

Original Article

Evaluation of long-term effects of nickel and benzo [a] anthracene contaminated diets in rats' kidney; mimicking human exposure from food

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Abstract: Objectives: Accumulative effects of heavy metals and polycyclic aromatic hydrocarbon could result in various toxicities. This study evaluated the effects of long-term exposure to low doses of nickel and benzo [a] anthracene on the kidney of rats, simulating human exposure through food. Methods: Thirty-six (36) Male rats weighing between 80-100 g were assigned into six groups of 6 animals each; Group A (normal), Group B1 and B2 (fed nickel contaminated feed for 12 and 24 weeks), Group C1 and C2 (fed benzo [a] anthracene contaminated feed for 12 and 24 weeks). Blood and kidney of the rats were harvested after animal sacrifice. Serum creatinine and urea concentration and renal Superoxide Dismutase (SOD) activity, GSH, MDA, protein carbonyl, and total protein concentration by spectrophotometric methods. While the concentration of 8-oxodeoxyguanosine in kidney was determined by ELISA method and protein carbonyl by colorimetric method. Renal histological analysis was done with H and E staining. Statistical analysis was performed with Statistical Package for Social Sciences (SPSS) and statistical significance was accepted 95 percent confidence level. Result: From the results, urea concentration increased significantly ($P < 0.05$) in the nickel exposed group after 24 weeks exposure whereas creatinine concentration increased significantly ($P < 0.05$) after 12 weeks of exposure when compared with the control. Comparison of the serum urea and creatinine level of the benzo [a] anthracene exposed group with the control showed no significant ($P > 0.05$) difference. Histological observations indicate glomerular atrophy and widened capsular space haemorrhagic areas, visceral and parietal layer of the Bowman's capsule, the proximal convoluted tubule in the nickel exposed group while the kidney of benzo (a) anthracene exposed rats showed deviation in the histo-architecture of the renal parenchyma as evidenced by glomerular atrophy and widened Bowman's capsular space and focal haemorrhagic areas. Protein thiol level and Superoxide dismutase activity was significantly ($P < 0.05$) depleted in the benzo [a] anthracene exposed groups. The levels of total protein, protein carbonyl, and 8-oxodeoxyguanosine were significantly ($P < 0.05$) elevated in the nickel and benzo [a] anthracene exposed groups. Conclusion: This study demonstrated the oxidative stress causing effects of benzo [a] anthracene and nickel in the kidney. It also shows that consistent exposure to low doses of the contaminants for a lifetime might result in renal oxidative stress with consequential loss of renal function.

Keywords: Kidney, oxidative stress, long-term, nickel, benzo [a] anthracene, histology

Introduction

The pollution of the natural environment with foreign chemical substances, known as pollutants, can have negative impacts on the ecosystem, causing changes, and damage [1]. Both wildlife and human populations are affected by exposure to a variety of these pollutants, leading to a range of biological effects [2].

Some examples of these pollutants include heavy metals, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs).

Among these pollutants, metals are particularly toxic to nearly all living organisms [35]. PAHs, on the other hand, can be generated during food processing through the pyrolysis of amino acids, fatty acids, and carbohydrates [3, 4]. As a result, PAH exposure to humans through the consumption of grilled meats, water, and smoked fishes is highly probable [5].

Nickel is a metallic and hard, malleable, white-silver element, primarily originating from factories and waste burning. It finds numerous applications, including hydrogenation of vegetable

Evaluation of long-term effects of nickel and benzo [a] anthracene on kidney

oils, production of heat-resistant dishes, coin manufacturing, and as a carrier for pharmaceutical compositions for treatment and drug delivery [6]. Consequently, exposure to this element could be unavoidable. Nickel and its compounds have been classified as carcinogenic based on the categorization of national toxicology in the United States and categorized as Group 1 carcinogens by the International Agency for Research on Cancer (IARC) [6, 7]. Increased exposure to nickel has been associated with various toxicities, such as pulmonary, cardiovascular, and renal toxicities. When transported in the blood, nickel can be deposited in tissues or excreted, primarily through urine, making the kidney a target organ for toxicity or carcinogenicity [8].

In literature, accumulation of heavy metal and PAH in foods such as vegetables, food crops and even aquatic organisms such as fish are well reported [9-11] thus food is a major source of human exposure [12-14]. We have previously reported that the accumulation level of the heavy metal and PAH in fish tissue caught from Ogun and Eleyele rivers pose carcinogenic risk based on human health risks evaluation [17, 18]. According to the studies, both the heavy metal and PAH even when present at levels below the recommended threshold could present significant risk to persistent consumers, therefore, the exact risk dose of the toxicants as obtained in the studies were validated experimentally.

The toxicity mechanism of nickel and PAHs, like benzo [a] anthracene, involves inducing oxidative stress, an adverse condition characterized by an elevation in cellular concentration of reactive oxygen species (ROS), such as singlet oxygen, superoxide anion, and hydroxyl radicals, generated from incomplete reduction of molecular oxygen [8]. This process leads to membrane lipid peroxidation, depletion of glutathione content, and DNA damage [15, 16]. In this study, the effects of long-term exposure to low doses of nickel and benzo [a] anthracene on the kidney of rats was evaluated simulating human exposure through food.

