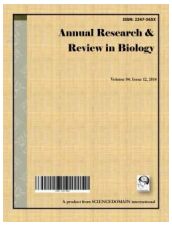

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Anti-inflammatory and Anti-oxidative Effects of Flavonoids-rich Extract of *Cymbopogon citratus* in Sodium Nitrite ($NaNO_2$) Induced Oxidative Stress in Wistar Rats

O. T. Adedosu[1*], G. E. Adeleke[1], J. A. Badmus[1], I. A. Ojeleye[1] and A. H. Fatoberu[1]

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author OTA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GEA and JAB managed the analyses of the study. Authors IAO and AHF managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Environmental exposure to radical generating agents from foods, drugs and cosmetics is a major concern in diseases associated with oxidative stress using natural products. This study investigated the effects of flavonoids-rich extracts of *Cymbopogon citratus* on oxidative and inflammatory markers in sodium nitrite exposed rats.
Study Design: Twenty four male Wistar rats averagely weighing 175 g used for this study were treated for one week, randomly selected into four groups, A-D. Group A (Control), Group B (Sodium nitrite only, 80 mg/kg.bw), Group C (treated extract at 100 mg/kg.bw. and intoxicated with sodium nitrite, 80 mg/kg.bw) and Group D (extract only, 100 mg/kg.bw).

**Corresponding author: E-mail: otadedosu@lautech.edu.ng, laniyidosu@yahoo.com;*

Methodology: Serum and liver total protein (TP) concentrations, Malondialdehyde (MDA) and Reduced glutathione (GSH) levels, Catalase (CAT) and Superoxide dismutase (SOD) activities, White blood cell count (WBC), C-Reactive protein (CRP) and Tumor necrosis factor alpha (TNF-α),were determined using international standardized methods.
Results: Results showed that Sodium nitrite significantly (*P* = .05) decreases liver total protein concentrations, GSH levels, CAT and SOD activities with corresponding significant (*P*=.05) increases in serum total proteins, WBC count, TNF-α, CRP and MDA levels. However, extract alone (group D) elicited significant (*P*=.05) increases in serum protein, WBC count, GSH level, CAT and SOD activities and significant decreases in serum TP,TNF-α, CRP, and MDA levels. Interestingly, the combined treatment (group C) showed similar trends with extracts as the parameters were significantly reversed to their control levels compared with group A and B.
Conclusion: Results are indicative of the toxic effects of sodium nitrite, its potential to induced apoptosis by up regulation of TNF-α and extract ability to boost anti-oxidant status, elicits modulatory, anti–inflammatory and anti-oxidative effects suggestive of its rich-flavonoids and medicinal values in preventing diseases associated with environmental toxins.

Keywords: Anti-inflammatory; anti-oxidant; Cymbopogon citrates; sodium nitrite.

1. INTRODUCTION

Accumulating evidences support the hypothesis that lipid peroxidation, inflammation, oxidative stress and oxidative damage to tissues and cellular molecules in humans are the major events in diseases and infections as well as ageing and most age-related diseases. The continuous efflux of free radicals and reactive oxygen species (ROS) from endogenous and exogenous sources results in continuous and accumulative oxidative damage to cellular components and alters many cellular functions. Among the biological target most vulnerable to oxidative damage are enzymes and proteins, lipid membranes and DNA [1]. Hydroxyl radicals, produced in mitochondrial compartments, are responsible for damage occurring in the mitochondrion but not the nucleus. Their high reactivity with biological molecules, which leads to their extremely short life span, does not permit their distribution within the intracellular environment and limits their effects to their site of formation. On the other hand H_2O_2 produced in mitochondria may interact elsewhere in the cytoplasm or in the nucleolus while cellular membranes are especially vulnerable to oxidation due to their high concentrations of unsaturated fatty acid. Their effects on lipids, usually called lipid peroxidation [2], while following protein oxidations, modified proteins are susceptible to many changes in their functions; these include chemical fragmentation, inactivation, and increased proteolytic degradation. Although DNA is a stable, well-protected molecule, ROS can interact with it and cause several types of damage: modification of DNA bases, single-and double-DNA breaks, loss of purines (apurinic sites), damage to the deoxyribose sugar, DNA-protein cross-linkage, and damage to the DNA repair system [3]. The events of cancer in human is also a complex process including cellular and molecular changes mediated by diverse endogenous and exogenous stimuli while it is well established that oxidative DNA damage is responsible for cancer development as cancer initiation and promotion are associated with chromosomal defects and oncogene activation induced by free radicals and ROS [4].

