DISRUPTION OF METABOLIC INTEGRATION OF PROTEIN IN CEREBRAL TISSUE DUE TO SUB-ACUTE CR(VI) EXPOSURE

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Abstract
Hypoglycaemia associated with probable anorexia and alteration of cerebro-cellular mass causes significant reduction in the body weight and cerebro somatic index in the sub-acute chromium [Cr(VI)] exposure at a dose of 10 mg/Kg body weight/day for a period of thirty days.Cr(VI) exerted significant impact on protein metabolism as evidenced by depletion of brain protein contents and increased level of free amino acid nitrogen. Proteolytic enzyme activities like trypsin, cathepsin and pronase were also significantly altered depending on the availability of their substrates. Additionally, the transaminase enzyme activities like GOT and GPT were also elevated in cerebral tissue after chromium exposure. Cr(VI) stimulates stress response in the intracellular settings and drags the cellular saturation into very vulnerable by competing growth efficient biomolecules and other sub-cellular components responsible for crucial physiological performance., like hormonal activity, metabolic integrity and summative physiological homeostasis in the organisms.

Keywords: Hexavalent chromium, protein depletion, proteolytic enzymes.

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1. INTRODUCTION

Ever changing food habit and environmental annoyance trigger proliferation of wide range of oxidative stress molecules in cellular micro-environment. These molecules of stress lineages significantly attack cellular materials, chromoso-mal and mitochondrial DNA resulting in genomic instability, post-translational modification of protein with subsequent alteration in bio-molecular metabolism, crucially responsible for synthesis, development and repair of the organism. Cooperative interaction of metabolic pathways is also interrupted due to excessive generation of free radicals and oxidative stress molecules. Hexavalent chromium interfere organ specific metabolic biochemistry and mitochondrial further more it also deteriorates probable mechanism of enzymatic and substrate level energy generation mechanism [Shil and Pal 2017, Pal and Shil 2018]. Neuronal cells are very delicate and subjective for oxidative stress molecules as they contain large amount of fats, heavy metal toxicity exerts significant role in the synthesis of free radicals and lipid peroxidation [Bagchi et al. 2002] and possess intensive intensity to hamper neural microenvironment after entering into the respective tissues. Cerebral maturation and development completes through several molecular mechanism in which different signalling and transcriptional molecules plays a crucial role [Luna and Sweeney 2004].

The present study thus illuminate on Cr(VI) induced alteration of protein metabolism and disruption of activity of cerebral proteolytic enzymes. The study will also reveal the how Cr(VI) is interact with different proteolytic enzymes and substrate in the cerebral tissue.

2. MATERIALSANDMETHODS

2.1. Materials

Chemicals like potassium dichromate ($K_2Cr_2O_7$), bovine serum albumin (BSA), trichloroacetic acid (TCA), sodium carbonate, sodium potassium tartarate, hemoglobin substrate, sucrose, urea, boric acid, hydrochloric acid (HCl), acetic acid, casein, ethanol, sodium cyanide, ninhydrin, leucine, isopropanol, methyl cello solve etc. were of analytical grade and purchased from Merck (India), SRL (India), Sigma–Aldrich (India). Biochemical kit S such as GPT,GOT, cholesterol (Total Cholesterol, LDL, HDL) and triglyceride were purchased from Coral clinical systems. Ultrapure water by Millipore was used throughout the experiment to avoid metal contamination in preparation of reagents.

2.2. Animals

To conduct the present experiment Swiss albino male mice (N=12) weighing 25-30gm were purchased from Chakraborty Enterprise, Kolkata, India, an authorised animal supplier nominated by Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Govt. of India.

According to the guideline of CPCSEA, animals were housed in polypropylene cages to be acclimatized in tolerable pathogen free laboratory setting for one week before starting the treatment. Drinking water was given to the animal *ad-libitum* throughout the treatment schedule. The animals were kept in the treatment room with sustaining 22˚C to 25˚C temperature and humidity (50%) with alternate light and dark coverage for 12 hours.

2.3. Experimental Design

Healthy and equal average sized body weight (30-35 gm) of mice were taken for the present study and divided into two groups namely control and chromium(VI)-treated group, each having six (N= 6) numbers of animals.

Control group:-Animals received 0.9% Na Clorally.

Chromium-treated groups: The animals of this group were treated with hexavalent chromium (as potassium dichromate, $K_2Cr_2O_7$ at a dose of 10 mg/kg b.w/day orally by oro-gastric feeding needle for a period of 30 days).

2.4. Animal Sacrifice

After the end of the treatment the mice were sacrificed by cervical dislocation as per rules and regulation of Institutional Animal Ethical Committee. Thereafter, blood was collected from the hepatic vein to separate serum, braintissues was taken out from all the animals, washed in ice-cold saline (0.9%) solution, blotted dry, weighed and kept at -20˚C until biochemical analyses were performed.