Method

Experimental rats

Thirty-six (36) Male Wistar Albino rats (80-100 g) were purchased from the animal house of

Ladoke Akintola University of Technology, Ogbomoso. The animals were kept in animal cages with wood shaves provided as beddings and housed in the animal house of the Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso. Animals were kept under normal light/dark cycle and were provided access to rodent growth breeding feed purchased from Glory veterinary feed mill and water *ad libitum* for the two weeks acclimatisation period. Standard protocol on animal handling and care was adhered to throughout the experimental period. The approval for experimentation was obtained from the ethical committee of the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso.

Preparation of contaminated feed

The animal control feed was prepared in accordance with standard diet preparation and made into granules. Proportion of the raw materials used in the feed formulation as indicated in **Table 1** was obtained from a commercial feed mill. The contaminated feed on one hand was prepared by blending the solution of the respective contaminant into the raw powder of the animal feed such that the quantity of the contaminant in one gram (1 g) feed was known, the feed was thereafter mixed manually and then with a mixer before turning into granular form. The choice of dose was based on the estimated daily intake (EDI) model of health risk evaluation calculated for nickel (Equation I) and Benzo (a) anthracene (Equation V) identified in the muscle of fish obtained from the Ogun and Eleyele Rivers and earlier reported in our laboratory [17, 18]. The highest concentration of the two toxicants in fish muscle (Ni; 3.72×10^{-5} mg), and Benzo (a) anthracene (0.39 μ g) that pose the most significant carcinogenic risk to the consumer was selected and prior to the blending of contaminant with the animal feed, the dose obtained from the Human health risk assessment was converted to the rat equivalent dose. The equivalent dose conversion coefficient between human to rats was estimated using the surface area as shown in Equation XII.

$$\text{Estimated Daily Intake (EDI)} = \frac{C \times IR}{BW_a}$$

$$\text{Rat Equivalent dose (mg/kg)} = \text{Dose to be converted} \times \frac{\text{Human Km}}{\text{Rat Km}} \dots \text{Equation XII}$$

Evaluation of long-term effects of nickel and benzo [a] anthracene on kidney

Table 1. Proportion of the rat feed components

Components	Quantity (kg)
Maize	4.375
Soybean	2.50
Groundnut Cake	0.95
Wheat over	3.15
Corn brown	0.65
Fish	0.315
Bone meal	0.44
Oyster shell	0.065
Lycine	0.015
Methionine	0.015
Salt	0.065

Glory vertynary feedmill.

From the equation, C is the concentration of contaminant identified in the fish muscle, IR is the ingestion rate of fish, BW_h is the average human body weight, Dose to be converted represents the EDI, Km_{human} is human surface area for 60 kg adult, while Km_{rat} is the rat surface area.

The equivalent dose obtained between human and rat for nickel was 0.000229 mg and Benzo (a) anthracene was 0.0024 mg.

Pilot experimental setup for feed quantification

Alongside the animal grouping, a pilot experimental set up of the animals was done. One animal each was respectively assigned into three (3) separate cages and given access to food and water *ad libitum*. The amount of food consumed daily was monitored and the average feed consumption was recorded. The average feed consumption was assumed as the quantity of feed each animal in the experimental set up will consume daily and was used as a guide to determine the amount of feed that will be blended with the desired dose of the contaminant. The set up was preserved until the end of experiment to note any possible changes in the feeding pattern of the experimental rats.

Experimental design

After the two weeks acclimatisation of the experimental rats, they were randomly assigned into two experimental setups consisting of short and long-term exposure. The Animals in the two experimental set up were respectively assigned into four groups of six rats each. The animals in the short-term setup were exposed

for 12 weeks while the animals in the long-term setup were exposed for 24 weeks. Throughout the experimental period, the rats were provided with the prepared diet and water as indicated in **Table 2.**

At each end point (the 12th and 24th week) of exposure, the rats were sacrificed by cervical dislocation after overnight fasting, the blood was collected through cardiac puncture into a plane sterile bottle for renal function analysis. The kidney was immediately harvested, and rinsed in phosphate buffered saline (pH 7.4): 0.0754 M Na₂HPO₄: 20.021 g and 0.0246 M NaH₂PO₄: 3.39 g were dissolved in 1 L of distilled water. The pH was adjusted with NaOH and HCL. Portions of the kidney was fixed in a 10% formalin buffer and used for histological analysis, whereas a portion was homogenised for the assessment of oxidative stress. The blood sample was allowed to clot and then centrifuged at 4000 rpm for 5 minutes, the serum was collected and analysed for renal function biomarkers. 10% (w/v) of the kidney was prepared in Phosphate Buffered Saline (PBS) (pH 7.4) by homogenization, thereafter centrifuged with a refrigerated centrifuge at 3000 rpm and 4°C for 10 minutes. The supernatant was collected and used.

Analysis of kidney function

Determination of serum creatinine concentration

Principle: The assay principle was based on creatinine reaction with picric acid in an alkaline medium to form a deep yellow complex. The amount of complex formed is directly proportional to the level of creatinine in the sample and was measured at 492 nm.

