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BIOCHEMICAL EFFECTS OF GEL AND LIQUID AIR FRESHENERS SOLD IN KANO ON SWISS ALBINO RATS (*Rattus norvegicus*)

Yakasai, A.U.[1] and Mohammed, B.[2]
[1]Department of Biological Sciences, Federal University Dutsinma
[2]Department of Microbiology, Bayero University, Kano
Correspondence author's e-mail: ummaabdulrahman@gmail.com Tel: 08033320620

Abstract
The study investigated the toxicity potential of air fresheners exposure on swiss albino rats and this was achieved through assessing changes in some biochemical parameters of the exposed animals. Eighty four male albino rats weighting between 120g and 140g were randomly divided into 21 groups of four animals per cage. Ten groups were exposed to gel air fresheners, each group with a concentration of 3g of a particular gel air freshener brand for 8 hours daily, another ten groups were exposed to liquid air fresheners, each group with a concentration of 3ml of a particular brand liquid air freshener daily for 4 weeks. At the end of the experiment, animals were euthenized and blood samples were collected for biochemical investigations using standard technique. Sera were separated and used to determine the effects of each air freshener exposure on the liver and kidney function. Liver function enzymes Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) increased significantly as compared with the control. Bilirubin, Creatinine and Serum Urea level also increases while the value of Total protein decreases significantly when compared with the corresponding control animals (P>0.05). The study revealed that air fresheners exposure may have toxic effect on liver and kidney of the albino rats. It is recommended to enhance good ventilation instead of air fresheners and remove sources of odors rather than mask it with chemicals.
Key words: Air freshener, Albino Rats, hepatotoxicity, renal impairment.

INTRODUCTION

A number of commercial products are widely used in a variety of indoor spaces to improve hygiene or sensory appeal, however many of such products have been discovered to be the major indoor air pollutants that may be responsible for harmful health effect. An Air Freshener is a deodorant that is used to eliminate odors either by a chemical reaction or by providing a refreshing masking scent (Kim *et al.,* 2015). Some of the different types of air fresheners include incense sticks, scented candles, aerosol, liquid, gel, and electric diffusers (BEUC, 2005). Air fresheners have become a staple in many homes, cars and offices, marketed with the promise of creating a clean, healthy, and sweet-smelling indoor atmosphere. But many of these products contain hazardous chemicals (Wickramasinghe *et al*., 2012). Benzene is contained in incense products and is released from liquid air fresheners. Formaldehyde, naphthalene, xylene, cresol, and ethanol are also contained in air fresheners, including scented candles and plug-in air fresheners (Norgaard *et al*., 2014). 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).

Spiller (2004) investigated the toxic effect of volatile substance for a 6 year period (1996-2001). He reported that three categories were responsible for the majority of deaths: gasoline (45%), air fresheners (26%), and propane/butane (11%). Anderson and Anderson (1997) reported that air freshener measured by the functional observational battery score cause sensory, pulmonary irritations and decreases in airflow velocity in addition to abnormalities of behavior measured by the functional observational battery score. 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). It has also been reported that air fresheners had a negative impact on lungs and heart histology (Mohammed and Yakasai, 2017), and recently Airaodion *et al.* (2020) reported that air-freshener induced renal problems. The present study was designed to investigate the possible toxic effects of gel and liquid air fresheners on kidney and liver of albino rats.

MATERIALS AND METHODS

Experimental Animals

A total of 84 male albino wistar rats ranged between 120 and 140 g 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 grower mash and water. The rats were allowed to acclimatized for two weeks before commencement of the experiment.

Ethical considerations

The internationally accepted National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals were observed (NIH, 2002).

Sourcing of the Air Fresheners

Twenty brands of air freshener were randomly selected and procured from Sabon Gari Market, Kano, Nigeria. Out of these twenty, ten were Gel air fresheners while the remaining ten were liquid air fresheners. The air fresheners were coded and stored at room temperature. The names of the air fresheners, the list of the active ingredients and expiry dates were obtained from the packages.

Distribution of Animals and Treatment

After two weeks of acclimatization, 84 animals were randomly divided into 21 groups of 4 animals each cage. Ten 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 ten 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 negative controls.

Sample Collection

After 28 days of the experiment, the animals were fasted overnight, and euthenized the next morning by cervical dislocation. Blood samples were collected in plain sterilized vials for serum separation. The sera were analyzed individually for each animal.