2.5. Preparation of tissue homogenate

The 5% brain tissue homogenate were prepared in0.1 M potassium phosphate buffer (pH 7.4) by using all glass homogenizer and kept frozen at -20ºC until biochemical analyses were performed.

2.6. Anthropometric parameters:

2.6.1. Body weight and cerebro-somatic index (CSI):

The body weight of each animal of each group was taken onward the commencement day of treatment and also noted periodically until

sacrifice to observe the changes of bodyweight in different groups. The organ weight (whole brain) of respective group of animals was also recorded after sacrifice of animals. From these, the organo-somatic index (OSI) of brain was calculated [Krishnaiah and Reddy, 2007].

$$Organo - somatic\ index = \frac{Weight\ of\ the\ organ\ (gm)}{Day\ 30th\ total\ body\ weight} \times 100$$

2.6.1. Tissue protein content in cerebral tissue

The tissue protein content was estimated according to the method of Lowry *et al.* (1951). To the 0.1 ml of tissue homogenate, 0.9 ml of redistilled water and 4.5 ml of alkaline copper reagent were added and kept at room temperature for 10 min. To this, 0.5 ml of Folin-ciacalteau reagent (1:2) was added and the purplish blue colour developed was read after 20 min at 640 nm. Protein content was expressed as mg%.

2.6.2. Free amino acid nitrogen content in cerebral tissue

To estimate the amount of free amino acid nitrogen in liver, brain and muscle the 5% tissue homogenate in 0.1 M phosphate buffer(pH 7.4) was used. The tissue homogenate was first dissolved in 0.67 (N) H_2SO_4 and 10% Na-tungstate to precipitate proteins and then centrifuged to get the protein free extract. The resultant supernatant was treated with cyanide acetate buffer and 3% ninhydrin solution as per the protocol proposed by Rosen *et al.* (1957). After that the solution was heated at 100^0C in a water bath for 5 minutes and immediately after cooling added with isopropanol to develop a violet colour. Optical density of that coloured solution was measured in a spectrophotometer at 570 nm.

2.6.3. Pronase activity in cerebral tissue

The pronase activity in different tissues (liver, brain and muscle)was estimated following the method of Barman (1974). A 5% tissue homogenate in sucrose solution was incubated at 40°C temperature with casein substrate for half an hour. The reaction was stopped by adding protein precipitating reagent solution. The mixture was then centrifuged to get the supernatant and the optical density of the resultant supernatant was taken at 280 nm wavelength. The tissue pronase activity was expressed as n moles of tyrosine formed/min/gm of protein.

2.6.4. Trypsin activity in cerebral tissue

To determine the activity of trypsin 5% tissue homogenate was used as proposed by Green (1953).In this method two separate tubes were taken, one containing a definite volume of tissue homogenate and 2.5 ml of Hb substrate which served as the sample tube and the other having 5% TCA and Hb substrate serving as the buffer blank. Both the tubes were incubated at 25°C for 30 minutes. After incubation 5% TCA was mixed to the sample tube to stop the reaction whereas 5% tissue homogenate was added to the tube marked as buffer blank. The resulting supernatant was taken for the measurement of released tyrosine content in a spectrophotometer at 280 nm wavelength. The enzyme activity was calculated as n moles of tyrosine produced/min/gm of protein.

2.6.5. Cathepsin activity in cerebral tissue

To examine the activity of cathepsin in different tissues the method of Pokrovsky (1989)was employed. According to this procedure the tissue homogenate (5%) was added with Hb substrate (4%) and incubated at 37°C for 1 hour. To stop the reaction 8% TCA was added and mixed well. In the same manner a buffer blank was also prepared in which TCA was added before incubation keeping all other ingredients in the mixture as like the sample tube. After precipitation of protein in the mixture, the supernatant was collected by centrifuging all the tubes at 3500 rpm for 10 minutes and optical density of samples was taken in a UV-vis spectrophotometer at 280 nm. Tissue cathepsin activity was expressed in terms of n moles of tyrosine produced/minute/gm protein.

2.6.6. Glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) activities

The transaminase enzyme activities in the studied tissues were determined following the method of Reitman and Frankel(1957). For this assay the standard kit (Coral clinical systems, Goa, India) was used to measure photometrically the colour intensity of the reaction mixture. Generally the colour was formed due to the chemical of reaction of alanine with α-ketoglutarate to form pyruvate in case of GPT, whereas the aspartate was used to react with α-ketoglutarate to originate pyruvate in case of GOT. After this enzymatic reaction all the tubes were allowed to incubate at 37°c for a specific period of time and absorption noted at 505 nm. Then the enzyme activities were in terms of units/mg of tissue at 505 nm.

