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Review

Overview of the Effects of *Moringa oleifera* Leaf Extract on Oxidative Stress and Male Infertility: A Review

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Abstract: According to a recent report by the WHO, 50–80 million people suffer with infertility. Amongst these populations, male counterparts account for 20–50% of infertility cases. The aetiology of infertility in men includes many factors such as psychological issues, lifestyle and environmental factors, hormonal disorders and chromosomal abnormalities. The pathophysiology of these aetiologies may be initiated by a local inflammatory reaction increasing reactive oxygen species (ROS) production, which can negatively affect the male reproductive system by altering the hypothalamic–pituitary–gonadotropin axis (HPG axis). Alteration of the HPG axis may affect testicular steroidogenesis, spermatogenesis, the Leydig cells and Sertoli cells, leading to poor semen quality. The prevalence of male infertility underscores the need for a thorough scientific investigation to identify treatable or reversible factors using plant adjuvants with antioxidative properties. Therefore, this review aims to provide an overview of the currently available knowledge on the aetiologies of male reproductive dysfunction, emphasising infertility risk factors, as well as elucidating the possible ways by which readily available alternatives, such as *Moringa oleifera* leaves, may mitigate male infertility by highlighting its role on the oxidative stress parameters, reproductive hormonal levels, testicular steroidogenesis and spermatogenesis, gene expression, weight and morphology of the testis and sperm parameters.

Keywords: *Moringa oleifera*; male infertility; oxidative stress; reactive oxygen species; testicular cells; semen quality

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1. Introduction

A global challenge affecting humankind lies in understanding, preventing and managing ever-increasing male infertility. The world health organization (WHO) defines infertility as the inability to conceive after a year of regular unprotected sexual intercourse [1]. Infertility affects all genders, and its prevalence among couples ranges from 10 to 15% globally, with male factors contributing 20–50% of all infertility cases [2,3]. In addition, male infertility causes substantial psychological and social distress and imposes a considerable economic burden on the health care system [3].

Many causes and risk factors contribute to the overarching incidence of male infertility, graded as idiopathic, acquired and congenital [3]. Previous reports estimate that about 30–50% of male infertility patients are idiopathic [2,4]. In addition, infertile couples experience many emotions, including depression, anger and shame. Furthermore, they are sometimes mocked, embarrassed and even pressured by peers, friends and parents, particularly in societies with high expectations for bearing children after marriage [4]. Acquired factors of male infertility include varicocele, testicular trauma, testicular torsion, recurrent urogenital infections and acquired secondary hypogonadism [5]. Among these factors,

varicocele is the most common cause of infertility in men, with a prevalence of 40% [5]. In addition, congenital causes of male infertility arise from the congenital bilateral absence of the vas deferens associated with cystic fibrosis, gene mutations and chromosomal abnormalities leading to the deterioration of testicular function and Y chromosome microdeletions resulting in isolated spermatogenic defects [6]. These risk factors may also cause excessive accumulation of reactive oxygen species (ROS) in cells and induce damage to the reproductive function by releasing inflammatory cytokines and, eventually, oxidative stress [7]. ROS affect sperm parameters directly or indirectly by impairing male reproductive hormones, cells and organs [7].

Some factors leading to male infertility can be surgically reversed or therapeutically ameliorated with drugs [8]. However, treatment options depend on the cause of male infertility, the patient's age, financial status, facilities available in a designated hospital and expertise [9]. The primary treatment for male factor infertility is intrauterine insemination (IUI), tubal and male ejaculatory duct cryosurgery, in-vitro fertilisation (IVF) and embryo transfer with or without intracytoplasmic sperm injection (ICSI) [9]. In addition, psychosexual counselling, vacuum constriction device (penis pump), diet, exercise, weight loss, approved phosphodiesterase type 5 (PDE5) inhibitors drugs, including Viagra (Sildenafil), Cialis (Tadalafil) and Levitra (Vardenafil) and Stendra (Avanafil), apomorphine and intracavernosal injection therapies as well as medicinal plants, such as Pausinystalia yohimbe and Tribulus terrestris, are used for the treatment of erectile dysfunction [8,10]. Furthermore, testosterone replacement therapy (TRT), aromatase inhibitors and human chorionic gonadotrophin (hCG) therapy [11,12] are used for the treatment of primary hypogonadism. Most of these male infertility treatment options have been deemed adequate. However, they also have their downsides regarding side effects, costs, invasiveness of the administration method and availability, particularly in African countries [8,9,11,12]. Therefore, medicinal herbs may be recommended to treat male reproductive impairment, as these herbs enhance reproductive functions [13,14].

M. oleifera (drumstick tree, horseradish tree, ben-oil tree or kelor tree) is of the family 'Moringaceae' [15–17]. It originates from the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan and can grow in subtropical and tropical climates [18]. In Southern Asia and Middle East Africa, the tree is mainly cultivated for its various nutraceutical and medicinal properties contained in different parts of the tree, such as seeds, roots, flowers, pots and leaves [19]. In vivo and in vitro studies have been conducted to determine the effects of *M. oleifera* leaves on the male reproductive system [14,20–23]. This review highlights the mitigating action of *M. oleifera* on some of the risk factors that lead to male infertility.

2. Male Infertility

There is increasing evidence of the progressive decline in human fertility in both developed and developing countries, with a variable prevalence [2]. For instance, the prevalence estimate of infertility is 6% in the United States of America (USA), 10–15% in the United Kingdom (UK) and 20–46% in sub-Saharan Africa, with the African continent having an overall infertility rate of 41.91% among males and females, in which male infertility contributes to 22.26% [3,6,24].

Male infertility is commonly caused by reduced semen quality characterised by low sperm count (oligozoospermia), impaired motility of the sperm (asthenozoospermia), decreased vitality of the sperm (necrozoospermia), impaired morphology of the sperm (teratozoospermia) or a combination of these parameters termed oligoasthenoteratozoospermia and azoospermia [2]. In total, 90% of male infertility problems are linked to sperm count, and there is a link between reduced sperm count and reduced semen quality [6]. In addition, a cohort study of South African men of reproductive age indicated in 34.2% of the sub-fertile men, 11.9% had severe azoospermia [25].

Several risk factors can disrupt semen quality leading to semen-related abnormalities [6]. Oxidative stress has been linked to male infertility due to enhanced reactive oxygen species

(ROS) production or decreased antioxidants. ROS leads to infertility through two fundamental mechanisms. Firstly, by inducing sperm membrane damage, which decreases sperm motility and its fertilisation capacity, as well as by altering the DNA molecule of sperm, resulting in the passage of defective paternal DNA on to the conceptus [7].

3. Oxidative Stress

Oxidative stress (OS) refers to an imbalance between the production and accumulation of ROS and the biological system's ability to detoxify these ROS in cells and tissues [26]. These ROS are radical and nonradical oxygen species that form through partial oxygen reduction within the mitochondria [27]. Oxidative stress contributes to multiple pathological conditions and diseases [28]. For example, moderate oxidative stress may cause cell dysfunction and altered behaviour, such as accelerated senescence, abnormal proliferation, dysregulated inflammatory responses and cell tumorigenesis. In contrast, high OS usually causes cell death (e.g., oncosis, apoptosis and autophagy) [29].

Cells have an antioxidant defence system that is made up of enzymes (e.g., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and non-enzymatic molecules (e.g., ascorbic acid, tocopherol, carotene) that can neutralise or scavenge ROS [30]. However, with the abundance of exogenous and endogenous sources of ROS, reproductive cells, in particular, lose the ability to balance the levels of oxidants and antioxidants over time, leading to damage to cellular constituents, such as lipids, DNA and proteins [31,32], which further complicates the functions of the reproductive cells and lead to male infertility [33].

For instance, OS is a significant cause of sperm cell dysfunction and contributes to the aetiology of male infertility due to the impairment of both spermatozoa's structural and functional integrity [34,35]. It disrupts the integrity of the DNA because of concurrent damage to proteins and lipids present in the sperm cell plasma membrane, affecting cell membrane fluidity and permeability [36]. The presence of high levels of DNA damage in human spermatozoa has been correlated with adverse clinical outcomes, including recurrent pregnancy loss, dominant genetic disorders and infertility.

