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Full Length Research Paper

Evaluation of Benz[A]anthracene-induced pulmonary toxicity in *Rattus norvegicus*

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Benz[a]anthracene is a polycyclic aromatic hydrocarbon (PAH) commonly found in the environment, capable of inducing an inflammatory response that may lead to pulmonary toxicity. Due to a lack of knowledge regarding the signs and pathological damages caused by benz[a]anthracene toxicity, there is a need to investigate its effects in a rat model. The determination of the median lethal dose (LD_{50}) involved nine rats using the up-and-down method, while twenty-four rats were used for the sub-acute toxicity study, divided into four groups of six. The first group received 3 ml/kg of physiological saline daily, and the second, third, and fourth groups were treated with benz[a]anthracene at doses of 12.5, 25, and 50 mg/kg/day, respectively, over a two-week period. Pre-treatment blood samples were collected on days zero (0) and 14 for hematological parameters and oncogenic biomarkers. Additionally, pre-treatment and weekly body weights were measured to calculate physiological parameters, total blood volume, and plasma volume using corresponding formulas. Morbid lung measurements were taken, and tissue samples were evaluated for histological changes. The acute toxicity study revealed that benz[a]anthracene has an LD_{50} of over 5000 mg/kg, although classical behavioral changes were observed at lower administered doses. After 14 days of benz[a]anthracene administration, there was a corresponding reduction in weight gain (-9.91 ± 10.77, 7.58 ± 9.00, 11.42 ± 9.17 and 31.23 ± 5.89% for 50, 25, 12.5 mg/kg, BW (body weight) and control groups, respectively) and inhibition of hematopoiesis with an increase in doses. A dose-dependent increase in CEA levels was observed across the groups (2.26 ± 0.29, 3.29 ± 0.52, 3.86 ± 0.26 and ng/Ml for 50, 25, 12.5 mg/kg and control groups, respectively), along with some dose-dependent gross and histopathological damages to the lung, such as congestion and damaged alveolar sacs with cellular infiltration.

Key words: Benz[a]anthracene, pulmonary toxicity, tumour marker, physiologic parameters.

INTRODUCTION

The environmental impact of any industrial or commercial activity is significant, as it results in the emission of pollutants such as noise, unpleasant odors, and volatile organic compounds, which have detrimental effects on the environment. Furthermore, the contamination of water and soil with hazardous materials, such as oil chemicals

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and hull paint, poses a significant threat to the environment. Additionally, human activities on land and in water have various environmental consequences. Finally, human activities such as transportation, industry, and climate change also contribute to environmental problems (Mesut 2021). Damage to the lungs is called pulmonary toxicity, or lung toxicity, which may be acute or chronic and can lead to pulmonary diseases. These diseases represent undesirable reactions that induce changes in the lungs or alter respiratory function, often caused by agents of medical or non-medical origin. Pulmonary toxicity of medical origin can result from the side effects of medicinal drugs and radiation (radiotherapy), while non-medical causes include exposure to chemical compounds and airborne particulate matter (Zuo et al., 2014; Mukhurjee and Agrawal 2017).

Atmospheric particulate matter, a component of air pollution, is primarily produced by car traffic, industrial production facilities, and cigarette smoking. Cigarette smoke is known to impose an oxidative burden and cause oxidative stress in the lungs (Kluchova and Tkacova, 2006). Pulmonary diseases associated with such toxicity include pneumonitis, lymph node swelling, alveolar hemorrhage, bronchitis, pneumonia, pleural effusion, pulmonary edema, pulmonary fibrosis, pulmonary arterial hypertension, acute respiratory distress syndrome, and very rarely, solitary pulmonary mass and lung failure (Reid and Inness, 2014).

Benz[a]anthracene (B[a]A), a polycyclic aromatic hydrocarbon (PAH), is produced through incomplete combustion of organic matter and has been linked to human cancer (IARC, 1984; U.S. EPA, 1990). Exposure to Benz[a]anthracene and other PAHs primarily occurs through ingestion and inhalation from smoking or second-hand smoke, air pollution with combustion products, or thermal processes in food preparation (such as grilling and roasting) and water pollution with combustion products (Gray and Hall, 2014). B[a]A is a carcinogenic constituent of tobacco smoke (Talhout et al., 2011), and exposure may also occur through the bioaccumulation of Benz[a]anthracene in consumed animals, such as livestock, and exposure to polluted air through exhaust fumes from burning fossil fuels (Wanda et al., 2010; Gray and Hall, 2014).

Given the considerable presence of Benz[a]anthracene in surface and drinking water, smoked foods, and edible aquatic organisms (U.S. EPA, 1987), we found it pertinent to evaluate its pulmonary toxicity in a laboratory animal model. Therefore, this study aimed to assess the extent of pulmonary toxicity in rats through the oral administration of varying doses of Benz[a]anthracene.

MATERIALS AND METHODS

Experimental animals

A total of thirty-six apparently healthy, young adult, male rats weighing 150 ± 15 g were used for the study. The rats were sourced from local breeders in Makurdi, Benue state, Nigeria and kept in perforated plastic cages. They were allowed to acclimatize for a period of two (2) weeks, before the commencement of the experiment. They were fed with standard feed (Grower^{R}) and water was provided ad libitum. The experiments were conducted according to international guiding principles for biomedical research involving animals [C.I.O.MS, 1985], and as recommended by ethical committee of the College of Veterinary Medicine, Joseph Sarwuan Tarka University, Makurdi.

Acute toxicity/limit dose test

The upper limit dose test was adopted to estimate the median lethal dose (LD_{50}) of Benz[a]anthracene [OECD, 2000]. The Lowest Observed Adverse Effect Level (LOAEL) and The No Observed Adverse Effect Level (NOAEL) were also estimated (Katsnelson et al., 2021). The rats were administered Benz[a]A orally using a metallic cannula and at each dose level, three rats were used with a default dose progression of *log* 3.05. B[a]A was administered at first dose of 5000 mg/kg/day followed by 3415, 1830 and 245 mg/kg while observing the possible signs of toxicities and mortality displayed by the rats. The rats were observed for any clinical signs for a period of 2 weeks after administration. Testing was completed after initial reversal in animal outcome and was also terminated when a dosage level per kg body weight was attained without mortality or signs of toxicity (Bruce, 1985; OECD, 2000).

