Afr. J. Med. Med. Sci. (2019) **48**, 329-337

Effect of trivalent chromium (Cr_2O_3) on stomach morphometry and some vital organs in male wistar rats

OA Odukanmi[1], O. Owoeye[2], AT Salami[1], OL Morakinyo[1], TO Iyiola[1] and SB Olaleye[1]

Laboratory for Gastrointestinal Secretion and Inflammation Research, Departments of Physiology[1] and Anatomy[2], College of Medicine, University of Ibadan, Ibadan, Nigeria

Abstract

Background: Trivalent chromium (Cr_2O_3) is required in trace amount and has health benefits. Its deficiency is linked to symptoms associated with endocrine and cardiovascular diseases. Its essentiality and presumed functions in body system is poorly understood. This study evaluated the effects of Cr_2O_3 on gross morphology of the stomach, liver, kidneys and brain of rats.

Methodology: Eighteen male Wistar rats (91.1 ± 4.2 g, 7 weeks old) were equally assigned to three groups: group 1 (control) received drinking water while groups 2 and 3 received 10 and 100 ppm Cr_2O_3 respectively for 12 weeks through drinking water. Animals were weighed weekly, sacrificed after 12 weeks and blood chromium concentration was determined and full blood count estimated. The stomach, colon, liver, kidney and brain were excised and weighed. Stomach was assessed for gross, histology and histomorphometry alterations. Liver, kidney and brain histology were also evaluated using standard methods.

Results: Blood chromium level was significantly higher in the group treated with 10 ppm Cr_2O_3 (0.17 ± 0.01ppm); 100 ppm (0.19 ± 0.01ppm) compared with control (0.11 ± 0.02 ppm). Platelet count was significantly lower in control (72.3 ± 3.1 x $10^3/\mu L$) compared to 10 ppm (107.7 ± 3.7 $10^3/\mu L$) and 100 ppm (101.3 ± 4.4 x $10^3/\mu L$). The stomach mucosa width was significantly high in group treated with 10 ppm (7097±130 μm) and 100 ppm (7306 ± 632 μm) compared with control (4623 ± 247 μm). Brain histology revealed few deranged cells in the chromium treated groups.

Conclusion: This study underscores possible stomach and few derangements in the brain cells from trivalent chromium treatment.

Keywords: *Trivalent chromium, Stomach, Brain, Histomorphometry, Rats*

Correspondence: Dr. O.A. Odukanmi, Laboratory for Gastrointestinal Secretion and Inflammation Research, Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria. Email: odukanmi@yahoo.com

Résumé

Contexte : Le chrome trivalent (Cr_2O_3) est requis en quantité minime et présente des avantages pour la santé. Sa carence est liée aux symptômes associés aux maladies endocriniennes et cardiovasculaires. Son caractère essentiel et ses fonctions présumées dans le système corporel sont mal compris. Cette étude évalue les effets du Cr_2O_3 sur la morphologie globale de l'estomac, du foie, des reins et du cerveau des rats.

Méthodologie : Dix-huit rats Wistar mâles (91,1 ± 4,2 g, âgés de 7 semaines) ont été également répartis dans trois groupes : le groupe 1 (témoin) a reçu de l'eau de boisson, tandis que les groupes 2 et 3 ont reçu respectivement par l'eau de boisson 10 et 100 ppm de Cr_2O_3 pendant 12 semaines. Les animaux ont été pesés chaque semaine, sacrifiés après 12 semaines et la concentration de chrome dans le sang a été déterminée et la formule sanguine complète estimée. L'estomac, le côlon, le foie, les reins et le cerveau ont été excisés et pesés. L'estomac a été évalué pour les altérations macroscopiques, histologiques et histomorphométriques. Les histologies du foie, des reins et du cerveau ont également été évaluées à l'aide de méthodes standard.

Résultats : Le taux de chrome sanguin était significativement plus élevé dans le groupe traité avec 10 ppm de Cr_2O_3 (0,17 ± 0,01 ppm). 100 ppm (0,19 ± 0,01 ppm) par rapport au contrôle (0,11 ± 0,02 ppm). La numération plaquettaire était significativement plus faible chez les témoins (72,3 ± 3,1 x $10^3/\mu L$) par rapport aux groupes à 10 ppm (107,7 ± 3,7 x $10^3/\mu L$) et à 100 ppm (101,3 ± 4,4 x $10^3/\mu L$). La largeur de la muqueuse gastrique était significativement élevée dans le groupe traité avec 10 ppm (7097 ± 130 μm) et 100 ppm (7306 ± 632 μm) par rapport au groupe contrôle (4623 ± 247 μm). L'histologie cérébrale a révélé peu de cellules perturbées dans les groupes traités au chrome .

Conclusion : Cette étude met en évidence un possible dérangement de l'estomac et peu de dérangements dans les cellules du cerveau à la suite d'un traitement au chrome trivalent.

