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AMELIORATIVE ROLE OF SELENIUM AND ZINC AGAINST REPRODUCTIVE TOXICITY DUE TO EXPOSURE TO A MIXTURE OF HEAVY METALS LEAD, CADMIUM AND ARSENIC IN MALE ALBINO RATS

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ABSTRACT: Introduction: Reproductive organs are the major target of heavy metal toxicity; oxidative stress plays a vital role in induction of such toxicity. Toxicity due to chronic exposure to a low dose mixture of three heavy metal lead (Pb), cadmium (Cd) and arsenic (As) and ameliorative potentials of two antioxidant micronutrients selenium (Se) and zinc (Zn) were evaluated in male albino rats. **Materials and Methods:** The animals were divided into four equal groups: i) control, ii) metal mixture, iii) mixture plus Se and iv) mixture plus Zn treated groups; all animals received respective treatment for ninety consecutive days. Body weight, relative organ weights, haematological and serum biochemical parameters, sperm characteristics, Steroidogenic enzymes, serum testosterone level, oxidative stress parameter sand testicular histology were evaluated. **Results:** There were significant alterations in body weight, relative organ weight, hematological and serum biochemical parameters in mixture treated groups indicative of systemic toxicity. There was marked decline in male sexual parameters; changes in oxidative stress parameters revealed associated oxidative stress. Supplementation with both Se and Zn along with metal mixture showed significant improvement in all the parameters. **Conclusion:** The study revealed that chronic exposure to a low dose mixture of three heavy metals Pb, Cd & As induced toxicity to the biological systems in male rats due to excess generation of free radicals and impairment of anti-oxidant defense mechanism. Supplementation with micronutrients Se and Zn could ameliorate the deleterious effects of exposure to such mixture.

INTRODUCTION: Increasing exposure to environmental pollutants is major concern faced by almost all living organism on the earth.

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Unrestricted use of chemicals of various forms in order to sustain ever-increasing developmental need are posing serious threats to the general and public health conditions in both developed and developing countries [1].

Out of different chemical pollutants associated with industrialization and anthropogenic activities most important are the heavy metals which form the natural compounds of the earth's crust. Sources of heavy metals as environmental pollutants include

ground water, industrial effluents, sewage discharge, insect or disease-causing agents applied to crops and many others [2]. Out of 90 naturally occurring heavy metals, As, Cd, Cr, Cu, Hg, Ni, Pb and Zn are listed as the most potent environmental pollutants even in minor quantity [3]. Most of the toxicological evaluations of these heavy metals so far are restrict to a single metal and at a very high exposure dose. However, both human and animal are being exposed to combination to various metals and often exposed to a chronic low exposure dose. In view of this, during recent times, the attention of scientific studies have focused more on assessment of combined low dose toxicity of heavy metals owing to their hazardous nature and potential presence in the natural sources in combinations [4]. In our laboratory, effect of combined low dose chronic exposure to a mixture of Pb, Cd and as was evaluated in female rats [5]. All these metals pose a great public health concern because of their high degree of toxicity and increase frequency of occurrence in combination. Lead is a ubiquitous environmental and industrial pollutant with a potent nephrotoxic effect.

