Tropical Journal of Natural Product Research

Available online at <u>https://www.tjnpr.org</u> Original Research Article



Acute (One-Hour) Inhalation toxicological study of Cashew Nut Shell (Anacardium occidentale L.) Fumes in Wistar Rats and Exotic Cockerel Chickens

Tunmise T. Oladipe^{1,*}, Oladunni O. Falola.², Abayomi A. Taiwo³, Paul I. Emeje⁴, Elizabeth Dangana³, Agenyi Omeje⁵, Favour Maha², Temitope Saliu², Augustina Clement², Nafisat Aliyu², Abayomi Ajayi⁵

¹Department of Biochemistry, Confluence University of Science and Technology Osara, Kogi State, Nigeria
 ²Department of Biological Sciences, Faculty of Natural Sciences, Prince Abubakar Audu University Anyigba, Kogi State Nigeria
 ³Department of Physiology, College of Health Sciences, Prince Abubakar Audu University Anyigba, Kogi State Nigeria
 ⁴Department of Medical Laboratory Sciences, College of Health Sciences, Prince Abubakar Audu University Anyigba, Kogi State Nigeria
 ⁵Department of Anatomy, College of Health Sciences, Prince Abubakar Audu University Anyigba, Kogi State Nigeria

ARTICLE INFO	ABSTRACT
Article history:	Local folks have reported that roasting cashew nuts has fatal effects on domestic birds, especially
Received 19 June 2024	chickens. This study compared the toxicological effect of acute (one-hour) inhalation of cashew
Revised 22 June 2024	nutshell fumes (CNSF) in Cockerel chickens and Wistar rats. Twenty-four mixed-sex Wistar rats
Accepted 31 July 2024	were divided into four groups; control, rats exposed to 500ppm/hr., 1250ppm/hr. and 2500ppm/hr.
Published online 01 September 2024	of CNSF for one (1) hour. The chickens were also divided into three groups of 6 chickens each; control (unexposed chickens), chickens exposed to 500ppm/hr. and 1000ppm/hr. of CNSF for one
	(1) hour. Hematological analysis as well as histological examinations of the lungs and heart of the
	Chickens and Rats were carried out. After one hour of exposure, the toxicity signs noted include;
	scratching, twitching, irregular breathing, and tremors. All rats exposed to 2500ppm/hr of CNSF
	died with moderate pulmonary congestion. Analysis of hematological parameters in the surviving
Copyright: © 2024 Tunmise <i>et al.</i> This is an open- access article distributed under the terms of the	animals showed a significant (P<0.05) increase in hemoglobin (Hb%), packed cell volume
	(PCV%) and white blood cells (WBCx109L) counts in both chickens and Wistar rats exposed to
<u>Creative Commons</u> Attribution License, which	CNSF compared with the unexposed control groups. No significant $(P>0.05)$ differences were
permits unrestricted use, distribution, and reproduction	found between male and female rats for all the hematological parameters measured. The results
in any medium, provided the original author and	also showed species-specific variations in all the hematological parameters. Histopathological
source are credited.	evaluation reflects a mononuclear inflammatory infiltration of the striated heart muscles and the

exposure to cashew nut fumes in animals and humans.

Keywords: Anacardium Occidentale, Fumes, Hematology, Histology, Inhalation Toxicology

septa of alveoli in the lungs. These results further highlight the possible adverse health effects of

Introduction

Cashew nut is the seed of the cashew tree *Anacardium* occidentale L.¹ The nut is one of the most important products of the cashew tree and one of the most beneficial nuts in international trade, ² because of its confectionery use. GC-MS and HPLC analysis of cashew nut revealed it's a rich source of nutrients such as; carbohydrates, protein, crude fat, fiber, free fatty acid, essential and non-essential amino acids, saturated fatty acid, and an insignificant amount of trans fat.² It is proven that cashew portions could be formed into a herb to treat snakebites and applied for cracked heels, or as a fungal treatment. The natural product, bark, and leaves are used for treating parasitic action, wounds, and rashes, and can be used as an antipyretic, for diarrhea.³

*Corresponding author. Email: topeoladipe@gmail.com; oladipett@custech.edu.ng_Tel: 2348149992660

Citation: Oladipe T.T, Falola O.O, Taiwo A.A, Emeje P. I, Dangana E., Omeje A, Maha F., Saliu T., Clement A, Aliyu N. and Ajayi A. Acute (One-Hour) Inhalation toxicological study of Cashew Nut Shell (*Anacardium occidentale L.*) Fumes in Wistar Rats and Exotic Cockerel Chickens. Trop J Nat Prod Res. 2024; 8(8):8244-8249 https://doi.org/10.26538/tjnpr/v8i8.47

