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Potential of Ginger (*Zingiber officinale*) Rhizome and Watermelon (*Citrullus lanatus*) Seeds in Mitigating Aspartame-Induced Oxidative Stress in Rat Model

E.V. Ikpeme, O.U. Udensi, E.E. Ekerette and U.H. Okon

Department of Genetics and Biotechnology, University of Calabar, PMB 1115, Calabar, Nigeria

Corresponding Author: E.V. Ikpeme, Department of Genetics and Biotechnology, University of Calabar, PMB 1115, Calabar, Nigeria

ABSTRACT

Aspartame (ASP) consumption has been reported to implicate oxidative stress and antioxidant supplements from plant origin are one of the safest ways of averting their effect in the body. The current study was aimed at ascertaining the ability of aspartame (ASP) to induce oxidative stress in rats as well as assessing the potential of ginger (*Zingiber officinale*) rhizome and watermelon (*Citrullus lanatus*) seeds extracts in averting the aspartame induced oxidative stress. This was done using sperm parameters and biochemical assays such as superoxide dismutase (SOD), glutathione peroxidase (GP_x), catalase, melonaldehyde (MDA), aspartate aminotransferase (AST), alanine aminotransferase and alkaline phosphate (ALP). Forty eight sexually matured male albino rats were divided into six groups (A-F) with eight rats in each group. Group A served as the positive control and received physiological saline. Group B served as the negative control and were administered with 1000 mg kg⁻¹ b.wt., of aspartame (ASP). Rats in group C were administered with 1000 mg kg⁻¹ b.wt., ASP+500 mg kg⁻¹ b.wt., of ginger extracts while rats in group D were administered with 1000 ASP+1000 mg kg⁻¹ b.wt., of ginger extracts. On the other hand, rats in group E received 1000 mg kg⁻¹ b.wt., ASP+500 mg kg⁻¹ b.wt., of watermelon seed extracts while rats in group F received 1000 mg kg⁻¹ b.wt., ASP+1000 mg kg⁻¹ b.wt., of watermelon seed extracts. The result revealed that administration of ASP reduced sperm viability, sperm count and increased sperm head abnormalities significantly ($p < 0.05$) while sperm motility was not affected by ASP administration in the rats. Superoxide dismutase and GP_x levels were increased significantly by ginger and watermelon seeds extracts. Although ginger extract reduced more of lipid peroxidation (MDA), watermelon seeds extract increased the activities of SOD, GP_x and reduced AST, ALT and ALP in the liver of rats at 1000 mg kg⁻¹ b.wt., than the extract of ginger. Thus, the extracts of these two medicinal plants are possible antioxidant reservoir and may provide reliable solution in averting oxidative stress pathologies.

Key words: Antioxidant, oxidative stress, aspartame, lipid peroxidation, *Zingiber officinale*, *Citrullus lanatus*

INTRODUCTION

Most of the traumatizing diseases affecting humanity today might be connected to our daily food intake, which we consume on the basis that they are nutritionally attractive or therapeutically valuable. The diseases emanating from food sources are basically due to the oxidative reaction in them which are also reported as the main cause of food deterioration (Duda-Chodak and Tarko, 2007). These oxidative reactions are responsible for the nutritional value losses as well as aroma,

taste and texture degradation (Duda-Chodak and Tarko, 2007). Consumption of such foods can upset cell homeostasis resulting in different diseases like tumour, heart failure, cataract, brain dysfunction and diabetes (Maniak and Targonski, 1996). Due to the insurgence of different forms of diseases in the society today, most of which are connected with what we consume, efforts have been made by scientists to discover ways of mitigating or completely eradicating the menace. One of the reliable ways in managing these oxidative reaction diseases is through the use of medicinal plants which are reported with therapeutic properties (Ikpeme *et al.*, 2013, 2014a).

