

## Effect of *Solenostemon monostachyus* on Antioxidant Biomarkers in Wistar Rats

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### Abstract

### Original Research Article

*Solenostemon monostachyus*, SOM, has aroused overwhelming attention, in recent years. There is growing concern whether SOM expresses its effect via a particular phytoconstituent, a combination of fractions, or otherwise. This study investigated the effect of both the ethanol extract and alkaloid fraction of SOM on some antioxidant biomarkers. Twenty-five adult Wistar rats were randomly grouped into five batches (n=5). Group I (control) received rodent feeds and water; Group II had 200mg/kg body weight of ethanol extract of SOM; Group III (400mg/body weight of ethanol extract). While groups IV and V, respectively, received, 33mg and 66mg/kg body weight of SOM alkaloid fraction. Serum levels of Superoxide dismutase (SOD), malondialdehyde (MAD) and catalase (CAT) enzymes were evaluated. The results showed significant elevation of SOD and CAT activity, respectively, while MAD levels were reduced by the effect of crude extract and alkaloid fraction. This suggests SOM's potential in maintaining cellular integrity and preventing tissue damage via anti-oxidative activity and reducing lipid peroxidation.

**Keywords:** Superoxidase dismutase, Malondialdehyde, Catalase, *Solenostemon monostachyus*, antioxidation.

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## 1.0 INTRODUCTION

The rapid increase in consumption of herbal remedies worldwide has been stimulated by several factors, including the notion that all herbal products are safe and effective. (Omoloye *et al.*, 2015)

*Solenostemon monostachyus*, SOM, has aroused overwhelming attention, in recent years (Okokon *et al.*, 2015). Its use in the ethno-medical treatment of sicknesses and diseases nearly cuts across all organ systems of the body. SOM is mainly administered orally, more so, in the form of a crude extract.

Like in any other herbal intake, adverse effects on the tissues and organ-systems cannot be ruled out. (Okaiyeto and Oguntibeju, 2021). There is growing concern whether SOM expresses its effect via a particular phytoconstituent, a combination of fractions, or otherwise (Okoko and Ere, 2015)

## 2.0 SOLENOSTEMON MONOSTACHYUS: A NOVEL HERB

*Solenostemon monostachyus* is an aromatic, medicinal plant that belongs to the family *Lamiaceae*. In English language, it is called Monkey potato. Its names among some ethnic groups in Nigeria include Ntorikwot (Ibibio) and Olojogbodun (Yoruba). The use of

*Solenostemon monostachyus* plant in traditional medicine by the people of Nigeria has been documented.(Okokon, *et al.*,2017) The aerial parts of the plant are used in various decoctions traditionally by the Ibibios of the Niger Delta of Nigeria to treat stomach ulcer, fever/malaria The plant has been reported to possess antioxidant (Okoko and Ere, 2012) antihypertensive (Fidele, Andre, Yao and Michel, 2012) and antimicrobial activities. Its anti-inflammatory and anti-nociceptive activities (Okokon, *et al.*,2020), and anti-microbial properties (Ekundayo *et al.*, 2006) and anti-ulcer as well as screening of its phytochemical constituents had been documented in literatures. Apart from the *S. monostachyus*, some plants and herbs including the leaf extract of *Carica papaya* (English name: pawpaw) show potent antioxidant activity. The leaf essential oil of *S. monostachyus* has been reported to contain;  $\beta$ -pinene, oct-1-en-3-ol,  $\beta$ -caryophyllene, octan-3-ol and (E,E)- $\alpha$ farnesene (Mvé-Mba *et al.*, 1994) in abundance and some of these compounds are sesquiterpenes. The presence of bioactive compounds is an affirmation of the use of *Solenostemon monostachyus* leaves in the management of various ailments, and can serve as a potential source of useful drugs. (Obichi *et al.*, 2015).

## 2.1 Origin and history of *Solenostemon monostachyus*

*Solenostemon monostachyus* which belongs to the family Lamiaceae (English name: Monkey potato; Ibibio name: Ntorikwot; Yoruba name: Olojogbodu) is an important edible herb which can be found mostly within the tropical countries (West and Central African). It's a valuable herb that thrives in both anthropogenic and rocky savannah environments. The plant is an erect, branched annual weed with a long inflorescence of violet flowers. It is slightly succulent, aromatic and grows up to 100 cm tall (Mye-Mba and Menut, 1994).