2.6.7 Protein carbonyl content:
Protein carbonyl content of cerebral tissue will be estimated according to the method of Stadtman & Levine [2000]. The samples was treated with an equal volume of 10mM 2, 4-DNPH in 2.5 M HCl and incubated for 30 minutes at room temperature by vortexing in every 15 minutes. After that samples are then treated with 20% TCA and allowed to keep in ice. After centrifugation, the supernatant was discarded. The pallets were then washed two times with ethanol/ethyl acetate (1:1 v/v). The final precipitate was dissolved in 6% SDS and allowed for centrifugation again. The optical density was then recorded at 370nm. The results were expressed as nmol of DNPH incorporated/mg protein based on the molar extinction coefficient of 22,000/M/cm.

2.7. Statistical Analyses
All results were expressed as means±S.E.M. To record the significance of differences paired 'Student's t test' was carried out for comparison between the two groups. P<0.05 was considered statistically significant.

3. RESULTS
3.1. Body weight: There was change(12.36% decrease; p<0.05) in the body weight of the experimental animals compared to the control group after 30 days of chromium exposure (**Table 1**).
3.2. Organo-somatic index (OSI): Significant decrease (40.85%; p<0.05) in cerebro-somatic index (CSI) was noticed after chromium exposure at the present dose and duration. On the contrary the cerebro-somatic index (CSI) remained unaltered following chromium treatment (**Table 1**).
3.3. Blood Glucose level: In this **Fig.1** it is expressed that blood glucose level significantly decreased in experimental mice at 56.26%(p<0.01) due to Cr(VI) intoxication.

3.4. Total protein: Change in total protein content reveals that chromium treatment at the present dose and duration significantly decreased the protein content in cerebral tissuesby19.47% (p<0.01), (**Fig 2**).

3. Free amino nitrogen: **Table 1** depicts the alteration in free amino acid nitrogen in observed tissues. The results indicate that chromium treatment increased the level of free amino nitrogen in all the tissues. The increase was found to be almost eight folds (p<0.05), in brain.

3.13. Pronase activity: Fig.3shows the change in pronase activity following exposure to chromium. The results indicate that the pronase activity significantly decreased in cerebral, tissues by 32.05% (p<0.05) after chromium exposure.

3.14. Trypsin activity: There was a significant increase in the activity of trypsin in cerebral, tissue of chromium-treated mice. The increased enzyme activity was accomplished by 138.3% (p<0.05), in brain(**Fig 4)**.

3.15. Cathepsin activity: The change in cathepsin activity is represented in **Fig 5** which reveals that chromium treatment decreased the enzyme activity by 18.8% (p<0.05), in brain.

3.16. GPT and GOT activities: Effects of chromium on tissue GPT and GOT activities reveal that GPT activity in the experimental group of mice was increased by 48.98% (p<0.05), in brain. Tissue GOT activities were also increased in chromium-exposed animals by 6.24% (p<0.01), in cerebral tissues **Fig 6, 7)**.

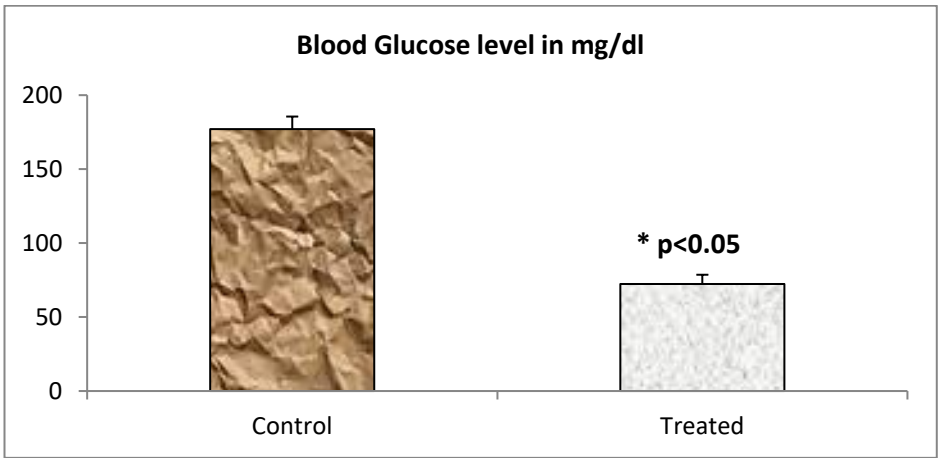

Fig 1: Effect of chromium on blood glucose level**.** Values are Means±S.E.M. p<0.05 is considered statistically significant.