Medicinal plants serve various purposes including the treatment of various disease conditions (Koffi *et al.*, 2009; Ikpeme *et al.*, 2014b). According to the World Health Organization; a medicinal plant is any herbal preparation produced by subjecting plant materials to extraction, fractionation, purification, concentration or other biological processes. Medicinal plants are reservoirs of biologically active compounds such as saponins, tannins, essential oils, flavonoids, phenols, alkaloids and other valuable chemical compounds. Interestingly, *in vivo* and *in vitro* studies of some of these plants revealed that they are good sources of antioxidants with inherent capacity to ameliorate oxidative stress linked diseases (Middleton *et al.*, 2000; Oluwole *et al.*, 2011; Koffi *et al.*, 2009; Ikpeme *et al.*, 2014a, b; Ekaluo *et al.*, 2015).

It is pertinent to note that most diseases today might either be directly or indirectly linked to oxidative stress. For instance, infertility, which is one of the major disease conditions facing humanity today is influenced by oxidative stress (Agarwal *et al.*, 2008). Other diseases such as cancer, asthma, cardiovascular diseases, diabetes, arthritis and inflammation are also reported to be associated with oxidative stress (Tripathy *et al.*, 2010; Kottaimuthu, 2008; Gomez-Flores *et al.*, 2008). This oxidative stress is a condition where there is an imbalance between antioxidants and free radical generation (reactive oxygen species) in the body. When this occurs, the generated free radicals, which are unstable atoms with unpaired valence electrons (Kadam *et al.*, 2010; Aluko *et al.*, 2013) attack biomolecules in the body transforming them into free radicals such as hydrogen peroxides (H_2O_2), hydroxyl radical (OH^-) and nitric oxide (NO). With time, the accumulations of these radicals metamorphose into oxidative stress induced diseases.

Aspartame [L-aspartyl-L-phenylalanine methyl ester ($C_{14}H_{18}N_2O_5$)], one of the most widely used artificial sweeteners in over 90 countries worldwide and in over 6000 products (Magnuson *et al.*, 2007) have been reported to induce oxidative stress (Walaa and Howida, 2015). It has been reported that aspartame facilitates intestinal hydrolysis and absorption of amino acids together with small amounts of free methanol (Burgert *et al.*, 1991) and as well been linked with neurological and behavioral disturbances (Humphries *et al.*, 2008), though most of these results yielded negative or inconclusive correlations (Magnuson *et al.*, 2007). On consumption, aspartame is rapidly and completely metabolized to 50% phenylalanine, 40% aspartic acid, 10% methanol and methanol is being increasingly recognized as a substance that damages the liver cells where it is oxidized to formaldehyde and later formate (Trocho *et al.*, 1998). These processes are accompanied by elevation of Nicotinamide Adenine Dinucleotide Hydrogenase (NADH) level and the formation of superoxide amino acid, which may be involved in lipid peroxidation (Poli, 1993; Parthasarathy *et al.*, 2006). Methanol intoxication is associated with mitochondrial damage and increased cellular proliferation resulting in increased production of oxygen radicals (Castro *et al.*, 2002). When there are no antioxidant supplements to counter these radicals, they attack and denature biomolecules like protein and lipid resulting in ailment. However, medicinal plants with antioxidant potentials are seen as reliable sources for the treatment of oxidative stress related pathologies.

Zingiber officinale is a herbaceous perennial plant comprising rhizomes, fibrous roots and aerial shoots. The rhizome of *Z. officinale* is one of the most widely used in the Zingiberaceae family and is a common condiment for various foods and beverages (Bhargava *et al.*, 2012). It is a strong antioxidant source and may either mitigate or prevent the generation of free radicals and is considered a safe herbal medicine. Gingerols derived from ginger have been shown to inhibit both prostaglandin and leukotrien biosynthesis (Kiuchi *et al.*, 1992; Aeschbach *et al.*, 1994). The anti-genotoxic properties of gingerols have been reported (Kim *et al.*, 2005; Nirmala *et al.*, 2007a, b; Rout *et al.*, 2015).