Mots - clés : *Chrome trivalent, Estomac, Cerveau, Histomorphométrie , Rats*

Introduction

A number of trace elements and heavy metals have direct effect on gastrointestinal tract when ingested

Results

Effect of trivalent chromium on body weight, relative organ weights and blood chromium level.

Figures 1, 2 and 3 describe the findings on percentage body weight, relative organ weight and blood chromium concentration after period of exposure to chromium respectively. There was no significant difference in the percentage body weight of chromium treated groups compared with the control. The relative organ weights of the two chromium groups also were significantly higher compared with control. However, the blood chromium level increased significantly ($p<0.05$) in the chromium treated groups, 10 ppm (0.17 ± 0.01ppm); 100 ppm (0.19 ± 0.01ppm) compared with control (0.11 ± 0.02 ppm).

Effect of trivalent chromium on haematological variables.

The result was not significant in all the variables measured except for the platelet counts that was significantly lower, $p<0.05$ in control ($72.3 \pm 3.1 \times 10^3/\mu L$) compared to 10 ppm ($107.7 \pm 3.7\ 10^3/\mu L$) and 100 ppm ($101.3 \pm 4.4 \times 10^3/\mu L$) (Table 1) respectively.

Effects of trivalent chromium on stomach gross morphology and histology.

There were no disruptions in the stomach mucosa integrity in the chromium treated rats compared with control at both gross and histology levels (Tables 2). However, there was significant increase ($p<0.05$)

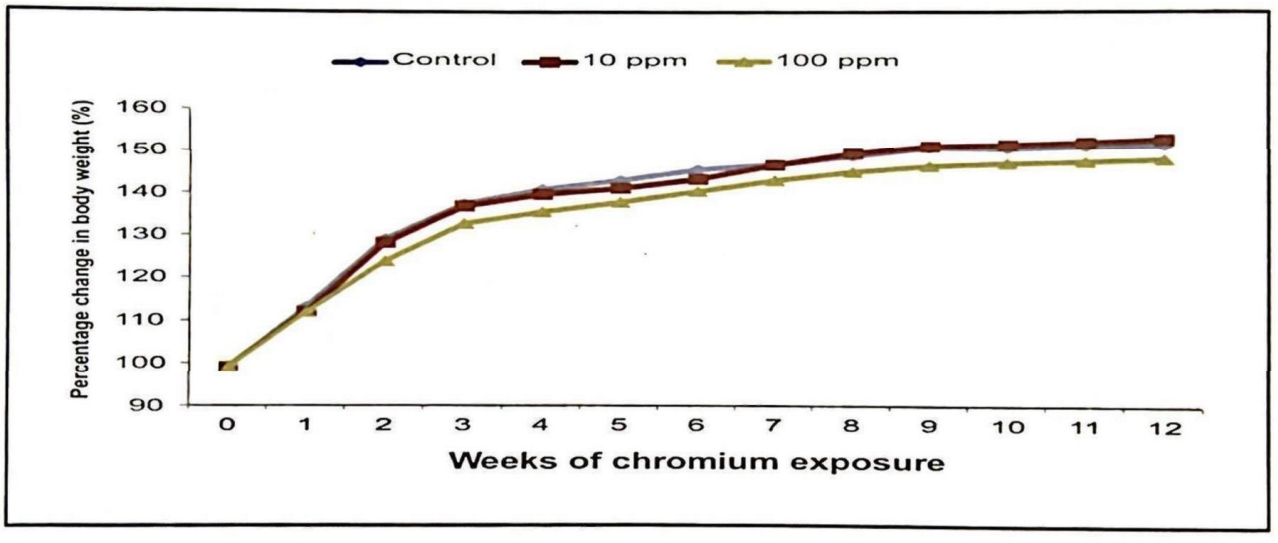


Fig. 1: Percentage body weight change following 12 weeks of chromium exposure.
No significant difference in chromium treated groups compared with control percentage body weight differences.