The term oxidative stress is a state of imbalance tissue oxidation refers to as condition in which cells are subjected to excessive levels of molecular oxygen or its chemical derivatives called reactive oxygen species (ROS), a persistent imbalance between antioxidants and pro-oxidants resulting in (often) irreversible cellular damage [5]. It is also known “as the bio molecular damage caused by attack of reactive oxygen upon the constituents of living organism” and the phenomenon associated with the action of free radicals and reactive metabolites in the organism [2]. Oxidative stress is involved in the process of aging, and various chronic diseases such as atherosclerosis [6], diabetes [7], and eye diseases. Oxidative stress and inflammation are the common persistent pathogenic factor mediating the appearance of insulin resistance (IR) as well as the passage from IR to overt Diabetes mellitus (DM), via impaired glucose tolerance (IGT), while producing the increased risk condition typical of pre diabetic and diabetic subjects resulting in atherosclerotic complications [7]. Inflammation is a protective attempt by the organism to remove the injurious

stimuli as well as initiate the healing process for the tissue. It is also a disorder involving localized increases in the number of leukocytes and a variety of complex mediator molecules [8], Prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation. Their biosynthesis has also been implicated in the pathophysiology of cardiovascular diseases, cancer, colonic adenomas and Alzheimer's disease [9].

Numerous agents have been linked with free radical and ROS generations during their metabolism apart from those produced via mitochondrial respiration chain leading to generations of pro-oxidants especially the so – called reactive oxygen species which notably includes free radicals [10]. These agents are numerous in foods, drugs, cosmetics and environmental toxicants in which humans are constantly exposed either deliberately or accidentally.

Sodium nitrite ($NaNO_2$), is a USDA-approved food preservative that gives cured meats such as ham, bacon and hot dogs their characteristics color and contributing to their flavor [11]. Periodically, controversy has emerged concerning the proposed link between sodium nitrite in cured meats and the endogenous formation of carcinogenic nitrosamines under certain conditions. Nitrates and nitrites toxicity are well documented in mammals including impairment of reproductive functions [12], hepatotoxicity [13], dysregulation of inflammatory responses and tissue injury [14], growth retardation [15], and endocrine disturbance [16]. Sodium nitrite is a white granular or crystalline solid which reacts with amines to produce carcinogenic nitrosamines. It exerts this effect by generation of free radicals that impair oxidant / antioxidant balance [17]. Increases in fetal and/or maternal met hemoglobin levels have been reported in sodium nitrite-treated guinea pigs, rats, and dairy cows, suggesting a potential for effects on the quantity of oxygen available to fetal tissues [15,18].

A major concern in considering the toxicity of Sodium nitrite is the induction of met hemoglobinemia – a condition in which there is a reduction in hemoglobin's ability to transport oxygen [18]. Nitrate is thought to be responsible for most of the toxic effects observed with excess nitrite ingestion. Nitric oxide free radical (NO), is generated from the nitrite by non–enzymatic method with the generation of peroxy-nitrite ($ONOO^-$) from reactions between nitric oxide and superoxide playing a critical role in the induction of inflammatory reactions and apoptosis [19,20].

Interestingly, the close association between oxidative stress, antioxidant status and environmental lifestyle –related diseases has become well known while aerobic organisms have evolved mechanisms to remove these highly reactive oxygen species to sustain life. Since oxidation reactions are crucial for life ,they can also be damaging; hence plants and animals maintain complex systems of multiple types of antioxidants, as oxidative stress is becoming an important part of many human diseases, the use of antioxidants in pharmacology is extensively studied especially from natural plants products where they are obtained as ingredients ,dietary supplements and herbs for promotion of health due to their rich bioactive constituents as chemists for years have realized their use due to their range of anti-oxidants constituents [21].

