CIBTech Journal of Zoology ISSN: 2319–3883
Online, International Journal, Available at http://www.cibtech.org/cjz.htm
2024 Vol.13, pp.182-199/Banani et al.
***Research Article** (Open Access)*

EFFECT OF CADMIUM ON IMMUNE SYSTEM, ROS AND REPRODUCTIVE HORMONE AND ON EXPRESSION OF MATRIX METALLOPROTEINASE OF ALBINO MICE

Banani Bindhani, Ettika Adhikary, Sayantika Saha, Ankita Singh and *Joydeep Das
Department of Zoology, Dinabandhu Andrews College, University of Calcutta,
Garia, South 24 Parganas 700084
**Author for Correspondence: joydeepdas914@gmail.com*

ABSTRACT
This study investigates the multifaceted impact of cadmium (Cd) exposure on the reproductive system of albino mice, with a particular focus on the expression of matrix metalloproteinase (MMPs), alterations in blood smear profiles, reproductive hormone levels, histological changes, and reactive oxygen species (ROS) levels. Cadmium, a pervasive environmental contaminant, is known for its toxicological effects on various biological systems. In this experimental setup, albino mice were exposed to cadmium chloride through oral administration over a specified period. The results revealed significant up regulation of MMPs, indicating enhanced extracellular matrix remodelling and potential tissue damage. Blood smear analysis showed morphological abnormalities in erythrocytes and leukocytes, suggesting hematotoxic effects. Reproductive hormone assays indicated disrupted levels of key hormones such as testosterone and oestrogen, correlating with impaired reproductive function. Histological examinations of reproductive tissues demonstrated structural alterations, including cellular degeneration and inflammation. Furthermore, there was a marked increase in ROS levels, highlighting oxidative stress as a critical mechanism underlying cadmium-induced reproductive toxicity. This study underscores the need for further research to elucidate the molecular pathways affected by cadmium and to develop targeted interventions to mitigate its adverse effects on reproductive health.

***Keywords**: Cadmium, Albino Mice, MMPs, ROS levels, Oestrogen, Testosterone*

INTRODUCTION
Cadmium, a non-essential element, enters human and animal bodies via different industrial products, environmental pollution and different contaminated foods. Cadmium (Cd) is a heavy metal having severe risks to human health (Godt *et. al.,* 2006). Cadmium occurs in low concentration in human diets and in cigarettes. In contaminated areas and in certain occupations, high human exposures occur. Cadmium is widely used in industry such as an anticorrosive agent, stabilizer in PVC products, colour pigment, a neutron – absorber in nuclear power plants and in the fabrications of nickel – cadmium batteries. Phosphate fertilizers also contain large amounts of cadmium (Larz, 2003). When Cadmium enters the body, it reaches the liver within the first 6 hrs. and binds to metallothionin which is mainly distributed to kidney and other and cause damage in the tissue. (Foulkes., 1990; Liu *et. al.,* 1996).

The effects of Cadmium in a concentration on male reproductive system, they showed the level of testosterone decreased significantly in high dose administered group. (Monsefi *et. al.,* 2008). The level of FSH, LH and Testosterone, Oestrogen level are decreased due to effect of Cadmium Chloride Monohydrate. There is detrimental effect observed on female reproductive system due to toxicity of Cadmium Chloride Monohydrate.

Free radical species affect all important components of cells such as lipids, proteins, carbohydrates and nucleic acids (Sarkar *et al.,* 1997). One of the most important effects of free radicals in oxidation of Poly-unsaturated fatty acids. In particular hydroxyl ($OH^{\cdot}$), peroxyl ($RO^{\cdot}$) and alkoxyl ($ROO^{\cdot}$) radicals play

important roles in the oxidation of PUFAs. As a result of free radical attack, lipids are oxidized and hence members are damaged (Sarkar *et. al.,* 1998).

Malondialdehyde (MDA), a well-known secondary product of lipid peroxidation after exposure to ROS and free radicals may be used as an indicator of cell membrane injury (Jacob *et. al.,* 1996). It has been reported that administration of Cadmium via different routes causes increased lipid peroxidation in membranes of erythrocytes and tissues such as liver, kidney and testis where MDA is used as an indicator of oxidative damage (Gutteridge 1995; Stohs *et. al.,* 2001). Intake of Cadmium results in consumption of Glutathione and protein binding sulfhydryl groups and subsequently the levels of free radicals such as hydrogen peroxide, hydroxide and superoxide are increased. Increased lipid peroxidation results in changes in intracellular stability. (Bagchi et al., 1996) reported that the levels of glutathione peroxidase (GSH-Px) were increased whereas a reduction was observed in the activity of Glutathione reductase in experimental Cadmium-induced toxicity.

Organisms that are used to explore the basic molecular mechanisms of the animal ontogeny called model organisms. When choosing a model organism for research, there are a number of different aspects to be considered like accessibility, cost, space requirements, and ease of handling. The laboratory mice are a small mammal of the order Rodentia which is bred and used for scientific research or feeders for certain pets. Laboratory rat are usually of the species *Mus musculus.* They are the most used mammalian research model and are used for research in genetics, physiology, psychology, medicine and other scientific disciplines. Mice have long served as the preferred species for research animal models due to their anatomical, physiological and genetic similarity to humans. Likewise, mice and humans each have approximately 30,000 genes of which approximately 95% are shared by all these species (Bryda 2013 May - Jun). The use of rodents for research purposes has economic advantages: mice are relatively small and require little space or resources to maintain, have short gestational times but relatively large numbers of offspring, and have fairly rapid development to adulthood and relatively short life spans. (Kara *et. al.,* 2005)

In this study, we used albino mice as an animal model to investigate the effects of high doses of Cadmium which can effect in reproductive system.

Matrix metalloproteinase (MMPs) compose a family of Zinc and Calcium dependent end peptidases responsible for the remodelling and degradation of ECM proteins, such as collagen, elastin, laminin, fibronectin and proteoglycans. Cadmium up regulated MMP-2 and MMP-14 expression.

We also discuss recent findings, regarding the molecular mechanisms by which environment toxicants (Cadmium) induce testicular injury via their initial actions at the Blood-testis Barrier to elicit subsequent damage to germ cell adhesion, thereby leading to germ – cell loss, reduced sperm count and male infertility and subfertility. Cadmium toxicity also reduces egg production and ruptures the germinal epithelium as well as many follicles which are disrupted by Cadmium.

MATERIALS AND METHODS

***Experimental Animal*:** Mice are both highly adaptable animals, which is probably led them to become so successful at procreating, surviving and spreading globally and also what led to their domestication. Their adaptability is also what has made them such common species in research. The proper care of these research animals is critical to the outcome of experiments. They were primarily housed in shoebox-type caging with a solid bottom that contains bedding material for 1 month. The typical shoebox cage provides 75 square inches of floor space, which is adequate for a maximum five adult mice.

A total of 7 Wister albino mice were used in this study. The average weight of the mice was about 20 grams. Then the animals were arranged into small groups, where one group consists 3 mice and other group consists 4 mice. Thus, total 2 small groups are named as “Control” (3 mice group), “Treatment” (4 mice group).

They were kept at a temperature range of 20-24°C. Proper amounts of nutrients required to sustain maximum growth of young mice. Moreover, nutrient requirements are not static; they change according

to developmental state, reproductive activity, and age. They were provided with food and water. For food, we made small dough of gram flour mixed with milk powder and water (as per requirement). Each cage was provided with 8 doughs every day and 125 ml of water in laboratory water feeding bottle. Thus, fresh food and water were served each and every day and was continued for about 7 days in the morning shift. Thus, they were allowed to acclimatize to the laboratory environment.

Cage cleaning is obviously necessary for good health and hygiene. Cleaning of cages was done every day as they urinate and defecate in a frequent manner. Top off water bottles (if ½ full or less) daily. Check that limits are free of debris before replacing water bottles. Bedding change – Monday and Thursday and Saturday. Clean the cage with water after transferring the mice to a new clean dry box and refill food and water.

Now, after 7 days, we started the Treatment with Cadmium Chloride Monohydrate with the treatment group. After 7 days 1 mouse from the control group and 1 mouse from the treatment group had died.

Control Cage – consisting of 2 mice (one male and one female)

Treatment Cage – consisting of 3 mice (two males and one female) treated with Cadmium Chloride Monohydrate.

Doses Selection: Cadmium Chloride Monohydrate was prepared by mixing it with distilled water in a 1:1 ratio.

