

Fluted Pumpkin and Oxidative Stress in Male Reproductive Organs: A Review

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Abstract

Oxidative stress plays a critical role in the pathophysiology of male reproductive dysfunction by impairing spermatogenesis, steroidogenesis, and overall testicular integrity. An imbalance between reactive oxygen species (ROS) and antioxidant defense systems can lead to lipid peroxidation, DNA damage, and apoptosis in reproductive tissues. Fluted pumpkin (*Telfairia occidentalis*), a widely consumed leafy vegetable in West Africa, is rich in bioactive compounds such as flavonoids, phenolics, vitamins (A, C, and E), and essential minerals with strong antioxidant properties. This review synthesizes current experimental evidence on the effects of fluted pumpkin on oxidative stress in the reproductive organs of male rats. Studies indicate that extracts and dietary inclusion of *T. occidentalis* enhance endogenous antioxidant enzymes—including superoxide dismutase, catalase, and glutathione peroxidase—while reducing markers of oxidative damage such as malondialdehyde in testicular tissues. Additionally, improvements in sperm quality, testosterone levels, and histo-architectural integrity of the testes have been reported. These findings suggest that fluted pumpkin exerts a protective effect against oxidative stress–induced reproductive toxicity in male rats. The review highlights the potential of *T. occidentalis* as a natural therapeutic agent for managing oxidative stress–related male infertility and underscores the need for further mechanistic and clinical studies to validate its translational relevance.

Keywords: Fluted Pumpkin, *Telfairia occidentalis*, Reproductive Organ, Antioxidant, Review.

1. Introduction

Male reproductive health is highly dependent on the integrity of the testes and accessory reproductive organs, which are particularly vulnerable to oxidative stress due to their high rate of cell division and abundance of polyunsaturated fatty acids in sperm membranes. Reactive oxygen species (ROS), when produced in excess or insufficiently neutralized by antioxidant systems, disrupt normal spermatogenesis, steroidogenesis, and sperm function. This oxidative imbalance has been implicated in a wide range of male fertility disorders, including reduced sperm count, poor motility, abnormal morphology, and impaired DNA integrity.

In experimental models, especially in male rats, oxidative stress is commonly induced by environmental toxicants, pharmaceuticals, heavy metals, pesticides, and metabolic disturbances. These stressors increase lipid peroxidation, protein oxidation, and DNA damage within the testes and epididymis, leading to degeneration of seminiferous tubules, reduced testosterone synthesis, and altered reproductive hormone profiles. As a result, oxidative stress–based animal models have become essential tools for investigating protective strategies against male reproductive toxicity.

Dietary antioxidants from plant sources have gained increasing attention as potential therapeutic agents due to their safety, availability, and multifaceted biological activities. One such plant is fluted pumpkin (*Telfairia occidentalis*), a leafy vegetable indigenous to West Africa and widely consumed in Nigeria and neighboring countries. Traditionally valued for its nutritional and medicinal properties, *T. occidentalis* leaves and seeds are rich in vitamins (A, C, and E), carotenoids, flavonoids, phenolic compounds, and essential minerals that contribute to its strong antioxidant capacity.

Recent experimental studies have demonstrated that *T. occidentalis* exhibits significant protective effects against oxidative damage in various tissues. In the context of male reproduction, evidence suggests that fluted pumpkin supplementation enhances endogenous antioxidant defenses, preserves testicular histo-architecture, and improves sperm parameters in rat models exposed to oxidative stress-inducing agents. These beneficial effects are attributed to its ability to scavenge free radicals, inhibit lipid peroxidation, and modulate redox-sensitive signaling pathways involved in cell survival and hormone regulation.

Given the growing prevalence of oxidative stress-related male infertility and the reliance on animal models to explore preventive and therapeutic strategies, it is important to critically evaluate the role of *T. occidentalis* in protecting reproductive organs against oxidative damage. Therefore, this review focuses on synthesizing current findings on the effects of fluted pumpkin on oxidative stress in the reproductive organs of male rats, highlighting its mechanisms of action, experimental outcomes, and potential relevance to human reproductive health.

2. Fluted Pumpkin

Commonly known as "ugu" in the traditional Nigerian Igbo language (Akoroda, 1990), it is a tropical plant cultivated in West Africa as a leaf vegetable and for its edible seeds. Common names for the plant include fluted gourd and ikong-ubong (in the Efik and Ibibio languages of Nigeria). *T. occidentalis* is a member of the family Cucurbitaceae and is indigenous to southern Nigeria (Akoroda, 1990). The fluted pumpkin grows in many nations of West Africa but is mainly cultivated in southeastern Nigeria, and it is used primarily in local dishes (soups) and herbal medicines (Okoli et al., 1983). Although the fruit is inedible, the seeds produced are high in protein and fat and can, therefore, contribute to a well-balanced diet. The plant is a drought-tolerant, dioecious perennial that is usually grown on a trellis. *T. occidentalis* is traditionally used by an estimated 30 to 35 million indigenous people in Nigeria, including the Efik, Ibibio, and Urhobo (Akoroda, 1990). However, it is largely used by the Igbo ethnic group of Nigeria (Okoli et al., 1993). Amongst the natives, the fluted pumpkin is noted to have healing properties and was used as a blood tonic to be administered to the weak or ill (Akoroda, 1990). It is also widespread in Southern Nigeria and was an asset to international food trades of the Igbo ethnic group (Akoroda, 1990).