Oxidative stress affects sperm function in two ways: by damaging the sperm nuclear and mitochondrial DNA (mtDNA), which is associated with shorter telomere length, formation of the oxidative base adduct 8-hydroxy-deoxyguanine (8-OHdG) and fragmentation of mitochondrial DNA; or by damaging the sperm plasma membrane and thus affecting sperm motility and its ability to fuse with the oocyte often leading to genome hypermutability, genetic instability, single-strand and double-strand breaks, aberrant DNA damage, Leydig cell dysfunction and finally leading to infertility (see Figure 1).

Furthermore, the risk factors that increase ROS in the male genital tract can create an imbalance in the production of oxidants and the scavenging capacity of antioxidant enzymes, consequently leading to OS, as illustrated in Figure 1. In addition, high levels of ROS may disrupt the hormonal balance that regulates male reproductive functions by acting on the hypothalamic–pituitary–gonadotropic (HPG) axis, thus reducing the secretion of LH and FSH from the anterior pituitary gland [37]. The reduced LH secretion results in failure to stimulate Leydig cells to produce sufficient testosterone [38], while reduced FSH negatively affects the release of androgen-binding protein (ABP), which helps in concentrating testosterone. This causes an overall decrease in Leydig cells' function and, in turn, affects the proteins mediating cholesterol uptake into the mitochondria, such as the steroidogenic acute regulatory (StAR) protein, or by increasing concentrations of inflammatory cytokines [39], and consequently decreasing circulating testosterone [40], which results in unregulated spermatogenesis and suppression of sexual behaviour [7].

In addition, obesity results in the excessive production of ROS by stimulating the adipocyte cells to produce leptin, the critical regulatory adipokine [41]. The increased leptin secretion may also decrease testosterone production by the Leydig cells through altering the endocrine regulation [42], which is mediated primarily through the hypothalamic–pituitary–gonadal (HPG) axis [43], which affects the release of hypothalamic GnRH,

FSH and LH. This will activate OS through cellular metabolisms, negatively impacting the differentiation processes of germ cells. The obesity-induced testicular OS explains this scenario.

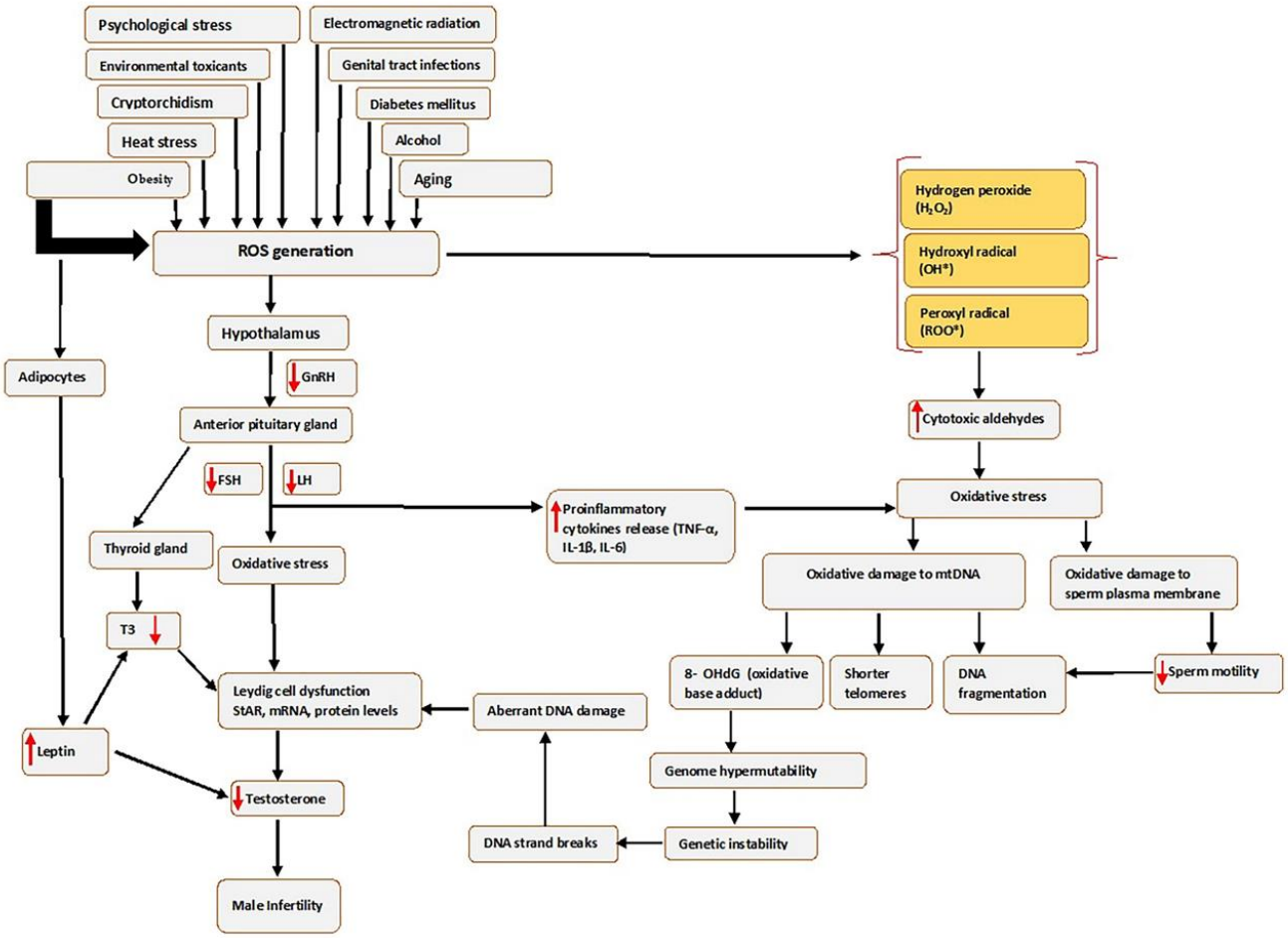

Figure 1. Causes of seminal oxidative stress and oxidative DNA damage. Various factors can lead to or affect the generation of reactive oxygen species (ROS) in the male germ line, which creates oxidative stress. Abbreviations: FSH-Follicle stimulating hormone; IL-6-interleukin 6; LH-luteinizing hormone; mRNA-messenger RNA; ROS, Reactive oxygen species; StAR-steroidogenic acute regulatory protein; TNF-α-Tumour necrosis factor alpha; T3, Triiodothyronine; 8 OHdG-8-hydroxy-2′-deoxyguanosine. Red arrows pointing up indicates increase; red arrows pointing downwards indicates a decrease.

Moreover, obesity may induce testicular OS via other potential mechanisms, such as increased fatty acid oxidation in mitochondria and peroxisomes by adipose tissue, which may lead to a higher generation of ROS. Thus, more elevated ROS mediates oxidative damage to biomolecules that include lipids, proteins and DNA. These cause oxidation of polyunsaturated fatty acids in the sperm membrane, loss of the mitochondrial membrane potential and single- and double-strand sperm DNA fragmentation (SDF) [44]. OS may also impact the hypothalamic–pituitary–thyroid (HPT) axis by decreasing the secretion of triiodothyronine (T3) and triiodothyronine (T4). Reduction in T3 lowers the levels of StAR, mRNA and protein in Leydig cells and reduces the generation of testosterone [45].

Spermatozoa are susceptible to OS because their plasma membrane contains an abundance of polyunsaturated fatty acids (PUFAs). The PUFAs in sperm are required to create fluidity, which is crucial for sperm motility, acrosome reaction and egg fertilisation [46]. An increase in unsaturated fatty acid content is associated with ROS generation that results in a decline in sperm motility [47] either through the ability of H_2O_2 to diffuse across the membranes and inhibit the activity of several enzymes crucial for the sperm movement [48],

or through inhibition of phosphorylation of axonemal proteins and subsequent sperm immobilisation [49]. High levels of ROS in the spermatozoa perpetuate a lipid peroxidation (LPO) cascade and ultimately drive these cells into a state of oxidative disintegration of DNA and proteins [50].