The medicinal properties attributed to ginger include anti-arthritic (Srivastava and Mustafa, 1992; Bliddal *et al.*, 2000), anti-migraine (Cady *et al.*, 2005), anti-thrombotic (Thomson *et al.*, 2002), anti-inflammatory (Thomson *et al.*, 2002; Penna *et al.*, 2003), hypolipidemic (Bhandari *et al.*, 2005; Fuhrman *et al.*, 2000) and anti-nausea properties (Portnoi *et al.*, 2003). According to Yeh *et al.* (2014), aqueous and ethanolic extract of ginger rhizome are effective free radical scavengers and metal chelators. Related researches have also shown antioxidant properties of ginger (Sekiwa *et al.*, 2000; Masuda *et al.*, 2004; Al-Assaf, 2012). *Citrullus lanatus* (water melon) is a fruit of a plant originally from a vine of South Africa. It produces about 93% water; hence the name "Watermelon". The seeds of *C. lanatus* are reported to contain various phytochemicals including phenols, saponins, tannins, flavonoids and alkaloids (Nwankwo *et al.*, 2014). One slice of water melon (about 1/16th of a melon) contains large amount of vitamin C and Beta-carotene which may probably fight against various forms of cancer (Nwankwo *et al.*, 2014). It is a good source of fibre for the prevention of colon and renal cancer (Gowda and Jalali, 1995). Emulsion obtained from the seed water extract of water melon is used to cure catarrh infections, disorders of the bowel, urinary passage infection and fever (Taiwo *et al.*, 2008). Nutritionally, it is also rich in citrulline, an effective precursor of L-arginine (Taiwo *et al.*, 2008). Phenolic compounds are constituents of both edible and non-edible parts of the plants. The seeds are sources of protein, tannins and minerals (Collins *et al.*, 2007). From the previous reports on the invaluable medicinal properties of *Zingiber officinale* and *Citrullus lanatus*, the current study was aimed at evaluating the ability of the two plants in mitigating oxidative stress induced by aspartame, which will be addition to catalogue of antioxidant herbals.

MATERIALS AND METHODS

Collection and identification of materials: Ginger (*Zingiber officinale*) rhizome and water melon (*Citrullus lanatus*) seeds were obtained from Watt Market, Calabar, Nigeria. They were taken to the Department of Botany, University of Calabar for identification and deposit of voucher copies. The rhizome of ginger and the seeds obtained from water melon were washed and dried under natural conditions and pulverized using a blender (Excella Eletroline, Model: CM/L7962804) to powdered form and stored in air-tight plastic containers. Growers mash from vital feed limited, Calabar was used as feed. Aspartame was sourced from Biotechnology resource company, Uk.

Experimental animal and administration of extract: A total of 48 sexually matured male albino rats weighing between 120+20 g were obtained from the animal house of the Department of Genetics and Biotechnology, University of Calabar, Calabar. They were allowed a period of acclimatization in well ventilated cages for 7 days. They were maintained under standard laboratory conditions and given water and feed (growers mash) *ad libitum* throughout the study.

Table 1: Distribution of experimental animals in the different treatment groups

Ginger			
A	B	C	D
Positive control fed with physiological saline	Negative control fed with 1000 mg kg ⁻¹ b.wt., ASP	1000 mg kg ⁻¹ b.wt., ASP+ 500 mg kg ⁻¹ b.wt., ginger extract	1000 mg kg ⁻¹ b.wt., ASP+ 1000 mg kg ⁻¹ b.wt., ginger extract
Water melon			
A	B	E	F
Positive control fed with physiological saline	Negative control fed with 1000 mg kg ⁻¹ b.wt., ASP	1000 mg kg ⁻¹ b.wt., ASP+ 500 mg kg ⁻¹ b.wt., water melon extract	1000 mg kg ⁻¹ b.wt., ASP+ 1000 mg kg ⁻¹ b.wt., water melon extract

ASP: Aspartame, BW: Body weight

Wood shavings were used as beddings and were changed every three days to maintain sanitary conditions within the cage. After the acclimatization period, the forty eight male rats were separated into two major groups (group administered with ginger and water melon respectively). The experiment was laid out in a 2×4 factorial using a Completely Randomized Design (CRD) with eight rats per group as summarize in Table 1. Treatment was via oral gavage on a daily basis for 65 days and at the end of the experiment the rats were sacrificed using diethyl ether.

