



Original Research Article

***Cymbopogon citratus* Stapf (DC) extract attenuates gasoline vapour-induced low-triiodothyronine syndrome, oxidative stress and lipid peroxidation in rats**

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ABSTRACT

Cymbopogon citratus Stapf (DC) (*Lemongrass*) is a widely distributed aromatic perennial plant that has the potential to mitigate xenobiotic-induced systemic disorders. However, whether *C. citratus* has any ameliorative effect on gasoline vapour (GV)-induced thyroid gland dysfunction has not been previously evaluated. Therefore, the present study aimed to assess the effect of *C. citratus* leaf decoctions on GV-induced thyroid gland disorders. Thirty-five Albino rats were segregated into 5 groups (n=7 per group). Animals in group 1 served as unexposed control, while animals in group 2 were exposed to GV alone for 4 weeks. Animals in groups 3, 4 and 5 in addition to being exposed to GV for 4 weeks, were treated with different concentrations of *C. citratus* leaf extracts for 2 weeks. Animals exposed to GV alone had significant decrease in serum levels of catalase, while serum levels of malondialdehyde significantly ($p < 0.05$) increased. Serum levels of thyroid stimulating hormone significantly ($p < 0.05$) increased and decreased in male and female rats respectively exposed to GV alone. Serum levels of triiodothyronine decreased in both male and female rats exposed to GV alone, whereas serum levels of thyroxine decreased only in female rats. In addition, exposure to GV alone caused significant alterations in the normal histo-morphology of the thyroid gland. Co-administration of *C. citratus* leaf decoctions caused reversal of these changes, as well. This study showed that *C. citratus* leaf extract has the potential to attenuate GV-induced thyroid gland disorders and oxidative stress due to its natural bioactive constituents.

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1. Introduction

Cymbopogon citratus (*C. citratus*) Stapf (DC), commonly known as lemongrass, is a tall aromatic perennial grass that grows worldwide, especially in the tropics and sub-tropics regions. It belongs to the Poaceae family, and has long slender leaves of about 90 cm long and 1.5 cm large (Ekpenyong, 2016). *C. citratus* has varied bio-constituents including phytochemicals (saponins, tannins, flavonoids, alkaloid, phenols and anthraquinones), minerals (calcium, iron,

magnesium, copper, manganese, selenium and zinc), vitamins (vitamins A, C, E, folate thiamine, niacin) and essential oil (EO) constituents (geranial, neral, linalool, nerol limonene, citronellol, geranyl acetate, catechol, luteolin, caffeic acid, apigenin, luteolin and kaempferol) (Ekpenyong et al., 2015). Because of its rich nutrient constituents, *C. citratus* is widely consumed and used in aromatic drinks and traditional cuisines, including teas, non-alcoholic beverages, ice creams, candies, pastries, and as flavoring and preservative agents in confections. Its EO is used as fragrance in the manufacturing of

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perfumes, soaps, detergents, face cleaners and creams (Praditvarn and Samhandharaksa, 1990). Recent evidence indicates the potential use of *C. citratus* essential oil as an alternative to synthetic food preservatives in domestic and industrial applications (Ekpenyong and Akpan, 2015). *C. citratus* decoction or infusion has long been used in traditional and Ayurvedic medicine for the treatment of oxidative stress (OS) related disorders (Sari et al., 2017), immune system dysfunction (Bao et al., 2015), bacterial (Ekpenyong, 2015), protozoal and fungal infections, cancer, inflammation (Yoon et al., 2010; Bachiega and Sforcin, 2011), diabetic mellitus (Bharti et al., 2012) and hypertension (Ekpenyong and Osim, 2016). Others, include treatment of helminthic, influenza related and pneumonia infections (Ekpenyong and Akpan, 2015; Ekpenyong, 2016). A brief survey of literature indicates that *C. citratus* has the ability to ameliorate several animal models of xenobiotic-induced systemic toxicities including gasoline vapor (GV)-induced renal toxicity (Ekpenyong and Akpan, 2015), hepatotoxicity (Ekpenyong and Bassey, 2016), hematotoxicity (Ekpenyong, 2017a), dyslipidemia (Ekpenyong and Oyebadejo, 2016), reproductive disorders (Ekpenyong, 2017b) and hyperglycemia (Ekpenyong et al., 2015). The other capabilities of this plant include cisplatin, hydrogen peroxide and carbon tetrachloride-induced liver damage (Arhoghro and Kpomah, 2013; Saleh 2013; Koh et al., 2012). However, its effect on GV-induced thyroid dysfunction has not been documented to date. Gasoline is a refined product of petroleum consisting of several hydrocarbons, additives and blending agents. It is widely used domestically and industrially as fuel in vehicles and aircrafts. 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 several organs pathologies (Azari et al., 2012) such as thyroid gland disorders. Nowadays, thyroid gland disorders are common worldwide. In Africa and Nigeria in particular, they represent the second most common endocrine disorders after diabetes mellitus (Ogbera et al., 2007; Salamu et al., 2016). The pattern of presentation may range from abnormal serum level of thyroid hormones (hypothyroidism or hyperthyroidism), to neck swelling (simple or malignant enlargement) or pain (thyroiditis). However, regardless of presentation, they are debilitating conditions due to the associated morbidity, psychological impact and effect on health related quality of life (Salamu et al., 2016). The etiology of thyroid disorder is complex and multi-dimensional and may include environmental and nutritional factors (Ogbera and Kuku, 2015). From environmental perspective, exposure to a number of environmental chemical pollutants, e.g. goitrogens, endocrine disrupters (natural or synthetic) are known to cause injury or perturbations of thyroid function through direct or indirect effect on thyroid gland or

