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EFFECT OF CADMIUM TOXICITY AND VITAMIN C AND E ON HEMATOLOGY AND SERUM BIOCHEMISTRY OF FEMALE WISTAR ALBINO RATS

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Abstract:

Cadmium is found all over the environment and it is consumed by organism directly or indirectly. It shows its toxic effect to almost all parts of body. Rats are divided in different groups and provided different doses of cadmium (3/5/7 mg/lit) through water ad libitum. Through hematology and serum biochemistry analysis of protein estimation, Urea estimation, Creatinine estimation, SGOT estimation, SGPT estimation, Acid phosphatase estimation, Alkaline phosphatase estimation, Serum hormone analysis takes place. This different analysis showing different defferent results. Level of effect depends upon does concentration of different groups. Vitamin C and E (100mg/kg b.w) shows their effect against cadmium toxicity in different groups also.

Keywords: *Cadmium, vitamin C and E, hematology and serum biochemistry, protein, Urea, Creatinine, SGOT, SGPT, Acid phosphatase, Alkaline phosphatase, Serum hormone.*

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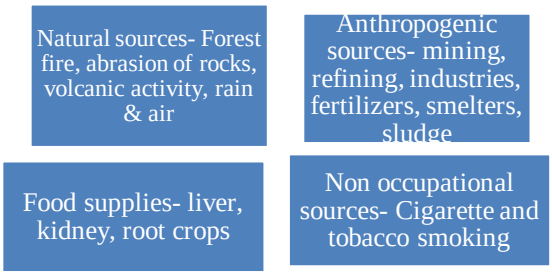
INTRODUCTION:

Accumulation of Cadmium is through combustion of fossil fuels, use of phosphate fertilizer, run-off from agricultural land and mining residues. It is widely used in Nickel–Cadmium batteries, metal plating, colour pigments, plastics, stabilizer in PVC products. It is used in the shipbuilding sector because it is oxidation-resistant. It is employed in the smelting of copper and nickel, the production of ceramics and glassware, refinement, the burning of municipal trash (particularly plastics and batteries containing cadmium), and glazes. In nuclear reactors, it also serves as a neutron absorber. When foods have an acidic pH, metal packaging may occasionally corrode and increase the amount of Cadmium in the food. Workers in the mining, paint, and battery industries are particularly exposed to high levels of Cadmium dust and fumes. The recycling and disposal of electronic and electrical trash (commonly known as "e-waste") has also been recognised as a possible significant source of cadmium exposure. It is reported that the main sources of non-occupational exposure to Cadmium are cigarette smoking and tobacco leaves among smokers and passive smokers. Smoking of cigarettes contributes between two and four μg of Cadmium per pack (Jarup and Akesson, 2009). Tobacco smoke containing approximately 1–2 μg of Cadmium causes reproductive impairments in smokers.

For non-smokers and non-occupationally exposed populations food is a primary source of Cadmium exposure. Cadmium is consumed by eating meat as liver and kidney. Fish, shellfish and mushrooms has been recorded to have Cadmium present in some regions. Certain species of oysters, scallops, mussels and crustaceans are food rich in cadmium. In several countries various cereals/grains, vegetables, meat and poultry organ meats and seafood (especially molluscs) are source of cadmium exposure. Application of municipal sewage sludge to agricultural soil can also be a significant source of cadmium. Sewage sludge from some United States 18 cities contained up to 100 mg/kg cadmium from sewage sludge that can be uptake by plants depending on soil conditions.



A significant heavy metal contaminant of global concern is cadmium. The International Register of Potentially Toxic Chemicals of the United Nations Environment Program has reviewed the toxicity of Cadmium and included it in the list of elements considered to be potentially dangerous at the global level. Human and animal consume Cadmium through contaminated food and drinking water and through tobacco smoking (WHO 1992; EFSA 2012).

Cadmium is responsible for numerous undesirable effects on health of animals and humans. The damage to the lungs in Cadmium-exposed workers was reported in the 1930s. Itai-itai disease was one of the conditions caused by chronic cadmium contaminated rice fields after World War II between 1960 to 1970 in Japan. It is well documented that cadmium worsens pulmonary inflammatory disease and impair lung function. Due to its absorption into the body through the gastro-intestinal tract, cadmium affects its function. Cadmium's exposure through the skin is less. Its solubility and absorption are affected by gastric and/ or intestinal pH. It is efficiently reabsorbed in kidneys, gets accumulates mostly in liver, kidney and testes (Cheng, 2012). It has a long biological half-life estimated from 10 to 30 years (Stawarz 2007, Satarug 2020).Accepted epidemiologic indicators of cadmium exposure include blood and urine. Kidney and diabetic nephropathy are caused by it. There is an increase in the excretion of protein, glucose, and amino acids in urine in cases of acute cadmium toxicity. Testicular destruction, hepatic and renal failure, as well as disturbances of the neurological and respiratory systems, are all documented effects of cadmium poisoning. (Ognjanovic et al., 2010).Cadmium has also been known as carcinogenic agent leading to the cancers of prostate, kidneys, pancreas and testicles (IARC, 2012, Kim 2015). Cardiovascular, skeletal, reproductive, and pulmonary disorders can be brought on by cadmium buildup in the body. By increasing the generation of reactive oxygen species (ROS), cadmium induces oxidative stress. Reactive oxygen species (ROS) causes damage to proteins, nucleic acids, lipids, membranes, and organelles, which may eventually lead to harmful diseases. The Agency for dangerous Substances and Disease Registry has listed cadmium as the seventh most dangerous compound (any material that can cause acute or chronic harm to the human body or is likely to do so).

Various pathological diseases are brought on by Cadmium intake. It has been identified as an environmental endocrine disruptor, which is defined as "exogenous substances or mixtures, that alter functions of the endocrine system and subsequently

cause adverse health effects in an intact organism, or its progeny, or (sub) populations." This is due to the fact that it is known to modulate the endocrine properties. (WHO/IPCS 2002). Cadmium causes peroxidation in membrane lipids, degradation of the antioxidant defence system, the emergence of inflammation, protein structure disorders and the oxidation of nucleic acids, and it negatively affects the DNA repair mechanism.

