JCHR (2023) 13(3), 497-509

Journal of Chemical Health Risks

www.jchr.org

ORIGINAL ARTICLE

Ameliorative Potentials of *Camellia sinensis* on Petrol Fumes-induced Oxidative Stress in Rats

Joy O. Uba[*1], Eugene N. Onyeike[2], Charity U. Ogunka-Nnoka[2], Catherine C. Ikewuchi[1, 2]

[1]*World Bank Africa Centre of Excellence in Public Health and Toxicological Research (ACE-PUTOR), University of Port Harcourt, Choba, Port Harcourt, Rivers State, Nigeria*

[2] *Department of Biochemistry, University of Port Harcourt, PMB, 5323, Port Harcourt, Rivers State, Nigeria*

Received: 8 August 2022 *Accepted: 25 October 2022)*

KEYWORDS

Inhalation;
Inflammation;
Aqueous;
Methanol

ABSTRACT: Volatile organic compounds found in petrol include benzene, toluene, ethylbenzene, and xylene (BTEX). Extract of *Camellia sinensis* (tea plant) leaf contains some secondary metabolites such as flavonoids, alkaloids, terpenoids saponins, anthraquinones, and tannins. Polyphenols in *Camellia sinensis* possess anti-atherosclerosis and cardioprotective, neuroprotective, anti-inflammatory, anti-carcinogenic, antibacterial, anti-diabetic, anti-tumor, anti-hypertensive, and hepato-protective effects. The study aimed to investigate the potential of *Camellia sinensis* in ameliorating lung damage, oxidative stress, and inflammation caused by petrol fumes. Forty-eight Wistar albino rats weighing between 140g-230g were randomized into 8 groups of 6 rats each consisting of control, petrol fumes only group, three aqueous drink groups of different doses, and three methanol extract groups of different doses respectively. The oxido-inflammatory responses and histopathological alterations in rat lungs following 6 hours of daily exposure for 30 and 60 days were recorded. Oxidative stress (superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT)) and inflammatory biomarkers namely: Tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6) were evaluated. The biochemical analyses showed that petrol fumes exposure resulted in significant ($P<.05$) increases in biomarkers of oxidative stress, pro-inflammation cytokines, and reduced GSH levels in rats as well histopathological alteration in lungs. The treated groups showed anti-oxidant properties by the elevation of antioxidant enzymes (CAT, SOD) and non-enzymatic antioxidant (gluthathione) and reduction of MDA levels as well as reversal of alterations in the lungs after histopathological analysis. This study showed that *Camellia sinensis* leaf aqueous and methanol extract have the potential to attenuate petrol fumes-induced oxidative stress due to its natural bioactive constituents.

INTRODUCTION

Petrol is a major source of fuel and exposure to petrol fumes is highly inevitable. This ranges from occupational exposure in the petrochemical industry to exposure to fumes in our homes. Petrol is a very volatile liquid and contains a mixture of aliphatic and aromatic hydrocarbons which will readily evaporate if left exposed. Toxicological effects following exposure to gasoline can be linked to volatile organic compounds (VOCs) present in gasoline, such as benzene, toluene, hexane, tetraethyl lead ethylene, and xylene [1]. Exposure to petrol fumes has been attributed to defects in organs such as the heart, lungs, skin, and kidneys as well as affects different forms of genotoxic, mutagenic, immunotoxic, carcinogenic and neurotoxic manifestations [2 - 6]. Some studies have further shown that fumes from petroleum products have an adverse

*Corresponding author: uba.joy@uniport.edu.ng (Joy O. Uba)
DOI: 10.22034/jchr.2022.1963766.1601

effect on hematological indices, stress hormones, antioxidant status, lung lipid peroxidation, plasma antioxidant defense system, and sex hormones of humans [7 - 10].

The lung is the first organ of contact with petrol fumes upon inhalation. Aside from being the major route of exposure [11], the respiratory tract is very vulnerable due to its high accessibility and excellent absorption surface which enables the hydrocarbons of petrol to be readily absorbed by the lungs [12,13]. The fumes disrupt and irritate the trachea, alveoli, and lung airways.

Camellia sinensis is also known as the tea plant. It is native to Asia and also grows in some parts of Africa such as The Mambilla Plateau, Taraba state, Nigeria, and Kenya. Since the lethal dose (LD_{50}) of *Camellia sinensis* was greater than 5000mg kg^{-1} [14], the plant is very safe to be used in diseased conditions since [15] indicated that LD_{50} greater than or equal to 5000 $mgkg^{-1}$ is biologically unimportant. The leaf extract had been reported to contain some secondary metabolites such as flavonoids, alkaloids, terpenoids, saponins, anthraquinones, and tannins [14, 16]. Polyphenols in *Camellia sinensis* possess anti-atherosclerosis and cardioprotective, neuroprotective, anti-inflammatory, anti-carcinogenic, antibacterial, anti-diabetic, anti-tumor, and anti-hypertensive and hepato-protective effects [16 - 18]. Methanol extract of *Camellia sinensis* possesses anti-oxidant, antidiabetic, antimicrobial, anti-inflammatory, and anti-hepatotoxic properties [19- 21].

MATERIALS AND METHODS

Plant preparation

The fresh leaves were collected from Mambilla Plateau Taraba state Nigeria. It was dried with the green tea drier at 120^{o}C for 55 minutes and ground to increase surface area and aid extraction. Dried leaves of *Camellia sinensis* were extracted with hot water to make the aqueous drink and 1000g of dried leaves were extracted in methanol, concentrated with a rotatory evaporator, and further dried to a paste using a water bath.

Animal treatment

Exposure to petrol, diesel, and kerosene vapors

The petrol was obtained from a Conoil filling station located in the University of Port Harcourt, Rivers State, Nigeria. Forty-eight (140g-230g) Wistar albino rats were randomized into 8 groups of 6 rats each and acclimatized for 1 week. G1 (control) G2 (test control; exposed to petrol fumes only), G3, G4, G5 were treated with aqueous (aq) drink of 500 mg kg^{-1} body weight (b.w), 1000 mg kg^{-1} b.w and 1500 mg kg^{-1} b.w respectively and then exposed to petrol fumes while G6, G7, G8 were treated with methanol (met) extract of 500 mg kg^{-1} b.w, 1000 mg kg^{-1} b.w and 1500 mg kg^{-1} b.w respectively and then exposed to petrol fumes. The test groups were pre-treated with the aqueous drink or methanol extract and then exposed to petrol fumes daily for 6 hours a day, 6 days a week for 60 days. They were exposed using the method described by [22] and also used by [23]. The animal cages were placed in the exposure chamber which measured 100×90×60cm. Two 1000ml graduated beakers each containing 500 ml of gasoline were placed in the exposure chamber one hour before animal exposure to ensure saturation of the chamber with petrol fumes. The tests were carried out twice at thirty days and sixty days.

Lipid peroxidation and anti-oxidant assay

Collection of lungs tissues for analysis

Chloroform anesthesia was administered to the animals twenty-four hours after the last exposure. Tissue samples were collected and homogenized immediately using phosphate buffer saline according to the method reported by [24]. Biochemical analyses were carried out for the measurement of catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), malondialdehyde (MDA), interleukin-6 (IL-6), tumor necrosis factor α (TNF-α).

Determination of oxidative stress level

Lung samples of rats were homogenized in phosphate buffer (0.1 M; pH 7.4) and assessed for biomarkers of oxidative stress. CAT and SOD levels were estimated using the procedure by [25, 26] respectively. The procedure by [27] was adopted for estimating GSH while the procedure of [28] was used to determine the

concentration of MDA. Assessments of TNF-α and IL-6 levels were performed using ELISA kits purchased from E-lab science Biotechnology (Beijing, China).

