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Molecular approaches based on investigating the therapeutic benefits of *Moringa oleifera*: Insights into biochemical and spermatological and metabolites studies

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ABSTRACT

This study aimed to investigate the potential of Moringa oleifera extract, an herbal treatment known to support male reproductive function, in improving sperm motility. Adult male guinea pigs were divided into four groups (n = 5 per group). Group 1 served as the control, while Group 2 was induced with subfertility using Carbendazim. Group 3 consisted of subfertile guinea pigs treated with *Moringa oleifera* extract, and Group 4 included subfertile guinea pigs treated with clomiphene citrate. Sperm motility parameters, including sperm counts (sperm/ml), rapid and progressive motility (sperm/ml), and sperm agglutination (%), were assessed using standard methods. In control group, guinea pigs exhibited significantly higher sperm counts (44.0 ± 0.89 x 10^6 sperm/ml) and sperm motility (57.6 ± 1.45 x 10^6 sperm/ml, rapid, progressive) compared to the Carbendazim-induced subfertile group ($p < 0.05$). Conversely, the subfertile group displayed significantly higher sperm agglutination (30 ± 1.26%) than the control group ($p < 0.05$). Treatment with *Moringa oleifera* L extract and clomiphene citrate resulted in improved sperm motility parameters, with both groups showing higher sperm counts and rapid, progressive motility, and lower sperm agglutination compared to the sub-fertile group. These findings suggest that Moringa oleifera extract may enhance sperm motility in male guinea pigs with carbendazim-induced subfertility, positioning herbal remedies as potential alternatives for treating male infertility. Furthermore, the study highlights the potential of Moringa oleifera as a therapeutic agent for male infertility by demonstrating its effectiveness in improving sperm motility and reducing sperm agglutination. These results underscore the importance of exploring herbal remedies as safer, natural alternatives to conventional treatments for addressing the subfertility issue. Further research is needed to discover the underlying molecular mechanisms and assess the clinical significance of these outcomes in the context of human male fertility.

1. Introduction

Male infertility (Mi) is a major generative health concern affecting couples worldwide (Chavda et al., 2024). In recent years, there has been a growing interest in alternative therapies for treating male infertility (Fasanghari et al., 2024). Male infertility involves isolating the root causes of modifications that may contribute to reduced spermatogenesis, quality, and reproductive function. Herbal production plays a role in cultivating overall health, repairing vitality, and promoting reproductive functions (Rathi et al., 2024; Al Khaldi and Al-khaldi, 2023) Herbs have gained prominence for their potential effects on male infertility and impact spermatogenesis, sperm motility, morphology and hormonal balance. Scientific statistics include *Ashwagandha (Withania somnifera), Shilajit (Asphaltum), Safed Musli (Chlorophytum borivilianum), and Gokshura (Tribulus terrestris)*. Herbal formulations should carefully consider the characteristics of the male reproductive system. (Singh et al., 2001; Sultana et al., 2022).

Current research suggests that bioactive complexes of herbs, such as alkaloids, flavonoids, and saponins, exert therapeutic effects on male reproductive factors (Krishnaiah, 2016 & Krishnaiah et al., 2011; Suresh et al., 2014, 2020). Clinical studies on the efficacy, safety, and mechanism of action of these herbal remedies have focused on male

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reproductive health. In herbal formulation practices, standardization and quality control measures are paramount to ensure consistent and reliable outcomes (Wang et al., 2023). Antioxidants, micro-and macro-nutrients, and essential fatty acids are important for spermatogenesis, sperm motility, and overall generative function. Diet nutrient deficiencies, oxidative stress (OS), hormonal disturbances (HD), male reproductive health (MRH). Antioxidants, found in fruits, vegetables, and whole grains, help counteract oxidative stress and protect sperm DNA from damage (Agarwal et al., 2016). OMF3acids, present in fish and flaxseeds, are known to affect sperm membrane integrity and motility (Attaman et al., 2012). In elements, and micro-nutrients influence testosterone levels for sperm production (Pilz et al., 2016). High intake of trans fats and saturated fats can lead to inflammation and oxidative stress, potentially affecting sperm (Afeiche et al., 2013). High diabetes can contribute to insulin resistance and hormonal imbalances and disturb reproductive hormone levels (Coleman-Jensen et al., 2015). Obesity is associated with impaired sperm production, decreased sperm quality, and increased oxidative stress (Sermondade et al., 2013).*Moringa oleifera,* a plant commonly known as drumstick tree or horseradish tree, has gained attention for its potential beneficial effects on various aspects of spermatological *Moringa oleifera* traditional use in different cultures aspect on medicinal properties (Shahid et al., 2020). Different parts of the plant, including leaves, seeds, and roots, have been utilised for their reputed health benefits. It is recognised for its nutritional richness and is a source of vitamins, minerals, and antioxidants. Recent scientific investigations have revealed the diverse bioactive compounds present in *M. oleifera* that contribute to its potential impact on male reproductive health. Studies have identified the presence of flavonoids, polyphenols, vitamins, and essential minerals in *M. oleifera* extracts (Sharifi-Rad et al., 2018). These compounds possess antioxidant and anti-inflammatory properties, which are believed to play crucial roles in protecting sperm cells from oxidative stress and preserving their viability and functionality (Muhammad et al., 2020).

Some studies suggest that *M. oleifera* extracts may contribute to improved sperm parameters, possibly through mechanisms involving hormonal balance, antioxidant defense, and mitochondrial function. These findings have spurred interest in evaluating the potential of M. oleifera as a natural intervention for men facing fertility challenges (Sulaiman et al., 2016; Ali et al., 2021). Inflammation and oxidative stress contribute to male infertility by compromising sperm function and integrity. *M. oleifera's* anti-inflammatory and antioxidant properties have garnered attention as potential factors in promoting healthy sperm function (Chaudhary et al., 2017). Inflammation and scavenging of harmful free radicals may help to create a more favorable environment for sperm development and maturation (Prabsattroo et al., 2015; Olaoye et al., 2021). In the current study, we attempted spermatological enhancements brought about by the utilization of *M. oleifera* leaf and flower extracts in male guinea pigs induced with subfertility.

2. Materials and methods

2.1. Plant collection and preparation of extract

M. oleifera were collected from the Erode (Dt) in Tamil Nadu. The collected plant parts were thoroughly washed with disH_2O water to remove impurities. Once dried, the plant parts were powdered using an electric blender to obtain a coarse powder. The powdered material was soaked in distilled water at a 1:1 ratio, which was selected based on prior research indicating its effective solubility and extraction efficiency. This mixture was mechanically shaken every 2 h at room temperature to ensure thorough extraction and prevent settling of the plant material. Finally, the prepared extract was stored in a refrigerator until further use.

2.2. Guinea pig selection and housing

Healthy adult male guinea pigs weighing 300–400 gm were selected for this investigation. The ethical committee approval number was JKKNCP/CERT/0126T22. This approval ensured that the study was conducted in accordance with the ethical guidelines and considerations for the welfare of the research subjects.

2.3. Investigational and group

Experimental Design and Grouping: The group size (n = 5 per group) was determined based on power analysis to ensure statistical significance. This approach was chosen to balance ethical considerations in the use of animals while maintaining robust and reliable results.

Animal Selection and Experimental Design: To minimise variability and ensure consistency, all guinea pigs included in the study were of similar age (3–4 months) and weight range (300–400 g). These criteria were chosen to control for age- and weight-related influences on the study outcomes.

Group 1: Control group - Animals in this group were fed distilled water orally for 20 days along with regular food, without any additional treatment.
Group 2: Carbendazim-treated group - Animals in this group received a single bolus dose of 50 mg/kg bavistin orally.
Group 3: Carbendazim-induced subfertile guinea pigs treated with *M. oleifera* extract - Animals in this group were administered the flower and leaf extracts of *M. oleifera* orally at a dose of 150 mL per body weight, divided into two doses per day.
Group 4: Carbendazim-induced subfertile guinea pigs treated with clomiphene citrate - Animals in this group were treated with the reference drug clomiphene citrate at a dose of 0.03 mg per body weight, divided into two doses per day. All groups were maintained for 20 days with ad libitum access to food and water throughout the study period.