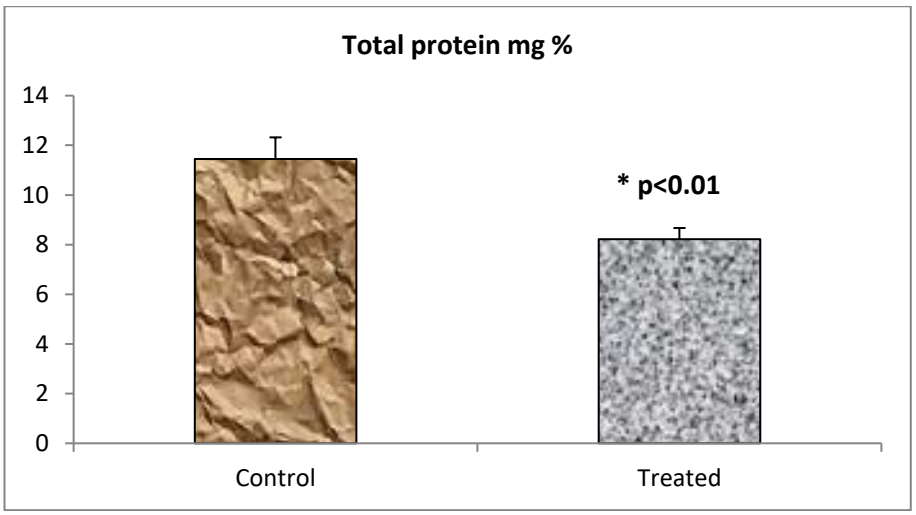

Fig. 2 Impact of Sub-acute Cr(VI) exposure on Total protein content. Values are Means±S.E.M. p<0.01 is considered statistically significant.

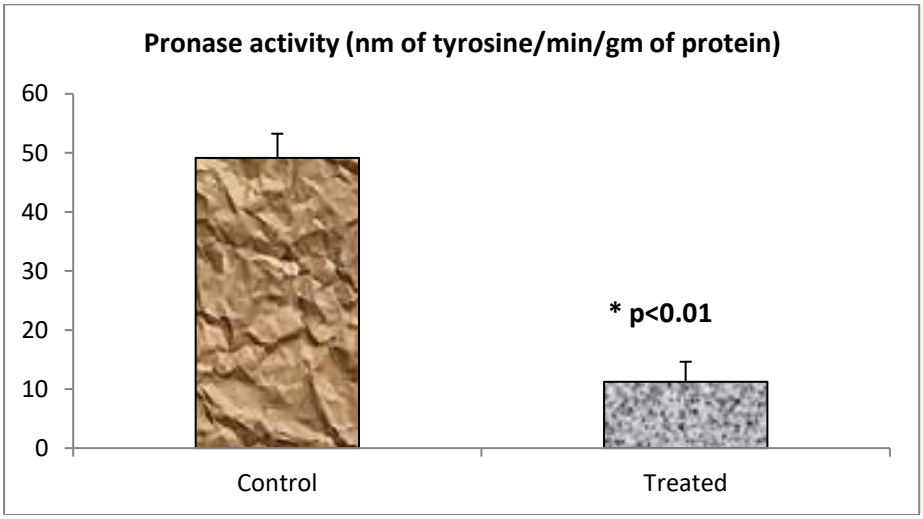

Fig. 3. Effect of Cr(VI) Pronase activity in the cerebral tissue. Values are Means±S.E.M. p<0.01 is considered statistically significant.

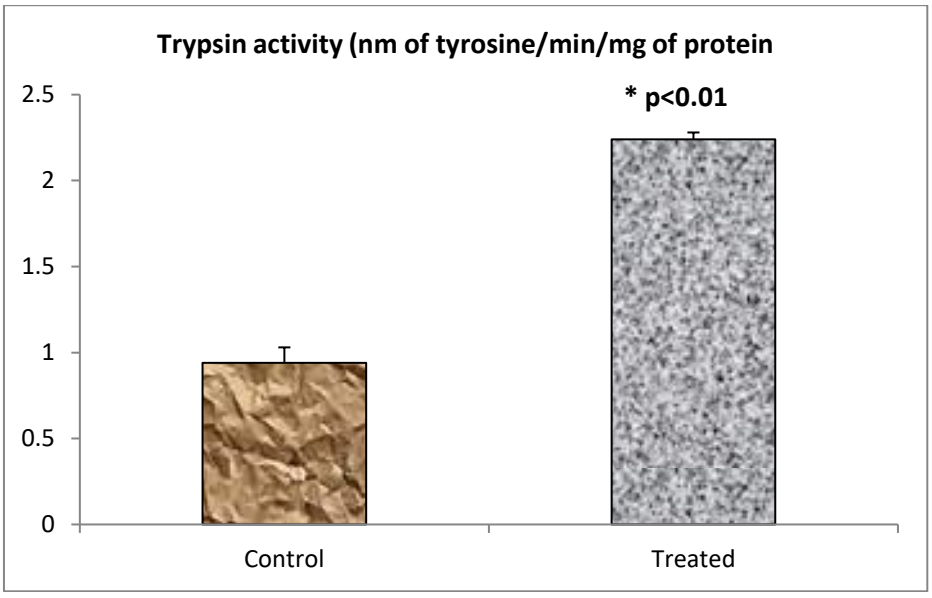

Fig. 4 Alteration of Trypsin activity due to sub-acute Cr(VI). Values are Means±S.E.M. p<0.01 is considered statistically significant.

