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Carica papaya seed extract slows human sperm

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Abstract

Ethnopharmacological relevance: Traditional healers use *Carica papaya* seeds as a remedy for diseases and as a contraceptive for men and abortion in women.
Material and methods: Semen samples from 35 healthy men were allowed to liquefy and subsequently incubated for 60 min in Human Tubular Fluid medium containing 1% bovine serum albumin with aqueous *C. papaya* seed extract at concentrations of zero, 0.025, 0.25, 2.5, 25, 250 and 2500 µg/ml. Afterwards, sperm were washed and used for assessment of capacitation and acrosome reaction, DNA fragmentation, vitality, motility, reactive oxygen species (ROS) and mitochondrial membrane potential (MMP).
Results: The extract showed no effects on straight-line velocity, linearity, straightness, beat-cross frequency and the percentage of capacitated, acrosome-reacted sperm. In contrast, vitality, total motility, progressive motility, curvilinear velocity, average-path velocity and the percentages of hyper-activated, ROS-positive and MMP-intact sperm decreased significantly ($P < 0.05$), while the percentage of DNA-fragmented sperm increased ($P < 0.05$).
Conclusions: Our data show that aqueous *C. papaya* seed extract significantly and negatively affects sperm motility parameters crucial for fertility; and thus, poses as a likely candidate for male contraception.

Keywords: contraceptive, *Carica papaya*, human sperm, DNA-Fragmentation, mitochondrial membrane potential

1. Introduction

Globally, almost 80% of the world's population rely upon contraceptives derived from traditional medicine (plants, and their extracts) because of their low cost and availability (Sabourian et al., 2016). One of the popular plants used for this purpose is *Carica papaya* (pawpaw or papaya). This plant is the third most popular tropical crop in the world. Though it originated from Central America and Southern Mexico, nowadays it is mainly cultivated in Brazil, India, and Mexico (Chávez-Pesqueira and Núñez-Farfán, 2017). Traditional healers use various parts of this plant to treat diabetes, inflammation, depression, and to regulate

blood pressure and cholesterol levels (Elgadir et al., 2014). Besides these main applications, the plant is also used as a contraceptive, an antiseptic, and an antimicrobial in Central America, South Asia and Africa (Elgadir et al., 2014). For instance, *Candida albicans* causes mucosal membrane diseases such as *Candida vaginitis* in women, and recently it has developed resistance to current treatment methods. Papaya seed extract has been used successfully as an alternative due to its antifungal activity, and specifically because of its ability to trigger reactive oxygen species (ROS) production and decrease mitochondrial membrane activity (Zhang and Chen, 2017).

Several studies have investigated the mechanism of action of the contraceptive effects of *C. papaya* in various animals such as monkeys (Lohiya et al., 2002), rabbits (Lohiya et al., 2000b), dogs (Ortega-Pacheco et al., 2011) and rats (Goyal et al., 2010). Lohiya et al., 2002, 2008 have shown that oral administration of 50 mg/kg/day of the chloroform and benzene chromatographic *C. papaya* seed extract resulted in long-term, reversible azoospermia with no significant side effects in langur monkeys. The treatment with chloroform extract (50 mg/kg/day) is effective after three months and reversible after 150 days (Lohiya et al., 2002). Furthermore, the treatment with extract deriving from a benzene chromatographic fraction decreased sperm concentration and normal sperm morphology, especially in the midpiece of the flagellum; this resulted in decreased sperm motility. Additionally, sperm mitochondrial activity, acrosome reaction (AR), and the hypo-osmotic swelling test were scored in the lowered infertile range in langur monkeys treated with *C. papaya* (Lohiya et al., 2002).

Moreover, chloroform and methanol fractionations of *C. papaya* seed extract negatively affected human sperm motility in a dose-dependent manner. After 20 min of exposure, all sperm were immotile (Lohiya et al., 2000a). Although chloroform and methanol are often used for the experimental extraction of plants, both solvents are highly toxic and difficult to eliminate from the extracts. For this reason, Medical Control Councils do not advocate utilizing herbal preparations extracted with these toxic solvents (Kermanshai et al., 2001). On the other hand, oral administration of aqueous, methanol, ethanol, ethyl acetate and chloroform extracts of *C. papaya* seeds have elucidated reversible contraceptive effects, whereas the aqueous and chloroform extracts were not toxic and did not impair libido in rats (Lohiya et al., 2002, 2006, 2008, 2017).