2.4. Weight measurement

The initial weights were measured before initiating any treatments, and the final weights were measured at the end of the 20-day period. Weight measurements were performed using a reliable weighing scale, and any changes in weight were noted for further analysis.

2.5. Blood collection and separation of blood serum

Blood samples were collected by venipuncture from control and guinea pigs. Collect an appropriate amount of blood (usually around 0.5–1 mL) in a syringe for blood collection tube containing an anticoagulant, such as EDTA.

2.6. Centrifugation and separation of serum

After blood collection, the sample was allowed to clot by leaving it undisturbed at room temperature for approximately 30 min. Once clotted, the sample was centrifuged at a suitable speed and duration to separate the serum from the clot and blood cells. at a centrifugation speed of approximately 1000-2000$\times g$ for 10–15 min is sufficient. After ensuring the separation of red blood cells or clots in the serum, the separated serum layer was carefully transferred into a clean, labelled tube using a pipette or transfer pipette. This serum is used for testing cholesterol serum protein level (Bradford, 1976), serum phospholipid (Bartlett, 1959), and blood sugar levels (Khan and Al-Amin., 2015).

2.7. Animal and tissue collection

After 20 days of treatment, the control and experimental guinea pigs were euthanised by decapitation, and weights of the testis, epididymis, and seminal vesicles were measured. The right testis and epididymis were fixed in Bouin's solution for histological analysis. The left testis,

epididymis, and seminal vesicles were used for biochemical analysis, including measurements of cholesterol, total protein, glycogen, and phospholipid.

2.8. Spermatological studies

2.8.1. Collection and dilution of sperm

The sperm that oozed out from the incision was quickly collected using a capillary tube up to the 0.05 µl mark. To facilitate further analysis, the collected sperm was diluted 200 times using phosphate-buffered saline (PBS). The diluted semen was thoroughly mixed to ensure homogeneity.

2.8.2. Sperm counts

Sperm counts were conducted following the method described by Kruger et al. (1986). A drop of diluted semen was placed on an improved Neubauer counting chamber, and a coverslip was carefully overlaid on the chamber to create a flat, even surface. The Neubauer chamber containing the semen sample was observed under a compound microscope at 450X magnification. Sperm within the central square of the Neubauer chamber were counted. The central square consisted of 25 large squares, and the volume of each large square was 0.1 mL. The sperm count was calculated using the following formula:

(Number of sperm in 25 squares / 25) x 10 x dilution factor
x 1000 sperm / mL

2.8.3. Assessment of sperm motility

Sperm motility was assessed using the hanging drop method. A drop of the diluted sperm was transferred onto a cover glass using a Pasteur pipette, which was inverted over a cavity slide to create a hanging drop. The edges of the cover slips were sealed with Vaseline to maintain the humidity. The hanging drop preparation was observed under a compound microscope at 450X magnification. Progressive motility, specifically the duration of the last motile sperm in minutes, was recorded at regular intervals. Two independent observers made separate hanging drop preparations for each animal, and their assessments were used to calculate an average for each animal. This approach ensured reliability and consistency in the evaluation of sperm motility (Chenniappan and Murugan, 2017).

2.8.4. Assessment of sperm vitality

A drop of semen was added to the mixture and thoroughly mixed using a Pasteur pipette. One drop of the resulting mixture was placed on a microscope slide, and a coverslip was carefully placed over it. The slide was observed under a compound microscope at 450X magnification.

2.8.5. Sperm morphology examination

Sperm morphology was observed using the eosin-nigrosin staining method. One or two drops of Eosin-Nigrosin stain was added to the collected semen on a clean and warm microscopic glass slide. Semen and stain were mixed thoroughly. Using the edge of another slide as a spreader, the mixture was evenly spread to obtain a thin film. The slide containing the thin film was air-dried and prepared for microscopy analysis. The prepared slides were observed under a microscope. At least 200 spermatozoa from different fields of view on the slide were examined for morphological features. Various aspects of sperm morphology, such as shape, size, and structural abnormalities, were assessed. At least 200 spermatozoa were examined, and their vitality was assessed based on the staining pattern. Spermatozoa with unstained heads were considered viable, while those with stained heads were considered non-viable (Kondracki et al., 2017)

2.8.6. Sperm agglutination test

Sperm agglutination was assessed using smear preparation and Leishman's staining method. A drop of diluted semen was smeared onto a clean microscope slide, fixed in ethanol, and stained with Leishman's stain. The slide was examined under a compound microscope at 450X magnification to identify any incidence of sperm agglutination, characterised by the clustering or sticking together of sperm cells. The agglutinated spermatozoa were recorded, and the incidence of sperm agglutination was determined based on microscopic observations. Sperm agglutination was determined by calculating the percentage of agglutinated spermatozoa out of the total sperm count observed (Kondracki et al., 2017).

2.8.7. Testis preparation for cholesterol, protein, and glycogen analysis

The collected testes were rinsed with ice-cold saline to remove excess blood and debris. A small portion of each testis was kept aside for protein (Lowry et al., 1951), glycogen (Dische., 1962), and cholesterol analysis.

2.8.8. Histological analysis

Histological analysis of the testes was performed using the paraffin method, as described by Humason (1962) (Figs. 3 and 4). Testis samples were dissected from both control and experimental rats, carefully washed in physiological saline to remove any debris, and cut into pieces of the desired size. The tissue pieces were fixed in Bouin-Hollande fixative for 24 h, followed by washing in 70% alcohol to remove excess picric acid. Dehydration was carried out using a graded series of alcohol, and the tissues were then cleared using xylene. After clearance, the tissues were infiltrated with molten paraffin, embedded, and sectioned into 3–5 µm thick slices using a rotary microtome. The sections were counterstained with Ehrlich's haematoxylin and eosin and mounted using DPX mounting for microscopic examination.

2.8.9. Investigation of the metabolites using GC-MS of M. oleifera

The ethanolic leaf extract of M. oleifera was identified by Gas chromatography–mass spectrometry (GC–MS) analysis using a GCMATE II GC–MS system equipped with a secondary electron multiplier (JEOL, Akishima, Tokyo, Japan). An Agilent 6890 N Network GC system was used for gas chromatography (Agilent Technologies, Santa Clara, CA, USA). Helium was utilised as the carrier gas, and the column used was a fused silica HP5 column with dimensions of 50 m × 0.25 mm (inner diameter). The sample was injected with a split ratio of 5:4, and the analysis proceeded under the following conditions: 20 min at 100 °C, followed by 3 min at 235 °C for column temperature. The injector temperature was maintained at 240 °C. The sample (1 µL) was evaporated using a splitless injector at 300 °C. The total duration of the analysis was 22 min. Compound identification was accomplished through gas chromatography coupled with mass spectrometry. This ensured the accurate identification of the compounds to reduce any error that could have been introduced by the kit. Thus, the molecular mass of the sample portion, formula, percentage composition, and peaks were compared with those of natural compounds in the spectrum archive NIST05. This process was useful for obtaining consistent readings of compounds identified in the samples.

2.8.10. Statistical analysis

The data are presented using the mean values and standard deviation (SD) to illustrate the extent of variation within the dataset. Statistical analyses were performed using one-way ANOVA to assess the differences between the experimental groups. This was followed by a post-hoc test (Tukey's HSD) to evaluate pairwise comparisons. Statistical significance was set at $p < 0.05$. All analyses were conducted using SPSS version 25.0 software. Relevant p-values have been reported alongside the results to ensure transparency and reproducibility.

