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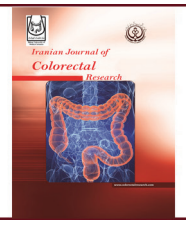

The Interplay of Neutrophils, Platelets, and Cytokines in the Healing Process of Acetic Acid-Induced Stomach Ulcer on Trivalent Chromium-exposed Mice

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Abstract

Background: Trivalent chromium is a trace element effective in treating experimental colitis and normal gastrointestinal tissues. We aimed to investigate the effect of trivalent chromium on the injured stomach in mice.
Methods: Sixty male slc:ddY mice were used. The animals were randomized into three groups of 20 mice (the control, 10ppm, and 100ppm). Five mice each of twenty were sacrificed on days 0 (for the cytokine study), 3, 7, and 14 and examined. Following anesthesia, the ulcer was induced with acetic acid via laparotomy and through the intraluminal route post-chromium exposure. The blood was obtained from cardiac puncture, and the stomach tissue was scored and excised for myeloperoxidase, catalase, superoxide dismutase, malondialdehyde, total nitrite, cytokines assays, and histology.
Results: Ulcer scores of the chromium-exposed group were reduced significantly compared to those of the control group. The platelets increased dramatically in 10- and 100 ppm compared to the control on day 1. There were decreasing neutrophils in the test groups compared with the control across the days of investigation. The malondialdehyde values (nmol/mg protein) significantly reduced in the 10ppm (6.42±0.24+; 3.70±0.42+) and 100ppm (5.22±0.47+; 2.95±0.31+) groups compared to the control (8.35±0.43; 4.53±0.48) on days 7 and 14 respectively. Superoxide dismutase increased, (P=0.0372, 0.0441, and 0.0421) on days 3, 7, and 14, respectively in the chromium groups compared with the control. Myeloperoxidase decreased significantly, P=0.0466, and 0.0383 on days 3 and 7 in the chromium groups compared with the control group, respectively. There was no significant change with Catalase and Nitrates assessed. IL-1α, TNF-α, and interferon-γ reduced while IL-10 increased in the mRNA chromium exposed.
Conclusion: Chromium exposure to mice upgrades the restoration of gastric ulcers in mice by inhibiting reactive oxygen radicals while promoting endogenous antioxidants

Keywords: Trivalent chromium, Reactive oxygen species, Inflammation, Cytokines, Healing, Ulcer

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Introduction

The preponderance of peptic ulcers has reduced over the years, mostly because of the reduction in *Helicobacter pylori* infections because of the availability of appropriate medications (1, 2). However, unethical use of medications such as the common non-steroidal anti-inflammatory drugs (NSAIDs) and associated ones such as acetylsalicylic acid (ASA) is linked to the perpetual increase in prevalence and complications of peptic ulcers (3, 4). Further, the gastrointestinal tract (GIT) plays host to lots of substances, some of which are beneficial and some of which are deleterious. Sometimes, the GIT can be exposed to naturally dangerous substances occurring in the form of heavy metals.

The typical heavy metals the gut is exposed to include lead, zinc, chromium, cadmium, nickel, copper, etc (5). They are mostly exposed to humans through natural means such as food and water contamination and various human activities (6). The activities of heavy metals in small amounts could help to sustain the homeostatic mechanisms of the multiple body systems, meaning they are not always harmful (6). However, when in excess, they could be toxic in their actions to the systems they have earlier helped stabilize (7). Nevertheless, some of these heavy metals have proven beneficial even to the gastrointestinal system and generally to the entire body system; evidence of this abounds from several previous research reports (8-11).

Trivalent chromium is a trace element naturally present in many foods, and its availability as a dietary supplement has been described as essential to humans (12). The 6+ valency is the hexavalent chromium ascribed as more toxic to humans and is usually a consequence of various industrial activities (13). The essentiality of trivalent chromium to human existence and its multiple roles has been queried in the recent past (14, 15), as its addition or its absence has not been linked directly with any ailment. The daily value (DV) for chromium is reportedly 35 mcg for adults and children aged four and older. The trivalent chromium used in this study is Chromium Picolinate - $Cr(C_6H_4NO_2)_3$, which is the more easily accessible form and and usually gotten from nutrients according to the US Food and Drug Administration (FDA) (16).

We used the $Cr(C_6H_4NO_2)_3$ for the current study because it aids the metabolism of the major nutrients in the body (8, 9, 17, 18), among other beneficial effects. Its effect on humans battling peptic ulcer disease has not yet been ascertained. However, in our laboratory recently, we reported its anti-inflammatory and reduction of oxygen species on experimental colitis in mice (8). We aimed to investigate this particular form of chromium on the effect of trivalent chromium on the recovery of gastric mucosa in experimental gastric ulcers and explores the mechanism attributable to the impact.

Material and Methods

Animal Care and Handling

Sixty 5-year-old male slc:ddY mice, with an average body weight of 26.1±1.9 g, were bought from SLC Incorporation, Japan, and used for the study following a period of acclimatization, in an appropriate animal house at the Maebashi Institute of Technology, Japan where the study was done. Food and water were provided *ad libitum*. During the post-acclimatization period, the mice were grouped into 3 of 20 mice each: control, 10ppm, and 100ppm. The control group had clean water freely, while groups 2 and 3 got 10- and 100 ppm chromium through drinking water for 12 weeks. Fifteen mice (5 per group) were sacrificed on days 0, 3, 7, and 14 for investigations. The large quantity of chromium exposed to the rats was chosen because of the poor bioavailability of the chromium generally within the tissues and supported by similar studies in mice (8, 19, 20).

Drugs and Chemicals Used for the Study

We purchased Sodium Nitrite, o-dianisidine dihydrochloride, and Hexa-decyl-trimethyl ammonium bromide from Tokyo Chemical Industry and procured trivalent chromium as Chromium Picolinate ($C_{18}H_{12}CrN_3O_6$) from Koshin Chemicals, Japan. Other chemicals used were of high analytical grades. RNA and Real-time PCR kits were purchased from Qiagen®, Japan, and Brilliant Agilent®, United Kingdom, respectively. The primers were gifted by qStandard®, London, as described in our previous report (8).

Induction of Ulcer Using Acetic Acid

We induced experimental gastric ulcers using acetic acid using the intraluminal method described previously by Amagase and Okabe (21), with slight modifications in fasted mice after the 12 weeks of chromium pre-treatment. In brief, 20 mice per group were fasted overnight (but were allowed to drink water) before the commencement of the procedures or gastric ulcer induction, as the case may be. When due, we opened the abdomen, and the stomach was exposed following anesthetizing with 2.5% isoflurane mice. The stomach walls were kept together by 6 mm diameter eye forceps and 0.1 ml, 50% vol acetic acid was injected into the luminal space between the forceps by a 1 mL syringe with a 25 G needle size. The process was timed for 45 seconds and the acid was drawn back into the syringe. The stomach was returned *in situ*, the peritoneum was irrigated with normal saline, and the abdominal wall was sutured in turn, first by a 4 0 catgut and the skin by a 4-0 nylon suture. Fifteen mice from each group were returned to their cages and allowed to recover from the surgery. Daily cleaning of the wound sites was done with the application of penicillin paste to prevent the suture sites from infection and 5 mice per group were taken

on day 0 for cytokine study only and days 3, 7, and 14 for cytokine and other assessments.