It is known to reduce growth and impair reproductive function, causes splenomegaly, damage to haemopoietic, central and peripheral nervous system. Chronic exposure to lead is aggregated with various neurological, hematological, immunological and hepatic disorders. It is reported to affect male reproductive system adversely causing altered spermatogenesis and testicular degeneration. Lead causes over production of reactive oxygen species (ROS), alters many biological activities at both molecular and cellular level inducing both systemic and organ-specific toxicity [6]. Exposure to cadmium has also been reported to elicit diverse toxic effects including nephrotoxicity, carcinogenicity, teratogenicity, and immunotoxicity. It causes reproductive toxicity either directly targeting gonads or indirectly by interfering with the hypothelamopituitary-gonadal axis [7]. Arsenic, a potent water contaminant, present in high concentration in water of South East Asian countries including India, is long being reported to be associated with developmental toxicity, cardiovascular diseases and cancer. It causes male reproductive toxicity and induces ovarian and uterine malfunction [8]. However, most of these studies lack detailed information on cofounders and their probable mode of action. Though exact mechanism of toxicity caused by combined low dose chronic exposure of these metals is still unknown, oxidative stress caused by each of these metals might be associated with their combined toxicity. Most of these metals might influence enzymatic antioxidant system by influencing the biological micro environment required for action of such enzymes [9]. Zinc and selenium are the two most important bio elements which have recently reported to have potential antioxidant effects against heavy metal-induced oxidative stress. Both Zn and Se were reported to provide protective effect in case of individual exposure to Cd, Pb and as in reproductive and other systems [10-12]. However, the protective effects of these anti-oxidants in condition of low dose chronic exposure to mixture of these heavy metals in male rat have not been evaluated so far. In the present study, an attempt has been made to evaluate toxic dynamic interactions of Pb, Cd and as in male rats and to evaluate the protective role of Se and Zn in them.

MATERIALS AND METHODS:

Animal: Male Wister albino rats (160-170 gm) were used for the study. The rats were housed in the animal house facility of Tripura University in clean and disinfected plastic cages and allowed to acclimatize to laboratory environment for seven days before the beginning of the experiment. Animals were maintained under controlled conditions of temperature (25 ± 2 ºC), humidity (50 ± 15%) and normal photoperiod (12-12 h light-dark cycles). The animals were fed with standard rat diet and allowed to drink water ad libitum. Ethical clearance for the study was obtained from Animal ethical committee of Tripura University (TU/IAEC/2015/X/1).

Test Chemicals: The water-soluble salts of cadmium (Cadmium chloride, $CdCl_2$), lead (Lead acetate; $Pb(C_2H_3O_2)_4$); arsenic (Sodium arsenate; Na_3ASO_4); Selenium (sodium selenate; Na_2SeO_4) and zinc (Zinc chloride; $ZnCl_2$) used for the study were obtained from Himedia Laboratory Pvt. Ltd., India .

Commercial kits for estimation of glucose, total protein, albumin, cholesterol, urea, creatinin serum glutamate pyruvate transaminase (SGPT), serum

glutamate oxaloacetate transaminase (SGOT) was purchased from coral system. Magnesium chloride, sodium hydroxide, p-nitrophenol, nitric acid, sodium nitrate was purchased from Sisco Research laboratory. Eosin solution was purchased from central drug home Pvt. Ltd and Harris heamatoxiline solution from Merk laboratory Pvt. Ltd.

Study Design: The animals were exposed to test chemicals for ninety (90) consecutive days. The animals were divided into four groups of six rats each and received test chemicals orally through gauges as follows:

Group I: Control/ water 2 ml/day.

Group II: Metal mixture (Na_3ASO_4 – 38.0 ppm; $Pb(C_2H_3O_2)_4$ – 22.0 ppm; $CdCl_2$- 9.8 ppm).

Group III: Metal mixture + Selinium (Na_2SeO_4- 10 ppm).

Group IV: Metal mixture + Zinc ($ZnCl_2$- 20 ppm).

During treatment, body weight and food intake were recorded at the interval of 15 days. At the end of treatment period, animals were sacrificed by cervical dislocation after an overnight fast and blood samples were collected immediately into tubes without anticoagulant.

A portion of fresh blood kept in heparinized tubes was used for various haematological studies. The blood vials were kept at room temperature for clotting and serum was separated. The separated serum was allocated into aliquots and stored at -20 ºC for biochemical analysis. The liver, kidney, testis, epididymis, seminal vesicle, prostate and vas deferens were removed, dissected out, weighted washed in ice-cold saline and used for further analysis of different parameters.

Hematological Analysis: Haematological parameters like total RBC, total WBC and platelet count and hemoglobin concentration were analyzed in a hematological analyzer (Aspen, PE 6800).