3. Results and discussion

The control group (Group 1) exhibited a significant increase in body weight from the initial value of 372 ± 5.5 g to 400 ± 14.14 g by the

Table 1
Effect of flower and leaf extract of *M. olifera* on body weight, testis weight, epididymis weight and seminal vesicle weight in carbendazim-induced sub-fertile male Guinea pigs 20 days of treatment.

Experimental group	Body weight (g)		Testis weight (mg)	Epididymis weight (mg)	Seminal vesicle weight (mg)
	Initial	Final			
Group.1-Control	372 ± 5.5[a]	400 ± 14.14 [a]	240 ± 10.94 [b]	92 ± 2.4 [c]	94 ± 2.75 [b]
Group.2-Carbendazim induced subfertile male guinea pigs	350 ± 14.14 [a]	320 ± 10.95 [b]	120 ± 6.3 [c]	34 ± 1.09 [c]	40 ± 1.78 [c]
Group 3-Carbendazim induced subfertile male guinea pig treated with *M. olifera*	325 ± 6.3 [a]	350 ± 16.7 [NS]	236 ± 1.78 [b]	85 ± 3.16 [c]	90 ± 1.78[b]
Group 4- Carbendazim induced subfertile male guinea pig treated with clomiphene citrate	340 ± 17.8 [b]	360 ± 6.63 [NS]	223 ± 1.67 [b]	82 ± 1.9 [b]	89 ± 1.4 [b]

All values are mean ± SD of three replicates; Level of Significance: [a] -P*<0.05; [b] -P**<0.01; [c] -P***<0.001 and NS-Not significant.

Table 2
Effect of flower and leaf extract of *M. olifera* extract on Cauda Epididymal Sperm quality in Carbendazim-Induced Subfertile Male Guinea Pigs after 20 Days of Treatment.

Experimental group	Sperm counts x 10^6 (sperm/ml)	Sperm mortality x 10^6 (sperm/ml)	Sperm agglutination (%)
Group.1- Control	44.0 ± 0.89[b]	57.6 ± 1.45 [b] (Rapid, progressive)	4 ± 1.4 [b]
Group.2-Carbendazim induced subfertile male guinea pigs	15.40 ± 1.62 [a]	9.4 ± 0.4 [b] (Rapid, progressive)	30 ± 1.26 [a]
Group 3-Carbendazim induced subfertile male guinea pig treated with ***M. olifera***	48.0 ± 1.26 [a]	68.0 ± 0.6 [b] (Rapid, progressive)	6 ± 1.09 [b]
Group 4- Carbendazim induced subfertile male guinea pig treated with clomiphene citrate	45.0 ± 2.28 [b]	61.2 ± 0.98 [b] (Rapid, progressive)	7 ± 0.89 [a]

All values are mean ± SD of three replicates; Level of Significance: a-P**<0.01 and b P***<0.001 and NS-Not significant.

study's end. The decrease in body weight observed in the *carbendazim-induced* subfertile group (Group 2) suggests *carbendazim* revelation and amendments in the health status of metabolic processes. Previous research has reported the influence of ecological toxins on body weight and overall health (Li et al., 2017a,b,c; Al-Khaldi et al., 2020). Remarkably, testis weight remained relatively stable, with no statistically significant change observed between the initial and final measurements (240 ± 10.94 mg). Similarly, the epididymal weight experienced only a slight increment from 92 ± 2.4 mg to 94 ± 2.75 mg. In Group 2, consisting of *Carbendazim*-induced subfertile male guinea pigs, set a key decrease of body weight from the initial 350 ± 14.14 g to 320 ± 10.95 g. Furthermore, the testis weight experienced a substantial reduction from 120 ± 6.3 mg to 34 ± 1.09 mg, accompanied by a significant decrease in epididymis weight from 40 ± 1.78 mg to 34 ± 1.09 mg. Similar results have been reported in studies showing exposure to reduced organ weights and reproductive dysfunction (Rehman et al., 2018; Prasad et al., 2019). The seminal vesicle weight declined from 40 ± 1.78 mg to 34 ± 1.09 mg (Table 1).

In Group 3, where *Carbendazim*-induced subfertile male guinea pigs were treated with *M. oleifera,* a mild reduction in initial body weight was observed (325 ± 6.3 g to 350 ± 16.7 g, not statistically significant), while testis weight and epididymis weight remained at 236 ± 1.78 mg and 85 ± 3.16 mg, respectively. These findings suggest that the herbal extract contributes to the recovery of reproductive organs, which could have implications for overall fertility potential. Similar reports effects on findings align with studies highlighting the potential benefits of herbal treatments and fertility medications (Tijani et al., 2016Tijani et al., 2016; Al-Quraishy et al., 2018; Acharya and Porwal, 2020; Atasever Arslan et al., 2023).

Seminal vesicle weight showed a slight increase from 85 ± 3.16 mg to 90 ± 1.78 mg. Group 4, comprising carbendazim-induced subfertile male guinea pigs treated with clomiphene citrate, demonstrated a modest increase in body weight (340 ± 17.8 g to 360 ± 6.63 g, not statistically significant). Moreover, the testis weight (223 ± 1.67 mg) and weights of the epididymis (82 ± 1.9 mg) and seminal vesicle (89 ± 1.4 mg) showed no significant differences throughout the study. The statistically significant changes in body and reproductive organ weights in certain groups (Groups 3 and 4) may be attributed to factors such as treatment duration, dosage, and differences. Similar observations have been reported in studies exploring the effects of herbal extracts on reproductive organs and function (Abdalla et al., 2017; Mabrouk et al., 2021). The study results provide an understanding of the complex interplay between environmental exposure, reproductive organ weights, and male fertility (Agrawal et al., 2016; Nadeem et al., 2020). Table 2 presents the effect of flower and leaf extracts of *M. oleifera* on cauda epididymal sperm quality in carbendazim-induced subfertile male guinea pigs after 20 days of treatment. The sperm parameters evaluated include sperm counts (x 10^6 sperm/ml), sperm motility (x 10^6 sperm/mL) characterized as rapid and progressive, and sperm agglutination (%). In the control group (Group 1), which did not receive Carbendazim treatment, a significantly higher sperm count was observed at 44.0 ± 0.89 x 10^6 sperm/mL compared to the Carbendazim-induced subfertile group (Group 2) with a sperm count of 15.40 ± 1.62 x 10^6 sperm/ml ($p < 0.001$). Furthermore, the control group displayed substantially greater sperm motility, with 57.6 ± 1.45 x 10^6 sperm/mL exhibiting rapid and progressive movement compared to the subfertile group (9.4 ± 0.4 x 10^6 sperm/mL) ($p < 0.001$). Sperm agglutination was significantly lower in the control group (4 ± 1.4%) than in the subfertile group (30 ± 1.26%) ($p < 0.001$). In Group 3, carbendazim-induced subfertile male guinea pigs treated with Moringa oleifera extract exhibited a remarkable improvement in sperm parameters. The sperm count increased significantly to 48.0 ± 1.26 x 10^6 sperm/mL compared to the subfertile group ($p < 0.001$). Moreover, the treated group displayed enhanced sperm motility (68.0 ± 0.6 x 10^6 sperm/ml, rapid and progressive), which was substantially higher than that in the subfertile group ($p < 0.001$). Furthermore, the extract led to a significant reduction in sperm agglutination (6 ± 1.09%) compared to that in the subfertile group ($p < 0.001$). These results suggest the potential stimulatory effects of the Moringa oleifera extract on sperm. The enhancement in sperm counts and motility can be attributed to the beneficial compounds present in *Moringa oleifera,* such as antioxidants and phytoestrogens, which have been reported to positively influence sperm quality (Ahmad et al., 2018; Ojewunmi et al., 2021a,b,c). In Group 4, sperm count increased significantly to 45.0 ± 2.28 x 10^6 sperm/ml compared to the subfertile group ($p < 0.001$). Likewise, sperm motility (61.2 ± 0.98 x 10^6 sperm/ml, rapid and progressive) was significantly higher than in the subfertile group ($p < 0.001$). However, sperm agglutination remained relatively high in this group at 7 ± 0.89%, although it was lower than that in the subfertile group ($p < 0.01$). These findings are consistent with the known effects of clomiphene citrate on sperm parameters (Anawar et al., 2017;). Table 3 presents the effect of *Moringa oleifera* extract on cauda epididymal sperm

Table 3
Effect of *M. olifera* extract on cauda epididymal sperm morphology and vitality in carbendazim induced subfertile male guinea pigs after 20 days treatment.