Biochemical Analyses

Alanine aminotransferase (ALT)) and Aspartate aminotransfersae (AST) were determined by Randox commercial enzyme kit according to the method of Reitmen and Frankel (1957). Alkaline phosphatase was determined by colorimetric end point method (Tiez, 1976), total protein was determined by biuret method (Tiez, 1995), creatinine was determined using the method adopted by Toora and Rejagopal (2002), the sulfanilic acid method, of Tietz and Shuey (1993) was employed for the measurement of bilirubin while serum urea level was measured by the method of (Fesus *et al.,* 1983).

Statistical Analysis

Data were expressed as mean ± standard deviation (X±S.D.). One-way ANOVA was used to find significant difference between control group and all the remaining groups exposed to air fresheners. The levels of significance were taken at $p < 0.05$. The statistical analyses was done using Sigma Stat software version 3.5.

RESULTS

Statistically significant elevate[ons in ALP, AST, and ALT were recorded in some groups. ALP increases significantly in groups 1, 2, 3, 4, 7, 8, 9, and 10 (Table 1), 12, 14, and 16 (Table 2). ALT increases significantly in groups 1, 2, 4, 5, 7, 8, 9 and 10 (Table 1), 15 and 20 (Table 2), while AST increases significantly in groups 2, 7, 8 (Table 1) and group 20 (Table 2). Total protein decreases significantly in all groups withury the exception of groups 6, and 7 (Table 1), 12, 18 and 19 (Table 2). Bilirubin increases significantly in groups 6, 8, and 9 (Table 1) 11, 15, 16 and 20 (Table 2), Creatinine increases significantly in groups 2, 5, 7, and 9 (Table 1) 13, 16, and 20 (Table 2), while the values of Serum urea increases significantly across all groups.

Table 1: Changes in Biochemical Parameters of Rats Exposed to Gel Air Fresheners

	Paramters						
Groups	**ALP (U/L)**	**AST (U/L)**	**ALT (U/L)**	**Total Protein (g/dl)**	**Bilirubin (mg/dl)**	**Creatinine (mg/dl)**	**Urea (mg/dl)**
1	63.64 ± 9.64*	78.00 ± 15.62	45.55 ± 9.19*	4.66± 1.02*	1.90 ± 0.45	1.04 ± 0.49	64.60 ±5.68*
2	95.46 ± 3.22*	138.20 ± 8.15*	80.0 ± 11.31*	2.09 ± 0.27*	1.50 ± 0.47	4.85 ± 0.99*	78.70 ±2.83*
3	68.19 ± 4.55*	87.70 ± 16.59	36.00 ± 9.89	3.73 ± 0.29*	1.80 ± 0.09	1.73 ± 0.49	62.60 ±2.86*
4	52.27 ± 3.22*	78.0 ± 15.60	55.00 ± 9.89*	4.60 ± 3.35*	1.70 ± 0.47	2.08 ± 0.99	90.87 ± 7.1*
5	38.64 ± 3.22*	87.70 ± 16.59	88.50 ± 7.78*	4.03 ± 0.31*	1.50 ± 0.61	3.81 ± 0.49*	76.79 ± 5.8*
6	32.96 ± 4.82	70.5 ± 26.20	36.50 ± 16.26	5.69 ± 1.02	3.30 ± 0.23*	1.74 ± 0.49	76.71 ± 2.9*
7	85.23 ± 8.04	129.60 ± 20.4*	75.50± 26.16*	5.69 ± 1.61	2.20 ± 0.16	4.12 ± 0.88*	74.71 ± 5.6*
8	64.78 ± 1.61*	120.90 ± 8.14*	45.50 ± 3.54*	2.38 ± 0.10*	3.20 ± 0.16*	1.74 ± 0.49*	64.6 ± 2.86*
9	78.41 ± 11.25*	87.74 ± 16.59	50.10 ± 2.69*	1.66 ± 0.28*	3.50 ± 0.07*	3.46 ± 0.98	64.60 ± 69*
10	61.37 ± 6.43*	55.50 ± 4.95	69.50 ± 3.54*	5.9 0 ± 0.44	1.60 ± 0.38	1.04 ± 0.49	66.60± 2.70*
control	18.18± 3.21	44.00 ± 15.80	14.5 ± 3.54*	8.87 ± 1.12	0.90 ± 0.23	0.73 ± 0.06	28.30 ±5.69