Pulmonary toxicity test

The rats were divided into 4 groups of six rats each. Group one rats were administered only normal saline, while group (II – IV) were treated daily with Benz[a]anthracene orally at 12.5, 25, and 50 mg/kg/day body weight respectively, for a period of two weeks. Pre-treatment blood samples were collected from each of the rats (3 ml) on day zero and thereafter on day 14 for the analysis of haematological parameters. Packed cell volume (PCV), red blood cells (RBC) count and total white blood cells (WBC) count were done as described by CSLI [2000], Pal et al. (2006), CSLI (2007), Praful and Godkar (2003), Cheesbrough (2009) respectively. Serum was harvested from the whole blood for biochemical analysis of tumour marker: CEA using Enzyme Linked Immunosorbent Assay (ELISA) method (Wild, 1994). However; the total blood volume (TBV) and plasma volume (PV) were calculated as described by Lee and Blaufox (1985) and Bijsterbosch et al. (1981) respectively.

$TBV\ (ml) = 0.06 \times BW \times 0.77$
$PV\ (ml) = 0.0291 * BW + 2.54$

where BW = body weight in gram.

The body weights were also used to calculate physiological parameters as described by Schmidt-Nielsen (1964) modified by Saganuwan (2017). Physiological parameters were calculated as follows:

(i) Body mass ratio (Kcal/day): $3.52\ W^{0.75}$, where W = body weight in gram.
(ii) O_2 consumption per kilogram ($L\ h^{-1}\ kg^{-1}$): $0.676 \times M_b^{-0.25}$
(iii) Heart rate (min^{-1}): $241 \times M_b^{0.25}$
(iv) Lung ventilation rate (liter h^{-1}): $20.0 \times M_b^{0.75}$
(v) Lung volume (liter): $0.063 \times M_b^{1.02}$
(vi) Respiration frequency (min^{-1}): $53.5 \times M_b^{-0.26}$, where Mb = body weight in kilogram

The rats were euthanized using 100 mg/kg of pentobarbital (Zatroch at al., 2017, AVMA, 2020). The lungs collected were fixed

Table 1. Toxicity and mortality value of acute toxicity of Benz[A]Anthracene.

Dose (mg/kg)	Benz[a]anthracene	
	Sign of toxicity	Mortality
5000	XXX	OOO
3415	XXX	OOO
1830	XXX	OOO
245	OO	OO

X = present, O = absent.

for histo-pathological examination (Drury et al., 1976).

Statistical analysis

Data generated from the study were expressed as mean ± standard error of the mean (S.E.M) and analyzed statistically using two-way analysis of variance (ANOVA). Significance was considered at $p \leq 0.05$, and Turkey's Post Hoc test was employed for post hoc analysis. The statistical analysis was performed using IBM SPSS version 24 computer statistical software (IBM, 2020).

RESULTS

Limit dose test

Following the administration of Benz[a]anthracene at doses of 5000, 3415 and 1830 mg/kg body weight, various behavioral changes were observed in the rats, including unstable motor activity, tonic extension, depression, stimulation, sedation, breathlessness, gasping, and squeaking. However, at the dose of 240 mg/kg body weight, no abnormal signs were noticed. Consequently, the lethal dose LD_{50} of benz[a]anthracene was determined to be >5000 mg/kg BW, with a probable LOAEL of 1037.5 ± 792.5 mg/kg BW and a NOAEL of 240 mg/kg (Table 1).

Post-mortem examinations revealed varying degree of pathology (Table 2) including pale and enlarged lungs with areas of focal necrosis. Similar pathological lesions were observed in other organs of rats administered different doses of Benz[a]anthracene, except for the 245 mg/kg dose. Hearts showed mild congestion, livers exhibited mild to moderate congestion in most animals (Figures 1C to E), except those administered Benz[a]anthracene at 245 mg/kg body weight (Figure 1B), and the spleens and kidneys displayed mild congestion.

Effect of varying doses of Benz[a]anthracene on the Rats' lung weight, body weight and the lung/body weight ratio

Statistically significant ($P < 0.05$) differences were observed in body weight on day 7 (F (3)=9.134) and day 14 (F(3)=4.577). The percentage change in body weight (F (3)=9.143) showed a progressive decrease in weight gain ranging from the control, 12.5, 25 mg/kg up to the 50 mg/kg dose (31.28 ± 7.87, 11.42 ± 9.17, 7.58 ± 9.00 and -9.91 ± 10.77 % respectively) Table 3. There was a significant decrease ($p<0.05$) in lung weight (F (3) =8.508) and lung/body weight ratio (F (3) =30.682) in 12.5 and 25 mg/kg BW groups compared to the control but the values at 50 mg/kg BW was significantly increased ($p<0.05$) compared to even the control group as shown in Table 3. There was a significant ($P < 0.05$) decrease in lung weight across the groups; between 12.5 and 50 mg/kg/day dose level (1.215 g), 25 and 50 mg/kg/day dose level (1.372 g), and between Control group and 50 mg/kg/day dose level (-1.507 g). There was also a significant ($P>0.05$) decrease in lung/body weight ratio across groups; between 12.5 mg/kg/day and 50mg/kg/day dose level (-0.874 g), 25 and 50mg/kg/day dose level (-0.932 g), and between control group and 50 mg/kg/day dose level (-0.480 g) as shown in Table 3.

Effects of Benz[a]anthracene on hematological parameters and oncogenic biomarker in rats

Table 4 shows the result of Benz[a]anthracene on hematological parameters and oncogenic biomarker in rats. There was no significant difference between the day 0 and day 14 values of TBV, PV and PCV parameters in the treated groups but there was a significant ($p<0.05$) increase in those parameter between day 0 and day 14 in the control group. However, the Carcino-embryonic antigen levels were significantly increases ($p<0.05$) in all the treatment groups compared to the control, although the values were still within the normal range Table 4.

Effect of Benz[a]anthracene on cardio-respiratory parameters of rats

Table 5 show the effect of varying doses of benz[a]anthracene on cardio-respiratory parameters of rats**.** There was a statistically-significant difference ($p<0.05$) in the BMR and LVR days 0 and 14 (F (3) =14.115, and 4.744 respectively), OCK and RF days 0, and 14 (F (3) = 29.228, and 5.208 respectively), HR days

Table 2. Description of postmortem lesions of Benz[A]Anthracene on lungs, heart, liver, spleen and kidney.