Experimental group	Sperm Morphology (%)		Sperm vitality (%)	
	Normal sperm	Abnormal sperm	Live sperms	Dead sperms
Group.1-Control	90 ± 3.34[a]	10 ± 0.89 [a]	80 ± 2.8 [a]	20 ± 1.78 [a]
Group.2-Carbendazim induced subfertile male guinea pigs	55 ± 3.68 [b]	45 ± 2.0 [a]	52 ± 1.26 [b]	48 ± 1.78 [b]
Group 3-Carbendazim induced subfertile male guinea pig treated with ***M. olifera***	89 ± 0.63[a]	11 ± 5.8[b]	87 ± 2.6 [a]	13 ± 3.28 [a]
Group 4-Carbendazim induced subfertile male guinea pig treated with clomiphene citrate	88 ± 3.16 [a]	12 ± 1.4 [a]	83 ± 1.26[b]	17 ± 2.0 [b]

All values are mean ± SD of three replicates; Level of Significance: a-P**<0.01 and b P***<0.001 and NS-Not significant.

morphology and vitality in carbendazim-induced sub-fertile male guinea pigs after 20 days of treatment. The parameters evaluated included the percentage of normal sperm, abnormal sperm, live sperm, and dead sperm. In the control group (Group 1), a significantly higher percentage of normal sperm was observed at 90 ± 3.34% compared to the carbendazim-induced sub-fertile group (Group 2) with only 55 ± 3.68% normal sperm ($p < 0.001$). Conversely, the subfertile group had a significantly higher percentage of abnormal sperm (45 ± 2.0%) than the control group ($p < 0.001$). Moreover, live sperm were substantially higher in the control group (80 ± 2.8%) than in the sub-fertile group (52 ± 1.26%) ($p < 0.001$). Dead sperm were significantly lower in the control group (20 ± 1.78%) than in the subfertile group (48 ± 1.78%) ($p < 0.001$). These results are consistent with the known adverse effects of carbendazim on sperm morphology and vitality, leading to sub-fertility (Li et al., 2017a,b,c; Al-Khaldi et al., 2020). In Group 3, carbendazim-induced sub-fertile male guinea pigs treated with *Moringa oleifera* extract exhibited a remarkable improvement in sperm morphology and vitality. The percentage of normal sperm increased significantly to 89 ± 0.63% compared to that in the subfertile group ($p < 0.01$). Conversely, the percentage of abnormal sperm decreased to 11 ± 5.8%, showing a marked improvement compared to that in the sub-fertile group ($p < 0.01$). Moreover, live sperm significantly increased to 87 ± 2.6% compared to the sub-fertile group ($p < 0.01$), while dead sperm decreased to 13 ± 3.28% ($p < 0.01$). In Group 4, the normal sperm was 88 ± 3.16%, which was similar to the control group. The abnormal sperm was 12 ± 1.4%, showing important development compared to the subfertile group ($p < 0.001$). Live sperm were 83 ± 1.26% and dead sperm were 17 ± 2.0%, indicating improvements in both parameters compared to the subfertile group ($p < 0.001$). These findings align with the known effects of clomiphene citrate in improving sperm morphology and vitality. Table 4 presents the effect of *Moringa oleifera* extract on serum cholesterol, protein, phospholipid, and blood sugar levels in carbendazim-induced subfertile male guinea pigs after 20 days of treatment.

Serum Cholesterol Levels: In the control group (Group 1), serum cholesterol levels were significantly higher at 30.30 ± 1.26 mg/dl compared to the Carbendazim-induced subfertile group (Group 2) with levels of 19.20 ± 0.89 mg/dl ($p < 0.001$). Treatment with *Moringa oleifera* extract (Group 3) led to a partial increase in serum cholesterol levels to 23.03 ± 2.4 mg/dl but remained lower than the control group. Clomiphene citrate treatment (Group 4) resulted in serum cholesterol levels of 24.54 ± 1.4 mg/dl.

Serum Protein Levels: In the control group (Group 1), serum protein levels were significantly higher at 5.8 ± 0.25 g/dl compared to the subfertile group (Group 2) with levels of 4.2 ± 0.7 g/dl ($p < 0.001$). Treatment with *Moringa oleifera* extract (Group 3) led to a substantial increase in serum protein levels to 6.2 ± 0.4 g/dl, approaching those of the control group. Clomiphene citrate treatment (Group 4) resulted in serum protein levels of 6.4 ± 0.25 g/dl.

Serum Phospholipid Levels: In the control group (Group 1), serum phospholipid levels were significantly higher at 50.20 ± 3.4 mg/dl compared to the subfertile group (Group 2) with levels of 23.32 ± 1.4 mg/dl ($p < 0.001$). Treatment with *Moringa oleifera* extract (Group 3) led to a substantial increase in serum phospholipid levels to 51.26 ± 2.28 mg/dl, while clomiphene citrate treatment (Group 4) resulted in levels of 50.12 ± 0.63 mg/dl, similar to the control group.

Blood Sugar Levels: In the control group (Group 1), blood sugar levels were significantly higher at 80 ± 1.4 g/dl compared to the sub-fertile group (Group 2) with levels of 50 ± 3.6 g/dl ($p < 0.001$). Group 3 led to an increase in blood sugar levels to 65 ± 2.28 g/dl, while clomiphene citrate treatment (Group 4) resulted in levels of 67 ± 1.89 g/dl, both significantly higher than the subfertile group ($p < 0.01$). These

Table 5
Effect of *M. olifera* extract on testis cholesterol, protein and glycogen levels in carbendazim induced subfertile male guinea pigs after 20 days treatment.

Experimental group	Testis cholesterol levels (mg/g)	Testis protein levels (mg/g)	Testis glycogen levels (mg/g)
Group.1-Control	32 ± 1.67[a]	45.20 ± 2.0 [a]	4.0 ± 0.2 [a]
Group.2-Carbendazim induced subfertile male guinea pigs	18 ± 1.26 [b]	20.30 ± 0.6 [b]	2.2 ± 0.3 [b]
Group 3-Carbendazim induced subfertile male guinea pig treated with ***M. olifera***	32 ± 1.4**	42.12 ± 1.4**	4.2 ± 0.2**
Group 4- Carbendazim induced subfertile male guinea pig treated with clomiphene citrate	31 ± 1.09 [a]	40.20 ± 1.26 [a]	4.1 ± 0.1 [a]

All values are mean ± SD of three replicates.
Level of Significance: [a]-P<0.01, [b]- P < 0.001 and NS-Not significant.

Table 4
Effect of *M. olifera* extract on serum cholesterol, protein, phospholipid and blood sugar level in carbendazim induced subfertile male guinea pigs after 20 days treatment.

