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ORIGINAL PAPER

Silica nanoparticles from melon seed husk improves atherogenic, hematologic and oxidative stress indices in male Sprague Dawley rats exposed to Ni, Al and Ni/Al mixtures

Chidinma P. Anyachor [1], Awolayeofori Dokubo [2], Doris N. Ajibo [3], Baridoo Donatus Dooka [1], Theresa C. Ugwu [4], Danladi C. Husaini [5], Orish E. Orisakwe [1,6]

[1] African Centre of Excellence for Public Health and Toxicological Research (ACE-PUTOR), University of Port Harcourt, Port Harcourt, Nigeria
[2] Department of Biochemistry Faculty of Science, Rivers State University, Port Harcourt, Nigeria
[3] Department of Experimental Pharmacology and Toxicology, Faculty of Pharmacy, University of Port-Harcourt, Port-Harcourt, Nigeria
[4] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Madonna University Elele, Rivers State, Nigeria
[5] Department of Pharmacology and Toxicology, Faculty of Health Sciences, University of Belize, Belmopan, Belize
[6] Advanced Research Centre, European University of Lefke, Lefke, Northern Cyprus, Mersin, Turkey

ABSTRACT

Introduction and aim. With the increased awareness from circular bioeconomy that focuses on 'no waste' generation mantra, various technologies have been developed to valorize these wastes into useful products, including melon seed husk. The aim of the study was to evaluate the effects of silica nanoparticles from melon seed husk (SiNPs MSH) against Ni, Al, and Ni/Al mixture-induced hematotoxicity and lipotoxicity in Sprague Dawley rats.

Material and methods. Fifty-six male Sprague Dawley, 6 to 8 weeks and weighing 220 to 250 g, were randomly allocated to eight groups (n=7). Group 1 received deionized water only (control), groups 2, 3 and 4 (exposed groups) received the Ni/Al mixture, 0.2 mg/kg Ni and 1.0 mg/kg Al, while groups 5 to 8 received the Ni/Al mixture, Ni, and Al plus 100, 200 and 400 mg/kg of SiNPs respectively for 90 days. Blood samples were collected for biochemical investigation.

Results. Ni, Al and Ni/Al groups showed significant ($p<0.05$) alteration ($p<0.05$) in the classic lipid profile, hematological and oxidative stress markers compared to the control. Co-administration with SiNPs did not show significant ($p>0.05$) difference in these parameters compared to the control.

Conclusion. MSH SiNPs reversed Ni, Al, and Ni/Al mixture mediated hemotoxicity and elevated superoxide dismutase, catalase, and glutathione probably via metal chelation.

Keywords. hematotoxicity, lipid profile, nickel and aluminum, oxidative stress, silica nanoparticles

Corresponding author: Orish E. Orisakwe, e-mail: orishebere@gmail.com

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Introduction

The processing of plant food products by the agri-food industry is now deemed useful given their enriched levels of bioactive components such as phenols, peptides, carotenoids, anthocyanins, fatty acids, fibers, and enzymes.[1] These bioactive compounds are often used in the production of functional foods, adjuvants, and drugs against acute and chronic diseases.[2] The presence of complex molecules such as cellulose, hemicelluloses, lignin and antinutritional compounds such as cyanogenic glycosides, oxalates, phytates, and trypsin inhibitors have hindered the utilization of these agro wastes, leading to large scale disposal in the environment and associated pollution.[3] With the increased awareness from circular bioeconomy that focuses on 'no waste' generation mantra, various technologies have been developed to valorize these wastes into useful products, including melon seed husk. Due to growing food waste (FW) issues and related environmental concerns, research into sustainable biotransformation of various types of FW and valorization of various agricultural waste has led to the research of a variety of high value commodities of interest over the past decades.[4] The utilization of melon seeds husk into various commodities of interest is limitless with a rich source of essential amino acids, vitamins and minerals, and fatty acids.[5] The abundance of noxious metals and metalloids in the earth crust, and their non-biodegradability confer on them the status of long environmental persistence from where they reach other environmental matrices naturally or through various anthropogenic activities.[6-8] Environmental pollution by heavy metals is now recognized as a huge toxicological menace.[9,6]

Aim

In view of occupational and daily human exposure to various mixtures of these metals and metalloids, and not just individual elements, the aim of the present study was to evaluate the ameliorative effects of silica nanoparticles from melon seed husk against Ni, Al, and Ni/Al mixture-induced lipotoxicity, atherogenic, hematological, and oxidative stress indices in male Sprague Dawley rats.

Material and methods

Collection of plant materials and chemicals

The melon seed husk was procured from the local market in Abuja, Nigeria and taken to the Department of Plant Science and Biotechnology, University of Port Harcourt for identification. All chemicals and reagents used for this study were analytical grade obtained from reputable companies.

