# SUB ACUTE TOXICITY STUDY OF SOME BRANDS OF AIR FRESHENERS SOLD IN KANO ON SWISS ALBINO RATS (*RATTUS NORVEGICUS*)

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## Abstract

Toxicity effects of some Air Fresheners sold in Kano were determined using standard technique by exposing Swiss Albino Rats (*Rattus Norvegicus*) to gel and liquid air fresheners eight hours daily for 28 Days. Changes in some physical and histological parameters were evaluated. Forty-four male albino rats weighing between 120g and 140g were randomly divided into 11 groups of 4 animals per cage. Five groups were exposed to gel air fresheners, (each group at a concentration of 3g) for 8 hours daily, another five groups were exposed to liquid air fresheners, (each group with a concentration of 3ml) daily for 4 weeks while the last group was considered as control. After termination of the experiment, animals were sacrificed and biopsies of heart and lungs of albino rats were collected in a sterile bottle containing 10% formal saline and transported to the laboratory for analyses. Results of the research indicated significant decrease ($P>0.05$) in final body weight of animals when compared with the final body weight of control, the rate of food intake also decreases quantitatively and movement of animals within the cage changes from normal to sluggish. Results of the histological analyses of lungs tissue have revealed areas of vascular congestion, heamorrhage and infiltration of leucocytes as the effects caused by air fresheners exposure, while sections of heart histology revealed mycocardial damage and haemorrhage as the effects induced by the effects of air fresheners inhalation. The study revealed that air fresheners exposures caused impairment in function and structure of albino rats heart's and lungs.

**Keywords:** Air freshener, Albino Rats, Toxicity, haemorrhage, histology.

## INTRODUCTION

The rise in popularity of air fresheners has outpaced awareness of the potential health threats from exposure to the effects of chemicals they may contain. Air fresheners are consumer products that emits fragrance to provide an aroma to a space, to mask an odor, or both (Steinemann, 2016). They are known to contain a number of different chemical agents in order to neutralize offensive odors and create a more pleasant scent (Gilbert, 2009). Some of the different types of air fresheners include incense sticks, scented candles, aerosol, liquid, gel, and electric diffusers (BEUC, 2005). The term air freshener may be misunderstood since these products do not considerably reduce air pollutants but rather add more substances to the air that have an odor strong enough to mask bad odors (kim *et al.,* 2015). Past Studies have shown that air fresheners emit over hundred different chemicals, including volatile organic compounds such as terpernes, benzene, formaldehyde, terpenoids, ethanol, formaldehyde, benzene, toluene, xylene and phthalate esthers (BEUC, 2005, NRDC, 2007, Steinemann, 2009, 2016). The components emitted from air fresheners are directly inhaled by the respiratory system through the nose to the alveoli; the eyes, nose, and skin are directly affected during the usage of air freshener (Kim *et al*., 2015). In addition the VOCs emitted by air fresheners react with ozone to produce secondary pollutants such as ultrafine particles; the particles formed by the reaction affect health in a manner dependent on particle diameter. Secondary pollutants also affect the respiratory system, central nervous system, and immune response (Kim *et al*., 2015).

A study in 2006 found that the prominent products of the reaction of terpenes found in air fresheners with ozone included formaldehyde, hydroxyl radical, and secondary ultrafine particles (EPA, 2006). In addition to this effect, it is associated with sensory irritation, most commercial air fresheners have low sensory threshold, and thus damaging health effect comes from sensory irritations that are caused by constant use of air fresheners (Wilkins *et al.,* 2007). Pollutants emitted from air fresheners are linked with damage to the brain, lungs, heart, reproductive system, immune system, and with cancer (Steinemann, 2009). Spiller (2004) investigates the toxic effect of volatile substance for 6year period (1996-2001). He reported that three categories were responsible for the majority of deaths: gasoline (45%), air fresheners (26%), and propane/butane (11%). In a U.S. cross-sectional study of 1054 individuals, 17.6% reported "headaches, breathing difficulties, or other health problems" when exposed to air fresheners (Caress and Steinemann, 2004). Among the 148 asthmatic study participants, 29.7% reported that air fresheners "make it difficult to breathe", while 37.2% reported finding "scented products" irritating. There are case reports of air freshener overdose from inhaling the spray at close range. This has caused rapid heartbeat that required hospitalization (Senthilkumaran *et al.,* 2011). The University of Bristol's Avon Longitudinal Study of Parents and Children (ALSPAC) found that exposure to volatile organic compounds through frequent use of air fresheners and other aerosols in the home was found to correlate with increased ear aches and diarrhea in infants, and with increased depression and headaches in their mothers (Farrow *et al.,* 2003). The present study was designed to investigate the possible changes in physical parameters and histological changes of lungs and hearts sections of albino rats exposed to gel and liquid air fresheners sold in kano.

## MATERIALS AND METHODS

### Study Site

The study was carried out at Biological Sciences Department of Bayero University Kano and the histological analyses were conducted at histology unit of Aminu Kano Teaching Hospital (AKTH) Kano.

### Experimental Animals Collection

A total of 44 male albino wistar rats ranged between 120 and 140g body weight were obtained from the animal house of Biological Sciences Department, Bayero University Kano. The animals were housed in a cage at room temperature and relative humidity, they were fed with palletized growers mash and water. The rats were allowed to acclimatize for two weeks before commencement of the experiment.

### Sourcing of the Air Freshener

Ten brands of air fresheners were randomly selected and procured from Sabon Gari Market, Kano Nigeria. Out of these ten air fresheners, five were Gel air fresheners while the remaining five were liquid air fresheners. The samples were stored at room temperature. The name of the air freshener, the list of the active ingredients and scented names were obtained from the packages.

