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In-vitro Evaluation of Potential Antioxidant Properties of *Eleusine indica* and *In-vivo* Visceral Organ Protective Effect of Higher-Dose of the Phytoextract in Normotensive Rats

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ABSTRACT
The therapeutic use of natural products from indigenous plants for ethnomedicinal purposes has grown tremendous interest among scientists to search for bioactive components that are beneficial to man. This study was aimed at determining the antioxidant properties of *Eleusine indica* (EI) root, and as well to evaluate its effect *in vivo*. The *in vitro* antioxidant properties were estimated by standard biochemical analytical assays. For *in-vivo* study, the food of experimental rats were improved with the addition of EI root extract at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg for 14 experimental days, while control rat was administered a saline solution (0.9%). On day 15, experimental rats were sacrificed for histological analysis. The impact of the extract on DPPH, NO, FRAP, TAC, H_2O_2 radicals were dependently and ascendingly concentrated, with DPPH and NO showing maximum antioxidant capability in conjunction with IC_{50} values. Data obtained from the histological assay in the present study, reinforced that the compounds present in the root of *Eleusine indica* (EI) possesses the potential of minimizing any deleterious effects associated with higher doses of the studied plant. In this case, antioxidant and histological tests showed that the goosegrass could protect animals from damage caused by toxic agent of plant origin. This study present proof that *Eleusine indica* (EI) root extract remain a possible source of natural antioxidant, and that the extract of the EI at higher doses, in this study, could represent phyto-remedial indicator in protecting the rats from toxic-induced damage of plant origin, indicating that the goosegrass exhibits protective action *in vivo*.

Keywords: *Eleusine indica*, biochemical, herbal therapy, antioxidant, plant extract

INTRODUCTION

Antioxidants exert positive effects on human health, as they protect the human body against harmful effects caused by reactive oxygen species, which damage macromolecules such as membrane lipids, proteins, and DNA and can lead to the development of several diseases, such as cancer and neurodegenerative, inflammatory, and heart diseases (Babber *et al*., 2011).

Recently, interest in the development of antioxidants from natural sources of both terrestrial and marine flora and fauna (Chakraborty *et al*., 2013) has increased considerably in the food and pharmaceutical industries. Red goosegrasss are one of the richest sources of natural antioxidants, including phenolic compounds, vitamins, and sulfated polysaccharides (Yangthong *et al*., 2009). In addition, in red goosegrasss, insoluble fibers are composed of cellulose and the soluble fibers are composed of sulfated galactans or soluble xylans. Fibers are mainly used as bulking and texturing agents, which are essential for the development of low calorie foods. A high intake of dietary fiber reduces the development of chronic diseases, such as diabetes, obesity, heart diseases, and cancer (Jimenez-Escrig *et al*., 2011).

Plants are being employed by humans to curtail various contagious and non-infectious illnesses since the dawn of time as a reliable source of therapy; and also represent the main source for many of orthodox medicines (Luciano-Montalvo *et al*., 2013). Plants, on the other hand, have long been employed as a food and medicine sources. Besides, they function in high-nutrient vegetable sources, but their different sections (seed, leaf, and fruit) are employed in remedial health utilization. Plant products' beneficial effects may be attributed to the bio-activities of their active ingredients (Dinda *et al*., 2007). There has recently been a growing interest in natural anti-reactive oxygen species, ensuring reduction in the harmful free radicals' effects on the human body. Because of the assurance of efficacy and protection, the free radical scavenging properties of medicinal plants, known as natural antioxidants, are used in a variety of medical applications (Al-Snafil, 2016). Consumption of fresh fruits and vegetables rich in plant polyphenols (antioxidants) as food has been documented to protect against several diseases including cancer, cardiovascular diseases, diabetes, asthma, etc. Pandy and Rizvi (2009), suggested that the mechanism of action of the secondary metabolites can be traced back to their antioxidant properties. An antioxidant works by halting these chain reactions by removing free radical intermediates and preventing oxidants from oxidizing useful molecules (Ferguson, 2010).

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Natural antioxidants, on the other hand, such as phytochemicals, are safer alternatives to synthetic antioxidants because they have a variety of therapeutic properties with little or no side effects (Saeed *et al*., 2012; Ayodele *et al*., 2013).

