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The effect of *Garcinia livingstonei* aqueous leaf extract on hyperglycaemic-induced human sperm cell: An *in-vitro* study

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ABSTRACT

Hyperglycaemia is the main instigator of the development of male infertility in men with diabetes mellitus (DM). The consequent production of excessive reactive oxidative species (ROS) and a compromised antioxidant defence system leads to testicular damage and a decline in sperm parameters. Supplementation with antioxidants sourced from medicinal plants is beneficial in the treatment of DM-related male infertility. However, a search for more efficacious and easily accessibly medicinal plants is paramount. *Garcinia livingstonei* is a well-known and accessible medicinal plant used in traditional practices for the treatment of diabetic complications. However, very scarce information on the plant extract's potential benefits exists in the literature. The current study investigated the effect of aqueous leaf extract of *G. livingstonei* on hyperglycaemic human male sperm cell parameters. Hyperglycaemia was induced using sperm media supplemented with 100 mM D-glucose over 24 h. Hyperglycaemia induction led to a significant ($P<0.05$) decline in sperm motility, vitality, DNA integrity, mitochondrial membrane potential (MMP), and acrosome integrity. The effect of the plant extract on spermatozoa was concentration-dependent, with the lowest concentration demonstrating the highest protective effects. A significant ($P<0.05$) improvement in all tested sperm parameters was observed after exposure of the hyperglycaemic spermatozoa to aqueous leaf extract of *G. livingstonei.* The effect of acarbose on all tested sperm parameters was comparable to that of the plant extract. The findings of this study suggest the potential therapeutic effect of *G. livingstonei* in the treatment of DM-related male infertility. However, the use of an appropriate dosage depending on the pathological target is paramount.

1. Introduction

Male infertility is defined as the inability of a male of child-bearing age to impregnate a fertile female after 12 months of intended unprotected sexual intercourse (Agarwal et al., 2021). The World Health Organisation (WHO) has reported the prevalence of 15–20% of infertility amongst couples (Chhikara et al., 2023). The prevalence of male infertility has risen to approximately 50% of the total infertility cases (Liu et al., 2023). Amongst other factors such as genetics, trauma, and lifestyle choices (Okonofua et al., 2022), diabetes *mellitus* (DM) is another causative factor associated with the development of male reproductive dysfunction (Barkabi-Zanjani et al., 2020). DM is characterised by prolonged hyperglycaemia and consequent oxidative stress that leads to tissue damage and complications such as cardiovascular disease, kidney disease, and male infertility (Andreadi et al., 2022; Pang et al., 2020; Winiarska et al., 2021). The prevalence of DM amongst the global population aged 20–79 years was estimated to 10.5% (536.6 million people) and is expected to rise to 12.2% by 2045 (Sun et al., 2022). Approximately 35% of DM- affected individuals are reported to be infertile (Zhu et al., 2023). Hyperglycaemia is linked to the decline in sperm parameters such as sperm motility, sperm vitality, mitochondrial membrane potential, and the fragmentation of sperm DNA, which leads to male infertility (Aksu et al., 2021; Papadopoulou et al., 2022). Male infertility is associated with socio-economic issues and depression in men (Njagi et al., 2020; Öztekin et al., 2019). The investigation of effective medication for the treatment of male infertility, with consideration of the causative factor, is necessary.

Hyperglycaemia is the main target in the treatment of diabetic

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complications (Taylor et al., 2021). Prolonged hyperglycaemia leads to excessive production of reactive oxidative species (ROS) through the mitochondria (Kaludercic and Di Lisa, 2020). Although the optimal production of ROS in the reproductive system is required in spermatogenesis and sperm maturation (Baskaran et al., 2021), excessive production of ROS in the testes is detrimental and can lead to male infertility (Takeshima et al., 2021). The antioxidant system maintains the redox status by neutralising the oxidative effect of free radicals, through the synthesis of enzymatic and non-enzymatic antioxidants (Chaudhary et al., 2023). Medicinal plants are a source of antioxidant bioactive compounds (Dar et al., 2023). Traditional medicine has recorded several plants used in the treatment of DM and male infertility (Teixeira et al., 2024). However, the study of the existing medicinal plants on animal sperm cells is limited. *Garcinia* species have been used in traditional medicine for weight loss and to aid in several ailments such as respiratory tract infections, tuberculosis, and other viral infections (Abdul-Rahman et al., 2023).

Garcinia livingstonei T. Anderson is another species of the *Garcinia* genus. It is a tall evergreen plant known in Limpopo for its benefit in the treatment of DM and its underlying complications(Ramadwa and Meddows-Taylor, 2023). In traditional medicine, *G. livinstonei* has been used to treat diseases similarly to other Garcinia extracts. However, the interest in the use of *G. livingstonei* leaves to treat DM complications has advanced and lacks more understanding (Ramadwa and Meddows-Taylor, 2023). The phytochemical analysis of *G. livingstonei* reveals the presence of phenolic compounds such as flavonoids, hydroxycinnamic acids, and benzophenones associated with antioxidant, anti-inflammatory, and hypoglycaemic effects linked to its benefit in the treatment of DM complications (Nethengwe et al., 2024). Although the hypoglycaemic effect of *G. livingstonei* has been reported as evidence for the potential anti-diabetic benefit of the extract, its direct effect on the hyperglycaemic sperm parameters has not been well uncovered. This study established the cellular hyperglycaemia model of the human sperm cell and investigated the effect of the aqueous leaf extract of *G. livingstonei* on sperm parameters of hyperglycaemia-induced human sperm cells.

2. Methods and materials

2.1. Plant harvest and extraction

The leaves of *G. livingstonei* were harvested from the Brackenridgea Nature Reserve at Thengwe village in Limpopo, South Africa. A sample of the plant was harvested and sent to the University of Venda for authentication. The process of authentication was carried out by Prof Tshisikhawe. The plant specimen was kept at the herbarium of the University of Venda, and a voucher was assigned (Voucher number: MNU002/10/22).

The leaves of the *G. livingstonei* were washed and left to dry in a shade for 5 days. The leaves were then hand-crushed before blending using a blender, to obtain a powder form. The powder was weighed to 100 g and mixed with 1000 ml of boiled water to prepare an aqueous extract. The mixture was left overnight (24 h) to allow extraction to occur. The liquid from the mixture was extracted and the plant material was discarded. The extract was filtered using a filter paper after which it was freeze-dried. A total yield of 35.48 g of the extract was stored at -20 °C.

2.2. Participants selection criteria

Ethical clearance for this study was obtained from Cape Peninsula University of Technology Research Ethics Committee (CPUT-REC) (Reference number: CPUT/HWS-REC 2024/H9), and the Biomedical Research Ethics Committee (BMREC) of the University of Western Cape (reference number: BM22/10/24). Male participants (n=25) between the ages of 18 and 40 were recruited, following ethical procedures, to voluntarily join the study. Participants with chronic diseases were excluded from the study. Baseline sperm parameters were assessed as a screening procedure, and all participants who did not meet the World Health Organisation (WHO) criteria were excluded from the study.

2.3. Collection of samples

Semen samples were collected through masturbation after 3–5 days of abstinence and incubated at 37 °C for liquefaction after the participants had agreed to join the study and had signed the consent form. Baseline sperm parameters were then assessed using the Sperm Class Analyser to classify sperm for selection. Semen samples were classified according to the WHO criteria where normozoospermic sperm had sperm concentration>15 million/mil, sperm count>39 million/ejaculate, and sperm motility>40%. After liquefaction, 3 μL of the sample was loaded into a Leja chamber slide, and all baseline parameters were analysed.

2.4. Hyperglycaemia induction

Sperm cells were washed and resuspended with 2–3 ml of sperm wash to achieve the desired concentration and incubated for 3 h for capacitation. A volume of 5 ml of the induction media (0.9 mM CaCl, 0.5 mM MgCl, 10 mM sodium lactate, 1 mM sodium pyruvate, 0.3% w/v bovine serum albumin (BSA), 1% v/v penicillin/streptomycin, 5 mM D-glucose (for optimal concentration), and 100 mM D-glucose (for hyperglycaemic concentration) was added into the cells to a final concentration of 10 mil/ml and incubated for 24 h. Samples in media were centrifuged and washed using a sperm wash before exposure to different treatments (Portela et al., 2015).

