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Research Article

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In vivo Toxicity Evaluation of Nigerian Bituminous Coal Fly Ash Following Repeated Administration to Albino Rats

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Abstract

Here, we investigated the biochemical profiles and in vivo toxicity of the Nigerian bituminous coal fly ash following 14 and 28 days of sample administration. The study was in response to the recent interest to diversify the energy generating sources in Nigeria and the proposed policy to explore the vast coal deposits to meets the gap. In the study, the biochemical indices showed a variable effect in the serum liver enzymes activity levels. The ALAT and SAP levels were observed to be mildly altered, while, an appreciable increase was recorded in ASAT concentrations. Similarly, a significant difference was observed in TB and ALB concentration in both study periods with less activity recorded for TP levels when compared to the control groups. The results further showed an insignificant activity levels in the serum electrolytes in both study periods. 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, haemorrhage in the kidney, pneumonitis in the lungs and wide spread goblets cells hyperplasia in the intestine. Despite the vast deposits of coal in Nigeria and the ongoing interest to explore same, these findings further support the rising concerns associated with the use and utilization of coal for energy generation and called for a holistic review of the policy with regards to the contribution of coal fly ash to anthropogenic activity.

Keywords: Coal fly ash; Bituminous coal fly ash; Serum biochemistry; Albino rats

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INTRODUCTION

Despite the global emphasis toward exploring green sources of energy, coal, relative to its abundance in nature remain integral parts in the global energy market [1]. World production of coal is about 3.5×10^{-9} Mta^{-1}, with an estimated 550 Mta^{-1} of fly ash generated from coal combustion processes in thermal power plants around the world [2,3]. In Nigeria, the exploration of the vast coal deposits is still at the infancy stage. Of recent, due to the fall in energy sufficiency, the Nigerian government has unveiled an energy program which includes among many, the utilization of the vast coal deposits to meet her energy demands production [4-6]. Based on the 2003 national energy policy in Nigeria, coal deposit was estimated to be about 639 million tonnes, consisting largely of ~49% subbituminous, 39% bituminous, and 12% lignitic coals [7,8]. However, while meeting the energy sufficiency, critical review into the environmental associated risk should form part of the entire efforts. Burning of coal and other fossil-based fuel contributes significantly to total lifecycle of greenhouse gases emissions and radiative forcing [9,10]. During the combustion of coal as energy source, over 70% is burned to produce electrical energy, firing of ceramic products and other by-products (fly ash, boiler slag and bottom ash) used mainly in the production of pavement materials [11]. Coal is sometimes carbonised to produce coal gas, coke, ammonia, coal tar and light-oil

products, from which an enormous number of chemical products and fly ash are produced along other effluents [12,13]. The major elemental compositions of coal fly ash consist mainly of silicon, aluminium, iron, calcium and the presence of some polycyclic aromatic hydrocarbons [14-19].

The ash contents value of coal sample depends on the combustion temperature at which the coal sample is burned [20]. The percentage ash content of the Nigerian bituminous coal sample burned at 500°C was reported to be about 10.7% [16,19,21]. More than 150 million tons of fly ash is produced annually worldwide from the combustion of coal in power plants, generating ~ 4–10 times more ash in the process [22,23]. The tunes of ash generated during the combustion processes are in most cases disposed of-landfill, thus, contributing to short or long term toxic effects when present above specific levels in the environment [24].

Though, use of electrostatic precipitator or a fabric filter reduces fly ash inhalation in power plants, this control-approach was however, observed to be limited in case of fly ash with an aerodynamic diameter in the range 0.1-1 µm [25]. As such, fly ash with smaller aerodynamic diameter could navigate over a long distance and easily extravasate into the body by inhalation, ingestions and via leaching into water sources [26-29].