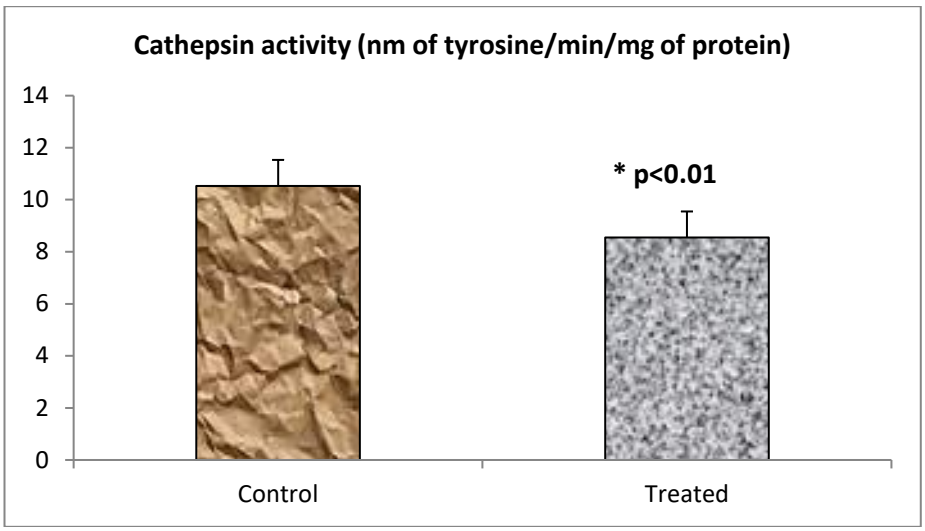

Fig. 5 Changes in Cathepsin activity due to sub-acute Cr(VI) exposure. Values are Means±S.E.M. p<0.01 is considered statistically significant.

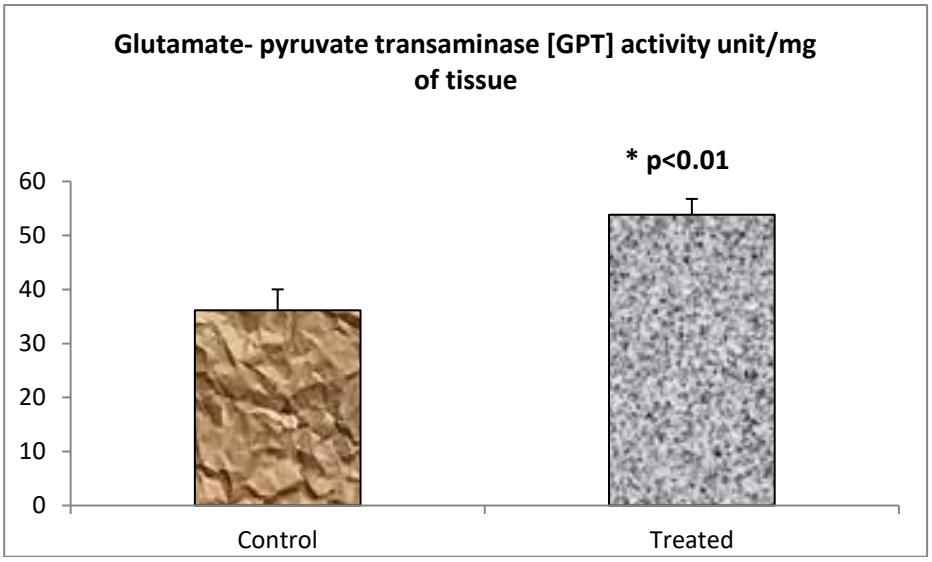

Fig. 6 Impact on GPT activities due to Cr(VI). Values are Means±S.E.M. p<0.01 is considered statistically significant.

