

Original Research Article

**BIOCHEMICAL CHANGES IN ALBINO RATS EXPOSED TO
RICE HUSK DUST AT ABAKALIKI RICE MILL
INDUSTRIAL AREA.**

ABSTRACT

Purpose: The effect of rice husk dust inhalation on some biochemical parameters was conducted on albino rats. **Method:** 100 albino rats were divided into five groups with each group containing twenty albino rats. Group A served as the control and were housed at the animal house away from rice dust while group B, C, and D were exposed to rice husk dust for two week, three weeks and four weeks respectively for dust inhalation. They were allowed access to rats feeds and deionized water *ad libitum*. Blood samples were collected from each group at the end of exposure by cardiac puncture. Glucose level was determined using the glucose reagent kit, acid phosphatase concentration was performed using reagent kits, creatinine and uric acid concentration was determined spectrophotometrically, the weight of the rats were recorded daily. **Results:** Result showed significant reduction in body weight of the exposed. The glucose level decreased significantly while the serum acid phosphatase and serum uric acid level showed a significant increase. Histological result showed various degrees of damage to the kidney of the exposed rats. **Conclusion:** We therefore conclude that rice husk dust inhalation induces glucose depletion, injury to the kidney and prostatic glands in the albino rat and impairs the renal function.

Key words: Rice husk dust, renal function, inhalation, prostate gland and exposure period.

INTRODUCTION.

Rice husk (or husk dust) are the coatings of seeds or the grain of rice which enables the grain to be protected especially during the growing season. Silica and lignin are the major hard material from which rice hull is formed and it is mostly indigestible to humans [1]. In Abakaliki rice mill industry, rice husk is removed from milled rice by winnowing which involve throwing the rice with its hull on the air for wind to blow away the hull while the heavy rice grain remain in the pan. This leads to the rice husk dust inhalation by the occupational workers and also those living around the industry. Some research has shown that rice husk dust inhalation might lead to liver function impairment [2]. Chemical irritant are contained in the particles generated from rice husk dust through microscopic view showing fine thorns that drops off from the sheath of the rice and can irritate the skin, throat, eyes and also lead to inflammation of the liver [3]. Other chemical constituent that might be present in rice husk dust which might attenuate liver damage among workers in industrial area includes some heavy metals which are present in the rice hull [4].

Since research has shown that rice husk dust contained some chemical and heavy metals [3,4], it is possible for these heavy metals to have toxic effect to some other organs of the body such as the prostate glands, kidneys, lungs among others. Prostatic acid phosphatase also known as prostatic specific acid phosphatase (PSAP), is an enzyme produced by the prostate in males. It may be found in increased amounts in men who have prostate cancer or other diseases, while the highest levels of acid phosphatase are found in metastasized prostate cancer. Diseases of the bone, such as Paget's disease or hyperparathyroidism, diseases of blood cells, such as sickle-cell disease or multiple myeloma or lysosomal storage diseases, such as Gaucher's disease, will show moderately increased levels. Certain medications can cause temporary increases or decreases in acid phosphatase levels. Its physiological function may be associated with the liquefaction process of semen [5]. Glucose on the hand is a simple monosaccharide found in plants. It is one of the three dietary monosaccharides, along with fructose and galactose that are absorbed directly into the bloodstream during digestion. An important carbohydrate in biology, cells use it as the primary source of energy and a metabolic intermediate. Glucose is one of the main products of photosynthesis and serves as fuel for cellular respiration [6]. Glucose is a common medical analyte measured in blood samples. Eating or fasting prior to taking a blood sample has an effect on the result. A high fasting blood sugar level may be a sign of prediabetes or diabetes mellitus [7,8] while reduced sugar level might be due to loss of appetite which might occur as a result of unfavorable change in ones pathological state.

