Effect of l-ascorbic acid on Nickel-Induced Alteration of Cardiovascular Pathophysiology in Wistar Rats. BIOL TRACE ELEM.RES (Springer)

Preprint *in* Biological Trace Element Research · July 2019

DOI: 10.1007/s12011-019-01829-w

CITATIONS
0

READS
174

6 authors, including:

Rachamalla Chandramouli Reddy
BLDE Deemed to be University
13 PUBLICATIONS **31** CITATIONS
SEE PROFILE

Basavaraj B Devaranavadagi
BLDE University
11 PUBLICATIONS **56** CITATIONS
SEE PROFILE

Shrilaxmi C Bagali
BLDE University
23 PUBLICATIONS **106** CITATIONS
SEE PROFILE

Raghavendra Kulkarni
BLDEA's College of Pharmacy
100 PUBLICATIONS **1,155** CITATIONS
SEE PROFILE

Springer

Dear Author

Here are the proofs of your article.

- You can submit your corrections **online,** via **e-mail** or by **fax**.
- For **online** submission please insert your corrections in the online correction form. Always indicate the line number to which the correction refers.
- You can also insert your corrections in the proof PDF and **email** the annotated PDF.
- For **fax** submission, please ensure that your corrections are clearly legible. Use a fine black pen and write the correction in the margin, not too close to the edge of the page.
- Remember to note the **journal title**, **article number**, and **your name** when sending your response via e-mail or fax.
- **Check** the metadata sheet to make sure that the header information, especially author names and the corresponding affiliations are correctly shown.
- **Check** the questions that may have arisen during copy editing and insert your answers/corrections.
- **Check** that the text is complete and that all figures, tables and their legends are included. Also check the accuracy of special characters, equations, and electronic supplementary material if applicable. If necessary refer to the *Edited manuscript*.
- The publication of inaccurate data such as dosages and units can have serious consequences. Please take particular care that all such details are correct.
- Please **do not** make changes that involve only matters of style. We have generally introduced forms that follow the journal's style.
- Substantial changes in content, e.g., new results, corrected values, title and authorship are not allowed without the approval of the responsible editor. In such a case, please contact the Editorial Office and return his/her consent together with the proof.
- If we do not receive your corrections **within 48 hours**, we will send you a reminder.
- Your article will be published **Online First** approximately one week after receipt of your corrected proofs. This is the **official first publication** citable with the DOI. **Further changes are, therefore, not possible.**
- The **printed version** will follow in a forthcoming issue.

Please note

After online publication, subscribers (personal/institutional) to this journal will have access to the complete article via the DOI using the URL:

`http://dx.doi.org/10.1007/s12011-019-01829-w`

If you would like to know when your article has been published online, take advantage of our free alert service. For registration and further information, go to: http://www.link.springer.com.

Due to the electronic nature of the procedure, the manuscript and the original figures will only be returned to you on special request. When you return your corrections, please inform us, if you would like to have these documents returned.

Metadata of the article that will be visualized in OnlineFirst

1	Article Title	**Effect of L-Ascorbic Acid on Nickel-Induced Alteration of Cardiovascular Pathophysiology in Wistar Rats**	
2	Article Sub- Title		
3	Article Copyright - Year	**Springer Science+Business Media, LLC, part of Springer Nature 2019 (This will be the copyright line in the final PDF)**	
4	Journal Name	Biological Trace Element Research	
5	Corresponding Author	Family Name	**Das**
6		Particle	
7		Given Name	**Kusal K.**
8		Suffix	
9		Organization	BLDE (Deemed to be University)
10		Division	Laboratory of Vascular Physiology and Medicine, Department of Physiology, Shri B. M. Patil Medical College, Hospital and Research Centre
11		Address	Vijayapura 586103, Karnataka, India
12		e-mail	kusaldas@bldedu.ac.in
13	Author	Family Name	**Reddy**
14		Particle	
15		Given Name	**R. Chandramouli**
16		Suffix	
17		Organization	BLDE (Deemed to be University)
18		Division	Department of Biochemistry, Shri B. M. Patil Medical College, Hospital and Research Centre
19		Address	Vijayapura 586103, Karnataka, India
20		Organization	BLDE (Deemed to be University)
21		Division	Laboratory of Vascular Physiology and Medicine, Department of Physiology, Shri B. M. Patil Medical College, Hospital and Research Centre
22		Address	Vijayapura 586103, Karnataka, India
23		e-mail	
24	Author	Family Name	**Devaranavadagi**
25		Particle	
26		Given Name	**Basavaraj**
27		Suffix	

28		Organization	BLDE (Deemed to be University)
29		Division	Department of Biochemistry, Shri B. M. Patil Medical College, Hospital and Research Centre
30		Address	Vijayapura 586103, Karnataka, India
31		e-mail	
32	Author	Family Name	**Yendigeri**
33		Particle	
34		Given Name	**Saeed M.**
35		Suffix	
36		Organization	Al-Ameen Medical College
37		Division	Department of Pathology
38		Address	Vijayapura 586108, Karnataka, India
39		e-mail	
40	Author	Family Name	**Bagali**
41		Particle	
42		Given Name	**Shrilaxmi**
43		Suffix	
44		Organization	BLDE (Deemed to be University)
45		Division	Laboratory of Vascular Physiology and Medicine, Department of Physiology, Shri B. M. Patil Medical College, Hospital and Research Centre
46		Address	Vijayapura 586103, Karnataka, India
47		e-mail	
48	Author	Family Name	**Kulkarni**
49		Particle	
50		Given Name	**Raghavendra V.**
51		Suffix	
52		Organization	BLDEA's SSM College of Pharmacy & Research Centre
53		Division	Department of Pharmaceutics & Pharmaceutical Technology
54		Address	BLDE University Campus, Vijayapura 586103, India
55		e-mail	

56	Schedule	Received	5 April 2019
57		Revised	
58		Accepted	10 July 2019

59 Abstract

Nickel, a widely used heavy metal is suspected as a cardiotoxic element. The aim of the present study was to assess the possible protective role of l-ascorbic acid on nickel-induced alterations of cardiovascular pathophysiology in male albino rats. Twenty-four albino rats (b.wt. 170–250 g) were randomized into four groups: control; l-ascorbic acid (50 mg/100 g b.wt., orally); $NiSO_4$