Histopathological study of rats' lungs

Lungs sample were fixed in 10% phosphate-buffered formaldehyde for 72 hours and were processed and sectioned at 4-5 μm thickness. The sections were stained with hematoxylin and eosin (H&E) stain and then mounted in a DPX medium for light microscopy. The slides were examined at different magnifications and the X100 magnifications were captured using a digital camera attached to the microscope.

Statistical analysis

Data were expressed as mean ± standard deviation (S.D). The results were analyzed using one- way analysis of variance (ANOVA) for (n=3) followed by Post hoc. The differences between groups were considered significant at $P < 0.05$.

RESULTS

Impact of Camellia sinensis aqueous drink and methanol extract on the regulation of oxidative stress markers in the lungs of petrol fumes-exposed rats

At 30 days, there was a significant ($P<0.05$) increase in CAT in aqueous (aq) 1000mg kg $^{-1}$ b.w, met 500mg kg $^{-1}$ b.w and methanol (met) 1000mg kg $^{-1}$ b.w groups when compared to control and petrol fumes only groups whereas met 1500mgkg $^{-1}$ b.w had a significant($P<0.05$) decrease when compared to petrol fumes only. For MDA there was a significant decrease ($P<0.05$) in all aqueous drink groups and met 500 mg kg $^{-1}$ b.w group when compared to the petrol fumes only group. GSH levels were significantly increased ($P<0.05$) in all test groups when compared to the control. GSH levels of aqu 1000,

met 500 and met 1000 mg kg-1 b.w groups were significantly ($P<0.05$) increased when compared to the petrol fumes-only group (Figure 1).

At 60 days, the CAT level of met500 mg kg $^{-1}$ was significantly increased ($P<0.05$) when compared to petrol fumes only group. The SOD levels of aq 500, aq 1000, and aq1500 mg kg $^{-1}$ b.w groups were significantly increased ($P<0.05$) when compared to the control and petrol fumes only group. For the MDA, petrol fumes-only group was significantly increased ($P<0.05$) but aqu 1500 was significantly reduced ($P<0.05$) when compared to the control whereas all treated groups were signifiicantly decreased when compared to the petrol fumes only group. GSH levels were significantly increased($P<0.05$) in aqu 1000 and aq 1500 mg kg $^{-1}$ b.w groups when compared to the control and the petrol fumes only group (Figure 2).

Impact of Camellia sinensis aqueous drink and methanol extract on the regulation of inflammatory markers in the lungs of petrol fumes-exposed rats

At 30 days, all groups treated with aqueous drink had significantly reduced ($P<0.05$) IL-6 levels when compared to control, and for TNF-α exposed(petrol fumes only), all aqueous drink treated groups, met 500 and met 1000 groups were significantly increased($P<0.05$) when compared to control (Figure 3).

At 60 days, the exposed and all treated groups had a significantly lower ($P<0.05$) IL-6 value when compared to the control whereas for TNF-α aq 500, met 1000 and met 1500 mg kg $^{-1}$ b.w groups were significantly increased ($P<0.05$) when compared to control (Figure 3).

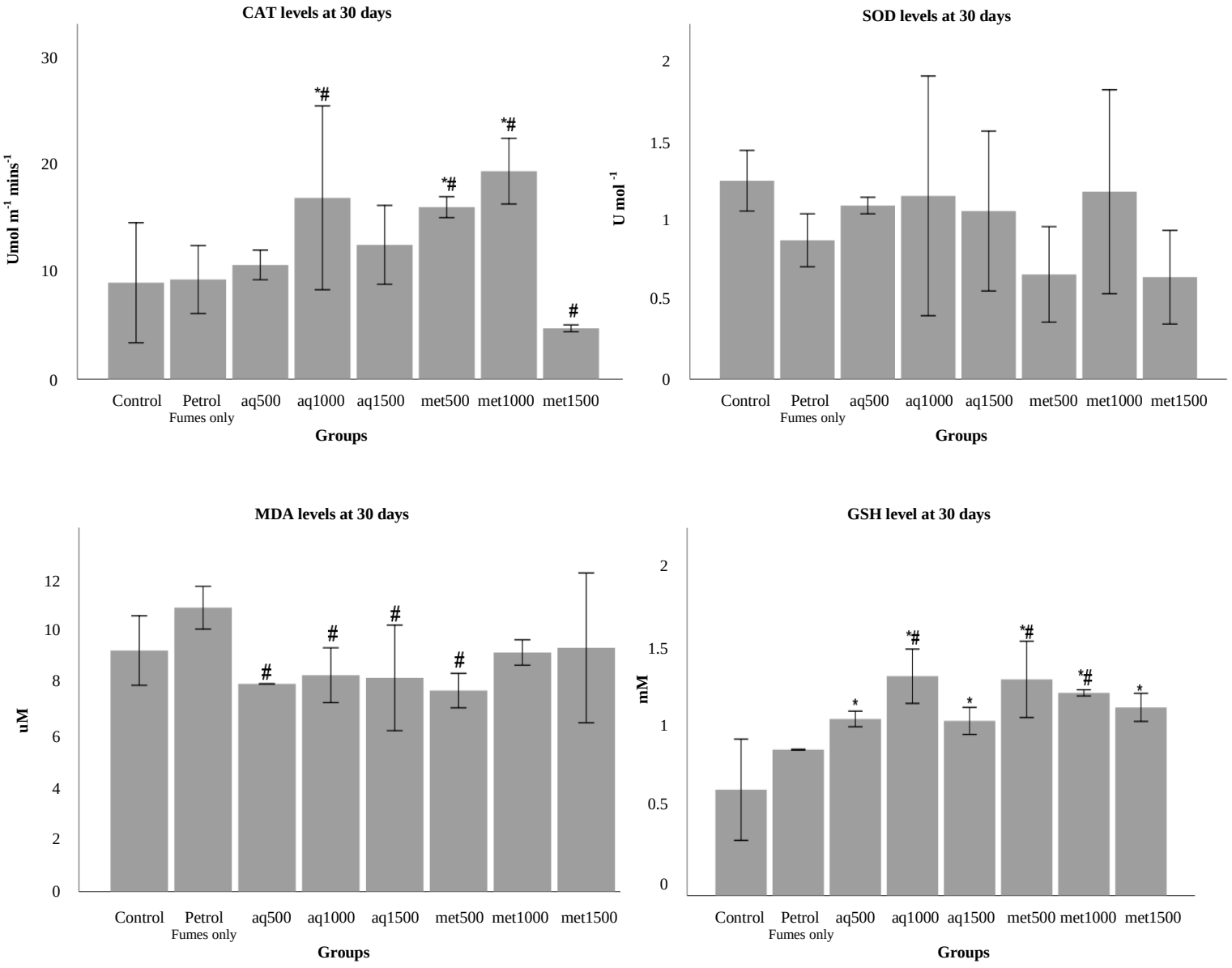

Figure 1. CAT, SOD, MDA, and GSH levels in the lung of rats following treatment with *Camellia sinensis* aqueous drink and methanol extract and exposure to petrol fumes for 30 days. Each bar represents the mean ± SD. *P<0.05 vs Control; #P<0.05 vs exposed (petrol fumes only).