Creatinine is a breakdown product of creatine which is an important part of muscle. Serum creatinine (a blood measurement) is an important indicator of renal health because it is easily-measured by-product of muscle metabolism that is excreted unchanged by the kidneys [9].. It is produced via a biological system involving phosphocreatine (also known as creatine phosphate) and adenosine triphosphate (ATP, the body's immediate energy supply) [10]. The creatine which should circulate to tissues and accumulate in the muscle where it serves as a high energy reservoir when phosphorylated to creatine phosphate is been degraded for excretion. Also, the high level of creatinine in the bloodstream from where it is supposed to be removed by the kidney is an indication of kidney impairment. Clinically, excretion of creatinine is used as a measure of both muscle mass and renal function. Uric acid otherwise called carbamide is an organic compound with the chemical formular $\text{Co}(\text{NH}_2)_2$. It serves an important function in the metabolism of nitrogen-containing compounds by animals and is the main nitrogen-containing substance in the urine of mammals. It is colourless and odourless solid, highly soluble in water and practically non-toxic. It is used by the body for nitrogen excretion. Due to the insolubility of uric acid in aqueous medium, it is possible that clinical conditions in which its elevated levels can result in deposition of sodium urate crystals primarily in joints. Hyperuricemia might also result because of increase levels of uric acid in the blood. Since uric acid is the end product of purin degradation in mammals, its excess level might indicate metabolic situations that might or might not be serious. Rice hull dust is a potential source of amorphous reactive silica and other chemical components which can be ingested by occupational workers. In this research, we aimed at providing information on how rice husk dust inhalation effects some biochemical parameters.

Methodology

Animals and Management (Exposure)

A total of twenty-four 100 albino rats weighing 170-200 were used for this research, the animals were grouped into A, B, C, D and group B, C and D were exposed to the rice husk dust at the Abakaliki rice mill for a period of one week, two weeks and four weeks respectively while group A which served as the control were kept in the department's animal house away from the rice mill industry. Each group contained twenty rats. The exposed rats were kept safe and away from the sun with the aid of the umbrella to mimic the natural environment under which occupational worker does they work. They were allowed access to grower's mash feed and deionized water *ad libitum*. At the end of exposure, the animals were sacrificed under light ether anesthesia and blood samples were collected for biochemical analysis.

Determination of biochemical / clinical parameters in serum.

The serum were separated from the blood samples using standard clinical procedures and were stored at -20°C until when the time for the analysis ACP (acid phosphatase), glucose levels, uric acid and creatinine levels. The glucose level were assayed by the colorimetric method outlined in the glucose oxidase kit of Quinnica Clinica Aplicada (Spain). Creatinine levels were assayed by colorimetric method with deproteinisation using kit (Syrbio / France). The method was based on the Jaffe reaction in which the coloured product was formed from creatinine and picric acid in alkaline solution while Serum uric acid determination was performed by an enzymatic method (Uricase) using kit (biomerieux /France). Acid phosphatase activities was determined using *MaxDiscovery™ Acid Phosphatase Assay Kit* which uses a unique colored reaction for specific visible detection of acid phosphatase activity within a ten minute time interval. The method involves the cleavage of naphthylphosphate by the enzyme to form naphthol and phosphate. The naphthol product hence reacts with Fast TR reagent included in the Reagent Mix to form a colored product. The amount of acid phosphatase in the various sample is quantified by measuring the absorbance at 405 nm within 5 minute time interval. To distinguish prostatic from nonprostatic acid phosphatase (TRAP) enzyme, sodium tartrate is added to the reaction to selectively inhibit the prostatic acid phosphatase.

Histopathological tissue processing

The histological tissue processing was carried out in the laboratory of the department of Morbid Anatom in accordance with the method of employed by [11]. It involved dehydration, where the specimen were routinely placed in ascending grades of serial dilutions of ethyl alcohol: first in 70%, then in 90% alcohol and finally in 100% (absolute) alcohol. Then dealcoholization (clearing , where the specimens were cleared in xylene. To replace the clearing agent (xylene), the tissues were impregnated in molten paraffin in a process called impregnation (infiltration). They were then in paraffin wax at 56°C in hot air oven for 24 hours after which each of the prepared paraffin blocks was trimmed to expose the embedded tissues to the cutting surface before sectioning. The blocks were clamped to a rotary microtome and cut at serial 5µm thick paraffin sections, which were then picked with a glass slide with 20% alcohol and gently placed in water bath. Each sections was picked from the water bath with a clean grease free slide and kept on a hot plate to dry. Deparaffinization and rehydration of the specimens were done before proceeding with the staining protocols. The specimens were stained with haematoxylin solution,

