' and 5'GC AGATGGCGTA TCGCTTCT3' respectively.

qPCR was performed using aliquots of cDNA and KAPASYBR FAST qPCR Master Mix (2X) kit. Each reaction was performed in a 25µl reaction volume containing 200nM of each amplified primer and 1ng or 2ng of cDNA. Expression of MT2 gene was compared with expression of RPL4 housekeeping gene along with Negative control (NTC).

The reaction was run in duplicates and carried out in CFX96 Touch™ Real-Time PCR detection System (BIO_RAD®) using the following cycling protocol: 95°C - 3min; 40 cycles of 90°C -3sec, 60°C -30sec and 95°C - 5sec. A Melting curve analysis was performed for each primer pair.

Statistical analysis

The data were analyzed using statistical package for social sciences (SPSS) software (version 16.0). Statistical comparison between means of different groups was carried out using independent t-tests.

The result of real time PCR was then calculated using:$\Delta Ct = Ct$ [Target]-Ct [Housekeeping] and $\Delta\Delta Ct = (\Delta Exp.)$ - $(\Delta Control)$. After using formula $2^{-\Delta\Delta Ct}$ the fold change of expression of MT2 gene was calculated. Those animal in which arsenic and chromium heavy metal were found to be within permissible range have been taken as control.

Results and Discussion

Blood and tissue samples were analyzed for heavy metals. The data obtained from AAS analysis of tissue homogenate of liver, kidney, pancreas, ovary and blood plasma has been categorized as heavy metal exposed and non-exposed groups which are presented in Figure 1.

In those animals where plasma concentration of Chromium and arsenic exceeded the permissible limit, tissue samples were also processed. In exposed group the level of chromium and arsenic in liver, kidney, pancreas, ovary and blood was found to be higher than the permissible limit.

Among different tissues and blood, the level of Chromium was high in ovary (2.3175±0.878) followed by Liver (1.0114±0.32), Kidney (0.6814±0.198), pancreas (0.5443±0.269), and Blood (0.2636±0.026) respectively. Whereas in Arsenic exposed group concentration (mean±S.E.) has been reported high in kidneys (0.8334±0.296ppm) followed by liver (0.705±0.20219ppm), pancreas (0.5575±0.008ppm), ovary (0.5523±0.007 ppm) and blood (0.5321± 0.02ppm).

Antioxidants

Various oxidative stress parameters of buffaloes in Chromium (Cr) and arsenic (As) exposed tissues and blood has been summarized in Figure 2.

The Overall mean±S.E value of MDA and activity of antioxidant enzymes Like SOD and Catalase in Cr and As exposed liver, kidney, pancreas, ovary, blood were found significantly ($p < 0.05$) high in all tissues and blood as compared to control non-exposed group (Figure 2).

Likewise, GPx activity of Chromium and Arsenic exposed tissue group was significantly higher than control. But activity of GPx in blood of both Cr and As exposed group has been found significantly lower than control group (Figure 2).

The overall mean level of GSH in erythrocytes of Chromium and arsenic exposed buffaloes was found to be 1.1543±0.205 µg/ml and 1.0879±0.177 µg/ml respectively which was lower ($p < 0.05$) than the control (2.5904±0.00896 µg/ml) as depicted in Table 1.

The concentration of vitamin C in plasma of Cr and As exposed group was 0.7377±0.0136 mg/dl and 0.26545±0.0188 mg/dl respectively (Table 1). The plasma level of Vitamin E in Cr, As metal exposed group of buffaloes was 0.38±0.01 mg/dl and 0.41±0.015 mg/dl which was found to be significantly ($p < 0.05$) lower as compared to control group (Table 1).

The total immunoglobulin in plasma of Cr and As exposed group decreased significantly ($p < 0.05$) as compared to control (Table 1) which was reported to be 0.3016±0.042 g/dl) and 0.3248±0.018 g/dl respectively.

Biochemical parameters

The glucose in Cr and As exposed buffaloes was 106.014±3.4 mg/dl and 109.620±1.792 mg/dl respectively which was significantly ($p < 0.05$) higher than control (Table 1). Plasma cholesterol in Cr and As exposed plasma was 61.2±3.775 mg/dl and 67.5471±2.399 mg/dl respectively.

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Alkaline phosphatase (ALP) were found to be significantly ($p < 0.05$) higher than control. Their respective level was 80.4714±6.796, 446.0257±55.084 and 139.4±17.019 U/L in Cr exposed group and 80.4594±6.572, 379.2553±36.331 and 125.9882±10.588 U/L in arsenic exposed plasma respectively (Table 1).