Preparation of melon seed husk ash

The melon husk was washed carefully and several times in running tap water and dried in direct sunlight for six days before further processing. The clean and dried husks were measured before loading. The accurate amount of melon seed husk (2 kg) was subjected to heat treatment by carefully packing them in batches in heat resistant petri dishes in muffle furnace and calcinated at a temperature of 750°C for six hours to produce an off-white ash which was cooled to room temperature to obtain the melon seed husk ash.

Synthesis of silica nano particles from melon seed husk ash

The sol-gel precipitation method was adapted to facilitate the precipitation from silica nanoparticles of the melon seed husk ash with slight modification.[10] The chemical reaction that provided the silica nanoparticles formation was expressed as shown in equations (1) and (2).

$SiO_2 + 2NaOH \quad Na_2SiO_3 + H_2O$ - (1)

$Na_2SiO_3 + H_2SO_4 \quad SiO_2 + NaSO_4 + H_2O$ - (2)

An equivalent sample weight of 5.00 g ash was carefully 0.5 N sodium hydroxide solution in 250 mL volumetric flask. The solution was heated for 30 minutes in a water bath and allowed to cool at room temperature prior to filtration. Hydrochloric acid was added dropwise manner until neutralized. The precipitated silica nanoparticles were carefully washed with double distilled and centrifuged at 5000 rpm for 10 minutes. The supernatant was discarded and pellets were dried in an oven at a temperature of 120° C for 24 hours and re-calcinated at 450° C for 1 hour to yield pure nanosilica. The characterization, particle size analysis of the silica nanoparticle from melon seed husk extract, X-ray (XRD) analytical pattern of the fabricated sample of silica nanoparticle from melon seed, the nano-silica size distribution range (DLS) and transmission electron microscopy of the silica nanoparticle from melon seed husk extract are shown in supplementary files 1, 2 and 3 respectively.

Experimental design

Fifty-six male Sprague Dawley rats free of disease and deformity, aged 6–8 weeks old; weighing 220 to 250 g was used in this study. The animals were randomly assigned to eight groups, each with seven animals, and were acclimatized for two weeks prior to the start of the study. The animals were kept in standard laboratory conditions (a daily light period of 12 h and temperature of 21±2°C) and were provided with *ad libitum* food and water. The experiment was carried out according to the guidelines of the Research Ethics Committee of the University of Port Harcourt. The accepted study protocol was assigned UPH/CEREMAD/REC/MM86/037 as the reference number.

Exposure and establishment of Ni, Al and Ni/Al-induced toxicity

To induce experimental poisoning, aluminum chloride and nickel chloride were administered orally at a dose of

1 mg/kg.[11] and 0.2 mg/kg body weight daily, respectively, for 90 days, respectively, three times a week.[12]

Treatment intervention protocol

The SiNP treatment groups were administered at concentrations of 100, 200 and 400 mg/kg.[13] Labeled low-dose, medium-dose, and high dose for 90 days by oral cannula and treated for 90 days as shown in Table 1.

Table 1. Treatment protocol for experimental animals

S/No.	Groups	Treatment Procedure	Identification
1	Normal control	Deionized water	Control
2	Ni/Al mixtures exposed	0.2 mg/kg Ni+1 mg/kg Al	Ni/Al mixtures
3	Ni only exposed	0.2 mg/kg Ni	Ni
4	Al only exposed	1 mg/kg Al	Al
5	Test 1	0.2mg/kg Ni+1 mg/kg Al +100mg/kg SiNPs	Ni/Al+100 mg/kg SiNPs+
6	Test 2	0.2 mg/kg Ni+1.0 mg/kg Al+200 mg/kg SiNPs	Ni/Al+200 mg/kg SiNPs+
7	Test 3	0.2 mg/kg Ni+1mg/kg Al + 400 mg/kg SiNPs	Ni/Al + 400 mg/kg SiNPs+
8	Test 4 (positive control)	100 mg/kg SiNPs only	100 mg/kg SiNPs

Collection of blood samples for serum biochemistry

After 90 days of treatment, rats in each group were sacrificed under pentobarbital (50 mg/kg IP) anesthesia, blood samples were collected from the abdominal artery in heparinized tubes and centrifuged to obtain plasma from which high-density lipoprotein (HDL-c) and low-density lipoprotein (LDL-c) + very low-density lipoprotein (VLDL-c) fractions were later obtained.[14] Simultaneously, additional blood samples were collected for hemology.[15] These samples were placed in tubes for serum separation, incubated at room temperature for 60–90 min and then centrifuged for 10 minutes.

