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Zinc and ascorbic acid treatment alleviates systemic inflammation and gastrointestinal and renal oxidative stress induced by sodium azide in rats

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Abstract

Background: Sodium azide (NaN_3) is a chemical of rapidly increasing economic importance but with high toxic attributes. In this study, the effects of zinc (Zn) and ascorbic acid (AsA) supplementation on sodium azide (NaN_3)-induced toxicity in the stomach, colon and kidneys were evaluated in Wistar rats. Twenty-eight rats were randomly allocated to four experimental groups as follows: group A (control) given distilled water only; group B (NaN_3 only, 20 mg/kg); group C (NaN_3 + zinc sulphate, $ZnSO_4$ 80 mg/kg); and group D (NaN_3 + AsA 200 mg/kg).

Results: NaN_3 was found to significantly ($p < 0.05$) induce increases in serum nitric oxide (NO), advanced oxidation protein products (AOPP), myeloperoxidase (MPO) and total protein levels, along with significant ($p < 0.05$) increase in gastric, colonic and renal malondialdehyde (MDA) and protein carbonyl (PCO) levels. In addition, NaN_3 induced significant ($p < 0.05$) reduction in superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione S-transferase (GST) activities in the colon and kidneys. Treatment with Zn or AsA caused significant ($p < 0.05$) reduction in serum levels of oxidative and inflammatory markers, as well as tissue PCO and MDA levels. Moreover, co-treatment with Zn or AsA significantly ($p < 0.05$) restored colonic and renal levels of antioxidant enzymes, reduced glutathione and protein thiols.

Conclusions: This study shows that Zn or AsA supplementation alleviated NaN_3 toxicity by suppressing systemic inflammation and preventing oxidative damage in the stomach, colon and kidneys of rats.

Keywords: Sodium azide, Zinc, Vitamin C, Oxidative stress, Stomach, Colon, Kidneys

1 Background

Sodium azide (NaN_3) is a crystalline, colorless, odorless and water-soluble solid commonly employed for use as preservative in aqueous laboratory reagents and as a gas generating component of automobile airbag systems and airplane ejection chutes [1]. The chemical may potentially exhibit an explosion hazard with emission of toxic fumes when exposed to heat [2]. It may also be converted to hydrazoic acid, a toxic gas, when it comes in contact with acid or solid metals such as lead or copper [3]. NaN_3 is extremely toxic to humans and can be taken up by inhalation or ingestion (accidental or suicidal), with hospital and laboratory workers at high risk of exposure [4]. Its use in automobile airbags has increased the potential for direct human exposure to the chemical via operations involving transportation, manufacturing, assembly and repair. Symptoms of sodium azide exposure include hypotension, dyspnea, headache, dizziness, nausea, vomiting and diarrhoea, which may progress into more fatal toxic effects such as seizure, coma and cardiopulmonary failure [1]. In recent times, NaN_3 has also been incorporated into herbicides,

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insecticides and pesticides, thereby constituting a potential environmental hazard to grazing animals as well as humans through contamination of groundwater and freshwater supplies [5].

The toxic effects of NaN_3 exposure arise principally from its ability to selectively inhibit cytochrome oxidase in the mitochondrial electron transport chain with resultant overproduction of reactive oxygen species (ROS) [6]. Consequently, there is a reduction in the ability of cells to utilize oxygen, as well as reduced generation of ATP, leading to cell death. Moreover, excessive ROS production in tissues exposed to NaN_3 may overwhelm endogenous antioxidant defences leading to the depletion of enzymic and non-enzymic antioxidant systems in different tissues [7, 8]. The gastrointestinal tract and kidneys are likely targets of NaN_3 toxicity being sites of contact for the absorption of NaN_3 and excretion of its metabolites, respectively. Injury to these tissues may arise from the direct effects of ROS overproduction, which potentially raises the permeability of the mitochondrial membrane via increased peroxidation of membrane lipids [9].

Advances in nutritional therapeutics have largely recognized the beneficial role of exogenous supplementation with potent antioxidants from dietary sources for the maintenance of cellular integrity and mitigation of endogenous antioxidant depletion during oxidative stress induced by xenobiotics [10]. Zinc (Zn) is an essential trace element with several biological functions, including its widespread role as cofactor for various metalloenzymes [11]. Its antioxidant activity is well recognized as it plays important roles in the scavenging of free radicals [12]. The antioxidant activities of Zn have been attributed to several mechanisms including its involvement in the catalytic action of copper/Zn superoxide dismutase; the stabilization of sulphhydryl groups, as well as the induction of glutamate-cysteine ligase, the rate-limiting enzyme for the de novo synthesis of glutathione [13]. Moreover, Zn exhibits antioxidant actions by facilitating the upregulation of expression of metallothionein, which promotes cellular antioxidant actions by binding redox-active metal ions such as iron, copper, mercury and chromium [14]. The ability of Zn to protect against free radical generation is believed to be important in the protection of cell membrane integrity and may be useful in the maintenance of gastrointestinal epithelial tight junction integrity and barrier function [15].

Ascorbic acid (AsA) is a well-recognized low-molecular-weight antioxidant found in several fruits and vegetables [16]. Ascorbate, the reduced form of vitamin C, is known to prevent cellular oxidative damage by scavenging oxidizing free radicals and harmful ROS such as hydroxyl radicals, hydrogen peroxide and singlet oxygen via donation of the hydrogen of its hydroxyl groups to form a relatively stable ascorbyl-free radical (Asc) [17]. Using such mechanism, AsA directly neutralizes ROS and prevents free radical damage. It also prevents lipid peroxidation via one-electron reduction of lipid hydroperoxyl radicals [18]. The antioxidant activities of AsA are believed to underlie its protective role in the healing and protection of the gastrointestinal tract from injurious insults, such as in peptic ulcer disease [19].