Data collection: The testes and epididymides were surgically removed and properly weighed (Grand G. Model: jj200ld = 0.01 g), while the semen was obtained for sperm analysis. Blood samples were collected via cardiac puncture into sterile tube for liver and oxidative stress enzyme analysis.

ESTIMATION OF SPERM QUALITY

Estimation of epididymides and testes weight: The epididymides and testes were dissected out at the end of the experiment and excess blood damped with cotton wool and placed in a clean weighing balance (Grand G. Model: jj200ld = 0.01 g) to record the weight.

Estimation of sperm motility: The sperm suspension was diluted in 2 mL of warmed buffered physiological saline and dropped on glass slides. This was viewed under light microscope to determine the percentage motile and non-motile sperm cells by their movement (WHO., 1992).

Estimation of sperm count: This was carried out according to the method of Ekaluo *et al.* (2009). The epididymal content was obtained by macerating within scissors known weights of the caput and cauda epididymis in a glass petri dish containing warmed buffered physiological saline in a ratio of 1:10 W/V. After vigorous pipetting, the suspension was separated from tissue fragments by filtering it through an 80 µm stainless mesh. A tissue free aliquot was loaded into the Neubauer haemocytometer (Deep 1/10, Labart, Germany). Five different counts were done for each sample and the mean were taken as the means count for each male rat in millions of sperm/mL.

Estimation of sperm viability: This was estimated using the improved one step eosin-nigrosin staining technique. A function of each suspension of the sperm samples was mixed with equal volume of eosin-nigrosin stain and air dried smears were prepared on glass slide for each samples according to Bjorndahl *et al.* (2003). The slides were coded randomly and examined under the microscope for percentage viability. Normal live sperm cells exuded the eosin-nigrosin while dead sperm cells took up the stain. Percentage viability was calculated based on the number of viable (live) sperm cells divided by the number of sperm cells within 30 min and multiply by 100.

Sperm head abnormalities: A portion of the sperm suspension was mixed with 1% eosin Y solution (10:1) for 30 min and air dried smears were prepared on glass slides for the sperm head abnormality test. The slides were examined for percentage sperm head abnormalities in every 200 spermatozoa observed on each slide and five air dried smears were prepared on glass slides for each sample. The percentage of sperm head abnormality was calculated according to Ekaluo *et al.* (2009).

Estimation of semen pH: The pH of semen was measured using a specially treated calibrated paper blot that changes colour according to the pH of the semen that it is exposed (Comhaire and Vermeulen, 1995).

Determination of oxidative stress enzyme activities: Oxidative stress enzyme activities in the blood samples were determined spectrophotometrically. Superoxide dismutase (SOD) was determined according to the method of Nishikimi *et al.* (1972). Similarly, catalase activities, lipid peroxidation (MDA) and glutathione (GSH) level were determined using the methods of Aebi (1984), Ohkawa *et al.* (1979) and Beutler *et al.* (1963), respectively.

Liver enzyme assay: Activities of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were determined based on the method described by Schmidt and Schmidt (1963).

Statistical analysis: All data collected on the different assays were subjected to analysis of variance (ANOVA) using Predictive Analytics Software (PASW), version 18.0. Significant means were separated using the least significant difference at 5% probability level.