differentiated in 1% acid alcohol and blued in tap water. Then the slide was stained with eosin and dehydrated by passing through 90% alcohol and then in absolute alcohol. After that, it was cleared in xylene, mounted with a synthetic mountant and covered with cover glass. Finally, microscopic photography of each of the stained specimen was taken with photomicrographic equipment.

RESULTS

Weight change

Table 1: Weight change in grams of mean $\pm$ standard deviation of both control and exposed rats. There were general decrease in the body weight of all the experimental group as compared to the sham control. This reduction weight of the exposed animals reflect a decline in the health condition of the exposed animals.

GROUP	A	B	C	D
	204.28 $\pm$ 4.49 ^a	193.57 $\pm$ 6.26 [*]	192.5 $\pm$ 7.78 [*]	180.95 $\pm$ 17.14 [*]
	192.14 $\pm$ 11.49 ^a	201.42 $\pm$ 5.94 ^a	182.5 $\pm$ 11.05 [*]	183.33 $\pm$ 19.05 [*]
	206.78 $\pm$ 11.53 ^a	187.85 $\pm$ 2.67 [*]	197.5 $\pm$ 11.56 [*]	200 $\pm$ 19.05
	210.71 $\pm$ 7.80 ^a	186.42 $\pm$ 4.75 [*]	186.78 $\pm$ 10.30 [*]	180.47 $\pm$ 12.75 [*]
	184.76 $\pm$ 11.58 ^a	196.42 $\pm$ 3.77	188.57 $\pm$ 10.08	180.47 $\pm$ 17.16 [*]
	210.47 $\pm$ 11.16	175.71 $\pm$ 5.34 [*]	168.57 $\pm$ 9.88 [*]	169.76 $\pm$ 14.27 [*]

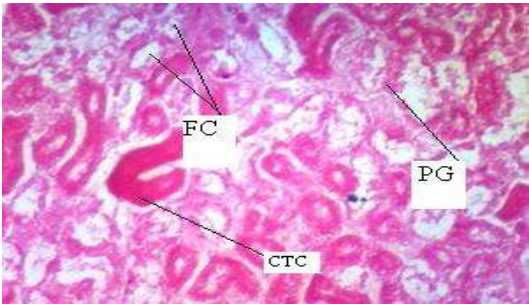
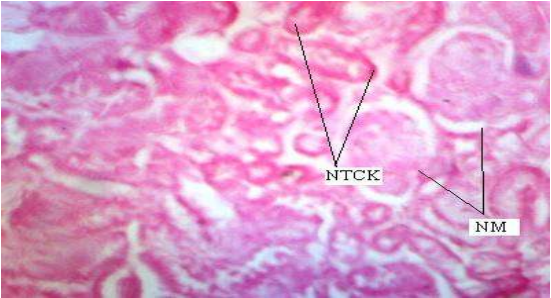
Value bearing the superscript * are statistically significant from the control.

Table 2: Shows the mean glucose concentration (mg/dl), acid phosphatase(u/l), uric acid(μ mol/L) and creatinine(μ mol/L) for the exposed and control of the albino rats. The result shows significant reduction in the glucose level which might be due loss of appetite by the exposed rats. The acid phosphatase enzyme which were supposed to be confined in the prostate gland tend to have leaked into the blood, hence its elevated level found in the serum. This might be due to an injury in the prostate thereby resulting in the leakage of the enzymes. Uric acid and creatinine level also were increased in the serum which shows that the renal system is unable to adequately control the absorption and secretion of these excretory products showing the harmfulness of inhalation of the rice husk dust to surrounding inhabitants.

