ALTERATION OF CEREBRAL CARBOHYDRATE METABOLISM DUE TO SUB-ACUTE EXPOSURE OF CR(VI)

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Abstract

Chromium exposure at a dose of 10 mg/Kg body weight/day for a period of thirty days produced hypoglycaemia and collapses the cerebral metabolic integrity and cellular metabolism in Swiss albino mice. Prognostic changes in feeding behavior, loss of appetite and possible bioaccumulation, stress induced neuronal damage by Cr(VI) in the cerebral tissue may cause the significant weight loss and alteration of CSI. The glycolytic activity was depressed as indicated by less accumulation of pyruvic acid in cerebral tissue. Additionally, chromium significantly inhibit the activities of succinate dehydrogenase and NADH dehydrogenase resulting in impaired oxidation of succinate *via* TCA cycle and also retardation of energy production through inhibition of mitochondrial respiratory chain. Moreover, the suppressed malate dehydrogenase activity in chromium stressed brain indicates less energy supply to cerebral tissue.

Keywords: Hexavalent chromium, glycolysis, TCA cycle, cerebro-somatic index, cellular energy generation

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1. INTRODUCTION

Industrial booming from twentieth century to current scenario environmental accumulation of volatile and persistent toxic metallic compound increased to unfathomable amount and drags the ecosystem, into a very vulnerable situation. Amongst them, hexavalent chromium [Cr(VI)] is released into the environment by means of human activities at stationary point sources in seven countries: South Africa, India, Kazakhstan, Zimbabwe, Finland, Brazil, and Turkey[Jacobs and Testa, 2005]. The primary transmit of this toxicant is inhalation [Bagchi et al. 2000]. The exposure and assimilation of Cr(VI) can happens also with the occasional and chronic ingestion through oral and dermal routs. Hazardous occupational accumulation may happens in the individual working in the wood preservation, welding and painting industries [Cunat, 2004]. Chromium compounds Cr (VI) have various industrial applications including tanning, corrosion inhibition, plating, glassware cleaning solutions, safety match manufacturing, metal finishing, and pigments where high concentrations of chromium (40–50,000 ppm) have been reported in the effluents of these industries [Park et al. 2004].

One of the most important features of chromium is its persistence in the environment and its change in form and valence. In fact, chromium is found in several valence form spredominately Cr(0), Cr(III), and Cr(VI)), yet only Cr(VI) has been found to be carcinogenic as well as mutagenic, and also abundant in natural water [Bagchi et al. 1997; Blasiak et al. 2000]. The toxic effects of hexavalent chromium are widely believed to be associated with the stimulation of free radical processes as well as the formation of highly reactive intermediates of Cr(VI) reduction [Valko et al. 2005]. It is well established that the brain with high lipid content and lower antioxidant levels as compared to other organs, is a major site of oxygen consumption, and also particularly susceptible to oxidative stress. A previous study of Bagchi et al. [1997] confirmed that the treatment with Cr(VI) could lead to lipid peroxidation in mammalian cerebral tissue[Bagchi et al. 2002]. Moreover, a number of studies reported that Cr(VI) hoarded in the rat hypothalamus, anterior pituitary, hepatic as well as muscular tissue implements toxic infestations among these tissues [Quinteros et al. 2007].

Tin the intracellular compartments he toxic manifestations of Cr(VI) mostly executed by interfering with the cytoplasm, nuclear macro and micro molecules. Thus the cellular materials infested with the toxic heavy metal acts radically and lead towards the irreversible damage and destruction to the respective tissue. [Tajima et al. 2010, Pan et al. 2012, Xiao et al. 2012].The present study thus elucidates some new information regarding carbohydrate metabolic efficacy in highly energetic cerebral tissues also to find out if there is any integration in their metabolic profiles that could be altered by chromium intoxication.

2. MATERIALSAND METHODS

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). 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 authorized 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% NaCl w/v orally.

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, brain tissues was taken out from all the animals, was hedin 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].

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

2.7. Biochemical analysis of cerebral tissue:

2.7.1. Cerebral pyruvic acid level

The cerebral pyruvic acid content was estimated according to the protocol of Segal*et al.* [1956]. Briefly, 10% tissue homogenate was centrifuged at 3000 rpm for 10 min with 5% TCA. The resultant supernatant was mixed with 1ml distilled water and 0.5 ml 2,4 DNPH and shaken it for 3 min. Then toluene was added and mixed vigorously by hand shaking for few min. After that added 2 ml of sodium carbonate and sodium hydroxide solution each to measure the optical density at 420 nm. The observed result was expressed as mg% of cerebral tissue.

