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ORIGINAL RESEARCH

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White tea aqueous extract counteracts cumene hydroperoxide-induced oxidative damage in human sperm: a comparative study with L-ascorbic acid

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Abstract

Background: Oxidative stress (OS) damages sperm by overwhelming antioxidant defences with excess free radicals, damaging seminal parameters and contributing to male infertility. Antioxidants from *Camellia sinensis* (white tea) are known to scavenge excessive reactive oxygen species (ROS), thereby protecting seminal parameters. Measuring OS using oxidative stress markers after antioxidant treatment is crucial for determining the redox state in biological samples and establishing an effective dosage. **Methods**: This study investigated the effect of white tea aqueous (0.465, 4.65, 46.5, 465 μg/mL) extract on normozoospermic samples following the induction of OS. Oxidative stress was induced using sperm wash media supplemented with 100 μM of cumene hydroperoxide at 37 °C for 60–120 minutes on motility and viability, as well as for 60 minutes on oxidation-reduction potential (ORP), ROS, mitochondrial membrane potential (MMP), and sperm-DNA-fragmentation (SDF) and acrosome reaction. **Results**: Cumene hydroperoxide-induced OS significantly decreased ($p < 0.05$) the integrity of the measured seminal parameters. The aqueous extract of white tea demonstrated protective effects on normozoospermic samples following the induction of OS, reducing DNA fragmentation, ORP, and ROS production while increasing intact MMP, vitality and motility ($p < 0.05$). Ascorbic acid had positive effects at a lower concentration, increasing acrosome reaction and lowering ORP ($p < 0.05$). At a higher concentration, it increased motility and sperm viability but also reduced acrosome reaction, MMP, and increased SDF ($p < 0.05$) with no significance for ROS ($p > 0.05$). **Conclusions**: White tea extract has a similar positive effect on sperm parameters as ascorbic acid, suggesting its potential in treating male infertility caused by OS.

Keywords

Oxidative stress; Seminal parameters; Antioxidant therapy; Herbal medicinal plants; Reactive oxygen species; Pro-oxidant

1. Introduction

Globally, couples become concerned when they cannot achieve pregnancy after 12 months of regular unprotected sexual intercourse without the use of contraceptives [1]. Historically, women were seen as the main culprits for infertility despite significant developments in human reproductive science over the past century [2]. It was only in the last four decades that research revealed that male-factor infertility contributes to about half of infertility cases, challenging the long-held stigma unfairly placed on women [2]. Infertility can be classified as primary or secondary [3]. Primary infertility refers to a couple that has never achieved pregnancy in their lifetime, while secondary infertility refers to a couple that has had biological progenies in their past [4]. Infertility affects approximately 20–30% of men worldwide, with 10–20% of these cases identified as idiopathic [5]. Males with idiopathic infertility are mostly diagnosed with oxidative stress (OS) compared to their fertile counterparts [6]. Oxidative stress is reported as the contributing factor to poor sperm quality and quantity, mainly due to the overproduction of free radicals [7]. The imbalance between the ROS and antioxidants results in oxidative damage to sperm parameters, including sperm motility, morphology, DNA integrity, and acrosome. The mitochondria, which serve as the energy source for sperm motility, are vulnerable to OS [8]. Disrupted mitochondria trigger cell death, disrupt signalling pathways, and ultimately compromise fertility. More so, OS is associated with inflammatory responses, which damage sperm function through the production of cytokines stimulated by excessive ROS levels [9].

A recent study challenged the notion that OS is the sole cause of male infertility, highlighting that reductive stress also con-

tributes to producing reactive oxygen species [10]. Excessive levels of reductants, often derived from dietary supplements, can lead to electron leakage and subsequent ROS formation [10, 11]. Moreover, the overuse of antioxidants as a treatment can induce reductive stress, which is equally damaging as OS [8]. This imbalance, known as the "antioxidant paradox", underscores the importance of maintaining balanced redox homeostasis for optimal male reproductive health [12]. These findings emphasise the need for targeted treatment strategies to address oxidative stress in male infertility effectively [13]. While male infertility remains a global social and psychological problem in many households, treatment for male infertility has evolved significantly over the past years [14]. Although traditional treatments such as anti-estrogens and androgens have proven ineffective, assisted reproductive technologies have demonstrated significant potential [15]. Intracytoplasmic sperm injection (ICSI) has become the most effective approach for addressing severe male infertility, providing the opportunity for fatherhood even in cases that were previously considered untreatable [16]. Although *in vitro* fertilisation (IVF) is effective and well-recommended, it is not easily accessible to individuals across all social and economic groups due to its high cost and limited availability [12]. Most of the population (60–80%) depends on medicinal plants and vitamins to treat medical conditions because they are more accessible, affordable, and easy to use than modern healthcare services [17]. Different parts of various plants are traditionally used to treat various aspects of male infertility, such as erectile dysfunction, sperm abnormalities, hormonal disorders, ejaculatory disorders, erectile dysfunction, and the absence or loss of libido [18]. However, while these alternatives are reported to be effective in counteracting the damaging effect of OS, their safety and mode of action are poorly reported [18]. White tea is derived from slightly processed tea from the young branch stem of *Camellia sinensis* [19, 20]. White tea has been shown to restore sperm quality and prevent oxidative damage in prediabetic rats, improving glucose tolerance, insulin sensitivity, and antioxidant capacity, which enhances sperm concentration, motility, and viability [20, 21]. These benefits are attributed to catechin polyphenols, particularly EGCG (epigallocatechin gallate), which are known for their antioxidant properties [22]. Vitamin C supplementation also improves sperm quality by protecting DNA from oxidative damage. In normozoospermic men, it reduced vitrification-induced damage [23], and daily doses of 2000 mg significantly enhanced sperm count, motility, and morphology in oligozoospermic men [24]. Its effects are linked to neutralising ROS and reducing oxidative DNA damage [25]. Several studies have utilised Vitamin C (L-ascorbic acid) as a reference antioxidant due to its well-known properties in reproductive biology [26, 27]. Its hydrophilic nature enables it to function effectively within the aqueous environment of spermatozoa, making it comparable to the polyphenolic compounds found in white tea [28]. This similarity provides a meaningful basis for evaluating the relative protective effects of these two agents against oxidative stress. Both Vitamin C and white tea extract primarily act as hydrophilic antioxidants, targeting intracellular reactive oxygen species (ROS) rather than extracellular ROS [29]. By acting within the cell, they help preserve sperm integrity and function under oxidative stress conditions [26]. This study, therefore, aims to determine the antioxidative effect of white tea and ascorbic acid on seminal parameters under oxidative stress conditions using cumene hydroperoxide.

2. Materials and methods

2.1 Plant harvest and extraction

Dilmah, Cape Town, South Africa (https://www.dilmahtea.co.za), generously provided loose white tea. To prepare the extracts, 20 g of the loose tea was infused in 1 L of freshly boiled distilled water for 5 minutes, then filtered through cheesecloth and Whatman filter papers (No. 4 and 1, Whatman, Maidstone, England). The resulting filtrate was freeze-dried, with the average yield of white tea being 3.72 g. The therapeutic concentration was determined based on an average adult male body weight of 80 kg and a daily tea consumption of six cups of tea daily [23]. This yielded an estimated dose of 46.5 mg/kg for white tea extract. For experimental purposes, this baseline dosage was then adjusted by multiplying it by factors of 10, 100, and 1000 to create different exposure concentrations. Serial dilutions (1:10) with appropriate media provided the final experimental concentrations of white tea: 0.465, 46.5, 4.65, 465 μg/mL. Human tubular fluid- bovine serum albumin (HTF-BSA) without extract was considered a negative control.

2.2 Source and recruitment of semen samples

This study obtained ethical clearance from the Biomedical Research Ethics Committee at the University of the Western Cape, Bellville, South Africa (BM22/5/1). Recruitment was conducted through convenience sampling and took place between 2022 and 2024. Semen samples were obtained from 25 healthy male volunteers (18–45 years) from the Western Cape Province after 2–3 days of abstinence.

2.2.1 Inclusion criteria

The study included semen samples from 25 healthy male volunteers aged 18–45 years from the Western Cape Province, who had an abstinence period of 2–3 days before sample collection. Included samples were from healthy individuals with no chronic diseases and no current use of chronic medications. The semen samples met World Health Organisation (WHO) guidelines for normozoospermic samples, with total motility $>$40%, progressive motility $>$32%, and sperm concentration $>15 \times 10^6$/mL.

2.2.2 Exclusion criteria

Volunteers undergoing treatments (including antioxidant therapy) that could potentially alter the results were excluded from the study. Participants were thoroughly screened for any medications or treatments taken before the study, and those that could impact the findings were excluded based on the disclosure and informed consent process, ensuring the integrity and reliability of the study results.

2.2.3 Semen preparation

Following sample collection, semen samples were liquefied at 37 °C, analysed for motility parameters using the Sperm Class Analyser 6.0 (SCA, Computer aided sperm class analyser, Microptics, Barcelona, Spain), and selected based on WHO guidelines [24] for normozoospermic samples (total motility >40%, progressive motility >32%, sperm concentration >15 × 10^6/mL). Semen samples were subjected to discontinuous (density) gradient centrifugation (80% and 40% media) to select sperm with >80% motility. After centrifugation at 300×g for 20 minutes, the pellet was washed, re-centrifuged at 300×g for 7 minutes, and then re-suspended in 2 mL of sperm wash media. The final sperm concentration was adjusted to 10–15 × 10^6 spermatozoa/mL.