Lipid profile markers determination

Lipid profile markers (total cholesterol, triglycerides, HDL-c, LDL-c, and VLDL-c) were measured enzymatically in a series of coupled reactions using a biochemical assay kit (Randox, Crumlin, United Kingdom). The enzymatic hydrolysis of cholesterol from its ester form by cholesterol esterase and further oxidation of cholesterol to produce hydrogen peroxide as a byproduct. Triglycerides are hydrolyzed to produce glycerol and oxidized using glycerol oxidase. Hydrogen peroxide (H_2O_2), the reaction by product was quantitatively measured at 500 nm.

Determination of atherosclerotic cardiovascular disease risk

Plasma atherogenic index, atherogenic coefficient, cardio risk ratio (Castelli's Index I and II), cardio protective index and small dense low density lipoprotein particles were used to estimate the estimation of atherosclerotic cardiovascular disease. AIP was calculated using equation (1), AC (2), CRI-I and II (3) and (4), cardio protective (5) and sdLDL-c (6) and sdLDL-c (5).

$$\text{Atherogenic index of plasma (AIP)} = Log\ \frac{TG}{HDL-c} \quad \text{- (1)}$$

$$\text{Atherogenic Coefficient (AC)} = \frac{(TC-HDL-c)}{HDL-c} \quad \text{- (2)}$$

$$\text{Castelli's risk Index (CRI-I)} = \frac{TC}{HDL-c} \quad \text{- (3)}$$

$$\text{Castelli's risk index (CRI- II)} = \frac{LDL-C}{HDL-c} \quad \text{- (4)}$$

$$\text{Cardio protective Index (CPI)} = \frac{sdLDL-C}{lbLDL-c} \quad \text{- (5)}$$

$$\text{sdLDL-c mg/dL} = 0.580\ (\text{non–HDL-C}) + 0.407\ (\text{dLDL-C}) - 0.719(\text{CLDL-c}) - 12.05 \quad \text{- (6)}$$

[non HDL-c=TC-HDL-c, large buoyant LDL-c (lbLDL-c)=LDL-c-sdLDL-c, and calculated LDL-c (CLDL-c)=TC-HDL-c- TG/5]

Oxidative stress markers

The lipid peroxidation which is marked by malondialdehyde (MDA), is evaluated by thiobarbituric acid reactive substances at 532 nm wavelength.[16] Nitric oxide (NO) was estimated at 540 nm wavelength.[17] Superoxide dismutase (SOD) activity was estimated by the rate of inhibition of pyrogallol auto-oxidation obtained by reporting the changes in absorbance, for 3 min (at 30 s intervals), at 420 nm.[18] Glutathione (GSH) levels and glutathione peroxidase (GPx) activity were determined at 450 nm and 340 nm, respectively.[19,20] A slight modification of technique was used for the catalase (CAT) activity assay, given that tissue catalase cleaves hydrogen peroxide which can be determined at 240 nm using a spectrophotometer.[21]

Statistical analysis

Data were expressed as mean±standard deviation (SD). Microsoft Xlstat 2014 (Microsoft, USA) was used in performing variance analysis and Kruskal-Wallis tests to check whether the concentration of the biomarkers was significantly different between groups. Data analysis involved performing descriptive statistics on metal and biomarkers concentration before ANOVA was used to establish whether there was significant difference in the concentration of heavy metals and biomarkers between groups.). Multivariate analysis was used to determine the level of association between dependent variables and independent variables in this study. All significant differences were at a $p<0.05$.

Results

The effect of SiNPs from melon seed husk MSH on the serum lipid profile

The effect of SiNPs on the serum lipid profile of rats exposed to Ni, Al, and Ni/Al mixtures is presented in Figure 1. Classic lipid parameters in groups exposed to Ni, Al, and Ni/Al mixtures showed significant ($p<0.05$) increase in the concentrations of TC, TG, LDL-c and VLDL-c, while significant ($p<0.05$) decrease in HDL-c concentration was observed compared to the control. The group co-administered with SiNP and Ni/Al mixtures (Ni/Al mixtures+100mg/kg SiNPs, Ni/Al mixtures+200 mg/kg SiNPs and Ni/Al mixtures +400 mg/kg SiNPs), showed no significant ($p>0.05$) difference in TC compared to the control, while significant ($p<0.05$) difference ($p <0.05$) was observed compared to the Ni, Al and Ni/Al exposed groups. There was a significant ($p<0.05$) decrease ($p <0.05$) in TG in the SiNP 100 mg/kg group compared to the control group. HDL-c values showed a significant ($p<0.05$) increase in test groups treated with Ni/Al mixture + 200 mg/kg SiNP and Ni/Al mixture + 400 mg/kg SiNP and were comparable to control. LDL-c showed a significant ($p<0.05$) decrease ($p <0.05$) in the Ni/Al mixture + 200 mg/kg SiNP, the Ni/Al mixture + 400 200 mg/kg SiNP and the Ni/Al mixture + 100 mg/kg SiNP compared to the exposed groups Ni, Al and Ni/Al. However, the group treated with LDL-cin 100 mg/kg of SiNP was significantly ($p<0.05$) different from the control. VLDL-c in all test groups treated with SiNP showed significant ($p<0.05$) decrease ($p <0.05$) compared to the Ni, Al and Ni+Al exposed groups.