Dose (mg/kg bw)	Description of post mortem picture of the organs				
	Lungs	Heart	Liver	Spleen	Kidney
5000	Generalized haemorrhage, enlarged macro nodular lesions	Normal	Moderately congestion	Moderately congestion	Moderately congestion
3415	Collapsed, generalized congestion, pale with macro nodular lesions	Normal	Mildly congested	Mild shrinkage	Normal
1830	Generalized haemorrhage with macro nodular lesions	Normal	Mildly congested	Mildly congested	Mildly congested
245	Collapsed and haemorrhagic	Normal	Normal	Mild congestion, enlarged	Normal

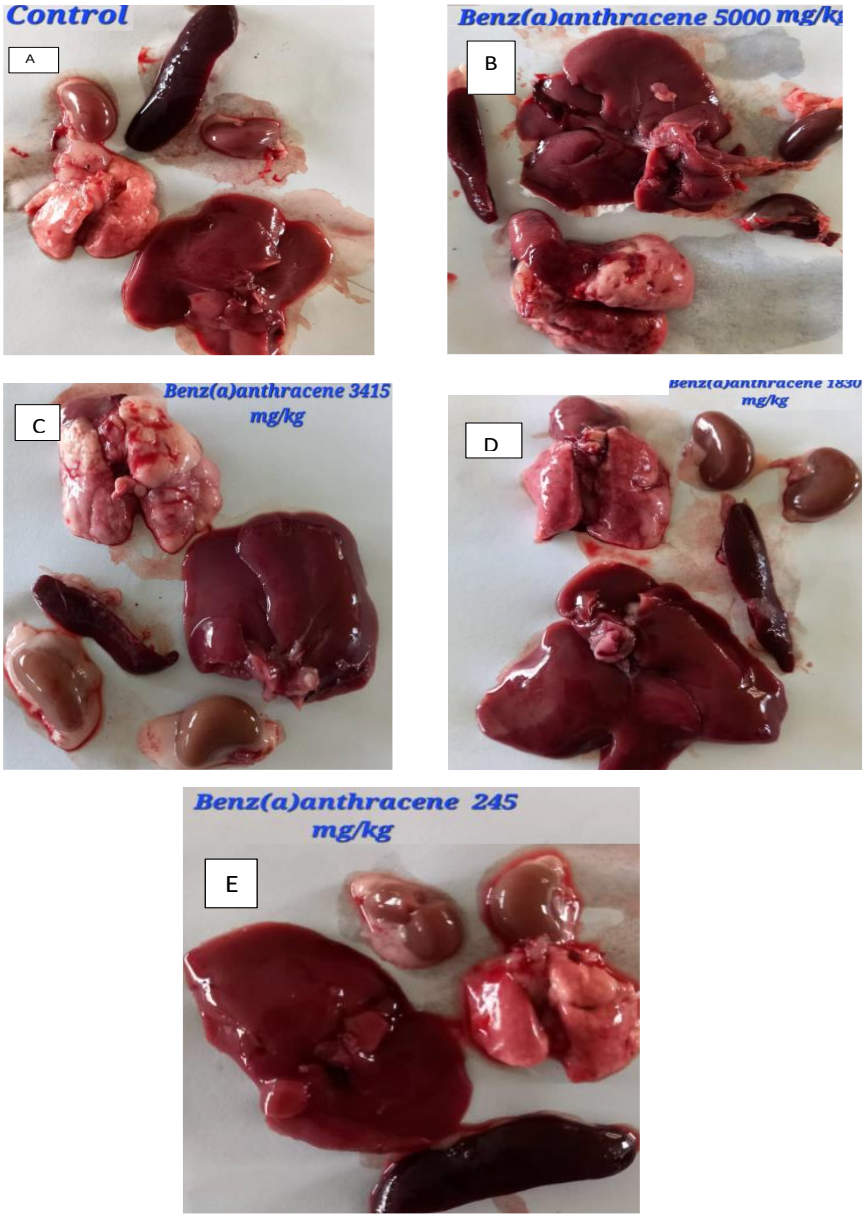

Figure 1. Gross lesions of the varying dose of benz(a)anthracene, the lungs, liver, kidney and the spleen.

Table 3. Effects of Ben[A]Anthracene on body weight, percentage change in weight, lung weight and lung/body weight ratio%.

Parameter (weight)	50 mg/kg/day	25 mg/kg/day	12.5 mg/kg/day	Control
day 0 body weight (g)	168.50 ± 16.48	137.50 ± 16.65	142.33 ± 9.48	122.43 ± 0.84
day 7 body weight (g)	140.20 ± 16.57*	136.00 ± 6.69*	153.60 ± 15.02*	140.93 ± 2.62
day 14 body weight (g)	139.8 ± 16.44*	141.00 ± 6.14*	158.40 ± 15.83*	160.72 ± 7.45
% change in body weight	-9.91 ± 10.77*	7.58 ± 9.00*	11.42 ± 9.17*	31.23± 5.89
Lung weight (g)	2.49 ± 0.46	1.12 ± 0.12*	1.28 ± 0.13*	1.50± 0.12*
Lung/body weight ratio %	1.72 ± 0.13	0.79 ± 0.07*	0.85 ± 0.03*	0.93 ± 0.04*

Control group: Physiological saline. M ± SEM: mean ± standard error of mean. Number per group: 6, $*P < 0.05$: significant difference in comparison with the control group, $**P < 0.05$: significant difference in comparison with the 50mg/kg/day dose level group.

Table 4. Effects Of varying doses of Benz[A]Anthracene on hematological parameters and oncogenic biomarker in rats.

Concentration of Benz[a]anthracene	TBV (mL)		PV(mL)		PCV (%)		RBC (X10^{12})		WBC (X10^{9})		CEA (ng/mL)
	DAY 0	DAY 14	DAY 0	DAY 14	DAY 0	DAY 14	DAY 0	DAY 14	DAY 0	DAY 14	DAY 14
50 mg/kg	7.78 ± 0.76	6.46 ± 0.76	4.98 ± 0.48	4.14 ± 0.48	53.43 ± 1.70	50.67 ± 2.68	9.08 ± 0.19	6.98 ± 0.11^{c}	1.45 ± 0.14	1.09 ± 0.06^{c}	2.26 ± 0.29^{b}
25 mg/kg	6.35 ± 0.77	6.51 ± 0.28	4.08 ± 0.48	4.18 ± 0.18	49.33 ± 0.71	50.83 ± 2.15	8.87 ± 0.14	6.98 ± 0.16^{c}	1.51 ± 0.14	1.13 ± 0.06^{c}	3.29 ± 0.52^{b}
12.5 mg/kg	6.58 ± 0.43	7.32 ± 0.73	4.22 ± 0.28	4.68 ± 0.46	52.83 ± 1.66	43.02 ± 8.85	8.84 ± 0.23	6.58 ± 0.73^{c}	1.34 ± 0.06	1.31 ± 0.23	3.86 ± 0.26^{b}
Control	5.65± 0.04	7.43 ± 0.34^{a}	33.64 ± 0.02	4.75± 0.22^{a}	29.83 ± 2.31	37.20 ± 1.65^{a}	3.71 ± 0.60	5.09 ± 0.28^{a}	4.17 ± 0.68	7.87 ± 0.88^{a}	0.52 ± 0.04