Some researchers investigated the effects of Nigerian bituminous coal fly ash in some haematological parameters [21]. However, no available efforts made to relate the biochemical indices with an *in vivo* histological findings. Furthermore, because of the differences in the composition of the coal fly ash, the temperature at which the ash is formed and the route of ingestion/accumulation; the physiological effects varies. Therefore, the purpose of this study was to investigate the *in vivo* effect of the Nigerian bituminous coal fly ash prepared under ashing temperature 500°C; by examining the effects under various dose concentrations based on some measured biochemical parameters and histology of the rats. However, in this manuscript, we hereby report our findings based on the serum biochemistry of the animal administered the coal fly ash. The study was conducted with respect to the following substances: Aspartate amino transferase (ASAT), Alanine amino transferase (ALAT), Serum Alkaline phosphatase (SAP), Total bilirubin (TB), Total protein (TP) and Albumin (ALB). The post-mortem examination was conducted on the excise tissue section of the liver, lungs, spleen, kidney and the intestine based on H & E technique. Though, the results generated from this study based on the measured biochemical parameters shows mild alteration in the biochemical indices when compared to the control. These changes however, provide us with some insights into the underlying effects and susceptibility of coal fly ash exposure *in vivo*.

MATERIALS AND METHODS

The bituminous coal sample used for this study was obtained from Omarako coalmines, Enugu State in Nigeria, made available by the National Metallurgical Development Center (NMDC), Jos and Plateau State. To obtain the desired coal ash sample, the dried pulverised coal sample was burned at 500°C for about 3 hours in an oven (Gallen Kamp, England). The ash samples were size fractionated using a mesh sieve (Endencott, England) of size range of 4 -5 µm. Following the ashing processes, a stock solution (300 mg/ml) of the sample were prepared by adding 3 g of the ash sample into 10 ml of phosphate buffered saline solution (PBS, pH 7.4 ± 0.2).

Animals and Treatment

Male albino rats of Wistar strain weighing 70-200 g obtained from the Wistar rat colony of the animal house of the University of Jos were used for the study. The rats were normal and free from any pathogen condition. They were fed with grower's mash (ECWA feeds Nigeria limited) and provided with water ad-libitum. All the experiments using animal models were conducted under the regulations set by the University of Maiduguri (Unimaid) ethics committee's guidelines for the care of laboratory animals.

In the experiment, a total of 42 rats were used. The rats were further divided into 3 sub -(A_1, A_2, A_3) groups of 12 rats each. The rats in the first group (A_1) were administered orally (by intubation) 100 mg/kg body weight of the ash, the second group (A_2) was administered 200 mg/kg body weight and the third group (A_3) 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. The control rats were administered buffered saline solution only. In the experiment, a total of 6 rats per group were sacrificed by cervical dislocation under anaesthesia on the 14^{th} day of treatment (primary study), while the remaining 6 rats in each group were sacrificed on the 28^{th} day of treatment cycle (secondary study).

Serum Biochemistry Analysis

At the end of treatment cycles, the rats were anesthetized and euthanized by cervical dislocation on days 14 and 28 of coal ash administration. The blood samples were collected from the jugular veins in the necks by cervical incision. Blood samples were centrifuged at 12,000 rpm for 5 minutes (Hettich Universal 11, Zentrifugen 72 Tuttlugen, Western Germany). The clear serum was analysed for Aspartate amino transferase (ASAT) and Alanine

amino transferase (ALAT) by Reitman and Frankel, (1957) methods. Serum alkaline phosphatase (SAP), by Klein et al, method. Total bilirubin (TB) was measured by Malloy and Evelyn, (1937) method. Total protein (TP) was analysed by a modified biuret method [30,31]. Serum Albumin (ALB) was by Doumas and Biggs, (1972) method. The Serum electrolytes, sodium (Na^+) and potassium (K^+), were measured by flame emission photometry method, while titrimetric method was used to determine bicarbonate (HCO^-_3) ions [32,33], and chloride Cl^- ion by Schales and Schales. The analyses of all the biochemical parameters were carried out at the chemical pathology laboratory of the University of Maiduguri Teaching Hospital, Maiduguri.

Histological Analysis

The samples of liver, spleen, kidney, intestine and lung were collected in 10% buffered formalin for post mortem histological processing and examination based on haematoxylin and eosin (H & E) techniques. Light microscopic investigation of the tissues was conducted to examine any histological changes.