Procedure: In pre-labelled test tubes, 100 µL of both the standard and samples were separately mixed with 1 ml of the working reagent (NaOH + Picric Acid + Creatinine Standard). Absorbance of the mixtures was read after 30 secs then again after exactly 120 seconds [19]. The serum creatinine concentration was estimated using the equation:

$$\text{Serum creatinine concentration} = \frac{\Delta_{\text{abs sample}}}{\Delta_{\text{abs standard}}} \times 177$$

Determination of serum urea concentration

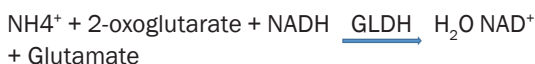
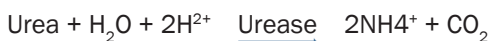
Principle: In the method, urea is hydrolysed in the presence of urease to produce ammonia

Evaluation of long-term effects of nickel and benzo [a] anthracene on kidney

Table 2. Grouping of experimental animals and the treatment

Groups	Treatment
Phase I (Experimental rats in this phase were fed with the contaminated feed for three months)	
A (Normal)	Normal control animals were fed with the uncontaminated feed
B (Nickel)	Animals in this group were fed with nickel contaminated feed for three months
C (Benzo [a] anthracene)	Animals in this group were fed with benzo (a) anthracene contaminated feed for three months
Phase II (Experimental rats in this phase were fed with the contaminated feed for three months)	
A2 (Normal)	Normal control animals were fed with the uncontaminated feed
B2 (Nickel)	Animals in this group were fed with nickel contaminated feed for six months
C2 (Benzo [a] anthracene)	Animals in this group were fed with benzo (a) anthracene contaminated feed for six months

and CO₂. The ammonia produced combines with 2-oxoglutarate and NADH in the presence of glutamine dehydrogenase (GLDH) to yield glutamate and NAD.



The decrease in absorbance due to the decrease of NADH concentration in unit time is proportional to the Urea concentration.

Procedure: The test tubes were pre-labelled as calibrator and samples. In the respective test tubes, 10 µL of sample and calibrator were respectively mixed with 1000 µL of the working reagent (Buffer + Enzyme Reagent + Standard). The mixtures were mixed properly and the initial absorbance (A1) was read after exactly 30 sec. and the final absorbance (A2) after additional 120 sec at 340 nm.

The Serum urea concentration was estimated using the equation:

$$\text{Serum urea concentration} = \frac{\Delta \text{abs sample}}{\Delta \text{abs calibrator}} \times \text{concentration of calibrator}$$

Estimation of super oxide dismutase (SOD) activity

Principle: The Super Oxide Dismutase activity was estimated following the protocol supplied in the enzyme kits. The assay method is based on the competition between pyrogallol autoxidation by O₂^{•-} and the dismutation of this radical by SOD. The autoxidation of pyrogallol in the presence of EDTA at pH 8.2 is 50%.

Procedure: For the analysis, 50 µL of tissue sample was mixed with 1 ml each of the assay

buffer and chromogen solution. The absorbance was immediately read at 420 nm and again after 1 minute. Blank preparation was also made with 50 µL distilled water excluding the sample. Changes in absorbance were respectively estimated for the blank and sample by subtracting the initial absorbance from the absorbance after 1 minutes [20, 21]. While the percentage inhibition of pyrogallol autoxidation was estimated by the following.

$$\% \text{ inhibition of Pyrogallol autoxidation} = \frac{\Delta S}{\Delta C} \times 100\%$$

$$\text{SOD activity (U/ml)} = \frac{\% \text{ inhibition of Pyrogallol autoxidation}}{50\%}$$

Estimation of reduced glutathione (GSH) concentration

Principle: Spectrophotometric determination of GSH concentration was based on reduction of 5,5'-dithiobis-(2-nitrobenzoic acid) to 2-nitro-5-mercaptobenzoic acid by the SH group of GSH [22]. The nitromercaptobenzoic acid anion produced has an intense yellow colour and its measurement at 412 nm estimates SH groups.

Procedure: Prior to analysis, 100 µL of Protein precipitation reagent was added to 400 µL of sample in a microcentrifuge tube. Mix and then centrifuge at 3,000 rpm for 5 minutes. The supernatant was used for the assay. 100 µL each of varied concentration of standard reagent (provided in the assay kit) and samples were separately mixed with 880 µL GSH dilution buffer. To the mixture, 20 µL GSH Chromogen was added and well mixed. The absorbance of both the standard and sample were measured within 5 minutes at 412 nm. To calculate the GSH concentration, a standard curve using the absorbance value of standard versus the GSH concentration for each standard. The equation of the line was determined

Evaluation of long-term effects of nickel and benzo [a] anthracene on kidney

Table 3. Standard curve preparation for GSH estimation

Standard	GSH Conc (mM)	Vol of distilled water (ul)	Source (uL)
S ₁	0	1000	-
S ₂	0.0625	500	500 OF S3
S ₃	0.125	500	500 OF S4
S ₄	0.25	500	500 OF S5
S ₅	0.5	500	500 OF S6
S ₆	1	500	500 of stock (2 mM solution)

Table 4. Standard curve preparation for MDA estimation

Standard	MDA Conc. (μM)	Vol. of dH ₂ O (μL)	Vol. of 20 μM MDA Stock (μL)
S ₀	0	400	0
S ₁	0.5	390	10
S ₂	1.0	380	20
S ₃	2.5	350	50
S ₄	5.0	300	100
S ₅	10.0	200	200
S ₆	15.0	100	300
S ₇	20.0	0	400

Estimation 8-OHdG (8-Hydroxydeoxyguanosine)

Principle: The method for the determination of 8-OHdG is a competitive-ELISA method. The ELISA microplate provided in the assay kit was already pre-coated with 8-OHdG. During the reaction process, the 8-OHdG in samples compete with a fixed amount of

using a linear fit method and was used for subsequent extrapolation of GSH concentration in each sample. Prior to use, 2 mM GSH standard provided in the assay kit was used to prepare series of standards as indicated in the **Table 3**.