TBV: Total blood volume; PCV: Packed cell volume; PV: Plasma volume; RBC: Red blood cells; WBC: White blood cells; CEA: Carcino-embryonic antigen. Control group: Physiological Saline. M ± SEM: mean ± standard error of mean. Number per group: 6, $^{a}P < 0.05$: significant increase in comparison with the pretreatment value, $^{c}P < 0.05$: significant decrease in comparison with the pretreatment value, $^{b}P < 0.05$: significant difference in comparison with the control group. .

Table 5. Effect of varying doses of Benz[A]Anthracene on cardio-respiratory parameters of rats.

Dose	Days	BMR (Kcal/day)	OCK (L $^{-}$h $^{-}$kg)	HR ($^{-}$min)	LVR (L $^{-}$h)	LV (L)	RF ($^{-}$min)
50 mg/kg/day	0	163.85 ± 12.21	0.60 ± 0.02	273.27 ± 6.99	29.44 ± 2.19	0.11 ± 0.01	47.11 ± 1.28
	14	142.34 ± 12.74	0.63 ± 0.02	260.61 ± 8.01	25.58 ± 2.29	0.09 ± 0.01	49.52 ± 1.63
25 mg/kg/day	0	140.43 ± 12.57	0.63 ± 0.02	259.32 ± 7.49	25.23 ± 2.26	0.09 ± 0.01	49.79 ± 1.44
	14	143.89 ± 4.74	0.62 ± 0.01*	262.37 ± 2.92*	25.86 ± 0.85	0.09 ± 0.004	49.01 ± 0.58*
12.5 mg/kg/day	0	144.74 ± 7.30	0.62 ± 0.01	262.66 ± 4.49	26.01 ± 1.31	0.09 ± 0.006	48.1 ± 0.89
	14	156.58 ± 11.74*	0.61 ± 0.02*	269.36 ± 6.72*	28.13 ± 2.11*	0.1 ± 0.01*	47.78 ± 1.23*
Control	0	129.56 ± 0.66	0.64 ± 0.00	253.50 ± 0.43	23.28 ± 0.12	0.08 ± 0.00	50.76 ± 0.09
	14	158.73 ± 5.53	0.60 ± 0.01	271.07 ± 3.16	28.51 ± 0.99	0.10± 0.01	47.38 ± 0.58

a = BMR: Body mass ratio; OCK: O_2 consumption per kilogram; HR: Heart rate; LVR: Lung ventilation rate; LV: Lung volume; RF: Respiratory frequency. Control group: Physiological saline, M ± SEM: mean ± standard error of mean, Number per group: 6 ,$*P < 0.05$: significant difference in comparison with the control group.

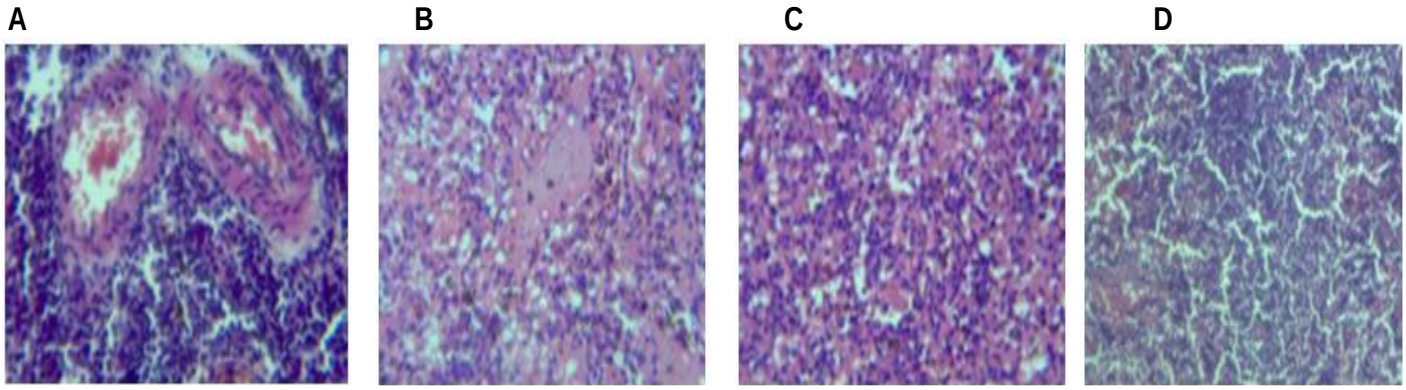

Figure 2. A) SPAD5000: Spleen tissue sections administered 5000 mg/kg body weight of Benz[a]anthracene. **B)** SPAD3415: Spleen tissue sections administered 3415 mg/kg body weight of Benz[a]anthracene. **C)** SPAD1830: Spleen tissue sections administered 1830 mg/kg body weight of Benz[a]anthracene. **D)** SPAD245: Spleen tissue sections administered 245 mg/kg body weight of Benz[a]anthracene.

