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Histopathological study on the effects of oral administration of aqueous leaf extracts of *cymbopogon citratus* on the frontal cortex of male sprague dawley rats

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ABSTRACT

This investigation was to evaluate the histopathological effects of oral consumption of the aqueous leaf extract of *Cymbopogon citratus* on the frontal cortex of Sprague Dawley (SD) rats. Ten male SD rats weighing between 150-230g were used. The rats were randomly assigned into two groups designated as groups A and B. Group A served as the control group while group B was the treatment. 300 mg/kg body weight of the aqueous leaf extract of *C. citratus* was administered once daily for 14 consecutive days (14d) orally through a sterilized orogastric tube, while the control rats received equal volume of phosphate buffered saline (PBS). The rats were sacrificed 24 hours after the last administration. The frontal cortices were excised, fixed in 10% formol calcium for 18 hours and were processed for routine light microscopic study using Hematoxylin and Eosin (H&E) method. The histological findings revealed that the extract treated sections showed cytoarchitectural distortions ranging from neuronal degeneration, distortion, vacuolations and evidence of necrotic bodies while the sections from the rats in group B conform to normal histological profile. These

findings suggest a deleterious and toxic effects of aqueous leaf extract of *C. citratus* on the frontal cortex of SD rats.

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1. Introduction

Nigeria is recognized worldwide for its vast fauna and flora biodiversity, which can be explored in several ways (i.e. culinary, medicinal, therapeutic, nutritional, e.t.c.) for the benefit of mankind. A wide range of plants/herbs species from Nigeria's flora have been used in folklore medicine for the treatment of several maladies both in the "Old and New world" (Adekomi *et al,* 2010).

Several investigators have been intensively investigating both the tropical and subtropical plant species with medicinal properties in order to assess the feasibility of developing natural, sustainable, and affordable "natural drugs" (Iwu , 2000; Orafidiya *et al,* 2001).

Cymbopogon citratus is a tall coarse grass with a strong lemon odor used for cooking, medicinal teas and potpourri. This plant commonly called lemon grass is an aromatic, perennial grass. It is a tropical plant, grown as an ornamental plant in many temperate areas with a maximum height of about 1.8 m and its leaves 1.9 cm wide covered with a whitish bloom. Like other members of the genus, *citratus* yields citral, a volatile oil with strong lemon fragrance. It is used in manufacture of perfumes, colored soaps and synthesis of vitamin A. Its oil is used as culinary flavoring, insect repellant and medicine. In Brazilian folk medicine it is believed to have anxiolytic, hypnotic and anticonvulsant properties (Blanco *et al,* 2009), but another study has reported no effect in Man (Leite *et al,* 1986).

Medicinal folklore use of *C. citratus* include antibacterial, antifungal antioxidant, antiseptic (externally), antispasmodic, bone and joint pain, carminative, cough, cold, colic, diarrhea, digestive disorders, diuretic, dyspepsia, edema, fever, flatulence, gastrointestinal disorders, gingivitis, headaches, hyperglycemia, muscle soreness, nervous conditions, ophthalmia, pneumonia, poultice, stiffness, stomachaches, taste enhancer, urinary system conditions, vascular disorders, water retention

The plant is known to contain a large number of phytochemical compounds which include: 0.2-0.4% essential oil; acetone; 4.3% ash; 3.7% calcium; alpha-camphorene; caprylic acid; caryophyllene; ceryl alcohol; chromium; 1,8-cineole; 0.1-0.34% citral; citronellal; citronellic acid; cobalt; cymbopogone; cymbopogonol; citrolnellol; 0.02% cymbopogonol (leaf wax); decanal; n-Decylaldehyde; diacetyl; dihydropsuedoionone; dipentene; farnesal; farnesol; 7.1% fat; furfural; geranic acid; geraniol; geranyl acetate; hexacosanol; iron; isopulegol; isovaleraldehyde; isovaleric acid; limonene; l-linalool; linalyl-acetate; luteolin; luteolin glycoside; magnesium; manganese; methyl heptenol; methyl heptenone; myrcene; neral; nerol; 2.1% phosphorus; alpha pinene; 2.3% potassium; 8.2% protein; quercetin; rutin; saponin; selenium; silicon; beta-sitosterol; sodium; alpha-terpineol; tin; triacontanol; zinc.

Several scientific investigations have demonstrated the sedative, CNS depressor, analgesic and anti-microbial activities of *C. citratus* leaves (Onawunmi *et al,* 1984; Lorenzetti *et al,* 1991; Mishra and Dubey, 1994; Negrelle and Gomes, 2007), and despite the widespread use of *C. citratus* in various parts of the world, not much has been reported in the literature about its toxicity on the brain. The few scientific reports available on *Cymbopogon* species were in connection with the efficacy of the essential oil of the plant (Onawunmi *et al,* 1984; Shadab *et al,* 1992; Dubey *et al,* 1997a, b; Bleasel *et al,* 2002; Koffi *et al,* 2009).