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

In Nigeria raw cashew nut oil is used as a direct tool for inscribing bodily tattoos in addition to other methods.⁴ To obtain the nut which is enclosed inside a kernel, it must be extracted from the double-walled shell by solvent extraction or by heat extraction such as roasting.⁵ In addition, roasting and frying are two of the most popular ways to improve the sensory qualities of nuts through heat processing.6 Irrespective of the extraction method, the cashew nut shell is known to contain a caustic viscous, dark liquid that contains mainly anacardic acid, cardol, cardanol, and other polymeric materials,⁷ hence there is a need for dehusking to get rid of the poisonous substances.8 Dehusking of cashews by roasting either for domestic consumption or commercial use could be hazardous to humans and other animals because the fumes arising during the roasting of cashews are very irritating to the face, nostrils, and throat.⁹ The mucous membranes of the mouth and throat are severely affected when they come into contact with shell oil or the irritating fumes emitted during roasting. It has been reported that poor occupational safety practices during Cashew shell oil (CSO) production, and shelling of kernels expose workers to raw or processed shell oil through dermal, nasal, and oral routes,⁴ which can lead to sensitization and dermatitis. Pollutants released during the process of burning cashew shells, include mainly, Carbon particles, Carbon monoxide, Nitrogen oxides, hydrocarbon residues, ozone(O3), nitrogen dioxide (NO2), dioxide Sulfur (SO2), polycyclic aromatic hydrocarbons (PAHs), fine particles such as particulate matter with an aerodynamic diameter equal to or less than 4µm (PM4.0), which is capable of penetrating the respiratory system with ease and reaching the pulmonary alveoli, causing serious respiratory diseases.¹⁰ The adverse health effects of exposure to fumes from the burning process of cashew nut shell have been studied, especially investigations looking at exposure of human with pre-existing respiratory diseases such as emphysema or asthma.¹¹ However, there is limited information regarding the toxicological profile of animals exposed to cashew nut shell fumes. Therefore, this study aims to determine and compare the toxicological effect of one-hour exposure to cashew nut shell fumes in Wistar rats and chickens.

Materials and Methods

Plant material

Dried samples of cashew seed (*A. occidentale*) were obtained from the Anyigba market at Ayingba, Kogi State, Nigeria. The cashew seeds were stored under open-air conditions.

Plant identification

The cashew nuts used for this study were collected in April 2023. The species *Anacardium occidentale* of the family of *Anacardiaceae Linn* was identified and authenticated in the department of Botany University of Ibadan, Oyo State Nigeria. The sample voucher number UIH-23435 was deposited at the University's herbarium.

Experimental Animals

A total of 24 Wistar rats of both sexes (12 males and 12 females) weighing 180±40g and 18 Chickens (Exotic cockerels) weighing 600±50g were used for this study. The rats were bred at the Animal House of the Faculty of Basic Medical Sciences, Prince Abubakar Audu University Anyigba Kogi State Nigeria. Day-old chicks were purchased at Anyigba market and housed at the animal house for two months before the experimental procedures. The animals were maintained under standard conditions (12 h light/dark cycles) before the experiment. Food (pellet from Animal Care Limited, Nigeria) and tap water were given *ad libitum*. The animals were cared for, in accordance with the recommendations provided in the "Guide for the Care and Use of Laboratory Animals" prepared by the University. The ethical approval for the research was obtained from the Research and Ethical Committee of the College of Health Sciences, Kogi State University Anyigba with approval number CHSREC/2021/0015.

Inhalation chamber design and operation

The exposure system for the inhalation toxicology consists of a fumegenerating chamber and a whole-body inhalation chamber that were serially connected via a polycarbonate plastic pipe. The cashew seed fume-generating chamber was made up of stainless steel to prevent contamination from metals. The whole-body inhalation chamber was made up of a transparent polycarbonate plastic which allowed observation of the animals during the experimental procedure. The chamber is rectangular with the following dimensions: length 59cm x width 34 cm x height 35cm. Cashew nuts were measured into the sealed fume-generating container and burnt under low heat continuously to generate fumes, that were released into the sealed inhalation chamber via a polycarbonate pipe. The exposure period was 1 hour per group of experimental animals as described by Makinwa *et al* (2019)⁹ with slight modifications.

Exposure procedure

Animals in each exposed group were placed in the chamber and were exposed to varying doses of CNSF per hour. Rats and chickens that were not exposed to cashew seed fumes serve as controls. Side examination of the exposure chamber was performed at 30-minute intervals to detect signs of toxicity such as chewing jaw movements, squinting, writhing, convulsion, yellowing of fur, restlessness, erection of fur, vocalization, exophthalmia, and behavioral abnormalities or even death. During each exposure procedure, we ensured the use of nose masks as a safety precaution to limit exposure to CNSF.