Assessment of Blood Parameters

Blood (1mL) was collected into heparinized bottles from 5 mice per group through cardiac puncture and then analyzed. The samples were run successively with a KX-21 hematological analyzer from Japan.

Assessment of the Microarchitecture of the Stomach Tissue Following Induction of Ulcer

After cardiac puncture to collect blood, the mice were sacrificed by exposure to excessive 2.5% isoflurane anesthesia. Stomach tissue sections for histology were fixed in 10% buffered formalin and set on paraffin wax blocks. Micro sectioning was done with a microtome and afterward stained with hematoxylin and eosin stains. An Accuscope microscope TS view, from China was used to portray images. Histomorphometry was used to quantify damage to the ultra features with a Motic Image plus 2.0 ML.

Homogenization of Stomach Tissues

Each stomach tissue (0.3 g) was homogenized in about 3 milliliters of sterile phosphate buffer solution at a P_H of 7.4 in a 10 ml tube put on ice, and centrifuged at the speed of 10,000 *g* for 10 min at a temperature of 4°C after applying tissue lysate. The resultant supernatant was transferred into 2 ml ultra clear centrifuge tubes, well labeled and frozen until used.

Assessment of Total Protein Level

The Biuret method was used to assay total protein levels as previously depicted (22) with minor alteration. Potassium iodide was added to the component to prevent the Cu^{2+} ions from precipitating. The supernatant from the stomach tissue homogenates constituted the soluble total protein fraction and was used for protein estimation. Appropriate dilution was done to reduce protein values to the sensitivity range of the Biuret method. Ratio 1:3 ml of the diluted samples and the Biuret reagent were mixed and incubated for 30 minutes at room temperature. The protein concentration was then determined from the absorbance read at 540 nm.

Determination of Biochemical Assays

Lipid peroxidation – this was assayed from the squashed stomach tissue as the earlier identified thiobarbituric acid reactive substances (TBARS) by Varshney and Kale (23). This determines the quantity of malondialdehyde (MDA) present in the homogenized mixture when read with a spectrophotometer at 532 nm.

Catalase activities – were determined as described by Sinha (24). The protocol highlighted the reduced dichromate to chromate in heated H_2O_2. The subsequent chromate was evaluated at 530 nm with a spectrophotometer.

Superoxide dismutase (SOD) – We adopted the method of SOD determination defined by Beauchamp and Fridovich (25) by adding 0.5 mL of the homogenized substance to 2.5 ml of 0.05 M carbonic acid buffer at pH of 10.2 and allowed to incubate in an appropriate cuvette. However, this method can determine total SOD activity and does not discriminate between SOD1 and SOD2. We activated the resultant solution with 0.3 mL of newly prepared 0.3 mM adrenaline. The progressive adjustment in the absorbance was observed at 480 nm and was documented every 30 seconds in 1 minute.

Total stomach tissue nitrite –This protocol to assay tissue nitrites strives on the diazotization reaction that applies sulfanilamide and N-1-naphthyl ethylenediamine dihydrochloride (NED) in acidic form and the previous procedure by Ignarro and colleagues (26) was adopted in this study.

Assessment of myeloperoxidase activity – The myeloperoxidase activity was determined from the stomach tissue homogenates as previously explained by Kim and co-workers (27) and represented as the U/mg protein of the tissue.

Determination of Total RNA and Real-Time PCR

We obtained total RNA from Qiagen, Japan, and the prescribed procedure was adopted as described previously (8). Briefly, we preserved 30 mg of stomach tissue in RNA stabilization reagent (DNAse treatment) before homogenization. Homogenates were centrifuged at 4°C for 3 minutes, and the supernatant was collected. The centrifuging was repeated for 2 minutes after ethanol and RNeasy were added to the mixture. RNA was then eluted with a spectrophotometer (Nanodrop® 2000) at 280nm. The procedure afterward applied the reverse transcription kit from Qiagen® to establish the cDNA using accepted kits, Master Mix. The primers are with the standards, Actb, Gapdh, and Rpl13 while the cytokines were Ifng, Il1a, Il6, Il10, and Tnf were used. The Fast Real-Time PCR System (Biosystems® 7500) was used to amplify the eight mRNA genes. The reference genes engaged were B-Actin, Gapdh, and Rpl13 while the five cytokines of interest were IL-1α, IL-6, IL-10, TNF-α, and IFN-λ. The three reference genes were applied to normalize the gene of interest (GOI) with the process explained earlier (28). The relative gene expression was estimated based on the 3 reference genes using the equation below:

Where,

$$\text{Relative Gene Expression} = \frac{(E_{\text{GOI}})^{\Delta Ct\ \text{G.O.I.}}}{GeoMean[(\text{E}_{\text{REF}})^{\Delta Ct\ REF}]}$$

REF- Reference Genes

GOI – Gene of Interest

$(E_{GOI})^{\Delta Ct\ G.O.I.}$-Quantity of Gene of Interest

$GeoMean[(E_{REF})^{\Delta Ct\ REF}]$- Geometric Mean of Relative Quantity of Reference Genes

Ethics Concerns

We adopted the Guide for the Care and Use of Laboratory Animals by the US National Institute of Health (29). We sought and obtained ethical clearance from the Animal Care and Use Committee of Maebashi Institute of Technology, Japan with approval number 15-009.

Statistical Analysis

Data were collated, cleaned, and reported as mean±SEM and analyzed by one-way ANOVA followed by Newman-Keul's comparison test with GraphPad Prism version 5 (GraphPad Software, San Diego, CA). Results were considered significant at P≤0.05.

Results

Effect of Chromium-exposure on the Stomach Macro- and Microarchitecture in Acetic Acid-induced Gastric Ulcers

Table 1, Figures 1 and 2 represent results for gross ulcer scores, histology, and stomach morphometry in control and test groups. The observed ulcer scores (mm^2) reduced significantly, (P=0.0092; 0.0219; 0.0421*)* days 3, 7 and 14 in the 10ppm (44.26±1.13; 27.42±2.49; 0.66±0.02) and 100ppm (37.70±2.04; 14.54±1.55; 0.48±0.00) compared with control (50.83±1.90; 45.33±3.23; 1.12±0.04), respectively. Mucosa width (µm) increased significantly (P=0.0074*;* 0.0118) on days 3 and 7 respectively and

Table 1: Gastric ulcer areas , histology scores and mucosa measurements after ulcer induction in chromium-exposed mice.

