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Effect of Vitamin E and Selenium (Se) Supplementation on Biochemical Parameters and Expression of Metallothionein (MT-2) in Heavy Metals Exposed Buffaloes

Himalaya Bhardwaj[1], Chanchal Singh[1], Shashi Nayyar[1], Sandeep Sodhi[2], Rajesh Jindal[1]

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ABSTRACT

Background: Farm animals may serve as bio-indicators of environmental pollution. Environmental heavy metals may disrupt the normal physiological and biochemical profile of the animals. The present study was planned to reduce the stress caused by heavy metal pollution by oral feeding of vitamin E and Selenium in heavy metals exposed buffaloes.

Methods: Twenty buffaloes were selected on the basis of blood levels of heavy metals and divided into exposed and non-exposed groups. Exposed animals (n=10) were orally supplemented with 20 ml/day of Cargill E care Se® containing vitamin E, 100 mg/ml and Se 0.5 mg/ml for 30 days. Antioxidants, biochemical parameters and the expression of metallothionein-2 were analyzed after supplementation on 0, 15 and 30 days.

Result: The levels of heavy metal were found to be elevated even after 30 days of supplementation. No significant alterations were observed in activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) after 30 days of supplementation. There was significantly ($p<0.05$) higher activity of blood glutathione and plasma Vitamin E. In supplemented group, plasma glucose, total cholesterol, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactic dehydrogenase (LDH), creatine kinase (CK) and total Immunoglobulin were found to be significantly reduced in supplemented group. Expression of metal binding protein, metallothionein-2 was found to be elevated in exposed animals despite supplementation with Vitamin E and Se for 30 days.

Key words: Antioxidants, Buffaloes, Heavy metals, Metallothionein, Se, Vitamin E.

INTRODUCTION

Overpopulation causes increase in food demand for food grains and livestock products. To full fill ever-increasing demand of food, dairy product and essential need of population modernization of industry is imminent and causes use of excessive fertilizer in agricultural land due to which increase in environmental concentration of heavy metals in water, feed, air and soil has taken place. (Sidhu *et al*., 2012; Singh *et al*., 2013b). Heavy metals constitute a very diversifying group of elements widely varied in their chemical properties and biological functions. The term heavy metals have been widely used as a group name for metals and metalloids that have been associated with contamination and potential toxicity. Heavy metals like zinc (Zn), copper (Cu), chromium (Cr) arsenic (As), cadmium (Cd) and lead (Pb) are potential bio accumulative toxins for the dairy production system (Li *et al*., 2005; Devasena *et al*., 2012). So, when ingested in large quantity via food chains get accumulated in the organism, which causes deleterious effect on animal and human health leading to toxicity. Farm animals, especially cattle and buffalo are very useful bio indicators of environmental pollution (FAO 2014; Dhaliwal *et al*., 2016). Heavy metals can upset the normal body function by production of reactive oxygen metabolites, hindering normal function by displacing the essential metal ion from the enzyme biomolecule leading to loss or inhibition of its activity (Lavicoli *et al*., 2009) and resulting in physiological and biochemical alterations in animals. Antioxidants reduce the damage caused by reactive oxidative species. (El-Demerdash *et al*., 2004). Selenium protects the cells against oxidative damage by the expression of selenoprotein genes and through anti-inflammatory mechanisms (Said *et al.,* 2014). Selenium is

[1]Department of Veterinary Physiology and Biochemistry, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004, Punjab, India.

[2]Department of Dairy Chemistry, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004, Punjab, India.

Corresponding Author: Himalaya Bhardwaj, Department of Veterinary Physiology and Biochemistry, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004, Punjab, India. Email: himalaya530@gmail.com

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also involved in detoxification of various heavy metals (Diplock *et al*., 1986). Vitamin E (α-tocopherol) being a chain-breaking antioxidant, protects cellular membranes and lipoprotein surfaces from lipid peroxidation (Al-Othman *et al*., 2011). The protective role of Vitamin E against the heavy metal toxicity in experimental animals has been well-established (Agarwal *et al*., 2010). When supplemented in combination selenium and vitamin E is highly effective in reducing storage and toxicity of ROS (Schwenke and Behr 1998).