	Glucose	Acid phosphatase	Uric acid	Creatinine
A (Control)	66.0 $\pm$ 2.37	1.69 $\pm$ 0.34	191.50 $\pm$ 14.73	75.47 $\pm$ 7.67
B (Week2)	65.83 $\pm$ 5.64	1.81 $\pm$ 0.41 [*]	213.67 $\pm$ 34.42 [*]	82.12 $\pm$ 23.18 [*]

C (Week3)	62.67 ± 4.18*	1.73 ± 0.40*	226.0 ± 34.44*	84.35 ± 16.64*
D (Week 4)	60.0 ± 5.22*	2.02 ± 0.19*	218.83 ± 35.10*	82.77 ± 10.91*

147



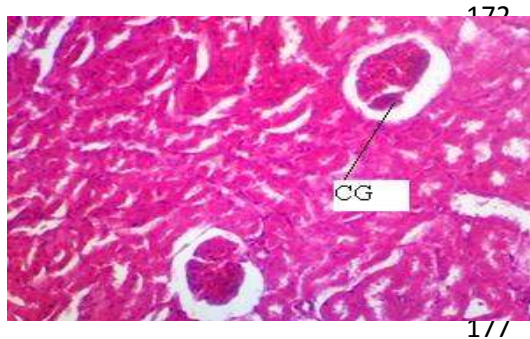
153 Plate A

Plate B

154 Plate A is the photomicrograph of the kidney of control rats
 155 showing normal glomeruli and normal tubular cells (NTCK] of
 156 the kidney while plate B which is the photomicrograph of
 157 kidneys of rats exposed for 7 days shows fatty changes (FC) in
 158 the background, the tubular cells appear congested (CTC) and
 159 the glomeruli appear pale (PG).Method of staining,(H &E X 40)
 160 Method of staining (H & E) X 40

161
162
163
164
165
166
167
168
169

170



177 Plate C

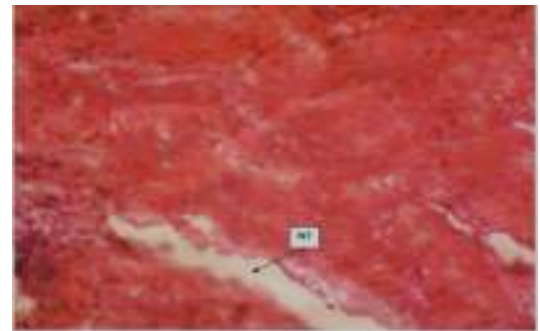
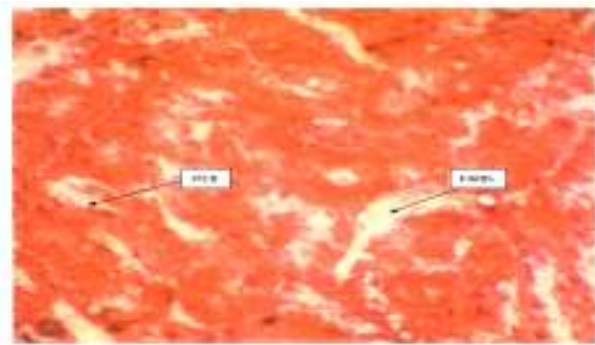


Plate D



184 Plate E

The photomicrographs of kidneys of rats exposed to rice dust for 14 days, 21 days and 28 days shown in plate C, D and E respectively revealed congested glomeruli (CG), necrotic tubules (NT), indistinct tubules cell border (ITCB) and enlargement of tubules with partial obstruction of the lumen (POL). The epithelial cells in plate E that suppose to align themselves along the tubular membrane are scattered. The glomerulus is contracted giving a focal widening Bowman space (FWBS) .Method of staining (H & E X40)

