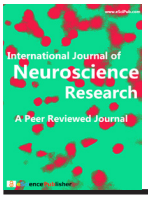
Research Article **IJNR (2017) 1:4**



International Journal of Neuroscience Research
(ISSN:2572-8385)

Effect of *Cymbopogon citratus* (Lemon Grass) on acute anoxic stress in mice

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ABSTRACT

Exposure to acute anoxic stress produces deleterious effects on the brain through the formation of oxidant molecules like reactive oxygen species. Thus, compounds with antioxidant property might demonstrate protective effect against the damaging effects of anoxic stress on brain cells. This study was carried out to evaluate the protective effect of Cymbopogon citratus, a medicinal plant with antioxidant property on convulsions induced by anoxic stress in mice. Male Swiss mice (20-22 g) were given *C. citratus* (25, 50 and 100 mg/kg, p.o), Panax ginseng (50 mg/kg, p.o) or vehicle (10 mL/kg p.o). Thirty minutes later, the animals were exposed to anoxic stress and the latency (s) to convulsion (anoxic tolerance time) was measured. Thereafter, the blood glucose level was measured using glucometer. The levels of malondialdehyde (MDA) and glutathione (GSH) were also determined in the brain homogenates of mice subjected to anoxic stress. *C. citratus* (25, 50 and 100 mg/kg, p.o) did not significantly ($p > 0.05$) delay the latency to anoxic convulsion and also failed to alter the brain concentrations of MDA and GSH in mice exposed to anoxic stress. However, *C. citratus* (25, 50 and 100 mg/kg, p.o) caused a significant and dose-dependent reduction in the blood glucose levels in anoxic stressed mice. These findings suggest that *C. citratus* has neither protective effect against convulsive episodes nor alter oxidative stress parameters induced by acute anoxic stress in mice. The decrease in blood glucose produced by *C. citratus* in anoxic condition may be unconnected with normalization of deregulation of plasma glucose level during stress responses.

Keywords: Cymbopogon citratus,Lemon Grass, anoxic stress

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How to cite this article:
Umukoro et al.,. Effect of Cymbopogon citratus (Lemon Grass) on acute anoxic stress in mice. International Journal of Neuroscience Research, 2017; 1:4.

eSciencePublisher
eSciPub LLC, Houston, TX USA.
Website: http://escipub.com/

Introduction

Anoxia is a condition of oxygen deprivation and is a very severe form of stressor, as it impairs various physiological and biochemical functions of the body. Although anoxic stress have been found to damage vital organs of the body, the brain is known to be more sensitive to oxygen deprivation [1-2]. In fact, it is one of the first organs to suffer from anoxic conditions, and dies quickly when deprived of adequate oxygen [3-4]. Brain injuries due to oxygen deprivation may occur in certain conditions that can interrupt the free flow of oxygen to the brain. For example stroke, cardiac arrest, anesthesia complications during surgery, suffocation, drowning, stragulation, carbon monoxide poisoning, electric shock and extreme asthmatic attacks can impair oxygen and nutrients delivery to the brain [5-8].

Although lacks of adequate oxygen delivery to the brain can cause various disabling effects, seizures are common manifestations seen in laboratory animals exposed to anoxic stress [4]. This type of seizure is often referred to as anoxic convulsion or anoxic tolerance time [4]. The ability of novel compounds to protect against convulsion induced by anoxic stress is judged by increase in anoxic tolerance time in rodents [4]. Thus, the increase in the threshold to anoxic convulsions or stress tolerance time indicates an effective adaptive responses or protection against anoxic conditions in rodents. Increased blood glucose level is also a common biomarker considered as an indicator of persistent stress [9] and reduction in hyperglycaemia shows efficient adaptation.