3.1. DNA Damage in Reproductive Cells

Reactive oxygen species cause various types of damage to DNA [1] due to their ability to oxidise the guanine base, which yields a pre-mutagenic 8-oxo-7,8-dihydroguanine (8-oxoG) [51]. 8-oxo-7,8-dihydroguanine (8-oxoG) can convert into single or double-strand breaks (SSBs or DSBs) [52]. DNA double-strand breaks induce several chromatin changes in the promoter, initiating permanent gene silencing in a small fraction of the repaired genes [53]. In the Leydig cells, the exogenous sources of ROS, such as heavy metals, particularly cadmium, showed that DNA damage caused by the accumulation of ROS might affect the expression and catalytic reactions of steroidogenic enzymes, such as 3βB-HSD, which is crucial for testosterone synthesis along with 17βB-HSD and P450c17A [54]. In addition, DNA damage in male germ cells impairs spermatogenesis, and mitochondrial DNA damage in sperm cells negatively affects motility associated with male infertility [55].

3.2. Lipid Peroxidation in Reproductive Cells

Lipid peroxidation (LPO) refers to the process whereby oxidants such as ROS attack the lipids, particularly the PUFAs, that involve hydrogen removal from carbon [56]. The LPO produces lipid hydroperoxides (LOOHs) as main products, and aldehydes, such as malondialdehyde (MDA), propanal, hexanal, and 4-hydroxynonenal (4-HNE), as secondary products [57]. In addition, lipid hydroperoxides may decompose in vivo through one-electron reduction and participate in initiation or propagation steps, induce new lipid hydroperoxides, and feed the lipid peroxidation process. All these mechanisms can contribute to peroxidative damage induction and loss of cell viability with increasing 7-OOH concentration in reproductive cells [58]. In spermatozoa, MDA molecules penetrate the cell membrane structure and impair the symmetric distribution of lipid membrane components. Lipid peroxidation disrupts the middle section of the sperm cell and causes loss of acrosome capacity for fertilisation [59].

3.3. Protein Oxidation in Reproductive Cells

Oxidative stress also causes damage to proteins, which involves modification of amino acids in a site-specific manner, fragmentation of the peptide chain, aggregation of the cross-linked reaction products, alteration of the electric charge, inactivation of an enzyme and susceptibility to proteolysis [26,56], which could disrupt enzymes responsible for steroidogenesis within the Leydig cells [33]. In addition, proteins can be damaged through post-translational modification processes such as oxidation facilitated by ROS [60]. For example, a study by Diemer et al. [61] demonstrated that ROS inhibited StAR protein post-transcriptionally in MA-10 Leydig cells, which inhibits cholesterol transport into the mitochondria and inhibits steroidogenesis.

4. *Moringa oleifera*

The *M. oleifera* tree is mainly cultivated for its various uses as an essential herb due to its nutraceutical and medicinal properties [62]. The different parts of the tree, such as the roots, flowers, fruits, seeds and leaves, are traditionally used to treat abdominal tumours, hysteria, scurvy, paralysis, helminthic bladder, prostate problems, sores and skin infections [19]. In addition, its leaves are commonly used as they contain many bioactive compounds such as nutrients and phytonutrients [63]. The properties of *M. oleifera* leaves are summarised in Figure 2.

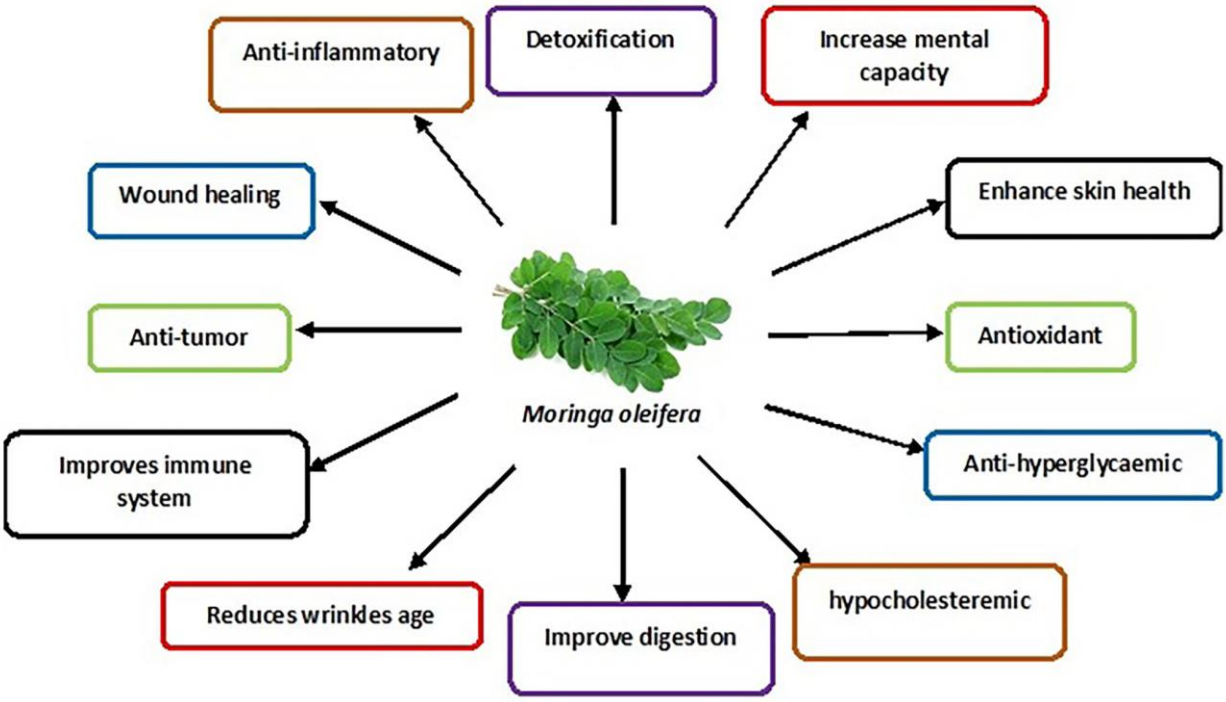

Figure 2. Schematic diagram showing various medicinal uses of *M. oleifera* leaves.

These nutrients include proteins, vitamins (E, C, beta-carotene, B-6), minerals (calcium, phosphorus, magnesium etc.) and fatty acids [64]. The intrinsic bioactive phytonutrients include flavonoids, phenolic acids and glycosides. Other groups include saponins, alkaloids, tannins, isothiocyanate and glucosinolate [17]. The flavonoid group mainly include quercetin and kaempferol, with a concentration of up to 137.81 and 106.75 mg/g, respectively, found in the *M. oleifera* leaves. The phenolic acid in the *M. oleifera* leaves includes ferulic acid, gallic acid, vanillic and ellagic acid, with chlorogenic acid being the most abundant [14,65].

M. oleifera leaves show significant protective effects against many diseases [16] and the widely persistent environmental toxins that disrupt cellular metabolic function [66]. *M. oleifera* is even used to treat neuro-dysfunctional diseases such as Alzheimer's, ischaemic stroke and epilepsy [14]. Studies have also confirmed its potential treatment in chronic diseases such as diabetes mellitus (insulin resistance and hyperglycaemia) [67] and high blood pressure [64]. In addition, *M. oleifera* is a preventive strategy for various conditions and diseases, e.g., preventing testosterone-induced benign prostate hyperplasia [67]. Furthermore, *M. oleifera* has been used to enhance male sexual functions, including libido, erectile dysfunction and testicular injury [14,68], the symptoms of primary hypogonadism that mostly leads to male infertility.