Statistical Analysis

The data was expressed as Mean ± SD. One-way ANOVA and student t-test was performed and the significance was set at $p<0.05$.

RESULTS AND DISCUSSION

The oral administration of the coal fly ash to the animal showed slight clinical changes in both the primary and secondary studies. The common clinical signs observed included restlessness and sneezing with profuse nasal discharge. Loss of appetite and slight drop in body weight was also observed towards the end of the treatment period. No death was recorded during the period of administration.

Effects of Coal Fly Ash Sample on the Liver Enzymes Activities

The values obtained for the activities of the liver enzymes, alanine amino transferase (ALAT), aspartate amino transferase (ASAT) and serum alkaline phosphatase (SAP) are presented in Figures1 and 2.

Primary study:

Except for group II, which produced an insignificant (P>0.05) increase in the serum ALAT concentration when compared to the control group, both group I and III rats showed significant (P<0.05) increase in the ALAT levels when compared to the control group. Furthermore, a significant (P<0.05) increase was observed in ASAT level in all the groups compared to the control group. Group II produced the highest level, when compared with groups I and III respectively. Consequently, insignificant (P>0.05) increase in serum SAP level were observed in all the treatment groups compared to the control group. Group II produced the highest concentration in SAP level, compared to groups I and III animals.

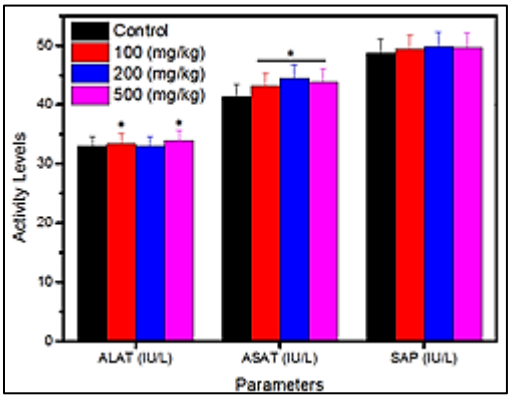

Figure 1: Mean serum ALAT, ASAT and SAP concentrations of albino rats exposed to different doses of coal fly ash sample, analysed following 14th days of sample administration

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

Secondary study:
A significant (P<0.05) increases in ALAT levels were observed in group I and III, while group II showed an insignificant (P>0.05) increase compared to the control group. The highest ALAT values were recorded in group III rats, when compared with other treatment groups. Aspartate amino transferase (ASAT) concentrations increased significantly (P<0.05) in groups I and II rats compared to the control group. Group III produced the lowest ASAT value when compared between the treated groups. Furthermore, an insignificant (P>0.05) increase was also observed in SAP concentration in all the treatment groups compared to the control group. The group exposed to 500 mg/kg body weight of the sample solution produced the highest SAP concentration, when compared with groups I and II animals.

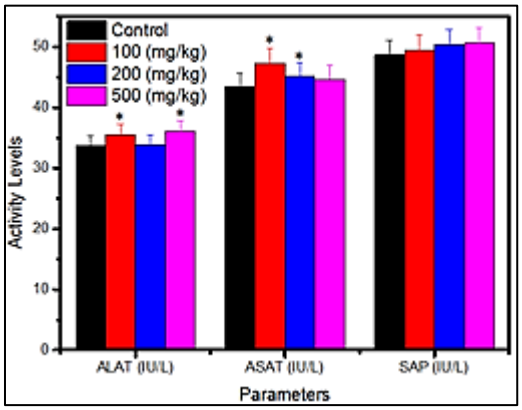

Figure 2: Mean serum ALAT, ASAT and SAP concentrations of albino rats exposed to different doses of coal fly ash sample, analysed following 28th days of sample administration

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

Effects of Coal Fly Ash Sample on TB, TP and ALB Concentrations
The result obtained for total bilirubin (TB), total protein (TP) and albumin (ALB) concentrations on administration of 100, 200 and 500 mg/kg body weight of sample A solution are presented in Figures 3 and 4.