Mucosa Parameter	3rd Day				7th Day				14th Day			
	Control	10 ppm	100 ppm	P value	Control	10 ppm	100 ppm	P value	Control	10 ppm	100 ppm	P value
Ulcer area (mm^2)	50.83 ±1.90	44.26 ±1.13+	37.70 ±2.04+	0.0092	45.33 ±3.23	27.42 ±2.49+	14.54 ±1.55+	0.0219	1.12 ±0.0	0.66 ±0.02+	0.48 ±0.00+	0.0421
Histology scores	4.01 ±0.27	3.12± 0.22+	2.94± 0.12++	0.0201	3.76 ±0.16	2.16 ±0.12+	2.02 ±0.19+	0.0287	1.23 ±0.11	1.07 ±0.09	0.99 ±0.14	0.086
Mucosa width (µm)	4331 ±188	6077 ±126++	6001 ±257+	0.0074	5377 ±201	6173 ±308+	6593 ±228+	0.0118	6140 ±255	5778 ±402	6381 ±277	0.0972
Pit Depth (µm)	711 ±38	865 ±32+	885 ±64+	0.0331	631 ±39	820 ±51+	838 ±41+	0.0288	799 ±49	843 ±34	882 ±76	0.0651

+Connote a significant difference at p≤0.05 when compared to the control, ++connote a significant change at P<0.01 when compared to the control

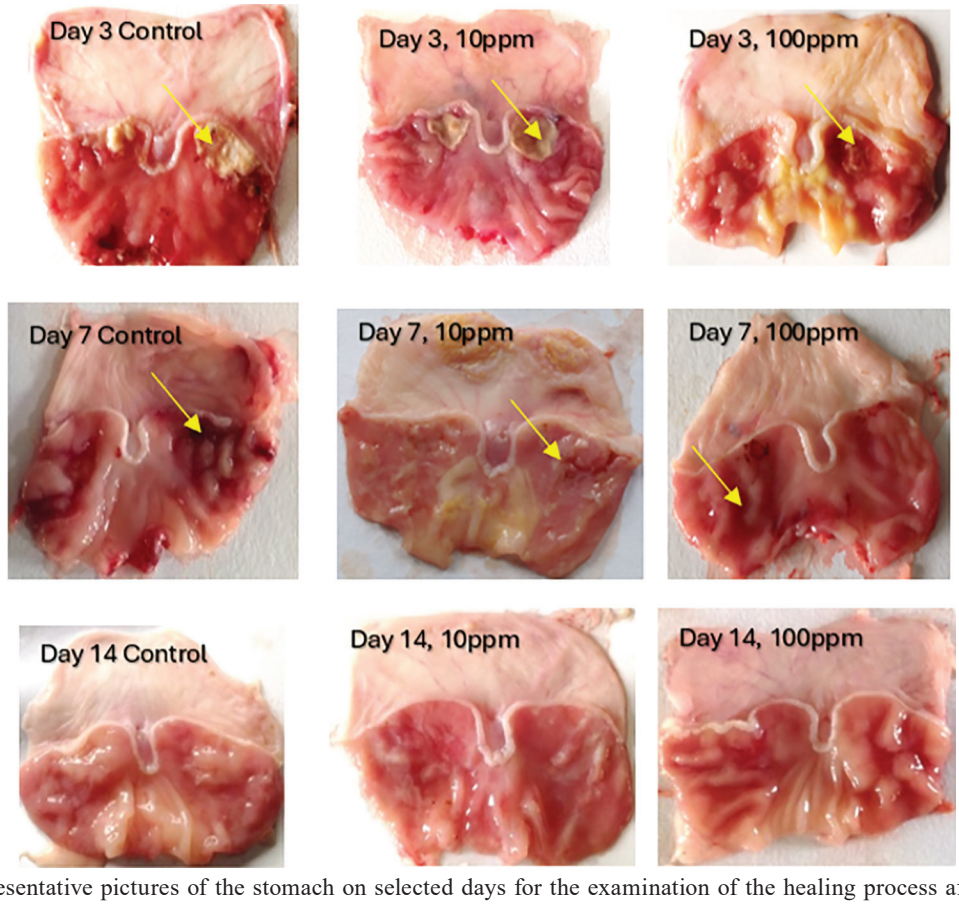

Figure 1: Representative pictures of the stomach on selected days for the examination of the healing process after induction of experimental gastric ulcer following period exposure to trivalent chromium. Yellow arrows indicated some of the identified ulcerated parts of the stomach.

the pit depth (µm) was significantly augmented in the 10ppm when likened to the control.

Effect of Oral Chromium on the Hematological Parameters on Acetic Acid-induced Gastric Ulcer

The differences are in the platelets, neutrophils, and lymphocyte counts, Table 2. The platelets count significantly increased in the chromium groups, 10ppm and 100ppm (167.7±20.1 x 10^3/µL, 161.0±12.9 x 10^3/µL; P=0.0482) on day 3 respectively compared to the control (123.7±17.6 x 10^3/µL). The lymphocyte count increased significantly (P=0.0326, 0.0331, and 0.0437) on days 3, 7 and 14 respectively in the chromium groups compared to the control (Table 2). A similar trend was observed in a reversal mode with the neutrophil count, with a decreased count in the chromium groups.

Exposure to Chromium on Stomach Oxidants, Antioxidants, and Inflammatory Markers in Experimental Gastric Ulcer in Mice

The biochemical variables are described in Table 3. The MDA reduced significantly (P=0.0418; 0.0367) in the test groups on days 7 and 14 in the chromium groups compared to the control respectively. The activities of SOD in the chromium-exposed groups increased significantly (P=0.0172; 0.0241) on days 3 and 7 compared to the control, respectively. However, the catalase activities were not significantly different after gastric ulcer induction. Neutrophil-Lymphocyte Ratio (NLR) significantly reduced (P=0.0287; 0.0291, 0.0376) on days 3, 7, and 14 respectively in chromium-exposed compared to control. Also, the activities of myeloperoxidase in the tissue were significantly reduced (P=0.0416, 0.0383) in the chromium-exposed compared to the control on days 3 and 7 respectively. However, we recorded no significance with total stomach nitrite.

