*The Journal of Anatomical Sciences*
*Email:* journalofanatomicalsciences@gmail.com

*J. Anat Sci 17(1) Mar*

# Effects of RHAD on the Hematology, Biochemistry and Histology of the Spleen and Kidneys in Wistar Rats

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## ABSTRACT

The discharge of rice husk ash dust (RHAD) into the air by rice mills caused air pollution with health implications. This industrial process has resulted in various diseases including chronic obstructive respiratory system, rhinitis, cough etc. This study assessed the effects of RHAD exposure on haematological, biochemical and histology of the spleen and kidneys in Wistar rats. A total of 30 rats were grouped into six. Groups I, II, and III served as controls. Groups IV, V and VI served as treatments, exposed to the RHAD daily by inhalation for 14, 28 and 56 days, respectively. At the end of the experiment, the rats were sacrificed and blood samples collected for kidney function test and haematological parameters. Kidneys and spleen were harvested, processed and stained with H and E stains for light microscopy. Data obtained were analysed using statistical software (Graph Pad Prism, v.9). Alterations were observed in some differential leucocyte counts, total leucocyte count (TLC) and packed cell volume (PCV). Kidney serum proteins showed changes in urea and creatinine levels, histological changes, were also observed. Rice husk ash dust results in alterations of haematological, biochemical and histology of the spleen and kidney.

**Keywords:** Hematology, histology biochemistry spleen and Kidneys

## INTRODUCTION

A single rice grain has an external husk layer, a bran layer and the endosperm (clean rice). Rice milling is the process of producing edible milled rice after separating the husk (20%), the bran layer (11%) and the starchy endosperm (69%). During these processes, mineralogical materials (fumes, dust and ash) get released into the atmosphere which constitutes toxic elements that may cause health risks[1]. De-husking is the process of removing the husk layer from the paddy by friction. It was originally done using mortar and pestle but, in modern rice mills, the paddy grain is passed between two abrasive surfaces that move at different speeds.

After the husk is separated from the paddy, it gets suctioned (aspirated) and then transported to a storage dump outside the milling plan. Rice mill dust contains RHAD and numerous amounts of contaminants which when inhaled can lead to respiratory illnesses [2-4]. Rice husk ash dust is the by-product of burnt rice husk and is an alkaline product [5]. It is mostly inhaled which causes irritation in the portal entry leading to respiratory tract diseases; it can also be absorbed through the skin and can also be swallowed[6].

Rice husk ash dust contains silicon, lead, zinc, aluminium, cadmium and other heavy metals; which are known to cause various types of toxicities [7]. Rice husk ash dust has airborne endo-toxins that cause inflammation in the broncho-pulmonary system. These airborne endo-toxins are very common in rice producing environment [8].

## MATERIALS AND METHODS

### *Experimental animals*

Thirty (30) Wistar rats were procured from Anatomy holdings Northwest University, Kano. They were maintained on normal pellet diet and portable drinking water *ad libitum* and were acclimatized for two weeks at the animal house before commencement of the experiment. Care and handling of animals was in accordance with national academy of sciences guide for the care and use of laboratory animals[9]; as well as Northwest University's ethical review committee's guide line on the use of laboratory animals.

*Acquisition of RHAD:* Rice husk ash dust was obtained from UMZA rice mills at Kwanar Dawaki, Kano.

***Experimental design***

A total of 30 Wistar rats were grouped into six groups (I, II, III, IV, V and VI). Groups I, II, and III served as controls for experiment days 14, 28 and 56 days, respectively. Groups IV, V and VI served as treatment, exposed to 5 g/kg of RHAD daily by inhalation using air pump (blacksmith bellows) for 14, 28 and 56 days, respectively. At the end of the experiment, the rats were sacrificed using ethyl-ether and cardiac puncture was performed to obtain blood samples for kidney function test and haematological parameters. The blood was then transferred into EDTA bottles to prevent it from coagulating. The kidneys and spleen were harvested, processed and stained with H and E stains for light microscopy.

***Histological processing of spleen and kidneys***

In order to examine the effect of RHAD on the spleen and kidneys, the tissues were immersed in 10% formalin. They were then grossed and put into a tissue cassette before they were processed using automated histological tissue processor. The tissues were sectioned at 5 µm and stained using Haematoxylin and Eosin Stains (H&E). Light microscope was used for visualization[10, 11].