Primary study:
A significant (P<0.05) increase in serum TB concentrations were observed in groups II and III rats compared to the control group, while an insignificant (P>0.05) increase were observed in group I compared to the control group. Group II rats produced the highest TB concentration, when compared to groups I and III animals respectively. A significant (P<0.05) decrease was also observed in serum TP concentration in all the treatment groups compared to the control. Group III rats produced the least result, compared to groups I and II. Significant (P<0.05) increase in serum ALB concentrations were also observed in all the treatment groups compared to the control group. The group exposed to 100 mg/kg body weight of the sample solution produced the highest ALB concentration when compared with the other treatment groups.

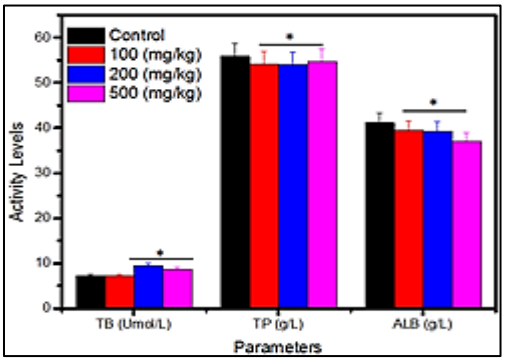

Figure 3: Mean serum TB, TP and ALB concentrations of albino rats exposed to different doses of coal fly ash, sample, analysed following 14th days of sample administration

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

Secondary study:

A significant (P<0.05) increase in serum TB were observed in all the treatment groups compared to the control group. The group exposed to 500 mg/kg body weight of the sample solution produced the least result, when compared to the other treatment groups. Similarly, a significant (P<0.05) decrease were observed in serum TP level in all the treatment groups when compared to the control group. Group II rats produced the least result in TP concentration when compared to the other treatment groups. In the same vain, a significant (P<0.05) decrease in serum ALB level were observed in all the treatment groups when compared to the control group. The highest ALB concentrations were recorded in group II rats when compared to the other treatment groups.

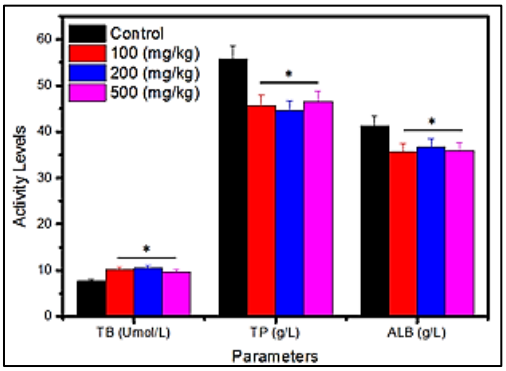

Figure 4: Mean serum TB, TP and ALB concentrations of albino rats exposed to different doses of coal fly ash, sample, analysed following 28th days of sample administration

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

Effect of Coal Fly Ash Sample on the Blood Electrolytes

The results obtained for the blood electrolytes sodium (Na^+), potassium (K^+), chloride (Cl^-) and bicarbonate (HCO^-_3) ions concentrations on administration of 100, 200 and 500 mg/kg body weight of sample A, are presented in Figures 5 and 6.

Primary study:

The effects of coal fly ash were observed to show an insignificant decrease (p>0.05) in sodium ion concentrations in the treated groups when compared to the control group. The group exposed to the highest dose produced the least effect. Similarly, the coal fly ash were observed to show an insignificant decrease (p>0.05) in K^+ ion level in the treated groups when compared to the control rats. The least effect was observed in group I rats when compared with other treatment groups. Furthermore, an insignificant decrease (P>0.05) in chloride and bicarbonate ion concentration were observed in the treated animals when compared to the control groups, with the groups exposed to the lowest dose, producing the highest effects, when compared to the other treated groups.