Eleusine indica (L.) Gaertn belongs to the family, Poacceae. The species, also known as Goosegrass, is a common annual turf and horticultural weed found throughout Florida. It grows well in compact, wet soil and competes successfully with warm-season and cool-season turf grasses especially with thin, open disturbed turf. It is considered an aggressively intrusive weed due to its vigorous growth and abundant seed production. Goosegrass can grow up to 3 feet tall (1 m) and spreads by reseeding itself (Steed *et al*., 2019).

The aim of this study was to evaluate potential antioxidant properties of *Eleusine indica* root, and to determine higher dose histological effect of the studied plant on visceral organ, liver and kidney in experimental rats.

Materials and Methods

Collection of plant material sample

The plant material (root of *Eleusine indica*) were collected in November 2022 within Okitipupa metropoly, Ondo State, Nigeria.

Sample preparation

Fresh root of *Eleusine indica* were separated from the plant, rinsed in water, and spread out on laboratory tables to dry at room temperature. The plant material was then transferred to a 40°C oven for 10 minutes, where it was reduced to fine powder with the help of a mechanical grinder.

Extraction of plant material

Two hundred gram (200 g) of powdered plant material was macerated in 1 liter of distilled water for 48 hours. The mixture was wrapped with porcelain cloth before being filtered with No. 1 Whatman filter paper. Before further testing, the filtrate was concentrated using a rotary evaporator and the raw concentrate was deposited in a refrigerator at 4^{o}C for further analytical use.

In vitro determination of antioxidant tests

Plant extract antioxidant ability was assayed. The DPPH radical scavenging, nitric oxide scavenging, FRAP, total antioxidant power, and hydrogen peroxide scavenging assays were used for the evaluation.

2,2-Diphenyl-1-picrylhydrazile (DPPH) radical scavenging assay

The DPPH scavenging capacity of the plant extract was calculated using the method described by Wintola and Afolayan (2011), with concentration changes. One milliliter of DPPH in distilled water (0.135 mM) was mixed with one milliliter of plant extract and standardized at concentrations ranging from 0.2 to 1 mg/mL. The mixture was thoroughly vortexed and left in the dark for 30 minutes at room temperature. A spectrophotometer was used to measure absorption at 517 nm. The plant extract DPPH scavenging ability was determined as follows: DPPH scavenging (percentage) = [(Abs control – Abs sample)/(Abs control)] x100, where Abs represents absorbent DPPH + aqueous. Abs sample represents absorbent DPPH radical + sample (extract/standard).

Nitric oxide (NO) scavenging activity

The method described by Lee *et al*. (2003) was used to determine the degree of inhibition of nitric oxide radical generation *in vitro*. At physiological pH, sodium nitroprusside in aqueous solution spontaneously produces nitric oxide, which reacts with oxygen to produce nitric ions at 546 nm spectrophotometrically. As reagents, sodium nitroprusside (100 mM), phosphate buffered saline (pH 7.4), and gray reagent (1% sulphanilamide, 2% H_3PO_4, and 0.1% napthylethylene dihydrochloride) were used. The reaction was triggered by the addition of 2.0 ml of sodium nitroprusside, 0.5 ml of PBS, and 0.5 ml of seed extract (50 mg), followed by 30 minutes of incubation at 25˚C. Griess reagent (0.5 ml) was added and incubated for an additional 30 minutes. Control tubes were made without the extract. A spectrophotometer was used to measure absorbance at 546 nm in comparison to a blank reagent (Genesys 10-S, USA).

Ferric reduction of antioxidant strength assay

The antioxidant potential of the extract was evaluated using the ferric reduction method described by Zhao *et al*. (2008). The reduction power increased as the absorbance of the reaction mixture increased.

Absolute antioxidant power assay (phosphomolybdenum: TAC)

Ohikhema *et al*. (2018) defined the phosphomolybdenum method for calculating total antioxidant ability. In brief, 0.3 mL of solvent extract and normal (0.025-0.4 mg/mL) were dissolved in 3 ML of reagent solution in test tubes (0.6 M sulfuric acid, 4Mm ammonium molybdate, and 28 mM sodium phosphate). The test tubes were sealed and incubated in a water bath at 95 degrees Celsius for 95 minutes. After allowing the mixture to cool to room temperature, the absorbency was measured at 695 nm. The test tubes were sealed and incubated in a water bath at 95 degrees Celsius for 95 minutes. After allowing the mixture to cool to room temperature, the absorbency was measured at 695 nm.

