Original Research Article

Short and Long-Term Exposure to Biomass Fuel (wood smoke) and Its Effects on Cardiovascular Risk Markers and Lipid Peroxidation of Male Albino Rats.

Abstract

Background: Prevalence of cardiovascular diseases (CVD) has surged rapidly in recent times. Exposure to particulate matter (PM) has been linked with increased cardiovascular morbidity and mortality. It is considered as one of the leading environmental risk factors of several diseases. Biomass smoke exposure has been shown to be associated with inflammation, coagulation, and lipid peroxidation, which are important factors in the development of CVD. Thus the purpose of this study was to evaluate the effect biomass fuel exposure on cardiovascular risk markers and lipid peroxidation.
Methods: The twenty adult male wistar rats were randomly assigned to two groups of ten animals each designated as groups A and B. Rats in group A served as control (exposed to fresh air) and group B exposed to inhalation of biomass smoke (wood smoke). The exposures were done using whole body exposure chambers 70cm x 60cm x 60cm measurement for six weeks, 6 days per week. . Five millilitres of blood sample were collected and serum extracted at the end of three and six weeks intervals. Serum concentrations of troponin I, CK-MB, hsCRP, myoglobin and MDA were determined using standard methods, while atherogenic indices were calculated using appropriate formula.
Results: The result shows significant increase in troponin I, CK-MB, hsCRP, myoglobin, CRR, AC, AIP and MDA at three weeks and six weeks relative to control, and these effects appears to be dependent on exposure duration.
Conclusion: The results suggest that repeated exposure to biomass fuel could potentiate the risk of CVD through elicitation and amplification of oxidative stress and cardio-inflammation, thus acting as significant risk factor for CVD.

Keywords: Biomass fuel, Cardiovascular Risk Markers, Lipid Peroxidation.

Introduction

Household air pollution from solid fuels is one of the leading risk factors for global disease burden accounting about 4.3% of global disability-adjusted life-years (DALYs) [1]. Cardiovascular diseases (CVDs) are the leading causes of morbidity and mortality globally accounting to about 31% of all global deaths [2]. Over 39% of the risk factor and causes of CVDs which have continued to increase in prevalence worldwide are unknown [3,4]. Air pollution exposure particularly particulate matter (PM) has been associated with increased cardiovascular morbidity and mortality [5,6]. Biomass smoke is one of the major source of PM and contributors of household air pollution worldwide. It is considered as one of the leading environmental risk factors of several diseases, such as Chronic obstructive pulmonary disease (COPD), acute lower respiratory disease and cardiovascular outcome, and is thought to cause 4 million deaths annually across the globe [7,8]). It's estimated that over 3 billion people rely on biomass fuels for domestic purposes [9]. Current predictions are that domestic consumption of biomass fuels will remain substantial for decades to come, particularly in

rural areas [10]. In developing countries women and children have the highest biomass smoke exposure due to cultural practices such as indoor cooking in housing with very poor air ventilation [11]. The absence of chimneys or pipes prevents the smoke venting outside and as such, particles become trapped and diffuse into the surroundings [11,12]. Biomass smoke has been shown to consist of over 200 different compounds, which includes a significant number of toxic compounds. Some of these include carbon monoxide (CO), varying sizes of PM, mostly PM10; sulphur and nitrogen oxides, polycyclic aromatic hydrocarbons (PAH), aldehydes, free radicals and non-radical oxidising species; and volatile organic compounds [10,11,13]. During the burning of these fuels, people indoors can be exposed with up to 30,000 µg/m3 of PM sized 10 µm or smaller [14], while stipulated concentration of PM10 exposure according to WHO guideline [15], is 50 µg/m3 for a 24 h period, which is extremely low compared to observed concentrations indoors where biomass fuels are burnt. Biomass smoke exposure has been shown to be associated with inflammation, coagulation, and lipid peroxidation, which are important factors in the development of CVD [13,16]. Gurgueira et al [17], reported increased reactive oxygen species concentration in the heart and lungs of rats exposed to concentrated ambient particles. Short-term exposures to diluted wood smoke have also been linked with increased arterial stiffness and decreased heart rate variability [18]. More so, exposure to PM from bush fires has been linked with out-of-hospital cardiac arrest [19,20]. Several studies has linked PM from biomass burning to increased oxidative potential which could contribute to cardiovascular mortality [21-23]. However, other studies reported no associations between short-term exposures to wood smoke and cardiac arrhythmia or increase in biomarkers of systemic inflammation [24,25]. Similarly, Henderson et al [26], reported no associations between forest fire smoke exposure and physician visits/hospital admissions for cardiovascular outcomes. Weichenthal et al [27], reported that short-term exposure to ambient PM concentrations from biomass smoke is associated with increase hospital admissions for myocardial infarction among elderly. In furtherance to that, this study was designed to evaluate the effect of exposure to biomass smoke on the serum levels of troponin I, creatine kinase-MB, high sensitive C-reactive protein, myoglobin, atherogenic index of plasma (AIP), Cardiac risk ratio (CRR), atherogenic coefficient (AC) and malondialdehyde (MDA) to ascertain possible cardiovascular risk involvement.