		(2.0 mg/100 g b.wt., i.p.); $NiSO_4$ with l-ascorbic acid. Cardiovascular electrophysiology, serum and cardiac tissue malondialdehyde (MDA), nitric oxide (NO), ascorbic acid, serum α-tocopherol and serum vascular endothelial growth factor (VEGF) were evaluated. Histopathology of cardiac and aortic tissues was also assessed. $NiSO_4$-treated rats showed a significant increase in heart rate, LF/HF ratio and blood pressure (SBP, DBP and MAP). A significant increase of serum MDA, NO and VEGF in $NiSO_4$ treatment with a concomitant decrease of serum ascorbic acid and α-tocopherol as compared to their respective controls were also observed. Simultaneous supplementation of l-ascorbic acid with $NiSO_4$ significantly decreased LF/HF ratio, BP and oxidative stress parameters, whereas ascorbic acid and α-tocopherol concentration was found to be increased. Histopathology of cardiac and aortic tissues showed nickel-induced focal myocardial hypertrophy and degeneration in cardiac tissue with focal aneurism in aortic tissues. Supplementation with l-ascorbic showed a protective action in both cardiac and aortic tissues. Results indicated the possible beneficial effect of l-ascorbic acid on nickel-induced alteration of the cardiovascular pathophysiology in experimental rats.
60	Keywords separated by ' - '	Nickel sulfate - Oxidative stress - Electrophysiology - VEGF - Cardiac tissue - L-ascorbic acid
61	Foot note information	Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Biological Trace Element Research
https://doi.org/10.1007/s12011-019-01829-w

Effect of L-Ascorbic Acid on Nickel-Induced Alteration of Cardiovascular Pathophysiology in Wistar Rats

R. Chandramouli Reddy[1,2] · Basavaraj Devaranavadagi[1] · Saeed M. Yendigeri[3] · Shrilaxmi Bagali[2] · Raghavendra V. Kulkarni[4] · Kusal K. Das[2]

Received: 5 April 2019 / Accepted: 10 July 2019

Abstract

Nickel, a widely used heavy metal is suspected as a cardiotoxic element. The aim of the present study was to assess the possible protective role of l-ascorbic acid on nickel-induced alterations of cardiovascular pathophysiology in male albino rats. Twenty-four albino rats (b.wt. 170–250 g) were randomized into four groups: control; l-ascorbic acid (50 mg/100 g b.wt., orally); $NiSO_4$ (2.0 mg/100 g b.wt., i.p.); $NiSO_4$ with l-ascorbic acid. Cardiovascular electrophysiology, serum and cardiac tissue malondialdehyde (MDA), nitric oxide (NO), ascorbic acid, serum α-tocopherol and serum vascular endothelial growth factor (VEGF) were evaluated. Histopathology of cardiac and aortic tissues was also assessed. $NiSO_4$-treated rats showed a significant increase in heart rate, LF/HF ratio and blood pressure (SBP, DBP and MAP). A significant increase of serum MDA, NO and VEGF in $NiSO_4$ treatment with a concomitant decrease of serum ascorbic acid and α-tocopherol as compared to their respective controls were also observed. Simultaneous supplementation of l-ascorbic acid with $NiSO_4$ significantly decreased LF/HF ratio, BP and oxidative stress parameters, whereas ascorbic acid and α-tocopherol concentration was found to be increased. Histopathology of cardiac and aortic tissues showed nickel-induced focal myocardial hypertrophy and degeneration in cardiac tissue with focal aneurism in aortic tissues. Supplementation with l-ascorbic showed a protective action in both cardiac and aortic tissues. Results indicated the possible beneficial effect of l-ascorbic acid on nickel-induced alteration of the cardiovascular pathophysiology in experimental rats.

Keywords Nickel sulfate · Oxidative stress · Electrophysiology · VEGF · Cardiac tissue · L-ascorbic acid

Introduction

Recent studies are showing a strong link between environmental pollutants and various cardiovascular diseases (CVD) [1–5]. Amid environmental heavy metal pollutants, nickel, a trace element with having a divalent cation, has enormous industrial applications. Because of its properties, it has been most widely used in electroplating of metals, battery manufacturing, as a catalyst, electrical and electronic industries, metallurgy and metal alloys preparations [6]. Nickel particles are widely distributed in the soil, air and water; its concentrations are excessive in industrial areas [7]. Nickel is a silver-white transition metal that belongs to the group VIIIb of the periodic table. Nickel concentrations in ambient air is minimal (approximately 6–20 ng/m^3) but higher concentrations (up to 150 ng/m^3) are found in the air polluted by anthropogenic sources [6]. Among the various nickel compounds, nickel sulfate contributes predominant nickel pollutant of ambient air. Nickel facilitates absorption of iron (Fe) in Fe^{3+} form in gastrointestinal tract (GIT) [8]. Nickel is considered as essential trace element for several animal species, but in humans deficiency manifestations have not been clearly mentioned [6]. In biological systems, absorption, distribution and clearance of nickel compounds mainly depend on their

✉ Kusal K. Das
kusaldas@bldedu.ac.in

[1] Department of Biochemistry, Shri B. M. Patil Medical College, Hospital and Research Centre, BLDE (Deemed to be University), Vijayapura, Karnataka 586103, India

[2] Laboratory of Vascular Physiology and Medicine, Department of Physiology, Shri B. M. Patil Medical College, Hospital and Research Centre, BLDE (Deemed to be University), Vijayapura, Karnataka 586103, India

[3] Department of Pathology, Al-Ameen Medical College, Vijayapura, Karnataka 586108, India

[4] Department of Pharmaceutics & Pharmaceutical Technology, BLDEA's SSM College of Pharmacy & Research Centre, BLDE University Campus, Vijayapura 586103, India

solubility in water and the route of exposure [6]. The absorption and toxic manifestations of nickel compounds depend on the solubility and order of absorption as follows: nickel carbonyl > soluble nickel compounds (chloride, nitrate, sulfate) > insoluble nickel compounds (oxides, sulphides) [9].

The degree of severity of nickel toxicities is manifested by its route and quantum of exposure [10]. It has been found that toxic manifestations of nickel usually occur through (1) increased ROS production and deprivation of the antioxidant defence system, and (2) alterations in the autonomic nervous system [1]. Nickel-induced increase of oxidative and nitrosative stress is well established and possible altered role of NOS2 (inducible nitric oxide synthase), NOS3 (endothelial nitric oxide synthase) and oxygen sensing cell signalling pathways is also found to be the potential factors to induce nickel toxicities [1]. Studies revealed that divalent cation (Ni^{2+}) influences cardiovascular functions and causes aortic hyper contraction, but exact physiological mechanisms are yet to be known [11].