Total protein and albumin was found to be 6.6343±0.175 g/dl and 2.8386±0.115 g/dl respectively in chromium exposed plasma and 6.6588±0.139g/dl and 2.8824±0.047 g/dl respectively in arsenic exposed buffaloes plasma which were significantly ($p < 0.05$) lower than non-exposed control group (Table 1).

The level of urea and creatinine was 32.5706±2.105 and 1.3112±0.059 mg/dl in arsenic exposed group and 35.5857±2.323 and 1.2757±0.107 mg/dl respectively in chromium exposed group which was significantly ($p < 0.05$) higher than the control group (Table 1).

Plasma lactate dehydrogenase (LDH) and Creatine Kinase (CK) activity in arsenic exposed group was 3239.2806±182.48 and 3015.6394±411.802 U/L respectively and in chromium exposed group these were 3389.43±365.369 and 4474.51±566.82 U/L respectively (Table 1).

Table.1 Metabolic profile of buffaloes environmentally exposed to heavy metals

Parameter	Control Group (n=10)	Arsenic exposed group (n=10)	Chromium exposed group (n=10)
Glucose (mg/dl)	51.188±0.35804	109.6206±1.792*	106.0143±3.4*
Total Cholesterol (mg/dl)	51.526±0.55412	67.5471±2.399*	61.2±3.775*
Triglycerides (mg/dl)	12.306±0.15207	10.4418±0.867	9.6829±1.067
ALT (U/L)	23.16±0.01414	80.4594±6.572*	80.4714±6.796*
AST (U/L	82.878±0.246	379.2553±36.331*	446.0257±55.084*
ALP (U/L)	72.396±0.39943	125.9882±10.588*	139.4±17.019*
LDH (U/L)	947.6±21.32276	3239.2806±182.48*	3389.43±365.369*
CK (U/L)	37.05±0.70711	3015.6394±411.802*	4474.51±566.82*
Total proteins (g/dl)	8.884±0.06088	6.6588±0.139*	6.6343±0.175*
Albumin (g/dl)	4.48±0.0114	2.8824±0.047*	2.8386±0.115*
Urea (mg/dl)	13.56±0.12083	32.5706±2.105*	35.5857±2.323*
Creatinine (mg/dl)	0.81±0.00707	1.3112±0.059*	1.2757±0.107*
Reduced blood glutathione (µg/ml)	2.5904±0.00896	1.0879±0.177*	1.1543±0.205*
Total immunoglobulin (g/dl)	1.024±0.0056	0.3248±0.018*	0.3016±0.042*
Vitamin E (mg/dl)	4.0872±0.004	0.41±0.015*	0.38±0.01*
Vitamin C (mg/dl)	1.2±0.0256	0.26545±0.0188*	0.7377±0.0136*

*indicates significant relationship ($P < 0.05$)