To our knowledge, the potentials of Zn or AsA to protect the gastrointestinal tract and kidneys against NaN_3-induced oxidative damage have not been explored. The present study was therefore designed (i) to evaluate the effect of NaN_3 administration on lipid and protein oxidation, thiol and antioxidant systems, including SOD, GPx and GST in the stomach, colon and kidneys; (ii) to evaluate the systemic oxidative/inflammatory responses to NaN_3 administration by measuring levels of NO, AOPP and the activity of myeloperoxidase (MPO) in the serum; and (iii) to investigate the potential protective roles of Zn and AsA against NaN_3.

2 Methods

2.1 Chemicals
Sodium azide (NaN_3), zinc sulphate, ascorbic acid, reduced glutathione (GSH), thiobarbituric acid (TBA), trichloroacetic acid (TCA), 1, 2-dichloro-4-nitrobenzene (CDNB), xylenol orange, sodium hydroxide, potassium hydroxide, hydrogen peroxide, guanidine hydrochloride, epinephrine 2, 4-dinitrophenylhydrazine, (5, 5´-dithio-bis-(2-nitrobenzoic acid) (DTNB) and 1-chloro-2, 4-dinitrobenzene (CDNB) were supplied from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of the highest purity commercially available.

2.2 Animals and experimental design
A total of 28 Wistar rats (aged 8-10 weeks and weighing between 120-140 g) were used in this study. They were obtained from [BLINDED FOR PEER REVIEW]. The animals were housed in a well-ventilated animal house facility under the same experimental conditions (photoperiod: 12 h light: 12 h dark cycle; room temperature: 24 ± 2 °C), during the acclimatization period (1 week) and the dosing period (9 days). Standard rat pellet and clean tap water were provided ad libitum throughout the duration of the experiments. The animals were randomized into four groups of seven each. The first group (A) received distilled water only and served as the control. The second group (B) received NaN_3 (20 mg/kg BW) by oral gavage. The third group (C) received both NaN_3 and $ZnSO_4$ (80 mg/kg BW by oral gavage), while the fourth group (D) received both NaN_3 and AsA (200 mg/kg BW by oral gavage). All treatments lasted for 9 days; the body weights of the animals were monitored prior to the commencement of dosing and immediately before

euthanasia. Percentage weight change was calculated with the formula: [(Final weight-initial weight)/initial weight] multiplied by 100. The doses of NaN$_3$ [20], ZnSO$_4$ [21] and AsA [22, 23] were selected on the basis of previous studies. The different compounds are all soluble in water and each was dissolved in normal saline as the vehicle for administration. Briefly, stock solutions for NaN$_3$ (10 mg/ml), ZnSO$_4$ (20 mg/ml) and AsA (50 mg/ml) were prepared by carefully measuring the specific amounts of each chemical and dispensing in appropriate volume of saline. All treatments were administered on the basis of individual weights of each rat (dose volumes ranging between 0.3 and 0.5 ml) by individuals that were blinded to the specific compounds and the allocation of animals to the different groups. The experimental protocols were performed in accordance with the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" [24], published by the National Institute of Health as well as institutional guidelines approved for animal care and use in research.

2.3 Biochemical estimations

At the end of the experiment, the rats were weighed and blood was collected from retro-orbital plexus into plain sample bottles and allowed to coagulate for about an hour. The blood samples were centrifuged at 3000 rpm (about 1790 g) for 10 min to separate the serum, which was stored as aliquots in Eppendorf tubes. The rats were euthanized by cervical dislocation and the stomach, colon and kidneys were immediately removed and weighed. The stomach was opened along the greater curvature while the colon was cut open longitudinally along the entire length. The tissues were all rinsed in ice-cold PBS. About 1 g of the stomach, colon and kidney was homogenized in 4 ml of 100 mM potassium phosphate buffer (pH 7.4) and the resulting homogenate was centrifuged at 10,000×g for 15 min at 4 °C in a refrigerated centrifuge. The resulting supernatant and serum were stored at − 20 °C prior to their use for the determination of the various markers of oxidative stress and antioxidant parameters.

2.4 Serum oxidative and inflammatory markers

2.4.1 Nitric oxide levels

Nitric oxide level in serum was measured as the content of nitrite using Griess reagent (consisting of 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulphanilamide and 2.5% phosphoric acid) as described by Olaleye et al. [25]. This method provides an indirect determination of NO involving the spectrophotometric measurement of its stable decomposition product, nitrite (NO_2^-). In brief, 20 μL of serum sample was mixed with 200 μL of Griess reagent and then incubated for 20 min. The absorbance

corresponding to nitrite concentration was measured at 490 nm using a micropipette plate reader. The actual concentrations were determined from a sodium nitrite ($NaNO_2$) standard curve and the nitric oxide content was expressed as μmol/L.

2.4.2 Advanced oxidation protein products

Advanced oxidation protein products (AOPPs) are formed by the reaction of plasma proteins with HOCl and chloramines during oxidative stress. The serum level of AOPP was measured using the method of Kayali et al. [26]. In brief, 0.4 mL of the serum was added to 0.8 mL phosphate buffer (0.1 M; pH 7.4), 0.1 mL potassium iodide (1.16M) and 0.2 mL acetic acid. The absorbance was recorded at 340 nm and the AOPP content was calculated using the extinction co-efficient of 261 cm^{-1}M^{-1}. The results were expressed as μmoles/mg protein.

2.4.3 Myeloperoxidase activity

Myeloperoxidase activity was determined using o-dianisidine as substrate, according to the method of Xia and Zweier [27]. O-dianisidine reacts in the presence of H_2O_2 to form a colored product, which can be estimated colorimetrically. Briefly, 2 ml of an O-dianisidine mixture (consisting of 0.53 mM O-dianisidine prepared in phosphate buffer, pH 6.0 and 0.15 mM H_2O_2) was added to 70 μL of serum. The absorbance of the reaction mixture was read at 0, 30 and 60 s at a wavelength of 450 nm using a microplate reader. Myeloperoxidase activity was calculated and expressed in μmol/min.

