The Effects of *Cymbopogon Citratus* Extract on Hydrocarbon-Induced Oxidative Stress and Haematotoxicity in Albino Rats

[1*]Ehikioya, Elizabeth Omoso & [1]Aghatise, Kevin E.
[1]Department of Medical Laboratory Science,
School of Basic Medical Sciences,
College of Health Sciences,
Igbinedion University, Okada, Nigeria.
*omoso.ehikioya@iuokada.edu.ng

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Abstract

Background*: The therapeutic use of medicinal plants and plant products to treat ailments and diseases is very popular in developing countries including Nigeria. Hence the need to scientifically analyse the ameliorating effects of these plants. Cymbopogon citratus is one of such medicinal plants, popular for its tea which is considered a healthy and refreshing beverage that is largely consumed worldwide as a substitute for other tea varieties because of its pleasant aroma and taste. Gasoline is a volatile liquid that contributes to significant environmental pollution and has been shown to be hazardous to the general population due to its ability to induce extensive adverse health effects including oxidative stress and haematotoxicity.*
Aim*: The aim of this study is to evaluate how a decoction created from lemongrass leaves (cymbopogon citratus) affects the consequences of gasoline vapour (GV) exposure.*
Methodology*: Random selection was used to pick thirty albino rats, which were then divided into six groups (n = 5 per group). Animals in group A served as unexposed controls, while animals in group B. They underwent exposure to gasoline vapour (GV) while concurrently being administered an extract derived from lemongrass leave (cymbopogon citratus). (250 mg/kg, 500 mg/kg, and 1000 mg/kg respectively) and animals in group F were exposed to GV and simultaneously received a co-administration of vitamin C (200 mg/kg) for the same duration.*
Result*: Exposure of animals to GV alone significantly ($p < 0.05$) increased malondialdehyde levels and decreased superoxide dismutase level and catalase levels. But there was no significant change in haematological parameters except for Mean Corpuscular Haemoglobin to the corresponding values in the control group. Supplementation with C. citratus leaf decoction and vitamin C reversed these GV-induced changes in oxidative stress markers in Groups C, D, E and F.*
Conclusion: *It can be seen that the decoction of Cymbopogon citratus leave and the supplementation of vitamin C exhibited a beneficial effect in mitigating oxidative stress induced by GV exposure in a rat model.*

Keywords*: Cymbopogon citratus, Oxidative Stress, haematotoxicity. Gasoline vapour*

Introduction

Gasoline is a complex mixture of hydrocarbons and additives and is primarily used in combustion engines as a motor fuel. It is also used as a diluent for paints and industrial solvents. Gasoline contains several compounds that are toxic to humans, such as benzene, toluene, ethylbenzene, and xylene (BTEX) as well as other additives (Ekpenyong and Asuquo, 2017).

Gasoline is obtained through the fractional distillation process of petroleum, and it has 5–10 carbon atoms. However, the relative concentrations of the components depend on the crude oil source and the refinery process. The volatile fractions of gasoline are released into the atmosphere at every stage of the production and marketing chains, thereby exposing workers to their potential adverse effects, including haematotoxicity (Yoon *et al.*, 2001; Udonwa *et al.*, 2009).

Several previous studies (Sahb, 2011; Tunsaringkarn *et al.*, 2013) have established a close association between exposure to gasoline compounds and haematotoxicity in humans. Potential mechanisms have been postulated for the haematotoxic effect of gasoline, including interference with cell cycle regulation and the expression of DNA damage/repair genes, oxidative stress genes, growth factor related genes, and haematopoiesis-related genes, as well as bone marrow toxicity, haemolysis, and increased formation of methaemoglobin (Udonwa *et al*, 2009). Central to these mechanisms is the induction of oxidative stress by gasoline component metabolites.

While multiple synthetic antioxidants like Vitamin A, C, and E have been demonstrated to alleviate gasoline-induced hematotocity to ameliorate gasoline-induced haematotoxicity in previous research (Sedky *et al*., 2015), adequate therapeutic coverage has been limited by the failures of modern medicine to provide holistic care and the disadvantages of multiple drug therapies as well as an inability to alleviate the pleotropic effects of the gasoline component metabolites. Therefore, recently, there has been renewed interest in medicinal plants owing to increased awareness of their health benefits. Antioxidants of plant origin typically do not induce the side effects associated with the use of synthetic antioxidants, such as butylatedhydroxytoluene (BHT), which has been found to cause haemorrhaging (Ndhlala *et al.*, 2010).

Medicinal plants are rich sources of nutrients and bioactive substances with anti-inflammatory, immunity-boosting, antioxidant, anti-carcinogenic, antimicrobial, anti-genotoxic, anti-apoptotic, and erythropoiesis-boosting effects. Thus, these plants have the potential to provide holistic therapeutic coverage, and thereby improve human health and prevent acute and chronic diseases (Ekpenyong *et al*., 2015). One such plant is Lemongrass (*Cymbopogon citratus*).