Estimation of MDA concentration

Principle: This assay is based on the reaction of a chromogenic reagent, 2-thiobarbituric acid, with MDA at 25°C. One molecule of MDA reacts with 2 molecules of 2-thiobarbituric acid via a Knoevenagel-type condensation to yield a chromophore with absorbance maximum at 532 nm. Prior to analysis, 100 μL of Protein precipitation reagent was added to 1000 μL of sample in a microcentrifuge tube. Mix and then centrifuge at 3,000 rpm for 5 minutes. The supernatant was used for the assay.

Procedure: In separate tubes, different concentration of standard (**Table 4**) and samples were mixed with indicator solution (1:1). For the blank, equal volume of sample and acid reagent was mixed. The mixture was incubated at room temperature for 45 minutes and thereafter, absorbance was measured at 532 nm [23]. Concentration of MDA was extrapolated from the standard curve plotted from **Tables 3, 5**.

8-OHdG on the solid phase supporter for sites on the Biotinylated Detection Ab specific to 8-OHdG. After the reaction, excess conjugate or unbound samples were washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated.

An enzyme-substrate reaction was started by the addition of TMB substrate solution to each well. The reaction was terminated by the addition of a stop solution and the colour change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of 8-OHdG in the samples was then determined by comparing the OD of the samples to the standard curve.

Procedure: Fifty (50 μL) of the standards, blank and sample were added to the respective wells followed by 50 μL of Biotinylated Detection Antibody working solution. The plate was covered with the sealer provided in the kit and incubated for 45 min at 37°C. After incubation, the solution was aspirated from each well, and 350 μL of wash buffer was added. The wash buffer was allowed to stand for 1 min before aspiration from each well and pat dry against clean absorbent paper. The washing step was repeated thrice. Immediately after washing,

Evaluation of long-term effects of nickel and benzo [a] anthracene on kidney

Table 5. Effects of nickel and benzo [a] anthracene on the redox status of rats' kidney after 12 and 24 weeks of exposure

Parameters	Duration (Weeks)	Group		
		Control	Nickel	Benzo (a) anthracene
Kidney				
SOD (U/ml)	12	0.42±0.07	0.52±0.03 [#]	2.99±0.35 [*]
	24	0.94±0.07	1.15±0.02 [#]	0.62±0.08 [*]
GSH (mM)	12	1.21±0.08	1.18±0.18 [#]	2.87±0.15 [*]
	24	1.46±0.09	1.79±0.04 [#]	1.89±0.19 [*]
MDA (uM)	12	31.83±1.87	31.14±2.52 [#]	26.72±2.53 [#]
	24	14.53±2.13	12.70±0.82 [#]	7.32±1.22 [*]

Values were expressed as mean ± SEM. ^{*}represent significant different with control. [#]represent insignificant different with control. Keys: Control: rats group fed with normal rats feed without any contamination. Nickel: rats group fed with nickel contaminated feed. Benzo (a) anthracene: rats group fed with benzo (a) anthracene contaminated feed.

100 µL of HRP conjugate working solution was added to each well and covered with a new sealer. The plate was incubated for 30 min at 37°C and thereafter, the solution was aspirated from the well. The wash process was again repeated 5 times. 90 µL of substrate reagent was added to the wells and the plate was covered with a new sealer. Another incubation step was followed for about 15 min at 37°C. During the process, the plate was protected from light. Sufficient time (maximum of 30 minutes) was allowed for colour development. 50 µL of the stop solution was added to each well and the optical density (OD value) of each well was read with a microplate reader at 450 nm.

Estimation of protein carbonyl content

Principle: The colorimetric assay was based on the reaction of the carbonyl group with 2,4-dinitrophenylhydrazine to form a reddish-brown precipitate. To measure the protein carbonyl content, the precipitate was dissolved and the absorbance was read at 370 nm.

Procedure: The sample was initially pre-treated by the addition of 450 µL of sample to 50 µL of reagent 2 (Sulfates) and was allowed to stand for 10 min at room temperature. This was followed by centrifugation for 10 min at 4°C and 11580 g. The supernatant was used for the detection of protein carbonyl. 100 µL of sample was mixed with 400 µL of reagent 3 (dinitrophenylhydrazine solution), while the control tube contain mixture of 100 µL of sample and 400 µL of reagent 4 (Acid Reagent). The mixture was incubated for 30 minutes at 37°C followed by the addition of 500 µL of reagent 5 (Protein precipitator). The mixture was centrifuged at 13780 g for 10 min at 4°C, and the

supernatant was discarded while the precipitate was kept. To the precipitate, 1 mL of anhydrous ethanol-ethyl acetate mixture was added, mixed, followed by another round of centrifugation at 13780 g and 4°C for 10 min. The supernatant was discarded and the precipitate was kept. The process was repeated 4 to 3 times and 1250 µL of reagent 6 (Denaturant), was added before incubation in a water bath at 37°C for 15 minutes. The tubes were thoroughly mixed to fully dissolve the precipitate. The dissolution was followed by another centrifugation round for 15 min at 4°C and 13780 g. The optical density of the supernatant was read at 370 nm. The protein concentration was also estimated in the supernatant and was used in the estimation of protein carbonyl. Protein carbonyl content was calculated using the formula below.