After proper dose selection, 35 mg/kg of cadmium chloride was injected on the surface of the gram flour dough by using micropipette and this dough were provided to treatment cages, whereas normal dough was provided to control cages. This procedure was continued for 7 days.

Sample collection: On the 8th day, all the control and treated mice were ready for dissection. They were placed on dissecting tray and are cut ventrally. After that, the testes (from males) and ovaries (from females), kidney, liver, as well as blood from heart ---were collected from both control and treated mice.

We took a few Eppendorf tubes and labelled some of them as CONTOL and rest of them as TREATED. Then we poured blood from control mice into the control tube and cadmium chloride treated blood into those tubes labelled as treated.

For histology purpose, we had cut out the target organs (testes and ovaries, liver, kidney), removed fat bodies from them, sliced them transversely by using a sharp blade and put those sections in Bouin's solution. But for other experiment, we kept the whole organs intact into some Eppendorf tubes.

Collection of Blood: Blood collection from the experimental animals is one of the important events in biomedical research. Here, blood is collected from the heart of mice by using 1ml Tuberculin syringe. During blood sample collection, the mice were terminally anaesthetized. We obtained about 0.1-1ml of blood from the mice depending on their size of hearts. Blood is preferably taken slowly from the LEFT VENTRICLE to avoid the collapsing of heart. (Andjelkovic *et. al.*, 2019)

Collection of Liver, Kidney, Testis and Ovary for histological studies: Liver, kidney, ovary and testis of each mouse was removed and fixed in 10% formalin solution and prepared using routine techniques for histology: Samples underwent dehydration by alcohol, clearing with xylol, embedding in paraffin wax, sectioning under 8 µm thicknesses, and staining with haematoxylin-eosin. Finally, photos were taken from the prepared slides under ZEISS microscope. (Monsefi *et. al.*, 2013)

Protein measurement (ROS): After homogenization of tissue sample (testis, ovary), supernatant was collected. After that it was mixed with 2ml of phosphate buffer solution (PBS) in an eppendorf. Then, it was centrifuged at 10,000 rpm for 10 minutes. After that, supernatant was collected.20µl of that sample and 1ml of Bradford reagent were mixed and collected in cuvette and incubated for 2-3 minutes. After that, protein samples were measured against BLANK (Bradford + double distilled water) in spectrophotometer. With respect to BLANK value, we have got OD values. By putting these values on a standard curve we have got protein concentration.

GST estimation was done by a kinetic method as described by Mannervik and Danielson, 1988 with slight modification. Briefly 10 µL of samples were transferred to wells of a 96 well plate in duplicates. 190 µL of substrate solution, consisting of 300mM phosphate buffered saline at pH 6.5, 200 mM of reduced

glutathione solution and 100 mM of CDNB in the ratio of 98:1:1, was added to each well. The plate was read in a multimode reader at 340 nm for 5 mins. ΔA_{340}/min was calculated by finding the difference between the initial read and final read and dividing it by 5 mins. GST activity was calculated by the following formula:

$$\text{GST Activity} = \frac{\frac{\Delta A340}{min} \text{x reaction volume}(200\mu L)\ x\ 1000}{extinction\ Coefficient\ (9.6)\ x\ sample\ volume\ (10\ \mu L) x\ protein\ (mg)}$$

Protein concentration was measured using nanodrop 2000c spectrophotometer taking absorbance of 2 µL sample at 260nm. (Kar *et. al.,* 2015)

Catalase activity was assayed following the method of Luck, 1974. H2O2-phosphate buffer (3.0ml) was taken in an experimental cuvette, followed by the rapid addition of 40µl of lysate and mixed thoroughly. The time required for a decrease in absorbance by 0.05 units was recorded at 240nm in a nanodrop spectrophotometer. The enzyme solution containingH2O2-free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240nm by 0.05 units. It was expressed as IU/mg protein.

Collection of Serum for hormonal assay: After blood collection, it is allowed to be clotted by leaving it undisturbed at room temperature for 15-30 minutes. Then the clots are removed by centrifuging it at 10000 RPM for 10 minutes in a refrigerated centrifuge machine. The resulting supernatant is designated as the SERUM. The estradiol concentrations were measured by ELISA based on Competitive enzyme immunoassay. Procedure is - Competitive ELISA, also known as inhibition ELISA, is a surface/plate based assay, where the plate is coated with capture antibodies reactive to the molecule of interest. Here, the sample (containing native molecule of interest) and enzyme conjugated recombinant protein (the competing molecule) are added to the coated wells. Since the amount of enzyme conjugated molecule in each well is constant, the level of native molecule in the sample will determine the binding ratio of enzyme conjugated molecule vs. native molecule. After an incubation period, any unbound antibody is washed off. Enzyme substrate (for example, TMB for HRP) is added to each well and will be transformed into a blue precipitate, the amount of which is linearly proportional to the amount of enzyme in the well. The precipitate is then turned into yellow by adding the acid stop solution and the concentration of yellow precipitate is read at 450nm for light absorbance (O.D. value). The O.D. is then used to calculate the amount of molecule of interest in each well, by comparing each sample well against the standard curve.

Determine the MMP level by Zymography: Matrix metalloproteinase (MMP) are zinc – containing metalloproteinase that can cleave any type of extracellular matrix (ECM) proteins (Verma *et. al.,* 2007; Visse *et. al.,* 2003) Two classes of MMP include – secreted MMP and membrane – bound MMP (Page – McCaw *et. al.,* 2007). MMP1 is primarily a secreted collagenase, cleaving collagen. Whereas, MMP2 is primarily a membrane – bound gelatinase breaking down the gelatin.All MMPs have a Signal peptide that targets the MMPs for secretion. A propeptide domain (containing a conserved Cys residue) and A catalytic domain (Glasheen *et. al.,* 2010). Some MMP subgroups also have a C – terminal hemopexin domain and a hinge region. Other MMP subgroups contain unique features such as a transmembrane domain, a cytoplasmic tail, and a membrane- type (MT) – loop, a glycosylphosphatidylinositol (GPI) anchor or a furin recognition site (MT – MMPs, and MMP – 11, - 21, -23A/B, and -28) fibronectin type II repeats (MMP -2 and – 9) and an N – terminal signal anchor, a cysteine array and an Ig – like domain (MMP – 23) (Morgunova *et. al.,* 1999). Zymography is defined as an electrophoretic technique that is used to study hydrolytic enzymes. We had performed two types of zymography. Gelatine zymography is used for the detection and analysis of gelatinase (MMP-2 and -9). Casein Zymography is used for the detection of collagenase (MMP-1, 8, 13).Both control and treated ovaries and testes are homogenized and centrifuged for 10 minutes in 2 ml of Phosphate Buffered Saline (PBS).Non reducing sample buffer added to each supernatant collected. Prepare 8% resolving gel containing gelatine and 8% resolving gel is prepared containing Casein. After 15 to 20 minutes, 4% stacking gel is prepared and the comb placed on the stacking gel. Wait for 20-25 minutes for the stacking gel to polymerize. Load the samples in each well after removing the comb and the gel was run at 160-170 V.Wash the gel in washing buffer for 30 minutes

and then keeps it in incubation buffer for 24 hrs. at 370° Cather gel is washed with distilled water and stained with Coomassie Blue (R-250) solution for 1 hr. to 3 hrs. at room temperature. Lastly, Place the gel in destaining solution for 10-15 minutes at room temperature.

RESULTS AND DISCUSSION

Results

Blood Smear Study: Cadmium is a heavy metal that can be toxic to many organs and systems. In our study we observed that there is a huge amount of RBC present in control group. In the control group WBCs are also active and engulf the foreign element. On the other hand, there is destruction and rupture of RBC found due to Cadmium toxicity. WBCs in the treatment group were so much active and lead to the process of Phagocytosis (Figure 1).