Expression of antioxidant enzymes and cellular proteins are altered by heavy metal exposure. Regulation and transport of heavy metals is carried out by metal binding proteins like metallothioneins (MTs). They are ubiquitous intracellular low molecular weight cysteine-rich metal binding proteins; MTs have four isoforms MT-1 to MT-4. MT-1 and MT-2 have been found to be most abundantly expressed in many tissues, particularly kidney, liver, pancreas and intestine whereas MT-3 and MT-4 are found principally in brain and skin (Wu *et al*., 2007; Kagi *et al.,* 1979; Kagi *et al*., 1987). These proteins bind firmly to heavy metals to decrease their toxicity (Klaassen *et al*., 2009,1999). In mammals, MTs have been found to bind zinc (Kagi 1991), in presence of excess copper or cadmium, zinc was reported to be replaced by these metals (Shaw *et al*., 1991). Due to availability of thiol groups in MTs, they bind a number of trace metals including cadmium, mercury, platinum and silver and protect cells and tissues against heavy metal toxicity. Very scarce data is available regarding heavy metal exposure in buffaloes. Therefore, the present study was planned to assess the levels of heavy metals (natural exposure), antioxidants, metabolic profile and metallothionein (MT-2) expression in buffaloes after treatment with antioxidant (Vitamin E and Se).

MATERIALS AND METHODS

Ethical approval

All experiments of the study were carried out by using adult female Murrah buffaloes (age 3-5 years). They were maintained in organized dairy farm by their owner in field conditions and provided with standard diet and ad libitum water. All the experiments were carried out according to the guidelines of the IAEC. The experiment protocols were approved by Institutional Animal Ethics Committee (IAEC), Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana.

Sample collection

Blood samples were collected from two separate buffalo farms to estimate heavy metals and other biochemical parameters. One farm was chosen in vicinity of highly polluted drainage channel (Buddha Nallha) area and other from the unexposed region of Ludhiana district, Punjab, India. After estimating the heavy metals in blood, animals were divided into control (un-exposed) and exposed groups. Both buffaloes farm were on daily food supply without any change, except supplementation which was given only to exposed bufalloe's farm. Those animals in which heavy metals were found to be within permissible range (ANZFA 2001; Puls 1994) have been taken as control. Exposed buffaloes were supplemented with Vitamin E and Se @20 ml/day and blood was collected on 0^{th}, 15^{th} and 30^{th} days post supplementation. For RNA isolation blood samples were collected in Tri-Reagent BD and store at -80°C (till processing).

Processing of samples

(a) Separation of plasma

The blood samples were centrifuged at 3000 rpm, for 30 min at room temperature to separate plasma. The plasma samples were stored in aliquots in vial free from any mineral at -20°C.

(b) 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 three time then these erythrocytes obtained after washing were hemolyzed with 9-fold volume of distilled water to prepare 10% hemolysate. Hemolysate was used to analyze reduced blood glutathione, superoxide dismutase (SOD), lipid peroxidation, catalase and glutathione peroxidase.

Determination of heavy metals in blood

Glassware decontamination

All glasswares used for heavy metals and micro minerals estimation were washed in detergent, soaked overnight in chromic acid and rinsed several time with triple distilled water and drying 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 10 ml with triple distilled water and used for estimation of heavy metals and micro minerals. The concentration of these were estimated by inductively coupled plasma optical emission spectrometer (PerkinElmer, Optima 2100DV) by using specific standard operating conditions meant for specific mineral. All these determinations were performed in duplicate.

Antioxidants and biochemical parameters

Estimation of lipid peroxidation in blood erythrocytes was estimated by the method based upon the reaction of thiobarbituric acid (TBA) with malonyldialdehyde (MDA) (Placer *et al*., 1966). The activities of superoxide dismutase (SOD), in blood was estimated by method based on principle that the nitrobluetetrazolium inhibits superoxide dismutase with reduced nicotinamide adenine dinucleotide (NADH) mediated by phenazoniummethosulphate under aerobic conditions (Nishikimi *et al*., 1972). The activity of erythrocytic glutathione peroxidase was measured by the method given by Hafeman *et al*. (1974), 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). Antioxidant levels [catalase, superoxide dismutase (SOD), lipid peroxidation (LPx), glutathione peroxidase (GPx), glutathione (GSH) and Vitamin E and C] were analyzed manually. The requisite stored plasma samples were evaluated for normal 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] by kits (Erba Manheim).