C. citratus belongs to the poaceae family and, thanks to its diverse bioactive compounds *Cymbopogon citratus,* an aromatic perennial tropical plant with global presence, features long slender leaves measuring 90cm in length and 1.5cm in width. When these leaves are crushed, they emit a mild ginger-like fragrance. This plant, boasts a wide range of nutritional, therapeutic, and cosmetic uses. It has long been used as a traditional medicine for its antibacterial (Wannissorn *et al.*, 2005), anti-carcinogenic (Puatonachokchai *et al*., 2002), anti-inflammatory (Abe *et al*., 2004), antioxidant, antimicrobial (Masuda et al, 2008), and

erythropoietic-boosting effects (Ekpenyong *et al*., 2005). The antioxidant effect of C. citrates has been described, and it has been proposed as an alternative treatment for numerous diseases (Koh *et al*., 2012).

Several synthetic antioxidant vitamins, including A, C, and E, have been shown to provide protective effects against gasoline vapour induced haematotoxicity (Udonwa *et al*., 2009, Sedky *et al*., 2015). These vitamins and other bioactive antioxidants are also found in vegetables, fruits, and other plants, such as *C. citratus*. However, their protective activities against gasoline vapour induced detrimental health effects, including haematotoxicity, have not been adequately investigated.

The use of crude oil products has increased globally due to increase in population of the world. This has resulted in increased exposure to the products. This is because man has invented a lot of equipment and materials which make use of the products in order to help him better his life. In a third world country like Nigeria who depends mostly on crude oil exploration for her foreign earning, there are no serious measures being put in place by the Government to checkmate pollution of the environment by crude oil products like gasoline. Regular usage of gasoline results in immediate medical effects on the users and is regarded as an environmental pollutant and toxin that negatively impacts organs such as the kidney, lungs, and liver. Its abundance in the atmosphere is due to its volatile nature (Koori *et al*., 2006; Ita and Udofia, 2013). Exposure to gasoline can occur through oral ingestion, skin contact, or inhalation (Ita and Udofia, 2013; Mahmood *et al*., 2013). Inhaling gasoline vapour has been observed to alter blood chemistry and trigger anaemia in experimental animals by causing bone marrow hypoplasia. This effect is attributed to the presence of heavy metals in gasoline (Okoro *et al*., 2006). This study seeks to investigate the effect of *Cymbopogon citratus* on the haematological disorders and oxidative stress caused by the inhalation of gasoline vapour in rats.

Materials and Methods

Research Design

Thirty (30) Mature albino rats were randomly chosen and divided into six groups, with each group consisting of 5 rats (n = 5 per group). This random allocation helps ensure that the groups are representative and that they any observed effects or results can be attributed to the experimental treatments rather than pre-existing differences among the animals. Animals in group A were served as unexposed controls, while animals in group B were exposed to gasoline vapour (GV) alone for 28 days. Animals in groups C, D, and E were exposed to GV and co-administered *C. citratus* leaf extract (250 mg/kg, 500 mg/kg, and 1000 mg/kg, respectively), and animals in group F were exposed to GV and co-administered vitamin C for the same time period. The rats were housed in six standard animal cages. Following a period of 14 days for acclimatization, they weights were recorded. This step is essential for tracking any changes in body weight that may occur during the experiment, which can be an indicator of overall health and the impact of experimental treatments. They were allowed free access to feed and drinking water.

Study Area

The study was carried out at Animal House in Benin City, Edo state, Nigeria.

Ethical Approval

Approval for this research was sought from the Ethics and research committee Ministry of Health, Edo State, Nigeria.

Experimental Animals

This study utilized thirty (30) mature albino rats with weights ranging from 120 to 190 grams. These rats were obtained from animal house of the Department of Anatomy, University of Benin, Benin City. The rats were given a daily diet of grower mash and provided with access to drinking water as part of their care and maintenance for the study. They were housed in well-ventilated conventional cages and given a two-week period to acclimatize to their environment before the start of the experiment. This acclimatization period helps ensure that the animals adapt to their surroundings and that the experiment's results are not influenced by stress or other factors related to their housing conditions.

Collection and Preparation of Plant Materials

Fresh lemon grass leaves were gathered in Benin and identified by a plant taxonomist in the Department of Plant Biology and Biotechnology, Dr. H. A. Akinnibosun. The plant was identified as *Cymbopogon citratus* and assigned a voucher number (UBH-C451) for reference and documentation.

The leaves were rinsed, air dried, and pulverized into powder using an electric blender. The powder was soaked with 4 L of hot water in a conical flask and allowed to stand for approximately 24 hours. After filtering the solution, the filtrate was evaporated to dryness by heating in a water bath at 40°C. The final solid extract was weighed with an electric balance. The prepared extract was stored in glass bottles at 4°C and dissolved in physiological saline.

Exposure to Gasoline Vapour

In the test group, the animals were exposed to gasoline vapour (GV) obtained from FAGCOOP refuelling station University of Benin, Benin City. In contrast, the rats in the control group were housed in cage free from gasoline vapour exposure. A calibrated 100-mL beaker was used to measure 50 mL of petrol which was used to soak cotton wool. In the test groups, gasoline-soaked cotton wool was placed inside the rat cages for the duration of 1 hour each day. This exposure to gasoline vapour was conducted for a total of 28 consecutive days as part of the experimental procedure. The control group (Group A) remained unexposed to gasoline vapour throughout the 28-days period. At the end of this duration, the animals were euthanized, and blood samples were collected via cardiac puncture using a 2 mL syringe. The blood was immediately transferred.

