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Lemongrass Leaf Extract Attenuates Hydrocarbon-Induced Oxidative Stress and Macrocytic Hypochromic Anaemia in Rats

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Abstract: The therapeutic use of medicinal plants and plant products to mitigate solvent/xenobiotic-induced haematological disorders has not been adequately explored. The aim of this study was to evaluate the effect of *Cymbopogon citratus* (*C. citratus*) leaf decoctions on gasoline vapour (GV)-induced haematological disorders and oxidative stress in rats. Seventy-two female rats weighing 180-200g were randomly selected and divided into six groups (n = 12 per group). Animals in group 1 served as unexposed controls, while animals in group 2 were exposed to gasoline vapour (GV) alone for 35 days. Animals in groups 3, 4, and 5 were exposed to GV and co-administered *C. citratus* leaf extract (500 mg/kg, 1000 mg/kg, and 1500 mg/kg, respectively), and animals in group 6 were exposed to GV and co-administered vitamin C (200 mg/kg) for the same time period. Exposure of animals to GV alone significantly ($p < 0.05$) decreased packed cell volume (PCV), haemoglobin concentration (HB), total red blood cells (RBC), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC). Total white blood cells (WBC), WBC lineages and malondialdehyde (MDA) levels significantly increased ($p < 0.05$) compared to the corresponding values in the control group. Supplementation with *C. citratus* leaf decoction and vitamin C reversed these GV-induced changes in haematological indices and MDA levels. Therefore, *C. citratus* leaf decoctions and vitamin C supplementation provided an ameliorative effect on GV-induced haematotoxicity and oxidative stress in a rat model of gasoline exposure.

Keywords: Lemongrass, Gasoline Fumes, Haematotoxicity, Amelioration, Rat

1. 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 [1].

Gasoline is derived from the fractional distillation 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 [2, 3].

Several previous studies [4-7] have established a close association between exposure to gasoline compounds and haematotoxicity in humans.

Several 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 [3]. Central to these mechanisms is the induction of oxidative stress by gasoline component metabolites.

Although various synthetic antioxidants (e.g. vitamins A, C, and E) have been shown to ameliorate gasoline-induced haematotoxicity in previous studies [8], 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 [9].

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 [10]. Thus, these plants have the potential to provide holistic therapeutic coverage, and thereby improve human health and prevent acute and chronic diseases [10]. One such plant is Lemongrsass (*Cymbopogon citratus* (*C. citratus*)).

C. citratus is an aromatic perennial tropical plant that grows worldwide. *C. citratus* has long slender leaves of about 90cm in length and 1.5cm in breadth, The leaves give off a slight aroma of ginger when crushed. It is a member of the Poaceae family, and owing to its varied bioactive constituents, has extensive nutritional, therapeutic, and cosmetic applications. It has long been used as a traditional medicine for its antibacterial [11], anticarcinogenic [12], anti-inflammatory [13], antioxidant, antimicrobial [14], and erythropoietic-boosting effects [10]. The antioxidant effect of *C. citratus* has been described, and it has been proposed as an alternative treatment for numerous diseases [15].

Several synthetic antioxidant vitamins, including A, C, and E, have been shown to provide protective effects against GV-induced haematotoxicity [3, 8]. These vitamins and other bioactive antioxidants are also found in vegetables, fruits, and other plants, such as *C. citratus*. However, their protective activities against GV-induced detrimental health effects, including haematotoxicity, have not been adequately investigated. The aim of the present study was to assess the effect of *C. citratus* decoction on GV-induced haematotoxicity in rats.

2. Materials and Methods

Fresh *C. citratus* leaves were obtained from an agricultural farm in Uyo, Akwa Ibom State, Nigeria a few days prior to utilization. Identification and authentication was performed by a taxonomist (ID UHH 4686/Uyo) in the Department of Botany at the University of Uyo.

The leaves were rinsed, sundried, and pulverized into powder using an electric blender to provide 400 g of material. The powder was soaked with 4 L of hot water in a conical flask and allowed to stand for approximately 10 h.

After filtering the solution through Whatman No. 2 filter paper, 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 (ACS-2E14; Surgifriend Medicals, Ltd., England), with a total yield of 30%. The prepared extract was stored in glass bottles at 4°C and was dissolved in physiological saline at 100 mg/mL.

2.1. Phytochemical/Nutritional Screening of C. Citratus Leaf Extract

The phytochemical analysis of the leaf extracts was carried out to determine the levels of tannins, phenolics, saponins, alkaloids, deoxysugars, and anthraquinones as described by Trease and Evans [16] and Sofowora [17]. Nutrient constituents were determined per 100g of extract as described previously [18].

