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A COMPARATIVE STUDY ON ANTIOXIDANT AND INHIBITORY EFFECT OF RAW AND BOILED GINGER (*ZINGIBER OFFICINALE* ROSCOE) ON FERROUS SULPHATE INDUCED OXIDATIVE STRESS IN RAT'S TESTES – *IN VITRO*

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ABSTRACT

It is believed that oxidative stress in the male germ line affects male fertility and has an impact on typical embryonic development. So far, the comparative antioxidant potencies and inhibitory effect between raw and boiled ginger have not been studied in detail and reported. Accordingly, this study aimed at evaluating and comparing the effects of ginger rhizome extracts on pro-oxidant-induced oxidative stress in rat's testes whilst reflecting on the total phenolic content, total antioxidant capacity and total flavonoid content of the extracts. After preparing the raw and boiled ginger's aqueous extracts, the antioxidant activities of the extracts were assessed by means of a spectrophotometric method, and HPLC was used to characterize the extracts. The result of HPLC characterization of these extracts reveals that chlorogenic acid, coumarin, gallic acid, caffeic acid, catechin, shogaol, gingerol, gingerenone, quercitrin, quercetin, kaempferol, and rutin are the major constituents of these extracts. Also, the result revealed that both extracts of ginger rhizome investigated in this study brought about a concentration-dependent decrease in the level of malondialdehyde (MDA) associated with $FeSO_4$- stressed testes homogenates. In addition, the extracts exhibited concentration-dependent NO, OH, DPPH, and ABTS radicals' scavenging abilities. The result of this study also showed that compared to raw ginger extract, boiled ginger extract has a considerably ($p < 0.05$) higher total phenolic content. The high levels of quercetin in these ginger extracts may be the cause of their possible antioxidative effects, and their capacity to scavenge free radicals may be the mechanism by which these potentials are effected. Aqueous extracts of ginger rhizome could be considered as good antioxidant therapeutic candidates for oxidative stress linked with male infertility.

1.Introduction

Infertility, being one of the challenges faced by humans is impacted by behavioral, genetic, genotoxic and environmental variables that decrease spermatogenesis at different phases and result in male infertility (Kamiński *et al.,* 2020). Many chemical medications have been used to treat infertility, however some of them have quite a few negative effects. The search for alternative therapies with less side effects and toxicity is therefore necessary (Ghajari *et al.,* 2022). Because it is efficient, affordable, safe, and readily available, herbal medicine and medicinal plants are being employed to treat a variety of diseases. Additionally, they contain strong antioxidant properties that can scavenge free radicals and enhance spermatogenesis (Rehman *et al.,* 2022). Infertility affects one in five males in Nigeria. Lately, quite a number of researches have been done to investigate the role

that oxidative stress (OS), one of the main factors affecting reproductive status, plays in male infertility. Reactive oxygen species (ROS) are required to preserve standard cell activities at physiological levels since oxygen is crucial for life (Sies *et al.,* 2022). On the other hand, oxygen breakdown products like ROS can be harmful to cell survival and function (Napolitano *et al.,* 2022). Although, cytosolic and mitochondrial free iron has the potential to significantly impair cellular function and integrity by acting as a catalyst in the synthesis of ROS, which has the ability to annihilate cellular nucleic acids, lipids, carbohydrates, and proteins, iron is physiologically required as a constituent of proteins, part of which are enzymes (Carocci *et al.,* 2018). By participating in Fenton reaction, iron (II) produces hydroxyl radical (*OH) when it reacts with hydrogen peroxide (H_2O_2), whereas iron (III) can restore iron (II) that takes part in the Fenton reaction when in reaction with superoxide (Charkoudian *et al.,* 2006). An excessive amount of ROS can cause a direct damage to fatty acids in biological membranes and trigger peroxidation of lipid. Spermatozoa constantly struggle with oxygen paradox, much like other aerobic cells (Ogbuewu *et al.,* 2010) due to the fact that it is rich in fatty acids that are polyunsaturated in nature, which makes them vulnerable to ROS attack and causes a drop in sperm motility (Udipi *et al.,* 2012).

The byproduct of lipid peroxidation is malondialdehyde (MDA). ROS break down polyunsaturated fatty acids during this process. The synthesis of this electrophilic aldehyde is utilized as a biomarker to quantify an organism's level of oxidative onslaught (Murray *et al.,* 2000). Nevertheless, increasing the antioxidant status of the body through increased fruit and vegetable eating is the most feasible and likely method to combat degenerative diseases. A significant class of secondary metabolites produced by plants is phenolic chemicals.

Since ancient times, numerous plants and their parts have been utilized to treat a variety of ailments. A member of the *Zingiberaceae* plant family, ginger (*Zingiber officinale*) is regarded as one of the most popular culinary seasonings. It has also been used medicinally for a very long time in Chinese traditional medicine (Kumar *et al.,* 2011). Ginger is grown throughout West Africa, the Caribbean and south-east Asia, with India and China thought to be its primary sources (El-hameed *et al.,* 2019). Several bioactive substances that could potentially have biological and pharmacological effects are present in ginger (Tomaino *et al.,* 2005).