2.3 Treatment

The aqueous extract of white tea, cumene hydroperoxide (310146/1, Sigma Aldrich, St. Louis, MO, USA), and L-ascorbic acid (A4403, Sigma Aldrich, St. Louis, MO, USA) were prepared in HTF-BSA. A stock solution of white tea (10 mg/mL) was prepared and serially diluted (1:10) to generate 10 different concentrations for initial motility screening. Four concentrations (0.465, 4.65, 46.5, 465 μg/mL) of the extract were selected for further investigation. The concentration of ascorbic acid (10 and 31.25 μM) was calculated using non-linear regression analysis in GraphPad Prism 10 for Windows (GraphPad Software, San Diego, CA, USA) based on an half-maximal effective concentration (EC50) curve with the maximum response expressed as a percentage [8].

The concentration of cumene hydroperoxide required to induce oxidative stress (100 μM) was determined using the ORP (mV) measurements and half-maximal inhibitory concentration (IC50) standard curve [8] derived from normozoospermic sperm samples. Following 1 h exposure to 100 μM cumene hydroperoxide, 100 μL sperm aliquots were centrifuged, and the supernatant was removed. The sperm pellets were then resuspended in 100 μL of the respective treatment concentrations. The negative control (NC) samples were treated with Bovine albumin serum-Human tubular fluid (HTF-BSA) alone (without extract or vitamin C), while positive control (PC) samples received cumene hydroperoxide only. All treated samples were incubated at 37 °C. Sperm motility, vitality, and kinematic parameters were assessed 60- and 120- minutes post-incubation to evaluate both immediate and prolonged effects of white tea extract on sperm movement and function. In contrast, intracellular ROS, MMP, DNA fragmentation, and acrosome reaction were evaluated at 60 minutes, as oxidative stress and mitochondrial function serve as early indicators of cellular stress, with changes in these parameters being most pronounced within the initial hour of exposure. This time point captures the acute effects on these sensitive cellular parameters.

2.4 Sperm motility and oxidation-reduction potential

Total motility was assessed at 60 and 120 minutes following oxidative stress induction with cumene hydroperoxide. The study evaluated the effects of various concentrations of white tea aqueous extract from *Camellia sinensis* and ascorbic acid on sperm parameters. Sperm motility was analysed using a computer-aided sperm class analyser (CASA) on a pre-heated microscope stage maintained at 37 °C. For each analysis, 2 μL of sperm suspension was loaded onto an 8-chamber Leja slide (Delfran, Johannesburg, South Africa). A minimum of 200 spermatozoa per sample was captured and analyzed by CASA. The measured parameters included total motility (%), progressive motility (%), curvilinear velocity (VCL; μm/s), straight-line velocity (VSL; μm/s), average path velocity (VAP; μm/s), linearity (LIN; %), straightness (STR; %), beat cross frequency (BCF; Hz), amplitude of lateral head displacement (ALH; μm), wobble (WOB; %), and hyperactivation (%) [30].

In addition to motility assessment, oxidation-reduction potential (ORP) was measured using galvanostat-based technology (MiOXSYS™ System; Electrochemical technology aided oxidation reduction potential analyzer, Aytu Bioscience, Englewood, CO, USA). Briefly, 30 μL of treated sample suspension was applied to the MiOXSYS sensor (LogixX Pharma, Theale, United Kingdom and UAB Caerus Biotechnologies, Berkshire, United Kingdom). The sensor was connected to the galvanostat, which provided the ORP value normalized to sperm concentration as static oxidation-reduction potential (sORP), expressed in millivolts per 10^6 sperm/mL (mV/10^6 sperm/mL).

2.5 Sperm viability

Sperm viability was assessed using eosin-nigrosin (E&N) staining (Sigma, St. Louis, MO, USA). A 50 μL aliquot of sperm suspension was mixed with an equal volume of E&N stain (1:1) and incubated at 37 °C for 15 minutes. The mixture was then smeared onto a glass slide and allowed to air-dry. Slides were examined under a light microscope (Zeiss, Oberkochen, BW, Germany) using a 100× oil immersion objective in bright field mode. Dead spermatozoa (stained red) and live spermatozoa (stained white) were differentiated and counted. A total of two hundred spermatozoa per sample were evaluated, and sperm vitality was expressed as the percentage of live spermatozoa [31].

2.6 Reactive oxygen species

Intracellular ROS production was assessed using dihydroethidium (DHE) [32]. Each sample was diluted with HTF-BSA (1:5) and centrifuged at 300×g for 10 minutes. The resulting pellets were resuspended in 100 μL of phosphate buffered saline (PBS) containing 20 μL of DHE stock solution (20 μM, pH 7.4) and incubated at 37 °C for 15 minutes. Following incubation10 μL of the suspension was smeared onto a Star Frost™ slide (Knittel Gläser, Braunschweig, NI, Germany) and examined using a fluorescence microscope (Zeiss, Oberkochen, BW, Germany; 1000×; 488 nm excitation and 590 emission filters). Spermatozoa with excessive ROS production exhibited bright orange fluorescence. A total of 200 spermatozoa per sample were evaluated, and results were expressed as the percentage of ROS-positive spermatozoa [33].

2.7 Capacitation and acrosome reaction

Capacitation and acrosome reaction were evaluated using the chlortetracycline (CTC; 24, Sigma-Aldrich, St. Louis, MI, USA) fluorescence assay. A stock solution of Hoechst 33258 (100 mg/mL; Sigma-Aldrich, St. Louis, MI, USA) was prepared and diluted 1:1000 in HTF, then added to treated samples at a 1:100 ratio in sperm wash media. Samples were incubated for 2 minutes at room temperature, then centrifuged at 900×g for 5 minutes with 2% polyvinylpyrrolidone (PVP40; 4 mL) in HTF. The CTC solution was freshly prepared and stored at 4 °C protected from light. Hoechst-treated spermatozoa (45 μL) were mixed with CTC solution (45 μL) and 8 μL of 12.5% paraformaldehyde (24898648, Sigma-Aldrich, St. Louis, MI, USA) in 0.5 M Tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl; pH 7.4). The mixture was placed on a slide, and a drop of 0.22 M 1,4-diazabicyclo (2.2.2) octane (DABCO, 280-57-9, Merck, Gauteng, South Africa) dissolved in glycerol and PBS (9:1) was added as an anti-fade medium. Slides were examined using an oil immersion objective lens (100×) under a fluorescence microscope (Zeiss). A total of 200 spermatozoa per sample were assessed and classified according to CTC staining patterns: non-capacitated with acrosome-intact, capacitated with acrosome-intact, and capacitated with acrosome-reacted [34].

2.8 Mitochondrial membrane potential

Sperm mitochondrial membrane potential was assessed using a 5,5′,6,6′-tetrachloro-1-1,1′,3,3′-tetraethyl benzimidazolyl carbocyanine iodide (JC-1; Sigma, St. Louis, MO, USA). According to the manufacturer's protocol, Slides were examined in a darkroom using a fluorescence microscope. A minimum of 200 spermatozoa per sample was evaluated. Spermatozoa were classified based on fluorescence: green fluorescence indicated disrupted mitochondrial membrane potential, while red fluorescence indicated intact mitochondrial membrane potential. Results were expressed as a percentage of spermatozoa with intact mitochondrial membrane potential.

2.9 Sperm DNA fragmentations

Sperm-DNA damage was evaluated using the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate (dUTP) nick-end labelling (TUNEL) assay (Detection System fluorescein; Promega, Mannheim, BW, Germany). Following with white tea and ascorbic acid, sperm samples were centrifuged at 300×g for 10 minutes, and the supernatant was discarded. The pellet was resuspended with phosphate buffer saline and smeared onto Superfrost slides (Knittel Gl€aser, Braunschweig, NI, Germany). Slides were fixed with freshly prepared 4% formaldehyde in PBS for 25 minutes at 4 °C, then washed in PBS for 5 minutes at room temperature. Spermatozoa were permeabilised using 0.2% Triton X-100 in PBS for 5 minutes and then rinsed twice with PBS at room temperature. After removing excess liquid, samples were equilibrated with 100 μL of equilibration buffer for 5 to 10 minutes. Subsequently, 20 μL of Terminal deoxynucleotidyl transferase (TdT) buffer was added, and slides were covered with a coverslip and incubated at 37 °C for 1 hour. Slides were then immersed in 2× saline sodium citrate (SSC) for 15 minutes, washed three times with distilled water, and examined under an epifluorescence microscope at 630× magnification. Results were expressed as the percentage of green-fluorescing (TUNEL-positive) spermatozoa [35].

2.10 Statistical analysis

Data analysis was performed using GraphPad Prism 10 for Windows (GraphPad Software, San Diego, CA, USA). The normality of data distribution was assessed using the D'Agostino-Pearson omnibus test. For normally distributed data, one-way analysis of variance (ANOVA) was performed, followed by Tukey's *post hoc* test for multiple comparisons. For non-normally distributed data, Sidak's multiple comparison tests were used. Statistical significance was set at $p < 0.05$. Data are presented as mean values with error bars representing standard deviations. Significant differences between groups were denoted by different letters above the bars, with groups sharing the same letters showing no significant difference. Significance was determined based on confidence intervals of mean differences, where intervals excluding zero indicated significant differences between groups.