Isolation of RNA

0.1% DEPC treated and properly sterilized laboratory wares were 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 Reagent BD (Sigma-Aldrich, USA) for blood. 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 OD_{260}/OD_{280} ratio >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. After isolation of RNA and synthesis of cDNA by using Reverse transcriptase PCR (RT-PCR) in thermo cycler. Real time PCR was run at 60°C temperature using specific MT-2 primer and reference gene primer. The oligonucleotide sequences for MT-2 forward and reverse primers are 5′ AAAGATTGCAAGTGCGCCTC 3′ and 5′ CACTTGTCCGAAGCCCCTTT 3′ respectively. RPL4 was used as the reference gene (Nygard *et al*. 2007) and forward and reverse primer sequence are 5′ TTGGAAACATGTGTC GTGGG3′ and 5′ GCAGATGGCGTATCGCTTCT3′ respectively. qPCR was performed using aliquots of amplified cDNA and KAPA SYBR FAST qPCR Master Mix (2X) kit. Each reaction was performed in a 25 µl reaction volume containing 200 nM of each amplified primer and 1 ng of cDNA. Expression of MT-2 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-3 min; 40 cycles of 90°C-3 sec, 60°C-30 sec and 95°C-5 sec.

Statistical analysis

The data were analyzed using statistical package for social sciences (SPSS) software (version 16.0). Multiple comparison of data were carried out by using one way ANOVA and statistical comparison between means of different groups were carried out by independent t-test. The result of real time PCR was then calculated using:ΔCt = Ct [Target]-Ct [Housekeeping] and $\Delta\Delta$Ct = (ΔExp.) - (ΔControl). After using formula $2^{-\Delta\Delta Ct}$ we have calculated the fold change of expression of MT2 gene.

RESULTS AND DISCUSSION

Effect of vitamin E and Se supplementation on plasma level of heavy metals and micro minerals in buffalo

Blood samples were collected from two different buffalo farms, analyzed for heavy metals using inductively coupled plasma optical emission spectrometer. Buffaloes being kept in non exposed area were found to have lower heavy metal levels. The concentration of Cr, Cd, Ni, Pb, As was found to be 0.0204±0.001, 0.028±0.0009, 0.0076±0.0007, 0.11±0.0091, 0.04±0.0063 ppm respectively (Fig 1a). However, samples collected from highly exposed Buddha Nallha region were found to have elevated heavy metals. The concentration of Cr, Cd, Ni, Pb, As was found to be 0.7575±0.19, 0.0555±0.031, 0.22±0.04, 0.593±0.31, 0.5865±0.02 ppm respectively (Fig 1a). Animals were divided into control (non-exposed) and exposed groups. The latter group was supplemented with Vitamin E and Se @20ml/day for 30 days. Blood was collected on 0th, 15th and 30th day post supplementation from exposed group (n=10) and control group (n=10) on 0th day, result were compared by independent t-test using SPSS (Fig 1a, 1b).

After supplementation of Vitamin E and Se, heavy metals such as lead (2.817±0.756 ppm), chromium (1.1865±0.4536 ppm) and cadmium (0.075± 0.015 ppm) were found to be gradually increasing even after 30th day of supplementation (Fig 1a). Among the heavy metals studied post treatment, few of them showed considerable variation. Nickel (ppm) was found to be decreased significantly as compared to its initial level prior to treatment at 0th day (0.219±0.044) which decreased significantly ($p<0.05$) after 30th day supplementation (0.111±0.018). There wae non significant difference was observed in arsenic level after 30th day of supplementation (Fig 1a), however, the levles of arsenic showed increasing trend. Prior to the treatment, the levels of micro minerals (Zn, Cu, Co, Fe) in control group were observed significantly lower as compared to exposed group. Exposed group also found with sub normal levels (ppm) of micro minerals *viz*. Zn (3.8415±0.9759), Cu (1.1355±0.1374), Co (0.033±0.0037) which were found lowered than permissible limit (Fig 1a) but Fe (81.1005±9.4165) was reported to be in normal range (Fig 1b). It was found that, the levels of micro minerals (Zn, Cu and Co) remained sub normal (except iron), lowered than the permissible limit in blood even after 30 day of supplementation of Vitamin E and Se @ 20 ml/day.

Previous studies have reported that heavy metal concentrations were very high in agricultural soil, fodder and water in Punjab, as a result of that these heavy metals *via* the food chain were recorded in blood of buffalo and other ruminants. (Dhaliwal *et al*., 2016; Dash *et al*., 2016; Yeotikar *et al*., 2018).

