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Effect of *Cymbopogon citratus* Decoctions on Gasoline Vapour-induced Reproductive Toxicity in Female Rats

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Aims: Recent research indicates that plant molecules, particularly those that are rich with antioxidant, anti-inflammatory and immune modulatory constituents, can modify and prevent the detrimental effects of gasoline compounds on reproductive endpoints. However, whether *C. citratus* decoction can alleviate gasoline vapour (GV)-induced derangement of female reproductive hormones has not yet been documented. In this study, the capacity of *C. citratus* decoction was evaluated for its ability to alleviate GV-induced reproductive toxicity in female rats.
Study Design: Seventy-two female Wistar albino rats weighing 185 ± 11.2 g were placed into six groups (n = 12 per group): the control (group 1, G1), GV alone (G2), GV plus *C. citratus* decoction (500 mg/kg; G3), (1000 mg/kg; G4), (1500 mg/kg; G5), and GV plus vitamin C (200 mg/kg; G6).
Place and Duration of Study: Department of Physiology, University of Uyo, Uyo, Akwa Ibom State, Nigeria. All groups were treated for 35 days.
Methodology: Serum levels of the female reproductive hormones progesterone (P_3) estradiol (E_2), luteinizing hormone (LH), and follicle stimulating hormone (FSH) as well as superoxide dismutase (SOD) and malondialdehyde (MDA; an oxidative stress marker) in the animals were assessed using standard procedures.
Results: The results showed that GV significantly ($p < 0.05$) decreased serum levels of P_3, E_2, LH,

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FSH, SOD and increased serum MDA levels compared to the levels in the control animals. However, co-administration of *C. citratus* at different doses to the animals in G3, G4, and G5 and vitamin C to the animals in G6 dose-dependently significantly ($p < 0.05$) increased the levels of the GV-reduced reproductive hormones and antioxidant enzyme and decreased the GV-increased oxidative stress marker levels to levels similar to those in the control group.
Conclusion: Thus, *C. citratus* decoction has an ameliorative effect on GV-induced reproductive dysfunction and oxidative stress.

Keywords: Attenuation; lemongrass; hydrocarbon; rats; reproductive toxicity.

1. INTRODUCTION

Gasoline is a mixture of several hydrocarbons, including aliphatics, aromatics, and several other branched saturated and unsaturated hydrocarbons, as well as many other additives. It is a volatile liquid and a ubiquitous hydrocarbon pollutant in the environment that is a particular problem for those living in close proximity to production, distribution, and retailing stations. Gasoline constituents particularly benzene, toluene, ethyl benzene, and xylene (BTEX) compounds are toxic to humans, and can affect many bodily functions [1,2].

Gasoline exposure is high among workers in oil exploration and retailing industries, and even in the general population during refuelling at self-service gasoline stations. In fact, it is estimated that 100 million people are exposed to gasoline constituents for a few minutes per week and approximately 100 minutes per year [3].

Interestingly, a toxic dose of gasoline can be inhaled within a very short period of time (seconds) [4]. It has been postulated that the air above an open barrel of gasoline in an unventilated out house on a “hot” day contains approximately 25,000 parts per million (ppm) of gasoline, the air around a tanker during bulk loading contains between 50–320 ppm, and the air around a petrol pump at service stations during vehicle refuelling contains 20–200 ppm [5,6].

Experimental and clinical data have established a close association between exposure to gasoline compounds and adverse reproductive endpoints [7,8,9], including changes in sexual behaviour, abnormal menstrual patterns and sex hormone profiles [10], decreased fertility, and interference with foetal development [11], leading to spontaneous abortion, birth defects, and intrauterine growth retardation [11]. Ugwoke et al. [8] reported structural disruptions in the uterus, fallopian tubes, and ovaries in female rats and spermatocytic arrest, chromosomal abnormalities, and altered fertility in male rats acutely exposed to GV. Increased risks of cytogenetic alterations and mutagenic effects in both somatic cells and embryonic tissues have also been reported [12]. A recent study suggested that medicinal plant products could ameliorate gasoline compound (BTEX)-induced ovarian disorders. Indeed, gasoline compounds are regarded as environmental endocrine and reproductive toxicants/disruptors [13,14] that can modulate or interfere with the production, release, transport, metabolism, binding, actions, and elimination of natural hormones in the body [15,16].