Table 2: Hematological parameters after ulcer induction in chromium-exposed mice

Blood Parameters	3rd Day				7th Day				14thDay			
	Control	10 ppm	100 ppm	P value	Control	10 ppm	100 ppm	P value	Control	10 ppm	100 ppm	P value
Packed Cell Volume (%)	41.3 ±1.0	41.0 ±2.1	40.1 ±0.13	0.115	43.0 ±1.9	43.3 ±2.0	44.5 ±2.2	cq	44.2 ±1.4	43.0 ±3.5	42.6 ±3.1	0.1129
Haemoglobin Concentration (g/dL)	13.9 ±0.1	11.4 ±2.1	11.1 ±2.2	0.0661	14.9 ±0.8	14.1 ±1.7	14.3 ±1.2	0.2441	14.4 ±0.9	13.8 ±1.9	13.2 ±1.7	0.3132
RBC count (x 10^6/µL)	7.9 ±1.3	7.1 ±0.5	7.3 ±0.9	0.3111	6.4 ±0.7	6.8 ±0.5	7.8 ±0.9	0.0819	7.6 ±0.9	7.8 ±0.7	7.2 ±0.5	0.0771
WBC count (x10^3/Ml)	7.9 ±0.9	8.2 ±2.1	8.1 ±2.5	0.0721	6.8 ±0.5	7.7 ±2.2	8.4 ±1.3	0.0551	5.8 ±0.9	5.7 ±1.1	4.9 ±1.3	0.1021
Platelets Count (x 10^3/µL)	123.7 ±17.6	167.7 ±20.1+	161.0 ±12.9+	0.0482	130.0 ±10.3	135.3 ±22.4	133.8 ±15.1	0.1883	99.0 ±7.1	105.5 ±22.4	110.2 ±11.1	0.1901
Lymphocyte Count (%)	30.3 ±2.7	42.3 ±2.9+	45.0 ±4.1+	0.0326	38.2 ±0.9	45.2 ±5.5+	42.4 ±1.5+	0.0331	51.2 ±0.9	59.4 ±4.5+	62.4 ±2.5+	0.0237
Neutrophil count (%)	64.0 ±1.9	55.0 ±2.1+	53.3 ±3.1+	0.0282	60.2 ±0.7	51.0 ±2.7+	54.3 ±2.5+	0.0141	47.2 ±1.2	38.0 ±1.3+	34.3 ±3.9+	0.0388
Monocyte (%)	2.0 ±1.0	3.0 ±1.5	1.9 ±0.9	0.2671	1.5 ±0.4	1.5 ±0.6	2.5 ±0.3	0.1611	1.6 ±0.3	1.8 ±0.6	2.0 ±0.2	0.1211
Eosinophil (%)	2.3 ±0.3	2.0 ±0.6	2.3 ±1.8	0.1330	2.5 ±0.4	2.4 ±0.7	2.0 ±0.4	0.0712	1.5 ±0.5	1.5 ±0.3	1.0 ±0.3	0.0667

+Connote a significant change at P≤0.05 when compared to the control

Table 3: Reactive oxygen species, antioxidants, and inflammatory markers after ulcer induction in chromium-exposed mice

Gastric tissue biochemical variables	Day 3				Day 7				Day 14			
	Control	10 ppm	100 ppm	P value	Control	10 ppm	100 ppm	P value	Control	10 ppm	100 ppm	P value
MDA (nmol/mg of protein)	7.47 ±0.42	6.03± 0.12 ns	6.64± 0.31 ns	0.0983	8.35 ±0.43	6.42± 0.24+	5.22± 0.47+	0.0418	4.53± 0.48	3.70± 0.42+	2.95± 0.31+	0.0367
SOD (µmol/mg protein)	699±42.8	891± 35.8+	912± 18.2+	0.0172	642 ±15.4	786± 26.9+	839± 22.5+	0.0241	467.4± 20.1	559.4± 22.9 ns	536.9± 20.7ns	0.0421
Catalase (µmol/ min /mg protein)	1560 ±250	1738± 223 ns	1710± 202 ns	0.2221	1537 ±163	1670± 132 ns	1740± 133 ns	0.3212	1078 ±187	1067± 168 ns	1193± 102ns	0.3319
Neutrophil-Lymphocyte ratio	4.22 ±0.51	2.96 ±0.22	2.76± 0.42	0.0287	2.46 ±0.15	1.59± 0.13	1.44± 0.14	0.0291	1.02± 0.17	0.62± 0.11	0.60± 0.11	0.0376
Myeloperoxidase (U/mg protein)	1.75 ±0.11	1.13 ±0.20+	1.05± 0.13+	0.0416	1.85 ±0.14	1.31± 0.12+	1.27± 0.12+	0.0383	0.66 ±0.1	0.40± 0.21ns	0.39± 0.14 ns	0.0621
Total Nitrite (µM/ mg tissue)	0.16 ±0.02	0.15 ±0.01 ns	0.15± 0.01 ns	0.1224	0.14 ±0.02	0.14± 0.02 ns	0.16± 0.01 ns	0.1109	0.12 ±0.02	0.13± 0.02 ns	0.12± 0.02 ns	0.2021

+Connote a significant change at P≤0.05 when compared to the control, ns connote not significant when compared to the control

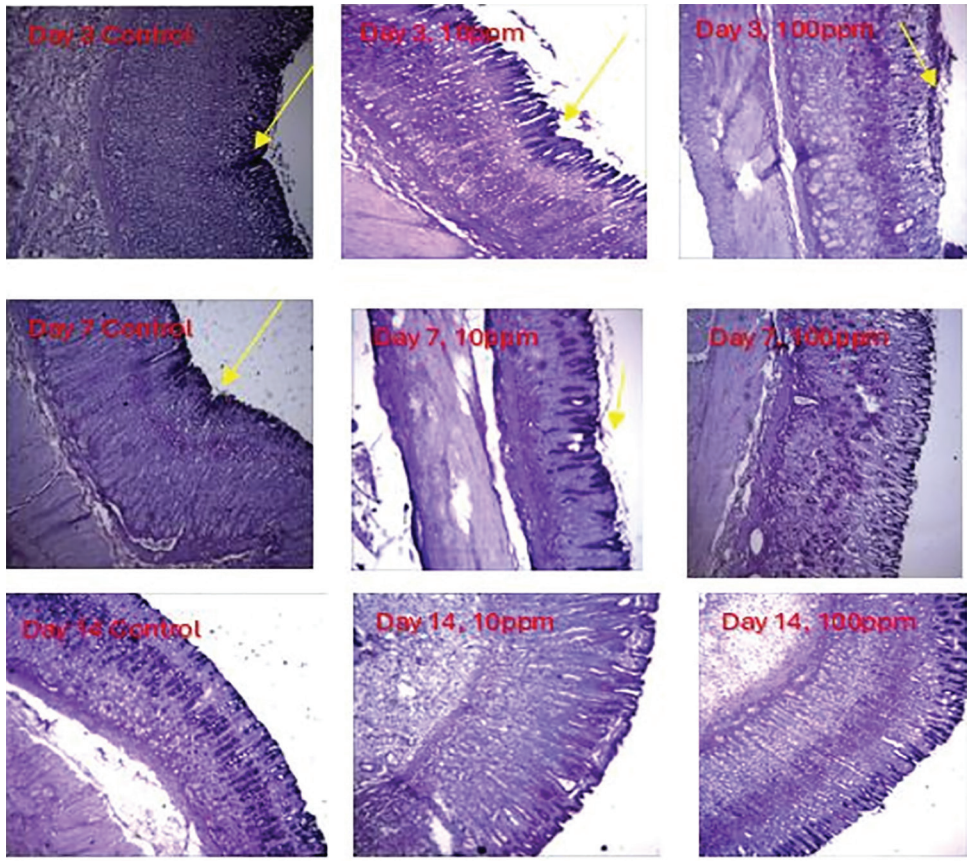

Figure 2: Representative photomicrographs of the stomach on selected days for the examination of healing process after induction of experimental gastric ulcer following period of exposure to trivalent chromium, H&E stain, 100x Mag. Yellow arrows show ulcer lesions in all the groups on day 3 and on day 7 for both control and 10 ppm chromium group.