***Estimation of serum creatinine and blood urea levels***

The kidney function test was conducted utilizing the Abcam assay kit for the determination of these tests (estimated serum urea nitrogen and serum creatinine test).

***Hematological analysis***

The packed cell volume (PCV) was determined by centrifuging EDTA treated blood in a haematocrit (capillary) tube. A capillary tube was gently inserted into the EDTA bottle at an angle that allowed the blood to enter into the tube. With the index at the top of the capillary tube, it was removed from the EDTA bottle and wiped clean with a cotton wool before placing it onto the plasticine tray. The centrifuge separates the blood into layers at 3000 RPM (revolutions per minute) for 5 minutes. The lengths of layers were measured using a hematocrit reader [12]. The white blood cell count (WBC) was done using micropipette and a haemocytometer (Neubauer counting chamber). 20 µL of the blood sample was mixed with 380 µL of Turk's solution in an empty sample bottle using a micropipette. The Turk's solution lyses red blood cells with the exception of nucleated RBCs, WBCs and platelets. The filled micropipette was placed at the edge of the chamber and gently discharged the diluted blood into the chamber. The cells were counted in the 4 large corner squares under low magnification (x10) [13].

**Blood smear (differential leukocyte count)**: A drop of blood was placed on a glass slide; a second slide was used to spread the drop of blood across the slide. It was left to dry and then was dipped in concentrated methanol first, then dipped in solution B which was eosin; an acidic solution that stains the basic part of the smear. Then it was dipped in solution A which was methylene blue; a basic solution that stains the acidic part of the smear. Finally, it was placed under a light microscope with a drop of immersion oil and the different types of leucocytes were counted based on their morphological features using a cell counter[14].

***Data analysis***

Data obtained from these studies were expressed as mean ± standard error of mean (SEM). T test was used to compare significant differences between groups at $P < 0.05$ in the different studied groups using Graph pad prism (V.9).

**RESULTS**

***Histological examination of the kidney and spleen following RHAD exposure***

The kidney control groups for experimental days 14, 28 and 56 days appeared to have their medulla and renal cortices intact as shown in Figure 1 (A, B and C). The RHAD treated groups however presented with the following pathological features as shown in figure 1 (D, E and F); distension of the renal tubules, vacoulations and congestion of glomerular capillaries.

The spleen control groups for experimental days 14, 28 and 56 days appeared to have all Red and White Pulps intact as shown in figure 2 (A, B and C). The RHAD treated groups however presented with the following pathological features as shown in figure 2 (D, E and F); reduced lymphocytes in the white pulp and congestion in the red pulp the as compared to the control.

***Biochemical analysis of urea and creatinine following RHAD exposure***

Both tests for blood Urea and serum Creatinine showed that there was increase in Urea and Creatinine levels of RHAD treated rats as compared to their controls and as presented in figure 2. The increase in Biochemical parameters of both tests is duration dependent.

***Effects of RHAD on some Hematological parameters***

Alterations were observed in some differential leucocyte counts (neutrophils, lymphocytes, monocytes and eosinophils), total leucocyte count (TLC) and Packed cell volume (PCV) when compared to the control. The packed cell volume and lymphocytes reduced when compared to their various controls. The neutrophils, monocytes and white blood cell count all increased in the RHAD treated rats when compared to their various controls (Table 1). The changes recorded in these parameters were observed to be based on the duration of administration.