disrupt thyroid hormone synthesis (Singh et al., 2000) and/or metabolism. The management of thyroid disorders may involve surgical and pharmacological modalities which may be suboptimal in some cases due to several constraints; therefore the search for alternative therapy is growing in the scientific community. The aims of the present study were first, to assess the effect of exposure to GV on the biochemical indices of thyroid gland function and thyroid histomorphology, second, to assess the effect of co-administration of *C. citratus* leaf decoctions on GV-induced thyroid disorders.

2. Experimental

2.1 Collection and preparation of *C. citratus* leaf extract

Fresh *C. citratus* leaves were collected from the medicinal farm of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, in the month of March, 2016 a day prior to utilization. The leaves were identified and authenticated by a taxonomist in the Department of Botany at the University of Uyo, Uyo. Voucher specimen No. UUPH33A was deposited at the herbarium in the Department of Botany at the University. The leaves were washed and air dried at room temperature, then sliced into smaller sizes and pulverized into powdery form with manual blender. Ethanol extract was prepared by macerating 2 kg of the powder into 95% ethanol for 72 h. The solution was thereafter filtered through Whatman No.2 filter paper, and the filtrate was concentrated to dryness at 45 °C in water bath. The final solid extract was weighed with an electronic weighing scale (METTLER TOLEDO) model AG204. A total yield of 28% (0.56 kg) was obtained. The extract was stored in glass bottles in a refrigerator at a temperature of 4 °C and dissolved in physiological saline at concentration of 100 mg/mL for use.

2.2. Determination of phytochemical and nutrient constituents

The phytochemical and nutritional analysis of the crude leaf extract were carried out using standard procedures to ascertain the presence of alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones reducing sugar, cardiac glycosides and others (Trease and Evans, 1989). The phytochemical screening included both qualitative and quantitative analyses. While the qualitative analysis indicated the presence of the phyto-constituents, the quantitative analysis assessed their actual concentrations. The concentrations of the phyto-constituents were determined as described by Hajir et al. (2016).

2.3. Determination of Median Lethal dose (LD₅₀) of *C. citratus* leaf extracts

Forty albino mice were divided into three groups and used for the determination of the LD₅₀ as described by Lorkes et al. (1983). Briefly, through intraperitoneal route, different doses of the leaf extracts, ranging between 500 mg/kg and 5000 mg/kg, were administered to the different groups, and the animals were observed for evidences of toxicity such as writing, excitation, paw licking, decreased motor activity, decreased limb tone, increased respiration, gasping, coma and death. The LD₅₀ was calculated as the square root of the product of the maximum dose producing 0% death and the minimum dose producing 100% death. Thus,

$$LD_{50} = \sqrt{AB} \quad (\text{Eqn. 1})$$

Where (A) and (B) respectively account for maximum dose producing 0% death and minimum dose producing 100% death. Therefore, the former equation could be considered as follows.

$$LD_{50} = \sqrt{AB} = 2600 \times 2800 \quad (\text{Eqn. 2})$$

2.4. Selection and segregation of animals

Thirty five (17 male and 18 female) albino rats weighing 200-250 g were obtained from the animal house at the Faculty of Pharmacy, University of Uyo, Uyo, Nigeria, kept in well ventilated cages for seven days to acclimatize, fed with standard animals chow (Vital Feeds, Grand Cereals Ltd., JOS), and finally allowed to have free access to water. After the acclimatization period, the rats were randomly divided into 5 groups (n=7 per group).

Group 1: Served as control group and animals were orally gavaged 2 mL of normal saline.

Group 2: Served as test group and animals were exposed to GV alone.

Group 3: Served as test group and animals were exposed to GV and concomitantly treated with low dose of the extract (0.6 mL) or (269.82 mg/kg).

Group 4: Served as test group and animals were exposed to GV and orally treated with medium dose of the extract (1.2 mL) or (539.63 mg/kg).

Group 5: Served as test group and animals were exposed to GV and orally treated with high dose of the extract (2 mL) or (809.45 mg/kg).

2.5. Exposure of the experimental animal to gasoline vapor

The animals in groups 2, 3, 4, and 5 were exposed to unleaded gasoline purchased from a Nigerian National Petroleum Cooperation (NNPC) refueling station on Itam-Ikot Ekpene Road in Uyo, Nigeria. They were exposed to GV in the exposure chambers (60 x 80 x 100) cm³ for 4 weeks. Briefly, 200 mL of gasoline was poured into a 250 mL beaker and the quantity evaporated after 8 hours was calculated as the difference between the initial and final volumes. After the exposure period, *C. citratus* leaf extracts were orally administered to animals in groups 3, 4 and 5 as low, medium and high doses, respectively for 2 weeks.

2.6. Biochemical Analysis

After the exposure period, animals were again weighed and anaesthetized with chloroform. Blood was collected by cardiac puncture for biochemical analysis including the determination of serum levels of thyroid hormones (triiodothyronine (T₃), thyroxine (T₄), and thyroid stimulating hormone (TSH) using Reitman and Frankel calorimetric methods.