Hydrogen peroxide (H_2O_2) scavenging activity

The percentage of H_2O_2 scavenging was calculated using the following equation: percentage of H_2O_2 scavenging = [(A0-A1)/A0] x 100, where A0 and A1 were the absorbance of the control and test extracts, respectively (Bozin *et al*., 2008).

***In Vivo* Experimental Assay**

Twenty-four rats (*Rattus novargicus*) of equal sex weighing 30–35 g, aged 30 days, purchased from the animal holding unit, Department of Anatomy, University of Benin, Nigeria, were used in this study. After the acclimation period, the rats were weighed and randomly divided into four groups, each containing six rats. The control group received a saline solution (0.9%, w/v) by gavage. Rats in the second group received aqueous root extract of *Eleusine indica* at the dose of 100 mg/kg body weight. The third and fourth groups of experimental rats received aqueous root extract of *Eleusine indica* at the doses of 200 and 400 mg/kg body weight daily. After 14 days of treatment, the rats were weighed and recorded. Finally, necropsy and histological analysis of experimental rats were carried out, and animals were euthanized by administration of high doses of anesthetic (20 mg/kg thiopental). The tissues and organs were examined macroscopically for visible abnormalities. Subsequently, the liver and kidneys of all animals were removed, weighed, and washed with PBS, pH7.4, to remove any red blood cells and clots, and divided into two equal parts. For histological analyses, one of these parts was fixed in buffered formaldehyde. After 24 h, the apparatus was embedded in paraffin, sectioned (5 μm in diameter), placed on glass slides, and stained with hematoxylin and eosin. The slides were examined through optical microscopy (20, 40, and 100x objective lens) for lesions and protective action, and interpretations were done as described by Neel *et al*. (2007).

The biological assay was developed in accordance with the ethical principles in animal experimentation, and the project was approved by the Ethics Committee on Animal Use (UBAU—Protocol 059/24).

Statistical analysis

The data obtained in biochemical assays were analyzed by one way ANOVA. Analytical determinations were made in triplicate and values considered to be significant at a significance level of 0.05 ($p < 0.05$). The mean and standard error of the mean are used to express the results.

Results

Antioxidant findings

Table 1 showed antioxidant activity *in vitro* results of *Eleusine indica* (EI) aqueous root extract. The experimental extract gotten by means of solvent, aqueous and proportion was capable of inhibiting the DPPH radical, NO scavenging, FRAP, TAC and HPSA. The potential for antioxidants varied greatly amongst the functional indexes used, and ranged from 29.91^{a}±7.96 with the DPPH method, 28.99^{a}±12.72 with the NO method, 7.60^{b}±1.65 with the FRAP method, 6.20^{b}±1.39 with the TAC method to 3.15^{c}±0.02 with the HPSA method.

Tables 1: *In vitro* antioxidant indices of *Eleusine indica* aqueous root extract

Chemicals	Level (% Concentration)
Ascorbic acid (Standard)	4.31%
DPPH Scavenging Activities (% inhibition)	29.91^{a}±7.96
Nitric Oxide (% Radical Scavenging Activity)	28.99^{a}±12.72
FRAP	7.60^{b}±1.65
Total Antioxidant Capacity	6.20^{b}±1.39
Hydrogen Peroxide Scavenging Activities	3.15^{c}±0.02

**P < 0.05 - Significant*; *Different letters in superscript down column showed significant differences in the chemical response when compared with value of the standard compound.*

Preliminary phytochemical assessments of *Eleusine indica* roots depicts availability of flavonoids, saponins, reducing sugar, alkaloids, mucilage, tannins, and cardiac glycosides; with varying concentrations, while anthraquinones and triterpenoids were absent or not detected

(Data unpublished). A good correlation has been observed between the polyphenol of the root of *Eleusine indica* and the antioxidant activity. Polyphenolic compounds and antioxidant activity in the present study, relayed the action of polyphenolic compounds in enhancing antioxidative effect and scavenge free radicals (Milliauskas *et al*., 2004; Mbaebe *et al*., 2012; Saeed *et al*., 2012; Kusonardiyani *et al*., 2016). The maximum antioxidant capacity of the aqueous extract of *Eleusine indica* root could be due to the presence of phenolic plant secondary metabolites, thereby suggesting that polyphenol compounds remains the primary contributors to the antioxidant capacity/ability possessed by *Eleusine indica* root extract.