The determination of phytochemical compounds from an aqueous extract of *C. papaya* seeds revealed the presence of tannins, steroids, terpenoids (Abayomi-Dada et al., 2016; Gadzama et al., 2016), saponins (Gadzama et al., 2016; Khan et al., 2012), phenols, flavonoids, ferric reducing antioxidant property (FRAP) (Abayomi-Dada et al., 2016), pro-anthocyanidins (Khan et al., 2012), alkaloids, anthraquinones and cardiac glycosides (Gadzama et al., 2016). Further determination of phytochemicals from *C. papaya* seeds by the use of other solvents such as methanol (Tariq et al., 2015), ethanol and chloroform resulted in similar compounds (Eke et al., 2014)

Considering initial encouraging reports and the need to investigate non-toxic solvents to extract the bioactive compounds from the seeds, there is merit in investigating the effects of *C. papaya* seed extract obtained after extraction using a safer solvent as a possible alternative contraceptive for men. Accordingly, the aim of this study was to explore details of the in vitro effects of an aqueous *C. papaya* seed extract on human sperm parameters.

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2. Materials and methods

Unless otherwise mentioned, all chemicals used were provided by Sigma (St. Louis, MO, USA).

2.1. C. papaya preparation

C. papaya seeds were provided by Neofresh, Nelspruit, South Africa, and labeled as Neo-65 F2. F2 denotes the second generation of the Neo-65 strain of *C. papaya* fruit (plant name has been checked with http://www.theplantlist.org). The botanical name is *Carica papaya L.* and belongs to the family of *Caricaceae*, a dicotyledonous polygamous plant; its common name is *Pawpaw*. It falls in the category of fruits and vegetables. There are no available herbarium records of the plant material and no further chemical fingerprints on the cultivar of *C. papaya* is performed by Neofresh. The seeds were washed, dried and ground to powder. Five grams of this powder was extracted for 72 h with 200 ml distilled water at 70 °C. The aqueous extract was then filtered using filter paper size 393 (Munktell Ahlstrom, Ahlstrom Munksjö, Helsinki, Finland) and frozen at −20 °C. The extract was eventually freeze-dried (ZIRBUS Technology GmbH, Bad Grund, Germany), which resulted in a bright brown powder (average of 545.9 mg extract/5 g of seeds).

2.2. Experimental design

The aqueous *C. papaya* seed extract was dissolved in Human Tubular Fluid medium (Quinn et al., 1985) supplemented with 1% bovine serum albumin (HTF-BSA) to make up the final concentrations of zero [control], 0.025, 0.25, 2.5, 25, 250 and 2500 μg/ml (Nayak et al., 2012). Sperm (2×106 sperm/200 μl) were incubated for 60 min at 37 °C, and motility, vitality, DNA-fragmentation, mitochondrial membrane potential (MMP), ROS, AR, and capacitation status were analysed using phase contrast and fluorescence microscopy (Nikon, DSFi1, Nikon Instruments Inc., New York, USA) at 100-X magnification under oil immersion, respectively. At least 200 sperm were counted for each sample where the sperm was equally distributed across the entire slide.

2.3. Semen sample preparation and measuring of motility

The Institutional Biomedical Research Ethics Committee at the University of the Western Cape, Bellville, South Africa (BMREC-UWC) approved the study. Thirty-five normozoospermic semen samples were obtained from healthy sperm donors (all semen donors were filled informed consent form before entering to the semen donor list) after 2–3 days of sexual abstinence. The average age of the donors was 23 ± 2.66 years and the average sperm concentration, motility and the volume of semen were 52.07 ± 5.11 million/ml, 59.48 ± 3.04%, and 2.73 ± 0.19 ml, respectively. After 20 min of liquefaction, samples were analysed for motility using the Sperm Class Analyzer (SCA, Version 4.1.0.1, Microptic S.L., Barcelona, Spain). Semen samples were washed three times with HTF-BSA and then sperm concentration was adjusted to 50×106 sperm/ml. Subsequently, samples were incubated in

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HTF-BSA with different concentrations of aqueous *C. papaya* seed extract for 60 min at 37 °C. Finally, total and progressive motility (%), curvilinear velocity (VCL, m/s), straight-line (rectilinear) velocity (VSL, m/s), average path velocity (VAP, m/s), linearity (LIN, %), straightness (STR, %), beat-cross frequency (BCF, Hz) and hyperactivation (%) were measured simultaneously using the SCA.

2.4. Evaluation of sperm vitality

The one-step eosin-nigrosin staining technique (World Health Organization, 2010) was used to evaluate the vitality 60 min after exposure of the sperm to the extract. Briefly, after mixing 50 µl of the sample with 50 µl of eosin-nigrosin, a smear was made on a glass slide and air-dried. Vital sperm are appearing white and were counted and calculated as percentage live sperm.