Cymbopogon citratus Stapf. (Lemon grass) is an aromatic plant belonging to the Gramineae family [10]. It is a tall, clumped perennial grass growing to a height of 1 m. The leaf-blade is linear, tapered at both ends and can grow to a height of 50 cm and width of 1.5 cm [11]. The leaf of *Cymbopogon citratus* is widely used in traditional medicine for the treatment of fever, digestive disorders, diabetes, inflammation and nerve disorders. Most of the biological effects ascribed to *Cymbopogon citratus* extracts have been attributed to its primary bioactive constituents, derived from its leaves and roots [11]. The chemical constituents isolated from the leaves and rhizomes of *C. citratus* including luteolin, isoscoparin, quercetin, kaempferol and apigenin have been reported to demonstrate diverse biological activities [12-15]. Specifically, previous studies have shown that *C. citratus* has anti-amoebic, antibacterial, antidiarrheal, antifilarial, antifungal and anti-inflammatory properties [13-16]. In addition, antimalarial, antimutagenic, antimycobacterial, hypoglycaemic, anticancer and neurobehaviorial activities exhibited by *C. citratus* have also been reported in literature [13-16]. Preclinical studies also showed that *C. citratus* demonstrated potent antioxidant activity, as it was shown to be efficient in scavenging free radicals [13, 16] suggesting a potential usefulness in conditions associated with oxidative stress-mediated tissue injury. Thus, this study was carried out to evaluate the effect of *Cymbopogon citratus* (Lemon grass) on acute anoxic stress in mice and the involvement of oxidative stress.

Materials and Methods

Laboratory animals

Male Swiss mice (20-22g) were used in the study. They were purchased from the Central Animal House, University of Ibadan; housed in plastic cages at room temperature and had free access to rodent pellet diet and water *ad libitum*. The animals were acclimatized for one week to the laboratory conditions before they were used for the study. The experimental procedures were done in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals.

Preparation *of Plant material*

The leaves of *Cymbopogon citratus* were obtained from the Botanical Garden, University of Ibadan, Nigeria and were sundried. The dried leaves were ground to powder using electric blender. The powdered leaf material (600 g) was soaked in distilled water for 24 h and then filtered using Whitman 3mm thick filter paper. The filtrate was then concentrated using rotary evaporator at 40^{o}C and the residue was dried in a desiccators before it was kept in a sterilized glass vial ready for use. The aqueous extract of *C. citratus* was dissolved in distilled water immediately before use. The doses of 25, 50 and 100 mg/kg of *C. citratus* used in the study were selected based information obtained from literature [17].

Drugs and Chemicals

Panax ginseng (Mason Vitamins Inc., Miami Lakes, FL 33014, USA), trichloroacetic acid–TCA (Burgoyne Burbidges & Co., Mumbai, India), thiobarbituric acid–TBA (Sigma, Germany), 5,5′-dithio-bis(2-nitrobenzoicacid)–DTNB (Sigma Aldrich, Germany) and Tris (hydroxymethyl)-amino-methane (Tris-buffer) (Hopkin & Williams Company, USA) were used in this study.

Experimental Procedures

Effect of *C. citratus* on anoxic convulsion

The effect of C. citratus on anoxic convulsion was carried out according to the method previously described by Caillard *et al.* [18]. On the experimental day, the animals were divided into seven different treatment groups (n = 6). Mice in group I received distilled water (10 mL/kg) and served as non-stress control. Mice in groups 2-4 were treated with the extract (25, 50 and 100 mg/kg) while group 5 received *Panax ginseng* (50 mg/kg). Thirty minutes after treatment, the animals were placed individually in air tight cylindrical vessel of 250 mL air capacity. Thereafter, the anoxic tolerance time was recorded and the animals were immediately removed from the vessel for recovery. The anoxic tolerance time was defined as the latency to first appearance of convulsion. The route of administration was oral.

Estimation of blood glucose in anoxic-stressed mice

The effect of *C. citratus* on blood glucose level in mice exposed to anoxic stress was done as previously described [19]. Immediately after the anoxic tolerance test, blood sample was obtained from the tail of each mouse, which was employed for the estimation of blood glucose level using a commercial glucometer (Accu-Chek Roche).