Experimental protocol

Wistar rats

Twenty-four (24) Wistar rats were divided into four groups of six animals each (3 male and 3 female rats). As follows

Group 1 (Control)- Wistar rats (male and female) control unexposed to CNSF. They were kept under normal room conditions for animals far from the experimental chamber

Group 2 (CNSF 500ppm/hr)- Wistar rats (male and female) exposed to 500ppm/hr. of cashew seed fumes for 1 hour

Group 3 (CNSF 1250ppm/hr) - Wistar rats (male and female) exposed to 1250ppm/hr. of cashew seed fumes for 1 hour

Group $\overline{4}$ (CNSF 2500ppm/hr) - Wistar rats (male and female) exposed to 2500ppm/hr. of cashew seed fumes for 1hour

Cockerel chickens

Eighteen (18) chickens were divided into three groups of six animals each. As follows

Group 1(Control)- Chickens control unexposed to CNSF. They were kept under normal room conditions for animals, far from the experimental chamber

Group 2(CNSF 500ppm/hr) - Chickens exposed to 500ppm/hr. of cashew seed fumes for 1 hour

Group 3(CNSF 1000ppm/hr) - Chickens exposed to 1000ppm/hr. of cashew seed fumes for 1 hour

Hematological determinations

At the end of the one-hour experiment, blood samples were collected from the animals by heart puncture into EDTA tubes, for the determination of Packed cell volume (PCV), Hemoglobin (HGB), White blood cells (WBC), platelets volume, Neutrophil (Neut), Lymphocytes (LYM), Mixed population of White Blood cells (MXD)-Monocyte, Basophil, Eosinophil. The hematological parameters were analyzed within 24 hours using an automated hematology autoanalyzer (Sysmex KX-21N) following the manufacturer's guidelines.

Histological analysis

The lung and heart tissues were examined histologically from the control (unexposed groups) and two exposure groups (500 ppm/hr and 12500 ppm/hr in rats and 500 ppm/hr and 1000 ppm/hr in chickens respectively) to CNSF in which most of the animals survived and from one exposure group (25000 ppm/hr) to CNSF from which all the rats died following I hr exposure to cashew nut fume. At necropsy, the lung and heart were fixed by inflating with 10% neutral buffered formalin, and dehydrated with descending grades of alcohol (100%, 90%, 80%, and 70%) accompanied by thorough rinsing in water. The tissues were then embedded in molten paraffin wax and sectioned at 5-6 microns, using a rotary microtome. The sections were examined microscopically after staining using hematoxylin for 5 minutes, rinsed thoroughly in water to remove excessive hematoxylin, and were briefly differentiated with 1% acid alcohol. Scott tap water was used to blue the nucleic for 5 minutes and rinsed in water before counter-staining with 1% aqueous eosin for 1 minute. The clearing was done with several changes of xylene and then mounted with DPX after which, the slides were examined under a light microscope at 10x and 40x magnification as described by Alabi et al., 12

Statistical analysis

Data were analyzed using SPSS package version 26.0 (SPSS Inc., Chicago, IL, USA) computer software. Values are expressed as the mean \pm SEM. Results were statistically analyzed by one-way analysis of variance (ANOVA) for differences between means of different groups, with the *P* of equal to 0.05 or less regarded as statistically significant. Student's T-test was used for the analysis of data between the two species of animals.

ISSN 2616-0684 (Print) ISSN 2616-0692 (Electronic)

Results and Discussion

There are four major routes of exposure through which toxic substances can enter the body: inhalation, dermal (or ocular) absorption, oral, and injection (intravenously or intramuscularly). Inhalation with related mortality rate is the most commonly reported.¹³ Gases and vapors are the most frequently inhaled substances; nevertheless, liquids and solids can also be inhaled in the form of finely divided mists, aerosols, or dust.¹³ Roasting of cashew nut shells promotes the release of various pollutants, such as aldehydes, ketones, and polycyclic aromatic

hydrocarbons (PAHs), which are capable of promoting significant pulmonary changes.¹⁰ In this study after one-hour exposure, the signs of toxicity observed at varying doses (500ppm/hr., 1000ppm/hr., 1250ppm/hr, and 2500ppm/hr.) of CNSF include; changes in motor activity, restlessness, licking, scratching, twitching, tremors, writhing, apathy, drowsiness. The animals did not recover to their normal state. Exposure to 2500ppm/hr. of cashew, seed fume resulted in the death of all the rats in the group within 1 hr. of exposure. As shown in Tables 1 and 2, there was a significant (P<0.05) increase in the serum Hb (g/dl) of chickens and rats exposed to CNSF for one hour compared with the unexposed control group.