## 2.2 Scientific classification of *solenostemon monostachyus*

The genus *Solenostemon* is in the family Lamiaceae in the major group, Angiosperms (Flowering plants). It is classified as follows: Kingdom (Plantae), Phylum (Tracheophyta), Class (Magnoliopsida), Order (Lamiales), Family (Lamiaceae), Genus (*Solenostemon*), Species (*monostachyus*, P. Beauv.). (Paton *et al.*,2019).

## 2.3 Phytochemical composition of *Solenostemon monostachyus*



Fig. 1: Image of *Solenostemon monostachyus* in its natural habitat (Paton *et al.*, 2019)

Phytochemicals are chemicals of plant origin The phytochemical screening of *S. monostachyus* leaf extract revealed the presence of saponins, tannins,

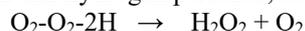
cyanogenic glycosides, flavonoids and alkaloids (Obichi, *et al.*, 2015).



Fig. 2: *Solenostemon monostachyus*  
Source: Wikipedia, 2022

### 3.0 ENZYMATIC ANTIOXIDANT BIOMARKERS: SUPEROXIDE DISMUTASE, MALONDIALDEHYDE, CATALASE

These enzymes play crucial role in neutralizing free radicals, reducing oxidative stress, and protecting against cell damage. Superoxide Dismutase (SOD), EC 1.15.1.1, is a potent intracellular enzymatic antioxidant and it catalyzes the conversion of superoxide anions to dioxygen and hydrogen peroxide, as follows.



Malondialdehyde (MDA) is a reactive aldehyde that is widely used as a biomarker of lipid peroxidation and oxidative stress. Because it contains two aldehyde groups, it can participate in several important chemical reactions, especially with nucleophiles like amines and thiols. Below are key reactions involving MDA:

#### 1. Formation of MDA from Lipid Peroxidation

MDA is produced during the oxidative degradation of polyunsaturated fatty acids (PUFAs) in cell membranes.

##### Steps:

**A. Initiation:** Free radicals ( $\bullet\text{OH}$ ,  $\bullet\text{O}_2^-$ ) extract a hydrogen from PUFA.

**B. Propagation:** Lipid radicals react with oxygen  $\rightarrow$  lipid peroxy radicals.

**C. Breakdown:** Peroxy radicals decompose  $\rightarrow$  MDA + other aldehydes.

#### 2. Reaction of MDA with Thiobarbituric Acid (TBA) — TBARS Assay

This is the most common method to measure MDA levels.

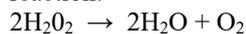
A. MDA reacts with 2-thiobarbituric acid (TBA) under acidic and high-temperature conditions.

B. This forms a pink chromogen that absorbs at 532–535 nm.

Malondialdehyde (MDA) assay is based on the reaction of MDA with thiobarbituric acid (TBA) forming an MDA-TBA<sub>2</sub> adduct that absorbs strongly at 532nm. This method is very relevant in estimating MDA levels in biological systems.

$\text{MDA} + 2 \text{ TBA} \rightarrow \text{MDA-TBA adduct (pink pigment)}$

On the other hand, catalase enzyme (CAT) catalyzes the quick breakdown of hydrogen peroxide, H<sub>2</sub>O<sub>2</sub>, to water and molecular oxygen. Catalase activity in a sample is measured as directly proportional to the rate of this reaction.



## 3.0 EXPERIMENTAL PROTOCOL

### 3.1 Plant Procurement

Fresh leaves of *Solenostemon monostachyus* were obtained from a botanical garden in Calabar, Cross River, in the month of March, 2024. The sample of the plant specimen was identified and authenticated by a botanist expert from the Department of Botany, University of Calabar, Nigeria.

### 3.2 Preparation of Ethanolic Plant Extract of SOM

Specifically, the leaves were sorted to eliminate any dead matter and other unwanted particles (Harborne,1998). They were air dried under shade at room temperature for 14 days and the leaves were blended with a manual hand blender to a powdered form. 100g of the fine powder was extracted using 80% ethanol (600ml) and allowed to stand for three (3) hours. It was then refrigerated at 4°C for three (3) days. A silk material was used to filter, and then a secondary filtration was done using Whatman's filter paper size number 2 was used to complete the process. The filtrate was then dried until a paste was formed which was stored (in a refrigerator) as a stock reference.