2.7. Determination of serum malondialdehyde and catalase levels

The serum malondialdehyde (MDA) level was determined by the double heating procedure as described by Draper and Hadley (1990). In this relation, 2.5 milliliters of trichloroacetic acid solution (10%) was added to 0.5 mL of serum in each centrifuge tube. Thereafter, the tubes were heated in a water bath at 100 °C for 15 minutes, cooled at room temperature and centrifuged at 1000_{xg} for 10 minutes. Two milliliters of the supernatant was mixed with 1 mL of thiobarbituric acid (TBA) solution (0.67% W/V) in a test tube. The tube was further heated at 100 °C for 15 minutes in a water bath and thereafter, the solution was analyzed spectrophotometrically at 532 nm. This was followed by computing the absorbance coefficient of the TBA-MDA complex and calculating the serum levels of MDA. Serum CAT was also determined spectrophotometrically.

2.8. Tissue processing and staining

The fixed tissues were dehydrated with different concentrations of alcohols (two changes of 70% and 95% of alcohol for 2 hours each and two changes of 100% or absolute alcohol for 2 h), cleared with xylene and impregnated with two changes of paraffin wax. The paraffin block was sectioned at 5 μm after cooling with ice bar. Slides were duly processed including rubbing with thymol containing albumen, staining with Haematoxylin and Eosin stains, deparaffining in two changes of xylene and hydrated through graded alcohols. Processed slides were carefully observed under the microscope at magnification of X400 and photomicrographs were obtained using the microscope camera linked to a computer.

2.9. Statistical analysis

One-way analysis of variance (ANOVA) followed by Duncan test were used to establish differences in the parameters between groups after computing the mean ± standard deviation (SD) of these parameters. The p value < 0.05 was considered statistically significant and the Statistical Package for Social Sciences (SPSS version 22.0) was used for data analysis.

3. Results and Discussion



3.1. Phytochemical and nutrient constituents of *C. citratus* leaf extract

The phytochemical screening of *C. citratus* leaf extract showed that it contains high concentrations of saponins, alkaloids, phlobatonnins, flavonoids,

moderate level of phenols and absence of tannins. Quantitatively, the concentrations of these phytoconstituents were as follows; 45%, 22.5%, 19%, 0.19% and 0.11% for saponins, phlobatonins, alkaloids, flavonoids and phenols, respectively (Table 1).

Table 1

Phytochemical constituents of *C. citratus* leaf extracts.

Constituents	Qualitative quantification	Quantitative quantification	Per 100 g
Saponins	+++	45%	45 g
Phlobatonnins	+++	19%	19 g
Alkaloids	+++	22.5%	22.5 g
Flavonoids	+++	0.19%	0.19 g
Cardiac glycosides	+++	8.7%	0.08 g
Phenols	++	0.11%	0.0011 g
Tannins	-	-	-

++=moderate concentration

+++=high concentration

These findings corroborate our previous representation (Ekpenyong et al., 2014) in which similar species of *C. citratus* herbs obtained from the same environment and subjected to similar thermal processing and extraction methods revealed the presence of phytochemical constituents similar to the above mentioned, and in relative similar concentrations. Several nutrients including electrolytes viz. sodium (Na⁺), potassium (K⁺), magnesium (Mg⁺), minerals (copper (Cu), manganese (Mn), selenium (Se), phosphorus (P), iron (Fe) along with vitamins like folate, niacin, pyridoxine, vitamins A, C and E were also detected (Ekpenyong et al., 2014).

The characterized bio-constituents in *C. citratus* leaf extract were responsible for the observed protective effects against GV-induced thyroid dysfunction in groups supplemented with the extracts, given that the protective effects were found only in these groups, and in accordance with the growing knowledge that the vast and versatile pharmacological activities of many medicinal plants are basically dependent on their phytochemical and nutrient constituents (Hussein and El-Assary, 2018).

3.2. Changes in oxidative stress (OS) markers following exposure to GV and treated with *C. citratus* leaf extracts

From the standpoint of pharmaco-dynamics, a recent review of literature suggests that GV disrupts endocrine functions through 3 patho-physiological processes: induction of OS, inflammation and immune system dysfunction (Ekpenyong and Akpan, 2017). Although immune system disorders and inflammation have

been found to contribute to GV-induced endocrine disorders, OS appears to play a central etiopathogenic role. Accordingly, significant increased and decreased serum levels of MDA and CAT respectively in the GV alone group, compared to levels in the control group, indicate OS mediated effects (Fig.s 1A&B and 2A&B). However, oral administration of *C. citratus* leaf extracts to GV exposed animals caused a reversal of these disorders. This observation suggests that *C. citratus* bioconstituents have the potential to attenuate GV-induced OS and are consistent with previous studies that showed that natural products that cause an increase antioxidant enzyme activities and a decrease in lipid peroxidation as observed in the present study may protect tissues or organs against xenobiotic-induced toxicity (Koh et al., 2012; Ekpenyong and Akpan, 2017). The various phytochemical constituents from *C. citratus* leaf extracts such as saponins, flavonoids, phenols and alkaloids have been reported for their antioxidative and chemopreventive activities. Interestingly, it was observed that the GV-mediated changes in markers OS were more marked in male than female rats supporting previous studies that demonstrated the existence of gender-related differences in the activities of cellular antioxidant enzymes in various important organs including the thyroid gland due to gender-related differences in various estrogen levels (Chen et al., 2011; Kander et al., 2017). Estrogen is known to act as an antioxidant by scavenging free radicals due to the presence of phenolic hydroxyl group in its structure (Kander et al., 2017).