Experimental group	Serum cholesterol levels (mg/dl)	Serum protein levels (g/dl)	Serum phospholipid levels (mg/dl)	Blood sugar levels (g/dl)
Group.1-Control	30.30 ± 1.26 [a]	5.8 ± 0.25 [a]	50.20 ± 3.4 [b]	80 ± 1.4 [b]
Group.2-Carbendazim induced subfertile male guinea pigs	19.20 ± 0.89 [b]	4.2 ± 0.7 [b]	23.32 ± 1.4 [b]	50 ± 3.6 [b]
Group 3-Carbendazim induced subfertile male guinea pig treated with ***M. olifera***	23.03 ± 2.4*	6.2 ± 0.4	51.26 ± 2.28**	65 ± 2.28*
Group 4-Carbendazim induced subfertile male guinea pig treated with clomiphene citrate	24.54 ± 1.4 [a]	6.4 ± 0.25 [a]	50.12 ± 0.63 [b]	67 ± 1.89 [a]

All values are mean ± SD of three replicates; Level of Significance: [a]-P<0.01, [b]- P < 0.001 and NS-Not significant.

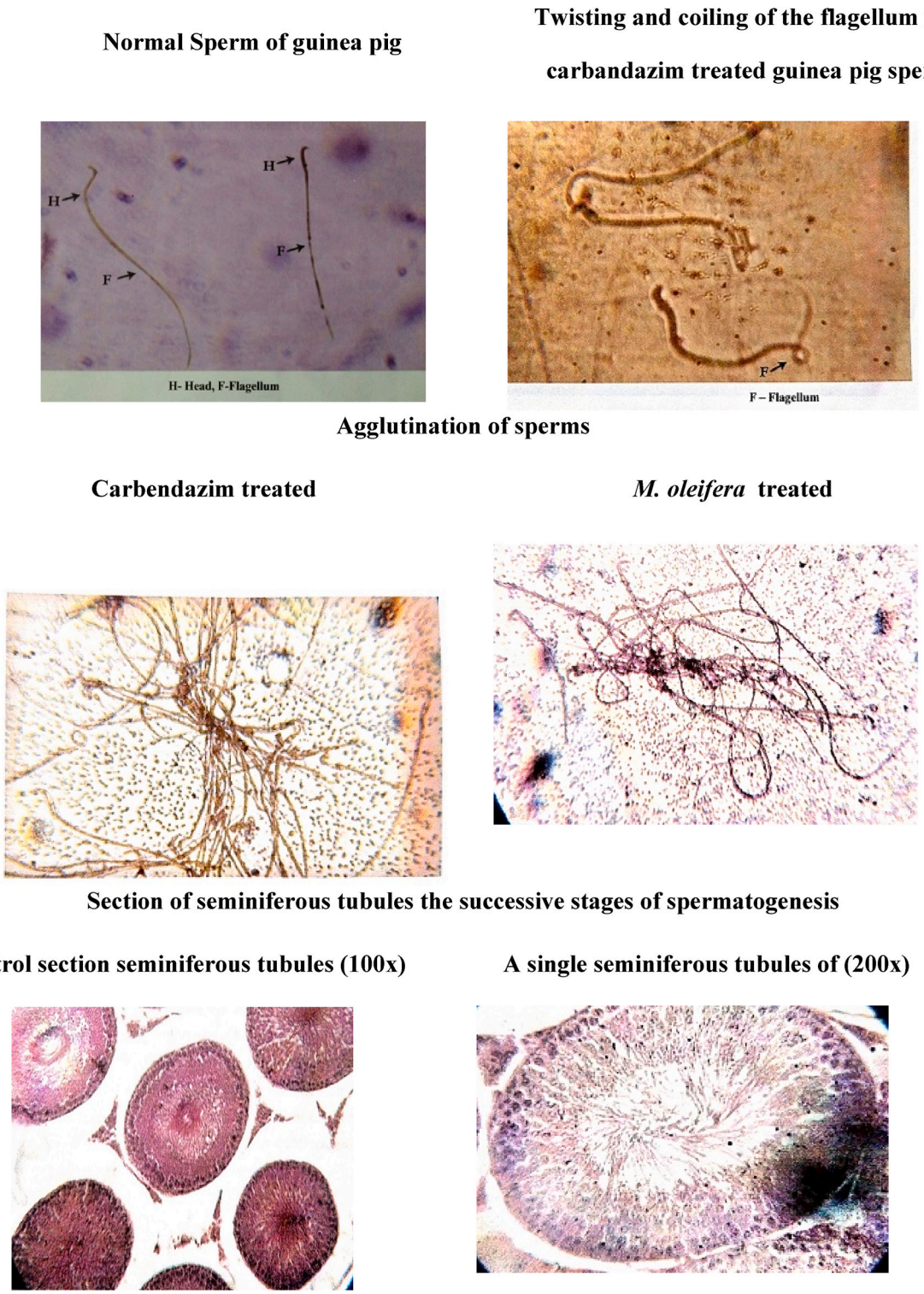

Fig. 1. Sperm morphology, agglutination and cross section of seminiferous tubules of Guinea pig treated with carbendazin, *M. oleifera* leaf extract and Clomiphene citrate.

results suggest that carbendazim exposure reduced blood sugar levels, and treatment with *Moringa oleifera* extract and clomiphene citrate partially improved blood sugar levels, although they remained lower than those of the control group after 20 days of treatment (Table 5).

3.1. Testis cholesterol levels

In the control group (Group 1), testis cholesterol levels were measured at 32 ± 1.67 mg/g. Carbendazim exposure significantly reduced these levels in the subfertile group (Group 2) to 18 ± 1.26 mg/g ($p < 0.001$). Treatment with *Moringa oleifera* extract (Group 3) effectively restored testis cholesterol levels to 32 ± 1.4 mg/g, similar to those of the control group. Clomiphene citrate treatment (Group 4) resulted in testis cholesterol levels of 31 ± 1.09 mg/g.

Testis Protein Levels: In the control group (Group 1), testis protein levels were measured at 45.20 ± 2.0 mg/g. The subfertile group (Group

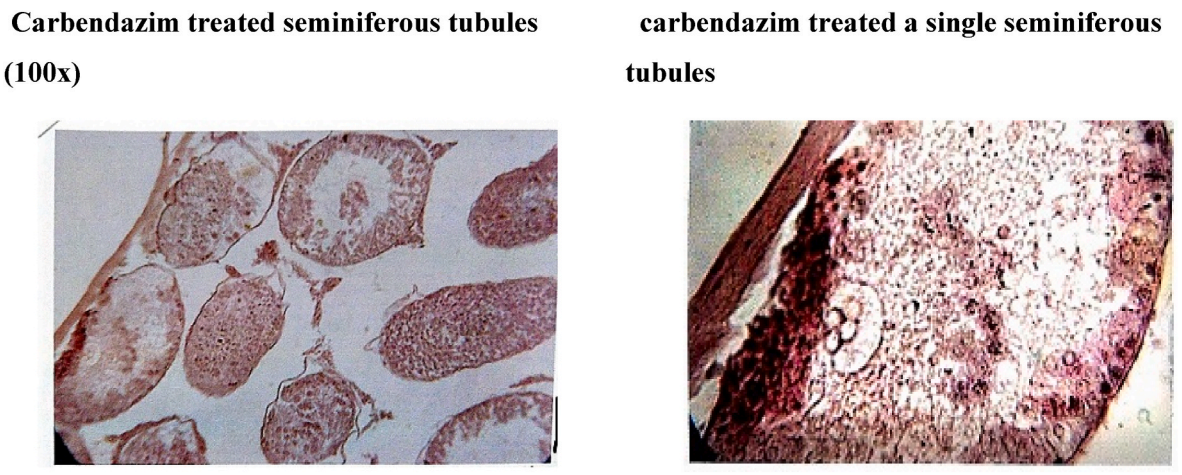

Fig. 2. Carbendazim –treated guinea pig showing fibrosis in the seminiferous tubules.

2) showed significantly lower levels of 20.30 ± 0.6 mg/g ($p < 0.001$) due to carbendazim exposure. Treatment with Moringa oleifera extract (Group 3) resulted in a substantial increase in testis protein levels to 42.12 ± 1.4 mg/g, while clomiphene citrate treatment (Group 4) led to levels of 40.20 ± 1.26 mg/g.