RESULTS

Effect of *Zingiber officinale* rhizome and *Citrullus lanatus* seed extracts on sperm profile of male rats: From Table 2, the results revealed that aspartame significantly reduced ($p < 0.05$) weight of the epididymides in rats treated with 1000 mg kg⁻¹ b.wt., of aspartame compared to rats treated with only physiological saline. However, administration of *Z. officinale* and *C. lanatus* extract increased the weight of epididymis more at 1000 mg kg⁻¹ b.wt. (0.57 g). The extract of both plants did not have significant effect ($p > 0.05$) on the testes of the rats except rats treated with 1000 mg kg⁻¹ b.wt., ASP+1000 mg kg⁻¹ b.wt., of *C. lanatus* seed extracts (1.37 g). There was no significant difference in sperm motility and semen pH of rats in all the groups after aspartame administration. Interestingly, aspartame administration (1000 mg kg⁻¹ b.wt.) caused significant ($p < 0.05$) reduction in sperm viability (55.67%), sperm count (35×10^6 mL⁻¹) and increased sperm head abnormalities (5.17%) compared to rats in other treatment groups (Table 2).

Effect of *Zingiber officinale* rhizome and *Citrullus lanatus* seed extracts on oxidative stress enzymes and liver functions of male rats: Aspartame administration reduced ($p < 0.05$) the level of superoxide dismutase of rats treated with 1000 mg kg⁻¹ b.wt., ASP (97.67 μ mol⁻¹) compared to rats in the group treated with physiological saline (152.33 μ mol⁻¹). It was observed that at 1000 mg kg⁻¹ b.wt., ASP+1000 mg kg⁻¹ b.wt., of *C. lanatus* seed extract, the level of SOD was boosted to 109 μ mol⁻¹ although not equivalent to the positive control (Table 3). The administration of the extracts also increased glutathione peroxidase (GP_x) and MDA

Table 2: Effect of *Z. officinale* and *C. lanatus* seed extract on sperm profile of male rats

Parameters	B		C (1000 mg kg ⁻¹ b.wt., ASP)		D (1000 mg kg ⁻¹ b.wt., ginger)		E (1000 mg kg ⁻¹ b.wt., ASP+500 mg kg ⁻¹ b.wt., watermelon)		F (1000 mg kg ⁻¹ b.wt., ASP+1000 mg kg ⁻¹ b.wt., watermelon)	
	A (positive control)	(1000 mg kg ⁻¹ b.wt., ASP)	(1000 mg kg ⁻¹ b.wt., ginger)	ASP+500 mg kg ⁻¹ b.wt., ginger)	ASP+500 mg kg ⁻¹ b.wt., ginger)	ASP+1000 mg kg ⁻¹ b.wt., ginger)	ASP+1000 mg kg ⁻¹ b.wt., ginger)	ASP+500 mg kg ⁻¹ b.wt., watermelon)	ASP+500 mg kg ⁻¹ b.wt., watermelon)	ASP+1000 mg kg ⁻¹ b.wt., watermelon)
Epididymides (g)	0.63±0.03 ^a	0.53±0.03 ^b	0.53±0.03 ^b	0.57±0.03 ^{ab}	0.57±0.03 ^b	0.53±0.03 ^b	0.53±0.03 ^b	0.57±0.03 ^{ab}	0.57±0.03 ^{ab}	0.57±0.03 ^{ab}
Motility (%)	76.00±2.08 ^b	55.67±2.33 ^a	64.67±2.60 ^a	70.67±1.76 ^a	70.67±1.76 ^a	64.33±2.33 ^a	64.33±2.33 ^a	69.33±3.48 ^a	69.33±3.48 ^a	69.33±3.48 ^a
Testes (g)	1.27±0.03 ^b	1.23±0.03 ^b	1.27±0.03 ^b	1.27±0.03 ^b	1.27±0.03 ^b	1.23±0.03 ^b	1.23±0.03 ^b	1.37±0.03 ^a	1.37±0.03 ^a	1.37±0.03 ^a
Viability (%)	84.33±2.33 ^a	61.67±1.67 ^c	72.33±1.45 ^b	77.33±1.45 ^b	77.33±1.45 ^b	72.33±1.86 ^b	72.33±1.86 ^b	76.33±1.86 ^b	76.33±1.86 ^b	76.33±1.86 ^b
Sperm count (10 ⁶ mL ⁻¹)	40.67±0.67 ^a	35.00±1.73 ^b	39.17±0.44 ^a	39.33±0.67 ^a	39.33±0.67 ^a	40.17±0.44 ^a	40.17±0.44 ^a	41.33±0.67 ^a	41.33±0.67 ^a	41.33±0.67 ^a
Sperm head abnormality (%)	3.17±0.17 ^b	5.17±0.17 ^a	3.33±0.17 ^b	3.33±0.17 ^b	3.33±0.17 ^b	4.17±0.60 ^{ab}	4.17±0.60 ^{ab}	3.50±0.5 ^b	3.50±0.5 ^b	3.50±0.5 ^b
pH	7.2±0.00 ^b	7.07±0.07 ^a	7.07±0.07 ^a	7.20±0.00 ^a	7.20±0.00 ^a	7.00±0.00 ^b	7.00±0.00 ^b	7.13±0.07 ^a	7.13±0.07 ^a	7.13±0.07 ^a