2.5 Hydrogen peroxide concentration, lipid peroxidation and protein oxidation

2.5.1 Hydrogen peroxide generation

H_2O_2 concentration in the stomach, colon and kidneys was measured by a colorimetric technique as described by Wolff [28]. Briefly, 50 μL of tissue supernatant was mixed with 2.5 mL of potassium phosphate buffer (0.1 M; pH 7.4), 250 μL ammonium ferrous sulphate, 100 μL sorbitol, 100 μL xylenol orange and 25 μL H_2SO_4. The mixture was thoroughly vortexed, incubated at room temperature for at least 30 min and the absorbance was recorded at 560 nm.

2.5.2 Lipid peroxidation levels

The level of lipid peroxidation in the tissues was measured as the concentration of malondialdehyde (MDA), an end product of lipid peroxidation, according to the method described by Varshney and Kale [29]. In brief, 0.4 mL of tissue supernatant was mixed with 1.6 mL of Tris-KCl buffer and 0.5 mL Trichloroacetic acid (TCA; 30% w/v) to precipitate proteins in the tissue sample. Thereafter, 0.5 mL of Thiobarbituric acid (TBA; 0.75% in 0.2 M HCl, w/v) was added to the mixture. The

mixture was heated in a water bath at 80°C for 45 min, then centrifuged at 1792×g for 15 min. The absorbance of the supernatant was measured at 532 nm and the MDA content was calculated with a molar extinction co-efficient of 1.56×10^5 cm^{-1}M^{-1}.

2.5.3 Protein carbonyl content
The content of protein carbonyls in the tissues were assayed using the method of Reznick and Packer [30]. Briefly, 100 μL of the tissue supernatant was initially incubated with 500 μL of 2, 4-dinitrophenylhydrazine (DNPH; 10 mM w/v in 2 N HCl) for 1 h at room temperature, while vortexing every 15 min. Thereafter, 500 μL of Trichloroacetic acid (TCA; 20 % w/v in distilled water) was added and the mixture was centrifuged at 1792×g at room temperature for 10 min to collect the protein precipitates as pellets. The pellets were washed twice with equal volume of ethyl acetate (v/v). The final precipitate was dissolved in 600 μL of guanidine hydrochloride solution (6 M) and was left to incubate for 15 min at 37 °C. The absorbance of the samples was measured at 370 nm and the protein carbonyl content was calculated based on a molar extinction coefficient of 2.2 $\times 10^4$ cm^{-1}M^{-1}.

2.5.4 Reduced glutathione and protein thiol levels
Reduced glutathione (GSH) content was estimated according to the method Jollow et al. [31] using sulfosalicylic acid (4 %, w/v) as protein precipitating agent and DTNB (5, 5′-dithio-bis-(2-nitrobenzoic acid) which develops a yellow color upon reaction with sulphhydryl groups. The absorbance was measured at 412 nm and GSH content was expressed as μmole per milligramme protein. The total thiol content in the samples was estimated according to the method of Ellman [32].

2.6 Antioxidant enzyme activities
2.6.1 GPx activity
The activity of GPx in the stomach, colon and kidney was assessed by the method of Rotruck et al. [33]. The reaction mixture consisted of 0.5 mL phosphate buffer, 0.1 mL of sodium azide, 0.1 mL of GSH, 0.1 mL of H_2O_2 and 0.5 mL of tissue supernatant, all of which were incubated for 10 min at 37 °C. The reaction was terminated by the addition of 0.5 mL of TCA and the mixture was then centrifuged at 3000 rpm for 5 min. Finally, 2 mL of K_2PHO_4 and 1 ml of DTNB were added to 1 mL of the supernatant and the absorbance was recorded at 412 nm.

2.6.2 GST activity
The ability of GST to catalyse conjugation of glutathione with the GST substrate, 1-chloro-2, 4-dinitrobenzene (CDNB) was assayed according to the method of Habig

et al. [34]. Briefly, 10 μL of tissue supernatant was mixed with 140 μL of phosphate buffer, 10 μL of GSH and 50 μL of CDNB. The absorbance was monitored at 340 nm over 3 min at 30-s intervals.

2.6.3 SOD activity
The activity of SOD was assayed by measuring the inhibition of auto-oxidation of epinephrine the method of Misra and Fridovich [35], with minor modifications. In brief, 30 μL of tissue supernatant was added to 2.5 mL of carbonate buffer (pH 10.2), followed by 300 μL of epinephrine acidified with HCl. The purpose of acidification was to keep epinephrine stable before use. The increase in absorbance at 480 nm was monitored every 30 s for 150 s.

2.6.4 Protein estimation
Protein concentration in the serum, stomach, colon and kidney homogenates was estimated using the Biuret method according to Gornal et al. [36], with bovine serum albumin (BSA) as standard.

2.6.5 Statistical analysis
Data were expressed as mean ± standard deviation and analysed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons among the groups in cases where F was significant. All data were analysed with the GraphPad Prism software (Version 7.00). Differences in mean values were considered statistically significant at p-values < 0.05.

3 Results
3.1 Body and organ weights
Changes in the body weights of the rats, as well as the weights of the stomach, colon and kidneys are presented in Table 1. There were increases in the body weights of all the rats within each group before and after the treatment period, as indicated by the average weight gain in the control group (12.99%), NaN$_3$ group (15.08%), NaN$_3$+Zn group (12.79%) and NaN$_3$+AsA group (16.77%). However, there were no significant differences in the average weight gained among the experimental groups, indicating that the treatments had no influence on the body weight changes in the rats. Similarly, there were no significant changes in the average weights of the stomach, colon or kidney across all the experimental groups.