Biochemical Analysis: To analyze the hepatic function and renal profile biochemical parameters such as serum glucose, serum protein, albumin, cholesterol, urea, creatinine, uric acid, SGPT and SGOT were measured by using commercial kits in a biochemical analyzer. Levels of enzyme alkaline phosphatase (ALP) and acid phosphate (ACP) were measured by the methods of Bessey *et al.* [12]

Analysis of Sperm Characteristics: Sperm were obtained from fresh right epididymis and sperm quality was counted using a hemocytometer and light microscopy. Sperm motility was determined on the basis of visual estimation of percentage of motile sperm under light microscopy 400x magnification [13]. The sperm abnormality was noted by visualizing abnormalities in head or tail under light microscopy and expressed as percentage.

Evaluation of Steroidogenic Parameters in Testis: Testicular cholesterol level was estimated by the method of Zlakis *et al* [1]. The activity of testicular steriodogenic enzymes Δ^5-3 β hydroxysteroid dehydrogenase(Δ^5-3β HSD) and 17β hydroxysteroid dehydrogenase (17β HSD) were assessed by the method of Talay *et al* [14].

Testicular Hormonal Assay: Plasma testosterone concentration was measured by enzyme immunoassay using commercial kit.

Lipid per Oxidation and Antioxidant Status: Lipid peroxidation level in testis was measured by a method using thiobarbituric acid reactive substance (TBARS) and estimation of protein carbonyls [15]. The activity of antioxidant enzyme superoxide dismutase (SOD) was determined by the method of Marklund and Marklund [16].

The enzyme catalase (CAT) activity was determined according to the method of Aebi *et al* [17]. Analysis of reduced glutathione (GSH) level was done by the method of Ellaman *et al.* [18] The measurement of activity of glutathione S-transferase (GST) was according to Hgib *et al.* [19] and glutathione peroxidase activity (GPx) was based on method of Pogdia and Valentine [20]. Protein content of the supernatant was estimated by using bovineserum albumin as standard [21].

Histopathological Examination of Testis: For microscopic evaluation, testis were fixed in 10% formal saline, embedded in paraffin, sectioned at 5 µm and stained with hematoxylin/eosin and studied under light microscope at 400x magnification.

Statistical Analysis: The data were presented as mean ± standard error of the mean (SEM). The data with normal distribution and homogeneous variance were analyzed for one-way analysis of variance (ANOVA) and ‘t’ test using statistical

software (SPSS 16.0), a P value ≤ 0.05 was considered statistically significant.

RESULTS: Analysis showed that treatment with metal mixture resulted in decline in percentage gain in body weight and amount of food consumption. Weight of male reproductive organs reduced at the end of treatment in animals of this group in comparison to the control group. However, there was a significant increase in weight of both liver and kidney in animals of mixture treated groups.

Supplementation with both Se and Zn reversed these changes; body weight gain, food consumption, relative weight of reproductive organs and both liver and kidney in animals of these two groups were nearer to the animals of control group **Table 1.**

TABLE 1: AMELIORATIVE EFFECT OF SELENIUM (SE) AND ZINC (ZN) ON METAL MIXTURE INDUCED TOXICITY ON BODY WEIGHT GAIN AND WEIGHT OF VITAL ORGANS OF ANIMALS

Parameters	Group			
	Group I	Group II	Group III	Group IV
Initial body weight (g)	177.3±2.5	172.1±1.8	172.6±1.7	176.0±2.1
Final body weight (g)	270.3±4.1	263.0±7.0	258.7±7.0	270.2±4.1
Body weight gain (%)	20.8±0.7	16.6±0.6a**	19.9±0.9b#d**	20.1±0.6c#e**f#
Food consumption (g/rat/day)	25.8±0.5	22.6±0.4a**	24.8±0.7b#d*	25.3±0.7c#e*f#
Weight of liver (g/100g/bw)	3.61±0.1	4.08±0.1a*	3.44±0.02b#d*	3.55±0.2c#e*f#
Weight of kidney (g/100g/bw)	0.44±0.01	0.55±0.02a**	0.40±0.02b#d*	0.43±0.03c#e**f#
Weight of testis (g/100g/bw)	2.05±0.1	1.53±0.1a**	1.92±0.1b#d*	2.06±0.1c#e**f#
Weight of epididymis (mg/100g/bw)	192.1±1.2	187.4±1.2a**	189.1±2.3b#d*	191.3±0.9c#e*f#
Weight of seminal vesicle (mg/100g/bw)	164.5±1.6	160.4±0.4a*	161.8±0.4b#d*	162.5±0.4c#e**f#
Weight of prostate (mg/100g/bw)	229.1±0.4	223.8±0.7a**	227.3±1.3b#d*	228.3±1.6c#e*f#