### Distribution of Animals and Treatment

After two weeks of acclimatization, the 44 animals were randomly divided into 11 groups of 4 animals each cage. Five groups each was exposed to a particular brand of gel air freshener at a concentration of 3g for 8 hours daily for 28 days (Akingbade *et al*., 2014). While for the other five groups (each group) was sprayed 3ml of a particular liquid air freshener once daily for 28 days following the method of Al Sahaf, (2012). Animals in the last group were considered as controls.

### Measurement and Observation of Physical Parameter

Weight of the rats were taken weekly for four weeks during the experimental period and finally before the sacrifice using Harris side arm balance, following the method of Ashade and Igbokwe (2014).

### Sample Collection

After 28 days of the experiment, the animals were fasted overnight, and sacrificed the next morning by cervical dislocation, the animals were dissected and the biopsies of lungs and heart of the albino rats were collected and immediately conveyed to the laboratory for analyses.

### Histological studies

The lungs and heart freshly dissected out from the sacrificed rat was fixed in 10% formal saline under tap water, dehydrated, cleared and embedded in paraffin wax (5860°C). The block was trimmed and sectioned at 4-5 µm thickness using a rotatory microtome. Permanent slides of transverse sections were stained with haematoxylin and eosin as per the standard method (Anwioro, 2010). Slides were observed using x10 magnification.

## Results

### Physical Changes Assessment

The effect of air fresheners exposure on physical parameters as recorded in table 4.2 caused significant decrease (P> 0.05) in the final body weight of the animals when compared with the final body weight of control animals, also activities of the animals in terms of movement within the cage also changes from normal to sluggish.

### Histological Changes Assessment

Exposing Rats to air Fresheners in the present study caused deleterious effects such as vascular congestion, infiltration of leucocytes and hemorrhage on the sections of the lungs histology of the exposed animals (Plate 1a-1k). Myocardial damage and hemorrhage were the effects observed from the sections of heart histology of the exposed animals (Plate 2a-2k).

**Table 1.0: Changes in Body Weight of Animals Exposed to Air Fresheners**

| Groups | Initial Weight (g) | Final Weight (g) |
|---|---|---|
| Group 1 | 135.0 ± 5.0 | 145.0 ± 5.0* |
| Group 2 | 132.5 ± 5.0 | 125.0 ± 5.0* |
| Group 3 | 135.0 ± 5.0 | 117.5 ± 2.5* |
| Group 4 | 135.0 ± 5.0 | 127.5 ± 2.5* |
| Group 5 | 127.5 ± 2.5 | 132.5 ± 2.5* |
| Group 6 | 127.5 ± 2.5 | 137.5 ± 2.5* |
| Group 7 | 127.5 ± 2.5 | 135.0 ± 5.0* |
| Group 8 | 135.0 ± 5.0 | 127.5 ± 2.5* |
| Group 9 | 127.5 ± 2.5 | 132.5 ± 2.5* |
| Group 10 | 137.5 ± 5.0 | 127.5 ± 2.5* |
| Group 11(Control) | 137.5 ± 2.5 | 165.5 ± 5.0 |

Values are expressed as mean ± SD (*) Significant at p<0.05

**Key:** NM – Normal
SL – Sluggish
Group 1-5: animals exposed to gel air fresheners
Group 6-10: animals exposed to liquid air fresheners.

**Sections of Lungs histopathogy**

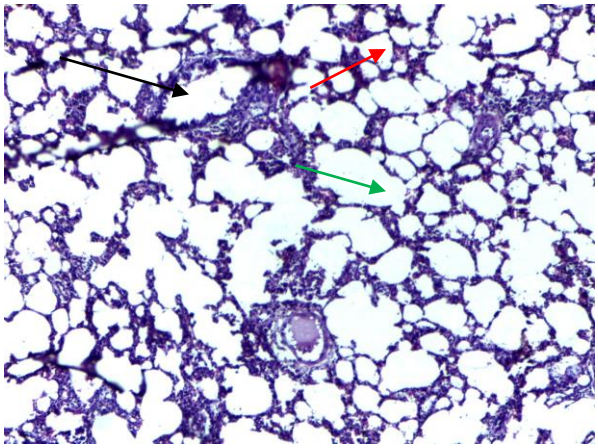

Plate 1: control animal lungs section showing normal alveolar sacs (red arrow), respiratory bronchioles (black arrow) and alveolar ducts (green arrow) (H&E x 100).

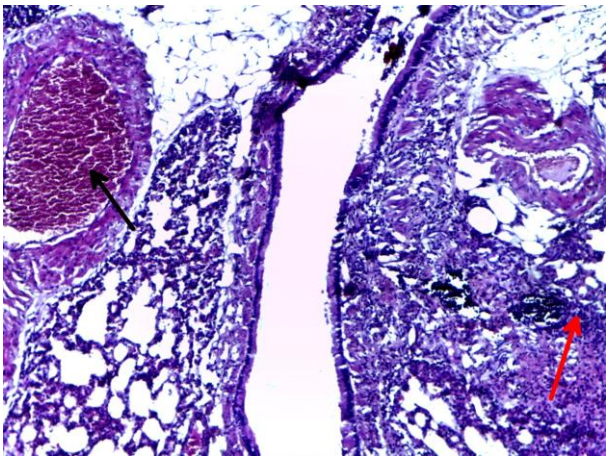

Plate 2: Group 1 animal lungs section (H&E x 100)

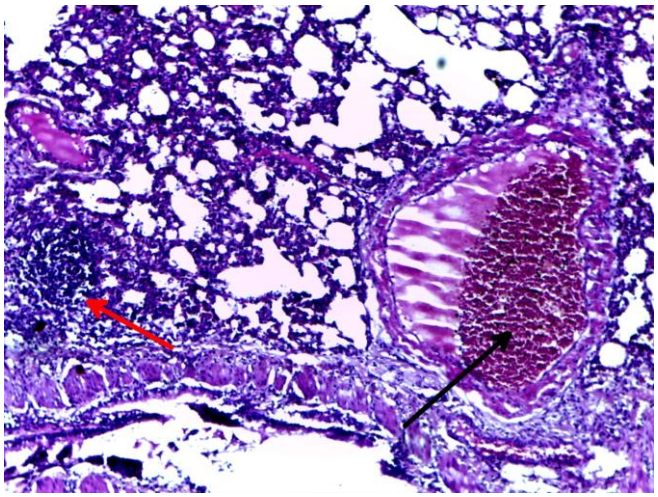

Plate 1c: Group 2 animal lungs section (H&E x 100)