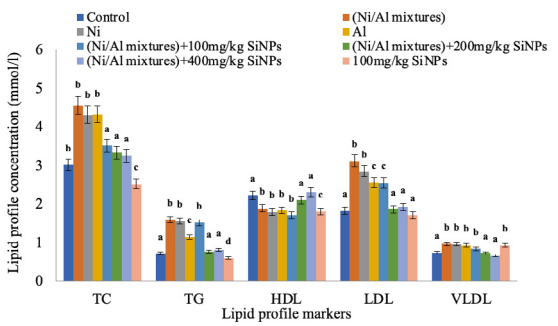

Fig. 1. Effect of SiNP from melon seed husk on classic lipid profile of rats exposed to Ni, Al and Ni/Al mixture (TC total cholesterol, LDL-c – low-density lipoprotein cholesterol HDL-c high-density lipoprotein cholesterol, VLDL-c very low-density lipoprotein cholesterol, TG triglyceride, values are expressed as mean±SD, superscripts with different letters are significantly different

The effect of SiNP on cardiovascular risk indices of rats exposed to Ni, Al and Ni/Al mixtures showed that AIP values ranged from -11.64 to -0.05 with an average value of -0.28 while sdLDL-c values range from -11.64 to -10.36 with an average value of -11.01 (mmol/L); however, risk values for atherosclerosis indicated low for exposed and treated groups, Table 2.

Table 2. The effect of SiNPs on the cardiovascular atherogenic risk indices of rats exposed to Ni, Al, and Ni/Al mixture *

Groups	AIP	AC	CRI-I	CRI-II	lbLDL-c	sdLDL-c	CPI
Control	-0.50	0.37	1.37	0.83	13.14	-11.32	-0.86
Ni/Al mixture	-0.08	1.42	2.42	1.65	14.04	-10.93	-0.78
Ni	-0.06	1.41	2.41	1.60	13.86	-11.02	-0.78
Al	-0.21	1.36	2.36	1.40	13.74	-11.19	-0.82
Ni/Al mixture+ 100mg/kg SiNPs	-0.05	1.06	2.06	1.48	13.57	-11.06	-0.82
Ni/Al mixture+ 200 mg/kg SiNPs	-0.44	0.58	1.58	0.88	13.21	-11.35	-0.86
Ni/Al mixture+ 400 mg/kg	-0.44	0.48	1.48	0.87	13.22	-11.30	-0.85
100 mg/kg SiNPs	-0.50	0.38	1.38	0.94	13.08	-11.37	-0.87

* AIP atherogenic index of plasma, AC atherogenic coefficient, CRI castelli risk index, lbLDL-c – large buoyant lipoprotein cholesterol, sdLDL-c, small dense lipoprotein cholesterol, CPI, cardioprotective index

Table 3. Multivariate regression analysis showing the association sdLDL-c with the classic lipid parameters model 1*

Parameter	β	*p*
Intercept	-12.05	3.72E-47
TC	-0.139	5.18E-43
TG	0.1438	3.26E-42
HDL-c	0.139	1.9E-42
LDL-c	0.407	2.82E-43

* values are significant, $p<0.05$, β – regression coefficient

Table 4. Multivariate regression analysis showing the association of sdLDL-c with cardiovascular atherogenic risk indices model 2*

Parameters	β	*p*
Intercept	-21.13	0.000114
AIP	0.58	0.003024
AC	-0.28	0.063182
Non-HDL-c	-0.13	0.119931
lbLDL	0.78	0.000972