Whenever any medication is ingested, the body system interacts with it in an attempt to get rid of any harmful toxins such medication may contain, most especially if the body cannot convert the foreign substance into useful components. These results into insults which are commonly manifested by changes in enzyme levels and alteration in the cellular make up of various affected organs. The toxicity could as well result in tissue or organ damage. The vital organs that are commonly affected are brain, liver, pancreas, and kidney among others (Dapar *et al.,* 2007). The aim of this study therefore, was to investigate the effect of the *C. citratus* on the histology of the frontal cortex of male SD rats as a marker of toxicity.

2. Materials and methods

2.1. Collection of plant and preparation of plant extracts

Fresh mature leaves *C. citratus* were harvested from lemon grass plant at the premises of the University of Ilorin Teaching Hospital, Ilorin, Kwara State, Nigeria. Identification of the plant was made at the Botany Department of the same University. The authenticated plant material was air-dried at room temperature at the herbarium of the University of Osun, Osogbo, Osun State, Nigeria. The air-dried leaves were weighed using Gallenkamp (FA2104A, England) electronic weighing balance and were grinded with automatic electrical Blender (model MS-223, China) to powdered form.

One-hundred and fifty-five grams of the grinded plant sample was later soaked in 1000 ml of PBS for 48 hours at room temperature, and was later filtered through cheese cloth and then through Whatman #1 filter paper, the filtrate was concentrated using a rotary evaporator (Rotavapor® R-210) at 42- 47°C.

2.2. Laboratory rats and feeding

Ten healthy adult male SD rats were randomly grouped into a control group A (n=5), and treatment group B (n=5). The body weights of the rats were documented on daily basis using a digital weighing scale (Saltun® EK5055Max).

The rats in the group B were administered orally with 300 mg/kg body weight of the aqueous leaf extract of *C. citratus* for 14d respectively, while the rats in group A received equal volume of PBS.

All the experimental rats were accommodated in clean cages of dimensions 33.0×20.5×19.0 cm situated in well ventilated standard housing conditions (temperature: 28–31^{o}C; humidity: 50–55%). All experimental procedures followed the recommendations provided in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and Published by the National Institute of Health (NIH, 1985). The rats were fed with standard rat chow at a recommended dose of 100 g/kg as advised by the International Centre of Diarrheal Disease Research, Bangladesh (ICDDR, B) daily. Drinking water was supplied *ad libitum*.

Twenty-four hours after the last administration, all the rats were sacrificed by cervical dislocation, the frontal cortices were carefully excised from the skulls of the rats and blotted dry on a filter paper. The frontal cortices were subsequently placed in specimen bottles containing 10% formol calcium for further histopathological studies.

2.3. Histological parameters

After fixing the frontal cortices of both the treated and control rats, the tissues were processed for routine H&E, embedded in paraffin wax; serial sections of 5µ thick were obtained using the Leitz Rotary microtome (Leitz 1512 Microtome). The sections were mounted in DPX and examined with the aid of the Olympus binocular light microscope (XSZ-107BN, No. 071771). The photomicrographs of each slide was taken with a Nikon Digital Camera DXM1200F (Nikon, Japan) for subsequent histological analysis.

3. Results and discussion

No morphological alterations were observed in the morphological outline of the frontal cortices of the rats in both the treatment and the control groups as the frontal cortices in both groups appeared morphologically normal.

Using the Olympus binocular light research microscope (XSZ-107BN, No. 0717721), the neurohistological assessment of the frontal cortices of the rats in the extract treated group displayed abnormal histological profile as there were varying degrees of degenerative changes such as vacuolations, gradual loss of cytoplasmic contents, and eccentric placement of the nucleus in the frontal cortices of the extract treated rats. In the sections obtained from the rats in the control group, there was no evidence of apoptotic bodies or degenerative changes, the histological profile was preserved. The sections obtained in the control group (Fig. 1, 2) conformed to normal histological features compared with the extract treated sections.

Search of available literature revealed dearth of published scientific report on the toxicity of *Cymbopogon citratus* on the frontal cortex in laboratory animal models and humans despite the widespread use of the plant in various herbal remedies. In this investigation, oral administration of aqueous leaf extract of *C. citratus* produced varying patterns of deleterious alterations on the frontal cortex of male SD rats.

Evidence from the present investigation showed that the plant extract is toxic and have adverse effects on the histology of the frontal cortex in male SD rats. It was observed that, oral administration of the aqueous leaf extract of *C. citratus* confers histological derangement and deleterious effects on the frontal cortex of the extract treated rats.