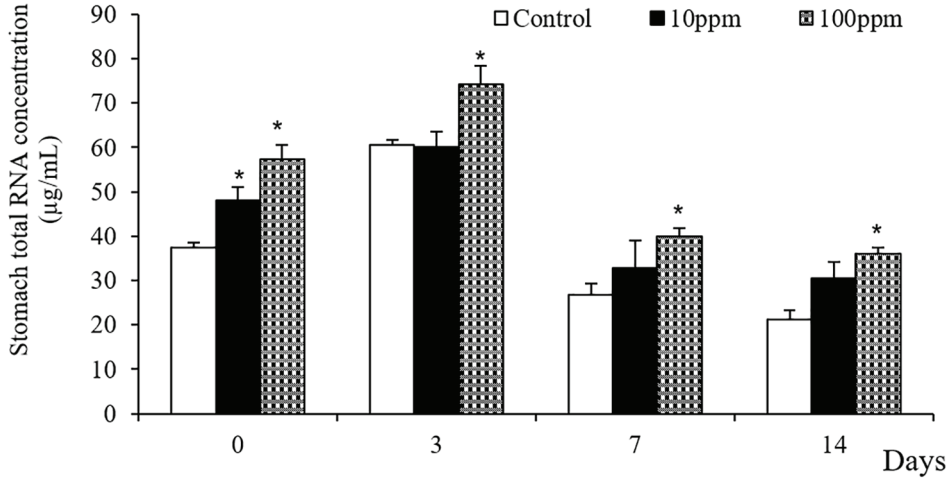

Figure 3: Stomach total RNA concentration in both pre- and post-ulcer evaluations following 12 weeks of exposure to chromium. *Significant at P<0.05 compared with control

Impact of Chromium Exposure on Stomach RNA

Total RNA (µg/mL) significantly increased on days 0, 3, 7, and 14, P=0.0014; 0.0197; 0.1263; and 0.0093 respectively in the 100ppm chromium group and on pre-ulceration (day 0) in the 10ppm group (P=0.0014) compared to the control (Figure 3).

Impact of Chromium-exposure and Chemical Injury on Stomach Pro- and Anti-inflammatory Cytokine

There were decreases in the copy number of the Il-1α (P=0.0036; 0.0233) and IFN-γ (P=0.0339, 0.0055) on days 3 and 7 of evaluation compared to the control (Figures 4 and 5, respectively), as well as a decrease in the normalized copy number of TNF-α on day 7 post-ulceration, P=0.0311 compared to the control (Figure 6). There was no significant change in the normalized mRNA copy number for IL-6 cytokine (Figure 7). However, the normalized copy number for IL-10 mRNA increased significantly, P=0.0001; 0.0002 compared to the control on days 3 and 7, post-injury respectively in the test groups compared to the control and also increased significantly (P=0.0026) on day 14 in the 100ppm compared to the control (Figure 8).

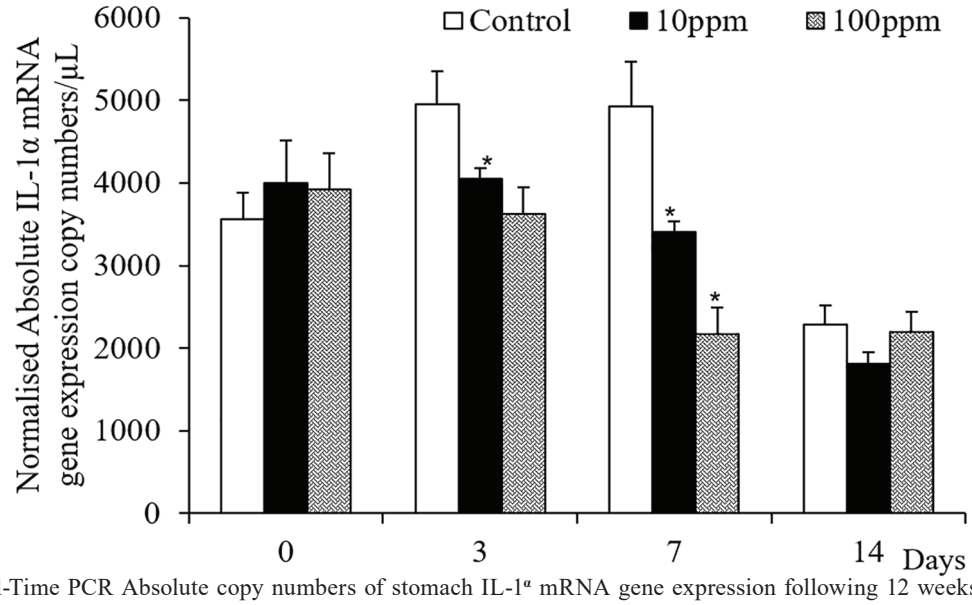

Figure 4: Real-Time PCR Absolute copy numbers of stomach IL-1ᵅ mRNA gene expression following 12 weeks of exposure to chromium and ulcer induction. Values were normalized with the three reference genes, B-actin, GAPDH, and RPL13. *Significant at P<0.05 compared with control

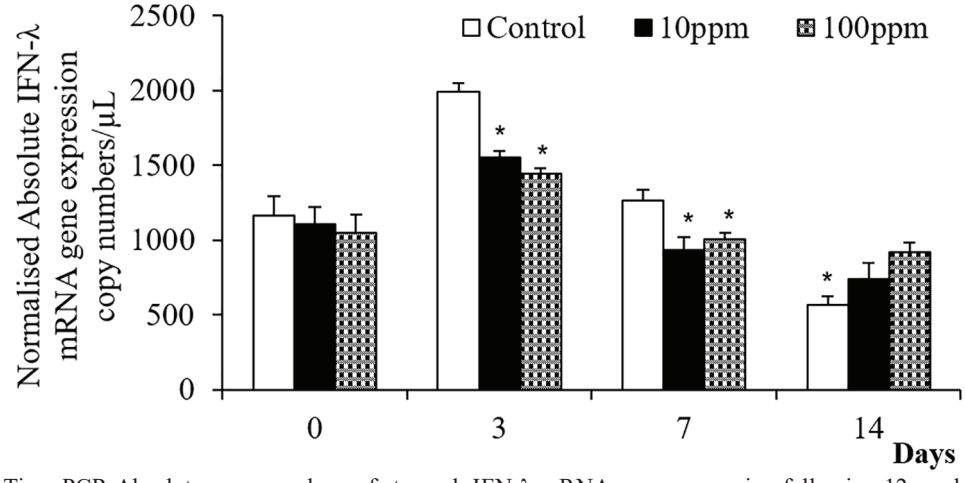

Figure 5: Real-Time PCR Absolute copy numbers of stomach IFN-**λ** mRNA gene expression following 12 weeks of exposure to chromium and ulcer induction. Values were normalized with the three reference genes, B-actin, GAPDH, and RPL13. *Significant at P≤0.05 compared with control