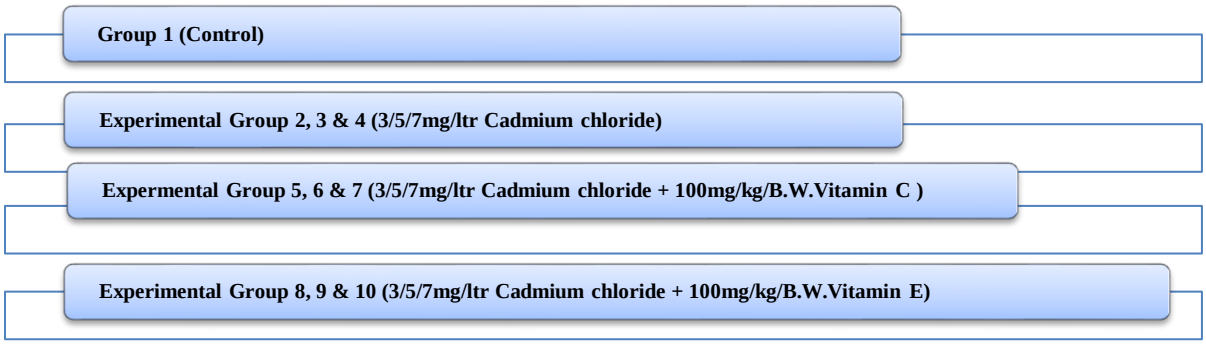
Cadmium affects cell proliferation and differentiation. Cadmium modifies gene expression and signal transduction, reduces activities of proteins involved in antioxidant defences, generate ROS and interferes with DNA repair mechanism and the induction of apoptotic cell death. At low concentrations, cadmium is claimed to impair cellular respiration and oxidative phosphorylation through binding to the mitochondria. (Patrick, 2003). Cadmium can affect apoptosis by modulating the cellular level of Ca^{2+} and caspases and nitrogen-activated protein kinases (MRPKs) activities in the cells.

Chromosome abnormalities, sister chromatid exchange, DNA strand breakage, and DNA-protein crosslinks in cell lines are all caused by cadmium. Chromosome deletions and mutations can also be caused by it (Joseph, 2009). It obstructs DNA restoration. Because of its toxicity, reduced glutathione (GSH) is depleted, sulfhydryl groups are bound to protein, reactive oxygen species (ROS) such superoxide ion, hydrogen peroxide, and hydroxyl radicals are produced at a higher rate, and oxidative stress is the outcome. It reduces activities of proteins involved in antioxidant defences as catalase, manganese-superoxide dismutase, and copper/zinc-dismutase (Filipic, 2012). Additionally, cadmium affects signal transmission and gene expression. The toxicity of cadmium has no effect on cells that have metallothionein. Conversely, cells that are unable to synthesise metallothionein are susceptible to cadmium toxicity (Han et al, 2015).

The amount of cadmium entering the body is dependent on its routes and ways through which a person is exposed to it. Cadmium easily enters the body through the gastro intestinal tract and is documented to affect it functions. Exposure through the skin is less since its constituents are more difficult for the skin to absorb. A diet high in meat and seafood can enhance the body's absorption of cadmium. Experimental animal's intestinal absorption of cadmium may be accelerated by a diet deficient in calcium and protein. The lungs air sacs can be reached by airborne cadmium particles. Cadmium is particularly easier to absorb if it is a gas or fume. Once in the body, cadmium is stored mainly in the bone, liver, and kidneys. Cadmium exposure can be measured by its presence in blood or urine. Blood is a good indicator of recent exposure of cadmium in a person. When cadmium is detected in urine it may indicates of total body burden.

MATERIAL AND METHODS:

Experiment design:



Autopsy

All animals were healthy and were taking normal diet before sacrificing them for experiment. After the completion of experiment, the rats were weighed and rats from all the groups were euthanized with an overdose of sodium pentobarbital (35 mg/kg, Sigma Aldrich) administered via intraperitoneal injection. The following instruments were used for autopsy-

i. scissors- straight, sharp, blunt and serrated of 14 cm
ii. fine scissors- cerama cut straight of 11.5 cm
iii. spring scissors-cutting edge of 14 cm and 6 mm
iv. forceps and callipers of standard size

After the death was confirmed, the fur was dampened with 70% alcohol before being cut to reduce the possibility of allergens. From the ovaries to the vulva, the entire female reproductive tract was removed by cutting through the pelvic girdle. By holding the ureters while cutting the dorsal connections the liver and both kidneys were removed. The liver, kidney, and ovaries were all put

into 10% neutral buffered formalin immediately. In dry tubes containing the anticoagulant EDTA, blood samples were independently taken from each animal by puncturing the heart with a needle 0.50 mm in diameter and 16 mm long. The blood samples were taken to laboratory for biochemical assays. Euthanasia and collection of blood was performed under the supervision of licensed veterinarian. Serum was separated individually from blood using centrifugation at 2000 rpm for 20 minutes and were preserved for biochemical analysis. Rest of the blood was processed at 4°C for hematological analysis. Approximately 5 ml serum from each experimental group was recovered. Serum was stored at -20 °C until further analysis. From the ovaries to the vulva, the entire female reproductive system was removed by cutting through the pelvic girdle. The removal, weighing and preparation of the ovaries for histopathological research was done.