Sample Collection

Following the exposure period, the rats were humanely euthanized by administering chloroform anaesthesia. Blood samples were collected via cardiac puncture into EDTA anticoagulant bottles, specifically for haematological analysis (Full blood count) and Lithium Heparin anticoagulant bottle for Oxidative stress analysis (SOD, CAT and MDA). Total blood count including total leucocyte and differential counts and other haematological parameters was measured using an Automated Haematology Analyser.

Measurement of malondialdehyde (MDA) (Varshney and Kale, 1990)

The simplest and most frequently used assay for lipid oxidation is the thiobarbituric acid or TBA assay was utilised for this study. Usually under strong acidic condition and heating, biological samples are reacted with TBA resulting in the formation of pink-coloured products. These products can be quantified using colorimetric or fluorometric methods as described by Draper and Hadley (1990) and Esterbauer and Cheeseman (1990). This approach is commonly used to assess the presence and quantity of certain compounds, such as malondialdehyde (MDA), which can be indicative of oxidative stress or lipid peroxidation in biological samples.

Measurement of Superoxide Dismutase (Misra and Fridovich,1972).

Superoxide dismutases (SODs) are a group of enzymes that facilitate the dismutation of superoxide radicals into oxygen and hydrogen peroxide.

In this method, adrenaline auto-oxidizes rapidly in aqueous solution to adrenochrome, whose concentration was determined at 420 nm using a spectrophotometer. The auto-oxidation of adrenaline depends on the presence of superoxide anions. The enzyme SOD inhibits the auto-oxidation of adrenaline by catalysing the breakdown of superoxide anion. The degree of inhibition is thus a reflection of the activity of SOD, and is typically determined in terms of one unit of the enzyme activity.

Full Blood Count

Full blood counts were done on the Mythic 18 fully automated haematology bench-top analyser using impedance technology for a complete blood count (CBC) and a 3-part white blood cell (WBC) differential. Samples were examined for the following parameters: total white blood cell count (WBC) and 3-part automated differential count, red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and platelet count (PLT).

Statistical Analysis

Data examination through mathematical methods was performed using the Statistical Package for Social Sciences (SPSS), version 20.0. Data obtained was analysed using descriptive statistics and reported as the mean standard deviation (SD). Independent t-test was done between Group A (Control) and Group B (Intervention), Group B (Intervention) and Group C, Group B (Intervention) and Group D, Group B (Intervention) and Group E and finally between Group B (Intervention) and Group F. Analysis of variance (ANOVA) was also be used. Differences with p values less than 0.05 will be considered statistically significant.

Results

Table 1: Descriptive distribution of the sampling grouping

Variable	Frequency (n =26)	Percent
Group A	5	19.2
Group B	4	15.4
Group C	4	15.4
Group D	4	15.4
Group E	5	19.2
Group F	4	15.4

Pre weight	
Mean ± SD (Kg)	134.65±13.278
Post weight	
Mean ± SD (Kg)	156.46±15.381

The mean (± SD) Malondialdehyde (MDA) of Positive Control group (6.3±1.69) was lower compared to Intervention group (38.7±13.56). There was statistical significant difference between mean values of the parameters in both groups (p = 0.001). The Superoxide Dismutase (SOD) of the Positive Control group (9.5±1.42) was higher compared to Intervention group (1.12±0.24). There was statistical significant difference between mean values of the parameters in both groups (p = <0.0001). The mean Catalase (CAT) of Positive Control group (223.2±27.7) was higher compared to Intervention group (103.6±12.9). There was statistical significant difference between mean values of the parameters in both groups (p = <0.0001).

The mean White blood cell (WBC) of Positive Control group (14.5±5.34) was higher compared to Intervention group (13.9±4.31). There was no statistical significant difference between mean values of both groups (p = 0.853). The mean Lymphocytes (LYM) of the Positive Control group (12.7±4.8) was higher compared to Intervention group (11.2±3.75). There was no statistical significant difference between mean values of both groups (p = 0.630). The mean Monocytes (MON) of Positive Control group (1.28±0.54) was lower compared to Intervention group (1.92±0.75). There was no statistical significant difference between mean values of the parameters in both groups (p = 0.177). The Granulocytes (GRA) of Positive Control group (0.54±0.28) was lower compared to Intervention group (0.72±0.30). There was no statistical significant difference between mean values of the parameters in both groups (p = 0.385).

The percentage Lymphocytes (LYM %) of the Positive Control group (87.5±4.62) was higher compared to percentage mean value of Intervention group (80.9±6.82). There was no statistical significant difference between percentage mean values of the parameters in both groups (p = 0.124). The percentage Monocytes (MON %) of positive control group (8.78±2.74) was lower compared to percentage mean value of Intervention group (13.9±5.25). There was no statistical significant difference between percentage mean values of the parameters in both groups (p = 0.099). The percentage mean value Granulocytes (GRA %) of the Positive Control group (3.66±1.95) was lower compared to percentage mean value of Intervention group (5.2±1.72). There was no statistical significant difference between percentage mean values of the parameters in both groups (p = 0.256).