3. Results

3.1 Kinematic parameters

Tables 1 and 2 present the effects of white tea aqueous extract and ascorbic acid on sperm kinematic parameters following cumene hydroperoxide-induced oxidative stress at 60 and 120 minutes. Treatment with various concentrations of white tea aqueous extract, ascorbic acid, and the negative control showed variable results when compared to the positive control.

The negative control showed a significant increase in some kinematic parameters, including VCL, VAP, VSL, ALH, STR, LIN, WOB and BCF at 60 and 120 minutes ($p < 0.0001$) compared to the positive control. Hyperactivation only increased at 120 minutes ($p < 0.05$). White also demonstrated comparable results of a significant increase when compared to the positive control at 60 and 120 minutes for VCL, VSL, VAP, STR, WOB and BCF ($p < 0.0001$). However, ALH reduced significantly at 60 and 120 minutes compared to the positive control ($p < 0.0001$), while LIN reduced significantly at 120 minutes and increased at 60 minutes ($p < 0.0001$). Hyperactivation showed no significance for both 60 and 120 minutes ($p > 0.05$).

For Ascorbic acid, STR and LIN increased significantly at both 60 and 120 minutes ($p < 0.0001$) compared to the positive control, while VAP, VSL and BCF showed a significant increase only at concentration 10 μM at 120 ($p < 0.0001$) with no significant change for 31.25 μM ($p > 0.05$). No significant difference was noted at 60 minutes for both concentrations ($p > 0.05$). Ascorbic acid (10–31.25 μM) increased VCL at 60 and 120 minutes ($p < 0.0001$), although at 31.25 μM no significant effect was noted at 60 minutes ($p > 0.05$) compared to the positive control. WOB was significantly higher at both 60 and 120 ($p < 0.0001$), with no significant difference for concentration 31.25 μM ($p > 0.05$) at 120 minutes. Lastly, Ascorbic acid significantly reduced ALH compared to the

TABLE 1. Summary of white tea statistics on kinematic parameters.

Kinematic	Hour	White tea aqueous extract (μg/mL)						*p*-value	
		NC	PC	0.465	4.65	46.5	465	NC	PC
VCL-µm/s									
	1	82 ± 2.1****	28 ± 2.6	70 ± 1.9****,#	73 ± 1.9****,###	68 ± 1.9****,#	72 ± 2.5****,###	0.0128	<0.0001
	2	82 ± 2.4****	30 ± 16	76 ± 2.5****	70 ± 1.6****,##	80 ± 3.2****	75 ± 2.4****	0.0081	0.0001
VAP-µm/s									
	1	44 ± 1.1****	16 ± 1.9	42 ± 0.7****	44 ± 1.3****	41 ± 0.8****	45 ± 1.6****	0.9999	<0.0001
	2	52 ± 1.1****	20 ± 2.3	44 ± 1.2****,###	42 ± 1.1****,####	47 ± 1.2****,#	44 ± 1.1****,###	0.0428	<0.0001
VSL-µm/s									
	1	30 ± 0.9****	10 ± 1.6	24 ± 1.6****,#	27 ± 1.3****	26 ± 1.9****	26 ± 2.4****	0.0252	<0.0001
	2	34 ± 0.9****	11 ± 1.4	26 ± 1.3****,##	26 ± 1.7****,##	28 ± 1.8****,#	23 ± 2.3****,####	0.0223	<0.0001
ALH-µm									
	1	36 ± 0.7****	14 ± 1.9	2.3 ± 0.1****,####	2.4 ± 0.1****,####	2.3 ± 0.03****,####	2.4 ± 0.1****,####	<0.0001	<0.0001
	2	14 ± 0.4****	7.0 ± 1.1	2.6 ± 0.1****,####	2.4 ± 0.1****,####	2.6 ± 0.1****,####	2.5 ± 0.1****,####	<0.0001	<0.0001
STR (%)									
	1	16 ± 0.4****	6.2 ± 1.3	54 ± 2.8****,#	60 ± 1.7****	57 ± 2.3****	54 ± 4.1****,#	0.0306	<0.0001
	2	55 ± 1.3****	19 ± 2.6	56 ± 1.6****	59 ± 2.1****	58 ± 2.4****	53 ± 4.5****	0.9999	<0.0001
LIN									
	1	36 ± 0.7****	14 ± 1.9	33 ± 1.4****	36 ± 1.2****	33 ± 1.9****	32 ± 3.1****	0.9999	<0.0001
	2	43 ± 0.9****	16 ± 2.1	2.6 ± 0.1****,####	2.4 ± 0.1****,##	2.6 ± 0.09****,##	2.5 ± 0.1****,####	0.0022	<0.0001
WOB									
	1	54 ± 0.6****	23 ± 2.1	60 ± 1.0****,#	58 ± 0.9****	59 ± 1.2****	62 ± 1.8****,###	0.0161	<0.0001
	2	49 ± 1.0****	19 ± 2.4	58 ± 0.8****,####	59 ± 1.2****,####	60 ± 1.2****,####	60 ± 0.8****,####	<0.0001	<0.0001
BCF									
	1	16 ± 0.4****	6.2 ± 1.3	15 ± 0.3****	16 ± 0.4****	15 ± 0.4****	16 ± 0.5****	0.9985	<0.0001
	2	14 ± 0.8****	3.9 ± 0.6	15 ± 0.4****	15 ± 0.4****	15 ± 0.4****	15 ± 0.5****	0.9786	<0.0001
HYP									
	1	8.5 ± 5.9	0.17 ± 0.1	0.29 ± 0.1	0.57 ± 0.2	0.71 ± 0.1	0.088 ± 0.04	0.146	>0.9999
	2	2.8 ± 0.5****	0.24 ± 0.1	0.052 ± 0.02####	0.061 ± 0.03####	0.1 ± 0.0####	0.01 ± 0.01####	<0.0001	0.0001

Values represented are the mean ± SD of spermatozoa from normozoospermic samples (n = 25) and semen samples exposed to the various concentrations of Camellia sinensis (White tea).

Abbreviations: ALH: amplitude of lateral head displacement; BCF: beat cross frequency; HYP: hyperactivation; LIN: linearity; STR: straightness; VAP: average path velocity; VCL: curvilinear velocity; VSL: straight-line velocity; WOB: wobble; NC: Negative Control (Bovine serum albumin-human tubular fluid); PC: Positive control (Cumene hydroperoxide). For one-way ANOVA, significance was represented by (****) $p < 0.0001$ when compared to the positive control. Hash tags represent: (#) $p < 0.05$, (##) $p < 0.01$, (###) $p < 0.001$, and (####) $p < 0.0001$ when compared to the negative control.

TABLE 2. Summary of ascorbic acid statistics on kinematic parameters.

Kinematic	Hour	Ascorbic acid extract (μM)				*p*-value	
		NC	PC	10	31.25	NC	PC
VCL-μm/s							
	1	82 ± 2.1****	28 ± 2.6	82 ± 2.1****,####	30 ± 4.4	<0.0001	<0.0001
	2	82 ± 2.4****	30 ± 16	58 ± 6.0##,****	49 ± 6.2####,**	0.0018	0.0029
VAP-μm/s							
	1	44 ± 1.1****	16 ± 1.9	15 ± 2.9####	21 ± 3.4####	<0.0001	<0.0001
	2	52 ± 1.1****	20 ± 2.3	31 ± 3.5####,**	24 ± 3.3####	<0.0001	0.0100
VSL-μm/s							
	1	30 ± 0.9****	10 ± 1.6	9.9 ± 1.9####	6.9 ± 1.1####	<0.0001	<0.0001
	2	34 ± 0.9****	11 ± 1.4	22 ± 2.9###,**	15 ± 2.4####	0.0001	0.0011
ALH-μm							
	1	36 ± 0.7****	14 ± 1.9	1.7 ± 0.2****,####	1.9 ± 0.2****,####	<0.0001	<0.0001
	2	14 ± 0.4****	7.0 ± 1.1	2.5 ± 0.2####,****	2.1 ± 0.2####,****	<0.0001	<0.0001
STR (%)							
	1	16 ± 0.4****	6.2 ± 1.3	54 ± 1.8##,****	53 ± 2.1###,****	0.0015	<0.0001
	2	55 ± 1.3****	19 ± 2.6	56 ± 3.0****	51 ± 1.9****	<0.0001	<0.0001
LIN							
	1	36 ± 0.7****	14 ± 1.9	25 ± 3.0###,**	23 ± 2.2####,*	0.0008	0.0012
	2	43 ± 0.9****	16 ± 2.1	31 ± 2.3****	23 ± 1.9#,*	0.014	0.0431
WOB							
	1	54 ± 0.6****	23 ± 2.1	39 ± 3.8###,****	39 ± 3.0###,***	0.0006	0.0001
	2	49 ± 1.0****	19 ± 2.4	47 ± 2.4##,**	43 ± 1.8####	0.0015	0.0020
BCF							
	1	16 ± 0.4****	6.2 ± 1.3	4.5 ± 1.0####	5.4 ± 1.3####	<0.0001	0.5603
	2	14 ± 0.8****	3.9 ± 0.7	8.4 ± 1.3##,**	6.5 ± 1.2####	0.0015	0.0020
Hyperactivation							
	1	8.5 ± 5.9****	0.17 ± 0.0	0.16 ± 0.04####	0.048 ± 0.02####	<0.0001	<0.0001
	2	2.8 ± 0.5****	0.24 ± 0.1	0.040 ± 0.02####	0.16 ± 0.05####	<0.0001	<0.0001

Values represented are the mean ± SD of spermatozoa from normozoospermic samples (n = 25) and semen samples exposed to the various concentrations of Ascorbic Acid. Abbreviations: ALH: amplitude of lateral head displacement; BCF: beat cross frequency; LIN: linearity; STR: straightness; VAP: average path velocity; VCL: curvilinear velocity; VSL: straight-line velocity; WOB: wobble; NC: Negative Control (Bovine serum albumin-human tubular fluid); PC: Positive control (Cumene hydroperoxide). For one-way ANOVA, significance was represented by (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$, and (****) $p < 0.0001$ when compared to the positive control. Hash tags represent: (#) $p < 0.05$, (##) $p < 0.01$, (###) $p < 0.001$, and (####) $p < 0.0001$ when compared to the negative control.

positive control at 60 and 120 minutes ($p < 0.0001$).