Very few studies have been conducted to know the toxic effects of divalent cations like nickel on cardiovascular health. Moreover, the effect of water-soluble antioxidant vitamins like l-ascorbic acid on nickel-induced cardiovascular abnormalities were found to be least investigated. Water-soluble chain breaking antioxidant vitamin C scavenges oxygen radicals in aqueous phase, but lipophilic antioxidants like alpha tocopherol (vitamin E) scavenge oxygen radicals within the membrane [12]. Vitamin C also acts as a co-antioxidant and facilitates regeneration of alpha tocopherol from its ascorbyl radical [12]. So, it is well known that some antioxidant vitamins like l-ascorbic acid (vitamin C) or α-tocopherol (vitamin E) may re-establish oxidant/antioxidant balance hence may provide additional defence against metal-induced cell injuries through oxidative stress [13, 14]. In many studies, it has been shown that vitamin C protects against cell death caused by various obnoxious stimuli and a major proportion of this protection has been associated with its antioxidant ability.

Hence, the present study was undertaken to explore the possible protective role of l-ascorbic acid on nickel-induced alterations of cardiovascular pathophysiology in experimental rats.

Materials and Methods

Experimental Design

Twenty-four healthy adult male albino Wistar rats (*Rattus novergicus*) of 8–10 weeks old with the weight of 170–250 g were procured from BLDE (Deemed to be University), Shri B.M. Patil Medical College, Hospital and Research Centre animal house. Experimental rats were acclimatized to standard laboratory conditions for 7 days before starting the experimentation. All standard laboratory conditions like temperature with 22 ± 2 °C and 12 h light-dark cycle were maintained throughout all days of the experiment. All the protocols were scrutinized and approved by the Institutional Animal Ethical Committee (Ref No: BLDE/BPC/641/2016-2017 dated 22 October 2016). Experimental animals were pair fed with normal laboratory diet and water provided ad libitum throughout the study period. All the experimental protocols were performed according to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Government of India [15]. All the experimental rats were divided into four groups and given respective interventions as shown in Table 1. Throughout the adaptation period and the experiment, the rats were accommodated in triplets in standard polycarbonate cages (24 in. × 12 in. × 8 in.) with stainless steel mesh on the top.

Gravimetry

Each animal body weight of all groups was measured on day 1 of intervention and the day of sacrifice by using Sartorious electronic balance (Model: Practum 1102-10IN) and percentage body weight gain was calculated. After sacrificing the experimental rats, heart tissue was dissected out and weighed immediately by using electronic balance (Sathyam digital scale, Model: H2F-A + 300). Organo-somatic index of heart was calculated by organ weight in gram per 100 g of animal body weight.

Electrophysiology

After 21 days of intervention, rats were kept overnight fasting and acclimatized to a small animal restrainer for assessment of non-invasive blood pressure (NIBP). Once rats acclimatized to restrainer, systolic and diastolic blood pressures were recorded by NIBP monitor with tail cuff sensor (Biopac NIBP200A model) connected to a PC. After that, rats were anaesthetised by injecting ketamine (60 mg/kg b.wt.; i.p.) and xylazine (6 mg/kg b.wt., i.p.) [18]. ECG was recorded by using an MP45 Biopac instrument with a PC-based BSL 4.1 (Biopac Student Lab 4.1) software. For ECG, bipolar electrodes were attached to the upper and lower limbs of the rats; heart rate variability (HRV) was analysed by using Kubios HRV software, version 2.0, developed by Department of Physics, University of Kuopio, Finland. Analysis of HRV was performed by using 5 min ECG R-R interval data. The following HRV parameters were measured: low frequency (LF) power (sympathetic activity), high frequency (HF) power (parasympathetic activity) and LF/HF ratio.

Animal Sacrifice and Sample Collection

After 21 days of the intervention, rats were kept overnight fasting, anaesthetized, recorded electrophysiological

t1.1 **Table 1** Experimental groups and the intervention

t1.2 Experimental groups	Dosage for 21 days
t1.3 Control	Placebo; oral gavage daily
t1.4 L-ascorbic acid	50 mg/100 g b.wt.; oral gavage daily [16]
t1.5 $NiSO_4$	2.0 mg/100 g b.wt.; i.p.; alternate day, 10 doses [17]
t1.6 $NiSO_4$ + l-ascorbic acid	$NiSO_4$ 2.0 mg/100 g b.wt.; i.p. alternate day, 10 doses + l-ascorbic acid 50 mg/100 g b.wt.; given by oral gavage daily

b.wt. body weight, *i.p.* intraperitoneal

parameters as said above and opened thoracic cage carefully, blood was collected by cardiac puncture and stored in a plain tube with clot activator. The samples were kept at room temperature for 45 min and centrifuged at 600×*g* for 15 min. Serum was separated, protected from light and stored at − 20 °C for further biochemical analysis. After blood collection, rats were sacrificed as per CPCSEA guidelines between 09.00 AM and 11.00 AM.

Oxidative Stress Markers and Antioxidant Vitamins

The concentration of lipid peroxidation product malondialdehyde (MDA) in serum and cardiac tissue homogenate samples was estimated by thiobarbituric acid (TBA) method, where MDA reacts with TBA in hot acidic conditions gives red coloured complex and this colour intensity was directly proportional to the concentration of MDA [19]. Nitrosative stress marker nitric oxide (NO) in its stable form nitrate in serum and cardiac tissue homogenate reduced to nitrite by cadmium reduction and forms coloured complex with N-napthylene diamine. The colour absorbance was measured by UV-Visible spectrophotometer (Shimadzu, Model: UV1800) [20, 21]. Ascorbic acid was estimated in serum and heart tissue homogenate by the method of Roe and Koether [22]. Serum alpha tocopherol concentrations were estimated by the method of Jargar et al. [23]. In the protein-free serum samples, alpha tocopherol was extracted into the xylene layer which reduces ferric to ferrous ions and forms red coloured complex with 2,2′-bipyridyl reagent. The intensity of red colour developed was red at 492 nm against blank by using microplate reader (Meril EIAQuant, Meril Diagnostics Pvt. Ltd., India) [23].

Molecular Markers

Serum VEGF was estimated by a commercially available ELISA kit (Biospespvt. Ltd., China) using a microplate reader (Model: Merilyzer EIAQUANT, Meril Diagnostics Pvt. Ltd).

Histopathology

After the blood collection, animals were sacrificed and cardiac tissue and thoracic aorta were dissected and washed in cold saline to remove the excess blood and then tissues were stored in 10% neutral-buffered formalin for histopathological evaluations. Paraffin blocks were made with fixed tissues and made sections of 3–5 μm thickness, deparaffinized, rehydrated and stained with haematoxylin and eosin (H&E) [24]. The stained tissue sections were observed under a photomicroscope and photographed (Olympus BH-2 with Samsung digital colour camera, Model No. SDC-242).