2.7.2. Lactate dehydrogenase (LDH) activity of cerebral tissue

The lactate dehydrogenase activity was estimated by the method of Bergmayer [1963];the activity of the enzyme was measured by the rate of consumption of pyruvate and reduced DPNH by 10% tissue homogenate. Decreased optical density at 340nm was measured in an UV-vis spectrophotometer for oxidation of DPNHat10 sec interval for 5 min. Enzyme activity was expressed in unit/min/mg of cerebral tissue.

2.7.3. Succinate dehydrogenase (SDH) activity in cerebral tissue

The SDH activity was measured spectro-photometrially by the reduction of potassium ferricyanide [$K_3Fe(CN)_6$] at 420 nm according to the method of Bandyopadhyay*et al.*[2014].One ml of the assay mixture contained 50 mM phosphate buffer (pH 7.4), 2% (w/v) BSA, 4 mM succinate, 2.5 mM $K_3Fe(CN)_6$ and a suitable aliquot of the enzyme. The enzyme activity was expressed as unit/min/mg tissue protein.

2.7.4. Isocitratede hydrogenase(IDH) activity in cerebral tissue

Mitochondrial IDH activity was measured according to the method of King [1965].To the 0.1 ml of Tris-HCl, 0.2 ml of trisodiumiso citrate, 0.3 ml of manganese chloride, 0.2 ml of mitochondrial suspension and 0.2 ml of NADP (0.2 ml of saline for control) were added. After 60 minutes of incubation, 0.001M of DNPH was added followed by 0.005 M of EDTA. Then 0.4N NaOH was added, OD taken at 420 nm in an UV spectrophotometer. The enzyme activity was expressed as unit/min/µgm of protein.

2.7.5. Malate dehydrogenase(MDH) assay in cerebral tissue

This enzyme activity was determined by the method of Mehler *et al.* [1948] using an assay mixture containing potassium phosphate buffer, 0.0076M oxaloacetic acid and 0.005 M NADH at pH 7.4. The reduction of NADH was measured at 340 nm for 3-5 min with 10 second interval and expressed the activity as mmoles of NADH oxidized/min/mg of protein.

2.7.6. . Assay of NADH: Ubiquinon C oxido-reductase activity in cerebral tissue

The activity of NADH: Ubiquinone C oxidoreductase was measured by the method of Minakami*et al.*[1962]. The reaction mixture contained 1 ml phosphate buffer,0.1 ml of potassium ferricyanide and 0.2 ml mitochondrial suspension in a total reaction volume of 3ml with distilled water. Freshly prepared (0.1%) NADH solution was added just before the addition of the enzyme except the control set. The change in the OD was measured at 420 nm for 3 min and the

enzyme activity was expressed as mmoles of NADH oxidised/min/mg of protein.

2.8. 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 accountable change (10.36% 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 (42.85%; p<0.05) in cerebro-somatic index (CSI) was noticed after chromium exposure at the present dose and duration (**Table 1**).

3.3. Blood Glucose level: In this **Fig.1** it is expressed that blood glucose level significantly decreased in experimental mice at 58.40%(p<0.01) due to Cr(VI) intoxication.

3.5. Pyruvic acid level in the tissues: It is indicated by **Fig. 2** that cerebral pyruvate content decreased by 45.38% (p<0.01) owing to chromium intoxication.

3.6. Lactate dehydrogenase activity: Fig. 3 reveals significant decreasing trends of lactate dehydrogenase activity in cerebral tissue due to sub-acute chromium toxicity by 37.14% (p<0.01) as compared to the control group.

3.7. Succinate dehydrogenase activity: It is revealed from **Fig. 4** that chromium treatment causes marked diminution of succinate dehydrogenase activity in cerebral tissue. The percentage decrease was noted as 63.58% (p<0.01) in cerebral tissue.

3.8.Isocitrate dehydrogenase activity: Fig. 5 represents appreciable increased activity of isocitrate dehydrogenase in brain tissue after chromium exposure at 37.93 %(p<0.05) in the present dose and duration.