3.2 Effect of treatments on packed cell volume and haemoglobin concentration
Administration of NaN$_3$ did not produce any significant changes in packed cell volume (PCV) and Hb levels compared with the control (Table 2). However, co-administration of NaN$_3$ with Zn produced a significant

Table 1 Effect of treatments on body, stomach, colon and kidney weights

	Control	NaN$_3$ only	NaN$_3$+Zn	NaN$_3$+AA
Body weight (initial)	129.00 ± 18.81	128.00 ± 14.41	126.00 ± 20.43	131.00 ± 8.22
Body weight (final)	144.00 ± 20.43	146.00 ± 4.18	140.00 ± 16.20	153.00 ± 10.96
Weight gain (%)	12.99	15.08	12.79	16.77
Stomach weight				
Absolute (g)	0.99 ± 0.21	1.05 ± 0.09	0.95 ± 0.11	1.09 ± 0.09
Relative (%)	0.69 ± 0.19	0.72 ± ± 0.08	0.68 ± 0.14	0.71 ± 0.05
Colon weight				
Absolute (g)	1.23 ± 0.28	1.32 ± 0.22	1.30 ± 0.17	1.30 ± 0.09
Relative (%)	0.85 ± 0.24	0.91 ± 0.16	0.93 ± 0.19	0.85 ± 0.09
Kidney weight				
Absolute (g)	1.14 ± 0.14	1.13 ± 0.12	1.09 ± 0.07	1.10 ± 0.18
Relative (%)	0.79 ± 0.17	0.78 ± 0.08	0.78 ± 0.13	0.72 ± 0.14

($p < 0.05$) increase in both PCV and Hb levels, while its co-administration with AsA produced a significant ($p < 0.05$) increase only in PCV levels, when compared with the NaN$_3$ group.

3.3 Effect of treatments on serum oxidative and inflammatory parameters

Treatment with NaN$_3$ caused a significant increase in the levels of total protein ($p < 0.01$), NO ($p < 0.0001$), AOPP ($p < 0.01$) and activity of MPO ($p < 0.0001$) in the serum compared with controls (Fig. 1), indicating a general state of systemic oxidative stress and/or inflammatory process. However, treatment with Zn or AsA prevented the increase in the levels of serum protein, NO, AOPP and activity of MPO induced by NaN$_3$. With Zn treatment, there was a significant reduction in serum NO ($p < 0.0001$), AOPP ($p < 0.01$) levels and MPO ($p < 0.001$) activity when compared with the NaN$_3$ group. Similarly, treatment with AsA significantly reduced serum levels of total protein ($p < 0.05$), NO ($p < 0.0001$), AOPP ($p < 0.0001$) and MPO activity ($p < 0.0001$).

3.4 Effect of treatments on hydrogen peroxide generation, lipid peroxidation and protein oxidation in the stomach, colon and kidney

Changes in the levels of H$_2$O$_2$, PCO and MDA as indicators of ROS production, protein oxidation and lipid peroxidation, respectively, are illustrated in Fig. 2. The levels of H$_2$O$_2$ ($p < 0.0001$ for kidney); PCO ($p < 0.0001$

Table 2 Effect of treatments on packed cell volume (PCV) and haemoglobin concentration (Hb)

	Control	NaN$_3$ only	NaN$_3$+Zn	NaN$_3$+AA
PCV (%)	35.33 ± 0.58	35.50 ± 1.73	39.00 ± 2.00[#]	38.00 ± 1.00[#]
Hb (g dL^{-1})	16.13 ± 0.70	15.34 ± 1.01	16.25 ± 0.78[#]	15.50 ± 2.69

Values are given as mean ± standard deviation for groups of seven rats each.
Significant differences: [#]$p < 0.05$ versus NaN$_3$ group

for stomach; $p < 0.01$ for colon and kidney) and MDA ($p < 0.0001$ for all tissues) in the NaN$_3$ group were significantly higher than those of the control group. However, the data showed that in the group treated with Zn, the levels of H$_2$O$_2$ ($p < 0.05$ for stomach; $p < 0.001$ for colon; $p < 0.0001$ for kidney), PCO ($p < 0.0001$ for stomach; $p < 0.001$ for colon; $p < 0.01$ for kidney) and MDA ($p < 0.0001$ for all tissues) were significantly lower than those of the NaN$_3$ group. In addition, the levels of H$_2$O$_2$ ($p < 0.05$ for colon; $p < 0.0001$ for kidney), PCO ($p < 0.0001$ for stomach; $p < 0.001$ for colon; $p < 0.05$ for kidney) and MDA ($p < 0.0001$ for all tissues) in the group treated with AsA were significantly lower compared with the NaN$_3$ group.

3.5 Effect of treatments on antioxidant enzyme activities in the stomach, colon and kidney

The activities of SOD, GPx and GST in the stomach, colon and kidney in all the groups are shown in Fig. 3. The activity of SOD was significantly increased ($p < 0.01$ for stomach), whereas its activity was significantly reduced ($p < 0.05$ for colon; $p < 0.0001$ for kidney) in the NaN$_3$ group compared with the control group. In similar fashion, GPx activity was significantly higher ($p < 0.0001$ for stomach), but significantly lower ($p < 0.0001$ for colon and kidney) in the NaN$_3$ group when compared with the control group. The activity of GST was significantly lower ($p < 0.05$ for stomach; $p < 0.0001$ for colon; $p < 0.01$ for kidney) in all tissues in the NaN$_3$ group compared with the control group.