Group I = Control group; Group II = metals mixture treated group; Group III = mixture treated and Se- Supplemented group; Group IV = mixture treated and Zn- Supplemented group. a= Group I *vs* Group II; b= Group I *vs* Group III; c=Group I vs Group IV; d= Group II *vs* Group III; e = Group II *vs* Group IV; f = Group III *vs* Group IV; * = p<0.05; ** = p<0.01; *** = p<0.001, # = Non significant. Values are expressed as mean ± SEM; animals (n) = 6/group.

Treatment with mixture of heavy metals (Pb, Cd & As) resulted in significant decrease in total RBC count (TRBC), Hb concentration, platelet count. There was a significant increase in total WBC count (TWBC) and clotting time in mixture treated rats. Both Se and Zn supplementation along with a mixture of metals prevented changes in heama-tological parameters **Table 2.**

TABLE 2: EFFECT OF SUPPLEMENTATION WITH SELENIUM (SE) AND ZINC (ZN) ON METAL MIXTURE INDUCED TOXICITY ON HEMATOLOGICAL PARAMETERS

Parameters	Group			
	Group I	Group II	Group III	Group IV
Total RBC count (millions/mm^3)	6.37±0.1	5.3±0.03a**	5.99±0.2b#d**	6.01±0.3c#e*f#
Haemoglobin (gm %)	13.85±0.4	11.97±0.4a*	13.09±0.3b#d#	13.20±0.1c#e**f#
Platelets count (lakhs/mm^3)	792.5±9.6	729.2±12.6a**	774.0±16.3b#d*	769.4±9.07c#e*f#
Total WBC count (thousand/mm^3)	6.37±0.1	7.05±0.2a**	6.29±0.2b#d**	6.28±0.3c#e*f#
Clotting time (minute)	4.5±0.1	5.2±0.1a**	4.28±0.1b#d**	4.5±0.1c#e*f#

Group I = Control group; Group II = metals mixture treated group; Group III = mixture treated and Se- Supplemented group; Group IV = mixture treated and Zn- Supplemented group. a= Group I *vs* Group II; b= Group I *vs* Group III; c=Group I *vs* Group IV; d= Group II *vs* Group III; e = Group II *vs* Group IV; f = Group III *vs* Group IV; * = p<0.05; ** = p<0.01; *** = p<0.001, # = Non significant. Values are expressed as mean ± SEM; animals (n) = 6/group.

Results of serum biochemical parameters revealed supplementation with both Se and Zn significantly prevented the increase in levels of glucose, cholesterol, urea, uric acid, creatinin and decrease in levels of total protein and albumin in mixture treated group. The activities of enzymes SGPT and SGOT increased significantly in mixture treated group in comparison to control group. Similarly, activities of enzymes ALP and ACP also increased significantly due to exposure to the metal mixture. All these effects were reversed nearer to the control group when Se and Zn were supplemented along with metal mixture **Table 3.**

TABLE 3: EFFECT OF SUPPLEMENTATION WITH SELENIUM (SE) AND ZINC (ZN) ON METAL MIXTURE INDUCED TOXICITY ON SERUM BIOCHEMICAL PARAMETERS