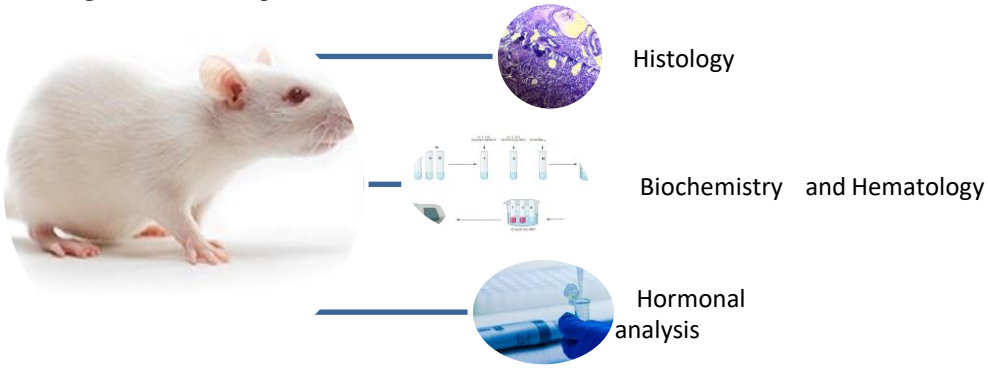


Fig. 1- Experiment layout

- **Hematological tests**

Haematological investigations included red blood cell count (RBC), white blood cell count (WBC), packed cell volume (PCV), platelet count (PLT), hemoglobin level (Hb)

1.The hemocytometer method was used to manually count the red and white blood cells.

2. Packed cell volume (PCV) was measured by the microhematocrit centrifuge by spinning at 12, 000xg for 5 minutes and read on hematocrit reader.

3. The platelet count (PLT) was measured by making a thin blood film and staining with Leishman stain.

4. Hemoglobin levels was determined by cyanmethemoglobin method using a CE4304 colorimeter (Cecil Instruments).

Serum biochemistry tests

(i) Protein estimation- Total protein in the tissues and serum were estimated according to Lowry's method (Lowry et al, 1954) as follows-

i. Ten mg tissue homogenates were precipitated by 1 ml TCA (10%) and then centrifuged at 5000× *g* for 10 min.
ii. After discarding the supernatant, the left-over was dissolved in 0.5 ml NaOH (0.1 N). An amount of 0.1 ml of the dissolved protein residue was taken and 5 ml of alkaline copper sulphate was added.
iii. 1 N Follin's reagent was added after incubating the mixture for five minutes, followed by another incubation in dark for ten minutes.
iv. By using BSA (Bovine Serum Albumin)as standard readings were taken at 620 nm using a Hitachi spectrophotometer against the reagent blank.

(ii) Urea estimation- Urea content was estimated by the procedure of diacetyl monoxime methods follows:

i. Serum samples were collected and 15% PCA to 0.5 mL of supernatant, 1 mL of acid mixture (1:3 sulfuric acid: phosphoric acid) and 0.5 mL of 2% diacetyl monoxime were added and vortexed.
ii. The contents were boiled for 30 minutes in boiling water bath and cooled to laboratory temperature.
iii. The reading of samples was taken at 480 nm against the reagent blank in a spectrophotometer.
iv. Urea content was expressed as µ moles of urea/gm wet weight of tissue.

Urea + Diacetylmonoxime
→ red coloured complex

(iii) Creatinine estimation- Creatinine estimation was performed according to the method as follows:

i. 1000 µl of Jaffe working solution was prepared by mixing 0.75 N NaOH and 1% picric acid in equal volume. It was added to each representative serum samples (1ml) from all experimental groups and standard and incubated at room temperature for fifteen minutes.

ii. Control samples were maintained to ascertain the accuracy of the results.
iii. The blank was prepared by adding 3 ml distilled water 1 ml of sodium hydroxide and 1 ml of picric acid.
iv. The intensity of the orange colour developed was measured by Hitachi spectrophotometer at 520 nm against the reagent blank.

(iv) SGOT estimation (serum glutamate oxaloacetate transaminase) (aspartate transaminase)- The serum SGOT enzyme was estimated according to the following method:

i. 1 ml of serum was mixed with 0.5 ml of glutamic-oxaloacetic transaminase (SGOT or AST) substrate and mixture was incubated for 1 h at room temperature.
ii. The reaction was stopped by adding 0.5 ml of 2, 4-dinitrophenyl hydrazine solution to the test tubes and allowed to stand for 20 minutes at room temperature.
iii. 10 ml of 0.4N NaOH was added for colour development, mixed and kept at room temperature for 20 minutes.
iv. The intensity of the developed colour was read by Hitachi spectrophotometer at 540 nm against a reagent blank.

(v) SGPT estimation serum glutamate pyruvate transaminase (alanine transaminase)- The serum SGPT enzyme was estimated according to the following method:

i. 0.1 ml of serum was mixed with 0.5 ml of glutamic-oxaloacetic transaminase (SGPT or ALT) substrate and mixture was incubated for 1 h at room temperature.
ii. The reaction was stopped by adding 0.5 ml of 2, 4-dinitrophenyl hydrazine solution was added to the respective test tubes and allowed to stand for 20 minutes at room temperature.
iii. 10 ml of 0.4NNaOH was added, mixed and kept at room temperature for 20 minutes for colour development.
iv. The intensity of the developed colour was read by Hitachi spectrophotometer at 540 nm against blank.

(vi) Acid phosphatase estimation-The enzyme activity was estimated by a commercially available Acid Phosphatase (ACP) Activity Assay Kit by Elabscience. The absorbance was measured by taking the OD value at 520 nm.

(vii) Alkaline phosphatase estimation-The enzyme was estimated by a commercially available Alkaline Phosphatase (ALP) Activity Assay Kit (Colorimetric) from LifeSpan Biosciences, Inc. The absorptions were recorded at 450nm in a microwell plate reader.

Serum hormone analysis

Estimation of estrogen, progesterone, Follicle stimulating hormone (FSH) and Luteinizing hormone (LH) were carried out using AccuBind enzyme-linked immunosorbent assay (ELISA) microwells purchased from Monobind Inc. Lake Forest CA USA. The estimation was performed according to the following steps:

i. 50 µl of each of the standards and serum samples were added to respective wells and incubated at room temperature for nearly an hour with 50 µl of HRP-conjugate enzyme and 50 µl antibody.
ii. After the incubation period, aspirating and decanting steps were done three times using wash buffer solutions.
iii. 50 µl of Substrate A and B were added to each well and incubated again at room temperature for further 15 minutes.
iv. 50 µl of stop solution was used to stop the reactions. The optical density (O.D) was measured at 450 nm using a microplate reader (Tecan, Switzerland)

Statistical analysis

All the obtained data were statistically analysed using SPSS package Results obtained were presented as Mean±SD for comparison of different experimental animal groups with control ones. To analyse the mean variations in the current study we used the one-way analysis of variance (ANOVA). p value <0.05 was considered significant.