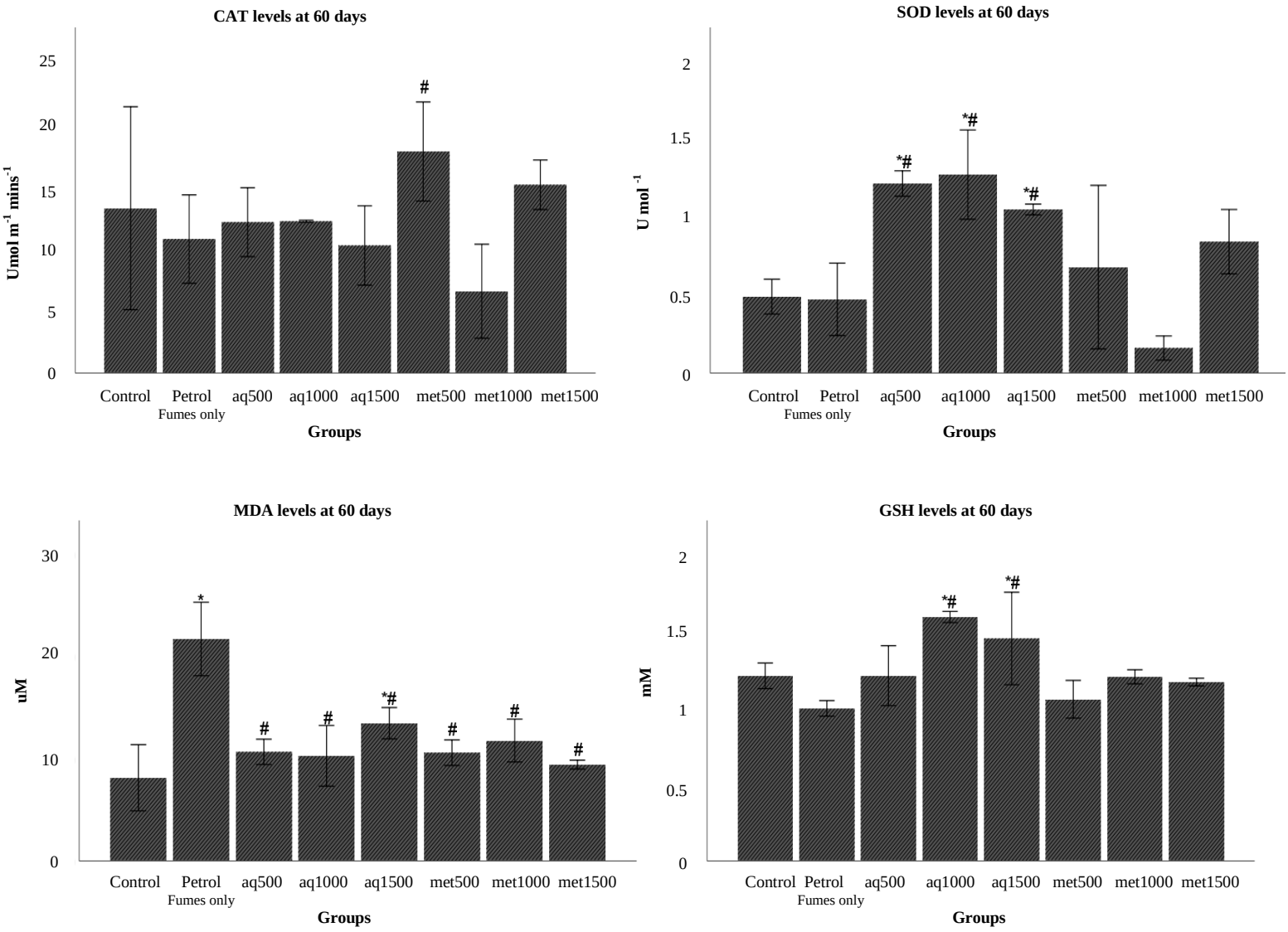

Figure 2 CAT, SOD, MDA, and GSH levels in the lung of rts following treatment with *Camellia sinensis* aqueous drink and methanol extract and exposure to petrol fumes for 60 days. Each bar represents the mean ± SD. *P<0.05 vs Control; #P<0.05 vs exposed (petrol fumes only).

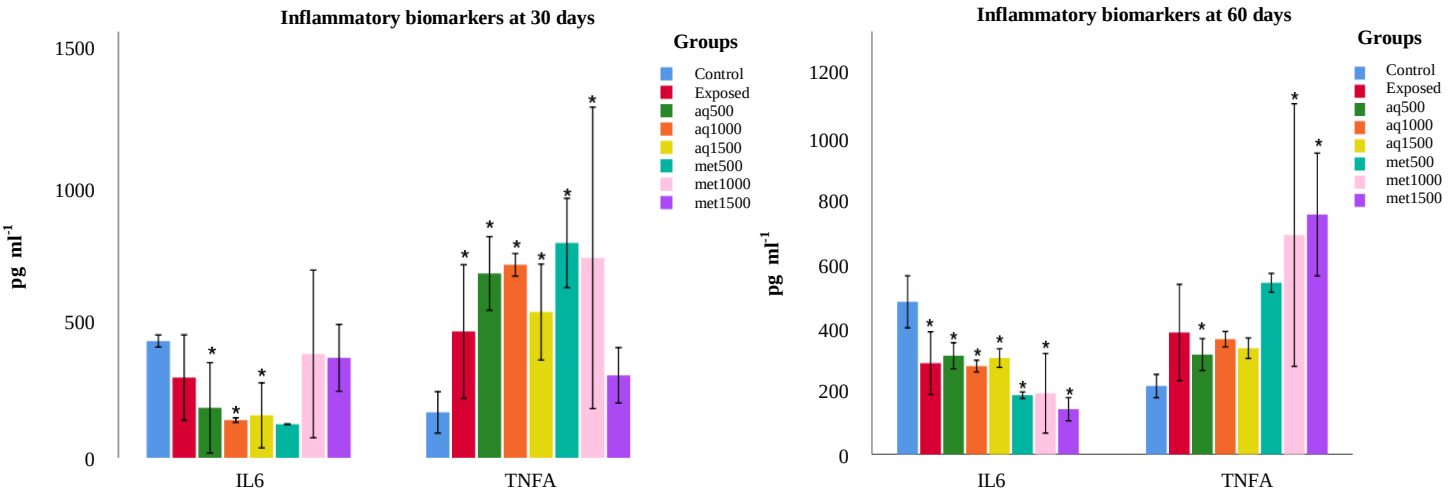

Figure 3. IL6 and TNF-α levels in the lung of rats following treatment with *Camellia sinensis* aqueous drink and methanol extract and exposure to petrol fumes for 30 and 60 days. Each bar represents the mean ± SD. *P<.05 vs Control; #P<.05 vs exposed (petrol fumes only). IL6, interleukin-6; TNF-α, tumor necrosis factor-alpha.

Time course effect on biomarkers of oxidative stress

In Table 1, CAT levels were significantly reduced (P<0.05) at 60 days when compared to 30 days in the met 1000 mg kg $^{-1}$ b.w group and were significantly increased (P<0.05) in the met 1500 mg kg $^{-1}$ b.w group. MDA levels were significantly increased (P<0.05) in petrol fumes only, aqu 500, aqu 1000, aq 1500 and met 500 mg kg $^{-1}$ b.w groups at 60 days when compared to 30 days. GSH levels were significantly increased (P<0.05) in control, aqu 1000, and aqu 1500 mg kg $^{-1}$ b.w groups at 60 days when compared to 30 days. SOD levels were significantly increased (P<0.05) in control and met 1000 mg kg $^{-1}$ b.w groups at 60 days when compared to 30 days.