Table 1: Hematological parameters of control and chickens exposed to cashew nut shell fumes (0	CNSF)

Groups/ CNSF Doses	(Hb)g/dl	% (PCV)	(WBC) x10 ⁹ /L	Platelet x10 ⁹ /L	Neut%	Lymph%	MXD%
1(Control)	7.50±0.54°	20.00±1.34°	17.20±1.86°	126.80±5.32	51.67±6.24	40.33±5.69	8.00 ± 1.97
2(500ppm/hr)	$6.98\pm0.78^{\circ}$	21.83±2.05°	18.94 ± 2.47	121.80±13.10	52.67±1.15	41.50±1.06	6.00 ± 1.00
3(1000ppm/hr)	$10.20{\pm}0.96^{ab}$	$27.83{\pm}2.06^{ab}$	$26.34{\pm}3.12^{a}$	117.67±9.93	60.17±3.35	33.33±3.17	6.50±0.62

The results are shown as means \pm SE (n=6). The different letters in the same column indicates statistical difference (p<0.05.) subscript a,b,c indicate a statistical difference (p<0.05) from groups 1, 2, 3 respectively. Hemoglobin (Hb), Packed cell volume (PCV), white blood cell count (WBC), Neutrophils (Neut), lymphocytes (LYM) and mixed population of white blood cells (MXD)

This increase may have resulted from oxygen deprivation and the need for more oxygen in the body. Reduced levels or oxygen deprivation may occur due to inhalation of particulates, carbon monoxides, and hydrocarbons in the CNSF which are released when cashew nuts are roasted. Carbon monoxide binds and hinders the ability of hemoglobin to bind and deliver oxygen to the body. Reduced oxygen binding capacity of hemoglobin may lead to subsequent oxygen deprivation in the body causing tiredness, dizziness, unconsciousness progressive heart problems, brain dysfunction, coma, and even death.^{14, 15} Excess levels of Hi, SHb, and HbCo usually occur due to inhalation of carbon particles, carbon monoxide, and hydrocarbon residues during the roasting of the cashew seed. This is supported by previous studies, 9,16 which reported that Hi, SHb, and HbCo levels were increased in the blood of chickens and rats exposed to cashew seed fumes. In addition, rats and chickens exposed to CNSF had a significantly (P<0.05) higher level of %PCV compared with unexposed groups. The elevated %PCV may be due to inhalation of smoke from the roasting of the cashew nuts. Smoke inhalation can thicken the blood by raising RBC production and decreasing plasma volume, potentially contributing to higher PCV. Furthermore, dehydration can cause fluid loss from the blood vessels, concentrating red blood cells and raising PCV. The higher % of PCV can also result from inflammation of the lungs by the CNSF. Exposure to CNSF significantly (P<0.05) increased the concentrations (as shown in Tables 1, 2, and 3) of WBC in both rats and chickens. The increase may have resulted from the stimulatory effect of the CNSF on the immune system and or defense mechanism elicited by the animal's immune system. The higher levels of the WBCs as seen in the CNSFexposed groups may be an indication of increased sensitization to an antigen or allergens. Previous studies have documented elevated WBCs in smokers;¹⁷ and rats exposed to smoke from mosquito repellant.¹⁸ WBCs are important in defending the body against infection, ¹⁹ thus, a higher level of the WBC and lymphocytes is an indication of increased sensitization to an allergen or antigen. These important key cells of the immune system are known to activate the production of antibodies to destroy evading pathogens. The platelets and serum levels of neutrophils, lymphocytes (LYM), and mixed population of white blood cells (MXD%) in chickens and rats exposed to CNSF for one hour were not significantly different from values in the unexposed control group. Although a previous study by Ayorinde et al., ¹⁸ reported higher serum concentrations of LYM and MXD% in rats exposed to mosquito coil smoke, when compared with the control for 2 weeks, the non-significant differences reported in the current study may be due to the short period (one hour) of exposure to the cashew nut shell fumes.

