Current Therapeutic Research 92 (2020) 100586

Contents lists available at ScienceDirect

Current Therapeutic Research

journal homepage: www.elsevier.com/locate/curtheres

Potential Protective Effects of Naringin on Oculo-Pulmonary Injury Induced by PM_{10} (Wood Smoke) Exposure by Modulation of Oxidative Damage and Acetylcholine Esterase Activity in a Rat Model

Jacob K. Akintunde, PhD[1,2,*], Joseph B. Abioye, MPH[2], Owen N. Ebinama, MPH[2]

[1] *Applied Biochemistry and Molecular Toxicology Research Group, Department of Biochemistry, College of Biosciences, Federal University of Agriculture,Abeokuta, Ogun State, Nigeria*
[2] *Toxicology and Safety Unit, Department of Environmental Health Sciences, Faculty of Public Health, College of Medicine, University of Ibadan, Ibadan, Oyo, Nigeria*

A R T I C L E I N F O

Article history:
Received 5 July 2019
Accepted 2 April 2020

Keywords:
Inhibition
Naringin
Ocular
Pulmonary
Toxicity
Wood smoke

A B S T R A C T

Background: Millions of households in the world depend on wood and biomass for cooking and heating. This dependence leads to undesirable toxic effects, such as ocular and pulmonary toxicity.
Objectives: The present study examined the potential oculoprotective and pulmonary protective activity of naringin (NRG), a naturally occurring flavonoid, against wood smoke (WS)-induced toxicity in a rat model.
Methods: Forty-eight adult male albino rats were randomly distributed into six (n=8) groups. All rats were fed, given water, and observed for 21 days, Group I (control) received only distilled water and no WS exposure, Group II was exposed to WS, Group III was exposed to WS and given 50 mg/kg/d α-tocopherol (vitamin E), Group IV was exposed to WS and given 80 mg/kg/day NRG, Group V was administered only 80 mg/kg/d NRG only, and Group VI was administered only 50 mg/kg/d vitamin E. WS exposure was for 20 min/d. The effect of NRG treatment on acetylcholinesterase activity, nitric oxide radical production, malondialdehyde level, and antioxidant enzymes (ie, superoxide dismustase and catalase) in WS-exposed rats was examined.
Results: Subchronic (21 day) exposure to WS induced ocular and pulmonary toxicity manifested by the infiltration of parenchyma, atrophy, and inflammation of the cells, which was correlated with alterations in antioxidant enzyme concentrations. Cell damage was associated with an increase in acetylcholinesterase activity and nitric oxide radical concentrations. The toxicity triggered by WS was modulated by the coadministration of NRG.
Conclusion: These results suggest that NRG treatment may be useful to reduce WS-induced oxidative stress and related ocular and pulmonary damage in rats. (*Curr Ther Res Clin Exp*. 2012; 73:XXX–XXX)

© 2020 The Author(s). Published by Elsevier Inc.
This is an open access article under the CC BY-NC-ND license.
(http://creativecommons.org/licenses/by-nc-nd/4.0/)

Introduction

Wood is commonly utilized as a major fuel source for domestic biomass combustion in developing countries.[1] Consumption of fuelwood is a traditional source of energy in more than half of the world's population. By burning logs, twigs, and other wood materials, rural dwellers use fuelwood as a source of energy.[2] The International Institute of Population Science reported that three-quarters (75%) of all households in Asian countries, particularly in India, use unprocessed biomass as their primary fuel for cooking and that more than 90% of these homes use either wood or animal dung as a source of fuel.[3] In Africa, daily consumption of firewood by rural communities, especially in Nigeria, was estimated at 27.5 million kilograms each day.[4]

Wood smoke (WS) is a complex mixture of numerous gases and ultrafine particles of varying diameter and inorganic/organic composition.[5] These particles are in the inhalable size range[6] and are too small to be filtered out by the nose and upper respiratory system. Hence, they accumulate in the lungs, causing structural damage. Wood burning stoves emit significant amounts of toxic compounds, including particulate matter with diameters $<10\mu m$

* Address correspondence to: Jacob K. Akintunde, PhD, Applied Biochemistry and Molecular Toxicology Research Group, Department of Biochemistry, College of Biosciences, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.
E-mail address: akintundejacob@yahoo.com (J.K. Akintunde).

https://doi.org/10.1016/j.curtheres.2020.100586
0011-393X/© 2020 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license.
(http://creativecommons.org/licenses/by-nc-nd/4.0/)

(PM_{10}), carbon monoxide, nitrogen oxides, sulfur oxides, aldehydes, polycyclic aromatic hydrocarbons, volatile organic compounds, and chlorinated dioxins.[7–9] Exposure to WS in humans is often associated with ocular[9] and pulmonary damage.[10] Exposure to high concentrations can cause acute lung failure in both humans and experimental animals and has become a major cause of lung cancer.[11] The World Health Organization estimates that indoor air pollution in developing countries accounts for 2.2 to 2.5 million deaths annually.[12] Subchronic exposure to WS particles has been shown to stimulate free radical production, resulting in oxidative deterioration of lipids and proteins, and DNA damage.[13]

Although millions of households worldwide depend on wood and biomass for their cooking and heating fuel needs, this activity leads to undesirable toxic effects on the ocular and pulmonary systems. There are few therapeutic possibilities to treat or prevent this development. However, there are concerted scientific efforts underway to develop therapeutic or prophylactic agents that will reverse or protect against ocular and lung toxicity induced by exposure to WS. However, in the absence of reliable and effective modern treatments, traditional efforts currently include exploring complementary or alternative medicines to treat WS-associated diseases.

Natural products such as flavonoid, carotenoid, and phenolic compounds may be effective in treating various causes of retinopathy and respiratory disorders. Flavonoids are inhibitors of aldose-reductase, which catalyzes the rate-limiting step in sorbitol synthesis.[14] Furthermore, flavonoids have shown some promise as anticancer agents,[15] inhibit breast cancer growth in a preclinical model by inducing autophagic cell death,[16] and enhance natural-killer-mediated toxicity against head and neck squamous cell carcinoma.[17] Additionally, natural plant products have shown potential to serve as alternative therapeutic agents to counter the side effects of various drugs. Naringin (NRG) (molecular formula C27H32O14 and molecular weight 580.4 g/mol) is an active flavanone glycoside derived from the flavanone naringenin and the disaccharide neohesperidose.[18] It is found abundantly in grapes and many citrus fruits.[19] When administered orally, it is hydrolyzed to its major metabolite, olite-NRG, by the enzymes α-rhamnosidase and β-glucosidase.[20] NRG exhibits many therapeutic and pharmacologic properties, including antimicrobial,[21] antimutagenic,[22] chemoprotective,[23] anticancer,[24] anti-inflammatory,[25] and antihypercholesterolemia effects.[26] Moreover, recent studies using a variety of assay systems suggest that NRG shows both in vivo and in vitro anti-inflammatory effects.[27,28] To the best of our knowledge, no previous studies were dedicated to evaluating the protective capacity of NRG against ocular and pulmonary toxicity of WS in rats. Therefore, the present study was designed to evaluate the ocular and pulmonary protective effect of NRG against subchronic toxicity of WS exposure in Wistar rats.

Materials and Methods

Chemicals and reagents

Substrates, including acetylcholine iodide, 5, 5-dithiobisnitrobenzoic acid, hydrogen peroxide, trichloro acetic acid, and thiobarbituric acid were purchased from Sigma (St Louis, Missouri). The vitamin E (α-tocopherol) supplement, and all other chemicals and reagents were of analytical grade.

Design of exposure chamber and wood combustor

The exposure chamber was fabricated using transparent plastic while the wood combustor was made using iron, with an outlet for the release of the smoke into the chamber and a 10 cm × 10 cm square oxygen inlet on the lid. The exposure chamber, of measurement 70 cm × 60 cm × 60 cm, was constructed to allow at least 20% ventilation, as stipulated by the Organization for Economic Co-operation and Development guidelines for inhalation toxicity. The burning process was initiated before exposure by igniting 3 kg Gmelina arborea wood with a lighter in the fabricated combustor. Filtered WS was diverted after kindling from the combustor into the exposure chamber using a metal host.