3.9. Malate dehydrogenase activity: Malate dehydrogenase activity changes in cerebral tissues, it is where as decreased in brain enzyme activity decreased by 58.07% (p<0.01) after exposure to chromium (**Fig. 6**).

3.10. Mitochondrial complex 1 activity: In **Fig: 7**activity of mitochondrial complex 1[NADH dehydrogenase] is represented which shows significant reducing trends in this enzyme complex by 41.05% (p<0.01)

4. DISCUSSION

Eco-toxicological elements are significantly recognized as carcinogenic, neuplastic, genotoxic and oxidative stress inducer in the cellular compartments. Non-biodegradable toxicants including heavy metals such as chromium, arsenic, lead etc. enter easily into the organisms through inhalation, drinking water or incidental chronic ingestion which ultimately accumulate inside the organisms affecting crucial bio-molecules and enzymatic functions[Soudani et al. 2013]. In the present investigation alteration of metabolic integrity among the carbohydrate, protein and fat in the brain tissue is noted after hexavalent chromium treatment. Chromium treatment appeared to have a significant effect in body weight as well as cerebro-somatic index (CSI) is observed in chromium exposed mice. These findings suggest that change in whole brain weight in relation to body weight is correlated and impacted due to chromium exposure at the present dose and duration. The reduction of the body weight and CSI after chromium exposure may be due to induction of cellular lesions and cerebral atrophy due to result of toxic injury imposed by chromium [Shil and Pal, 2019].Chromium is associated with overproduction of free radicals and other stress molecule lineages, consequently responsible for cellular hypertrophy and cellular damage [Ben Hamida et al. 2016] that may be one of the important causes of alteration of body weight and CSI. Cerebral tissue is heavily structured with fatty acid and very much susceptible to oxidative stress and free radicals, accumulation of Cr(VI) in the brain tissue makes cerebral tissue more vulnerable when there is deficit in cellular defense system due to metabolic disintegration in cellular micro-environment.

Chromium exposure causes significant decrease in blood glucose level at the present dose and duration. It is suggested that hypoglycaemia following chromium exposure may result from renal glycosuria caused by impairment of renal re-absorption of glucose. Chromium-induced acute nephrotoxicity had been demonstrated by Hegazy et al.[2016], [Shil and Pal, 2019]. It is reported that kidney is the major target organ for toxicants, and within it the proximal tubule epithelium is the most important target site of toxicant-induced cell damage [Shil and Pal, 2022].The depressing effect of chromium on cerebral tissue pyruvic acid

content significantly supports hypoglycaemic situation in the organism and the non-availability of substrate molecules for the glycolytic mechanism. Cr(VI) cause cessation principle glycolytic enzymes in the cellular compartments and produce energy deficient state in the accumulated tissues [Shil and Pal, 2022]. Reduced pyruvate content also depicts about the weakening cerebral metabolic state and the overall status of lactate/pyruvate ratio and neurotransmitter glutamate and Gama amino butyric acid (GABA). The reduced pyruvate content also reveals the like of glucose that was produced by increased glycogenolytic activities of the mentioned tissues was released immediately to blood, and this in turn may reduce the accumulation of pyruvic acid in hepatic and muscular tissues by diminishing the glycolytic activity. Variation in the bioenergetics flux in the cerebral tissue may be due to unusual activities of enzymes and substrate level in the circulation as well as in the cellular level which are directly involved in the metabolic pathways and simultaneously altered cellular energy components in the exposed organisms [Ryberg and Alexander 1990]. Retardation of glycolytic activity may also result from reductive conversion of hexavalent chromium to trivalent chromium-ATP complex formation that behaves as competitive inhibitor for various ATP dependent enzymes and several kinases involved in glycolysis as well as TCA cycle [Lipperd et al. 1995, Ahmed et al. 2011]. Additionally, this decrease in glycolytic activity in observed tissues may be an effect of hypoglycaemia induced by chromium. LDH, being a key enzyme of metabolic link between glycolysis and TCA cycle involves in conversion of pyruvate to lactate and thus acts as a good indicator of cellular damage. Suppressed LDH activity by chromium may result from decreased availability of pyruvic acid as substrate in hepatic tissue. Another suggestive mechanism of reduced LDH activity may be the leakage of this enzyme from the hepatic tissue as a result of toxic injury imposed by chromium. Inhibited LDH activity by hexavalent chromium was also observed in teleost fish hepatocytes and in renal tissue at sub-chronic exposure [Venugopal and Reddy 1992] and also in hepatocytes of the African catfish at sub-lethal dose of exposure [Kori-Siakpere et al. 2012].