2.5. Treatment

The plant extract and acarbose were both prepared in HTF-BSA. To prepare different concentrations of the plant extract, a stock solution (1 mg/ ml) was prepared. A 10-times serial dilution of the stock solution (1 mg/ml) was done to prepare 10 different concentrations that were tested on sperm cells to assess motility. Four concentrations (0.001, 0.01, 0.1, and 1 μg/ml) of the extract were then selected for the study. Acarbose was prepared at a concentration of 100 μg/ml). After 24 h of hyperglycaemia induction, 100 μL aliquots were centrifuged, and the supernatant was discarded. A volume of 100 μL of the different treatments was added, with normal and hyperglycaemic control cells treated with HTF-BSA, and the acarbose group treated with acarbose. Treated samples were incubated at 37 °C for 1 h, then centrifuged for 10 min at 300x g.

2.6. Total motility

Total motility was assessed after induction of hyperglycaemia and exposure to the different concentrations of the aqueous leaf extract of G. *livingstonei*. A volume of 3 μL of each sample was loaded into each chamber of the 8-chamber Leja slide. The slide was analysed under a microscope using computer-assisted semen analysis (CASA), with a total of 200 sperm cells analysed.

2.7. Vitality

Sperm vitality was determined using the eosin-nigrosin dye technique. The sperm sample and eosin-nigrosin dye (Sigma Aldrich, St Louis, MO) were mixed at a 1:1 ratio and incubated at 37 °C for 15 min. The sample mixture was smeared on a slide and allowed to air dry. The slide was viewed under a light microscope (Zeiss, Oberkochen, Germany) using the oil immersion objective (100 ×). At least 200 sperm cells were analysed with white cells appearing white, and dead cells appearing pink (Shalaweh et al., 2015).

2.8. Mitochondrial membrane potential

A mitochondrial staining kit (Sigma-Aldrich Inc., St Louis, MO, USA). was used to assess mitochondrial membrane potential according to the manufacturer's instructions. After treatment, the cell suspension (100 μL) was stained by the MMP staining solution (80 μL of distilled water, 20 μL of JC-5 buffer, and 0.5 μL of frozen MMP 200x stock solution) at a 1:1 ratio and incubated at 37 °C for 20 min in the dark. After incubation, the samples were centrifuged at 500x g and 5–7 °C for 5 min and the supernatant was discarded. The cell suspension was resuspended with 100 μL of cool JC-1 buffer prepared by 80 distilled water and 20 μL JC-5 buffer and centrifuged again at 500x g and 5–7 °C for 5 min. The supernatant was discarded, and the cells were resuspended with more than 100 μL of the JC-1 buffer. A volume of 5–10 μL of the suspension was placed on a frosted slide and covered by a cover slip. The slide was analysed in the dark under a Nikon Eclipse 50i fluorescence microscope (Zeiss, Oberkochen, Germany). Assessment of at least 200 sperm cells was done and a percentage of sperm cells with intact mitochondria was recorded, with green-stained sperm cells considered to have non-intact mitochondria and red-stained sperm cells with intact mitochondria.

2.9. DNA fragmentation

DNA fragmentation was assessed following the protocol of the GoldCyto DNA kit (Goldcyto Biotech Corp, Guangzhou, China). After the exposure of sperm cells to the different treatments, cell suspensions were resuspended with HTF-BSA to a final concentration of 10 mil/ml. A volume of 30 μL of the sample was added into a pre-heated (90 °C -100 °C for 5 min followed by 37 °C for 5 min) agarose Eppendorf. A volume of 20 μl of the sample in the agarose gel was placed on a clean slide and covered with a cover slip. The slide was left in the fridge at 4 °C for 5 min and the cover was gently removed. The slide was incubated horizontally in different solutions (acid denaturation solution for 7 min, lysis solution for 25 min, distilled water for 5 min, 70% ethanol for 2 min, and 90 % ethanol for 2 min) consecutively at room temperature. The slide was left to dry at room temperature and stored in the dark until staining. To stain, the slide was placed in solution A for 1 min, then solution B for 2 min, then washed gently in tap water and left to dry at room temperature. The slide was viewed under a fluorescent microscope (Zeiss, Oberkochen, Germany) with 488 nm and 510–530 nm excitation and emission filters, respectively with an oil immersion objective (400 ×), scoring 200 sperm cells for analysis. Sperm cells with no halo, or with a halo similar or smaller than 1/3 of the minor diameter of the middle part of the sperm head (core) were considered fragmented. Sperm cells with a halo higher than 1/3 of the minor diameter of the core were considered non-fragmented.

2.10. Capacitation and acrosome reaction

The chlortetracycline (CTC) fluorescence assay was conducted to assess acrosome reaction and capacitation. The sperm cells were resuspended in HTF-BSA after treatment, and 1 μL of Hoechst 32,258 was added. The samples were incubated for 2 min in Hoechst 32,258 after which 400 μL of 2 polyvinylpyrrolidone (PVP40) was added, and centrifuged for 5 min at 900x g and 37 °C. After the supernatant was discarded, 45 μL of 750 μM CTC solution (130 mM NaCl, 5 mM cysteine, 20 mM Tris-HCl, pH 7.8) and 8 μL of Tris-HCl solution (12.5% w/v paraformaldehyde, 0.5 M Tris-HCl, pH 7.4) were added into the cell suspension. A volume of 10 μL was placed on a frosted slide, covered with a cover slip, and analysed under a Nikon eclipse 50i fluorescence microscope (Zeiss, Oberkochen, Germany) At least 200 sperm cells were analysed and categorized into three patterns. Non-capacitated, acrosome-intact sperm (F-pattern) were represented by sperm cells with fluorescence over the entire head. Capacitated, acrosome-intact sperm (B-pattern) were represented by sperm cells with fluorescence over the sperm head excluding the post-acrosomal region. Sperm cells with no fluorescence over the head were categorized as capacitated, acrosome-reacted sperm cells (AR pattern) (Shalaweh et al., 2015).

2.11. Reactive oxidative species

Reactive oxidative species were determined through the dihydroethidine (DHE) staining assay following the methods of Shalaweh et al. (2015). DHE stock solution (20 μM) was prepared in PBS. After the treatment of sperm cells, the cell suspension was resuspended with 20 μL of DHE solution and 180 μL of PBS and incubated for 15–20 min at 37 °C. After incubation, 10 μL of the sample was placed on a clean frosted slide, covered with a cover slip, and analysed under a Nikon eclipse 50i fluorescence microscope (Zeiss, Oberkochen, Germany) with oil immersion. A total of 200 sperm cells were analysed, with red-stained sperm cells indicating excessive production of ROS and non-stained sperm cells indicating very little or no ROS production (Shalaweh et al., 2015).

2.12. Superoxide dismutase activity

Superoxide dismutase (SOD) activity in the plasma of the spermatozoa was determined following the methods of Brannan and colleagues, (1981). A 6-hydroxydopamine hydrobromide (6-OHD) solution (50 μl of perchloric acid, 10 ml of distilled water, and 4 mg of 6-OHD) was freshly prepared. Diethylene triamine pentaacetic acid (DETAPAC) was prepared by adding 2 mg of DETAPAC to 50 ml of the 100 mM phosphate buffered saline (PBS) buffer. A volume of 10 μl of each sample and blank was loaded into the wells in triplicates and 15 μl of 6-OHD was added to each well. DETAPAC (170 μl) was added into the wells and the plate was read immediately at 490 nm in an Omega Spectrum plate reader (Thermo Fisher Scientific, Waltham, MA, USA). SOD activity was estimated by calculating the 50% inhibition of auto-oxidation 6-OHD by superoxide free radical.