Although the proposed molecular mechanisms through which GV induces reproductive toxicity may vary [17], including initiation of oxidative cell injury [18], suppression of rapidly growing cells (e.g. bone marrow cells by gasoline metabolites) [19,20], and impairment of hypothalamic-pituitary axis functions, accumulated data favour the induction of oxidative stress as the central pathophysiological mechanism [18].

Oxidative stress disturbs the antioxidant defence system, and through several intermediary mechanisms, it affects numerous bodily functions, including reproductive endpoints. Consequently, supplementation with antioxidants (synthetic or natural) may abate or reverse GV-induced reproductive toxicity. Several synthetic antioxidant minerals and vitamins, including vitamins A, C, and E, have been used to mitigate the deleterious effects of GV exposure in experimental models [21-24].

Accordingly, Uboh et al. [17] studied the effect of vitamin C on GV-induced reproductive toxicity and found that supplementation with vitamin C significantly reversed the reduced serum levels of sex hormones (progesterone and estradiol) to levels within the normal range and had an ameliorative effect on ovarian tissues in exposed

female rats. Their findings are consistent with several other studies in the literature [7-9].

Because of the inability of synthetic antioxidant monotherapies to address the pleotropic effects of gasoline metabolites on the reproductive system and the drawbacks of multiple drug therapies, there is a fervent search for non-pharmacological means, specifically natural products, to reduce gasoline-induced reproductive toxicity and circumvent the limitations of multiple drug therapy. The numerous bioactive constituents present in natural products could mitigate the multi-dimensional effects of gasoline metabolites in gasoline-induced reproductive toxicity. This hypothesis is supported in a study conducted by Sirotkin et al. [2]. In that study, the authors found that administration of an extract from a medicinal plant (Yucca) restored ovarian cell function and attenuated the toxic effect of gasoline (BTEX) compounds. *Cymbopogon citratus* (*C. citratus*) is one such plant that has been explored for its antioxidant constituents and potentials [25].

C. citratus belongs to Poaceae family of plants and is widely distributed worldwide, especially in tropical and sub-tropical countries. It contains diverse bioactive constituents, including phytochemicals (saponins, tannins, flavonoids, phenols, anthraquinones, alkaloids, and deoxysugars) [26]; nutrients (proteins, moisture, ash, crude fibre, fat, and carbohydrates); electrolytes (potassium, calcium, copper, magnesium, manganese, selenium, phosphorus, iron, and zinc); and vitamins (folate, niacin, pyridoxine, and vitamins A, C, and E), and essential oil constituents (myrcene, neral, geraniol, linalool, limonene astral) [27]. Most of these constituents have recognized antioxidants anti-inflammatory and immune boosting effects.

However, whether *C. citratus* can ameliorate GV-induced reproductive toxicity has not yet been documented. Therefore, the present study aimed to accurately assess the effect of *C. citratus* decoction on gasoline-induced reproductive toxicity in female rats.

2. MATERIALS AND METHODS

2.1 Identification and Extraction

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 in the Department of Botany at the University of Uyo. A voucher specimen (IDUHH4686/Uyo) was deposited in the herbarium for reference purposes. 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℃. 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℃ and was dissolved in physiological saline at 100 mg/mL.

2.2 Phytochemical Screening of *C. citratus* Leaf Extract

The phytochemical analysis of the leaf extracts was carried out using standard procedures to determine the presence of tannins, phenolics, saponins, alkaloids, deoxysugars, and anthraquinones [28,29]. The concentrations of phytoconstituents were determined as described by Hajir et al. [30].

2.3 Experimental Animals

Seventy-two mature female Wistar albino rats weighing 185 ± 11.2 g were obtained from the animal house at the Faculty of Basic Medical Sciences, University of Uyo, Nigeria. The rats were divided into six groups with 12 rats per group, as follows:

Group 1: Control group, unexposed
Group 2: Test group exposed to GV only
Group 3: Test group exposed to GV and concomitantly treated with *C. citratus* decoction (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
Group 6: Test group exposed to GV and concomitantly treated with vitamin C (200 mg/kg)

2.4 Determination of LD50

The median lethal dose (LD50) was determined using standard methods [31]. From the LD50

(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℃], 45% humidity, with a 12-h light/dark cycle). All animals were fed normal rat pellets 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.