Selection and burning of G arborea wood

The authors purchased Melina wood (G arborea) from a commercial wood seller near the University College Hospital, Ibadan, Nigeria. This is because Melina wood is commonly used as the raw material for energy production, including wood fuel, pulp, and saw-logs. About 3 kg Melina wood was used to generate the WS introduced into the chamber. The burning process was initiated by igniting 3 kg wood to maintain continuity of the fire and stable concentrations of carbon monoxide and PM_{10} during the exposure period.[29]

Determination of PM_{10} and carbon monoxide levels

The PM_{10} concentration in the exposure chamber was monitored using a PM_{10} monitor (Portable Particulate Monitor, PD5 1500 model, USA) that was suspended in the WS chamber for the 21 daily 20-minute sessions. A carbon monoxide monitor was also suspended in the exposure chamber to measure the level of carbon monoxide during the 21 daily 20-minute sessions. The authors recorded results weekly (Tables 1 and 2).

Ophthalmologic examination

After the WS exposure, an ophthalmoscope was used to examine the ophthalmological features of the rats' fovea, iris, cornea, and lens.

Experimental protocols

Forty-eight male Wistar albino rats, weighting between 200 g and 300 g, were procured from the University of Ibadan animal house and housed in cages at the vivarium within the Institute of Molecular Research and Advanced Technology, University College Hospital, Ibadan. The rats were acclimatized for 2 weeks and fed with standard chow pellets and water ad libitum. The rats were kept in iron cages with dimensions of 30 cm × 15 cm × 25 cm at room temperature (25°C). After acclimatization, they were weighed and grouped into 6 different groups containing 8 (n = 8) rats each:

- Group, I (control) served as the unexposed group and was given a single oral daily dose of 0.5 mL distilled water for 21 days,
- Group II (WS) served as an exposed group and was exposed to 441.2 µg/m^3 PM_{10} from WS for 20 minutes once daily for 21 days,
- Group III (WS + 50 mg/kg vitamin E) was co-treated daily with a single daily oral dose of vitamin E at 50 mg/kg body weight and exposed to 441.2 µg/m^3 PM_{10} from WS for 20 minutes daily for 21 days,
- Group IV (WS + 80 mg/kg NRG) was co-treated daily with a single daily oral dose of NRG at 80 mg/kg body weight and exposed to 441.2 µg/m^3 PM[10] from WS for 20 minutes daily for 21 days,
  Group V (80 mg/kg NRG only) received a single daily dose of NRG at 80 mg/kg body weight for 21 days, and
- Group VI (50 mg/kg vitamin E only) received a daily single daily dose of vitamin E at 50 mg/kg body weight for 21 days.

Groups II through IV were exposed to WS for 20 minutes daily and all the groups were given the same food and water treatment to ensure identical conditions. The dose of 80 mg/kg NRG and 50 mg/kg vitamin E (negative control) was selected based on previous studies.[30,31] Animal care and handling were performed according to the institutional guidelines for the protection of animal welfare during experiments.[32] On termination of the 21-day experiment, rats were fasted overnight and, after 12 hours, they were sequentially anesthetized with inhaled diethyl ether and humanely put to death.

Preparation of lung and ocular tissue homogenates for biochemical assays

Tissue homogenate was prepared by the method described by Akintunde.[14] Each rat's lung and eye was weighed and then homogenized with 4 mL 10% w/v Tris-HCl buffer (0.1 M, pH 7.4) using a homogenizing machine (REMI Homogenizer, Mumbai, india). Each lung and ocular tissue homogenate was centrifuged at 10,000 × g for 15 minutes at 4°C. Afterward, the lung and ocular supernatants were immediately separated into various aliquots for different biochemical assays (pellets were discarded).

Biochemical examinations

Acetylcholinesterase activity assay

The activity of acetylcholinesterase (AChE) was determined by the modified method of Ellman[33] and expressed as nmol AChE per minute per milligram of protein. Briefly, 100 µL 3.3 mM 5,5-dithiobis(2-nitrobenzoic) acid was mixed in 0.1 M phosphate buffer (pH 8.0) and mixed with 100 µL either lung or ocular postmitochondrial fraction, followed by an additional 500 µL phosphate buffer (pH 8.0), and incubated for 20 minutes at 25°C. Afterward, acetylthiocholine iodide (100 µL 0.05 mM solution) was mixed as the substrate, and AChE activity was quantified by measuring the changes in optical density at 412 nm.

Determination of nitrite and nitrate as markers of nitric oxide production

Tissue nitrite and nitrate were measured as an index of nitric oxide radical production. Nitrite and nitrate levels were quantified using a method based on the Griess reaction.[34] Samples were initially deproteinized with Somogyi reagent, then total nitric oxide (nitrite + nitrate) was measured using a spectrophotometer at 545 nm after reduction of nitrate to nitrite by copperized cadmium granules. A standard curve was established with a set of serial dilutions (10^{-8} to 10^{-10} mol/L) of sodium nitrite. The resulting curve was then used to calculate the unknown sample concentrations, with results expressed as millimoles per gram of tissue.

Lipid peroxidation

Lipid peroxidation was quantified as MDA according to the method described by Ohkawa and colleagues[35] and expressed as micromoles per milligram of protein.

Superoxide dismutase activity

Superoxide dismutase (SOD) was determined according the method of Misra and Fridovich.[36]

Catalase activity

Catalase (CAT) activity was assayed by the method of Sinha[37] and expressed as moles of hydrogen peroxide consumed per minute per milligram of protein.

Table 1
Measurement of weekly particulate matter of PM_{10} (µg/m^3) exposed to the control and experimental albino rats.

Exposed group	Week 1 (µg/m^3)	Week 2 (µg/m^3)	Week 3 (µg/m^3)
CG	0.11	0.11	0.11
WS	441.2 ± 1.3	441.0 ± 1.6	441.2 ± 1.3
WS + Vit E	441.2 ± 1.3	441.2 ± 1.3	441.0 ± 1.1
WS + NRG	441.2 ± 1.3	441.0 ± 1.1	441.5 ± 1.5
NRG	0.11	0.11	0.11
Vit E	0.11	0.11	0.11

Table 2
Measurement of weekly carbon monoxide (ppm) exposed to the control and experimental albino rats

Exposed group	Week 1(ppm)	Week 2 (ppm)	Week 3 (ppm)
CG	0.00	0.00	0.00
WS	786 ± 77.8	797 ± 83.2	755 ± 92.9
WS + Vit E	734 ± 66.9	793 ± 61.8	774 ± 80.5
WS + NRG	710 ± 103.5	777 ± 48.7	767 ± 108.5
NRG	0.00	0.00	0.00
Vit E	0.00	0.00	0.00

Histologic examination

The lungs and ocular tissues were quickly removed after anesthetization of rats and were fixed with 10% neutral buffered formalin solution. Histologic sections were prepared, stained with hematoxylin and eosin, and then examined under a microscope. The examiner of the histology slides was blinded to treatment group.

Statistical analysis

The data are expressed as mean (SD). A 1-way ANOVA was used to analyze the results, and the Duncan multiple test was used for the post hoc analysis. The Statistical Package for Social Science version 20.0 for Windows (IBM-SPSS Inc, Armonk, NY) was used for the analysis and the least significant difference was accepted at $P < 0.05$.

