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Evaluation of Biochemical Parameters under Oxidative Stress in Experimental Rats Exposed to Cadmium toxicity and the Protective Role of Zinc and Vitamin C

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Abstract: ***Aim: In the present study we aimed to determine the protective effects of Zinc and Vitamin C both individually and in combination in diminishing the biochemical changes in the test tissues (liver and kidney) under Cadmium (Cd) intoxication. Method: For this purpose, Wistar strain male albino rats were treated with Cadmium Chloride ($CdCl_2$) at a dose of 1/10th LD50 / 96 h i. e.22.5 mg/Kg body weight for an experimental period of 7, 15 and 30 day time interval. Then 15d Cd treated rats were divided into three groups. The first group received Zn (12 mg/Kg) alone, second group Vitamin-C (200 mg/Kg) alone and a third group received both Zn and Vitamin-C again for 7, 15 and 30 d long sojourn. Results: Cd induced toxicity rats showed significant changes in serum biochemical parameters along with concomitant increase in LPO content. Further Zn and Vitamin-C supplementation to Cd-treated rat tissues showed decrease in the biochemical parameters. Conclusion: From the present study we conclude that Zinc and Vitamin C are prominent nutrients that alter the biochemical processes and decrease the Cd body burden. Zn and Vitamin C supplementation either individually or in combination showed much protective effects in combating Cd stress in selected test tissues of male albino rat.***

Keywords: Cadmium, Albino rat, Zinc, Vitamin C, AST, ALT, ALP, Urea and Creatinine

1.Introduction

Cadmium (Cd) is naturally occurring heavy metal present in atmosphere with widespread industrial applications and hence humans get exposed to Cd toxicity by various modes. Sub lethal levels of Cd alters, biochemical processes and the subsequent change in tissues lead to abnormal histopathological changes, alteration in the antioxidants status and metallothionein content [1, 2]. Changes in serum enzyme activities might show profound impact on the animal metabolic status and promotes organ damage. Activities of serum enzymes-Aspartate aminotransferase [AST], Alanine Aminotransferase [ALT], Alkaline phosphatases [ALP], Urea [UR] and Creatinine [CRE] showed rise under toxic effects [2, 3]. Hence Biochemical parameters are essential to determine the extent of organ damage occurred under toxic conditions.

ALT is a cytoplasmic enzyme that catalyzes the transfer of amino groups between L-alanine and glutamate and forms a pyruvate and L-glutamate found mostly in liver and trace amounts in heart, skeletal muscle and kidney. Enhanced levels of ALT results in liver cirrhosis and acute hepatitis. ALT is more specific to diagnose liver diseases and acute renal disease [4].

AST is cytoplasmic and mitochondrial enzyme that catalyzes the transfer of amino and keto groups between α-amino acids and α-keto acids (from aspartate to ∝ -Ketoglutarate). AST is non-specific enzyme present in liver, heart, kidney, brain, blood (leucocytes and erythrocytes), pancreas, lungs, cardiac and skeletal muscles. As AST is not tissue specific its enhancement might shows less impact on liver and other organs [5].

ALP promotes protein synthesis and found in bone, placenta, lining the hepatic biliary ducts, kidney, gastro intestinal tract and bile canaliculi of the liver. An enhancement in ALP might increases the serum bilirubin secretion and promotes biliary obstruction, liver cirrhosis and hepatic tumors [6]. Tumours in kidney and liver indicate that there might be rise in ALP levels.

Urea gets synthesized in the organism due to Urea cycle by amino acids oxidation or from ammonia and is the end product of protein metabolism. When proteins gets break down ammonia is formed that is converted to Urea in liver and is excreted through kidneys. Enhanced levels of blood Urea (normal level 10-40 mg/dl) help to diagnose glomerulo nephritis, diabetic coma, obstruction in urinary tract and polycystic kidney [7].

Creatinine is a prominent renal health indicator because it is measured byproduct produced under muscle metabolism and gets eliminated by the kidneys in unchanged form [8]. Hence Creatinine is eliminated from the blood by the kidneys, principally due to glomerular filtration, but if the product is not filtered in the kidney properly, Creatinine levels in blood arise. Hence Creatinine content is a diagnostic tool to assess kidney function and is not influenced by endogenous and exogenous factors as in case of Urea.