Gingerols, protodioscin, saponins, zingibrene, gingerdiol, and schogaols are the most significant components of ginger (Sakr and Badawy, 2011). Ginger, is frequently used to flavor food and treat a variety of illnesses. The presence of many bioactive chemicals in ginger may be related to its ability to promote health. According to Palatty *et al.* (2013), ginger has significant anti-inflammatory, antiemetic, antithrombotic, anti-hepatotoxic, and antioxidant properties that can help with nausea and vomiting (El-Morsy Ibrahim and Al-Shathly, 2015). Similar to natural antioxidants like retinol, ascorbic acid and tocopherol, which can shield DNA from damage and other essential biomolecules from oxidation, improve the quality of sperm and thus fertility in men, the antioxidant content of herbal medicines is crucial for enhancing antioxidant defense and reducing oxidative state (Rajeev *et al.,* 2006). The antioxidant (El-hameed *et al.,* 2019) and androgenic activity (Kamtchouing *et al.,* 2002) of ginger may be responsible for its beneficial impact on male fertility. Gingerol, which gives ginger its flavor (Semwal *et al.,* 2015), shogaols, and certain phenolic ketone derivatives are the major antioxidants in ginger (Pittler, 2004).

Although numerous studies claimed that ginger enhanced sperm motility, viability, and testosterone concentration while lowering malondialdehyde (MDA), which in turn reduced lipid peroxidation and increased reproductive success (Khaki et al., 2009; Memudu *et al.,* 2012; Ghlissi *et al.,* 2013); furthermore, Morakinyo *et al.* (2008) hypothesized that ginger's protodioscin and saponins could raise levels of libido, luteinizing hormone (LH), and testosterone, which was crucial for the traditional medical approach to treating sexual

dysfunction; in addition, Sabik and El-Rahman (2009) discovered that ginger could raise testosterone, estrogen, and pregnenolone levels in men as well as their sexual potency; the potential of ginger in the treatment and averting oxidative stress-related reproductive problems is yet poorly understood. Therefore, in this *in vitro* study, we evaluate and compare the effects of raw and boiled ginger rhizome extracts on pro-oxidant-induced oxidative stress in rat's testes.

2. Materials and Methods

2.1. Chemicals and reagents

The water utilized for this investigation was glass distilled, and all of the chemicals and reagents were procured from Sigma-Aldrich Chemie GmBH and Sigma-Aldrich Co.

2.2. Sample Collection

Fresh ginger rhizomes were purchased fresh from a local market in Ilorin metropolis, Kwara State, South Western Nigeria. The samples were authenticated in the Department of plant Science and Biotechnology, Ekiti State University, Ado Ekiti, Nigeria with voucher number UHAE 2021054.

2.3. Sample preparation

Fresh ginger rhizomes of the Roscoe kind were cleaned, peeled, and then cut into cubes. It was then divided into two equal pieces. The initial portion of ginger was air dried at room temperature (37 degrees Celsius) to make raw ginger powder, which was then made by grinding raw dried ginger into powder. The second portion of the prepared ginger was cooked at medium heat for one hour to make the boiled ginger powder. The ratio used is 1 gram of ginger rhizome to 100 milliliters of distilled water. After boiling, boiled ginger together with the water was blended in a blender to create a homogeneous mixture. Once blended, the mixture was lyophilized to obtain boiled dried ginger powder. The two ginger samples (raw and boiled) were stored in tightly closed plastic jars and kept in freezer until use.

2.4. Preparation of aqueous extract

Separately, the ground ginger samples were soaked in distill water for 48 hours before being filtered. The crude extract needed for HPLC-DAD characterization was then obtained by freeze drying and rotary evaporation of the filtrate.

2.5. Ethical clearance

The use of animals in this research was authorized by the Ekiti State University ethics committee (reference number: ORDI/AD/EAC/23/127). The animals were treated humanely at all times throughout the experiments, as outlined in the Guide for the Care and Use of Laboratory Animals published by the National Academies of Science and the National Institutes of Health.

2.6. Experimental animals

Male Wistar strain albino rats weighing 200–300 g were purchased from the breeding colony of Department of Veterinary Medicine, University of Ibadan, Nigeria. Rats were maintained at 25°C, on a 12 h light/12 h dark cycle, with free access to food and water. They were acclimatized under these conditions for 1–2 week before the experiment. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

2.7. Preparation of Tissue Homogenates

Under mild diethyl ether anaesthesia, the rats were decapitated, and the testes and liver tissues were separately swiftly removed, stored on ice, and weighed. This tissue was separately homogenized in cold saline (1/10 w/v) in a Teflon glass homogenizer (Mexxcare, mc14 362, Aayu-shi Design Pvt. Ltd., India) with around 10 strokes at roughly 1200 rev/min. The homogenate was centrifuged for 10 minutes at 3000 g (KX3400C Kenxin International Co. Hong Kong) to produce a pellet that was discarded and a low-speed supernatant (SI) that was preserved for the lipid peroxidation assay (Belle et al., 2004).