Histological Analysis

Figure 1 showed histological sections of livers. In the negative control group (Figure 1(a)), normal hepatocytes with preserved cytoplasm and nucleus was observed. The same characteristics were observed in the liver of the animals treated with EI (Figure 1(c) and Figure 1(d)). This indicated that the phytoextract of EI is not toxic to the animals at the dose levels of 200 mg/kg and 400 mg/kg. In contrast, the liver sections of rats that received 100 mg/kg of the EI phytoextract (Figure 1(b)) contain pyknotic nuclei, vacuolized cells, liver damage with mild to moderate hepatocellular degeneration, and necrosis. When 200 mg/kg and 400 mg/kg dose levels of the EI phytoextract (Figure 1(c and d)) were administered, these parameters of hepatocellular degeneration, and necrosis were all decreased, indicating hepatic remedial effect of phytoextract of the studied plant at higher-doses used in this study.

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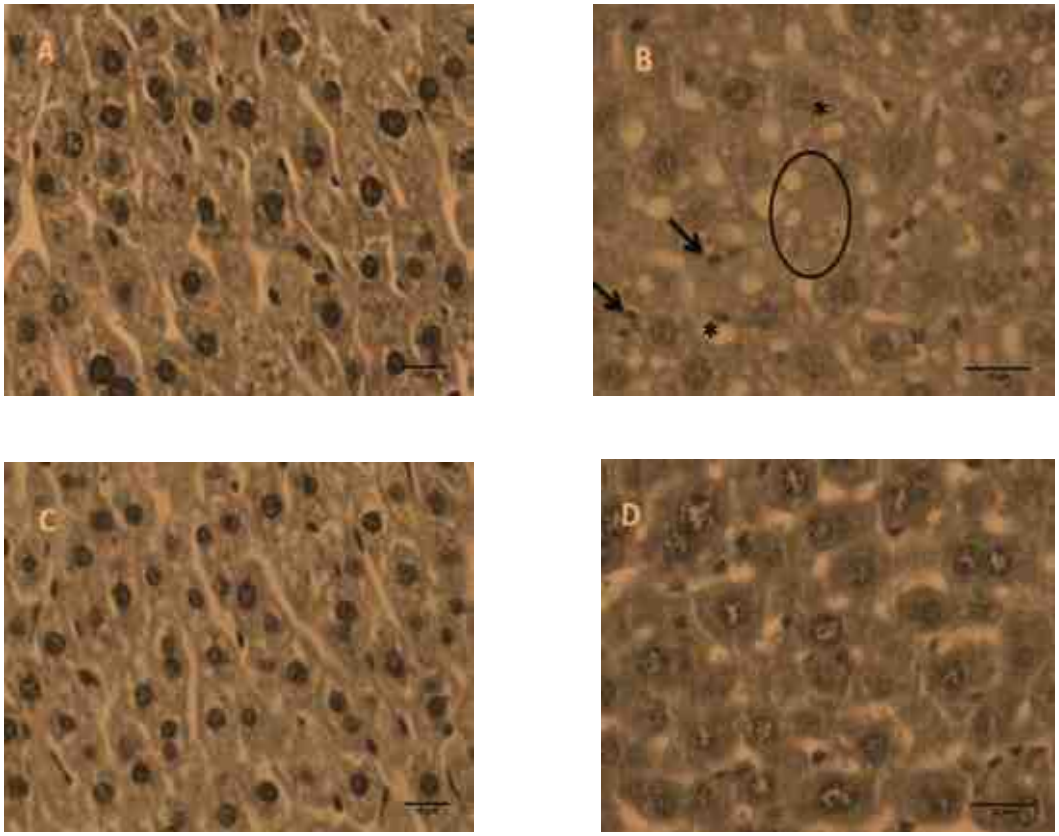

Figure 1: Histopathological changes in the rat's liver (hematoxylin and eosin stain). (a) Control rat liver. (b) Rat liver treated with 100 mg/kg body weight. (c) Rat liver treated with 200 mg/kg body weight. (d) Rat liver treated with 400 mg/kg body weight, magnification 20x. Bar: 10 μm. **Circle:** necrosis; **arrow:** pycnosis; **asterisk:** cell vacuoles.