Abarikwu et al. 2022, in their work, revealed that fluted pumpkin has the potential of protecting the testes against atrazine-induced testicular injury in adult male albino Wistar rats. Similarly, Abarikwu et al. (2019) also reported that the aqueous extract of fluted pumpkin could be used as potential adjuvant in chemotherapy to combat the testiculo-toxic effect of busulfan on the testes.

2.1. Classification of *T. Occidentalis*

Kingdom: *Plantae*

Clade: *Tracheophytes*

Clade: *Angiosperms*

Clade: *Eudicots*

Clade: *Rosids*

Order: *Cucurbitales*

Family: *Cucurbitaceae*

Genus: *Telfairia*

Species: *T. occidentalis*

2.2. Nutritional Value

Considered an "oil seed," the fluted gourd is high in oil (30%) (Akoroda, 1990). Shoots of *T. occidentalis* contain high levels of potassium and iron, while seeds are composed of 27% crude proteins and 53% fats (Aiyelaagbe & Kintomo, 2002). The leaves contain a high number of antioxidants and hepatoprotective and antimicrobial properties (Nwanna, 2008). The large (up to 5 cm), dark-red seed is rich in fat and protein and can be eaten whole, ground into powder for a kind of soup, or made into a fermented porridge. The plant contains high amounts of phosphorus, making it useful for keeping off the onset of kidney diseases like kidney stones. It consists of seed part that has high oil content and high amounts of characterized results in anti-oxidative properties such as oleic acid, vitamin A, alkaloids, tannins and linoleic acid, which makes it capable of treating infertility in males by boosting the functionality of the testicles for an increased sperm count (Akanbi et al., 2007).

2.3. Ethno-pharmacological Properties of Fluted Pumpkin

2.3.1. Hematological Activity

The high content of iron and proteins in the leaf of *Telfairia occidentalis* is proof of its potent effect on hematological activities. According to Alada 2000 and Odede et al. (2010), it was observed that there was a dose-dependent increase in all the measured hematological parameters (packed cell volume, red blood cells, white blood cells, and hemoglobin concentration) in rats fed with fluted pumpkin. Osuntoki & Sanusi 2007 reported that human erythrocytes exposed to hypotonic and heat stress were stabilized by the extract of fluted pumpkin in a dose-dependent manner.

2.3.2. Anti-cancer Activity

Scientific reports have shown that fluted pumpkin possesses anti-cancer properties. Okokon et al. (2012) reported that the crude extract of the seed of fluted pumpkin showed anti-cancer property by prominently inhibiting oxidative burst activity in isolated whole blood polymorphonuclear cells (PMNs) and mononuclear cells (MNCs). Ejike and Ezeanyika (2011) documented that the extract of fluted pumpkin reduced the mean relative prostate weight and protein content (mg/tissue) of the rats' prostates and serum prostatic acid phosphatase, thus inhibiting the induction of benign prostatic hyperplasia (BPH) in rats. The leaves of fluted pumpkin showed the presence of phenolic compounds, flavonoids, phytosterols, tannins, saponins, chlorophyll, and glycosides, which have been reported to exhibit its chemo-suppressive activity (Okokon et al. 2012).

2.3.3. Anti-inflammatory Activity

The seed extract of *Telfairia occidentalis* showed significant anti-inflammatory activity against xylene-induced oedema and exhibited a dose-dependent inhibition of pain in acetic acid-induced writhing, formalin-induced hind paw licking, and thermal-induced pain models (Okokon et al. 2012).

2.3.4. Male fertility activity

Fluted pumpkin improved seminal fluid analysis and testes histology in a daily dosage manner, indicating near-complete morphological restoration and accelerated spermatogenesis (Nwangwa et al., 2007). The leaf extract of fluted pumpkin increased sperm motility, sperm viability, and sperm count in rats (Saalu et al., 2010). The seed oil at a dose of 400 mg/kg also improved semen parameters but had no effect on testicular histology, testosterone, and luteinizing hormone level of rats (Akang et al., 2014). Incorporating fluted pumpkin seeds into the diet of selected test rats resulted in a slight reduction in biochemical amputation and an enhancement in testicular secretory capability in rats, indicating that it is effective in preventing synthetic andropause (Ejike et al., 2013). Other components of the plant, such as arginine, vitamin C, and zinc, may also play important roles since studies have shown that nutritional therapies with zinc, vitamin C, vitamin E, and arginine proved to be of benefit in the treatment of male infertility. The beneficial effect of the plant on male fertility may be as a result of the antioxidant components of the plant since antioxidants such as vitamins A, C, and E have been reported to have good protective effects on the testis (Akang et al., 2011).