Medicinal plants are important source of new chemical substances with potential therapeutic effects, research into plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs [9]. Because existing synthetic molecule like non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors that increase the incidence of adverse cardiovascular thrombotic effects [22], have certain limitations.

Epidemiological studies have shown that the consumption of vegetables and fruits can protect humans against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species [23]. Many plants including fruits and vegetables are recognized as sources of natural antioxidants that can protect against oxidative stress and thus play an important role in the chemoprevention of diseases that have their etiology and pathophysiology in reactive oxygen species [24,25]. Hence, there is need to focus on the scientific exploration of herbal bioactive agents having fewer side effects as these positive effects are believed to be attributable to their antioxidants properties especially now that many developing countries are busy using these natural products for prevention, treatment and management of several diseases.

Cymbopogon citratus (Lemon grass) is an aromatic perennial tall grass with rhizomes and densely tufted fibrous root. It has short underground stems with ringed segments, coarse, green slightly leathery leaves in dense clusters [26]. The plant is a native herb from India and is cultivated in other tropical and subtropical countries [27]. It is used as traditional folk medicine in the treatment of nervous, gastrointestinal disturbances, fevers and hypertension [28]. Lemon grass is also a folk remedy for coughs, elephantiasis, flu, gingivitis, headache, leprosy, malaria, ophthalmia, pneumonia and vascular disorders. It is principally taken as tea to remedy digestive problem, diarrhea and stomach ache [26], rheumatism and other joint pains. The infusion or decoction of aerial parts of lemon grass has wide spread used in folk medicine [26]. Although there are limited scientific data on the medical claims of lemon grass, it has been used in folk medicine for treatment of several ailments world-wide [26]. It is pertinent however, since several studies have evolved to measure total antioxidant capacity of biological systems to investigate the involvement of oxidative stress/antioxidant status in pathological conditions or to evaluate the functional bio availability of many dietary antioxidants of plant origin in other related conditions with the possible outcome on the revelation of their mechanism(s) of action, this study was therefore aimed at investigating the effects of flavonoids –rich extract of *Cymbopogon citratus* on the anti-oxidant status and some inflammatory parameters in sodium nitrite exposed rats.

2. METHODOLOGY

2.1 Materials

Materials used in this study include; electronic weighing balance, triple weighing balance, measuring cylinders, 2 mL and 5 mL syringes and needles, beakers, test tubes, conical flasks, serum bottles, water bath, pH meter, spatula, centrifuge, thermometer, disposable gloves, tissue papers, micropipette, washing brushes, detergents, separating funnels, refrigerator, spectrophotometer, dissecting sets, mortar and pestle, stopwatch, test tube racks.

2.2 Reagents

All the reagents used were of good and high analytical quality mostly from Sigma USA, these are Tris buffer, Potassium chloride, Adrenaline, Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), Distilled water, Methanol, Chloroform, Ethyl-acetate, n-Hexane, Laboratory Kits for the quantitative determination of total protein, C-reactive protein Kits, TNF-α kits, Normal saline, Washing buffer and homogenizing buffer.

2.3 Plant Material and Preparation of Flavonoids-rich Extract

Cymbopogon citratus (Lemon grass) was collected at Ladoke Akintola University of Technology Teaching and Research farm and identified at the Botany Unit of the Department of Pure and Applied Biology, of the same institution with herbarium voucher number LHO 285 deposited. The plant was air dried in the laboratory and powdered after dryness with 800 g of the powdered leaves soaked in 5000 mL of 70% methanol for 72 hours, and was filtered using filter paper after this period. The filtrate of the extract was concentrated to dryness between 35-40ºC to obtain dry crude methanol extract residue. This was stored in a desiccator to remove any methanol remaining in it. About 50 g of the crude methanol extract of *Cymbopogon citratus* was weighed into a beaker and 25 mL of distilled water was added and stirred, while n-Hexane, Chloroform, Ethyl acetate were consequently used as washing solvents in a separating funnel using liquid –liquid chromatographic methods as modified by [29], to obtain the flavonoids-rich ethyl acetate extract used for this study. Ethyl acetate fraction of the extract was used for this experiment based on evidence obtained from the previous research findings that ethyl acetate fraction has the highest percentage of flavonoid of all other extracts [30].