Smoke inhalation" is a generic term that refers to a potential exposure to a wide variety of substances because of the complex chemistry of heat decomposition and pyrolysis. Smoke contains particulate matter which is formed from incomplete combustion of organic material, usually less than 0.5 μ m in size.¹³ The entire respiratory tract can be endangered by smoke inhalation from fires,¹³ and inhalation of toxic gases such as carbon monoxide (CO), hydrogen sulphide (HS), sulphur dioxide (SO), and SPM (aerosols) in smoke.²⁰ Thus, small particles can easily reach the terminal bronchioles and can initiate an inflammatory reaction, leading to bronchospasm.²¹ A study by Uche and Otimize revealed that cigarette smoking and exposure to cigarette smoke compromise lung function with a decrease in peak expiratory flow rate.22 The findings in this study indicated moderate pneumonitis (Figures 1 and 4) in rats and chickens that were exposed to CNSF. pneumonitis is a respiratory tract disease that is characterized by general inflammation of lung tissue. It usually occurs when an irritant such as mold, bacteria, fungi, or chemicals found in animal fur, bird droppings, hot tubs, contaminated foods, or humidifiers causes lung inflammation. Previous studies,^{20, 23} had documented pneumonitis as a respiratory disease in birds. The moderate pneumonitis reported in the current study may have resulted from the mixture of pollutants and particulate matter from the combustion of the cashew nut shell. Previous studies have revealed that combustion exhaust gases from Cashew nutshell (CNS) promote the release of various pollutants, such as aldehydes, ketones and polycyclic aromatic hydrocarbons (PAHs), which are capable of promoting significant pulmonary changes, 10 as well as total particulate matter (TPM), which can easily penetrate the respiratory system and reach the pulmonary alveoli, resulting in respiratory diseases, 10such as chronic obstructive pulmonary disease (COPD). Histological assessment of CNSF exposed rats (male and female) lungs relative to unexposed rats (control) corroborated the findings of the hematological assay (Fig 3 and 4).

Groups/ CNSF Doses	(Hb)g/dl	% (PCV)	(WBC) x10 ⁹ /L	Platelet x10 ⁹ /L	Neut%	Lymph%	MXD%
1(Control)	9.40±0.86°	29.67±2.35°	2.10±0.20 ^{bc}	348.40±44.97	43.33±5.62	57.80±1.91	4.50±0.50
2(500ppm/hr)	11.56±0.62	28.50±3.21°	8.14 ± 0.72^{ac}	244.00±67.76	45.40 ± 7.92	55.66±8.01	3.33±0.67
3(1250ppm/hr)	13.47±1.03ª	$40.50{\pm}3.30^{ab}$	4.23±0.03 ^{ab}	283±00±47.82	26.80±4.74	70.60 ± 5.18	2.80±0.73

Table 2: Hematological parameters of control rats (group1) and rats exposed to CNSF (groups 2 and group 3)

The results are shown as means \pm SE (n=6). The different letters in the same column indicates statistical difference (p<0.05.) subscript a, b, c indicate a statistical difference (p<0.05) from groups 1, 2, 3 respectively. Hemoglobin (Hb), Packed cell volume (PCV), white blood cell count (WBC), Neutrophils (Neut), lymphocytes (LYM) and mixed population of white blood cells (MXD)

Groups /	Sex	(Hb)g/dl	% (PCV)	(WBC) x10 ⁹ /L	Platelet x109/L	Neut%	Lymph%	MXD%
CNSF Doses								
1(Control)	1F	8.40 ± 0.85^{cef}	25.67 ± 2.40^{f}	2.13±0.35 ^{cd}	346.00±29.00	35.50±4.50	60.50±3.50	4.67±0.88
	1 M	10.90 ± 1.20	33.67±2.40	2.00 ± 0.00^{cd}	350.00±80.25	39.67 ± 2.40	56.00 ± 2.00	4.33±0.67
2(500ppm)	2F	12.10±0.20 ^a	27.67 ± 4.70^{f}	$7.65{\pm}1.65^{abef}$	232.00±115.32	33.67±6.06	62.67 ± 7.50	3.67±1.45
	2M	11.20±1.05	$29.33{\pm}5.36^{\rm f}$	$8.47{\pm}0.84^{abef}$	262.00±74.00	63.00±0.00	34.00±0.00	3.00±0.00
3(1250ppm)	3F	$12.40{\pm}1.40^{a}$	37.00±4.00	$3.80{\pm}1.00^{cd}$	273.00±54.87	29.33±6.33	67.33±7.21	3.33±2.08
	3M	14.65±1.55 ^a	44.00±5.00 ^{acd}	4.75±0.50 ^{cd}	299.00±116.00	32.00±0.00	67.00±0.00	2.00±0.00

The results are shown as means \pm SE (n=3). The different letters in the same column indicates statistical difference (p<0.05.) subscript a, b, c, d, e, f indicate a statistical difference (p<0.05) from groups 1F, 1M, 2F, 2M, 3F, 3M respectively. F=female rats, M=male rats. Hemoglobin (Hb), Packed cell volume (PCV), white blood cell count (WBC), Neutrophils (Neut), lymphocytes (LYM) and mixed population of white blood cells (MXD)