The mean Red blood cell (RBC) of the Positive Control group (5.7±0.52) was lower compared to Intervention group (6.3±0.18). There was no statistical significant difference between mean values of the parameters in both groups (p = 0.052). The mean Haemoglobin (HGB) concentration of the Positive Control group (13.4±1.43) was slightly lower compared to Intervention group (13.8±0.70). There was no statistical significant difference between mean values of both groups (p = 0.647). The mean Haematocrit (HCT) of the Positive Control group (38.2±3.8) was lower compared to Intervention group (40.0±1.41). There was no statistical significant difference between mean values of both groups (p = 0.408).

The Mean cell volume (MCV) of the Positive Control group (55.6±10.8) was slightly higher compared to Intervention group (55.0±1.90). There was no statistical significant difference between mean values of the parameter in both groups (p = 0.924). The Mean cell haemoglobin

(MCH) of the Positive Control group (23.46±.63) was higher compared to Intervention group (21.6±1.16). There was statistical significant difference between mean values of the parameter in both groups (p = 0.018). The Mean cell haemoglobin (MCHC) concentration of the Positive Control group (38.5±1.33) was lower compared to Intervention group (39.1±1.01). There was no statistical significant difference between mean values of the parameter in both groups (p = 0.432).

The Platelet (PLT) of the Positive Control group (528.4±125.6) was lower compared to Intervention group (575.2±69.6). There was no statistical significant difference between mean values of the parameter in both groups (p = 0.529).

Table 2: Independent t-test of Group A and Group B

Variables	Group A (Positive Control)	Group B (Intervention)	*Test Statistics	p-value
MDA (µ/ml)	6.3±1.69	38.7±13.56	5.379	**0.001**
SOD (µ/mg)	9.5±1.42	1.12±0.24	-11.480	**<0.0001**
CAT (µ/g)	223.2±27.7	103.6±12.9	-7.876	**<0.0001**
WBC (10^3µl)	14.5±5.34	13.9±4.31	-0.192	0.853
LYN (10^3µl)	12.7±4.8	11.2±3.75	-0.503	0.630
MON (10^3µl)	1.28±0.54	1.92±0.75	1.500	0.177
GRA (10^3µl)	0.54±0.28	0.72±0.30	0.927	0.385
LYM%	87.5±4.62	80.9±6.82	-1.749	0.124
MON%	8.78±2.74	13.9±5.25	1.901	0.099
GRA%	3.66±1.95	5.2±1.72	1.237	0.256
RBC (10^6µl)	5.7±0.52	6.3±0.18	2.335	0.052
HGB (g/dl)	13.4±1.43	13.8±0.70	0.479	0.647
HCT%	38.2±3.8	40.0±1.41	0.879	0.408
MCV(µm^3)	55.6±10.8	55.0±1.90	-0.099	0.924
MCH (pg)	23.46±0.63	21.6±1.16	-3.086	**0.018**
MCHC (g/dl)	38.5±1.33	39.1±1.01	0.833	0.432
PLT (10^3/µl)	528.4±125.6	575.2±69.6	0.663	0.529

*Independent t-test

The Malondialdehyde (MDA) of the Negative Control group (38.7±13.56) was higher compared to Intervention group (17.9±1.82). There was statistical significant difference between mean values of the parameter in both groups (p = 0.023). The Superoxide Dismutase (SOD) of Negative Control group (1.12±0.24) was lower compared to Intervention group (3.28±0.67). There was statistical significant difference between mean values of the parameter in both groups (p = 0.001). The Catalase (CAT) of the Negative Control group (103.6±12.9) was lower compared to Intervention group (127.3±8.62). There was statistical significant difference between mean values of the parameter in both groups (p = 0.023).

The mean White blood cell (WBC) of the Negative Control group (13.9±4.31) was higher compared to Intervention group (12.5±4.08). There was no statistical significant difference between mean values of the parameter in both groups (p = 0.648). The Lymphocytes (LYN) of experiment parameter in the Negative Control group (11.2±3.75) was higher compared to Intervention group (10.7±3.91). There was no statistical significant difference between mean values of both groups (p = 0.630). The Monocytes (MON) of the Negative Control group

(1.92±0.75) was higher compared to Intervention group (1.22±0.22). There was no statistical significant difference between mean values of both groups (p = 0.124). The Granulocytes (GRA) of the Negative Control group (0.72±0.30) was higher compared to Intervention group (0.50±0.14). There was no statistical significant difference between mean values both groups (p = 0.234).

The percentage Lymphocytes (LYM %) of the Negative Control group (80.9±6.82) was lower compared to percentage mean value of Intervention group (85.42±4.22). There was no statistical significant difference between percentage mean values of the parameters in both groups (p = 0.303). The percentage Monocytes (MON %) of experiment parameter in the Negative Control group (13.9±5.25) was higher compared to percentage mean value of Intervention group (10.4±3.15). There was no statistical significant difference between percentage mean values of the parameters in both groups (p = 0.297). The percentage Granulocytes (GRA %) of experiment parameter in the Negative Control group (5.2±1.72) was higher compared to percentage mean value of Intervention group (4.17±1.11). There was no statistical significant difference between percentage mean values of the parameters in both groups (p = 0.356).