2.5. Detection of DNA-fragmentation

Sperm DNA integrity was determined by means of the Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay (DeadEnd™ Fluorometric TUNEL System, Promega Corporation, Madison, USA) established by Henkel et al. (Henkel et al., 2010; Shalaweh et al., 2015). After incubation with the *C. papaya* seed extract, samples were centrifuged for 5 min at 250xg. Thereafter, the supernatant was removed, and the pellet re-suspended in 50 µl phosphate buffered saline (PBS, Oxoid, Basing Stock, Hampshire, England), then 10 µl smears were made on Superfrost slides and allowed to airdry. Subsequently, slides were fixed for 25 min at 4 °C in 4% formaldehyde prepared in PBS and washed for 5 min at 25 °C in PBS. Further, samples were permeabilized for 5 min with 0.2% Triton X-100 prepared in PBS and washed in PBS at 25 °C twice. Slides were then equilibrated in 100 µl equilibration buffer for 10 min. Subsequently, slides were incubated with TdT for 1 h at 37 °C. The reaction was then stopped by immersing the slides in the 2-X saline-sodium citrate (SSC) buffer in deionized water for 15 min at 25 °C. Finally, slides were washed three times in distilled water and TUNEL-positive sperm exhibiting green fluorescence were counted and reported as percentage TUNELpositive sperm (Fig. 1A).

2.6. Detection of reactive oxygen species

The intracellular ROS production of sperm can be detected using the dihydroethidium (Molecular Probes, Eugene, OR, USA) (DHE) stain (Shalaweh et al., 2015). Briefly, after incubation with the seed extract, semen samples were centrifuged for 5 min at 250xg, the supernatant was discarded, and the pellet re-suspended in 100 µl PBS with 20 µl of a 20 µM DHE stock solution in PBS. Samples were incubated at 37 °C for 15 min in the dark. Finally, ROS-positive sperm identified with a red fluorescence of the sperm head were counted and reported as percentage ROS-positive sperm (Fig. 1B).

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2.7. Detection of capacitation and acrosome reaction

Capacitation and AR were determined using the chlortetracycline fluorescence assay (CTC) as established by Kholkute (Kholkute et al., 1992). Sperm samples were centrifuged for 5 min at 250xg. Pellets were re-suspended in 100 µl of HTF without BSA and contained 1 µl of Hoechst 33258 prepared in 1000 µl. Subsequently, samples were incubated for 2 min at 25°C and centrifuged for 5 min at 600xg with 4 ml of 2% polyvinylpyrrolidone. After centrifugation, pellets were re-suspended in 45 µl HTF without BSA and mixed with 45 µl of CTC solution. The CTC solution was prepared in 130mM NaCl, 5mM cysteine and 20mM Tris hydrochloride in 50 ml distilled water (pH 7.8). The final solution was kept at 4 °C. Finally, sperm were analysed based on their respective fluorescence distribution. Those with uniform yellow/green fluorescence covering the whole sperm head were counted as non-capacitated, acrosome-intact. Sperm without or dull fluorescence over the whole sperm were counted as capacitated, acrosome-reacted, and sperm with a fluorescence-free band in the post acrosomal region were counted as capacitated, acrosome-intact (Fig. 1C). The relevant percentages were calculated.