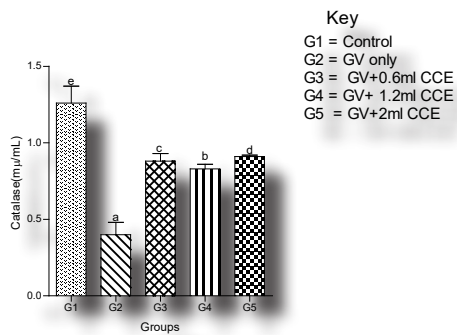
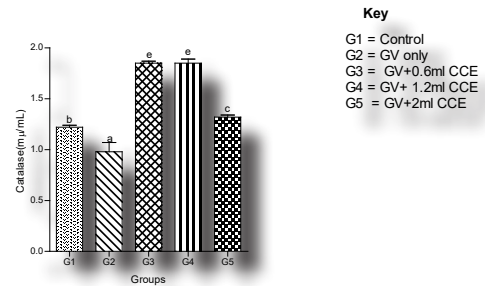
(A)

(B)


Fig. 1. A & B: Effect of *C. citratus* decoctions on GV-induced changes in serum catalase levels in male (A) and female (B) rats. Values are expressed as Mean \pm SEM at $p < 0.05$ relative to control. Different letters indicate significant difference, while similar letters represent non-significant difference.

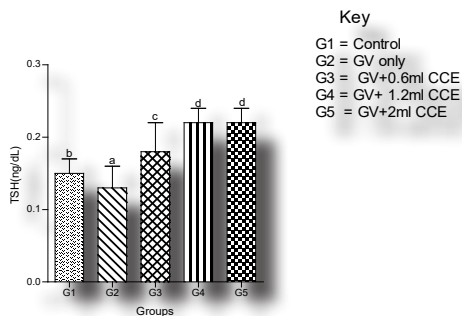
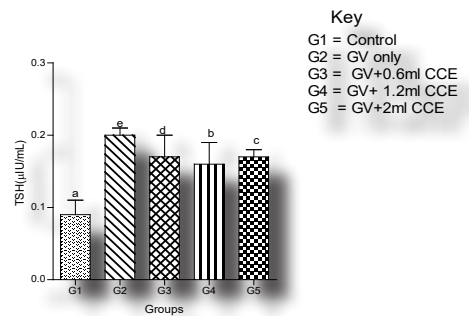
(A)

(B)


Fig. 2. A & B: Effect of *C. citratus* decoctions on GV-induced changes in serum MDA levels in (A) Male, (B) Female rats. Values are expressed as Mean \pm SEM at $p < 0.05$ relative to control. Different letters indicate significant difference, while similar letters represent non-significant difference.

3.4. Ameliorative effects of *C. citratus* extracts on thyroid function indices of GV exposed animals

Also, administration of *C. citratus* extracts to animals in GV-exposed groups reversed the GV-induced thyroid disorders, supporting the anti-oxidative

effects of the extract. For instance, exposure to GV alone caused a significant decrease in serum TSH levels in female rats, while a significant increase in serum TSH level was observed in male rats. Administration of *C. citratus* extracts reversed these changes in both male and female rats (Fig. 3A & B).

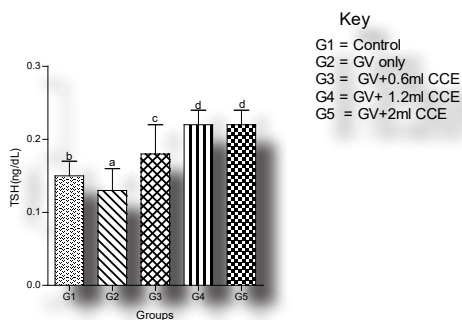
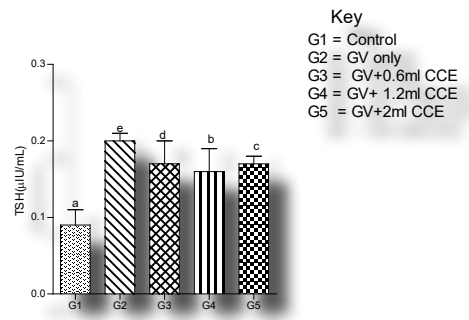
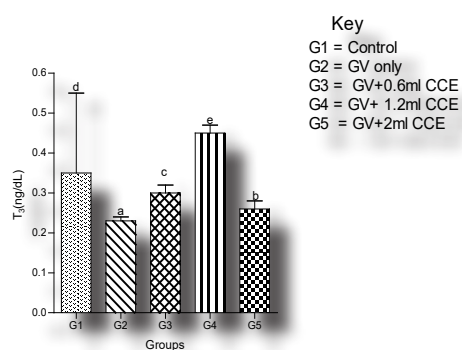
(A)

(B)


Fig. 3. A & B: Effect of *C. citratus* decoctions on GV-induced changes in serum TSH levels in (A) Female, (B) Male rats. Values are expressed as Mean \pm SEM at $p < 0.05$ relative to control. Different letters indicate significant difference, while similar letters represent non-significant difference.