Figure 2 showed histological sections of the kidneys. The kidneys of animals in the control group (Figure 2(a)) and those treated with 200 and 400 mg/kg body weight phytoextract of the studied plant EI (Figure 2(c) and Figure 2(d)) presented well-preserved glomerulus. In contrast, kidney sections from rats treated with phytoextract of the studied plant EI at the dose of 100 mg/kg body weight (Figure 2(b)) contained renal tubules characterized by necrosis and loss of the glomerular borders, which are suggestive of inflammation and intense vascularization. However, the histopathological lesions observed following administration of 100 mg/kg body weight were minimized with the administration of 200 and 400 mg/kg body weight phytoextract of the studied plant EI (Figure 2(c) and Figure 2(d)).

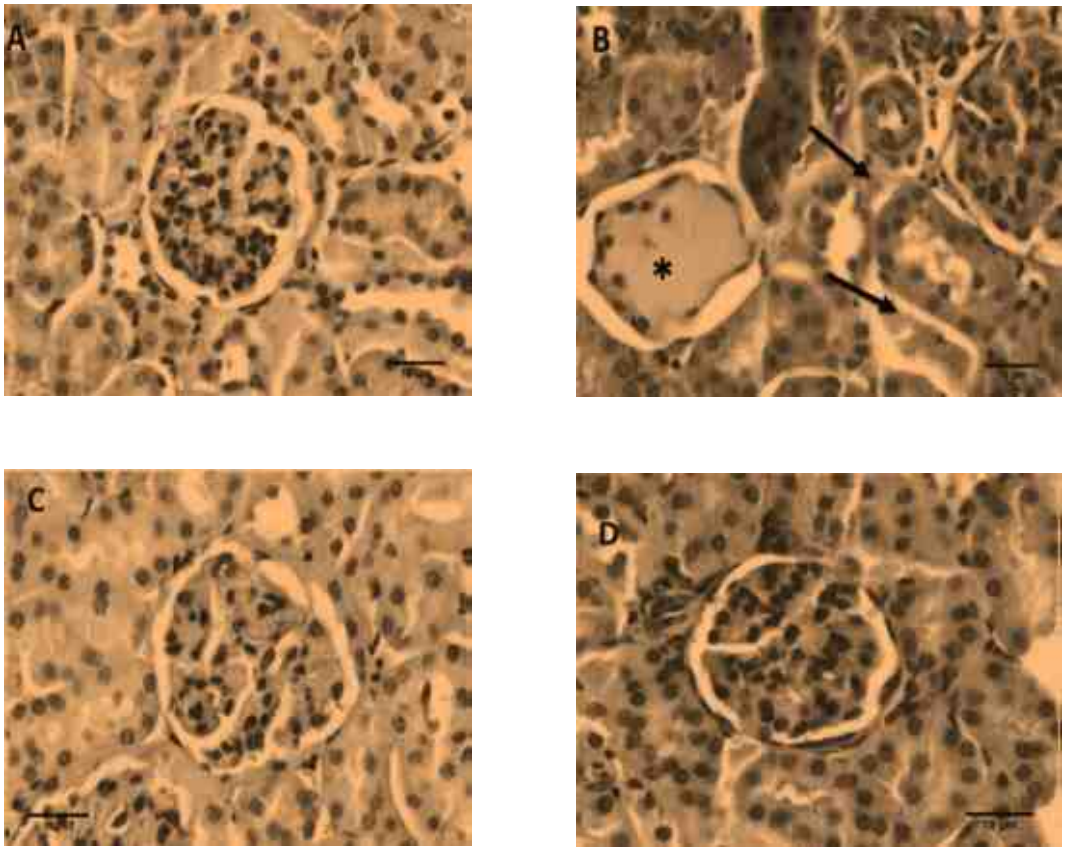

Figure 2: Histopathological changes in the rat's kidney (hematoxylin and eosin stain). (a) Control rat kidney. (b) Rat kidney treated with 100 mg/kg body weight. (c) Rat kidney treated with 200 mg/kg body weight. (d) Rat kidney treated with 400 mg/kg body weight, magnification 40x. Bar: 10 μm. **Asterisk:** necrosis; **arrow:** hematosis (intense vascularization).