The present data indicated that rats treated with Zn in combination with NaN$_3$ exhibited significant improvements in the activities of SOD ($p < 0.05$ for kidney), GPx ($p < 0.001$ for kidney) and GST ($p < 0.05$ for stomach and colon) in some tissues, compared with those treated with NaN$_3$ alone. Furthermore, there was a significant increase in the activities of SOD ($p < 0.05$ for kidney),
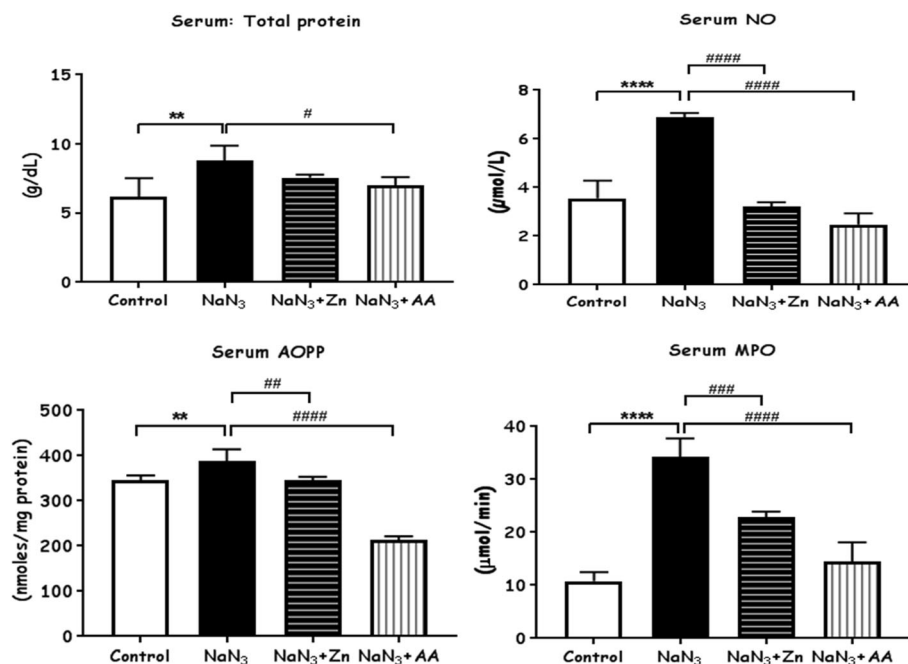
Fig. 1 The effects of Zn sulphate (Zn) and AsA (AA) on serum protein, nitric oxide (NO), advanced oxidation protein products (AOPP) levels and myeloperoxidase (MPO) activity in rats treated with sodium azide (NaN_3). Values are given as mean ± standard deviation ($n = 7$). Significant differences: *NaN_3; NaN_3 + Zn; NaN_3 + AA versus control group (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$). #NaN_3 + Zn; NaN_3 + AA versus NaN_3 group (#$p < 0.05$; ##$p < 0.01$; ###$p < 0.001$; ####$p < 0.0001$)

GPx ($p < 0.0001$ for kidney) and GST ($p < 0.05$ for all tissues) in the group treated with AsA and NaN_3 compared with those treated with $NanN_3$ alone.

3.6 Effect of treatments on reduced glutathione and protein thiol concentrations in the stomach, colon and kidney

The levels of GSH and protein thiols in the stomach, colon and kidneys are presented in Fig. 4. There was a significant reduction in the levels of GSH ($p < 0.01$ for stomach; $p < 0.05$ for colon) and protein thiols in the kidney ($p < 0.05$) of rats exposed to NaN_3 compared with the control group. With Zn treatment, however, there was a significant elevation in the levels of GSH ($p < 0.0001$ for stomach) and protein thiols ($p < 0.01$ for stomach and kidney), compared with the NaN_3 group. Similarly, AsA treatment produced a significant increase in GSH ($p < 0.01$ for kidney; $p < 0.05$ for colon and kidney) in all tissues compared with the NaN_3 group. However, treatment with AsA did not improve protein thiol levels compared with the NaN_3 group.

4 Discussion

There is increasing recognition of the toxicological relevance of NaN_3 as an important environmental and occupational toxicant [20]. However, its toxic effects on gastrointestinal and renal tissues as possible targets of chemical toxicity from NaN_3 exposure have not been well studied [37]. Studies have implicated the inhibition of mitochondrial respiratory chain with excessive production of free radicals, ROS and subsequent oxidative stress as the major mechanism of tissue damage induced by NaN_3 [38]. Therefore, in the present study, we have determined the effects of supplementation of rats with dietary antioxidants, Zn and AsA on NaN_3-induced markers of oxidative injury and antioxidant status in the stomach, colon and kidney, as well as markers of inflammation in serum. To our knowledge, this is the first report evaluating the protective effects of Zn and AsA against NaN_3 toxicity.