### 3.3. Acid-base extraction of alkaloid fraction of SOM.

Alkaloid constituents were selectively extracted with acid-base extraction approach (Harborne,1998), with minor modification (Anza, *et al.*,2022). Root powder (400 g) was extracted with 97 % ethanol (3 x 1.5L) for 24 h at room temperature by maceration while shaking by electronic shaker at a speed of 230 rpm at room temperature. The solution was filtered and concentrated using a vacuum rotary evaporator to yield a crude extract. The residue of ethanol extract was redissolved in chloroform (100 mL) and then extracted with aqueous H<sub>2</sub>SO<sub>4</sub> 5% to pH 3-4 (3 x 50 mL) in a separatory funnel to remove lipids, acidic and neutral metabolites. The aqueous phases were combined and basified with NH<sub>4</sub>OH 5% to pH 11 and extracted with chloroform (3 x 100 mL). The chloroform phases were combined, dehydrated with anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated by using a rotary evaporator to afford 6.9 g of an alkaloid fraction. Finally, the presence of alkaloids was checked by Dragondroff and Mayer's reagent chemical test.

### 3.4 Animal Groupings

Twenty-five (25) Wistar rats (both sexes), 220 ± 40 grams body weight, were randomly assigned to five batches (n=5). Group I: Control (rat, chow, water *ad libitum*). Group II: 200 mg SOM / kg body weight (rat chow, water *ad libitum*, *ethanol extract*); low dose SOM {ETHANOL LD} Group III: 400 mg SOM /kg body weight (rat chow, water *ad libitum*, *ethanol extract*); high dose SOM {ETHANOL HD} Group IV: 33 mg alkaloid /kg body weight (rat chow, water *ad libitum*, *alkaloid fraction*); low dose alkaloid {ALKALOID LD} Group V: 66 mg ALKALOID/kg body weight (rat chow, water *ad libitum*, *alkaloid fraction*); high dose alkaloid {ALKALOID LD}

## 4.0 ANTIOXIDANT ENZYME ACTIVITIES AND LIPID PEROXIDATION ASSAYS

### 4.1 Superoxide Dismutase (SOD) Activity

#### 4.1.2 Principle:

Superoxide dismutase (SOD) activity was determined using a modified method of Marklund and Marklund (1974). The assay is based on the ability of SOD to inhibit the autoxidation of pyrogallol. SOD

catalyzes the dismutation of the superoxide radical ( $O_2^{\bullet-}$ ) into molecular oxygen and hydrogen peroxide.

#### 4.13 Preparation of Tissue Homogenate:

Tissue homogenates were treated with the non-ionic detergent Triton X-100 (1% final concentration) and incubated on ice for 30 minutes to ensure complete release of the Cu–Zn SOD enzyme.

#### 4.14 Assay Procedure:

The reaction mixture (final volume: 1 ml) consisted of 500  $\mu$ l of 0.1 M sodium phosphate buffer (pH 8.0; 50 mM), 33  $\mu$ l of 3.3 mM EDTA (0.1 mM), 60  $\mu$ l of 8.1 mM pyrogallol (0.48 mM), and an appropriate volume of tissue homogenate containing 10  $\mu$ g of protein. The increase in absorbance was monitored at 420 nm for 2 minutes at 25°C against a reagent blank lacking tissue homogenate.

#### 4.15 Calculation of SOD Activity:

SOD activity was expressed as units per milligram of protein. One unit of SOD was defined as the amount of enzyme required to produce 50% inhibition of pyrogallol autoxidation.

## 4.2 Determination of Lipid Peroxidation (Malondialdehyde, MDA)

Lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels using a modified method of Armstrong and Al-Awadi (1991).

#### 4.21 Assay Procedure:

A 1 ml aliquot of sample or standard was mixed with 3 ml of TCA–TBA–HCl reagent and thoroughly vortexed. The mixture was heated in a boiling water bath for 15 minutes, cooled to room temperature, and centrifuged at 1000  $\times$  g for 10 minutes. The absorbance of the resulting supernatant was measured at 535 nm against a reference blank.

#### 4.22 Calculation of MDA Concentration:

MDA concentration was calculated using a molar extinction coefficient of  $1.56 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  and expressed as units per gram of tissue.

#### 4.3 Determination of Catalase Activity

Catalase activity was determined following the method described by Cohen *et al.* (1970). The assay is based on the decomposition of hydrogen peroxide ( $H_2O_2$ ) by catalase and subsequent reaction of residual  $H_2O_2$  with potassium permanganate ( $KMnO_4$ ).