Table 4: Hematological parameters of control Chickens and control Wistar rats

Group/	(Hb)g/dl	% (PCV)	(WBC) x10 ⁹ /L	Platelet x109/L	Neut%	Lymph%	MXD%
Animal Specie							
Control Chickens	7.50±0.55	20.00±1.34b	17.20±1.86 ^b	126.80±5.32ª	51.66±6.24	40.33±5.69 ^a	8.00±1.97
Control Rats	9.40±0.86	29.67±2.35ª	2.10±0.20 ^a	348.40 ± 44.97^{b}	43.33±5.62	57.80±1.91 ^b	4.50 ± 0.50

The results are shown as means \pm SE (n=6). The different letters in the same column indicates statistical difference (p<0.05.) subscript a and b indicate a statistical difference (p<0.05) from control chickens and rats respectively. Hemoglobin (Hb), Packed cell volume (PCV), white blood cell count (WBC), Neutrophils (Neut), lymphocytes (LYM) and mixed population of white blood cells (MXD)=

There was a mononuclear inflammation of the septa of alveoli indicative of moderate pneumonitis in rats (both male and female) exposed to 500ppm/hr. and 2500ppm/hr. of CNSF for 1 hr. The heart histology of chickens and rats exposed to the CNSF at different doses for one hour is suggestive of mild and moderate myocarditis (Figures 2 and 3) compared with the control rats which showed essentially normal striated muscles. Data from previous studies have shown that exposure to fumes altered the morphology of the cardiac cells and tissues. A Study by Amakiri et al.²⁰ revealed that the hearts of chickens exposed to the flame and fumes of kerosene showed waviness of cardiac myocardial muscle cells, while Ou and Ramos 24 reported that aromatic and polycyclic hydrocarbons are known persistent environmental contaminants identified as vascular toxins in experimental animals, and are known to initiate atherogenic process in aorta of animals. These findings agree with the observations in this study. Comparative analysis of the hematological parameters in the chickens and rats (for both control and those exposed to 500ppm of CNSF) showed the same pattern of variations for all the parameters. All the experimental chickens (both control and chickens exposed to 500ppm of CNSF)) had lower Hb, %PCV, Lymph, and platelets compared with the Wistar rats.

The rats (both control and rats exposed to 500 ppm of CNSF) had lower White blood cell count (WBC), Neutrophils, and

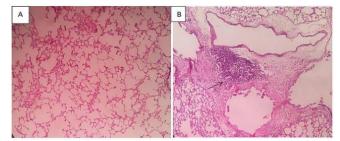


Figure 1: Photomicrograph of the lung sections of control (unexposed) and Cockerel Chickens exposed to CNSF for 1hour (H and E X10). A=control Chickens showing normal alveoli spaces filled with air. B = Chickens exposed to 500ppm/hr. of CNSF for 1 hr. showing mononuclear inflammation of the septa of alveoli (moderate pneumonitis)

ISSN 2616-0684 (Print) ISSN 2616-0692 (Electronic)

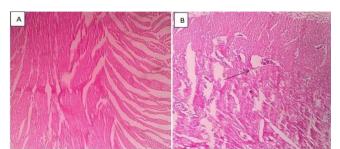


Figure 2: Photomicrograph sections of the Heart of control (unexposed) and Cockerel Chickens exposed to CNSF for 1hour (H and E X10). A=control Chickens showing essentially normal striated muscles. B =Chickens exposed to 500ppm/hr for 1 hr. showing mononuclear inflammatory infiltration of the striated muscles (moderate myocarditis)

Mixed population of white blood cells (MXD) levels compared with the chickens. Species-specific differences could have important implications in acute inhalation toxicity testing, because of differences in the pattern of deposition of the test substance and variation in the specific pathways by which the compound is cleared from the lungs.²⁵ The deposition of inhaled substances depends upon airflow dynamics such as the gross anatomy and geometry of airways in both the upper and lower respiratory tract, airway dimensions (e.g., length and diameter) ²⁶ and respiratory physiology (e.g., breathing mode and ventilation rates), which is different across species.²⁵

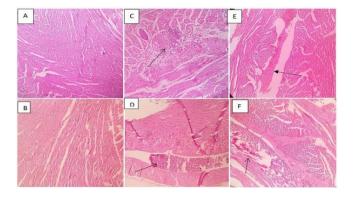


Figure 3: Photomicrograph sections of the Heart of control unexposed and rats exposed to CNSF. A and B =control Male and Female rats showing essentially normal striated muscles. C and D = Male and Female rats exposed to 500ppm/hr. E and F Male and Female rats exposed to 2500ppm/hr. of cashew nut fumes respectively for 1 hr. showing mononuclear inflammatory infiltration of the striated muscles (moderate myocarditis)