* values are significant, $p<0.05$, β – regression coefficient

Multiple regression analysis performed with sdLDL-c concentration as a dependent variable and TC, TG, HDL-c and LDL-c as independent variables, Table 3. This stepwise regression analysis was identified as (model 1) and significant variables ($p<0.05$; $R2=1$) and the standard error of the estimate as ($4.61x10^{-16}$ mmol/L). The best fit of the linear regression equation was as follows:

$$y=-12.05 -0.14TC+0.14TG+0.14HDL-c+0.407 LDL-c \quad - (1)$$

Multiple regression analysis with the concentration of sdLDL-c as the dependent variable AIP, AC, Non-HDL, and lbLDL-c as independent variables was performed Table 4. This stepwise regression analysis was identified as (model 2) and significant variables (p<0.001; R^2=0.99) and the standard error of the estimate was 0.0083 mmol/L. The best fit of the linear regression equation was as follows:

y=-21.13 +0.58AI-0.28AC-0.133NonHDL-c+0.78lb

Table 5. Effect of SiNPs from melon seed husk on the hematological profile of rats exposed to the Ni, Al and Ni/Al mixture*

Groups	PCV	WBC	RBC	Hb	Platelet
Control	48.00±5.66^{a}	16.95±3.46^{a}	8.35±0.21^{a}	15.65±1.48^{a}	726.50±45.96^{a}
Ni/Al mixture	25.50±2.12^{b}	22.60±3.39^{b}	4.25±0.07^{b}	11.25±0.21^{b}	513.00±59.40^{b}
Ni	25.50±4.95^{b}	22.55±0.78^{b}	3.65±0.49^{b}	10.85±0.21^{b}	531.50±28.99^{b}
Al	28.00±5.78^{b}	20.60±2.26^{b}	3.40±0.42^{b}	10.75±0.35^{b}	517.00±63.64^{b}
Ni/Al mixture + 100 mg/kg SiNPs	48.50±2.12^{a}	13.70±3.96^{ac}	8.15±0.21^{a}	16.00±0.57^{a}	640.00±32.53^{ab}
Ni/Al mixture + 200 mg/kg SiNPs	48.50±2.12^{a}	16.00±4.53^{a}	8.00±0.85^{a}	15.25±0.92^{a}	682.00±77.78^{ab}
Ni/Al mixture + 400 mg/kg SiNPs	50.50±2.12^{a}	15.s75±4.67^{a}	8.65±0.07^{a}	16.55±1.34^{a}	705.00±84.85^{a}
100 mg/kg SiNPs	48.50±4.95^{a}	11.35±1.34^{c}	8.30±0.00^{a}	14.85±0.78^{a}	627.00±56.57^{ab}

***** White blood cell, PCV – packed cell volume PCV, hemoglobin Hb, red blood cell, P – platelet, values expressed as mean±SD, superscripts with different letters are significantly different

Table 6. Effect of SiNPs from melon seed husk on the hematological profile of rats exposed to the Ni, Al and Ni/Al mixture*

Groups	N	L	M	E	MCV	MCH	MCHC
Control	13.00 ±2.83^{a}	82.00 ±0.71^{a}	2.00 ±1.41^{a}	1.00 ±0.00^{a}	57.10 ±5.23^{a}	18.65 ±1.20^{a}	32.75 ±0.92^{a}
Ni/Al mixture	23.50 ±2.83^{b}	71.00 ±4.24^{b}	3.50 ±0.71^{b}	2.00 ±1.41^{b}	52.30 ±3.54^{b}	17.90 ±0.99^{b}	30.55 ±0.35^{b}
Ni	22.50 ±3.54^{b}	72.00 ±5.65^{b}	3.00 ±1.41^{b}	2.50 ±0.71^{b}	51.15 ±1.77^{b}	17.40 ±0.14^{b}	30.25 ±1.20^{b}
Al	24.50 ±2.12^{b}	70.50 ±3.54^{b}	3.50 ±0.71^{b}	1.50 ±0.71^{a}	53.10 ±2.97^{b}	16.10 ±0.85^{b}	29.95 ±0.21^{b}
Ni/Al mixture + 100 mg/kg SiNPs	15.50 ±0.71^{a}	80.50 ±4.24^{a}	2.50 ±2.12^{a}	1.50 ±0.71^{a}	61.30 ±4.10^{a}	19.00 ±1.56^{a}	32.70 ±0.28^{a}
Ni/Al mixture + 200 mg/kg SiNPs	14.00 ±4.24^{a}	81.50 ±4.95^{a}	2.00 ±0.00^{a}	1.50 ±0.71^{a}	58.00 ±5.66^{a}	18.40 ±1.13^{a}	31.70 ±1.27^{a}
Ni/Al mixture + 400 mg/kg SiNPs	16.50 ±3.54^{a}	80.50 ±4.24^{a}	1.00 ±0.00^{a}	1.00 ±0.00^{a}	57.90 ±5.09^{a}	18.20 ±0.28^{a}	33.50 ±2.26^{a}
100 mg/kg SiNPs	13.00 ±1.41^{a}	84.00 ±2.83^{a}	2.00 ±1.41^{a}	1.00 ±0.00^{a}	57.00 ±0.42^{a}	18.30 ±0.28^{a}	33.80 ±1.41^{a}

* N neutrophils, L – lymphocytes, M monocytes, E – eosinophils, MCV – mean corpuscular volume, mean corpuscular hemoglobin, MCHC – mean corpuscular hemoglobin concentration of MCHC, values are expressed as mean±SD, superscripts with different letters are significantly different

The effect of SiNPs on the hematological profile of rats exposed to Ni, Al, and Ni/Al mixtures

The effect of SiNPs on the hematologic profile of rats exposure to Ni, Al, and Ni/Al mixtures is presented in Tables 5 and 6.