Parameters	Group			
	Group I	Group II	Group III	Group IV
Serum glucose (mg/dl)	106.7±2.0	121.5±3.5a**	109.4±2.3b#d*	108.2±2.8c#e*f#
Protein (g/dl)	7.43±0.1	5.37±0.1a**	7.08±0.2b#d**	6.91±0.2c#e**f#
Albumin (mg/dl)	4.37±0.1	3.29±0.1a**	4.26±0.1b#d**	4.27±0.1c#e**f#
Cholesterol (mg/dl)	63.6±2.2	73.2±0.9a**	64.3±0.5b#d**	63.4±1.1c#e**f#
Urea (mg/dl)	44.4±1.1	57.29±1.5a**	45.93±0.9b#d**	43.9±0.7c#e**f#
Uric acid (µmol/L)	329.2±2.6	442.3±4.8a*	327.4±3.8b#d**	325.1±1.8c#e**f#
Creatinine (mg/dl)	0.68±0.01	1.47±0.05a**	0.63±0.04b#d**	0.63±0.03c#e**f#
SGOT (U/ml)	64.16±1.4	73.04±0.9a**	63.34±1.0b#d**	63.40±1.0c#e**f#
SGPT (U/ml)	51.25±1.2	57.71±0.9a**	52.59±0.9b#d*	51.36±0.7c#e**f#
ALP (U/ml)	5.51±0.05	7.40±0.05a**	5.36±0.06b#d**	5.44±0.05c#e**f#
ACP (U/ml)	2.48±0.1	3.14±0.02a**	2.46±0.08b#d**	2.42±0.05c#e**f#

Group I = Control group; Group II = metals mixture treated group; Group III = mixture treated and Se- Supplemented group; Group IV = mixture treated and Zn- Supplemented group. a= Group I *vs* Group II; b= Group I *vs* Group III; c=Group I *vs* Group IV; d= Group II *vs* Group III; e = Group II *vs* Group IV; f = Group III *vs* Group IV; * = p<0.05; ** = p<0.01; *** = p<0.001, # = Non significant. Values are expressed as mean ± SEM; animals (n) = 6/group. SGOT = Serum Glutamate Oxalacetate Transaminase; SGPT = Serum Glutamate Pyruvate Transaminase; ALP = alkaline phosphatase; ACP = acid phosphatise.

Administration of metal mixture in male rats significantly reduced sperm concentration, sperm motility and increased percentage of dead and abnormal sperm as compared to the control group.

Supplementation with Se and Zn along with the metal mixture could restore the concentration and motility of sperm and reduced the percentage of dead and abnormal sperms **Table 4.** The activities of steroidogenic enzymes Δ^5-3β HSD and 17β HSD reduced due to administration of metal mixture. Along with this, there was a significant increase in testicular cholesterol levels in animals of this group in comparison to the control animals. There was a decrease in serum levels of steroid hormone testosterone in mixture treated animals.

Supplementation with both Se and Zn increased the activities of both the steroidogenic enzymes, reduced the levels of cholesterol in testis and increased serum levels of male sex hormone testosterone **Table 4.**

TABLE 4: EFFECT OF SUPPLEMENTATION WITH SELENIUM (SE) AND ZINC (ZN) ON METAL INDUCED TOXICITY ON SPERM PARAMETERS, STERODOGENIC PARAMETERS (TESTIS) AND SERUM HORMONE

	Group I	Group II	Group III	Group IV
Sperm concentration (million/epidiaymis)	28.29±0.51	21.33±0.41a**	28.26±0.31b#d**	28.22±0.37c#e**f#
Sperm Motility (%)	85.18±0.46	63.88±1.10a**	84.14±0.50b#d**	85.22±0.35c#e**f#
Sperm abnormalities (%)	2.71±0.17	8.01±0.13a*	2.54±0.20b#d**	2.42±0.14c#e**f#
Testicular Cholesterol (mg/gm tissue)	13.55±0.1	17.42±0.2a**	13.29±0.2b#d**	13.28±0.15c#e**f#
3β-HSD (Δ OD/min/mg protein)	37.74±0.5	20.44±.03a**	36.31±0.6b#d**	36.83±0.4c#e**f#
17β-HSD (Δ OD /min/mg protein)	37.99±0.5	21.00±0.3a**	36.81±0.4b#d**	37.45±0.4c#e**f#
Testosterone (ng/ml)	3.75±0.07	2.37±0.09a**	3.59±0.07b#d**	3.49±0.07c#e**f#