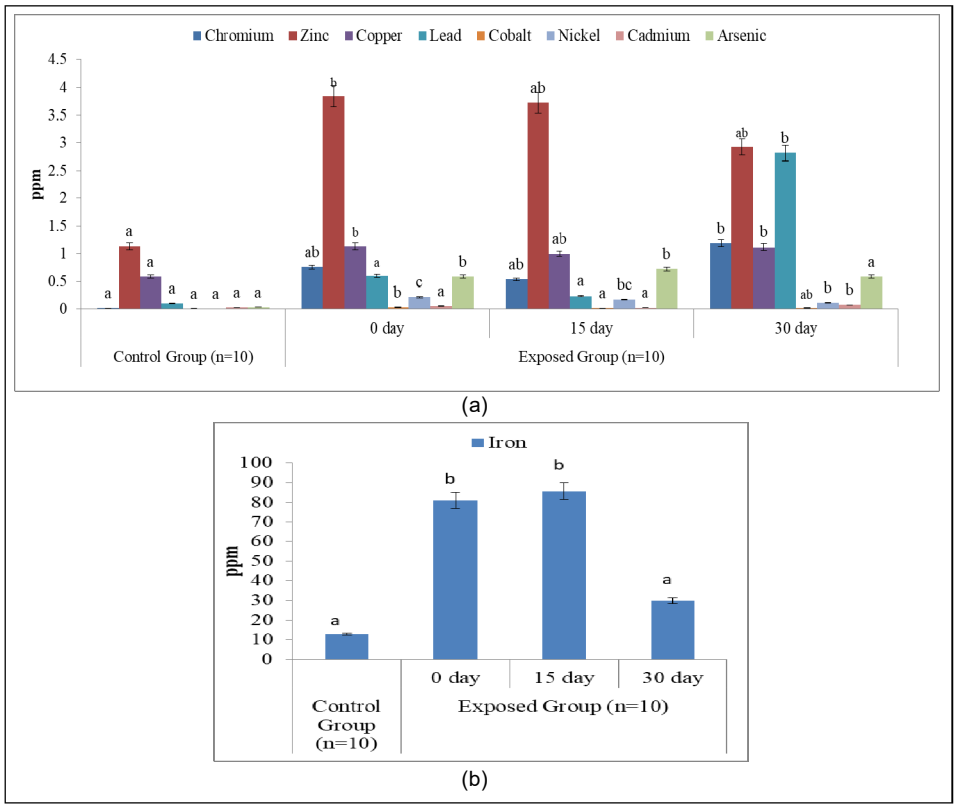

Fig 1: (a-b): Plasma concentration of heavy metal/micro mineral (mean±S.E.) in heavy metals exposed and control buffaloes supplemented with vitamin E and Se.

[a,b,c]Columns superscripted with different letters are significantly ($p<0.05$) different.

Several other studies in animals revealed that decrease in micro-minerals could ensue due to increase in heavy metal concentrations. One of the study conducted on pigs reported that dietary arsenic fed to the crossbred pigs resulted in decreased ($p<0.05$) serum Fe concentration but plasma Cu levels remained unaffected (Wang *et al*., 2006). Roy *et al*. (2009) reported non-significant decrease in blood Cu level in Arsenic (As) exposed goats (2 mg sodium arsenite/kg BW *i.e*. equivalent to 38 ppm). Dietary As caused a marked accumulation of Cu in the kidney of rats (Shiguang and Beynen 2000) and a reduction of Cu concentrations in the liver (Uthus 2001), plasma and blood cells (Elsenhans *et al*., 1987; Hunder *et al*., 1999). Thompson *et al*. (1991) reported that excess As might be attributed to direct toxic effect and secondary deficiency of other trace elements like Zn, Cu, Mn to precipitate indirect toxicity. Contrary to the present study, Roy *et al*. (2009) reported non-significant increase of plasma Zn level in As exposed goats. Their report suggested that the essential elements might have contributed to the protection of animals from the effects of heavy metal exposure, while their deficiency may aggravate toxicity.

Effect of vitamin E and Se supplementation on oxidative stress markers

A non-significant difference was observed in MDA level in exposed group (0th day) when compared to that of control group and significant decrease ($p<0.05$) in lipid peroxidation (nmol/mg Hb) was observed in exposed group after 15 days (2.139±0.1701) of supplementation and increased non-significantly after 30 days (2.5722±0.4844) of supplementation yet lower than the unsupplemented control group (Fig 2a). Decrease in MDA level significantly ($p<0.05$) on 15th day of supplementation might be protective effect of Vitamin E and Se from heavy metal stress. Non-significant increase of MDA on 30th day might be due to persistence of heavy metals in the system, which led to the production of free radicals and consequently lipid peroxidation. Study conducted on rats revealed that, feeding of mercuric chloride lead to increase in lung MDA but when supplemented with vitamin E plus mercuric chloride significant decrease in MDA was reported (Celikoglu *et al*., 2015).

Activity of antioxidant enzymes in unexposed control and heavy metal exposed buffaloes was noted before beginning of the supplementation trial. SOD, CAT, GPx on 0 day in control group was found to be 2.2792±0.1755, 0.0136±0.0012, 4.0662±0.122 U/mg and in exposed group was 2.763±0.5014, 0.1857±0.046 and 1.610597±0.205 U/mg (Fig 2b-2c) respectively. It was observed that SOD was elevated as compared to control but non-significant on 0th, 15th and 30th day of supplementation with Vitamin E and Se in contrary GPx and CAT were significantly high as compared to control (Fig 2b-2c).