Treatment with NaN_3 resulted in elevated gastric, colonic and renal levels of H_2O_2, MDA and protein carbonyls. MDA is a by-product of lipid peroxidation while protein carbonyls are products of irreversible non-enzymatic oxidation of proteins [39, 40]. Specifically, protein carbonylation often results in loss of protein function, including antioxidant enzymes [41]. In the present study, NaN_3 treatment also reduced the tissue activities of antioxidant enzymes (SOD, GPx and GST), levels of GSH and protein thiols, especially in the colon and kidneys. SOD is essential for the conversion of the superoxide radical to H_2O_2, while GPx reduces the cyto-toxicity of H_2O_2 by converting it to water and oxygen [42]. Reduction in the activities of these enzymes may produce further increase in ROS production and
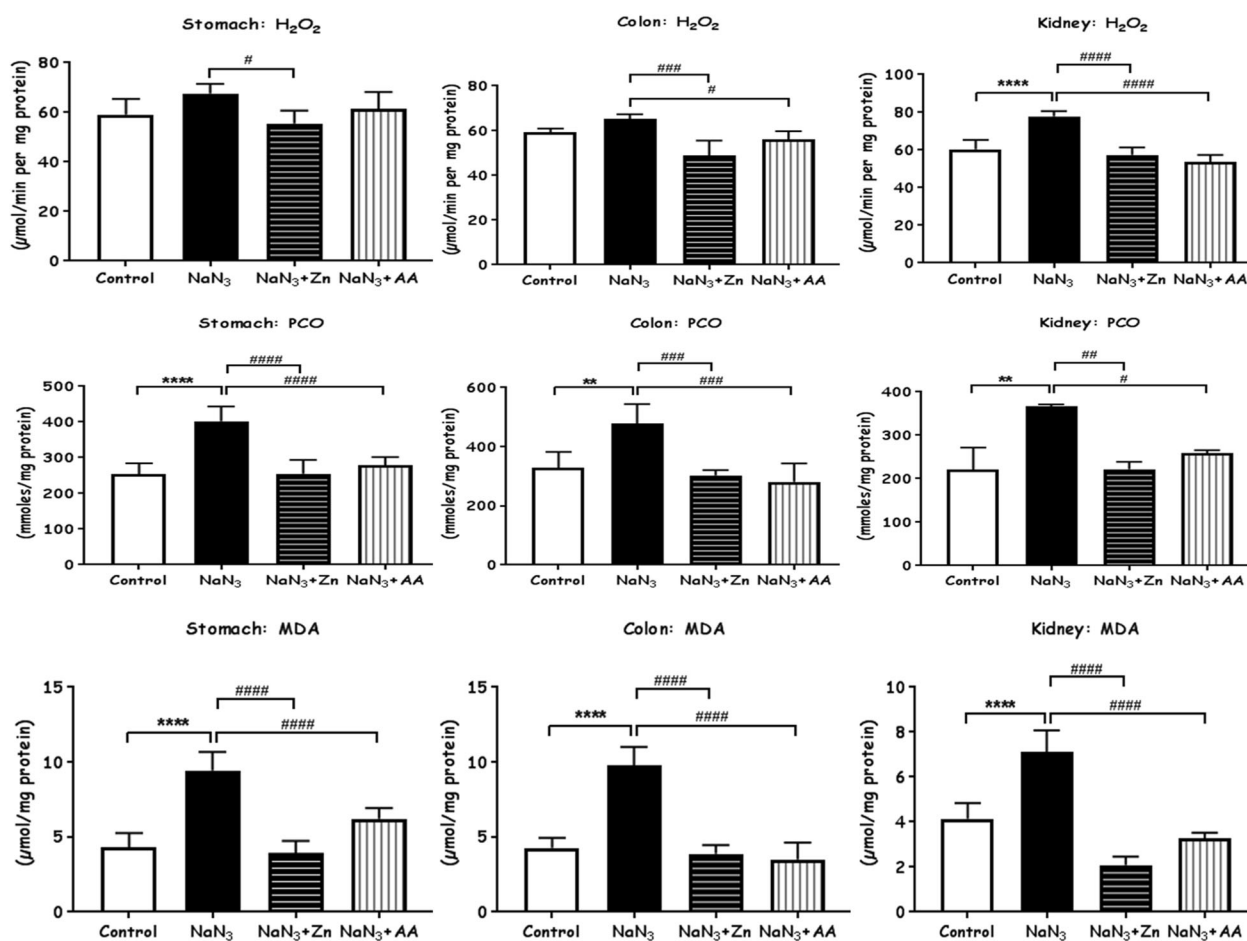
Fig. 2 The effects of Zn sulphate (Zn) and AsA (AA) on hydrogen peroxide (H_2O_2), Protein carbonyl (PCO) and malondialdehyde (MDA) levels in stomach, colon and kidney of rats treated with sodium azide (NaN_3). Values are given as mean ± standard deviation ($n = 7$). Significant differences: *NaN_3; NaN_3 + Zn; NaN_3 + AA versus control group (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$). #NaN_3 + Zn; NaN_3 + AA versus NaN_3 group (#$p < 0.05$; ##$p < 0.01$; ###$p < 0.001$; ####$p < 0.0001$)

aggravation of oxidative stress, disruption of cellular membrane integrity with eventual damage to cells [43].

Furthermore, NaN_3 treatment induced a significant elevation in serum levels of NO and AOPP, along with increased activity of myeloperoxidase (MPO). These responses suggest a more generalized oxidative and inflammatory response, aside from the observed effects on the GIT and kidneys. The enzyme MPO is often released from activated neutrophils, monocytes and tissue macrophages at sites of inflammation. It exerts its activity by utilizing H_2O_2 to oxidize several halides, such as chloride and bromide ions forming hypochlorous and hypobromous acid, respectively, which are important in neutrophil-dependent killing of pathogens [44]. The formation of AOPPs has also been associated with inflammatory and oxidative stress conditions in which plasma proteins, particularly albumin and fibrinogen, tend to react with hypochlorous acid (formed from MPO-catalysed reactions) and chloramines forming various di-tyrosines and carbonyl-containing protein products [45, 46]. Similarly, reactive oxygen/nitrogen species such as NO are known to be produced in large amounts during inflammation [47]. When present in excessive amounts, NO and its derivatives such as peroxynitrite (OONO-) are toxic and can cause tissue damage [48].

