International Journal of Biochemistry Research & Review

20(2): 1-10, 2017; Article no.IJBCRR.38602
ISSN: 2231-086X, NLM ID: 101654445

Systemic Effects of Nigerian Bituminous Coal Fly Ash in Albino Rats: Serum Biochemistry and Histopathological Evaluation

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Authors' contributions

This work was carried out in collaboration between all authors. Author IBB designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SHG and IDL managed the analyses of the study. Authors DYS, TSM and YM managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2017/38602
Editor(s):
(1) Halit Demir, Professor, Department of Chemistry, Faculty of Art and ScienceYuzuncu, Yil University, Turkey.
Reviewers:
(1) Hilal Colak, Istanbul University, Turkey.
(2) Esmeralda Carrillo Delgado, Biosanitary Institute of Granada, Biopathology and Regenerative Medicine Institute, University of Granada, Spain.
(3) Maha Zaki Rizk, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/22523

Original Research Article

Received 2nd December 2017
Accepted 25th December 2017
Published 30th December 2017

ABSTRACT

In this study, the *in vivo* toxicity index of Nigerian bituminous coal fly ash prepared under a burning temperature of 900ºC was evaluated following oral administration to Albino rats. The effect of the sample prepared at this temperature was compared under various dose (100, 200 and 500 mg/kg body weight) concentrations and systematically relate the effect on the serum electrolytes (Na^+, K^+, Cl^-, HCO^-_3) activity levels, the liver enzymes (ASAT, ALAT, SAP), Total bilirubin (TB), Total protein (TP) and Albumin (ALB) respectively. From the results, the fly ash was observed to induced significant alteration in the TB, TP and ALB concentrations in both study periods, with no apparent disturbances recorded in the liver enzymes and serum electrolyte concentrations when compared to the control. Furthermore, the fly ash was observed to profoundly induce pathological lesion in the lungs and the intestine, with mild histological changes observed in the liver and spleen. While, no

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significant changes observed in the kidney in the primary study, the fly ash in the secondary study was observed to induce tubular degeneration in the kidney. The results further showed that, the Nigerian bituminous coal fly ash, irrespective of the treatment groups demonstrated a time-dependent effects histologically and in serum biochemistry profiles. Thus, further confirmed that, the reactivity of the coal fly ash, though independent of the sampling dose might be attributed to the organic and inorganic constituents in the fly ash interacting with the rat's normal metabolic pathways, initiating and triggering toxic-induced effects histological and physiologically. The results further provide us with additional information on the *in vivo* effects and susceptibility of Nigerian bituminous coal fly ash and the need to explore same for energy generation under control and regulated combustion processes.

Keywords: Coal fly ash; bituminous coal fly ash; serum biochemistry; albino rats.

1. INTRODUCTION

The physiological and mineralogical characterization of Nigerian bituminous coal fly ash as investigated and reported by some researchers were observed to vary in content based on the temperature at which the coal sample is burned [1,2], which according to other findings represents additional environmental concerns [3]. From the investigation conducted by some researchers [4], about 10.7% ash content were generated following burning of Nigerian bituminous coal at 500^0C temperature and the characterization shows the coal generated at this temperature contains some polycyclic aromatic hydrocarbons and trace elements. Same author generated 6.3% ash content on increasing the coal burning temperature to 900ºC. At this temperature, it was observed that the fly ash consists mostly of trace metals [2]. Reports shows that, these species emitted as primary pollutants have high solubility's to be directly removed by dissolution in liquid water droplets and then deposited to the ground by wet deposition [5,6]. These processes were observed to depends on the total concentration of the trace elements in fly ash, particle distribution and the mobility of the elements to be transformed into reactive secondary forms [5–7].

The degree of toxicity of coal fly ash are directly influence by several factors which includes among others, temperature of the coal ash formation, particle dose and size distribution and to more significant extent on the elemental species adsorbed by the fly ash particles [6]. The fate of these species being ingested in relation to their non-specific binding to cell membrane and intracellular proteins are found to be actively involved in facilitating the formation of reactive oxygen (ROS) species or reactive nitrogen (RNS) species [8,9]. The reactive species either in their primary or secondary forms has the mobility to be readily absorbed after ingestion or inhalation and translocate into the reticulum endothelium systems, triggering toxic-induced effects [10].