Table 1. CAT, SOD, MDA, and GSH levels in the lung of rats following treatment with *Camellia sinensis* aqueous drink and methanol extract and exposure to petrol fumes for 30 and 60 days. Each value represents the mean ± SD. *P<0.05 when compared to the corresponding value at 30 days. CAT, catalase; SOD, superoxide dismutase; MDA, malondialdehyde; GSH, glutathione.

GROUPS	CAT		MDA		GSH		SOD	
	30 (Days)	60 (Days)	30 (Days)	60 (Days)	30 (Days)	60 (Days)	30 (Days)	60 (Days)
1(control)	9.05±5.63	13.25±8.15	9.20±1.33	8.14±3.24	0.64±0.31	1.21±0.08*	1.24 ± 0.19	0.49 ± 0.11*
2(petrol fumes only)	9.35±3.19	10.77±3.55	10.83±0.82	21.72±3.60*	0.88±0.00	0.99± 0.05	0.87 ± 0.17	0.47 ± 0.23
3(500 mg kg $^{-1}$ bw aqu drink +petrolfumes)	10.72±1.39	12.13±2.77	7.92 ± 0.00	10.68±1.24*	1.07±0.05	1.21± 0.20	1.09 ± 0.05	1.22 ± 0.08
4(1000 mg kg $^{-1}$ bw aqu drink +petrolfumes)	17.02±8.63	12.20±0.09	8.26 ± 1.05	10.29±2.97*	1.33±0.16	1.59±0.04*	1.15 ± 0.75	1.28 ± 0.29
5(1500 mg kg $^{-1}$ bw aqu drink + petrol fumes)	12.61±3.70	10.25±3.19	8.15 ± 2.02	13.48±1.53*	1.06±0.08	1.45±0.30*	1.05 ± 0.50	1.05 ± 0.03
6(500mg kg $^{-1}$ bw methanol extract + petrol fumes)	16.14±0.99	17.81±3.98	7.67 ± 0.66	10.60±1.25*	1.31±0.23	1.06±0.12	0.67 ± 0.30	0.68 ± 0.53
7(1000mg kg $^{-1}$ bw methanol extract + petrol fumes)	19.52±3.08	6.56± 3.78*	9.12 ± 0.48	11.78± 2.10	1.23±0.02	1.20±0.05	1.17 ± 0.64	0.16 ± 0.08*
8(1500 mg kg $^{-1}$ bw methanol extract + petrol fumes)	4.77 ± 0.32	15.13±1.99*	9.30 ± 2.86	9.43 ± 0.43	1.14±0.08	1.17±0.03	0.64 ± 0.29	0.84 ± 0.21

Time course effect on biomarkers of inflammation

In Table 2, IL6 levels were significantly increased ($P<0.05$) in aqu 1000, met 500, and met 1500 mg kg $^{-1}$ b.w groups at 60 days when compared to 30 days. TNF-α levels were significantly reduced ($P<0.05$) in aqu 500, aqu 1000 mg kg $^{-1}$ b.w groups and significantly increased ($P<0.05$) in met 1500 mg kg $^{-1}$ b.w group at 60 days when compared to 30 days.

Table 2. IL6 and TNFA levels in the lung of rats following treatment with *Camellia sinensis* aqueous drink and methanol extract and exposure to petrol fumes for 30 and 60 days. Each value represents the mean ± SD. *P<.05 when compared to the corresponding value at 30 days. IL6, interleukin-6; TNF-**α**, tumor necrosis factor-alpha.

GROUPS	IL-6		TNF-α	
	30 DAYS	60 DAYS	30 DAYS	60 DAYS
1(control)	430.26 ± 22.09	480.88 ± 82.07	170.98 ± 75.42	215.41 ± 36.76
2(petrol fumes only)	297.49 ± 155.39	287.53 ± 98.95	464.92 ± 242.85	384.10 ± 151.60
3(500 mg kg $^{-1}$ bw aqu drink +petrolfumes)	187.50 ± 164.35	310.89 ± 40.99	676.08 ± 133.91	619.92± 331.83*
4(1000 mg kg $^{-1}$ bw aqu drink +petrolfumes)	142.62 ± 8.28	278.13 ± 18.48*	707.54 ± 41.07	363.19 ± 24.14*
5(1500 mg kg $^{-1}$ bw aqu drink + petrol fumes)	159.96 ±118.35	303.52 ± 29.32	535.76 ± 174.40	334.46 ± 32.32
6(500mg kg $^{-1}$ bw methanol extract + petrol fumes)	127.79 ± 1.53	186.54 ±10.21*	786.97 ± 162.45	540.76 ± 29.59
7(1000mg kg^{-1} bw methanol extract + petrol fumes)	383.34 ± 304.84	192.44 ±125.37	732.85 ± 548.21	691.29 ± 413.79
8(1500 mg kg $^{-1}$ bw methanol extract + petrol fumes)	369.13 ± 121.67	142.35 ± 36.41*	305.66 ±100.75	755.95 ± 193.36*

Histopathological alterations in lungs of petroleum fumes exposed - rats treated with Camellia sinensis aqueous drink and methanol extract

Sections of the lung tissue show the different morphologic effects of different exposure and treatment on the intra-alveolar bronchioles (blue), vascular changes (red), and the alveolar interstitium (yellow). At 30 days, in both the aqueous drink and methanol extract treated groups (Figures 4 and 5), control (A) showed normal bronchiole whereas test control (B) showed distended vessels with congestion (red), widespread interstitial inflammation (yellow), congestion and hemorrhage, peribronchial lymphocytic aggregate (blue). C (500mg kg $^{-1}$ b.w) and D (1000 mg kg $^{-1}$ b.w) showed a slightly recovering state when compared to test control. E (1500 mg kg $^{-1}$ b.w) showed peribronchial lymphocytic infiltrate and edema (blue), distended vessel (red), focal interstitial inflammation edema, and hemorrhage (yellow). At 60 days, control (A) showed normal bronchiole whereas test control (B) showed a critical case of peribronchial inflammation with lymphocytic aggregate (blue) distended vessels with congestion (red), focal interstitial inflammation, edema, hemorrhage (yellow). For the aqueous drink, C (500 mg kg $^{-1}$ b.w), D (1000 mg kg $^{-1}$ b.w) and E (1500 mg kg $^{-1}$ b.w) shows a better recovery state when compared to test control (Figure 6). For the methanol extract, C (500 mg kg $^{-1}$ b.w) shows a recovery state, D (1000 mg kg $^{-1}$ b.w) showed peribronchial lymphocytic infiltrate and edema (blue), distended vessel (red), focal interstitial inflammation edema and hemorrhage (yellow) which may be due to alcoholic effect and E (1500 mg kg $^{-1}$ b.w) showed peribronchial lymphocytic aggregate (blue), distended vessels with congestion (red) and mild interstitial inflammation (yellow) (Figure 7).