Results

Constituents of WS

The common mixture of gas pollutants detected in the exposed chamber was PM_{10} and carbon monoxide, as shown in Tables 1 and 2. The concentration of PM_{10} pollutant in the WS to which patients were exposed in groups II to IV was approximately identical across the groups (441.2 µg/m^3). The environments of unexposed groups, Group I (control), Group V (NRG only), and Group VI (vitamin E only) showed 0.11 µg/m^3 (Table 1). Similarly, the quantity of carbon monoxide to which the rats were exposed through WS for 21 days is presented in Table 2. Mean (SD) carbon monoxide concentration readings for Group II (WS only) showed 786 (77.8) ppm (week 1), 797 (83.2) ppm (week 2), and 755 (92.9) ppm (week 3); readings for Group III (WS + vitamin E) showed 734 (66.9) ppm (week 1), 793 (61.8) ppm (week 2), and 774 (80.5) ppm (week 3); readings for Group IV (WS + NRG) showed 710 (103.5) ppm (week 1), 777 (48.7) ppm (week 2), and 767 (108.5) ppm (week 3). These carbon monoxide concentration values demonstrate that all the rats were exposed to similar amounts of carbon monoxide.

Furthermore, the oculo-clinical examination indicated that the exposed rats manifested eyes abnormalities, including redness and inflammation of the cornea as well as cloudiness of the lens when compared with the control and co-treated group.

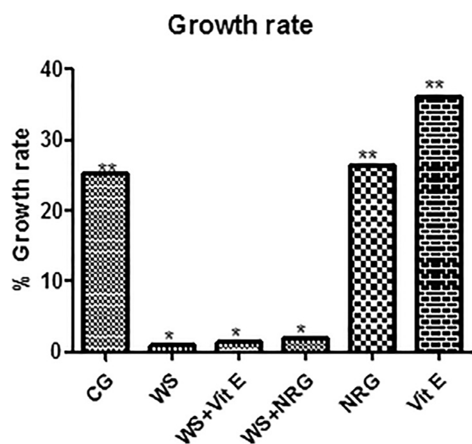

Figure 1. Effect of naringin on growth rate pattern in animals exposed to 441.2 μg/m^3 P.M$_{10}$ from wood smoke for 21 days, Mean ± S.D, (n = 8). Bars with different asterisks are significantly different (P < 0.05).

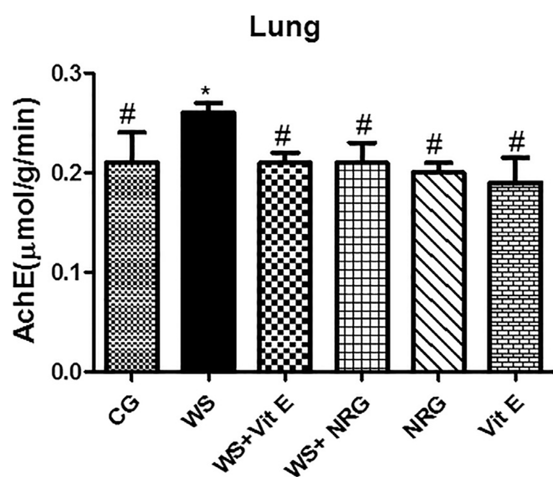

Figure 2. Effect of Naringin on lung acetylcholinesterase (AChE) activity in animals exposed to 441.2 μg/m^3 P.M$_{10}$ from wood smoke for 21 days, Mean ± S.D, (n = 8). Bars with different asterisks are significantly different (P < 0.05).

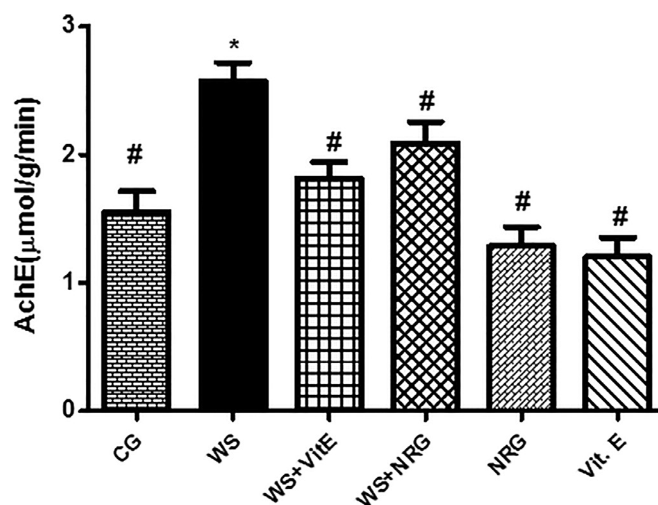

Figure 3. Effect of Naringin on ocular acetylcholinesterase (AChE) activity in animals exposed to 441.2 μg/m^3 P.M$_{10}$ from wood smoke for 21 days, Mean ± S.D, (n = 8). Bars with different asterisks are significantly different (P < 0.05).

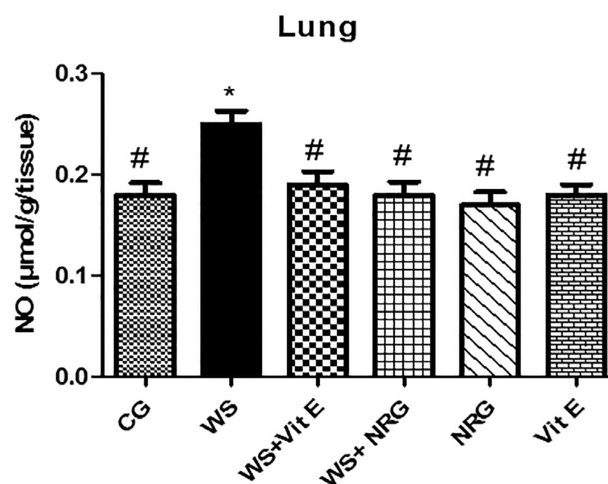

Figure 4. Effect of Naringin on nitric oxide (NO) level in animals exposed to 441.2 μg/m^3 P.M$_{10}$ from wood smoke for 21 days, Mean ± S.D, (n = 8). Bars with different asterisks are significantly different (P < 0.05).

Effect of NRG on growth rate in rats exposed to WS

The effect of NRG on the growth patterns of rats exposed to WS is presented in Figure 1. The growth rate of the exposed rats showed a significant ($P < 0.05$) reduction when compared with the control and co-treated groups.

Effect of NRG on the activity of AChE in pulmonary and ocular tissues

Figure 2 shows the effect of NRG administration (80 mg/kg body weight) on AChE activity in lung and ocular cells. The study showed that the subchronic exposure to WS containing 441.2 μg/m^3 PM$_{10}$ and 797 ppm carbon monoxide for 21 days significantly ($P < 0.05$) increased pulmonary AChE activity in relation to control and co-treated groups. Conversely, lung AChE activity was significantly ($P < 0.05$) reduced by flavonoid-NRG (80 mg/kg) and vitamin E (50 mg/kg) when compared with Group I (WS only). A similar trend was found in the activity of the ocular AChE (Figure 3). There was a substantial elevation ($P < 0.05$) of ocular AChE activity in rats exposed to WS. However, co-treatment of NRG and vitamin E at doses of 80 mg/kg NRG and 50 mg/kg vitamin E, respectively, significantly ameliorated the ocular AChE activity.

Effect of NRG on lung nitric oxide production

The measured nitric oxide production across the treatment groups is depicted in Figure 4. The level of nitric oxide in PM$_{10}$ WS-exposed rats' lung tissue was significantly higher ($P < 0.05$) than in the control group. By contrast, co-treatment with NRG (80 mg/kg body weight) and vitamin E (50 mg/kg body weight) significantly ($P < 0.05$) lowered nitric oxide levels in the lungs to normal.