MATERIALS AND METHODS

Experimental Animals

Twenty adult male albinos' rats (wistar strain) seven weeks old, that weighed 130±10g obtained from the Animal Breeding Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, were used as the experimental animals. The rats were kept in cages for two weeks allowed to acclimatize to Animal House of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus and were allowed free access to food and water *ad libitum*. The protocol was in line with the guidelines of the National Institute of Health (NIH) (NIH Publication 85-23, 1985) for laboratory animal care and use. Thereafter, the animals

were randomly (while controlling for weight differences) distributed into two groups of ten animals each based on the exposure.

Biomass

The biomass Melina wood (*G arborea*) was purchased from commercial wood seller at Nnewi City, Anambra State, Nigeria.

Design of Exposure Chamber and Wood Combustor

The exposure chamber was fabricated using plywood (China OSB), while the wood combustor was made using iron, with an outlet for the release of the smoke into the chamber and a square oxygen inlet on the lid (10cm x10cm). The exposure chamber measures 70cm x 60cm x 60cm as previously described by Uboh *et a*l[28] and Akintunde *et al* [29], was constructed to allow at least 20% ventilation, as stipulated by the Organisation for Economic \Co-operation and Development guidelines for inhalation toxicity. The burning process was initiated first before exposure by igniting 4kg Gmelina arborea wood with a lighter in a fabricated combustor. The wood smoke was diverted from the combustor into the exposure chamber using a metal host.

Experimental Design

The twenty (20) adult male wistar rats obtained from the animal breeding unit of the faculty of Veterinary Medicine, University of Nigeria, Nsukka, after two weeks of acclimatization to the animal house were randomly (while controlling for weight differences) assigned to two groups of ten animals each designated as groups A and B. Rats in group A served as control (exposed to fresh air) and group B exposed to biomass smoke (wood smoke). The exposures were done using whole body exposure chambers 70cm x 60cm x 60cm measurement. The animals in group B were exposed to biomass smoke by igniting 4kg *Gmelina arborea* wood with a lighter in a fabricated combustor. The wood smoke was diverted from the combustor into the exposure chamber using a metal host. The animals in group B were exposed to 1005ug/m^3 of PM_{10} from wood smoke for 1h/day, 6 day/week for 6 weeks. The PM concentration in the chamber was monitored using a PM_{10} monitor (portable particulate monitor PCE-RCM 10, PCE Deutschland GmbH). At the end of each exposure day, the animals were transferred to biomass smoke free section of the experimental animal house. Body weight of animals and mortality data were routinely monitored. The study lasted for 6 weeks and blood samples were collected at the end 3 weeks and 6 weeks intervals by ocular and cardiac puncture respectively for the biochemical analysis.

Table 1: Summary of Exposures

Groups	Exposures
Group A (control)	Exposed to fresh air
Group B (biomass)	Exposed to 1005± 6.3ug/m^3 h-1 Kg-1 of biomass smoke

Collection of blood sample

The blood samples were collected at three weeks and six weeks intervals. At end of three weeks and six weeks the animals were fasted overnight, anesthetized with chloroform and blood samples collected by ocular and cardiac puncture respectively into plain sample tubes.

Serum samples were separated 1 h after extraction of blood by centrifugation at 3000 g for 10mins and stored in at -30^{o}c. Biochemical analyses on the serum samples were done 24 h after sample collection. Biochemical analyses were carried out for the measurement of serum levels of troponin I, CK-MB, hsCRP, myoglobin, AC, CRR, AIP and MDA.