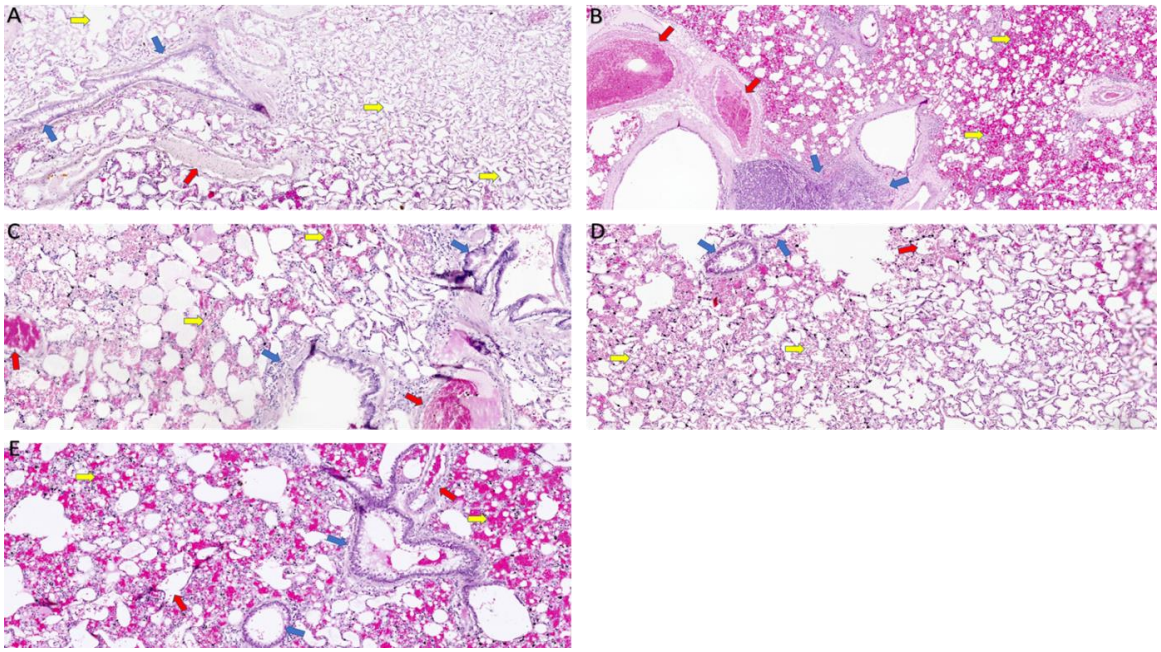

Figure 4 (a) Photomicrograph of G1 (control) at 30 days (b) Photomicrograph of G2 (exposed to petrol fumes only) at 30 days (c) Photomicrograph of G3 (500 mg kg $^{-1}$ b.w aqueous extract + petrol fumes) at 30 days (d) Photomicrograph of G4 (1000mg kg $^{-1}$ b.w aqueous extract + petrol fumes) at 30 days (e) Photomicrograph of G5 (1500mg kg $^{-1}$ b.w aqueous extract + petrol fumes) at 30 days showing intra-alveolar bronchioles(blue), vascular changes(red) and the alveolar interstitium(yellow). All photomicrographs were stained with hematoxylin and eosin (H&E) stain and viewed at x100 magnification.

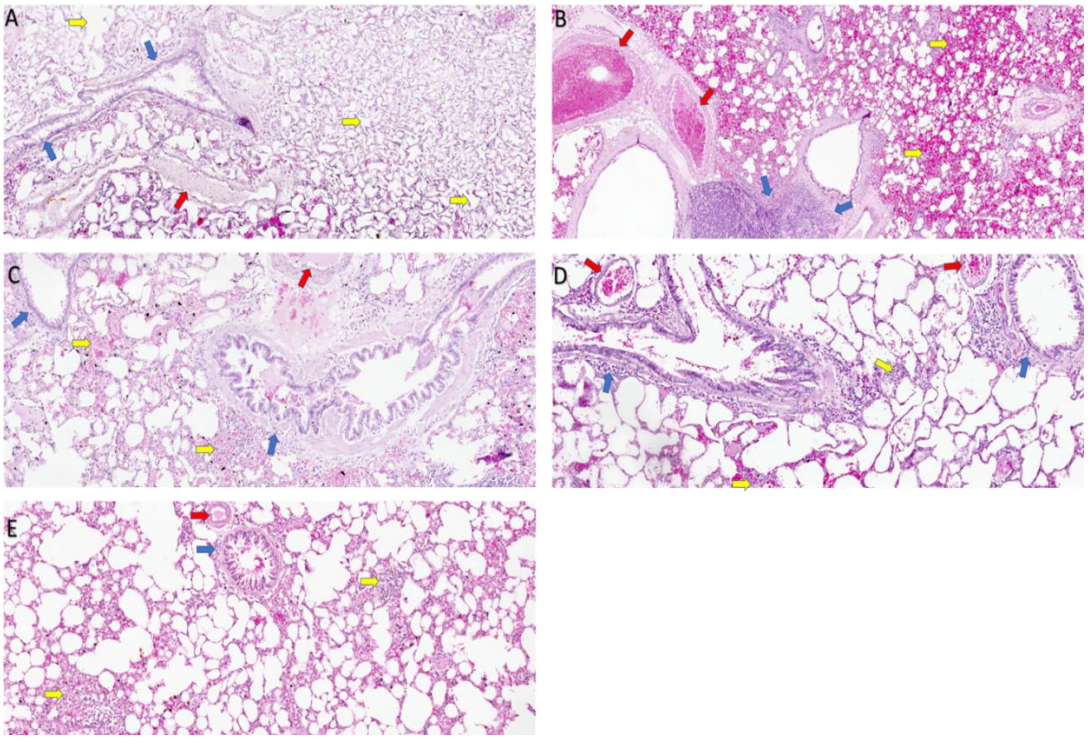

Figure 5. (a) Photomicrograph of G1 (control) at 30 days (b) Photomicrograph of G2 (exposed to petrol fumes only) at 30 days (c) Photomicrograph of G3 (500mg kg $^{-1}$ b.w methanol extract + petrol fumes) at 30 days (d) Photomicrograph of G4 (1000mg kg $^{-1}$ b.w methanol extract + petrol fumes) at 30 days (e) Photomicrograph of G5 (1500mg kg $^{-1}$ b.w methanol extract + petrol fumes) at 30 days showing intra-alveolar bronchioles (blue), vascular changes (red) and the alveolar interstitium (yellow). All photomicrographs were stained with hematoxylin and eosin (H&E) stain and viewed at x100 magnification.