In our previous study, we reported the *in vivo* toxicity of Nigerian bituminous coal fly ash based on some measured biochemical indices and post-mortem investigation of some selected excise tissue section of albino rats. The coal fly ash used for the study was prepared at a burning temperature of 500ºC and administered to the subjects for 14 and 28 days [11]. In the study, the effect of the coal fly ash following the exposure to the subjects, though observed not to be dose dependent, exerted some degree of disturbances in the biochemical indices and in the H & E examination. Histological, the liver in the secondary study shows cloudy degeneration of the hepatocytes, while no obvious pathological lesion observed in the same organs in the primary study. Other histological changes observed includes splenic hyperplasia, hemorrhage in the kidney, pneumonitis in the lungs and wide spread goblets cells hyperplasia in the intestine [11].

This present investigation is an extension of the previous study [11] and conducted particularly to evaluate the *in vivo* toxicity of the Nigerian bituminous coal fly ash burned at a higher temperature of 900ºC. Since the composition and ash content of the fly ash were reported to varies with respect to burning temperature [2,4], we assumed that this study will provides us with more insight weather coal burning temperature participated in defining the underlying mechanisms leading to increased susceptibility. The investigation was conducted histologically by examining the liver, spleen, lungs, kidney and intestine of the treated rats and systematically relates the finding to some measured

biochemical indicators (liver, kidney and intestinal biomarkers) relevant to the hypotheses underlying the toxicity of particulate matters associated with the Nigerian bituminous coal fly.

2. MATERIALS AND METHODS

2.1 Sample Preparation

The dried pulverised bituminous coal sample obtained from Omarako coal mines, Enugu State in Nigeria, was burned at 900ºC for about 4 hours in an oven (Gallen Kamp, England) to form the ash sample. The ash samples were further size fractionated to obtain a particle with a size distribution range of 4–5 um. A stock suspension of the ash sample with a concentration of 300 mg/ml was prepared by dispersing the ash in phosphate buffered saline solution (PBS, pH 7.4 ± 0.2).

2.2 Animals and Treatment

A total of 42 male Albino Rats of Wistar strain weighing 70 – 200 g were used for this study. The animals were made available by the Wistar rat colony of the animal house of the University of Jos. All the experiment involving animal care and utilization were conducted under the regulations set by the University of Maiduguri (Unimaid) ethics committee's guidelines. The grouping and treatments used in this experiment followed the same protocol described in our previous study [11]. Briefly, 100 mg/kg body weight of the ash was administered orally (by intubation) to the rats in group I, the group II rats were administered 200 mg/kg body weight and the rats in group III received 500 mg/kg body weight of the sample ash solution. The remaining 6 rats of the total of 42 rats were used as the experimental control group.

2.3 Serum Biochemistry Analysis and Histopathological Evaluation

Following the 14 and 28 days of sample administration, the rats were anesthetized and euthanized by cervical dislocation. Blood samples were collected and the liver, spleen, lungs, kidney and the intestine were also harvested for Haematoxylin and Eosin (H & E) histological examination. Both the serum biochemistry and the post-mortem histological processing and examination were conducted following the same protocol previously described elsewhere [11]. The data was expressed as Mean ± SD. One-way ANOVA and student t-test was performed and the significance was set at $p < 0.05$.

3. RESULTS

3.1 Clinical Observations

In the study, the oral administration of the coal fly ash burned at 900ºC to the animal showed slight clinical changes in both the primary and secondary studies. A slight drop in body weight was observed in all the treatment groups with obvious sign of restlessness and loss of appetite compared with the control groups. Despite the clinical sign observed, no death was recorded throughout the study periods.

3.2 Effects of Coal Fly Ash Sample on the Liver Enzymes Activities

The results of the alanine amino transferase (ALAT), aspartate amino transferase (ASAT) and serum alkaline phosphatase (SAP) activity levels following the exposure of the coal fly ash to the rats are presented in Fig. 1(a & b).

From the results as observed in the primary study (Fig. 1a), an insignificant (P> 0.05) increase in ALAT level was recorded in both group I & III rats, with only group II showing a slight significant (P< 0.05) increase when compared to the control group. Similarly, a significant (P< 0.05) increase was also observed in ASAT level in groups I and II rats, while an insignificant (P> 0.05) increase was recorded for group III when compared to the control group. The group exposed to 500 mg/kg body weight of the sample, produced the least ASAT concentration when compared amongst the treatment groups. In the case of SAP level, an insignificant (P> 0.05) increase was observed in all the treatment groups compared to the control group, with the highest value recorded in group II rats when compared to the other treatment groups. The effects of the coal fly ash on the livers enzymes activity levels following the 14 days' exposure were observed not to be dose dependent.