Furthermore, when compared to the negative control, white tea demonstrated variable results. VAP, LIN and hyperactivation were significantly lower at 120 minutes ($p < 0.05$), with no significant change at 60 minutes ($p > 0.05$). At 60 minutes, white tea (0.465, 465 μg/mL) significantly increased STR compared to the negative control ($p < 0.05$) with no significant effect at 120 minutes ($p > 0.05$). At 60 minutes, VCL significantly decreased ($p < 0.05$) and only at 4.65 μg/mL for 120 minutes compared to the negative control ($p < 0.05$). ALH were significantly low at 60 and 120 minutes ($p < 0.05$). VSL decreased significantly at 60 (0.465 μg/mL) and 120 minutes ($p < 0.05$). No significance was notable for BCF at both 60 and 120 minutes ($p > 0.05$).

Treatment with ascorbic acid resulted in a decrease in VAP, VSL, ALH, WOB, BCF and hyperactivation compared to the negative control at both 60 and 120 minutes ($p < 0.0001$). Furthermore, VCL showed a similar average to the negative control at 60 minutes at the 10 μM concentration, with no significant difference observed at 31.25 μM ($p > 0.05$). However, at 120 minutes, VCL was significantly lower at both concentrations compared to the negative control ($p < 0.001$). More so, STR increased significantly at 60 minutes with no change at 120 minutes, for both concentrations ($p < 0.05$).

In addition, at 60 minutes LIN reduced significantly for both concentrations, and only at 31.25 μM ascorbic acid for 120 minutes ($p < 0.05$).

Negative mean differences (MD) values represent an increase in the parameter, and 95% confidence intervals that do not include 0 represent a significant difference. Negative values indicate an increase in the parameter, while 95% confidence intervals that do not include 0 signify significant differences. The table includes comparisons between the negative control (NC) and positive control (PC) groups. The results represent the MD between treatment groups. The effects of white tea extract at concentrations of 0.465, 4.65, 46.5, and 465 μg/mL, as well as ascorbic acid at 10 and 31.25 μM, are shown.

3.2 Total motility

The effect of white tea aqueous extract and ascorbic acid on total motility is shown in Fig. 1A. Treatment with white tea aqueous extract and the negative control significantly increased total motility at 60 minutes ($p < 0.05$) and 120 minutes ($p < 0.001$) compared to the positive control. Conversely, ascorbic acid treatment showed no significant changes in motility at either time point compared to the positive control ($p > 0.05$).

When compared to the negative control, white tea extract at 465 μg/mL significantly decreased motility at 60 minutes ($p < 0.01$), while concentrations of 0.465, 4.65, and 465 μg/mL showed significant decreases at 120 minutes ($p < 0.05$). Ascorbic acid demonstrated lower motility compared to the negative control at both 60 and 120 minutes ($p < 0.0001$). No time-dependent effects were observed for either white tea aqueous extract or ascorbic acid ($p > 0.05$) (**Supplementary Table 1**).

White tea aqueous extract exhibited notably higher motility percentages compared to ascorbic acid (10–31.25 μM) at 60 minutes ($p < 0.0001$), except at 465 μg/mL, where the difference was not significant ($p > 0.05$). At 120 minutes, white tea extract maintained higher motility compared to 31.25 μM ascorbic acid across all concentrations except at 465 μg/mL. Compared to 10 μM ascorbic acid, white tea extract at 0.465 and 46.5 μg/mL showed significantly increased motility at 120 minutes, while 465 μg/mL showed no significant differences.

3.3 Progressive motility

The percentage of progressive motile sperm was assessed following the induction of oxidative stress (Fig. 1B). The negative control significantly increased progressive motility compared to the positive control at both 60 and 120 minutes ($p < 00001$). White tea aqueous extract significantly increased progressive motility compared to the positive control at 60 minutes for concentrations of 0.465 and 4.65 μg/mL ($p < 0.0001$) and at 120 minutes for concentrations of 0.465, 4.65, and 46.5 μg/mL ($p < 0.0001$). However, the highest concentration (465 μg/mL) showed no significant improvement at either time point ($p > 0.05$). Ascorbic acid demonstrated significantly decreased progressive motility compared to the negative control at 60 and 120 minutes ($p < 0.0001$; **Supplementary Table 1**). Time-dependent changes were observed for white tea aqueous extract, with significant decreases at 0.465 and 4.65 μg/mL ($p < 0.05$), and a significant increase at 46.5 μg/mL ($p < 0.05$). White tea showed significantly higher progressive motility than ascorbic acid at 0.465, 4.65, and 46.5 μg/mL ($p < 0.05$) at 60 minutes, but not significant at 465 μg/mL. At 120 minutes, significant differences were observed only at 46.5 μg/mL ($p < 0.05$).

3.4 Sperm viability

The percentage of viable sperm following oxidative stress induction is presented in Fig. 1C and **Supplementary Table 1** at 60 and 120 minutes. The negative control, white tea aqueous extract, and ascorbic acid significantly increased sperm viability compared to the positive control ($p < 0.0001$), except for ascorbic acid at 31.25 μM at 60 minutes, which showed no significant increase ($p > 0.05$). Compared to the negative control, white tea treatment demonstrated a significant concentration-dependent increase in viable sperm at both time points ($p < 0.0001$), except at the highest concentration (465 μg/mL), which showed no significant difference ($p > 0.05$). Conversely, ascorbic acid treatment resulted in a significantly lower sperm viability compared to the negative control at 60 minutes ($p < 0.0001$) while showing no significant difference at 120 minutes ($p > 0.05$).

No significant differences were observed at 4.65 and 465 μg/mL. Overall, white tea demonstrated concentration- and time-dependent decreases in viable sperm at 60 and 120 minutes ($p < 0.05$), whereas ascorbic acid showed concentration- and time-dependent increases in viable sperm percentages ($p < 0.05$).

When compared to ascorbic acid (10–31.25 μM), white tea treatment yielded significantly higher percentages of viable sperm at 60 minutes ($p < 0.05$). At 120 minutes, white tea at concentrations 0.465, 4.65 and 465 μg/mL was significantly lower than 10 μM ascorbic acid ($p < 0.05$), while concentration 46.5 μg/mL showed no significant difference ($p > 0.05$). Compared to 31.25 μM ascorbic acid, white tea at 0.465 μg/mL showed a significantly lower percentage of viable sperm, while 46.5 μg/mL showed a significantly higher percentage ($p < 0.05$). No significant differences were observed at 4.65 and 465 μg/mL. Overall, white tea demonstrated concentration- and time-dependent decreases in viable sperm at 60 and 120 minutes ($p < 0.05$), whereas ascorbic acid showed concentration- and time-dependent increases in viable sperm percentages ($p < 0.05$).

3.5 Oxidation-reduction potential

The oxidation-reduction potential and oxidative stress levels are presented in Fig. 2. Treatment with the negative control, white tea aqueous extracts, and ascorbic acid resulted in significant reductions in both ORP and static-ORP levels compared to the positive control ($p < 0.05$). Ascorbic acid treatment significantly decreased ORP and static-ORP levels compared to the negative control ($p < 0.05$). A concentration-dependent increase in ORP and static-ORP was observed with increasing white tea aqueous extract concentrations ($p < 0.05$). Although a similar trend was noted for ascorbic acid, the increase was not statistically significant ($p > 0.05$). When comparing white tea extract to ascorbic acid at 10 and 31.25 μM, ORP and