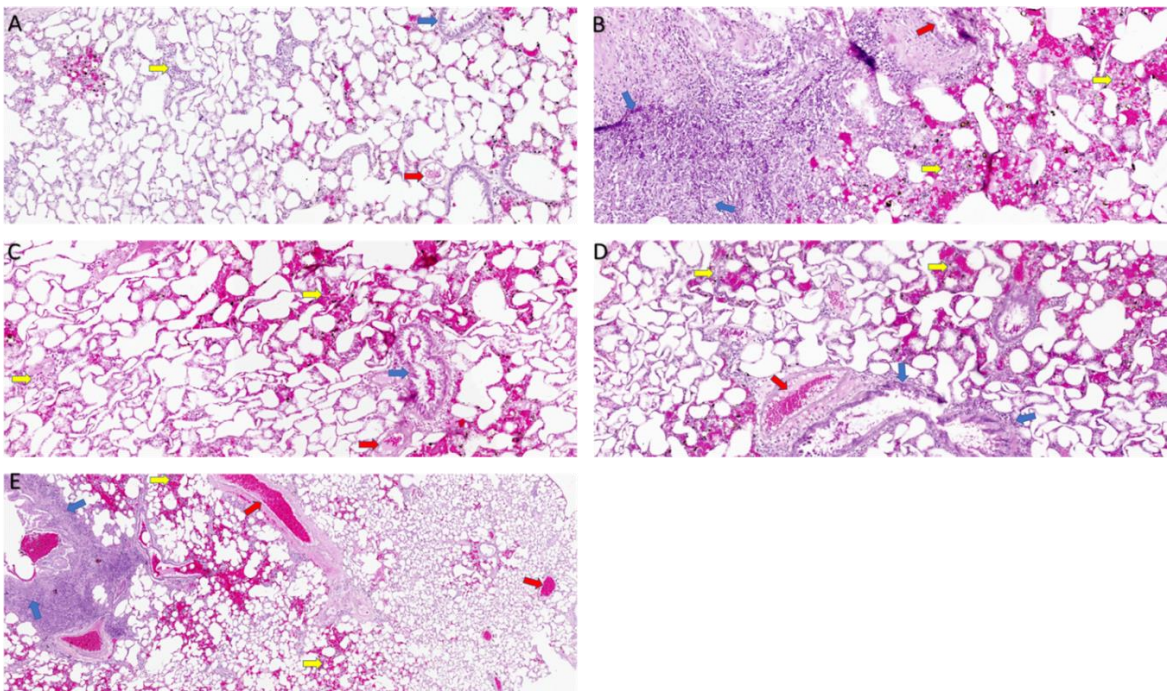

Figure 6 (a) Photomicrograph of G1 (control) at 60 days (b) Photomicrograph of G2 (exposed to petrol fumes only) at 60 days (c) Photomicrograph of G3 (500 mg kg $^{-1}$ b.w aqueous extract + petrol fumes) at 60 days (d) Photomicrograph of G4 (1000 mg kg $^{-1}$ b.w aqueous extract + petrol fumes) at 60 days (e) Photomicrograph of G5 (1500 mg kg $^{-1}$ b.w aqueous extract + petrol fumes) at 60 days showing intra-alveolar bronchioles (blue), vascular changes (red) and the alveolar interstitium (yellow). All photomicrographs were stained with hematoxylin and eosin (H&E) stain and viewed at x100 magnification.

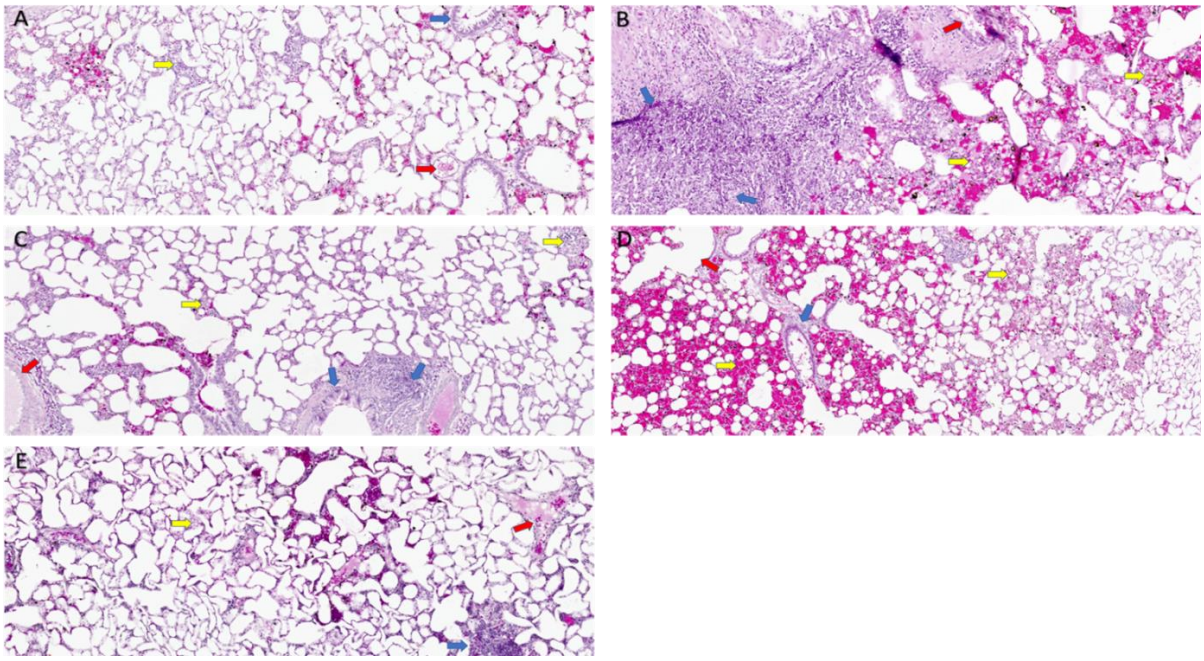

Figure 7. (a) Photomicrograph of G1 (control) at 60 days (b) Photomicrograph of G2 (exposed to petrol fumes only) at 60 days (c) Photomicrograph of G3 (500 mg kg $^{-1}$ b.w methanol extract + petrol fumes) at 60 days (d) Photomicrograph of G4 (1000 mg kg $^{-1}$ b.w methanol extract + petrol fumes) at 60 days (e) Photomicrograph of G5 (1500 mg kg $^{-1}$ b.w methanol extract + petrol fumes) at 60 days showing intra-alveolar bronchioles (blue), vascular changes (red) and the alveolar interstitium (yellow). All photomicrographs were stained with hematoxylin and eosin (H&E) stain and viewed at x100 magnification.

DISCUSSION

Oxidative stress results from the accumulation of reactive oxygen species following an increase in the production of these species and a decline in the ability of antioxidants to scavenge them. The antioxidants are either enzymes such as superoxide dismutase (SOD) and catalase (CAT) or non-enzymes such as glutathione (GSH). Hence measuring the levels of these antioxidants after exposure to a toxicant is a way to determine the

extent of oxidative damage to the cell. In this study, the exposure of experimental animals to petrol fumes triggered oxidative stress. This may be due to the presence of the volatile organic compounds: benzene, toluene, ethylbenzene, and xylene. The antioxidant enzymes SOD and CAT serve as a defense in eradicating free radicals produced by oxidative stress. SOD reduces the superoxide anion (O^{2-}) to hydrogen peroxide (H_2O_2) and oxygen (O^2) whereas CAT works with the non-enzymatic antioxidant glutathione to reduce H_2O_2 to water (H_2O) and oxygen. The increased MDA level and decreased SOD, CAT, and GSH in the petrol fumes-only group suggests that exposure to petrol fumes led to oxidative stress in the lungs by consuming the enzymatic and non-enzymatic antioxidants. The alterations in pro-inflammatory cytokines in this study indicate that there was oxidative stress and inflammatory responses mediated tissue injury in the lung of rats in the petrol fumes-only group. This was further confirmed in the histopathological result. This is in agreement with various studies where petrol fumes exposure resulted in decreases in CAT, SOD and GSH, and an increase in MDA [23, 29 - 31]. Pre-treatment of albino rats with different doses of *Camellia sinensis* aqueous drink or methanol extract increased CAT, SOD, and GSH levels and decreased MDA levels in the lungs when compared to the petrol fumes-only group. Therefore petrol fumes may be a pro-oxidant that leads to oxidative stress and causes oxidative damage whereas *Camellia sinensis* showed some antioxidant properties as well as reduced extent of cellular damage due to oxidative stress. Phytochemicals found in *Camellia sinensis* leaves obtained from the same location as the one used in this study include flavonoids, saponins, alkaloids, steroids, triterpenes, cardiac glycosides, and tannins [14] with flavonoids being the most abundant. The major polyphenol constituents of the tea plant are flavan3-ols otherwise known as catechins such as epigallocatechin (EGC), epigallocatechin-3-gallate (EGCG), epicatechin (EC), epicatechin-3-gallate (ECG) with EGCG as the most abundant and active compound [32]. Flavonoids have high antioxidant activity as they possess a hydroxyl (OH-) group which acts as a hydrogen donor and also can donate electrons via resonance from their conjugated double bonds to reactive oxygen and nitrogen species thereby inhibiting the formation of free radicals [33]. Flavonoids also possess anti-inflammatory properties [34]. Other phenolic compounds are known to be metal chelators which reduce free radicals formation [35]. They also inhibit cytochrome P450 isoforms such as xanthine oxidase, lipo-oxygenases, and cyclooxygenase which are important in the formation of free radicals [36]. Catechins especially produce phase II detoxification enzymes such as Glutathione-S-transferase (GST) which in turn leads to an increase in GSH levels [37].