Furthermore, the values obtained on the 28^{th} day (secondary study) as shown in Fig. 1b following the administration of the coal fly ash showed an insignificant (P> 0.05) increase in ALAT concentrations in all the treatment groups when compared to the control group, with the highest value recorded in group III rats when compared amongst the treatment groups. An insignificant (P> 0.05) increase in ASAT levels were also observed in all the treatment groups when

compared to the control group, with the least result observed in group II rats when compared to the other treatment groups. Similarly, an insignificant (P> 0.05) increase were also observed in SAP levels when compared to the control group. Group exposed to 200 mg/kg body weight of the sample produced the highest effects in SAP level when compared to groups I and III animals.

3.3 Effects of Coal Fly Ash on TB, TP and ALB Concentrations

The values obtained for serum total bilirubin (TB), total protein (TP) and albumin (ALB) concentrations are presented in Fig. 2 (a & b). As observed in the primary study (Fig. 2a), a significant (P< 0.05) increase in TB levels were recorded in groups II and III rats, while an insignificant (P> 0.05) increase was observed in groups I rats, when compared to the control group. The highest TB concentrations were observed in group II when compared to group I and III rats respectively. A significant (P< 0.05) decrease was also observed in TP levels in all the treatment groups when compared to the control group, with the least value recorded in group I rats when compared to the other treatment groups. Furthermore, significant (P< 0.05) decrease were observed in ALB levels in all the treatment groups with the highest value recorded in group III rats when compared to groups I and II rats respectively.

In the secondary study (Fig. 2b), a significant (P< 0.05) increase was observed in serum TB levels in all the treatment groups when compared to the control group. The highest value was observed in group II rats when compared amongst the treatment groups. A significant (P< 0.05) decrease was observed in serum TP level in all the treatment groups when compared to the control group with the group exposed to 500 mg/kg body weight of the sample recording the highest value when compared amongst the treatment groups. Furthermore, a significant (P< 0.05) decrease were also observed in ALB level in all the treatment groups when compared to the control group. Group III rats showed the highest ALB concentration when compared between the treatment groups.

3.4 Effect of Coal Fly Ash Sample on Serum Electrolytes

The values obtained for the following blood electrolytes sodium (Na^+), potassium (K^+), chloride (Cl^-) and bicarbonate (HCO^-_3) ions concentration are presented in Fig. 3(a & b). The results from the primary study (Fig. 3a) showed an insignificant decrease (p>0.05) in sodium ion concentrations in groups I and II while group III produced an insignificant increase (p>0.05) when compared to the control. A similar insignificant (p>0.05) decrease in K^+ ion level was also observed in the treated animals when compared to the control rats, with the highest effect recorded in the group exposed to the highest dose. Furthermore, a general insignificant decrease (p>0.05) was also observed in the chloride and bicarbonate ion concentrations on the administration of coal ash to albino rats when compared to the control animals.

In the secondary study (Fig. 3b), an insignificant decrease (p>0.05) in Na^+ and K^+ ion concentrations was observed in the treated groups when compared to the control animals. The highest Na^+ ion level was recorded in group II rats, while group III rats produced the highest K^+ ion concentrations. Similarly, a significant decrease (p<0.05) in Cl^- ion level was observed in groups I and II rats, while an insignificant decrease (P>0.05) were observed in group III rats when compared to the control group. The albino rats exposed to 500/kg body weight of coal fly ash, showed a slight significant decrease (p<0.05) in HCO_3^- ion level, while, an insignificant decrease (p>0.05) were observed in groups I and II rats when compared to the control group. The group exposed to the lowest dose, produced the highest HCO_3^- ion concentration when compared to other treated animals. The effects of the coal fly ash on these blood electrolytes were observed not to be dose dependent.

3.5 Effect of Coal Fly Ash on the Tissues

The postmortem examination of the H & E tissue sections of the liver, kidneys, lungs and the intestine following the administration of 100, 200 and 500 mg/kg body weight of coal fly ash are presented in Fig. 4(a –o).

