

Therapeutic Effects of Fluted Pumpkin (*Telfairia Occidentalis*) Seed Extract in Aging Induced Oxidative Stress in Testis of Male Wistar Rats

Chisanum King Wofuru-Nyenke*¹

¹Department of Biochemistry, Faculty of Science, University of Port Harcourt, Nigeria.

*Correspondence: chisanumnyenke@gmail.com

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Abstract

The aim of this study is to ascertain the potency of fluted pumpkin seed extract (FPSE) in ameliorating effects caused by oxidative stress in reproductive organs of aged male rats, taking into consideration CAT (Catalase), LPO (Lipid peroxidation), and GSH (Glutathione) as oxidative stress biomarkers. Histopathology of the testes was also examined. 60 male Wistar rats, of which 40 of them are adult males with average weight of 273g and 20 young male rats having an average weight of 87g were separated into 6 groups containing 10 rats each, with the 10 young rats grouped into 1 group Group 1 (young control) received 0.2 ml of corn oil, Group 2 (adult control) received 0.4 ml of corn oil, Group 3 (50 mg/kg FPSE) received 1.4 ml of the extract, Group 4 (100 mg/kg FPSE) received 2.8 ml of the extract, Group 5 (200 mg/kg FPSF) received 5.5 ml of the extract, and Group 6 (testosterone) received 0.14 ml of testosterone administered intraperitoneally once a week for 40 days. Groups 3, 4, and 5 received their treatment twice a week. From this research, it was deduced that the level of CAT, LPO, and GSH increased in Group 2 (adult control) when compared with Group 1 (young control) but decreased statistically in Group 3 (50 mg/kg FPS extract), Group 4 (100 mg/kg FPS extract), Group 5 (200 mg/kg FPS extract), and Group 6 (testosterone), with Group 3 (50 mg/kg FPS extract) having the most reduction. The histopathology showed damage to the epithelium and reduced germ cells in the testes of the aged rats, which was restored in the groups that received the plant extract and testosterone. In conclusion, the information presented in this study suggests that consumption of FPS extract at low doses may be advantageous in reducing the harm done to the reproductive organs of old male rats by oxidative stress.

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

1. Introduction

Reactive oxygen and nitrogen species (RONS) are produced by several endogenous and exogenous processes, and their negative effects are neutralized by antioxidant defenses. Oxidative stress occurs from the imbalance between RONS production and these antioxidant defenses. Aging is a process characterized by the progressive loss of tissue and organ function. The oxidative stress theory of aging is based on the hypothesis that age-associated functional losses are due to the accumulation of RONS-induced damages. At the same time, oxidative stress is involved in several age-related conditions (i.e., cardiovascular diseases (CVDs), chronic obstructive pulmonary disease, chronic kidney disease, neurodegenerative diseases, and cancer), including sarcopenia and frailty.

Reactive oxygen species are generated mainly as byproducts of mitochondrial respiration; mitochondria are the primary target of oxidative damage and play an important role in aging. Emerging evidence has linked mitochondrial dysfunction to a variety of age-related diseases, including neurodegenerative diseases and cancer (Yan, 2014). Aging is characterized by a progressive decline in the efficiency of biochemical and physiological processes, the functional maintenance of tissue homeostasis, and an increasing susceptibility to diseases. Aging is a multifactorial process,

which is genetically determined and influenced epigenetically by environment (Kirkwood, 2005). In recent years it has been well known that oxidative stress may play important roles in elderly health, and, in particular, there is increasing evidence that aging might be caused by the potential and harmful effects of an accumulation of oxidative damage caused by reactive species (RS, in particular reactive oxygen species, or ROS, and reactive nitrogen species, or RNS). This evidence is supported by the "free radical theory of aging" proposed by Harman (Harman, 1956).

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, and reduction of cell junctions, along with a marked reduction in number (Jiang et al., 2014).