2.2. Experimental Animals

Seventy-two mature female Wistar albino rats weighing 180 –200g were obtained from the animal house at the Faculty of Basic Medical Sciences, University of Uyo, Nigeria. The rats were randomly divided into six groups with 12 rats per group as follows: Group 1, normal (unexposed) control; Group 2, Test group exposed to GV only; Group 3, Test group exposed to GV and concomitantly treated with *C. citratus* extract (500 mg/kg; low dose) daily, Group 4, Test group exposed to GV and concomitantly treated with (1000 mg/kg; medium dose) daily; Group 5, Test group exposed to GV and concomitantly treated with (1500 mg/kg; high dose) daily; and Group 6, Test group exposed to GV and concomitantly treated with vitamin C (200 mg/kg).

2.3. Determination of LD_{50}

The median lethal dose (LD_{50}) was determined as previously described [19, 20]. From the LD_{50} (5000 mg/kg), the low, medium, and high doses were calculated using a standard formula as 500 mg/kg, 1000 mg/kg, and 1500 mg/kg, respectively.

The rats were acclimatized for one week before the starting the experiment. Each animal was housed in a standard wooden cage with wood shavings as bedding, which was regularly replaced. The experiment was performed under standard laboratory conditions (at room temperature [28 ± 8°C], 45% humidity, with a 12-h light/dark cycle). All animals were fed normal rat pellets (Bendel Feed and Flour Mill Ltd, Benin) and allowed free access to food and water throughout the experimental period.

All research protocols were performed at the University of Uyo according to Nigerian and international laws governing the acceptable use of laboratory animals.

The protocol was approved by the Institutional Animal Care and Use Committee.

2.4. Exposure to GV

The animals in the test group were exposed to unleaded gasoline purchased from a Nigerian National Petroleum Cooperation (NNPC) refuelling station on Itam - Ikot Ekpene Road in Uyo, Nigeria. The rats in the exposed groups were housed in their cages and exposed to GV in an exposure chamber (80 × 60 × 100 cm). Rats in the control group were kept in a GV-free section of the experimental house. Two calibrated 500-mL beakers each containing 100 mL of petrol were put in the modified chamber where the exposed groups

were placed, and then the rats were allowed to inhale the GV in the chamber for 6 h (9 am–3 pm) daily for 35 consecutive days. At the end of the exposure period, the gasoline was removed; the initial and final volumes were recorded before and after exposure, respectively. The daily difference in volume was used to estimate the relative vapour exposure. The average exposure was approximately 80 mL/day.

2.5. Treatment of Rats with C. citratus Leaf Extracts and Vitamin C

Rats in groups 3, 4, and 5 were treated with decoctions prepared from 500 mg/kg, 1000 mg/kg, and 1500 mg/kg of *C. citratus* extract, respectively, while rats in group VI were treated with 200 mg/kg (i.e. the normal prophylactic dose) of vitamin C [21]. The *C. citratus* decoctions and water-solubilized vitamin C were administered by oral gavage (using an intragastric syringe) for the final 14 days of the 35-day GV exposure.

After the exposure period, the rats were sacrificed by anaesthetizing with chloroform. Blood was collected by cardiac puncture into specimen bottles with and without anti-coagulant for haematological analysis and MDA estimation respectively. Serum samples were stored at – 80°C until analysis.

Total blood count including total leucocyte and differential counts and other haematological parameters were measured within 2h of blood sample collection using the SYSMEX KX-21 Automated Haematology Analyzer (Kobe, Japan).

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS), version 20.0.

Data obtained were analysed using descriptive statistics and reported as the mean ± standard deviation (SD). Analysis of variance (ANOVA) was also used. Duncan's multiple range test was used to compare results between groups, determine the direction of significance, and to analyse the effect of the *C. citratus* decoction on the haematoxicity of GV. Differences with *p* values less than 0.05 were considered statistically significant.

3. Results

Analysis of the bioactive natural constituents of the *C. citratus* leaf extract in the present study showed moderate levels of saponins, tannins and flavonoids and relatively low levels of alkaloids, and anthraquinones. Nutritional constituents as determined previously [10] revealed the presence of crude protein (2.5 g), crude fibre (2.92 g), carbohydrate (36.02 g), potassium (420 mg), sodium (14.30 mg), iron (18.4 mg), magnesium (32.10 mg), zinc (2.35 mg), copper (0.368 mg), selenium (1.32 mcg), vitamin C (3.65 mg), pyridoxine (0.07 mg) and folate (78 mcg).