Values are presented in $\bar{x} \pm SE$, ^{a,b,c,d,e,f}Mean with different superscript along the same horizontal array are significantly different (p<0.05) from each other

Table 3: Effect of *Z. officinale* and *C. lanatus* seed extract on oxidative stress enzymes and liver functions of male rats

Parameters	B (1000 mg kg ⁻¹ b.wt., ASP)		C (1000 mg kg ⁻¹ b.wt., ginger)		D (1000 mg kg ⁻¹ b.wt., ginger)		E (1000 mg kg ⁻¹ b.wt., ASP+500 mg kg ⁻¹ b.wt., watermelon)		F (1000 mg kg ⁻¹ b.wt., ASP+1000 mg kg ⁻¹ b.wt., watermelon)	
	A (positive control)	(1000 mg kg ⁻¹ b.wt., ASP)	(1000 mg kg ⁻¹ b.wt., ginger)	ASP+500 mg kg ⁻¹ b.wt., ginger)	ASP+500 mg kg ⁻¹ b.wt., ginger)	ASP+1000 mg kg ⁻¹ b.wt., ginger)	ASP+500 mg kg ⁻¹ b.wt., watermelon)	ASP+500 mg kg ⁻¹ b.wt., watermelon)	ASP+1000 mg kg ⁻¹ b.wt., watermelon)	ASP+1000 mg kg ⁻¹ b.wt., watermelon)
SOD (μ mol ⁻¹)	152.33±0.88 ^a	97.67±0.88 ^d	87.33±0.88 ^f	93.00±0.58 ^e	93.00±0.58 ^e	103.67±0.88 ^c	103.67±0.88 ^c	109.00±0.58 ^b	109.00±0.58 ^b	109.00±0.58 ^b
GPx (μ g ⁻¹ Hb)	69.20±1.24 ^c	63.23±1.07 ^e	43.50±1.93 ^d	48.37±1.62 ^d	48.37±1.62 ^d	86.73±1.47 ^b	86.73±1.47 ^b	94.97±3.55 ^a	94.97±3.55 ^a	94.97±3.55 ^a
CAT (n mol ⁻¹)	1.30±0.06 ^a	0.70±0.06 ^b	0.50±0.06 ^c	0.70±0.06 ^b	0.70±0.06 ^b	0.30±0.06 ^d	0.30±0.06 ^d	0.40±0.06 ^{cd}	0.40±0.06 ^{cd}	0.40±0.06 ^{cd}
MDA (nmol mL ⁻¹)	0.80±0.06 ^c	2.33±0.09 ^e	1.23±0.09 ^d	0.09±0.06 ^c	0.09±0.06 ^c	2.77±0.09 ^b	2.77±0.09 ^b	3.23±0.15 ^a	3.23±0.15 ^a	3.23±0.15 ^a
AST (μ L ⁻¹)	24.48±1.38 ^e	38.23±1.09 ^c	42.21±0.83 ^a	32.44±1.25 ^d	32.44±1.25 ^d	43.62±1.30 ^b	43.62±1.30 ^b	21.35±0.93 ^e	21.35±0.93 ^e	21.35±0.93 ^e
ALT (μ L ⁻¹)	1.82±0.57 ^e	21.97±1.15 ^c	24.24±1.09 ^b	19.49±0.34 ^d	19.49±0.34 ^d	34.24±0.57 ^b	34.24±0.57 ^b	19.52±0.91 ^d	19.52±0.91 ^d	19.52±0.91 ^d
ALP (μ L ⁻¹)	89.73±0.76 ^b	94.40±0.65 ^a	84.98±0.89 ^e	74.37±1.01 ^d	74.37±1.01 ^d	45.26±0.78 ^e	45.26±0.78 ^e	21.71±0.81 ^f	21.71±0.81 ^f	21.71±0.81 ^f