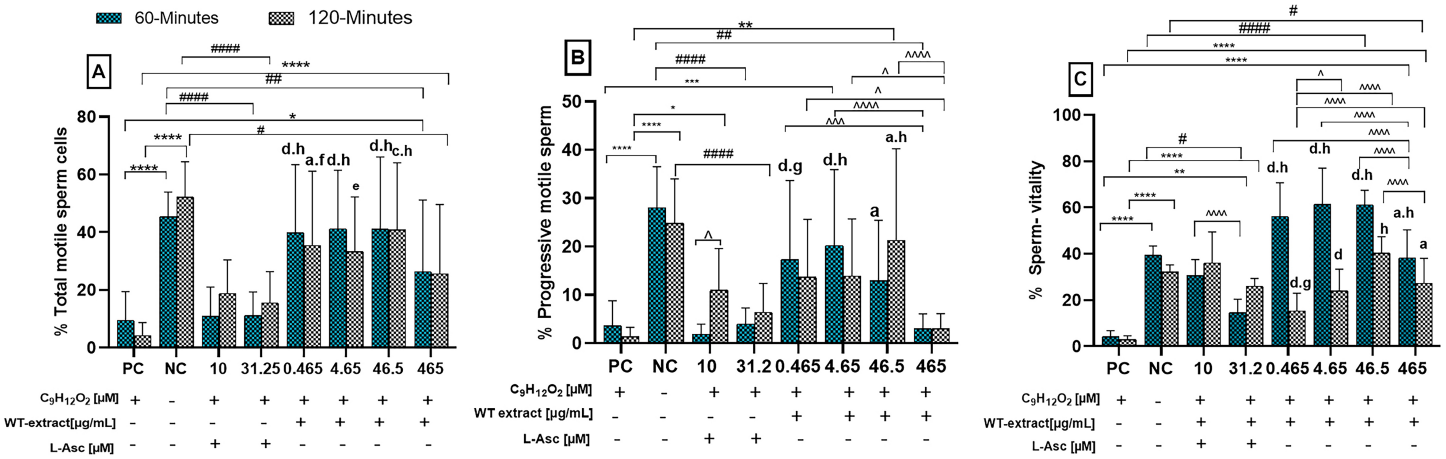

FIGURE 1. The effect of aqueous extract of white tea and ascorbic acid on (A) Total motility and Progressive motility (B) and Vitality (C). Data for normozoospermic semen samples (n = 25) is presented as mean ± SD. Statistical significance was represented using one-way ANOVA, where (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$, and (****) $p < 0.0001$ indicate significance compared to the positive control, while ($^{\#}$) $p < 0.05$, ($^{\#\#}$) $p < 0.01$, and ($^{\#\#\#\#}$) $p < 0.0001$ indicate significance compared to the negative control. Two-way ANOVA-multiple comparison significance for 60 and 120 minutes is denoted by (^) $p < 0.05$, (^^^) $p < 0.001$, and (^^^^) $p < 0.0001$. Comparisons between ascorbic acid concentrations and white tea aqueous extract are denoted by (d) $p < 0.05$, (c) $p < 0.01$, and (a) $p < 0.0001$ for 10 μM, while for 31.25 μM, significance is indicated by (e) $p < 0.05$, (f) $p < 0.01$, (g) $p < 0.001$, and (h) $p < 0.0001$. PC: Positive control; NC: Negative Control; WT: White tea; $C_9H_{12}O_2$: cumene hydroperoxide; L-ASc: L-ascorbic acid.

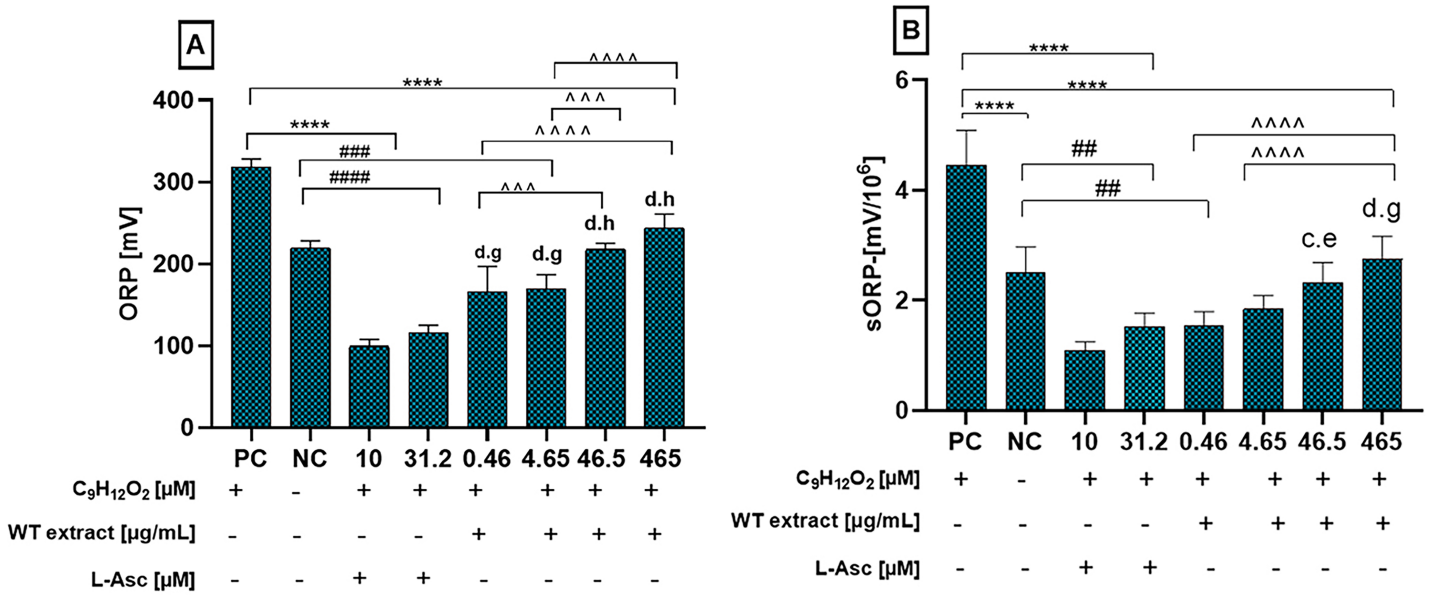

FIGURE 2. Oxidation-reduction potential (A) and (B) static oxidation-reduction potential for White tea and Ascorbic acid. Data for normozoospermic semen samples (n = 25) is presented as mean ± SD. Statistical significance was measured using one-way ANOVA, where (****) $p < 0.0001$ indicate significance compared to the positive control, while ($^{\#\#}$) $p < 0.01$, ($^{\#\#\#}$) $p < 0.001$, and ($^{\#\#\#\#}$) $p < 0.0001$ indicate significance compared to the negative control at 60 minutes. Multiple comparison significance is denoted by (^^^) $p < 0.001$, and (^^^^) $p < 0.0001$. Comparisons between ascorbic acid concentrations and white tea aqueous extract are denoted by (d) $p < 0.05$, (c) $p < 0.01$ for 10 μM, while for 31.25 μM, significance is indicated by (e) $p < 0.05$, (g) $p < 0.001$, and (h) $p < 0.0001$. PC: Positive control; NC: Negative Control; ORP: oxidation-reduction potential; WT: White tea; $C_9H_{12}O_2$: cumene hydroperoxide; L-ASc: L-ascorbic acid.

static-ORP levels were significantly higher in white tea-treated samples ($p < 0.05$).

Negative MD values represent an increase in the parameter, and 95% confidence intervals that do not include 0 represent a significant difference. Negative values indicate an increase in the parameter, while 95% confidence intervals that do not include 0 signify significant differences. The table includes comparisons between the negative control (NC) and positive control (PC) groups. The results represent the mean differences (MD) between treatment groups. The effects of white tea extract at concentrations of 0.465, 4.65, 46.5, and 465 μg/mL, as well as ascorbic acid at 10 and 31.25 μM, are shown.

3.6 Mitochondrial membrane potential

The impact of oxidative stress on mitochondrial membrane potential is illustrated in Fig. 3A, showing the percentage of intact MMP. The negative control, white tea aqueous extract and ascorbic acid significantly increased intact MMP compared to the positive control ($p < 0.05$). When compared to the negative control, lower concentrations of white tea extract (0.465, 4.65, 46.5 μg/mL: $p < 0.05$) and ascorbic acid (10 μM: $p < 0.01$) demonstrated significant increases in intact MMP. Conversely, the highest concentration of white tea extract (465 μg/mL) showed a significant decrease ($p < 0.05$), while 31.25 μM ascorbic acid showed no significant change ($p < 0.05$; **Supplementary Table 2**). White tea extracts at 0.465, 4.65, and 46.5 μg/mL showed significantly higher intact MMP compared to 31.25 μM ascorbic acid ($p < 0.0001$), whereas 465 μg/mL showed no significant difference ($p > 0.05$). Compared to 10 μM ascorbic acid, white tea at 46.5 μg/mL significantly increased intact MMP ($p < 0.01$), while 465 μg/mL resulted in a significant decrease ($p < 0.0001$). A concentration-dependent increase in intact MMP was observed for white tea (0.465–46.5 μg/mL, $p < 0.001$), followed by a decrease at 465 μg/mL ($p < 0.0001$). Ascorbic acid showed a concentration-dependent decrease ($p < 0.0001$).

3.7 Reactive oxygen species

Intracellular ROS production following oxidative stress is illustrated in Fig. 3B. The negative control, white tea aqueous extract and ascorbic acid significantly reduced intracellular ROS levels compared to the positive control ($p < 0.0001$). Compared to the negative control, white tea aqueous extract at 0.465, 4.65, and 46.5 μg/mL demonstrated significant decreases in ROS levels ($p < 0.001$), while the highest concentration (465 μg/mL) showed no significant difference ($p > 0.05$). Similarly, Ascorbic acid treatment showed no significant change compared to the negative control ($p > 0.05$). Within the white tea treatment groups, a significant decrease in ROS levels was observed between 0.465 and 4.65 μg/mL ($p < 0.01$; **Supplementary Table 2**), while a significant increase was noted between 4.65, 46.5 and 465 μg/mL ($p < 0.0001$). No significant differences were observed between ascorbic acid concentrations ($p > 0.05$).