2.8. Lipid peroxidation and thiobarbituric acid reactions

The Ohkawa *et al.* (1979) method was used to conduct the lipid peroxidation assay. In a nutshell, one hundred milliliter of a fraction was combined with thirty milliliters of freshly generated 250 mM $FeSO_4$, thirty milliliters of zero-point one molar Tris-HCl buffer, and one hundred milliliter of ginger aqueous extract. Prior to incubation at thirty-seven degrees Celsius for an hour, the volume was filled to a capacity of 300 L with water. By adding three hundred liters of eight-point one percent sodium dodecylsulphate to the solution that contains the fraction, the colour reaction was developed. Following this, five hundred liter each of acetic acid/hydrochloric acid (with a pH of 3.4) and five hundred liters of zero-point eight percent TBA were added. For one hour, the resulting solution was incubated at one hundred degrees Celsius. At 532 nm, the reactive species of thiobarbituric acid were evaluated for absorbance. MDA output was measured as a percentage (%). Using non-linear regression analysis, the EC_{50} (concentration of extract needed to suppress 50% of malondialdehyde generated) value was determined.

2.9. DPPH free radical scavenging ability

The extract's capacity to scavenge DPPH (1,1-diphenyl-2 picrylhydrazyl) free radical was assessed according to Gyamfi *et al.,* (1999). In a nutshell, the extracts were diluted appropriately (one milliliter) and combined with one milliliter of zero point four millimolar DPPH radical-containing methanolic solution. This reaction mix was then allowed to stand in the dark for thirty minutes, and the absorbance was taken at 516 nm. The test samples were not used in the control, which was performed using a 2 mL DPPH solution. DPPH free radical scavenging ability was calculated using the mathematical expression:

DPPH scavenging ability (%) = [(absorbance of control − absorbance of samples)/ absorbance of control] × 100. (1)

2.10. 2,2-azinobis(3-ethylbenzo-thiazoline-6-sulfonate) (ABTS*) scavenging ability

The ginger rhizome extracts' capacity to scavenge ABTS* was assessed using the Re *et al.* (1999) method. In the absence of light, an aqueous solution of ABTS (7 mmol L) was combined with 2.45 mmol L of $K_2S_2O_8$ to generate ABTS*.

2.11. Degradation of deoxyribose (Fenton's reaction)

Using the Halliwell and Gutteridge (1981) method, the ginger rhizome extracts' capacity to stampede iron (II)/hydrogen peroxide -triggered breakdown of deoxyribose was tested. In a nutshell, newly obtained extracts of ginger (zero – one hundred liter) was introduced to the reaction solution consisting of eight hundred liter of distilled water, one-hundred and twenty liters of twenty millimolar deoxyribose, four hundred liters of zero-point one molar phosphate buffer, forty liters of twenty millimolar hydrogen peroxide, and forty liters of five-hundred millimolar of Iron (II) tetraoxosulphate (VI). After 30 minutes of incubation at thirty-seven degrees Celsius, the reaction mixture was halted by introducing five hundred liters of two-point eight percent trichloroacetic acid solution, which was then followed by four hundred liters of zero-point six percent TBA solution. The tubes were then subjected to heat for 20 minutes in water bath at one hundred degrees Celsius. The extracts' % capacity to neutralize hydroxyl radicals was determined using the mathematical expression below whilst the absorbance was taken at five hundred and thirty-two nanometers:

[(Absref - Abssample) / Absref] x 100 (2)

2.12. Nitric oxide scavenging assay

The Igbinosa *et al.* (2011) method was used to assess the extracts' ability to scavenge nitric oxide. To (100-400 L) of plant extract, 25 mM sodium nitroprusside was added in one milliliter of zero-point five phosphate buffer saline (having a pH of 7.4) before being vortexed. The resulting solution was then incubated for two hours a thirty-seven degree Celsius before being combined with one milliliter of Griess reagent

(equivalent quantities of one percent sulphanilic acid produced in two percent orthophosphoric acid and 0.01% (w/v) naphthylenediamine dichloride) before being incubated for 30 minutes at twenty-five degrees Celsius. The absorbance was quantified at 546 nm, and the mathematical expression below was used to determine the amount of the inhibitory effect of the ginger rhizome extracts on NO radical:

NO scavenging activity (%) = [(Abs control – Abs sample)] / (Abs control) x 100

3. Results and Discussion

Peroxidation of lipids associated with cellular membranes is one of the main causes of cellular damage in living organisms exposed to oxidative onslaught (Repetto *et al.,* 2012). As demonstrated in this study, incubating rat testes homogenates in the presence of iron (II) tetraoxosulphate (VI) ($FeSO_4$) led to a noticeably higher MDA level (263.24 % Control) in the testes (Figure 1). These results are consistent with our past reports on how Fe^{2+} interacts with the testes, in which it was demonstrated that Fe^{2+} is a very strong instigator of lipid peroxidation in the testes (Akomolafe *et al.,* 2012). This finding is somewhat in-line with the earlier study by Akintunde *et al.* (2013), which found greater levels of MDA in rat testes subjected to repeated consumption of leachate samples containing higher quantities of mixed metals than allowed. Catalytically, iron (II) can drive one-electron transfer events that produce reactive hydroxyl radical (•OH), which is produced from hydrogen peroxide via the Fenton reaction; which may account for the enhanced peroxidation of lipid in the presence of $FeSO_4$ observed in figure 1. Additionally, iron breaks down lipid peroxides, producing radicals of peroxides and alkoxides, as a result, which encourages the progression of peroxidative damage of lipids (Zago *et al.,* 2000). The MDA level of the $FeSO_4$-stressed testes homogenate did, however, significantly decrease upon the addition of raw and boiled ginger aqueous extract, with the littlest MDA synthesis recorded at the highest concentration of all extracts (1.00 mg/ml) (Figure 1). The inherent chemicals may form complexes with iron (II) to prevent them from driving the peroxidation of lipids, which would explain the mode of inhibition of $FeSO_4$-induced peroxidation of lipid, or possibly the plant chemicals have sucked up radicals liberated in Fe^{2+}-driven reaction (Oboh *et al.,* 2007).

Table 1. Effective concentration causing 50% antioxidant ability (EC_{50} values) of 1,1-diphenyl-2 picrylhydrazyl (DPPH), 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS*), hydroxyl (OH*), nitric oxide (NO*) radical scavenging abilities of and inhibition of FeSO4-Induced lipid peroxidation of aqueous extract by aqueous extract of raw and boiled ginger rhizome.

	EC_{50} values (µg/ml)		
Parameters	Raw	Boiled	BHT
DPPH Radical Scavenging Ability	96.78 ± 2.30^a	78.25 ± 1.20^b	51.70 ± 2.13^c
ABTS* Radical Scavenging Ability	81.96 ± 1.20^a	57.05 ± 2.01^b	42.90 ± 2.41^c
NO* Radical Scavenging Ability	66.74 ± 2.12^a	48.07 ± 1.14^b	36.78 ± 2.08^c
OH* Radical Scavenging Ability	77.11 ± 2.04^a	66.80 ± 1.11^b	49.85 ± 0.89^c
FeSO4-induced lipid peroxidation (Testis)	780.7 ± 1.18^a	592.7 ± 2.12^b	394.2 ± 2.13^c
FeSO4-induced lipid peroxidation (Liver)	564.0 ± 2.20^a	511.9 ± 1.32^b	493.5 ± 1.21^c

Values represent means ± standard deviation of triplicate readings. Superscripts with the same letters along the same column are not significantly different ($P > 0.05$). BHT = Butylated hydroxytoluene

According to the EC_{50} value (Table 1), the concentrations of the raw and boiled ginger rhizome extracts that will have a 50% inhibitory effect on $FeSO_4$-induced peroxidation of lipid in testes, are 780.7 µg/ml and 592.7 µg/ml respectively. This speculates that the boiled ginger rhizome extract has a greater potential to reduce lipid peroxidation. Our discoveries are in line with a related study by El-hameed *et al.* (2019), who demonstrated that aqueous ginger rhizome extract reduces lipid peroxidation and enhances male rabbit fecundity.