Succinate dehydrogenase, isocitrate dehydro-genase and malate dehydrogenase are the important enzymes of the TCA cycle that play a crucial role in ATP generation in all vital organs of the individuals. Hexavalent chromium exposure at the present dose and duration exerts negative impact on succinate dehydrogenase enzyme activity thus hampering the ATP production because this enzyme not only plays important role in TCA cycle but also a potential component of the mitochondrial electron transport chain. So, suppressed energy production may trigger anaerobic metabolism in cerebral tissue following chromium treatment. That TCA cycle is impaired by chromium treatment was also evident from the earlier observation[Molina–Jijon et al. 2011]. Insufficient compensation by fermentation and inhibition of cellular respiration by hexavalent chromium imbalance the nucleotide pool and ultimately obliterate the homeostasis of the energy status in chromium intoxicated organs of the experimental animals [Abreu et al. 2013].

Moreover, hexavalent chromium exerts powerful inhibition on mitochondrial dehydrogenases such as NADH dehydrogenase (mitochondrial complex I) and succinate dehydrogenase(mitochondrial complex II)which in turn causes depletion of NADH pool from the tissue [Bianchi et al. 1982, Ryberg and Alexander 1990, Rossi et al. 1988]. In the present study both of the seen zyme activities were significantly decreased in the brain tissue due to chromium toxicity, indicating significant deterioration of the intracellular NADH pool and ATP production in that specific tissue. Less availability of NADH may also contribute to retardation of anaerobic conversion of pyruvate to lactate *via* suppressed activity of LDH.

Change in malate dehydrogenase (MDH) activity in mitochondrial isolate reveals that chromium exposure causes reduction in the enzymatic activity in the cerebral tissue. Apart from being a TCA cycle enzyme, the cytosolic MDH also helps in gluconeogenesis to produce glucose from non-carbohydrate source. In this regard, oxaloacetate, the TCA cycle intermediate which is produced from pyruvate in the mitochondria by pyruvate carboxylase is reduced to malate before leaving the inner mitochondrial membrane and mitochondrial MDH helps in this reduction process. Decreased mitochondrial MDH activity in brain by chromium exposure indicates suppressed metabolic conversion of malate to oxaloacetate in TCA cycle, which may contribute low energy supply to those specific tissues as a result of toxic insult. Reduced glucose supply to brain tissue may also contribute to less production of glycolytic intermediates in those tissues resulting in impairment of energy yielding mechanism and consequent cerebral dysfunctions after chromium intoxication.

5. CONCLUSION

From the overall study it is thus suggested that chromium exposure at sub-lethal dose for a period

of thirty days produces hypoglycaemia. The diminished pyruvic acid content in cerebral tissue indicates retardation of glycolytic activity in tissue level following exposure to chromium. Chromium exposure significantly altered the TCA cycle enzyme activities. Inhibition of SDH and NADH dehydrogenase by chromium indicates impaired oxidation of succinate *via* TCA cycle and also retardation of energy production through mitochondrial respiratory chain. The MDH activity in brain was also decreased indicating less energy production in those tissues.