2.13. Catalase antioxidant activity

Catalase activity in the plasma of sperm cells was measured using the procedure followed by Brannan and colleagues, (1981). An H_2O_2 solution was prepared by adding 50 μl of H_2O_2 to 10 ml of 100 mM phosphate buffered saline (PBS) buffer. The sample and blank were loaded into wells of a 96-well UV Greiner plate. A volume of 170 μl of PBS was added to each well, after which the H_2O_2 reagent (75 μl) was added. The plate was read immediately in an Omega Spectrum plate reader (Thermo Fisher Scientific, Waltham, MA, USA) at 232 nm.

2.14. Protein content determination

A modified version of the protocol followed by Kummari and colleagues (2022) was used to determine total protein content. The Bradford reagent was used to determine protein concentration in the plasma obtained from the spermatozoa. A protein standard dilution series (0.25, 0.5, 1, 1.4, and 2 mg/ml) was prepared 1% SDS and 2 mg/ml stock solution of bovine serum albumin (BSA) protein, as the standard protein. A volume of 5 μl of the blank, standard concentrations and the samples was loaded in the wells of a 96-well plate. The Bradford reagent (250 μl) was then added to the wells. The plate was incubated in the dark for 30 min after a gentle shake. The plate was read using an Omega Spectrum plate reader (Thermo Fisher Scientific, Waltham, MA, USA) at 595 nm (Kummari et al., 2022).

2.15. Statistical analysis

A two-tailed paired T-test (and nonparametric tests) was performed for statistical analysis was performed using GraphPad Prism version 5. The mean points were represented by a column mean with vertical error bars. Letters were placed on top of mean points to denote significance

(CI intervals excluding zero), with different letters in different points representing significant differences between the points. Significance was determined using confidence intervals of mean differences. Confidence intervals that do not include zero represented significant differences between groups. Statistical analysis was done using GraphPad Prism version 5.

3. Results

3.1. Reactive oxidative species production

The antioxidant effect of G. *livingstonei* aqueous leaf extract on hyperglycaemia-induced human spermatozoa is depicted in Fig. 1A and Table 1. A significant (MD: -16.22; CI: -24.10, -8.340) increase in ROS was observed in untreated hyperglycaemic sperm cells compared to control sperm cells. The level of ROS was significantly reduced after the treatment of the sperm cells with 0.001 μg/ml (MD: 27.62; 95% CI: 19.74, 35.50), 0.01 μg/ml (MD: 21.72; 95% CI: 13.84, 29.60), and 0.1 μg/ml (MD: 13.30; 95% CI: 5.420, 21.18) of the extract compared to the untreated hyperglycaemic sperm cells. However, sperm cells treated with the highest concentration (1 μg/ml) of the extract showed no significant (MD: 5.50; 95% CI: -2.380, 13.38) reduction in ROS compared to the untreated hyperglycaemic sperm cells. Treatment of the hyperglycaemic sperm cells with acarbose significantly (MD: 16.38; 95% CI: 8.500, 24.26) reduced the level of ROS compared to the untreated hyperglycaemic cells. The level of ROS in sperm cells treated with acarbose was significantly (MD: -11.22; 95% CI: -19.12, -3.360) higher compared to the level of ROS in sperm cells treated with the lowest concentration of the plant extract.

3.2. Mitochondrial membrane potential

The mitochondrial membrane potential (MMP) of sperm cells before and after induction of hyperglycaemia, and after administration of the aqueous leaf extract of G. *livingstonei*, is depicted in Fig. 1B and Table 1. The number of mitochondria-intact sperm cells was significantly (MD: 10.50; 95% CI: 1.778, 19.22) reduced following hyperglycaemia induction, compared to the control sperm cells. A significant increase in the number of sperm cells with intact mitochondrial was observed after the treatment of the sperm cells with 0.001 (MD: -17.68; 95% CI: -26.40, -8.958), 0.01(MD: -15.96; 95% CI: -24.68, -7.238) and 0.1 μg/ml (MD:

Table 1
Representation of the mean differences (MD) between different treatment groups depicting the effect of hyperglycaemia on the production of reactive oxidative species (ROS) and mitochondrial membrane potential (MMP), and the effect of different concentrations 0.001, 0.01, 0.1, and 1 μg/ml of the plant, and acarbose. Negative MD values represent an increase in the parameter, and 95% confidence intervals that do not include 0 represent a significant difference. NC: normal control; DC: diabetic control, ACA: acarbose.

	ROS-positive		Intact MMP	
Groups	Mean difference	95% CI of difference	Mean difference	95% CI of difference
NC vs DC	-16.22	(-24.10, -8.340)	10.50	(1.778, 19.22)
DC vs Q-0.001	27.62	(19.74, 35.50)	-17.68	(-26.40, -8.958)
DC vs Q-0.01	21.72	(13.84, 29.60)	-15.96	(-24.68, -7.238)
DC vs Q-0.1	13.30	(5.420, 21.18)	-9.480	(-18.20, -0.7575)
DC vs Q-1	5.500	(-2.380, 13.38)	-2.320	(-11.04, 6.402)
DC vs ACA	16.38	(8.500, 24.26)	-10.06	(-18.78, -1.338)
Q-0.001 vs Q-0.01	-5.900	(-13.78, 1.980)	1.720	(-7.002, 10.44)
Q-0.001 vs Q-0.1	-14.32	(-22.20, -6.440)	8.200	(-0.5224, 16.92)
Q-0.001 vs Q-1	-22.12	(-30.00, -14.24)	15.36	(6.638, 24.08)
Q-0.001 vs ACA	-11.24	(-19.12, -3.360)	7.620	(-1.102, 16.34)
Q-0.01 vs Q-0.1	-8.420	(-16.30, -0.5403)	6.480	(-2.242, 15.20)
Q-0.01 vs Q-1	-16.22	(-24.10, -8.340)	13.64	(4.918, 22.36)
Q-0.01 vs ACA	-5.340	(-13.22, 2.540)	5.900	(-2.822, 14.62)
Q-0.1 vs Q-1	-7.800	(-15.68, 0.07972)	7.160	(-1.562, 15.88)
Q-0.1 vs ACA	3.080	(-4.800, 10.96)	-0.5800	(-9.302, 8.142)
Q-1 vs ACA	10.88	(3.000, 18.76)	-7.740	(-16.46, 0.9824)

Negative MD values represent an increase in the parameter, and 95% confidence intervals that do not include 0 represent a significant difference. NC: normal control; DC: diabetic control, ACA: acarbose.

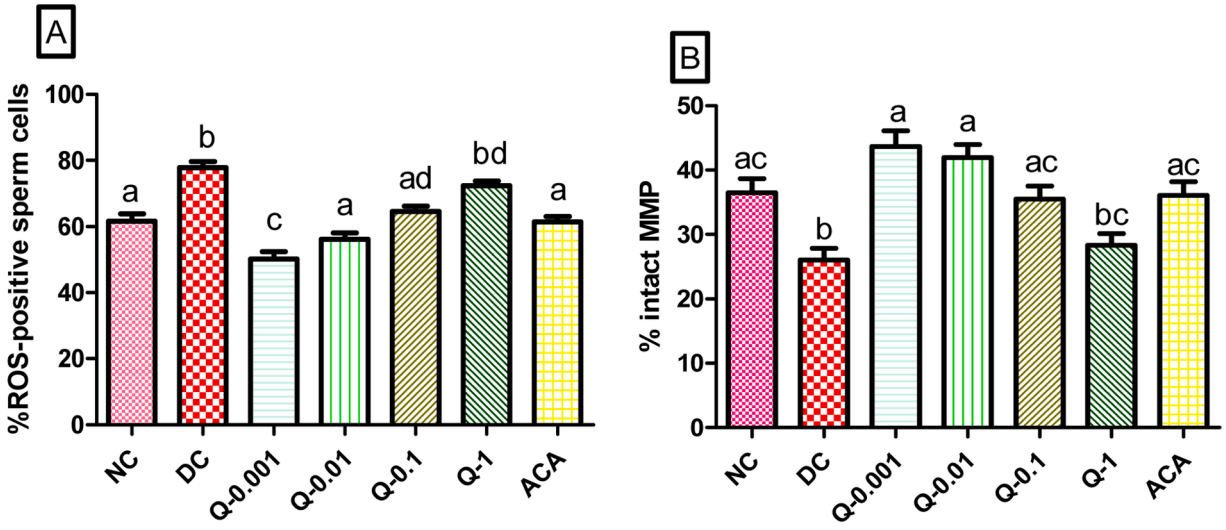

Fig. 1. The effect of 0.001, 0.01, 0.1, and 1 μg/ml concentrations of aqueous leaf extract of *G. livingstonei* on A). reactive oxidative species (ROS) production and B). mitochondrial membrane potential (MMP), in hyperglycaemic sperm cells. Data is represented as ±SEM of 200 sperm cells. Significance is denoted by the letters on each bar, with letters different from each other showing significance (95% confidence interval excluding 0) while same letters show no significance (95% confidence interval including 0). NC: normal control; DC: diabetic control, ACA: acarbose.