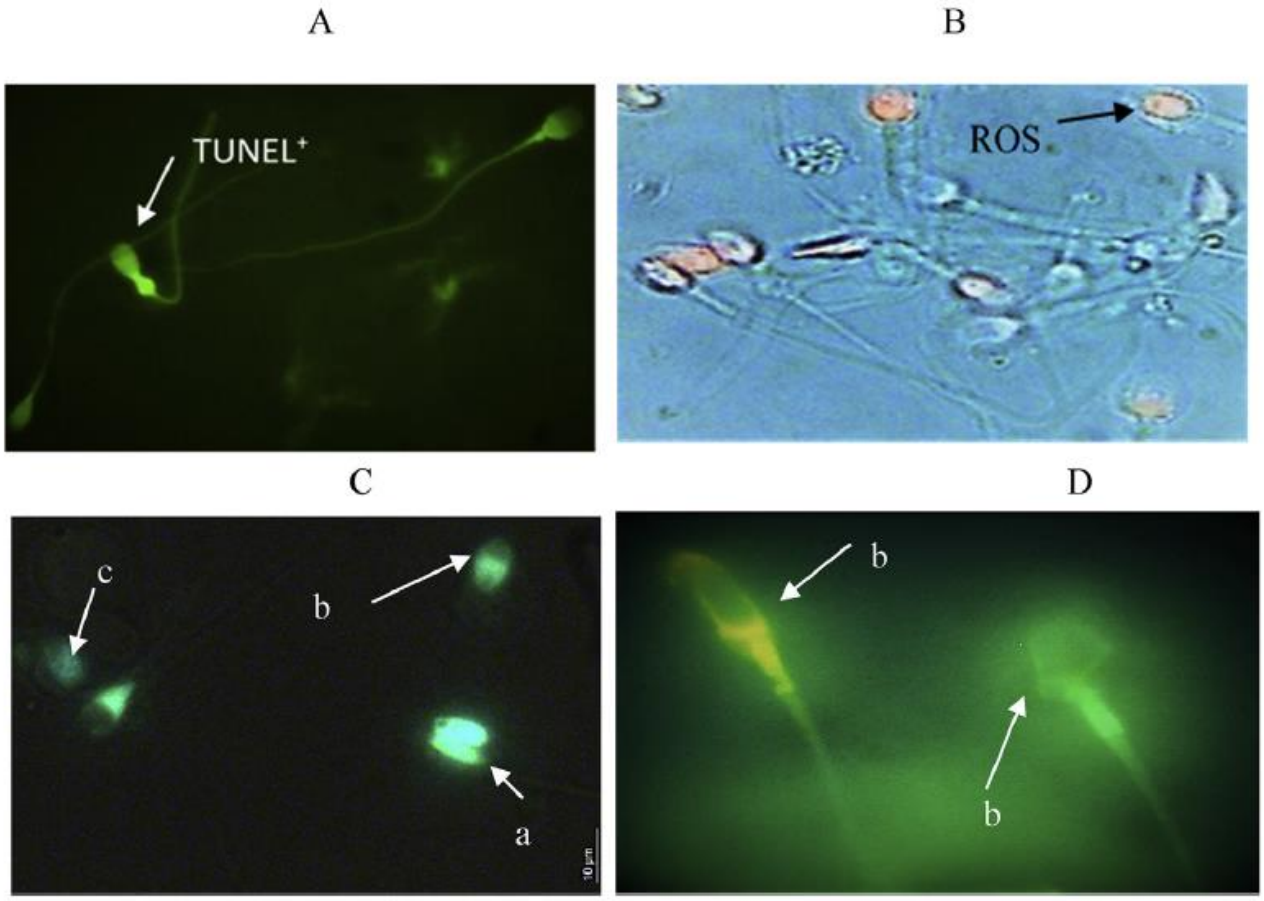

Fig. 1. Sperm with green fluorescence were counted as TUNEL-positive sperm (Fig. 1A). ROS-positive sperm stained with DHE, identified with a red fluorescence of the sperm head (Fig. 1B). Sperm stained with CTC with uniform green fluorescence covering the whole sperm

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head were counted as non-capacitated, acrosome-intact (a), Sperm without or dull fluorescence over the whole sperm head were counted as capacitated, acrosome-reacted (b), and sperm with a fluorescence-free band in the post-acrosomal region were counted as capacitated, acrosome-intact (c, Fig. 1C). Sperm stained with DePsipher, with green fluorescence in the midpiece of the flagellum was counted disrupted MMP (Arrow a), whereas sperm with reddish fluorescence was counted intact MMP (Arrow b, Fig. 1D). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.8. Determination of sperm mitochondrial membrane potential

MMP was measured using a protocol established by Henkel et al. (2012). After experimental incubation of sperm, samples were centrifuged for 5 min at 250xg. Then, pellets were re-suspended in the DePsipher incubation buffer (Trevigen®, Gaithersburg, Maryland, USA) prepared according to the manufacturer's instructions. Reaction buffer (10-X) was diluted to 1-X using distilled water and 20 µl/ml of stabilizer were added to increase the stability of the dye. A final concentration of 5 µg/ml was obtained by adding 1 µl of DePsipher to the prepared 1-X reaction buffer. After re-suspension, sperm were incubated for 20 min at 37 °C and centrifuged for 5 min at 250xg to remove the supernatant. Pellets were then re-suspended in 100 µl of pre-warmed 1-X reaction buffer. Sperm displaying a green fluorescence in the mid-piece of the flagellum were regarded as having disrupted MMP, whereas sperm showing reddish fluorescence were regarded as having intact MMP (Fig. 1D). The percentage of sperm with intact MMP was reported.

Table 1
The effects of different concentrations of the aqueous *C. papaya* seed extract on sperm parameters. Data were analysed by Kruskal-Wallis test. For all variables with the same letter, the difference between the means was not statistically significant. Different letters indicate variables that were significantly different. Data are presented as mean ± SEM.