Effect of flavonoid antioxidant treatment on pulmonary MDA and ocular MDA following sub-chronic exposure to 441.2 μg/m^3 PM$_{10}$ from WS

Levels of MDA measured in both lung and ocular tissues as a measure of oxidative damage, are shown in Figures 5 and 6. The study found that pulmonary MDA levels were elevated in WS exposure groups when compared with controls following the subchronic exposure to 441.2 μg/m^3 PM$_{10}$ from WS for 21 days (Figure 5). The significant hike ($P < 0.05$) in pulmonary MDA was downregulated by co-treatment with NRG (80 mg/kg) and vitamin E (50 mg/kg) compared with the control group (Figure 5). The ocular MDA levels were also significantly ($P < 0.05$) increased fol-

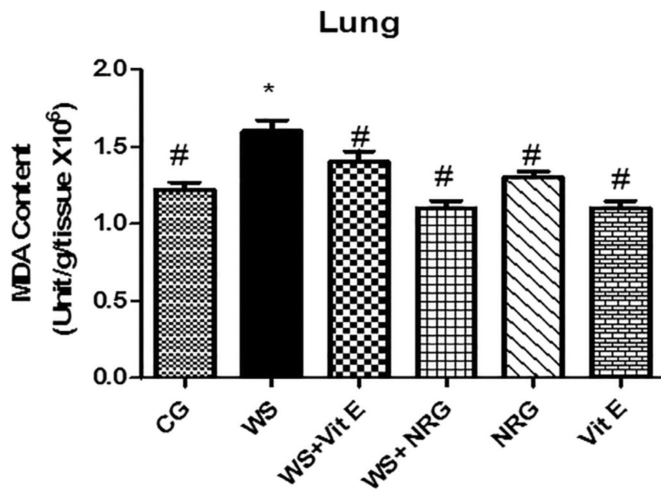

Figure 5. Effect of Naringin on pulmonary malonaldehyde (pMDA) level in animals exposed to 441.2 µg/m^3 P.M$_{10}$ from wood smoke for 21 days, Mean ± S.D, (n = 8). Bars with different asterisks are significantly different (P < 0.05).

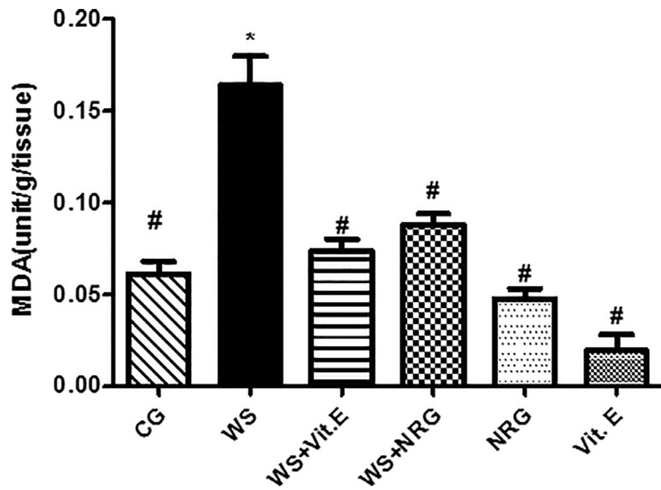

Figure 6. Effect of Naringin on ocular malonaldehyde (oMDA) level in animals exposed to 441.2 µg/m^3 P.M$_{10}$ from wood smoke for 21 days, Mean ± S.D, (n = 8). Bars with different asterisks are significantly different (P < 0.05).

lowing 441.2 µg/m^3 PM$_{10}$ exposure to WS for 21 days (Figure 6). Co-treatment with flavonoid NRG and vitamin E significantly (P < 0.05) blunted the increase, maintaining normal ocular MDA levels and restoring ocular membrane integrity.

Effect of NRG on WS-induced alterations in oxidative stress biomarkers SOD and CAT

The enzymatic antioxidant proteins in the WS exposed rat lung and ocular postmitochondrial homogenates are presented in Figures 7 through 10. The results showed that pulmonary SOD activity was significantly (P < 0.05) upregulated by 21-day WS exposure (Figure 7) relative to the corresponding control and co-treated groups. An inverse relationship was detected in lung SOD activity in the groups co-treated with NRG (80 mg/kg) and vitamin E (50 mg/kg) (as shown in Figure 7). Additionally, ocular SOD activity was significantly (P < .05) depleted by 21-day WS exposure when compared with the control (Figure 8). Co-treatment with NRG and vitamin E during the 21 days noticeably (P < 0.05) restored the ocular SOD activity to normal (Figure 8).

Furthermore, as shown in Figure 9, pulmonary CAT activity was remarkably increased by 21-day WS exposure when compared

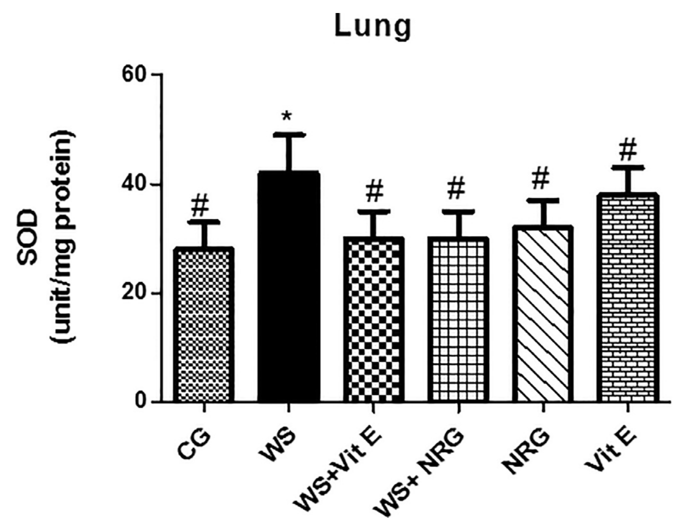

Figure 7. Effect of Naringin on lung superoxide dismutase (SOD) activity in animals exposed to 441.2 µg/m^3 P.M$_{10}$ from wood smoke for 21 days, Mean ± S.D, (n = 8). Bars with different asterisks are significantly different (P < 0.05).

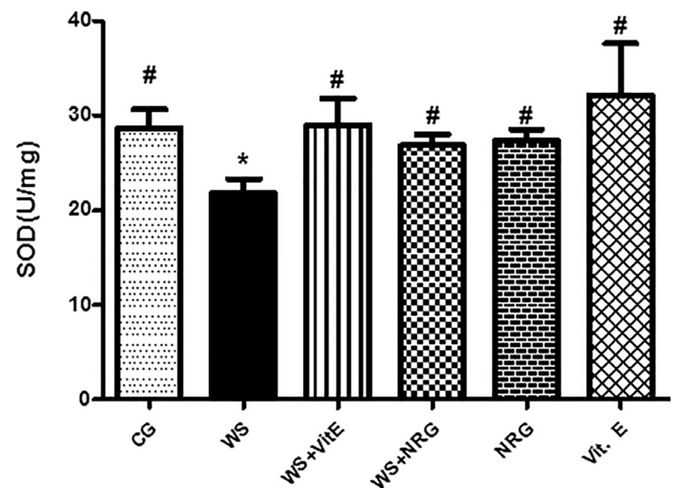

Figure 8. Effect of Naringin on ocular superoxide dismutase (SOD) activity in animals exposed to 441.2 µg/m^3 P.M$_{10}$ from wood smoke for 21 days, Mean ± S.D, (n = 8). Bars with different asterisks are significantly different (P < 0.05).

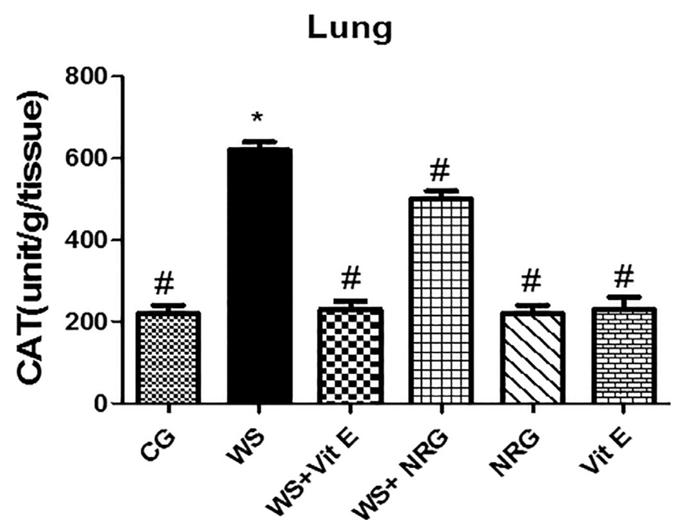

Figure 9. Effect of Naringin on lung catalase (CAT) activity in animals exposed to 441.2 µg/m^3 P.M$_{10}$ from wood smoke for 21 days, Mean ± S.D, (n = 8). Bars with different asterisks are significantly different (P < 0.05).