3.8 DNA fragmentation

The percentage of sperm with fragmented DNA following the oxidative stress induction is presented in Fig. 4A. Treatment with white tea aqueous extract and ascorbic acid significantly reduced the percentage of fragmented DNA compared to the positive control ($p < 0.0001$). However, when compared to the negative control, both ascorbic acid ($p < 0.0001$) and white tea aqueous extract ($p < 0.01$) showed a significant increase in the percentage of sperm with fragmented DNA (Fig. 4A; **Supplementary Table 2**). White tea at 4.65, 46.5 μg/mL demonstrated lower percentages of DNA fragmentation ($p < 0.05$) compared to ascorbic acid at 10 and 31.25 μM ($p < 0.05$). A concentration-dependent decrease in the DNA fragmentation was observed for white tea extract at 0.465, 4.65, and 46.5 μg/mL, followed by an increase at 465 μg/mL ($p < 0.05$). In contrast, ascorbic acid exhibited a concentration-dependent increase in the percentage of fragmented sperm ($p < 0.001$).

3.9 Capacitation and acrosome reaction

The acrosome reaction and acrosome intact in sperm following oxidative stress induction is illustrated in Fig. 4B,C. Treatment with white tea aqueous extracts and ascorbic acid significantly increased the percentage of acrosome-reacted and acrosome intact sperm compared to the positive control ($p < 0.0001$). However, when compared to the negative control, both white tea ($p < 0.0001$) and ascorbic acid ($p < 0.001$) showed significant decreases in the percentage of acrosome-reacted sperm, except for white tea at 46.5 μg/mL ($p > 0.05$), which showed no significant difference. Ascorbic acid at 10 μM significantly increased the percentage of acrosome-reacted sperm compared to white tea at 0.465, 4.65, and 465 μg/mL ($p < 0.05$). Conversely, white tea at 46.5 μg/mL showed a significantly higher percentage of acrosome-reacted sperm when compared to ascorbic acid at 10 μM ($p < 0.05$; **Supplementary Table 2**). For capacitated acrosome intact sperm white tea significant decreased compared to the negative control. Ascorbic acid showed a significant increase in the percentage of acrosome intact sperm compared to the negative control ($p < 0.05$; Fig. 4C).

Ascorbic acid at 31.25 μM showed a significant decrease in acrosome-reacted sperm compared to white tea at 46.5 μg/mL ($p < 0.0001$), with no significant differences observed when compared to white tea at 0.465, 4.65, and 465 μg/mL ($p > 0.05$). Additionally, ascorbic acid exhibited a significantly higher percentage of acrosome-intact sperm compared to white tea across all concentrations (0.465, 4.65, 46.5, and 465 μg/mL). A concentration-dependent increase in acrosome-reacted sperm was observed for white tea between 0.46 μg/mL and 46.5 μg/mL ($p < 0.05$), followed by a significant decrease between 46.5 μg/mL and 465 μg/mL ($p < 0.0001$).

4. Discussion

Oxidative stress, induced by organic hydroperoxide like cumene hydroperoxide (CHP), causes significant damage to spermatozoa [36]. In humans, excessive ROS production can adversely affect key sperm parameters, including motility,

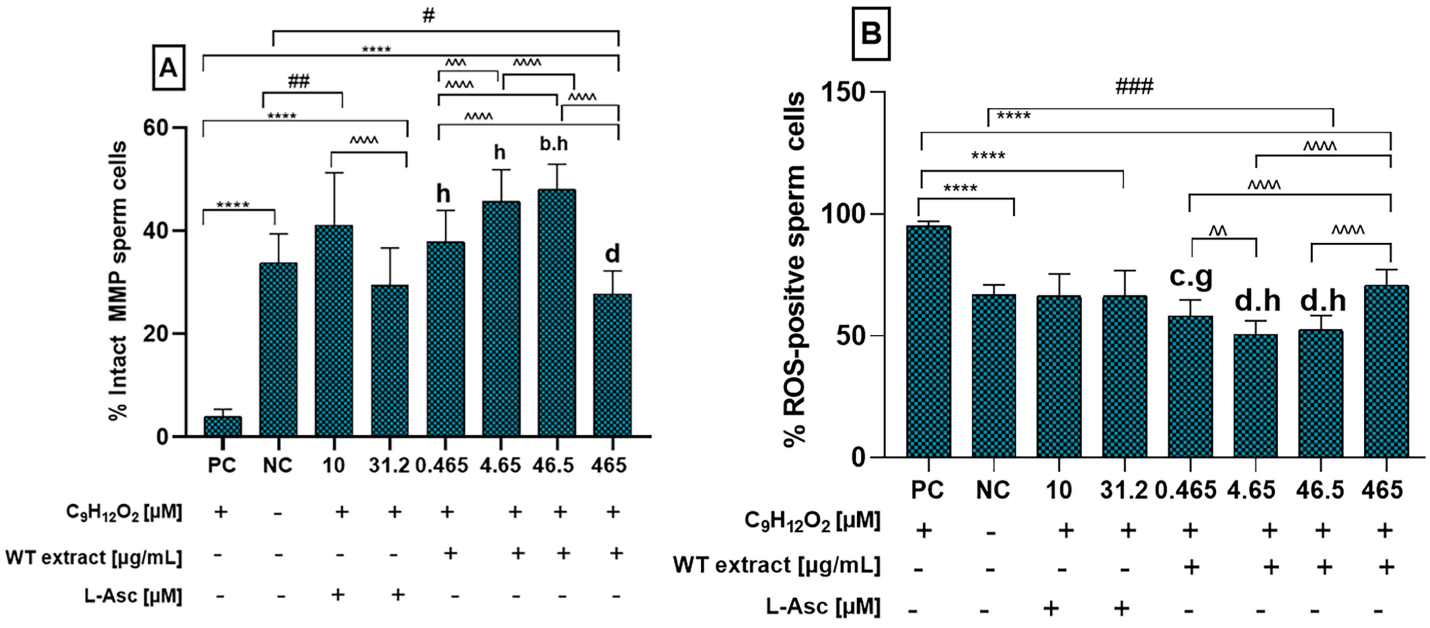

FIGURE 3. The effect of aqueous extract of white tea and ascorbic acid on (A) Intact MMP, (B) ROS-positive sperm. Data for normozoospermic semen samples (n = 25) is presented as mean ± SD. Statistical significance was measured using one-way ANOVA, where (****) $p < 0.0001$ indicate significance compared to the positive control, while ($^{\#}$) $p < 0.05$, ($^{\#\#}$) $p < 0.01$, and ($^{\#\#\#}$) $p < 0.001$ indicate significance compared to the negative control at 60 minutes. Multiple comparison significance is denoted by (^^) $p < 0.01$, (^^^) $p < 0.001$, and (^^^^) $p < 0.0001$. Comparisons between ascorbic acid concentrations and white tea aqueous extract are denoted by (d) $p < 0.05$, (c) $p < 0.01$, and (b) $p < 0.001$ for 10 μM, while for 31.25 μM, significance is indicated by (g) $p < 0.001$, and (h) $p < 0.0001$. PC: Positive control; NC: Negative Control; ROS: Reactive oxygen species; WT: White tea; $C_9H_{12}O_2$: Cumene hydroperoxide; L-ASc: L-ascorbic acid.