2.3.5. Hepatoprotective property

Both aqueous and ethanolic extracts of fluted pumpkin leaf have hepatoprotective properties, although the aqueous extract was more effective than the ethanolic extract, which could be attributed to the higher antioxidant activity of the aqueous extract compared to the ethanolic extracts of fluted pumpkin leaves (Oboh, 2005). Fluted pumpkin proved to be a better vegetable in preventing garlic-induced hepatotoxicity compared with the other vegetables used in the study (Oboh, 2006).

2.3.6. Antibacterial activity

The antibacterial activity of the leaf of fluted pumpkin against selected intestinal pathogens was investigated using the agar diffusion technique. The extract showed a higher antibacterial activity against *E. coli*, *S. faecalis*, and *S. typhi*. MIC was 0.5, 5.0, and 500 mg/mL for *E. coli*, *S. typhi*, and *S. faecalis*, respectively (Oyewole et al., 2012). Similarly, the ethanol leaf extract had a higher inhibitory effect against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus sp.* (Oyewole et al, 2012).

2.3. Fluted Pumpkin Seed

The seeds are eaten roasted or boiled and are also sometimes used as soup thickeners (Okoli and Mgbeogu, 1983). The seeds contain 13% oil and are used for cooking, marmalade manufacturing, and cookie formulations (Giami & Barber, 2004). The oil of *T. occidentalis* seeds has a high iodine content and a high content of unsaturated fatty acids when compared to palm oil. The seed oil is also suitable for the manufacturing of soaps, paints, and varnishes (Nworgu et al., 2007). The fermented seeds of fluted pumpkin are used in the production of "Ogiri u gu," a locally made custard (Christian, 2007). Seed residue after oil extraction is also used as animal feed (Ajayi et al., 2005). Fluted pumpkin seeds have been reported to be rich in proteins (Akwaowo et al., 2000). There is, however, a major limitation in the utilization of many plant seeds, including *T. occidentalis* seeds, because they contain anti-nutritional factors such as enzyme inhibitors, allergens, lectins, and other naturally occurring substances that may influence diet intake, digestibility, absorption, and metabolic processes in animals and humans. There have been reports that the existence of these anti-nutritional factors in food causes growth inhibition as well as digestive and histological perturbations in laboratory models (Chunmei et al. 2010).



Figure 1: Fluted pumpkin pod and seeds (Source: Chunmei et al., 2010).

As a good source of protein, fluted pumpkin seeds play an important role in the world of food production for both humans and animals. However, the seeds contain many kinds of anti-nutritional factors, such as lectin, trypsin inhibitors, phytates, and tannins. Some of these anti-nutritional factors, such as saponins, oxalates, and phytates, are removed by washing, soaking, and parboiling, but others, such as lectins and trypsin inhibitors, are more resistant and will require higher temperatures and prolonged cooking in order to be inactivated. This study, therefore, was aimed at investigating the effects of some anti-nutritional factors in the seeds of *Telfairia occidentalis* (processed and unprocessed) on growth performance and nutrient metabolism in rats (Kuku et al., 2014).

According to Christian (2007), *T. occidentalis* seeds have 31.38% crude fat, 2.02% ash, 50.08% carbohydrate, and a fiber content of 2.12%, while Akwaowo et al. (2000) reported 56.24% crude fat, 1.80% crude fiber, 1.60% ash, and 16.10% carbohydrate. Kuku et al. (2014) reported the presence of 18.4% fat, 2.41% carbohydrate, 1.53% ash, and 0.88% crude fiber content in the seeds of *T. occidentalis*.

2.4. Oxidative Stress

In humans, oxidative stress is thought to be involved in the development of ADHD, cancer, Parkinson's disease, Lafora disease, Alzheimer's disease, atherosclerosis, heart failure, myocardial infarction, fragile X syndrome, sickle-cell disease, lichen planus, vitiligo, autism, infection, chronic fatigue syndrome (ME/CFS), and depression (Jiménez-Fernández et al., 2015) and seems to be characteristic of individuals with Asperger syndrome. However, reactive oxygen species can be beneficial, as they are used by the immune system as a way to attack and kill pathogens. Short-term oxidative stress may also be important in the prevention of aging by induction of a process named mitohormesis (Gems & Partridge, 2008).