AST, ALT and ALP enzymes present in the liver tissue gets secreted from the liver when their concentration enhances due to toxic insult. Hence their secretion enhances in blood due to damage of liver cells, oxidative stress generated in organism, hepatic damage and inefficiency of hepatocytes to work properly due to toxic

insult and enhances serum enzymes [9]. AST, ALT and ALP levels are useful parameters to detect the extent of hepatic damage. Creatinine and Urea levels are the key indicators for estimating the kidney damage [10].

Hence in the present study we made an attempt to determine the impact of Zinc and Vitamin C supplementation on altering the Biochemical parameters that dysregulated under Cd induced toxic effects.

2.Materials and Methods

Chemicals:

Cd as cadmium chloride (CdCl2), Zn as Zinc Chloride and vitamin-C was purchased from Merck (Dormstadt, Germany). All other chemicals which were used in the present study were obtained from the standard chemical companies like Sigma Chemical Co. (St Louis, MO, USA) and SD Fine Chemicals, India. The chemicals used in this study were of the highest purity.

Animals:

Three months-old Wistar strain male albino rats weighing 180 ± 20 g were chosen for the present study. The animals were obtained from Sri Venkateswara Traders, Bangalore, Karnataka, India and were kept in stainless steel mesh cages, housed under standard laboratory conditions (23 2C, 50 20% relative humidity, 12h light-dark cycle) with standard rat chow (SaiDurga Feeds and Foods, Bangalore, India) and drinking water *ad libitum*. The rats were acclimatized to the laboratory conditions for 10 days. The protocol and animal use has been approved by the Institutional Animal Ethics Committee (Resol. No.58/2012/ (i) /a/CPCSEA/IAEC/ SVU/AUR - VK), Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Experimental design:

After acclimatization, the rats were divided into two groups, namely control and experimental. Control rats received only deionized water without the Cd. The experimental rats were treated with cadmium chloride at a dose of 1/10th LD50 / 96 h i.e. 22.5 mg/Kg body weight for 7, 15 and 30 days (d) time intervals. Then 15d Cd treated rats were divided into three groups. The first group received Zn (12 mg/Kg), second group vitamin-C (200 mg/Kg) alone and a third group supplemented with both Zn and vitamin C for again 7, 15 and 30d long sojourn.

After specific time intervals, all the control and experimental rats were anesthetized, blood samples were collected by cardiac puncture. Sacrifice animals by cervical dislocation and the liver, kidney tissues were removed. Serum samples were separated by centrifugation at 2000 rpm for 20 min. Serum samples were used for biochemical analysis. The liver and kidney was weighed to their nearest mg using Shimadzu Electronic Balance and used for experimental study. Biochemical analyses were performed by following methods:

AST (E. C: 2.6.1.1): Reitman and Frankel (1957) [11]

ALT (E. C: 2.6.1.2): Reitman and Frankel (1957) [11]

ALP (E. C: 3.1.3.1): Kind and King (1954) [12]

Urea: Natelson (1971) [13]

Creatinine: Faulkner *et al*. (1976) [14]

The organ weight was calculated and presented as follows:

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Whole body weight (g)}} \times 100$$

3.Results

Changes in Body and Organ weights (liver and kidney weights) induced by Cadmium (Cd) in *Wistar*Rats:

Administration of cadmium chloride at a dose of 1/10 LD_{50} i. e. Sub lethal dose declined the body weight and relative weights of liver and kidney of male albino rats treated for 30 days duration. The body weight and organ weights of rat liver and kidney, HistoSomatic Index (HSI) of organs under Cd administration were calculated and tabulated in Table.1. Further Cd induced changes in body and organ weights were subjected to different treatments (Zn alone, Vit-C alone and Zn + Vit-C combined supplementation) are estimated and tabulated in Table 2.

The results obtained showed statistically significant changes ($p<0.05$) over the control. After supplementation with trace element (Zinc) and Vitamin C individually and in combination profoundly ameliorated the Cd induced alterations and improved the body and organ weights. The HSI of test tissues (liver and kidney) showed significant ($p<0.05$) changes in all the experimental groups except Cd-treated group of rats.

Changes in Biochemical Parameters under Cadmium treatment and Zn, Vit-C, Zn + Vit-C supplementation in *Wistar* Rats:

The biochemical parameters such as AST, ALT, ALP, Urea and Creatinine were determined in the control rat and experimental group of rats for 7d, 15d and 30d time interval. The results showed that Cd treatment significantly ($P < 0.05$) enhanced the serum ALT, AST, ALP, Urea and Creatinine levels when compared to the control rats are presented in Table 3. On the other hand, Zinc and Vit-C significantly ($P < 0.05$) reduced the serum ALT, AST, ALP, Urea and Creatinine when compared to the Cd treated rat group and presented in Table 4.