Biochemical Analysis

The cardiac markers were determined by chemiluminescence immunoassay (CLIA) using Maglumi 600 reagent kits manufactured by Shenzhen New Industries Biomedical Engineering Co., Ltd, 4F, Weames Tech Bldg, Science and Industry Park, Nanshan, Shenzhen, China 518057. Troponin I was determined by the method of Cummins *et al* [30], CK-MB by Pierce and Jaffe, [31], hsCRP by McPherson and Pincus, [32] and myoglobin by Mair et al.,[33]. Malondialdehyde concentration was determined by method of Jentzsch et al.,[34]. The atherogenic indices were calculated from lipid profile as described by Dobiasova [35]:

- Cardiac Risk Ratio (CRR) = TC/HDLC
- Atherogenic Coefficient (AC) = TC-HDLC/HDLC
- Atherogenic Index of Plasma (AIP) = Log(TG/HDL)

Statistical analysis

Data collected were subjected to Independent-Samples T-Test. In order to test whether or not significant differences existed between groups. Paired-Samples T-Test was used to compare significant difference between 3 weeks and 6 weeks of exposure. The mean±SD of each parameter was taken for each group. Test probability value of $p<0.05$ was considered significant. The analyses were carried out on SPSS for Windows version 23.0.

Ethical Consideration

The study protocol was in line with the guidelines of the National Institute of Health (NIH) (NIH Publication 85-23, 1985) for laboratory animal care and use.

Results

Table 2 shows the comparison of values of troponin I, CK-MB, hsCRP, myoglobin, CRR, AC, AIP and MDA after 3 weeks exposure to biomass smoke. The mean troponin I, CK-MB, hsCRP, myoglobin, CRR, AC, AIP and MDA level was highest in test group B and lowest in control. The mean troponin I, CK-MB, hsCRP, myoglobin, CRR, AC and MDA value of the test group B compared to control were statistically significantly (p=.001,.001,.001,.001,.001, .001 and .001 respectively), while the mean AIP value of test group B compared to control were statistically similar(p=.078).

Table 3 depicts the comparison of values of troponin I, CK-MB, hsCRP, myoglobin, CRR, AC, AIP and MDA after 6 weeks exposure to biomass smoke. The mean troponin I, CK-MB, hsCRP, myoglobin, CRR, AC, AIP and MDA level was highest in test group B and lowest in control. The mean troponin I, CK-MB, hsCRP, myoglobin, CRR, AC and MDA value of the test group B compared to control were statistically significantly (p=.001,.001,.001,.001,.001, .001 and .001 respectively), while the mean AIP value of test group B compared to control were statistically similar(p=.695).

Table 4 depicts the comparison of 3 weeks and 6 weeks exposure values of troponin I, CK-MB, hsCRP, myoglobin, CRR, AC, AIP and MDA. The mean troponin I, CK-MB, hsCRP, myoglobin, CRR, AC, AIP and MDA level was highest in 6 weeks exposure and lowest in 3 weeks exposure. The mean troponin I, hsCRP, myoglobin, CRR, AC, AIP and MDA value of 6 weeks exposure compared to 3 weeks exposure were statistically significantly (p=.001,.032,.005, .008,.004,.032,and .001 respectively), while the mean CK-MB value of 6 weeks exposure compared to 3 weeks exposure were statistically similar(p=.102).

Table 2: Comparison of Biochemical Parameters after 3 Weeks of Exposure

Parameters	----------Groups----------			
	Group A (Control)	Group B (Biomass)	t-value	p-value
Troponin I (ng/l)	16.11 ±1.04	35.17 ±3.87	-15.012	0.001*
CK-MB (ng/ml)	1.85 ±0.22	3.55 ±0.14	-11.307	0.001*
hsCRP (ng/ml)	195.71 ±34.04	359.19 ±52.15	-8.176	0.001*
Myoglobin (ng/ml)	22.09 ±3.22	42.99 ±3.26	-14.030	0.001*
CRR	1.61 ±0.26	2.21 ±0.25	-5.057	0.001*
AC	0.64 ±0.27	1.20 ±0.24	-4.573	0.001*
AIP	-0.24 ±0.11	-0.13 ±0.12	-1.878	0.078
MDA (µmol/l)	1.37 ±0.07	1.88 ±0.07	-14.854	0.001*