Different types of oxidative stress biomarkers have been identified and may provide important information about the efficacy of the treatment, guiding the selection of the most effective drugs/dose regimens for patients and, if particularly relevant from a pathophysiological point of view, acting on a specific therapeutic target (Ilaria et al., 2018). Chemically, oxidative stress is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defenses, such as glutathione (Schafer & Buettner, 2001). The effects of oxidative stress depend upon the size of these changes, with a cell being able to overcome small perturbations and regain its original state. However, more severe oxidative stress can cause cell death, and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis (Lennon et al., 1991).

2.4.1. Oxidative Stress Biomarkers

The biomarkers that can be used to assess oxidative stress have been attracting interest because the accurate assessment of such stress is necessary for the investigation of various pathological conditions, as well as to evaluate the efficacy of drugs. Assessment of the extent of oxidative stress using biomarkers is interesting from a clinical standpoint. The markers found in blood, urine, and other biological fluids may provide information of diagnostic value, but it would be ideal if organs and tissues suffering from oxidative stress could be imaged in a manner similar to CT scanning and MR imaging.

DNA Fragmentation

DNA fragmentation is the separation or breaking of DNA strands into pieces. It can be done intentionally by laboratory personnel or by cells or can occur spontaneously. Spontaneous or accidental DNA fragmentation is fragmentation that gradually accumulates in a cell. It can be measured by, e.g., the Comet assay or by the TUNEL assay. Men with sperm motility defects often have high levels of sperm DNA fragmentation (Belloc et al., 2014). The degree of DNA fragmentation in sperm cells can predict outcomes for in vitro fertilization (Simon et al., 2010) (IVF) and its expansion, intracytoplasmic sperm injection (ICSI). The sperm chromatin dispersion test (SCD) and TUNEL assay are both effective in detecting sperm DNA damage (Gorczyen et al., 1993). Using bright-field microscopy, the SCD test appears to be more sensitive than the TUNEL assay (Zhang et al., 2009). Its main unit of measurement is the DNA Fragmentation Index (DFI). A DFI of 20% or more significantly reduces the success rates after ICSI (Speyer et al., 2010).

Catalase

Catalase is a key enzyme that uses hydrogen peroxide, a nonradical ROS, as its substrate. This enzyme is responsible for neutralization through decomposition of hydrogen peroxide, thereby maintaining an optimum level of the molecule in the cell, which is also essential for cellular signaling processes. A catalase (E.C. 1.11.1.6) is one of the most important antioxidant enzymes. It is present in almost all aerobic organisms. Catalase breaks down two hydrogen peroxide molecules into one molecule of oxygen and two molecules of water in a two-step reaction. (Deisseroth & Dounce, 2008). Catalase deficiency or malfunctioning is associated with many diseases such as diabetes mellitus, vitiligo, cardiovascular diseases, Wilson disease, hypertension, anemia, some dermatological disorders, Alzheimer's disease, bipolar disorder, and schizophrenia. It has been reported that an anomaly of catalase activity is inherited in acatalasemia, which is a rare genetic disorder (also known as Takahara disease).

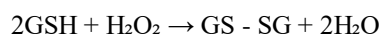
Lipid Peroxidation

Free radical attacks on unsaturated fatty acids in the cell membranes made up of lipids result in lipid peroxidation. The end product of the lipid peroxidation reaction is malondialdehyde (MDA), which is most times analyzed with thiobarbituric acid (TBA) (DeZwart et al., 1993). Reported by Forman et al. (2008), lipid peroxidation plays a major role in various disorders and diseases such as cardiovascular diseases, cancer, neurodegenerative diseases, and even aging, with increasing evidence indicating the involvement of in vivo oxidation in these conditions. Pali et al. (2008), however, postulated that specific lipid peroxidation products exert various biological functions in vivo, such as regulating gene expression, signaling, activating receptors, and adaptive responses. Literature has it that lipid peroxidation causes abnormality in the middle of sperm cells as well as loss of acrosome capacity for fertilization. The breakdown of the peroxides of unsaturated fatty acids leads to the production of malonaldehyde (MDA) acids. MDA is used as a biomarker to ascertain the rate of oxidative damage to lipids that differs depending on biotic and abiotic stress (Muller et al., 2007).

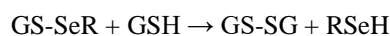
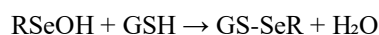
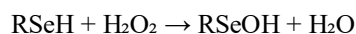
Glutathione Peroxidase

Glutathione Peroxidase (GPx) (EC 1.11.1.9) is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage (Muthukumar & Nachiappan, 2010). The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water (Muthukumar et al., 2011).