Reduced glutathione level on 0th day in control and heavy metal exposed group was 2.6±0.017 and 4.431±1.0687 µg/ml respectively. It was found that after Vitamin E and Se supplementation the blood glutathione level increased significantly ($p<0.05$) after 30th day (9.7338±0.5486 µg/ml) (Fig 2d).

Non-enzymatic antioxidant vitamins E and C were estimated to monitored post treatment effects. Vitamin E level (Fig 3) was 9.7±0.0046 mg/dl on 30th day in exposed group post supplementation which was found to be significantly ($p<0.05$) higher as compared with control (3.7±0.0002 mg/dl). Plasma levels of Vitamin C was found significantly higher ($p<0.05$) prior to supplementation on 0th day in exposed group but non-significant increase was observed on 30th day after oral supplementation of Vitamin E and Se (Fig 2e).

Antioxidant enzymes has been considered to be the first line of cellular defense against oxidative damage. Nandi *et al*. (2005) reported the non-significant changes ($P>0.05$) in blood SOD and CAT activity in rats fed with as (10ppm) which was comparable with our studies. Some authors have been reported non-significant ($P>0.05$) decrease of SOD and CAT activity in goat kids fed with arsenic and arsenic with vitamin E for 90 days (Patel *et al*., 2009; Vaswani *et al*., 2010). Flora (1999) and Ramanathan *et al*. (2002) recorded significant increase ($p<0.05$) in SOD and CAT activity in brain