Figure.1 Arsenic and chromium concentration (PPM) in tissues and blood samples of slaughtered buffaloes (Mean±S.E.)
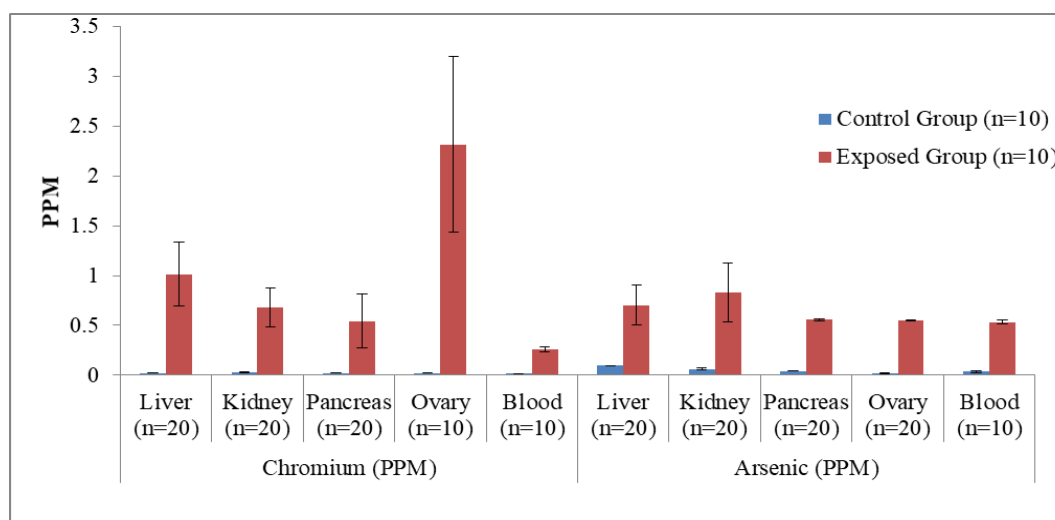
Figure.2 Oxidative stress marker in Buffaloes, (a) Catalase (U/mg). (b) Glutathione peroxidase (u/mg), (c) MDA (nmol/mg), (d) Superoxide dismutase (U/mg), (e) Reduced Blood Glutathione (µg/ml)