In a similar manner, serum levels of T3 decreased significantly ($p < 0.05$) in animals exposed to GV alone

compared to levels in the control and other treatment groups (Figs. 4A & B).

(A)



(B)

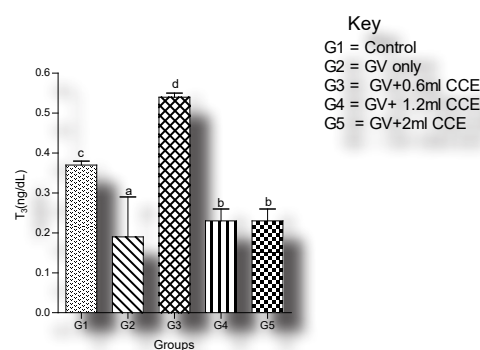
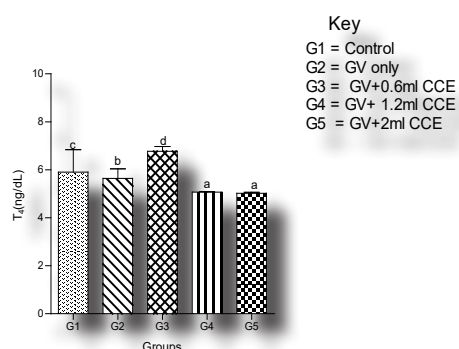


Fig. 4 A & B: Effect of *C. citratus* decoctions on GV-induced T3 disorders in (A) Male, (B) Female rats. Values are expressed as Mean \pm SEM at $p < 0.05$ relative to control. Different letters indicate significant difference, while similar letters represent non-significant difference.

The results also showed that serum T4 level decreased significantly ($p < 0.05$) in male rats in groups 2, 4 and 5, but increased significantly in group 3. In female

rats, serum T4 levels increased significantly ($p < 0.05$) in groups 2 and 4 and decreased in other treatment groups (Fig.s 5A & B).

(A)



(B)

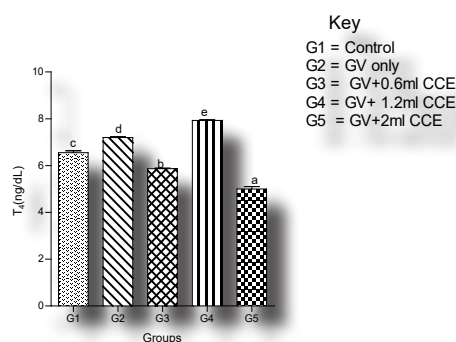


Fig. 5 A & B: Effect of *C. citratus* decoctions on GV-induced T4 disorders in (A) Female, (B) Male rats. Values are expressed as Mean \pm SEM at $p < 0.05$ relative to control. Different letters indicate significant difference, while similar letters represent non-significant difference.

These observations are consistent with a plethora of research which shows that medicinal plants are good sources of phytochemical and nutritional compounds that possess robust antioxidant activities (Altemimi et al., 2017; Mohammadhosseini et al., 2017; Wansi et al., 2018; Sarker and Nahar, 2018; Venditti and Bianco, 2018; Wansi et al., 2019; Mohammadhosseini et al., 2019). A growing number of multi-level studies have shown that the phytochemical and essential oil constituents from *C. citratus* leaf extracts have impressive antioxidant effects, and have been found to ameliorate many drugs/chemicals induced OS and associated systemic disorders, including cisplatin-induced hepatotoxicity (Arhoghro, 2012), carbon tetra chloride-induced hepato-toxicity (Koh et al., 2012), hydrogen peroxide-induced liver injury (Rahim et al., 2014), isoprenoid-induced cardio-toxicity and lipid-peroxidation in rats (Gayathri et al., 2011). Others, include GV-induced nephro-toxicity (Ekpenyong and Akpan, 2017), hepato-toxicity (Ekpenyong and Bassey, 2016), hematotoxicity (Ekpenyong, 2017a), reproductive toxicity (Ekpenyong, 2017b), metabolic disorders and cardiovascular disease risk reduction in rats (Ekpenyong et al., 2016). The antioxidant effects of *C. citratus* bioconstituents have also been found to cause a reduction in oxidative risk in post menopausal women (Gelatti et al., 2016). Attenuation of gentamycin-induced nephro-toxicity due to the

activities of flavonoid compounds in *C. citratus* leaf extracts (Ullah et al., 2013) and amelioration of adenine-induced nephro-toxicity (Said et al., 2019) have also been reported. Common features of these studies were improvements in serum levels of antioxidant markers (superoxide dismutase (SOD), glutathione (GSH), MDA, CAT), markers of inflammation e.g., inhibition of cytokine production, tumor necrosis factor alpha, production of interleukin 1beta, induction IL-6, suppression of cyclooxygenase and inhibition of prostaglandin production and histo-pathological disorders due to its phenolic content (Francisco et al., 2013; Said et al., 2019). In one study, *C. citraus* leaf extracts restored the activities of all the antioxidant enzymes including CAT, SOD and GSH which were initially suppressed by the paracetamol-induced toxicity (Cheel et al., 2005). According to Cheel et al. (2005) and Balakrishnan et al. (2015), the phytochemicals and essential oil constituents from *C. citratus* leaf extracts can act as radical scavengers, reducing agents, hydrogen donors and signet oxygen quenchers. The present study findings are corroborated by the above mentioned and fills a knowledge gap about the effect of GV on thyroid gland biochemical status and histomorphological features, as well as the chemopreventive effects of *C. citratus* leaf extracts (Fig.s 1-10).