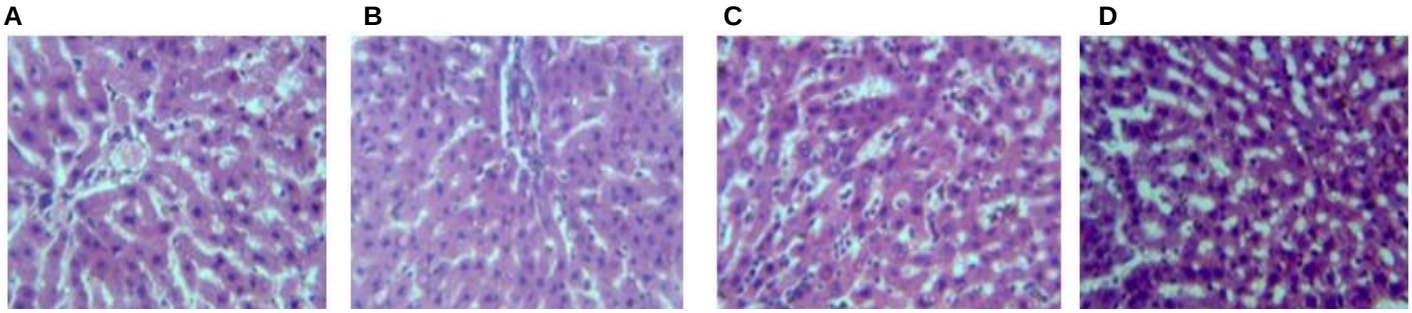

Figure 3. A) LVAD5000: Liver tissue sections administered 5000 mg/kg body weight of Benz[a]anthracene. **B)** LVAD3415: Liver tissue sections administered 3415 mg/kg body weight of Benz[a]anthracene. **C)** LVAD1830: Liver tissue sections administered 1830 mg/kg body weight of Benz[a]anthracene. **D)** LVAD245: Liver tissue sections administered 245 mg/kg body weight of Benz[a]anthracene.

0, and 14 (F (3) = 20.084, and 5.028 respectively), LV days 0, and 14 (F (3) =11.791, and 4.563 respectively), LV days 0, and 14 (F (3) =11.791, and 4.563 as presented in Table 5. Tukey post-hoc test revealed significant (p<0.05) increase on day 14 between 12.5 mg/kg/day dose level and Control group (+44.96 Kcal/day on BMR); between both 12.5 and 25 mg/kg/day dose level and Control group (+28.57, and +21.58 $^{-}$min respectively on HR); between 12.5 mg/kg/day dose level and Control group (+8.07 l $^{-}$h on LVR) and between 12.5 mg/kg/day dose level and Control group (+0.037 L on LV). A significant (p<0.05) decrease on OCK and RF was observed between both 12.5 and 25 mg/kg/day dose level and Control group (-0.071, and -0.056 liter $^{-}$h^{-}kg respectively on OCK) and 12.5 and 25 mg/kg/day dose level and Control group (-5.87 and -4.64 $^{-}$min respectively on RF).

Histolopathologic result

Spleen: The spleen tissue with AD 245 mg/kg showed mild reactive changes. AD 1830 mg/kg revealed severe granulomatous tissue. AD 5000 mg/kg showed lymphoid hyperplasia (Figure 2).

Liver: Liver tissue with AD 245 mg/kg showed regenerative and degenerative changes. AD 1830 mg/kg exhibited mild hepatic toxicity. AD 5000 mg/kg displayed regenerative changes (Figure 3).

Heart: Heart tissue with AD 1830 mg/kg and AD 5000 mg/kg showed no pathology (Figure 4).

Kidney: Kidney tissue with AD 245 mg/kg showed mild renal toxicity. AD 1830 mg/kg and AD 5000 mg/kg showed progressive renal toxicity (Figure 5).

Lung: Lung tissue with AD 245 mg/kg showed mild pulmonary reactive changes. AD 1830 mg/kg showed no pathology. AD 5000 mg/kg showed mild pulmonary toxicity (Figure 6). Overall, liver, kidney, and lung showed dose-dependent toxicity.

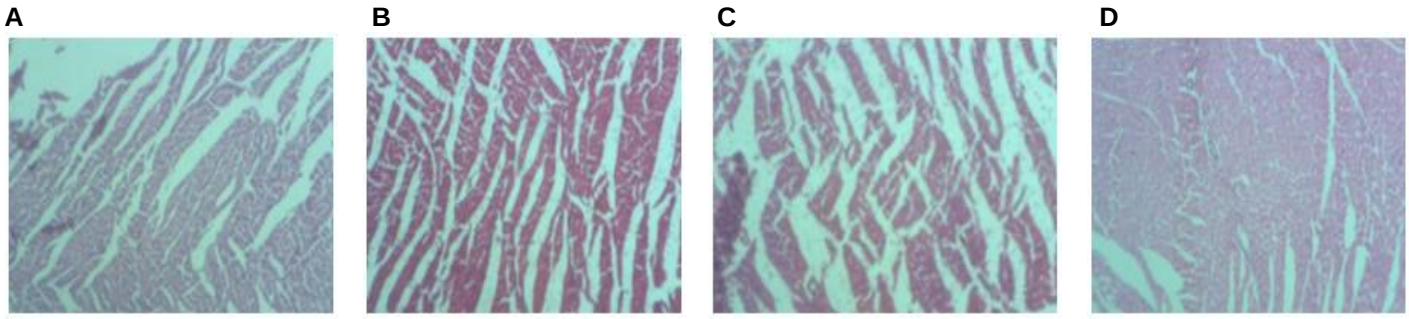

Figure 4. A) HTAD5000: Heart tissue sections administered 5000 mg/kg body weight of Benz[a]anthracene. **B)** HTAD3415: Heart tissue sections administered 3415 mg/kg body weight of Benz[a]anthracene. **C)** HTAD1830: Heart tissue sections administered 1830 mg/kg body weight of Benz[a]anthracene. **D)** HTAD245: Heart tissue sections administered 245 mg/kg body weight of Benz[a]anthracene.

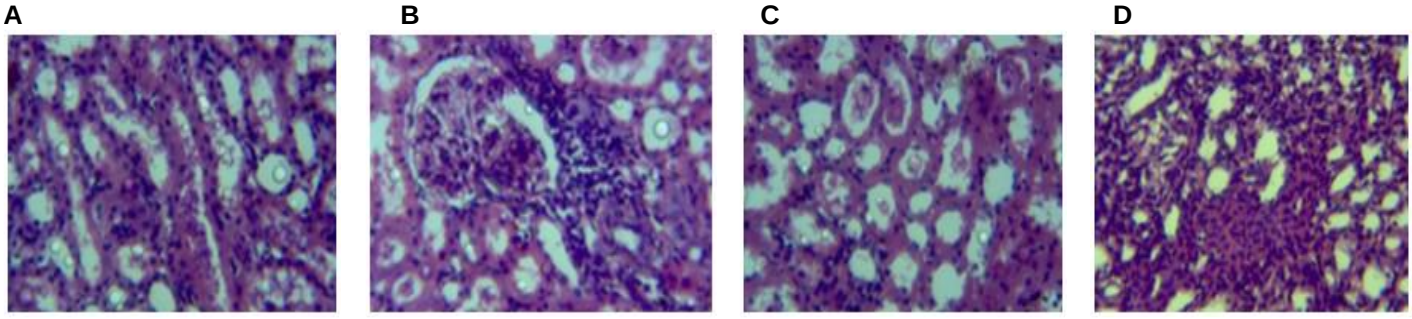

Figure 5. A) KDAD5000: Kidney tissue sections administered 5000mg/kg body weight of Benz[a]anthracene. **B)** KDAD3415: Kidney tissue sections administered 3415mg/kg body weight of Benz[a]anthracene. **C)** KDAD1830: Kidney tissue sections administered 1830mg/kg body weight of Benz[a]anthracene. **D)** KDAD245: Kidney tissue sections administered 245 mg/kg body weight of Benz[a]anthracene.