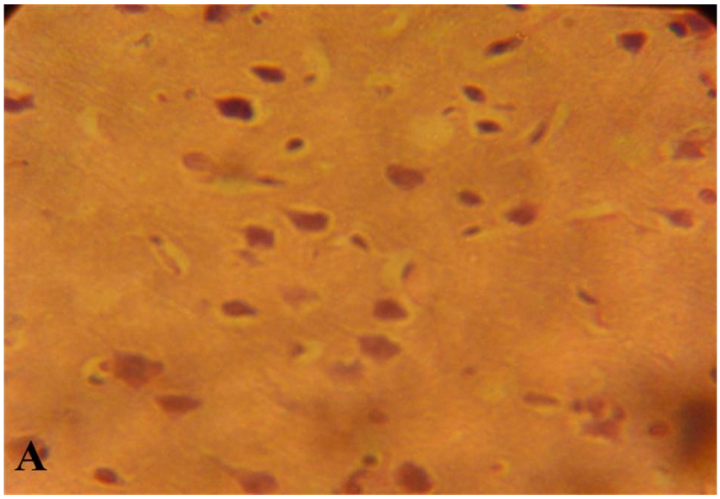

Fig. 1. Photomicrograph of the frontal cortex of the rat in group A (control) showing well preserved histological outline (H&E x400).

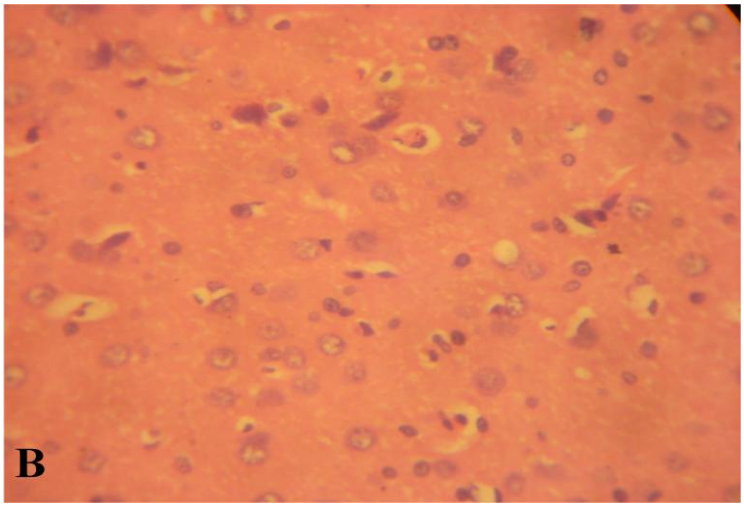

Fig. 2. Photomicrograph of the frontal cortex of the rat in group B (treated) showing neurons with varying degrees of vacuolations and cytoarchitectural distortions (H&E x400).

In the Brazilian folk medicine, *C. citratus* is consumed as a remedy to various maladies of the nervous system (Blanco *et al,* 2009). The histopathological outcome from this investigation does not agree with the potential of the plant as a remedy to nervous disorders.

Many herbal preparations have been found to exhibit deleterious effects on the functional integrity of many organs of the body. The tea made from the leaves of *C. citratus* has been observed to cause a recurrence of contact dermatitis in one case (Bleasel *et al,* 2002). According to Pereira *et al.* (2009), *C. citratus* is not a considerable effective agent in the prevention of various neurological diseases associated with oxidative stress. The significance of this in orthodox and traditional practice needs to be evaluated.

Cell death occurring pathologically or accidentally is regarded as necrotic and could result from extrinsic implications and/or disturbances to the cell and these may include toxic or traumatic effects (Farber *et al.,* 1981). Processes involved in cellular necrosis which may lead to cell death include compromise and/or disruption of the structural and functional potentials of the various membranes in and within the cell. Necrosis of the cell is not induced by intrinsic stimuli to the cells as observed in programmed cell death, but by an abrupt environmental disturbances and deviation from the normal physiological conditions, factors and functions (Ito *et al.,* 2003). The type of cell loss and the particular part of the organ affected determines the symptoms associated with individual disease (Waters, 1994).

The histopathological alterations observed in this investigation as characterized by histological damage to frontal cortex of male SD rats could have been as a result of direct toxicity or could have resulted from the release of toxic substances from other organs like the liver and kidneys into the blood which may have circulated to the brain. It could also have occurred as a result of the compromising effects of the phytochemical make up of the plant.

This investigation confirmed that oral administration of the aqueous leaf extract of *C. citratus* confer toxic and disruptive interference on cellular integrity of the frontal cortex in male SD rats.

To the best of our knowledge, this is the first study reporting the effect of *C. citratus* on the frontal cortex of male SD rats.

4. Conclusion

In conclusion, data obtained from this study show that the oral administration of aqueous leaf extract of *C. citratus* on the frontal cortex at the dose administered to the rats has deleterious effect on the cytoarchitecture of the frontal cortex. Considering the toxicity of *C. citratus*, herbal practitioners and herbal product users should be properly educated on this especially when they recommend and use this plant in the management and treatment of medical deviations like malaria, headache and stress-related conditions.

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