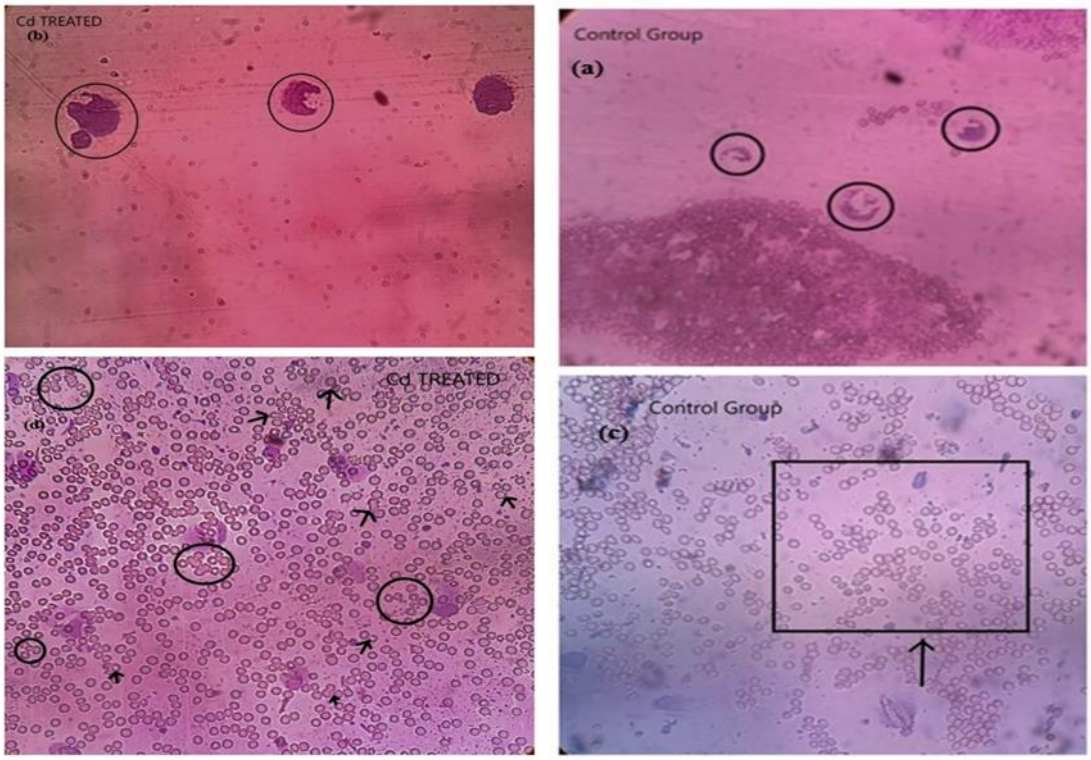

Figure 1A: photomicrograph of blood smears of control and treatment mice. (a) shows numerous WBC engulfing foreign element (phagocytosis)(marked with circles) of control group, (b) shows active WBC of treatment group showing phagocytosis (marked with circles), (c) shows RBC in the blood smear of control group showing round shaped normal RBC (marked with square), (d) shows RBC in the blood smear of treatment group showing disrupted and ruptured RBC (marked with circles).

Histological Studies

In case of Liver, Light microscopic examination of liver sections of control group of albino mice shows the presence of central vein, sheets of hepatocytes, sinusoids and portal veins are clearly visible. The liver lobules are also visible (Figure 2a). On the other hand the Cadmium Chloride Monohydrate treatment group shows various effects. It shows cellular damage on the liver lobule. The central vein and portal veins are also visible very clearly in the treatment group (Figure 2b).

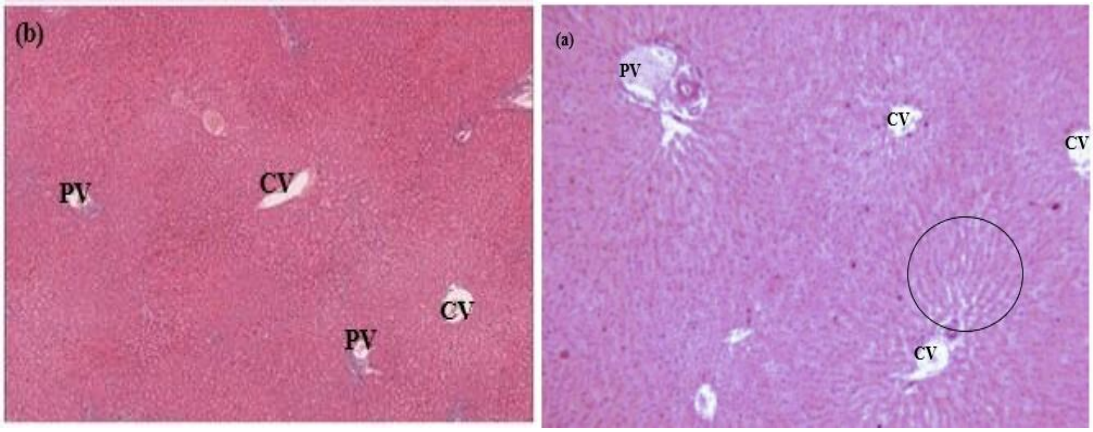

Figure 2a: A photomicrograph of liver sections of Control group of mice (a) shows portal vein and central vein, sinusoids (marked with circle) (PV = portal vein, CV= Central vein) (b) shows central veins and portal veins (PV=portal veins, CV = Central vein)

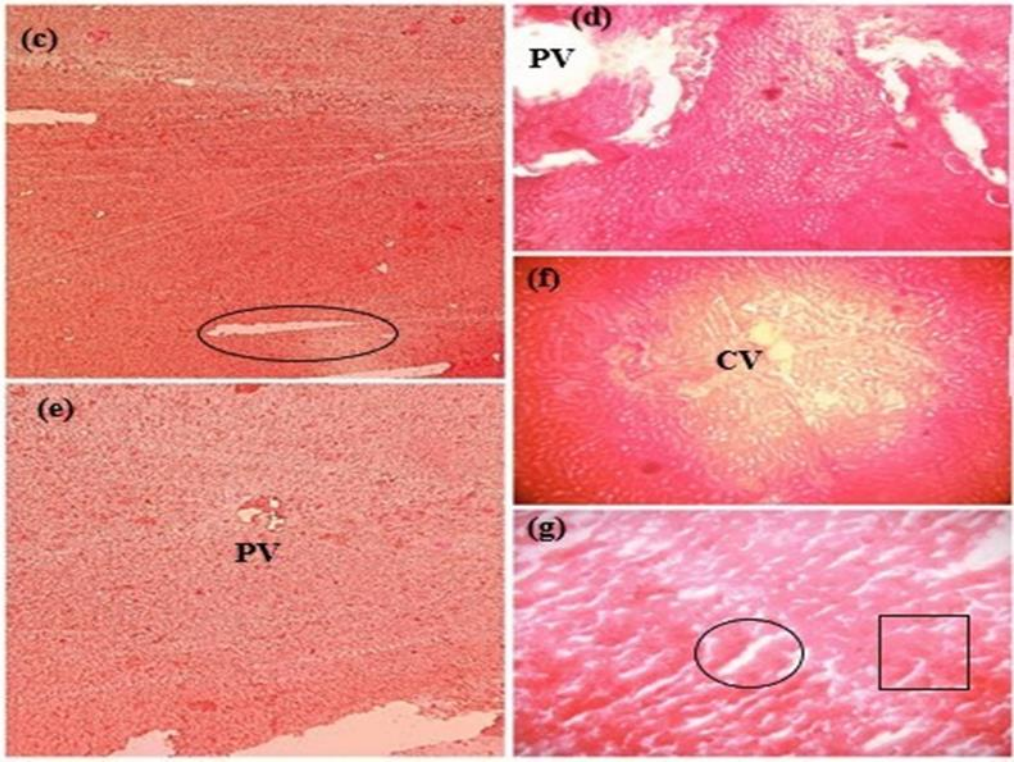

Figure 2 b: A photomicrograph of liver sections of Cadmium Treatment group of mice (c) shows central vein (signified with circle), (d) and (e) shows liver lobule with portal vein (PV= portal vein), (f) shows liver lobule of central vein (CV= central vein), (g) shows sinusoids signified with circle and hepatocytes (marked with square shape).