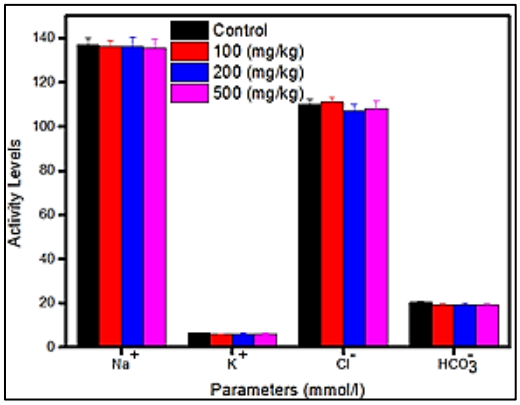

Figure 5: Mean serum Na^+, K^+, Cl^-, and HCO_3^- concentrations of albino rats exposed to different doses of coal fly ash, sample, analysed following 14th days of sample administration

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

Secondary study:
An insignificant decrease ($p>0.05$) in Na^+ ion level was observed in the experimental animals. The results were observed not to be dose dependent when compared between the treated groups. Similarly, an insignificant decrease ($p>0.05$) was also recorded in K^+ ion level in the treated animals when compared to the control rats. The chloride ion concentrations in the treated animals, showed a significant decrease ($p<0.05$), in group I rats and an insignificant decrease ($p>0.05$) in groups II and III rats, when compared to the control group. The results were observed not to be dose dependent when compared amongst the treated groups. However, a significant decrease ($p<0.05$) in HCO^-_3 ion level were recorded in all the groups, when compared to the control group.

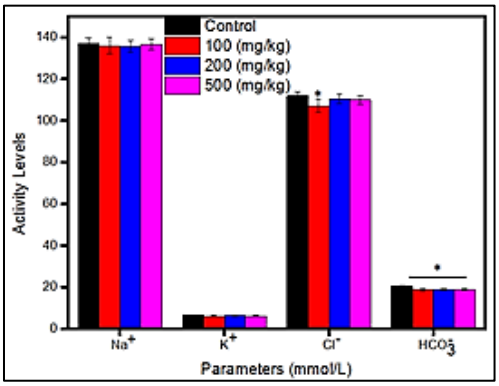

Figure 6: Mean serum Na+, K+, Cl-, and HCO_3^- concentrations of albino rats exposed to different doses of coal fly ash, sample, analysed following 28th days of sample administration

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

The Effect of Coal Fly Ash on the Tissues
The histology of the liver, kidneys, lungs and the intestine following the administration of 100, 200 and 500 mg/kg body weight of the sample are presented in Figure 7.

Primary study:
The albino rats exposed to the coal fly ash did not reveal any histological changes in the liver and spleen in all the treated groups, when observed under light microscope. The liver when compared to the control groups shows normal hepatocytes (T) and central vain (CV) and similarly, the spleen when compared to the control groups shows a normal red pulp (RP). The histology of the lungs tissues in all the treated animals, showed pneumonitis (arrow), characterized by infiltration of mononuclear cells. In the same vain, the histology of the control groups shows normal alveolus (AL) and bronchiole (B). Furthermore, hemorrhage in the cortex (arrows) was observed in the kidney on exposure of the coal fly ash to albino rats in all the treated groups, with the control groups showing normal tubular lumen (TL), tubular epithelium (TE) and renal corpuscles (RC). However, when compared to the control groups which shows normal mucosal villi (V) and lumina propria (P), the epithelium of the intestine of the albino rats in all the treated groups, showed goblets cell hyperplasia.

Secondary study:
Under light microscopic study, wide spread cytoplasmic vacuole degeneration of the hepatocyte (arrow), were observed in the liver of the treated rats in group. While, mild spleenic hyperplasia (V) in the red pulp was observed in the histology of the spleen of the experimental animals. Furthermore, severe bronchitis with infiltration of mononuclear cells into the lumen and the wall of a bronchiole (arrow), and necrosis of bronchial epithelium lining and bronchial cartilage (X) were observed in the lungs of all the treated animals. And similarly, hemorrhage in the cortex (arrow) was observed in the kidney in all the treated animals. Necrosis of the epithelial cells of the villi, marked goblets cell hyperplasia (arrow) and eosinophilic infiltration of the lamina propria (R) were observed in the intestine of the group exposed to the coal fly ash.