5. Studies on the Effects of *M. oleifera* Leaf Extracts on Male Reproductive Function

5.1. Sperm Parameters

M. oleifera significantly affects the improvement of sperm characteristics; this is demonstrated by the Egyptian buffalo bulls fed with 4% and 8% concentrations of *M. oleifera* leaves in their diet [69]. Furthermore, *M. oleifera* has been shown to reduce sperm abnormality in male Swiss mice fed with 4% and 8% *M. oleifera* in their diet [70]. In addition, rabbit bucks administered with 200 and 400 mg/kg B.W. showed an increase in sperm viability, sperm membrane integrity and sperm motility [20]. Finally, Bali bulls fed with a diet containing 15% *M. oleifera* demonstrated increased sperm motility [14].

5.2. Hormonal Levels

M. oleifera significantly increased serum testosterone levels and gene expressions for luteinizing hormone and follicle-stimulating hormone in Rabbit bucks [20]. However, in buffalo bulls [69] and Bali bulls [14], there is only an increase in testosterone with no effect on LH and FSH. Contrastingly, *M. oleifera* leaf extract increased the concentrations of FSH and LH in New Zealand white (NZW) rabbit bucks. The extracts also increased semen volume, sperm count and motility [71].

5.3. Testis

Administration of *M. oleifera* improved the testicular structure of rabbit bucks. High doses of moringa leaves led to germinal hyperactivity of cells, such as the increase in the number of cells at all stages of spermatogenesis, increased density of spermatids, prominence of spermatozoa in the lumen of seminiferous tubules and wider interstitial areas with normal Leydig cells compared to non-supplemented rabbit bucks [20]. Additionally, a significant increase in seminiferous tubule diameter, height and epithelium area, type A spermatogonia and spermatogenic efficiency, as well as the increased number of Sertoli cells and total spermatogenic cells was demonstrated following treatment in male rats [72]. In addition, a significant increase in relative testis, epididymis and seminal vesicle weight, and diameter of the seminiferous tubules was observed in male-treated mice [21].

5.4. Male Reproductive Cells: Leydig Cells and Sperm Cells

M. oleifera leaf extracts (10, 50, 100, 250, 500 and 1000 ug/mL) on TM3 Leydig cells increased the levels of testosterone under stimulatory conditions of hCG. However, the testosterone increase was seen only in 500 and 1000 ug/mL concentrations under basal conditions [22]. In addition, *M. oleifera* also demonstrated its antioxidative effects by increasing the glutathione concentration in the cells exposed to 250 ug/mL of the extract [22].

M. oleifera leaf extracts were also observed on sperm cells with varying concentrations (0.625; 6.25; 62.5; 625 ug/mL). The findings indicated that *M. oleifera* inhibited the formation of sperm intracellular ROS at 62.5 and 625 ug/mL, reduced the percentage of sperm with DNA fragmentation and increased the percentage of incapacitated and intact acrosome spermatozoa at 625 ug/mL [73].

6. Effects of *M. oleifera* Leaf Extracts on Male Reproductive System Constituents following Exposure to Male Infertility Risk Factors

Table 1 summarises the effect of the *M. oleifera* on the male reproductive system following exposure to various infertility risk factors.

Table 1. Effects of *M. oleifera* leaves on damage induced by male infertility risk factors in the male reproductive system.

Male Infertility Risk Factors	Oxidative Stress Parameters	Hormonal Levels	Sperm Parameters	Gene Expression	Testicular Histology Examination	Authors
Heat stress	↑ TAC, ↓ GST	↑ Testosterone levels	↑ sperm quality (sperm concentration with intact acrosome, total sperm output, motility and viability).		↑ the normal morphology and number of the tubular epithelial cells, germinal Sertoli cells, spermatogonia, spermatocytes, early spermatids, late spermatids and spermatozoa. ↓ Leydig cells and Sertoli cells pyknosis.	[74–77]
Electromagnetic radiations	↓ MDA, ↑ SOD and ↑ CAT	↑ Serum testosterone levels	↑ Epididymal sperm count and motility. ↓ sperm defects (pyriform head, detached head, coiled tails and multiple abnormalities).		↓ degeneration in some parts of the seminiferous tubules and ↑ the number of Leydig cells.	[78–80]

Table 1. *Cont.*

Male Infertility Risk Factors	Oxidative Stress Parameters	Hormonal Levels	Sperm Parameters	Gene Expression	Testicular Histology Examination	Authors
Environmental toxicants	↓ testicular tissue GST activity and MDA. ↑ GPx. ↑ SOD, ↑ CAT	↑ Testosterone. ↑ serum FSH and LH	↑ sperm motility, ↑ sperm viability, ↑ sperm count and ↓ sperm abnormalities.	↑ *StAR* gene, ↑ cytochrome p450o17 subfamily A (*CYP17A*), ↑ *CYP11A1* and ↑ *HSD17B3* genes of the steroidogenic hormones. ↓ expression of *CYP19A1* aromatase gene.	↓ weight of the reproductive organ. ↑ elongated spermatids and spermatozoa, ↑ the epididymal histological integrity, ↑ sperm density and ↓ congestion and interstitial oedema in the seminal vesicle and prostate gland.	[81–85]
Obesity	↓ MDA, ↑ SOD, ↑ CAT and ↑ GSH	↑ Testosterone, ↑ FSH and ↑ LH	↑ sperm count, ↑ sperm motility, ↓ immotile spermatozoa, ↓ primary and secondary sperm abnormalities.			[86,87]
Diabetes	↓ TBARS, ↑ SOD, ↑ CAT, ↑ GSH and ↑ Ascorbic acid	↑ LH, ↑ FSH and ↑ testosterone	↑ sperm count and ↑ sperm mobility.		↑ mean number of spermatogonia in the seminiferous tubules, ↑ population of the round (normal) spermatids. ↑ diameter of the seminiferous tubules, ↑ nuclear diameter of the Leydig cells and ↑ weight of the epididymis.	[88,89]
Therapy and medications (HAART)		↑ FSH, ↑ LH and ↑ testosterone	↓ the sperms with abnormal morphology, ↑ semen quality (sperm progressivity, sperm volume, sperm motility, sperm count and viability).		↑ testicular weight. ↑ normal testicular morphology.	[90]
Alcohol					↑ myoid living cells, spermatogenic living cells, spermatogonia, spermatocytes, spermatids and spermatozoa and lumen filled with semen.	[91]
Psychological stress		↓ PDE-5 activity, ↑ testosterone and ↓ corticosterone			↓ Reduced Leydig cells disruption ↑ interstitial Leydig cells and ↑ spermatozoa in the seminiferous tubule lumen.	[92]
Aging			↑ sperm count and ↑ normal sperm morphology.			[93]
Cryptorchidism	↓ GGT activity, ↑ SOD activity and ↓ MDA	↑ testicular testosterone	↑ sperm count, ↑ germ cell count.		↑ testicular weight, ↓ the abnormal appearance of the testes. ↓ abnormal appearance of the seminiferous epithelium.	[94,95]
Food			↑ sperm motility.			[96]

↑ = Increase; ↓ = Decrease. Abbreviations: CAT, catalase; GST, glutathione -S-transferase; GGT, gamma-glutamyl transferase; HAART, highly active antiretroviral therapy; FSH, follicle stimulating hormone; GSH, glutathione; GPx, glutathione peroxidase; LH, luteinizing hormone; MDA, malondialdehyde; PDE-5, Phosphodiesterase-5; SOD, superoxide dismutase; StAR, steroidogenic acute regulatory protein; TAC, total antioxidant capacity; TBARS, thiobarbituric acid reactive substances.

6.1. Environmental Toxicants and Heavy Metals

Environmental toxicants have adverse effects on male reproductive potential. For example, these contaminants affect the quality of the semen. In addition, they disrupt the Leydig and Sertoli cells, disrupting DNA integrity, hormone biosynthesis, gene expression and epigenetic modifications [7]. These contaminants also reduce testis size, leading to testicular dysfunction [11]. Table 2 summarises the respective treatment of *M. oleifera* on different models, length and mode of treatment.

Table 2. Male infertility risk factors studied, *M. oleifera* extract, model of the study, length and mode of treatment.