In case of Kidney, Light microscope examination of Kidney sections of control group of albino mice shows that there is presence of Bowman's capsule. On the other hand, the treatment group shows cortex and medulla portion. (Figure 3)

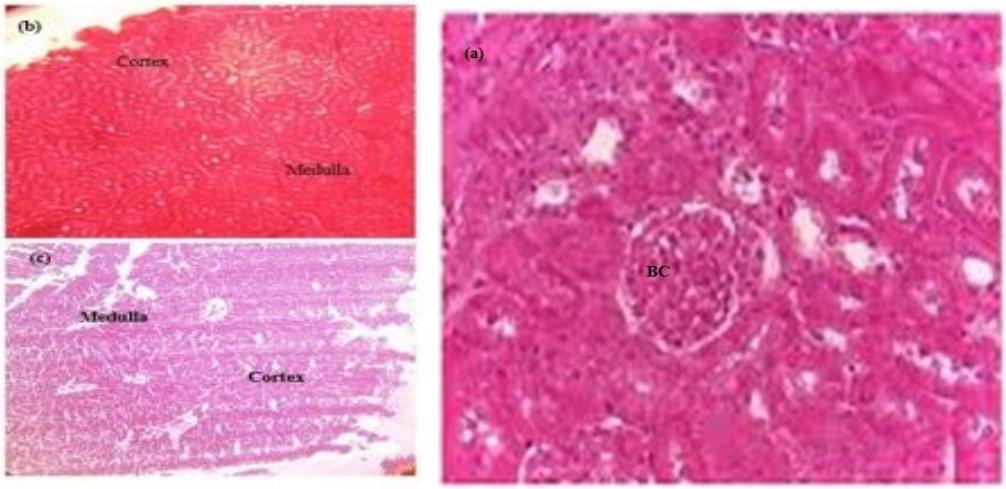

Figure 3 A: photomicrograph of Kidney sections of both control and treatment mice (a) shows Bowman's capsule of control group (BC= Bowman's capsule), (b) and (c) shows cortex and medulla portion of kidney section of treatment group

In case of Testis: Light microscopic examination of Testis sections of control group of albino mice shows that seminiferous tubule and lumen are clearly visible. On the other hand, treatment group has degenerated seminiferous tubule. It also shows poor germinal epithelium layer. Control group shows proper Leydig cells and sertoli cells. But treatment group shows disrupted cells. (Figure 4)

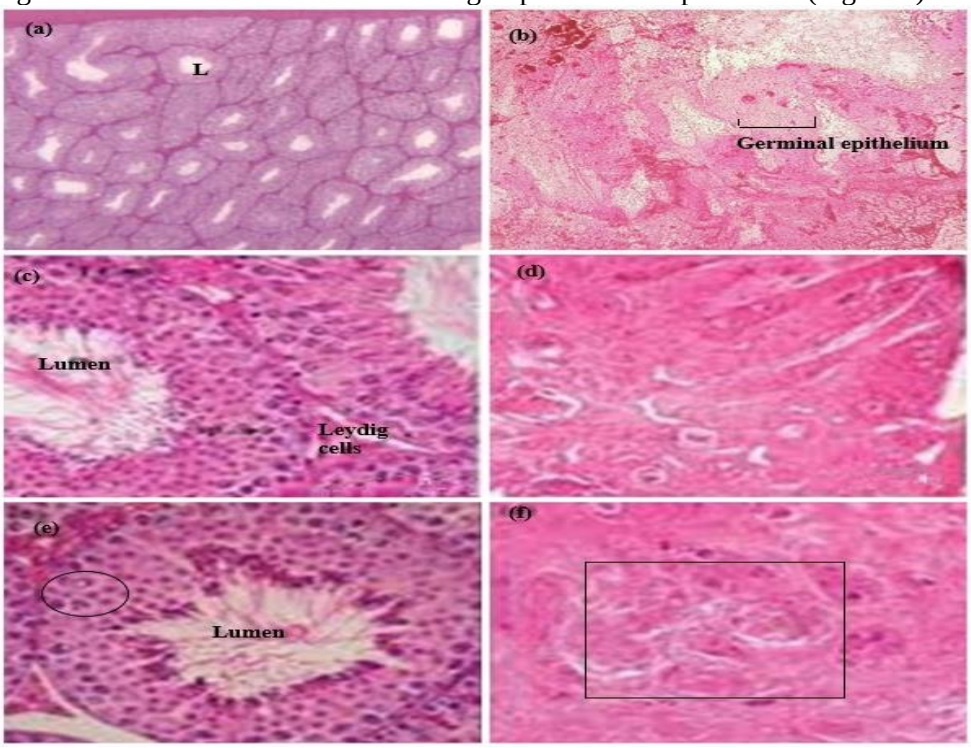

Figure 4 A: photomicrograph of Testis sections of control and treatment group of mice (a) shows seminiferous tubule showing lumen (L=Lumen) of control group, (b) shows disrupted germinal epithelium, (c) shows lumen and Leydig cells are clearly visible of control group, (d) shows disrupted sertoli cells of treatment group, (e) shows spermatocyte (marked with circle) and lumen of control group (f) shows ruptured seminal vesicle of treatment group (marked with square).

In case of Ovary, Light microscopic examination of ovarian sections of control group of albino mice shows that germinal epithelium and follicles. On the other hand treatment group shows disrupted outline of germinal epithelium. Graafian follicle also observed in the sections of control group. It also observed in the treatment group sections (Figure 5).

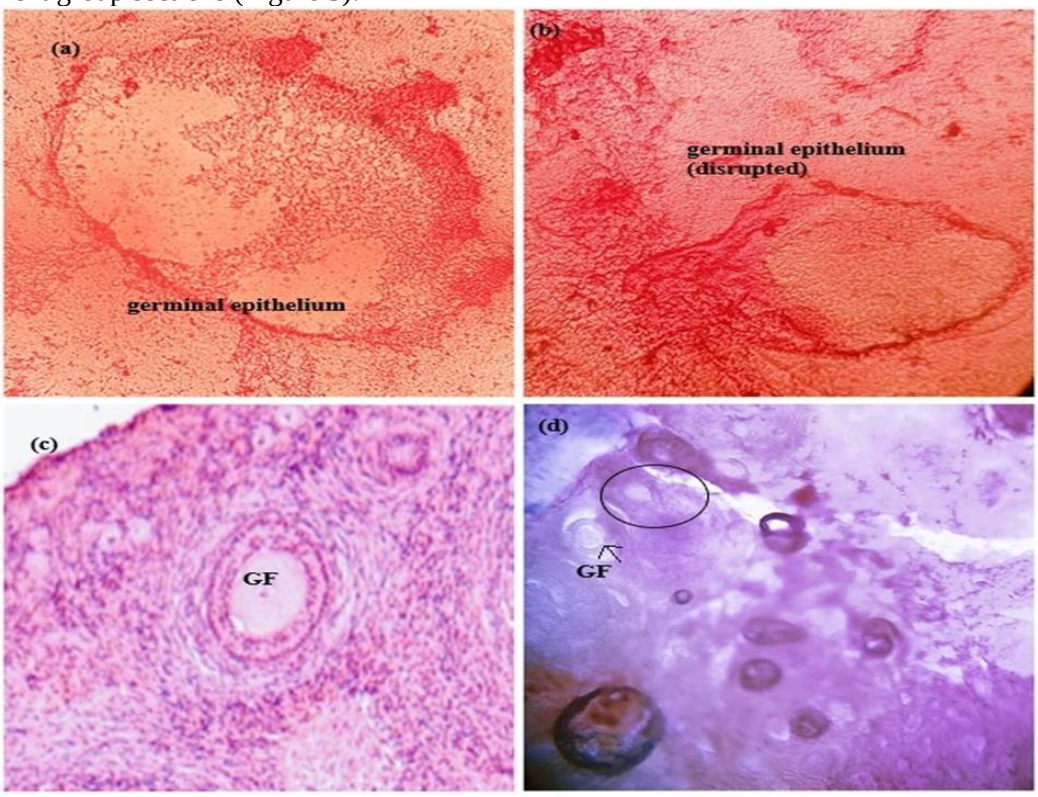

Figure 5: A photomicrograph of Ovarian sections of control and treatment group of mice (a) shows germinal epithelium of control group, (b) shows disrupted germinal epithelium of treatment group, (c) shows graafian follicle of control group (GF = graafian follicle), (d) shows graafian follicle and other ovarian follicle (signified with circle) of treatment group (GF= graafian follicle)

Determine ROS level

Table 1: Different Expression Levels of Catalase Enzyme in Control and Treated Testis of Albino Mice

Male	**Testis**				
				Mean	**SD**
Control	**1.988**	**1.966**	**1.833**	**1.929**	**0.083**
Cadmium Chloride Monohydrate	**1.023**	**0.988**	**1.009**	**1.006**	**0.017**
	Mean		**SD**		
Control	**1.929**		**0.083**		
Cadmium Chloride Monohydrate	**1.006**		**0.017**		

To determine the value of Catalase most of the harmful effects of heavy metals on human health are mediated through oxidative stress. Cadmium is an extremely toxic heavy metal and has been shown to generate considerable amount of reactive oxygen species (ROS). However, in our study we able to inhibit the activity of both GSH and catalase enzymes. In our study there is clearly visible that Catalase enzyme is decreased in the treatment group in both male and female. The mean value of control group in ovary is 1.071 and standard deviation is 0.041(Table 2). But in the treatment group the mean value is 0.704 and SD is 0.023(Table 2). In case of testes the mean value is 1.929 and SD value is 0.083(Table 1). But in the Cadmium treatment group the mean value is 1.006 and SD value is 0.017(Table 1). Using Student t test the value of catalase in testis and ovary is highly significant under 1% level of significance (Figure 6 and 7).