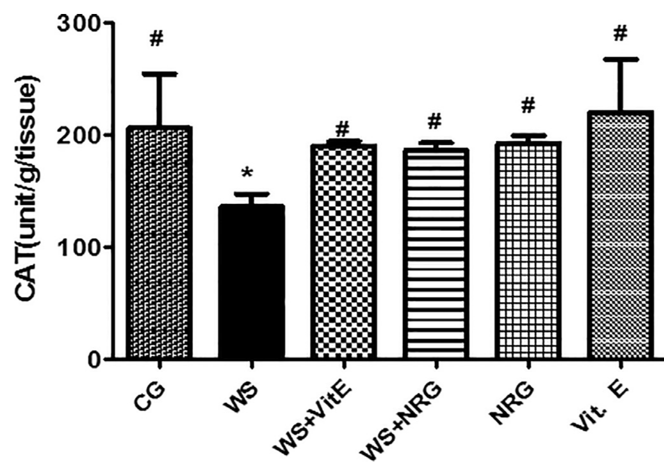

Figure 10. Effect of Naringin on ocular catalase (CAT) activity in animals exposed to 441.2 µg/m^3 P.M$_{10}$ from wood smoke for 21 days, Mean ± S.D, (n = 8). Bars with different asterisks are significantly different (P < 0.05).

with the corresponding control. Once again, co-treatment with the flavonoid NRG (80 mg/kg) and vitamin E (50 mg/kg) significantly (P > 0.05) prevented this increase (Figure 9). Co-treatment with vitamin E (50 mg/kg) showed a particularly effective, significant (P < 0.05) effect in rat lungs (Figure 9). Lastly, Figure 10 shows that ocular CAT activity was substantially (P < 0.05) suppressed by 21 days of WS exposure that was reversed by co-treatment with NRG and vitamin E in the eye tissue.

Effect of NRG on histopathological WS-caused changes in lung and ocular cells

The control group showed moderate focal infiltration of the parenchyma with moderate alveolar hyperplasia (Figure 11A). Subchronic WS exposure caused infiltration of the parenchyma and inflammation of lung cells (Figure 11B). There were also hemorrhagic lesions as evidenced by focal areas of thrombosis in the bronchioles. The lung cells in WS-exposed rats treated with vitamin E (50 mg/kg body weight) showed moderate granulation around the bronchioles with moderate focal area of thrombosis and a polyp (Figure 11C). Additionally, there was moderate infiltration of the parenchyma by inflammatory cells, along with moderate hyperplasia with cytological atypia (Figure 11C). The lung cells of WS-exposed rats treated with NRG (80 mg/kg) showed a moderate area of lymphoid aggregates with moderate inflammation and moderate thrombosis (Figure 11D). WS-exposed rats treated with only NRG (80 mg/kg) displayed moderate areas of lymph node damage with moderate inflammation and moderate hemorrhagic lesions and moderate vascular sclerosis (Figure 11E). In comparison, WS-exposed rats treated only with vitamin E (50 mg/kg) showed moderate hemorrhagic lesions with slight infiltration of the lung parenchyma by inflammatory cells (Figure 11F).

The histopathologic examination of ocular cells in control group rats showed normal architecture without any signs of ocular lesions (Figure 12A). Histopathologic examination revealed that subchronic WS exposure to 441.2 µg/m^3 WS PM$_{10}$ without co-treatments led to atrophy and neovascularization of ocular cells (Figure 12B). The ocular cells of rats co-treated with vitamin E (50 mg/kg) showed no visible lesions or any deviation from the normal architectural structure (Figure 12C). The ocular cells of WS-exposed rats co-treated with NRG (80 mg/kg) also showed no lesions or deviation from the normal architectural structure (Figure 12D). Similarly, the ocular cells of WS-exposed rats treated with either NRG only or vitamin E only showed no lesions or deviation from the normal architectural structure (Figure 12E and 12F, respectively).

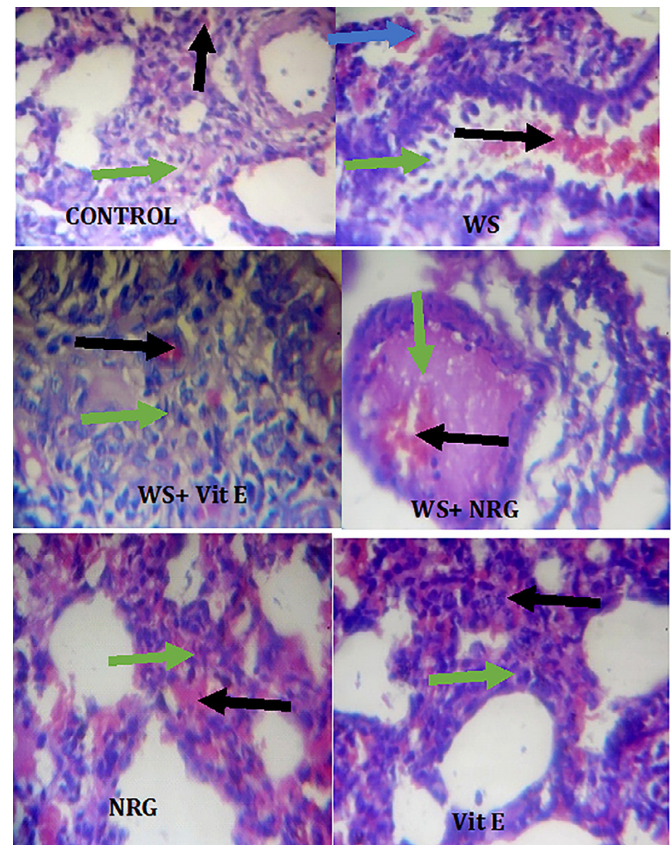

Figure 11. Lung histopathology changes on rat treated with NRG and Vit E in wood smoke induced oculo-pulmonary toxicity (× 400). **CONTROL (a):** Moderate focal area of lymph node (black arrow), moderate disseminated congestion (blue arrow), and moderate focal infiltration of the parenchyma with moderate area of alveolar hyperplasia (green arrow) **WS (b):** The lung cells show infiltration of the parenchyma by inflammatory cells (green arrow). Extensive hemorrhagic lesion and focal area of thrombosis (blue arrow) with hemorrhagic lesion in the bronchiole (black arrow). **WS + Vit E (c):** Moderate focal area of granulation tissue around the bronchiole (black arrow), moderate focal area of thrombosis and polyp (blue arrow) and moderate hyperplasia with cytological atypia (green arrow). **WS + NRG (d):** Moderate area of lymphoid aggregate (blue arrow), moderate inflammation (black arrow) and moderate thrombosis (green arrow). **NRG (e):** Moderate area of lymph node with moderate inflammation (black arrow), moderate haemorrhagic lesion (green arrow) and moderate vascular sclerosis (blue arrow). **Vit E (f):** Moderate haemorrhagic lesion (black arrow) and inflammatory cells (green arrow).