Statistical Analysis

All the data obtained from control and experimental groups were analysed for the level of significance by using one-way analysis of variance (ANOVA) followed by Tukey's post hoc t-test, by using Windows-based SPSS software version 15.0. The p value < 0.05 is considered statistically significant.

Results

Gravimetry

Figure 1 shows that $NiSO_4$-treated rats gained the least % body weight during 21 days of intervention period when compared to all other groups. Both control and l-ascorbic acid-treated rats showed greater % body weight gain among all groups. Rats simultaneously treated with l-ascorbic acid and $NiSO_4$ showed little improvement in body weight gain when compared to animals treated with $NiSO_4$ alone but it is not statically significant.

Organo-Somatic Index

Figure 2 shows a significantly higher cardiac organo-somatic index of $NiSO_4$ alone-treated rats when compared to control and l-ascorbic acid groups. Rats simultaneously treated with l-ascorbic acid and $NiSO_4$ did not show a statistically significant decrease when compared to $NiSO_4$ alone-treated rats.

Electrophysiology

Heart Rate Variability and Heart Rate

Table 2 shows the LF, HF, LF/HF ratio and heart rate of all groups of experimental rats. Results showed a significant increase of LF power, a decrease of HF power and an increase of LF/HF ratio and heart rate (HR) in $NiSO_4$ alone-treated rats when compared to all other groups. Rats simultaneously treated with $NiSO_4$ and l-ascorbic acid show a significant decrease

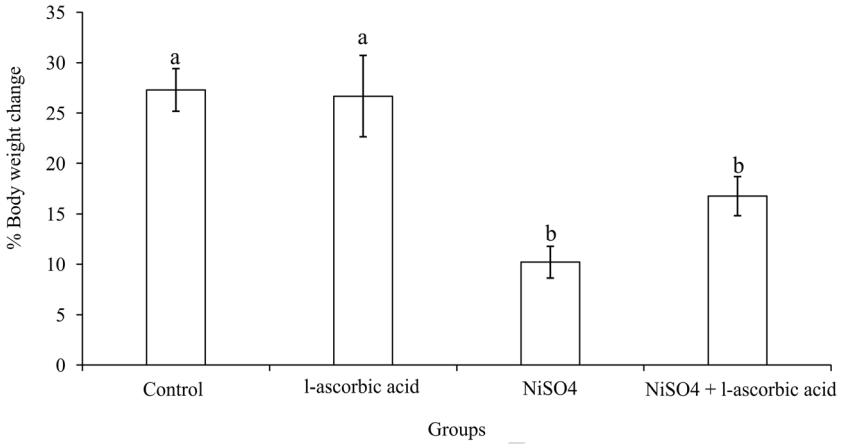

Fig. 1 Comparison of % body weight gain in control, l-ascorbic acid, $NiSO_4$ and $NiSO_4$ + l-ascorbic acid. $n = 6$ rats in each group. Values with different superscripts (a, b) are significantly different from each other ($p < 0.05$)

of LF, an increase of HF and a decrease of LF/HF ratio and HR as compared to nickel alone-treated rats.

Blood Pressure

Table 3 shows significantly increased SBP, DBP and MAP in $NiSO_4$ alone-treated rats whereas simultaneous supplementation of l-ascorbic acid with $NiSO_4$ shows a significant decrease in blood pressure parameters.

Oxidative Stress Markers and Antioxidant Vitamins

Table 4 shows a significant increase in serum and cardiac tissue MDA concentrations in $NiSO_4$ alone-treated rats in comparison to control and l-ascorbic acid groups, whereas rats supplemented with l-ascorbic acid and $NiSO_4$ showed a significant decrease in MDA concentration. Serum and cardiac tissue nitric oxide concentrations in $NiSO_4$ group showed a significant increase when compared to control and l-ascorbic acid groups. Supplementation of l-ascorbic acid with $NiSO_4$ significantly decreased heart tissue NO concentration whereas serum NO concentration remained unchanged as compared to $NiSO_4$ alone-treated group.

Serum and cardiac tissue ascorbic acid concentrations are found to be significantly decreased in $NiSO_4$-treated rats when compared to control and l-ascorbic acid groups. $NiSO_4$ + l-ascorbic acid-treated group of rats showed a significant increase in serum ascorbic acid concentrations but no significant increase was found in case of cardiac tissue l-ascorbic acid concentration as compared to $NiSO_4$ alone-treated rats. $NiSO_4$ alone-treated rats showed a significant decrease in serum α-tocopherol concentration when compared to control and l-ascorbic acid groups, but the group simultaneously treated with $NiSO_4$ and l-ascorbic acid did not show any significant changes as compared to $NiSO_4$ alone-treated group.

Molecular Marker

Figure 3 shows a significant increase of serum VEGF concentration in $NiSO_4$ alone-treated rats when compared to control and l-ascorbic acid-treated rats, whereas rats supplemented with l-ascorbic acid with $NiSO_4$ showed significantly decreased serum VEGF concentration when compared to $NiSO_4$ alone-treated rats.

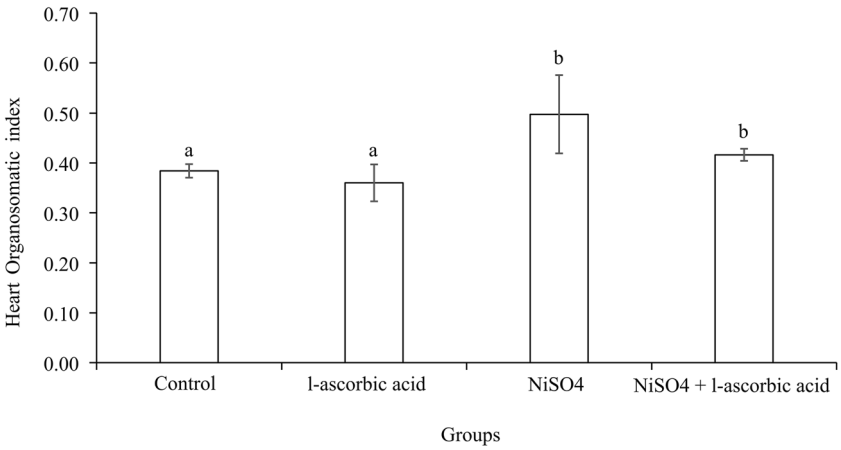

Fig. 2 Comparison of the organosomatic index of cardiac tissue in control, l-ascorbic acid, $NiSO_4$ and $NiSO_4$ + l-ascorbic acid. $n = 6$ rats in each group. Values with different superscripts (a, b) are significantly different from each other ($p < 0.05$)