2.5 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. 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* decoction, respectively, while rats in group 6 were treated with 200 mg/kg (i.e. the normal prophylactic dose) of vitamin C [17]. 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.

2.6 Biochemical Assay

After the exposure period, the rats were sacrificed by anaesthetizing with chloroform. Blood was collected by cardiac puncture for biochemical analyses, including measurement of serum female reproductive hormone profile (progesterone [P3], estradiol [E2], follicle stimulating hormone [FSH], and luteinizing hormone). Serum malondialdehyde (MDA) and superoxide dismutase (SOD) levels were determined using the double heating method of Draper and Hadley as described elsewhere [32].

2.7 Statistical Analysis

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 reproductive toxicity of GV. Differences with p values less than 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

The result of this study showed that exposure to gasoline compounds is a significant risk for reproductive function impairment, and that, the use of plants and plant products, particularly those with potent antioxidant, anti-inflammatory and immune modulatory compounds (such as *C. citratus*) can alleviate GV-induced adverse reproductive endpoints. Evidence for this contention is twofold: first, the significant decreases in the serum levels of reproductive hormones (E_2, P_3, LH, and FSH) and antioxidant enzyme (SOD) and a significant increase serum MDA in animals exposed to GV alone compared to the corresponding levels in control animals; second, the reversal of these changes following the co-administration of *C. citratus* leaf decoction at 500 mg/kg, 1000 mg/kg, and 1500 mg/kg and vitamin C to animals in groups 3, 4, 5 and 6, respectively.

These results confirm previous speculation that gasoline compounds down regulate ovarian steroid hormones levels via several unexplained mechanisms, including inhibition of increased hypothalamic GnRH/pituitary gonadotropin production [17] as evidence by the significant decrease in E_2, P_3, LH, and FSH levels.

These observations corroborate previous studies showing that inhalation of toluene by rats was

associated with a reduction in the hypothalamic levels of gonadotropins and alteration of FSH and LH. Likewise, xylene exposure led to decrease in plasma P_3 and E_2. Oxidative cell injury is the postulated pathophysiologic mechanism underlying gasoline compound-induced reproductive toxicity [18]. For example, one gasoline compound, toluene, has been shown to decrease glutathione peroxidase activity and increase lipid peroxidation in ovarian tissues, resulting in ovarian cell damage [18]. Therefore, administration of antioxidant substances (synthetic or natural) could inhibit or even reverse the toxic effects of gasoline-induced oxidative stress and subsequent reproductive dysfunction. This hypothesis is supported in studies by Uboh et al. [17] and Sirotkin et al. [2], which showed that vitamin C had a ameliorative effect on GV-induced impaired reproductive function in rats, and that Yucca extract modified and abated the effect of benzene on ovarian hormone release, respectively.

Our results showed increased oxidative stress in animals exposed to GV alone as evidenced by a significant increase in MDA which is a common marker of lipid peroxidation [33] and decrease in antioxidant enzyme (SOD) levels, evidence of oxidative stress. Elevated MDA indicates peroxidative damage to cell membranes and other lipid-derived macromolecules [4]. Similar to previous studies [4], an increase in the lipid peroxidation product (MDA) was correlated with decrease in superoxide dismutase (SOD). This creates an imbalance between pro-oxidants and antioxidants, which has been shown to be associated with adverse female reproductive endpoints [34], including a reduction in the reproductive hormone profile [35]; increased risk of abortion; low birth weight [35], and intrauterine growth retardation (IUGR) [11]. Others include structural disruption in the uterus, fallopian tubes, and ovaries [8]; disordered menstrual pattern [10]; ovarian hypo and hyperplasia [36,37]; and reduced hypothalamic levels of gonadotropin releasing hormone (GnRH) and plasma gonadotropin levels [2,38].

In support of this observation, Su et al. [39] have also reported that some medicinal plants products, either alone or in combination, have the potential to restore reproductive organ degeneration by improving the histo-morphology of the ovaries, uterus, and vagina and modulating E_2 imbalance and neurotransmitter disorders.

Likewise other studies have shown that medicinal plant products can abate gasoline (BETX) compounds-induced ovarian dysfunction, including changes in the release of P_3, insulin growth factor-1 (IGF-1), and prostaglandin F (PGF), and prevent the effects of benzene on ovarian hormone (P_3 and E_2) release.