RESULTS:

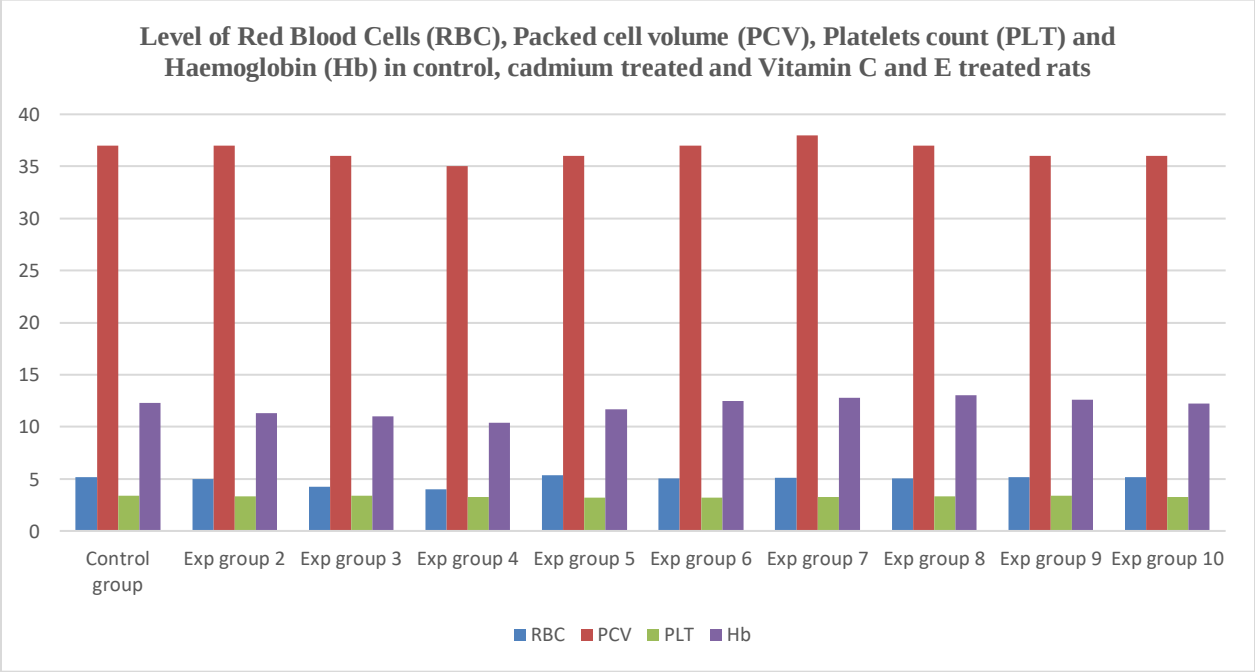
Hematology results-

A significant decrease (P <0.05) in red blood count (RBC), packed cell volume (PCV) and haemoglobin (Hb) was recorded in animals treated with cadmium as compared to control. RBC, PCV and Hb showed improvement when animals were treated with cadmium and Vitamin C and E.No significant difference in efficacy is observed between Vitamin C and E. WBC count showed significant increase (P <0.05) in cadmium treated animals. Platelet counts decreased in experimental group treated with cadmium only. Platelet count improved in animals treated with cadmium + Vitamin C and E (Table 3).

Table 1- Haematological changes in control and experimental groups of female Wistar Albino rats

Parameters	Group 1 (control)	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10
RBC (10^6/µl)	5.16± 1.0	5.00 ± 1.0	4.26± 1.1	4.00± 1.0	5.36± 1.2	5.05± 0.9	5.10± 0.6	5.05± 1.0	5.18± 0.6	5.19± 0.2
WBC (per mm^3)	7607±2100	7687±2805	7720±2761	7800±2060	7780±2800	7770±2675	7607±2005	7689±2805	7769±2695	7890± 2712
PCV (%)	37± 8.6	37± 5.9	36± 8.5	35± 7.4	36± 6.7	37± 8.3	38± 3.7	37± 6.5	36± 5.5	36±3.4
PLT (10^5/µl)	3.40± 1.0	3.29± 0.9	3.37± 0.8	3.23± 1.1	3.19± 0.8	3.20± 0.7	3.25± 1.0	3.30± 1.2	3.38± 1.0	3.24± 1.4
Hb (g/dl)	12.29± 1.8	11.29± 0.9	11.00± 0.7	10.39± 0.9	11.67±1.0	12.45± 0.9	12.80±1.1	13.04± 0.5	12.58±1.3	12.24± 1.1

Table 1- Haematological changes in control and experimental groups of female Wistar Albino rats