Model of the Study	Concentration of Moringa Leaf Extracts	Length of Treatment	Mode of Treatment	Male Infertility Risk Factors	Authors
Adult male Wistar rats	100, 200 and 400 mg/kg ethanolic leaf extracts (daily)	14 days	Intragastric gavage	Heat stress	[76]
V-line Rabbit bucks	50, 100 and 150 mg/kg B.W. ethanolic leaf extracts (daily)	12 weeks	Oral	Heat stress	[74]
White rats (Rattus norvegicus)	100, 200 and 400 mg/kg *M. oleifera* leaf extracts (daily)	14 days	Gastric gavage	Heat stress	[77]
Rabbit bucks	2.5%, 5% and 7.5% *M. oleifera* diet (daily)	24 weeks	Oral	Heat stress	[75]
Adult Sprague Dawley male rats	500 mg/kg *M. oleifera* extracts (daily)	56 days	Intragastric gavage	Cadmium chloride-induced toxicity	[82]
Adult male Wistar rats	500 mg/kg and 750 mg/kg extracts (daily)	35 days	Oral gavage	Cadmium-induced oxidative stress	[85]
Male Wistar rats	50 mg/kg *M. oleifera* leaf extracts (daily)	8 weeks	Oral	Chromium-induced toxicity	[81]
Mature albino male rats	200 mg/kg leaf extracts (daily)	6 weeks	Oral	Melamine-induced testicular toxicity	[84]
Adult male Wistar rats	100 mg/kg leaf extracts (daily)	16 days	Oral	Lead-induced testicular damage	[83]
Male Sprague-Dawley rats	200 mg/kg leaf extracts (daily)	8 weeks	Oral	Electromagnetic radiations	[78]
Male Wistar rats	200 mg/kg leaf extracts (daily)	1 month	Gavage	Radiofrequency-Electromagnetic radiation (RF-EMR)	[79]
White Albino male rats	200 mg/kg leaf extracts (daily)	6 days	Oral	Electromagnetic field	[80]
Adult male Wistar albino rats	300 mg/kg leaf extracts (daily)	14 weeks	Oral	Obesity (High-fat diet-induced obesity)	[86]
Young men, Omnivores	350 mL of 10 g MLP, diet (10–30 g L.P.) daily	14 weeks	Oral	Obesity	[87]
Swiss albino mice	200 mg/kg leaf extracts (daily)	21 days	Oral	Diabetes	[89]
Adult Wistar albino rats	100, 250 and 500 mg/kg (daily)	60 days	Oral	Diabetes	[88]
Adult male Wistar rats	200 and 300 mg/kg (daily)	28 days	Oral	Therapy and Medication (HAART therapy)	[90]
Male Wistar rats	200 mg/kg and 500 mg/kg (daily)	3 weeks	Oral	Cryptorchidism	[95]
Male Albino rats	200 mg/kg (daily)	2 weeks	Oral	Cryptorchidism	[94]
Male mice	1200, 600 and 300 mg/kg (daily)	6 weeks	Oral gastric swab	Food additive	[96]
Male Wistar rats	10 mg/kg, 50 mg/kg and 250 mg/kg (daily)	7 days	Oral	Psychological stress-induced	[92]
Male Wistar rats	400 mg/kg (daily)	2 weeks	Gastric gavage	Alcohol	[91]
Male Wistar rats	50 mg/kg ethanol extract of Moringa leaves dissolved in 0.5 mL of 0.5% carboxymethylcellulose (CMC) diet	30 days	Oral	Ageing	([93]

Some environmental toxicants damage the reproductive system via the induction of oxidative stress [11]. *M. oleifera* leaf extracts in animal models exposed to environmental toxicants have demonstrated their antioxidative capacity by significantly increasing superoxide dismutase (SOD) and catalase (CAT) levels and reducing malondialdehyde (MDA) molecules [85]. *M. oleifera* also increased the testicular levels of glutathione peroxidase (GPx) [81,84] and reduced glutathione S-transferase (GST) activity [82].

M. oleifera leaf extract increased the concentration of serum testosterone, FSH and LH [84,85]. *M. oleifera* also increased testicular testosterone levels in male rats [81,82]. Additionally, *M. oleifera* leaf extracts upregulated CYP11A1 and HSD17B3 genes of the steroidogenesis hormones downregulated CYP19A1 aromatase gene [84] and upregulated StAR and cytochrome p450c17 subfamily A (CYP17A) genes [82].

M. oleifera leaf extracts also demonstrated its beneficial effects on semen quality of male rats by increasing the percentage of viable and normal spermatozoa [85], sperm count, sperm motility and reduced sperm abnormality [82,84] and reduced sperms with morphological damage [81].

M. oleifera leaf extracts in male rats exposed to environmental toxicants increased the weight, volume of the testis [81] and gonadosomatic index [84]. The extracts also preserved the Leydig cells and prevented the disruption of the seminiferous tubules by heavy metals [83]. More so, *M. oleifera* leaf extract increased elongated spermatids and spermatozoa in the seminiferous tubules. The epididymal histological integrity and sperm density were also improved, while congestion and interstitial oedema reduction were noted [82]. Lastly, *M. oleifera* leaf extract reduced the vacuolisation of germinal epithelium [84].

6.2. Electromagnetic Radiations

There is evidence the long-term exposure to electromagnetic radiation causes an increase in the production of reactive oxygen species (ROS) in organs of the reproductive system [7]. These radiations have been reported to reduce sperm motility, cause sperm defects, increase peroxidation, histological aberrations in the testicular tissue and testicular tissue atrophy [97]. Additionally, these radiations affect the HPA axis by increasing the adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary gland, which stimulates the production of cortisol form the adrenal cortex [7]. Cortisol then suppresses testosterone secretion from the Leydig cells [98].

Studies on the effects of *M. oleifera* leaf extracts on male Wistar rats exposed to electromagnetic radiation have demonstrated a reduction in oxidative damage in vivo (see Table 1), as indicated by the reduced MDA, increased CAT and SOD activities [78,79]. In addition, *M. oleifera* leaf extracts also increased serum testosterone levels [79], increased sperm parameters (epididymal sperm count and motility) and reduced sperm defects (pyriform head, detached head, coiled tails and multiple abnormalities) [78].

In another study, *M. oleifera* leaf extracts on adult white albino male rats exposed to an electromagnetic field reduced the degeneration of some parts of the seminiferous tubules. As a result, they increased the number of Leydig cells [80]. In addition, the testes' morphology was almost normal, as seen in the histoarchitecture of the seminiferous tubules and Sertoli cells [80].

6.3. Heat Stress

Heat stress on the testis enhances the production of ROS and reduces the enzyme defence system activities, reducing the semen quality of males. Heat stress also increases NADPH oxidase activity and disrupts the homeostasis of mitochondria of the reproductive cells [7]. Additionally, heat stress negatively impacts the male HPG-axis through impairment of the normal release of hypothalamic GnRH and LH and FSH from the pituitary gland, decreasing serum circulating testosterone. In the testis, heat stress leads to Leydig cell apoptosis and the reduction in testicular testosterone synthesis [7].

A study conducted on human subjects to determine the correlation between heat stress and semen quality among male workers in the steel industry found that workers exposed to heat had poor semen quality compared to unexposed subjects [11]. Additional studies observed that increased scrotal temperature in fertile men decreases sperm quality and temper with sperm morphology [11].

A study on the effects of *M. oleifera* extract (100, 200 and 400 mg/kg) on adult male Wistar rats exposed to heat stress for 14 days indicated an improvement in tubular epithelial cells, germinal Sertoli cells, spermatogonia, spermatocytes, early spermatids, late spermatids and spermatozoa, which led to an improvement of the histopathology of the testis [76].

Another study on Spanish maternal line (V-line) rabbit bucks showed an increase in hormonal testosterone levels and increased sperm quality, including increased sperm concentration with intact acrosome, total sperm output, motility and viability [74]. Additionally, rabbit bucks orally supplemented with *M. oleifera* at different concentrations (50, 100 and 150 mg/kg) for 12 weeks had a lower rectal temperature [74]. In White rats exposed to hot temperatures, *M. oleifera* prevented the harmful effects of high temperature on Leydig cells and Sertoli cells, indicated by the reduction of testicular cell pyknosis [77].