Sperm Motion Parameters	Control	0.025 µg/ml	0.25 µg/ml	2.5 µg/ml	25 µg/ml	250 µg/ml	2500 µg/ml	Kruskal-Wallis test (P Value)
Total Motility (%)	48.7 ± 1.9	44.9 ± 2.0	42.4 ± 2.3A	42.8 ± 2.3A	43.2 ± 2.1A	42.9 ± 2.1A	39.02 ± 1.9AB	0.027
PR (%)	30.76 ± 1.7	26.87 ± 1.9	25.83 ± 2.1	27.05 ± 1.9	25.67 ± 1.7	25.86 ± 1.7	23.61 ± 1.7A	0.037
VCL (µm/s)	103.6 ± 3.2	102.2 ± 3.7	103.75 ± 3.6	103.66 ± 3.7	102.63 ± 4.6	99.53 ± 3.2A	92.04 ± 2.8AB	0.008
VSL (µm/s)	48.86 ± 2.0	47.43 ± 2.2	47.40 ± 2.4	48.20 ± 2.4	46.04 ± 2.2	44.28 ± 2.3	41.72 ± 1.5	0.145
VAP (µm/s)	60.08 ± 2.0	58.15 ± 2.3	58.76 ± 2.5	59.01 ± 2.4	57.72 ± 2.5	55.45 ± 2.4A	52.25 ± 1.8A	0.021
LIN (%)	47.14 ± 1.1	46.30 ± 1.4	45.42 ± 1.4	46.70 ± 1.1	45.51 ± 1.3	45.01 ± 1.4	46.63 ± 1.4	0.755
STR (%)	76.05 ± 1.1	75.66 ± 1.1	75.34 ± 1.2	76.88 ± 1.1	74.98 ± 1.3	74.85 ± 1.2	76.27 ± 1.3	0.633
BCF (Hz)	22.14 ± 0.4	21.49 ± 0.4	22.13 ± 0.6	21.72 ± 0.4	21.53 ± 0.5	20.83 ± 0.4	20.62 ± 0.5	0.093

PR, Progressive Motility; VCL, Curvilinear Velocity; VSL, Straight Line Velocity; VAP, Average-Path Velocity; LIN, Linearity; Straightness; BCF, Beat Cross Frequency.

2.9. Statistical analysis

All data were analysed using the MedCalc statistical software version 18.2.1 (MedCalc Software bvba, Ostend, Belgium). All data were first checked for normal distribution using the Kolmogorov–Smirnov test. If data were normally distributed, one-way ANOVA and other

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relevant parametric tests were employed. In case data were not normally distributed, the Kruskal-Wallis test and non-parametric tests were applied. Results are reported as mean ± SEM and a P-value of less than 0.05 was regarded as statistically significant.

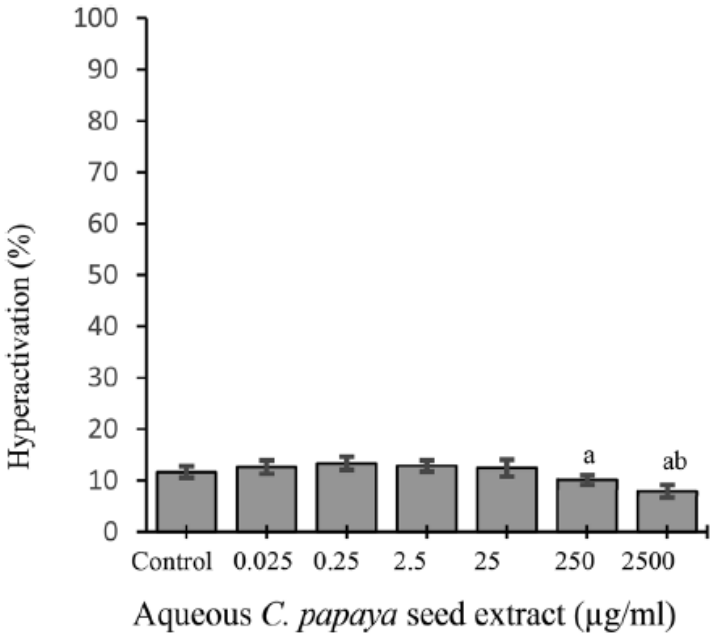

Fig. 2. Effect of aqueous *C. papaya* seed extract on human sperm hyperactivation; higher concentrations of aqueous *C. papaya* seed extract decreased sperm hyperactivation significantly after 1-h of incubation (250 and 2500 µg/ ml, respectively). Data were analysed using the Kruskal-Wallis test and are reported as mean ± SEM. For all variables with the same letter, there is no difference between the means. When two variables have different letters, the difference is significant ($P < 0.05$).