Exposure of experimental animals to GV alone caused significant decreases in PCV (p=0.00), haemoglobin (Hb) concentration (p=0.004), RBC (p=0.03), platelet (PL) counts, and reticulocyte counts compared to corresponding levels in control animals.

Co-administration of *C. citratus* decoction at various concentrations (500 mg/kg, 1000 mg/kg, and 1500 mg/kg) and vitamin C (200 mg/kg) increased PCV, Hb, and RBC, while PL and reticulocyte counts significantly decreased compared to corresponding levels in GV-alone group (Table 1).

Table 2 shows that exposure of the experimental animals to GV-alone caused significant decreases in MCH and SOD levels, and a non-significant decrease in MCHC levels compared to corresponding levels in the control group. Also, MCV and MDA levels significantly increased following exposure to GV alone. Co-administration of *C. citratus* decoction at various concentrations (500 mg/kg, 1000 mg/kg, and 1500 mg/kg) and vitamin C (200 mg/kg) caused a significant increase in MCH levels, and significant decreases in MCV and MDA levels. Non-significant decreases in MCHC and SOD levels were found in animals co-treated with 500 mg/kg and 1000 mg/kg of C. citrates decoctions, while non-significant increases in MCHC and SOD levels were found in animals co-administered with 1500 mg/kg of *C. citratus* decoction and 200 mg/kg of vitamin C respectively.

Total WBC counts and WBC lineages including neutrophil counts (p=0.000), lymphocyte counts (p=0.004), monocyte counts (p=0.00), eosinophil counts (p=0.003) and basophil counts significantly increased in animals exposed to GV alone compared to corresponding levels in the control animals.

Supplementation with *C. citratus* decoctions and vitamin C reversed these GV-induced changes in total WBC and WBC lineages (Table 3).

Table 1. *Effect of C. citratus decoctions and ascorbic acid supplementation on some haematological profile of GV- induced haematotoxicity model in rats.*

Group	PCV (%)	Hb (mgd/L)	RBC ($X10^6/mm^3$)	PL (%)	Reticulocyte counts (%)
1	40.75 ± 1.89^c	13.58 ± 2.81^a	7.33 ± 0.42^b	645.33 ± 63.13^d	153.50 ± 7.99^a
2	21.15 ± 1.06^a	7.05 ± 1.79^b	6.40 ± 0.28^a	662.50 ± 13.54^c	190.00 ± 8.49^b
3	28.17 ± 1.17^b	9.39 ± 1.87^c	6.50 ± 0.24^a	509.50 ± 10.64^b	180.50 ± 8.17^b
4	27.78 ± 1.83^b	9.26 ± 1.11^c	6.60 ± 0.38^a	597.50 ± 14.41^c	151.33 ± 9.16^a
5	26.15 ± 1.43^b	8.72 ± 1.62^c	6.98 ± 0.37^b	330.33 ± 10.66^a	154.50 ± 5.572^a
6	28.25 ± 2.99^b	9.42 ± 1.600^c	7.07 ± 0.19^b	576.0 ± 13.07^c	152.60 ± 5.10^a

The different superscripts are significant at 5% ($p < 0.05$),
PVC (Packed cell volume), Hb (Haemoglobin),
RBC (Red blood cells), PL (Platelets).

Table 2. *Effect of C. citratus decoctions and ascorbic acid supplementation on red blood cell and oxidative stress markers.*

Parameters	MCH (pg)	MCHC (mm^3)	MCV (ft)	SOD (mmol/L)	MDA (mmol/L)
Groups					
1	17.83 ± 1.47^b	33.47 ± 2.04^b	48.10 ± 2.74^a	10.530 ± 1.240^b	5.81 ± 0.55^a
2	15.00 ± 1.41^a	30.40 ± 4.44^b	54.90 ± 2.08^b	7.064 ± 1.520^a	11.10 ± 0.41^d
3	18.17 ± 1.94^b	25.92 ± 4.52^a	45.68 ± 2.64^a	7.840 ± 1.380^a	9.03 ± 0.45^c
4	17.50 ± 2.07^b	27.92 ± 3.72^a	50.35 ± 2.66^a	8.24 ± 2.24^a	7.24 ± 0.39^b
5	18.00 ± 0.89^b	31.53 ± 1.87^b	47.40 ± 1.38^a	9.76 ± 2.81^b	7.07 ± 0.39^b
6	18.83 ± 2.32^b	31.30 ± 1.48^b	46.80 ± 2.20^a	10.23 ± 2.88^b	4.18 ± 3.27^a

Same letters mean not significantly different ($p > 0.05$),
Different letters means significantly different ($p < 0.05$).