6.4. Obesity

Obesity affects the male reproductive function by increasing the leptin hormonal level, which activates the HPG axis by stimulating the secretion of GnRH, LH and FSH. However, leptin can also negatively affect the male reproductive function by suppressing testosterone secretion through its receptor isoforms in the gonads [7].

The peripheral adipose tissue in obese individuals can increase the aromatisation of serum testosterone into oestradiol, suppressing the HPG axis through negative feedback

inhibition [99]. Increased oestradiol is commonly seen in obese people, and reduces sex hormone-binding globulin (SHBG), FSH and inhibin B [100]. Hence, obesity is positively correlated with reduced testicular volume, semen quality, spermatogenesis, hypogonadism and erectile dysfunction [100,101]. Testicular damage in patients with high body mass index (BMIs) includes alterations in sperm parameters, notably reduced sperm count, concentration, motility, progressiveness, disturbed sperm morphology and sperm DNA fragmentation [7,101].

Some damaging effects of obesity on male reproductive functions may be attributed to the accumulation of ROS. Increased levels of ROS in obese people and obesity-induced animal models are correlated with impairment of testicular function and the HPA-axis [7]. In induced obese rats, *M. oleifera* leaf extracts reduced the damaging effects of oxidative stress by lowering MDA concentration, which is a marker of lipid peroxidation. In addition, they increased antioxidant enzymes such as SOD, CAT and GSH [86].

Additionally, *M. oleifera* leaf extract (300 mg/kg) increased serum testosterone, FSH and LH, sperm count and sperm motility. In contrast, immotile spermatozoa and primary and secondary sperm abnormalities decreased in induced obese rats [86]. Therefore, *M. oleifera* leaf extracts could be a potential solution to fertility problems that are associated with obesity.

6.5. Diabetes Mellitus

It is estimated that about 382 million people globally are living with diabetes mellitus [102]. Diabetes mellitus can result in various medical complications; however, the most common complication is male fertility [103]. Studies have shown that 51% of diabetic males show signs of infertility, which indicates that diabetes mellitus leads to alterations in male reproductive function [102,103].

The serum levels of insulin and glucose in diabetic males affect the maturation of sperm cells on the acrosome and plasma membrane [102], which subsequently affects sperm viability, motility and morphology [104]. Insulin and glucose levels also play essential roles in the sugar movement in sperm cells, which indirectly control motility during capacitation and fertilisation [103].

Glucose is a crucial requirement in spermatogenesis and acrosome reaction and is diffused into the mammalian testicular cells via a concentration gradient utilising specific enzyme transporter known as glucose transporters (GLUTs). The family of these glucose transporters comprises 14 members, categorised into groups of three based on similarities of their sequences. Therefore, reduced insulin levels result in the inactivity of GLUT2 function, which results in low glucose levels and subsequent low energy, thereby reducing the rate of spermatogenesis and motility [105]. Furthermore, *M. oleifera* leaf extracts in male animal models exposed to diabetic conditions show its ameliorative effects on the male reproductive system.

M. oleifera leaf extracts on male albino Swiss mice exposed to diabetic conditions for 21 days increased sperm count and motility [89]. Diabetes mellitus can affect spermatogenesis by indirectly affecting the epithelium of the seminiferous tubules and reducing FSH [7]. Reduced FSH result in poor activation of Sertoli cells, which leads to impaired spermatogenesis [103]. In some cases of diabetes mellitus (T2DM), there is inhibition of the HPG-axis, which leads to low production of FSH and LH in response to GnRH, as well as reduced testosterone levels as a result of diminishing Leydig cells of the testis [7]. Fortunately, *M. oleifera* has an androgenic effect on the male reproductive system in vitro [22]. *M. oleifera* leaf extract on male Wistar rats induced with diabetes increased LH, FSH and testosterone levels, which were reduced by the damaging effects of diabetes mellitus [88].

Oxidative stress is regarded as the leading cause of reproductive deficiency in patients with diabetes mellitus [106]. Diabetes mellitus induces oxidative stress through elevated levels of ROS, lipid peroxidation [102] and reduced total antioxidant capacity (TAC) in the seminal fluid [107]. This increases the DNA fragmentation of sperms, alteration in ATP synthesis of the mitochondria and decreases sperm motility [102]. *M. oleifera* leaf extract

reduced lipid peroxidation and increased the capacity of the antioxidant defensive system by increasing SOD, CAT, GSH and ascorbic acid in the testes of diabetes mellitus-induced rats. This indicates that *M. oleifera* may ameliorate the effects of diabetes on male fertility through the enhancement of the antioxidant defence system of the testis [88].

Hyperglycaemic conditions have also been correlated with decreased germ cell population, epithelial cell clusters, reduced stereocilia and lipid vacuolisation in the testes [88]. A reduced seminal vesicle weight, mass and weight of the testis, number of Leydig cells, diameter of the seminiferous tubules and height of the germinal epithelium in hyperglycaemic conditions were also shown in another study [102]. *M. oleifera* showed ameliorative effects on the testicular histology of diabetic rats by increasing the mean number of spermatogonia in the seminiferous tubules and increasing the population of the round (normal) spermatids. *M. oleifera* also increased the diameter of the seminiferous tubules, the nuclear diameter of the Leydig cells [88] and the weight of the epididymis [89].

6.6. Therapy and Medications: Highly Active Antiretroviral Therapy

The increment in global access to highly active antiretroviral therapy (HAART) has significantly improved the management of HIV/AIDS [108]. HAART positively impacts AIDS patients' survival with a considerable decline in morbidity and a reduction in new HIV cases. HAART involves a combination of two or more antiretroviral drugs to treat HIV infection. The drugs mainly combined are nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) and protease inhibitors (P.I.s) or non-nucleoside reverse transcriptase inhibitors (NNRTIs). The first line of HIV-1 defence contains a combination of two NRTIs (tenofovir and emtricitabine) and NNRTIs (efavirenz) [109]. Despite its high success rate, chronic use of NRTIs is followed by many adverse effects. Clinical practice and experimental studies have shown the negative consequence of the long-term use of HAART on male fertility [110]. The use of HAART leads to extensive apoptotic degeneration of germinal cells and metabolic disruption of testicular cells. HAART is also extensively involved in generating free radicals leading to oxidative stress damage. Studies on the animal model showed HAART administration reduced sperm parameters by distortion of testicular cell architecture and induction of DNA fragmentation of the testicular tissue. Hence, disturbing spermatogenesis and steroid synthesis [111]. It has also been indicated that exposure to HAART causes degeneration of the seminiferous tubules, spermatids necrosis and deformation of spermatocytes [112].

Long-term exposure to HAART in patients with HIV-1 impairs sperm parameters by causing mitochondrial DNA depletion [109]. NRTIs prevent the replication of the virus; however, they also cause the depletion of mitochondrial DNA by inhibiting DNA polymerase, which leads to a decrease in the production of proteins involved in the electron transport chain, such as cytochrome c oxidase [113]. Hence, this causes damage to sperm motility and progressiveness [114]. Additionally, in HIV-1-infected men, HAART results in lower ejaculate volume and abnormal morphology of spermatozoa [114]. Impairment of sperm parameters may also be due to increased testicular oxidative stress induced by HAART [112].

M. oleifera increased the levels of FSH, LH and testosterone hormone in male Wistar rats exposed to HAART therapy. *M. oleifera* also reduced the percentage of sperm with abnormal morphology and increased semen quality, characterised by fast sperm progressivity, increased sperm volume, increased motility of sperm and increased sperm count and viability [90]. Additionally, *M. oleifera* (100 and 300 mg/kg) increased the testicular weight and restored the microarchitecture of testicular morphology altered during HAART treatment [90]. Therefore, *M. oleifera* shows predominant effects in alleviating the side effects of HAART therapy.