The Red blood cells (RBC) of the Negative Control group (6.3±0.18) was higher compared to Intervention group (5.77±0.72). There was no statistical significant difference between mean values of the parameter in both groups (p = 0.165). The mean Haemoglobin (HGB) concentration of the Negative Control group (13.8±0.70) was higher compared to Intervention group (12.5±0.94). There was no statistical significant difference between mean values of both groups (p = 0.074). The Haematocrit (HCT) of the Negative Control group (40.0±1.41) was higher compared to Intervention group (37.0±2.8). There was no statistical significant difference between mean values of the parameter in both groups (p = 0.107).

The Mean cell volume (MCV) of the Negative Control group (55.0±1.90) was lower compared to Intervention group (57.8±5.5). There was no statistical significant difference between mean values of the parameters in both groups (p = 0.374). The Mean cell haemoglobin (MCH) of experiment parameter in the Negative Control group (21.6±1.16) was slightly lower compared to Intervention group (21.8±1.46). There was statistical significant difference between mean values of the parameters in both groups (p = 0.838). The Mean cell haemoglobin (MCHC) concentration of the Negative Control group (39.1±1.01) was higher compared to Intervention group (37.7±1.25). There was no statistical significant difference between mean values of both groups (p = 0.134).

The mean Platelet (PLT) of the Negative Control group (575.2±69.6) was lower compared to Intervention group (660.5±55.4). There was no statistical significant difference between mean values of the parameter in both groups (p = 0.104).

Table 3: Independent t-test of Group B and Group C

Variables	Group B (Negative Control)	Group C (Intervention)	*Test Statistics	p-value
MDA (µ/ml)	38.7±13.56	17.9±1.82	3.029	**0.023**
SOD (µ/mg)	1.12±0.24	3.28±0.67	-5.971	**0.001**
CAT (µ/g)	103.6±12.9	127.3±8.62	-3.048	**0.023**
WBC (10^3µl)	13.9±4.31	12.5±4.08	0.480	0.648
LYN (10^3µl)	11.2±3.75	10.7±3.91	0.175	0.867

MON (10^3µl)	1.92±0.75	1.22±0.22	1.790	0.124
GRA (10^3µl)	0.72±0.30	0.50±0.14	1.322	0.234
LYM%	80.9±6.82	85.42±4.22	-1.127	0.303
MON%	13.9±5.25	10.4±3.15	1.143	0.297
GRA%	5.2±1.72	4.17±1.11	1.000	0.356
RBC (10^6µl)	6.3±0.18	5.77±0.72	1.580	0.165
HGB (g/dl)	13.8±0.70	12.5±0.94	2.158	0.074
HCT%	40.0±1.41	37.0±2.8	1.897	0.107
MCV(µm^3)	55.0±1.90	57.8±5.5	-0.960	0.374
MCH (pg)	21.6±1.16	21.8±1.46	-0.214	0.838
MCHC (g/dl)	39.1±1.01	37.7±1.25	1.733	0.134
PLT (10^3/µl)	575.2±69.6	660.5±55.4	-1.916	0.104

*Independent t-test

The Malondialdehyde (MDA) of the Negative Control group (38.7±13.56) was higher compared to Intervention group (12.09±1.34). There was statistical significant difference between mean values of the parameters in both groups (p = 0.008). The mean Superoxide Dismutase (SOD) of the Negative Control group (1.12±0.24) was lower compared to Intervention group (5.09±0.92). There was statistical significant difference between mean values of Superoxide Dismutase in both groups (p = <0.0001). The mean Catalase (CAT) of the Negative Control group (103.6±12.9) was lower compared to Intervention group (151.7±6.82). There was statistical significant difference between mean Catalase values in both groups (p = 0.001).

The mean White blood cell (WBC) of the Negative Control group (13.9±4.31) was higher compared to Intervention group (8.47±3.14). There was no statistical significant difference between mean values in both groups (p = 0.087). The mean Lymphocytes (LYN) of the Negative Control group (11.2±3.75) was higher compared to Intervention group (7.17±2.53). There was no statistical significant difference between mean values of both groups (p = 0.122). The Monocytes (MON) of the Negative Control group (1.92±0.75) was higher compared to Intervention group (0.97±0.44), but no statistically significant difference (p = 0.072). The mean Granulocytes (GRA) of the Negative Control group (0.72±0.30) was higher compared to Intervention group (0.30±0.18), but no statistically significant difference (p = 0.056). The percentage Lymphocytes (LYM %) of Negative Control group (80.9±6.82) was lower compared to percentage mean value of Intervention group (85.12±3.87), but no statistically significant difference (p = 0.323).

The percentage mean Monocytes (MON %) of Negative Control group (13.9±5.25) was higher compared to percentage mean value of Intervention group (11.30±2.79), but the difference was not statistically significant (p = 0.416). The percentage Granulocytes (GRA %) of the Negative Control group (5.2±1.72) was higher compared to percentage mean value of Intervention group (3.57±1.18), but the difference was not statistically significant (p = 0.171).

The mean Red blood cells (RBC) of the Negative Control group (6.3±0.18) were higher compared to Intervention group (5.98±0.42), but the difference was not statistically significant (p = 0.155). The mean Haemoglobin (HGB) concentration of the Negative Control group (13.8±0.70) was slightly higher compared to Intervention group (13.5±0.42), but the difference was not statistically significant (p = 0.530). The mean Haematocrit (HCT) of the Negative

Control group (40.0±1.41) was higher compared to Intervention group (39.7±1.50), but the difference was not statistically significant (p = 0.816).