Camellia sinensis is also rich in vitamins C and E which possess antioxidant properties. Vitamin C is relevant in breaking the chain and neutralizing OH-, superoxide, and H_2O_2 radicals, and reducing lipid peroxidation [38]. Vitamin E neutralizes singlet oxygen molecules thus preserving the cell membrane [39]. An increase in enzymatic and non-enzymatic antioxidants as seen in *Camellia sinensis* treated groups means a decrease in free radicals.

Tumour necrosis factor α (TNF-α) is important to produce cytokines in the cell [40] as well as regulating the production of cytokines. In this study, TNF-α increased in the petrol fumes-only group and all treated groups when compared to the control at 30 days whereas at 60 days the levels reduced in all aqueous drink-treated groups when compared to petrol fumes only group. Increases in TNF-α levels may be due to increased nitric oxide (NO) level that forms peroxynitrite when superoxide anions are present [41,42]. At 30 days, IL6 levels decreased in all aqueous drink-treated groups and the met 500 $mg\ kg^{-1}$ b.w group but increased in the higher doses of methanol extract (1000$mg\ kg^{-1}$ and 1500$mg\ kg^{-1}$). This may be due to an increased alcohol effect associated with an increased dose of methanol extract whereas, at 60days, all treated groups reduced significantly when compared to the control. At 60 days, the increase in IL6 seen in the aqueous drink-treated groups when compared to the petrol fumes-only group may be due to liver regeneration since IL6 is essential for liver regeneration [43]. The antioxidant defense system protects the cells and tissues from the harmful effects of free radicals [44, 45] thus if the antioxidants are depleted, cell and tissue integrity are lost. This could explain the edema, peribronchial lymphocytic infiltrate, and distended vessel of lung tissue seen in the histopathological study of the

lungs after exposure to petrol fumes. A study by [46] reported that exposure to petroleum hydrocarbons impairs type II pneumocytes which leads to decreased production of surfactant and alveolar collapse, ventilation‐perfusion mismatch as well as hypoxemia. This consequently leads to interstitial inflammation, hemorrhagic alveolitis, intra‐alveolar hemorrhage and edema, bronchial necrosis, hyperemia, and vascular necrosis [46]. These histological changes may be due to a combination of mechanisms involving the inhibition of enzymatic and non-enzymatic antioxidants, increased generation of reactive oxygen and nitrogen species, and inflammatory responses.

GSH plays an important role in neutralizing oxidative stress-induced injury in the epithelial cells of the lungs and abating the pro-inflammatory processes [47]. The findings from this study reveal that inhalation of petrol fumes is a risk factor in pulmonary pathologies however intake of *Camellia sinensis* provides a protective effect against oxidative stress and inflammation-induced pathologies. The benefits were more with the *Camellia sinensis* aqueous drink and low dosage (500mg kg^{-1} b.w) of methanol extract. Caution should however be applied when using higher doses (1000mg kg^{-1}, 1500 mg kg^{-1} b.w) of methanol extract.

ACKNOWLEDGEMENTS

The authors acknowledge the management of Mambilla Beverages Nigeria Limited for the supply of *Camellia sinensis* plant from their farm as well as Mr Bulus Votapwa for his contribution in ensuring proper collection and drying of samples.

ETHICAL CONSIDERATION

This work was approved by the University of Port Harcourt Ethics Committee after a critical review of the methodologies. This approval with number UPH/CEREMAD/REC/MM72/092 was gotten before the research commenced.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1.Kirchstetter T.W., Singer B.C., Harley R.A., Kendall G.R., Hesson J.M., 1999. Impact of California reformulated gasoline on motor vehicle emissions. 2. Volatile organic compound speciation and reactivity. Environmental Science & Technology. 33(2), 329-36.

2. d' Azevedo P.A., Tannhauser M., Tannhauser S.L., Barros H.M., 1996. Hematological alterations in rats from xylene and benzene. Veterinary and Human Toxicology. 38(5), 340-4.

3. Smith J.H., Mallett A.K., Priston R.A., Brantom P.G., Worrell N.R., Sexsmith C., Simpson B.J, 1996. Ninety-day feeding study in Fischer-344 rats of highly refined petroleum-derived food-grade white oils and waxes. Toxicologic Pathology. 24(2), 214-30.

4. Raabe G.K., Wong O., 1996. Leukemia mortality by cell type in petroleum workers with potential exposure to benzene. Environmental Health Perspectives. 104(suppl 6), 1381-92.

5. Ross D., 1996. Metabolic basis of benzene toxicity. European Journal of Haematology. 57(S60), 111-118.

6. Rothman N., Li G.L., Dosemeci M., Bechtold W.E., Marti G.E., Wang Y.Z., Linet M., Xi L.Q., Lu W., Smith M.T., Titenko- Holland N.,1996. Hematotoxocity among Chinese workers heavily exposed to benzene. American Journal of Industrial Medicine. 29(3), 236-46.

7. Abdrabouh A.E., 2019. Liver disorders related to exposure to gasoline fumes in male rats and role of fenugreek seed supplementation. Environmental Science and Pollution Research. 26(9), 8949–57.

8. Owagboriaye F.O., Dedeke G.A., Aladesida A.A., Bamidele J.A., Olooto W.E., 2018. Assessment of the effect of gasoline fume on stress hormones, antioxidant status and lipid peroxidation in albino rat. Journal of king Saud University-science. 30(3), 393-9.

9. Imo C., Uhegbu F.O., Ifeanacho N.G., 2015. Effect of exposure to inhalation of selected petroleum products on liver function of male albino rats: A comparative study. IOSR Journal of Environmental Science, Toxicology and Food Technology. 19, 2319-99.

10. Ogunneye A.L., Omoboyowa D.A., Sonibare A.L., Adebusuyi A.J., Faniran T.P., 2014. Hepatotoxic and nephrotoxic effects of petroleum fumes on petrol

attendants in Ibadan, Nigeria. Nigerian Journal of Basic and Applied Sciences. 22(3-4), 57-62.

11. Ahmed F.E., 2001. Toxicology and human health effects following exposure to oxygenated or reformulated gasoline. Toxicology letters. 123(2-3), 89-113.

12. Ezzat A.R., Riad N.H., Fares N.H., Hegazy H.G., Alrefadi M.A., 2011. Gasoline inhalation induces perturbation in the rat lung antioxidant defense system and tissue structure. IJESE. 1, 1-4.

13. Tardif R., Liu L., Raizenne M., 2004. Exhaled ethanol and acetaldehyde in human subjects exposed to low levels of ethanol. Inhalation Toxicology. 16(4), 203-7.

14. Ukwubile C.A., Malgwi T.S., Dibal M.Y., Bababe A.B., 2020. Phytochemical Composition and Toxicity Evaluation of *Camellia Sinensis* (L.) O. Kuntze (Theaceae) (Green Tea) Leaves Collected from Mambila Beverages Ltd Nigeria. International Journal of Medicinal Plants and Natural Products. 6(2), 7-13.