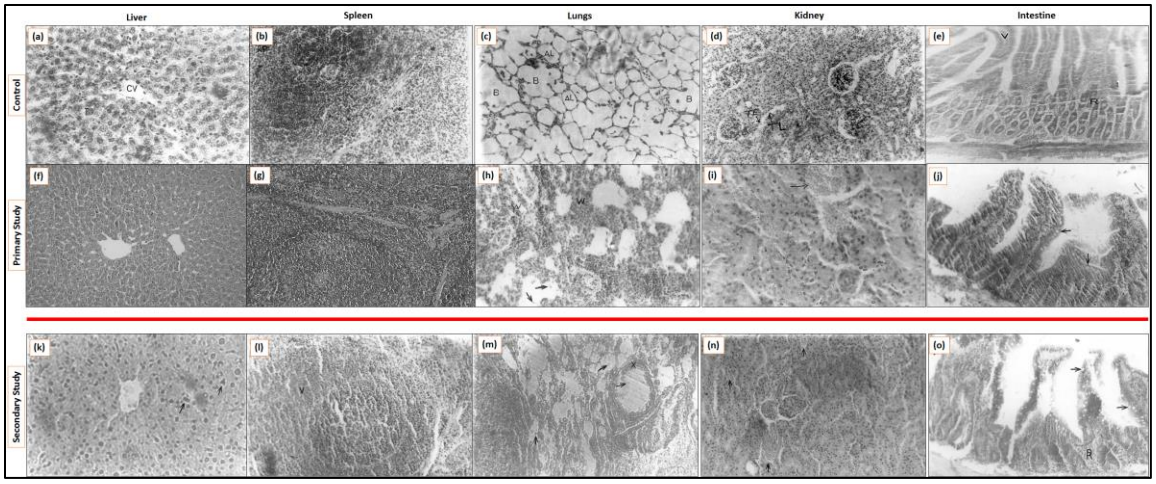

Figure 7: Light micrograph of tissue section of liver, spleen, lungs, kidney and intestine of Albino Rats exposed to coal fly ash sample. Showing the control (a-e), the results from the primary study (f-l), and the results from the secondary study (k-o). H and E stain. (X 200)

DISCUSSION

Coal fly ash is reported to contain large number of organic and inorganic elements or compounds [14-16,19,21] which are reported to interact with many biological systems and impede their normal metabolic activities [34]. The physicochemical characterization of the Nigerian bituminous coal fly ash collected at 500°C temperature as documented by Ogugbuaja and James, contains some trace elements. And are reported to contribute to atmospheric concentrations of particulate matter and gaseous pollutants such as SO_2 and NO_2 [35]. This elements or particulates are reported to be readily absorbed after ingestion or inhalation and the primary site of their toxicity are in regions where metabolism or excretion of toxic metabolites frequently occurs [36]. Thus, the fate of these particulates in relation to their non-specific binding to cell membrane and intracellular proteins are found to be actively involved in facilitating the formation of ROS/RNS [37].

Biochemistry measurements especially the liver enzymes often recognized to reflect liver membrane integrity or oxidative stress induction phenomenon, which often initiate transient disturbance in the normal levels of ALAT and ASAT [38-41]. Alanine amino transferase (ALAT) is recognized to be a liver specific enzyme; on the other hand ASAT might be non-specific index because it is distributed not only in the liver but also in the heart, skeletal muscle, kidney, intestine and the brain [41]. It was expected that the coal fly ash could trigger a severe imbalance in the liver enzymes integrity, this study however, shows only a slight significant ($P<0.05$) increase in the concentrations of the serum ALAT in rats of group I and III following the 14 and 28 days of samples administration. Onyeyili et al. reported the bioaccumulation of trace element in the liver of rabbits exposed to coal fly ash sample. These are presumably expected to cause cell injury in the liver and hence an imbalance in the liver enzymes. However, the histology of the liver in the primary study was observed to appear normal under light microscopic investigation. Cornelius, reported that hepatic cell functions may be altered and result in an abnormal value for ALAT and other specific liver function tests even though histological changes may not be obvious because of the rapid regenerative ability of the liver. The rapidly regenerative tendency and functional reserve of the liver to fight toxic elements could be the probable reason for the liver in most of the experimental groups to appear normal despite the little disturbances in the concentrations of ALAT. Contreras-Zentella and Hernández-Muñoz, reviewed that, an increase in serum liver enzymes activities does not necessary have to reflect hepatic malfunction and research shows fraction of the hepatic release of enzymes depends largely on hemodynamic changes in the liver. Except otherwise overwhelmed, the estimation of the presence or absence of hepatic mal function is compelled by the tremendous functional reserve of the liver and its power of regeneration [38-43]. The liver cells produce multiple defence mechanisms against free radicals by secreting enzymes such as superoxidismutase, catalase, glutathione reductase and vitamin A and E which serve as anti-oxidant [44,45], blocking free radical formation and terminates radical toxicity induce potentials [45-48].