The Mean cell volume (MCV) of the Negative Control group (55.0±1.90) was lower compared to Intervention group (57.35±4.37), but the difference was not statistically significant (p = 0.373). The Mean cell haemoglobin (MCH) of the Negative Control group (21.6±1.16) was lower compared to Intervention group (22.6±1.53), but the difference was not statistically significant (p = 0.307). The Mean cell haemoglobin (MCHC) concentration of the Negative Control group (39.1±1.01) was lower compared to Intervention group (39.55±0.35), but the difference was not statistically significant (p = 0.512).

The mean Platelet (PLT) of the Negative Control group (575.2±69.6) was lower compared to Intervention group (715.2±67.29), the difference was statistically significant (p = 0.028).

Table 4: Independent t-test of Group B and Group D

Variables	Group B (Negative Control)	Group D (Intervention)	*Test Statistics	p-value
MDA (µ/ml)	38.7±13.56	12.09±1.34	3.904	**0.008**
SOD (µ/mg)	1.12±0.24	5.09±0.92	-8.299	**<0.0001**
CAT (µ/g)	103.6±12.9	151.7±6.82	-6.589	**0.001**
WBC (10^3µl)	13.9±4.31	8.47±3.14	2.041	0.087
LYN (10^3µl)	11.2±3.75	7.17±2.53	1.801	0.122
MON (10^3µl)	1.92±0.75	0.97±0.44	2.182	0.072
GRA (10^3µl)	0.72±0.30	0.30±0.18	3.365	0.056
LYM%	80.9±6.82	85.12±3.87	-1.076	0.323
MON%	13.9±5.25	11.30±2.79	0.874	0.416
GRA%	5.2±1.72	3.57±1.18	1.555	0.171
RBC (10^6µl)	6.3±0.18	5.98±0.42	1.625	0.155
HGB (g/dl)	13.8±0.70	13.5±0.42	0.666	0.530
HCT%	40.0±1.41	39.7±1.50	0.243	0.816
MCV(µm^3)	55.0±1.90	57.35±4.37	-0.963	0.373
MCH (pg)	21.6±1.16	22.6±1.53	-1.115	0.307
MCHC (g/dl)	39.1±1.01	39.55±0.35	-0.697	0.512
PLT (10^3/µl)	575.2±69.6	715.2±67.29	-2.891	**0.028**

*Independent t-test

The mean Malondialdehyde (MDA) level of the Negative Control group (38.7±13.56) was higher compared to Intervention group (7.02±1.45). There was statistical significant difference between mean values of both groups (p = 0.001). The mean Superoxide Dismutase (SOD) of the Negative Control group (1.12±0.24) was lower compared to Intervention group (7.65±1.25). There was statistical significant difference between mean values of both groups (p = <0.0001).

The mean Catalase (CAT) of the Negative Control group (103.6±12.9) was lower compared to Intervention group (184.9±11.2). There was statistically significant difference between mean values of the parameters in both groups (p = <0.0001).

The mean White blood cell (WBC) of the Negative Control group (13.9±4.31) was lower compared to Intervention group (16.2±4.39), but the difference was not statistically significant (p = 0.462). The mean Lymphocytes of the Negative Control group (11.2±3.75) was lower

compared to Intervention group (14.5±3.77), but the difference was not statistically significant (p = 0.239). The Monocytes (MON) of the Negative Control group (1.92±0.75) was higher compared to Intervention group (1.28±0.54), but the difference was not statistically significant (p = 0.177). The Granulocytes (GRA) of the Negative Control group (0.72±0.30) was higher compared to Intervention group (0.42±0.24), but the difference was not statistically significant (p = 0.144).

The percentage Lymphocytes (LYM %) of the Negative Control group (80.9±6.82) was lower compared to percentage mean value of Intervention group (89.76±2.64). There was statistical significant difference between percentage mean values of both groups (p = 0.031). The percentage Monocytes (MON %) of the Negative Control group (13.9±5.25) was higher compared to percentage mean value of Intervention group (7.64±1.68). There was statistically significant difference between percentage mean values of both groups (p = 0.038). The percentage Granulocytes (GRA %) of the Negative Control group (5.2±1.72) was higher compared to percentage mean value of Intervention group (2.60±1.0). There was statistically significant difference between percentage mean values of both groups (p = 0.024).

The Red blood cells (RBC) of the Negative Control group (6.3±0.18) was higher compared to Intervention group (6.09±0.47), but the difference was not statistically significant (p = 0.324). The mean Haemoglobin (HGB) concentration of the Negative Control group (13.8±0.70) was higher compared to Intervention group (13.3±0.78), but the difference was not statistically significant (p = 0.432). The mean Haematocrit (HCT) of the Negative Control group (40.0±1.41) was higher compared to Intervention group (38.8±1.64), but the difference was not statistically significant (p = 0.286).

The Mean cell volume (MCV) of the Negative Control group (55.0±1.90) was lower compared to Intervention group (55.7±3.29), but the difference was not statistically significant (p = 0.715). The Mean cell haemoglobin (MCH) of the Negative Control group (21.6±1.16) was lower compared to Intervention group (22.0±0.67), but the difference was not statistically significant (p = 0.536). The Mean cell haemoglobin (MCHC) concentration of the Negative Control group (39.1±1.01) was lower compared to Intervention group (39.5±1.25), but the difference was not statistically significant (p = 0.689).