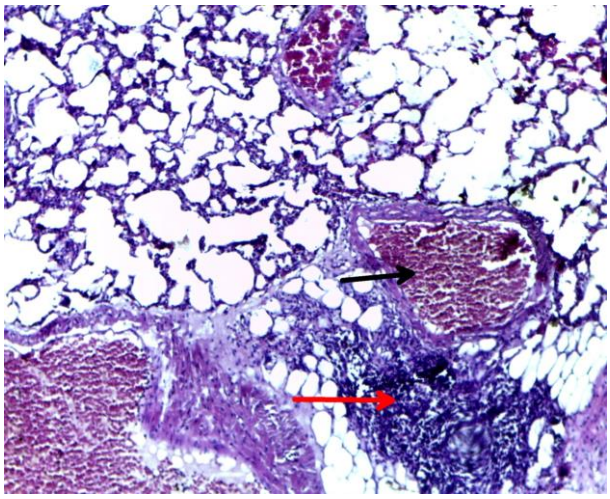

Plate 1d: Group 3 animal lungs section (H&E x 100).

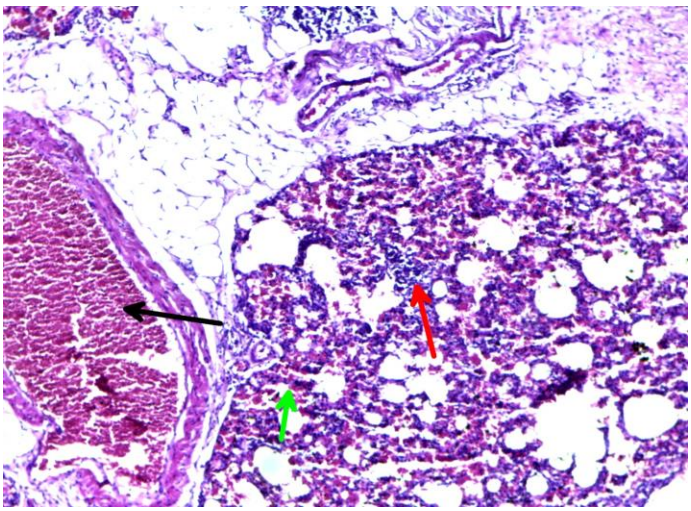

Plate 1e: Group 4 animal lungs section (H&E x 100).

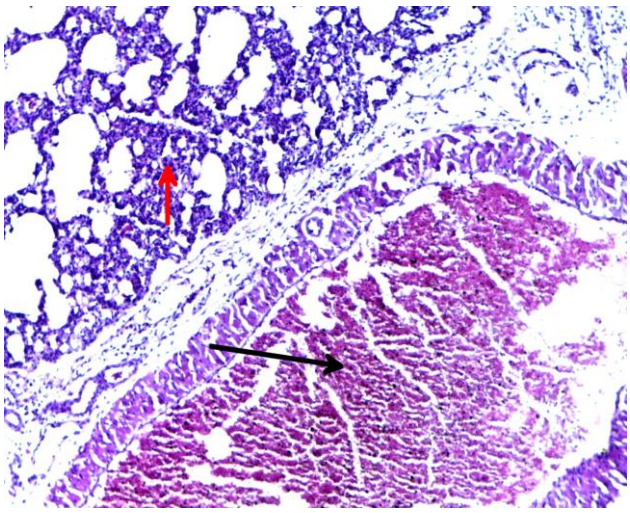

Plate 1f: Group 5 animal lungs section (H&E x 100).

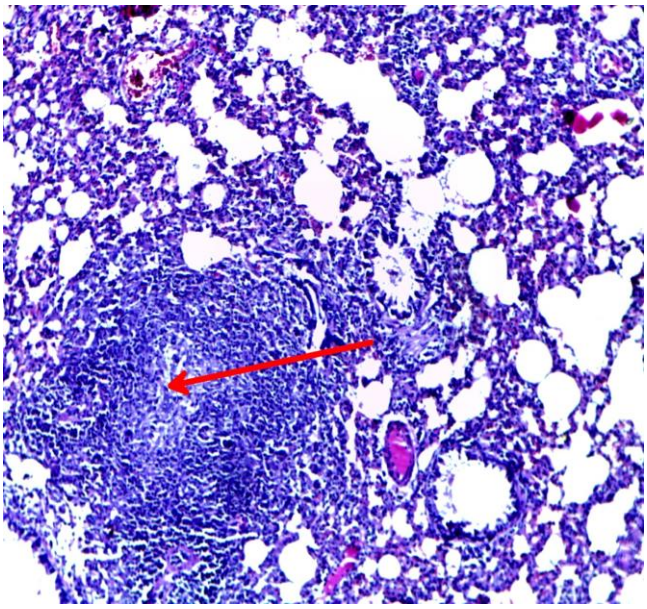

Plate 1g: Group 6 animal lungs section (H&E x 100).