Table 2: Changes in Biochemical Parameters of Rats Exposed to Liquid Air Fresheners

	Parameters						
Groups	**ALP (U/L)**	**AST (U/L)**	**ALT (U/L)**	**Total Protein (g/dl)**	**Bilirubin (mg/dl)**	**Creatinine (mg/dl)**	**Urea (mg/dl)**
11	30.68 ± 14.5	64.00 ± 16.90	27.50 ± 9.2	4.90± 2.20*	3.40 ± 0.25*	2.08 ± 0.98	72.70 ± 2.00*
12	50.0 ± 3.20*	64.00 ± 16.90	36.13 ± 2.8	5.90 ± 2.40	1.90 ± 0.16	2.43 ± 0.48	68.70 ± 5.69*
13	26.1 4 ± 11.50	45.00± 19.80	26.59 ± 2.2	3.10 ± 1.16*	1.70 ± 0.29	3.81 ± 0.48*	76.70 ± 4.02*
14	65.91 ± 9.70*	67.50 ± 12.00	32.0 ± 9.90	4.80 ± 1.50*	1.70 ± 0.61	1.74 ± 1.47	70.70 ± 8.57*
15	30.68 ± 14.50	75.74 ± 33.60	57.00 ±7.0*	2.69 ± 0.60*	3.10 ± 0.61*	2.78 ± 0.98	74.70 ± 2.86*
16	44.32 ± 14.40*	78.0 ±15.60	28.65 ± 5.2	3.50 ± 0.29*	4.70 ± 0.99*	4.45 ± 0.41*	62.60 ± 2.70*
17	27.68 ± 3.80	87.09 ± 39.70	25.84 ± 1.18	2.17 ± 0.20*	2.30 ± 0.54	1.39 ± 0.99	68.6 0 ± 5.64*
18	38.80 ± 9.89	70.5 ± 26.16	36.00 ± 2.83	5.20 ± 1.75	1.60 ± 0.16	1.74 ± 0.49	70.70 ± 8.56*
19	37.37 ± 3.22	78.00 ± 15.56	29.50 ± 6.36	5.40 ± 1.17	1.80 ± 0.14	2.10 ± 1.04	74.70 ± 8.56*
20	74.09 ± 8.35*	120.90 ± 8.14*	69.50 ± 3.54*	1.55 ± 0.14*	3.40 ± 0.25*	3.47 ± 1.47*	70.70 ± 2.86*
control	18.18 ± 3.21	44.00 ± 15.80	14.5 ± 3.54	8.87 ± 1.12	0.90 ± 0.23	0.73 ± 0.06	28.30 ± 5.69

Values are expressed as mean ± standard deviation. Values with * indicates significant difference at $P < 0.05$

DISCUSSION

An obvious sign of hepatic injury is leakage of cellular enzymes into plasma. When the liver cell plasma membrane is damaged, a variety of enzymes normally located in the cystosol are released into blood stream, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are the most often used and most specific indicators of hepatic injury and represent markers of hepatocellular necrosis (Akunna *et al*., 2011). In the present study ALP increases significantly in groups 1, 2, 3, 4, 7, 8, 9, and 10 within the groups exposed to gel air fresheners and also in groups 12, 14, 16 and 20, for the groups exposed to liquid air fresheners. AST increases significantly in groups 2, 7 and 8 for gel air freshener groups and group 20 which is a liquid air freshener exposed group. ALT increases significantly in groups1, 2, 4, 5, 7, 8, 9, and 10 for the groups exposed to gel air fresheners, and groups 15 and 20 which are exposed to liquid air fresheners. The increase in liver function enzymes test in the present study is in accordance with the study of Al-Sahaf (2012) who finds significant increase in ALP, ALT and AST in rabbits exposed to air freshener spray for 4 weeks. Similar observations have been made by Airaodion *et al.* (2020) after exposing rats to gel air freshener for 4 weeks. Air fresheners contain hydrocarbons as part of ingredients, hydrocarbons inhalation is hepatotoxic, the toxicity results after the hydrocarbon under goes metabolism thereby inducing free radicals which subsequently bond with hepatic macromolecules and ultimately cause lipid peroxidation, the active metabolites and its triggered lipid peroxidation might be involved in the main mechanism by which air fresheners damage hepatocytes (Akunna *et al.,* 2011).