C. citratus and vitamin C have recently received attention as natural and synthetic antioxidants, anti-inflammatory and immune modulators respectively, with great potential to alleviate drug/chemical-induced oxidative stress, inflammation and immune perturbation and associated disorders [17,25,40-42]. In a study by Rahim et al. [41] to evaluate the effect of *C. citratus* aqueous extract and vitamin E on hydrogen peroxide-induced reproductive function impairment in male rats, *C. citratus* significantly increased the body, testicular and epididymal weight, and testosterone and GSH levels. Improvement in values of semen characteristics and reduction in serum and testicular MDA levels were also observed. Supplementation with the *C. citratus* aqueous extract also caused improvement in testicular histopathological alteration. The antioxidant activity of *C. citratus* extract is due to its constituent phytochemicals (saponins, tannins, polyphenols, and flavonoids), vitamins (vitamins A, C, and E), minerals and trace elements (Zn, Cu, Mg, Se, and Fe), and essential oil components (geraniol, myrcene, linalool, limonene, β-ionone, and citral) [43] (Table 1).

Some of these constituents provide direct antioxidant effects, while others, such as Se, Cu, Mn, and Zn, do not have antioxidant effects themselves, but are required for the activity of some antioxidant enzymes [32]. Vitamin C acts as a first-line antioxidant in plasma [44]. It inhibits LDL oxidation and maintains normal endothelial function. The rich antioxidant nutrient composition of *C. citratus* has been exploited for the management of several drug/chemical-induced oxidative stress-mediated adverse health effects including cisplantin, carbon tetrachloride, and hydrogen peroxide-induced liver damage [25,39,40] respectively, and isoprenoid-induced cardiotoxicity and lipid peroxidation [45] in rats. Here we showed that *C. citratus* leaf extract has an ameliorative effect on GV-induced reproductive system dysfunction, partly due to its rich antioxidative anti-inflammatory and immune modulatory constituents and their activities (Table 2).

Table 1. Phytochemical constituents of *C. citratus* leaf extract

Composition	Tannins	Saponins	Flavonoids	Alkaloids	Phenols	Anthraquinones
Concentration	++	++	++	+	++	+

Key: + Present, ++ Moderate

Table 2. Effect of *C. citratus* decoctions on GV -induced derangement in serum female sex hormone profile

Groups	FSH (mμ/mL)	LH (m μ /mL)	Estradiol (ng/mL)	Progesterone (ng/mL)	SOD (mmol/L)	MDA (mmol/L)
G1 (control)	5.14±2.02^{b}	13.12±2.47^{b}	56.22 ± 6.72^{c}	36.08 ± 8.22^{c}	10.530±1.240^{b}	5.81± 0.55^{a}
G2	2.32±1.04^{a}	8.06 ± 3.14^{a}	25.24 ± 5.22^{a}	16.02 ± 9.11^{a}	7.064±1.520^{a}	11.10±0.41^{c}
G3	3.08±0.99^{b}	10.14±2.02^{b}	28.38±18.20^{a}	20.82 ± 6.54^{a}	7.840 ± 1.380^{a}	9.03 ± 0.45^{c}
G4	3.86±0.52^{b}	10.84±1.82^{b}	32.42 ± 6.47^{b}	26.42 ± 5.85^{b}	8.24 ± 2.24^{a}	7.42 ±0.39^{b}
G5	4.06±1.22^{b}	11.24±1.22^{b}	38.06±3.04^{b}	28.26 ± 4.61^{b}	9.76 ± 2.81^{b}	7.07 ±0.39^{b}
G6	4.16±0.78^{b}	12.06±3.72^{b}	40.42±4.82^{b}	30.22 ± 5.88^{c}	10.23 ± 2.88^{b}	4.18 ±3.27^{a}

Similar letters/alphabet mean not significantly different ($p > 0.05$),
Different letters mean significantly different ($p < 0.05$),Values are reported as mean ± SD.
FSH: follicle stimulating hormone; LH: luteinizing hormone; SOD: superoxide dismutase; MDA: malondialdehyde

4. CONCLUSION

Results of the present study indicate that *C. citratus* decoction has an ameliorative effect on GV-induced reproductive dysfunction and oxidative stress.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The author hereby declares that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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