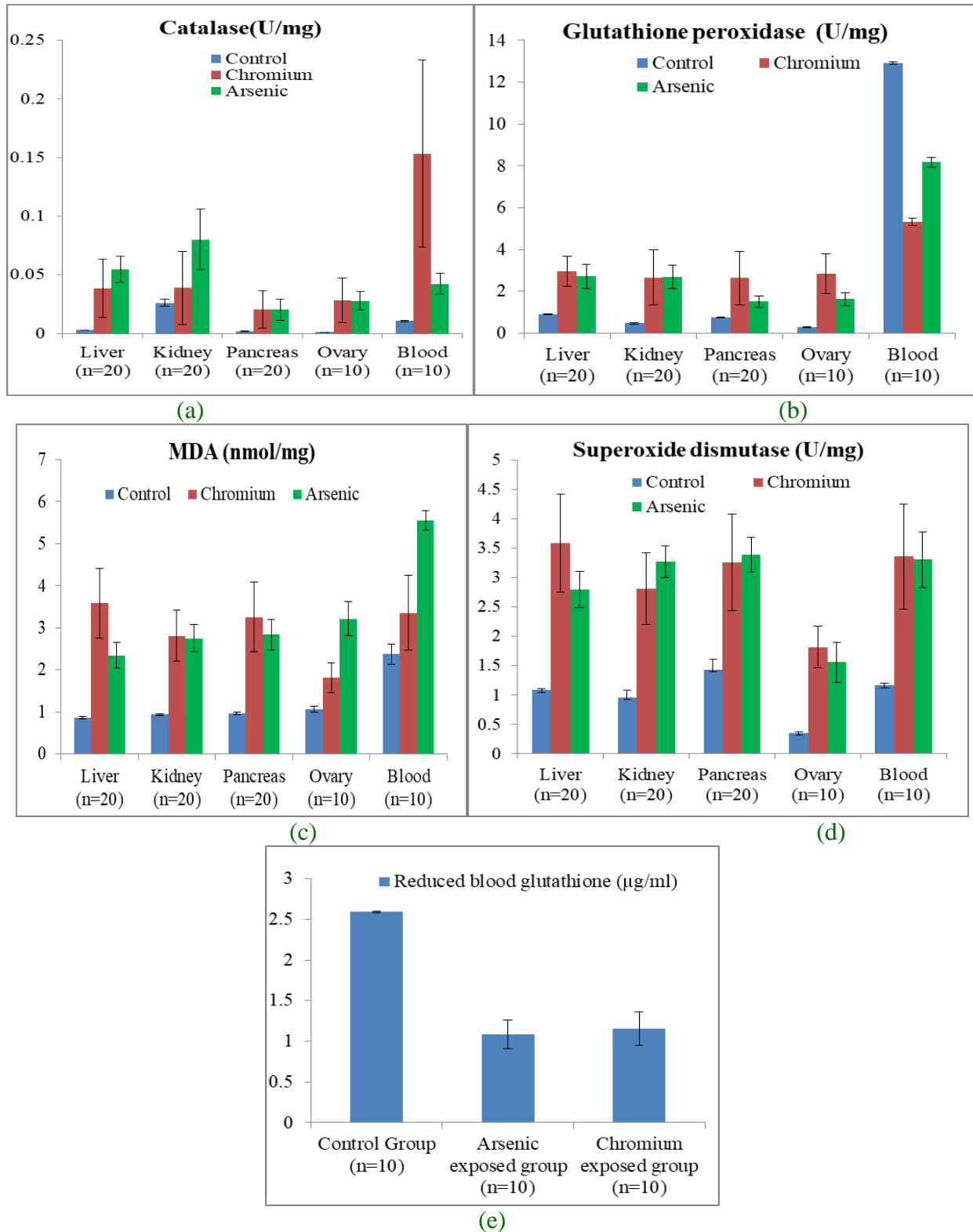
Figure.3 Agarose gel image of PCR product of Metallothionein in different tissue and blood (well 1 liver & 3 –kidney, well 4,5 pancreas; well 6,7 ovary and 10,11 well of blood)
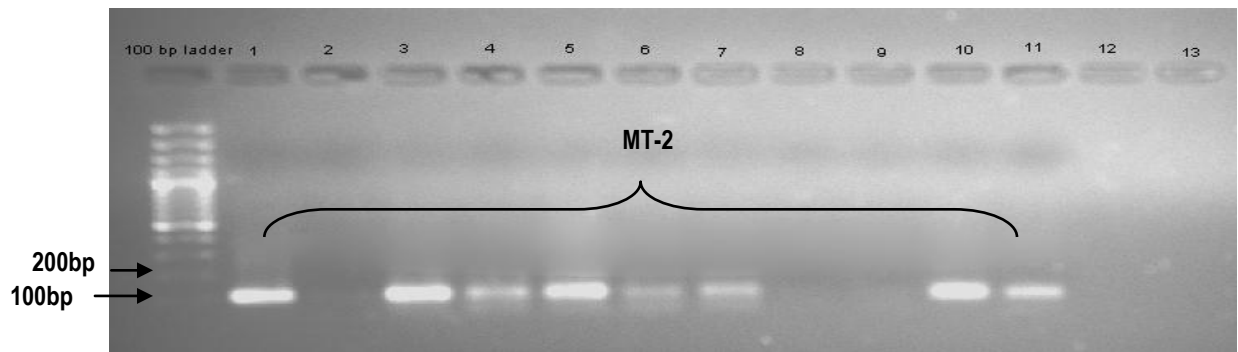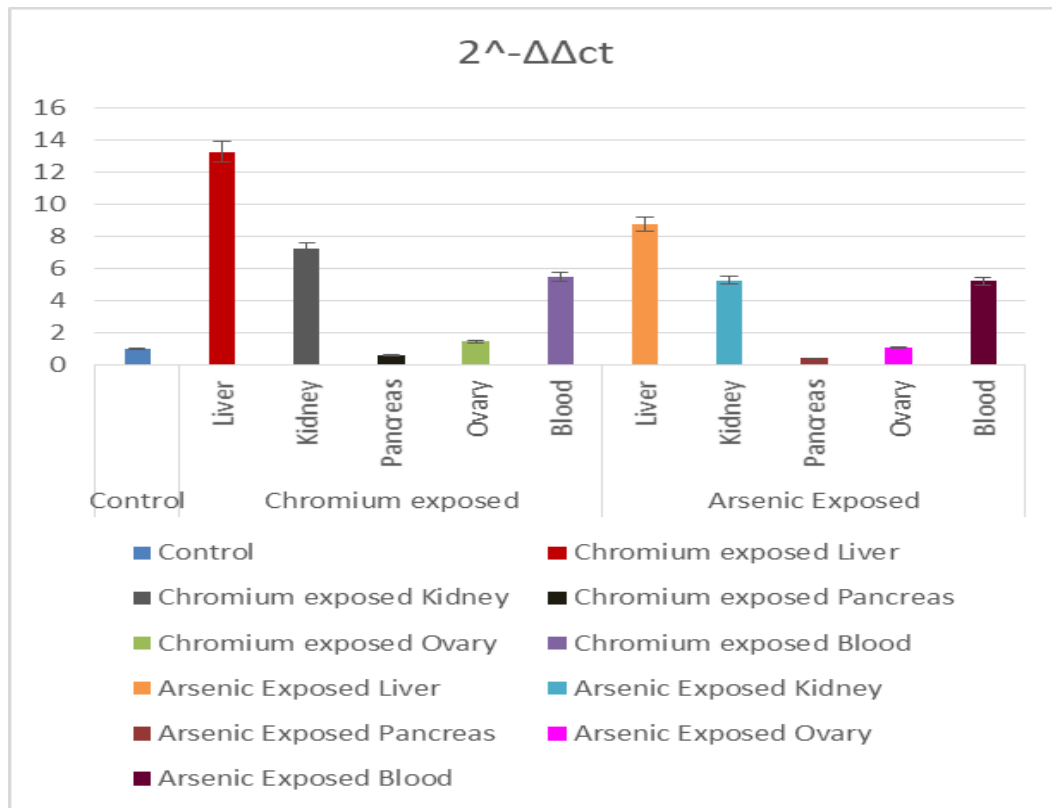