Preparation of brain tissue for biochemical studies

The animals were sacrificed under ether anaesthesia and the brains were rapidly removed. Thereafter, each brain was weighed and homogenized with 10% w/v phosphate buffer (0.1M, pH 7.4). Each brain tissue homogenate was separated into 2 portions and used for determination of the brain concentrations of *glutathione (GSH) and* malondialdehyde (MDA).

Determination of glutathione (GSH) concentration

The concentrations of GSH were measured in the aliquots of brain tissue homogenates according to the method previously described by Moron *et al*. [20]. Equal volume (0.4 mL) of brain tissue homogenate (0.4 mL) was added to 20% TCA (0.4 mL) and then centrifuged using a cold centrifuge at 10,000 rpm at 4°C for 20 min. The supernatant (0.25 mL) was added to 2 mL of 0.6mM DTNB and the final volume was made up to 3 mL with phosphate buffer (0.2M, pH 8.0). The absorbance was then read at 412 nm against blank reagent using a spectrophotometer. The concentrations of reduced GSH in the brain tissues were expressed as micromoles per gram tissue (µmol/g tissue).

Determination of malondialdehyde (MDA) concentrations

The brain concentration of MDA was estimated according to the method of Adam-Vizi and Seregi [21]. Distilled water (0.5 mL) and10% TCA (1 mL) were added to 0.5 mL of each brain tissue homogenate and centrifuged at 3000 rpm for 10 min. Then, 0.1 mL TBA (0.375%) was added to 0.9 mL of the supernatant. The mixture was placed in a water bath at 80ºC for 40 min and then allowed to cool to room temperature. Afterward, the absorbance of the supernatant was measured at 532 nm using a spectrophotometer. The MDA concentration was calculated using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and values were expressed as µmoles of MDA per gramme tissue.

Statistical analysis

The data obtained were all expressed as mean ± S.E.M (standard error of mean) and analyzed with Graph Pad Prism software version 5.00.Statistical analysis of data was done using One-way ANOVA, followed by Newman-keuls post-hoc test. P-values less than 0.05 ($p<0.05$) were considered statistically significant.

Results

Effect of C. citratus on anoxic convulsion

The effect of *C. citratus* on the latency to convulsions induced by anoxic stress in mice is shown in Figure 1. As shown in Fig 1, oral administra-

tion of the extract (25-100 mg/kg) did not significantly ($p > 0.05$) delay the latency to convulsion in mice exposed to acute anoxic stress. However, pre-treatment with *Panax ginseng* (50 mg/kg) significantly ($p < 0.05$) delay the latency to convulsion induced by anoxic stress (Fig. 1).

C. citratus reduces blood glucose level in mice subjected to anoxic stress

As shown in Table 1, the increase in blood glucose level produced by acute anoxic stress was significantly ($p < 0.05$) reduced by the aqueous extract of *C. citratus* (25-100 mg/kg) in a dose-dependent manner. *Panax ginseng* (50 mg/kg) also produced a significant decrease in the blood glucose level in mice exposed to acute anoxic test (Table 1).

Effect of *C. citratus on* brain concentrations of GSH *and* MDA

Figures 2 and 3 showed the effect of *C. citratus* on the brain concentrations of GSH and MDA in mice exposed to acute anoxic stress. Oral administration of the extract (25-100 mg/kg) did not caused a significant ($p < 0.05$) alterations of the brain content of GSH and MDA in anoxic-stressed mice (Figures 2 and 3).

Discussion

The results of this study showed that aqueous leaf of *C. citratus* did not prolong the latency to convulsions induced by anoxic stress in mice. The levels of the biomarkers of oxidative stress were not affected by the aqueous leaf extract of *C. citratus as* it did not significantly modify the concentrations of MDA and GSH in the brain of mice exposed to acute anoxic stress. However, the extract significantly reduced the blood glucose levels in anoxic stressed-mice in a dose-dependent manner.