Protein carbonyl content (nmol/mgprot) =

$$\frac{(A1 - A2)}{(\epsilon \times d)} \div (Cpr \times \frac{V1}{V2}) \times 10^6 \times f$$

A1: the OD value of sample, A2: the OD value of control, ϵ : the molar extinction coefficient of carbonyl (22000 L/mol/cm), d: the optical path of cuvette (0.8 cm), V1: the total volume of reaction system (1.25 mL), V2: the volume of sample added to the reaction system (0.1 mL), Cpr: the protein concentration of the sample supernatant (mgprot/L), 10^6 : unit conversion (1 mol/L = 10^6 nmol/mL), f: dilution factor of sample before tested.

Estimation of protein thiol content

Principle: To estimate the protein thiol content of the sample, the total thiol or sulfhydryl groups (SH) and non-protein thiol (GSH) con-

Evaluation of long-term effects of nickel and benzo [a] anthracene on kidney

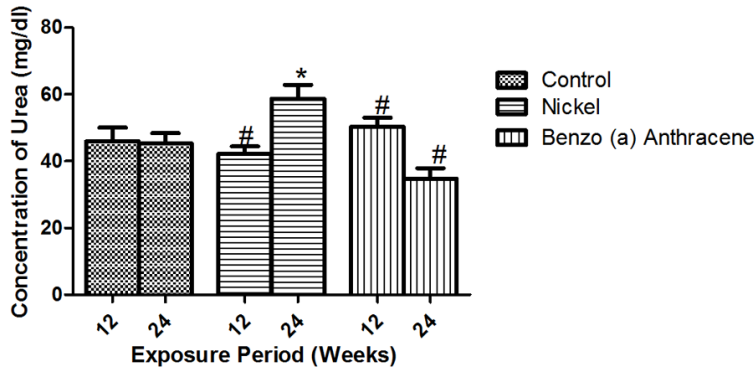


Figure 1. Effects of nickel and benzo [a] anthracene on the urea concentration of rats at week 12 and 24 of exposure. Values were expressed as mean \pm SEM. *represent significant different with control. #represent insignificant different with control. Keys: Control: rats group fed with normal rats feed without any contamination. Nickel: rats group fed with nickel contaminated feed. Benzo (a) anthracene: rats group fed with benzo (a) anthracene contaminated feed.

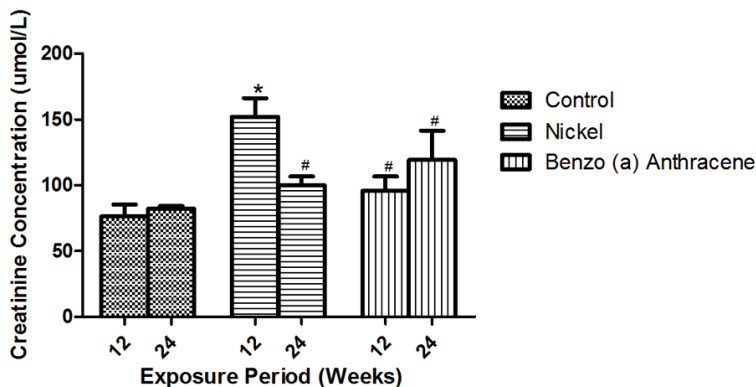


Figure 2. Effects of nickel and benzo [a] anthracene on the creatinine concentration of rats at day 12 and 24 weeks of exposure. Values were expressed as mean \pm SEM. *represent significant different with control. #represent insignificant different with control. Keys: Control: rats group fed with normal rats feed without any contamination. Nickel: rats group fed with nickel contaminated feed. Benzo (a) anthracene: rats group fed with benzo (a) anthracene contaminated feed.

centration were measured by the methods of Hu [24]. The assay principle is based on the interaction of thiol with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) which forms a highly coloured anion with maximum peak at 412 nm.

Procedure: Twenty-five (25 μ L) of sample was mixed with 1000 μ L Tris-EDTA buffer (0.25 mmol/L Tris base, 20 mmol/L EDTA, pH 8.2), and the initial absorbance was read at 412 nm. After initial absorbance measurement, 25 μ L DTNB solution (10 mmol/L in absolute methanol) was added and after 15 minutes, the absorbance was read while DTNB only was used as blank. Reduced glutathione was used

as sulfhydryl group standard and the concentration was expressed as mmol/L. After obtaining the total thiol content, the protein thiol concentration in the sample was calculated by subtracting the GSH level from the total thiol level.

Histological analysis

The kidney section was stained in Harris haematoxylin for 5 minutes, it was then rinsed in water. The rinsed section was differentiated in 1% acid alcohol briefly, rinse under tap water for 10 minutes. Counter stain in 1% aqueous eosin for 3 minutes, rinse in water, and dehydrated through ascending grades of alcohol (70%, 80%, 90%, and absolute). The dehydrated section was cleared in xylene, and then mounted with a dpx mountant. Lesions in the kidney were observed under light microscope and interpreted by an expert.

Statistical analysis

All data in this study were presented as mean \pm SEM, statistical difference between mean was performed by one-way analysis of variance using Statistical Package for Social Sciences (SPSS) and the differences between the mean was observed using Duncan's Multiple Test. To analyse the influence of time and treatment on the biological data, two-way analysis of variance was performed and statistical significance was accepted when $P < 0.05$ (95% confidence level).