The H & E microgram of the control rats as shown in Fig. 4(a) shows normal central vein (CV) and hepatic cells (arrow) in the liver, while mild cloudy degeneration of hepatocyte was observed in the liver following the 14 days of coal fly administration (Fig. 4g). Further histological evaluation of the excised tissues in the primary study did not reveal any histological changes under light microscopic study in the kidney (Fig. 4i) and spleen (Fig. 4g) of the treated animals

when compared to the control groups. Similarly, in the primary study, severe pneumonitis (W) of the lungs was observed in the treated groups (Fig. 4h) when compared to the control (c) rats showing normal bronchiole (B) and alveolus (AL). In the intestine, the coal fly ash induces eosinophilic enteritis into the lamina propria (A) with necrosis of the epithelial lining of the villi (V) in the treated groups (Fig. 4.j) when compared to the control rats showing normal lamina propria (R) and villi (V).

In the secondary study following the 28 days of sample administration, the histology of the liver of the albino rats exposed to coal fly ash shows a mild cloudy degeneration of hepatic cells (arrow) and congested central vein (CV) under light microscope (Fig. 4k). Similarly, the histology of

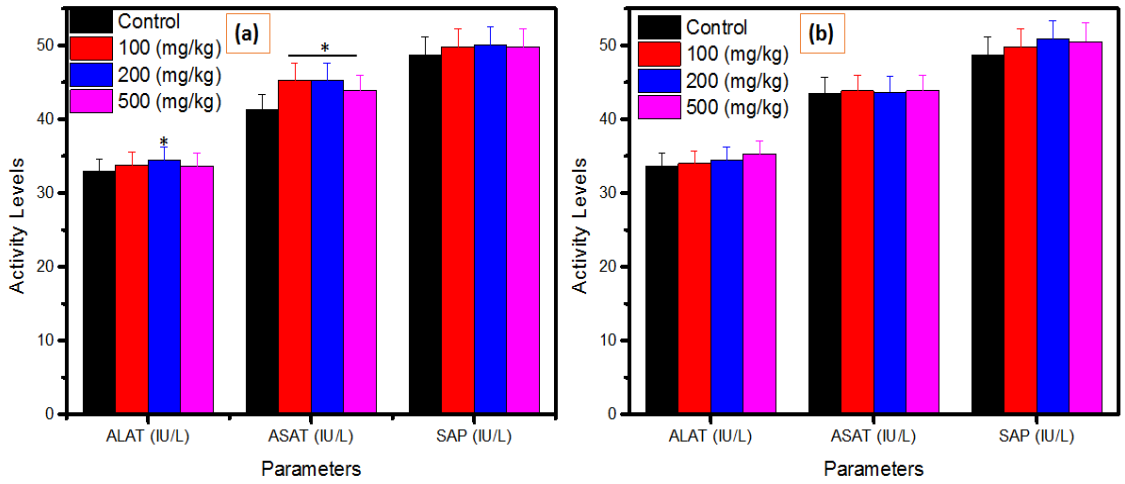

Fig. 1. Mean serum ALAT, ASAT and SAP concentrations of albino rats exposed to different doses of coal fly ash, analysed following (a) 14th days and (b) 28th days of sample administration

*Results is presented as mean ± SEM. From the figure, * corresponds to significant (p<0.05) values*