Table 7. Percentage change in the hematological profile of rats exposed to Ni/Al mixtures, Ni and Al with respect to the control, test groups co-administrated with SiNP*

Groups	PCV	WBC	RBC	Hb	Platelets	N	L	M	E	MCV	MCH	MCHC
Control	46.44	-44.66	54.44	31.28	22.96	-53.26	11.96	-81.82	-50	11.65	7.55	7.30
CombSiNPs	45.14	-29.31	54.89	30.03	28.36	-80.77	13.21	-66.67	-100	8.61	8.13	7.63
SiNPs only	45.70	-93.11	54.62	26.26	16.99	-80.77	15.28	-66.67	-100	8.45	6.37	10.53

* test groups co-administered with SiNP (CombSiNP) (HMM + 100 mg/kg SiNP, HMM + 200 mg/kg SiNP, HMM + 400 mg/kg SiNP) and SiNPs only (100 mg/kg SiNP), PCV packed cell volume, WBC – white blood cell, RBC red blood cell, N – neutrophils, L lymphocytes, M – monocytes, E eosinophils, MCV – mean corpuscular volume, MCH mean corpuscular hemoglobin, MCHC mean corpuscular haemoglobin concentration

There were significant (p<0.05) changes (p <0.05) in all measured hematological parameters in the groups exposed to Ni, Al and Ni/Al mixtures compared to the control and test groups coadministered with SiNPs. The WBC values in the Ni/Al mixture + 100 mg/kg SiNP and 100 mg/kg SiNP, platelets (Ni/Al mixture + 100 mg/kg SiNP, Ni/Al mixture + 200 mg/kg SiNP and 100 mg/kg SiNP only) groups decreased significantly (p<0.05) in comparison to the control. In Ni/Al mixtures, Ni and Al exposed groups Lymphocytes (L), MCV, MCH and MCHC had a significant (p<0.05) decrease (p <0.05) while neutrophils (N), monocytes (M) and eosinophils (E) significant (p<0.05) increased significantly (p <0.05) compared to the control. The average % reduction was observed to be in the following order: 54.9% (RBC)<-45% (PCV)<-30.1% (Hb)< 28% (platelets)<13.21% (lymphocytes)<8.6% (MCV) < 8.13% (MCH) < 7.6% (MCHC). While the average percentage elevation was observed to be in the following order and +16.04% (E) <29.10% (WBC) <66.67 %(M) < 80.76% (N) with respect to the control. The test groups had an average increase in WBC to be 60.0% (Ni/Al mixture+100 mg/kg SiNP) and 93.1% (100 mg/kg SiNPs) while platelets had an average of 59.6 % for the test groups (Ni/Al mixture + 100 mg/kg SiNP, Ni/Al mixture + 200 mg/kg SiNP and 100 mg/kg SiNP) respectively with respect to the metals and Ni/Al, Ni and Al only exposed groups. Coadministration of SiNPs caused significant (p<0.05) increase in PCV, RBC, Hb and platelet counts, while significant (p<0.05) reduction comparable to the control was observed for WBC, N, M and E. The percentage reduction for WBC was observed for -29.31 which was the three times the value obtained for the SiNPs only treated

group (-93.11) and 1.5 times the control group (-44.66), respectively. The E% reduction was twice the value. The value of N was over 60 % reduction. The % increase for PCV and RBC were greater than 40%, Hb and platelets greater than 20%, L, MCV, MCH and MHCH were greater than 5% as shown in Table 7.

The effect of SiNPs on the oxidative stress markers in the blood of rats exposed to Ni, Al, and Ni/Al mixtures

The effect of SiNPs on oxidative stress markers in the blood of rats exposed to Ni, Al, and Ni/Al mixtures is presented in Figure 2.