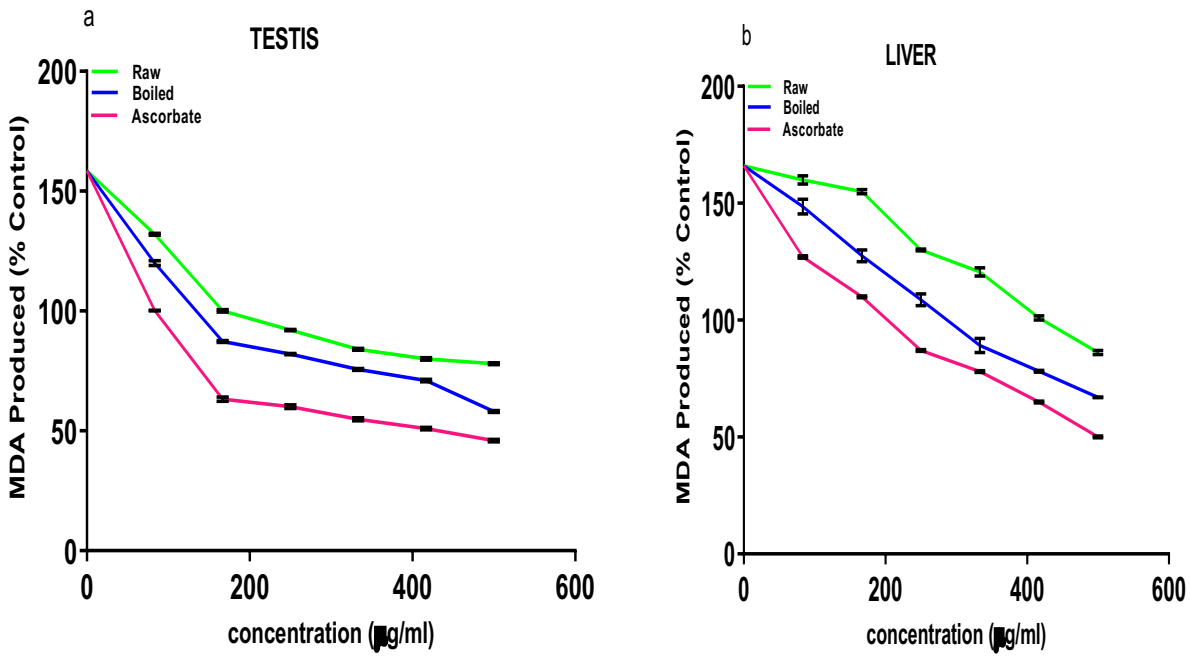

Figure 1. $FeSO_4$-induced MDA inhibition of aqueous extract of raw and boiled ginger rhizome in rat (a) testicular and (b) liver homogenates. Values represent means ± standard deviation of triplicate readings.

Table 2. Phenolic constituents of aqueous extract of raw and boiled ginger rhizome

Constituents	**Fresh ginger rhizome (mg/g)**	
	Raw	**Boiled**
Chlorogenic acid	12.96 ± 0.83^{a}	14.45 ± 0.32^{a}
Coumarin	29.05 ± 1.24^{b}	29.64 ± 0.07^{b}
Gallic acid	8.32 ± 0.67^{c}	8.72 ± 0.89^{c}
Caffeic acid	3.41 ± 0.43^{d}	3.72 ± 0.46^{d}
Catechin	2.17 ± 0.43^{e}	2.46 ± 0.12^{e}
Shogaol	0.66 ± 0.31^{f}	0.79 ± 0.14^{f}
Gingerol	0.72 ± 0.37^{f}	0.90 ± 0.29^{f}
Gingerenone	0.09 ± 0.02^{g}	0.69 ± 0.22^{f}
Quercitrin	0.73 ± 0.03^{f}	1.10 ± 0.03^{f}
Quercetin	95.14 ± 0.19^{h}	97.09 ± 0.04^{g}
Kaempferol	25.96 ± 0.64^{i}	28.42 ± 0.48^{b}
Rutin	7.20 ± 0.22^{c}	9.51 ± 0.33^{c}
Luteolin	2.02 ± 0.01^{e}	3.61 ± 0.31^{d}

Values represent means ± standard deviation of triplicate readings. Superscripts with the same letters along the same row are not significantly different ($P > 0.05$).

Many significant phenolic and non-phenolic plant chemicals have been linked to the antioxidant capabilities of plants (Cheplick *et al.,* 2007). In this study, the ginger extracts were characterized using HPLC-DAD, and the results are presented in table 2. The major components of the raw and boiled ginger rhizome aqueous extracts were found to be chlorogenic acid, coumarin, gallic acid, caffeic acid, catechin, shogaol, gingerol, gingerenone, quercitrin, quercetin, kaempferol, and rutin, with quercetin being the predominating phytochemical (95.14 ± 0.19 mg/g and 97.09 ± 0.04 mg/g for raw and boiled ginger rhizome extracts respectively). The extremely high quantity of quercetin in the ginger rhizome extracts may therefore be responsible for protecting testes tissue from $FeSO_4$-induced lipid peroxidation. Overall, the result shown in table 2 indicates that the boiled ginger extract contains more of each phytochemical constituent than the raw ginger extract does. This might have to do with the rupture of cells brought on by boiling them at a high temperature, which eventually causes the release of cellular components.

Table 3. Ferric Reducing Antioxidant Property (FRAP) and Total Antioxidant Capacity (TAC) of aqueous extract of raw and boiled ginger rhizome

	Ginger rhizome		
Parameters	Raw	Boiled	BHT
Ferric Reducing Antioxidant Property (mg/g)	0.50 ± 0.01^{a}	0.75 ± 0.01^{a}	1.04 ± 0.01^{b}
Total Antioxidant Capacity (mg/100mg)	9.46 ± 0.66^{a}	11.92 ± 0.86^{b}	15.65 ± 0.93^{c}

Values represent means ± standard deviation of triplicate readings. Super-scripts with the same letters along the same column are not significantly different (P > 0.05). BHT = Butylated hydroxytoluene.