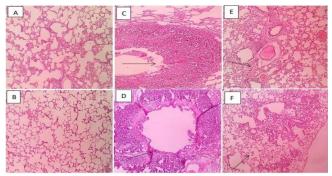


Figure 4: Photomicrograph sections of the Lungs of control unexposed rats and rats exposed to CNSF. A and B = control Male and Female rats showing normal alveoli spaces filled with air. C and D = Male and Female rats exposed to 500ppm/hr., and E and F Male and Female rats exposed to 2500ppm/hr. of cashew nut fumes respectively for 1 hr. showing mononuclear inflammation of the septa of alveoli (moderate pneumonitis)

The higher toxicity of some pesticides or toxicants documented in birds than in mammals had been reported to be due to the lower activity of avian metabolic enzymes.²⁷ In this study the higher level of WBC, Neut, and MXD% recorded in the CNSF-exposed chickens compared with the rats may be due to lower activity of avian metabolic enzymes, differences in gross anatomy and geometry of airways as well as higher level of susceptibility and sensitivity to respiratory pathogens. It has been reported that Avian responses to inhalation pollution include; respiratory distress, increased detoxification efforts, elevated stress levels, immunosuppression, behavioral changes, and impaired reproductive success.²⁸

Table 5: Hematological parameters of Chickens and Rats exposed to 500ppm of cashew nut shell fumes (CNSF)

CNSF Doses / Animal specie	(Hb)g/dl	% (PCV)	(WBC) x10 ⁹ /L	Platelet x10 ⁹ /L	Neut%	Lymph%	MXD%
500ppm/hr. Chickens	6.98±0.78 ^b	21.83±2.05	18.94±2.47 ^b	121.80±13.10	52.66±1.15	41.50±1.06	6.00±1.00 ^b
500ppm/hr Rats	11.56±0.62 ^a	28.50±3.21	8.14±0.72 ^a	244.00±67.76	45.40 ± 7.92	55.66 ± 8.01	3.33±0.67 ^a

The results are shown as means \pm SE (n=6). The different letters in the same column indicates statistical difference (p<0.05.) subscript a and b indicate a statistical difference (p<0.05) from chickens and rats exposed to 500ppm of CNSF respectively. Hemoglobin (Hb), Packed cell volume (PCV), white blood cell count (WBC), Neutrophils (Neut), lymphocytes (LYM) and mixed population of white blood cells (MXD)

Conclusion

In conclusion, these findings have shown an extent of toxicity due to cashew nut fumes after one-hour acute exposure. The study therefore suggests caution in exposure to cashew nut fumes chronically that may result in cardiac and respiratory problems such as coughing, wheezing, shortness of breath, bronchitis, and asthma.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

 Agboola-Adedoja MO, Adelusi AA, Ogunwolu QA, Ugwu CA, Alli MA, Adesanya KA and Akinpelu AO. Cashew production, consumption and utilization: Implication on health of end users. World J of Adv Res and Rev, 2022; 14(01), 182–186