3. Results

3.1. Sperm motility

Summary statistics of the motility parameters are provided in Table 1. Results show that total motility, progressive motility, VCL, and VAP decreased significantly (P=0.04, P=0.03, P=0.008, and P=0.02, respectively). Contrary, values for VSL, LIN, STR, and BCF were not affected by the treatment. Although hyperactivation increased slightly, but not significantly, at low concentrations of the extract, values decreased again at 25 µg/ml and the difference to the control became significant at higher concentrations of the extract (P=0.001; Fig. 2).

3.2. MMP and DNA-fragmentation

While *C. papaya* treatment of sperm caused a dose-dependent decrease in the percentages of MMP-intact sperm ($P < 0.0001$; Fig. 3A), the percentage of DNA-fragmented sperm increased significantly ($P < 0.0001$), reaching double the number of spermatozoa with damaged DNA at the highest concentration of the extract compared to the control (Fig. 3B).

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3.3. Vitality and percentages of ROS-positive sperm

Incubation of sperm for 1 h with the selected concentrations of the aqueous *C. papaya* seed extract resulted in a dose-dependent, significant decrease in sperm vitality (P=0.03; Fig. 4A). Percentage of ROS-positive sperm significantly increased in a dose-dependent manner (P=0.04; Fig. 4B). The increase in the percentage of ROS-positive sperm correlated positively with the increase in the percentage of DNA fragmented sperm (r=0.187; P=0.004) and inversely with the percentage of MMP-intact sperm (r=−0.082; P=0.211).

3.4. Capacitation and acrosome reaction

There was a slight decrease in the percentage of non-capacitated, acrosome-intact sperm, but it was not statistically significant (Table 2).

4. Discussion

To the best of our knowledge, this is the first study investigating the effects of an aqueous *C. papaya* seed extract on human sperm functions. Sperm motility is crucial to fertilize oocytes as in vivo sperm have to swim vigorously in the female reproductive tract to pass through cervix and uterus to reach the ampulla in the fallopian tube (Suarez, 2008). In addition, sperm motility is essential for the penetration of the oocyte vestments. In the fallopian tube, in close vicinity to the oocyte, sperm undergo hyperactivation, a process which is essential for the male germ cells in order to be able to fertilize the eggs. As a result of this process, the flagellar beat changes direction and is increasing force. Hyperactivation and capacitation enable spermatozoa to penetrate the cumulus oophorus and zona pellucida (ZP) (Suarez, 2008).

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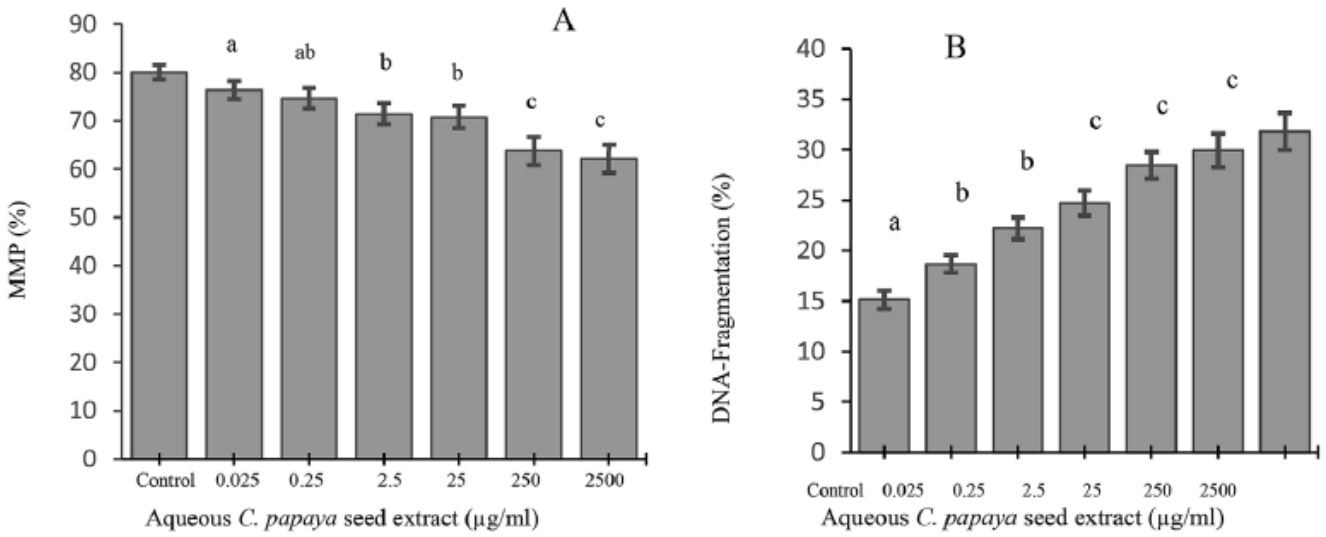

Fig. 3. Effect of aqueous *C. papaya* seed extract on human sperm mitochondrial membrane potential (MMP); MMP (A) and DNA-fragmentation (B) of sperm incubated with aqueous *C. papaya* seed extract is significantly affected in a dose dependent manner. The data were analysed using the Kruskal-Wallis test. For all variables with the same letter, there is no difference between the means. When two variables have different letters, the difference is significant ($P < 0.05$).