The results of the present study showed that supplementation of rats with either Zn or ascorbic significantly prevented the NaN_3 –induced oxidative alterations in GIT and renal tissues, while also reducing systemic inflammation. Specifically, treatment with either Zn or AsA treatment produced a marked reduction in tissue levels of H_2O_2, PCO and MDA, along with a reduction in serum levels of NO, AOPP and MPO. Furthermore, both Zn and AsA produced a significant enhancement of the activities of the antioxidant enzymes SOD, GPx and GST. These results are in agreement with those of Djeffal et al. [23] and Rawi et al. [49], which
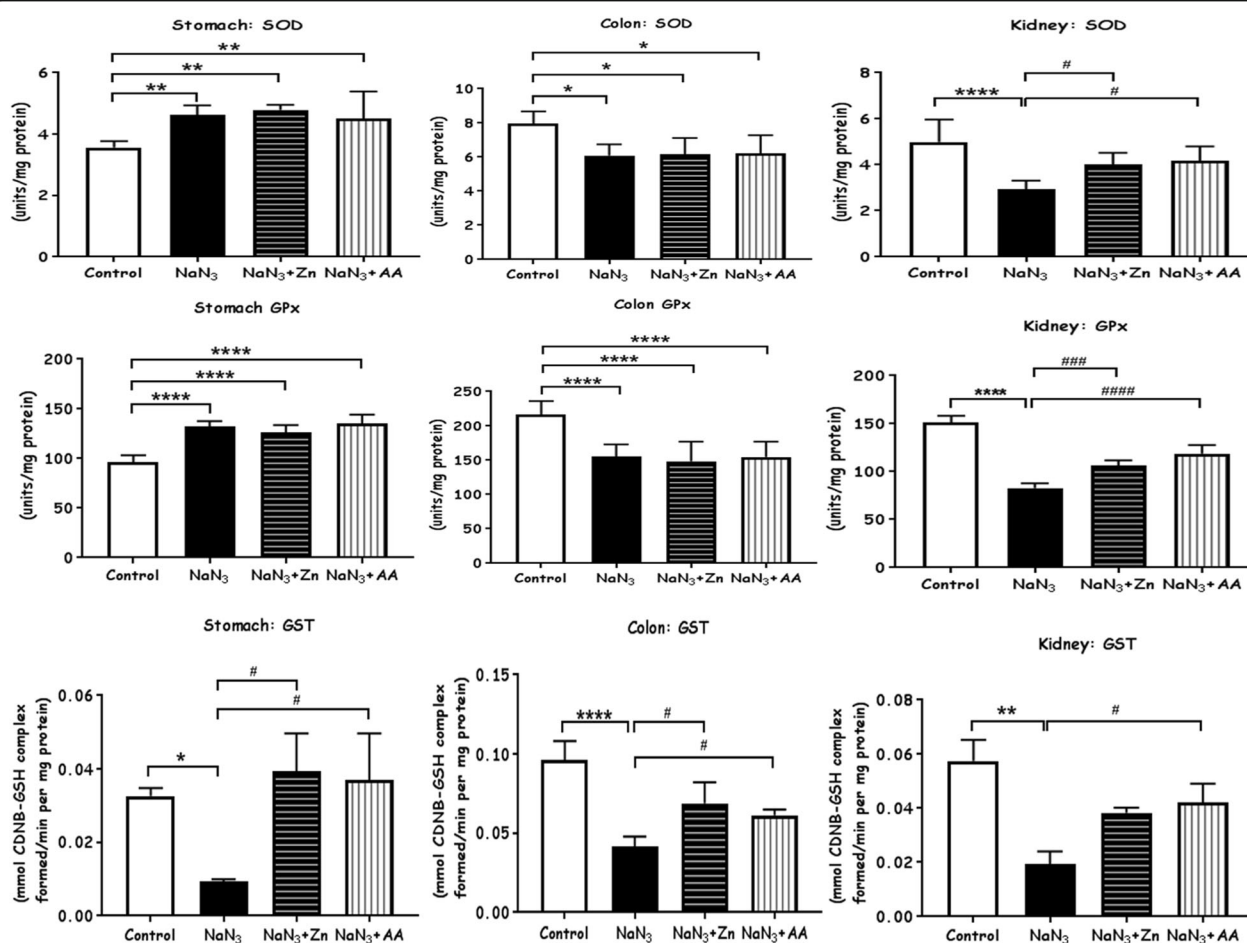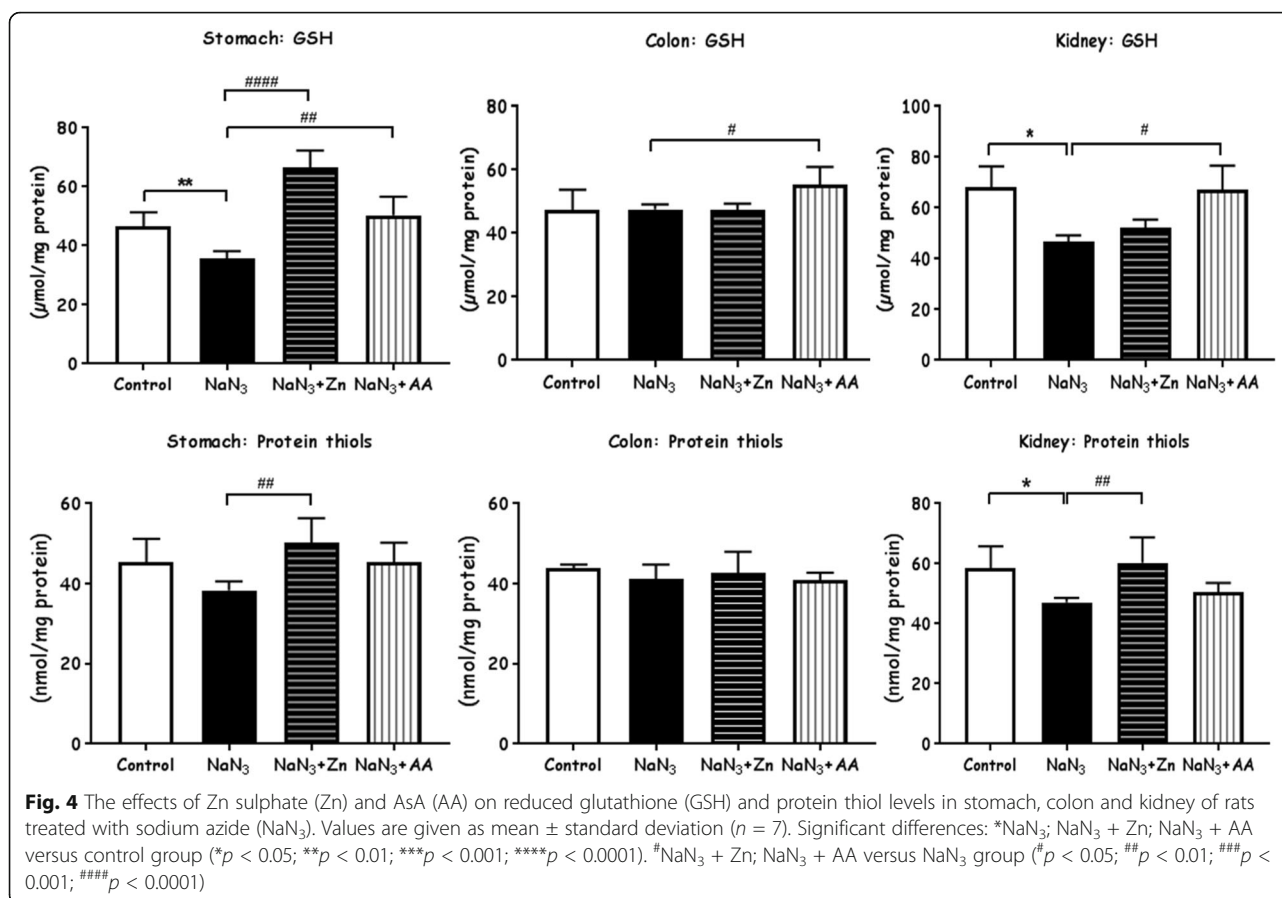
Fig. 3 The effects of Zn sulphate (Zn) and AsA (AA) on the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione S-transferase (GST) in stomach, colon and kidney of rats treated with sodium azide (NaN$_3$). Values are given as mean ± standard deviation (n = 7). Significant differences: *NaN$_3$; NaN$_3$ + Zn; NaN$_3$ + AA versus control group (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$). #NaN$_3$ + Zn; NaN$_3$ + AA versus NaN$_3$ group (#$p < 0.05$; ##$p < 0.01$; ###$p < 0.001$; ####$p < 0.0001$)