The mean Platelet (PLT) of the Negative Control group (575.2±69.6) was lower compared to Intervention group (652.8±79.43), but the difference was not statistically significant (p = 0.169).

Table 5: Independent t-test of Group B and Group E

Variables	Group B (Negative Control)	Group E (Intervention)	*Test Statistics	p-value
MDA (µ/ml)	38.7±13.56	7.02±1.45	5.277	**0.001**
SOD (µ/mg)	1.12±0.24	7.65±1.25	-10.109	**<0.0001**
CAT (µ/g)	103.6±12.9	184.9±11.2	-10.089	**<0.0001**
WBC (10^3µl)	13.9±4.31	16.2±4.39	-0.778	0.462
LYN (10^3µl)	11.2±3.75	14.5±3.77	-1.287	0.239
MON (10^3µl)	1.92±0.75	1.28±0.54	1.500	0.177
GRA (10^3µl)	0.72±0.30	0.42±0.24	1.644	0.144
LYM%	80.9±6.82	89.76±2.64	-2.697	**0.031**
MON%	13.9±5.25	7.64±1.68	2.544	**0.038**

GRA%	5.2±1.72	2.60±1.0	2.857	**0.024**
RBC (10^6µl)	6.3±0.18	6.09±0.47	1.062	0.324
HGB (g/dl)	13.8±0.70	13.3±0.78	0.834	0.432
HCT%	40.0±1.41	38.8±1.64	1.155	0.286
MCV(µm^3)	55.0±1.90	55.7±3.29	-0.380	0.715
MCH (pg)	21.6±1.16	22.0±0.67	-0.651	0.536
MCHC (g/dl)	39.1±1.01	39.5±1.25	-0.417	0.689
PLT (10^3/µl)	575.2±69.6	652.8±79.43	-1.533	0.169

*Independent t-test

The Malondialdehyde (MDA) of the Negative Control group (38.7±13.56) was higher compared to Intervention group (13.9±1.55). There was statistically significant difference between both groups (p = 0.011). The Superoxide Dismutase (SOD) of the Negative Control group (1.12±0.24) was lower compared to Intervention group (5.0±0.87). There was statistical significant difference between mean values of both groups (p = <0.0001). The mean Catalase (CAT) of the Negative Control group (103.6±12.9) was lower compared to Intervention group (153.4±9.96). There was a statistically significant difference between mean values of both groups (p = 0.001).

The White blood cells (WBC) of the Negative Control group (13.9±4.31) was higher compared to Intervention group (9.55±2.5), but the difference was not statistically significant (p = 0.133). The lymphocytes count of the Negative Control group (11.2±3.75) was higher compared to Intervention group (8.2±2.03), but the difference was not statistically significant (p = 0.203). The mean Monocytes count of the Negative Control group (1.92±0.75) was higher compared to Intervention group (0.97±0.48), but the difference was not statistically significant (p = 0.078). The mean Granulocytes (GRA) of the Negative Control group (0.72±0.30) was higher compared to Intervention group (0.37±0.15), but the difference was not statistically significant (p = 0.088).

The percentage Lymphocytes (LYM %) of the Negative Control group (80.9±6.82) was lower compared to percentage mean value of Intervention group (86.0±3.08), but the difference was not statistically significant (p = 0.216).

The percentage Monocytes (MON %) of Negative Control group (13.9±5.25) was higher compared to percentage mean value of Intervention group (10.1±2.50) but the difference was not statistically significant (p = 0.242). The percentage Granulocytes (GRA %) of the Negative Control group (5.2±1.72) was lower compared to percentage mean value of Intervention group (12.3±17.11) but the difference was not statistically significant (p = 0.438).

The Red blood cells (RBC) of the Negative Control group (6.3±0.18) was slightly higher compared to Intervention group (6.28±0.33) but the difference was not statistically significant (p = 0.693). The mean Haemoglobin (HGB) concentration of the Negative Control group (13.8±0.70) was slightly higher compared to Intervention group (13.3±0.50) but the difference was not statistically significant (p = 0.293). The mean Haematocrit (HCT) of the Negative Control group (40.0±1.41) was higher compared to Intervention group (37.4±2.09) but the difference was not statistically significant (p = 0.085).

The Mean cell volume (MCV) of the Negative Control group (55.0±1.90) was higher compared to Intervention group (54.6±2.85) but the difference was not statistically significant (p = 0.802). The Mean cell haemoglobin (MCH) of the Negative Control group (21.6±1.16) was slightly higher compared to Intervention group (21.15±0.38) but the difference was not statistically significant (p = 0.490). The Mean cell haemoglobin (MCHC) concentration of the Negative Control group (39.1±1.01) was slightly higher compared to Intervention group (38.85±1.58) but the difference was not statistically significant (p = 0.742).

The mean Platelet (PLT) of the Negative Control group (575.2±69.6) was lower compared to Intervention group (606.5±135.1), but the difference was not statistically significant (p = 0.695).