Cd treated group of rats showed statistically significant increase ($p<0.05$) when compared with control group of rats. Similarly the Cd-administered rats treated with zinc and vitamin-C showed decrement when compared to Cd

treated rats and all the values are found to be significant ($p<0.05$).

4.Discussion

The biochemical studies pertain to the impairment in functionality of vital organs under toxic conditions due to Cd induced toxic stress [15]. Cd toxicity induced blood samples were collected for biochemical study of AST, ALT, ALP, serum Urea and Creatinine content. Further body weight and relative organ weights are determined which are index of the extent of organ damage. The enhancement of above parameters in the present study paved a way to determine the toxicity of cadmium in male albino rats and the activity of Zn and Vit-C in suppressing Cd induced toxic effects.

The decline in the body weight and organ weight of the animal might be due to the toxic effects of cadmium, and it might disturbs the normal metabolic status of organism. Hence animal might suffer due to oxidative stress and may decrease the body weight and organ weight [16, 10].

Under the supplementation of Zinc and vitamin C the animal restored the body weight and organ weight in the present study suggesting that the nutrients used in the present study might activate the metabolism in the organism and suppress the oxidative stress generated [16, 17].

In mammalian toxicological studies, the enhanced content of AST, ALT and ALP under Cd intoxication are marker enzymes of hepatic damage and hepatic lesions that caused leakage of these enzymes from the liver cytosol into the blood stream. The present study supported that cadmium induced stress in the organism by increasing the levels of biochemical parameters along with enhancement of LPO content. The present study results are in consonance with the studies of [18, 19 and 20] suggesting that Cd enhanced the levels of AST, ALT, ALP, Urea and Creatinine.

Rise in biochemical parameters may indicate toxic accumulation, impaired carbohydrate metabolism and an organ function disruption, in addition significant increase in the above parameters under Cd treated group when compared to control group is an indication of organ injury [21]. Further the supplementation of Zinc and vitamin-C is known to reduce the raised levels of Creatinine and Urea in Cd treated rats are proven by the previous studies [18, 10, 3].

Elevation in serum Creatinine and Urea levels might be due to oxidative insult generated in organism, renal infiltration, damage of renal tubule and renal dysfunction. Hence the present study results suggested that kidney damage under cadmium toxic stress impaired the levels of serum Creatinine and Urea. Hence the Urea and Creatinine levels are useful parameters to detect the extent of renal damage. Elevated Kidney function markers concentration in serum under Cd treatment might accredit to decline the glomerular filtration rate in kidney tissue and promote kidney tubules dysfunctionality [22].

Both Urea and Creatinine levels enhances in the blood, due to excessive protein intake or toxic stress and are considered as major waste products. As the breakdown of proteins enhances the formation of ammonia is initiated and gets converted into Urea in the liver tissue and gets excreted through kidneys. Under normal metabolic conditions those levels are less and get eliminated through the kidneys efficiently. But under toxic insult the blood Urea and blood Creatinine levels enhances in the blood and reaches the kidneys, and kidney gets damaged due to toxic stress and fails to remove them from body. Hence under toxic insult their content raises in blood promoting renal failures, congestive heart failure, kidney diseases, myocardial ischemia, dehydration, diabetes mellitus, gastrointestinal bleedings, and obstruction of urinary tract.

Deficiency of Zn and Vit-C supplementation to animal might enhances the renal markers-serum Urea and Creatinine, and may show profound impact on kidney function and effects glomerular filtration rate [23]. Vitamin C is a prominent dietary antioxidant known to enhance glomerular filtration rate by reducing glomerular microvascular ischemia.

Zinc is the vital nutrients that promotes protein synthesis, stabilizes cell membranes and reduces protein degradation thus produce fewer amounts of Urea and Creatinine [24]. Transaminases enzymes increase in the cadmium intoxicated rat serum promoting liver injury, due to enzymes leakage from the tissue into the plasma. Zn might stabilize the hepatic cell membrane and protect hepatocytes from injury and reduces AST, ALT, ALP, Urea and Creatinine content [10].