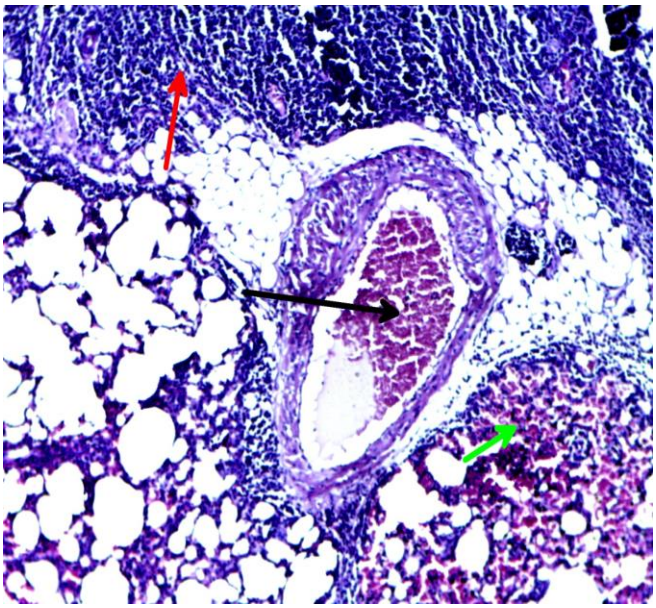

Plate 1h: Group 7 animal lungs section.

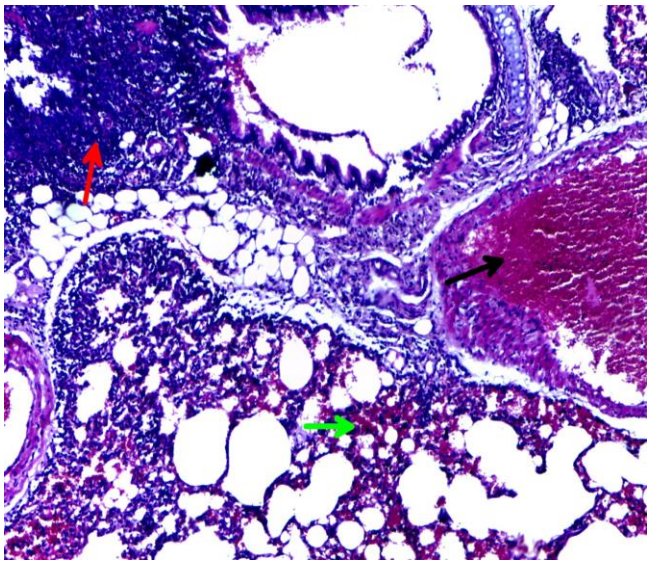

Plate 1i: Group 8 animal lungs section (H&E x 100).

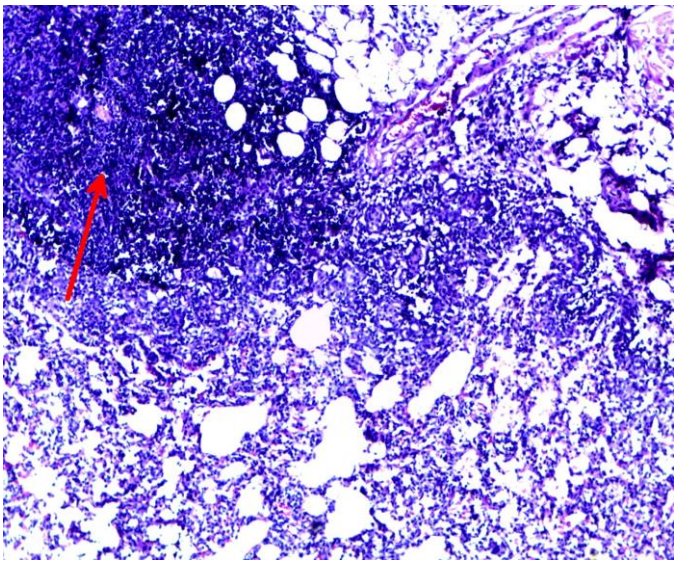

Plate 1j: Group 9 animal lungs section (H&E x 100).

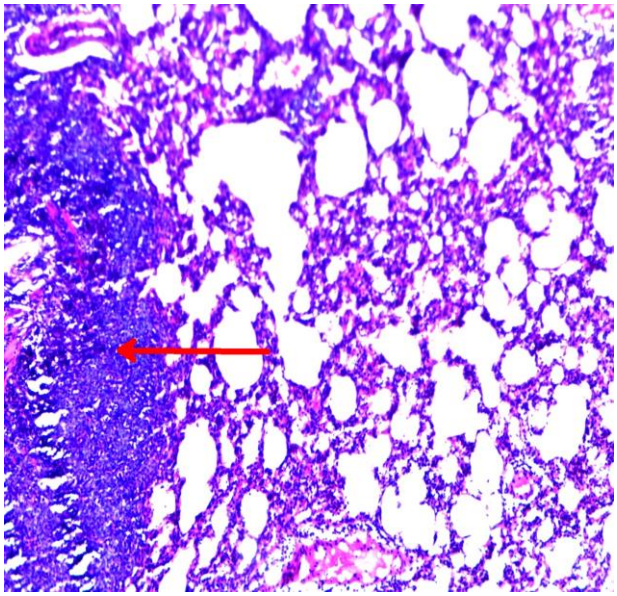

Plate 1k: Group 10 animal lungs section (H&E x 100).

**Keys:** green arrow represents hemorrhage, red arrow represents infiltration of leucocytes and black arrow represents vascular congestion.

Sections of heart histopathology.

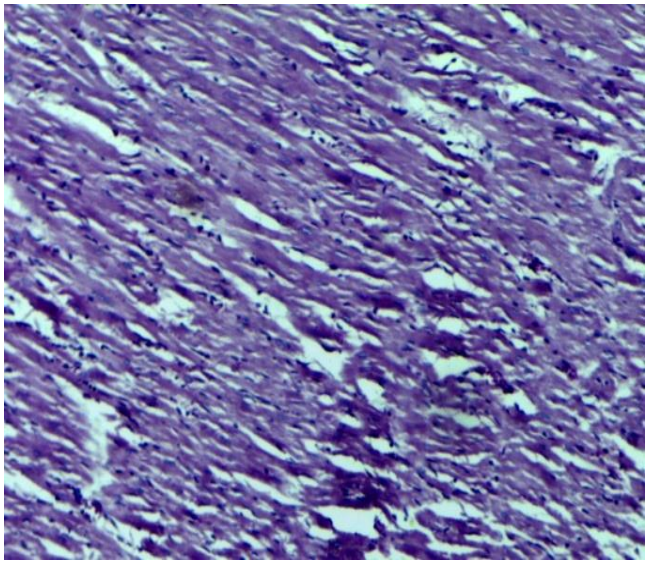

Plate 2a: Control animal heart section showing normal arrangement of myocardial tissue (H&E x 100).