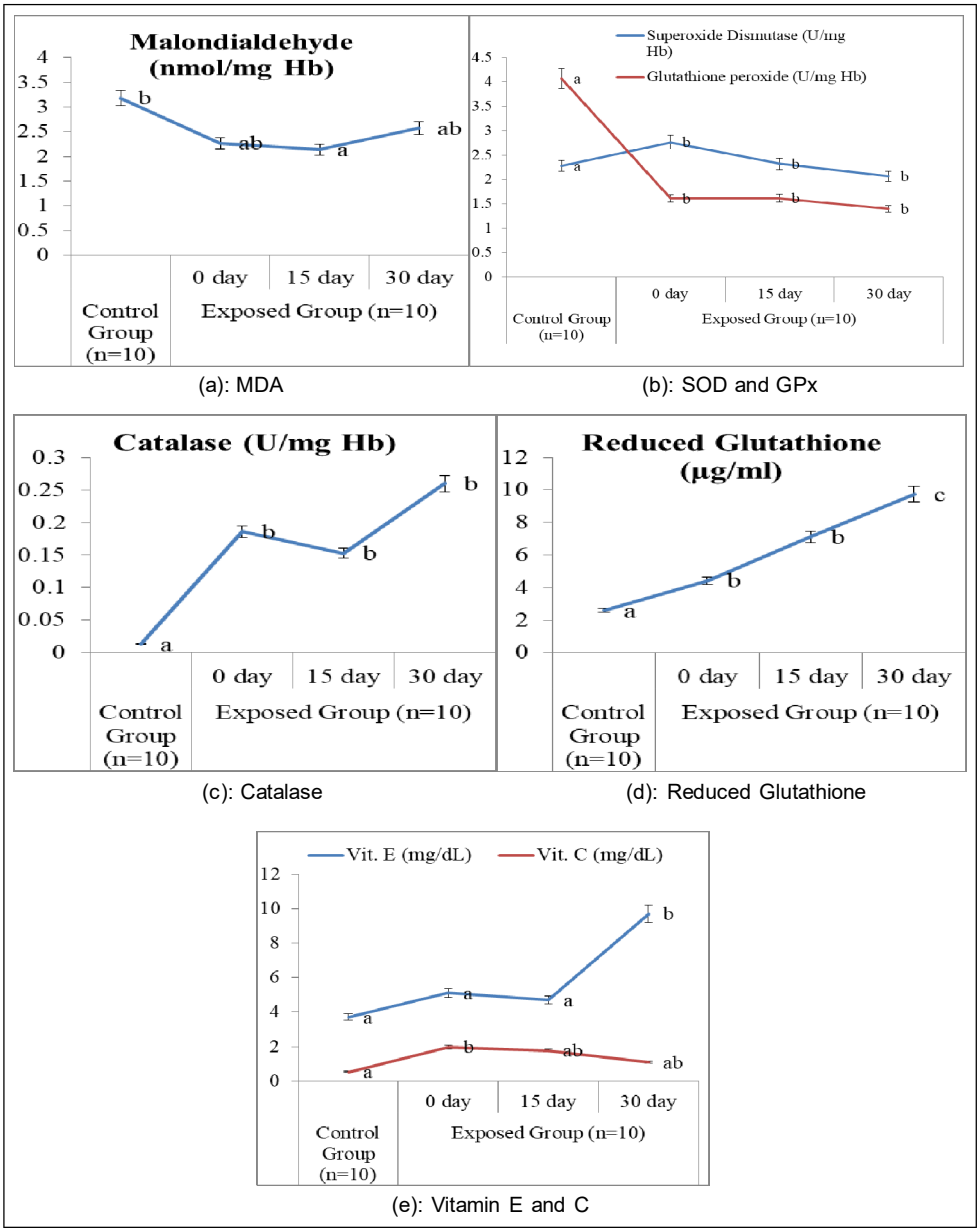

Fig 2: (a-e): Oxidative parameters (mean±S.E.) in control and exposed blood sample of buffaloes supplemented with Vitamin E and Se. [a,b,c]Columns superscripted with different letters are significantly ($p<0.05$) different.

tissue in As with α-tocopherol treated group when compared to exclusively As exposed rats which was found to be in agreement with the present study. Nandi *et al*. (2008) reported that the supplementation of ascorbic acid (an antioxidant) for 8 weeks restored blood SOD and CAT activities towards normalcy in As exposed rats, which is in accordance with our findings. Studies conducted on mice has revealed that there was significantly decrease in CAT, GSH-Px activities and GSH in nickel treatment. So when supplemented with vitamin E with or without vitamin c showed significant decrease in toxic effect and increase in antioxidant status (Dahdouh *et al.*, 2016).

Our findings indicated that, gradual increase in heavy metals in exposed buffaloes even though they were supplemented with Vitamin E and Se @ 20ml/day for 30 days. Drastic alterations in the antioxidant enzyme activity status was not noticed. These enzymes remained in uniform range that could be due to the effect of supplementation. In contrast to our finding, Karaboduk *et al*. 2015 reported that mercuric chloride exposure in mice led to decreased SOD, catalase, GPx activity as compared to control. But the reversal in the activity had been noticed after treatment with Vitamin E and Se. A significant rise in GPx, catalase, SOD and decreased in MDA were reported post treatment with Vitamin E and Se. Layachi and Kechrid (2012) have found that cadmium induced rats showed reduction in antioxidant activity which was increased by ameliorative effect of vitamin E and C.