#### 4.31 Assay Procedure:

The rate of  $H_2O_2$  decomposition was assessed spectrophotometrically by measuring the residual permanganate at 480 nm. Catalase activity was directly proportional to the rate of decrease in  $H_2O_2$  concentration.

#### 4.32 Preparation of Reagents:

Phosphate buffer (pH 7.4) was prepared by dissolving appropriate quantities of disodium phosphate and potassium dihydrogen phosphate in distilled water. The pH was adjusted using 0.1 M hydrochloric acid, and the final volume was made up to 1000 ml. A 6 M sulphuric acid solution was prepared by carefully adding 163.2 ml of concentrated sulphuric acid (98%) to distilled water with continuous stirring, followed by dilution to a final volume of 1000 ml. A 0.01 M potassium permanganate solution was prepared by dissolving 0.158 g of  $KMnO_4$  in 100 ml of distilled water.

Hydrogen peroxide (30 mM) was prepared fresh by adding 0.1 ml of 10%  $H_2O_2$  to approximately 80 ml of 0.05 M phosphate buffer (pH 7.4), and then the volume was adjusted to 100 ml with the buffer solution.

#### 4.33 Assay Protocol

To measure catalase activity, 0.5 ml of the sample was mixed with 5 ml of 30 mM  $H_2O_2$  in an ice-cold test tube, and the reaction was stopped after 3 minutes by adding 1 ml of 6 M  $H_2SO_4$  followed by mixing. The resulting solution was then measured for absorbance at 480 nm against distilled water within 30–60 seconds. A blank solution was prepared in the same way as the test but with 0.5 ml of distilled water instead of the sample. A spectrophotometric standard was also prepared by adding 1 ml of 0.01 M  $KMnO_4$  to a tube containing 5.5 ml of 0.05 M phosphate buffer (pH 7.4) and 1 ml of 6 M  $H_2SO_4$  solution, and its absorbance value was measured at 480 nm.

#### 4.34 Calculation of Catalase Activity

The catalase activity was then calculated using the formula below:

$$\text{Catalase Activity (Kc)} = \text{Log}_{10} \times 2.03$$

Where:

So = Absorbance of standard – Absorbance of blank

Ss = Absorbance of standard – Absorbance of test

#### 4.35 Statistical Analysis

All results are presented as mean  $\pm$  standard error of mean. Data were analyzed using one way of variance (ANOVA), followed by the least significant difference (LSD) procedure for significant F values. A  $p < 0.05$  was considered significant. Computer Software (SPSS and Excel Analyzer) was used for the analysis.

## 5.0 RESULTS

### 5.1 Effect of SOM on Anti-oxidant Biomarkers

#### 5.12 Effect of SOM on Superoxidase Dismutase Enzyme (SOD), Fig. 3

The activity of SOD was assayed, and the values (pg/ml) were as follows:  $61.00 \pm 1.95$ ,  $308.20 \pm 3.35$ ,  $459.00 \pm 0.89$ ,  $465 \pm 0.73$  and  $378 \pm 1.47$ , respectively, for control, ELD, EHD, ALD and AHD.

(ELD= ethanol Extract low dose, 200mg/body weight; EHD= ethanol Extract high dose, 400mg/body weight; ALD= alkaloid fraction low dose, 33mg/body weight; alkaloid fraction high dose, 66mg/body weight)