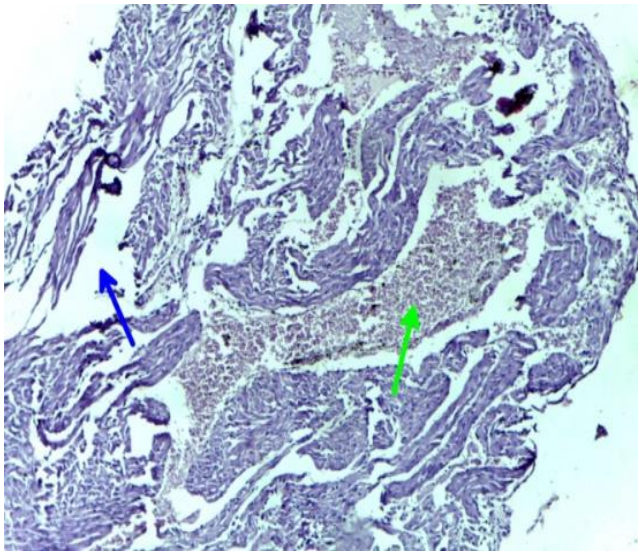

Plate 2b: Group 1 animal heart section (H&E x 100).

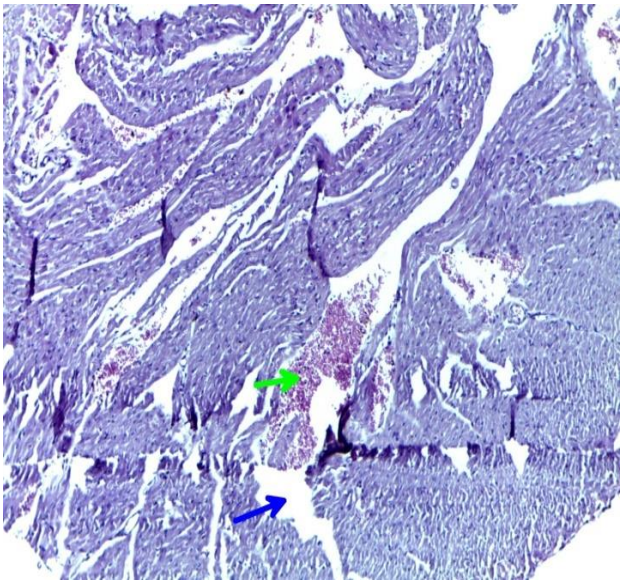

Plate 2c: Group 2 animal heart section (H&E x 100).

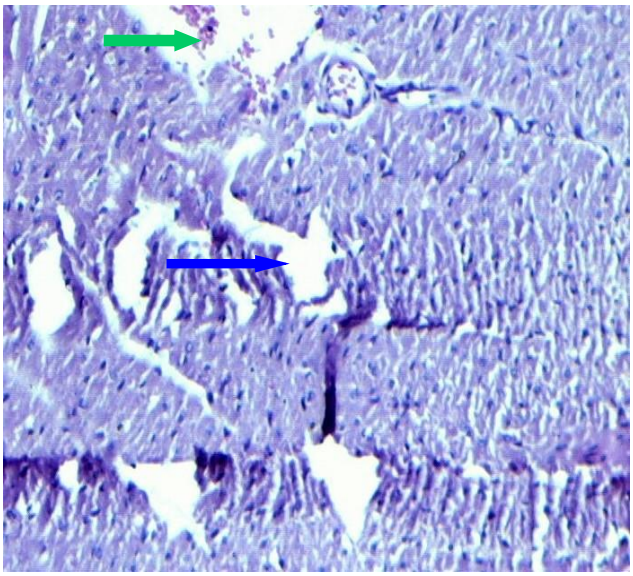

Plate 2d: Group 3 animal heart section (H&E x 100).

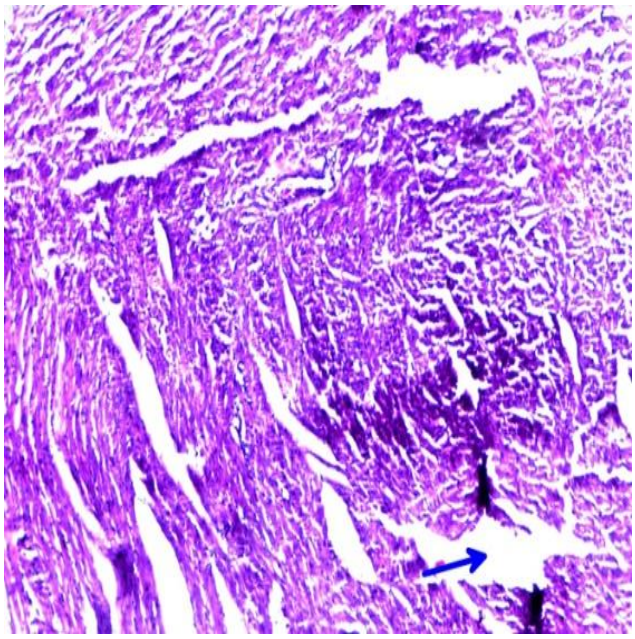

Plate 2e: Group 4 animal heart section (H&E x 100).