Table 4. Total phenolic and total flavonoid contents of aqueous extract of raw and boiled ginger rhizome

	Ginger rhizome	
Parameters	Raw	Boiled
Total Phenolic content (mgGAE/g)	30.57 ± 1.59^{a}	35.97 ± 0.35^{b}
Total Flavoniod content (mgQE/g)	20.17 ± 0.71^{a}	29.58 ± 0.63^{b}

Values represent means ± standard deviation of triplicate readings. Superscript with the same letters along the same column are not significantly different (P > 0.05). GAE = Gallic acid equivalent. QE = Quercetin equivalent.

The ferric reducing antioxidant property, total antioxidant capacity, as well as the total flavonoid and phenolic contents of boiled and raw ginger rhizome extracts is presented in Tables 3 and 4. When compared to the raw ginger extract (30.57 mg/QE), the boiled ginger extract had a considerably ($p < 0.05$) higher phenolic content (35.97 mg/QE). The total flavonoid content, ferric reducing antioxidant property and total antioxidant capacity of these ginger extracts showed the same trend of results. Strong antioxidants, phenolic substances can neutralize free radicals, mask metal catalysts in form chelation, and activate antioxidant enzymes in addition to impeding oxidases (Cheplick *et al.,* 2007). According to Ozgen *et al.* (2016), quercetin, the bioflavonoid that predominates in these extracts, is a potent antioxidant whose effects have been attributed to its aromatic rings, which contains not less than one hydroxyl group each, linked by a bridge of three carbons to form a heterocyclic six-membered ring (Hanasaki *et al.,* 1994). The release of these compounds into water during boiling may account for the drop in total phenolic and total flavonoid concentrations seen in the boiled ginger extract.

In order to protect cells from damage, antioxidants can either stop the synthesis of free radicals, counterbalance or scavenge those synthesized naturally by the body, or lessen or chelate the transition metal content of food (Ami *et al.,* 2003; Kamdem *et al.,* 2013). The ability of these extracts to neutralize DPPH, ABTS, OH, and NO radicals was evaluated in an effort to identify the primary mechanism by which the aqueous extracts of raw and boiled ginger rhizome protect testes tissue against $FeSO_4$-induced lipid peroxidation. Figures 2, 3, 4 and 5 illustrate the concentration-dependent radical scavenging properties of ginger rhizome boiled and raw aqueous extracts.

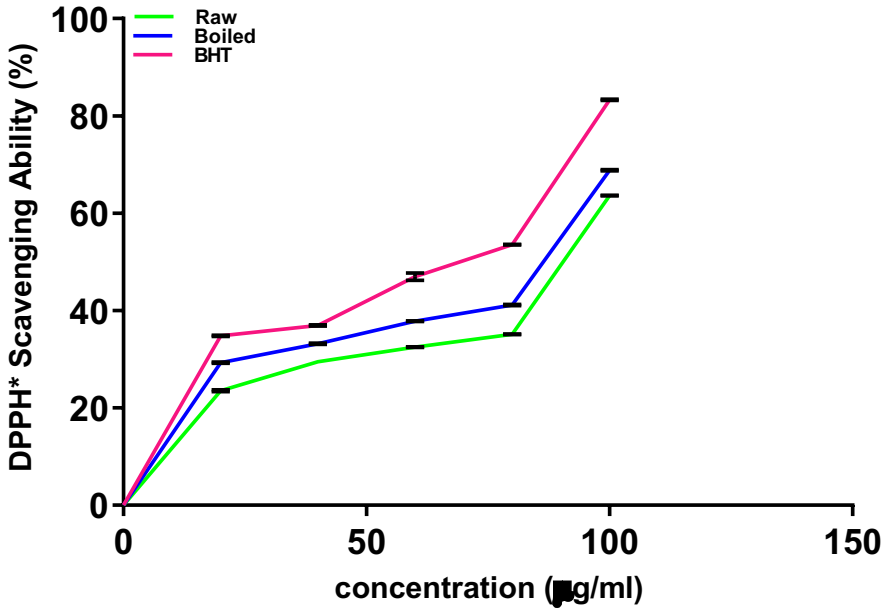

Figure 2. 1,1-diphenyl-2 picrylhydrazyl (DPPH) radical scavenging ability of aqueous extract of raw and boiled ginger rhizome. Values represent means ± standard deviation of triplicate readings.