Testis Glycogen Levels: In the control group (Group 1), testis glycogen levels were measured at 4.0 ± 0.2 mg/g. Carbendazim exposure in the subfertile group (Group 2) led to a decrease in glycogen levels to 2.2 ± 0.3 mg/g ($p < 0.001$). Treatment with *Moringa oleifera* extract (Group 3) resulted in a significant increase in testis glycogen levels to 4.2 ± 0.2 mg/g, similar to those of the control group. Clomiphene citrate treatment (Group 4) led to glycogen levels of 4.1 ± 0.1 mg/g. A comprehensive assessment of sperm morphology, sperm agglutination, and seminiferous tubules was conducted among the experimental groups (Fig. 1). Guinea pigs exposed to carbendazim (Group 2) displayed notable aberrations in sperm morphology, characterised by an increased proportion of abnormal sperm and elevated sperm agglutination. Moreover, examination of seminiferous tubules in this group revealed significant fibrosis, indicative of compromised testicular health and impaired spermatogenesis. These findings underscored the detrimental effects of carbendazim on sperm quality and testicular tissue. Guinea pigs (group 3) exhibited marked improvements across these parameters. These results strongly suggest the potential therapeutic effects of *Moringa oleifera* in enhancing sperm quality and mitigating testicular damage caused by carbendazim exposure. and observed in Guinea pigs treated with clomiphene citrate (Group 4).In Fig. 2, the focus shifts to the histological examination of the seminiferous tubules. Carbendazim-exposed Guinea pigs (Group 2) displayed seminiferous tubules afflicted with fibrosis and substantial damage, indicating severe disruptions in spermatogenesis. Conversely, Guinea pigs treated with *Moringa oleifera* (Group 3) showed seminiferous tubules with less damage and a closer-to-normal histological appearance, affirming the protective attributes of *Moringa oleifera* on testicular tissue. Similarly, Clomiphene citrate-treated Guinea pigs (Group 4) demonstrated seminiferous tubules with a histology more akin to normalcy than the carbendazim group. Carbendazim, a widely used fungicide, induced significant subfertility in an experimental guinea pig model. This suggests that *Moringa oleifera* extract holds promise as a natural alternative for managing subfertility, offering a potential therapeutic option with fewer side effects. The exact mechanisms underlying the beneficial effects of *Moringa oleifera* extract require further investigation. Phytoestrogenic, antioxidant, and anti-inflammatory properties contribute to the restoration of sperm quality and protection of testicular tissue.

3.2. Interpretation on GC-MS of Moringa oleifera

GC-MS interpretation was conducted using the database of National Institutes of Standards and Technology (NIST) with more than 62,000 patterns. Spectrum of unknown components stored in the NIST library. The GC-MS characterization of the *Moringa oleifera* fraction is presented in Table. Here 10 compounds were identified and reported. The polyphenolic-rich fraction of *Moringa oleifera had the* highest flavonoid

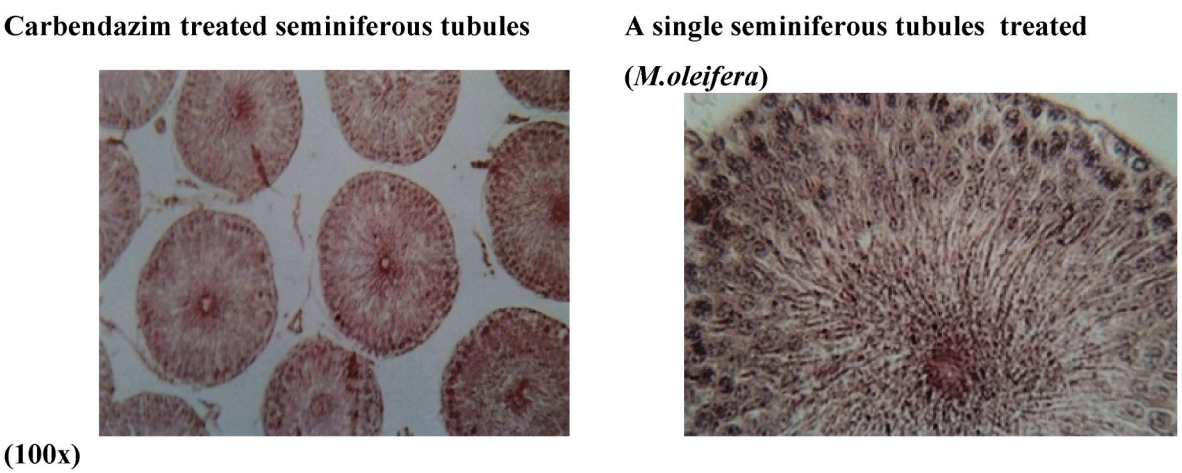

Fig. 3. Carendazim induced subfertile male guinea pig treated with *M.oleifera* showing less damage in seminiferous tubules of testis and with a normal histology (100x).

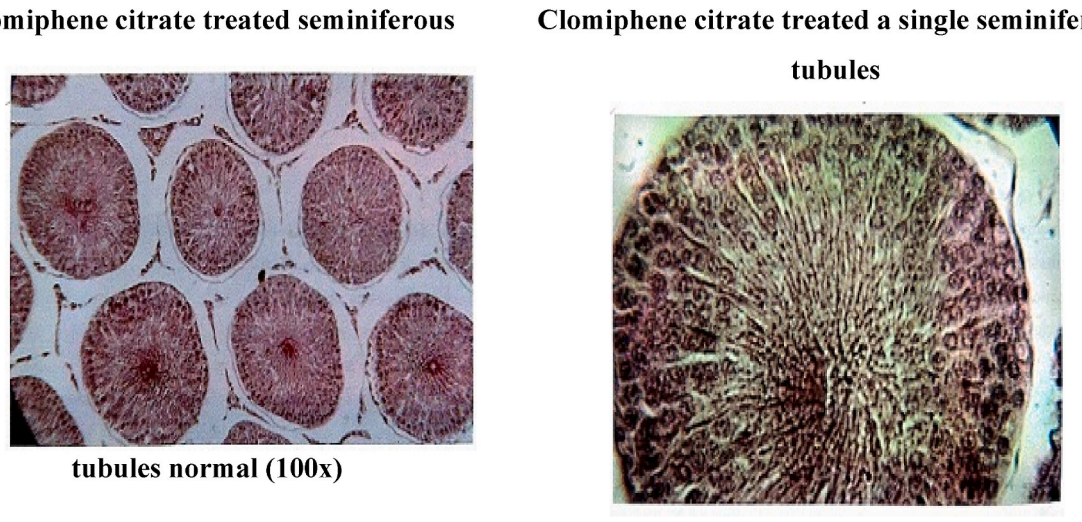

Fig. 4. Seminiferous tubules of a carbendazim induced subfertile male guinea pig.