demonstrated that Zn and AsA were able to prevent free radical damage as well as stimulate several antioxidant enzymes. The ability of these dietary nutrients to protect against lipid and protein oxidation is attributable to their reported antioxidant activities which may occur via direct scavenging of free radicals or the enhancement of endogenous antioxidants [16, 22]. Zn is an essential trace element which exerts its antioxidant activities by activating the synthesis of antioxidant proteins and enzymes such as GSH and SOD, as obtained in the present study. In addition, Zn is known to reduce the activities of pro-oxidant and pro-inflammatory enzymes such as inducible nitric oxide synthase (iNOS) and NADPH oxidase [50]. This may explain the observation of profound reduction of NO levels in the serum of rats treated with Zn in the present study. Moreover, the antioxidant actions of Zn may also derive from its ability to compete with and displace redox-active metals (e.g. Fe and Cu), from reactions that produce hydroxyl radicals from

H$_2$O$_2$ [51]. AsA is a well-known water-soluble vitamin which, as an antioxidant, has the ability to scavenge water-soluble oxygen and nitrogen free radicals as well as highly reactive by-products of lipid peroxidation, thereby protecting cellular compartments against oxidative damage [52].

Blood parameters such as PCV and Hb are important indicators for the evaluation of the stress of chemical exposures [53]. In this study, NaN$_3$ exposure did not produce any significant changes in PCV and Hb levels, in relation to the control. Similar results were obtained by Agbasi et al. [54], which reported that NaN$_3$ induced no changes in PCV, Hb and RBC values in rats, after 21 days of administration. However, our results indicated that both Zn and AsA protected erythrocyte integrity with significant enhancement in packed cell volume (PCV) and haemoglobin (Hb) concentration, compared with the levels in the control and NaN$_3$-treated rats. The improvement of PCV and Hb following supplementation

Fig. 4 The effects of Zn sulphate (Zn) and AsA (AA) on reduced glutathione (GSH) and protein thiol levels in stomach, colon and kidney of rats treated with sodium azide (NaN$_3$). Values are given as mean ± standard deviation ($n = 7$). Significant differences: *NaN$_3$; NaN$_3$ + Zn; NaN$_3$ + AA versus control group (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$). #NaN$_3$ + Zn; NaN$_3$ + AA versus NaN$_3$ group (#$p < 0.05$; ##$p < 0.01$; ###$p < 0.001$; ####$p < 0.0001$)

with Zn or AsA may be related to their involvement in haemoglobin synthesis [55]. Zn is known to be directly involved in haemoglobin synthesis by influencing the activity of δ-aminolevulinic acid dehydratase (ALAD), an enzyme involved in haem synthesis [56]. AsA, on the other hand, exerts rather indirect roles on haemoglobin synthesis by mobilizing haemosiderin stores of iron and also promoting iron absorption, which promotes haem synthesis [55]. The results of this study are corroborated by findings reported by Rahfiludin and Ginandjar [57] who found that Zn and AsA supplementation resulted in increased Hb and haematocrit levels in human patients.

The present data on body and organ weights indicate that there were no significant differences in the average body weight gain across all the groups, while the weights of the stomach, colon and kidneys also remained unaltered among the various groups. This implies that the increases in body weight could not be attributed to the chemicals administered.

In contrast, Somade et al. [58] reported reductions in brain and lung weights in rats treated with a single intra-peritoneal injection of rats with NaN$_3$ (20 mg/kg). These contrasting observations may reflect differences in susceptibility of various organs to NaN$_3$ toxicity, as well

as the effect of different routes of administration of NaN$_3$ in the two studies. It is noteworthy, however, that all rats in the different experimental groups gained weight over the duration of the experiment, as indicated by increases in the average weights of the groups.

5 Conclusions

From the results of this study, it can be concluded that NaN$_3$ produced severe alterations in the oxidative and inflammatory status of the gastric, colonic and renal tissues of rats. However, supplementation with the dietary nutrients, Zn and AsA, effectively decreased oxidative stress, lipid and protein oxidation and systemic inflammation by mechanisms including the reduction of free radical/ROS generation, direct scavenging of already produced ROS and enhancement of endogenous antioxidant defence. Dietary treatment with either zinc or ascorbic acid may, therefore, be beneficial in alleviating tissue oxidative damage induced by environmental toxicants such as sodium azide.

Abbreviations
NaN$_3$: Sodium azide; AsA: Ascorbic acid; Zn: Zinc; AOPP: Advanced oxidation protein products; PCO: Protein carbonyls; MPO: Myeloperoxidase; NO: Nitric oxide; H$_2$O$_2$: Hydrogen peroxide; GST: Glutathione S-transferase; GPx: Glutathione peroxidase; SOD: Superoxide dismutase; GSH: Reduced

glutathione; TCA: Trichloroacetic acid; TBA: Thiobarbituric acid; DTNB: 5, 5′-Dithio-bis-(2-nitrobenzoic acid); CDNB: 1-Chloro-2, 4-dinitrobenzene (CDNB); DNPH: 2, 4-Dinitrophenylhydrazine; PCV: Packed cell volume; Hb: Haemoglobin concentration; ROS: Reactive oxygen species

Availability of data and materials
All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
In this study, the experimental protocols involving animals were performed in accordance with the criteria outlined in the "Guide for the Care and Use of Laboratory Animals", published by the National Institute of Health as well as guidelines approved by the Animal Care and Use Research Ethics Committee of the University of Ibadan, Nigeria. No specific reference number was assigned.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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