Table 3. *Effect of C. citratus decoctions and ascorbic acid supplementation on total white blood cell and white blood cell lineages of GV-induced haematotoxicity model in rat.*

Group	Total WBC ($x10^3/mm^3$)	Neutrophil Counts (%)	Lymphocyte Counts (%)	Monocyte Counts (%)	Eosinophil Counts (%)	Basophil Counts (%)
1	3.13 ± 1.11^a	0.23 ± 0.11^a	3.43 ± 2.69^a	0.07 ± 0.02^a	0.11 ± 0.00^a	0.00 ± 0.00^a
2	12.75 ± 1.77^c	1.00 ± 0.28^c	7.90 ± 0.99^c	0.20 ± 0.01^b	0.13 ± 0.04^c	0.10 ± 0.14^c
3	10.72 ± 1.08^b	1.07 ± 0.23^c	6.67 ± 0.82^c	0.11 ± 0.05^b	0.03 ± 0.02^a	0.06 ± 0.10^b
4	9.55 ± 0.51^b	0.55 ± 0.34^a	5.58 ± 0.87^b	0.11 ± 0.05^b	0.03 ± 0.01^a	0.01 ± 0.01^a
5	9.40 ± 0.53^b	0.42 ± 0.30^a	5.92 ± 0.50^b	0.08 ± 0.03^a	0.06 ± 0.08^b	0.01 ± 0.01^a
6	9.27 ± 0.31^b	0.75 ± 0.12^b	7.00 ± 0.41^c	0.07 ± 0.02^a	0.00 ± 0.00^a	0.00 ± 0.00^a

The different superscripts are significant at 5% ($p < 0.05$).

4. Discussion

In agreement with the findings of previous studies, results of the present study showed that exposure to GV decreased several erythropoiesis-modulated haemocytic variables (PCV, Hb, RBC, MCH, and MCHC) and caused significant increases in reticulocyte counts, MCV and MDA levels and total WBC and WBC lineage cell (neutrophils, lymphocytes, monocytes, eosinophils and basophil) counts, compared to the control group [1]. However, when *C. citratus* decoction (500 mg/kg, 1000 mg/kg, and 1500 mg/kg) or vitamin C (200 mg/kg) was co-administered to the animals in groups 3, 4, 5, or 6, respectively, the GV-induced changes in the haematological parameters and MDA were reversed in a dose-dependent manner. Obviously, the aforementioned changes in haematological parameters (specifically significant increases in MDA, MCV, and decreases in PCV, Hb, MCH, and MCHC) of animals exposed to GV alone indicate GV-induced lipid peroxidation (evidence of oxidative stress), and macrocytic hypochromic anaemia.

The present data provide additional evidence to support previous epidemiological and clinical studies that demonstrated a close association of exposure to gasoline compounds with haematotoxicity [2, 4-7] and the erythropoiesis-boosting (haematoprotective) effect of *C. citratus* leaf extracts [10]. Although several mechanisms have been posited to underlie GV-induced haematoxicity, induction of oxidative stress is central to the pathophysiology of GV-induced haematotoxicity. Exposure to GV is associated with increased generation of reactive metabolites, leading to oxidative stress and oxidative damage to RBC [8]. Other effects include covalent binding and damage to DNA, impaired enzyme activities, cell-cycle suppression, myelosuppression, and aplastic anaemia [2]. Numerous established studies have confirmed that the most common cause of macrocytic anaemia is impaired DNA synthesis. However, the significant increase in reticulocyte count in the present study indicates associated hemolysis.

Furthermore, oxidative damage to DNA can cause activation of proto-oncogenes, suppression of p53, and leukaemia [4]. Suppression of growth factor- and haemopoiesis-related genes has also been observed [5]. Some gasoline compounds, such as benzene, have been shown to cause degeneration of bone marrow, aplastic anaemia, leukaemia, and changes in the dynamics of erythropoiesis [3, 22, 23]. Therefore, the ameliorative effect of *C. citratus* on GV-induced haematotoxicity observed in the present study is partly due to the synergistic effects of its antioxidative, haematoprotective and erythropoiesis-boosting bioactive constituents against these GV-mediated pathological processes leading to qualitative and quantitative deficiency of Hb, total RBC counts and RBC indices. Evaluation of the phytochemical and nutritional constituents of fresh *C. citratus* leaf extract in a previous and present 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 [10], and essential oil constituents (geraniol, myrcene, linalool, limonene, β-ionone, and citral), show anti-oxidative effects in human and animal cells [24]. Other important nutrients identified in *C. citratus* extract include carbohydrates, protein, and fat. These nutrient-rich constituents are precursors of erythropoiesis, and the anti-oxidative constituents can alleviate GV-induced oxidative stress and suppress other pathophysiological processes leading to GV-induced haematotoxicity.