Vitamin-C significantly reduces liver functional transaminases (AST and ALT) and alkaline phosphatase (ALP) enzymes activity, Urea and Creatinine content in serum [3, 4]. Zn and Vitamin-C are prominent antioxidants and nutritive substances known to induce the synthesis of liver enzymes and might prevent the organ damage. Vitamin C supplementation for 28 days showed decline in cadmium content in the test tissues-liver, kidney and testis rats [25].

Decline in liver function marker enzymes (AST, ALT and ALP) and kidney function markers (Urea and Creatinine) when compared to the Cd intoxicated rats [4]. Cd intoxication might increase the bilirubin content that further enhances AST and ALT levels indicating liver damage [26]. Zn supplementation is known to alter the AST, ALT, Urea and Creatinine content that got enhanced upon Cd intoxication providing hepatoprotective and nephroprotective effects [10]. Cd might declines the Zn content in the blood, and enhances the Cd toxic effects.

AST, ALT, ALP, Urea and Creatinine levels enhanced in Cd treated rats and increased the oxidative insult in kidney. Cd administration thus impairs the excretory function of the kidney and also causes liver damage. Hence the raised levels of AST, ALT, ALP, Urea and Creatinine under Cd toxicity might enhance the oxidative stress in organism and promotes organ damage. Disrupted biochemical metabolism, promoted under Cd-induced

toxic effects by enhancement in the concentration of AST, ALT, ALP, Urea and Creatinine are corrected by the addition of Zn and Vit-C individually and in combination. The combined supplementation showed statistically significant results when compared to the individual supplementation and promoted hepatoprotective and nephroprotective effects.

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Table 1: Body and Relative organ weights of Control and Experimental rats

S. no	Parameter	Control	Cadmium		
			7days	15days	30days
1	Initial body weight (g)	218.42±0.13	225.12±0.06	224.33±0.20	236.49±0.18
2	Final body weight (g)	248.56±0.12 **PDC**	188.40±0.10 -24.20	172.45±0.09 -30.62	162.39±0.09 -34.66
3	Body weight gain/loss (g) in 7, 15, 30d	30.14±2.45	-36.72±1.00	-51.48±1.09	-74.10±2.13
4	Liver weight (g)	11.15±0.36 **PDC**	5.69±0.51 -48.96	6.18±0.64 -44.57	4.82±0.64 -56.77
5	HSI of Liver	4.48	3.02	3.58	2.97
6	Kidney weight (g)	2.68±0.52 **PDC**	1.33±0.46 -50.37	1.28±0.46 -52.23	1.19±0.41 -55.59
7	HSI of Kidney	1.07	0.70	0.74	0.73

All Values are Mean ± SD of six individual observations.
PDC: Percent deviation over control group.
HSI: Histo Somatic Index

Table 2: Body and Relative organ weights of Cd treated rats and after supplementation with Zinc, Vitamin-C, and Zn+ Vit-C

S. no	Parameters/ Serum	Control 15d Cd	Zinc			Vitamin-C			Zn+Vitamin-C		
			7days	15days	30days	7days	15days	30days	7days	15days	30days
1	Initial body weight (g)	224.33 ±0.20	228.63 ± 0.12	232.57 ±0.08	238.47 ±0.10	225.46 ±0.24	231.84 ±0.06	223.43 ±0.07	232.38 ±0.18	221.71 ±0.11	224.53 ±0.08
2	Final body weight (g)	172.4 ±0.09 **PDC**	256.27 ±0.11 +48.64	263.55 ±0.11 +53.02	267.67 ±0.10 +55.26	248.44 ±0.08 +44.10	253.22 ±0.16 +46.87	256.86 ±2.59 +48.99	261.79 ±0.09 +51.85	267.51 ±0.17 +55.16	271.45 ±0.12 +57.45
3	Body weight gain/loss (g) in 7, 15, 30d	51.48 ±1.09	27.64 ±0.97	30.98 ±1.21	29.20 ±0.82	22.98 ±1.02	21.38 ±0.73	33.43 ±1.41	29.41 ±1.81	45.8 ±0.81	46.92 ±1.08
4	Liver weight (g)	6.18 ±0.64 **PDC**	8.17 ±0.69 +32.20	8.36 ±0.59 +35.27	8.56 ±0.55 +38.51	7.31 ±0.77 +18.28	7.48 ±0.80 +21.03	7.62 ±0.71 +23.30	9.24 ±1.06 +49.51	9.31 ±0.55 +50.64	9.42 ±0.80 +52.42
5	HSI of Liver	3.58	3.19	3.17	3.20	2.94	3.35	2.95	3.52	3.48	3.47
6	Kidney weight (g)	1.28 ±0.46 **PDC**	1.83 ±0.50 +42.96	1.88 ±0.42 +46.87	1.91 ±0.44 +49.21	1.62 ±0.47 +26.56	1.68 ±0.49 +31.25	1.71 ±0.54 +33.59	1.98 ±0.84 +54.68	2.12 ±0.72 +65.62	2.23 ±0.67 +74.21
7	HSI of Kidney	0.74	0.71	0.71	0.71	0.65	0.66	0.66	0.75	0.79	0.82