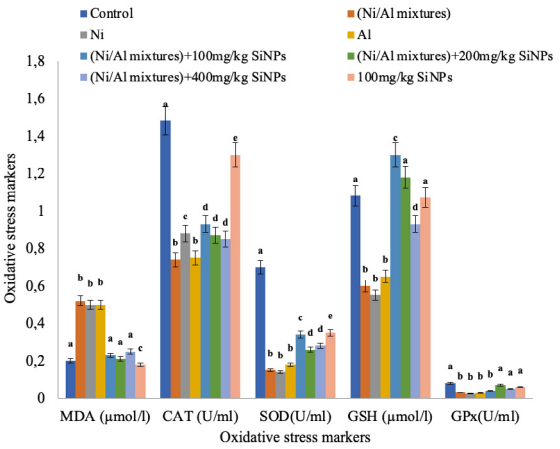

Fig. 2. SiNP treatment on oxidative stress markers in rats exposed to Ni, Al and Ni/Al mixture, values are expressed as mean±SD, superscripts with different letters are significantly different

In the Ni, Al and Ni/Al mixture exposed groups, there was a significant ($p<0.05$) increase ($p<0.05$) in MDA levels compared to the control and the groups co-administration with SiNP. CAT, SOD, GPx, and GSH concentrations decreased significantly ($p<0.05$) in groups exposed to Ni, Al and Ni/Al mixture only compared to control and test groups co administered with SiNP. Coadministration of Ni, Al, Ni/Al mixture groups with SiNPs showed remarkable reduction in MDA levels and increase in SOD, CAT, and GPx.

Discussion

Nickel (Ni) is a known environmental toxicant that can be found in various sources such as air pollution, industrial emissions, and tobacco smoke.[22-25] On the other hand, Al is a widely used element and can be found in various food additives, medications, and cosmetic products.[26-28] There has been a surge in public health concerns regarding its potential impact on human health, including disruption of normal functioning of lipid metabolism, the driving force behind progression of cardiovascular disease (CVD) hematotoxicity, and induction of oxidative stress markers.[29]

Previous studies have evaluated the effect of Ni and Al on their potential effects on lipid metabolism, including their impact on LDL, VLDL, TC, and TG levels in rats.[30,31] In this study, rats exposed to Ni, Al, and Ni/Al mixtures had significant increase in TC, TG, LDL-c, and VLDL-c while the HDL-c showed a significant decrease. These observations may be linked to interference in the synthesis and metabolism of lipoproteins that are responsible for transporting cholesterol and other lipids throughout the body.[32,33] Ni-induced lipotoxicity may involve interference with enzymes that play a critical role in lipid synthesis and metabolism.[34] Ni exposure has been linked to inhibition of major enzymes such as acetyl-CoA carboxylases and fatty acid synthases, essentially required for the synthesis of triglyceride and cholesterol.[35] The mechanisms behind Al effects on the lipid profile may also be attributed to interference with key enzymes involved in lipid metabolism and transport, as well as expression of genes responsible for lipid metabolism.[36,37] In the test group co-administration with SiNPs, the lipid profile markers were similar to the control. HDL-c is regarded as a good cholesterol crucial for the transportation of cholesterol from peripheral tissues to the liver for metabolism. The specific effects of Ni and Al on HDL-c may have occurred through reverse cholesterol transport.

Lipid oxidation emanates from an abnormal lipid profile, since increased LDL-c levels are deemed the main contributor to atherosclerosis.[38] Elevated plasma levels of LDL and VLDL-c seen in noncommunicable diseases NCDS like hypertension and obesity, are now known as the primary health issues globally.[39] The different ratios or combinations of these lipid profiles are vital tools used in the prediction and detection of high-risk individuals than just the lipid parameters alone.[38] There are three ratios of lipid profile indices namely atherogenic coefficient (AC), atherogenic index of plasma (AIP), and Castelli's risk indices that employed as markers of lipid atherogenic risk (CRIs). Calculated lipid fraction ratios have tended to replace the more conventional lipid profile parameters in clinical settings in the assessment of cardiovascular risk since the atherogenic index specifies the degree of the probable rate of atherosclerosis as a marker of CVD.[40,41]

Changes in lipid metabolism can significantly affect cardiovascular health and increase the risk of developing heart disease.[42,43] In this study, an attempt to better characterize the atherogenic potential of the measured lipid profile, the following parameters as indices for cardiovascular atherogenic risk markers were integrated. AIP, AC, cardiorisk ratio index (CRR-I), cardio-protective index (CPI), high buoyant lipoprotein cholesterol (lbLDL-c), and small dense lipoprotein cholesterol (sdLDL-c). The indices suggested a low risk of atherosclerosis with classification values that fall within the range of -0.3 to 0.1 (low risk), 0.1 to 0.24 (moderate) and >0.24 (high) for AIP.[44,45]