190 DISCUSSION

191 The present result showed that body weight and serum glucose level decreased significantly in
192 those rats exposed to rice dust inhalation while there was a significant increase in the serum acid
193 phosphatase activities , serum uric acid level and serum creatinine level relative to the control.
194 The blood glucose level appears to decrease significantly among the experimental animals
195 exposed to rice husk dust. The decrease were found to be severe as the period of exposure
196 increases. This is related to works done by [12] where dietary administration of 0.5 or 1% rice
197 hull smoke extract to alloxan-induced diabetic mice caused a decrease in blood glucose level
198 and increased both serum insulin and hepatic glycogen level. These changes resulted in restoring
199 glucose-regulating enzyme levels to control values. The body weight of the animals also

decreased significantly relative to the control groups. This might explain the decrease in the blood glucose levels as the inhalation of this dust might lead to a wide variety of changes including loss of appetite and hence reduction in body weight. Oxidative stress might have also been induced by the rice husk dust inhalation just as smoke inhalation does as reported by [13].

On the other hand, acid phosphatase activities was elevated in the serum as evidenced by the result. This enzyme are suppose to be confined to the prostate gland, therefore, the high activities of this enzyme in the blood might suggest that an injury has been caused to the prostate as a result of the inhalation of the rice husk dust, hence its leakage into the blood. This can invariably lead to prostatic cancer. This is to say that a lesion on the prostate gland can result to disruption in the reading of base sequence in nucleic acid synthesis thereby leading to uncontrolled cell growth[14]. Serum uric acid level and creatinine concentration in the blood was also significantly elevated in exposed group than in the control group. The increase was dose dependent. This is similar to findings by [15] where rabbits where exposed to industrial fumes (smoke) and it was after 28 days that the rabbits started recovering from excessive secretion of uric acid into the blood, this is possibly due to the inability of the renal system to functionally control the absorption and or excretion (homeostasis) of uric acid [16]. *Because uric acid insoluble in aqueous medium, its high level can lead to a clinical conditions that might lead to the deposition of sodium urate crystals mainly inthe joints. In fact the exposed groups had a clinical condition called hyperuricemia.*

Our result also reveals that there was a significant increase in creatinine level in the first two weeks, but subsequently, a significant decrease was observed but still not compared to the control sample, this can be explained by works from [17] where a sub-acute toxicity experiment was conducted on wistar rats and it was observed that on subsequent administration of a particular dose (50mg/kg) of Chromium, the creatinine level which was formerly increasing started decreasing, this is probably due to the recovery of activity by the renal system thereby maintaining the normal clearance rate of the kidney. *The creatine when circulated to tissues, accumulates in the muscle where it serves as a high energy reservoir when phosphorylated to creatine phosphate. Here this creatine is been degraded to creatinine for excretion. Moreover, the high level of creatinine which should be removed by the kidney in the bloodstream is an indication of kidney impairment. Clinically, excretion of creatinine is used as a measure of both muscle mass and renal function. Its increase in the blood of the rats exposed to rice husk dust is an indication of a compromised pathological state.* Furthermore, creatinine level may increase when angiotensin- converting enzyme (ACE) inhibitors (ACEI) or angiotensin 11 receptor antagonist or angiotensin receptor blockers (ARB) are taken. Using both ACE1 and ARB concomitantly will increase creatinine levels to a greater degree than either of the two drugs would individually [18]. In a recent Japanese study a lower serum creatinine level was found to be associated with an increase risk for the development of type 2 diabetes in Japanese men²⁰. Rice husk dust contained various heavy metals as well as other organic compounds that might elicit a wide degree of toxicity to the body [3]. The histological examination of the kidney showed varying degrees of damage to the kidney ranging from congestion of tubular cells, partial obstruction of the lumen, scattered epithelial cells and necrotic tubules.

CONCLUSION

We therefore conclude that rice husk dust inhalation result to certain level of toxicity to the body, individuals working at the rice mill industry are therefore advised to reduce the amount of rice husk dust ingestion by engaging in some preventive measures such as the use of nose mask during work hour.