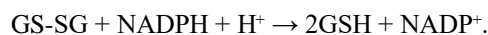
The main reaction that glutathione peroxidase catalyzes is



where GSH represents reduced monomeric glutathione, and GS-SG represents glutathione disulfide. The mechanism involves oxidation of the selenol of a selenocysteine residue by hydrogen peroxide. This process gives the derivative with a selenenic acid (RSeOH) group. The selenenic acid is then converted back to the selenol by a two-step process that begins with a reaction with GSH to form the GS-SeR and water. A second GSH molecule reduces the GS-SeR intermediate back to the selenol, releasing GS-SG as the byproduct. A simplified representation is shown below: (Bhabak & Mugesh, 2010).



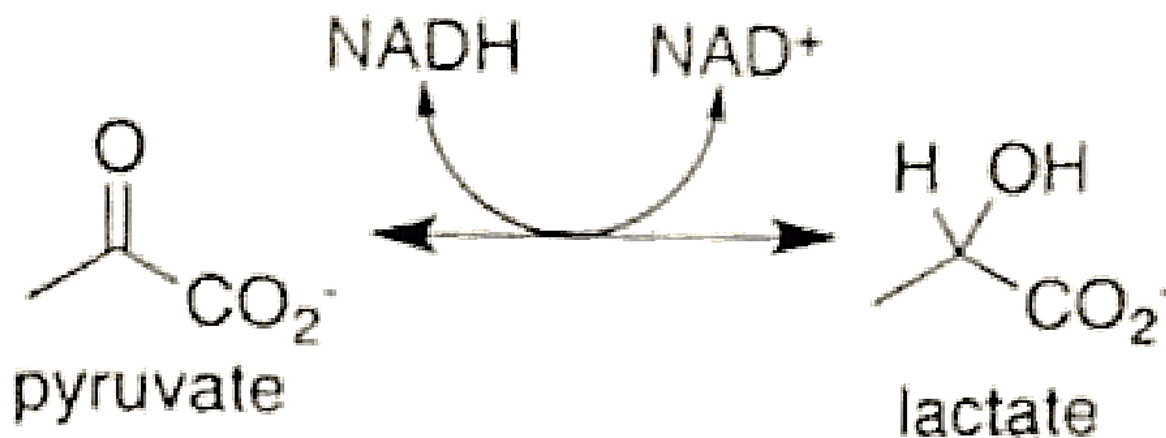
Glutathione reductase then reduces the oxidized glutathione to complete the cycle:



Lactate Dehydrogenase

Lactate dehydrogenase (LDH) is an enzyme found in nearly all living cells. LDH catalyzes the conversion of lactate to pyruvate and back, as it converts NAD^+ to NADH and back. A dehydrogenase is an enzyme that transfers a hydride from one molecule to another (Holmes and Goldberg, 2009). LDH exists in four distinct enzyme classes. This article is specifically about the NAD(P)-dependent L-lactate dehydrogenase. Other LDHs act on D-lactate and/or are dependent on cytochrome c: D-lactate dehydrogenase (cytochrome) and L-lactate dehydrogenase (cytochrome) (Zhang et al., 2014). LDH is expressed extensively in body tissues, such as blood cells and heart muscle. Because it is released during tissue damage, it is a marker of common injuries and diseases such as heart failure (Madern, 2002).

Lactate dehydrogenase catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD⁺. It converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply, and it performs the reverse reaction during the Cori cycle in the liver. At high concentrations of lactate, the enzyme exhibits feedback inhibition, and the rate of conversion of pyruvate to lactate is decreased. It also catalyzes the dehydrogenation of 2-hydroxybutyrate, but this is a much poorer substrate than lactate (Madern, 2002).



Reaction catalyzed by lactate dehydrogenase



In medicine, LDH is often used as a marker of tissue breakdown, as LDH is abundant in red blood cells and can function as a marker for hemolysis. A blood sample that has been handled incorrectly can show false-positively high levels of LDH due to erythrocyte damage (Spriet et al., 2000). It can also be used as a marker of myocardial infarction. Following a myocardial infarction, levels of LDH peak at 3-4 days and remain elevated for up to 10 days. In this way, elevated levels of LDH (where the level of LDH1 is higher than that of LDH2, i.e., the LDH flip, as normally, in serum, LDH2 is higher than LDH1) can be useful for determining whether a patient has had a myocardial infarction if they come to doctors several days after an episode of chest pain (Millar et al., 1969).