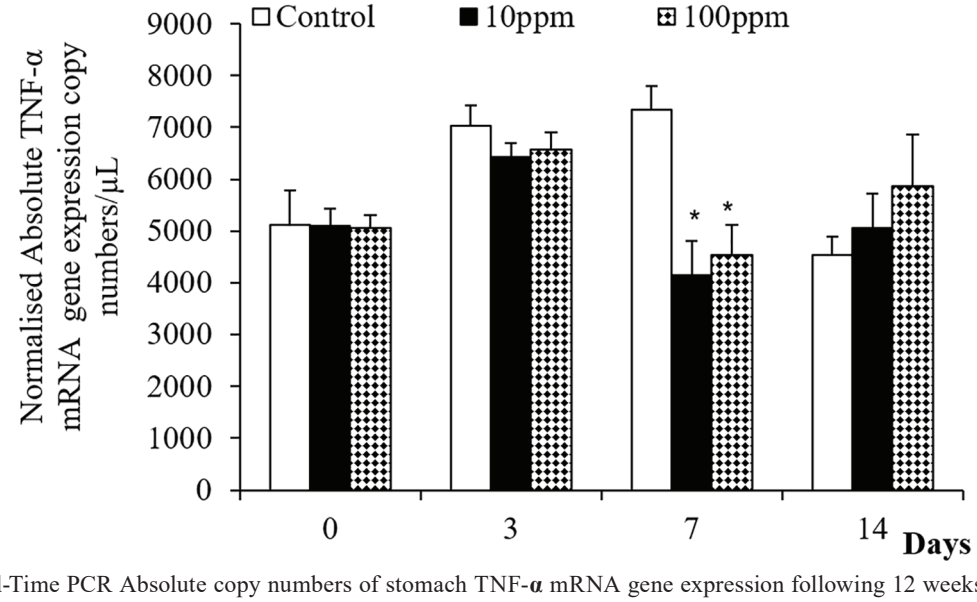

Figure 6: Real-Time PCR Absolute copy numbers of stomach TNF-**α** mRNA gene expression following 12 weeks of exposure to chromium and ulcer induction. Values were normalized with the three reference genes, B-actin, GAPDH, and RPL13. *Significant at P≤0.05 compared with control

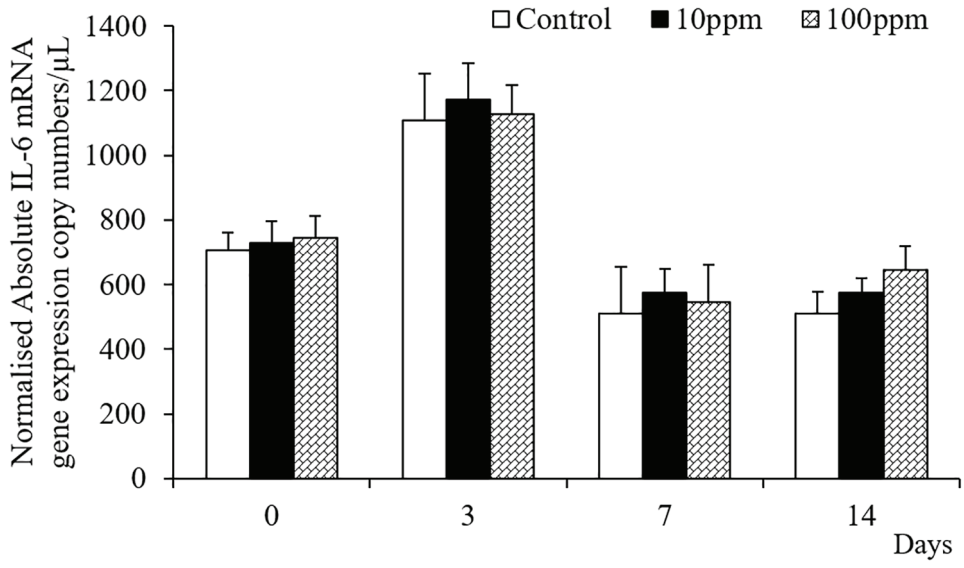

Figure 7: Real-Time PCR Absolute copy numbers of stomach IL-6 mRNA gene expression following 12 weeks of exposure to chromium and ulcer induction. Values were normalized with the three reference genes, B-actin, GAPDH, and RPL13. *P≤0.05 compared with control

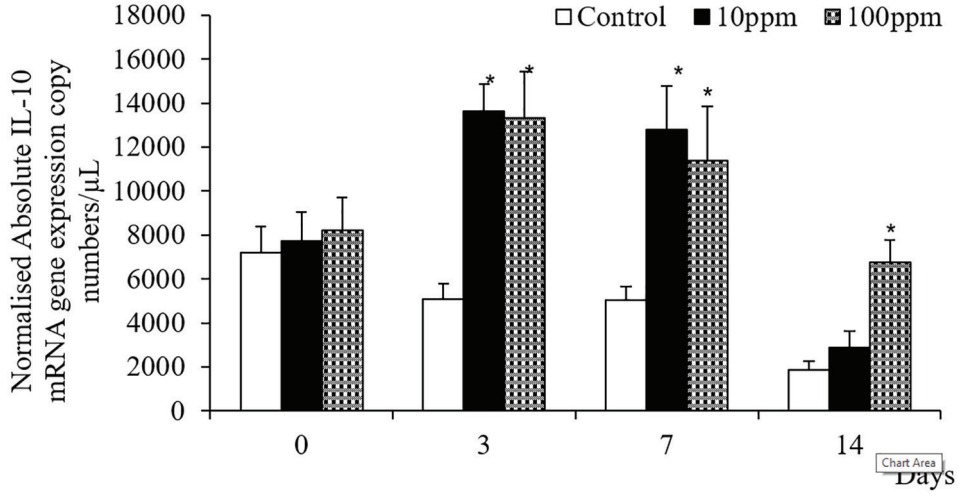

Figure 8: Real-Time PCR Absolute copy numbers of stomach IL-10 mRNA gene expression following 12 weeks of exposure to chromium and ulcer induction. Values were normalized with the three reference genes, B-actin, GAPDH, and RPL13. *Significant at P≤0.05 compared with control

Discussion

This study investigated the impact of exposure to oral trivalent chromium in experimental gastric ulcers to ascertain the possible mechanisms of its effects. In this study, the chromium-exposed groups reduced the harmful impact of acetic acid on the stomach mucosa by fortifying the mucosa thickness before the injury and reducing ulcer scores. The observation from the histology and histomorphology further corroborated the protection and the hastened healing observed with the chromium groups. In our previous reports, we highlighted the improved healing properties and the preservation of the typical architecture of the mice's colon after exposure to chromium for the same number of weeks (8, 19).

This protective effect against the chemotoxic agent-acetic acid could have been through many associated mechanisms. By releasing circulating inflammatory cells such as neutrophils, the inflammatory pathways could be one of these suspected paths. Neutrophils are the first line of inflammatory cells released after the early phase of injury before the onset of healing within the first 48 hours and fall subsequently within another 36 hours (30). Neutrophils released are expected to have mobilized certain interleukins towards the wound sites, thereby helping to institute inflammatory processes essential for wound healing. Initially thought to be harmful to wound sites when recruited and after the battle, they do commit suicide and release many cytokines that could further sustain inflammation and delay healing (31). This view has been flawed and could be debated against, particularly with sterile injury, as neutrophils provide pro-inflammatory and anti-inflammatory responses towards healing phases (32)neutrophils invade sites of inflammation to eradicate pathogens and clear debris. Traditionally, neutrophils were thought to cause collateral tissue damage before dying at the site. However, the presence of neutrophil infiltration

into sterile injuries (in the absence of infections. The increase was persistent in our study, which could be essential for the faster healing reported.