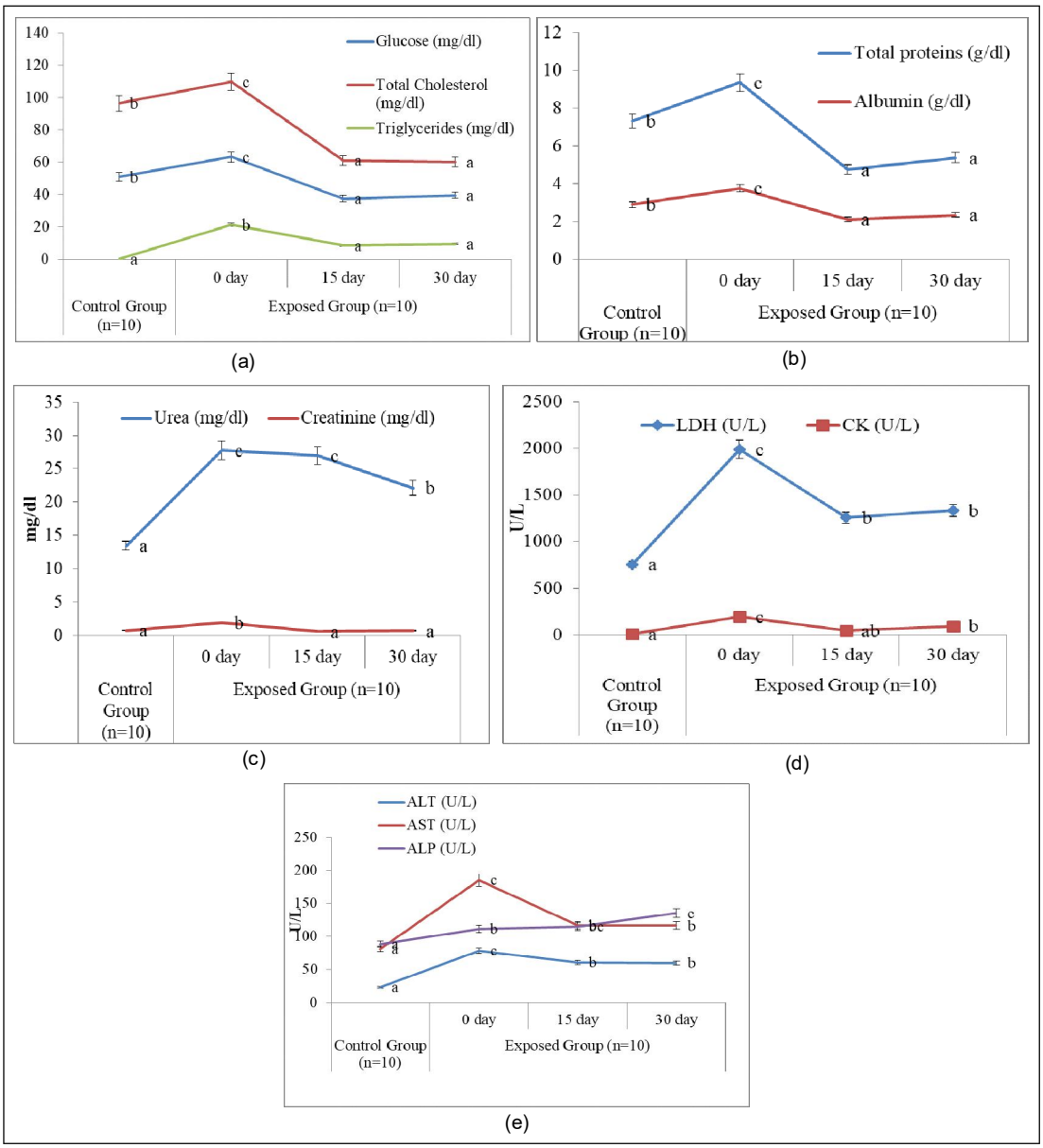

Fig 3: (a-e): Biochemical parameters (mean±S.E.) in control and exposed blood sample of buffaloes supplemented with Vitamin E and Se. [a,b,c]Columns superscripted with different letters are significantly ($p<0.05$) different.

Similar study done by Al-Attar (2010) on mice revealed that when treated with vitamin E increased activity of GSH and SOD was observed, which was comparable to our present findings. Balanced activity in SOD, CAT, GPx and increase in GSH level after vitamin E supplementation might be due to its role in scavenging free radicals *i.e.* superoxide, hydroxyl radicals, peroxyl and alkoxyl radicals resulted from damage caused by heavy metals, before they attack membrane lipids leading to removal of cell damaging free radicals.

Effect of vitamin E and Se supplementation on biochemical profile

The plasma glucose, cholesterol, triglycerides, total protein, albumin, urea and creatinine in control animals were found to be 50.878±0.3813, 96.422±0.0961, 11.47±0.0494, 7.314±0.1715, 2.904±0.0676, 13.432±0.036 and 0.76±0.051 mg/dl respectively (Fig 3a-c). The activity of non-antioxidant enzymes, AST (81.442±0.4547 U/L), ALT (23.372±0.1589 U/L), ALP (88.578±0.1605 U/L), LDH (755.2±38.9479 U/L) and CK (6.64±0.0707 U/L) was found to be in normal range (Fig 3d-e).

Biochemical parameters in the heavy metal exposed group were found to be elevated before supplementation of vitamin E and Se. On day 0 level of glucose, total cholesterol, triglycerides was 63.239±1.9748, 109.59±4.0203 and 21.384± 2.6023 mg/dl respectively (Fig 3a). After supplementation with Vitamin E and Se @20ml/day to heavy metal exposed group initial analysis of biochemical profile revealed significantly higher level of plasma blood glucose, total cholesterol, triglycerides. Total protein (9.356±0.2708 g/dl) and albumin (3.766±0.1767 g/dl) was also found to be significantly high on day 0 (Fig 3b). Activity of liver enzyme like AST (185.543±11.972 U/L), ALT (78.647±7.45 U/L) and ALP (111.505±4.1775 U/L) was found to be increased when compared with control or non-exposed group (Fig 3e). Our findings have indicated that heavy metals exposed group supplemented with Vitamin E and Se @20ml/day resulted in significant decrease in Glucose, total cholesterol and triglycerides as compared to the initial day 0^{th} status. Supplementation of Vitamin E and Se also affected ($p<0.05$) the activity of ALT, AST, LDH, albumin, urea, and creatinine. Total protein concentration was found to be decreased ($p<0.05$) after 30^{th} day of supplementation (Fig 3b). Studies conducted on mice has revealed that serum glucose, total protein, ALT, AST and ALP was significantly increased in nickel treated mice (Dahdouh *et al.*, 2016) which was in agreement with our present findings.