In a previous study [10], the authors showed findings

similar to those of the present study: that consumption of infusions prepared from *C. citratus* extracts was associated with a significant increase in PCV, HB, and RBC in human volunteers.

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., [25], Koh et al., [15], and Rahim et al. [26] 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., [27] have observed that *C. citratus* extract produces marked attenuation of isoprenoid-induced cardiotoxicity and lipid peroxidation in rats. Common findings in these studies were improvement in the levels of antioxidant enzymes (SOD, CAT, and GSH) and suppression of the oxidative stress marker MDA. Findings of the present study are consistent with the aforementioned studies and provide support for the antioxidant potential of *C. citratus* decoction in mitigating GV-induced haematotoxicity.

While the erythropoiesis–modulated haemocytic variables significantly decreased in GV–alone group, total WBC and differentials showed the converse. The increase in total WBC was largely due to increase in the numbers of circulating neutrophils, eosinophils, and lymphocytes. These findings are similar to those previously observed in acute and chronic inflammatory processes and confirm the induction of inflammation as a pathogenic step in GV – induced haematotoxicity. Studies by others have shown that exposure to GV could induce local inflammatory processes and subsequently chronic inflammation. This is evidenced by increase markers of acute inflammation such as increase mucosal neutrophils, mast cells, interleukin–8, leukocytosis, myeloperoxidase, and glutathione [28, 29].

Neutrophil polymorphs are the first cells involve in many acute inflammatory lesions, followed by increase monocyte counts which become phagocytic and large in tissues and are called macrophages. Lymphocytes increase in the later stages of acute inflammation and persist in chronic inflammation giving a mixed picture of acute and chronic inflammatory cells as observed in the present study. Conversely, co-administration of *C. citratus* decoctions to rats in G3, G4 and G5 and Vitamin C to G6 caused decreases in total WBC and fluctuations in WBC differentials.

These findings confirm a previous human study [10] with similar results, and indicate the anti-inflammatory potential of *C. citratus* extract as previously reported. Carbajal et al., [30] found that hot water extract of dried *C. citratus* leaves elicited anti-inflammatory effects against the carrageen-induced edema. Likewise it was demonstrated that, intraperitoneal (i.p) administration of 5 ml of *C. citratus* oil suppressed leukolyte recruitment into the peritoneal cavity including neutrophil accumulation [13]. Also, 0.0125% to 0.1% *C. citratus* oil was found to suppress the activation of neutrophils by the tumor necrosis factor (TNF-α) [13].

In a related study by Gbenou et al. [31], *C. citratus* EO displayed significant dose dependent edema (formol-induced) inhibition effect over time, and a strong analgesic and antipyretic properties similar to that induced by 50mg/kg of acetyl salicylate, further demonstrating its anti-inflammatory effect. The mechanisms underlying the anti-inflammatory actions of *C. citratus* extract is posited to include inhibition of a complex immune-inflammatory mechanism and prostaglandin biosynthesis leading to pyrogens and prostaglandins release respectively [31]. These effects are comparable to the effect of salicylate drugs and non-steroidal anti-inflammatory drugs [31, 32]. Also, *C. citratus* EO is conceived to block the excitation of neuronal ends induced by pro-inflammatory substances. Aldehyde and Ketone molecules are the major molecules in *C. citratus* EO responsible for its anti-inflammatory effects.

5. Conclusion

The results of this study demonstrate that *C. citratus* decoction has ameliorative effects on GV-induced oxidative stress and haemato-toxicity in rats owing to its anti-oxidative, anti-inflammatory and erythropoiesis boosting activities. The results also suggest a greater effect of gasoline-induced haematotoxicity on erythropoiesis-modulated haemocyte variables. This is consistent with the results of a previous study [10] and provides additional support for the hypothesis that the *C. citratus* mediated increase in PCV, HB, and RBC may be elicited by an effect on the stimulant cytokine EPO [10]. Further studies to evaluate the effect of *C. citratus* decoction on erythropoietin (EPO) activities are needed.

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