6.7. Cryptorchidism

Cryptorchidism is characterised by the failure of one or both testes to descend into the bottom of the scrotum. Instead, the testes are located along the usual route of testic-

ular descent, which may have an intra-abdominal, inguinal, suprascrotal or high scrotal position. The abnormal positioning of the testis induces hyperthermia, which is detrimental to the process of spermatogenesis [115]. There is evidence that 10% of infertile men are cryptorchid, and 98% of these cryptorchid men are azoospermic. In patients with cryptorchidism, there is also a prominent depletion of the Leydig cells each month due to insufficient secretion of gonadotropic hormones [116]. There is also impairment of germ cell maturation, reduced sperm cell density and tubular and interstitial damage [117].

The temperature in undescended testicles might influence the testicular environment and spermatogenesis through oxidative stress induction [118]. Fortunately, *M. oleifera* leaves exhibit enormous antioxidant properties. In male Wistar rats induced with bilateral cryptorchidism, *M. oleifera* leaf extract increased SOD activity, reduced GGT activity [95] and the levels of MDA [94]. This leads to increased sperm count, germ cell count, testicular testosterone and testicular weight [95]

Histological evaluation of the testes of cryptorchid rats indicated a widened seminiferous tubule lumen with an absence of spermatozoa strands and degenerative alterations in the epithelium of the seminiferous tubules, which was suggested by indistinct Sertoli cells and loss of germ cells. Administration of a low dose of *M. oleifera* improved the appearance of the testes. At the same time, a high dose of *M. oleifera* attenuated the appearance of the seminiferous epithelium and interstitium with mild degenerative changes characterised by very few tubules with normal germ cell layers visible [95].

6.8. Psychological Stress

Psychological stress has been revealed as one of the causes of idiopathic male infertility, confirmed by studies based on the correlation between stress and impaired semen quality [119]. Psychological stress is explained as a less comfortable surge of emotions coupled with alterations in an individual's biochemistry, physiology and behaviour [120]. These alterations lead to changes in reproductive functions, reducing libido, sexual performance and overall functions of the reproductive system [119].

M. oleifera leaf extracts in male Wistar rats subjected to the 12 h immobilisation of stress for seven days improved sexual performance by decreasing the intromission latency and increasing intromission frequency on the stressed male rats, and also suppressed the activity of monoamine and phosphodiesterase type 5 (PDE-5) [92].

Psychological stress in animals and humans increases corticosterone and cortisol, respectively, and increases apoptosis of the Leydig cells, subsequently reducing testosterone levels; this leads to changes in Sertoli cells and the blood–testis barrier, causing spermatogenesis arrest [7,120]. Treatment of stressed rats with *M. oleifera* reduced corticosterone and increased testosterone levels, number of Leydig cells and spermatozoa. In addition, *M. oleifera* in these stressed rats resulted in a more organised seminiferous epithelium with more interstitial Leydig cells and more spermatozoa in the seminiferous tubule lumen [92].

6.9. Food Additives

In many studies, men who consume healthy food such as fish, fruits, vegetables, legumes, whole grains, and omega-3- and omega-6-fatty acids have increased semen quality as compared to men consuming caffeine, red meat, processed meat, pizza, sugary drinks, and sweets, etc. in their diet [121]. The latter-mentioned foods typically contain monosodium glutamate, a widely used food additive found in many ingredients and processed foods that can be obtained in every market and grocery store [122].

Monosodium glutamate (MSG) has been associated with different toxicities and linked to obesity, metabolic disorders, and detrimental effects on the reproductive organs [122]. Monosodium glutamate toxicity can be linked to its ability to act on the glutamate receptors and release neurotransmitters playing a crucial role in normal pathological and physiological processes [123]. Due to the ability of MSG to influence cells of the reproductive system, MSG can cause sperm alterations, histological alterations, hormonal imbalance, and oxidative damage, which eventually leads to abnormalities in reproductive function [123].

Additionally, MSG leads to sperm membrane dysfunction, sperm DNA damage and sperm motility impairment [124]. These can be attributed to the effects of MSG in the induction of oxidative stress within the testes, marked by increased MDA and reduced GSH [125]. A study on male mice orally administered with monosodium glutamate food additive for 30 days showed a reduction in sperm motility of the rats, which was improved with the administration of *M. oleifera* leaf extracts at 300 and 600 mg/kg doses [96]. Although this is the only study on the effects of *M. oleifera* on monosodium glutamate-induced reduction of sperm quality, *M. oleifera* can serve as a potential treatment for male infertility that might occur as a result of poor diet.

6.10. Alcohol

Chronic abuse of alcohol can eventually lead to atrophy of the testicles, germ cell degeneration, reduced lumen size of the seminiferous tubules, an abundance of lipid droplets and apoptosis of the Sertoli cells [7]. Sertoli cells are the most prone to damage by alcohol due to their cross-links with the Leydig cells, which are also affected by the effects of alcohol, leading to the disruption of male fertility [7]. Male rats exposed to alcohol showed variously atrophied and damaged cells in the seminiferous tubules, depleted spermatogonia, spermatocytes, spermatids, spermatozoa, and the lumen filled with semen, degenerated intratesticular Leydig cells and interstitium [91].

Administration of *M. oleifera* leaves (400 mg/kg) on male Wistar rats exposed to alcohol resulted in the normal histological architecture of the testis indicated by numerous seminiferous tubules containing swollen myoid living cells, spermatogenic living cells, spermatogonia, spermatocytes, spermatids, spermatozoa, the lumen filled with semen and normal interstitial Leydig cells in between the seminiferous tubules [91]. These results demonstrated the protective action of *M. oleifera* leaves and its preventive and reversibility of alcohol-induced testicular injuries.

6.11. Ageing

The ageing process may be correlated with an increase in endogenous sources of ROS, which diminishes the anti-oxidant enzyme activities, and Leydig cells are mainly prone to this effect. Excessive growth in ROS leads to alterations of the DNA and membrane potential in Leydig cells, disrupting the synthesis of testosterone [7]. Ageing also diminishes the number of LH receptors on each of the Leydig cells, leading to the inability of the LH to activate the StAR gene, leading to less production of testosterone [7].

It has already been established that reduced testosterone synthesis leads to the loss of Sertoli cell function in spermatogenesis [7], indicating that the effects of ageing on the Leydig cells may indirectly affect spermatogenesis leading to disruption of sperm count as well as sperm morphology. The study of *M. oleifera* on the sperm count and morphology in old rats (18–19 months old) showed an improvement in sperm count and morphology after oral administration of *M. oleifera*, which indicates its beneficial effects in the treatment of reproductive changes induced by ageing [93].

7. Mechanism of Action of *M. oleifera* Extract on Oxidative Stress and Male Fertility

Moringa oleifera extracts possess anti-oxidative, anti-inflammatory, anti-diabetic, anti-obesity and anti-apoptotic properties [86,126], which have been attributed to its polyphenols, flavonoids (particularly quercetin and kaempferol), phenolic acids, caffeoylquinic acid and isothiocyanates [127,128]. The alkaloids, flavonoids, saponins, triterpenoids/steroids and tannins in *M. oleifera* extract are powerful anti-oxidants that acts to prevent new free radicals and chain reactions and protects the cells from oxidative damage [129]. Figure 3 shows some mechanisms through which *M. oleifera* extract inhibit the damaging effects of oxidative stress, thereby preventing male infertility.