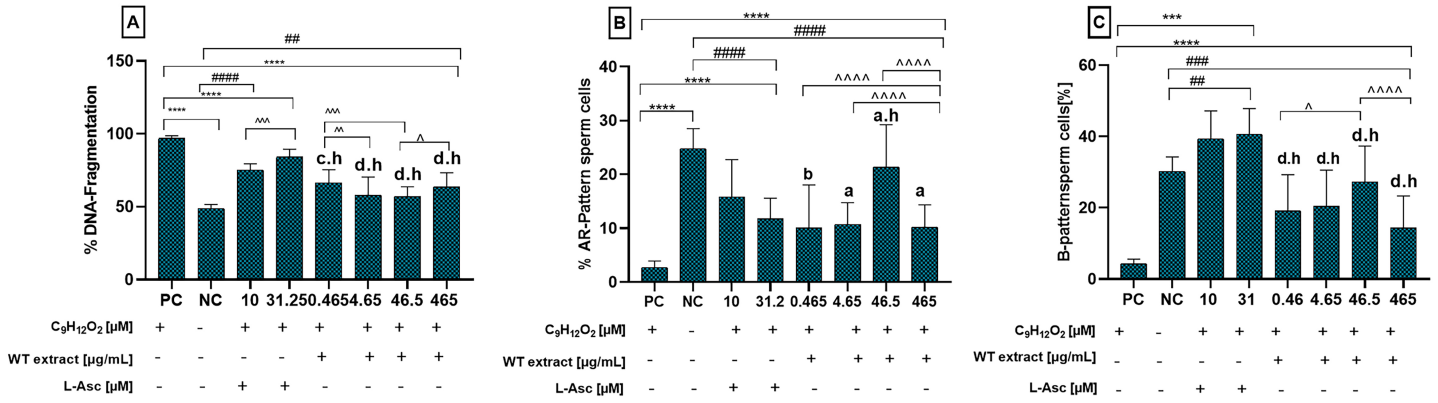

FIGURE 4. The effect of aqueous extract of white tea and ascorbic acid on sperm DNA-fragmentation (A), Acrosome reacted sperm (B) and (C) Acrosome intact sperm. Data for normozoospermic semen samples (n = 25) is presented as mean ± SD. Statistical significance was measured using one-way ANOVA, where (***) $p < 0.001$, and (****) $p < 0.0001$ indicate significance compared to the positive control, while ($^{\#\#}$) $p < 0.01$, ($^{\#\#\#}$) $p < 0.001$, and ($^{\#\#\#\#}$) $p < 0.0001$ indicate significance compared to the negative control at 60 minutes. Multiple comparison significance is denoted by (^) $p < 0.05$, (^^) $p < 0.01$, (^^^) $p < 0.001$, and (^^^^) $p < 0.0001$. Comparisons between ascorbic acid concentrations and white tea aqueous extract are denoted by (d) $p < 0.05$, (c) $p < 0.01$, (b) $p < 0.001$, and (a) $p < 0.0001$ for 10 μM, while for 31.25 μM, significance is indicated by (h) $p < 0.0001$. PC: Positive control; NC: Negative Control; AR: Acrosome reaction; WT: White tea; $C_9H_{12}O_2$: Cumene hydroperoxide; L-ASc: L-ascorbic acid.

viability, morphology, acrosome integrity, and DNA quality [29]. While low levels of ROS may enhance sperm-oocyte fusion, higher levels—especially with prolonged exposure—can lead to DNA fragmentation and lipid peroxidation, ultimately impairing fertility by reducing sperm motility and compromising embryo development [9]. To counteract oxidative stress, antioxidant supplementation has emerged as a promising strategy for improving male fertility. Numerous studies have demonstrated that herbal medicinal plants, along with antioxidants like ascorbic acid (vitamin C), can enhance semen quality, improve sperm functionality, and positively influence reproductive outcomes [18, 24, 25]. However, recent research has raised concerns about excessive antioxidant intake, as high doses can paradoxically exhibit pro-oxidant effects, leading to DNA damage and compromised fertility even in healthy individuals [37]. The present study further supports these findings regarding the damaging effects of oxidative stress. However, the relatively small sample size (n = 25) limits the generalizability of the findings, as it reduces statistical power and may not fully capture the variability within larger populations. While the extracts demonstrated a promising ability to enhance seminal parameters, further studies with a larger sample size are required to validate these preliminary findings further.

The induction of oxidative stress with cumene hydroperoxide resulted in a significant increase in ORP, excessive reactive oxygen species production, poor outcomes in sperm motility, viability, mitochondrial membrane potential, acrosome integrity, and DNA fragmentation. The increase in poor motility, viability, acrosome, mitochondrial and DNA integrity was notably observed in higher concentrations of white tea extract and ascorbic acid treatment groups than in the negative control group.

The negative control in our study highlighted the inherent production of reactive oxygen species, a crucial factor for sperm production and maturation. However, it also illustrated the susceptibility of sperm cells to oxidative stress. When oxidative stress occurs, it triggers lipid peroxidation. ROS interact with lipids in the sperm cell membrane, leading to the abstraction of electrons and subsequent damage to the membrane's structure [38, 39]. This disruption compromises cell membrane integrity, activates cell death pathways, and impairs sperm function. Ultimately, this culminates in cell death through autophagy, a process where the cell recycles its damaged or dysfunctional components, which can inadvertently remove essential cellular elements and activate apoptotic pathways [40, 41]. Moreover, oxidative stress can result in mitochondrial membrane permeabilisation and reduced mitochondrial membrane potential [42, 43]. This decrease in adenosine triphosphate (ATP) synthesis negatively affects axonemal dynein adenosine triphosphatase (ATPase) activity, leading to impaired motility of sperm flagella. The resulting ATP deficit further activates intrinsic apoptotic pathways, culminating in the death of sperm cells [44]. This sequence of events significantly compromises fertility potential by reducing the number of viable spermatozoa available for fertilisation, thus exacerbating issues related to male factor infertility. Our study also demonstrated that exposure to cumene hydroperoxide significantly increases ROS production, creating a cascade of oxidative damage. This oxidative damage results in the activation of cellular stress pathways, leading to the opening of the mitochondrial permeability transition pore (MPTP) and a collapse of mitochondrial membrane potential [44]. The ensuing decline in ATP production, combined with the oxidative damage, activates the intrinsic apoptotic pathway, characterised by the release of cytochrome c and the activation of caspase-3, ultimately resulting in reduced sperm motility and cell death [44].

The detrimental impact of ROS on sperm cells highlights the need for practical therapeutic approaches to enhance male fertility. One promising strategy is the use of antioxidants to counteract oxidative stress. Research has demonstrated that antioxidants can significantly reduce ORP levels in human embryo culture media. Notably, cysteine, ascorbic acid, and β-mercaptoethanol effectively lower ORP to physiological levels [38].

ORP is a comprehensive marker of oxidative stress in semen and is measured using the MiOXSYS system. There is a negative correlation between ORP levels and conventional semen parameters, as well as DNA fragmentation [14, 45]. As such, ORP measurement has emerged as a robust diagnostic tool for male infertility, with well-defined cut-off values that can distinguish fertile men from those who are infertile [46]. One of the key advantages of ORP measurement is that it requires only a tiny amount of semen sample and provides rapid results, making it a valuable addition to routine male infertility assessments [47]. Additionally, assessing ORP can help identify patients who may benefit from antioxidant treatment and allows for ongoing monitoring of treatment efficacy.

Notable findings in various *in vivo* and *in vitro* studies have highlighted the antioxidant properties and composition of *Camellia sinensis* (white tea) [23, 42, 48] and ascorbic acid (Vitamin C) [27]. White tea, in particular, has been shown to contain a high concentration of catechins, although its overall antioxidant capacity may be somewhat lower compared to ascorbic acid due to fewer non-catechin antioxidants [24]. The antioxidant components of white tea have garnered interest for their ability to scavenge free radicals and inhibit oxidative stress, making them potential therapeutic agents for improving male fertility. The treatment of cumene hydroperoxide on sperm cells with an aqueous extract of white tea demonstrated a concentration-dependent effect in reducing oxidative stress. Antioxidants are crucial in counteracting oxidative stress, with studies indicating a positive correlation between antioxidant concentration and sperm parameters [49]. Notably, catechins in the white tea extract may be responsible for the observed reduction in ROS and the increase in intact mitochondria. The white tea aqueous extract exhibited significant antioxidant activity at lower concentrations (4.65 and 46.5 μg/mL), as well as with lower doses of ascorbic acid (10 μM). The lower ORP and static oxidation-reduction substantiated the potential values recorded at these concentrations, indicating that the antioxidant properties of both the extract and ascorbic acid were most effective at these levels.

However, a paradoxical effect emerged at higher concentrations. Administration of high dosages (465 μg/mL) of white tea extract and 31.25 μM of ascorbic acid appeared to induce a pro-oxidant effect, leading to increased free radicals and

elevated ORP values. This surge in free radicals resulted in mitochondrial damage, particularly at higher concentrations in both treatment groups. This consequently triggered a cascade of harmful effects, including a decreased percentage of live sperm over 60 and 120 minutes compared to the negative control. The mitochondrial damage caused by the pro-oxidant effects of high-dose white tea extract can have severe implications for cellular function and overall health. Such damage disrupts the mitochondrial membrane potential, impairs energy production, increases ROS generation, and activates apoptotic pathways. As a result, cells experience reduced energy levels, heightened oxidative stress, and increased damage to cellular components such as lipids and proteins. While research on the cytotoxicity of white tea in human reproduction remains limited, studies on *Camellia sinensis* have indicated potential side effects, including hepatotoxicity and gastrointestinal disorders, particularly when consumed on an empty stomach [50]. Notably, acute toxicity studies in rats have shown no significant adverse effects at doses up to 2000 mg/kg body weight [51].

Ascorbic acid plays a crucial role in protecting human sperm from oxidative damage. Studies, such as those by [52], have demonstrated that higher levels of ascorbic acid in seminal fluid are associated with reduced oxidative damage in sperm. However, the protective effects of ascorbic acid are concentration-dependent and can vary from species to species. Research indicates that lower ascorbic acid concentrations can enhance sperm motility and viability, whereas higher concentrations may be detrimental [53]. This finding is reflected in the present study, where administering low concentrations of ascorbic acid specifically, 10 μM, to oxidative stress-induced sperm cells resulted in a significant increase in both the number of intact mitochondria and the percentage of live sperm over time when compared to the positive control. While there was an improvement in mitochondrial integrity and cell viability at this lower concentration, no concentration-dependent significance concerning ROS levels was observed as concentrations increased.

Nevertheless, it is noteworthy that ascorbic acid-treated cells exhibited lower ROS levels than the negative and positive controls. This highlights the importance of cautious usage and dosing of antioxidants such as ascorbic acid and white tea. Although both are essential for maintaining a balance between oxidants and antioxidants, high doses can act as pro-oxidants, potentially disrupting normal cellular functions [54]. Therefore, appropriate dosing is critical, with recommendations suggesting lower preventive doses for healthy individuals and higher therapeutic doses for specific treatments [54].