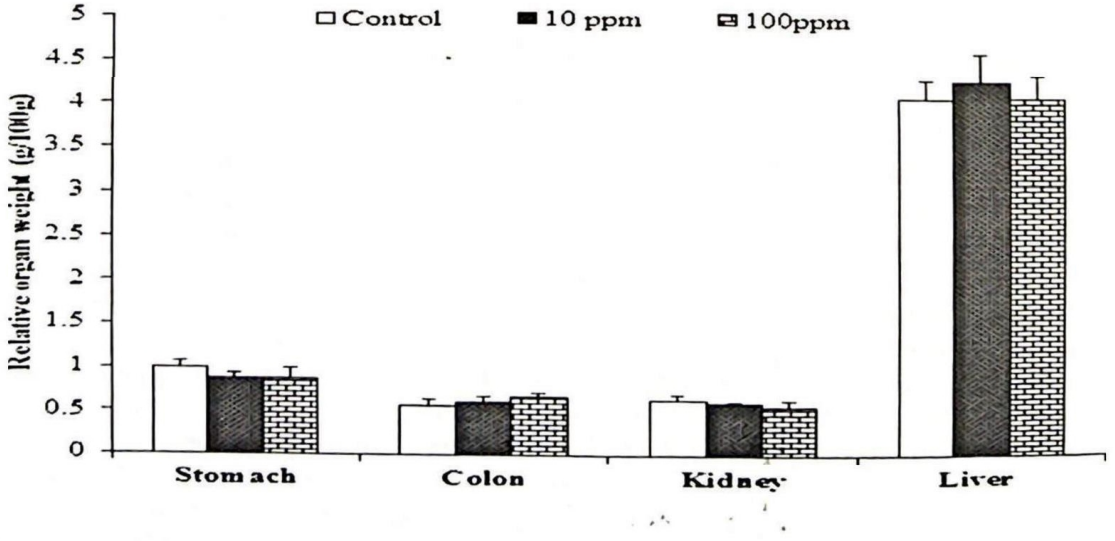


Fig. 2: Mean relative organ weights following 12 weeks of chromium exposure. No significant difference in test groups compared with control in organ weights

and /or indirect impact on other body organs, thereby presenting health challenge and health in general. The heavy metals which are majorly metalloids could induce toxicity at minimal exposure dose [1]. Human exposure to these metals is on exponential increase particularly due to increasing environmental pollution from industrial, domestic and technological activities [2, 3], especially in developing world.

`Chromium, a trace element and heavy metal (transitional metal) occurs naturally in two main forms either as trivalent or hexavalent chromium [4]. The trivalent form is the focus of this study and it is available food substances such as meat and vegetables among others [5]. Trivalent chromium is an essential nutrient in both animals and human, and it plays important role in glucose, fat and protein metabolism by potentiating the action of insulin [6] whereas; hexavalent chromium exposure in humans is reportedly toxic to the exposed tissues [7, 8]. Over 300,000 workers are estimated to be exposed annually to hexavalent chromium especially in places of work [6]. Non-occupational exposure occurs through food and drinking water whereas, occupational exposure occurs majorly through inhalation [9].

The increase in consumption of trivalent chromium supplement [10] has been reported with dearth of information on its effects on the stomach majorly. The inadequate information has created vital gap in the knowledge base for possible interaction between the stomach and chromium. In this study, we evaluated the effect of chromium consumption on gross morphology of the stomach, liver, kidneys and brain of rats.

Materials and methods

Chemicals and drugs

Trivalent chromium (Cr_2O_3) (analytical grade) was obtained from Koshin Chemicals, Japan.

Animals and treatment protocol.

Eighteen male Wistar rats (91.1 ± 4.2 g, 7 weeks old) were purchased from the Preclinical Animal House, College of Medicine, University of Ibadan and were used for the experiments. Rats were acclimatized under standard laboratory conditions, fed with Ladokun® feeds with free access to water. They were grouped into 3: group 1, control (n=6) were allowed free access to clean drinking water and groups 2 and 3, were administered oral 10 ppm, (n=6) and 100 ppm (n=6) Cr_2O_3 respectively through their drinking water for 12 weeks. The study was carried out in conformation to the Guidelines of the National Institute of Health - *Guide for the Care and Use of Laboratory Animals [11].*

Termination of the experiment and harvest of organs

The stomach and metabolic organs; liver, colon and kidneys were removed and weighed following laparotomy under cocktail of both xylazine (0.0005 ml/g b.wt) and ketamine (0.015 ml/g b.wt) anaesthesia.

Determination of blood chromium

After 12 weeks of oral exposure to Cr_2O_3, 2 ml of blood was collected from retro-orbital sinus of rats and was quickly transferred into a test tube and subsequently digested with 2 ml of nitric acid (HNO_3) overnight. The digested blood was placed in water-bath and heated for 30 min at 100°C and allowed to cool to room temperature. The sample was made up to 15 ml with addition of 12 ml distilled water and was filtered. The chromium concentration of the filtrate was now read at 530 nm using flame atomic absorption spectrophotometer (FAAS).

Haematological Analysis

Blood samples were obtained from all animals through retro-orbital sinus for full blood count [12].