15. Varadharajan V., Janarthanan U.K., Krishnamurthy V., 2012. Physicochemical, phytochemical screening and profiling of secondary metabolites of Annona squamosa leaf extract. World Journal of Pharmaceutical Research. 1(4), 1143-64.

16. Chan E.W., Lim Y.Y., Chew Y.L., 2007. Antioxidant activity of Camellia sinensis leaves and tea from a lowland plantation in Malaysia. Food Chemistry. 102(4), 1214-22.

17. Fuggetta M.P., Lanzilli G., Tricarico M., Cottarelli A., Falchetti R., Ravagnan G., Bonmassar E., 2006. Effect of resveratrol on proliferation and telomerase activity of human colon cancer cells in vitro. Journal of Experimental and Clinical Cancer Research. 25(2), 189.

18. Kim Y., Goodner K.L., Park J.D., Choi J., Talcott S.T., 2011. Changes in antioxidant phytochemicals and volatile composition of Camellia sinensis by oxidation during tea fermentation. Food Chemistry. 129(4), 1331-42.

19. Dey A., Chattopadhyay S., Jana S., Giri M.K., Khatun S., Dash M., Perveen H., Maity M., 2018. Restoration of uterine redox-balance by methanolic extract of Camellia sinensis in arsenicated rats. Acta Biologica Szegediensis. 62(1), 7-15.

20. Dzotam J.K., Kuete V., 2017. Antibacterial and antibiotic-modifying activity of methanol extracts from six Cameroonian food plants against multidrug-resistant enteric bacteria. BioMed Research International. 2017,1-19.

21.Thitimuta S., Pithayanukul P., Nithitanakool S., Bavovada R., Leanpolchareanchai J., Saparpakorn P.,2017. Camellia sinensis L. Extract and its potential beneficial effects in antioxidant, anti-inflammatory, anti-hepatotoxic, and anti-tyrosinase activities. Molecules. 22(3), 401.

22. Uboh F.E., Ebong P.E., Eka O.U., Eyong E.U., Akpanabiatu M.I., 2005. Effect of inhalation exposure to kerosene and petrol-fumes on some anaemia-diagnostic indices in rats. Global Journal of Environmental Sciences. 4(1), 59-63.

23. Owagboriaye F.O., Dedeke G.A., Ashidi J.S., Aladesida A.A., Olooto W.E., 2018. Effect of gasoline fumes on reproductive function in male albino rats. Environmental Science and Pollution Research. 25(5), 4309-19.

24. Akpanabiatu M.I., Igiri A.O., Eyong E.U., Eteng M.U., 2003. Biochemical and histological effects of Eleophorbia drupifera leaf extract in Wistar albino rats. Pharmaceutical Biology. 41(2), 96-9.

25. Fossati P., Prencipe L., Berti G., 1980. Use of 3, 5-dichloro- 2 - hydroxybenzenesulfonic acid / 4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clinical Chemistry. 26(2), 227-31.

26. Marklund S., Marklund G., 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European journal of biochemistry. 47(3), 469-74.

27. Ellman G.L, 1959. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics. 82(1), 70-7.

28. Wright J.R., Colby H.D., Miles P.R., 1981. Cytosolic factors which affect microsomal lipid peroxidation in lung and liver. Archives of Biochemistry and Biophysics. 206(2), 296-304.

29. Owumi S.E., Elebiyo T.C., Oladimeji B.N, 2021. Oxido inflammatory responses and histological alterations in rat lungs exposed to petroleum product fumes. Environmental Toxicology. 36(1), 132-43.

30. Odewabi A.O., Ogundahunsi O.A., Oyalowo M., 2014. Effect of exposure to petroleum fumes on plasma

antioxidant defense system in petrol attendants. British Journal of Pharmacology and Toxicology. 5(2), 83-8

31. Azeez O.M., Akhigbe R.E., Anigbogu C.N., 2012. Exposure to petroleum hydrocarbon: implications in lung lipid peroxidation and antioxidant defense system in rat. Toxicology International. 19(3), 306.

32. Hugel H.M., Jackson N., 2012. Redox chemistry of green tea polyphenols: therapeutic benefits in neurodegenerative diseases. Mini Reviews in Medicinal Chemistry. 12(5), 380.

33. Santos-Buelga C., Feliciano A.S., 2017. Flavonoids: from structure to health issues. Molecules. 22(3), 477.

34. Ramesh P., Jagadeesan R., Sekaran S., Dhanasekaran A., Vimalraj S., 2021. Flavonoids: Classification, Function, and Molecular Mechanisms Involved in Bone Remodelling. Frontiers in Endocrinology. 12. , 779638.

35. Croft K.D, 1998. The chemistry and biological effects of flavonoids and phenolic acids a. Annals of the New York Academy of Sciences. 854(1), 435-42.

36. Parr A.J., Bolwell G.P., 2000. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. Journal of the Science of Food and Agriculture. 80(7), 985-1012.

37. Bernatoniene J., Kopustinskiene D.M., 2018. The role of catechins in cellular responses to oxidative stress. Molecules. 23(4), 965.

38. Lampiao F., 2012. Free radicals generation in an in vitro fertilization setting and how to minimize them. World Journal of Obstetrics and Gynecology. 1(3), 29-34.

39. Rahim S.M., Taha E.M., Mubark Z.M., Aziz S.S., Simon K.D., Mazlan A.G., 2013. Protective effect of Cymbopogon citratus on hydrogen peroxide-induced oxidative stress in the reproductive system of male rats. Systems Biology in Reproductive Medicine. 59(6), 329-36.

40. Parameswaran N., Patial S., 2010. Tumor necrosis factor-α signaling in macrophages. Critical Reviews™ in Eukaryotic Gene Expression. 20(2), 87–103.

41. Cavicchi M., Gibbs L., Whittle B.J., 2000. Inhibition of inducible nitric oxide synthase in the human intestinal epithelial cell line, DLD-1, by the inducers of heme oxygenase 1, bismuth salts, heme, and nitric oxide donors. Gut. 47(6), 771-8.

42. Nussler A.K., Billiar T.R., Liu Z.Z., Morris Jr S.M., 1994.Coinduction of nitric oxide synthase and argininosuccinate synthetase in a murine macrophage cell line. Implications for regulation of nitric oxide production. Journal of Biological Chemistry. 269(2), 1257-61.

43. Schmidt-Arras D., Rose-John S., 2016. IL-6 pathway in the liver: from physiopathology to therapy. Journal of Hepatology. 64(6), 1403-15.

44. Otitoju O., Onwurah I.N., 2007. Glutathione S-transferase (GST) activity as a biomarker in ecological risk assessment of pesticide contaminated environment. African Journal of Biotechnology. 6(12), 1455-1459 .

45. Amer M., Metwalli M., Abu El Magd Y., 2002. Skin diseases and enzymatic antioxidant activity among workers exposed to pesticides. EMHJ-Eastern Mediterranean Health Journal. 8 (2-3), 363-373.

46. Prasad R., Karmakar S., Sodhi R., Karmakar S., 2011. Bilateral hemorrhagic pleural effusion due to kerosene aspiration. Lung India: Official Organ of Indian Chest Society. 28(2), 130.

47.Rahman I., MacNee W., 2000. Oxidative stress and regulation of glutathione in lung inflammation. European Respiratory Journal. 16(3), 534-554.