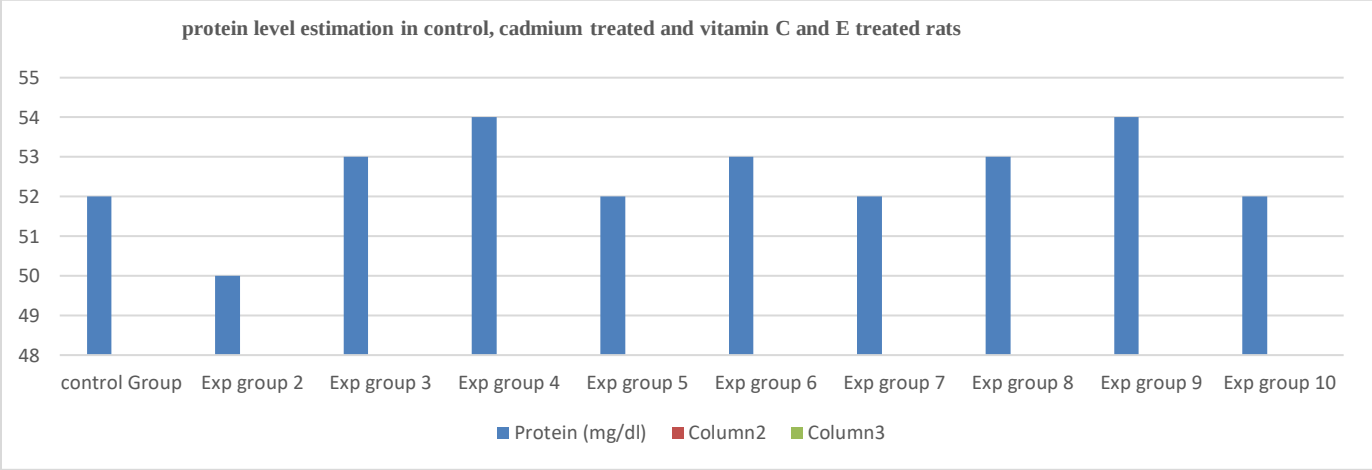
4.4 Serum biochemistry results-

i. Protein estimation

The protein content was determined by method of Lowry et al., 1954. The colour intensity is directly proportional to the concentration of the protein present in the serum sample. The results are expressed as mg/dl. As indicated in table below the high readings were obtained in groups receiving Cadmium alone (P<0.001) and maximum protein level was observed in the group that received high dose of cadmium chloride (7mg/ltr).

Table 2- Protein estimation in control and experimental groups of female Wistar Albino rats. The data are expressed as the mean ± SD. Each value represents the mean of six serum samples

Parameters	Group 1 (control)	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10
Protein estimation (mg/dl)	52 ± 1.72	50 ± 1.22	53 ± 1.62	54 ± 1.92	52± 1.21	53 ± 1.49	52 ± 1.92	53± 1.78	54± 1.22	52± 1.20

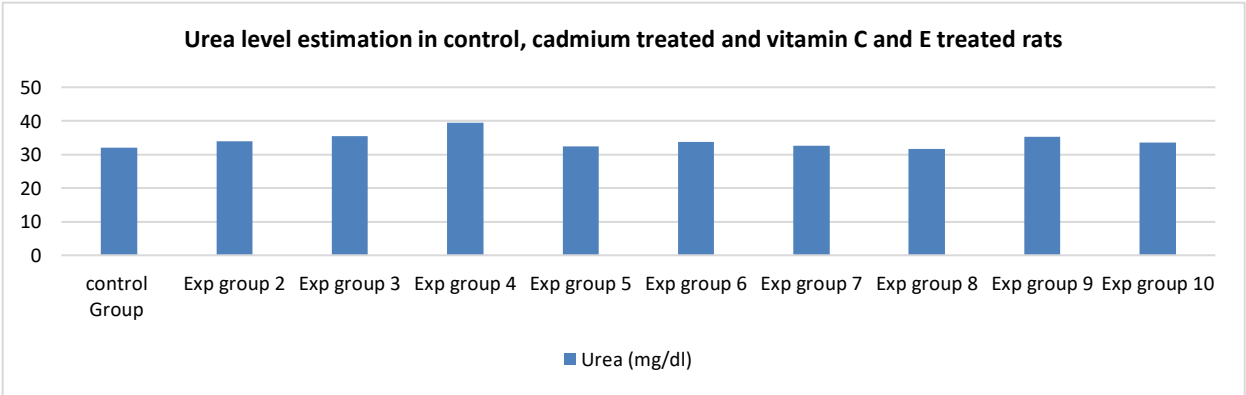


ii. Urea estimation-

Serum urea levels were increased in rats given cadmium treatment. The serum urea levels were recorded maximum in experimental group given high dose of cadmium (7mg/ltr). The high readings of serum urea level in cadmium treated rats is a marker of acute toxicity induced by cadmium.P value came out be <0.05.

Table 3- Urea estimation in control and experimental groups of female Wistar Albino rats. The data are expressed as the mean ± SD. Each value represents the mean of six serum samples

Parameters	Group 1 (control)	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10
Urea estimation (mg/dl)	31.94 ±0.32	33.83 ±0.28	35.47 ±0.95	39.47 ±0.70	32.47 ±0.73	33.77 ±0.02	32.63±0.28	31.57 ±0.72	35.28 ±0.27	33.47 ±0.09

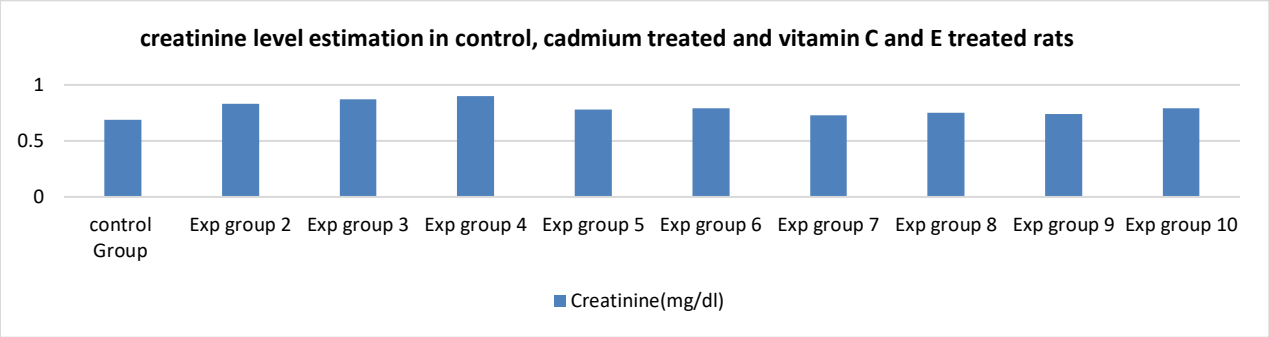


iii. Creatinine estimation-

When creatinine reacts with picric acid in an alkaline medium it gives an orange colour that shows maximum absorbance at 520 nm. The colour intensity is directly proportional to the concentration of the creatinine present in the serum sample. Maximum creatinine level is observed in the group of rats given high cadmium dose, since creatinine is a biomarker for the toxicity in liver. The results are expressed as mg/dl.P value came out be <0.05.