All Values are Mean ± SD of six individual observations.
PDC: Percent deviation over control group.

Table 3: Changes in the serum enzymes and blood parameters in control and Cadmium treated rats

S. no.	Parameters / Serum	Control 15d Cd	Cadmium		
			7days	15 days	30days
1	AST (IU/ml/hr)	46.67± 0.05	51.97^{c}±0.06	55.96^{b}±0.10	59.88^{a}±0.39
2	ALT (IU/ml/hr)	29.24±0.09	32.92^{c}±0.16	35.63^{b}±0.06	38.14^{a}±0.09
3	ALP (IU/ml/hr)	70.73±0.09	78.84^{c}±0.07	84.43^{b}±0.08	90.26^{a}±0.08
4	CREATININE (g/lit)	3.26±0.06	5.96^{a}±0.10	8.01^{a}±0.11	10.34^{a}±0.07
5	UREA (g/lit)	19.52±0.07	21.62^{c}±0.10	22.98^{b}±0.08	24.43^{a}±0.07

All Values are Mean ± SD of six individual observations.
Values are statistically significant at (a) $p<0.001$, (b) $p<0.01$, (c) $p< 0.05$.
PDC: Percent deviation over control group.

Table 4: Changes in the serum enzymes and blood parameters in control and Cadmium treated rats and after supplementation with Zinc, Vitamin C and Zn+Vit-C

S.no.	Parameter / Serum	Control 15d Cd	Zinc			Vitamin-C			Zn+Vitamin-C		
			7days	15days	30days	7days	15days	30days	7days	15days	30days
1	AST IU/ml/hr	55.96[b] ±0.10	48.71[c] ±0.11	44.45[b] ±0.10	40.32[a] ±0.28	49.35[c] ±0.34	46.11[b] ±0.14	42.23[a] ±0.08	47.78[c] ±0.08	43.34[a] ±0.09	38.71[a] ±0.11
2	ALT IU/ml/hr	35.63[b] ±0.06	30.28[b] ±0.09	28.34[b] ±0.09	26.24[a] ±0.14	31.12[c] ±0.13	29.29[b] ±0.09	27.36[a] ±0.07	30.82[c] ±0.17	26.37[a] ±0.07	24.84[a] ±0.09
3	ALP IU/ml/hr	84.43[b] ±0.08	74.76[c] ±0.09	71.64[b] ±0.09	65.86[a] ±0.14	75.87[c] ±0.17	72.84[c] ±0.08	66.15[a] ±0.26	72.53[c] ±0.09	69.76[b] ±0.42	63.93[a] ±0.39
4	CREATININE g/lit	8.01[b] ±0.11	6.43[b] ±0.07	5.36[a] ±0.07	3.96[a] ±0.11	6.80[b] ±0.08	5.82[a] ±0.11	4.37[a] ±0.09	5.90[a] ±0.07	4.52[a] ±0.08	3.17[a] ±0.07
5	UREA g/lit	22.98[b] ±0.08	18.14[b] ±0.14	16.64[a] ±0.08	15.63[a] ±0.06	18.75[b] ±0.09	17.32[a] ±0.11	16.01[a] ±0.06	17.77[a] ±0.13	16.28[a] ±0.07	14.58[a] ±0.39

All values are Mean ± SD of six individual observations.
Values are statistically significant at (a) $p<0.001$, (b) $p<0.01$, (c) $p< 0.05$
PDC: Percent deviation over control group.
HSI: Histo Somatic Index
AST: Aspertate Amino transferase
ALT: Alanine Amino transferase
ALP: Alkaline Phosphatase