Values are presented in $\bar{x} \pm SE$, ^{a,b,c,d,e,f}Mean with different superscript along the same horizontal array are significantly different (p<0.05) from each other, SOD: Superoxide dismutase, GPx: Glutathione peroxidase, CAT: Catalase, MDA: Malonaldehyde, AST: Aspartate aminotransferase, ALT: Alanine amino transferase, ALP: Alkaline phosphate

significantly while the level of catalase was reduced by aspartame treatment in all the groups. From Table 3, the results show that the administration of aspartame increased the level of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP) in the rats. However, AST was reduced in rats treated with 1000 mg kg⁻¹ b.wt., ASP+1000 mg kg⁻¹ of *C. lanatus* (21.35 μ L⁻¹) which was the same with rats treated with physiological saline (24.48 μ L⁻¹). The level of ALT was the same in rats treated with 1000 mg kg⁻¹ b.wt., ASP+1000 mg kg⁻¹ b.wt., of *Z. officinale* and *C. lanatus* (19.49 and 19.52 μ L⁻¹). At 1000 mg kg⁻¹ b.wt., ASP+1000 mg kg⁻¹ b.wt., of *C. lanatus*, the level of ALP was significantly reduced (21.71 μ L⁻¹) than rats in other groups.

DISCUSSION

Increased level of lipid peroxidation is taken as direct evidence for oxidative stress (Matsumoto *et al.*, 1999; Choudhary and Devi, 2014). Thus, the elevated level of lipid peroxidation after aspartame administration in the rats suggests that aspartame can induce oxidative stress. This alteration after aspartame administration may be due to metabolite methanol of aspartame. Methanol, a metabolite of aspartame is primarily metabolized by oxidation to formaldehyde and then to formate, processes which are accompanied by the formation of superoxide anion and hydrogen peroxide. The formation of these compounds cause lipid peroxidation with damage to polysaturated fatty acids and reduction of membrane fluidity which is essential for proper functioning of the cells (Walaa and Howida, 2015). Our results on oxidative stress and antioxidant defense system showed that aspartame administration reduced superoxide dismutase (SOD), glutathione peroxidase (GP_x) and catalase levels in the rats (Table 3). However, administration of *Z. officinale* and *C. lanatus* extracts successfully improved the levels of these enzymes compared to the negative control (rats administered with 1000 mg kg⁻¹ b.wt., of aspartame). Elevated lipid peroxidation (MDA) was observed in the aspartame treated rats but treatment of rats with the extracts of the two plants had a reverse effect, especially extracts of *Z. officinale* at 100 mg kg⁻¹ b.wt. This could be an indication that these two medicinal plants have the inherent potentials to mitigate oxidation processes caused by aspartame administration in the rats.

Superoxide dismutase constitutes an important link in the biological defense mechanism through conversion of endogenous cytotoxic superoxide radicals to H₂O₂ and O₂⁻ which are deleterious to polyunsaturated fatty acids and proteins (Murray *et al.*, 2003). Catalase and glutathione peroxidase further detoxify H₂O₂ into H₂O and O₂ (Murray *et al.*, 2003). Thus, SOD, catalase and GP_x function mutually as enzymatic anti-oxidative defense mechanism to counter the deleterious effect of ROS. It thus suggests that reduction in the level of these enzymes can lead to oxidative stress. As evidence in the results of this study, aspartame administration reduced the level of these enzymes in the rats, however, treatment of the rats with extract of *Z. officinale* and *C. lanatus* significantly increase their levels dose dependently. The extract of *C. lanatus* increased the level of SOD and GP_x than *Z. officinale* at the same concentrations suggesting their possible usage in mitigating oxidative stress.