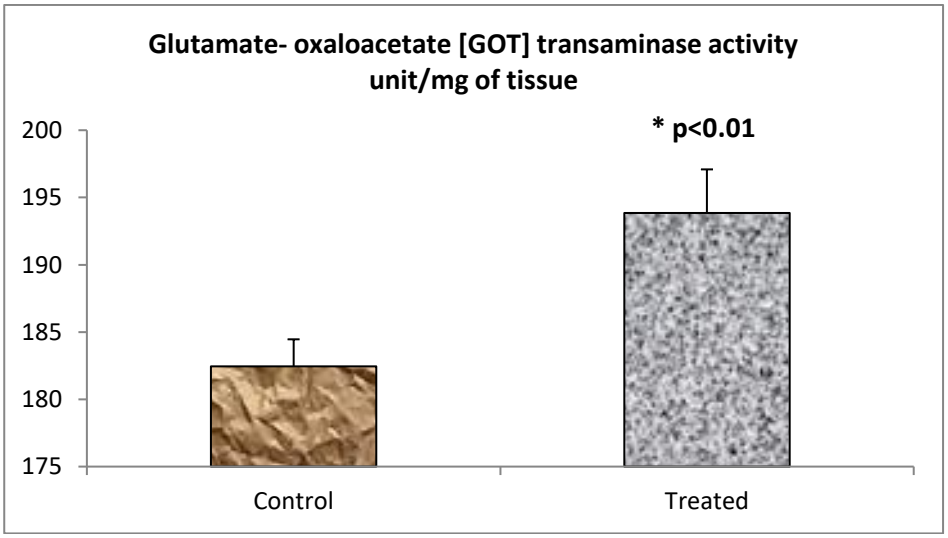

Fig.7 Alteration of GOT activities due to sub-acute Cr(VI) exposure. Values are Means±S.E.M. p<0.01 is considered statistically significant.

Table1: Changes in the Body weight and Organo-somatic index of mice exposed by chromium

Group of animals	Body weight (gm)	Free amino nitrogen level mg%	Organo-somatic Index
			Brain
Control (N=6)	39.34±2.11	0.98±0.07	2.98±0.34 P<0.05
Treated (N=6)	32.58±0.91 P<0.05	2.98±0.34 P<0.05	0.24±0.02 P<0.05

[Values are means±S.E.M. Figures in the parentheses indicate number of animals. p<0.05 is considered statistically significant]

4. DISCUSSION

Metal toxicity leads to significant alteration in the metabolic and physiological occurrences in the organ and cellular micro-environments. Heavy metal and toxicant enters into the physiological system by mimicking the activity of ligends, active cataion and anions with the help transporter proteins. Upon the entry to the cellular setting all these heavy metal cause irreversible reaction with the bio molecules of cytoplasmic as well as nuclear macromolecular crowd, all these detrimental reactions escort towards micro-molecular instability, genetic aberration, translational modification and sub-cellular dysfunctional, Which might be the principle contributing factor for significant weight loss in the Cr(VI) exposed organisms.

Cr(VI) a recognized EDC and a persistent chemicals that reacts with the endocrine system and interferes with overall growth, development, metabolic and digestive systems. Subsequently cause tissue damage, cellular necrosis and hormonal dysfunctions in the target organs. The significant reduction of cerebro-somatic index may be due to toxic bioaccumulation of Cr(VI) and possible neuronal damages due to Cr(VI) exposure.

The present study explores that total protein content was declined in the cerebral tissue due to exposure of Cr(VI). Protein depletion in the tissue indicates the physiological strategy in order to meet the energy demands or to adapt itself to the changed metabolic system which may lead to the stimulation of degradation processes such as proteolysis and utilization of degraded products for increased energy metabolism [Begam and Vijaya raghavan 1996, Palanisamy 2011]. The reduction of total protein content may be due to breakdown of tissue proteins under the effect of heavy metal which in turn increased the free amino acid nitrogen concentration in various tissue[Shakoori et al. 1994]. The present study is in resemblance with the observation of Chandravarthy and Reddy [1994]which revealed a remarkable decrease in total protein content with increased activities of transaminases and proteases in experimental animals on exposure to heavy transitional metal. The current study further establishes that the sub-acute Cr(VI) exposure elevates free amino nitrogen content in cerebral tissue significantly. Enhancement of free amino acid nitrogen level in cerebral tissue may be ascribed to the enhanced accumulation of protein degradation products after chromium intoxication or may be due to mobilization of free amino acids from peripheral tissues like kidney to the liver and brain to promote gluconeogenesis to meet the demand of energy. It is reported that there is an appreciable decline in different biochemical constituents in various tissues offresh water fish under chromium stress[Vutukuru 2005]. Kori-Siakpere et al.[2006]observed that the plasma protein was lowered in experimental animals exposed to sub-lethal dose of heavy metal [Kori-Siakpere et al. 2006].However, increased proteolytic activity of trypsin,a lysosomal enzyme, in brain indicates the increased rate of proteolysis and supports the total protein depletion and elevated free amino nitrogen content in cerebral tissue after chromium intoxication. Heavy metal toxicity can lead to alteration of the structure, permeability and integrity of cell membranes resulting in diffusion of their enzymes into cytosol where it allows them to enhance their functional activities[Sternlib et al. 1976]. Hence high activity of proteases like trypsin in the organs of the experimental animals may be due to the damage caused by Cr(VI) at cellular level, especially in cerebral tissue. According to the study of Obrian et al. [2003] cellular structure of all vital organs is very much susceptible to the Cr(VI) toxicity. Enhanced protease activity induces proteolysis in the respective tissue and elevates the free amino acids which may act as osmotic and ionic effectors to carry electrostatic symmetry among peripheral medium and blood [Schmit-Nielson 1975, Prasath et al. 2008].