Table 6: Independent t-test of Group B and Group F

Variables	Group B (Negative Control)	Group F (Intervention)	*Test Statistics	p-value
MDA (µ/ml)	38.7±13.56	13.9±1.55	3.626	**0.011**
SOD (µ/mg)	1.12±0.24	5.0±0.87	-8.526	**<0.0001**
CAT (µ/g)	103.6±12.9	153.4±9.96	-6.110	**0.001**
WBC (10^3µl)	13.9±4.31	9.55±2.5	1.738	0.133
LYN (10^3µl)	11.2±3.75	8.2±2.03	1.430	0.203
MON (10^3µl)	1.92±0.75	0.97±0.48	2.126	0.078
GRA (10^3µl)	0.72±0.30	0.37±0.15	2.035	0.088
LYM%	80.9±6.82	86.0±3.08	-1.381	0.216
MON%	13.9±5.25	10.1±2.50	1.297	0.242
GRA%	5.2±1.72	12.3±17.11	-0.831	0.438
RBC (10^6µl)	6.3±0.18	6.28±0.33	0.414	0.693
HGB (g/dl)	13.8±0.70	13.3±0.50	1.152	0.293
HCT%	40.0±1.41	37.4±2.09	2.060	0.085
MCV(µm^3)	55.0±1.90	54.6±2.85	0.262	0.802
MCH (pg)	21.6±1.16	21.15±0.38	0.736	0.490
MCHC (g/dl)	39.1±1.01	38.85±1.58	0.345	0.742
PLT (10^3/µl)	575.2±69.6	606.5±135.1	-0.411	0.695

*Independent t-test

Discussion

A total of 30 albino rats were used for to carry out the experiment. But towards the conclusion of the experiment, 26 rats survived. The average pre weight of the rats was 134.65g and the average post weight was 156. 46g. In agreement with the findings of Ekpenyong and Asuquo (2017), the findings from this study indicated that exposure to gasoline vapour resulted in a notable elevation in MDA levels. From this research it has been observed that exposure to gasoline vapour also caused a significant decrease in SOD and CAT level. Apart from MCH, there were no significant alterations observed in haematological parameters. This means exposure to gasoline vapour did not cause haemotoxicity in the rats. Perhaps, this might be as a result of the time of exposure of the gasoline vapour. According to Okonkwo et al, changes in haematological parameters in albino rats were higher in test groups that were exposed for a

longer period. In this study, the rats underwent daily exposure to gasoline vapour for duration of 1 hour each day.

When *C. citratus* decoction (250 mg/kg, 500 mg/kg, and 1000 mg/kg) or vitamin C (200mg/kg) was co-administered to the animals in groups C, D, E, or F, respectively, the GV-induced changes in the MDA, SOD and CAT were reversed. This indicated that *C. citratus* was able to ameliorate the effect of GV-induced toxicity. The aforementioned changes in Oxidative stress parameters (specifically significant increases in MDA, and decrease in SOD and CAT) of animals exposed to GV alone indicate GV-induced lipid peroxidation leading to an increased MDA level. The study also pointed out that superoxide dismutase (SOD) and catalase (CAT) were involved in reacting with the reactive oxygen species (ROS) generated due to the inhalation of gasoline vapour. All these are evidence of oxidative stress caused by inhalation of gasoline vapour. MDA is produced as a decomposition product when lipids undergo oxidation. Hence, the increase in MDA levels, decrease in SOD and Catalase were as a result SOD dismutating O_2 - into H_2O_2 to avoid accumulation to toxic level. Catalase which is one of the most abundant peroxisomal proteins in mammalian cells, then converts H_2O_2 into H_2O and O_2.

The ameliorative effect of *C. citratus* on GV-induced oxidative stress observed in this study is due to its antioxidative activity. Evaluation of the phytochemical and nutritional constituents of fresh *C. citratus* leaf extract in a previous study showed that several *C. citratus* constituents, including phytochemicals (Saponins, polyphenols, tannins, flavonoids, and alkaloids); vitamins (vitamins A, C, E, folate, thiamine, niacin, pyridoxine, and riboflavin); minerals and trace elements (magnesium, zinc, copper, selenium and iron),electrolytes (Ekpenyong and Bassey, 2016), show antioxidative effects in human and animal cells (Saeio, 2011). Other important nutrients identified in *C. citratus* extract include carbohydrates, protein, and fat. These antioxidative constituents can alleviate GV-induced oxidative stress and suppress other pathophysiological processes.

Indeed, there are numerous reports on the antioxidant activity of *C. citratus* as well as its potential to inhibit many drug/chemical-induced adverse health effects. For example, studies by Arhoghro *et al*., (2012), Koh *et al*., (2012), and Rahim *et al*., (2014) demonstrated the ameliorative effect of *C. Citratus* leaf extracts on chemical-induced liver damage (by cisplatin, carbon tetrachloride, and hydrogen peroxide, respectively). Additionally, Gayathri *et al*., (2011) have observed that *C.citratus* extract produces marked attenuation of isoprenoid induced cardiotoxicity and lipid peroxidation in rats.

Conclusion

The results of this study demonstrate that *C. Citratus* decoction and vitamin C have ameliorative effects on GV-induced oxidative stress in rats owing to its anti-oxidative activities. The results also suggest the oxidative effect of gasoline inhalation but not a significant haematotoxic effect in albino wistar rats for the period of exposure. Further studies to identify the active ingredient or ingredients responsible for the antioxidant activities of *C. Citratus* decoction are needed.

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