Table 4- Creatinine estimation in control and experimental groups of female Wistar Albino rats. The data are expressed as the mean ± SD. Each value represents the mean of six serum samples

Parameters	Group 1 (control)	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10
Creatinine estimation (mg/dl)	0.69 ± 0.04	0.83 ±0.18	0.87 ±0.07	0.90 ±0.05	0.78 ±0.17	0.79 ±0.02	0.73 ±0.08	0.75 ±0.38	0.74 ±0.09	0.79 ±0.85

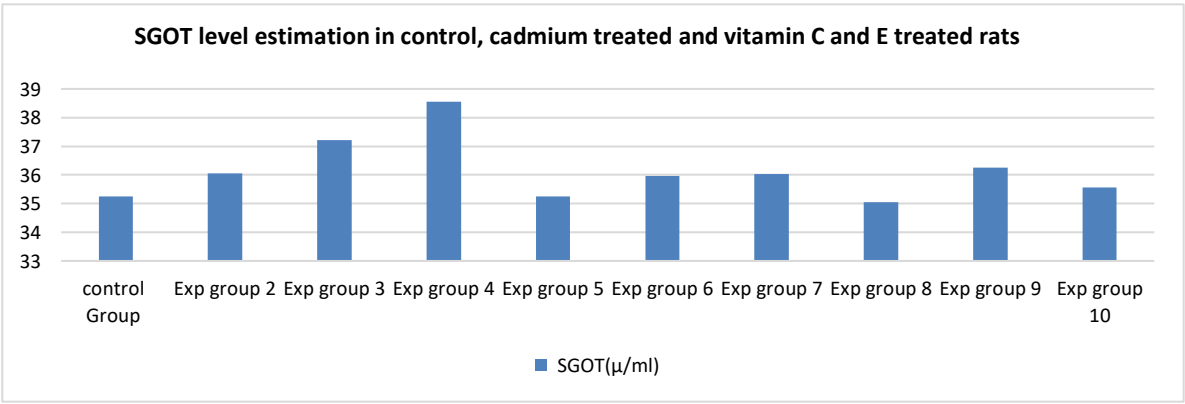


iv SGOT estimation

The levels of SGOT enzyme increased in the rat group treated with high cadmium dose (7mg/ltr). A significant improvement is observed in rats treated with Vitamin C and E.P value came out be <0.05.

Table 5- SGOT estimation in control and experimental groups of female Wistar Albino rats. The data are expressed as the mean ± SD. Each value represents the mean of six serum samples

Parameters	**Group 1 (control)**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**	**Group 7**	**Group 8**	**Group 9**	**Group 10**
SGOT (µ/ml) estimation	**35.25±2.23**	**36.06 ±1.2**	**37.21 ± 1.96**	**38.56±2.40**	**35.25±2.23**	**35.96±3.47**	**36.02±1.23**	**35.05±2.02**	**36.25±1.99**	**35.57±2.59**

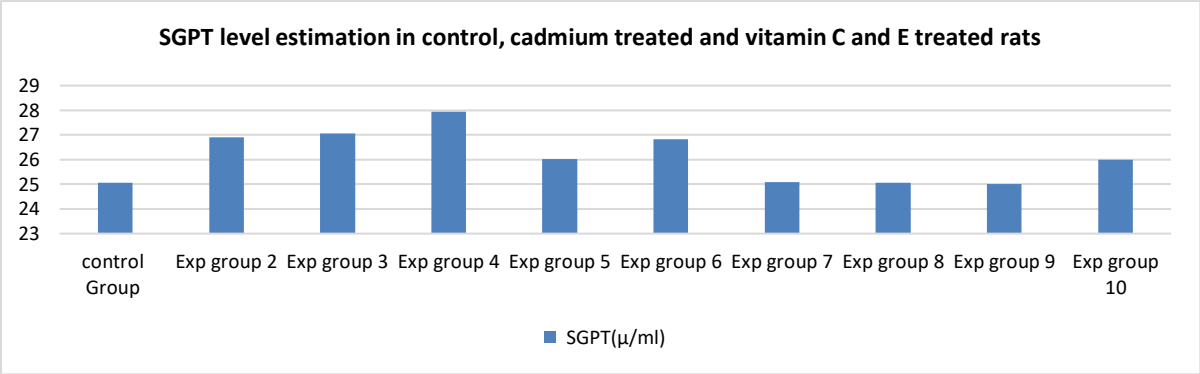


v. SGPT estimation

The levels of SGPT enzyme increased in the rat group treated with high cadmium dose (7mg/ltr). A significant improvement is observed in rats treated with Vitamin C and E.P value came out be <0.05.

Table 6- SGPT estimation in control and experimental groups of female Wistar Albino rats. The data are expressed as the mean ± SD. Each value represents the mean of six serum samples

Parameters	Group 1 (control)	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10
SGPT (µ/ml) estimation	25.06 ± 2.2	26.89 ± 1.3	27.05 ±1.9	27.94 ± 2.6	26.02 ±1.34	26.83 ±1.9	25.09 ±1.2	25.06 ±2.3	25.02 ±1.4	25.99 ±2.2