*= significant at p<0.05,CRR=cardiac risk ratio, AC=atherogenic coefficient, AIP=atherogenic index of plasma, MDA=malondialdehyde, hsCRP=high sensitive C-reactive protein, CK-MB=Creatine kinase-MB

Table 3: Comparison of Biochemical Parameters after 6 Weeks of Exposure

Parameters	----------Groups----------			
	Group A (Control)	Group B (Biomass)	t-value	p-value

Troponin I (ng/l)	16.47 ±1.03	41.40 ±3.76	-20.164	0.001*
CK-MB (ng/ml)	2.10 ±0.25	4.07 ±0.53	-10.297	0.001*
hsCRP (ng/ml)	212.69 ±31.24	448.40 ±95.36	-7.385	0.001*
Myoglobin (ng/ml)	23.24 ±3.49	50.96 ±4.50	-14.724	0.001*
CRR	1.77 ±0.25	2.72 ±0.28	-7.490	0.001*
AC	0.80 ±0.24	1.76 ±0.28	-7.659	0.001*
AIP	-0.06 ±0.15	-0.03 ±0.07	-0.399	0.695
MDA (µmol/l)	1.36 ±0.05	2.26 ±0.19	-14.245	0.001*

*= significant at p<0.05,CRR=cardiac risk ratio, AC=atherogenic coefficient, AIP=atherogenic index of plasma,MDA=malondialdehyde, hsCRP=high sensitive C-reactive protein, CK-MB=Creatine kinase-MB

Table 4: Comparison of Biochemical Parameters between 3 weeks and 6 weeks Exposure

	Duration of Exposure			
Parameters	**3 weeks**	**6 weeks**	**t-Value**	**P-value**
Troponin 1	35.17±3.87	41.40±3.76	-5.263	0.001*
CK-MB	3.55±0.41	4.07±0.53	-1.880	0.102
hsCRP	359.19±52.15	448.40±95.36	-2.681	0.032*
Myoglobin	42.99±3.26	50.96±4.50	-4.072	0.005*
CRR	2.21±0.25	2.72±0.28	-3.705	0.008*
AC	1.20±0.24	1.76±0.28	-4.177	0.004*
AIP	-0.13±0.12	-0.03±0.07	-2.666	0.032*
MDA	1.88±0.07	2.26±0.19	-8.706	0.001*

*= significant at p<0.05, CK-MB= Creatine kinase MB, hsCRP= High sensitive C-Reactive protein, CRR=Cardiac Risk Ratio, AC=Atherogenic coefficient, AIP=Atherogenic index of Plasma.

Discussion

Wood smoke is one of the major source of particulate matter (PM) and a major contributor of household air pollution worldwide. People living in developing countries are becoming more vulnerable to the adverse health effect due to over dependency on woods as alternative source of energy for cooking and heating. There appears to be dearth of information regarding cardiovascular systemic changes in rats and other mammals induced by exposures to environmental toxicants like biomass smoke. Although exposure to PM has been linked with increased cardiovascular morbidity and mortality [5,6]. Therefore, this study sought to investigate whether short and long term exposure to wood smoke affects cardiovascular risk