-9.48; 95% CI: -18.20, -0.7575) concentrations of the aqueous leaf extract of G. *livingstonei*. The percentage of mitochondrial-intact sperm cells treated with the highest tested concentration (1 μg/ml) of the extract was not significantly (MD: -2.32; 95% CI: -11.04, 6.402) different compared to that of untreated hyperglycaemic sperm cells. Treatment of hyperglycaemic sperm cells with acarbose also caused a significant (MD: -10.06; 95% CI: -18.78, -1.338) increase in the number of sperm cells with intact mitochondria.

3.3. Capacitation and acrosome reaction

The results from the chlortetracycline (CTC) assay are represented in Fig. 2 and Table 2. There was a significant (MD: -26.32; 95% CI: -33.35, -19.29) increase in capacitated and acrosome reacted (AR-pattern) sperm cells in hyperglycaemic sperm cells compared to the normal control cells. The induction of hyperglycaemia resulted in a significant decrease in non-capacitated and contained intact acrosomes (F-pattern) (MD: 16.72; 95% CI: 7.545, 25.94) and capacitated, acrosome-intact (B-pattern) (MD: 9.58; 95% CI: 2.099, 17.06) sperm cells in comparison to the normal control sperm cells. In addition to this, AR pattern sperm cells were dominant in the hyperglycaemic cell group, while most of the sperm cells in the normal control group showed the F-pattern. A concentration-dependent increase in a decrease in AR-pattern sperm cells compared to untreated hyperglycaemic cells was observed post-treatment with 0.001 (MD: 17.12; 95% CI: 10.09, 24.15), 0.01 (MD: 16.10; 95% CI: 9.075, 23.13), 0.1 (MD: 2.12; 95% CI: -4.905, 9.145), and 1 μg/ml (MD: 4.54; 95% CI: -2.485, 11.57) concentration of the aqueous leaf extract of G. *livingstonei*, with the lowest concentration, exhibiting

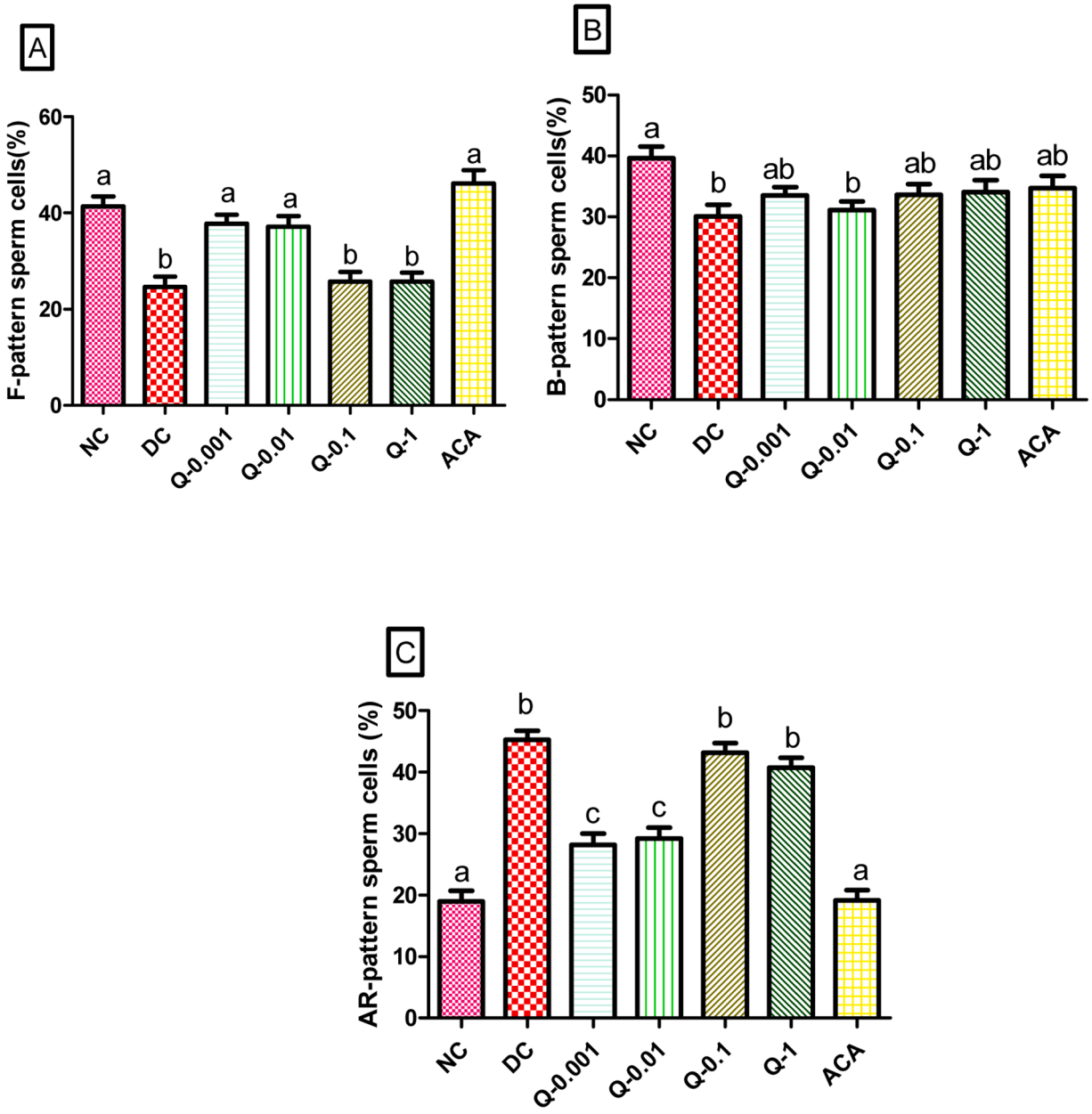

Fig. 2. The effect of 0.001, 0.01, 0.1, and 1 μg/ml concentrations of aqueous leaf extract of G. *livingstonei* on the percentage of (A). non-capacitated with intact acrosomes (F-pattern), (B). capacitated, acrosome-intact (B-pattern), and (C). Capacitated and acrosome reacted (AR-pattern) in hyperglycaemic sperm cells. Data is represented as ±SEM of 200 sperm cells. Significance is denoted by the letters on each point, with letters different from each other showing significance (95% confidence interval excluding 0) while the same letters show no significance (95% confidence interval including 0). NC: normal control; DC: diabetic control, ACA: acarbose.

Table 2
Representation of the mean differences (MD) between different treatment groups depicting the effect of hyperglycaemia on capacitation and acrosome reaction, and the effect of different concentrations 0.001, 0.01, 0.1, and 1 μg/ml of the plant, and acarbose.