Another blood cell judged necessary in healing processes is the platelet, and its indices have been used in several studies to support the healing mechanism. Apart from their role in preventing blood loss through the prompt formation of clots (30), their importance in wound healing has been emphasized (33). Its interaction with inflammation and immune responses is also crucial for wound healing processes, and their inflammasome interactions have been described with appropriate immune responses toward salvaging injured tissues (33). Activating platelets in wound healing is essential to process possible degranulations (34, 35), after which it releases substances to consolidate the repair of damaged tissues (36). The raised platelets in this current study were at the peak of inflammation day - day 3 following injury compared to the control but began a reduction to the baseline in subsequent days. The initial raised value of platelets consolidated the activity of the neutrophils throughout the days of the study. In acute inflammation, depending on the cause or the extent of damage, sterile or wounds with contaminants, platelets may be raised for up to 14 days after the injury (37). The gross limitation of platelets in synthesizing protein reduced its roles in promoting healing (38). Still, its prowess in acute inflammation to enhance programmed cell deaths and promote repairs is well-known (39). The chromium-treated groups elicited raised platelet values that are substantial enough to hasten the repair of the injured stomach mucosa in this study compared to the control group with ulcers and no chromium exposure.

Oxidative stress constitutes an essential interaction between the pro-antioxidants and their antioxidant counterparts, which usually results in both molecular and cellular injury (40, 41). Many heavy metals were implicated in generating reactive oxygen species (ROS) harmful to the human system with similar mechanisms (42). However, trivalent chromium exposed to the gut reduced MDA, an indicator of low lipid peroxidation after introducing the chemical irritant in our study compared to the control group. ROS formation in gastric ulcers and all inflammatory processes is well known. However, in the present study, MDA value was lower, and SOD activity was higher in trivalent chromium pre-treated groups. Otherwise, Catalase activity did not increase, which means that hydrogen-peroxide, as a product of SOD activity, decomposes by other enzymes, for instance, Glutathione peroxidase (GPx). This observation is similar to our initial results in experimental colitis, where trivalent chromium reduced MDA and increased SOD following induction of colitis in mice (8). Superoxide dismutase is the initial line of protection against reactive oxygen species-instituted injuries (43), which subdue inflammation and promote healing in injured tissues where ROS hold sway (44). The increase in SOD in our study indicates the level of stress faced in combating the effect of ROS.

To achieve effective wound healing, adequate and appropriate interactions between cytokines, extracellular matrix, and many cell types are essential. The interplay between blood cells released to wound sites and interactions with ROS and cytokines in wound healing processes are crucial and cannot be downplayed. Within the wound bed, neutrophils and platelets facilitate the release of cytokines that may help promote the inflammatory process or inhibit it, as the case may be (45). Neutrophils cleanse the wound and debride while distributing pro-inflammatory cytokines, and other growth factors (46). In our study, the IL-1α, TNF-α, and interferon-γ reduction in the chromium-exposed groups, especially on the days peaking inflammatory processes instead, suggest a protection mechanism in that interaction. The anti-inflammatory cytokine, IL-10, was elevated from day 3 until at least day 7 post-injury. A plethora of information implicates the cytokine IL-10 in handling tissue inflammatory processes, and recently, its role in fibrogenesis during wound healing has been emphasized (47). Interleukin-10 families maintain epithelial integrity through their host defense mechanism approach (47).

Limitations: the major limitation of our study was the time of exposure which could be made longer in order to further determine other probable effects as well as limited funds available did not allow for additional biomolecular studies.

Conclusion

We observed improved healing in the trivalent during a sterile stomach mucosa injury through many cellular inputs from the interplay of the blood neutrophils and platelets on suppressing reactive oxygen species and their interactions with anti- and pro-inflammatory cytokines. The stomach mucosa healing observed in this study from the exposure to oral trivalent chromium is due to the potentiation of IL-10 and suppression of TNF-α and interferon-γ while inhibiting reactive oxygen radicals in the presence of increased superoxide dismutase, neutrophils, and platelets. We suggest that pre-treatment with trivalent chromium protects the gastric mucosa and promotes the healing of injured gastric ulcers in mice.

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Authors' Contribution

OAO: Collected, collated, and interpreted data; sourced for funding; project administration; drafted and edited the article. SBO: Conceptualize the project, supervise the work, and review the manuscript. Both authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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References