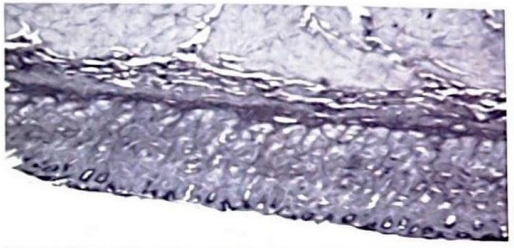
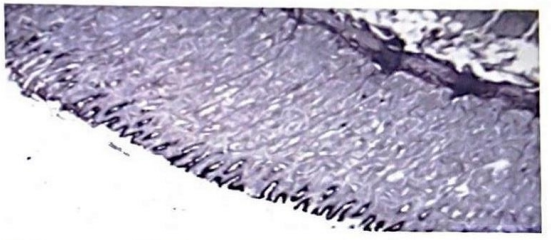
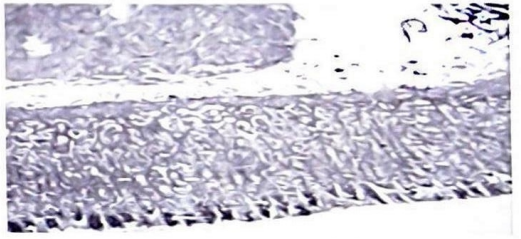
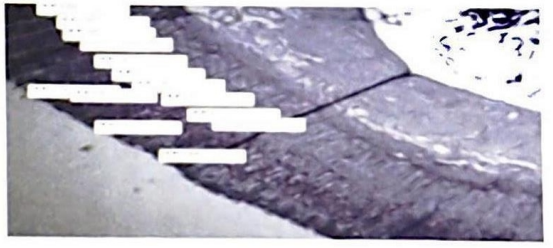
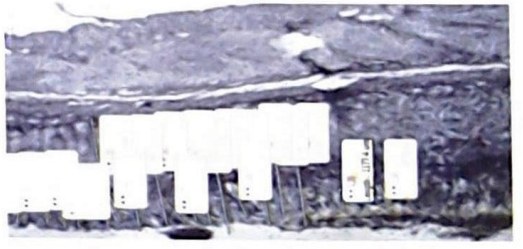
Histological and Histomorphometrical Evaluation

Ketamine (100 mg/kg) was injected intraperitoneally and cervical dislocation done to sacrifice the animals, followed by harvesting of the tissues. Sections of stomach, liver, and kidney were fixed in 10% buffered neutral formalin. The tissues were processed and embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin (H&E) according to the method described by Bancroft and Gamble [13]. Accuscope TS view, China was used to capture images and to evaluate morphological changes and stomach histomorphometry. From each section of the stomach, ten fields per mucosa were randomly selected and measured using image J in each slide per field and the average of the fields was determined[14]. Slides from the rat brain were examined under Leica DM 500 digital light microscope (Germany) and images captured with Leica ICC50 E digital camera (Germany) using an objective lens (x 40) and an ocular lens (x 10).

Statistical analysis

Results were expressed as Mean ± SEM and one-way ANOVA with Newman-Keuls comparison *post hoc* test was adopted using GraphPad Prism version 5.0 for Windows (GraphPad software Inc., San Diego, CA), $p<0.05$ was considered significant.

Table 2: Effects of oral trivalent chromium on the stomach gross, histology and histomorphometry in rats.

	Control	10 ppm	100 ppm
Macroscopic			
Microscopic			
Histomorphometry			
Gross mucosa lesion scores (mm^2)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Histology scores	0.24 ± 0.11	0.28 ± 0.11	0.20 ± 0.09
Mucosa width (µm)	4623 ± 247.5	7097 ± 130**	7306± 632**
Pit depth (µm)	893.9 ± 25.8	922.6 ± 29.2	936.1± 66.9

**significant difference at $p<0.01$ compared with control (Mag. X 100). H &E stain

Table 1: Effects of chromium exposure on some haematological variables

	Control	10 ppm	100 ppm
Packed Cell Volume (%)	41.1 ±1.2	41.7±1.3	41.9±0.7
Haemoglobin Conc. (g/dL)	13.5 ±1.3	12.3±0.9	13.1±0.7
Red Blood Cell count(x 10^6/μL)	7.1 ± 0.3	7.4±0.3	7.0±0.1
White Blood Cell count(x 10^3/μL)	2.2 ± 0.3	2.5±0.2	2.6±0.3
Platelets Count (x 10^3/μL)	72.3 ± 3.1	107.7±3.7+	101.3±4.4+
Lymphocyte Count (%)	64.2 ± 1.3	62.3±9.7	63.0±2.2
Neutrophil count (%)	30.8 ± 2.1	32.33±5.3	33.3±3.1
Monocyte (%)	0.3 ± 0.1	2.1±1.17+	2.0±0.7+
Eosinophil (%)	2.0 ± 0.3	2.0±1.0	2.0±0.3