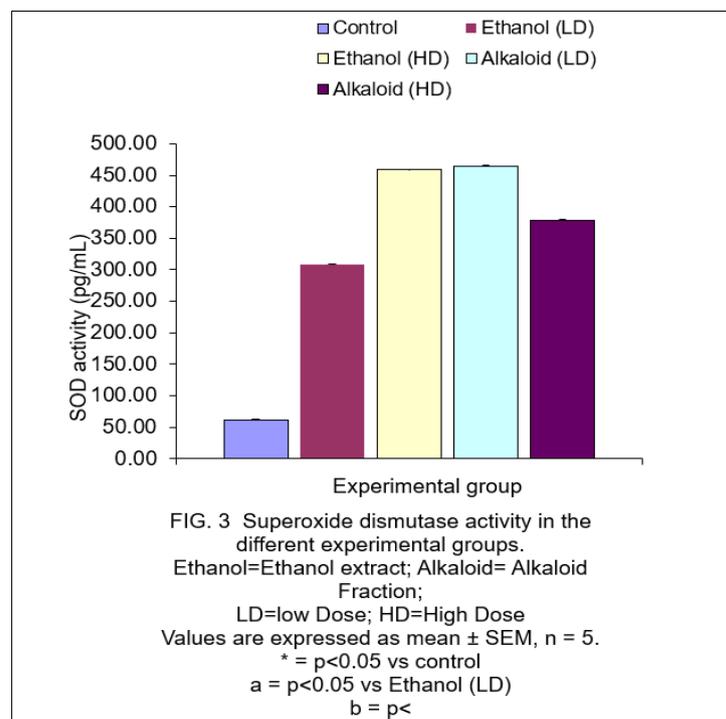
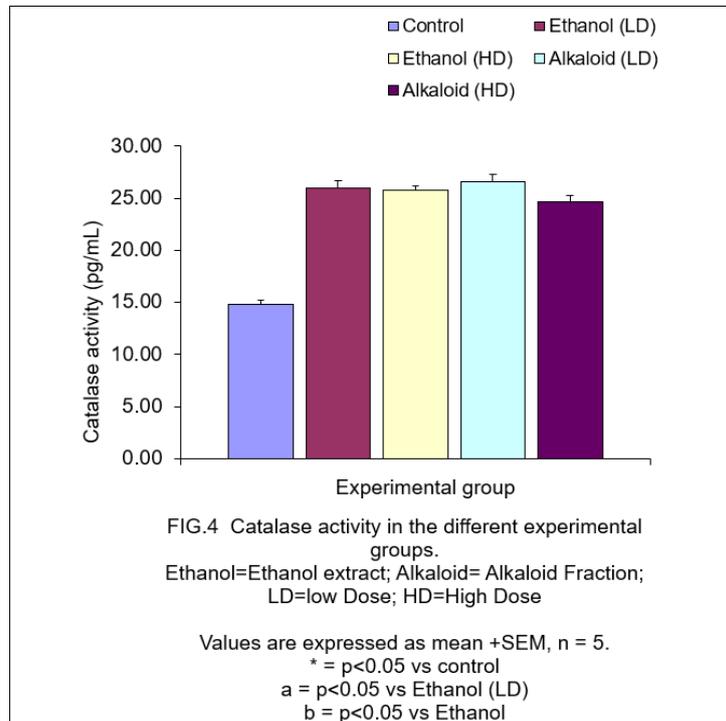
**5. 13 Effect of SOM on Catalase Enzyme (CAT), Fig. 4**

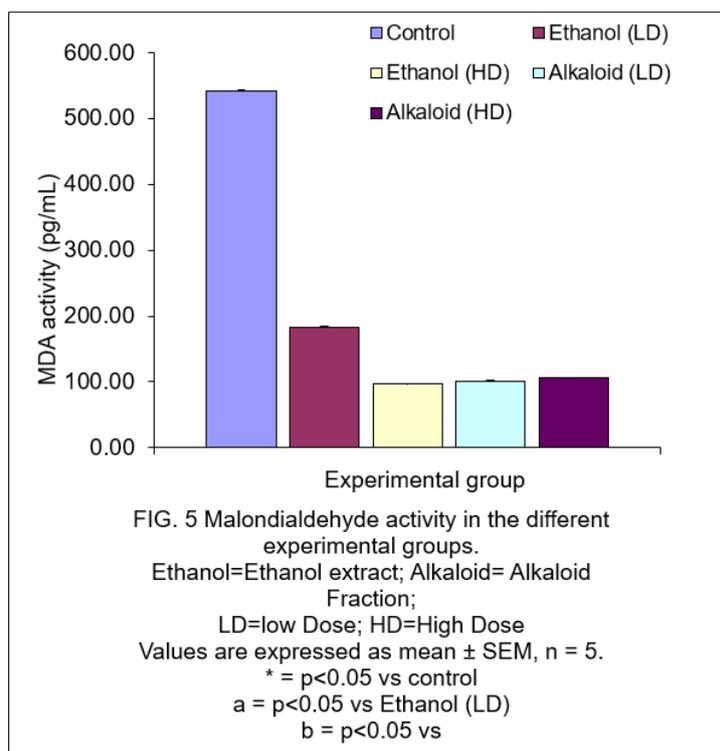
The corresponding levels for CAT were 14.80 ± 0.37, 26.00 ± 0.71, 25.80 ± 0.37, 26.6 ± 0.65 and 24.7

± 0.52 pg/ml for control, ELD, EHD, ALD and AHD, respectively.