- Nkwocha CC, Uchendu ON, Amujiri NA. Phytochemicals and Chemical Compositions of Raw Cashew (Anarcardium occidantale) Nuts Sourced from Enugu State, South Eastern Nigeria. Trop J Nat Prod Res. 2020; 4(12):1039-1044. doi.org/10.26538/tjnpr/v4i12.3
- Zarqa I, Muhammad A, Muhammad MS, Marium A, Muhammad D, Aamir S, Muhammad TK, Hina A, Fahad S, Muhammad R. Medicinal Uses of Cashew (Anacardium occidentale): Review J of Sci Tech and Res (JSTAR) (2021).2, (1) 1-10
- Owumi SE, Fatoki JO, Gbadegesin MA and Odunola OA. Clastogenic and toxicological assessment of cashew (Anacardium occidentale) nut bark extracts in Wistar rats. ACTABIOCHIMICAPOLONICA Vol. 2015; 62 (3)563– 567
- Kumar PP, Paramashivappa R, Vithayathil PJ, Subba RPV and Srinivasa RA. Process for isolation of cardanol from technical cashew (Anacardium occidentale L.) nut shell liquid. J of Agric and Food Chem. 2002; 50:4705-4708.
- Adriana D and Diogo PC. A Mini-Review on Almonds and Cashew Nuts: Processing Impact, Phytochemical and Microbiological Properties, and Implications on Human Health. *American Journal of Food Science and Technology*. 2022, 10(5), 233-238. DOI: 10.12691/ajfst-10-5-4
- 7. Ikeda R, Tanaka H, Uyama H, and Kobayashi S. Synthesis and curing behaviors of cross-linkable polymer from cashew nut shell liquid. Polymer. 2002; **43**:3475-3481.
- Master A, Santosh KD, and Sachidananda P. Eco-Consciousness for Poisonous and Injurious Plants Among Urban Dwellers of Bhubaneswar, Orissa. J. Hum. Ecol. 2006; 19(4): 239-248
- 9. Makinwa TT, Ode TA, Alabi TD, Akinmosin BF, and Akeshinro AA. Toxicological evaluation of Exposure to Cashew (*Anacardium occidentale*) seed fume on serum Methemoglobin, Carboxyhemoglobin, Sulphahemoglobin, and Lactate Dehydrogenase activities in Chickens. Biokemistri. 2019; **31**(2) 53 – 62
- Josino JB, Serra DS, Gomes MDM, Araújo RS, de Oliveira MLM and Cavalcante FSA. Changes of respiratory system in mice exposed to PM4.0 or TSP from exhaust gases of combustion of cashew nut shell. Envt toxicol and pharm. 2017; 56:1-9.
- Fladimir LG, Gilvan RS, Daniel SS, Rinaldo SA, Mona Lisa MO and Francisco SÁC. Effects of Exposure to Total Particulate Matter of Cashew Nuts Shell in the Respiratory System of Mice with Chronic Obstructive Pulmonary Disease. Aust J of Bas and Appl Sci. 2019;**13**(1): 71-79.
- 12. Alabi TD, Brooks N I, and Oguntibeju OO. Antioxidant status and hepato-protective role of Anchomanes difformis in streptozotocin-induced diabetes in male Wistar rats. Herba Pol, 2020; 66(1):18-36
- Gorguner M and Akgun M. Acute Inhalation Injury. EAJM. 2010; 42: 28-35
- Ranney HM and Sharma V. Structure and Function of Hemoglobin. In Beutler E, Lichtman M et al (eds) William's Hematology (6th edition) McGraw Hill: 2000; pp. 345-353.
- 15. Bateman DN. "Carbon Monoxide". Med. 2003; **31** (10):233.
- 16. Makinwa TT, Ibiyemi DA, Alabi TD, Okunade MB, Oniwon WO, Adeusi TE Activities of Lactate Dehydrogenase, Methemoglobin, Carboxyhemoglobin and Sulphahemoglobin Concentrations in the Blood of Albino Rats Exposed to Cashew Seed Fume. Open Access Lib J. 2016; 3: 2284.
- Sunyer J, Munoz A, Peng Y, Margolick J, Chmiel JS, Oishi J, Kingsley L, Samet JM. Longitudinal relation between smoking and white blood cells. Am. J. Epid. 1996; 144: 734-741.
- 18. Ayorinde AF, Oboh BO, Olutanjo OA, Alinba AC, and Odeigah PC. Some toxicological effects of commonly used

mosquito repellant in Lagos State Nigeria. Res. J. Environ. Toxicol. 2014; 8 (1) 46-52

- Aboderin FI and Oyetayo VO. Hematological studies of rats fed different doses of probiotic, *Lactobacillus plantarum*, isolated from fermenting corn slurry. *Pak. J. Nutr.* 2006; 5:102-105
- Amakiri AO, Owen O and Iboh JI. Histopathology of the organs of broiler chickens exposed to flame and fumes of kerosene burning. Bulletin of animal health and production in Africa. 2010; 58; 268.
- 21. Ainsile G. Inhalational injuries produced by smoke and nitrogen dioxide. Respir Med 1993; 87: 169-74. 34.
- 22. 22.Uche KO and Otimize FM. Modulation in Airway Smooth Muscle Reactivity and Improvement in Lung Function of Cigarette Smokers and Passive Non-smokers Following Garcinia kola Treatment: http://www.doi.org/10.26538/tjnpr/v1i4.7. Trop. J Nat. Prod. Res. (*TJNPR*) 2017; 1(5), 209–212.
- 23. Gino L. Diseases of Poultry; fungal infections. Avian Diseases Manual. 6th edition 2023; pp 1-2
- 24. Ou X and Ramos KS. Modulation of aortic protein phosphorylation by benzo(a)pyrene: implication in PAH-induced atherogenesis. J of Biochem Toxicol. 1992; **7**(**3**)147-154
- Movia D, Bruni-Favier S and Prina-Mello A. In vitro Alternatives to Acute Inhalation Toxicity Studies in Animal Models. A Perspective. Front. Bioeng. Biotechnol. 2020; 8:549.
- Hofmann W, Koblinger L and Martonen TB. Structural differences between human and rat lungs: implications for Monte Carlo modeling of aerosol deposition. Health Phys. 1989; 57, 41–46
- Katagi T and Fujisawa T. Acute toxicity and metabolism of pesticides in birds. J. Pestic. Sci.2021; 46(4) 305-321
- Sanderfoot OV and Holloway T. Air pollution impacts on avian species via inhalation exposure and associated outcomes. Environ. Res. Lett. 2017; (12)083002