In the present study, total protein level decreases significantly in groups 1, 2, 3, 4, 5, 8, and 9 for the groups exposed to gel air fresheners and groups 11, 13, 14, 15, 16, 17 and 20 for the groups exposed to liquid air fresheners when compared with the controls. This is in agreement with the findings of Firdaus *et al*. (2014) who recorded similar decrease in total protein in rats exposed to sulphur di oxide (an air pollutant) for 30 days. A significant increase in AST and ALT level is also correlated with decrease in serum total protein due to inhalation of toxicant in albino rats. Present findings are corroborated by the findings of Mustafa et al. (1984) who suggested that decrease in serum total protein may be an indicator of inflammation due to extensive pulmonary injury. According to Srivastava *et al.* (1995) and Teitz (1986), toxicants have been shown to affect the liver tissues resulting into the reduction of total serum protein. In the present study bilirubin increases significantly in groups 6, 8, and 9 for gel air freshener groups and in groups 11, 15, 16 and 20 for animals exposed to liquid air fresheners. Abousalem *et al*. (2012) also recorded significant decrease in total protein and increased bilirubin level in rats exposed to benzene (one of the constituents of air fresheners). Increase in bilirubin concentration in the present study is an indication that too much haemoglobin is being destroyed. Bilirubin can be elevated due to haemolysis or due to dyserythopoiesis (Wickramasinghe *et al.,* 2012).

Serum Creatinine and Urea are important biomarkers in assessing kidney function. In the present study Creatinine increases significantly in groups 2, 5, 7, and 9 for groups exposed to gel air freshener and group 13, 16 and 20 for the groups exposed to liquid air freshener. Serum Urea increases significantly in all the 20 groups exposed to air fresheners when compared with the control group. Similar observations have been made by Airaodion *et al.* (2020) who recorded significant increase in urea and creatinine concentrations after exposing rats to gel air freshener for 4 weeks. This is in agreement with the findings of Abousalem *et al*. (2012) who recorded a significant increase in urea and creatinine concentrations and a decrease in total protein concentrations after exposing rats to benzene (one of the major constituents of air freshener) for 60 days. High elevation of urea and creatinine is an indication of kidney impairment. Urea is a major nitrogenous end product of protein and amino acid catabolism and creatinine is a breakdown product of creatinine phosphate in the muscle (Kamal, 2014). According to Olufansho *et al.,* (2014) hydrocarbons (some of the air freshener ingredients) induces renal injury by combining with renal proteins and artery as haptene to induce auto unfavorable cytokine profile and predisposing to T cell mediated renal injury. Thus the significant rise in serum creatinine observed in this study most probably represents the increased production of creatinine to meet the energy demand following severe oxidative stress caused by the chemical components of the air-freshener (Airaodion *et al*., 2020).

CONCLUSION

The present study revealed that gel and liquid air fresheners may have toxic effects on liver and kidney of albino rats by causing increased levels of serum enzymes (ALP, AST, ALT, Bilirubin, Creatinine and Urea) and a decrease in total protein. This could be used as potential biomarkers for air fresheners induced hepatotoxicity and kidney impairment by causing alterations in the levels of liver and kidney

enzymes. It is recommended to use ventilation instead of air fresheners and remove sources of odors rather than mask with chemicals.

REFERENCES

Abousalem, M ., Elgerwi, A., and El-Mashad. (2012). Biotoxic and Haematoxic effect of air pollutants at a benzene station on albino rats. *InternationaL Journal of Veterinary and animal Advances.* 4(3):187-190.

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.

Airaodion, A.I., Alabi, O.J., Ogbuagu, E.O., Atiba, F.A., and Olawoyin, D.S. (2020). Nephro and Hepatotoxic Effect of Air-Freshener in Wistar Rats. *Asian Journal of Research in Nephrology* 3(2): 1-9, 2020; Article no.AJRN.58662

Akunna, G.G., Saalu, O.S., Ogunlade, B., Adefolaju, G.A., and Bello A.J. (2011). The effects 2 Nigerian made parfumes on the liver of adult wistar Rat. *Journal of Medical Sciences,* 11:220-225.

Al Sahaf, 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.

Begum, G., and Vijayaraghavan, S. (1995). In vivo toxicity of dimethoate on proteins and transaminases in the liver tissue of fresh water fish, Clarias batrachus (Linn) *Journal of Environmental Contamination and Toxicology*. 54:370375

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

Butler, A. R. (1975). The jaffe reaction Identification of colored species; *Clinical Chemistry Act* 59:227

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

Cohen, A., Jansseen, S., and Solomon, G. (2007). *Hidden hazards of air fresheners* Downloaded from http://www.NRDC.org.