t2.1 **Table 2** Effect of l-ascorbic acid supplementation on $NiSO_4$-induced alterations of heart rate variability (HRV) parameters like LF, HF, LF/HF ratio and heart rate

t2.2 Groups	LF (Power n.u)	HF (Power n.u)	LF/HF ratio	Heart rate (BPM)
t2.3 Control	49.87 ± 1.60^a	49.37 ± 1.52^a	1.01 ± 0.06^a	255.70 ± 6.97^a
t2.4 L-ascorbic acid	49.09 ± 1.10^a	49.04 ± 1.22^a	0.99 ± 0.02^a	325.07 ± 8.92^a
t2.5 $NiSO_4$	59.54 ± 1.73^b	41.89 ± 1.24^b	1.42 ± 0.01^b	364.03 ± 20.26^b
t2.6 $NiSO_4$ + l-ascorbic acid	53.40 ± 3.08^a	46.33 ± 3.17^a	1.15 ± 0.14^a	270.82 ± 26.94^a
t2.7 *p* value	0.001	0.006	0.001	0.037

Horizontal values are mean ± SD of six observations in each group: control, l-ascorbic acid, $NiSO_4$ and $NiSO_4$ + l-ascorbic acid. Values with different superscripts (a, b) are significantly different from each other ($p < 0.05$)

LF low frequency, *HF* high frequency, *BPM* beats per minute

Histopathology of Cardiac Tissue and Aorta

Histopathology of Cardiac Tissue

Figure 4 shows sections of the cardiac tissues of all groups. H&E-stained tissue sections of control and l-ascorbic acid-treated rats studied under the microscope show normal histological features like myocardium composed of branching and anastomosing, striated muscle fibres arranged in parallel fashion, separated by capillaries, myocardial fibres are connected to each other by intercalated discs and coronary arteries appear normal. $NiSO_4$ alone-treated tissue sections show focal myocardial hypertrophy, degeneration, capillary congestion and moderate arteriosclerosis of coronary arteries. Rats simultaneously treated with l-ascorbic acid and $NiSO_4$ show some histological improvements in architecture by showing myocardium composed of branching and anastomosing striated muscle fibres arranged in parallel fashion, separated by capillaries; there is no evidence of arteriosclerosis and coronary arterial congestion.

Histopathology of Aorta

Figure 5 showing aortic tissue sections of all groups. H&E-stained thoracic aortic tissue sections of control and l-ascorbic acid-treated rats studied under the microscope show large-sized artery normal histological structures and no evidence of arteriosclerosis, atherosclerosis, arteritis, calcification, aneurysm and dysplasia. But $NiSO_4$ alone-treated rats' aortic tissue sections show mild thickening of tunica intima, medial sclerosis with hyperplastic smooth muscle cells and focal aneurysm. Tissues sections of rats simultaneously treated with $NiSO_4$ and l-ascorbic acid show near normal architecture with only mild thickening of tunica media.

Discussion

Results indicate that $NiSO_4$ has an adverse effect on % body weight gain of rats. Many theories and pathways have been proposed about the control and maintenance of the body composition. But the present results of our study are supported by electrophysiological parameters. In accordance with the experimental findings of Bray et al. (2000), increased sympathetic activity reduces food intake and our study also showed increased sympathetic activity in $NiSO_4$-treated rats [25]. But l-ascorbic acid supplementation in $NiSO_4$-treated rats has shown statistically insignificant body weight gain when compared to $NiSO_4$ alone-treated rats. After 21 days of intervention with $NiSO_4$ alone, an increased cardiac mass showed when compared to control and l-ascorbic acid groups (Fig. 2). This increased cardiac mass could be due to cardiac hypertrophy in response to increased mean arterial pressure (supported by histopathology reports and MAP values) [26,

t3.1 **Table 3** Effect of l-ascorbic acid supplementation on $NiSO_4$-induced alterations of Blood Pressure Values

t3.2 Groups	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)
t3.3 Control	124.55 ± 3.23^a	102.22 ± 3.93^a	110.80 ± 4.41^a
t3.4 L-ascorbic acid	118.28 ± 6.62^a	97.20 ± 5.66^a	120.36 ± 0.51^a
t3.5 $NiSO_4$	166.11 ± 7.71^b	136.80 ± 12.67^b	146.56 ± 10.67^b
t3.6 $NiSO_4$ + l-ascorbic acid	133.78 ± 15.44^a	106.01 ± 12.38^a	115.23 ± 12.66^a
t3.7 *p* value	0.001	0.004	0.004

Horizontal values are mean ± SD of six observations in each group: control, l-ascorbic acid, $NiSO_4$ and $NiSO_4$ + l-ascorbic acid. Values with different superscripts (a, b) are significantly differ from each other ($p < 0.05$)

SBP systolic blood pressure, *DBP* diastolic blood pressure, *MAP* mean arterial pressure

t4.1 **Table 4** Effect of l-ascorbic acid supplementation on $NiSO_4$-induced alterations of serum and cardiac tissue stress markers and antioxidant vitamins

t4.2 Parameters	Control	L-ascorbic acid	$NiSO_4$	$NiSO_4$ + l-ascorbic acid	*p* value
t4.3 Serum MDA (μM/L)	1.63 ± 0.29^a	1.27 ± 0.13^a	2.4 ± 0.39^b	1.38 ± 0.17^a	0.003
t4.4 Cardiac tissue MDA (μM/g of tissue)	3.48 ± 0.87^a	2.94 ± 1.07^a	13.72 ± 0.67^b	6.99 ± 1.00^c	0.000
t4.5 Serum NO (μM/L)	6.11 ± 0.19^a	7.78 ± 1.44^a	12.22 ± 2.26^b	10.01 ± 1.49^b	0.000
t4.6 Cardiac tissue NO (μM/g of tissue)	26.0 ± 5.86^a	23.61 ± 3.64^a	82.25 ± 8.27^b	52.60 ± 8.19^c	0.000
t4.7 Serum ascorbic acid (mg/dL)	0.81 ± 0.03^a	1.28 ± 0.14^b	0.61 ± 0.02^c	1.28 ± 0.13^b	0.000
t4.8 Cardiac tissue ascorbic acid (mg/g of tissue)	4.76 ± 1.25^a	7.69 ± 1.93^a	3.23 ± 1.64^b	5.12 ± 1.43^b	0.050
t4.9 Serum α-tocopherol (μg/mL)	2.86 ± 0.38^a	3.46 ± 0.85^a	1.32 ± 0.30^b	1.39 ± 0.06^b	0.001

Horizontal values are mean ± SD of six observations in each group: control, l-ascorbic acid, $NiSO_4$ and $NiSO_4$ + l-ascorbic acid. In each column, values with different superscripts (a, b, c) are significantly different from each other ($p < 0.05$)

MDA malondialdehyde, *NO* nitric oxide

27]. But simultaneous supplementation of l-ascorbic acid in $NiSO_4$ administered group showed statistically insignificant reduction in cardiac mass. So further study may be required to know the beneficial effect of long-term supplementation with l-ascorbic acid in nickel-induced decrease of % body weight gain and cardiac hypertrophic changes.