Generally, studies have shown that heavy metals are associated with increased atherogenic indices.[46-48] However, some can also lead to a lower AIP. An AC value greater than 3 is considered an abnormal value of cardiovascular risk.[44] The values computed for the exposed and treated groups were lower than the low 3 and reflect the atherogenic potential of the entire lipoprotein fraction to be low. Castelli risk indices CRI-I and CRI-II, CPI are both atherogenic and protective lipid fractions to evaluate cardio atherogenic disease (CAD).[49] However, the lipid indices obtained for these markers were below the reference limit of (>4 and >3, respectively).[50] sdLD-cL has been shown to be more atherogenic than lbLDL-c to promote the formation of atherosclerotic plaques, sdLDL-c provides longer time and more chances of its penetration into the subendothelial space.[50] sdLDL-c is more sensitive to oxidation; stronger binding ability to proteoglycans and glucosamines located in the endothelium lining.[51-53] Decrease in the sdLDL-c may be attributed to rapid inhibition of oxidative modification of sdLDL-c in the treated group.[51] This can prevent accumulation of oxidized LDL-c that are associated with plaque instability in coronary and carotid artery disease and other metabolic syndrome.[52] Furthermore, elevated sdLDL-c is generally accompanied with reduced HDL-c and elevated levels of TG.[54] There was a significant correlation and association of classic lipid markers and atherogenicity in Ni/Al exposed groups and reduced levels of atherogenic lipids and a lower AIP in SiNPs.

Ni and Al can exert a negative impact on the hematological profile of humans. Several studies have reported the influence of these elements on the hematopoetic system through different routes of exposure.[55-58] In this study, rats exposed to Ni, Al, Ni/Al mixture had significant alterations in the hematologic profile. Elevated exposure to Ni is associated with potential hemolysis.[59-61] Al exposure impacts red blood cell production and disrupts iron homeostasis, absorption, transportation, and iron utilization, ultimately affecting packed cell volume levels in rats.[62-64] Rats exposed to Ni, Al, and Ni/Al and Ni/Al mixtures had significant increase in neutrophils, monocytes, and eosinophils compared to control, as well as decreased platelet counts that can further lead to platelet dysfunction and impairment.[65-67] Co administration of SiNPs tended to attenuate hemolysis. Nutraceuticals are known to ameliorate Ni and Al mediate hematotoxicity in rats.[68-70] SiNPs may contain antioxidants and metal chelating bioactive compounds.[71-73] SiNPs can chelate Ni and Al ions to accelerate fecal elimination and offer at least the benefit of SiNPs in the present study.[74] Ni and Al exposure can trigger inflammation and hematotoxicity that can be ameliorated by SiNPs treatment. [52,71]

MDA is considered a marker of lipid peroxidation. Other studies have shown that Ni and Al exposure is accompanied by elevated MDA levels.[75-77] In this study there was a significant decrease in CAT, SOD, GPx and GSH) and increased MDA after exposure to Ni/Al, Ni, Al. Ni exposure decreases the activity of antioxidants in tissues just like Al exposure.[74-82]

Conclusion

The toxicity of the Ni, Al and Ni/Al binary mixtures can cause a decrease in the HDL-c and increase in TC, TG, LDL-c, and VLDL-c cholesterol in rats, which may be attributed to interference with key enzymes involved in the metabolism and transport as the well as expression of genes responsible for lipid metabolism. Ni, Al Ni/Al mixtures may interfere with hematopoiesis via oxidative stress mechanism.

SiNPs may ameliorate Ni, Al, and Ni/Al-mediated lipotoxicity via antioxidant activity, and metal chelating activity suggesting a beneficial role of SiNPs in attenuating cardiovascular risk. Together, SiNPs from melon seed husk can improve atherogenic, hematological and oxidative stress indices in male Sprague Dawley rats exposed to Ni, Al, and Ni/Al mixture.

Declarations

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Authors' contributions

Conceptualization, O.E.O.; Methodology, C.P.A., A.W., D.N.A., B.D.D. and T.C.U.; Validation, O.E.O.; Formal Analysis, C.P.A., A.W., D.N.A., B.D.D. and T.C.U.; Investigation, C.P.A. and B.D.D.; Data Curation, C.P.A., A.W., D.N.A., B.D.D. and T.C.U.; Writing – Original Draft Preparation, C.P.A., A.W., D.N.A., B.D.D. and T.C.U.; Writing – Review & Editing, A.W., D.N.A., T.C.U. and O.E.O.; Supervision, O.E.O.; Project Administration, O.E.O.

Conflicts of interest

The authors confirm that there were no conflicts of interest.

Data availability

All data have been provided.

Ethics approval

The experiment was carried out according to the guidelines of the Research Ethics Committee of the University of Port Harcourt. The accepted study protocol was assigned UPH/CEREMAD/REC/MM86/037 as the reference number.

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