Results

Effect of nickel and benzo (a) anthracene exposure on the renal function of rats

The serum urea and creatinine concentration of the rats fed with either nickel or benzo (a) anthracene contaminated feed was measured as indices of renal function and the result is presented in **Figures 1** and **2** respectively. No

Evaluation of long-term effects of nickel and benzo [a] anthracene on kidney

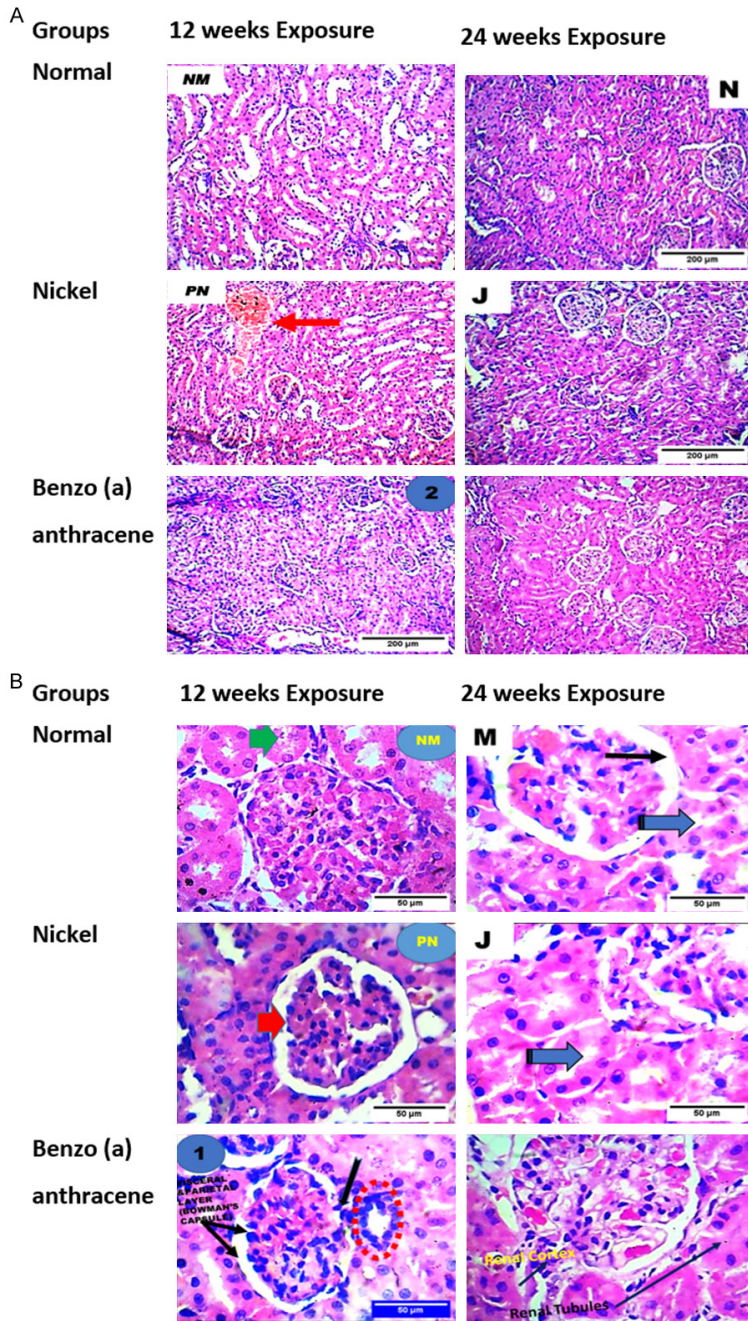


Figure 3. A: Representative photomicrographs of hematoxylin and eosin-stained renal tissue of rats exposed to nickel and benzo [a] anthracene for 12 weeks and 24 weeks respectively. Scale Bars - 200 µm. Glomerular atrophy and widened capsular space haemorrhagic areas, (red arrow), visceral and parietal layer of the Bowman's capsule. B: Representative photomicrographs of hematoxylin and eosin-stained renal tissue of rats exposed to nickel and benzo [a] anthracene for 12 weeks and 24 weeks respectively. Scale Bars - 50 µm. Glomerular atrophy and widened capsular space haemorrhagic areas, (red arrow), visceral and parietal layer of the Bowman's capsule, the proximal convoluted tubule (striped blue arrow), the eosinophilic proximal convoluted tubule (green arrow).

significant ($P>0.05$) difference was observed in the serum urea concentration of rats fed with

nickel and benzo (a) anthracene contaminated feed for 12 weeks. When the rats were left on nickel contaminated feed for 24 weeks, significant ($P<0.05$) increase was observed in the serum urea concentration whereas, the groups left on benzo (a) anthracene contaminated feed showed insignificant ($P>0.05$) difference in the serum urea concentration when compared with the control. In **Figure 2**, serum concentration of creatinine in the groups fed benzo (a) anthracene for 12 weeks differs insignificantly ($P>0.05$) from the control, whereas, the concentration in the group fed nickel contaminated feed increased significantly ($P<0.05$). When the rats were fed with nickel and benzo (a) anthracene contaminated feed no significant ($P>0.05$) difference was observed in the serum creatinine concentration compared with the control.