6. ACKNOWLEDGEMENT

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7. CONFLICT OF INTEREST

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

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Figures and figure legends

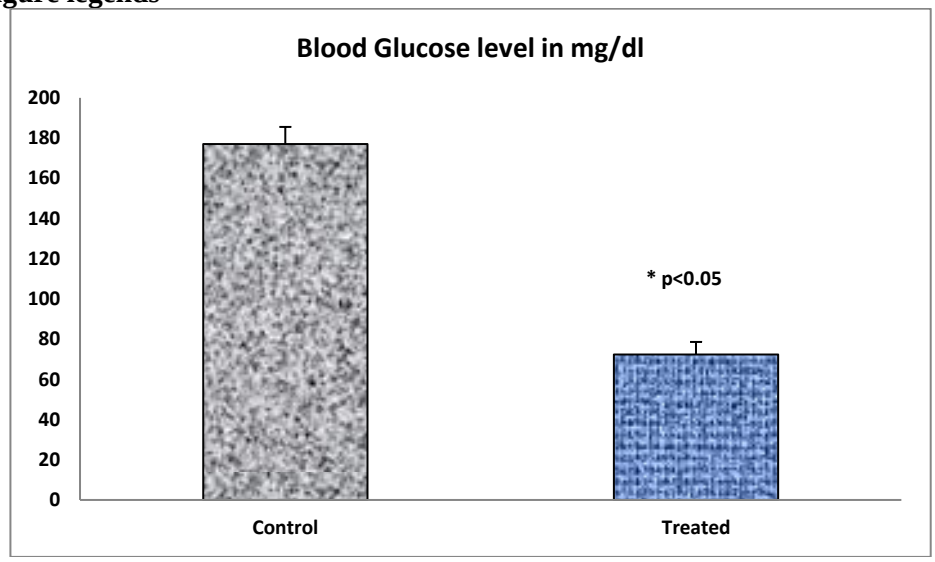

Fig 1: Effect of chromium on blood glucose level
Values are Means±S.E.M. p<0.05 is considered statistically significant.

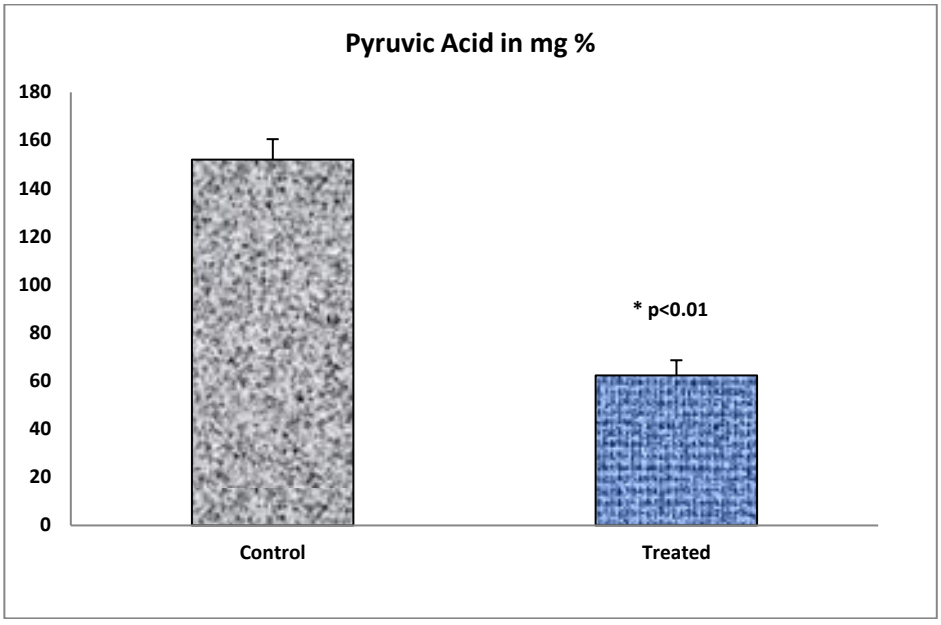

Fig 2: Effect of chromium on liver pyruvic acid content
Values are Means±S.E.M. p<0.01 is considered statistically significant.