Discussion

Millions of households around the world depend on wood and other forms of biomass as their cooking and heating fuel.[1] The generated smoke and particulate matter from these anthropogenic sources are dangerous to the eyes and lungs.[2,8,9] This study found that subchronic (21 day), short-term (20 min/d) exposure of rats to 441.2 µg/m^3 PM$_{10}$ and 797 mg/L carbon monoxide from WS elevated both lung and ocular AChE activity and decreased growth rate patterns. This suggests that WS exposure in mammals could be a risk factor for diseases characterized by ocular and pulmonary dysfunction, including catarrh, glaucoma, emphysema, and bronchial fibrosis.[38–40] The results suggest that both dopaminergic and cholinergic pathways of ocular and pulmonary cells were compromised. Previous studies have reported that individuals with retinopathy and glaucoma can display disordered lung dopaminergic/cholinergic homeostasis, followed by decreased pulmonary immunoproteins.[41,42] We measured the effect of WS on the activity of AChE because it is among the crucial neurotransmitter signaling molecules implicated in the regulation of retinogenesis.[43] Additionally, the significant reduction of growth rate after 21 days of

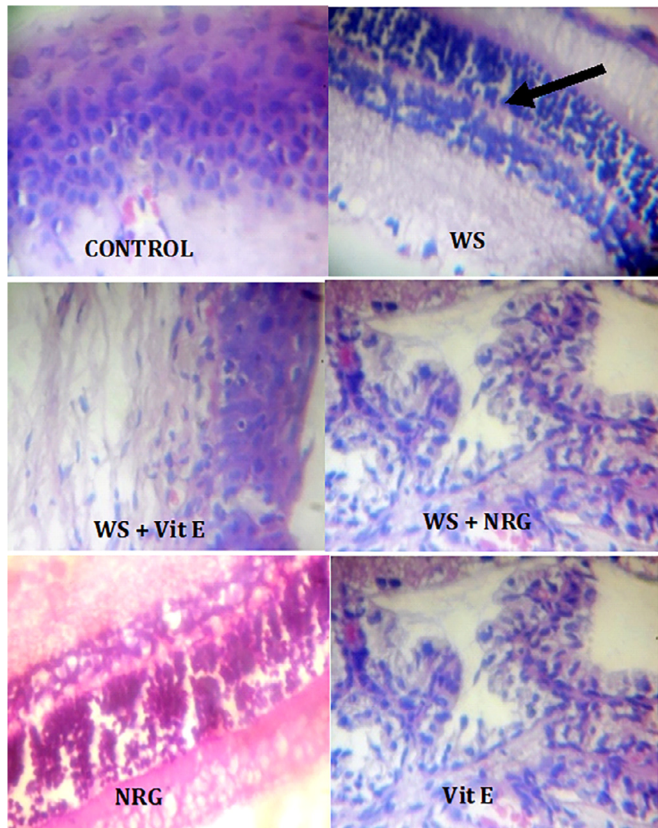

Figure 12. Ocular histopathology changes on rat treated with NRG and Vit E in wood smoke induced oculo-pulmonary toxicity (× 400). **CONTROL (a):** No lesions to architectural structure **WS (b):** Development of atrophy and neovascularization (arrow) **WS + Vit E (c):** No lesions to architectural structure **WS + NRG (d):** No lesions to architectural structure **NRG (e):** No lesions to architectural structure **Vit E (f):** No lesions to architectural structure.

WS exposure suggests the WS exposure caused organ toxicity.[44] Potent, naturally occurring antioxidant products and their analogs have been reported to be effective in the management of ocular infections associated with several metabolic disorders in mammals.[45] Therefore, we investigated the ability of NRG (4,5,7-trihydroxy flavanone 7-rhamnoglucoside), an active flavone glucoside found in grapes and many citrus fruits, at a dose of 80 mg/kg to mitigate the effects of subchronic toxicity from WS exposure in rats. The administration of both NRG and a positive control drug, α-tocopherol (ie, vitamin E), significantly regulated both lung and ocular AChE activity; a biomarker of toxicity. The protective effect of NRG treatment on this biomarker along with the protective effects of NRG therapy against distended alveoli (emphysema), bronchial necrosis, and ocular injuries triggered by WS exposure suggest NRG has prophylactic efficacy against WS exposure. Consistent with this, previous studies have shown that NRG and vitamin E can act as cytoprotection and repair mitochondrial dysfunction, which is a contributor to cellular pathogenesis and lung impairment.[46,47] Additionally, other studies have suggested that flavonoids, particularly vitamin E, have the ability to reduce inflammation, mutation, and oxidation, suggesting that flavonoids might be effective for treating or preventing catarrh and lung failure.[48,49] NRG and vitamin E have also been reported to be potent antioxidants, eliciting strong protection against lens and glial cell necrosis,[22] aqueous/ vitreous humor damage,[15] and ultraviolet light-induced catarrh and glaucoma.[61] Lastly, recent studies have reported that NRG reduced 3-nitropropionic acid induced inflammation through the modulation of nuclear reactor factor-2-driven antioxidant release element gene expression, followed by the reduction of tumor necrosis factor-α, cyclooxygenase-2, and inducible nitric oxide synthase expression causing the depleted production of proinflammatory mediators.[25,31]

Modulation of pulmonary nitric oxide could be a useful measure of tissue viability. Accumulation of the nitric oxide radicals in the body causes metabolic disorders, particularly lung fibrosis and ocular injury.[9] Additionally, nitric oxide is among the major mediators of toxin-induced oxidative tissue injury.[50] However, the mechanism by which nitric oxide triggers a series of events leading to bronchopulmonary fibrosis is not fully understood. The present study showed that repeated (21 daily), short-term (20 minute) exposures to 441.2 μg/m^3 PM_{10} from WS significantly increased pulmonary nitric oxide levels, as well as altered lung structure (described as hemorrhagic alveolitis, interstitial inflammation, intra-alveolar hemorrhage, edema, hyperemia, bronchial necrosis, and vascular necrosis).[51] Concomitant treatment with NRG (80 mg/kg) and/or vitamin E (50 mg/kg) significantly decreased these nitric oxide levels, and demonstrating significant protection against WS-mediated pulmonary toxicity. However, these results also suggest that NRG could also act as an anti-inflammatory agent, because nitric oxide radical depletion is reported to be cytoprotective in vitro in many cell types such as heart cells,[50] neurons and glial cells,[52] epithelial cells, and retinal cells.[9]

The toxicity from exposure to the carbon monoxide, particulate matter, and other organic compounds in heating fuels has recently generated many concerns.[5] WS constituents are metabolized via glucuronidation and sulfation reactions occurring principally in the lungs and the eyes. This produces water-soluble metabolites that are excreted in urine or tears. During the metabolic conversion of various WS constituents by the microsomal CYP-450 enzyme system, highly reactive intermediate metabolites are produced.[53] These highly toxic metabolites come into direct contact with ocular and lung proteins, triggering the depletion of cellular proteins.[54] When these metabolites bind to cellular proteins, lipid peroxidation is initiated, leading to ocular[9] and pulmonary injury.[55] The generation of reactive oxygen species precedes intracellular lipid peroxidation, oxidization of proteins, and DNA damage in the lung and the eyes.[55]

Current evidence suggests that intracellular lipid peroxidation is implicated in optic neuropathy, retinopathy, losses in vision, and damage to lung cell membrane integrity.[56,57] Substantial elevations in the concentrations of MDA in both ocular and lung tissue are associated with ocular and pulmonary toxicity.[12,57] Consistent with previous studies,[56,57] exposure to 441.2 μg/m^3 PM_{10} from WS in our study led to alterations in the eyes as well as pulmonary enzymatic antioxidants (eg, CAT and SOD) in rats. There was also substantial peroxidation of ocular and pulmonary membrane lipids of exposed rats. The altered enzymatic antioxidant status could be the mechanism responsible for the ocular and pulmonary toxicity observed. Free radical generation is known to have harmful effects on the cells, tissues, and organs. NRG can scavenge several types of free radicals.[47,58] The co-treatment of WS exposed rats with NRG significantly restored the activity of SOD and CAT in both the eye and the lung. The normalization of these enzymes' activities is a potential explanation for the protection provided by NRG administration in rats against both ocular and pulmonary oxidative stress induced by WS, as reported in previous studies.[59,60]

The histological cytotoxicity examinations demonstrated that subchronic WS exposure caused infiltration of the parenchyma and inflammation of the lung cells. The focal areas of thrombosis are evidence of hemorrhagic lesions in the bronchioles.[62] The histology results supported the view that NRG protected the lung cells from hemorrhagic lesion induced by WS exposure. Atrophy and neovascularization of ocular cells are evidence of chronic ocular injury and retinal necrosis.[63,64] Co-intake of NRG and/or vitamin E inhib-

ited this atrophy and neovascularization, thereby alleviating ocular inflammation and damage.