Table 2: Different Expression Levels of Catalase Enzyme in Control and Treated Ovary of Albino Mice

Female	**Ovary**				
				Mean	**SD**
Control	**1.043**	**1.051**	**1.112**	**1.071**	**0.041**
Cadmium Chloride Monohydrate	**0.667**	**0.721**	**0.713**	**0.704**	**0.023**
	Mean		**SD**		
Control	**1.071**		**0.041**		
Cadmium Chloride Monohydrate	**0.704**		**0.023**		

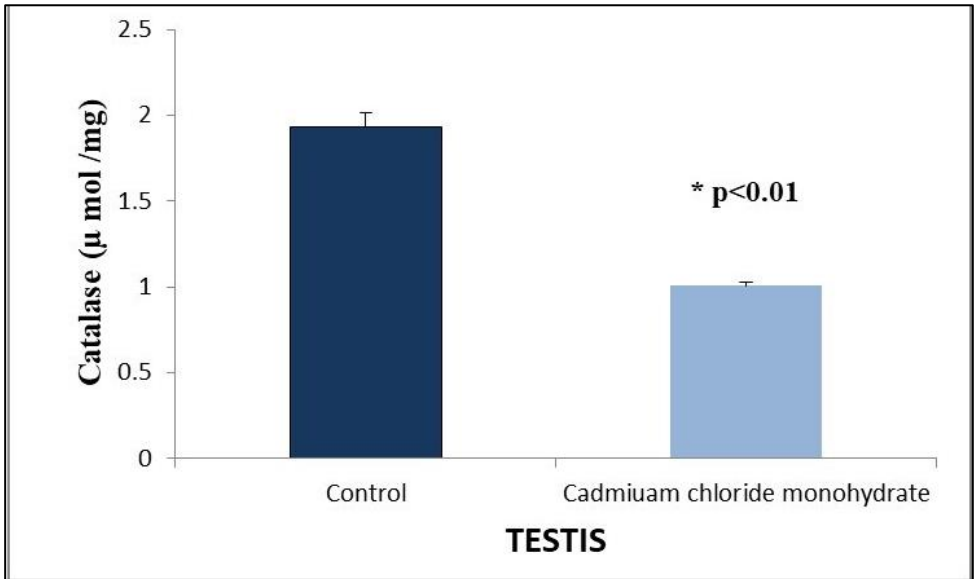

Figure 6: Relationship between Catalase Level of Control as well as Treatment samples from testis of Albino mice

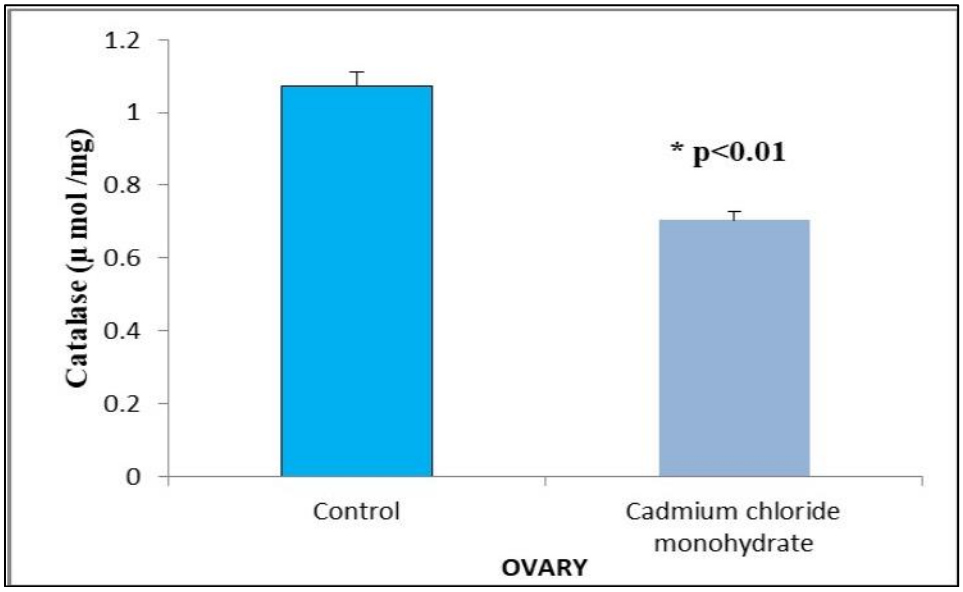

Figure 7: Relationship between Catalase Level of Control as well as Treatment samples from ovary of Albino mice

To determine the value of GSH: Cadmium Chloride Monohydrate exposure has been shown to have detrimental effects on the Glutathione (GSH) levels in the testes and ovaries of mice. Cadmium is a heavy metal with high affinity for thiol groups, which are abundant in antioxidant molecules like GSH.GSH is a crucial antioxidant that protects cells from oxidative damage caused by reactive oxygen species (ROS). However, in our study we would observe the level of GSH in cadmium treatment group as well as control group. In our study there is clearly visible that GSH enzyme is decreased in the treatment group in both male and female. The mean value of control group in ovary is 35.03 and standard deviation is 0.632(Table 4).But in the Cadmium treatment group the mean value is 22.263 and SD is 0.249(Table 4). In case of testes the mean value is 39.99 and SD value is 0.487(Table 3).But in the Cadmium treatment group the mean value is 32.96 and SD value is 0.717(Table 3). Using Student t test the value of catalase in testis and ovary is highly significant under 1% level of significance (Figure 8 and 9).

Table 3: Different Expression Levels of GSH (GLUTATHIONE) Enzyme in Control and Treated Testis of Albino Mice

Male	**Testis**				
				Mean	**SD**
Control	**40.22**	**40.32**	**39.43**	**39.99**	**0.487**
Cadmium Chloride Monohydrate	**33.78**	**32.45**	**32.65**	**32.96**	**0.717**
	Mean		**SD**		
Control	**39.99**		**0.487**		
Cadmium Chloride Monohydrate	**32.96**		**0.717**		

Table 4: Different Expression Levels of GSH (GLUTATHIONE) Enzyme in Control and Treated Ovary of Albino Mice

Female	Ovary				
				Mean	SD
Control	34.66	35.76	34.67	35.03	0.632
Cadmium Chloride Monohydrate	22.45	22.36	21.98	22.263	0.249
	Mean		SD		
Control	35.03		0.632		
Cadmium Chloride Monohydrate	22.263		0.249		

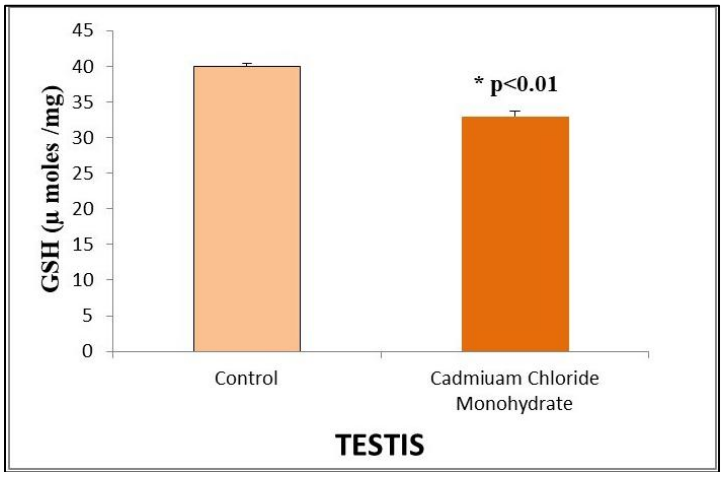

Figure 8: Relationship between GSH Level of Control as well as Treatment samples from testis Albino mice

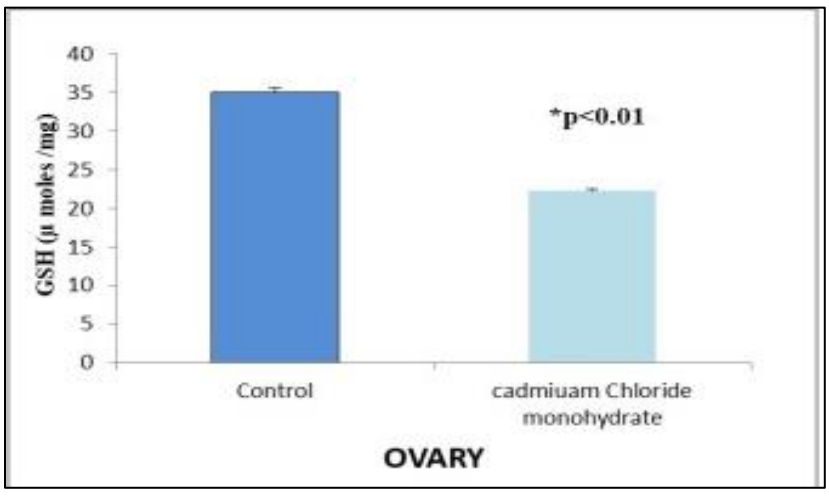

Figure 9: Relationship between GSH Level of Control as well as Treatment samples from ovary of Albino mice

Hormonal assay

Generally, the mice injected with $CdCl_2$ presented various degrees of anorexia appetite. We mix the Cadmium Chloride Monohydrate with the food balls of mice for the experiment. First of all we examined the effects of $CdCl_2$ upon the serum levels of various hormones related to reproductive activity. As shown in Table when mice were treated with $CdCl_2$ for 7 days, the levels of Estradiol and Testosterone were significantly decreased at the dose of $CdCl_2$, compared to that of the control group. On the other hand, the serum levels of FSH and LH were significantly decreased compared to control group.