markers and lipid peroxidation. The result showed that exposure to wood smoke significantly increase serum levels of troponin I, CK-MB and myoglobin which are indicator of myocardial injury and cardiotoxicity. The myocardium responds to any injury that causes disruption of its sarcolemma membrane by releasing cytoplasmic pool of biomarkers such as myoglobin, creatine kinase and troponins [36]. These markers are released, so that the blood levels rise rapidly above its cut off points. This is then followed by a more protracted release of biomarkers from the disintegrating myofilaments that may continue for several days. Troponin-I is the biomarker of choice for detection of cardiac injury. Cardiac troponin I is more sensitive and specific than CK-MB and myoglobin [37]. The cardiac troponin found in blood may not only be due to cell death; but could also result from reversible myocardial injury [37]. Mechanistic studies have shown that necrosis is not essential for cardiac troponin release and that even preload and integrin stimulation both have shown to cause proteolysis and cardiac troponin release [37]. There is sparse literature report on wood smoke induced changes in cardiac marker probably due to strict regulations governing the use of biomass fuel in most countries. The increase in serum levels of troponin I, CK-MB and myoglobin found in this study is indicative of possible cardiovascular risk involvement of wood smoke exposure and this finding appears to be dependent on duration of exposure; the higher the exposure duration, the higher the risk of cardiovascular event and vice versa. This result is in agreement with the findings of Abderrahim et al [38] and Das et al [39], who both reported significant increases in cardiac markers upon exposure to biomass smoke. Similar findings have also been reported upon exposure to environmental toxicant [40,41]. High sensitivity C-reactive protein (hs-CRP) an acute phase protein has been implicated in various inflammatory conditions. Studies has shown that increased risks of developing cardiovascular disease (CVD) are associated with elevated high sensitivity C-reactive protein and has been considered as markers of low-grade systemic inflammation [42,43]. There are many evidences to suggest that atherosclerosis is an inflammatory disease [44,45]. The increase in serum levels of hs-CRP found in this study is indicative of possible systemic inflammation associated with cardiovascular event as a result of biomass smoke exposure. This result is in tandem with the findings of Jianmin et al [46] and Dauchet et al [47]. Atherogenic indices are strong indicators of the risk of heart disease: the higher the value, the higher the risk of developing cardiovascular disease and vice versa [35,48]. According to Usoro et al [48], low atherogenic indices are protective against coronary heart disease while higher atherogenic indices increase the risk. From the result obtained it's apparent that exposure to biomass smoke significantly increased the atherogenic indices; CRR, AC and AIP indicating the likely role in cardiovascular disease. These findings is in tandem with Uboh et al [49] and Ubani et al [50], who reported significant increase in atherogenic indicies of wistar rats exposed to environmental toxicants. Smoking has been suggested to be one of the factors playing a role in oxidative stress through its generation of reactive oxygen species [51]. Oxidative stress has been implicated in the pathogenesis of several diseases including cardiovascular diseases. Increased MDA is an indicator of lipid peroxidation and thus oxidative stress [52]. The significant increase in MDA concentration recorded with exposure to biomass smoke indicates possible increase lipid peroxidation initiated by free radicals generated by their toxic contents which could be deleterious to the cells and organs. The significant increase in MDA found in this study agrees with the report of Ujowundu et al [53]. The exact

mechanisms by which biomass smoke induce cardio-toxicity are poorly understood. These effects could be attributed to inflammation and oxidative stress. Inflammation plays a significant role in the pathophysiology of atherosclerosis. The inflammatory cells types found in atheroma include monocytes-derived macrophages and lymphocytes. Macrophages present in atherogenous plaque leads to the release of mediators like cytokines and chemokines which in turn increase the plasma concentration of CRP which amplify inflammatory and procoagulant responses [54]. Among other inflammatory markers studied for prediction of coronary accidents in healthy adult CRP appears to be the most powerful inflammatory marker of future cardiovascular risk [55-62]. Increased reactive oxygen species concentration has been reported upon exposure to PM [17,63]. The significant increase in level of malondialdehyde found in this study connotes the role of oxidative stress in cardio-toxicity upon exposure to wood smoke.

Conclusion

In conclusion, the results of this work suggest that repeated exposure to biomass fuel could potentiate the risk of cardiovascular disease through elicitation and amplification of oxidative stress and cardio-inflammation, thus acting as significant risk factor to cardiovascular disease. Hence those occupationally exposed to biomass smoke should ensure appropriate use of personal protective equipment, in addition should have regular medical check-up to ascertain their health condition.

COMPETING INTERESTS

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

1. Lim, S.S, Vos, T, Flaxman, A.D, Danaei, G, Shibuya, K, Adair-Rohani, H (2012). A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. **380**:2224-2260.
2. http://www.who.int/mediacentre/factsheets.
3. Collins, D.R, Tompson, A.C, Onakpoya, I.J, Roberts, N, Ward, A.M, Heneghan, C.J (2017). Global cardiovascular risk assessment in the primary prevention of cardiovascular disease in adults: systematic review of systematic reviews. *BMJ open*. **7**:e013650.
4. World Health Organization. Global health risks: mortality and burden of disease attributable to selected major risks. *Geneva*: World Health Organization; 2009.
5. Anakwue, R.C, Anakwue, A.C (2014). Cardiovascular Disease Risk Profiling in Africa: Environmental Pollutants are not on the Agenda. *Cardiovasc Toxicol*. **14**:193-207.