The present experiment on HRV in nickel-treated rats considered as a clinically approved indicator of cardiac autonomic balance [28]. The results clearly indicated a vago-sympathetic imbalance in nickel-treated rats by sympathetic overdrive with concomitant under drive of parasympathetic actions. The increase of heart rate in $NiSO_4$ treatment in the present study further indicates a possible increase in sympathetic stimulation. These autonomic malfunctions may also be due to nickel-initiated inflammatory response, increased oxidative stress and decreased availability of antioxidants [29]. Altered sympatho-vagal balance due to $NiSO_4$ administration was significantly improved by the supplementation of antioxidant l-ascorbic acid, suggesting that concurrent response of oxidative stress involved in the modulation of cardiac autonomic function [1].

In this study, we have also observed an increase of SBP, DBP and MAP in the rats treated with $NiSO_4$. This may also be due to the generation of reactive oxygen species (ROS) and cyclooxygenase 2 (COX2)-dependent endothelium contracting factors (EDCFs) by endothelial cells [1, 4, 11, 27]. Golovko et al. (2003) reported that nickel ion induces vasoconstriction in the isolated canine coronary artery by enhancing $CaCl_2$ influx into vascular smooth muscle cells [30, 31]. These increased concentrations of intracellular smooth muscle Ca^{2+} could lead to the increased arterial tone which in turn leading to an increase of blood pressure [32]. However, simultaneous supplementation of l-ascorbic acid with $NiSO_4$ significantly improved the altered blood pressure parameters in rats, as l-ascorbic acid is found to be a potent antioxidant which improves endothelial functions by reducing the oxidative stress, suppressing endothelial cell apoptosis by cytokines and tumour necrosis factor-α (TNF-α) and angiotensin II [33].

Increased oxidative stress in the physiological system is considered to be a key factor underlying cardiovascular dysfunction, hence increase of serum and heart tissue MDA with the decrease of l-ascorbic acid and alpha tocopherol in serum and decrease of l-ascorbic acid in heart tissues in nickel treated rats ascertain cardiotoxicities due to nickel-induced oxidative stress [12]. Nickel is a known atherogenic substance which may exert its effects by increasing the ROS production which in turn increases lipid peroxidation by the increase of oxygen

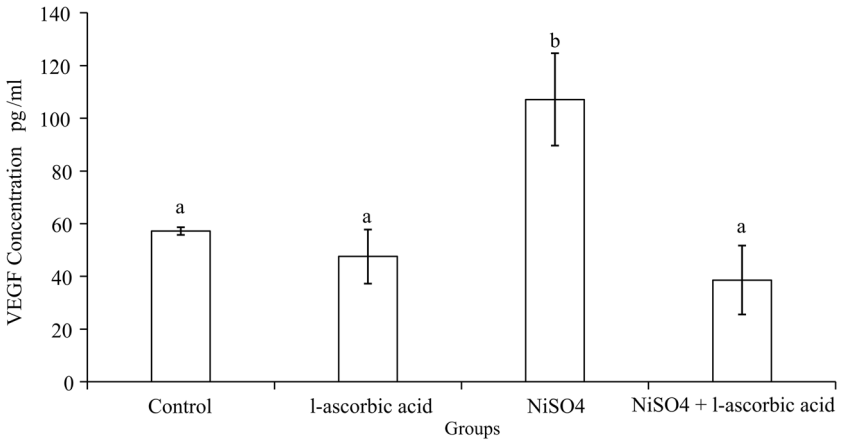

Fig. 3 Comparison of VEGF concentration in control, l-ascorbic acid, $NiSO_4$ and $NiSO_4$ + l-ascorbic acid. $n = 6$ rats in each group. Values with different superscripts (a, b) are significantly different from each other ($p < 0.05$)

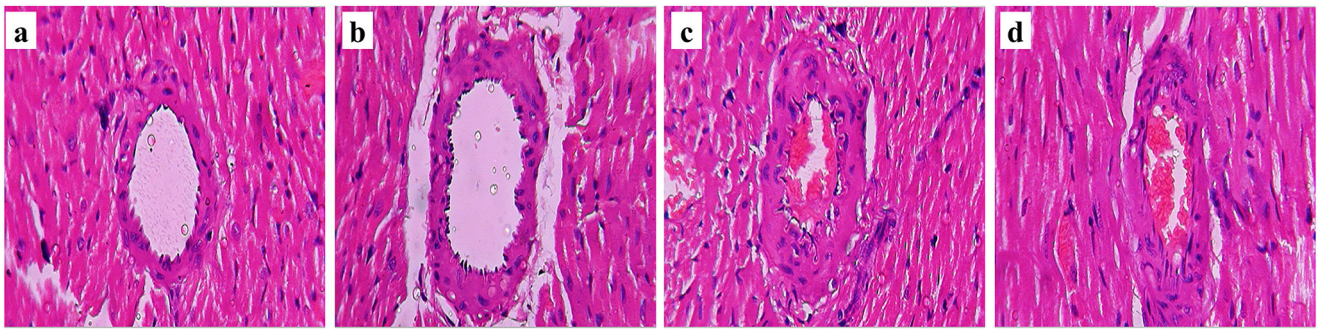

Fig. 4 Haematoxylin and eosin-stained photo-micrographs of cardiac tissue with coronary artery from **a** control (×40); **b** l-ascorbic acid (×40); **c** $NiSO_4$ (×40); **d** $NiSO_4$ + l-ascorbic acid (×40)

free radicals and overutilization of membrane antioxidants [34]. Supplementation of l-ascorbic acid in nickel-treated rats was found to be beneficial to counteract nickel-induced cardiotoxicities due to oxidative stress.