Effect of nickel, and benzo (a) anthracene on the kidney histology of exposed rats

Figure 3A, 3B respectively represent low and high-power light photomicrographs of renal tissues of rats exposed to nickel and benzo (a) anthracene stained in hematoxylin and eosin stain. In the low magnification power (scale Bars - 200 µm) observed was the renal cortex comprising the renal corpuscles (glomerulus and Bowman's capsule) and renal tubules. Displayed in the photomicrograph included the glomerular (Bowman's capsular's space) visceral and parietal layer of the Bowman's capsule, the eosinophilic proximal convoluted tubule (green arrow) with heterochromatic nuclei

and presence of brush border with acidophilic cells, the distal convoluted tubule (black arrow).

Evaluation of long-term effects of nickel and benzo [a] anthracene on kidney

Table 6. Effects of nickel and benzo [a] anthracene on the level of protein, protein thiol, and carbonyl in the kidney of rats after 12 and 24 weeks of exposure

Parameters	Duration (Weeks)	Group		
		Control	Nickel	Benzo (a) anthracene
Kidney				
Protein Carbonyl ($\times 10^{-5}$ nmol/mgprot)	12	0.22 \pm 0.05	0.33 \pm 0.04 [#]	0.72 \pm 0.01 [*]
	24	4.63 \pm 0.46	4.45 \pm 0.16 [#]	3.33 \pm 0.54 [#]
Protein thiol (mM)	12	1.13 \pm 0.16	0.88 \pm 0.24 [#]	1.16 \pm 0.21 [#]
	24	2.65 \pm 0.13	2.18 \pm 0.04 [*]	2.06 \pm 0.13 [*]
Total Protein (g/dl)	12	3.61 \pm 1.01	3.25 \pm 0.30 [#]	3.77 \pm 0.22 [#]
	24	2.58 \pm 0.10	2.48 \pm 0.03 [#]	8.90 \pm 1.48 [*]

Values were expressed as mean \pm SEM. ^{*}represent significant different with control. [#]represent insignificant different with control. Keys: Control: rats group fed with normal rats feed without any contamination. Nickel: rats group fed with nickel contaminated feed. Benzo (a) anthracene: rats group fed with benzo (a) anthracene contaminated feed.

After the 12 weeks of exposure, the normal control group showed a normal general histoarchitectural pattern of the renal cortex and the distal convoluted tubule (black arrow) with less intensely stained cells which lack brush borders appear intact. Evidenced in the nickel and benzo (a) anthracene exposed groups included glomerular atrophy and widened capsular space (red arrow). Other observations included congested renal tubules (proximal and distal convoluted tubules), infiltrated renal parenchyma by red inflammatory cells, dilated renal tubules, pyknotic renal parenchymal cells, signs of haemorrhage, and concentrated mesangial cells towards a point. Extension of metal and PAH exposure up to 24 weeks revealed the histoarchitecture of the renal cortex showing the renal corpuscles comprising tuft of capillaries (glomerulus) and the renal tubules. The glomerular (Bowman's capsular space) (black arrow), visceral and parietal layer of the Bowman's capsule, the proximal convoluted tubule (striped blue arrow) with acidophilic cells, brush broader and clear lumen, the smaller distal convoluted tubule with less intensely stained cells which lack brush borders also are well delineated in all groups. Renal mesangial cells and juxtaglomerular cells could also be seen in all groups. In the benzo (a) anthracene exposed group there appears to be some deviation in the histoarchitecture of the renal parenchyma as evidenced by glomerular atrophy and widened Bowman's capsular space and focal haemorrhagic areas (red arrow) which are suggestive of glomerulonephritis (Scale Bars - 50 μ m). On the contrary, representative of the normal and nickel exposed groups showed normal architecture without any significant observable alteration as seen in both magnifications: the renal cortex

shows normal glomeruli with normal mesangial cells and capsular spaces, the renal tubules appear normal; clear and not congested, the interstitial spaces appear normal with well-defined profile.

Effect of nickel, and benzo (a) anthracene exposure on the renal redox status of rats

The result in **Table 5** shows the renal Superoxide Dismutase (SOD) activity, reduced glutathione (GSH) and Malondialdehyde (MDA) concentration of rats fed nickel and benzo (a) anthracene contaminated feed for 12 and 24 weeks. There was a significant ($P < 0.05$) increase in the SOD activity of rats fed with benzo (a) anthracene contaminated feed at week 12, but decreased significantly ($P < 0.05$) at week 24 whereas no significant ($P > 0.05$) difference was observed in the group fed nickel contaminated feed at week 12 and 24 in comparison with the control.

GSH concentration increased significantly ($P < 0.05$) in the group fed benzo (a) anthracene contaminated feed for 12 and 24 weeks when compared with the control. In the groups fed nickel contaminated feed, no significant difference ($P > 0.05$) was observed in the concentration of GSH at week 12 and 24 respectively. Similarly, the MDA concentration decreased significantly ($P < 0.05$) in the rats group fed benzo (a) anthracene contaminated feed for 24 weeks, whereas the concentration was insignificantly ($P > 0.05$) different from the control when fed to rats for 12 weeks. The concentration of MDA in the nickel fed group, showed no significant ($P > 0.05$) difference when compared with the control.