2.4 Experimental Animals and Groupings

Male albino rats of Wistar strain used for this study were obtained from the animal house of Ladoke Akintola University of Technology, Ogbomoso, Oyo State. The animals were handled and treated based on Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria, guidelines on ethics and conducts for handling experimental animals which conforms with the international standards. They were acclimatized in the laboratory for two weeks before any experimental work was undertaken. They were fed with normal feed and water ad-libitum and their weights were monitored. The

animals were randomly selected into four groups; A-D, with six animals in each group (Table 1). These were treated as follows:

Group A- Control, administered with 0.1 mL of normal saline

Group B- Administered with normal saline (0.1 mL) and sodium nitrite only (80 mg/kg body weight) made into 0.1 mL with distilled water intra peritoneally at three days interval

Group C- Received extract daily at 100 mg/kg body weight with 80 mg/kg body weight of Sodium nitrite at three days interval.

Group D-Administered only with 100 mg/kg body weight of the extract daily as positive control.

Table 1. Protocol table for various groups and treatments

Groups	Treatment
Group A (Control)	Feeds and normal saline only
Group B(Sodium nitrite only)	Normal saline + 80 mg/kg bodyweight of sodium nitrite
Group C(Extract +Sodium nitrite)	100 mg/kg bodyweight of extract +80 mg/kg bodyweight of Sodium nitrite
Group D(Extract only)	100 mg/kg bodyweight of extract daily.

2.5 Preparation of Liver Homogenate and Blood Serum

The experimental animals were sacrificed at the end of the administration period on the 9th day by cervical dislocation. The animals were carefully open and the blood was drained from the heart using a syringe and needle (heart puncturing). The liver was excised and placed in a separate pre-weighed beaker containing 5mL of washing buffer. The liver was thoroughly washed several times in separate cold washing buffer to remove hemoglobin which may inhibit the activity of the enzymes. All these procedure were carried out at 4ºC. The washed liver was then weighed and 1 g of this was transferred to a beaker containing 4 mL of homogenizing buffer and homogenized using glass homogenizer. The homogenate was stored at 4ºC. The blood was collected in small serum bottles and was centrifuged at 4000 rpm for 10 minutes to collect the serum. The serum collected was stored at 4ºC.

2.6 Biochemical Studies

Various samples were prepared from the blood and the liver for different assays. Serum total proteins were determined according to the Biuret method [31], while quantitative determination of C Reactive proteins (CRP) and Tumor necrosis factor-alpha (TNF-α) were determined using solid phase Enzyme Linked Immunosorbent Assay (ELISA) designed to measure CRP and TNF-α in cell culture supernatant, serum and plasma. This assay employs the quantitative sandwich enzyme immunoassay technique with an antibody specific for CRP and TNF-α pre-coated onto a micro plate using Ray Biotech diagnostic kits based on the principle of the interaction between antibody and antigen to quantify the CRP and TNF-α in the serum. Total white blood cell (WBC) counts were determined by acetic acid dilution of the whole blood which facilitate hemolysis of mature erythrocytes and enhance leukocyte counting. The liver homogenates were used to assay for the following indices; Malondialdehyde (MDA) were estimated spectrophotometrically by thiobarbituric acid-reacting substances (TBARS) as described by the procedure of Varshney and Kale [32]. Determination of reduced glutathione (GSH) concentration was done using the method described by Anderson [33], while superoxide dismutase (SOD) and catalase (CAT) activities were determined by the methods of Misra and Fridovich [34] and Aebi [35] respectively. Tissue total protein was determined by the same method as earlier reported.

2.7 Statistical Analysis

The results were reported as means ± SD from six repeated determinations and evaluated with data obtained and analysed using analysis of Variance (ANOVA). Value of *P*=.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

The constant exposure of humans to free radicals has been likened to being irradiated with these chemicals at low levels from the environment all the time, while accumulation of these radicals in our cellular structures causes a wide variety of degenerative diseases which may eventually lead to premature or sudden death as

over sixty known diseases and disorders have been associated with exposure to free radicals some of which are products of cigarette, smokes, herbicides, high fats, pesticides, smog, car exhaust and drugs. In this study the toxic effects of sodium nitrite as well as the possible roles of the flavonoids-rich extracts of *Cymbopogon citratus* leaves on the toxic effects of sodium nitrite were investigated in rats using data from various biochemical indices investigated.