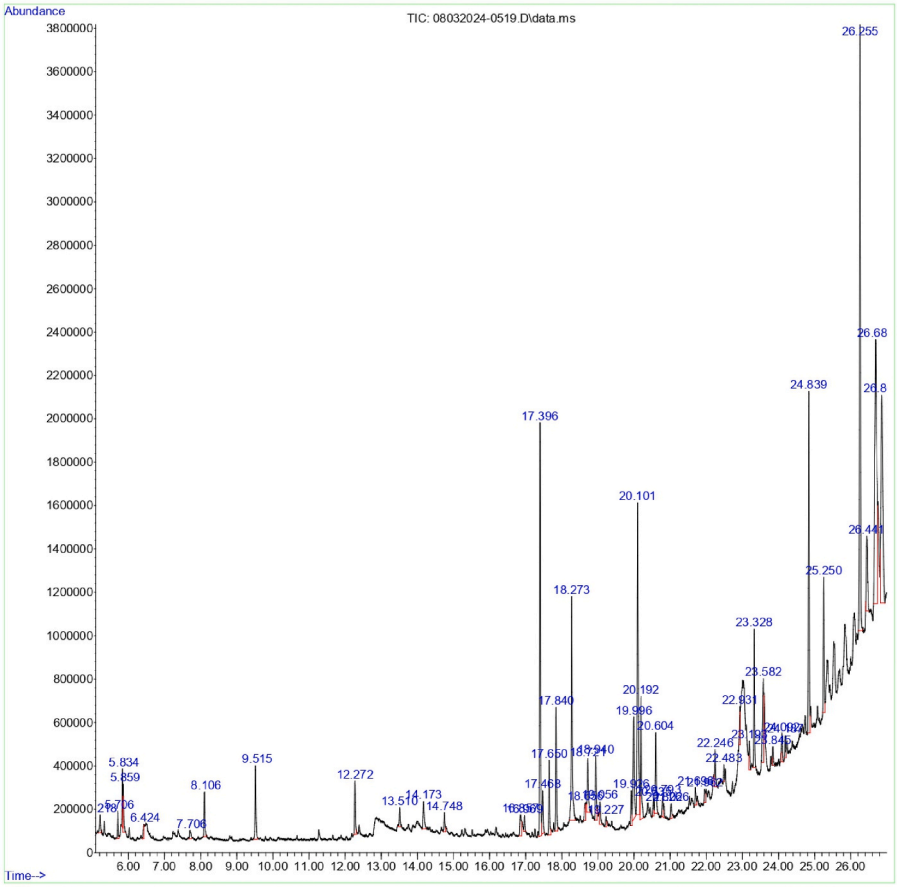

Fig. 5. GC-MS analysis of metabolites of Moringa oleifera.

content and exhibited the strongest anti-diabetic antioxidant activity. Gas chromatography-mass spectrometry (GC-MS) analysis was used to determine the chemical composition that may contribute to this activity. GC-MS analysis showed a variety of phenolic compounds (Fig. 5). The antioxidants show a key role in scavenging free radicals and reducing oxidative stress, which can contribute to sperm damage and impaired function. Anti-inflammatory effects may assistance generate a favorable micro-environment for spermatogenesis. Research suggests that may influence hormonal balance, particularly testosterone levels. Testosterone is a key hormone involved in sperm production, and its optimal levels are important for sustaining a healthy sperm count (Zhou et al., 2019) Phyto-constituents support testosterone regulation of sperm production (Ali et al., 2014). Chronic inflammation and immune dysfunction are associated with male infertility. *The anti-inflammatory* and immunomodulatory properties of *M. oleifera* may contribute to creating a conducive environment for spermatogenesis by reducing

inflammation and immune system balance (Pari et al., 2002; Andersen et al., 2016). *Moringa oleifera* presents a promising avenue for enhancing male reproductive health and addressing spermatological aspects. Its rnutritional content, antioxidant properties, and hormonal modulation make it a compelling candidate for improving sperm quality, sperm count, and overall fertility potential.

4. Conclusion

This study shows that *Moringa oleifera* extract can be used in prostate pathological conditions to transform male fertility by increasing sperm motility and decreasing sperm agglutination in a carbendazim-induced subfertility model. Some studies raise hope for the use of these herbal remedies for male infertility, as seen in the findings of this research. Further studies should be directed toward the identification of molecular targets of the effects observed, finding the bioactive compounds in Moringa oleifera producing the effects, and assessing the effects of *Moringa oleifera* in other animal models. Human studies are also required to give credence to a treatment benefit claim and to prove efficacy without causing harm in large populations.

CRediT authorship contribution statement

Sudha Sankar: Data curation, Conceptualization. **Subramaniam Umavathi:** Conceptualization. **Ekambaram Gayathiri:** Writing – review & editing. **Palanisamy Prakash:** Writing – review & editing.

Data availability

The data will be made available upon request.

Ethical approval

Ethical committee approval for this study was obtained, and the reference number is JKKNCP/CERT/0126T22.

Funding

NIL.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

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References

Abdalla, H., Abramowski, A., Aharonian, F., Benkhali, F.A., Angüner, E.O., Arakawa, M., Arrieta, M., Aubert, P., Backes, M., Balzer, A., Barnard, M., 2017. TeV gamma-ray observations of the binary neutron star merger GW170817 with HESS. Astrophys. J. Lett. 850 (2), L22.

Acharya, R., Porwal, A., 2020. A vulnerability index for the management of and response to the COVID-19 epidemic in India: an ecological study. The Lancet Global Health 8 (9), e1142–e1151.

Afeiche, M., Williams, P.L., Mendiola, J., Gaskins, A.J., Jørgensen, N., Swan, S.H., Chavarro, J.E., 2013. Dairy food intake in relation to semen quality and reproductive hormone levels among physically active young men. Hum. Reprod. 28 (8), 2265–2275.

Agarwal, A., Majzoub, A., Esteves, S.C., Ko, E., Ramasamy, R., Zini, A., 2016. Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios. Transl. Androl. Urol. 5 (6), 935.

Ahmad, A., et al., 2018. Evaluation of the effect of Moringa oleifera seed powder on spermatogenesis in the rat. J. Basic Clin. Physiol. Pharmacol. 29 (3), 299–306.

Ali, S., Gladieux, P., Rahman, H., Saqib, M.S., Fiaz, M., Ahmad, H., de Vallavieille-Pope, C., 2014. Inferring the contribution of sexual reproduction, migration and off-season survival to the temporal maintenance of microbial populations: a case study on the wheat fungal pathogen P uccinia striiformis f. sp. tritici. Mol. Ecol. 23 (3), 603–617.

Ali, K., Berman, G., Zhou, H., Deng, W., Faughnan, V., Coronado-Voges, M., McPhee, R., 2021. Evaluation of mRNA-1273 SARS-CoV-2 vaccine in adolescents. New Engl. J. Med. 385 (24), 2241–2251.

Al Khaldi, M.R., Al-khaldi, M., 2023. A systematic review on infertility in men due to sperm DNA damage: causes investigations, & management. American Journal of Medical Science and Innovation 2 (2), 79–89.

Al-Khaldi, A.A., et al., 2020. Impact of carbendazim fungicide on reproductive performance in male mice. Andrologia 52 (7), e13629.

Al-Quraishy, S., Dkhil, M.A., Al-Shaebi, E.M., Abdel-Baki, A.A.S., Araúzo-Bravo, M.J., Delic, D., Wunderlich, F., 2018. Gene expression of the liver of vaccination-protected mice in response to early patent infections of Plasmodium chabaudi blood-stage malaria. Malaria J. 17, 1–16.

Andersen, J.M., Rønning, P.O., Herning, H., Bekken, S.D., Haugen, T.B., Witczak, O., 2016. Fatty acid composition of spermatozoa is associated with BMI and with semen quality. Andrology 4 (5), 857–865.

Atasever Arslan, B., Kaya, B., Ülküseven, B., 2023. Synthesis, SARS-CoV-2 3CL main protease inhibitor, anti-inflammatory, and wound-healing effects of a zinc (II)-thiosemicarbazone complex. Appl. Organometall. Chem. 37 (4), e7030.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Chaudhary, S., Kanwar, R.K., Sehgal, A., Cahill, D.M., Barrow, C.J., Sehgal, R., Kanwar, J.R., 2017. Progress on Azadirachta indica based biopesticides in replacing synthetic toxic pesticides. Front. Plant Sci. 8, 610.

Chavda, V.P., Sonak, S.S., Balar, P.C., Vyas, K., Palandurkar, P., Mule, K., Patel, K., Hala, V., Vaghela, D.A., Acharya, D., Vaishnav, A., 2024. Reviving fertility: phytochemicals as natural allies in the fight against non-genetic male infertility. Clinical Complementary Medicine and Pharmacology 4 (1), 100128.