	F-pattern		B-pattern		AR-pattern	
Groups	Mean difference	95% CI of difference	Mean difference	95% CI of difference	Mean difference	95% CI of difference
NC vs DC	16.74	(7.545, 25.94)	9.580	(2.099, 17.06)	-26.32	(-33.35, -19.29)
DC vs Q-0.001	-13.12	(-22.32, -3.925)	-3.440	(-10.92, 4.041)	17.12	(10.09, 24.15)
DC vs Q-0.01	-12.54	(-21.74, -3.345)	-1.040	(-8.521, 6.441)	16.10	(9.075, 23.13)
DC vs Q-0.1	-1.080	(-10.28, 8.115)	-3.560	(-11.04, 3.921)	2.120	(-4.905, 9.145)
DC vs Q-1	-1.100	(-10.30, 8.095)	-4.000	(-11.48, 3.481)	4.540	(-2.485, 11.57)
DC vs ACA	-21.48	(-30.68, -12.28)	-4.660	(-12.14, 2.821)	26.14	(19.11, 33.17)
Q-0.001 vs Q-0.01	0.5800	(-8.615, 9.775)	2.400	(-5.081, 9.881)	-1.020	(-8.045, 6.005)
Q-0.001 vs Q-0.1	12.04	(2.845, 21.24)	-0.1200	(-7.601, 7.361)	-15.00	(-22.03, -7.975)
Q-0.001 vs Q-1	12.02	(2.825, 21.22)	-0.5600	(-8.041, 6.921)	-12.58	(-19.61, -5.555)
Q-0.001 vs ACA	-8.360	(-17.56, 0.8352)	-1.220	(-8.701, 6.261)	9.020	(1.995, 16.05)
Q-0.01 vs Q-0.1	11.46	(2.265, 20.66)	-2.520	(-10.00, 4.961)	-13.98	(-21.01, -6.955)
Q-0.001 vs Q-1	11.44	(2.245, 20.64)	-2.960	(-10.44, 4.521)	-11.56	(-18.59, -4.535)
Q-0.001 vs ACA	-8.940	(-18.14, 0.2552)	-3.620	(-11.10, 3.861)	10.04	(3.015, 17.07)
Q-0.1 vs Q-1	-0.02000	(-9.215, 9.175)	0.2494	(-7.921, 7.041)	2.420	(-4.605, 9.445)
Q-0.1 vs ACA	-20.40	(29.60, -11.20)	0.6235	(-8.581, 6.381)	24.02	(16.99, 31.05)
Q-1 vs ACA	-20.38	(-29.58, -11.18)	0.3741	(-8.141, 6.821)	21.60	(14.57, 28.63)

Negative MD values represent an increase in the parameter, and 95% confidence intervals that do not include 0 represent a significant difference. NC: normal control; DC: diabetic control, ACA: acarbose; F-pattern: non-capacitated with intact acrosomes; B-pattern: capacitated, acrosome-intact; AR-pattern: capacitated and acrosome reacted.

more effect. Hyperglycaemic sperm cells treated with ACA showed a significant reduction in the number of AR-pattern (MD: 26.14; 95% CI: 19.11, 33.17) sperm cells and an increase in F-pattern (-21.48; 95% CI: -30.68,-12.28) sperm cells. No significant difference was observed in the number of B-pattern cells after treatment with both the plant extract and ACA as observed in Table 2.

3.4. DNA fragmentation

The percentage of DNA-fragmented sperm cells was recorded to determine the effect of hyperglycaemia on spermatozoa DNA integrity, and the therapeutic effect of the aqueous leaf extract of G. *livingstonei* on hyperglycaemia-induced DNA fragmentation. The results are depicted in Fig. 3A and Table 3. A significantly (MD: -19.42; 95% CI: -29.03, -9.809) higher number of cells with fragmented DNA was observed with hyperglycaemia induction compared to normal control cells. However, a significantly lower number of sperm cells with DNA fragmentation was observed after treatment of the sperm cells with 0.001 (MD: 26.40; 95% CI: 16.79, 36.01), 0.01 (MD: 15.62; 95% CI: 6.009, 25.23), 0.1 (MD: 22.76; 95% CI: 13.15, 32.37), and 1 μg/ml (MD: 11.62; 95% CI: 2.009, 21.23) concentration of the of G. *livingstonei* aqueous leaf extract. Treatment with ACA significantly (MD: 18.64; 95% CI: 9.029, 28.25) reduced DNA fragmentation in hyperglycaemic cells. As observed in Fig. 3A and Table 3, the effect of acarbose on the DNA of hyperglycaemic sperm cells was comparable to the effect caused by the different concentrations of the plant extract.

3.5. Vitality

To assess the viability of sperm cells in different treatment groups, the percentage of live sperm cells in different treatment groups was compared and the results are depicted in Fig. 3B and Table 3. The induction of hyperglycaemia was followed by a significant (MD: 22.10; 95% CI: 13.06, 31.14) reduction in the percentage of live sperm cells

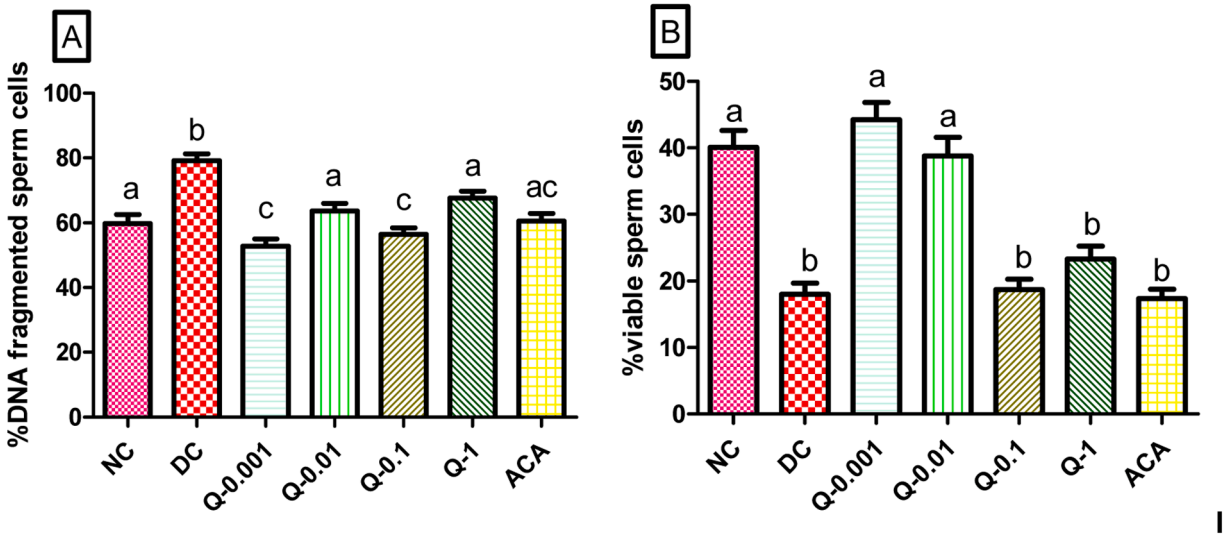

Fig. 3. The effect of 0.001, 0.01, 0.1, and 1 μg/ml concentrations of aqueous leaf extract of *G. livingstonei* on (A). DNA fragmentation and (B). vitality in hyperglycaemic sperm cells. Data is represented as ±SEM of 200 sperm cells. Significance is denoted by the letters on each point, with letters different from each other showing significance (95% confidence interval excluding 0) while the same letters show no significance (95% confidence interval including 0). NC: normal control; DC: diabetic control, ACA: acarbose.

Table 3
Representation of the mean differences (MD) between different treatment groups depicting the effect of hyperglycaemia on sperm cell DNA fragmentation and vitality, and the effect of different concentrations 0.001, 0.01, 0.1, and 1 µg/ml of the plant, and acarbose. Negative MD values represent an increase in the parameter, and 95% confidence intervals that do not include 0 represent a significant difference. NC: normal control; DC: diabetic control, ACA: acarbose.