Anoxia is a more severe form of stressor and can results in the death of brain cells [21].. The increased susceptibility of the brain cells to oxygen deprivation is known to be related to its high metabolic rate and low antioxidant defence systems [1-2]. Although lacks of adequate oxygen delivery to the brain can cause various disabling effects, seizure is one of the major behavioral phenotypes measured in laboratory animals exposed to anoxic stress [4, 18]. This type of seizure is often referred to as anoxic convulsion or anoxic tolerance time [4, 18]. The ability of novel agents to protect against convulsion induced by anoxic stress is judged by increase in anoxic tolerance time in rodents [4]. Thus, the increase in the threshold to anoxic convulsions or stress tolerance time indicates effective adaptive responses or protection against anoxic conditions in rodents [4,18]. Compounds with adaptogenic effect are known to protect the brain cells against anoxic stress and to delay the latency to anoxic convulsions in mice [4, 19, 22]. However, in this study, oral administration of aqueous leaf extract of *C. citratus* did not increase the threshold to anoxic convulsions in mice, which suggests lack of adatogenic property.

Stress has been shown to activate the hypothalamic-pituitary-adrenal axis, which leads to increase in concentrations of cortisol in plasma and brain tissues [23]. High concentrations of cortisol have been found to damage brain cells through the formation of free radicals and depletion of antioxidant systems [1, 23]. Thus, the protection of brain cells against the damaging effect of free radicals underlies one of the mechanisms of action of adaptogens. Adaptogens have been described as compounds of plant origin that increase the capacity of an organism to cope with stress and they act in a non-specific manner to increase the resistance of the organisms against aversive situations [24]. Adaptogens are efficient in scavenging free radicals and are known to possess neuroprotective activity [22, 24]. However, in this study, acute administration of the extract did not exhibit antioxidant activity in anoxic-stressed mice and the contribution of this finding to its ineffectiveness in combating anoxic convulsions in mice needs further investigations.

During response to stress, increased plasma cortisol also promotes mobilization of fats and carbohydrate from storage sites, which lead to increase in blood glucose levels. Increased blood glucose level is commonly considered as a potential indicator of stress [25] . Thus, suppression of blood glucose levels by novel compounds in mice exposed to stress is an indication of adatogenic property. However, the relevance of the finding that the extract lowered blood glucose level in anoxic-stressed mice needs further studies. It is important to note that previous studies have shown that the leaf extract of *C. citratus*

Table 1: Effect of *C. citratus* on blood glucose level in mice subjected to anoxic stress

Treatment	Dose (mg/kg)	Blood glucose(mg/dl)
Control	-	308.8± 27.9
C. citratus	25	200.8 ± 29.6 *
C. citratus	50	181.7 ± 13.8 *
C. citratus	100	172.5 ± 11.7 *
Ginseng	50	259.3 ± 6.5*

Each represents mean ± S.E.M for 6 animals per group. *P < 0.05 compared to stress control (ANOVA followed by Newman Keuls test).

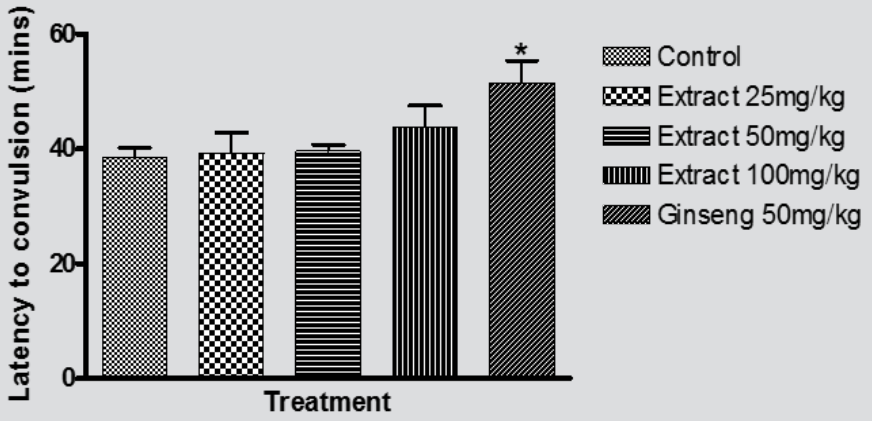

Fig. 1 Effect of C. citratus on the onset of convulsion in anoxic stressed mice. Each column represents mean ± S.E.M for 6 animals per group. * < 0.05 compared to stress control (ANOVA followed by Newman Keuls test).