Δ5 3β hydroxysteroid dehydrogenase; 17β – hydroxysteroiddehydrogenase .Group I = Control group; Group II = metals mixture treated group; Group III = mixture treated and Se- Supplemented group; Group IV = mixture treated and Zn- Supplemented group. a= Group I *vs* Group II; b= Group I *vs* Group III; c=Group I *vs* Group IV; d= Group II *vs* Group III; e = Group II vs Group IV; f = Group III *vs* Group IV; * = p<0.05; ** = p<0.01; *** = p<0.001, # = Non significant. Values are expressed as mean ± SEM; animals (n) = 6/group.

There was a significant increase in the levels of MDA and protein carbonyl, indicators of lipid peroxidation, in animals treated with mixture of heavy metals. Along with this, there was a significant decline in activities of antioxidant enzymes SOD, CAT, GST, GPx and levels of GSH in these animals. Supplementation with Se and Zn improved the condition by reducing levels of MDA and protein carbonyl; and increasing the activities of enzymes SOD, CAT, GST,GPx and levels of GSH **Table 4.**

TABLE 4: EFFECT OF SUPPLEMENTATION WITH SELENIUM (SE) AND ZINC (ZN) ON METAL MIXTURE INDUCED TOXICITY ON TESTIS OXIDATIVE AND ANTIOXIDANTS MARKERS

Parameters	Group			
	Group I	Group II	Group III	Group IV
MDA concentration (nmol/mg protein)	29.94±0.6	32.60±0.6a*	29.52±0.6b#d**	29.34±0.7c#e**f#
Protein carbonyl (nmol/ mg protein)	0.69±0.02	0.78±0.02a*	0.68±0.03b#d*	0.67±0.01c#e**
SOD (µmol/mg protein)	3.25±0.13	2.42±0.15a**	3.13±0.13b#d**	3.16±0.13c#e**f#
CAT (µmol H_2O_2 consumed /min /mg protein)	9.28±0.14	8.43±0.14a**	9.19±0.14b#d**	9.08±0.04c#e**f#
GST (µmoles/min/mg protein	2.82±0.02	2.61±0.09a*	2.80±0.02b#d*	2.81±0.01c#e*f#
GSH (µmol/mg protein)	3.03±0.25	1.68±0.11a**	2.88±0.08b#d**	2.89±0.03c#e*f#
GPX (unit/mg protein)	21.61±0.3	16.67±0.34a**	20.93±0.3b#d**	21.57±0.25c#e*f#

MDA = Malondialdehyde; GST = Glutathione S transferase; SOD = Superoxide Dismutase; CAT = Catalase; GSH = Reduced glutathione; GPx = Glutathione peroxidase. Group I = Control group; Group II = metals mixture treated group; Group III = mixture treated and Se- Supplemented group; Group IV = mixture treated and Zn- Supplemented group. a= Group I *vs* Group II; b= Group I *vs* Group III; c=Group I *vs* Group IV; d= Group II *vs* Group III; e = Group II *vs* Group IV; f = Group III *vs* Group IV; * = p<0.05; ** = p<0.01; *** = p<0.001, # = Non significant. Values are expressed as mean ± SEM; animals (n) = 6/group.

Histopathology of testis in mixture treated group showed degenerated seminiferous tubules, reduced number of leydig cells and spermatozoa in tubules. Supplementation with Se and Zn showed almost normal architecture of the testis in comparison to metal mixture treated group **Fig. 1.**