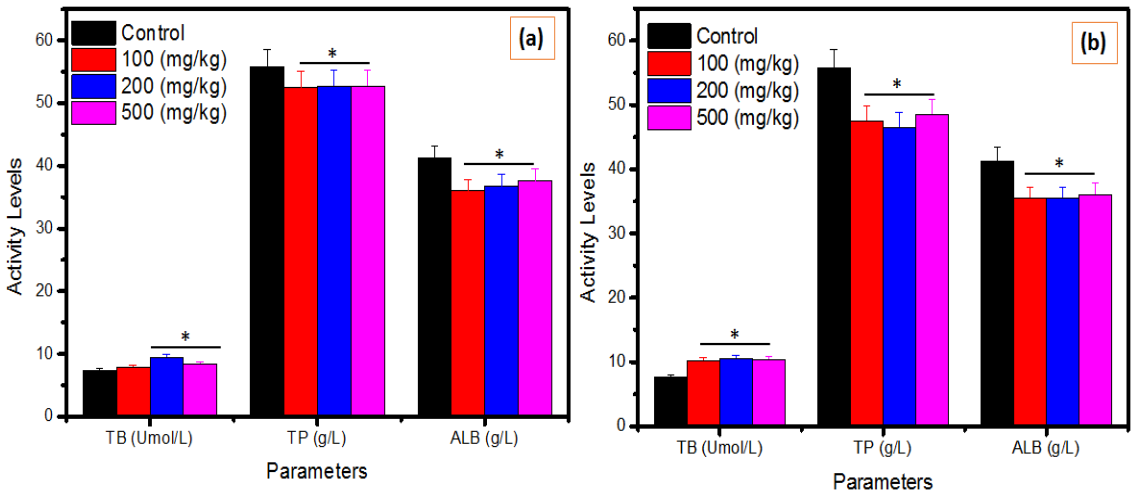

Fig. 2. Mean serum TB, TP and ALB concentrations of albino rats exposed to different doses of coal fly ash, sample, analysed following (a) 14th days and (b) 28th days of sample administration.

*Results is presented as mean ± SEM. From the figure, * corresponds to significant (p<0.05) values*

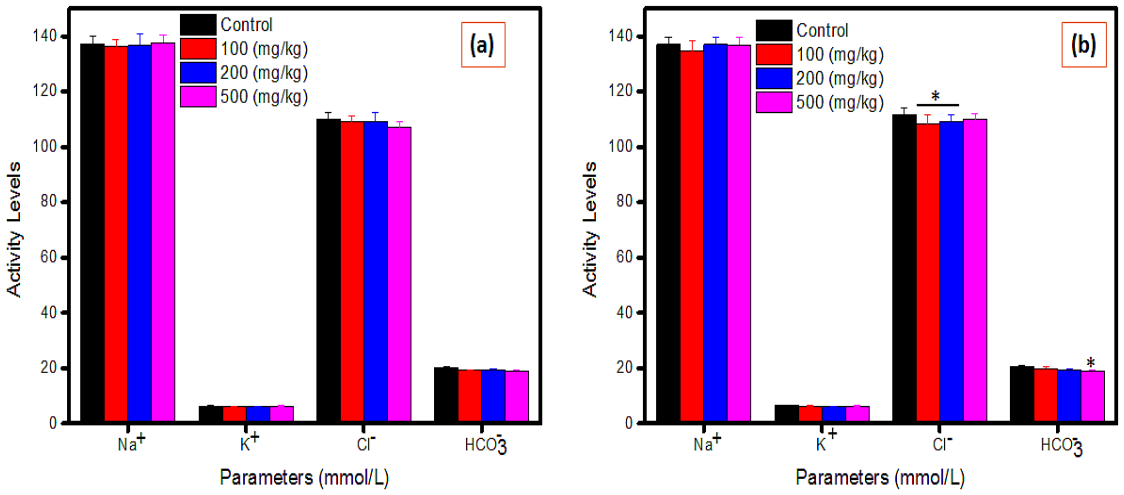

Fig. 3. Mean serum Na^+, K^+, Cl^- and HCO^-_3 ions concentration of albino rats exposed to different doses of coal fly ash, sample, analysed following (a) 14th days and (b) 28th days of sample administration.

*Results is presented as mean ± SEM. From the figure, * corresponds to significant ($p<0.05$) values*