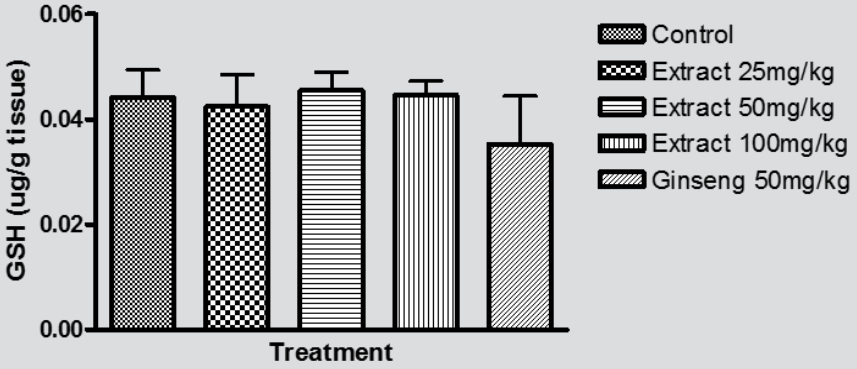

Fig. 2: Effect of C. citratus on the brain level of GSH in mice exposed to anoxic stress
Each column represents mean ± S.E.M for 6 animals per group. There are no significant differences between treatment groups (p> 0.05 compared to Control (ANOVA followed by Newman Keuls test).

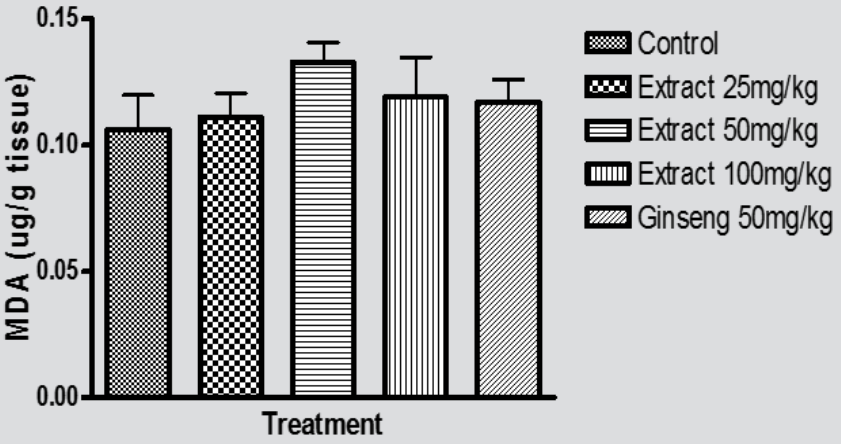

Fig. 3: Effect of C. citratus on the brain concentration of MDA in mice subjected to anoxic stress
Each column represents mean ± S.E.M for 6 animals per group. There are no significant differences between treatment groups (P > 0.05 compared to control (ANOVA followed by Newman Keuls test).

lowered blood glucose level in diabetic rats [16]. Thus, the fall in blood glucose level in mice exposed to acute anoxic stress may likely be related to its potent hypoglycaemic effect rather than normalization of glucose homeostasis in stressful conditions.

Conclusions

The results of this study suggest that C. citratus did not demonstrate protective effect against convulsive episodes nor alter oxidative stress parameters induced by acute anoxic stress in mice. However, the decrease in blood glucose level produced by *C. citratus* in anoxic condition may be unconnected with normalization of deregulation of plasma glucose level during stress responses.

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