Besides OS mediated effects, GV is also posited to cause damage to tissues through the induction of inflammatory reactions (Francisco et al., 2013; Said et al., 2019) and immune system dysfunction (Bao et al., 2015) probably due to the link between OS and immune dysfunction (Cachofeiro et al., 2008). Hence, *C. citratus* leaf extracts could have also exerted its ameliorative effects through its anti-inflammatory and immune system modulatory activities. This notion is supported by previous studies that demonstrated the anti-inflammatory and immune boosting activities of *C. citratus* leaf extract bioconstituents, especially the phenolic content (Hosch et al., 2003; Yoon et al., 2010; Xiao et al., 2015; Said et al., 2019). According to Hosch et al. (2003), *C. citratus* water extract inhibited the production of interleukin 1 beta (IL-1 β), but induced IL-6 production, while the EO prevented cytokine production *in vitro* due to the activities of linalool oxide and epoxy-linalool found in the water extract and geranial from the EO. Francisco et al. (2013) demonstrated the anti-inflammatory effect of *C. citratus* leaf extract and attributed it to the presence of high quantities of phenolic compounds in its chemical profile. In addition, in a study by Said et al. (2019), *C. citratus* leaf extract inhibited tumor

necrosis factor alpha as a marker of inflammation. *C. citratus* extract was reported to have prevented the release of IL-6, while citral from *C. citratus* EO inhibited IL-1 β , and IL-10 (Bachiega and Sforcin, 2011). Citral from *C. citratus* also caused the suppression of cyclooxygenase (COX)-2, an enzyme responsible for the prostaglandin (PG) synthesis (Katsukawa et al., 2010). Limonene found in *C. citratus* EO inhibited the production of PGE₂ (Yoon et al., 2010), while alpha-terpineol and alpha-caracrol-induced a higher COX-2 activity inhibition (Pongprayoon et al., 1997). Saponins inhibit circulatory and tissue rennin-angiotensin-aldosterone system (RAAS) (Hiwatashi et al., 2010; Chen et al., 2013). Bao et al. (2015) showed how a polysaccharide (CCPS) from *C. citratus* leaf extracts improved the immunity of tumor-bearing mice.

3.5. Changes in histomorphology of the thyroid gland following exposure to GV

The photomicrograph of a transverse section of the thyroid gland of the healthy rats in group 1 (control) showed normal histo-architectural arrangements of thyroid cells. The presence of thyroid follicles and tiny blood vessels were also observed (Fig. 6).

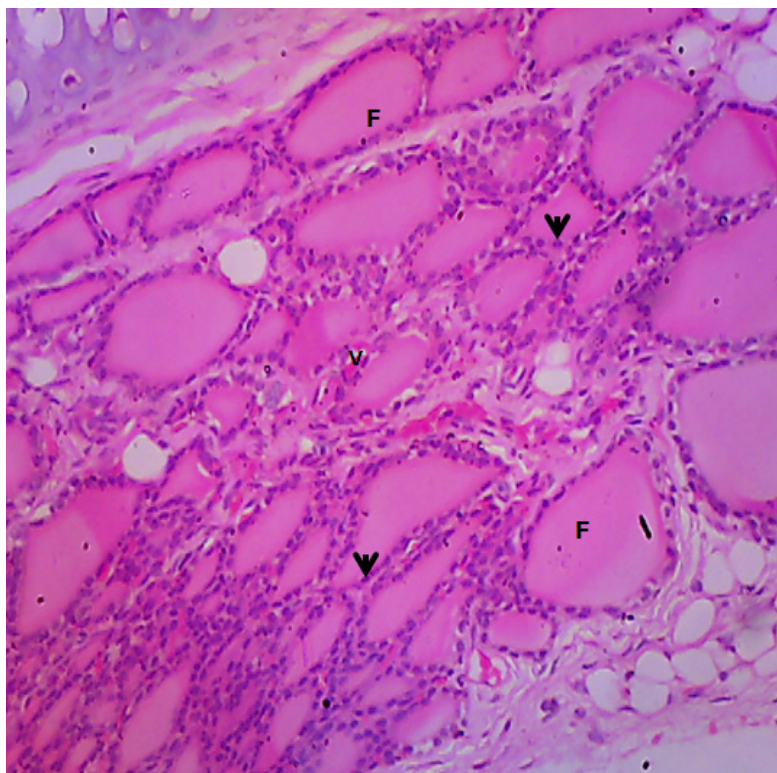


Fig. 6: Histological section of thyroid gland (Haematoxylin & Eosin Stain) of control group at magnification (X400) demonstrating thyroid follicles (F) lined by cubical follicular cells that exhibit rounded nuclei (arrow head), tiny blood vessels (V) present.