2.4.2. Oxidative Stress and Male Infertility

Oxidative stress is an important factor for the development of male infertility because of the very high rate of cell division and mitochondrial oxygen consumption in testicular tissue as well as the comparatively higher levels of unsaturated fatty acids in this tissue than in other tissues. Moreover, the level of oxygen pressure is low due to the weakness of the testicular artery; therefore, there is a severe cell competition for oxygen. Therefore, the testicular tissue and male reproductive system are particularly susceptible to oxidative stress (Nematollah et al., 2017). Most problems that threaten the health of the reproductive system, especially testicular function, are associated with free radical-induced oxidative stress. Life-threatening attacks of free radicals may cause arterial occlusion and serious damage to the reproductive system cells, and consequently defects in spermatogenesis. In other words, increased production of free radicals and peroxides as well as a weakened antioxidant defense system lead to oxidative stress (Halliwell, 2006).

15% of couples have infertility, which is characterized as the inability to conceive naturally after 12 months or more of consistent, unprotected sexual activity (Aitken, 2020). Nearly 50% of cases of infertility are caused by male factors, which contribute equally to the problem (Wagner et al., 2018). According to WHO recommendations, the diagnosis of male infertility is frequently based on routine semen parameter analysis; yet, a significant part of infertile guys go undiagnosed, being viewed as idiopathic or unexplained cases. Numerous investigations pointed to oxidative stress as a newly discovered role in unexplained male infertility. This state is defined by an imbalance between reactive oxygen species (ROS) generation and antioxidant defense systems. (Cito et al., 2020). As spermatozoa mature, chromatin compaction, motility, chemotaxis, sperm capacitation, hyperactivation, acrosome response, and oocyte contact are all promoted. At physiological levels, ROS are linked to the development of sperm fertilization capabilities (Du Plestis et al., 2015). Sperm damage is often caused by an excessive ROS production. In fact, spermatozoa are very vulnerable to oxidation because of the abundance of membrane unsaturated fatty acids and the absence of cytoplasmic antioxidant enzymes (Agarwal et al., 2017), with concomitant negative consequences on sperm quality/functioning. (Barati et al., 2020).

2.4.3. Effect of Oxidative Stress on Sperm Morphological Characteristics

Damage to Sperm Morphological Characteristics:

Various parameters of sperm, such as count, motility, and morphology, are significantly susceptible to free radicals, and hence free radicals can reduce sperm fertility (Saleh & Agarwal, 2002). The free radical-induced oxidative stress contributes significantly in producing and increasing abnormal sperm and decreasing sperm count and transformation and fragmenting sperm DNA.

These changes in sperm DNA result in infertility. In this regard, incubation of spermatozoa under high oxygen pressure reduces the rate and motility of sperm; however, adding catalase to the culture medium prevents this effect (Aitken & Clarkson, 1987). Increased production of hydrogen peroxide (H_2O_2) by spermatozoa under high oxygen pressure may be a factor for reducing sperm motility (Baker & Aitken, 2004). Moreover, the reactive oxygen species (ROS) produced by leukocytes or spermatozooids have fatal effects on sperm function in infertile men. Thus, the ROS produced in the sperm must be inactivated continuously such that ROS concentration constantly remains low enough to allow for the normal performance of cells, because a lack of balance between the production and elimination of ROS causes oxidative stress in sperm and subsequently reduces fertility potential. Researchers believe that the sperm is more susceptible to oxidative stress than other cells due to the limited amount of cytoplasm in a mature sperm and the concentration of ROS-suppressing antioxidants in the sperm as well as high levels of unsaturated fatty acids in the sperm structure (Saleh & Agarwal, 2002).

Lipid Peroxidation of Sperm Plasma Membrane:

Lipid peroxidation in the cell membrane can disrupt fluidity and permeability of cell membranes and damage all cells. In other words, when the cell membranes are damaged by free radicals, their protective cell is lost, and thus the total cell is exposed to risk. In this regard, increased production of ROS induces lipid peroxidation in spermatozoa, which reduces sperm combination with oocyte and increases spermatozoa ability to bind to the transparent area (zona pellucida) (Said et al., 2005). As well, lipid peroxidation caused abnormality in the middle section of sperm and loss of acrosome capacity of fertilization. Malondialdehyde (MDA) molecules cause asymmetric distribution of lipid membrane components by penetrating into the cell membrane structure. Notably, the rate of lipid peroxidation is determined according to the resulting product of secondary failure of primary lipid hydroperoxides (Said et al., 2005).

Damage to sperm DNA

Oxidative stress causes an increase in DNA breakdown (WHO, 1999). Besides that, evidence indicates that fragmentation of DNA, commonly seen in infertile people's spermatozoa, is due to the high concentration of ROS in the sperm (Bennetts & Aitken, 2005). Therefore, a main reason for infertility is believed to be excessive production of ROS or decreased antioxidant capacity in semen that causes oxidative stress conditions and ultimately decrease in sperm motility, increase in sperm death, and fragmentation of DNA (Said et al., 2005)

2.4.4. Factors That Induce Testicular Oxidative Stress

Infection

Oxidative stress due to inflammation and inflammatory disease is a common predisposing factor for male infertility, testicular infection causes a significant decrease in production of testosterone and disturbance in spermatogenesis (Reddy et al., 2006), analysis of microarray data on the genes expression in infertile men's testicles indicates increased expression of inflammatory genes (Allen et al., 2004), a study showed that the oxidative stress induced by intraperitoneal administration of lipopolysaccharide (Ips) caused stimulation of lipid peroxidation in leydig cell membrane and considerable increase in steroid making and beta-hydroxyl dehydrogenase activity, besides, this administration caused mitochondrial and leydig cell dysfunction and specifically, inhibition of cholesterol transport activity (Husain & Somani, 1998).