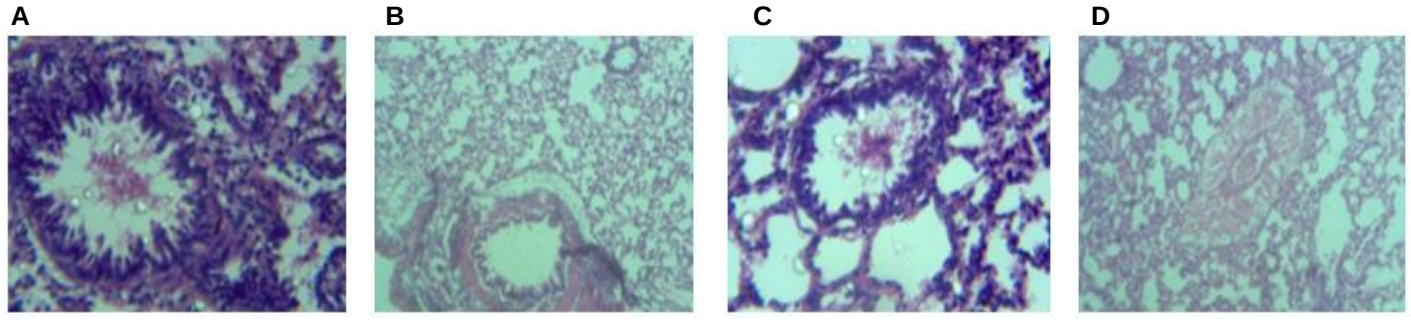

Figure 6. A) LUAD5000: Lung tissue sections administered 5000 mg/kg body weight of Benz[a]anthracene. **B)** LUAD3415: Lung tissue sections administered 3415 mg/kg body weight of Benz[a]anthracene. **C)** LUAD1830: Lung tissue sections administered 1830 mg/kg body weight of Benz[a]anthracene. **D)** LUAD245: Lung tissue sections administered 245 mg/kg body weight of Benz[a]anthracene.

The spleen had varying degrees of reactive changes. The heart tissue remained unaffected. Histopathology of untreated rat and rats treated with varying doses of Benz[a]anthracene on the Rats' lung (Plate 1). This part shows pathologies within the respiratory architecture comprising of tissue hemorrhage detailing red blood cells extravasations, in the bronchioles lumen (Bs), alveoli sacs (AS), blood vessels (V) and capillaries (V) of the interstitial connective tissue; adjacent lymphoid aggregation, peribronchioles inflammatory cells (L) infiltration and inflammatory cells activation within the alveoli sacs as well as mild alveoli septal distortion (Plate 2). This reveals increased lymphocytic cells (LT) aggregation and inflammatory cells which infiltrates into

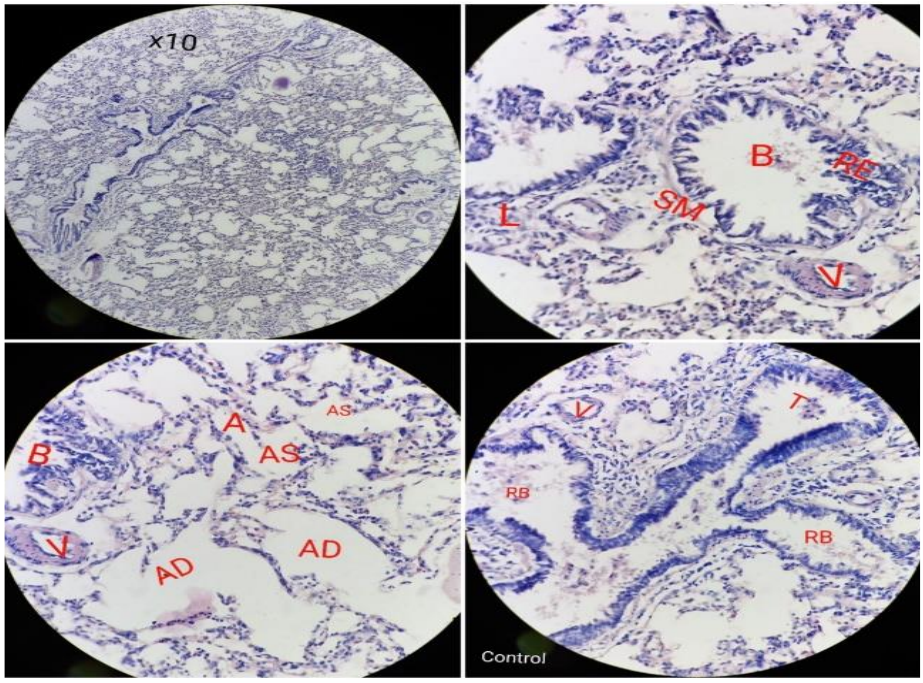

Plate 1. Histopathology of untreated rat showing non pathologic lung architecture showing bronchus (B), terminal bronchioles (T) with its underlying unremarkable ciliated pseudostratified columnar epithelium (RE) disposed within several alveoli sacs (AS) lined by simple epithelium. RB = respiratory bronchioles; T = terminal bronchioles; L = mononuclear cells; AD = alveoli duct; AS = alveoli sac; A = alveoli; V = pulmonary vein; B = bronchi; SM = smooth muscle layer; E = respiratory epithelium.