To determine the level of Estradiol, Estradiol is a very important hormone in female body. It can regulate various activities in females (Table 5). In our study we could observe the level of estradiol in comparison to control and treatment mice (Figure 10). Using Student t test the value of estradiol is highly significant under 0.1% level of significance.

Table 5: Different expression of Estradiol hormone of control as well as treatment samples of albino mice.

Estradiol	Control (pg/ml)	Cadmium Chloride Monohydrate (pg/ml)
	60	28
	62	27
	61	31
Mean Value	61	28.667

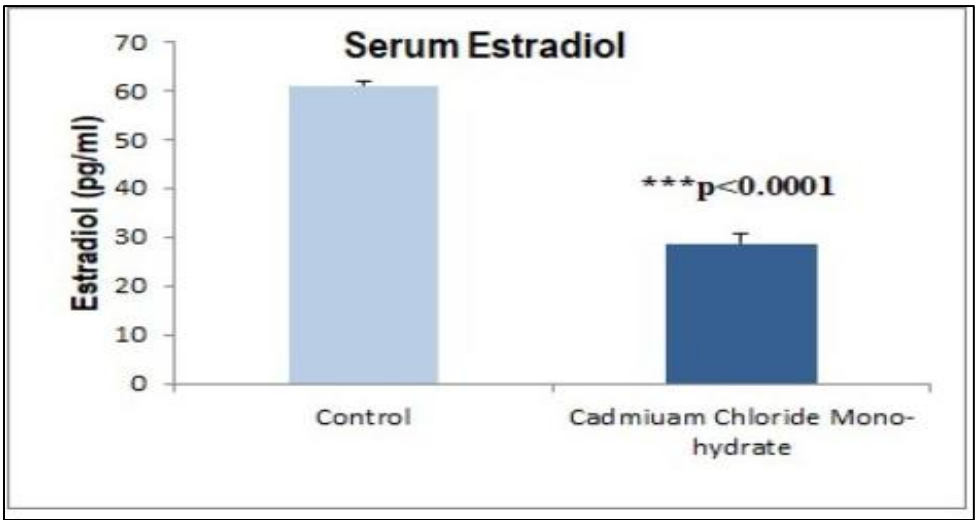

Figure 10: Relationship between Serum Estradiol Level of Control as well as Treatment samples from Albino mice

To determine the level of FSH, FSH (Follicular Stimulating Hormone**)** is a very important hormone. It can regulate various activities (Table 6). In our study we could observe the level of FSH in comparison to control and treatment mice. (Figure 11) Using Student t test the value of FSH is highly significant under 0.1% level of significance.

Table 6: Different expression of FSH hormone of control as well as treatment samples of albino mice.

FSH	Control (µ IU/ml)	Cadmium Chloride Monohydrate (µ IU /ml)
	12	4.3
	13	5.1
	11.5	4.4
Mean value	12.167	4.6

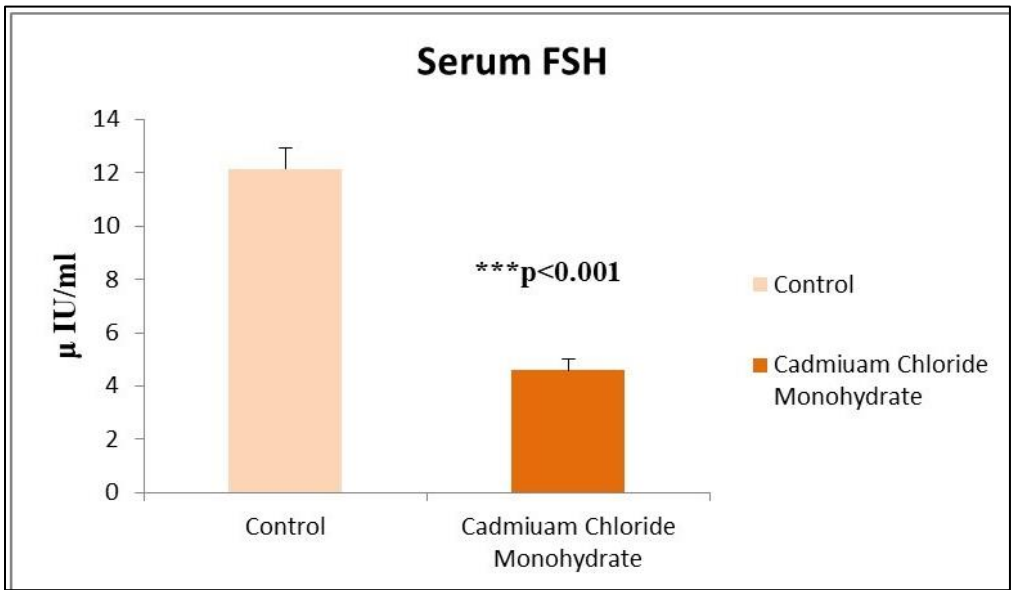

Figure 11: Relationship between Serum FSH Level of Control as well as Treatment samples from Albino mice

To determine the level of LH, LH (Luteinizing Hormone**)** is a very important hormone. It can regulate various activities (Table 7). In our study we could observe the level of LH in comparison to control and treatment mice (Figure 12). Using Student t test the value of FSH is highly significant under 0.1% level of significance.

Table 7: Different expression of LH hormone of Control as well as Treatment samples from Albino mice

LH	Control (µ IU/ml)	Cadmium Chloride Monohydrate (µ IU /ml)
	0.65	0.31
	0.72	0.29
	0.76	0.28
Mean value	0.71	0.293

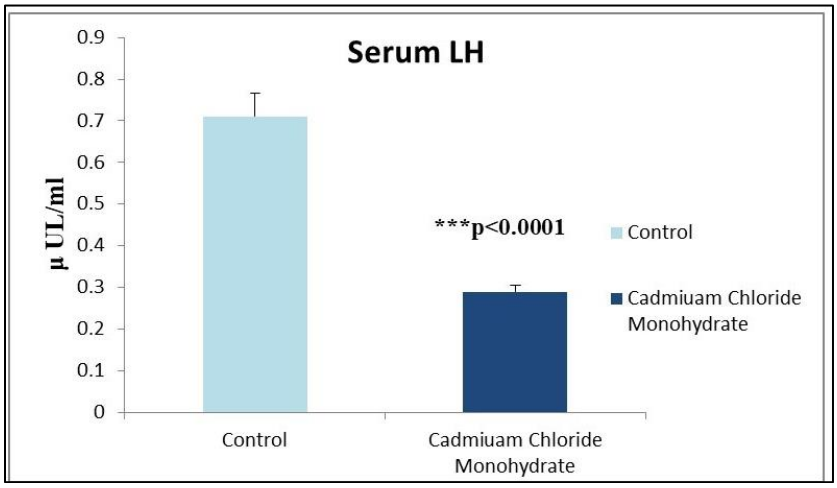

Figure 12: Relationship between Serum LH Level of Control as well as Treatment samples from Albino mice

To determine the level of Testosterone, Testosterone is a very important hormone in male body. It can regulate various activitiesin males (Table 8). In our study we could observe the level of LH in comparison to control and treatment mice. (Figure 13). Using student t test the value of testosterone is highly significant under the 0.1% level of significance.

Table 8: Different expression of Testosterone hormone of Control as well as treatment samples from albino mice.