Furthermore, a contrary histological result was observed in the liver of the rats exposed to the coal fly ash following the 28 days of sample administrations. The continues administration of the samples for 28 days increased the bioaccumulation and translocation of the particulate in the liver cells [49], resulting to a wide spread cytoplasmic vacuole degeneration of the hepatocyte. The histological changes in the liver observed in the secondary study

compared to the results recorded in the primary study could be due to the continues and repeated administration of the coal fly ash to the subjects [50,51]. Reports shows that rats exposed to particulates for 28 days showed enhanced biochemical activity levels and histopathological changes [50]. This finding further explain the significant alteration in the ALAT levels and degeneration of the hepatocytes observed in the secondary study. Since the expression of ASAT is not liver specific, the significantly higher ($P<0.05$) levels of ASAT observed in all the groups in the primary study and group I and II in the secondary study, in the absence of obvious liver damage as observed in the primary study could be related to possible kidney and intestinal dysfunction caused by the coal ash samples. Furthermore, the insignificant ($P>0.05$) increase in serum SAP levels on exposure to coal fly ash could be from kidney and intestinal dysfunction caused by the coal fly ash samples. Davy et al. reported that the intestine, bone and the kidney are the richest source of SAP, and ASAT is distributed not only in the liver but also in the heart, skeletal muscle, kidney, intestine and the brain [41,52] which may increase when these tissues are damaged.

Further study shows a significant ($P<0.05$) decrease in the serum TP and ALB activities levels following the exposure of coal fly ash to the rats. This characteristic decrease, though observed not to be dose dependent could also be attributed to possible histopathological changes in the intestine and kidney. Relating the activity of ASAT, ASAP, TB and ALB histologically, we observed that the coal fly ash samples administered to the experimental rats show histopathological changes in the intestine. The effect is represented by generalised goblet cell hyperplasia, mild necrosis of epithelial cells of the villi and eosinophilic infiltrations. The effect of the coal ash samples on the intestine may be attributed to the localized activities of the immobilized elements in the coal fly ash. These elements when ingested into the body interact with the carrier protein, probably through the sulfhydril group (-SH), which are embedded in the intestinal membrane causing local irritation in the mucosal lining [53,54]. Specifically, trace elements such as Fe, Cu. Al, As, Cr, and Hg readily bind readily to –SH groups or amino residue and directly or indirectly increase lipid peroxidation processes and impair amino pyrine metabolism, translating to the induction of toxicity. Thus having a direct impact toward inducing intestinal and other tissue damage [48,55,56].

The observed rise in total bilirubin in most of the experimental groups in this study could be from the activities of the heme moiety of the haemoglobin released from senescent erythrocytes that are destroyed in the reticuloendothelial system, notably in the Spleen. Bilirubin may increase in severe haemolytic disease or when proteins binding drugs or metals displace bilirubin from albumin [57]. These suggestions are also supported by Hoffman et al. who reported that, for each mole of heme catabolised, one mole of CO, bilirubin and ferric ions are produced. The albino rats exposed to the coal fly ash were observed to course spleenic hyperplasia. The phagocytic cells of the spleen both free and attached remove foreign particles, bacteria, degenerating leukocytes and erythrocytes and consequently serve as an organ of blood distribution [57,58]. In pathological condition as observed in this work, the spleen is mostly enlarged and usually leads to expansion of the red pulp with increased sequestration and when pronounced leads to premature destruction of the formed elements in the blood, and consequently, accumulation of these product in the spleen [59]**;** the result is known as hypersplenism