According to Kottaimuthu (2008), free radicals attack unsaturated fatty acids in the membranes resulting in membrane lipid peroxidation which decreases membrane fluidity, leakage of enzyme and loss of receptor activity as well as damage membrane proteins leading to cell inactivation. As lipid peroxidation progressively increased, antioxidant defense system decrease equivalently (Tiwari and Rao, 2002) resulting in oxidative stress. This suggests that the administration of aspartame might have weakened the liver membrane of the rats with subsequent penetration and elevation of AST, ALT and ALP in the liver. Linking this to the results of sperm parameters

obtained in this work, it was revealed that aspartame administration significantly reduced the epididymis weight, sperm count and viability and increased percentage sperm head abnormality in the rats. These alterations in sperm profile of the rats may be as the result of oxidative stress induced by elevated lipid peroxidation and liver enzyme activities in the rats which are reported to facilitate reproductive pathologies (Lanzafame *et al.*, 2009; Desai *et al.*, 2010) including sperm damage and reduction of sperm motility. Interestingly, the treatment of the rats with extracts of *Z. officinale* and *C. lanatus* was able to reverse these effects comparably with the positive control. Although the administration of aspartame did not cause any significant change in the motility of the sperm cells, however, the reduction of sperm count and viability caused by aspartame administration is of great panic because if the amount of sperm cells are compromised the number of these cells available to make their way to the oocyte may be too few therefore reducing fertilization efficiency. The process of fertilization requires only viable sperm cells; thus, even when the cells are mobile and can make their way to oocyte, the unviable state of the cells may not guarantee successful capacitation process and may implicate infertility.

According to Walaa and Howida (2015), aspartame administration induces oxidative stress by generation of oxygen radical which initiates oxidation processes. This oxidation process might not be unconnected with the sperm head abnormality result observed by aspartame administration in the rats. This could as well be responsible for the observed reduction in sperm viability. In recent times medicinal plants have been reported as antioxidants reservoir and can act to reduce and scavenge reactive oxygen species (Kukic *et al.*, 2006; Uyoh *et al.*, 2013; Ikpeme *et al.*, 2013, 2014b). These antioxidants act as sensors to regulate the concentration of ROS as well as finding equilibrium between the physiological actions of ROS on sperm cells and their adverse effect on cell physiology (Drevet, 2006). Our results thus show that treating rats with the extracts of *Z. officinale* and *C. lanatus* might probably reduce oxidation process in the spermatozoa significantly by balancing the available antioxidants and ROS. This portrays the inherent medicinal properties of these two plants and will be a reliable source for mitigating oxidative stress.

CONCLUSION

From the result of this study, it can be concluded that aspartame can induce oxidative stress in rats. Ginger rhizome and watermelon seed extracts are antioxidants reservoir with the potency to ameliorate aspartame-induced oxidative stress in rats. Comparatively, the extract of *Citrullus lanatus* reduced oxidative stress activities in the rats especially at 1000 mg kg⁻¹ b.wt., than *Zingiber officinale* as evidenced in the biochemical and sperm profile assays. It is recommended that the consumption of beverages and food products with aspartame additive should be reduced as prolonged consumption and accumulation could result in oxidative stress. The seeds of *Citrullus lanatus* (watermelon) proved very potent in averting induced oxidative stress over *Zingiber officinale* rhizome in this study. Thus, it is recommended that the seeds of this plant should also be consumed while eating the fruit rather than discarding them which is very common practice among its consumers. Lastly, with the findings of this study in rat model, clinical works are required to further validate the current findings.

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