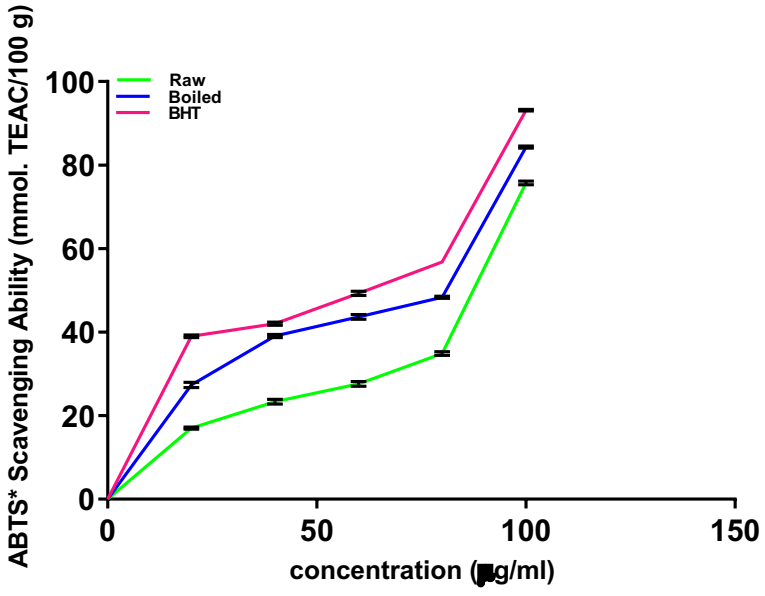

Figure 3. 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging ability of aqueous extract of raw and boiled ginger rhizome. Values represent means ± standard deviation of triplicate readings.

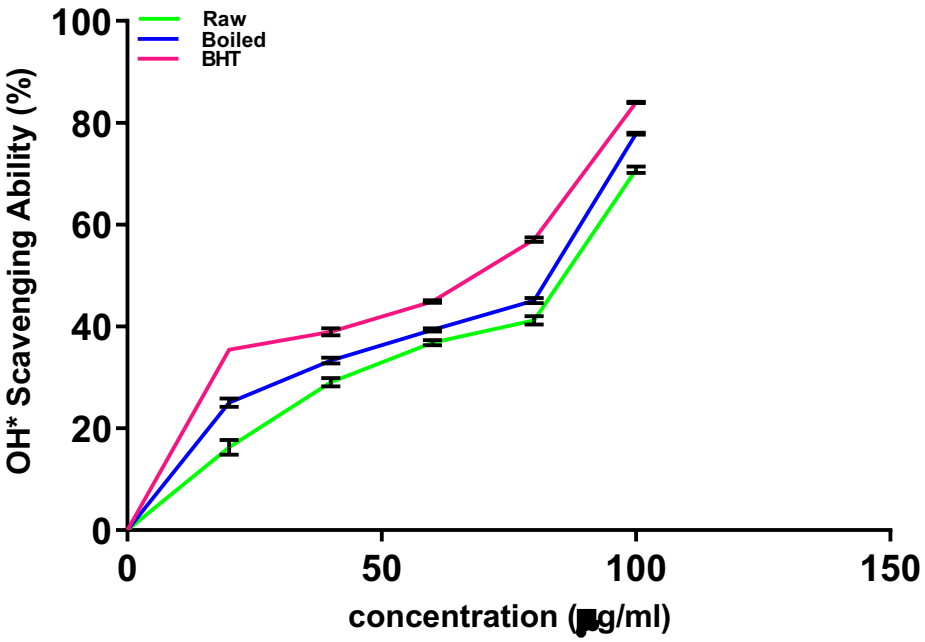

Figure 4. Hydroxyl (OH) radical scavenging ability of aqueous extract of raw and boiled ginger rhizome. Values represent means ± standard deviation of triplicate readings.

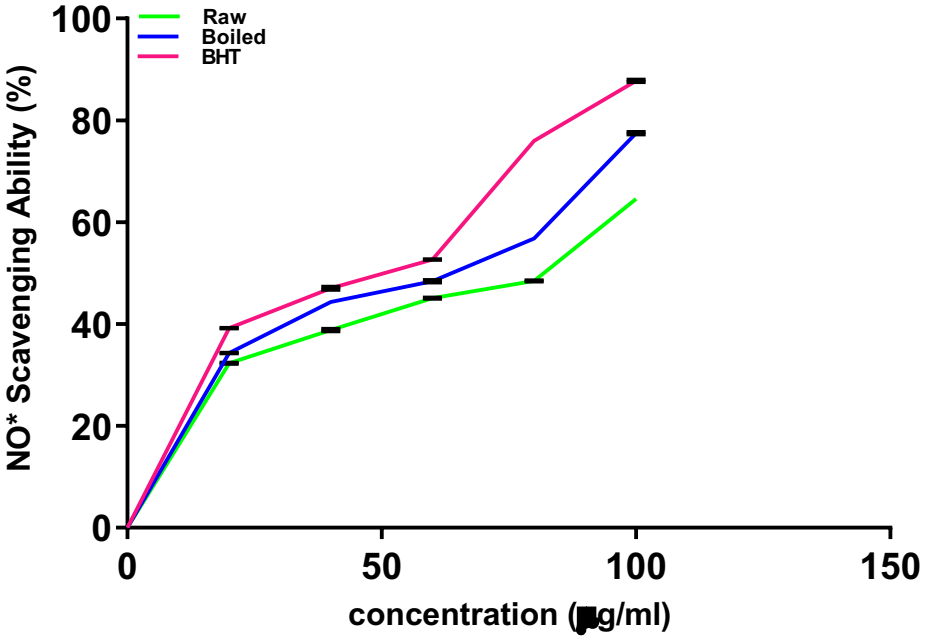

Figure 5. Nitric oxide (NO) radical scavenging ability of aqueous extract of raw and boiled ginger rhizome. Values represent means ± standard deviation of triplicate readings.