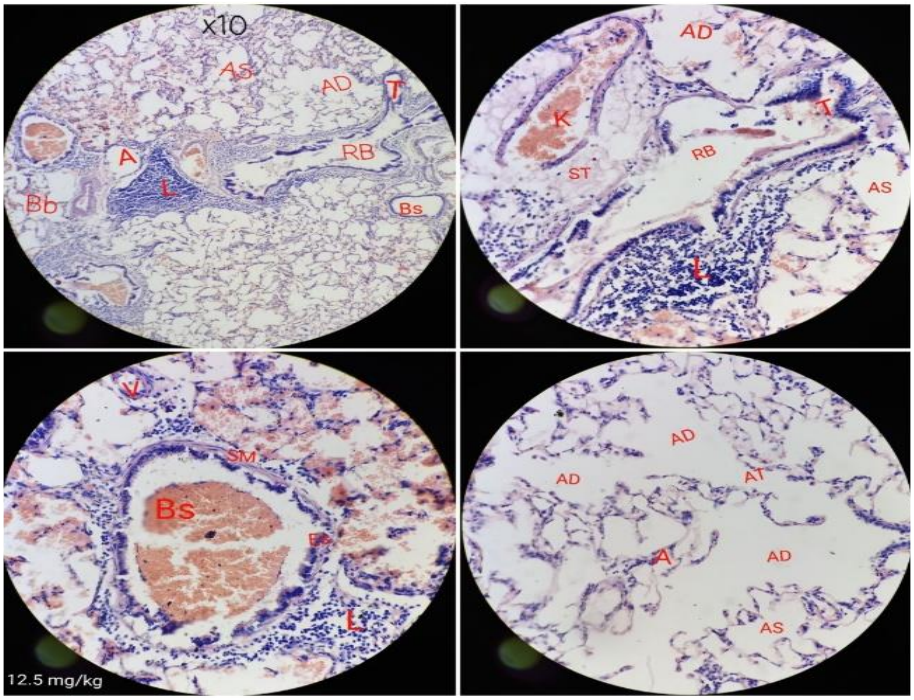

Plate 2. Histopathology of rats treated with 12.5 mg/kg body weight benz(a)anthracene. RB = Respiratory bronchioles; T = terminal bronchioles; L = inflammatory cells; AD = alveoli duct; AS = alveoli sac; A = alveoli; K = pulmonary artery; Bs = segmental bronchus; SM = smooth muscle layer; Es = segmental epithelium; V = pulmonary capillaries; ST = fibrous septa.

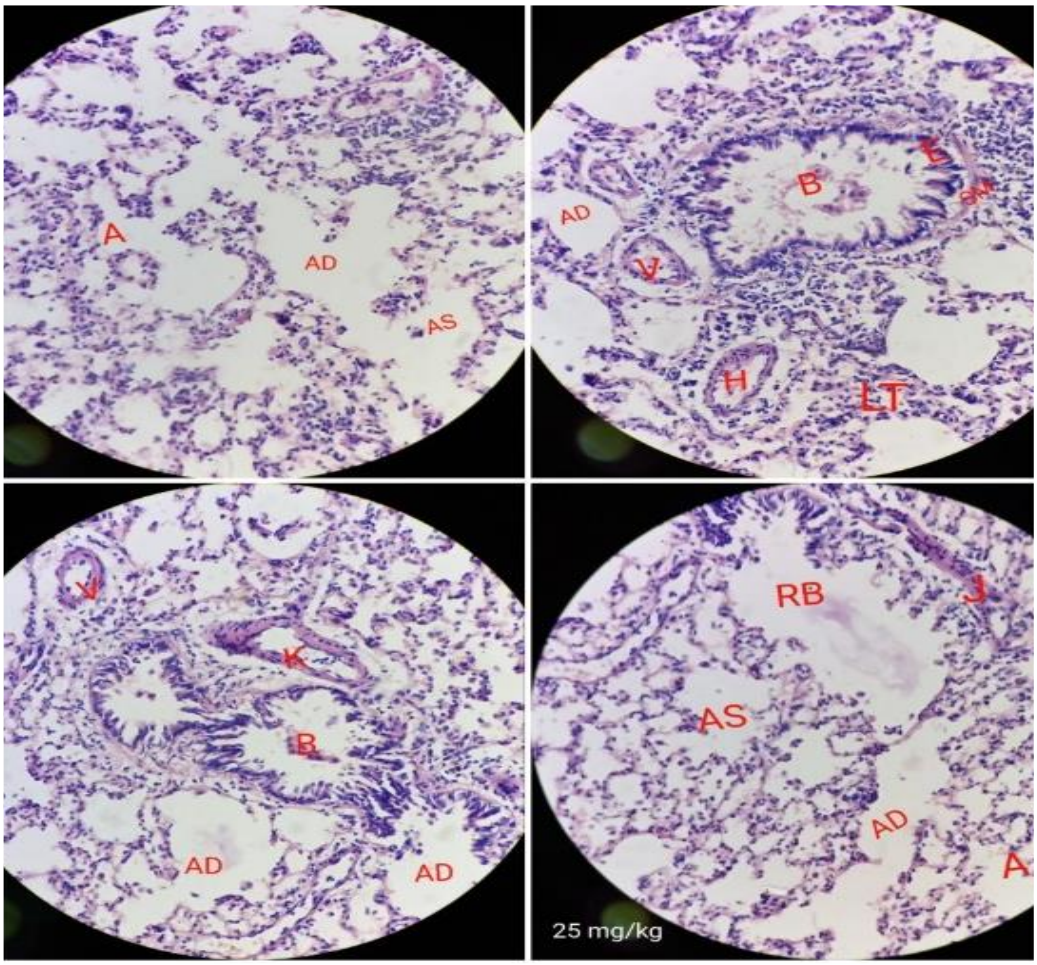

Plate 3. Histopathology of rats treated with 25 mg/kg body weight benz(a)anthracene. RB = Respiratory bronchioles; LT = lung tissue; AD = alveoli duct; AS = alveoli sac; A = alveoli; V and H = pulmonary artery and vein; B = bronchioles; SM = smooth muscle layer; E = respiratory epithelium; K = large arteriole; J = cartilages.

the blood vessels, lumen of the alveoli sacs (AS) and peribronchioles leading further distortion of the bronchioles muscular walls (SM); alveoli sacs (AS) constriction and dilation as well as alveoli septal connective tissue distortion (Plate 3). This shows increased inflammatory cell (L) infiltration causing destruction of the bronchioles muscular wall (SM) and alveoli wall. This results in alveoli sacs (AS) constriction. Also seen are bronchioles epithelial cells (E) hyperplasia, interstitial connective tissue (C) destruction, and congestion within the capillaries (V) (Plate 4).