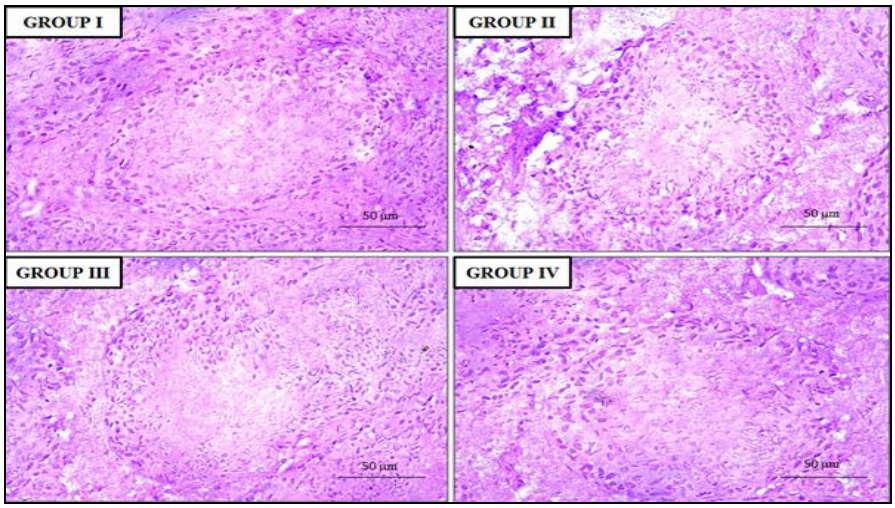

FIG. 1: TESTICULAR SECTION OF RATS FROM THE FOUR EXPERIMENTAL GROUPS STAINED WITH H & E STAIN AND VIEWED UNDER 40X MAGNIFICATION (SCALE BAR=50 MM). GROUP I- CONTROL GROUP; GROUP II- METAL MIXTURE TREATED GROUP; GROUP III- SE SUPPLEMENTED GROUP AND GROUP IV- ZN SUPPLEMENTED GROUP

DISCUSSION: The present study was carried out to determine the protective capacity of both selenium (Se) and zinc (Zn) to restore the damage caused to reproductive and other systems in male rats exposed chronically to a low dose combination of heavy metals Pb, Cd and As. Our observations of decline in percentage gain in body weight, amount of food consumption, relative weight of male reproductive organs and an increase in weight of liver and kidney clearly indicated a deleterious effect of metal mixture on general as well as reproductive health of the treated animals. Our previous study in female rats also showed similar effects on reproductive and other organ systems of the treated animals [22].

Exposure to heavy metals is generally known to reduce body weight gain by compromising food efficiency [23]. Both hepatomegaly and increase in weight of kidney are the signs of body's adaptive mechanism to combat the general toxic effect of the metal mixture [24]. Restoration of percentage gain in body weight and weight of other organs in both Zn and Se supplemented animals revealed the protective role of both the micronutrients against such toxicity. Results of our hematological study revealed the disturbance in hematopoietic system with decrease in TRBC count, platelet count and Hb concentration. These may lead to anemia, coagulation disorders and other hemorrhagic disturbances in treated animals.

These findings are in agreement with findings of Nicoli *et al.,* which showed that Pb and Cd intoxication leads to decrease erythrocytes, hemoglobin and hematocrit [25]. Arsenic is often taken up by RBC and WBCs upon absorption, leading to hematological changes such as microcytic anemia, elevated eosinophil and basophilic stippling of RBC [26]. Kenston *et. al.,* in their study with mixture of eight common heavy metals (Zn, Cu, Mn, Cr, Ni, Cd, Pb and Hg) observed abnormalities in haematological system of treated male and female rats [27]. In our study, the effects on hematological system due to exposure to the mixture of heavy metals (Pb, Cd and As) were ameliorated by simultaneous supplementation of animals with Se and Zn. There is a change in the energetic profile parameters with an increase in glucose and cholesterol and decrease in protein and albumin in mixture treated animals.

The increase in serum glucose is a common finding in heavy metal toxicity which is usually connected with inhibition of insulin release from beta cells and or block of glucose utilization by cells, might be associated with altered glucagon secretion [28]. Elevation of cholesterol level might be associated with activation of cholesterol synthetic enzymes with simultaneous suppression of cholesterol catabolic enzymes [29]. Decrease in serum protein and albumin levels might be due to the changes in protein synthesis and or metabolism in mixture of heavy metals treated animals**.** Increase in levels of urea, uric acid and creatinine along with increase in activities of SGOT and SGPT is indicative of both hepatotoxic and nephrotoxic effects of the metal mixtures in treated animals. This is further supported by the observation of increased activities of hepatic and renal enzymes ACP and ALP [30].