Groups	%DNA fragmentation		%Vitality	
	Mean difference	95% CI of difference	Mean difference	95% CI of difference
NC vs DC	-19.42	(-29.03, -9.809)	22.10	(13.06, 31.14)
DC vs Q-0.001	26.40	(16.79, 36.01)	-26.24	-35.28 to -17.20
DC vs Q-0.01	15.62	(6.009, 25.23)	-20.80	-29.84 to -11.76
DC vs Q-0.1	22.76	(13.15, 32.37)	-0.7000	-9.738 to 8.338
DC vs Q-1	11.62	(2.009, 21.23)	-5.280	-14.32 to 3.758
DC vs ACA	18.64	(9.029, 28.25)	0.6800	-8.358 to 9.718
Q-0.001 vs Q-0.01	-10.78	(-20.39, -1.169)	5.440	-3.598 to 14.48
Q-0.001 vs Q-0.1	-3.640	(-13.25, 5.971)	25.54	16.50 to 34.58
Q-0.001 vs Q-1	-14.78	(-24.39, -5.169)	20.96	11.92 to 30.00
Q-0.001 vs ACA	-7.760	(-17.37, 1.851)	26.92	17.88 to 35.96
Q-0.01 vs Q-0.1	7.140	(-2.471, 16.75)	20.10	11.06 to 29.14
Q-0.001 vs Q-1	-4.000	(-13.61, 5.611)	15.52	6.482 to 24.56
Q-0.001 vs ACA	3.020	(-6.591, 12.63)	21.48	12.44 to 30.52
Q-0.1 vs Q-1	-11.14	(-20.75, -1.529)	-4.580	-13.62 to 4.458
Q-0.1 vs ACA	-4.120	(-13.73, 5.491)	1.380	-7.658 to 10.42
Q-1 vs ACA	7.020	(-2.591, 16.63)	5.960	-3.078 to 15.00

Negative MD values represent an increase in the parameter, and 95% confidence intervals that do not include 0 represent a significant difference. NC: normal control; DC: diabetic control, ACA: acarbose.

compared to the normal control sperm cells. The effect of the treatment with *G. livingstonei* aqueous leaf extract on the sperm cell viability was concentration-dependant. A significant increase in live cells was observed after treatment with 0.001 µg/ml (MD: -26.24; 95% CI: -35.28,-17.20) and 0.01 µg/ml (MD: -20.80; 95% CI: -29.84, -11.76) concentration of the plant extract compared to the untreated hyperglycaemic sperm cells. There was no significant difference in sperm cell vitality compared to the untreated hyperglycaemic sperm cells after treatment with 0.1 µg/ml (MD: -0.70; 95% CI: -9.738, 8.338) and 1 µg/ml (MD: -5.28; 95% CI; -14.32, 3.758) concentration of the plant extract. Similarly, the treatment of the hyperglycaemic cells with ACA showed no significant (MD: 0.68; 95% CI: -8.358, 9.718) difference in sperm cell vitality compared to the untreated hyperglycaemic sperm cells.

3.6. Motility

The percentage of motile sperm cells was assessed to determine the effect of hyperglycaemia on the motility of sperm cells, and the therapeutic effect of the aqueous leaf extract of G. *livingstonei* on the motility of the sperm cells. The total motility results were recorded and represented in Fig. 4 and Table 4. A significantly (MD: 21.17; 95% CI: 9.457, 32.88) lower percentage motility was observed in untreated hyperglycaemic sperm cells compared to normal control cells. After treatment with the lowest concentration of the plant extract, the total motility of sperm cells was significantly (MD: 15.32; 95% CI: -26.76, -3.886)

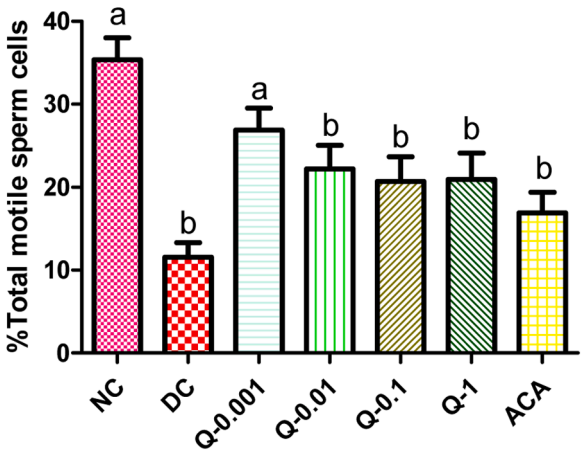

Fig. 4. The effect of 0.001, 0.01, 0.1, and 1 µg/ml concentrations of aqueous leaf extract of G. *livingstonei* on motility of hyperglycaemic sperm cells. Data is represented as ±SEM of 200 sperm cells. Significance is denoted by the letters on each point, with letters different from each other showing significance (95% confidence interval excluding 0) while the same letters show no significance (95% confidence interval including 0). NC: normal control; DC: diabetic control, ACA: acarbose.

Table 4
Representation of the mean differences (MD) between different treatment groups depicting the effect of hyperglycaemia on sperm cell motility and the effect of different concentrations of 0.001, 0.01, 0.1, and 1 µg/ml of the plant, and acarbose. Negative MD values represent an increase in the parameter, and 95% confidence intervals that do not include 0 represent a significant difference. NC: normal control; DC: diabetic control, ACA: acarbose.

Groups	Mean difference	95% CI of difference
NC vs DC	23.79	(12.35, 35.23)
DC vs Q-0.001	-15.32	(-26.76, -3.886)
DC vs Q-0.01	-10.63	(-22.07, 0.8107)
DC vs Q-0.1	-9.129	(-20.57, 2.310)
DC vs Q-1	-9.395	(-20.83, 2.044)
DC vs ACA	-5.350	(-16.79, 6.089)
Q-0.001 vs Q-0.01	4.697	(-6.742, 16.14)
Q-0.001 vs Q-0.1	6.196	(-5.243, 17.63)
Q-0.001 vs Q-1	5.930	(-5.509, 17.37)
Q-0.001 vs ACA	9.975	(-1.464, 21.41)
Q-0.01 vs Q-0.1	1.499	(-9.940, 12.94)
Q-0.001 vs Q-1	1.233	(-10.21, 12.67)
Q-0.001 vs ACA	5.278	(-6.160, 16.72)
Q-0.1 vs Q-1	-0.2664	(-11.71, 11.17)
Q-0.1 vs ACA	3.779	(-7.660, 15.22)
Q-1 vs ACA	4.046	(-7.393, 15.48)

Negative MD values represent an increase in the parameter, and 95% confidence intervals that do not include 0 represent a significant difference. NC: normal control; DC: diabetic control, ACA: acarbose.

increased compared to the untreated hyperglycaemic cells. However, no significant difference in total motility was observed after treatment with 0.01 µg/ml (MD: -10.63; 95% CI: -22.07, 0.8107), 0.1 µg/ml (MD: -9.129; 95% CI: -20.57, 2.310), 1 µg/ml (MD: -9.395; 95% CI: -20.83, 2.044) concentrations of the plant extract and acarbose (MD: -5.350; 95% CI: -16.79, 6.089) caused no significant difference in total motility compared to the untreated hyperglycaemic sperm cells.