Figure.4 Fold Change Expression of Metallothionein in Chromium and Arsenic exposed organ and blood of Buffalo

Expression of MT-2

After standardization of PCR, specific gene band (MT-2) was checked on 2% agarose gel against 100bp ladder (Figure 3), the images were tasked using gel doc. Fold change expression of metallothionein in different tissue, and blood has been illustrated in figure 4. Mean Ct (cycle threshold) value of metallothionein-2 in arsenic exposed liver, kidney, pancreas, ovary and blood were 21.3725, 19.7225,

22.6175, 23.845, and 19.258 respectively. In Chromium exposed liver, kidney, pancreas, ovary and blood mean Ct (cycle threshold) value of MT-2 were 21.0475, 20.9, 24.455, 24.2 and 22.8595 respectively. Fold change expression of MT-2 in arsenic exposed animal was depicted highest in liver (8.76) followed by nearly equal expression in blood (5.18) and kidney (5.257) and down-regulated expression was showed by pancreas (0.397). Among Chromium exposed organs, liver (13.246) showed maximum up-regulated expression followed by kidney (7.235), Blood (5.488) and ovary (1.4349). Fold change expression of pancreas (0.6137) showed down regulation compared to control group.

The concentration of heavy metals like arsenic and Chromium have been reported to be very high in agricultural soil, fodder and water in Punjab, India (Dash *et al.,* 2016; Yeotikar *et al.,* 2018). These heavy metals enter the food chain and get accumulated in the system of buffaloes and other ruminants (Dhaliwal *et al.,* 2016; Dash *et al.,* 2016; Yeotikar *et al.,* 2018). The present study reported high level of As and Cr in blood (above than permissible limit) which was similar to several other research reported these heavy metals in blood of cattle and buffalo in different part of India (Dey *et al.,* 1997; Swarup *et al.,* 1993, 1997; Dash *et al.,* 2016; Yeotikar *et al.,* 2018). High amount of Chromium and arsenic were also reported in serum samples of buffalo consuming fodder grown in land irrigated by polluted water (Raj *et al.,* 2006; Dash *et al.,* 2016; Yeotikar *et al.,* 2018). Likewise, findings have also been reported where they found that the highest Cr concentration (15.763 mg/kg) in the liver of cow and the lowest (0.075 mg/kg) was in the meat of chicken (EI-Salam *et al.,* 2013). In Current study, Arsenic were found to be high in Kidney, followed by liver, pancreas and ovary tissue.

In the current study, significant (p<0.05) elevated MDA level was observed in the tissues and blood of Cr exposed buffaloes which could be due to an increased oxidative stress of these heavy metals on tissues and blood resulting in production of free radicals which allow them to attack lipid membrane and result in an increase in lipid peroxidation. Similar findings have been reported by other authors where high level of MDA was found in blood of heavy metal exposed buffaloes (Rana *et al.,* 2010; Dhaliwal *et al.,* 2016; Yeotikar *et al.,* 2018).

The activity of free radicals and oxidative stress can be assessed by endogenous antioxidants in blood and tissues (Roy *et al.,* 2013). Free radical scavenging enzymes such as SOD, CAT, and GST are the first line of cellular defense against the toxic effects of ROS (Priscilla and Price, 2009) and they are widely used as biomarkers of oxidative stress (Uzun *et al.,* 2010). Studies have revealed that chromium treatment has resulted in increased activities of SOD and CAT in the liver and kidneys of Rats (Patlolla *et al.,* 2009). The increase in tissue and blood SOD activity was might be to protect cellular DNA, proteins and cell membranes from oxidative stress. Since SOD catalyzes the dismutation of superoxide anion to H_2O_2, which is in turn the substrate of CAT, this fact could explain the observed increment of the two enzyme activities. As these enzymes have a protective role against oxygen free radical-induced damage, their induction can be understood as an adaptive response to oxidative stress.

SOD activity also reflected the intensity of the stress because of toxic action (Patlolla *et al.,* 2009). In contrast to current findings, few other studies reported decrease in SOD and CAT activity in heavy metal exposed buffaloes that might be due to enhanced

production of superoxide radicals during heavy metal metabolism. Different heavy metals have been reported to significantly inhibit the antioxidant enzyme activities in blood of exposed buffalo and cattle (Dhaliwal *et al.,* 2016; Yeotikar *et al.,* 2018).