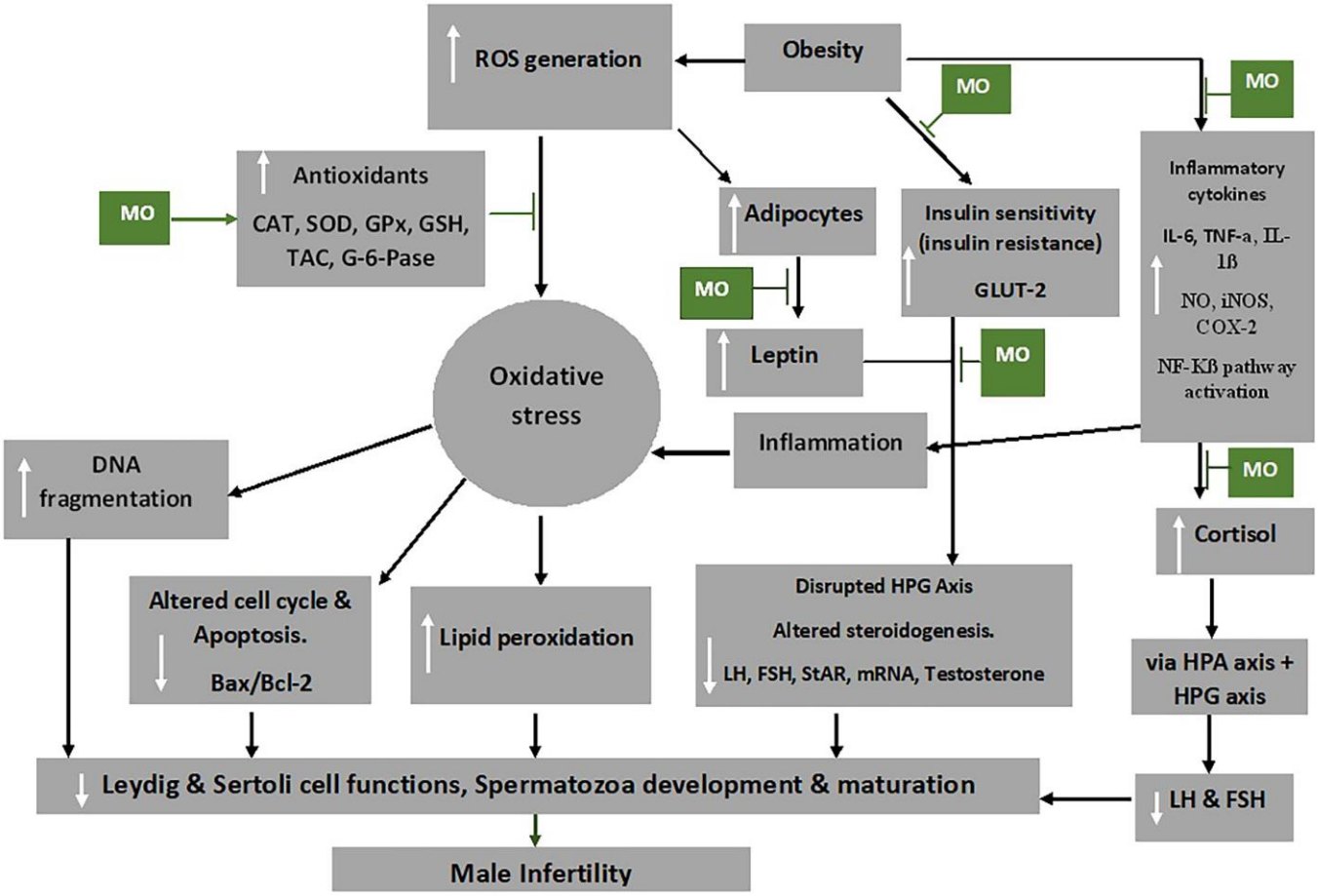

Figure 3. Mechanism of action of *Moringa oleifera* (MO) extract on oxidative stress and male infertility. Abbreviations: CAT-catalase; FSH-follicle stimulating hormone; COX-2-cyclooxygenase -2; GLUT2-glucose transporter 2; G-6-pase-glucose 6-phosphatase; GPx-glutathione peroxidase; GSH-glutathione; HPA-hypothalamic–pituitary–adrenal axis; HPG-hypothalamic–pituitary–gonadal axis; IL-6-interleukin 6; iNOS-inducible nitric oxide synthase; LH-luteinizing hormone; mRNA-messenger RNA; NF-Kβ-nuclear factor kappa-light-chain-enhancer of activated B cells; NO-nitric oxide; ROS-reactive oxygen species; SOD-superoxide dismutase; StAR-steroidogenic acute regulatory protein, TAC-total anti-oxidant capacity; TNF-α-tumour necrosis factor alpha. White arrows pointing up indicates increase; white arrows pointing downwards indicates a decrease. ⊣ Indicated the regulatory/inhibitory role of Moringa oleifera extract.

Obesity activates the adipocytes to further produce leptin and could lead to leptin resistance, which has been shown to inhibit the GnRH neurons due to the suppression of KISS1 neuron activities and increased NPY levels [130]. This, consequently, affects the HPG axis, by the impairment of the release of GnRH, FSH and LH, and ultimately impairs the functions of the reproductive cells, testosterone release and development and maturation of the spermatozoa [131]. Besides the reduction of food intake, the anti-obesity and anti-hyperglycaemic activities of *M. oleifera* are brought about by the reduction of leptin levels by down regulating mRNA expression of leptin and resistin [132]. Additionally, hyperglycaemia may occur due to oxidative stress associated with obesity as it impedes the functioning of insulin and glucose utilisation by peripheral tissue [133]. Flavonoids, particularly quercetin, in the extracts has been shown to act as an apical inhibitor of glucose transporter 2, demonstrating its anti-hyperglycaemic activity [134]. Furthermore, the anti-hyperglycaemic activity of the plant extract is noted by the inhibition of α-glucosidase, pancreatic α-amylase and intestinal sucrose [135].

Inflammatory cytokines, including IL-1β and TNF-α, can increase the production of prostaglandin E2 (PGE-2), nitric oxide (NO), inducible NO synthase (iNOS), cyclooxygenase-2 (COX-2) and microsomal PGE synthase-1 (mPGES-1) as well as their expression in target cells [136]. *M. oleifera* has also been shown to reduce the production of inflammatory cytokines, such as of TNF-α and IL-6 [126], as well as inhibit the expression of RelA, a gene involved in NF-kB p65 signalling during inflammation [137].

Cortisol and leptin, respectively, produce a primary and secondary negative feedback mechanism for the HPA axis [138], which is critical in maintaining equilibrium under stressful conditions. Chronic psychological stress, for instance, can bring about dysregulation of cortisol [139]. The high levels of ROS result in the release of cortisol (stress hormone), which is usually activated by the HPA axis. The HPA axis, through its communication with the HPG axis, decreases the release of LH, FSH and, ultimately, testosterone [38,140]. In addition, the HPT axis is also affected by oxidative stress and consequently decreases T3 production from the thyroid gland and decreases the circulating testosterone through HPT–HPG axes cross-talk [7].

High levels of ROS disrupt the inner and outer mitochondrial membrane by the induction of cytochrome c protein and activation of caspases and apoptosis [141]. *Moringa oleifera* extract down regulates caspase 3 and the activation of pathways of NF-kB and phosphatidylinositide 3-kinase/protein kinase B (P13K/AKT) and suppress testicular apoptosis by down regulating Bax expression [70,142,143], thereby preventing male infertility. NF-kB promotes the transcription of genes engaged in apoptosis of male germ cells, which may result from stimulation of Bax/Bcl2 and initiation of caspases [144]. In addition, it has been demonstrated that mechanisms that reduce ROS production ensure that the Bcl-2 inhibitor gene of apoptosis protects the cells [141].

8. Conclusions

Pre-testicular activities (such as hormonal regulation by the endocrine system), testicular activities (such as spermatogenesis, steroidogenesis, Leydig cell, germ cell and Sertoli cell proliferation) and post-testicular activities (such as ejaculation and erectile activities) can be affected by the disrupted balance between the reactive oxygen species and the anti-oxidant defence system in the male reproductive system. The unregulated generation of reactive oxygen species from endogenous and exogenous sources can interfere with the HPG axis and all pathways of the male reproductive system, leading to a reduction in semen quality, which causes male infertility. However, *M. oleifera* can alleviate all the impacts of reactive oxygen species on the male reproductive system and improve semen quality, increasing libido, erection and ejaculatory function by directly acting on the pathways of male reproductive function or utilising its anti-oxidative, anti-inflammatory, anti-hyperglycaemic and anti-apoptotic properties without toxicities at correct doses. Hence, M. oleifera may be a potential alternative to treating male infertility.

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