In the present study, we observed an increase in serum and cardiac tissue NO concentrations in nickel-treated rats may be due to the overexpression of inducible nitric oxide synthase (i-NOS) [34]. Increased levels of nitric oxide can react with the superoxide anion (O^{-}_{2}) and form peroxynitrite anion (ONOO–). Peroxynitrite is a potent oxidant and may trigger lipid peroxidation, inhibit mitochondrial electron transport and oxidize thiol compounds [35]. i-NOS gene expression reported being increased in conditions like cellular hypoxia, oxidative stress and pro-inflammatory conditions [36]. Nickel mediated activation of the transcription factors NF-κB and STAT-1α reported being an essential step for the i-NOS induction in most of the cells [32]. Supplementation of l-ascorbic acid may decrease the NO production by blocking the NF-κB pathway in nickel-treated rats.

In many studies, it has been observed that nickel or any other heavy metals create cellular hypoxia by binding to the heme portion of oxygen sensing molecules [34]. The present study indicated that nickel-induced increase of VEGF, potent oxygen sensing growth factor causes cellular hypoxia, vascular damage, triggers hypoxia cell signalling and angiogenesis pathways to protect tissue from further damage [37]. Supplementation of l-ascorbic acid in nickel-treated rats decreased oxygen free radicals which in turn decrease cellular damage and protect heme-containing oxygen sensing biomolecules in physiological systems including the cardiovascular system.

Histopathological studies of cardiac tissues along with coronary artery and aorta show cardiovascular remodelling due to nickel toxicity. The results also indicate a possible association between nickel and vascular architecture by the development of hypertrophy and hyperplasia in vessels, expansion of the endothelial and adventitial layers along with increased arterial wall thickness [36]. Nickel-induced oxidative, nitrosative stress and concomitant decrease of antioxidant defensive agents are responsible for changes in cardiovascular pathophysiology and affect structural and functional homeostasis. But supplementation of l-ascorbic acid mediated improvements of both heart and aortic tissue architectures in nickel-treated rats may be because of decreased oxidative, nitrosative stress and increased antioxidant agents [14].

Conclusion

Results suggest that heavy metal like nickel impairs cardiac autonomic functions, vascular functions, oxidant-antioxidant balance and oxygen sensing cell signalling mechanism (VEGF). Further, nickel sulfate was also found to have an adverse impact on cardiac and aortic tissue histopathology.

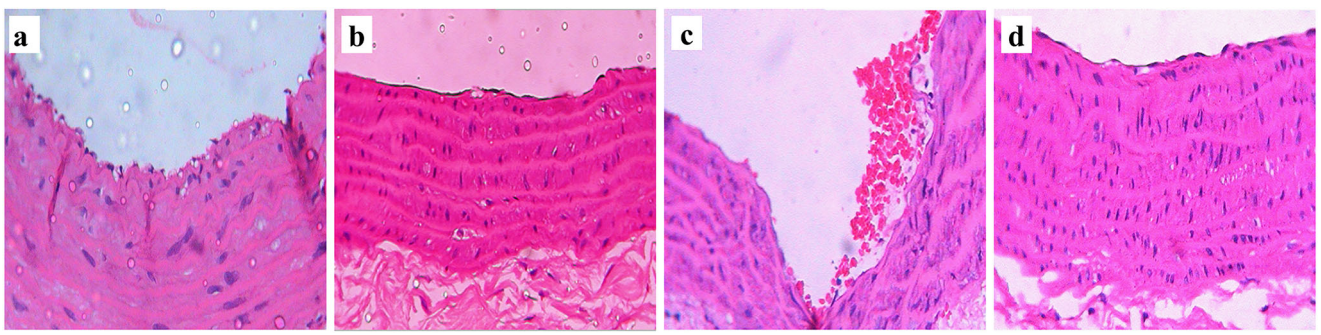

Fig. 5 Haematoxylin and eosin-stained photo-micrographs of the thoracic aorta from **a** control (×40); **b** l-ascorbic acid (×40); **c** NiSO4 (×40); **d** $NiSO_4$ + l-ascorbic acid (×40)

Supplementation of l-ascorbic acid, a potent antioxidant, was found to be beneficial against nickel-induced cardiovascular toxicities. The results obtained in this study may have clinical value in humans and the effect of l-ascorbic acid on nickel-induced cardiovascular toxicities deserves further exploration.

Funding The first (RCR) and the last (KKD) author acknowledge the financial support from the Life Sciences Research Board (LSRB), Defence Research and Development Organisation (DRDO), Ministry of Defence, Government of India [R&D/81/48222/LSRB-285/EPB/2014 dated 18 July 2014], VGST (VGST-KFIST/1230/2015-16 dated 22 Jun 2016), Government of Karnataka, India.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval Institutional Animal Ethical Committee (IAEC) approval was taken from BLDE Association's Shri Sanganabasava Mahaswamiji College of Pharmacy & Research Centre, BLDE (Deemed to be University), Vijayapur, Karnataka, India (Ref No: BLDE/BPC/641/2016-2017 dated 22 October 2016) as per institutional CPCSEA (Committee for the Purpose of Control and Supervision of |Experiments on Animals), Ministry of Environment, Forests and Climate Change, Government of India Reg. No. 1076/PO/ERs/S/07 CPCSEA dated 20 August 2014.