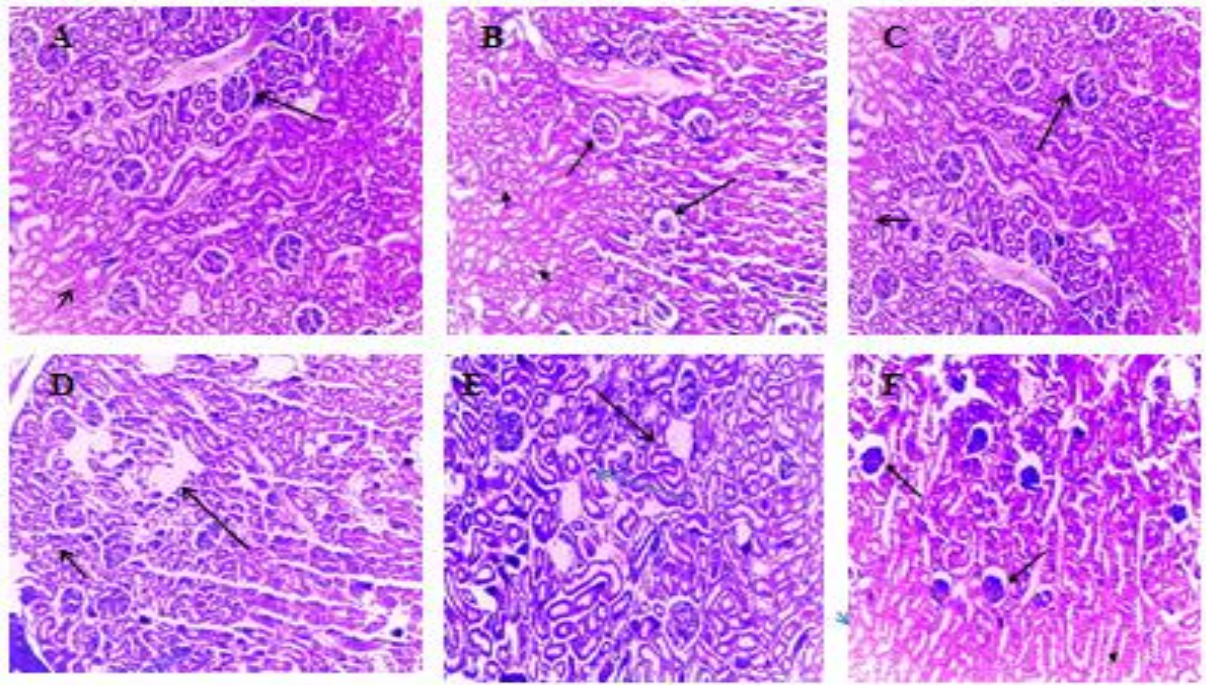


**Figure 1:** Micrograph of Section of the Kidneys of Wistar rat. H&E ×400. A, B &C = sections of the kidney of the controls representing 14 days, 28 days & 56 days respectively, Showing (short and long arrows) normal histology of the Kidney. D, E & F = sections of the kidneys of the rats treated with 5 g of RHAD representing 14 days, 28 days & 56 days respectively, showing (short and long arrows) glomerular dilations and tubular necrosis of the Kidney.