1. Lassen A, Hallas J, De Muckadell O.B.S. Complicated and uncomplicated peptic ulcers in a Danish county 1993-2002: a population-based cohort study. *Am J Gastroenterol* 2006; 101: 945–953.
2. Xia B, Xia H.H.X, Ma C.W, et al. Trends in the prevalence of peptic ulcer disease and Helicobacter pylori infection in family physician-referred uninvestigated dyspeptic patients in Hong Kong. *Aliment Pharmacol Ther* 2005; 22: 243–249.
3. Schiffmann L, Kahrau S, Berger G, et al. Colon perforation in an adolescent after short-term diclofenac intake. *ANZ J Surg* 2005; 75: 726–727.
4. McCarthy D.M. Prevention and treatment of gastrointestinal symptoms and complications due to NSAIDs. *Best Pract Res Clin Gastroenterol* 2001; 15: 755–773.
5. Lambert M, Leven B, technology RG-E science and, et al. New methods of cleaning up heavy metal in soils and water. *Citeseer*, http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.400.2031&rep=rep1&type=pdf (accessed 14 October 2021).
6. Jaishankar M, Tseten T, Anbalagan N, et al. Toxicity, mechanism and health effects of some heavy metals. *Interdiscip Toxicol* 2014; 7: 60.
7. Losacco C, Perillo A. Metal-induced oxidative Stress and Cellular Signaling Alteration in Animals. *Iran J Appl Anim Sci* 2018; 8: 367–373.
8. Odukanmi O. A, Salami A.T, Koda K, et al. Trivalent Chromium Promotes Healing of Experimental Colitis in Mice by Suppression of Inflammation and Oxidative Stress. *J Biosci Med* 2017; 5: 108–126.
9. Mertz W. Interaction of chromium with insulin: A progress report. *Nutr Rev* 1998; 56: 174–177.
10. Lv H, Lin Y, Liu P, et al. Protective effects and potential underlying mechanisms of sodium copper chlorophyllin against ethanol-induced gastric ulcer in mice. *Acta Biochim Biophys Sin (Shanghai)* 2019; 51: 925–933.
11. Yazdanpanah K, Parhizkar B, Sheikhesmaeili F, et al. Efficacy of zinc sulfate in peptic ulcer disease: A randomized double-blind clinical trial study. *J Clin Diagnostic Res* 2016; 10: OC11–OC15.
12. Chromium - Health Professional Fact Sheet, https://ods.od.nih.gov/factsheets/Chromium-HealthProfessional/ (accessed 17 October 2021).
13. Sazakli E, Villanueva C.M., Kogevinas M, Maltezis K, Mouzaki A. Chromium in Drinking Water: Association with Biomarkers of Exposure and Effect. *Int J Environ Res Publ Heal* 2014; 11: 10125–10145.
14. Vincent J.B, Lukaski H. C. Chromium. *Adv Nutr* 2018; 9: 505–506.
15. Scientific Opinion on Dietary Reference Values for chromium. *EFSA J*; 12. Epub ahead of print 1 October 2014. DOI: 10.2903/J.EFSA.2014.3845.
16. Federal Register : Food Labeling: Revision of the Nutrition and Supplement Facts Labels, https://www.federalregister.gov/documents/2016/05/27/2016-11867/food-labeling-revision-of-the-nutrition-and-supplement-facts-labels (accessed 17 October 2021).
17. Vincent J.B. The potential value and toxicity of chromium picolinate as a nutritional supplement, weight loss agent and muscle development agent. *Sports Med* 2003; 33: 213–230.
18. Nakada M, Demura S. The effect of past and present lifestyle, nutrition habits, and gender on bone mineral density. *Health (Irvine Calif)* 2010; 02: 713–721.
19. Odukanmi O.A, Owoeye O, Salami A. T, Morakinyo O.L., Iyiola O.T and Olaleye S.B. Effect of trivalent chromium (Cr2O3) on stomach morphometry and some vital organs in male wistar rats | African Journal of Medicine and Medical Sciences. *Afr J Med Med Sci* 2019; 48: 329–337.
20. Kareema H, Dohan A-H, Abbas H. Effects of Potassium Dichromate on Reproduction and Fertility in Albino Female Mice. *Int J PharmTech Res* 2016; 2016: 205–213.
21. Amagase K, Okabe S. Acetic acid ulcers: a new method for producing solitary chronic ulcers in rat stomachs by intraluminal application of acetic acid solution. *InflammoPharmacology 2002 104* 2002; 10: 385–389.
22. Gornall AG, Bardawill CJ, David MM. Determination of serum proteins using the biuret reaction. Epub ahead of print 1949. DOI: 10.1016/S0021-9258(18)57021-6.
23. Varshney R, Kale R.K. Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. *Int J Radiat Biol* 1990; 58: 733–743.
24. Sinha A.K. Colorimetric assay of catalase. *Anal Biochem* 1972; 47: 389–394.
25. Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal Biochem* 1971; 44: 276–287.
26. Ignarro, L.J., Buga, G.M., Wood, K.S., Byrns, R.E., and Chaudhuri, G. 1987. Endothelium-Derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc. Natl. Acad. Sci.* U.S.A. **84**(24): 9265–9269
27. Kim H, Wei Y, Lee JY, et al. Myeloperoxidase Inhibition Increases Neurogenesis after Ischemic Stroke. *J Pharmacol Exp Ther* 2016; 359: 262.
28. Vandesompele J, De Preter K, Pattyn F, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol* 2002; 3: 1–12.
29. Animals NRC (US.) C for the U of the G for the C and U of L. Guide for the Care and Use of Laboratory Animals. *Guid Care Use Lab Anim.* Epub ahead of print 27 December 2011. DOI: 10.17226/12910.
30. Qing C. The molecular biology in wound healing & non-healing wound. *Chinese J Traumatol* 2017; 20: 189–193.

31. Phillipson M, Kubes P. The neutrophil in vascular inflammation. *Nat Med* 2011; 17: 1381–1390.
32. Peiseler M, Kubes P. More friend than foe: The emerging role of neutrophils in tissue repair. *J Clin Invest* 2019; 129: 2629–2639.
33. Hottz ED, Paula Monteiro AT, Bozza FA, et al. Review Article Inflammasome in Platelets: Allying Coagulation and Inflammation in Infectious and Sterile Diseases? Epub ahead of print 2015. DOI: 10.1155/2015/435783.
34. Andia I, Sanchez M, Maffulli N. Tendon healing and platelet-rich plasma therapies. *Expert Opin Biol Ther* 2010; 10: 1415–1426.
35. Halpern BC, Chaudhury S, Rodeo SA. The Role of Platelet-Rich Plasma in Inducing Musculoskeletal Tissue Healing: *https://doi.org/101007/s11420-011-9239-7* 2012; 8: 137–145.
36. Davis VL, Abukabda AB, Radio NM, et al. Platelet-rich preparations to improve healing. Part II: platelet activation and enrichment, leukocyte inclusion, and other selection criteria. *J Oral Implantol* 2014; 40: 511–521.
37. Demidova-Rice TN, Hamblin MR, Herman IM. Acute and impaired wound healing: pathophysiology and current methods for drug delivery, part 1: normal and chronic wounds: biology, causes, and approaches to care. *Adv Skin Wound Care* 2012; 25: 304–314.
38. Zimmerman G.A, Weyrich A.S. Signal-Dependent Protein Synthesis by Activated Platelets New Pathways to Altered Phenotype and Function. *Arterioscler Thromb Vasc Biol* 2008; 28: s17.
39. Parrish W.R. Physiology of Blood Components in Wound Healing: an Appreciation of Cellular Co-Operativity in Platelet Rich Plasma Action. *J Exerc Sport Orthop* 2017; 4: 1–14.
40. Conti V, Izzo V, Corbi G, et al. Antioxidant Supplementation in the Treatment of Aging-Associated Diseases. *Front Pharmacol*; 7. Epub ahead of print 12 February 2016. DOI: 10.3389/FPHAR.2016.00024.
41. Hwang J, Jin J, Jeon S, et al. SOD1 suppresses pro-inflammatory immune responses by protecting against oxidative stress in colitis. *Redox Biol* 2020; 37: 101760.
42. Balali-Mood M, Naseri K, Tahergorabi Z, et al. Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic. *Front Pharmacol* 2021; 12: 227.
43. Kangralkar, V.A, Patil, S.D, Bandivadekar R. Oxidative stress and diabetes: a review. *Int J Pharm Appl* 2010; 1: 38-45.
44. Porfire A.S, Leucuţa SE, Kiss B, et al. Investigation into the role of Cu/Zn-SOD delivery system on its antioxidant and anti-inflammatory activity in rat model of peritonitis. *Pharmacol Rep* 2014; 66: 670–676.
45. Satish L, Kathju S. Cellular and Molecular Characteristics of Scarless versus Fibrotic Wound Healing. *Dermatol Res Pract*; 2010. Epub ahead of print 2010. DOI: 10.1155/2010/790234.
46. Schultz G.S, Chin G.A, Moldawer L, et al. Principles of Wound Healing. *Diabet Foot Probl* 2011; 395–402.
47. Sziksz E, Pap D, Lippai R, et al. Fibrosis Related Inflammatory Mediators: Role of the IL-10 Cytokine Family. *Mediators Inflamm*; 2015. Epub ahead of print 2015. DOI: 10.1155/2015/764641.