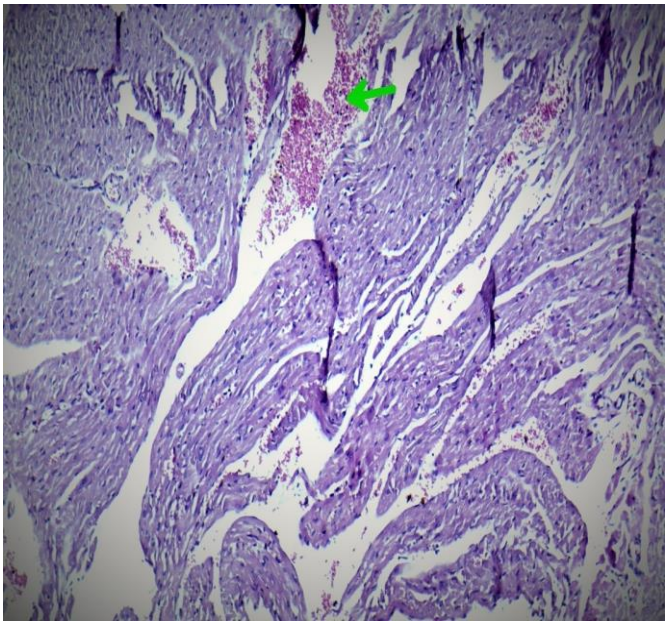

Plate 2f: Group 5 animal heart section (H&E x 100).

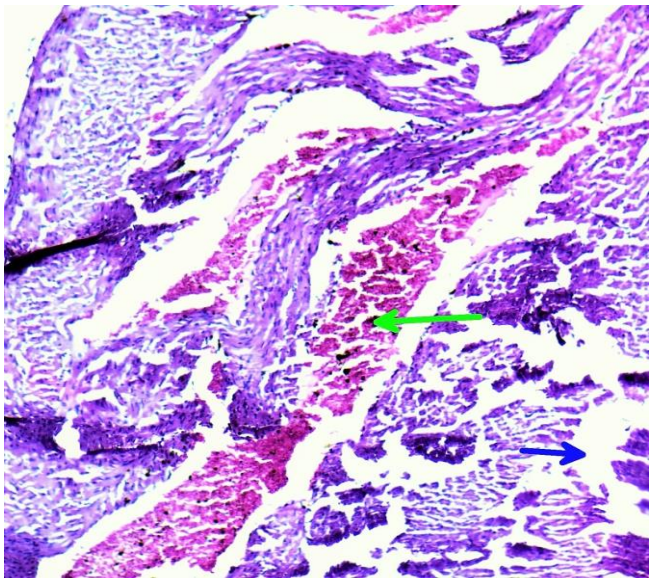

Plate 2g: Group 6 animal heart section (H&E x 100).

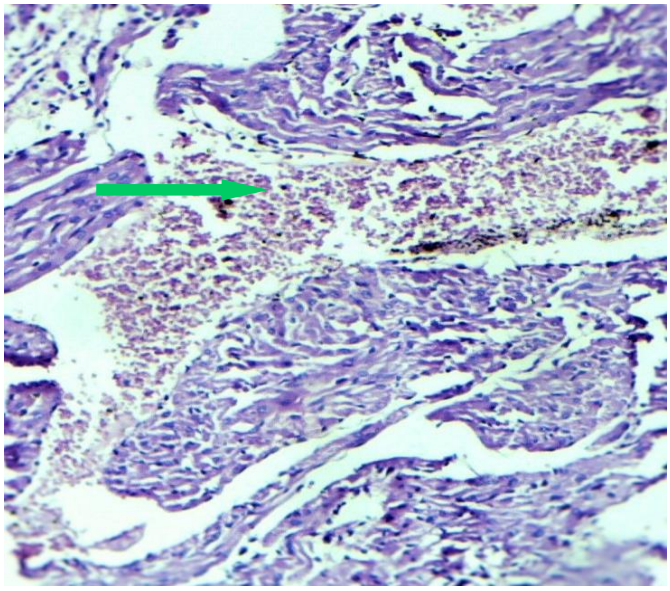

Plate 2h: Group 7 animal heart section (H&E x 100).

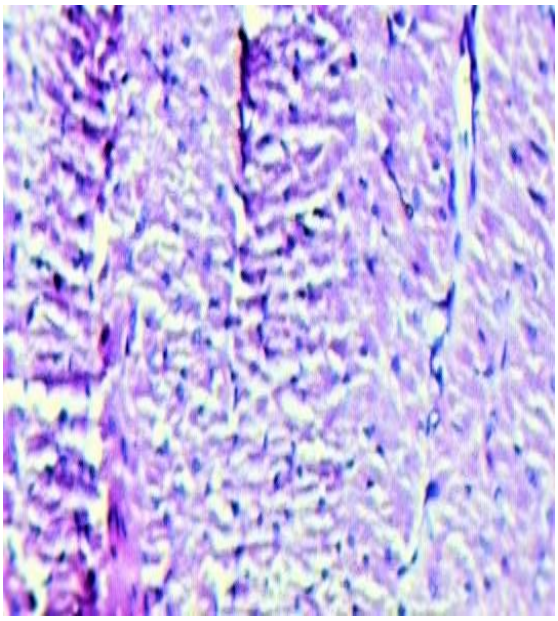

Plate 2i: Group 8 animal heart section (H&E x 100).

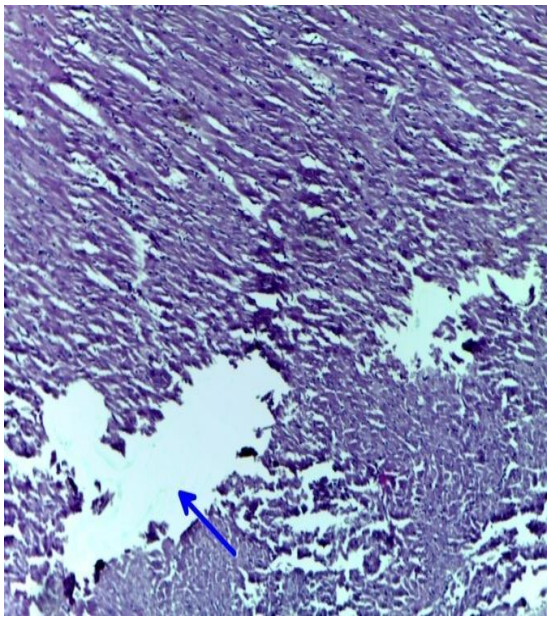

Plate 2j: Group 9 animal heart section (H&E x 100).