Testosterone	Control (pg/ml)	Cadmium Chloride Monohydrate (pg/ml)
	73	31
	78	32
	72	34
Mean Value	**74.333**	**32.333**

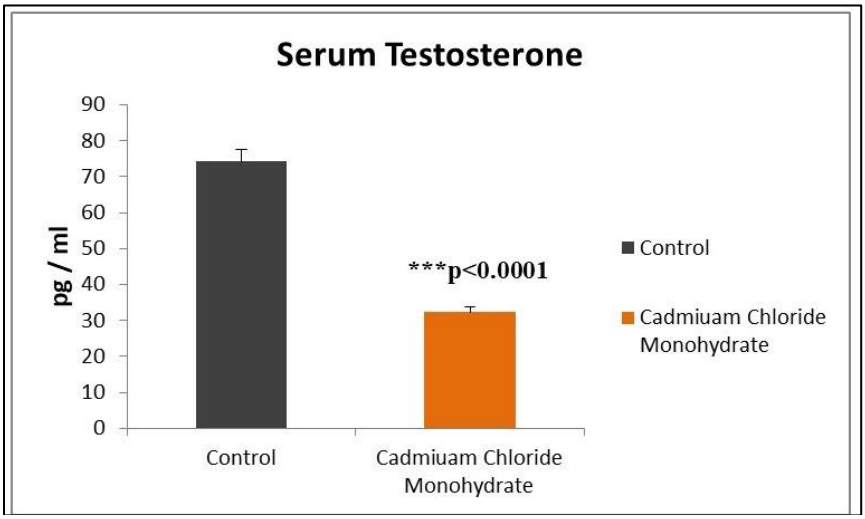

Figure 13: Relationship between Serum Testosterone level of Control as well as Treatment samples from Albino mice

The mean value of FSH in control mice is 12.167 µ IU/ml and in treatment mice is 4.6µ IU/ml.The mean value of LH in control mice is 0.71µ IU/ml and in treatment mice is 0.293 µ IU/ml. The mean value of

Estradiol in control mice is 61 µ IU/ml and in treatment mice is 28.667 µ IU/ml. The mean value of Testosterone in control mice is 74.333 µ IU/ml and in treatment mice is 32.333 µ IU/ml. Using Student t test the values of Estradiol, FSH, LH and Testosterone are highly significant under 0.1% level of significance.

Determine the MMP level by Zymography

Mice that were exposed to Cadmium in their food present alterations in the activity of MMP2 and MMP9 within their tissue extracts. Testis extracts from 7 days -treated mice exhibited a significant reduction of 30% in the gelatinolytic activity of the active form of MMP2, when compared to untreated mice (92, 82, 72 and 62 kDa, respectively). MMP9 activity was detected for this lobe. CdCl2 in the zymography incubation buffer was able to inhibit 80% and 100%, respectively, of the MMP2 and MMP9 activities in the testes extracts from untreated animals. (Figure 14 and 15)

Gelatine Zymography

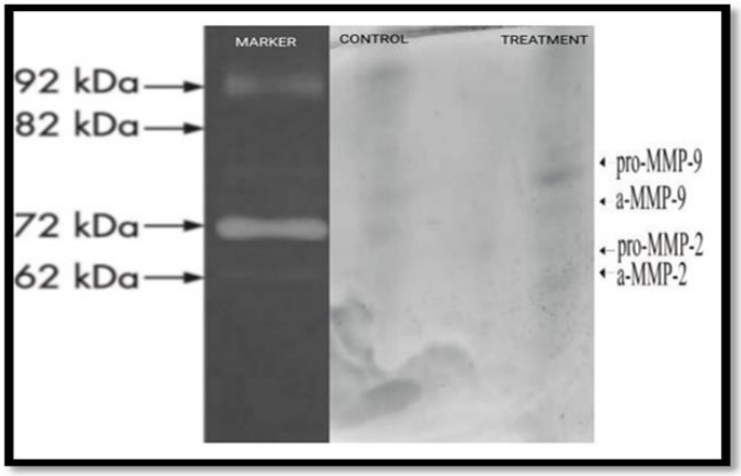

Figure 14: MMP9 and MMP2 is shown by the Gelatine zymography. In the figure there is clearly visible that band is appeared in between 72kDa (kDa = kilo Dalton) in treatment group. On the other hand Control band is appeared at about 92 kDa.

Casein Zymography

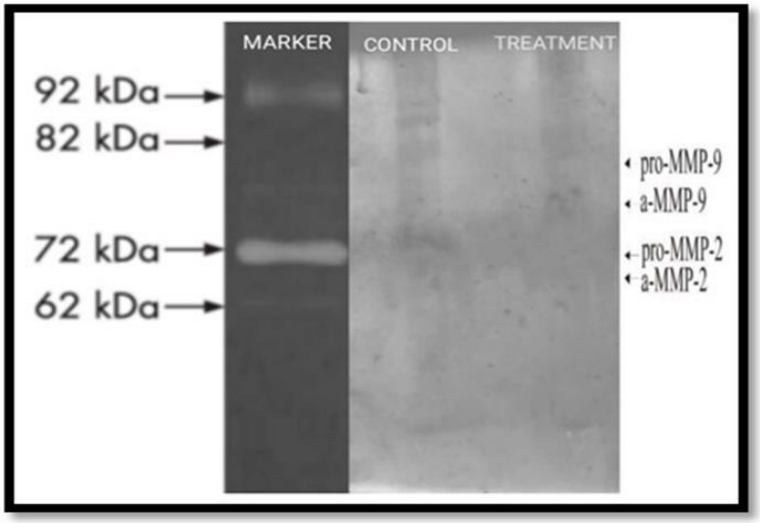

Figure 15: MMP9 and MMP2 is shown by the Casein zymography. In this figure bands are visible in both control and treatment group. In the control group bands are visible at about 82 kDa and in the treatment group bands are visible in between 92kDa and 82 kDa.

DISCUSSION

Cadmium (Cd) is a heavy metal that can be toxic to many organs and systems, including the blood. Studies have shown that cadmium exposure can have a variety of effects on the blood cells of mice, including: Cadmium exposure can also cause an increase in the number of white blood cells, which is a sign of inflammation. This may be due to the fact that cadmium can damage cells and tissues, which triggers the immune system to send white blood cells to repair the damage. Some studies have shown that cadmium exposure can also decrease the function of red blood cells. Red blood cells are responsible for carrying oxygen throughout the body, so a decreased in their function can lead to anaemia and oxidative stress. The severity of these effects will depend on the dose and duration of cadmium exposure. However, even low levels of cadmium exposure can have negative effects on blood cells.

Cadmium exposure can have detrimental effects on the histology of the liver in albino mice, acting as a liver toxin. Cadmium disrupts the normal structure and function of liver cells (hepatocytes). This can lead to cell death (necrosis), degeneration, and depletion of these cells. Cadmium disrupts the liver's antioxidant defences, leading to an imbalance of free radicals and oxidative stress. This oxidative stress can further damage liver cells. These histological changes are often accompanied by biochemical changes in the blood, such as elevated levels of liver enzymes indicative of damage.

Cadmium is a heavy metal that can have a toxic effect on many organs in the body, including the kidneys. When albino mice are exposed to cadmium, it can cause a number of histological changes in the kidneys. In the kidneys, cadmium can cause necrosis of the cells in the proximal tubules, which are responsible for reabsorbing water and nutrients from the urine. These histological changes can lead to a number of functional problems in the kidneys. Cadmium can damage the glomeruli, which are the tiny filters in the kidneys that remove waste products.

Cadmium exposure has been shown to have a detrimental effect on the histology of testes in albino mice.

Seminiferous tubules: These tubules are responsible for sperm production. Cadmium exposure can lead to a reduction in the diameter of these tubules, disrupting spermatogenesis (sperm production).

Sertoli cells: These cells support and nourish developing sperm cells. Cadmium can damage Sertoli cells, leading to decreased sperm production and quality.

Leydig cells: These cells produce testosterone, the male sex hormone. Cadmium exposure can disrupt Leydig cell function, leading to decreased testosterone levels.

Blood-testis barrier: This barrier protects developing sperm cells from harmful substances in the bloodstream. Cadmium can damage the blood-testis barrier, allowing toxins to reach the sperm cells and further impair spermatogenesis.

The severity of these effects depends on the dose and duration of cadmium exposure.

Cadmium, a heavy metal, has been shown to have detrimental effects on the histology of ovaries in albino mice.

Disruption of Follicular Structure: Cadmium disrupts the normal development of follicles, which are fluid-filled sacs that house maturing eggs. This can lead to a decrease in the number of healthy follicles.