**5.14 Effect of SOM on Malondialdehyde (MDA) Fig. 5**

The concentration of MDA was 543.00 ± 0.86, 183.40 ± 0.51, 96.80 ± 1.24, 101.2 ± 0.84 and 105.6 ± 0.57 pg/ml, respectively, for control, ELD, EHD, ALD and AHD groups.





## 6.0 DISCUSSION

### 6.1 Effect on Antioxidant Biomarkers

Antioxidants are enzymatic or non-enzymatic agents that can prevent undesired oxidation through their reaction with reactive oxygen species (ROS) or oxidation intermediates (Sipoloni *et al.*, 2025). As highlighted earlier, enzymatic antioxidants include SOD, catalase, glutathione peroxidase and glutathione reductase. Non-enzymatic antioxidants include vitamins E and C,  $\beta$ -carotene, GSH and flavonoids.

In this study, both Extract and Alkaloid fraction of SOM raised the serum levels of superoxide dismutase (SOD) and catalase (CAT) enzyme, while decreasing the concentration of malondialdehyde (MDA). This is compatible with antioxidant activity of the plant. Phytoconstituent analysis (Koikoibo, 2024, Olohigbe, *et al.*, 2018) has shown the presence of ascorbic acid (12.6 %) and squalene (7.5%), bioactive compounds with high antioxidant activity. In 2024, Du, Ma and Gao reported that Squalene, an unsaturated triterpene compound, enhances antioxidant enzyme activity in intestinal mucosa, reduces the expression levels of inflammatory factors, decreases infiltration of inflammatory cells, repairs damaged intestinal epithelial barriers, and promotes colonic health by inhibiting NF- $\kappa$ B and Nrf-Keap1 signaling pathways.

SOM has been reported to possess anti-oxidative and anti-inflammatory bioactive compounds like flavonoids, polyphenols, and vitamins. These components are effective in scavenging free radicals and mitigating oxidative stress, contributing to tissue repair and preventing further damage (Akinmoladun, *et*

*al.*,2021). This finding supports the therapeutic potential of SOM in managing oxidative tissue injury, as seen in various studies where plant-derived anti-oxidants reduced cellular injury and inflammation in hepatic tissues (Adedapo, *et al.*,2019). The antioxidant agents present in SOM may contribute to the maintenance of cellular integrity and help prevent oxidative damage, a thought similarly expressed by Okoro, *et al.*,2020.

Okoko and Ere (2012) showed that the extract of *S. monostatychnus* possessed significant abilities to reduce lipid peroxidation and haemolysis in erythrocytes induced by hydrogen peroxide when compared with the ability of ascorbic acid to do the same. Some bioactive agents in the plant protect cells from oxidative stress and reduce the risk of chronic diseases (Adegbola *et al.*,2017) Oxidative stress may be due to reactive oxygen species (ROS) which can be modulated by either endogenous or exogenous antioxidant nutrients (Gekpe, *et al.*,2023).

### 6.2 Limitations

This report excludes the effect of SOM on specific organs like the liver, brain, lungs and heart. Neither does it involve histological and molecular basis of function.

## 7.0 CONCLUSION

SOM has antioxidant properties, with relatively better effect via the alkaloid fraction compared with the ethanol crude extract, in the Wistar rat. This might be a beneficial therapeutic use in the human biological system.

## 8.0 RECOMMENDATION

It is expedient to explore some relevant aspects of function at the molecular and tissue levels in further studies. SOM may be developed as a novel drug alternative in complementary medicinal therapeutics and diagnosis.

## 9.0 ETHICAL APPROVAL

Ethical approval was duly obtained from the Faculty Animal Research Ethics Committee (FAREC-FBMS) of the Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, Cross River State of Nigeria. The approval code was 302PHY3724 contained in a letter of approval with reference number, FAREC/PA/[UC/049].

All procedures used in this study were in accordance with the guidelines of the above committee, and also in compliance with the care and use of the laboratory, as outlined in the Helsinki (1964) declaration and adopted by Kurihara, *et al.*, 2024.

## AUTHORS' CONTRIBUTIONS

The trio of WK, IDE and OAO developed the concept, experimental design and laboratory protocol, as well as the manuscript. Other authors did procurements, literature review, collation of results, data analysis and editing/approval of the final write-up.

**Conflict of Interest:** The authors declare no conflict of intellectual property.

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