6. Pope, C.A 3rd, Burnett, R.T, Thurston, G.D, Thun, M.J, Calle, E.E (2004). Cardiovascular Mortality and long-term exposure to particulate air pollution: epidemiological evidence of general pathophysiological pathways of disease. *Circulation*.109:71-77
7. Salvi, S, Barnes, P.J (2010). Is exposure to biomass smoke the biggest risk factor for COPD globally? Chest , **138**: 3–6.
8. Martin, W.J, Glass, R.I, Balbus, J.M, Collins, F.S (2011). A major environmental cause of death. Science, **334**: 180–181.
9. Bank, T.W (2011). Household Cookstoves, Environment, Health and Climate Change: A New Look at an Old Problem; The World Bank: Washington, DC, USA.
10. Jain, A.; Ray, S.; Ganesan, K.; Aklin, M.; Cheng, C.Y.; Urpelainen, J (2016). Council on energy, environment and water. In Access to Clean Cooking Energy and Electricity: Survey of States in India (ACCESS); Council on Energy, Environment and Water: New Delhi, India, Volume 1.
11. Gordon, S.B, Bruce, N.G, Grigg, J, Hibberd, P.L, Kurmi, O.P, Lam, K.B, Mortimer, K, Asante, K.P, Balakrishnan, K, Balmes, J,(2014). Respiratory risks from household air pollution in low and middle income countries. Lancet Respir. Med. 2: 823–860.
12. Smith, K.R.; Bruce, N.; Balakrishnan, K.; Adair-Rohani, H.; Balmes, J.; Chafe, Z.; Dherani, M.; Hosgood, H.D.; Mehta, S.; Pope, D (2014). Millions dead: How do we know and what does it mean? Methods used in the comparative risk assessment of household air pollution. Annu. Rev. Public Health, 35: 185–206.
13. Naeher, L.P.; Brauer, M.; Lipsett, M.; Zelikoff, J.T.; Simpson, C.D.; Koenig, J.Q.; Smith, K.R (2007). Wood smoke health effects: A review. Inhal. Toxicol. 19: 67–106.
14. Sussan, T.E.; Ingole, V.; Kim, J.-H.; McCormick, S.; Negherbon, J.; Fallica, J.; Akulian, J.; Yarmus, L.; Feller-Kopman, D.; Wills-Karp, M (2014). Source of biomass cooking fuel determines pulmonary response to household air pollution. Am. J. Respir. Cell Mol. Biol. 50: 538–548.
15. WHO, (2006) World Health Organization. Fuels for life, household energy and health, Rehfuess E, WHO Library Cataloguing-in-Publication Data.
16. Barregard, L, Sällsten, G, Gustafson, P (2006). Experimental exposure to wood-smoke particles in healthy humans: effects on markers of inflammation, coagulation, and lipid peroxidation. Inhal Toxicol. 18:845–853.
17. Gurgueira, S.A, Lawrence, J, Coull, B, Murthy, G.G, González-Flecha, B (2002). Rapid increases in the steady-state concentration of reactive oxygen species in the lungs and heart after particulate air pollution inhalation. Environ Health Perspect, 110(8):749-55.
18. Unosson, J, Blomberg, A, Sandström, T (2013). Exposure to wood smoke increases arterial stiffness and decreases heart rate variability in humans. Part Fibre Toxicol. 10:20.
19. Dennekamp, M, Straney, L.D, Erbas, B (2015). Forest fire smoke exposures and out-of-hospital cardiac arrests in Melbourne, Australia: a case-crossover study. Environ Health Perspect. 123:959–964.
20. Haikerwal, A, Akram, M, Del Monaco, A (2015). Impact of fine particulate matter (PM2.5) exposure during wildfires on cardiovascular health outcomes. J Am Heart Assoc. 4: e001653.
21. Kurmi, O.P, Dunster, C, Ayres, J.G, Kelly, F.J (2013). Oxidative potential of smoke from burning wood and mixed biomass fuels. Free Radic Res. 47:829–835.
22. Heo, J, Schauer, J.J, Yi, O, Paek, D, Kim, H, Yi, S.M (2014). Fine particle air pollution and mortality: importance of specific sources and chemical species. Epidemiology. 25:379–388.
23. Bates, J.T, Weber, R.J, Abrams, J, (2015). Reactive oxygen species generation linked to sources of atmospheric particulate matter and cardiorespiratory effects. Environ Sci Technol. 49:13605–13612.
24. Stockfelt, L, Sallsten, G, Almerud, P, Basu, S, Barregard, L (2013). Short-term chamber exposure to low doses of two kinds of wood smoke does not induce systemic inflammation, coagulation or oxidative stress in healthy humans. Inhal Toxicol. 25:417–425.
25. Langrish, J.P, Watts, S.J, Hunter, A.J (2014). Controlled exposures to air pollutants and risk of cardiac arrhythmia. Environ Health Perspect. 122: 747–753.