Layachi and Kechrid (2012) have reported oxidative liver injury in rats exposed to cadmium characterized by increased serum glucose, ALT and ALP activities. Similar studies have been conducted by Das *et al.* (2012), goat supplemented with arsenic (As) reported significant increased ($p<0.05$) AST and ALT activities, vitamin E supplementation at higher doses showed a protective effect ($p<0.05$). Plasma total protein was found to be decreased ($p<0.05$) but creatinine increased periodically in all As supplemented groups.

Effect of vitamin E and Se supplementation on expression study of metallothionein (MT-2)

Previous studies have been indicated the role of metal binding protein, metallothionein (MT) in heavy metal metabolism. The alternations in expression pattern of MTs could be a marker in determining heavy metal exposure in animals. Keeping this in view the abundance in animals, MT-2 expression was analyzed in blood cells. MT-2 expression was studied in control and heavy metal exposed buffaloes. Exposed buffaloes, which were supplemented with vitamin E and Se, corresponding expression of MT-2, was monitored during supplementation and after completion of the trial. The fold change expression of MT-2 after supplementation was 5.507±0.047 on 0th and 7.8349±1.12 on 30^{th} day which was found to be significantly ($p<0.05$) high as compared to control (Fig 4). The concentration of few of the heavy metals fluctuated during the supplementation period. It was observed that some of the heavy metals decreased on 15^{th} day after supplementation that resulted in non-significant decrease in MT-2 fold expression but at the end of trial after 30^{th} day of supplementation level of heavy metals were also increased. As vitamin E and Se was supplemented level of heavy metal remained unabated and despite supplementation up-regulation of MT-2 gene expression was reported.

Studies conducted on mice revealed that arsenic exposure resulted in up-regulation of MT-2 gene expression which could be due to decrease in micro-minerals and increase in heavy metal concentration (Kreppel *et al.*, 1993). No studies are available in buffaloes that could correlate metallothionein expression pattern with heavy metals. We observed proportionate elevation in MT-2 expression despite of feeding Vitamin E and Se supplementation, which could imply that feeding vitamin E and Se might not be affecting the cellular accumulation of heavy metals. Studies conducted on Grass Carp (fish) observed that administration

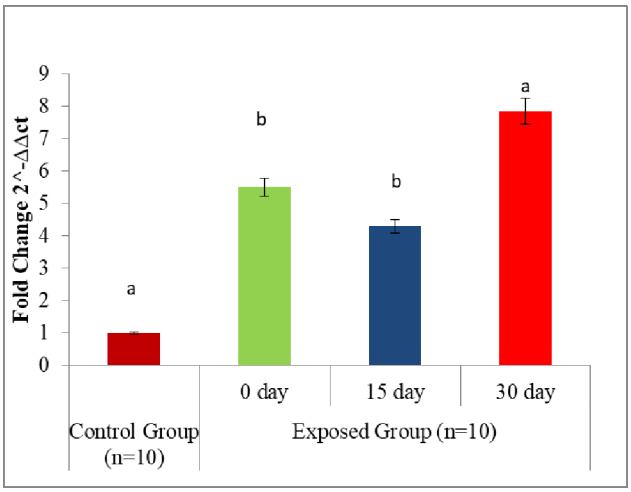

Fig 4: Fold change expression of metallothionein (MT-2) in control and heavy metals exposed buffaloes supplemented with Vitamin E and Se on 0^{th}, 15^{th} and 30^{th} days.

with Cadmium (Cd) resulted in liver toxicity, which was expressed as increase in malondialdehyde (MDA) percentage of hepatocyte apoptosis and apoptosis-related gene mRNA transcript expression. Treatment with vitamin E and MT lead to protection against Cd-induced hepatotoxicity by decreasing Cd contents, lipid peroxidation, and histological damage and reducing the percentage of hepatocyte apoptosis by regulating related mRNA transcript expression. Therefore, it was found that Vitamin E and MT treatments could moderate/lessen Cd-induced hepatotoxicity through their antioxidative and antiapoptotic effects. MT had more powerful effect than vitamin E. (Duan *et al*., 2018).

CONCLUSION

It can be concluded that Vitamin E and Se supplementation to heavy metal exposed buffaloes may ameliorate oxidative stress and biochemical response as indicated by reduction in malondialdehyde and increased reduced glutathione level. However, the levels of heavy metals in blood remain unaffected despite of Vitamin E and Se supplementation.

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