Our results show a dose-dependent decrease of motility and hyperactivation after 1 h of exposure to the extract. The physiological pathway of these important sperm functions are regulated via calcium influx, enhanced cyclic adenosine monophosphate (cAMP) and protein kinase A levels, and the phosphorylation of non-essential amino acid tyrosine proteins (O'Flaherty et al., 2006). However, hyperactivation can also occur independently of acrosomal responsiveness to prepare sperm to undergo AR (Rotem et al., 1992). The increase in cAMP levels is triggered by ROS, which suppress tyrosine phosphatase causing tyrosine phosphorylation (O'Flaherty et al., 2006). In the present study, aqueous *C. papaya* seed extract reduced hyperactivation and intracellular ROS-production while AR and capacitation were unaffected.

Mitochondria are organelles that play a critical role in sperm cell activity especially motility by providing energy via ATP. Dysfunction of mitochondria by a break-down of the MMP will have a direct negative effect on sperm motility (Agnihotri et al., 2016). The present study shows that the MMP and motility decreased in a dose-dependent manner during the 1 h incubation of sperm to the aqueous *C. papaya* seed extract. Additionally, another study has shown that sperm parameters such as sperm concentration, motility, and normal morphology were negatively related to DNA-fragmentation, apoptosis, and MMP (Sharbatoghli et al., 2012). Furthermore, a decrease in MMP induced apoptosis through the opening of pores in the mitochondrial membrane, subsequently changing the transmembrane potential by depolarization of the membrane (Ly et al., 2003). This leads to a loss of oxidative phosphorylation as well as the mitochondrial release of several proapoptotic proteins such as cytochrome c into the cytosol. In turn, this triggers the activation of caspase-9 through the formation of the multimeric apoptosome complex and subsequently once caspase-3 is in action, apoptotic cell death is inevitable (Ly et al., 2003).

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Moreover, reduction of the MMP is among the changes encountered during early reversible stages of cell death and is preceded by cytochrome c release in several cell types (Zhang and Chen, 2017). In the present study, the MMP significantly decreased in a dose-dependent manner whereas the percentage of DNA fragmented sperm increased; these findings coincide, with the observed increase in ROS-positive sperm.