Conclusions

Subchronic WS exposure to significantly increased cellular AChE activities and nitric oxide levels in rat ocular and pulmonary tissues that were prevented or ameliorated by coadministration of NRG and/or vitamin E. This suggests that NRG administration could promote the activity of ocular and lung antioxidant enzymes and inhibit AChE and nitric oxide in lung and eye tissue, which are the primary target organs of WS exposure. Further, the results suggest that NRG administration may have therapeutic potential for patients exposed to PM_{10} WS. Further studies to elucidate both the molecular mechanism of action of NRG, any possible long-term adverse effects, and most effective dosing regimens and combinations are needed before starting clinical testing in human beings.

Acknowledgments

Jacob K. Akintunde (MPH) provided the figure creation, study design, data interpretation, supervision of the study and writing of the manuscript. Joseph B. Abioye (MPH) provided the literature search and data collection, and Owen N. Ebinama (MPH) provided the literature search and data collection.

Conflicts of Interest Statement

The authors have indicated that they have no conflicts of interest regarding the content of this article.

References

1. Forchhammer L, Møller P, Riddervold IS, Bønløkke J, Massling A, Sigsgaard T, Loft S. Controlled human wood smoke exposure: oxidative stress, inflammation and microvascular function. *Particle Fibre Toxicol*. 2012;9:7.
2. Migliaccio C, Manderly J. Biomass smoke exposures: Toxicology and animal study design. *Inhal Toxicol*. 2009;20:1–4.
3. Dandona R, Pandey A, Dandona L. A review of national health surveys in India. *Bull World Health Organ*. 2016;94(4):286–296.
4. Zaku SG, Kabir A, Tukur AA, Jimento IG. Wood fuel consumption in Nigeria and the energy ladder: A review of fuel wood use in Kaduna State. *J Pet Technol Altern Fuels*. 2013;4(5):85–89.
5. Cassee FR, Héroux M, Gerlofs-Nijland ME, Kelly FJ. Particulate matter beyond mass: recent health evidence on the role of fractions, chemical constituents and sources of emission. *Inhal Toxicol*. 2013;25(14):802–812.
6. Lippmann M, Chen LC, Gordon T, Ito K, Thurston GD. National Particle Component Toxicity (NPACT) Initiative: integrated epidemiologic and toxicologic studies of the health effects of particulate matter components. *Res Rep Health Eff Inst*. 2013(177):5–13.
7. Goulaouic S, Foucaud L, Bennasroune A, Laval-Gilly P, Falla J. Effect of polycyclic aromatic hydrocarbons and carbon black particles on pro-inflammatory cytokine secretion: impact of PAH coating onto particles. *J Immunotoxicol*. 2008;5(3):337–345.
8. Dilger M, Orasche J, Zimmermann R, Paur HR, Diabaté S, Weiss C. Toxicity of wood smoke particles in human A549 lung epithelial cells: the role of PAHs, soot and zinc. *Arch Toxicol*. 2016;90(12):3029–3044.
9. Karakoçak BB, Patel S, Ravi N, Biswas P. Investigating the Effects of Stove Emissions on Ocular and Cancer Cells. *Sci Rep*. 2019;9(1):1870.
10. Hawley B, Volckens J. Proinflammatory effects of cookstove emissions on human bronchial epithelial cells. *Indoor Air*. 2013;23(1):4–13.
11. Whitehouse AL, Miyashita L, Liu NM, Lesosky M, Flitz G, Ndamala C, Balmes JR, Gordon SB, Mortimer K, Grigg J. Use of cleaner-burning biomass stoves and airway macrophage black carbon in Malawian women. *Sci Total Environ*. 2018;635:405–411.
12. Balcan B, Akan S, Özsancak Uğurlu A, Ceyhan B. Altered pulmonary functions due to biomass smoke in a rural population of Turkish women: a descriptive study. *Tuberk Toraks*. 2018;66(2):122–129.
13. Claxton LD. The history, genotoxicity, and carcinogenicity of carbon-based fuels and their emissions. Part 2: solid fuels. *Mutat Res Rev Mutat Res*. 2014;762:108–212.
14. Akintunde JK. Functional Oil from Black Seed Differentially Inhibits Aldose-reductase and Ectonucleotidase Activities by Up-regulating Cellular Energy in Haloperidol-induced Hepatic Toxicity in Rat Liver. *J Oleo Sci*. 2017;66(9):1051–1060.
15. Bhattacharya S, Muhammad N, Steele R, Kornbluth J, Ray RB. Bitter Melon Enhances Natural Killer Mediated Toxicity against Head and Neck Cancer Cells. *Cancer Prev. Res.*. 2017;10(6):337–344.
16. Muhammad N, Steele R, Isbell TS, Philips N, Ray RB. Bitter melon extract inhibits breast cancer growth in preclinical model by inducing autophagic cell death. *Oncotarget*. 2017;8(39):66226–66236.
17. Bhattacharya S, Muhammad N, Steele R, Peng G, Ray RB. Immunomodulatory role of bitter melon extract in inhibition of head and neck squamous cell carcinoma growth. *Oncotarget*. 2016;7(22):33202–33209.
18. Barreca D, Gattuso G, Bellocco E, Calderaro A, Trombetta D, Smeriglio A. Flavanones: Citrus phytochemical with health-promoting properties. *Biofact.*. 2017;43(4):495–506.
19. Arul D, Subramanian P. Inhibitory effect of naringenin (citrus flavonone) on N-nitrosodiethylamine induced Phytochem hepatocarcinogenesis in rats. *Biochem Biophys Res Commun*. 2013;434:203–220.
20. Yoshida H, Takamura N, Shuto T, Ogata K, Tokunaga J, Kawai K, Kai H. The citrus flavonoids hesperetin and naringenin block the lipolytic actions of TNF-alpha in mouse adipocytes. *Biochem Biophys Res Commun.*. 2010;394(3):728–732.
21. Bharti S, Rani N, Krishnamurthy B, Arya DS. Preclinical evidence for the pharmacological actions of naringin: a review. *Planta Med*. 2014;80:437–451.
22. Du G, Jin L, Han X. Naringenin: a potential immunomodulator for inhibiting lung fibrosis and metastasis. *Cancer Res.*. 2009;69:3205–3321.
23. Chtourou Y, Gargouri B, Kebieche M, Fetoui H. Naringin abrogates cisplatin-induced cognitive deficits and cholinergic dysfunction through the down-regulation of AChE expression and ions signaling pathways in hippocampus of aged rats. *J Molecular Neurosci*. 2015;56:349–362.
24. Long R, Mao K, Ye X, Yan W, Huang Y, Wang J. Surface Facet of Palladium Nanocrystals: A Key Parameter to the Activation of Molecular Oxygen for Organic Catalysis and Cancer Treatment. *J Am Chem Soc*. 2013;135:3200–3207.
25. Lunzhao Y, Shasha M, Dabing R. Phytochemistry and bioactivity of Citrus flavonoids: a focus on antioxidant, anti-inflammatory, anticancer and cardiovascular protection activities. *Phytochem Rev*. 2017. doi:10.1007/s11101-017-9497-1.
26. Alam MA, Subhan N, Rahman MM, Uddin SJ, Reza HM, Sarker SD. Effect of citrus flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action. *Adv Nutr*. 2014;5:404–417.
27. Li H, Yang B, Huang J, Xiang T, Yin X, Wan J, Luo F, Zhang L, Li H, Ren G. Naringin inhibits growth potential of human triple-negative breast cancer cells by targeting beta-catenin signaling pathway. *Toxicol Lett*. 2013;220:219–228.
28. Golechha M, Sarangal V, Bhatia J, Chaudhry U, Saluja D, Arya DS. Naringin ameliorates pentylenetetrazol-induced seizures and associated oxidative stress, inflammation, and cognitive impairment in rats: possible mechanisms of neuroprotection. *Epilepsy Behav*. 2014;41:98–102.
29. Yohannes T, Shashibhushan PS, Jennifer EF, Justin K, Edward BB, Philip MF, Jacob DM, Fletcher FH, Joe LM. *Toxicol Sci.*. 2002;65(1):115–125.
30. Nianhu L, Zhanwang X, Paul HW, Jianxin Z, Shang-You Y. Therapeutic potentials of naringin on polymethylmethacrylate induced osteoclastogenesis and osteolysis, in vitro and in vivo assessments. *Drug Design Dev and Ther*. 2014;8:1–11.
31. Gerson SP, Carlton AT, Marcos CC, Ivone AD, Elisângela CB, Karen PC. A Comparative Study of the effects of Vitamins C and E in the Development of Sarcoma 180 in Mice. *J cancer*. 2013;4:724–725.
32. Public Health Service (PHS). *Public Health Service Policy on Humane Care and Use of Laboratory Animals*. Washington, DC: US Department of Health and Human Services (PL 99-158. Health Research Extension Act, 1985); 1996.
33. Perry N, Houghton P, Theobal D, Jenner P, Perry E. *In vitro* activity of S. lavan–dulaefolia (Spanish sage) relevant to treatment of Alzheimer's disease. *J Pharm Pharmacol.*. 2000;52:895–902.
34. Cortas NK, Wakid NW. Determination of inorganic nitrate in serum and urine by a kineticcadmium-reduction method. *Clin Chem*. 1990;36:1440–1443.
35. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analy Biochem*. 1979;95:351–358.
36. Misra H, Fridovich I. The role of superoxide anion in the auto-oxidation of epinephrine and a simple assay of superoxide dismutase. *Toxicol Biol Chem*. 1989;2417:3170.
37. Sinha KA. Calorimetric assay of catalase. *Analy Biochem*. 1972;42:389–394.
38. Sheila KW, Michael NB, Jennifer SL, Debra AS, David JL, Heather A. Is Household Air Pollution a Risk Factor for Eye Disease. *Int J Environ Res Public Health*. 2013;10(11):5378–5398.
39. Gordon SB, Bruce NG, Grigg J, Hibberd PL, Kurmi OP, Lam KH. Respiratory risks from household air pollution in low and middle income countries. *Lancet Respir Med*. 2014;2(10):823–860.
40. Lee A, Kinney P, Chillrud S, Jack D. A Systematic Review of Innate Immunomodulatory Effects of Household Air Pollution Secondary to the Burning of Biomass Fuels. *Ann Glob Health*. 2015;81(3):368–374.
41. Huerta C, García Rodríguez LA, Möller CS, Arellano FM. The risk of obstructive airways disease in a glaucoma population. *Pharmacoepidemiol Drug Saf*. 2001;10(2):157–163.
42. Zhang WZ, Butler JJ, Cloonan SM. Smoking-induced iron dysregulation in the lung. *Free Radic Biol Med.*. 2019;133:238–247.
43. Muramatsu I, Uwada J, Masuoka T, Yoshiki H, Sada K, Lee KS, Nishio M, Ishibashi T, Taniguchi T. Regulation of synaptic acetylcholine concentrations by acetylcholine transport in rat striatal cholinergic transmission. *J Neurochem*. 2017;143(1):76–86.
44. Julian CL, Jeffrey B. Mechanisms Limiting Body Growth in Mammals. *Endocr Rev*. 2011;32(3):422–440.