The present study further depicts the decreasing activities of other important proteases such as cathepsin and pronase in the brain with sub-acute Cr(VI) exposure. Cathepsin and pronase are the vital lysosomal proteolytic enzymes that accomplish protein degradation and facilitate

enrichment of amino acid pool in various tissues for maintaining crucial metabolic attributes such as cellular repair, energy generation and utilisation of degraded products [Palanisamy 2011]. It is established that proteins and proteolytic enzymes are very much sensitive to the heavy metal poisoning [Jacobs et al.1977] and Cr(VI), being one of them is widely suspected to impose organ toxicity, genotoxicity, chromosomal aberration, mutational changes and DNA-DNA cross strand etc. which may prevent enzyme formation or may enhance depletion of proper metabolic inter mediate from the respective tissue [Zithcovitch 2011]. Less availability of substrates in the above mentioned tissues may be one of the causative factors of Cr(VI) induced retardation of pronase and cathepsin activities. Alteration in physico-chemical properties of proteins may involve excess production of reactive oxygen species, and chromium, being a free radical generator [Quinteros et al. 2008] may contribute to them. These in turn may attribute to reduced level of desired tissue proteins for pronase and cathepsin actions. Alteration of these specific proteolytic enzyme activities after chromium exposure is a unique observation of the present study.

Amino-transferases contribute an important role in amino acid catabolism and play key role in nitrogen metabolism and energy mobilization [Calabrese et al. 1977]. Transaminases such as GPT and GOT activities in brain increased significantly in chromium toxicity. This may be due to increased accumulation of free amino acid nitrogen in those tissues which may contribute more substrates for gluconeogenesis to compensate hypoglycaemia induced by chromium. Increased transaminase activity in chromium toxicity in specific organ is also reported earlier [Soudani et al. 2012, Kim and Kang 2016] reflecting adverse effects of chromium at tissue level.

5. ACKNOWLEDGEMENT

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6. CONFLICT OF INTEREST

The author strongly declare that there is no conflict of interest in publishing the data in the present form.

7. REFERENCES

1. Abreu, P.L., Ferreira, L.M.R., Urbano, A.M., 2014. Impact of hexavalent chromium on mammalian cell bioenergetics, phenotypic changes, molecular basis and potential relevance to chromate-induced lung cancer. Biometals27, 409-443.
2. Ahmad, M.K., Syma, S., Mahmood, R., 2011.Cr(VI) induces lipid peroxidation, protein oxidation and alters the activities of antioxidant enzymes in human erythrocytes. Biol. Trace. Elem. Res. 144, 426–435.
3. Allain, C.C., 1974. Enzymatic determination of total cholesterol. Clin. Chem. 204, 440-470.
4. Bagchi, D., Vuchetich, P.J., Bagchi, M., Hassoun, E.A., Tran, M.X., Tang, L., Stohs, S.J., 1997.Induction of oxidative stress by chronic administration of sodium dichromate [chromium VI] and cadmium chloride [cadmium II] to rats. Free. Rad. Biol. Med. 22, 471-478.
5. Bagchi, D., Balmoori, J., Bagchi, M., Ye, X., Williams, C.B., Stohs, S.J., 2000.Role of p53 tumor suppressor gene in the toxicity of TCDD, endrin, naphthalene, and chromium (VI) in liver and brain tissues of mice. Free Rad. Biol. Med. 28, 895-903.
6. Bandyopadhyay, D., Dutta, M., Chattopadhyay, A., Bose, G., Ghosh, A., Banerjee, A., Ghosh, A.K., Mishra, S., Pattari, S.K., Das, T., 2014. Aqueous bark extract of Terminalia arjuna protects against high fat diet aggravated arsenic-induced oxidative stress in rat heart and liver, involvement of antioxidant mechanisms. J. Pharm. Res. 8, 1285-1302.
7. Bergmeyer, H.U., 1983. Methods of Enzymatic Analysis, 3rded.Verlag Chemie, Weinheim.
8. Belyaeva, E.A., 2010. Mitochondrial respiratory chain inhibitors modulate the metal-induced inner mitochondrial membrane permeabilization. Acta.Biochim.Pol.57, 435-41.
9. Calabrese, A., Thurberg, F.P., Gould, E., 1977. Effects of cadmium, mercury and silver on marine animals. Mar. Fish. Rev. 39, 5-11.
10. Chandravarthy, V.M., Reddy, S.L.N., 1994. In vivo recovery of protein metabolism in gill and brain of fresh water fish Anabas scadens, after exposure to lead nitrate. J. Environ. Biol. 151, 75-82.
11. Green, N.M., Work, E., 1953. Pancreatic trypsin inhibitor: Reaction with trypsin. Biochem. J. 54, 347–352.
12. Harper, A. H., 1985. Review of Bio Chemistry, 20thed. Lange Medical, California.
13. Harabawy, A.S., Mosleh, Y.Y., 2014.The role of vitamins A, C, E and selenium as