High Testicular Temperature

Any factors that cause abnormal increases in testicular temperature are associated with testicular oxidative stress. Besides that, increased testicular temperature is associated with decreased SOD and function of catalase (Ahotupa & Huhtaniemi, 1992). The exposure of sperm germ cells to increased temperature consequently increases the H₂O₂ level and is associated with an increased rate of apoptosis. Therefore, an increase in the catalase level can largely prevent cell death, as it decreases the H₂O₂ level (Ikeda et al., 1999).

Ageing

A study demonstrated that in rats, as age increases, the expression of enzymatic and non-enzymatic antioxidants decreases, which leads to increased damage due to oxidative stress. Besides that, the level of glutathione, an antioxidant, decreases in older mice (Syntin et al., 2001). According to the findings of different studies, aging causes degenerative changes in testicular tissue and a decline in sperm quality in rodents. In this regard, certain vacuoles are seen in the germinal cells of testicular tissue in laboratory mice with an increase in age. In addition, the number of germinal cells decreased in mice testicles (Luo et al., 2006). Despite different studies conducted on the effect of age on the increase in testicular oxidative stress, further research should be conducted to establish the association between aging, oxidative stress, and testicular function.

2.5. Testicular Aging

Aging is accompanied by numerous changes in the function of the endocrine system. Many of these changes are very pronounced, readily detectable, and have been thoroughly documented (Hermann et al., 2000). Recent studies have focused more on the aging-related dysregulation observed in the inflammatory and oxidative status of the testis (Frungieri et al., 2018), which is in line with the commonly accepted oxidation-inflammation theory of aging described for several extragonadal tissues (De la Fuente & Miquel, 2009). In general terms, this theory suggests that the aging process is linked to chronic oxidative stress, which affects all cells of the organism, especially immune cells. As men age, testicular function declines gradually, affecting not only their fertilizing capacity but also their overall health status and quality of life (Pataky et al., 2021; Corona et al., 2013). However, consensus on the importance of the study of the reproductive aging process, as well as the communication of such a topic to the general public, has been, thus far, very limited (Matzkin et al., 2021).

Reactive oxygen species and the resulting oxidative stress are intimately related to apoptosis; the increased apoptotic events that have been described in the human testis during aging (Jiang et al., 2014) could also be, at least in part, explained by the accumulating ROS in aged testes. As degenerating abnormal cells that arise upon aging, (such as germ cells) are phagocytosed by Sertoli cells, an accumulation of lipid droplets in the Sertoli cell cytoplasm ensues (Paniagua et al., 1987) However, this is not the only change that has been reported in Sertoli cells during aging. Multiple morphological abnormalities have been described for this cell population, including multinucleation (Paniagua et al., 1987), mitochondrial metaplasia (Paniagua et al., 1991), and reduction of cell junctions, along with a marked reduction in number (Paniagua et al., 1987).

2.6.1 Animal Models for the Study of Testicular Aging.

Given the poor, and sometimes ethically impeded, access to fresh, disease-free testicular tissue from old men, it has been rather difficult to obtain data on isolated testicular cell population physiology and the corresponding underlying regulatory mechanisms involved in their proposed impaired function during aging. Although there is conflicting evidence about the extent to which aging is a process that is similar across all organisms or particular to each species (Cohen, 2018). The integrative understanding of aging implies that a diversity of model organisms will be essential to achieve a full understanding of the aging process. In fact, model organisms have been vital to the common goal of identifying and understanding the molecular, cellular, and environmental factors affecting longevity and improving health span (Matzkin et al., 2021). While many different non-human organisms have been used to explore the aging process (e.g., yeast, roundworms, and fruit flies), rodents such as mice and rats (Zhang et al., 2020) are routinely the models of choice. From the perspective of aging biology, several life characteristics make rodents an extremely appealing group for comparative studies, from the diversity in their maximum lifespans to the many similarities they share with aging in humans. Furthermore, their short lifespans (compared to humans) and the ability to control environmental exposure create opportunities for regulatory upregulation of lifespan (Matzkin et al., 2021).

Naturally aged rats have also been employed in testicular aging studies, showing decreased serum testosterone levels alongside signs of increased testicular inflammation and interstitial senescence (Zhang et al., 2020).