DISCUSSION

The lung is exposed to environmental pollutants, and due to its function, it stands at a high risk of insults from these pollutants. Several polycyclic aromatic hydrocarbons (PAHs), including Benz[a]anthracene, are among the common pollutants that can readily affect the lungs. Talhout et al. (2011) reported that PAHs are highly toxic and can generate high levels of reactive oxygen species (ROS). This present study revealed that the LD50 of Benz[a]anthracene is above 5000 mg/kg, although several systemic and behavioral disorders were observed across the various doses of Benz[a]anthracene administered. The LD_{50}, LOAEL and NOAEL values indicated that Benz[a]anthracene is classified as a practically non-toxic agent (Loomis and Hayes, 1996). However, when considering the signs of toxicity alone and not mortality in this study, the LD_{50} of benz[a]anthracene was calculated to be 1037.5 ± 792.5 mg/kg BW, as suggested by Katsnelson et al. (2021) that pathologic effects may have no real threshold at all. This aligns with the report of Saganuwa (2016), indicating that with significant improvements in animal welfare, evident signs of toxicity are considered as relevant endpoints instead of death for determining LD_{50}. This approach

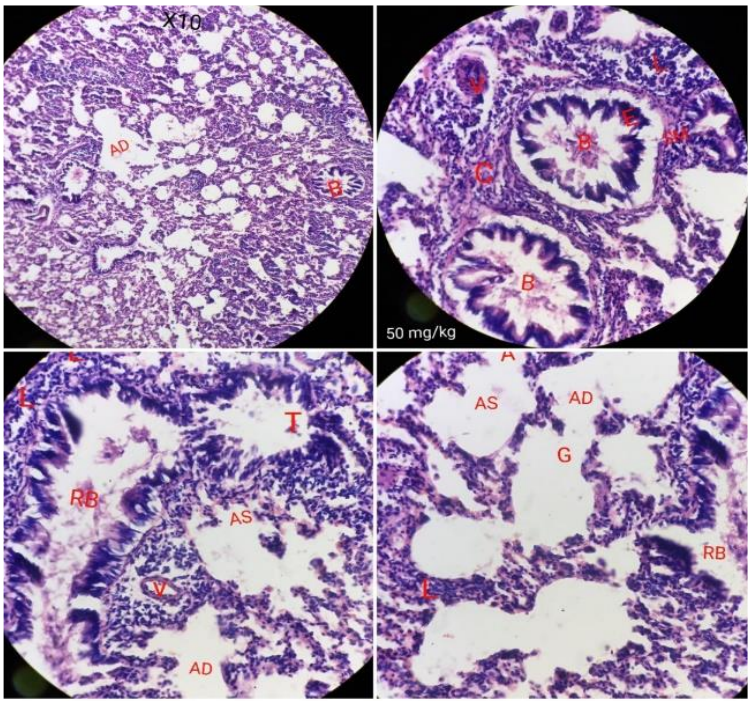

Plate 4. Histopathology of rats treated with 50 mg/kg body weight benz(a)anthracene. RB = Respiratory bronchioles; T = terminal bronchioles; L = inflammatory cells; AD = alveoli duct; AS = alveoli sac; A = alveoli; V = capillaries; B = bronchioles; SM = smooth muscle layer; E = respiratory epithelium; C = connective tissue.

provides more information on target organs and possible mechanisms of toxicity. The gross pathologic lesions observed in various organs of the treated rats are indicative of the potential of Benz[a]anthracene to induce systemic damages even without acute mortality. This is particularly significant considering that exposure to this compound may lead to severe systemic damages such as cardiac, hepatic, or renal failure and even cancer after prolonged exposure. Akuru et al. (2019) reported an increase in liver and kidney enzymes in rats treated with Benz[a]Anthracene.

The inversely proportional decrease in weight gain with increased doses of Benz[a]anthracene across the treatment group could be attributed to the damaging effect on tissues or a reduced feed conversion rate caused by Benz[a]Anthracene. Although there was no significant decrease in hematologic parameters across the Benz[a]anthracene treatment groups, the consistent increase in hematologic parameters in the control group suggests a possible inhibitory effect of Benz[a]anthracene on the hematopoietic system. Animal studies indicate that exposure to bay-region polycyclic aromatic hydrocarbons, including Benz[a]anthracene, can damage the hematopoietic system, leading to progressive anemia as well as agranulocytosis (Robinson et al., 1975; Cawein and Sydnor, 1968). CEA results showed a directly proportional increase in values with an increase in the dose of Benz[a]anthracene, suggesting that Benz[a]anthracene could be carcinogenic, especially with prolonged exposure or increased dose.

Benz[a]anthracene has been reported to induce cancer in various organs, including the skin and mammary glands (Forcados et al., 2020; Narayanankutty et al., 2020). Although there were no significant changes in the cardiopulmonary parameters, possibly due to the body's capacity to compensate for deficiency and distress, the slight changes in values observed suggest ongoing pathologic changes. If allowed to persist for a prolonged duration, these changes could potentially disrupt the system and eventually lead to death.

Histological findings revealed pulmonary pathologies, including reduced alveolar space, damaged alveolar sacs, and infiltration of interseptal space with mononuclear cells, suggesting active inflammatory activities. No pathology was observed in the control group, and respiratory vessels were unremarkable, with less than 2% of the tissue body showing alveolar sac constriction and mild inflammatory changes. This confirms the pulmonary distress observed in the rats exposed to varying doses of benz(a)anthracene. The histopathologic lesions observed on several organs of the rats, confirm evidently, that the compound benz(a)anthracene has enormous toxic potentials causing cellular inflammatory and oxidative changes.

Therefore benz(a)anthracene should be carefully handled as an industrial chemical and be monitored carefully in the environment to control possible pollution of either air or water since the compound can easily get into the body through oral, inhalation or dermal route. Thus, potent antioxidants like organoselenium compounds [1-isopropyl-3-methylbenzimidazole-2-selenone (Se I) and 1,3-di-p-methoxybenzylpyrimidine-2-selenone (Se II)] can be resourceful in managing environmental toxicants like Benz[a]anthracene (Talas et al., 2009).

Conclusion

Benz[a]anthracene, a common environmental pollutant, possesses a high LD50 but causes harmful health impacts on different organs, notably triggering inflammation, cellular oxidation and carrying a risk of cancer induction. Additionally, it exhibits a growth-retarding effect. While these pathological effects were observed to be dose-dependent, there is a crucial need to monitor and control environmental pollution from this agent to prevent intoxication. Further studies should investigate the effects of this common pollutant over chronic exposure and its impact on other bodily systems such as the nervous and reproductive systems.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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