+ Significantly different at $p<0.05$ compared with control

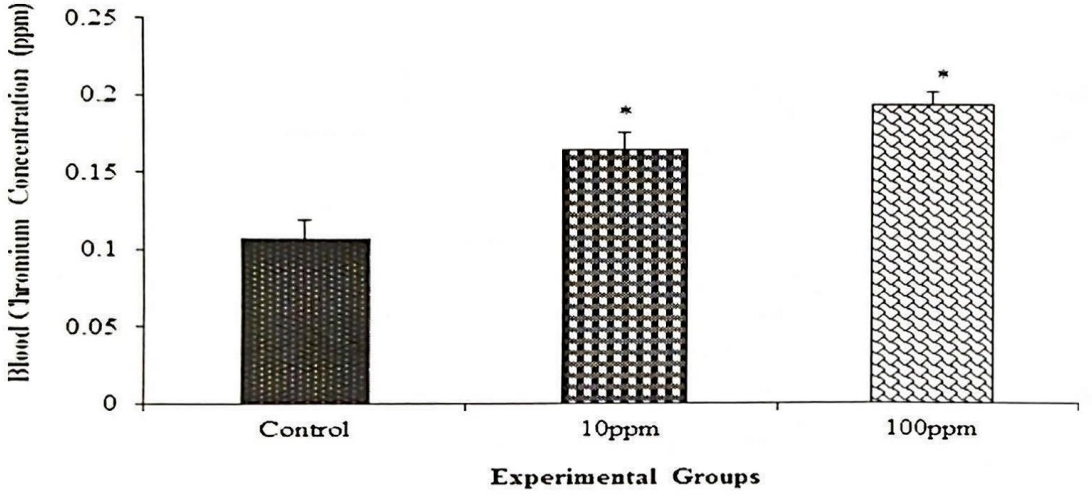


Fig. 3: Mean blood chromium concentration following 12 weeks of chromium exposure.
* Significant at $P<0.05$ compared with the control

in the stomach mucosa width of animals exposed to 10 ppm (7097 ± 130 μm) and 100 ppm (7306 ± 632 μm) chromium compared with control (4623 ± 247 μm).

Effects of trivalent chromium on the histology of the liver, kidney and brain.

Liver (plate 1) and kidney (plate 2) histology shows no significant distortion in the chromium exposed groups compared with control. Plates 3, 4 and 5 display sections of cornu ammonis, dentate gyrus and cerebral cortex of the rats' brain respectively.. The three layers of the CA3 (stratum oriens, stratum pyramidalis, stratum radiatum) and the pyramidal neurons of the cornu ammonis 3 (CA3) of the hippocampal retained their normal architecture in all the groups. However, the granule neurons of the dentate gyrus were distorted in the chromium groups compared with the control. There were few pyknotic (dead) cells in the cerebral cortex region more in the chromium treated. Other brain areas examined were essentially normal as compared with control.

Discussion

Heavy metals and trace elements play significant roles in health and disease conditions of man [15]. The burden of these metals on cellular metabolism is enormous and could be harmful, while in some instances it might be vital for sustaining physiologic metabolic activities of cells and tissues. Excess or deficiencies of some heavy-trace metals have been implicated in some cancer burdens [16, 17]. However, trivalent chromium has no reported risk in the development or progression of gastric diseases from ingestion. This study was conducted to assess the impact of trivalent chromium on the structure of

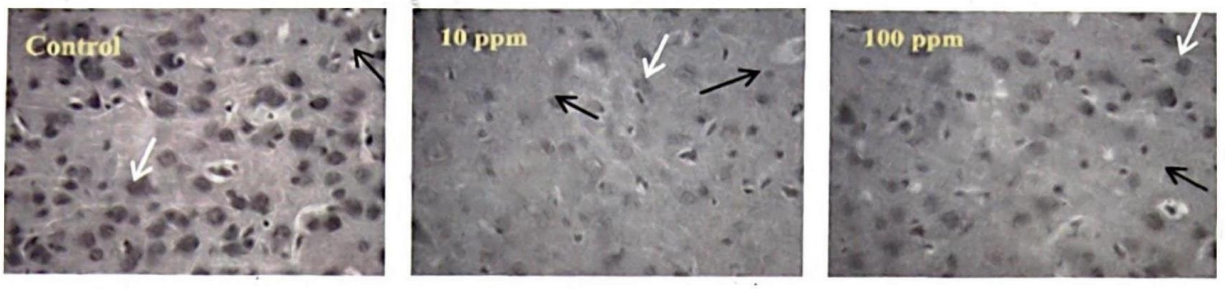


Plate 5: Representative stained sections of the cerebral cortex of rats after oral chromium treatment for 12 weeks. The neuronal cells in control and chromium groups appear essentially normal (white arrows), except for a few pyknotic cells across the groups but appear more in the test groups (black arrow) (Mag. X 400)

the stomach, brain, colon, liver and kidney, in view of the recent increase in its consumption rate [10].

Findings from the present study revealed no significant changes in body weight throughout the period of trivalent chromium administration. The result is in agreement with previous findings from Bunting *et al.*, [18] as well as Mathison and Engstrom [19] and Swanson *et al.*, [20] who found no positive effect of chromium on body weight. However, Chang and Mowat [21], Moonsie-Shageer and Mowat [22] and Kegley *et al.*, [23] reported a gain in weight. Pittler *et al.*, [24] from their studies agreed to loss of body weight following chromium supplementation.