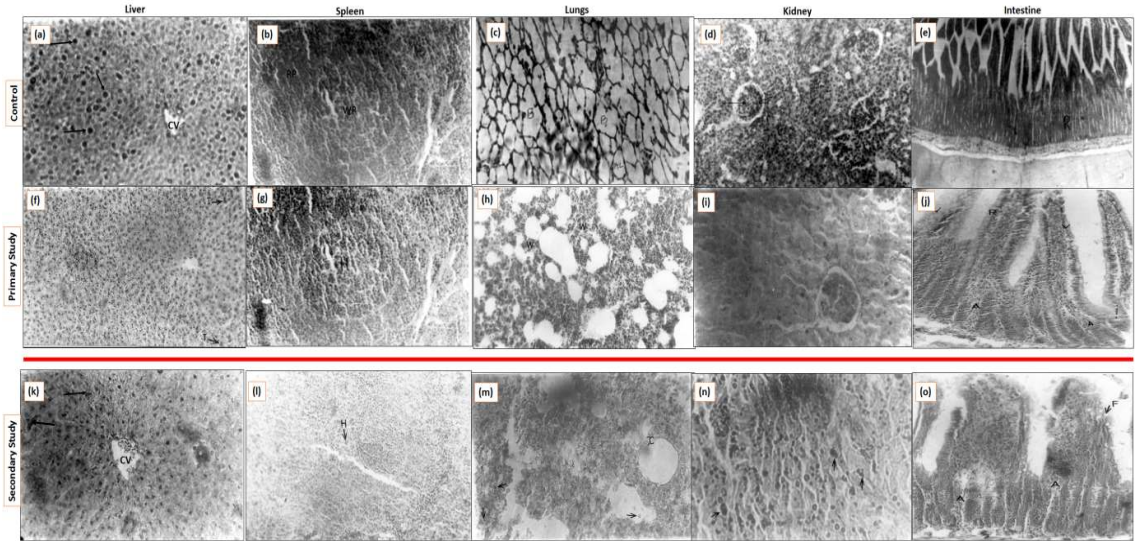

Fig. 4. Light micrograph of tissue section of liver, spleen, lungs, kidney and intestine of Albino Rats exposed to coal fly ash sample. Showing the control (a-e), the results from the primary study (f-l), and the results from the secondary study (k-o). H and E stain. (X 200)

the spleen, showed eosinophilic splenitis with marked infiltration of eosinophils (H) into the red pulp (Fig. 4l). Furthermore, severe pneumonitis and bronchitis with the degeneration of the bronchial wall (X) and mild infiltration of mononuclear (arrow) cells were observed in the lungs of the treated animals (Fig. 4m). The kidney showed, focus of tubular degeneration and necrosis (arrow) in the treated animals (Fig. 4n), while the intestine showed eosinophilic enteritis (A) and goblet cell hyperplasia (arrow) in the treated rats, (Fig. 4o).

4. DISCUSSION

The presence of both organic and inorganic substances in coal fly ash as relates to several findings contributes immensely to a number of anthropogenic activities [6,12-14]. These particulates or elements find their way via

leaching or direct inhalation/ingestion and based on some non-specific binding chemistry with cell membrane and proteins matrixes induces both transient/or pronounced disturbances in the normal metabolic activities of a biological systems [5,14,15].

In this study, based on the light microscopic investigation, the liver of the treated rats when compared to the control was observed to be mildly affected by the coal fly ash. A cloudy degeneration of the hepatocytes was observed in both study periods. Despites the pathological lesion induced by the coal fly ash in the hepatic cells, no remarkable abnormalities were observed in the liver enzymes activity levels. This finding is in deviation from the results obtained from the previous study where the coal fly ash prepared under burning temperature of 500ºC was seen to have triggered slight significant disturbances (not dose dependent) in the liver enzymes in some of the treated groups [11]. The cloudy degeneration of the hepatocytes could be a reversible effect due to the regenerative tendency and functional reserve of the hepatic cells to detoxify both endogenous and exogenous substances [16–18]; hence the insignificant changes recorded in the liver enzymes activities. Liver cells are highly resilient with an exceptional versatility, except otherwise overwhelmed, the estimation of the presence or absence of hepatic mal-function is compelled by the tremendous functional reserve of the liver to terminates radical toxicity induce potentials [19, 20-24].

Similar to the observation recorded following the exposure of coal fly ash prepared under burning temperature of 500ºC to Albino rats on the serum TB, TP and ALB activity levels [11]; a comparable effects was also observed on exposure of the coal fly ash prepared under the burning temperature of 900ºC. In this study, following the administration of the coal fly ash prepared under burning temperature of 900ºC, a significant ($P< 0.05$) decrease in the serum TP and ALB were observed on exposure to the rats. This characteristic decrease, though observed not to be dose dependent could be attributed to the histopathological changes observed in the intestine and kidney. Eosinophilic infiltration, goblet cells hyperplasia and necrosis of epithelial lining of the villi in the intestine might result in the mal - absorption and or loss of protein in the urine, which could impair the proper absorption of protein across the intestinal membrane resulting in hypoproteinemia and hypoalbunemia. This observation falls in line with the suggestions that hypoproteinemia and hypoalbunemia could arise due to (a) impaired synthesis or poor protein intake, (b) increased catabolism because of tissue damage and infiltration, (c) reduced absorption of amino acids caused by mal absorption Syndrome, (d) protein loss in urine due to renal damage as observed in this study and (e) altered distribution that may sequester large amount of albumin and protein, in the estravascular compartment (edema) [24].