From the results obtained in Table 2, it was observed that group B (Animals treated with Sodium nitrite only) elicit a significant (*P*=.05) increase in the level of serum protein concentration compared with other treated groups. However, the combined treatment group C, (Extract + Sodium nitrite) showed significant (*P* =.05) decreases in serum protein concentration compared with group B. While the group D (Extract only) showed the least level of serum protein concentration with no significant differences (p>.05) compared to controls. The increase in the serum protein concentration may be suggested to be a result of the toxic effect of sodium nitrite on the organ (Liver) which makes more protein to be released into the blood from the liver leading to significant increases in the serum protein concentrations since proteins serves as a major constituents of membranes which are possible targets for attack by ROS especially the OH^- and nitrogen –reactive radicals which predominantly caused protein damage [36].

Proteins are present in all body fluids, but it has been observed that there is always increased rates of incorporation of radioactive amino acids into proteins in the serum during inflammation, such as those described for the major acute phase inflammatory-protein of the rats, the increase in the serum concentration of the acute phase reactants during inflammation is due to increased rates of synthesis as the site specific oxidative damage of some of the susceptible amino acids of proteins is regarded as the major cause of metabolic dysfunction during pathogenesis [37].

Inversely, the results of the protein concentrations of the liver (Table 2); showed that group B animals elicits significant (*P*=.05) decrease in the tissue protein concentration compared with controls (group A). However, in the combined treatment (group C), no significant (p>.05) increased in tissue protein concentration was observed compared with group B. The decrease in the tissue protein concentration may be as a result of the toxic effect of the toxicant (Sodium nitrite) on the liver which may encourage protein hydrolysis and its subsequent release into the blood, causing significant decreases in protein concentrations in the liver [36].

Furthermore, Pro-inflammatory cytokines, such as interleukin 6 (IL-6) and interleukin 8 (IL-8), are important inducers of white blood cell (WBC) production. It can be speculated that the elevated amount of these cytokines is responsible for higher WBC count in diabetes or cardiovascular disease. Adipose tissue is a great source of inflammatory factors, such as IL-6 and C-reactive protein (CRP), which also are well established markers of systemic inflammation [38]. In Table 3, group B (Animals treated with Sodium nitrite only) showed significant (*P*=.05) increases in concentration of White Blood Cell count compared with group A and C. While there was a significant (*P*=.05) decrease in the concentration of White Blood Cell in Group C (Combined Treatment) compared with group B, suggestive of its modulatory effects on the toxicant. This result also implies that administration of toxicant (Sodium nitrite) causes inflammation as it leads

Table 2. Total protein concentrations in the serum and liver homogenates of various treatment groups

Treatment Group	Total protein concentration (Serum) Mg/dl±SD	Total protein concentration (Liver) Mg/dl±SD
Group A (Control)	1.294.±0.315	1.237±0.308
Group B (Sodium nitrite only)	2.291*±0.393	0.880*±0.040
Group C (Extract +Sodium nitrite)	1.613±0.259	1.037±0.359
Group D (Extract only)	1.187±0.396	1.153±0.201

Values are given as mean and standard deviation of six determinations.
**Values differ significantly from control (P =.05)*

to more secretion of White Blood Cell by the immune system to combat the foreign agents. Also, the increased White Blood Cell concentrations in group D (Extract only) may be suggestive of dosage of the extract which may cause it to act as pro-oxidants.

Similarly Tumor Necrosis Factor (TNF) is an intercellular chemical messenger, or cytokine, that is involved in the inflammatory process. Its primary role is the regulation of immune cells. TNF is produced by various blood cells, such as T lymphocytes, or white blood cells, known as macrophages and monocytes, which are responsible for the body's immune response.