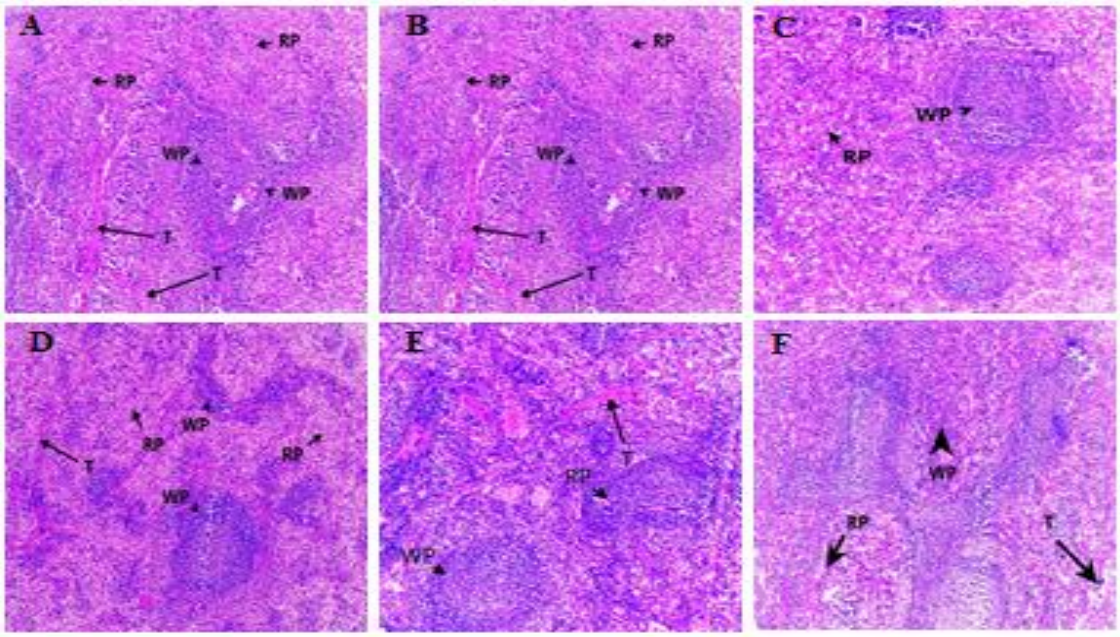


**Figure 2:** Micrograph of Section of the Spleen of Wistar rats. H&E ×400. A, B &C = sections of the spleen of the controls representing 14 days, 28 days & 56 days respectively (long & short arrows) showing white pulp (WP), Red pulp (RP) and Trabeculae. D, E & F = sections of the spleen of the rats treated with 5g of RHAD representing 14 days, 28 days & 56 days respectively, showing (short and long arrows) depleted lymphocytes in white pulp (WP) and congestion of the red pulp.

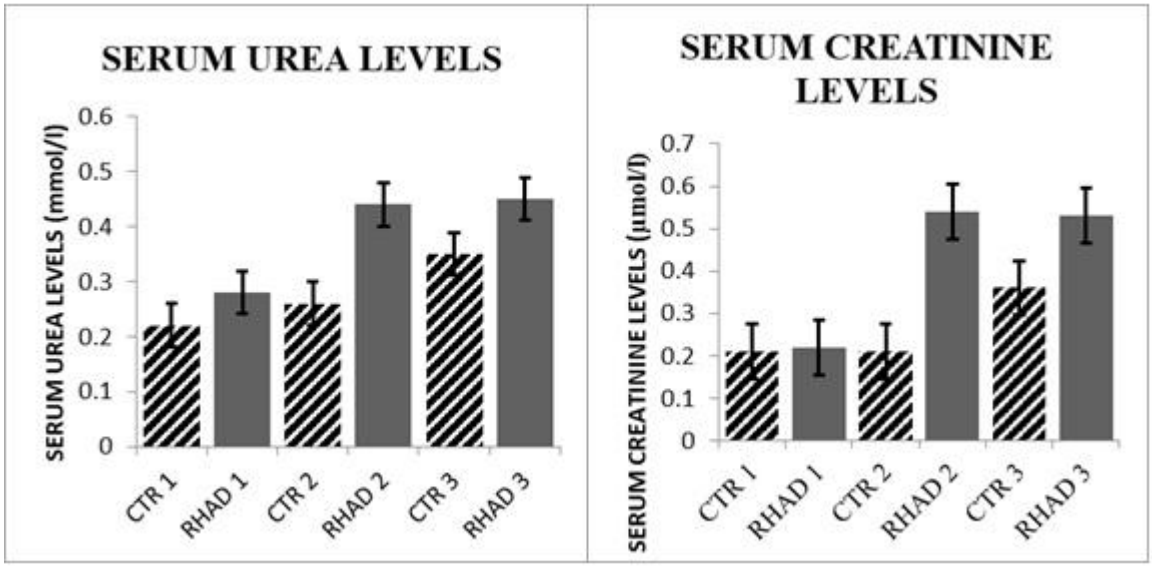


**Figure 3:** Blood urea and serum creatinine levels of Wistar rats exposed to 5 g of RHAD for 14, 28 and 56 days respectively. CTR = Control, RHAD = Rice Husk Ash Dust. Asterisk (*) = statistically significant difference (P≤0.05).