A key antioxidant method of action is thought to be the hinderance of the chain-starting step (Gulcin, 2020). DPPH, a type of free radical, changes to a diamagnetic molecule upon its acceptance of electron or hydrogen (Je *et al.,* 2009). When defining antioxidant action that has to do with free radical-scavenging, tendencies of electron or hydrogen donation are

crucial considerations (Hu *et al.,* 2000). Our research showed that ginger rhizome extracts, both boiled and raw, neutralized DPPH in a concentration-dependent fashion as presented in Figure 2. But boiled ginger extract is more effective at scavenging DPPH radicals than raw extract is (Table 1). Due to its unusual electron configuration, DPPH exhibits a prominent absorption band in visible spectroscopy at a wavelength of 517nm. The decolorization that results is stoichiometric with regard to the quantity of electrons accepted as the absorption disappears, as the odd electron pairs up in the presence of free radicals (Fauconneau *et al.,* 1997). The ability of both extracts to neutralize DPPH radicals can therefore be linked to their potential to donate hydrogen. The extracts' capacity to scavenge free radicals is what causes the DPPH radical to be bleached.

As in this work, the capability to neutralize DPPH radicals is frequently used in various experimental activities that examine the antioxidant potential of a test compound. However, sample solubility and color interference have been observed to limit its use as a measure of antioxidant capacity (Dorman *et al.,* 2004). Therefore, in order to assess the antioxidative capacities of the ginger extracts, we conducted the ABTS assay as a complementary assay for free radical scavenging ability. According to Shalaby and Shanab (2013), the blue-green dye, ABTS is predominantly reactive with sulfhydryl group-containing compounds, phenolics, and other antioxidants. ABTS is a comparatively stable free radical. Its test, which is essentially a decolorization assay, relies solely on the formation of the ABTS radical monocation. Instead of the free radical formation occurring continuously at the introduction of antioxidant compound, in this case, the radical cation is typically produced prior to the addition of the antioxidant test component. Table 1's EC_{50} values for the extracts demonstrate that the boiled ginger rhizome has more capacity to scavenge free radicals than its raw counterpart. The enhanced ability of the extracts to scavenge ABTS* radicals (Figure 3 and Table 1) may be linked to the polyphenolics therein's capacity to donate hydrogen, which finally stops or inhibits the generation of ABTS*. The higher ABTS* scavenging capability of the boiled extract could be due to a stronger capability of its inherent polyphenolics to donate hydrogen, which eventually prevent/inhibit the production of ABTS radical.

Additionally, this study's findings demonstrated that the extracts exhibited a concentration-dependent capacity to neutralize the OH* generated by the breakdown of deoxyribose in the Fenton reaction (Figure 4 and Table 1). This finding shows that the extract can be utilized in place of synthetic antioxidants to treat the hydroxyl radical's oxidative activity. However, as shown in table 1, the boiled ginger rhizome extract demonstrated a much better OH* radical scavenging ability than the raw ginger rhizome extract based on the EC_{50} values of these extracts. Nitric oxide (NO) radical, a highly reactive substance, produced from sodium nitroprusside at physiological pH has been shown to have the capability of altering the structural components of cell and thus its functionality (Ashokkumar *et al.,* 2008). The ginger rhizome's aqueous extracts reduced NO* production in a concentration-dependent fashion (Figure 5). The ability of the ginger rhizome extracts to scavenge reactive oxygen species and its derivatives is what causes them to have an inhibitory effect on NO radicals (Packer, 1997). The EC_{50} values in Table 1 show that boiled ginger extract has a considerably ($p < 0.05$) stronger NO radical scavenging activity than raw ginger extract.

4. Conclusions

Abundance of quercetin in these aqueous ginger extracts may be responsible for extracts' ability to protect testes tissues from $FeSO_4$-induced lipid peroxidation. This ability may be due to quercetin's capacity to scavenge free radicals. Reactive oxygen species-related reproductive cellular damage may be managed or treated with the help of this plant.

5.References

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Ethical clearance

The use of animals in this research was authorized by the Ekiti State University ethics committee (reference number: ORDI/AD/EAC/23/127). The animals were treated humanely at all times throughout the experiments, as outlined in the Guide for the Care and Use of Laboratory Animals published by the National Academies of Science and the National Institutes of Health.