Epithelial Damage: The outer layer of the ovary, called the ovarian surface epithelium, can become damaged by cadmium exposure. This damage can manifest as proliferation, formation of finger-like projections, and even cell death.

Reduced Corpus Luteum: Corpus luteum is a structure formed after ovulation that produces progesterone, a hormone essential for pregnancy. Cadmium exposure can lead to a decrease in the number of corpus luteum, potentially affecting fertility.

Other Changes: Other histological changes observed in cadmium-exposed ovaries include oedema (fluid accumulation), disorganization of the ovarian stroma (connective tissue), and congestion of blood vessels.

Many studies show that cadmium chloride exposure can lead to testicular damage, reduced sperm count, and altered hormone production. When cadmium enters the testes, it disrupts cellular processes and increases the production of free radicals, which are harmful molecules. Studies have shown a decrease in catalase activity in the testes with prolonged exposure (Figure 6). Studies have shown that cadmium

chloride can inhibit the activity of catalase in the ovaries (Figure 7). This inhibition can lead to increased oxidative stress in the ovaries, which can damage cells and impair ovarian function. The decrease of catalase in ovaries can lead to reduced egg quality, disrupted ovulation and increased risk of ovarian cancer.

A decrease in GSH levels may make cells more susceptible to oxidative stress, which can lead to cell death. Cadmium chloride monohydrate (CdCl2) exposure has been shown to have detrimental effects on the glutathione (GSH) levels in the ovaries of mice (Figure 9). In the ovary, GSH safeguards developing follicles and oocytes (egg cells) from oxidative stress. When $CdCl_2$ exposure reduces GSH levels, it weakens the ovary's antioxidant defence system. This can lead to increased oxidative stress, potentially harming ovarian function and fertility. Studies have found that exposure to cadmium chloride leads to a significant decrease in GSH levels in testicular tissue (Figure 8). This depletion of GSH is believed to be a major contributor to the testicular damage caused by cadmium's depletion can also lead to apoptosis, or programmed cell death, in testicular cells. Overall, cadmium chloride exposure can have a significant negative impact on testicular health by depleting GSH levels and increasing oxidative stress.

In order to explore the effect of Cd exposure on mice, we recorded the body weight changes and found that the body weight of the Cd-treated group was significantly higher than that of the Control group. As compared to control mice the mean value of FSH, LH, Estradiol and Testosterone is decreased. Studies show that both ovary and uterus are known to accumulate Cd to a great extent. Also, it is known that in the experimental animals exposed to Cd, the delay in the experimental animals exposed to Cd, the delay in the estrous cycle, the retardation of follicular growth and development and the inhibition of ovulation occurred leading to temporary sterility. Previously reported that the serum level of LH was greatly decreased in the rats administered subcutaneously with a single dose of 5 – 10 mg/Kg Body Weight of Cd. However, our results revealed that the serum levels of FSH and LH were significantly decreased between control and Cd – treated groups in 7 – days exposure. The serum testosterone levels of the Cd treated group were lower than those of the control group. These data indicated that Cd exposure can reduce the serum testosterone levels and affect the testis function. There are many factors that affect testosterone synthesis, including changes in genes related to testosterone synthesis and changes in organelle function closely related to testosterone synthesis.

Cadmium exerts a real carcinogenic stimulus to the testes. It induces activity of MMPs in this organ. Our study is to describe that $CdCl_2$ inhibits the MMP2 and MMP9 gelatinolytic activity. We have demonstrated that cadmium also down regulated the activity of MMPs in the testicles, which could contribute to an imbalance in reproductive success, given the important role of these peptidases on the motility of spermatozoids. Moreover, the toxic effects of this metal on the testis may be also related to an impairment of MMPs activities in these tissues, suggesting that further investigation about cadmium toxicity should take into account its impact on MMPs.

ACKNOWLEDGEMENT

We would like to express my gratitude to our Principal Dr. Somnath Mukhopadhyay of Dinabandhu Andrews College for allowing us to undertake this research work.

REFERENCES

Godt J, Scheiding F, Siestrup CS, Esche V, Brandenburg P, Reich A and Groneberg DA, (2006). The toxicity of Cadmium and resulting hazardous for human health. *Journal of Occupational Medicine and Toxicology*, **1** 22 *DOI: 10.1186/1745-6673-1-22*

Larz J (2003). Hazards of heavy metal contamination. *British Medical Bulletin.*

Foulkes EC (1990). Transport of heavy metals by kidney. Toxicology Letters, **53** 29-31.

Liu J, Liu Y, Michalska AE, Choo KH and Klaassen CD (1996). Distribution and retention of Cadmium in metallothionein I and II null mice. *Toxicology and Applied Pharmacology*, **136** 260-268.

Monsefi M, Alaee S, Moradshahi A and Rohani L (2010). Cadmium- induced infertility in Male Mice. *Environmental Toxicology*, **25**(1) 94-102 *DOI: https://doi.org/10.1002/tox.20468*
Sarkar S, Yadav P and Bhatnagar DJ (1997). Cadmium –induced lipid peroxidation and the antioxidant system in rat erythrocytes: the role of antioxidants. *Journal of Trace Elements in Medicine and Biology*, **11** 8-13.
Sarkar S, Yadav P and Bhatnagar DJ (1998). Lipid Peroxidative damage on Cadmium exposure and alterations in antioxidant system in rat erythrocytes: A study with relation to time. *BioMetals*, **11** 153-157.
Jacob RA and Burri BJ (1996). Oxidative damage and defence. *The American Journal of Clinical Nutrition*, **63** 985-990.
Gutteridge JM (1995). Lipid Perodixation and Antioxidants as biomarkers of tissue damage. *Clinical Chemistry*, **41** 1819-1828.
Stohs SJ, Bagchi D, Hassoun E and Bagchi M (2001). Oxidative mechanisms in the toxicity of Chromium and Cadmium ions. *Journal of Environmetal Pathology, Toxicology and Oncology*, **20** 77-88.
Bagchi D, Bagchi M, Hassoun E and Stohs SJ (1996). Cadmium induced excretion of urinary lipid metabolites, DNA damage, Glutathione depletion and hepatic lipid peroxidation in Sprague – Dawley rats. *Biological trace element research*, **52** 143-154.
Bryda EC (2013 May – Jun). The Mighty Mouse: The Impact of Rodents on Advances in Biomedical Research. *Missouri Medicine*, **110**(3) 207-211.
Kara H, Karatas F and Canatan H (2005). Effect of Single Dose Cadmium Chloride Administration on Oxidative Stress in Male and Female Rats. *Turkish Journal of Veterinary and Animal Sciences*, **29** 37-42.
Andjelkovic M, Djardjevic AB, Antonijevic E, ANtonifevic B, Stanic M, Stevuljevic JK, Kalimanovska VS, Jovanovic M, Boricic N, Wallace D and Bulat Z (2019). Toxic Effect of Acute Cadmium and Lead Exposure in Rat Blood, Liver and Kidney. *International Journal of Environmental Research and Public Health*, **16** 274.
Monsefi M and Fereydouni B (2013). The Effects of Cadmium Pollution on Female Rat Reproductive System. *Journal of Infertility and Reproductive Biology*, **1**(1) 1-6.
Kar R, Garg S, Halder S, Galav V, Chandra N and Mehndiratta M (2015 April – June). Cadmium Exposure Induces Oxidative Stress By Decreasing Expression of Antioxidant Enzymes in Mice Liver. *International Journal of Clinical Biochemistry and Research*, **2**(2) 89-96.
Verma RP and Hansch C (2007). Matrix metalloproteinases (MMPs): Chemical Biological functions and (Q) SARs. *Bioorganic and Medicinal Chemistry*, **15** 2223-2268.
Visse R and Nagase H (2003). Matrix metalloproteinases and tissue inhibitors of metalloproteinases; Structure, function and biochemistry. *Circulation Research*, **92** 827-839.
Page – McCaw A, Ewald AJand Werb Z (2007). Matrix metalloproteinases and the regulation of tissue remodelling. *Nature Reviews Molecular Cell Biology*, **8** 221-233.
Glasheen BM, Robbins RM, Piette C, Beitel GJ and Page – McCaw A (2010). A matrix metalloproteinases mediates airway remodelling in *Drosophila*. *Developmental Biology*, **344** 772-783.
Morgunova E, Tuuttila A, Bergmann U, Isupov M, Lindqvist Y, Schneider G and Tryggvason K (1999). Structure of human pro-matrix metalloproteinase – 2; Activation mechanism revealed. *Science*, **284** 1667-1670.