GPx activity in exposed tissues was found to be significantly (p<0.05) high. Conversely, its activity in heavy metal exposed blood was significantly (p<0.05) low which indicates inhibition of body defense system. We also report the similar findings in accordance with the previous studies which reveal the reduction in GSH caused by oxidative stress due to increased presence of heavy metals. The decrease in concentration of GSH makes cells more prone to oxidative injuries (Kumar and Padhy, 2013). The decrease in level of GSH and increase in level of MDA were found to be in agreement with previous studies in cattle blood (Dhaliwal *et al.,* 2016). The present findings reported significant (p<0.05) low levels of Vitamin C and E in the exposed group, which may be due to utilization of vitamin C in defense mechanism during oxidative stress (Pathan *et al.,* 2006; Joshi *et al.,* 2013).

The present study also explored the influence of As and Cr heavy metals on some biochemical markers. It has been found that glucose, total Cholesterol, urea and creatinine was significantly (p<0.05) higher in heavy metal exposed group. Elevated levels of glucose in metal exposed group might be due to disturbed glucose metabolism. The glucocorticoid system might have been hampered by exposure to heavy metals which plays important role in carbohydrates, lipid and protein metabolism (Kaltreider *et al.,* 2001). Increased glucose and plasma cholesterol level with decrease in total protein and albumin has been reported due to high exposure of different

heavy metals like arsenic and lead exposed ruminant and mice (El- Nekeety *et al.,* 2009; Rana *et al.,* 2010a; Mohajeri *et al.,* 2014; Dash *et al.,* 2018). Significant (p<0.05) decrease in total protein in heavy metals exposed buffaloes might be due to toxic effects of these heavy metals on protein synthesis. The reduced levels of albumin reported in the present study might be due to liver dysfunction caused by heavy metals (As, Cr) as albumin is produced by liver. The reduced levels of total protein and albumin has been reported in heavy metal exposed fishes (Panigrahi *et al.,* 2016; Javed *et al.,* 2017). The elevated level of creatinine and urea in heavy metal exposed buffaloes might be due to nephrotoxic metals like As, Pb, Cr etc.

We report significant (p<0.05) higher level of plasma AST, ALT, ALP activities which clearly suggest the hepatic dysfunction in heavy metal exposed buffaloes. Plasma non anti-oxidant enzyme activity like LDH and CK were very high indicating muscle injuries or occurrence of anaerobic respiration due to stress in exposed buffaloes (Dash *et al.,* 2016; Kaneko *et a1.,* 1997; Aslani *et al.,* 2012). Similar findings of elevated plasma hepatic and muscle enzymes due to various heavy metals have been reported in ruminants and mice (El- Nekeety *et al.,* 2009; Rana *et al.,* 2010a; Mohajeri *et al.,* 2014; Dash *et al.,* 2018)that was comparable to our study.

Likewise, significant (p <0.05) decreased immunoglobulin might be attributed to compromised immune system due to heavy metals (Dash *et al.,* 2016). The decreased total immunoglobulin observed in chromium and arsenic exposed buffaloes could be caused by disturbances in immune regulation and abnormal function of white blood cells which resulted in loss of immune function due to heavy metal exposure (Chen *et al.,* 2003; Dash *et al.,* 2016).

To assess the exposure of As and Cr in buffaloes metal binding protein, metallothionein-2 (MT-2) expression was studied as a biomarker. It was found that the fold expression of metallothionein-2 in arsenic and chromium exposed group was significantly (p<0.05) up- regulated in liver tissues followed by kidney, blood and ovary but pancreas showed down-regulated expression as compared to control group. It was also observed that chromium exposed group had higher expression of MT-2 as compared to As exposed group. The up-regulation of MT-2 expression in various tissues and blood could be to prevent animal from oxidative stress by scavenging the free radicals generated due to increase in toxic/permissible level of arsenic and chromium (Ruttkay-Nedecky *et al.,* 2013). Similar study has been reportedin arsenic exposed human beings and the findings revealed lowered expression of MT in blood as compared to tissues (Liu *et al.,* 2007). Marked increase in metallothioneinwas also observed in liver of mice injected with lead and nickel (Šveikauskaitė *et al.,* 2014) which was in accordance with the current study. Several studies also showed that cells which contain excessive metallothionein are resistant to heavy metal like Cadmium (Ruttkay-Nedecky *et al.,* 2013; Karin *et al.,* 1983). Increase in expression of this metal binding protein could prevent the tissue damage by binding with As and Cr and from oxidative stress caused by different heavy metals.

Acknowledgement

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