Furthermore, the significant ($P< 0.05$) increase in total bilirubin levels could be ascribed to the morphological changes induced by the coal fly ash in the spleen of the Albino rats. The H & E sections of the spleen shows eosinophilic splenitis with marked infiltration of eosinophils into the red pulp which could probably be the reasons behind the raise in the TB levels. The trace element or metabolites present in the fly ash that easily binds to proteins following non-specific chemistry are reported to have the capacity to catabolize the heme-moity in the senescent erythrocytes. Thus, initiating possible displacement of bilirubin from albumin; releasing CO, bilirubin and ferric iron in the process [25–27].

The observed tubular degeneration and necrosis in the kidney of the experimental rats exposed to the coal ash samples may point directly to the activities of the trace elements or substances found in the coal ash samples. The histological lesion induced by the fly ash on the kidney didn't triggered significant ($p>0.05$) turbulence in the serum electrolytes activities. The liver in this case might have played a detoxifying role in reducing the toxicity potentials of the substances in the coal fly ash. This process allowed to a large extend the excretion of the already detoxified substances by the kidney, with minimum impairment to the normal electrolyte ion exchange in the distal tubule [28,29]. Hence, the insignificant activity in the electrolyte levels recorded in this work.

As described in our previous work, where coal fly ash burned at 500ºC was observed to have induced pathological changes in the lungs [11]; in this study, the coal fly ash prepared under a burning temperature of 900ºC was observed to trigger similar lung injury in all the experimental groups irrespective of sample dose. Based on the microscopic investigation, pneumonitis and bronchitis with infiltration of mononuclear cell into

the lumen were observed in the lungs of the albino rats. The elements in the coal fly ash through a gaseous-blood exchange processes in the lungs [30] are translocated and transformed following cyclic oxidation and reduction chemistry into reactive species or secondary metabolites [31,32]. This species or metabolites in their reactive forms bind covalently to DNA inducing lung injury in the process [32-34].

Contrary to the observation made by some researchers [15, 34,35], the coal fly ash collected at 500ºC as described in our previous study [11] and the ash sample burned at 900ºC used in this present study were observed to show similar effect on both the biochemical indices and the H & E tissue sections of the treated animals. These observations therefore suggested that, the observed biochemical effects and pathological lesion were independent of the temperature at which samples were burned. This observation agreed with other findings [36], which suggested that the biological reactivity of fly ash could be attributed, more probably to the combustion efficiency rather than coal type and fly ash sampling temperature. Cytotoxicity of the coal fly ash as suggested and reported in another study [37], to be due to the physical nature of the particles and/or presence of inorganic compounds. While pneumoconiosis, as observed in this study could be due to (a) the amount of dust retained in the lungs and airways, (b) the size, shape and buoyancy of the particles, (c) particle solubility and physiochemical reactivity [26]. Therefore, it could be said that, the reactivity of the coal fly ash though independent of the fly ash sampling temperatures might be attributed to the ability of both the organic and inorganic constituents in the coal ash to initiate and induce the formation of free radicals particularly activated reactive oxygen species, by Fenton like reaction [6,35].

5. CONCLUSION

Fly ash generated from the Nigerian bituminous coal prepared under a burning temperature of 900^0C was observed to produce similar biochemical and histological effects in the rats *in vivo*, irrespective of the sample dose. The biochemical indices showed that ALAT, ASAT and SAP were not significantly increased, while, a significant difference was observed in TB, TP and ALB concentration in both study periods when compared to the control groups. Similarly, the fly ash used in this study induced some pathological lesion in the rat's tissues.

Though, the results did not provide significant difference in the liver enzymes and serum electrolytes chemistry in both study periods, the study however shows a mild degeneration of hepatic cells and tubular necrosis in the kidney. Which further suggest that the Nigerian bituminous coal fly ash induce mild time-dependent effects in the liver, spleen and kidney, but profoundly induce significant time-dependent changes histologically in the excise tissue of the lungs and intestines.

From the outcome of the study, it could be said that, the toxicity of the coal fly ash could be harmonized as a direct response owed to the physical nature of the particles and / or presence of inorganic compounds. While the pneumoconiosis as observed in this study could be attributed to (a) the amount of dust retained in the lungs and airways, (b) the size, shape and buoyancy of the particles, (c) particle solubility and physiochemical reactivity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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