3.7. Total protein content and antioxidant enzyme activity

The effect of the aqueous leaf extract of *G. livingstonei* on antioxidant enzymes and total protein content in the plasma obtained from the sperm cells was determined, and the results are demonstrated in Fig. 5. There was no significant change in protein content of the plasma after the induction of hyperglycaemia. There was no significant difference in

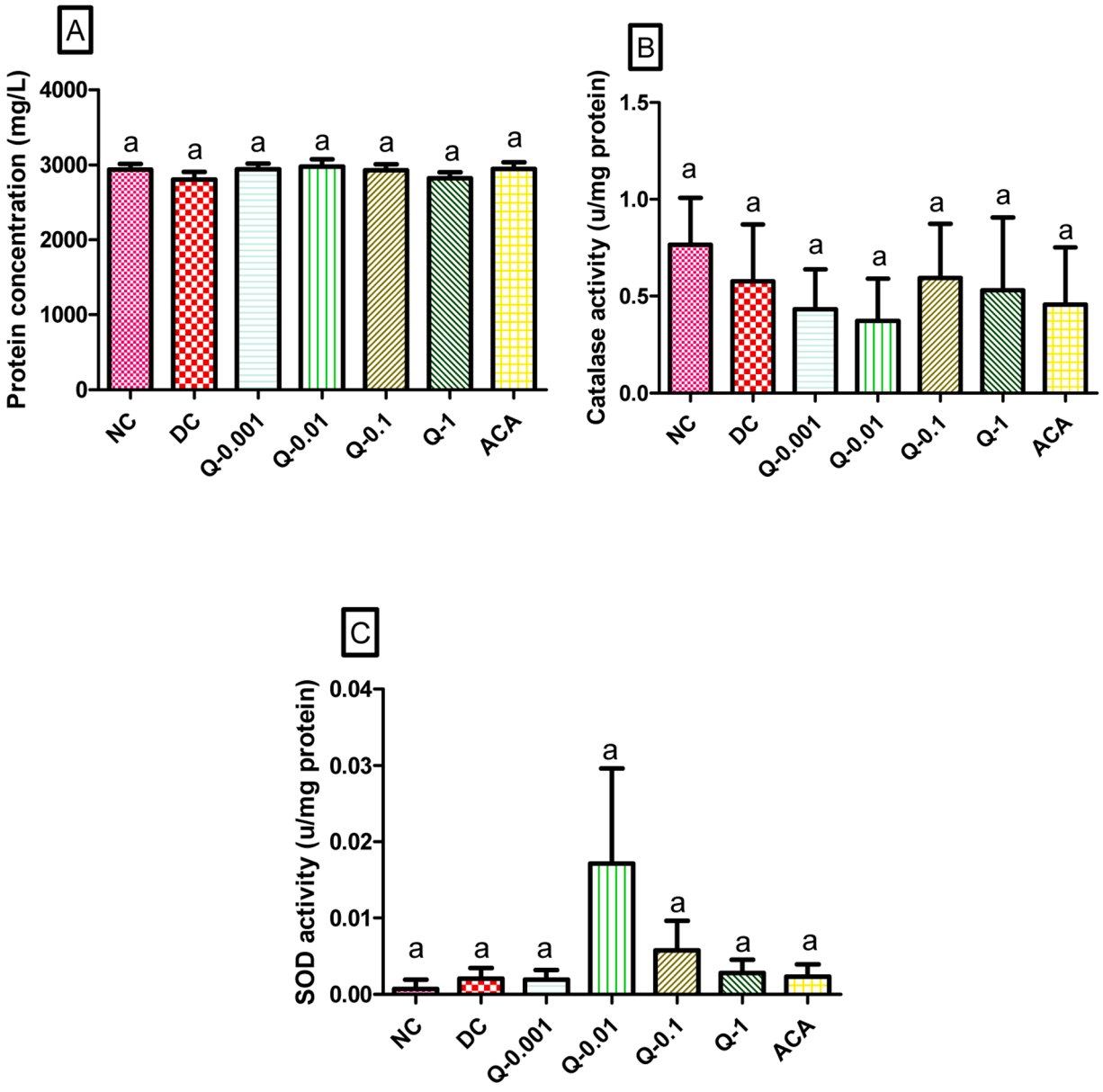

Fig. 5. The effect of 0.001, 0.01, 0.1, and 1 μg/ml concentrations of aqueous leaf extract of G. *livingstonei* on A. total protein concentration, B. catalase activity, and C. SOD activity in the plasma of hyperglycaemic sperm cells. Data is represented as ±SEM. Significance is denoted by the letters on each point, with letters different from each other showing significance (95% confidence interval excluding 0) while the same letters show no significance (95% confidence interval including 0). NC: normal control; DC: diabetic control, ACA: acarbose, SOD: superoxide dismutase.

both catalase and SOD activity after exposure of the cells to high glucose conditions. There was no significant effect exhibited on the total protein content, catalase, and SOD activity in the plasma of hyperglycaemic cells, after treatment with the plant extract. Also, no significant change was observed after treatment with acarbose.

4. Discussion

Prolonged hyperglycaemia causes excessive production of ROS in the mitochondria and auto-oxidation of glucose molecules (González et al., 2023). The abnormal increase in the production of free radicals compromises the antioxidant system and leads to oxidative stress (González et al., 2023). Hyperglycaemia leads to the upregulation of a protein (Calpain-1), which reduces the activity of ATP synthase and leads to an increase in ROS (Kaludercic and Di Lisa, 2020). Free radicals accumulate in the semen due to the reaction of glucose with proteins, and the formation of AGEs (Tian et al., 2020). More than 60% of sperm cells under hyperglycaemic conditions have been found to express RAGEs and consequently contain excessive ROS (Tian et al., 2020). This present study corroborates the previous findings, as the induction of hyperglycaemia led to the excessive production of ROS compared to the normal control sperm cells.

The production of ROS in normal control cells reveals the normal production of ROS for sperm production and maturation, and the vulnerability of sperm cells to oxidative stress. The oxidation of the sperm cell lipid membrane leads to the damage and death of sperm cells via autophagy (Tian et al., 2020). In addition to this, oxidative stress leads to the formation of pores in the mitochondrial membrane and a decrease in mitochondrial membrane potential (Kaludercic and Di Lisa, 2020). The reduction in ATP in the sperm cells can lead to a reduction in sperm cell motility and apoptosis. Amongst other complications of the male reproductive function that lead to infertility, sperm cell death leads to the reduction of the chance of fertilisation (Chianese and Pierantoni, 2021). It is evident from the present study that hyperglycaemia led to a

reduction in the number of viable cells. The reduction in viability of hyperglycaemic cells compared to normal control cells can be connected to the excessive production of ROS which leads to autophagy and apoptosis of sperm cells (Sadeghi et al., 2020).

It can be deduced from the reported disruptive effect of ROS on sperm cells, that the amelioration of oxidative stress in the male reproductive system could be a therapeutic target in the treatment of hyperglycaemic-related male reproductive dysfunction. *G. livingstonei* has been previously reported for its antioxidant potential associated with its high polyphenol content, and its high antioxidant capacity (Nossier et al., 2023). In our previous study, an HPLC-MS analysis showed the abundant presence of antioxidants such as hydroxycinnamic acids, benzophenones, and flavonoids which led to a high antioxidant capacity in the aqueous leaf extract of *G. livingstonei* (Nethengwe et al., 2024). The treatment of hyperglycaemic sperm cells with the aqueous leaf extract of *G. livingstonei* showed a concentration-dependent oxidative stress-reducing effect. Polyphenols in medicinal plants reduce oxidative stress by neutralising and deactivation of the action of ROS. The presence of polyphenolic compounds in *G. livingstonei* could be potentially responsible for the reduction of ROS observed in this study model. However, the highest antioxidant effect of G. *livingstonei* was observed at the lowest concentration(0.001 μg/ml) of the extract. The administration of a toxic dosage of the extract possibly led to damage to the mitochondrial membrane, resulting in more production of ROS. Although the plant extract showed high antioxidant capacity as demonstrated by the significant reduction in ROS, the activity of antioxidant enzymes remained the same amongst the different study groups. The increase in the production of ROS is therefore linked to the pathways leading to the production of ROS rather than the compromised antioxidant system. It can be deduced that the antioxidant activity of the plant extract is inclined to the neutralisation of ROS than the activation of antioxidant enzymes.