antioxidants against genotoxicity and cytotoxicity of cadmium, copper, lead and zinc on erythrocytes of Nile tilapia, Oreo chromisniloticus. Ecotoxicol. Environ. Safety 104, 28-35.
14. Hegazy, R., Salama, A., Mansour, D., Hassan, A., 2016. Reno protective Effect of Lactoferrin against Chromium-Induced Acute Kidney Injury in Rats: Involvement of IL-18 and IGF-1 Inhibition. Plos One. 11, 1-18.
15. Jacobs, J.M., Carmicheal, N., Cavanagh, J.B., 1977. Ultra structural changes in the nervous system of rabbits poisoned with methyl mercury. Toxical. Appl. Pharmocol. 39, 249–261.
16. Jacobs, J.A., Testa, S.M., 2005. Overview of chromium VI) in the environment: background and history. Chromium VI) handbook. CRC Press, USA.
17. Kim, J. H., Kang, J. C., 2016. The chromium accumulation and its physiological effects in juvenile rockfish, Sebastes schlegelii, exposed to different levels of dietary chromium (Cr 6+) concentrations. Environ. Toxicol. Pharm. 41, 152-158.
18. King, J., 1965. Isocitrate dehydrogenase, Practical Clinical Enzymology. Nostrand Co., London.
19. Kori-Siakpere, O., Ake, J.E.G., Avworo,U.M.,2006. Sub-lethal effects of Cadmium on some selected haematological parameters of Heteroclarias A Hybrid of Heterobran chusbidorsalis and Clarias gariepinus**.** Inter. J. Zool. Res. 2, 77-83.
20. Krishnaiah, C., Reddy, K.P., 2007.Dose-dependent effects of fluoride on neuro chemical milieu in the hippocampus and neocortex of rat brain.Fluoride40, 101-10.
21. Lowry, O.H., Rose brough, N.J., Farr, A.L., Randall, R.J., 1951.Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265-275..
22. O'Brien, T., Xu, J., Patierno, S.R., 2001. Effects of glutathione on chromium-induced DNA crosslinking and DNA polymerase arrest. Mol. Cell. Biochem.222, 173–182.
23. Palanisamy, P., Sasikala, G., Mallikaraj, D., Bhuvaneshwari N., Natarajan. N., 2011. Haematological changes of fresh water food fish, Channastriataon exposure to Cleistanthus collinussuicidal plant extract. Res. J. Pharma. Biol. Chem. Sci. 2, 812-816.
24. Pokrovsky, A.A., Archakov, A.I., Lyubimt seva, O.N., Strove, E.A., Makarova, V.O., 1989. Laboratory Manual In Biochemistry 1st ed. Mir Publisher, Moscow.
25. Prasath, P.M.D., Arivoli, S., 2008. Bio-chemical study of freshwater fish Catlacatla with reference to mercury chloride. Iran. J. Environ. Health Sci. Eng. 5,109-116.
26. Quinteros, F.A., Machiavelli, L.I., Miler, E.A., Cabilla, J.P., Duvilanski, B.H., 2008. Mechanisms of chromium (VI)-induced apoptosis in anterior pituitary cells. Toxicology 249, 109-115.
27. Reitman, S., Frankel, S., 1957.Determination of serum glutamic oxaloactic and glutamic pyruvic transaminase. Am. J. Clin.Pathol.28, 56-60.
28. Schmit–Nielson, B., 1975.Comparative physiology cellularion and volume regulation. J. Exp. Zool.194, 107–220.
29. Soudani, N., Rafrafi, M., Ben-Amara, I., Hakim, A., Troudi, A., Zeghal, K.M., Ben Salah H, Boudawara T, Zeghal N. 2013. Oxidative stress-related lung dysfunction by chromium (VI), alleviation by Citrus aurantium L.J. Physiol. Biochem. 69, 239-253.
30. Shakoori, A.R., Javed Iqbal, M., Latif Mughal, A., Syed Shahid A., 1994.Biochemical changes induced by inorganic mercury on the blood, liver and muscles of fresh water fish, Ctenopharyng odonidella. J. Ecotoxicol. Environ. Monit.4, 81–92.
31. Sternlieb, I., Goldfischer, S., 1976. Heavy metals and lysosomes. Lysosomes in biology and pathology, 5th ed. American Elsevier Publishing Company, New York.
32. Stadtman ER, Levine RL. Protein oxidation. Ann N Y Acad Sci. 2000;899:191-208.
33. Vutukuru, S.S., 2005. Acute effects of Hexavalent chromium on survival, oxygen consumption hematological parameters and some biochemical profiles of the Indian Major carp, Labeorohita. Int. J. Environ. Res. Public Health.23, 456-462.
34. Zhitkovich, A., 2011. Chromium in drinking water: sources, metabolism, and cancer risks. Chem. Res. Toxicol, 24, 1617-1629.