Sperm motility is crucial to the male reproductive system's fertility potential. Successful fertilisation occurs when sperm cells exhibit progressive motility towards the egg cell [55]. However, a significant reduction in sperm motility can adversely impact fertility chances [56]. In the present study, we observed that prolonged oxidative stress decreased sperm cell motility. These findings are consistent with previous research indicating that oxidative stress negatively affects motility and compromises male reproductive function. Human sperm cells depend on physiological levels of ROS and mitochondrial oxidative phosphorylation to produce quality sperm and adenosine triphosphate, which is essential for motility [57]. Notably, physiological ROS levels are vital for regulating sperm motility. Low to moderate levels of ROS facilitate key processes, such as sperm capacitation, the activation of motility proteins, and the maintenance of sperm membrane fluidity. Moreover, ROS play a significant role in inducing tyrosine phosphorylation of proteins associated with sperm motility, modulating cyclic adenosine monophosphate (cAMP) signalling pathways, and influencing the redox state of sperm proteins. These processes collectively impact the activity and motility of sperm cells [58].

The present study demonstrated that treatment of spermatozoa with white tea aqueous extract and ascorbic acid exerted concentration-dependent effects on sperm motility, kinematics, and progressive motility. Notably, lower concentrations of these treatments resulted in higher percentages of total motility, progressive motility, and improved kinematic parameters. Conversely, higher concentrations decreased sperm motility over time, likely due to potential concentration-dependent toxicity and the effects of prolonged incubation periods. These findings resonate with existing literature, which indicates that prolonged incubation of human spermatozoa at body temperature can significantly alter sperm function and motility [59]. Specifically, studies have shown that extended incubation reduces motile sperm count and impaired movement quality [60]. This decline in motility is often accompanied by increased levels of ROS and phosphatidylserine externalisation, indicating compromised membrane integrity [60].

Also, prolonged incubation can increase DNA fragmentation and decrease mitochondrial membrane potential [61]. Our study also suggests that when used at lower concentrations, white tea aqueous extract and ascorbic acid can counteract ROS's damaging effects while preserving crucial seminal parameters. Previous research supports these findings, showing that white tea intake can restore sperm concentration, motility, and viability in pre-diabetic rats by enhancing testicular antioxidant potential and reducing oxidative stress [18]. The high catechin content in white tea, particularly (-)-epigallocatechin-3-gallate, contributes to its potent antioxidant properties [62].

Similarly, ascorbic acid supplementation has been recognised for its ability to enhance sperm motility and viability across various studies. For example, adding 0.5% L-ascorbic acid to Tris egg yolk extender significantly improved kinematic motility and recovery rates of sexed sperm in Friesian Holstein bulls [63]. Furthermore, vitamin C supplementation increased total and progressive sperm motility and viability in rats under stress conditions [64]. Notably, ascorbic acid (5 mM) used as a semen additive has been found to improve post-thaw semen quality in cattle bulls, enhancing live spermatozoa count, acrosomal integrity, and hyperosmotic swelling test results while simultaneously reducing sperm abnormalities and oxidative stress [65].

Sperm capacitation and acrosome reaction are crucial processes for fertilisation, occurring naturally in the female reproductive tract or induced *in vitro* [48, 66]. Oxidative stress can induce a spontaneous acrosome reaction in sperm cells, leading to a premature release of acrosomal enzymes [67]. This is because oxidative stress activates protein kinases, such as pro-

tein kinase C (PKC), which trigger a cascade of events leading to the acrosome reaction [68]. Premature sperm capacitation and acrosome reaction, induced by oxidative stress, can be detrimental to fertility. The premature release of acrosomal enzymes can lead to their inactivation or degradation before reaching the egg, reducing the chances of successful fertilisation [69]. Additionally, capacitated sperm have a shorter lifespan, and premature capacitation can impair sperm-egg interaction, ultimately decreasing fertility [69].

This study investigated the effects of white tea aqueous extract and ascorbic acid on the oxidative stress-induced acrosome reaction. Both substances demonstrated antioxidant properties, effectively scavenging reactive oxygen species and reducing oxidative stress. However, the findings revealed that higher concentrations of white tea aqueous extract and ascorbic acid could disrupt the delicate balance of ROS necessary for sperm capacitation, leading to decreased acrosome-reacted sperm. This indicates that while antioxidants can mitigate oxidative stress-induced acrosome reactions, excessive antioxidant activity may harm capacitation. Interestingly, the differences in the impact of white tea aqueous extract and ascorbic acid on acrosome integrity can be attributed to their distinct antioxidant mechanisms. Ascorbic acid is a potent antioxidant, actively scavenging ROS and reducing oxidative stress, while the white tea aqueous extract contains a variety of antioxidants that may exert a more nuanced effect on ROS levels [65, 70, 71]. Overall, these findings suggest that antioxidants can modulate both capacitation and the acrosome reaction in sperm cells, with effects being highly dependent on concentration and the mechanisms of action. A delicate balance of ROS is required for proper capacitation; thus, excessive antioxidant activity can disrupt this balance.

Additionally, traditional sperm analysis parameters—such as motility, concentration, and morphology—offer limited insights into fertility status [72]. Sperm DNA fragmentation plays a crucial role in the production, maturation, and transfer of genetic material to the egg cell, highlighting its significance in reproductive science research [73, 74]. Notably, white tea extract has been reported to protect against acrylamide-induced DNA damage in male rats [75]. Although not explicitly focused on white tea, research on oral antioxidant treatment with vitamins C and E demonstrated a significant reduction in sperm DNA fragmentation in men with unexplained infertility [76]. In this study, white tea extract exhibited antioxidant effects in protecting sperm DNA, particularly at concentrations of 0.465 and 10 μM of ascorbic acid, which correlated with improved sperm viability, mitochondrial membrane potential, and ROS levels. However, high concentrations of white tea extract and ascorbic acid resulted in significant DNA damage, likely due to redox imbalance. ROS can damage DNA through various mechanisms, including modifying all bases, creating base-free sites, frame-shift mutations, eliminating DNA cross-links, and chromosomal aberrations [77]. The high prevalence of single-strand and double-strand DNA breaks has also been linked to oxidative stress. Evidence suggests ROS can trigger gene mutations, including polymorphisms and point mutations, decreasing semen quality [78].

Nevertheless, some studies indicate that the relationship between DNA fragmentation and ROS is not always straightforward. This raises the possibility that DNA fragmentation caused by high antioxidants may be solely responsible for cell death. At the same time, ROS may directly damage nuclear DNA within spermatozoa under certain conditions [79]. White tea aqueous extract emerged as a more effective antioxidant than ascorbic acid in protecting sperm quality, at the concentrations used in this study, demonstrating significant improvements across various parameters. White tea extract showed enhanced sperm kinematic parameters, including VCL, VAP, VSL, ALH, STR, WOB, and BCF, and increased total and progressive motility.

Additionally, white tea extract improved mitochondrial membrane potential, ROS levels, and decreased DNA fragmentation. It also exhibited a higher percentage of viable sperm, acrosome-reacted sperm, and acrosome-intact sperm compared to the positive control. The superior antioxidant effects of white tea extract can be attributed to its unique polyphenolic composition, which includes catechins, theaflavins, and thearubigins. These polyphenols work synergistically to neutralise free radicals, reduce oxidative stress, and protect sperm cells from damage.

In contrast, although a potent antioxidant, ascorbic acid has limited bioavailability and stability, reducing its efficacy in protecting sperm quality. Ascorbic acid's antioxidant activity may also be overwhelmed by its pro-oxidant effects at high concentrations, further contributing to its relatively lower effectiveness than white tea. Additionally, the limited antioxidant capacity of ascorbic acid may be exhausted more quickly, making it less effective in providing long-term protection against oxidative stress [80].

5. Limitations

This study has several limitations that warrant consideration. The *in vitro* design may not fully reflect *in vivo* conditions, and the long-term effects of white tea on sperm function were not assessed. Additionally, the specific bioactive compounds responsible for the observed improvements in seminal parameters were not identified, and the measurement of extracellular ROS was not included. Future research should focus on isolating and characterising the bioactive compounds in white tea, evaluating its long-term effects on sperm function, and establishing its clinical efficacy and safety profile to bridge the gap between laboratory findings and clinical practice.

6. Conclusions

The current study suggests that white tea extract holds potential as a natural antioxidant for improving sperm function and reducing oxidative stress, thereby enhancing male fertility.

AVAILABILITY OF DATA AND MATERIALS

Data will be made available at a reasonable request.

AUTHOR CONTRIBUTIONS

NBT—writing–original manuscript draft, methodology, data analysis, statistical software, data visualisation, presentation,

conceptualisation. TKM—writing–review and editing, validation, supervision. RH—writing–review and editing, validation, supervision. CSO—writing–review and editing, validation, data analysis, data visualisation, presentation, conceptualisation, supervision and securing funding.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the University of the Western Cape Ethics Committee (BM22/5/1). All participants received project information and provided informed consent for the experiments.

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CONFLICT OF INTEREST

Ralf Henkel is employed at LogixX Pharma. Dilmah tea company donated us with tea for the study. The other authors declare that no conflict of interest could be perceived as prejudicing the impartiality of the research reported.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found, in the online version, at https://oss.jomh.org/files/article/20171189146751303 68/attachment/Supplementary%20material.docx.

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