REFERENCES

- 1 Mitani., Naniki., Jian., Feng., Takashy and Iwashitao . Identification of the silicon in xylem sap of rice. *Journal of plant cell physiology* 2005; 46: 279-283.
- 2 Czech, W., Krutmann,J., Budnik, A., Schopf, E and Kapp, A. Induction of intercellular adhesion molecules-1 (1 CAM), expression in normal human eosinophils. *Investigation dermatology* 1993; 100: 417-423
- 3 Kim W. J., Terada N., Nomura T., Takahashi R., Lee S. D., park J .H and Kanno A. Effect of formaldehyde on the Expression of Adhesion Molecule In nasal Micro vascular Endothelial cells: The role of Formaldehydes in the Pathogenesis of sick Building syndrome. *Journal of Experimental Allergy* 2002; 32: 287-295.
- 4 Ajaml M., Rao R.A., Anwar S. Ahmad J and Ahmad R. Adsorption Studies on Rice Husk: Removal and Recovery of Cd 11, Zn, Cr, Hg, Co, As from Waste Water and Rice Husk dust. *Journal of Bioresources and technology* 2003; 86: 147-149
- 5 Walter, F. and Boron, D. *Medical Physiology: A Cellular And Molecular Appraoch*. Elsevier/Saunders press. 5th edition 2003. 13-18p
- 6 Clark, D. and Sokoloff, L. *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*, Lippincott, 11th edition 2000. 637–670p
- 7 Stephen, T. J and Arai, H. Prostatic Acid phosphatase Degrades Lysophosphatidic acid in Seminal Plasma. *Journal of Clinical Medicine* 2004; 571: 197–204.
- 8 Roy, M.A. and Marcelli, M. Changes in Androgen receptor Nongenotropic signaling Correlate with Transition of LNCaP cells to Androgen Independence. *Cancer Research Journal* 2007; 64: 7156–7168.
- 9 Fraser-Reid, Bert . "Van't Hoff's Glucose". *England Journal of Chemistry* 2009; 77 (39): 8-18.
- 10 Allen, P. Creatinine Metabolism and psychiatric disorder: Does creatinine supplementation has therapeutic value. *Neuroscience Bio-behaviour review* 2010; 36(5): 1442 – 1462.
- 11 Afaf, A.E and Hanan, A.T. Chlorpyrifos (from different sources) : Effect of testicular biochemistry of male albino rats. *Journal of American Science* 2010;6(7):252-261
- 12 Alexanda, W.J., Smith, D.S. and Ratliff, T.L. Measurement of Prostate-specific Antigen in Serum as a Screening Test for Prostate Cancer. *New England Journal of Medicine* 2001; 324:1156-1161.

279 13 Myung, S.P., Leopaldo, C.C., Bryan, S.J., Williams, W.B., Victor, R.R and Micheal, A.D.
280 Assessment of oxidative stress in lungs from sheep after inhalation of wood smoke. SciencDirect
281 2004; 195:97-112

282 14 Busch, C., Hanssen, T.A., Wagener, C. and Brink, B. Down-regulation of CEACAM1 in Human Prostate
283 Cancer: Correlation With Loss of Cell Polarity, Increased Proliferation Rate, and Gleason Grade 3 to 4
284 Transition. Journal of Human Pathology 2002; 33:290–298.

285 15 Washington, R.E., Andrew, R.M., Mutter, R.L. Emergency Departmental visits for adults with Diabetes.
286 Agency for Health care Research and Quality, Rockville 2010; 5(19):1371 – 1384.

287

288 16 Genega, E.M., Hutchinson, B., Reuter, V.E. and Gaudin, P.B (2000)."Immunophenotype of High-grade
289 Prostatic Adenocarcinoma and Urothelial Carcinoma". Journal of Medical 2000; 2:45-53

290 17 Harita N, Hayashi T, and Sato K.K. "Lower Serum creatinine is a New Risk Factor of type 2 Diabetes: the
291 Kansai Health Care Study". Diabetes Care 2009; 32 (3): 1186-1191.

292 18 Gross, J.I., Azevedo, M.I., Silveiro, S.P., Conani, L.H., Caramori, M.L. and Zelmanovitaz,K.. Diabetic
293 Nephropathy. Diagnosisi, Prevention, and Treatment, "Diabetes Care 2005 ;28 (1): 164-176.

294