Supplementation with either Se or Zn could reverse both these systemic toxic effect and impairment of function of liver and kidney due to treatment of mixture of heavy metals. Chronic exposure to mixture of heavy metals showed a decrease in testicular weight and weight of accessory sex organs. The decrease in testicular weight is indicative of altered spermatogenesis as observed in histopathological examination of testicular sections. This is supported by our observations on sperm concentration, motility and viability. On the other hand, the activities of testicular steroidogenic enzyme Δ^5 3β HSD and 17β HSD were decreased along with the accumulation of steroid precursor cholesterol indicating decline in steroidogenic activities of testis. This has been ultimately resulted in decreased serum testosterone level in exposed animals. It might be the cause for alteration in spermatogenic activities in testis as testosterone plays a key role in growth and maturation of testicular germ cells for maintenance of proper spermatogenesis [31]. Decrease in testicular testosterone level further resulted in decrease in the weight of accessory sex organs in treated animals as testosterone is known to play a major role in maintenance and structural integrity and functional activities of accessory organs**.** Various studies suggested an interaction of heavy metals hypothalamo-hyphophysis axis controlling spermatogenesis resulting into changes in testicular architecture and spermatogenesis in treated rats [32].

In the present study, heavy metal mixture intoxicated rats revealed a significant accumulation of MDA and protein carbonyl and a significant decline in activities of SOD, CAT, GST, GPx and levels of GSH. Accumulation of MDA and protein carbonyl is an indicator of lipid peroxidation which causes impairment of membrane structure and function. SOD protects cells against toxic effects of superoxide anions. While CAT catalyzes the reductions of hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals. GSH acts as natural antioxidant and potential reducing agent which helps in detoxification and excretion of heavy metals [33]. GSH is capable of reducing any disulfide bond formed within the cytoplasmic protein to cystiene by serving as an electron donor. In the reduced state, the thiol group of cystiene is able to donate a reducing equivalent to unstable molecules, such as ROS.

In the process of GSH is converted to its oxidized form glutathione disulfide (GSSG) by the enzyme glutathione peroxidase (GPx) and oxidized form of glutathione by the enzyme glutathione reductase (GR). A decreased level of reduced glutathione and its associated enzyme activity is often used as measure of a cellular toxicity and indicative of oxidative stress in the cells or tissues [34]. Therefore, it can be postulated that the deleterious effects observed in our study might have been mediated through oxidative stress caused by the accumu-

lation of $O_2^{\cdot}$ and conversion of H_2O_2 to $OH^{\cdot}$; both of which are reported to be the ultimate toxicants for various transition metals and xenobiotics. This was further supported by our observation after supplementation with two micronutrients with potent antioxidant properties Se and Zn. Our results revealed the supplementation with either Se or Zn could ameliorate the deleterious effects of metal mixture on both general and reproductive toxicity in treated rats and improved the oxidative stress markers. Se supplementation increases the activities of Se dependent enzymes such as GSH-Px. This may decrease free radical-mediated lipid peroxidation and regenerate GSH [35]. Se inhibits the oxidative damage to spermatozoa, gene knock out studies on selenoproteins showed that abnormal spermatozoa occur due to knock out and it affects semen quality and fertility [36]. The ability of Zn to reduce the toxic effects of metals on body is reasonable since Zn has been shown to be essential for structure and function of large number of macromolecules and is also essential for several enzymatic reactions [37]. Various studies have reported that Zn can reduce the toxic effects of metal mixtures including the mixture of Pb, Cd and As [38]. Zn has potent antioxidant properties which helps it to act as an ameliorative agent in metal-induced toxicity [39].

CONCLUSION: Collectively our observations revealed that both Se and Zn has potent antioxidant activities and both are capable of scavenging the potential reproductive and general toxicity caused by exposure to a low dose mixture of heavy metals Pb, Cd and As. None the less, further studies are required to explore the molecular mechanism behind their repairing capacities.

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