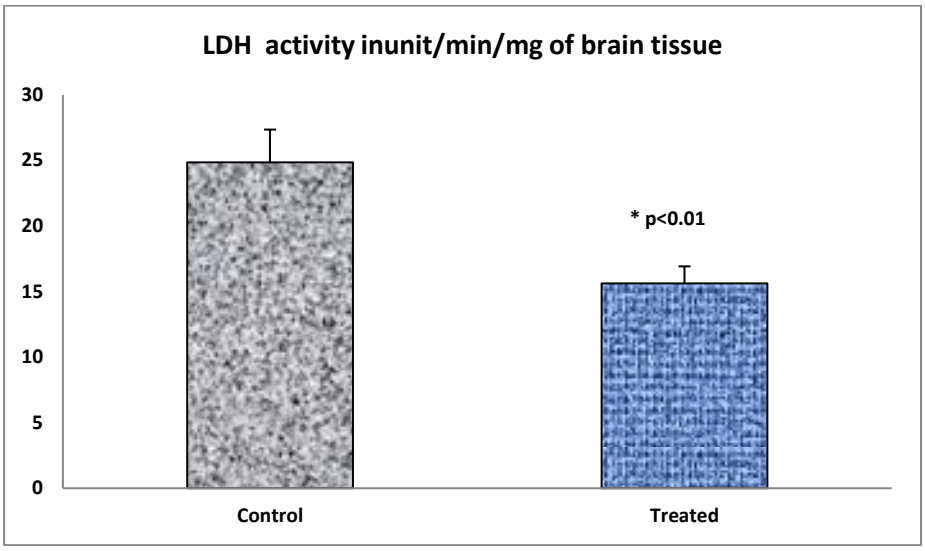

Fig 3: Effect of chromium on liver lactate dehydrogenase activity
Values are Means±S.E.M. p<0.01 is considered statistically significant.

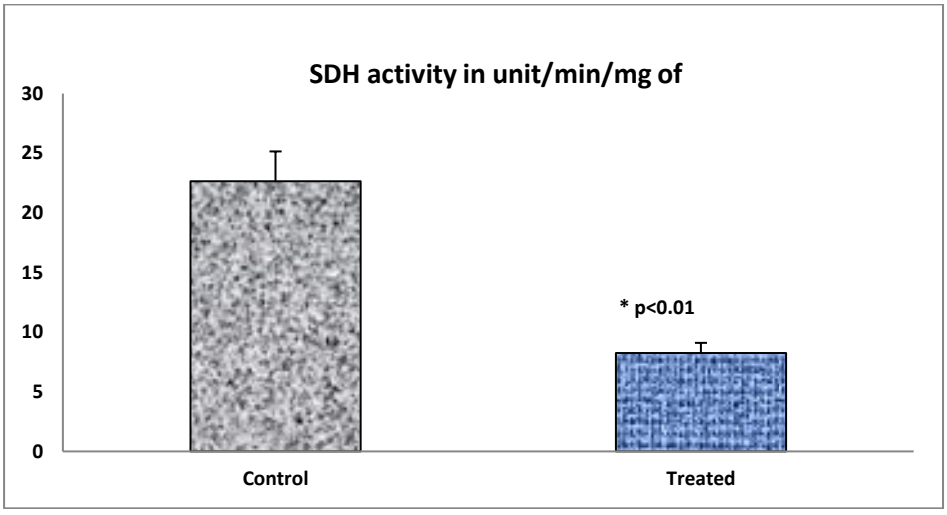

Fig 4: Change in SDH activity following exposure to chromium
Values are Means±S.E.M. p<0.05 and p<0.01 are considered statistically significant.

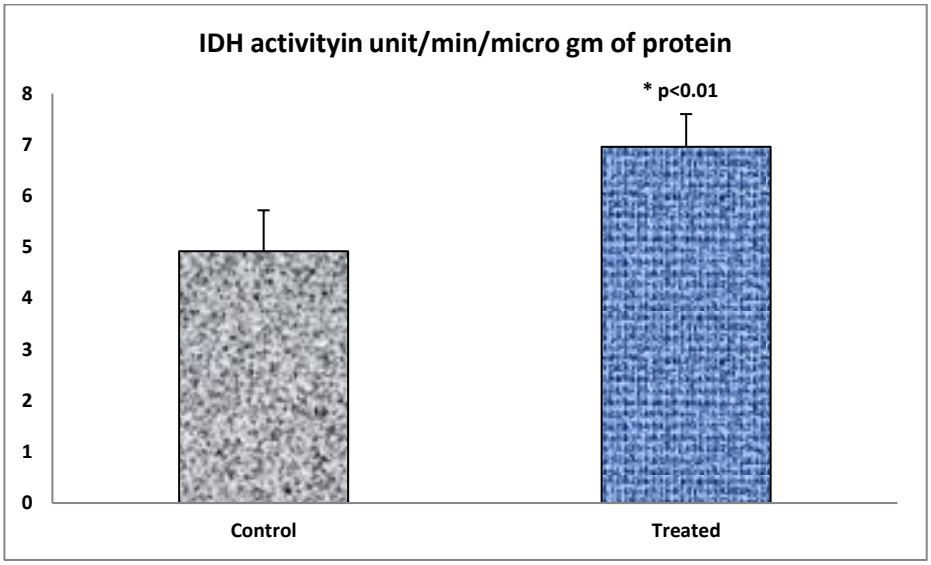

Fig 5: Change in IDH activity following chromium exposure
Values are Means±S.E.M. p<0.01 is considered statistically significant.