Whereas a transverse section of the thyroid gland of rats in group 2 showed an abnormal histo-architecture of thyroid cells including the presence of numerous compacted thyroid follicles with almost disappearing

colloids and hypertrophy of the thyroid cells. The histomorphological changes observed in thyroid gland sections of animals in the GV alone group compared to control group are typical of OS-mediated lesions (Fig. 7).

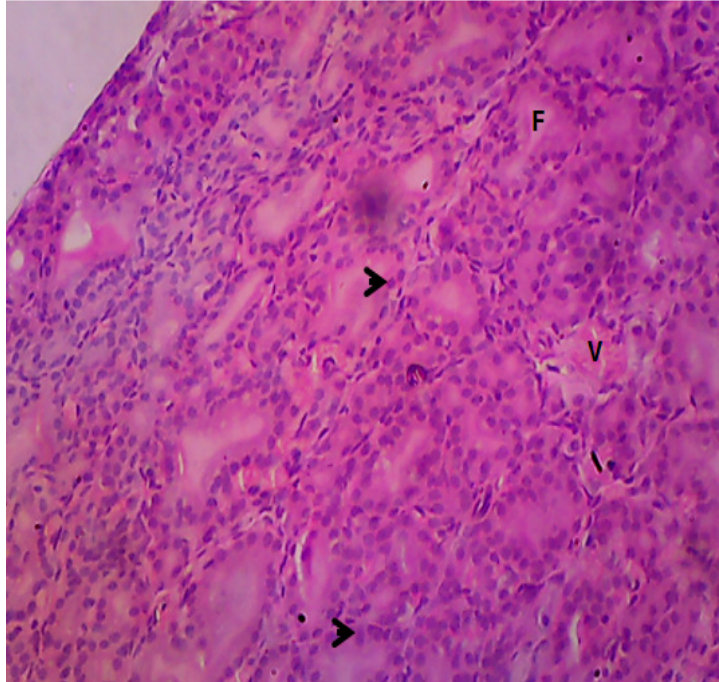


Fig. 7. Histological section of thyroid gland (Haematoxylin & Eosin Stain) of GV only group 2 at magnification (X400) demonstrating thyroid follicles (F) lined by cubical follicular cells that exhibit rounded nuclei (arrow head), and congested blood vessels (V). The section displays evidence of hypertrophy with numerous compacted follicles with almost disappearing colloid.

Pathophysiologically, OS-mediated decreased production of active hormone (T3), leads to negative feedback effect involving the stimulation of hypothalamic-pituitary axis and causing increase TSH production. High TSH level stimulates the deiodinase enzyme to increase the conversion of T4 to T3. This action caused an increase in the production of hydrogen peroxide which further leads to increase in OS burden to thyroid tissue causing more injury to thyroid cells and leading to the histomorphological changes observed in GV alone group in the present study.

3.6. Effect of different concentrations of *C. citratus* leaf extracts on the histomorphology of the thyroid gland of animals exposed to GV.

Treatment with different concentrations (269.82 mg/kg, 539.63 mg/kg and 809.45 mg/kg) of *C. citratus* leaf extracts caused improvements in GV-induced pathological changes in the histomorphology of thyroid gland including reactivation of thyroid follicles and blood capillaries (Figs 8 and 9), and numerous compressed thyroid follicles due to cellular hyperplasia (Fig. 10).

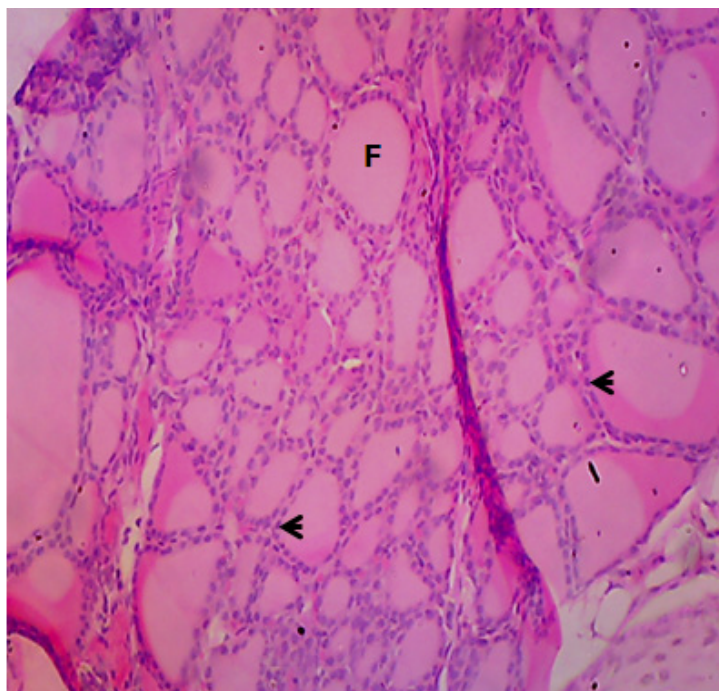


Fig. 8. Histological section of thyroid gland (Haematoxylin & Eosin Stain) of group 3 at magnification (X400) demonstrating thyroid follicles (F) lined by cubical follicular cells that exhibit