Tumor necrosis factor α (TNF-α) has been shown to play a critical role in the pathogenesis of inflammatory bowel disease (IBD). TNF-α blockers are biological agents that specifically target this key cytokine in the inflammatory process and have become a mainstay in the therapy of inflammatory bowel diseases [39]. In Table 3, the significant (*P*=.05) increases in the TNF-α level in group B (Animals treated with Sodium nitrite only) was considered to be as a result of manifestation of inflammation. Whereas, the decreased level of TNF-α in Group C (Extract +Sodium nitrite) and D (Extract only) are indicative of the protective, modulatory and mopping-up effects of the extract against the toxic effects of Sodium nitrite –induced free radicals generations respectively, and generally by been able to boost the anti-oxidant status of the animal as a possible mechanism. While the basic mechanism of action is by inhibiting the secretion of NO and pro-inflammatory cytokine tumor necrosis factor TNF-α [40]. Also the ability of Sodium nitrite to up regulate TNF-α is suggestive of its potential to induce Apoptosis.

In the same vein, C-reactive protein (CRP) is widely used as systemic biomarker for diagnosing acute and chronic inflammation. CRP is produced by hepatocytes in response to inflammatory cytokines, particularly, interleukin 6 from the tumor microenvironment. Preoperative CRP levels are parallel to the progression or pathological stages or identification of groups of patients with chronic inflammation [41].

From the results (Table 3), group B (Animals treated with Sodium nitrite only) showed significant (*P*=.05) increases in serum concentration of CRP compared with other treatment groups. Group C (extract + Sodium nitrite) however showed significant (*P*=.05) decreases compared with group B, while group D (Extract only) showed significant (*P*=.05) decreases compared with other groups. The increase in the level of CRP concentration in group B may probably be as a result of manifestation of inflammation, While the decreased noted in Group C compared to Group B may be suggested to be a result of modulatory effect of the extract and its antioxidants effects against inflammation induced by uncontrolled free radical generation also resulting in lipid peroxidation [40].

Furthermore, Malondialdehyde (MDA) is known to be a good biomarker of lipid peroxidation. Lipid peroxidation leads to breakdown of lipids, formation of a wide array of primary oxidation products, such as conju-dienes or lipid hydro peroxides and secondary products including MDA [42], as a result of free radical-induced oxidative stress and its marker. In Table 4, group C (Extract + Sodium nitrite) showed significant (*P*=.05) decreases in MDA compared to Group B (Sodium nitrite only) which significantly increase (*P*=.05) MDA levels compared to other treated groups. The significant increases in the MDA level in group B may not be unconnected with the result of the toxic effect of Sodium nitrite which induces radical formation in cellular environment leading to lipid peroxidation [42]. Interestingly, group D which showed significant (*P*=.05) decreases in MDA level is indicatives of the antioxidant properties of the extract, the effects of which were reflected in group C as it protect and modulates these toxic effect of the toxicant. The extract exhibition of antioxidant properties may be by decreasing reactive oxygen species production during Sodium nitrite metabolism thus limiting MDA formation and lipid peroxidation, as well as, by increasing superoxide dismutase activity and glutathione formation [40].

Lipid peroxidation in liver cells could, over a prolonged periods of time, lead to liver diseases and diabetes [43]. Peroxides formed during lipid peroxidation processes can decompose into a vast array of toxic carbonyl products such as malondialdehyde (MDA), playing among other a role in the carcinogenesis process by interacting with cellular DNA, yielding DNA-MDA adducts that appear to be pro mutagenic [9]. However, this may have been averted by the ability of the flavonoids-rich extract to inhibit ROS formation as well as stimulates SOD and CAT production [44].

Table 3. White blood cell (WBC) counts, serum tumor necrotic factor-alpha (TNF-α) and C-reactive protein (CRP) concentrations of various treatment groups

Treatment group	WBC counts (mm^3)±SD.	TNF-α concentration (ng/mL)±SD.	C-reactive protein (CRP) concentration (ng/mL)±SD.
Group A (Control)	$46.616x10^3±7.8x10^3$	$1.66x10^3±3.2x10^2$	$2.8x10^5±4.19x10^4$
Group B (Sodium nitrite only)	$56.733x10^3 ±7.1x10^{3*}$	$3.05x10^3±3.22x10^{2*}$	$4.70x10^5±2.5x10^{4*}$
Group C (Extract +Sodium nitrite)	$22.117x10^3±7.52x10^{3*}$	$2.63x10^3±1.6x10^{2*}$	$3.12x10^5±3.77x10^{4*}$
Group D (Extract only)	$55.244x10^3±5.8x10^{3*}$	$1.96x10^3±1.29x10^2$	$3.59x10^5±1.6x104$