45. Gregório BM, De Souza DB, de Morais Nascimento FA, Pereira LM, Fernandes-Santos C. The potential role of antioxidants in metabolic syndrome. *Curr Pharm Des*. 2016;22(7):859–869.
46. Ulaganathan M, Jyotirmoi A, Geeta DL, Surendra KS, Amit KD, Balaram G. Effects of vitamin E on mitochondrial dysfunction and asthma features in an experimental allergic murine model. *J Appl Physiol.*. 2009;107(4):1285–1292.
47. Piantadosi C.A., Suliman H.B. Mitochondrial Dysfunction in Lung Pathogenesis. Annu Rev Physiol. 79:495-515.
48. Yue L, Yao H. Mitochondrial dysfunction in inflammatory responses and cellular senescence: pathogenesis and pharmacological targets for chronic lung diseases. *Br J Pharmacol*. 2018;173(15):2305–2318.
49. Zhao G, Li K, Chen J, Li L. Protective Effect of Extract of *Bletilla Striata* on Isoflurane Induced Neuronal Injury By Altering PI3K/Akt Pathway. *Transl Neurosci*. 2018;9:183–189.
50. Das A, Jaman K, Singh AV. Antimicrobial and antioxidant activities of *Callistemon linearis* DC Leaf extract. *Pharmacol*. 2008;3:875–881.
51. Sundaram C, Shantveer GU, Megha SU, Roshni TP, Aruna KP, Manmadha TR. Pulmonary zygomycosis: A clinicopathological study. *Lung India*. 2011;28(1):25–29.
52. Belyanskaya L, Weigel S, Hirsch C, Tobler U, Krug HF, Wick P. Effects of carbon nanotubes on primary neurons and glial cells. *Neurotoxicol*. 2009;30(4):702–711.
53. Rawal S, Coulombe RA. Metabolism of aflatoxin B1 in turkey liver microsomes: the relative roles of cytochromes P450 1A5 and 3A37. *Toxicol and Applied Pharmacol*. 2011;254(3):349–354.
54. Gorguner M, Akgun M. Acute Inhalation Injury. *Eurasian J Med*. 2015;42(1):28–35; Kerger B.D., Fedoruk M.J. Pathology, toxicology, and latency of irritant gases known to cause bronchiolitis obliterans disease: Does diacetyl fit the pattern? Toxicol Rep. 2015; 2:1463-1472..
55. Sood A. Performing a lung disability evaluation: how, when, and why? *J Occup Environ Med. Suppl.*. 2014;10:S23–S29.
56. Małgorzata N, Andrzej G. The Role of the Reactive Oxygen Species and Oxidative Stress in the Pathomechanism of the Age-Related Ocular Diseases and Other Pathologies of the Anterior and Posterior Eye Segments in Adults. *Oxid Med Cell Longev*. 2016;2016.
57. Saccà SC, Cutolo CA, Ferrari D, Corazza P, Traverso CE. The Eye, Oxidative Damage and Polyunsaturated Fatty Acids. *Nutrients.*. 2018;10(6):E668.
58. Ung L, Pattamatta U, Carnt N, Wilkinson-Berka JL, Liew G, White AJR. Oxidative stress and reactive oxygen species: a review of their role in ocular disease. *Clin Sci (Lond)*. 2017;131(24):2865–2883.
59. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. *J Nutr Sci.*. 2017;5:e47.
60. Zhu F, Qiu X, Wang J, Jin Y, Sun Y, Lv T, Xia Z. A rat model of smoke inhalation injury. *Inhal Toxicol.*. 2016;24(6):356–364.
61. Akintunde JK, Farouk AA, Mogbojuri O. Metabolic treatment of syndrome linked with Parkinson's disease and hypothalamus pituitary gonadal hormones by turmeric curcumin in Bisphenol-A induced neuro-testicular dysfunction of Wistar rat. *Biochem Biophys Rep*. 2019;17:97–107.
62. Akintunde JK, Woleola MT. Impairment of neuro-renal cells on exposure to cosmopolitan polluted river water followed by differential protection of *Launea taraxacifolia* in male rats. *Comparative Clin. Pathol.*. 2019;28(2) Article in press. doi:10.1007/s00580-019-02898-y.
63. Maalouf S, El-Sabban M, Darwiche N, Gali-Muhtasib H. Protective effect of vitamin E on ultraviolet B light-induced damage in keratinocytes. *Mol Carcinog*. 2002;34(3):121–130.
64. Jin Y, Zhang C, Zhang R, Ren J, Chen S, Sui M. Pulmonary edema following central nervous system lesions induced by a non- mouse-adapted EV71 strain in neonatal BALB/c mice. *Virol J*. 2017;14(1):243.