Chenniappan, K., Murugan, K., 2017. Therapeutic and fertility restoration effects of Ionidium suffruticosum on sub-fertile male albino Wistar rats: effects on testis and caudal spermatozoa. Pharm. Biol. 55 (1), 946–957. https://doi.org/10.1080/13880209.2016.1278453.

Coleman-Jensen, A., Rabbitt, M.P., Gregory, C., Singh, A., 2015. Household Food Security in the United States in 2014.

Fasanghari, M., Keramat, A., Tansaz, M., Moini, A., Chaman, R., 2024. Effect of alternative and complementary medicine on male infertility: an umbrella review. Health Sci Rep 7 (6), e2118. https://doi.org/10.1002/hsr2.2118.

Humason, G.L., 1962. Animal Tissue Techniques.

Kondracki, S., Wysokińska, A., Kania, M., Górski, K., 2017. Application of two staining methods for sperm morphometric evaluation in domestic pigs. J Vet Res 61 (3), 345–349. https://doi.org/10.1515/jvetres-2017-0045.

Krishnaiah, N.V., 2016. Varietal resistance breaking ability and insecticide resistance developing ability of BPH-Is there any relation between the two? Mol. Entomol. 7.

Krishnaiah, D., Sarbatly, R., Nithyanandam, R., 2011. A review of the antioxidant potential of medicinal plant species. Food Bioprod. Process. 89 (3), 217–233.

Kruger, TF, Menkveld, R, Stander, FSH, Lombard, CJ, Van der Merwe, JP, van Zyl, JA, Smith, K, 1986. Sperm morphologic features as a prognostic factor in in vitro fertilization. Fertility and Sterility 46 (6), 1118–1123.

Li, X., et al., 2017a. Toxic effects of carbendazim on male reproductive system in Sprague-Dawley rats. Environ. Toxicol. 32 (3), 894–905.

Li, X., et al., 2017b. Toxic effects of carbendazim on male reproductive system in Sprague-Dawley rats. Environ. Toxicol. 32 (3), 894–905.

Li, X., et al., 2017c. Toxic effects of carbendazim on male reproductive system in Sprague-Dawley rats. Environ. Toxicol. 32 (3), 894–905.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent.

Mabrouk, M.M., Mansour, A.T., Abdelhamid, A.F., Abualnaja, K.M., Mamoon, A., Gado, W.S., Matter, A.F., Ayoub, H.F., 2021. Impact of aqueous exposure to silver nanoparticles on growth performance, redox status, non-specific immunity, and histopathological changes of Nile Tilapia, Oreochromis niloticus, challenged with Aeromonas hydrophila. Aquaculture Reports 21, 100816.

Muhammad, S., Long, X., Salman, M., 2020. COVID-19 pandemic and environmental pollution: A blessing in disguise? Sci. Total Environ. 728, 138820.

Nadeem, M., Bahadar, S., Gull, A.A., Iqbal, U., 2020. Are women eco-friendly? Board gender diversity and environmental innovation. Bus. Strat. Environ. 29 (8), 3146–3161.

Ojewunmi, O., et al., 2021a. Effects of Moringa oleifera seed extract on testicular morphology and sperm parameters in adult Wistar rats. Andrologia 53 (1), e13855.

Ojewunmi, O., et al., 2021b. Effects of Moringa oleifera seed extract on testicular morphology and sperm parameters in adult Wistar rats. Andrologia 53 (1), e13855.

Ojewunmi, O., et al., 2021c. Effects of Moringa oleifera seed extract on testicular morphology and sperm parameters in adult Wistar rats. Andrologia 53 (1), e13855.

Olaoye, A.B., Ologunde, C.A., Molehin, O.R., 2021. Comparative antioxidant analysis of *moringa oleifera* leaf extracts from south western states in Nigeria. Futur. J. Pharm. Sci. 7, 68. https://doi.org/10.1186/s43094-021-00204-8.

Pilz, S., Gruebler, M., Gaksch, M., Schwetz, V., Trummer, C., Hartaigh, B.O., Verheyen, N., Tomaschitz, A., Maerz, W., 2016. Vitamin D and mortality. Anticancer Res. 36 (3), 1379–1387.

Prabsattroo, T., Wattanathorn, J., Iamsaard, S., Somsapt, P., Sritragool, O., Thukhummee, W., Muchimapura, S., 2015. Moringa oleifera extract enhances sexual performance in stressed rats. J. Zhejiang Univ. Sci. B 16 (3), 179.

Prasad, A., Bharathi, V., Sivalingam, V., Girdhar, A., Patel, B.K., 2019. Molecular mechanisms of TDP-43 misfolding and pathology in amyotrophic lateral sclerosis. Front. Mol. Neurosci. 12, 25.

Rathi, I., Mavi, A., Shannawaz, M., Saeed, S., Yadav, A., Hasan, S., 2024. Effectiveness of ayurveda intervention in the management of infertility: a systematic review. Cureus 16 (4), e57730. https://doi.org/10.7759/cureus.57730.

Sermondade, N., Faure, C., Fezeu, L., Shayeb, A.G., Bonde, J.P., Jensen, T.K., Van Wely, M., Cao, J., Martini, A.C., Eskandar, M., Chavarro, J.E., Koloszar, S., Twigt, J. M., Ramlau-Hansen, C.H., Borges Jr., E., Lotti, F., Steegers-Theunissen, R.P., Zorn, B., Polotsky, A.J., La Vignera, S., Eskenazi, B., Tremellen, K., Magnusdottir, E. V., Fejes, I., Hercberg, S., Lévy, R., Czernichow, S., 2013. BMI in relation to sperm count: an updated systematic review and collaborative meta-analysis. Hum. Reprod. Update 19 (3), 221–231. https://doi.org/10.1093/humupd/dms050.

Shahid, M., Nadeem, M., Bakhat, H.F., 2020. Environmental toxicology and associated human health risks. Environ. Sci. Pollut. Control Ser. 27, 39671–39675.

Sharifi-Rad, M., Varoni, E.M., Iriti, M., Martorell, M., Setzer, W.N., del Mar Contreras, M., Sharifi-Rad, J., 2018. Carvacrol and human health: A comprehensive review. Phytother. Res. 32 (9), 1675–1687.

Singh, S., Nair, V., Gupta, Y.K., 2001. Antioxidant activity of Withania somnifera and its protective effect against DNA damage and hydroxyl radical induced cell death. Phytomedicine 8 (6), 454–460.

Sultana, M., Rownok, M.H., Sabrin, M., Rahaman, M.H., Alam, S.N., 2022. A review on experimental chemically modified activated carbon to enhance dye and heavy metals adsorption. Cleaner engineering and technology 6, 100382.

Suresh, S., Kavitha, C.N., Babu, S.M., 2014. Antioxidant activity of Sida cordifolia L. aerial dried leaves. Sci. World J. 2014, 612450.

Suresh, D., Srinivas, A.N., Kumar, D.P., 2020. Etiology of hepatocellular carcinoma: special focus on fatty liver disease. Front. Oncol. 10, 601710.

Tijani, L.A., Akanji, A.M., Agbalaya, K., Onigemo, M., 2016. Comparative effects of graded levels of Moringa leaf meal on haematological and serum biochemical profile of broiler chickens. J. Agric. Sci. Sri Lanka 11 (3).

Wang, H., Chen, Y., Wang, L., Liu, Q., Yang, S., Wang, C., 2023. Advancing herbal medicine: enhancing product quality and safety through robust quality control practices. Front. Pharmacol. 14, 1265178. https://doi.org/10.3389/fphar.2023.1265178.

Zhou, M., Wang, H., Zeng, X., Yin, P., Zhu, J., Chen, W., Li, X., Wang, L., Wang, L., Liu, Y., Liu, J., 2019. Mortality, morbidity, and risk factors in China and its provinces, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 394 (10204), 1145–1158.