The use of ACA as a standard drug is associated with its capacity to reduce ROS and increase antioxidant capacity (Arya et al., 2023). This is well depicted in this present study, as the administration of ACA on hyperglycaemic sperm cells led to the reduction of ROS production compared to the untreated hyperglycaemic cell. Although ACA exhibits an antioxidant effect, sperm cells treated with ACA showed low cell viability with a non-significant difference compared to the non-treated hyperglycaemic cells. This suggests that the death of sperm cells in hyperglycaemic conditions are not entirely caused by oxidative stress. Previous studies have reported relevant factors that could possibly lead to sperm cell death in hyperglycaemic conditions such as AGEs reaction with the sperm cells. Although the toxicity of ACA on sperm cells is not well-documented. A similar hypoglycaemic drug, metformin, has been recorded for its toxicity on sperm cells leading to reduced viability and this effect could be exhibited by ACA (Alzain et al., 2020). Following the treatment of sperm cells with different concentrations of the aqueous leaf extract of G. *livingstonei*, sperm cell viability was increased in the lower concentrations of the extract. These findings are in correlation with the reduced ROS production in the lower-concentration extract group and show that the increase in ROS can consequently lead to a reduction in cell viability. The toxicity of G. *livingstonei* following a relative increase in dosage depending on cell type has not been concealed, hence the lower viability of sperm cells at higher concentrations (Nethengwe et al., 2024).

Sperm motility is a vital parameter of the male reproductive system in fertility. Fertilisation is achieved when sperm cells are progressively motile towards the egg cell. A drastic reduction in sperm motility reduces the chance of fertility. In the present study, prolonged hyperglycaemia led to the reduction of sperm cell motility as compared to the normal control sperm cells. These findings support the findings from previous studies that hyperglycaemia leads to reduced motility and compromises male reproductive function. Human sperm cells rely solely on mitochondrial oxidative phosphorylation for the production of ATP as fuel for sperm motility (Barbagallo et al., 2021). In normal glycaemic conditions, the sperm mitochondria utilise glucose to produce ATP for the normal motility of sperm cells. During glucose metabolism, a low number of free radicals are produced in aid of sperm maturation. However, in hyperglycaemic conditions, excessive glucose molecules undergo oxidative phosphorylation which overloads the mitochondria and produces excessive ROS (Barbagallo et al., 2021). Mitochondrial damage caused by hyperglycaemia leads to less ATP being produced, and the reduction of percentage sperm motility. Similarly, the present study shows the reduction of MMP in hyperglycaemic sperm cells and a resultant decrease in sperm motility. As observed initially in this study, the reduction in MMP of the sperm cells can be associated with the hyperglycaemia-induced oxidative stress.

Addition of G. *livingstonei* leaf extract to the sperm cells showed a concentration-dependent effect on the sperm motility. Lower concentration of the plant extract yielded a higher percentage of sperm motility. The possible toxicity of the plant extract at higher concentrations led to the interruption of sperm cell motility. The reduction of ROS by the extract led to an increase in MMP in comparison to the non-treated hyperglycaemic cells, which increased ATP and sperm motility.

Capacitation and acrosome reaction are pre-requisites for achieving fertilisation of the oocyte (Chhikara et al., 2023). Capacitation of the sperm cells begins in the female reproductive tract after ejaculation (*in vivo*). In an in vitro setting, different inducing factors such as calcium ionophore are used to initiate capacitation and acrosome reaction (De Villiers et al., 2022). However, slow and less capacitation occurs spontaneously due to the growth media, and incorrect handling of the sample. Although capacitation and acrosome reaction are indicators of possible fertilisation, premature capacitation, and acrosome reaction are both worrying phenomena and can lead to reduced fertility. In the present study, no inducing factors were used, hence the sperm cells suitable for fertilisation were expected to have less capacitation and contain intact acrosomes. Due to spontaneous capacitation and possible contamination in sample handling, a few cells in the normal control group were capacitated and contained reacted acrosomes. However, the comparison between normal control sperm cells and the hyperglycaemic sperm cells showed increased capacitation and a decrease in acrosome integrity in hyperglycaemic sperm cells. Although a minimum amount of ROS is required for cell capacitation and the initiation of acrosome reaction, fluctuation of the production of ROS in the sperm cells can interfere with these processes (Sanocka and Kurpisz, 2004).

Lipid peroxidation of sperm cell membranes instigated by oxidative stress interferes and blocks chemical and biophysical changes that initiate appropriate capacitation and acrosome reaction in conditions suitable for these fusion processes (Benko et al., 2022). In a previous study that investigated the effect of oxidative stress in cryopreserved sperm cells, the presence of ROS was associated with premature capacitation and acrosome reaction (Benko et al., 2022). Similarly, this present study shows premature capacitation and acrosome reaction in hyperglycaemic sperm cells, possibly caused by the excessive production of ROS. The increase in non-capacitated and acrosome-intact sperm cells after the administration of lower concentrations of the aqueous leaf extract of *G. livingstonei* on hyperglycaemic sperm cells reveals the antioxidant effect of the extract and further suggests the therapeutic effect on fertilisation fusion processes, associated with the reduction of ROS. The disruption of the sperm cell membranes and interference with the capacitation process reduce sperm motility (Balbach et al., 2023). The correlation between sperm motility and acrosome integrity is apparent in this study as sperm cells with reduced acrosome integrity showed reduced sperm motility.

The information obtained from sperm analysis parameters such as motility, concentration, and morphology is limited to conclude the fertility status (Liu et al., 2023). Sperm DNA fragmentation plays a huge role in the production, maturation, and transfer of genetic material to the egg cell, hence its in-found importance in reproduction science research (Liu et al., 2023). DNA fragmentation is common in fewer sperm cells during maturation and movement through the urethra and

vaginal lining (Nielsen et al., 2023). However, increased sperm DNA fragmentation reduces sperm motility and is detrimental to male fertility (Qi et al., 2024). Oxidative stress is an instigator of sperm DNA fragmentation (Nielsen et al., 2023). The increase in sperm DNA fragmentation of hyperglycaemic sperm cells in this study reveals the contribution of high glucose levels to disrupted fertility. Hyperglycaemic-induced production of ROS is implicated in the fragmentation of sperm DNA in this study. A negative correlation between sperm DNA fragmentation and total sperm motility was observed in this study, similar to a previous study (Qi et al., 2024). The neutralisation of free radicals in hyperglycaemic sperm cells was expected to reduce sperm DNA fragmentation. The antioxidant effect of *G. livingstonei* leaf extract was expressed, and a resultant concentration-dependent reduction in sperm DNA fragmentation was observed after treatment with the extract. The lower concentrations of the extract showed maximum antioxidant activity, hence the lower ROS and reduced sperm DNA fragmentation. The reduction of sperm DNA fragmentation in higher concentrations of the extract regardless of high ROS production suggests the alternative protective effect of the extract against sperm DNA fragmentation.

5. Conclusion and limitations

The underlying pathology of male reproductive dysfunction in DM is associated with a decline in multiple interconnected sperm parameters. Therefore, the assessment of the underlying molecular pathways that are associated with male infertility is paramount to finding a suitable treatment and dosage. For instance, this study shows that at lower concentration, the extract significantly reduced sperm DNA fragmentation compared to the higher concentration of the extract, indicating the necessity of appropriate dosage of medicinal plant extracts in the treatment of any disease. The appropriate use of *G. livingstonei* aqueous leaf extract holds therapeutic potential in the treatment of male reproductive complications related to hyperglycaemia and oxidative stress. However, this study model is an *in vitro* study and does not completely mimic diabetes mellitus and the male reproductive system. This study aimed at investigating the effect of the plant extract independent of any standard drug, and due to ethical concerns, the study could not include untreated diabetic individuals as a control. With these findings, future studies can focus on the coupled effect of *G. livingstonei* aqueous leaf extract and a standard drug on diabetic individuals *in-vitro* or *in vivo*.

Research data

Data will be made available on reasonable request.

CRediT authorship contribution statement

Murendeni Nethengwe: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Data curation, Conceptualization. **Kunle Okaiyeto:** Writing – review & editing, Validation, Supervision, Conceptualization. **Chinyerum S. Opuwari:** Writing – review & editing, Validation, Supervision, Conceptualization. **Oluwafemi O. Oguntibeju:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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