Values are given as mean and standard deviation of six determinations.
* *Values differ significantly from control group (P =.05)*

Table 4. Liver Malondialdehyde (MDA) and reduced glutathione (GSH) concentrations of various treatment groups

Treatment group	Malondialdehyde (MDA) concentration (Mg/dl)±SD	Reduced glutathione (GSH) concentration (Mg/dl)±SD
Group A (Control)	2.100±0.189	1.580±0.014
Group B(Sodium nitrite only)	5.130±0.620*	1.071±0.020*
Group C(Extract +Sodium nitrite)	3.110±0.887	1.600±0.051
Group D(Extract only)	2.010±0.124	1.750±0.035

Values are given as mean and standard deviation of six determinations.
* *Values differ significantly from control (P =.05)*

Table 5. Superoxide Dismutase (SOD) and Catalase (CAT) activities in the liver of various treatment groups

Treatment group	SOD activity (U/mg Protein)±SD	CAT activity (U/mg Protein)±SD
Group A (Control)	0.301±0.042	0.200±0.010
Group B (Sodium nitrite only)	0.195±0.079*	0.100±0.011*
Group C (Extract +Sodium nitrite)	0.250±0.010	0.151±0.095
Group D (Extract only)	0.401±0.082*	0.225±0.007

Values are given as mean and standard deviation of six determinations.
* *Values differ significantly from control (P =.05)*

Also, reduced glutathione (GSH) is a water-soluble tripeptide composed of the amino acids glutamine, cysteine, and glycine. The thiol group is a potent reducing agent, rendering GSH the most abundant intracellular small molecule thiol, reaching millimolar concentrations in some tissues. Results of Its determination in this study as antioxidant index are shown in Table 4. From the results, group B (Animal treated with Sodium nitrite only) showed significant (*P*=.05) decrease in the GSH level compared with other treatments group, which implies that the free radicals generated by Sodium nitrite suppresses the concentration of GSH [45]. In group C (Extract + Sodium nitrite), GSH concentrations was significantly increased compared with group B which showed the extract modulatory effect on the toxicant. While group administered with the extract alone (group D), showed its ability to boost the antioxidant status with elevated levels of GSH due to its bioactive contents which are majorly bioflavonoids. GSH plays a role in other cellular reactions, including, the glyoxalase system, reduction of ribonucleotides to deoxyribonucleotides, regulation of protein and gene expression via thiol:disulfide exchange reactions [46].

Further evaluation of the tissue activities of the Superoxide dismutase (SOD) and Catalase (CAT) in all the treatment groups revealed the toxic effects of Sodium nitrite on the two

parameters as these were significantly (*P*=.05) decreased in group B animals (Table 5), an indication of the ability of the free radical generated by nitrite to suppress the activity of these enzymes [47]. However, the administration of the extract singly (group D) as well as its combinations with sodium nitrite (group C) revealed the antioxidant, modulatory and protective potentials of the extract as the activities of these enzymes were significantly increased.

3.1 Results

The results of this study are shown in Tables 2-5.

4. CONCLUSION

The results of this study indicates that the flavonoids–rich extract of *Cymbopogon citratus* leaves exhibits potent antioxidant and anti-inflammatory activities against sodium nitrite induced toxicity ,justifying its flavonoids-rich bioactive components and its usage in folk medicine and also a potential lead for possible development into phyto drugs for chemoprevention, treatment and management of inflammation and other conditions related with cellular oxidative stress induced by toxins or toxicants obtained from the environment (exogenous) and endogenous sources as humans are continuously exposed to different kinds of chemicals such as food additives, industrial chemicals, pesticides and other undesirable contaminants in the air, food and soil. This study therefore support the use of the plant as dietary supplements for boosting antioxidant status as well as remedy for prevention of oxidative stress associated with diseases and ageing while the characterization of the flavonoids types in the plant needs further investigation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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