Earlier reports where studies were conducted for periods beyond twelve months of chromium administration, reported no change in the relative organ weights following period of chromium treatments when compared with control [19, 20]. Chromium supplements have been claimed to reduce body fat and increase lean (muscle) mass [25]. A review of 24 studies that examined the effects of 200 to 1,000 mcg/day of chromium (in the form of chromium picolinate) on body composition reported no significant benefits on body weight [25]. In another clinical trial, findings show insignificant change in body weight after chromium supplementation [24].

The blood chromium level is similar with the findings of Anderson and Kozlovsky [26] who reported a significant increase in blood chromium after supplementation for three months. The haematological indices did not change significantly except for the increased platelet count in chromium exposed groups. Increased platelets protect the mucosa against anti-platelets agents. Platelets contain many agents that help in promoting tissue growth and neovascularization including vascular endothelial growth factor (VEGF), transforming growth factor-b and platelet factor-4 [27, 28]. The increased platelet count reported could be an important factor constituting protection to the stomach mucosa of rats in chromium groups.

Normally, gastric mucosa and other mucosae are constantly regenerated with continuous mucosal cell proliferation, and this also depends on the degree of assaults involved. Mucosa of the GI tract is maintained by ongoing cell renewal and any deviation from the replicative processes might compromise both the structural and functional integrity [29]. The observed increase in mucosa width of the chromium group could be as a result of reaction to the chromium water over time and might be a protective factor for the stomach mucosa. This may be a coping mechanism which could permit the gastric mucosa to withstand frequent exposure to damaging factors or agents.

The brain tissues evaluated appeared essentially normal in all the groups but for few necrotic neurons which was expressed more in the chromium groups. Krikorian *et al.*, [30] reported a beneficial effect on cognitive functions of laboratory rats following supplementation with trivalent chromium. It was also reported that chromium supplementation attenuated post-stroke infarction in rats [31].

This present study and findings underscore possible stomach tissue toxicities from trivalent chromium administration to rats. The increase in mucosa architecture in the stomach tissue might suggest cyto-protection to the mucosa integrity when exposed to oral trivalent chromium. It could also side line possible toxicity to other tissues examined. In the case of the brain tissue, it is certainly not clear what the effect might be on cognition, memory and behavior if evaluated. A prolonged period of chromium administration could be more revealing.

References

1. Duffus JH. Heavy metals-a meaningless term? Pure Appl Chem., 2002; 74(5):793–807.

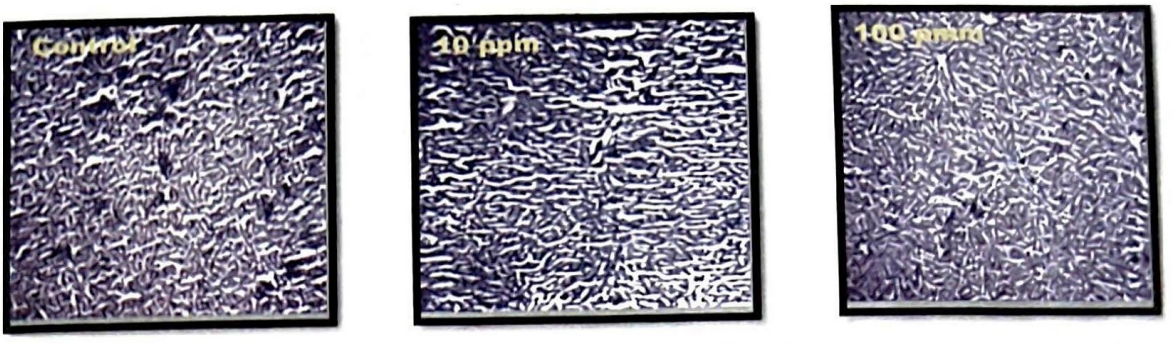


Plate 1: *Representative micrographs of transverse section of rats' liver (Mag. X100), after exposure to chromium. Tissue stained with H&E. No visible lesion is seen in the liver parenchyma in all groups.*