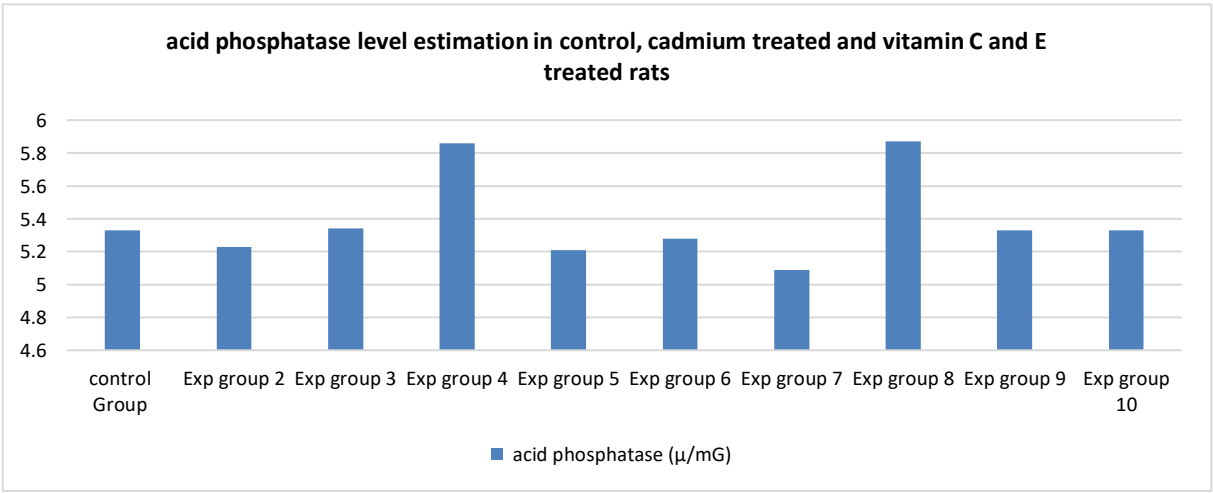


vi. Acid phosphatase estimation

Enzyme acid phosphatase was elevated in cadmium treated groups with maximum increase in the rats that were administered high dose cadmium (7mg/ltr). Group treated with cadmium chloride and Vitamin C and E showed slight improvement in the level of enzyme (P<0.5)

Table 7- Acid phosphatase estimation in control and experimental groups of female Wistar Albino rats. The data are expressed as the mean ± SD. Each value represents the mean of six serum samples

Parameters	Group 1 (control)	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10
Acid phosphatase (µ/mg) estimation	5.33 ± 0.75	5.23± 0.58	5.34± 0.93	5.86± 0.28	5.21± 0.90	5.28± 0.37	5.09± 0.54	5.87± 0.75	5.33 ± 0.75	5.33 ± 0.75

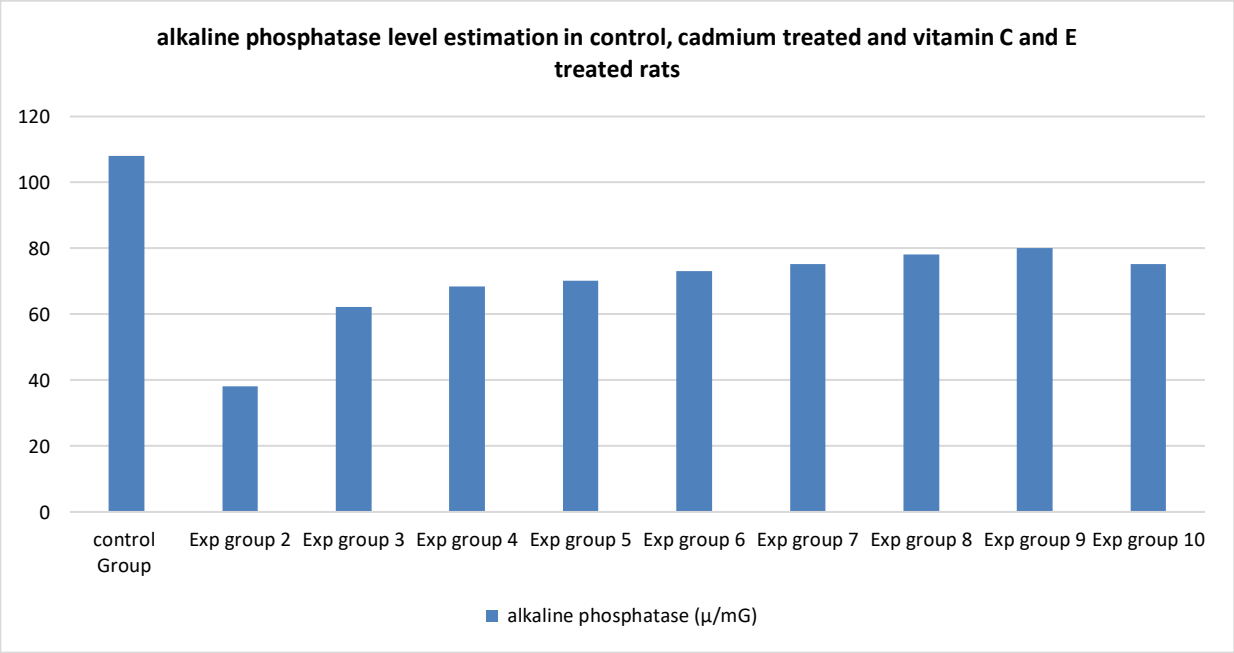


vii. Alkaline phosphatase estimation

Serum alkaline phosphatase had increased significantly ($P<0.05$) in all the groups treated with cadmium, particularly the group treated with 7mg/ltr cadmium dose. Vitamin C and E prevented the effects of Cadmium on the treated rats.

Table 8- Alkaline phosphatase estimation in control and experimental groups of female Wistar Albino rats. The data are expressed as the mean ± SD. Each value represents the mean of six serum samples

Parameters	Group 1 (control)	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10
Alkaline phosphatase estimation	108.12 ±8.14	38.19 ±2.15	62.13 ±3.75	68.34 ±3.49	70.13 ±3.74	73.14 ±4.18	75.18 ±4.42	78.11 ±4.49	80.13 ±4.13	75.12 ±8.14