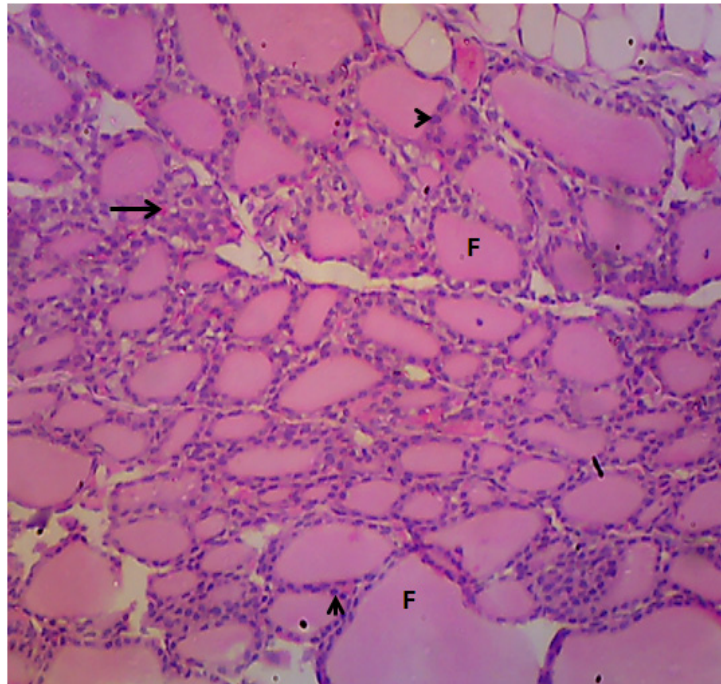


Fig. 9. Histological section of thyroid gland (Haematoxylin & Eosin Stain) of group 4 at magnification (X400) demonstrating thyroid follicles (F) lined by cuboidal follicular cells that exhibit rounded nuclei (arrow head). Note the tiny blood capillaries (arrow).

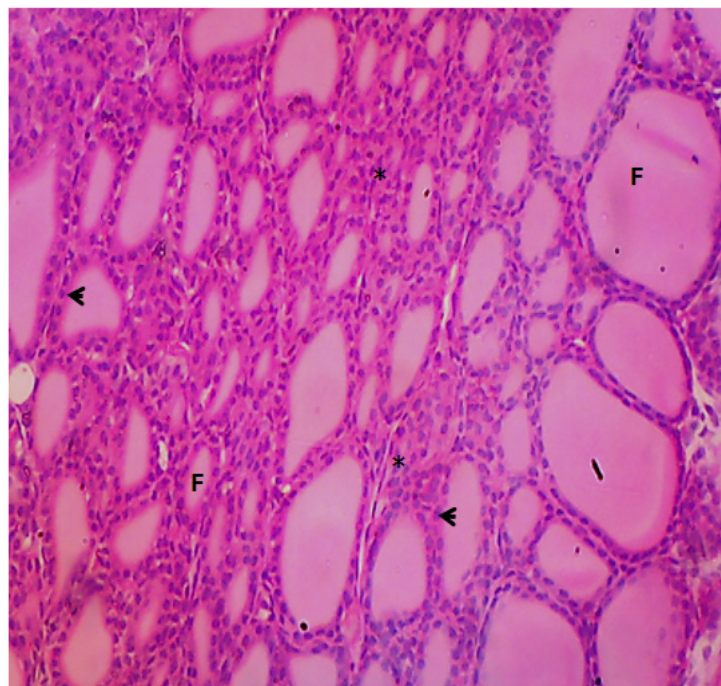


Fig. 10. Histological section of thyroid gland (Haematoxylin & Eosin Stain) of group 5 at magnification (X400) demonstrating numerous compressed thyroid follicles (F) due to cellular hyperplasia (*), the follicles are lined by cubical follicular cells that exhibit rounded nuclei (arrow head).

4. Concluding remarks

The present study findings showed that *C. citratus* leaf extracts have protective effects against GV-induced thyroid disorders due to its antioxidant and radical-scavenging constituents. These observations support the current use of natural products as effective chemopreventive agents. In the last two decades, the search for novel natural sources of chemoprevention has grown remarkably. Several plants bioactive

compounds have been reported to alleviate chemical-induced tissue damage likely due to their antioxidant, anti-inflammatory and immuno-modulatory activities. The characterized bioconstituents from *C. citratus* leaf extracts and the essential oil constituents have been individually reported for their antioxidant, anti-inflammatory and immunity boosting activities. In this context, Liu Zhong et al. (2007) demonstrated the antioxidant effect of flavonoids from *C. citratus* leaf extract. Several authors have independently reported



phenolic compounds as the major active ingredients of natural products with robust antioxidant and chemopreventive effects (Diaz et al., 2012; Sieniawska et al., 2013). Saponins also inhibited circulatory and tissue renin angiotensin aldosterone system (Hiwatashi et al., 2010). A polysaccharide (CCPS) from *C. citratus* improved immunity in rats (Bao et al., 2015). Linalool and epoxy-linalool found in *C. citratus* EO caused the inhibition of cytokine production in vitro. Francisco et al. (2013) demonstrated the anti-inflammatory effects of phenolic compounds. Therefore, the findings of the present study are credited to the synergistic effects of all antioxidant, anti-inflammatory and immunomodulatory constituents present in *C. citratus* leaf extracts. It seems that in continuation of the present, further studies should target the identification and evaluation of the specific role of the individual chemopreventive components of *C. citratus* leaf extract.

Conflict of interest

The authors declare that there is no conflict of interest

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