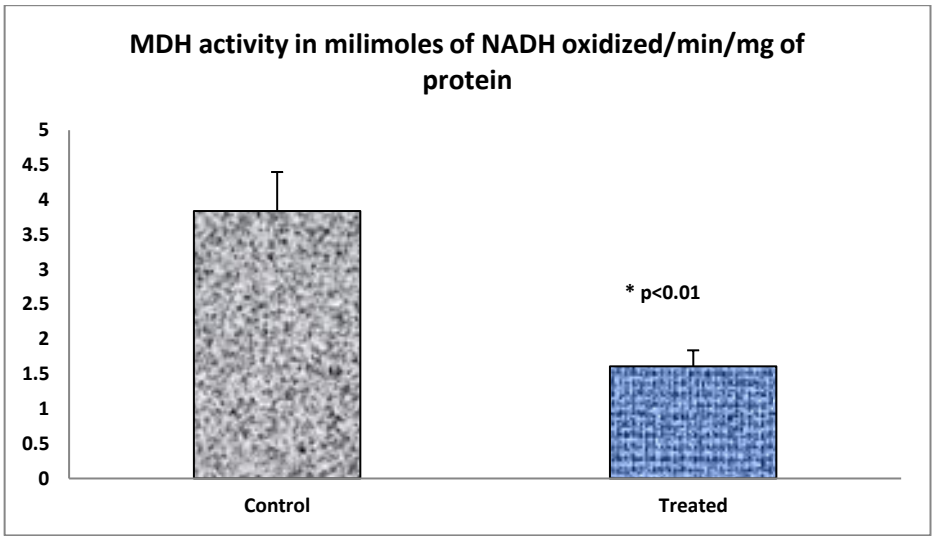

Fig 6: Effect of chromium on tissue MDH activity
Values are Means±S.E.M. p<0.01 is considered statistically significant.

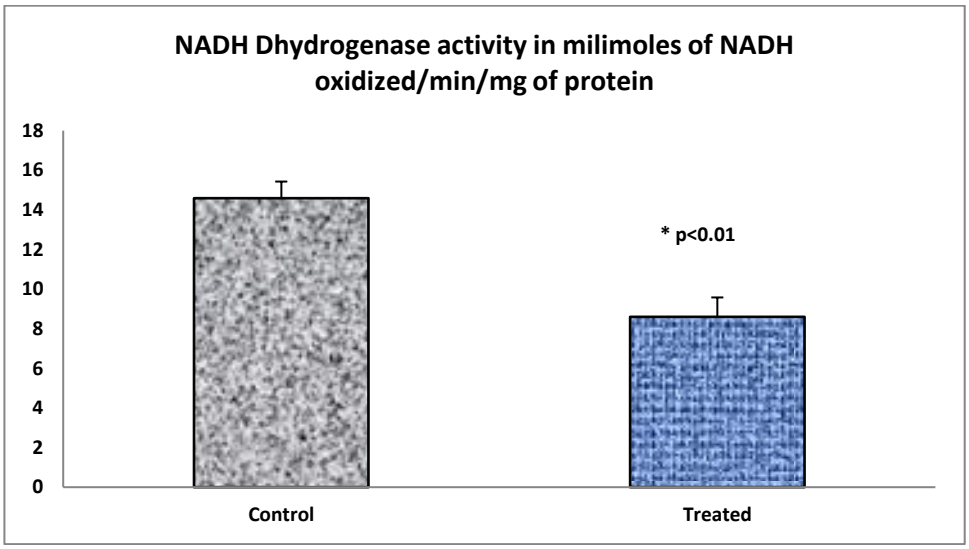

Fig 7: Effect of chromium on tissue NADH dehydrogenase activity
Values are Means±S.E.M. p<0.01 is considered statistically significant.

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

Group of animals	Body weight (gm)	Organo-somatic Index
		Brain
Control (N=6)	38.34±2.11	0.42±0.06
Treated (N=6)	32.58±0.91 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]