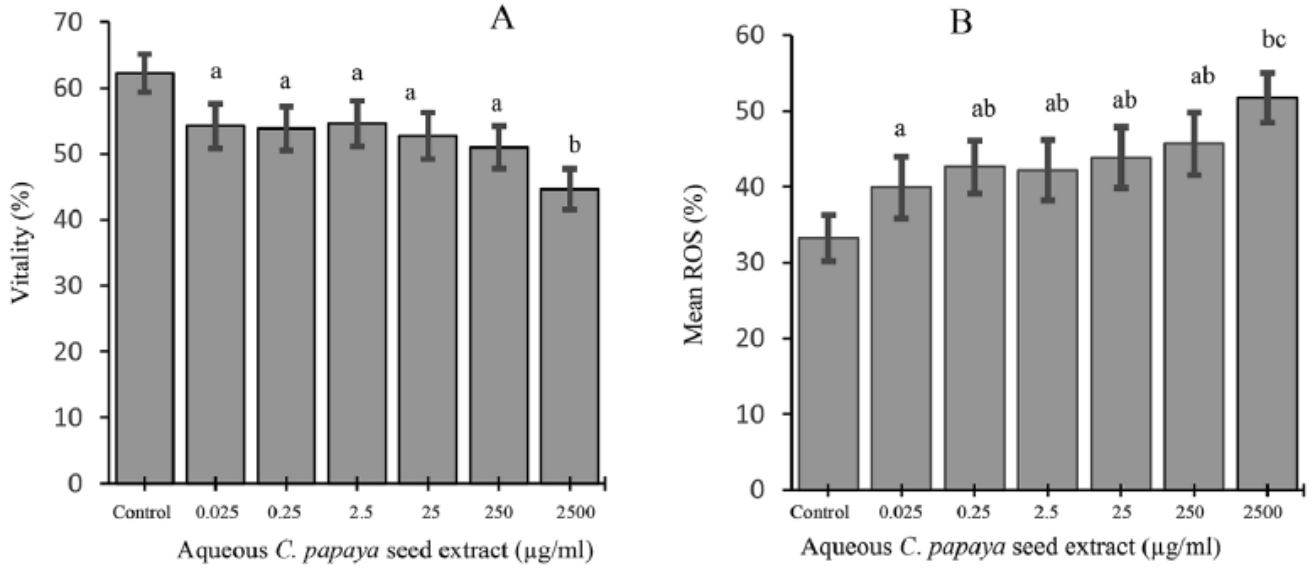

Fig. 4. Effect of aqueous *C. papaya* seed extract on human vitality (A) and sperm ROS production (B). A: Sperm vitality decreased 1-h after exposure to aqueous C. papaya seed extract. Data were analysed using the Kruskal-Wallis test. B: Sperm ROS production increased significantly after incubation with aqueous *C. papaya* seed extract. Data were analysed using ANOVA one way test. For all variables with the same letter, there is no difference between the means. When two variables have different letters, the difference is significant ($P < 0.05$).

Table 2

Effect of aqueous *C. papaya* seed extract on capacitation and AR. After 1 h of incubation in aqueous *C. papaya* seed extract, sperm capacitation and AR show no change when data were analysed by Kruskal-Wallis test. Data presented as mean ± SEM.

Sperm capacitation and acrosome reaction alteration	Control	0.025 µg/ml	0.25 µg/ml	2.5 µg/ml	25 µg/ml	250 µg/ml	2500 µg/ml	Kruskal Wallis test (P Value)
A	4.58 ± 0.95	5.24 ± 1.00	6.74 ± 1.29	7.78 ± 1.75	9.35 ± 2.28	9.20 ± 2.27	8.33 ± 1.98	0.978
B	16.73 ± 1.23	18.36 ± 1.44	17.94 ± 1.46	17.54 ± 1.49	16.80 ± 1.42	17.29 ± 1.47	17.59 ± 1.85	0.987
C	78.68 ± 1.85	76.38 ± 2.22	75.31 ± 2.39	74.66 ± 2.80	73.84 ± 3.20	73.50 ± 2.99	74.07 ± 3.35	0.986

A, non-capacitated, acrosome-intact; B, capacitated, acrosome-intact; C, capacitated, acrosome-reacted sperm.

Steamed distillation extraction of papaya seeds has been shown to represent a rich and highly pure source of the bioactive compound BITC (Zhang and Chen, 2017). Exposure of *Candida albicans* to papaya seed extract led to a significant increase in ROS production and a rapid collapse of the MMP (Zhang and Chen, 2017). A similar effect was seen after exposure

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of human spermatozoa to aqueous papaya seed extract in this present study. Thus, one can assume that BITC is also the bioactive compound leading to the inactivation of the mitochondria respiratory chain with a subsequent increase in sperm ROS-production and its consequences, in this present study.

In addition, another study has also shown that BITC promoted ROS formation, a process which induces cell death via apoptosis in human breast cancer cells (Wu et al., 2011). BITC resulted in increased DNA fragmentation in human melanoma cells (Huang et al., 2012) and has also been shown to increase the level of apoptosis-associated factors such as ROS formation and a breakdown of the MMP. Thus, BITC, together with increasing ROS levels could induce mitochondrial membrane depolarization, which in turn is one of the initial intracellular events of apoptosis (Ly et al., 2003). Furthermore, BITC has been described as a proliferation inhibiting agent as it can cause a cell cycle arrest and/or induce apoptosis in cancer cells (Srivastava and Singh,
2004).

Besides the anti-fertility effects, papaya has been shown to contain many bioactive compounds that can inhibit the level of lipid peroxidation including α-tocopherol, ascorbic acid, beta carotene, flavonoids, vitamin B1, papain and niacin. These compounds have been shown to scavenge 80% of hydroxyl radicals and increase superoxide dismutase activity in the cortex and hippocampus (Mehdipour et al., 2006). Thus, the negative effects of the extract on sperm capacitation and AR might be inhibited during the experimental short-term incubation.

In conclusion, aqueous *C. papaya* seed extract negatively affected sperm motion parameters including motility, hyperactivation, increased sperm ROS production, MMP, and DNA-fragmentation. These effects might be due to the bioactive compound BITC, which is present in high amounts in aqueous *C. papaya* seed extract of ripe papayas.
Whereas, the percentages of capacitated and AR sperm did not change significantly; this is possibly because seeds extract contains different phenolic compounds, vanillic acid, and vitamin C with antioxidant activities in aqueous *C. papaya* seed extract (Panzarini et al., 2014). Currently, the chemical identification of the bioactive compounds is underway as infrared spectroscopy showed the presence of hydroxyl groups (-OH), bonded carbonyl groups (-CHO) and C–H single bonds (Ghaffarilaleh, unpublished).

Conflicts of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at
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