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.

Fesus, P.D., Pressac, M., Braconnier, F., and Aymard, P. (1983). Automated determination of urinary Na, K, inorganic phosphate, urea, and creatinine without sample dilution, with the RA-XT. *Clinical Chemistry*. 35: 481-483.

Firdaus, F. Nighat, B. Bechan, S. and Ravi S. P. (2014) . Toxicological evaluation of sulphur dioxide in albino rat, Rattus norvegicus. *Journal of Biochemistry esearch,* Volume 2(2) page 18-26.

Kamal, A. (2014). Estimation of blood urea (BUN) and serum creatinine level in patients of renal disorder. *IJFALS*: 4(4):199 – 202.

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

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

Mohammed, B. and Yakasai, U.A. (2017). Sub acute toxicity study of some brands of air fresheners sold in kano on swiss albino rats (rattus norvegicus). *International Journal of Advanced Academic Research* Vol. 3, (5) 19-32.

Mustafa, M.G. Elsayed, N.M., Vondohlen, F.M., Hassett C.M., Pasttethwait E.M, Quinn C.L., Grahm J.A., Gardner D.E. (1984). A comparison of biochemical effects of nitrogen dioxide ozone and their combination in mouse lung. *Journal of Applied Toxicology and Pharmacology*. 72(1):82-90.

National Institute of Health, NIH (2002). Animals in Research", public health service policy on Human Care and Use of Laboratory Animals, Health Research Extension Act

of 1985, *Public Law* pp. 99-158, reprinted 2002.

Norgaard, A.W., Kudal, J.D., Sorensen, V., Koponen, and Wolkoff. (2014). Ozone Initiated VOCs and particle emissions from a cleaning agent and an air Freshener risk assessment of acute air ways effects. *Journal of Environmental Sciences.* 68,209-215.

Okoro, A., Mani, E. J., Ibu, J. O., Akpogomeh B. A. (2000). Effect of petroleum products inhalation on some haematological indices of fuel attendants in calabar metropolis,Nigeria. *Nigerian journal of Physiological Sciences.* 21 (1-2): 71-75.

Olufunsho, A. Ademola, A. S. and Alade (2014). Evaluation of haematological, hepatic and renal function of petroleum tanker drivers in Lagos, Nigeria. *Journal of African Health Sciences.* 14(1): 178-18 available online at http://dx.doi.org/10.4314/ahs.v14i1.27.

Ovuru, S. S., and Ekweozor, I. K. E. (2004). Haematological changes associated with crude oil ingestion in experimental rabbits. *Afr. J. Biotech.* 3: 346-348.

Ravnskor, U. (2005). Experimental glomerulonephritis induced by hydrocarbons exposure. A systemic review*. BMC nephrology*; 6:15 6.

Reitman, S., and Frankel, S.(1957). A colorimetric method for the determination of serum glutamic oxalocetic and glutamic pyruvic transaminase: *American Journal of Clinical Pathology*. 28: 56-63.

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.

Srivastava, S.J., Singh, N.D., Srivastava, A.K, Sinha, R. (1995). Acute toxicity of malachite green and its effects on certain blood parameters of catfish H. fossilis. *Journal of Aquatic Toxicology.* 31:241-247.

Tietz, N.W. (1976). *Fundamentals of Clinical Chemistry,* Philadelphia: W. B. Saunders Co., p. 1028.

Tietz, N. Wand Shuey, D. F. (1993); Lipase in serum the elusive enzyme; an overview.*Clinical Chemistry*. 39; 746-756.

Teitz, N.W. (1995). *Clinical Guide to Laboratory Tests.* 3rd Edition. WB Saunders Company. Philadelphia. PA pp 518-519

Toora, B.D. and Rejagopal, G. (2002). Measurement of creatinine concentration by Jaffe's Reaction*. Indian Journal of Experimental Biology*. 2002;40(3):352–354.

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.

Wickramasinghe, A.P., Karunaratne, D.G., Sivakanesan, R.. (2012). PM (10)-bound polycyclic aromatic hydrocarbons: biological indicators, lung cancer risk of realistic receptors and 'source-exposure effect relationship' under different source scenarios. *Chemosphere.* 87 (11): 1381-1387.