**Table 1:** Effects of RHAD on some Hematological parameters of Wistar Rats

| TESTS | CTR 1 | RHAD 1 | CTR 2 | RHAD 2 | CTR 3 | RHAD 3 |
|---|---|---|---|---|---|---|
| PCV (%) | 42.0±1.1 | 39.4±0.5 | 43.8±1.9 | 38.4±0.9*** | 41.0±0.7 | 38.4±1.8* |
| WBC (x10$^9$/L) | 13.3±1.1 | 14.6±1.0 | 13.1±1.0 | 15.6±0.5$^b$ | 11.3±0.70 | 15.8±0.9* |
| NEU (x10$^9$/L) | 15.0±0.18 | 19.0±0.47*** | 16±0.18 | 19.0±0.25** | 14.2±0.17 | 17.6±0.24*** |
| LYM (x10$^9$/L) | 84.0±026 | 81.8±0.55** | 85.0±0.26 | 83.0±0.37 | 83.8±0.12 | 78.8±0.25*** |
| EOS (x10$^9$/L) | 0.8 ± 0.032 | 1.0±0.063 | 0.6±0.04 | 1.0±0.063 | 0.8±0.049 | 0.9±0.057 |
| MON (x10$^9$/L) | 3.2±0.16 | 5.4±0.05*** | 5.2±0.05 | 3.6±0.14 | 4.0±0.12 | 5.8±0.05*** |

Values are mean ± SEM, SEM = Standard Error of Mean, CTR = Control, RHAD = Rice Husk Ash Dust 1, 2 & 3 = treatments (14, 28 & 56 days), MON= monocytes, LYM = lymphocytes, NEU = neutrophils, EOS = eosinophils, WBC = white blood cell count, PCV = Packed cell volume. Values in the same row bearing different superscript are statistically significant. Asterisk (*) are statistically significant at p≤0.05

## DISCUSSION

Rice husk Ash dust contains trace amounts of heavy metals such as; silica, zinc, lead, aluminum, chromium etc. these metals have been shown to cause toxicity in different biological tissues [15].

Result obtained from this research showed that rice husk ash dust caused histopathological changes in the kidneys and spleen of the experimental rats.The likely explanation for the occurrence of these lesions is that during chronic and acute exposure to cadmium and other heavy metals, the oxygen uptake is significantly reduced by the whole body and different tissues resulting in the exposed rats turning towards anaerobic metabolism[16, 17]. The histopathological changes recorded in this studies is in line with earlier researches conducted [18, 17] which found histopathological changes following administration of mercury and cadmium respectively in soft tissues of rats.

The increase in blood urea and serum creatinine levels witnessed in this study cannot be unconnected with heavy metal deposits in the tissues. These accumulated metals caused damage to the renal tubules resulting in increased creatinine levels.

[19, 18]. The sustained increase in creatinine levels is in accordance with earlier researches [20 21 18] which recorded increased creatinine levels after administration of different heavy metals.

We recorded decrease in packed cell volume and in lymphocyte count. These findings are in agreement with number of studies [22 23 18]. These might be due to inactivation of $Na^+$ - $k^+$ ATpase by generated free radicals which in turn allows entry of $Ca^{2+}$into the cell. This causes mechanical fragility of the plasma membrane and shortened the RBC life subsequently removing it from circulation[24, 25 18]. The present study also reported decrease in lymphocyte count. This report is in consistence with earlier findings [18 17]. This might be due to the effect of various heavy metals present in RHAD on lymphocytopoesis.

Similarly, a study carried out to determine the health effects of RHAD in Malaysians [26] on physical examination, total and differential white cell counts, lung function tests etc. shows raised WBC count has been implicated in Leukemia, however this studies observed decrease in WBC count. This findings correlate with that of earlier researchers [18]. Eosinophils, basophils and monocytes have low percentage in blood and constantly enter certain tissues in normal states and circulate in the blood in relatively low numbers. These cells further move from blood to tissue due to the presence of RHAD in tissues. This may probably be the reason why we reported low eosinophils and monocytes as compared to neutrophils and lymphocytes.

## CONCLUSION

Rice husk ash dust results to alterations in haematological, biochemical and histology of the spleen and kidney. These changes manifested as vacoulations and distension of the renal tubules, increase in urea and creatinine levels and increase in WBC, neutrophils, eosinophils and monocytes. While PCV and lymphocytes decreased.

**Acknowledgement**

The contribution made by Northwest University Clinic in processing our blood samples on time is well acknowledged. We also wish to thank the management of UMZA rice mills, Kano for the acquisition of rice husk ash dust. Finally, we say a big thank you to our research assistants and technical support group with special recognition to Dr. Agbon for his advice on manuscript development.

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