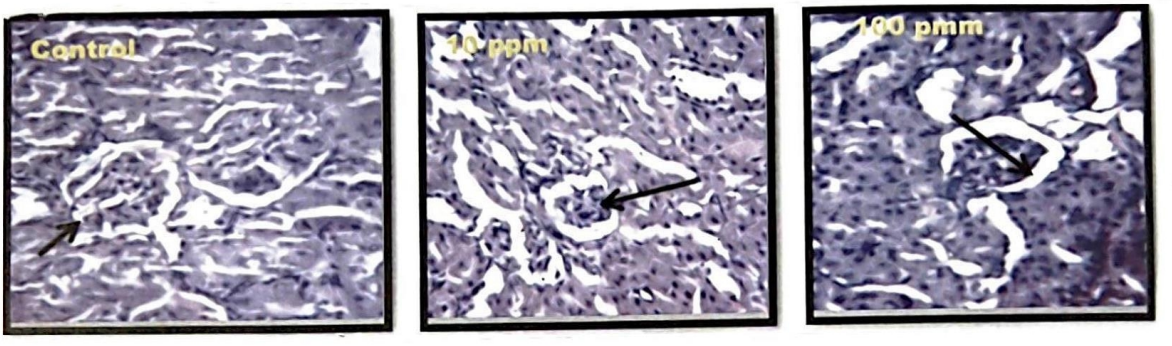


Plate 2: *Representative micrographs of the transverse section of kidney after trivalent chromium exposure (Mag. X 100). Tissue stained with H & E. There is no visible lesion seen in all groups, the glomerulus apparatus (black arrows) appeared normal in all groups.*

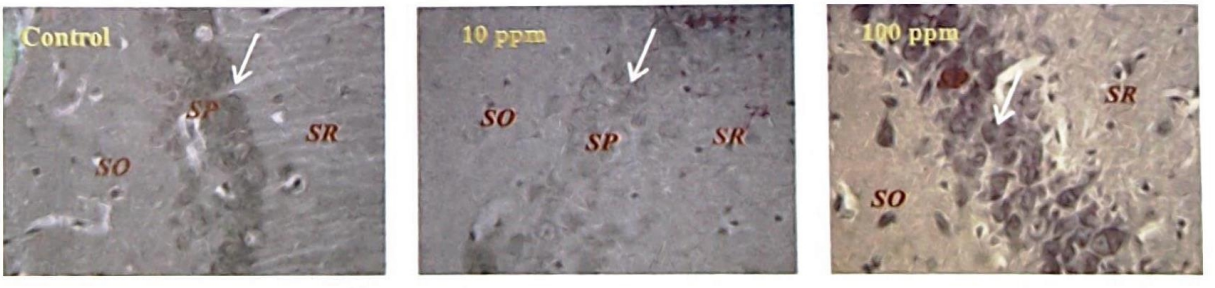


Plate 3: *Representative stained sections of the Cornu Ammonis 3 of hippocampus after oral chromium treatment for 12 weeks. The neurons appear normal in all groups (white arrows) – (SO- stratum oriens, SP- stratum pyramidalis, SR- stratum radiatum) (Mag. X 400).*

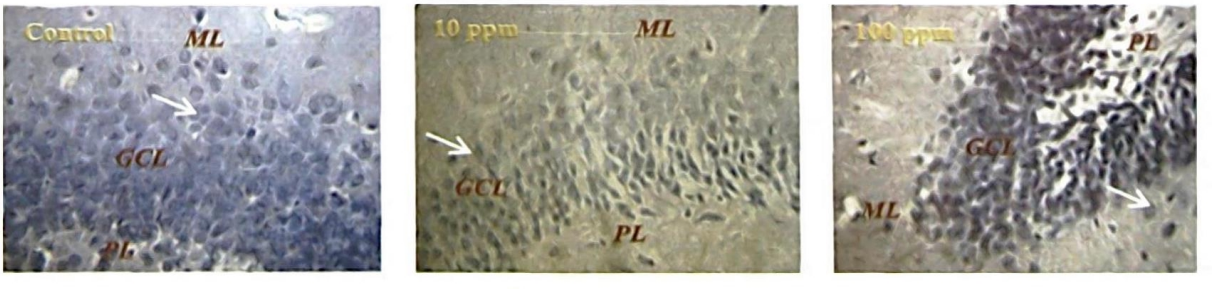


Plate 4: *Representative stained sections of the dentate gyrus of hippocampal formation of rats after oral chromium treatment for 12 weeks. The control and chromium treated hila cells appear normal with normal Purkinje cell staining. The granule cell layer (GCL) appears mildly distorted in the chromium groups. ML and PL represent the molecular layer and polymorphic layer (Mag. X 400).*

29. Saber A and Bayumi EK. Age-Related Gastric Changes. J. of Surgery. 2016; 4(2-1): 20-26.
30. Krikorian R, Eliassen JC, Boespflug EL, Nash TA and Shidler MD Improved cognitive cerebral function in older adults with chromium supplementation. Nutr Neurosci. 2010, 13(3):116-122.
31. Chen WY, Mao FC, Liu CH, *et. al.* Chromium supplementation improved post-stroke brain infarction and hyperglycemia. Metabolic Brain Disease, 2016; 31(2): 289–297.