26. Henderson, S.B, Brauer, M, Macnab, Y.C, Kennedy, S.M (2011). Three measures of forest fire smoke exposure and their associations with respiratory and cardiovascular health outcomes in a population-based cohort. Environ Health Perspect. 119:1266–1271.
27. Weichenthal, S, Kulka, R, Lavigne, E, van Rijswijk, D, Brauer, M, Villeneuve, P.J, Stieb, D, Joseph, L, Burnett, R.T (2017). Biomass Burning as a Source of Ambient Fine Particulate Air Pollution and Acute Myocardial Infarction. Epidemiology, 28(3):329-337.
28. Uboh, F.E., Akpanabiatu, M.I, Eyong, E. U., Ebong, P.E Eka, O.O (2005). Evaluation of toxicological implications of inhalation exposure to kerosene fumes and petrol fumes in rats. Acta Biol Szeged, 49(3-4):19-22.
29. Akintunde, J.K, Abioye, J.B, Ebinama, O.N (2020). Potential Protective Effects of Naringin on Oculo-Pulmonary Injury Induced by PM10 (Wood Smoke) Exposure by Modulation of Oxidative Damage and Acetylcholine Esterase Activity in a Rat Model. Curr Ther Res Clin Exp. 92:100586.
30. Cummins, B, Auckland, M.L, Cummins, P (1987). Cardiac specific troponin radioimmunoassy in the diagnosis of acute myocardial infarction. Am. Heart J,113:1333-1344.
31. Pierce, G.f, Jaffe A.S (1986).Increased Creatine Kinase-MB in the absence of acute myocardial infarction. Clin Chem. 32:2044-2051.
32. McPherson, R.A, Pincus, M.R (2007). Henry's clinical diagnosis and management by laboratory methods. 21.New Delhi: Elsevier. P. 209.
33. Mair, J, Artner-Dworzak, E, Lechleitner, P, Morass, B, Smidt, J, Wagner, I, Dienstl, F, Puschendorf, B (1992). Early diagnosis of acute myocardial infarction by a newly developed rapid immunoturbidimetric assay for myoglobin. Br Heart J., 68(5): 462–468.
34. Jentzsch, A. M, Bachmann, H, Furst, P, Biesalski, H.K (1996). Improved Analysis of Malondialdehyde in Human Body Fluids, Free Rad. Biol. Med. 20: 251-256.
35. Dobiásová, M. (2004). "Atherogenic Index of Plasma [log(triglyceride/HDL-Cholesterol)]: Theoretical and Practical Implications". Clin Chem. 50(7): 1113-1115.
36. Ertl G, Frantz S (2005). Healing after myocardial infarction. Cardiovasc Res. 66:02.
37. Babuin L, Jaffe A.S (2005). Troponin: the biomarker of choice for the detection of cardiac injury. CMAJ : Canadian Medical Association Journal = Journal de l'Association Medicale Canadienne. 173(10):1191-1202.
38. Abderrahim, N, Suhail, AS, Priya Y, Sumaya B, Javed Y, Badreldin H.A (2017). Chronic exposure to water-pipe smoke induces cardiovascular dysfunction in mice. Am. J physiol, 312(2): H329-H339.
39. Das A, Dey N, Ghosh A, Das S, Chattopadhyay DJ, Chatterjee IB (2012) Molecular and cellular mechanisms of cigarette smoke-induced myocardial injury: prevention by vitamin C. PLoS One 7:e44151
40. Panagiotakos, D.B, Pitsavos, C, Stefanadis, C (2007). Chronic exposure to second hand smoke and 30 days prognosis of patients hospitalized with acute coronary syndromes. The Greek study of acute coronary syndromes. Heart 93:309-312.
41. Azeez, O, Anigbogu, C, Akhigbe, R, Saka, W (2015). Carditoxicity induced by inhalation of petroleum products. Journal of African Association of Physiological Sciences. African Association of Physiological Sciences (AAPS) 2015.
42. Danesh J, Collins R, Appleby P, Peto R (1998). Association of fibrinogen, Creactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. JAMA; 279:1477–1482.