Discussion

Phytochemicals such as phenolic compounds are potent antioxidants, with the ability to inhibit tumor progression and manage free radical damage induced by toxins, viruses, and microbes amongst others (Omage *et al*., 2017). They have an astringent property; they can hasten wound healing as well as repair inflamed mucous membranes (Omage *et al*., 2017). They can therefore heal burns and ulcers. They also induce tumor regression and act as antibiotics (Omage *et al*., 2017). The presence of these phytochemicals in *E. indica* (EI) lends credence to the use of this plant for the management of diseases.

The phytochemicals present in the *Eleusine indica* roots extract (Data unpublished) used in this study are likely to be responsible for the observed *in vitro* antioxidant properties in this study. In this study, *E. indica* root extract displayed the ability to bleach DPPH, scavenge hydrogen peroxide, and reduce ferric ions. The DPPH results showed that the aqueous extract had a higher antioxidant potential than the standard, ascorbic acid.

Oxidative stress, caused by the lack of the biological system's ability to neutralize excessive free radical products has been linked to a variety of human diseases and aging (Lee *et al*., 2003; Liu *et al*., 2014). Antioxidants (free radical scavengers) are substances that interact with and neutralize free radicals, preventing them from causing cell damage in the biological system (Diplock *et al*., 1998). Antioxidants are produced by the body and used to neutralize free radicals. Endogenous antioxidants are the antioxidants that occur naturally in the body. However, the body also relies heavily on external (exogenous) food sources to obtain the remainder of the antioxidants it requires (Valko *et al*., 2007). It is believed that these plants can prevent or protect tissues from the harmful effects of free radicals (Osawa and Kato, 2005). Beneficial medicinal impacts of plant materials are basically the outcome of interactions between secondary metabolites found in the plant; through the additive or synergistic action of multiple chemical compounds at a single or multiple target sites linked with a physiological process (Briskin, 2000).

Histopathological studies were performed in rats to assess the effect of higher-dose phytoextract of the studied plant on liver and kidney tissues and to verify whether tissue damage is reduced following administration of the higher-dose phytoextract in association with the goosegrass (EI) toxicity effect at a low dose as observed in this study. The roots of *E. indica* are consumed by humans; therefore, it was not surprising that the aqueous extract of *E. indica* used in this study was not harmful to rats even when consumed at a high doses of 200 and 400 mg/kg body weight. At a low dose of 100 mg/kg, the studied plant extract caused cellular degeneration of the visceral organs studied, liver and kidney; which later brought remedial effects on the visceral organs following the administration of higher doses of 200 and 400 mg/kg body weight. These results support the data obtained from the antioxidant assays in the present study and reinforced that the compounds present in the goosegrass (EI), when applied at higher-doses used in the present study, could minimize the deleterious effects of toxic substances of plant origin in animal experimental model, justifying human circumstances. As in the present study, Wu *et al*. (2013) evaluated the protective effect of sulfated polysaccharides from the brown alga *Hizikia fusiformis* in the liver of rats and also obtained positive results. Also, Rodrigues *et al*. (2011) observed that extracts from the red alga *Hypnea musciformis* also protected the renal tissue from CCl4-induced damage. The study stated that the protective action of the alga is mainly due to the presence of the antioxidant sulfated polysaccharides in the extracts. Therefore, we believe that *E. indica* (EI) protected the renal tissues of the animals via the potential antioxidants synthesized by it.

In conclusion, the sets of experimental results gotten in this research have given a scientific verdict that the studied plant, had a significant impact on the protection of both the livers and the kidneys of rats from damage that might be caused by toxic substances of plant origin, indicating that the *Eleusine indica* studied, exhibited a protective action *in vitro* and *in vivo*, possibly due to its antioxidant capacity. It deserves being recommended to expand its application in the field of health maintenance.

Authors' contributions

Methodology: Adekunle Orimisan Ojatula; Investigation: The trio Authors; Writing-original draft-review and editing: Adekunle Orimisan Ojatula; Supervision: Adekunle Orimisan Ojatula.

Potential conflicts of interest

Author does not portray any potential conflict of interest.

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