References

1. Lippmann M, Ito K, Hwang JS et al (2006) Cardiovascular effects of nickel in ambient air. Environ Health Perspect 114:1662–1669. https://doi.org/10.1289/ehp.9150
2. Zhang Z, Chau PYK, Lai HK, Wong CM (2009) A review of effects of particulate matter-associated nickel and vanadium species on cardiovascular and respiratory systems. Int J Environ Health Res 19:175–185. https://doi.org/10.1080/09603120802460392
3. Cosselman KE, Navas-Acien A, Kaufman JD (2015) Environmental factors in cardiovascular disease. Nat Rev Cardiol 12:627–642. https://doi.org/10.1038/nrcardio.2015.152
4. Vallejo M, Ruiz S, Hermosillo AG et al (2006) Ambient fine particles modify heart rate variability in young healthy adults. J Expo Sci Environ Epidemiol 16:125–130. https://doi.org/10.1038/sj.jea.7500447
5. Gold DR, Litonjua A, Schwartz J et al (2000) Ambient pollution and heart rate variability. Circulation 101:1267–1273
6. Barceloux DG, Barceloux D (1999) Nickel. J Toxicol Clin Toxicol 37:239–258. https://doi.org/10.1081/CLT-100102423
7. Das KK, Dasgupta S (1997) Alteration of testicular biochemistry during protein restriction in nickel treated rats. Biol Trace Elem Res 60:243–249. https://doi.org/10.1007/BF02784444
8. Nielsen FH (1980) Effect of form of iron on the interaction between nickel and iron in rats: growth and blood parameters. J Nutr 110:965–973. https://doi.org/10.1093/jn/110.5.965
9. Sunderman FW, Hopfer SM, Sweeney KR et al (1989) Nickel absorption and kinetics in human volunteers. Proc Soc Exp Biol Med 191:5–11
10. Das KK, Reddy RC, Bagoji IB et al (2019) Primary concept of nickel toxicity—an overview. J Basic Clin Physiol Pharmacol 30:141–152. https://doi.org/10.1515/jbcpp-2017-0171
11. Wani SA, Khan LA, Basir SF (2018) Role of calcium channels and endothelial factors in nickel induced aortic hypercontraction in Wistar rats. J Smooth Muscle Res 54:71–82. https://doi.org/10.1540/jsmr.54.71
12. Niki E (1987) Interaction of ascorbate and a-tocopherol. Ann N Y Acad Sci 498:186–199
13. Das K, Dhundasi S, Jargar J, Yendigeri S (2014) Protective effect of vitamin E (a-tocopherol) on nickel-induced alteration of testicular pathophysiology in alloxan-treated diabetic rats. Int J Clin Exp Physiol 1:290. https://doi.org/10.4103/2348-8093.149762
14. Das KK, Das SN, DasGupta S (2001) The influence of ascorbic acid on nickel-induced hepatic lipid peroxidation in rats. J Basic Clin Physiol Pharmacol 12:187–195. https://doi.org/10.1515/JBCPP.2001.12.3.187
15. Das KK, Dasgupta S (2002) Effect of nickel sulfate on testicular steroidogenesis in rats during protein restriction. Environ Health Perspect 110:923–926. https://doi.org/10.1289/ehp.02110923
16. Husain K, Sugendran K, Pant SC et al (1992) Biochemical and pathological changes in response to hyperoxia and protection by antioxidants in rats. Indian J Physiol Pharmacol 36:97–100
17. Borders EPV (1983) Myocardial changes induced by nickel as in association with cadmium. Rev Ig Bacteriol Virusol Parazitol Epidemiol Pneumoftiziol 32:51–56
18. Kawahara Y, Tanonaka K, Daicho T et al (2005) Preferable anesthetic conditions for echocardiographic determination of murine cardiac function. J Pharmacol Sci 99:95–104. https://doi.org/10.1254/jphs.FP0050343
19. Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Methods Enzymol 52:302–310. https://doi.org/10.1016/S0076-6879(78)52032-6
20. Mohamed AA, Mubarak AT, Fawy KF, El-Shahat MF (2008) Modification of AOAC method 973.31 for determination of nitrite in cured meats. J AOAC Int 91:820–827
21. Moshage H, Kok B, Huizenga JR, Jansen PLM (1995) Nitrite and nitrate determinations in plasma: a critical evaluation. Clin Chem 41:892–896. https://doi.org/10.1016/S0584-8547(97)00141-9
22. Roe JH, Kuether CA (1943) The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. J Biol Chem 147:399–407
23. Jargar JG, Hattiwale SH, Das S et al (2012) A modified simple method for determination of serum α-tocopherol (vitamin E). J Basic Clin Physiol Pharmacol 23:45–48. https://doi.org/10.1515/jbcpp-2011-0033
24. Drury RAB (1986) Cellular pathology technique. J Clin Pathol 39:467–468
25. Bray GA (2000) Reciprocal relation of food intake and sympathetic activity: experimental observations and clinical implications. Int J Obes Relat Metab Disord 24(Suppl 2):S8–S17
26. Hunter JJ, Chien KR (1999) Signaling pathways for cardiac hypertrophy and failure. N Engl J Med 341:1276–1283. https://doi.org/10.1056/NEJM199910213411706
27. Julian RJ (2007) The response of the heart and pulmonary arteries to hypoxia, pressure, and volume. A short review. Poult Sci 86:1006–1011. https://doi.org/10.1093/ps/86.5.1006
28. Chuang H-C, Hsueh T-W, Chang C-C et al (2013) Nickel-regulated heart rate variability: the roles of oxidative stress and inflammation. Toxicol Appl Pharmacol 266:298–306. https://doi.org/10.1016/J.TAAP.2012.11.006
29. Kasprzak K (2003) Nickel carcinogenesis. Mutat Res Mol Mech Mutagen 533:67–97. https://doi.org/10.1016/j.mrfmmm.2003.08.021
30. Koller A, Rubányi G, Ligeti L, Kovách AG (1982) Effect of verapamil and phenoxybenzamine on nickel-induced coronary vasoconstriction in the anaesthetized dog. Acta Physiol Acad Sci Hung 59:287–290

31. Golovko VA, Bojtsov IV, Kotov LN (2003) Single and multiple early afterdepolarization caused by nickel in rat atrial muscle. Gen Physiol Biophys 22:275–278
32. Forstermann U, Sessa WC (2012) Nitric oxide synthases: regulation and function. Eur Heart J 33:829–837. https://doi.org/10.1093/eurheartj/ehr304
33. Das KK, Das SNDS (2010) Nickel: molecular diversity, application, essentiality and toxicity in human health. In: Biometals: molecular structures, binding properties and applications. Nova Science Publishers Inc, pp 33–58
34. Detmar M, Brown LF, Berse B et al (1997) Hypoxia regulates the expression of vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) and its receptors in human skin. J Invest Dermatol 108:263–268. https://doi.org/10.1111/1523-1747.ep12286453
35. Hattiwale SH, Saha S, Yendigeri SM et al (2013) Protective effect of L-ascorbic acid on nickel induced pulmonary nitrosative stress in male albino rats. Biometals 26:329–336. https://doi.org/10.1007/s10534-013-9617-3
36. Pautz A, Art J, Hahn S et al (2010) Regulation of the expression of inducible nitric oxide synthase. Nitric Oxide 23:75–93. https://doi.org/10.1016/j.niox.2010.04.007
37. Das KK, Honnutagi R, Mullur L et al (2019) Heavy metals and low-oxygen microenvironment—its impact on liver metabolism and dietary supplementation. In: Dietary interventions in liver disease. Elsevier, pp 315–332

AUTHOR QUERY

AUTHOR PLEASE ANSWER QUERY.

Q1. Please check if the affiliations are presented correctly. Amend if necessary.