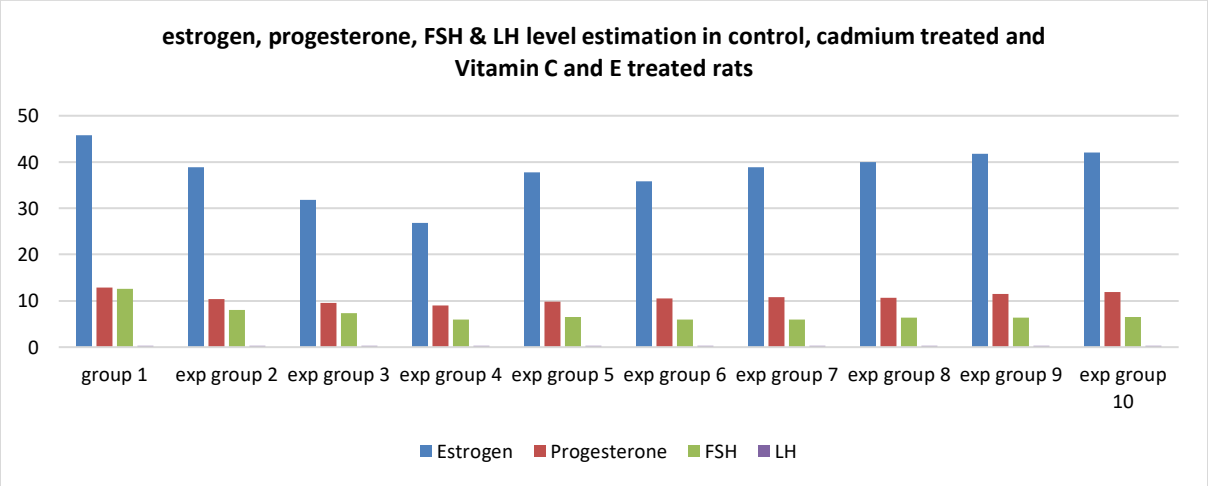


1.5 Serum hormone results

Serum estrogen and progesterone declined significantly after female rats were given different Cadmium treatments. However, serum estrogen and progesterone level improved non significantly in all the groups of Cadmium+ Vitamin C and E treatments. Levels of FSH decreased significantly in the group given cadmium only. The maximum decrease of FSH was observed in the highest dose of cadmium chloride (7mg/ltr). The levels of FSH improved slightly in the groups treated with cadmium chloride and Vitamin C and E. However, Vitamin E showed better improvement of FSH levels as compared to Vitamin C. LH levels were also decreased in groups treated with various cadmium chloride doses and maximum decrease was observed for group of experimental rats given high dose of cadmium chloride (7/mg/ltr). Slight improvement is recorded in the rats with cadmium chloride and Vitamin C and E. No significant difference is recorded between the efficacy of Vitamin C and E.

Table 9- Serum hormone estimation in control and experimental groups of female Wistar Albino rats

Parameters	Group 1 (control)	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10
Estrogen (ng/dl)	45.83 ± 1.47	38.87± 0.75	31.83 ± 0.47	26.83 ± 1.42	37.83 ± 0.89	35.83 ± 1.74	38.83 ± 1.49	39.93 ± 0.97	41.83 ± 1.29	42.09± 1.62
Progesterone (ng/dl)	12.83 ± 0.47	10.38± 0.26	09.58± 0.09	8.95 ± 0.16	9.83 ± 0.79	10.52± 0.47	10.83 ± 0.65	10.69± 0.28	11.49± 0.47	11.95± 0.52
FSH (ng/dl)	12.60± 0.63	8.05± 0.15	7.38 ± 0.26	5.95± 0.22	6.47± 0.17	6.01± 0.59	6.00± 0.26	6.42± 0.16	6.38 ± 0.36	06.54± 0.20
LH (ng/dl)	0.29± 0.04	0.28 ± 0.04	0.27± 0.03	0.25± 0.04	0.29± 0.06	0.27± 0.04	0.25± 0.03	0.28 ± 0.05	0.26± 0.06	0.27± 0.04



DISCUSSION:

In our study an elevated level of creatinine and urea in rats treated with different cadmium concentration was observed as compared with the control rat group. The urea and creatinine levels are used as serum biochemical markers in kidney function (Sahu et al., 2020). Important metabolic tasks like detoxification, storage, and excretion of xenobiotics and their metabolites are carried out by the liver and kidney, respectively. It is possible to link the elevated serum levels of urea and creatinine in mice exposed to various concentrations of cadmium chloride to the kidney's oxidative stress caused by cadmium, which in turn causes a large increase in urea and creatinine in blood (Aughey et al. 1984) administered cadmium at 50 mg/L of drinking-water to rats for 6 months and also found histological evidence of renal toxicity with a renal cortical cadmium concentration of 50 µg/g.Reports given by Girolami et al. 1989 also supports findings of our study where urea level was elevated as a result of Cadmium induction. Similar results were reported by Renugadevi and Prabu in an experiment where Cadmium was responsible for an elevated level of creatinine (Renugadevi and Prabu, 2009).Shatti, 2011 reported that rise in creatinine level is an indication of renal–tubular damage due to Cadmium-induced nephrotoxicity. In experimental group of rats given Vitamin C and E as treatment the level of creatinine and urea declined indicating that the extraneous supplementation of Vitamin C and E can protect the rats from cadmium induced oxidative stress. Similar result of an increase ($P < 0.05$) in the concentration of serum urea and creatinine is reported by Alsudani, 2022. Serum urea and creatinine was also found to be increased significantly ($p < 0.05$) in cadmium rats however, a significant ($p < 0.05$) reduction in creatinine was observed in Cadmium administered rats by Ijaz et al 2021. Urea and creatinine biomarkers for the evaluation of hepatic damage under Cadmium toxicity.

A significant decrease ($P < 0.05$) in red blood count (RBC), packed cell volume (PCV), haemoglobin (Hb) and platelet counts were recorded in animals treated with cadmium as compared to control. Kidney being the critical target organ in mammals, including humans, exposed for long periods to small amounts of cadmium. Haematological parameters showed improvement in animals treated with cadmium + Vitamin C and E. Similarly, protein, urea, creatinine, SGOT, SGPT, alkaline and acid phosphatase were significantly elevated ($P < 0.05$).

To reduce the Cadmium induced toxicity beneficial effects of vitamin C and E have been evaluated in several studies. Vitamin C and E showed good efficacy in controlling the urea and creatinine levels in kidney.

The results obtained in the present study pertaining to haematology, serum biochemistry, ovarian, liver and kidney histopathology and serum hormonal analysis clearly demonstrate that co- administration of vitamin C and E had nearly the same protective effects against Cadmium toxicity at the tissue level. They both have beneficial effects in the amelioration of Cadmium toxicity. Therefore, substances which are enriched with natural antioxidants should be provided to overcome xenobiotic toxic stress. Further studies are necessary to understand other possible protective effects of the above selected agents in subjects exposed to Cadmium and to establish the exact pathways or reactions at the cellular level.

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