2.6.2 Intercessions to Reverse Aging-related Testicular Damage

According to Matzkin et al., 2021, there is no available information about successful interventions that have been implemented in elderly men to improve testicular function. For this reason, this review will only focus on those reports that have addressed some aspects of testicular aging using either naturally, chemically induced, or genetically accelerated aged animals (Matzkin et al., 2021).

With age, renal synthesis of 1,2,5-dihydroxy-vitamin D₃, or calcitriol (the active form of vitamin D), declines (Jeremy et al., 2019). Vitamin D supplementation has been found to alleviate the manifestations of male reproductive aging. Jeremy et al. (2019) administered vitamin D (40-400 U/kg) subcutaneously twice a week for 6 weeks to D-gal-induced aged rats. Their studies show that vitamin D supplementation resulted in a decrease in apoptotic germ cells and an increase in proliferating germ cells (Jeremy et al., 2019). Vitamin D also significantly decreased testicular peroxidation and increased the activities of antioxidant enzymes SOD and Catalase (Jeremy et al., 2019).

2.7 Spermatogenesis

All of the procedures necessary to produce gametes are referred to as spermatogenesis. Male fertility depends on the testis' ability to produce a significant quantity of healthy spermatozoa through the intricate spermatogenesis process. Three primary phases may be broken down into this process: mitosis, which multiplies spermatogonial (immature cells); meiosis, which reduces the number of diploid chromosomes to haploid; and the entry of type b spermatogonial into the prophase of the first meiotic division (Nwangwa et al., 2007)

Spermatogenesis entails the creation of haploid spermatids from the tetraploid initial spermatocytes through meiotic cell division and the differentiation of spermatogonial stem cells into spermatocytes through mitotic cell division. In the succeeding stage of spermatogenesis, known as spermiogenesis, spermatids produce spermatozoa. These essential processes, in which meiosis occurs before mitotic proliferation during spermatogenesis, are seen in a wide range of organisms (Reddy et al., 2006).

Throughout an animal's reproductive lifetime or season, sperm are continuously produced by spermatogenesis. The final products (sperm) are ejected from the organ (spermiation), and spermatogenic stem cells start to produce the subsequent round of sperm. Therefore, the beginning of spermatogenesis and spermiation must be coordinated in order to preserve the continuum of sperm production. In most mammals, it takes 30 to 40 days to produce spermatozoa from spermatogenic stem cells (Clermont, 1972)

The mature male gametes in many sexually reproducing species are called spermatozoa. So spermatogenesis is in males, and oogenesis is in females.

Spermatogenesis, which is crucial for sexual reproduction, is highly dependent on ideal conditions to take place successfully. The process necessitates high local concentrations of testosterone, which are maintained by androgen-binding protein found in the seminiferous tubules (Zohani et al., 2012).

The Sertoli cells are in close proximity to the spermatogenic cells throughout all stages of differentiation and are thought to support the developing sperm cells' structural and metabolic needs. Light microscopic examination reveals that a single Sertoli cell extends from the basement membrane to the lumen of the seminiferous tubule, albeit it is challenging to discern the cytoplasmic processes (Clermont, 1972).

3. Conclusion

Oxidative stress is a major contributor to male reproductive dysfunction, primarily through its damaging effects on testicular structure, sperm quality, and hormonal balance. Evidence from experimental studies in male rats demonstrates that fluted pumpkin (*Telfairia occidentalis*) possesses strong antioxidant and cytoprotective properties that mitigate oxidative damage in reproductive organs. Its rich content of flavonoids, phenolics, vitamins, and essential micronutrients enhances endogenous antioxidant enzyme activities, reduces lipid peroxidation, and preserves the histo-architectural integrity of the testes and epididymis.

Collectively, the reviewed studies indicate that dietary inclusion or extract administration of *T. occidentalis* improves sperm count, motility, morphology, and testosterone levels in oxidative stress–induced models. These findings support the view that fluted pumpkin acts not only as a nutritional supplement but also as a functional therapeutic agent against redox-mediated reproductive toxicity.

Despite these promising results, most evidence remains preclinical and derived from animal models. Variations in extraction methods, dosages, treatment duration, and oxidative stress inducers limit direct comparison across studies and hinder definitive conclusions about optimal use. Therefore, future research should focus on standardized experimental designs, detailed mechanistic investigations, and translational studies to determine safety, efficacy, and relevance in human male reproductive health.

In summary, *Telfairia occidentalis* represents a valuable natural resource with significant potential in the prevention and management of oxidative stress–related male infertility. Its integration into evidence-based nutritional and therapeutic strategies could contribute meaningfully to improving reproductive outcomes, particularly in regions where the plant is readily available and culturally accepted.

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