43. Benderly M, Haim M, Boyko V, Tanne D, Behar S, Matas Z (2007). Creactive protein distribution and correlates among men and women with chronic coronary heart disease. Cardiology; 107:345–353.
44. Ross R (1999). Atherosclerosis is an inflammatory disease. Am Heart J;138:S419–420.
45. Libby P (2002). Inflammation in atherosclerosis. Nature ;420:868–874.
46. Jianmin L, Qiwei L, Kimberly F-P, Raheema M-K, Lonnie R, Hans R, Paul M, Mohamadi S (2011). Relationship between Biomarkers of Cigarette Smoke Exposure and Biomarkers of Inflammation, Oxidative Stress, and Platelet Activation in Adult Cigarette Smokers. *Cancer Epidemiol Biomarkers Prev*; 20:1760-1769.
47. Dauchet L, Hulo S, Cherot-Kornobis N, Matran R, Amouyel P, Edme J.L, Giovannelli J (2018). Short-term exposure to air pollution: Association with lung function and inflammatory markers in non-smoking, healthy adults. Environmental international, 121(1):610-619.
48. Usoro, C. A. O., Adikwuru, C. C., Usoro, I. N., Nsonwu, A. C. (2006). Lipid Profile of Postmenopausal Women in Calabar, Nigeria. Pakistan Journal of Nutrition. 5: 79-82.
49. Uboh, F.E, Akpanbiatu, M.I, Eteng, P.M.U, Ebong, E, Umoh, I.B (2008). Toxicological effects of exposure to gasoline vapours in male and female rats. Internet J Toxicol.4:40–45.
50. Ubani, C.S, Joshua, P.E, Ogbonna, U.S (2008). Biochemical Effects of Diesel on Serum Lipid Profile of Albino Rats. Animal Research International 5(3): 923 – 927.
51. Burke A, FitzGerald G.A (2003). Oxidative stress and smoking-induced vascular injury. Prog Cardiovasc Dis 2003;46:79–90.
52. Chessman KH, Slater TF (1993). An Introduction to Free Radical Biochemistry. Br Med Bull 49(3): 481- 493.
53. Ujowundu C.O, Igwe K.O, Agha N.C, Okechukwu R.I (2014). Toxicological Studies in Albino Rats Maintained on Fish Smoked With Firewood and Waste Tyre Materials. J Environ Anal Toxicol 4: 258.
54. Pasceri V, Willerson J.T, Yeh E.T.H (2000). Direct proinflammatory effect of C-reactive protein on human endothelial cells. Circulation; 102:2165–2168.
55. Yeh E.T.H, Anderson H.V, Pasceri V, Willerson J.T (2001). C-reactive protein: linking inflammation to cardiovascular complications. Circulation. 104:974–975.
56. Willerson J.T (2002). Systemic and local inflammation in patients with unstable atherosclerotic plaques. Prog Cardiovasc Dis. 44:469–478.
57. Ridker P.M, Hennekens C.H, Roitman-Johnson B, Stampfer M, Allen J (1998). Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. Lancet. 351:88–92.
58. Nakagomi A, Feedman S.B, Geczy C.L(2000). Interferon and lipopolysaccharide potentiate monocyte tissue factor induction by C-reactive protein. Relationship with age, sex, and hormone replacement treatment. Circulation. 101:1785–1791.
59. Verma S, Wang C-H, Li S-H, Dumont S.A.S, Fedak P.W.M, Badiwala M.V (2002). A self-fulfilling prophecy: C-reactive protein attenuates nitric oxide production and inhibits angiogenesis. Circulation. 106:913–919.
60. Braunwald E (1997). Sahttuck lecture-cardiovascular medicine at the turn of the millennium: triumph, concerns and opportunities. N Engl J Med. 337:1360–1369.
61. Blake G.J, Ridker P.M (2001). Novel clinical markers of vascular wall inflammation. Circ Res. 89(9):763–771.
62. Blake G.J, Ridker P.M (2002). Inflammatory biomarkers and cardio-vascular risk prediction. J Intern Med. 252(4):283–294.

63. Forchhammer L , Møller P , Riddervold IS , Bønløkke J , Massling A , Sigsgaard T , Loft S (2012) . Controlled human wood smoke exposure: oxidative stress, inflammation and microvascular function. Particle Fibre Toxicol . 2012;9:7 .