2. Bradl H. Heavy Metals in the Environment: Origin, Interaction and Remediation Volume 6. London: Academic Press; 2002.
3. He ZL, Yang XE and Stoffella PJ. Trace elements in agro-ecosystems and impacts on the environment. J Trace Elem Med Biol., 2005; 19(2–3):125–140. [PubMed: 16325528]
4. Shrivastava R, Upreti RK, Seth PK and Chaturvedi UC. Effects of chromium on the immune system. FEMS Immunology and Medical Microbiology, 2002; 34:1-7.
5. National Institute of Health. Chromium dietary supplement facts sheet, 2013; 1-7.
6. Goyer RA. Toxic effects of metals. In: Klaassen, CD., editor. Cassarett and Doull's Toxicology: The Basic Science of Poisons. New York: McGraw-Hill Publisher; 2001; 811-867.
7. Costa M. Toxicity and carcinogenicity of Cr (VI) in animal models and humans. Crit. Rev Toxicol., 1997; 27:431–442. [PubMed: 9347224]
8. Shelnutt SR, Goad P and Belsito DV. Dermatological toxicity of hexavalent chromium. Crit. Rev Toxicol., 2007; 37:375–387. [PubMed: 17612952]
9. Langård S and Vigander T. Occurrence of lung cancer in workers producing chromium pigments. Br J Ind Med., 1983; 40(1):71–74. [PubMed: 6824603]
10. Nutrition Business Journal. NBJ's Supplement Business Report. Penton Media Inc., San Diego, CA. 2003.
11. National Institute of Health. Guide for the Care and Use of Laboratory Animals. NIH Publication number 85-23 revised, 1985: US Department of Health, Education and Welfare Bethesda,MA.
12. Dacie JV and Lewis SM. In Practical Haematology 7th edition ELBS with Churchill Livingstone. Longman group UK. 5-82:160-175, 1994.
13. Bancroft JD and Gamble M. Theory and practice of histology techniques (6th ed). Philadelphia, PA: Churchill LivingstoneElsevier.2008;83–134.
14. Eyarefe OD, Emikpe BO and Arowolo FO. Small bowel responses to enteral and glutamine administration following massive small bowel resection in rabbit. Afr. J. Med. Med. Sci., 2008; 37:309-314
15. Emre O, Demir H, Dogan E, *et al.* Plasma Concentrations of Some Trace Element and Heavy Metals in Patients with Metastatic Colon Cancer. J. of Cancer Therapy, 2013, 4: 1085-1090.
16. Cavusoglu K, Cakir A.S., Kurtman C, *et al.*,. The De- termination of Alteration in Plasma Trace Element Levels of Patients with Lung Cancer Received Radiotherapy. Firat Univer Med J. of Health Sci, 2008; 22 (4): 211-222.
17. Cobanoglu U, Demir H, Sayir F, *et al.*, .Some Mineral, Trace Element and Heavy Metal Concentrations in Lung Cancer. Asian Pac J. of Cancer Prev. 2010; 11(5):1383-1388.
18. Bunting LD, Fernandez JM, Thompson DL Jr and Southern LL. Inûuence of chromium picolinate on glucose usage and metabolic criteria in growing Holste in calves. J. Anim. Sci., 1994; 72:1591–1599.
19. Mathison GW and Engstrom DF. Chromium and protein supplements for growing ûnishing beef steers fed barley-based diets. Can. J. Anim. Sci., 1995; 75:549–558.
20. Swanson KC, Harmon DL, Jacques KA, *et. al.* Efficacy of chromium-yeast supplementation for growing beef steers. Anim Feed and Sci Technol, 2000; 86: 95–105.
21. Chang X and Mowat DN. Supplemental chromium for stressed and growing feeder calves. J. Anim. Sci. 1992; 70: 559–565.
22. Moonsie-Shageer S and Mowat DN. Effect of level of supplemental chromium on performance, serum constituents, and immune status of stressed feeder calves. J. Anim. Sci., 1993; 71:232–238.
23. Kegley EBJ, Spears W and Brown TT Jr. Effect of shipping and chromium supplementation on performance, immune response, and disease resistance of steers. J. Anim. Sci., 1997; 75:1956–1964.
24. Pittler MH, Stevinson C and Ernst E. Chromium picolinate for reducing body weight: Meta-analysis of randomized trials. Int J. of Obesity, 2003; 27:522–529.
25. Vincent JB. The potential value and toxicity of chromium picolinate as a nutritional supplement, weight loss agent and muscle development agent. Sports Med., 2003; 33:213-30.
26. 6Anderson RA and Kozlovsky AS. Chromium intake, absorption and excretion of subjects consuming self-selected diets. Am J of Clin Nutr. 1985; 41:1177–1183.
27. Maloney JP, Silliman CC, Ambruso DR, *et al.* In vitro release of vascular endothelial growth factor during platelet aggregation. Am. J. Physiol. 1998; 275: H1054–H1061.
28. Von Hundelshausen P, Weber KS, Huo Y, *et al.* RANTES deposition by platelets triggers monocyte arrest on inflamed and atherosclerotic endothelium. Circulation, 2001; 103: 1718–1720.