Table 6 reflects the effects of rats' exposure to nickel, and benzo (a) anthracene for 12 and 24

Evaluation of long-term effects of nickel and benzo [a] anthracene on kidney

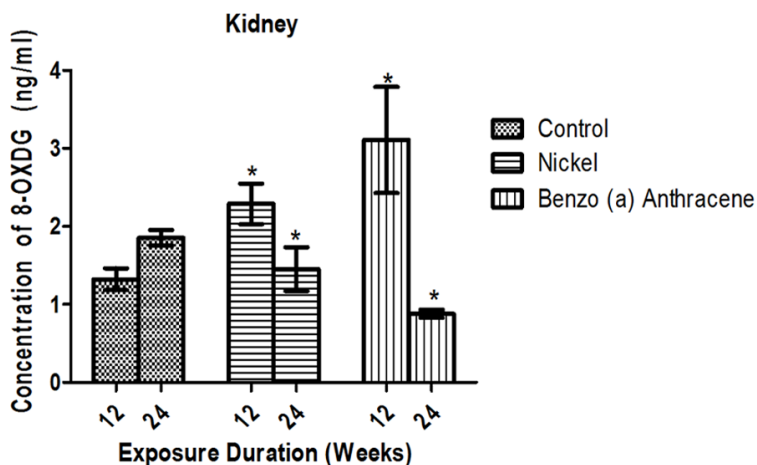


Figure 4. Effects of nickel and benzo [a] anthracene on the concentration of 8-OXDG in the kidney of rats after 12 and 24 weeks of exposure. Values were expressed as mean \pm SEM. *represent significant different with control. #represent insignificant different with control. Keys: Control: rats group fed with normal rats fed without any contamination. Nickel: rats group fed with nickel contaminated feed. Benzo (a) anthracene: rats group fed with benzo (a) anthracene contaminated feed.

weeks on the renal protein carbonyl, protein thiol, total protein, and 8-oxodeoxyguanosine concentration. The result showed, that significant ($P < 0.05$) increase was only observed in the renal protein carbonyl concentration of rats group fed benzo (a) anthracene contaminated feed for 12 weeks whereas no significant ($P > 0.05$) difference was observed in the nickel-exposed group when compared with the control at weeks 12 and 24. Protein thiol on the other hand decreased significantly ($P < 0.05$) in the group fed benzo (a) anthracene and nickel contaminated feed for 24 weeks, while the concentration at week 12 differs insignificantly ($P > 0.05$) with the control. No significant ($P > 0.05$) difference was observed in the renal total protein concentration of rat groups fed nickel contaminated feed for 12 and 24 weeks as well as the group fed benzo (a) anthracene contaminated feed for 12 weeks when compared with the control. The concentration when the rats were fed benzo (a) anthracene contaminated feed for 24 weeks increased significantly ($P < 0.05$) when compared with the control. In **Figure 4**, the concentration of 8-oxodeoxyguanosine increased significantly ($P < 0.05$) when the rats were fed with nickel and benzo (a) anthracene contaminated feed for 12 weeks, whereas, the concentration decreased significantly ($P < 0.05$) in the two groups when the exposure lasted for 24 weeks.

Discussion

Because of the role of kidney in the excretion of waste especially the metabolites of xenobiotic metabolism, its integrity could be compromised and consequently result in its abnormality or loss of function [7, 8, 25, 26]. Many reports of the reno-toxicity effects of metals and PAH have shown that the mechanisms usually involve a compromise in the oxidative status that could further aggravates through the activation of the inflammatory pathway [27-30]. In this study, evaluation of the possible reno-toxicity effects of low dose of the selected toxicants over 24-week exposure duration was performed.

The overall result showed only a slight change in the renal function integrity most especially demonstrated in the renal histo-architecture. However, nickel exhibited the most significant renal toxicity effect as demonstrated by the serum level of creatinine and urea. The reason for this effect of nickel might be due to its accumulative capacity in the kidney [8]. The kidney contains high amount of metallothionein, a metal binding protein with high affinity for nickel [27, 31]. Specific changes observed in the renal histology were nephritis that is characterized by the widening of the Bowman's capsule and few inflammatory cells infiltration [32, 33].

Thiols, such as protein, and the non-protein (GSH) is important target of heavy metals [26]. The SH-groups in thiols are critical for neutralization of free radicals [26] therefore, this class of molecules constitute an integral part of cellular antioxidant system and are involved in the maintenance of cellular redox stability [25]. In an attempt to maintain cellular homeostasis, the level of thiols may be increased in response to oxidative stress, and may as well be overwhelmed as result of the loss in the replenishing ability of cell during exaggerated oxidative stress [34]. Increased in the kidney GSH concentration of rats exposed to benzo [a] anthracene might be due to adaptive response where-

Evaluation of long-term effects of nickel and benzo [a] anthracene on kidney

as the reduction in the protein thiol by nickel and benzo [a] anthracene might be due to oxidative stress. Other studies have shown suppression of kidney thiol levels after exposure to heavy metals [8, 26]. The oxidative causing effects of benzo [a] anthracene and nickel in the kidney was also made clear by the increases observed in the level of the DNA oxidative products (8-OHdG). Overall observation of the effects of nickel and benzo [a] anthracene on the kidney of rat shows that consistent exposure for a lifetime might result in renal oxidative stress with consequential loss of renal function.

Conclusion

Overall result of the study showed that low dose of nickel over prolonged exposure time resulted in the enlargement of the nephron and nephritis. Therefore, control of the environment from pollution is very vital in the preservation of health. Inclusion of antioxidant in diets could also be encouraged to mitigate the possible effects of exposure to environmental concern contaminants.

Disclosure of conflict of interest

None.

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Evaluation of long-term effects of nickel and benzo [a] anthracene on kidney

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