Investigation into the activities of the serum electrolytes (Na^+, K^+, Cl^-, and HCO^-_3) concentrations in this study following the exposure of the coal fly ash to albino rats shows an insignificant ($P>0.05$) activity levels in most of treated groups. The effect was observed not to be dose dependent. Logically, the haemorrhage observed in the cortex of the kidney is expected to lead to some disturbance in the serum electrolytes activity, however, the experimental results in this study shows insignificant impairment in their activity levels except in the HCO^-_3 levels observed in the secondary study. One possible explanation in the insignificant results observed in the primary study could be attributed to the biodegradation activity of the ingested samples by the liver. Thus, resulting to the excretion of mostly already detoxified isolated particulates by the kidney, thereby allowing the kidney to carry out an effective electrolyte distribution and normal exchange of Na^+ and K^+ ions for H^+ ion and the conversion of HCO^-_3 ion in the distal tubule. The efficient permeability of the glomerular endothelium [60] ensure rapid urinary elimination of the particulates [61,62], thus, allowing the glomerular capillaries to filter and excrete the isolated particulates from the plasma [63]. However, the continues administration of the coal fly ash samples for 28 days possible overwhelmed the filtration processes of the glomerular capillaries, thus initiating some disturbances in the normal conversion of HCO^-_3 ion and consequently leads to the loss of HCO^-_3 ion due to poor re - absorption by the distal tubule [64-66].

In this study, the Nigerian bituminous coal fly ash was observed to cause lung injury in all the experimental groups irrespective of sample dose. Other related study reported that, rats exposed to fly ash for 28 days showed high concentrations of Cd in lungs, indicating that after absorption in the lungs Cd was transported to these extrapulmonary organs. And further hypothesised that some of the metals present in the fly ash may have being translocated via gaseous-blood exchange processes [51]. Pneumonitis and bronchitis with infiltration of mononuclear cell into the lumen were observed in the lungs on exposure to the Nigerian bituminous coal fly ash to albino rats in this study. The coal fly ash used in this study as reported by Ogugbuaja and James) and Ogugbuaja et

al. contains organic compounds and trace elements. The ability of these elements to participate in free radical formation could be the possible reason for the observed lung injury.
Research shows that, polycyclic aromatic hydrocarbons (PAHs) and trace metals are metabolised by cytochrome P-450 oxidase to secondary metabolites; this metabolites are reported to readily bind covalently to DNA inducing lung injury in the process [67-69]. Kehrer et al. further highlighted that this compound undergoes cyclic oxidation and reduction in the lungs resulting in the generation of excess reactive oxygen species, thereby disturbing normal metabolic activity leading to lung injury and pulmonary edema. Experimental reports showed that particulate matters have a very high mobility to cross the capillary-to-alveolar barrier in rats and mouse favoring the initiation of free radicals [70-73]. The free radicals trigger the alveolar macrophages to release lysosomal enzymes and produce different mediators, which include chemo tactic (cytokine) agent, which consequently initiate tissue injury [68,73-75]. Animals exposed to particulate were observed to have high concentrations of the particle in the pulmonary alveolar macrophages in the alveolar lumen of the lungs [76]. This observation agreed with the histophatological changes observed in the lungs in this study.

CONCLUSION

The results in this study showed that, the Nigerian bituminous coal fly ash sample collected at ashing temperatures of 500°C exerts some levels of toxicity. The biochemical indices showed some degree of disturbances in the level of ALAT and ASAP. Similarly, a significant difference was observed in TB, TP and ALB concentration in both study periods when compared to the control groups. The effects were found not to be dose dependent. The *in vivo* study following the exposure of the Nigerian bituminous coal fly ash shows some changes in the histology of the animal models. Based on the results of this study, it is suggested that particulates from the coal combustion processes acquired active mobility and thus exerted some toxicological responses following the repeated oral administration. The results further highlighted the toxicity potentials of the Nigerian bituminous coal fly ash and thus, emphasize the need to apply safety indices in the exploration and utilization of the bituminous coal deposit in Nigeria

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