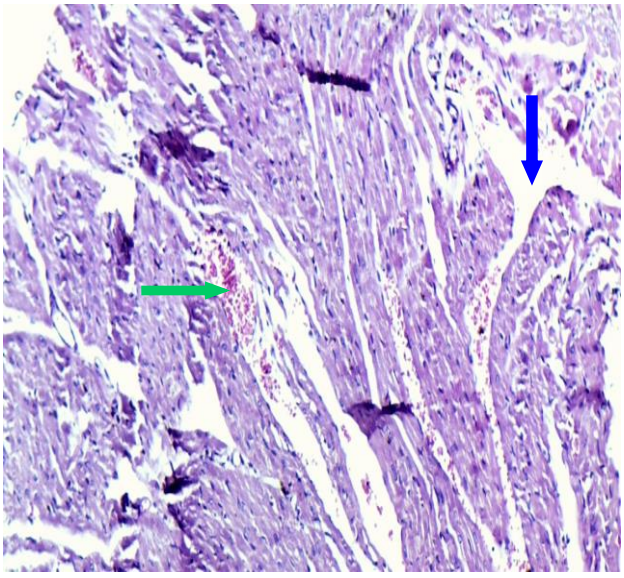

Plate 2k: Group 10 animal heart section (H&E x 100).

**Keys:** green arrow represents hemorrhage, while blue arrow represents myocardial damage.

**Discussion**

Exposing albino rats to gel and liquid air fresheners, in the present work caused significant difference in the final body weight of the animals when compared with the final body weight of the control animals. The increase in body weight of control animals is an indication that the animals are still in the active growth condition during the study period (Saalu *et al*., 2008). The decrease in body weight of groups 2, 3, 4, 8, and 10 animals is attributed to decrease in food intake as observed during the experimental period. The reduction in body weight might also be due to the effects of fragrance and parfum ingredients that caused interruption in absorption and metabolism of food nutrients essential for health (Gentry *et al*., 2014). Similar decrease in body weight of animals has been recorded by Akingbade *et al*. (2014) after exposing albino rats to incense air fresheners for four weeks. It was also observed in the present study that the activity of the animals in terms of movement within cage changes from normal to sluggish during the experimental period. The above change is attributed to the effect of fragrance chemicals that causes a host of physical and neurological problems such as dizziness, headache, and fatigue (William, 2004).

The histological analyses of lungs section of the exposed animals show areas of vascular congestion, hemorrhage and infiltration of leucocytes. All the lungs sections were affected with one or more of the listed disorders (plate 1a-1k). The present study is in agreement with the study of Anderson and Anderson (1997), who found that air freshener causes sensory, pulmonary irritations, decreased expiratory air flow velocity and neurotoxicity in laboratory mice which was indicated by the alterations of functional observational battery used in the field of study. Pulmonary irritation has also been observed in rats exposed to limonene, ozone reaction products (Sunil *et al*., 2007). In this experiment, rats were exposed for 3 hours to 6 ppm limonene (a common component of air fresheners). The pulmonary vascular congestion observed in the exposed rats could be due to the effects of toxic chemicals in the air fresheners. Research has shown that exposure to volatile organic compounds causes pneumonitis resulting in decreased surfactant production; the decrease in surfactant results in alveolar collapse, ventilation perfusion, mismatch and hypoxemia (Michael, 2015). The hypoxia in lung tissue might possibly results in more vascular congestion. The intra alveolar hemorrhage noticed in the exposed rats could be explained by the increased vascular permeability, this vascular permeability was a result of release of polypeptide mediators from the pulmonary cells (Hosan *et al.,* 2010). In addition to that, air fresheners emission results in the formation of particulate matter that resulted in impairment of endothelial function due to increased oxidative stress (Kim *et al*., 2015). Cellular infiltration of leucocytes observed in the present study could be regarded as a defense mechanism by the lung against the toxic effects of air fresheners. Infiltrating cells help in the swift of the rapid removal of foreign particles such as tissue debris and red cells thus paving the way for regeneration (Hosan *et al*., 2010). Myocardial damage and hemorrhage were the effects observed in the heart of animals exposed to air fresheners in the present study, although group 8 animal heart section was not affected (Plate 2a-2k). A recent study in Europe found a link between long-term use of air fresheners in the home and altered heart function; those with lung disease were more affected (Mehta *et al*., 2012). There is case report of air freshener overdose from inhaling the spray at close range which has caused rapid heartbeat that required hospitalization (Senthikumaran *et al.,* 2012). The study of Michael (2015) has shown that exposure to aromatic hydrocarbon (some of the major ingredients in air freshener) can result in cardiotoxicity, most importantly the myocardium become sensitized to the effects of catecholamines which can predispose the patient to tachydysrhythmias. Formation of ultrafine particles by reaction of ozone with air freshener compounds may directly cause

cardiac dysfunction as well as pulmonary and inflammatory symptoms through reactive oxygen species production (Zhou *et* al., 2014). Repercussion injury may also cause intra myocardial hemorrhage by erythrocytes extravasation through severely damaged endothelial walls (Lotan *et al*., 1992). According to Steinemann (2009), Pollutants emitted from air fresheners are linked with damage to the brain, lungs, heart, reproductive system, immune system, and with cancer, everyone is vulnerable, especially children.

## Conclusion

It could be concluded from the present study that gel and liquid air fresheners has toxic effect on albino rats by causing toxicity in physical parameters such as decrease in body weight, loss of appetite and dizziness. Thus, the results of the histological analyses revealed that air freshener exposure induced injury in the lungs and heart of albino rats by causing alterations in the structural integrity of the histological sections.

It is recommended to use ventilation instead of air fresheners. Good ventilation system helps to reduce the need for air fresheners by eliminating the need to mask odors and naturally eliminates unpleasant odors. Air fresheners may hide a problem such as mold even if the musty odor is hidden by the fragrance in air freshener, the mold spores can still be a health problem. It is best to address the actual problem rather than use a deodorant to hide it.

**References**

Akingbade, A.M., Saalu, I.C., Oyebanji,O.O., Oyeniran, D.A, and Akunna, G.G. (2014). Rhodinol based Incense Testiculotoxicity in Albino Rats: Testicular histology, Spermatogenic and Biochemical Evaluations. *Journal of pharmacology and Toxicology,* 9:68-81

Al Sahhaf.Z.Y (2012). Hematological and biochemical effects of an air freshener in Rabbits. *Journal of American Sciences*; 8(8):7073. ISSN: 1545-1003.Downloaded from http://www.jofamericanscience.org.

Anderson, R.C, and Anderson, J.H. (1997). Toxic effects of air freshener emissions. *Archive of Environmental Health.* 52(6):433-441.

Anwioro, O.G (201O). *Histochemistry and Tissue Pathology:* principle and technique Second edition, 2010

Ashade, O.O., and Igbokwe, L. (2014). Haematological and biochemical assessment of composite wood extract in Albino Rats ( male wistar strain). *Journal of natural sciences research.* ISSN 2224. 3186 vol 4, No 18, 2014.

BEUC, (2005) Burea European Des Union de Consummateurs. Study on air fresheners. *Emission of chemicals by air fresheners test on 74 consumer products sold in Europe.* Adopted by the SCHER. European commission Health and Consumer Protection Directorate General.

Caress SM, Steinemann AC. (2004). A national population study of the prevalence of multiple chemical sensitivity. *Archive of Environmental Health, 59*(6):300-305.

EPA, (2006) Environmental Protection Agency. *Air freshener petition to EPA*

Farrow, A. Taylor, H. Kate, G. J. (2003). "Symptoms of Mothers and Infants Related to Total Volatile Organic Compounds in Household Products". Archives of Environmental Health: *An International Journal* **58** (10): 633–641.

Gentry, N., Top, E.N., Snitily, M.V., Casey, C.A., and Preheim, L.C (2004). A rat model to determine the biomedical consequences of concurrent ethanol ingestion and cigarette smoke exposure. Alcoholism: *Journal of Clinical Experiment Resources*. 28:1120-128

Gilbert, S.(2009). *Air Fresheners Toxipedia*. Available online at http/user/Download/Air Freshener-Toxipedia.html.

Hosan, A. Usama, A.G., and Hanan, S.S. (2010). Histological, Histochemical, Immunohistochemical study of Adult Male Albino Rat's Lung Following Exposure to Air Pollution. *Egyptian Journal of Histology.* Vol. 33 No 1, 140-155.

Kim, S., Hong, S.H., and Bong, C.K. (2015). Characterization of air freshener emission: The potential health effect. *Journal of toxicological sciences.* Available at http://www. Ncbi.nlm.nih.gov/pubmed/26354370.

Lotan, C.S., Bouchard, A., Cranney, G.B., Bishop, S.P., and Pohost, G.M. (1992). An assessment of post reperfusion myocardial hemorrhage using proton NMR imaging at 1.5 T", circulation. *Journal of Histology* Vol. 86, No 3, 1018-1025.

Mehta, A.J., Martin, A., Schaffner, E., Berthelemy, J.C., Carballo, D., and Gaspoz, T.R (2012). Heart rate variability in association with frequent use of household sprays and scented products in SAPALDIA. *Environmental Health Perspective 1*20: 958-964.

Michael, D.L. (2015). Hydrocarbon Toxicity, *a Review*. Available at http//www.emedicine.medscape.com/article

Natural Resource Development Council (NRDC), (2007). *Health risk of Air Freshener.* Downloaded from http:/user/downloads/health risk of Air Fresheners-Health scans.

Saalu, L.C., Jewo, P.I., Fadeyibi, I.O and Ikverowo, S.O, (2008).The effects of unilateral varicocele on the contralateral testicular amino acid determination. Impaired renal function or increased tissue protein break down are associated with increased urea nitrogenlevel.

Senthikumaran, S. Meenakshisundaram, R. Andrew, D.M. Namasivayam, B. and Ponniah M.D. (2012). Ventricular fibrillation after exposure to air freshener- Death just a breath away. *Journal of Electrocardiology* 45: 164-166.

Spiller H.A. (2004): Epidemiology of volatile substance abuse (VSA) cases reported to US poison centers. *American Journal of Drug and Alcohol Abuse*. 30 (1): 155-65. 22.

Steinemann, A.C.(2009). Fragranced consumer products and undisclosed ingredients. *Environmental Impact Assessment Review* 2008;29(1): 32-38.

Sunil V.R., Laumbach R.J., and Patel K.J (2007). Pulmonary effects of inhaled limonene ozone reaction products in elderly rats. *Journal of Applied Pharmacology and toxicology* 222:211-220.

William, R.M. (2004). *Fragrance alters mood and brain chemistry*. Available at www.the powerhour.com/news4/fragrance.html.

Wilkins, K., Wolkoff, P., Knusdsen, H,N., and Clausen, P.A (2007). The impact of Information on perceived air quality: *organic Vs synthetic building materials Indoor air.* 117, 130-134.

Zhou, P., Guo, J., Zhou, X., Zhang, W., Liu, L., Liu, Y., and Lin, K (2014). PM 2.5, PM 10 And health risk assessment of heavy metals in typical printed circuit roards manufacturing workshop. *Journal of Environmental Sciences,* 26, 2018-2026.