Normal levels of RS are essential for various cellular mechanisms (Sena and Chandel, 2012). High levels of ROS/RNS would be detrimental to cells and have been thought to contribute to aging and the pathogenesis of numerous age-related diseases (Malinin et al., 2011). Antioxidant reserve (RAO) controls the production of ROS. Reactive oxygen species, in particular hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\cdot-}$), are physiologically produced in a number of cellular reactions, including the iron-catalyzed Fenton reaction (Barbusinsky, 2009) and by various enzymes, such as lipoxygenases, peroxidases, NADPH oxidase, and xanthine oxidase. An excess of oxidation and/or a lack of antioxidants causes a condition of oxidative stress (OS) (Cornelli, 2009). The OS condition can be temporary or prolonged depending on the response of the antioxidant defense network. A prolonged OS condition can cause damage to all the macromolecular constituents of the body, such as lipids, protein and DNA (Sanders & Greenamyre, 2013) and is ultimately an emerging risk factor for human health and for the development of many diseases (diabetes, cancer, cardiovascular and neurodegenerative diseases, aging) (Cornelli, 2009). The aim of this study is to ascertain the potency of FPSE (fluted pumpkin seed extract) in ameliorating effects caused by oxidative stress in reproductive organs of aged male rats. The objectives include to determine the effect of FPSE (fluted pumpkin seed extract) on stress enzymes (CAT, LPO, GPx) in aged Wistar rats; to determine the effect of FPSE on aged Wistar albino rats. FPSE (fluted pumpkin seed extract) on the histology of testes of aged Wistar rats. The results of this research work will show the protective and beneficial effects of fluted pumpkin seeds against oxidative stress on the testes of aged male Wistar rats. This study will justify the need of a fluted pumpkin seed-based diet for therapeutic purposes on the male reproductive organ of patients.

2. Methodology

2.1 Materials

| | |
|----------------------------|--------------------------------------------|
| Thiobarbituric acid (TBA) | Sigma-Aldrich, Chemical Co., St. Louis, MO |
| Glutathione | Sigma-Aldrich, Chemical Co., St. Louis, MO |
| Sodium azide | Sigma-Aldrich, Chemical Co., St. Louis, MO |
| Ellman's reagent | Sigma-Aldrich, Chemical Co., St. Louis, MO |
| Pregnenolone | Sigma-Aldrich, Chemical Co., St. Louis, MO |
| Trichloroacetic acid (TCA) | Sigma-Aldrich, Chemical Co., St. Louis, MO |

| | |
|----------------------------------------------|--------------------------------------------|
| Nicotinamide Adenine Dinucleotide (NAD) | Sigma-Aldrich, Chemical Co., St. Louis, MO |
| Sulfanilamide | Sigma-Aldrich, Chemical Co., St. Louis, MO |
| 3,3',5,5'-Tetramethylbenzidine (TMB) | Sigma-Aldrich, Chemical Co., St. Louis, MO |
| 5,5-dithio-bis (2-nitro-benzoic acid) (DTNB) | Sigma-Aldrich, Chemical Co., St. Louis, MO |
| N-(1-naphthyl) ethylenediamine dichloride | Sigma-Aldrich, Chemical Co., St. Louis, MO |
| sodium pyrophosphate | Sigma-Aldrich, Chemical Co., St. Louis, MO |
| 1,1,3,3-tetraoxy propane | Sigma-Aldrich, Chemical Co., St. Louis, MO |

2.2 Equipment:

| | |
|-----------------------------------------------|-----------------------|
| Spectrophotometer | BIOBASE: BK-(D560) |
| Centrifuge | BIOBASE: BK-THR16K |
| ELISA reader | BIOBASE: EL-10A |
| Weighing balance | Mettler Toledo: AB204 |
| Light microscope: Labomed Lx 40 magnification | |
| Water bath | Jinotech: HH-W420 |
| Water distiller | |
| pH meter | CyberScan 500 |
| Incubator | Techmel: TT-9022A |
| Soxhlet extractor | |
| Microwave oven. | |

2.3 Preparation of Fluted Pumpkin Seed Extract

Raw and matured pods of fluted pumpkin were bought from Aluu boundary market in Port Harcourt, Nigeria. The pods were cut open, and the seeds were collected, sorted (to remove bad ones), and washed. The seeds were air-dried, deshelled, and then ground into powder. Flours obtained from the seeds and weighing 2 kg were defatted with n-hexane in a Soxhlet apparatus for 8 hours and then air-dried on aluminum trays at room temperature. The hexane fraction was collected from the FPS mare extract and stored in the refrigerator. The defatted FPS mare extract was then soaked in absolute ethanol for 72 h, after which they were sieved using a muslin cloth and thereafter filtered with Whatman paper. The collected filtrate was thickened at low pressure to constant weight in a Soxhlet extractor at 45°C, and the brownish residue was dried in an oven at 40°C. The weight of the extract was 4.22% (w/w). To prepare the hexane-rich fraction, the brownish extract was mixed with the hexane fraction, and the mixture was suspended in an equimolar volume of water and hexane (50 ml) for 24 h with constant intermittent shaking. After 24 h, the partitioned hexane layer was collected with a separating funnel. After three cycles of successive separation with hexane, the resulting hexane-fraction extracts were pooled together and evaporated under a vacuum using a rotary evaporator to yield a brownish-dark residue. The extract was dried in an oven at 40°C and kept in the freezer until it was used. The final yield of the extract was 5.43% (w/w). The extraction yield was calculated as follows:

% Extraction yield = $\frac{\text{Weight (g) of the dry extract after solvent extraction}}{\text{Weight (g) of dry plant sample before extraction}} \times 100\%$

Weight (g) of dry plant sample before extraction

2.4 Animals

Sixty (60) male Wistar rats (*Rattus norvegicus*), 50 aged rats (219 plus/minus 33 g; 6 months old), and 10 young rats (87 ± 18 g; 2 months) were bought from an animal breeder and kept in the Department of Biochemistry, University of Port Harcourt animal house. The rats were allowed one week to acclimatize to the environment and had unrestricted access to normal rat chow and water ad libitum. They were kept in plastic cages with dry sawdust as bedding material under standard conditions of temperature (23 ± 1 degree C), humidity (50-60%), and a 12-hour light/12-hour dark cycle throughout the 40-day study period. The study received authorization of the Department of Biochemistry Research Ethics Committee on 18 January 2022 with an ethical approval reference

number UPI/BCHREC/2022/009; and the methodology for the handling of the animals was also in accordance with the requirements of the National Institute of Health Guidelines for Animal Care and Use of Laboratory Animals (Publication Number 85-23). The rats were divided into 6 groups of 10 rats each. The testosterone group was administered testosterone (15 mg/kg⁻¹ body weight) intraperitoneally, once a week for 40 days. The FPS group was treated with FPS extract (50, 100, and 200 mg/kg⁻¹ body weight) orally, twice a week, while the aged and young controls were administered with corn oil (2 ml/kg⁻¹ body weight) orally, twice a week for 40 days. The ages of the rats at 2 and 6 months were selected because the 8-week-old rats are young animals and the 24-week-old rats are adult animals, and they exhibit differences in the capacity of their testes to respond to oxidative stress. At the end of the study, the rats were weighed and killed, blood samples were collected, and tissue samples were removed, washed in ice-cold 1.15% potassium chloride, and damped gently in-between Whatman paper before they were weighed and processed for biochemical assays.

2.5 Experimental Design

Sixty (60) mature male Wistar albino rats were separated into 6 groups containing 10 rats each, with 10 young rats grouped into 1 group.

Group 1: Young control - 0.2 ml (corn oil)

Group 2: Adult control 0.4 ml (corn oil)

Group 3: 50 mg/kg PPSE (Fluted pumpkin seed extract) - 1.4 ml

Group 4: 100 mg/kg FPSE (Fluted pumpkin seed extract) - 2.8 ml

Group 5: 200 mg/kg FPSE (Fluted pumpkin seed extract) - 5.5 ml

Group 6: Testosterone - 0.14 ml

Groups 2-5 were all adult rats, with Group 1 having only young rats. Group 6 received 0.14 ml of testosterone intraperitoneally once a week for the 40 days of treatment. Group 3, Group 4, and Group 5 orally received 1.4 ml of 50 mg/kg b/w, 2.8 ml of 100 mg/kg b/w, and 5.5 ml of 200 mg/kg b/w, respectively, of fluted pumpkin seed extract twice a week throughout the period of treatment. Group 2 was the adult control; the rats received 0.4 ml of corn oil orally, and Group 1, being the young control, received 0.2 ml of corn oil orally. Administration to Group 5 and Group 6 was done twice a week. Administrations were done for a total of 40 days.

2.5.1 Animal Sacrifice and Collection of Reproductive Organs:

The body weight of the rats was recorded prior to sacrifice after the last day of treatment. The rats were anesthetized with chloroform followed by decapitation. After cutting a side in the neck, the blood was carefully collected into a labeled specimen bottle until the animal stopped breathing and the blood stopped flowing, and serum was obtained from the blood samples for the various biochemical assays. The paired testes were dissected out quickly and washed in 1.15% ice-cold potassium chloride (KCl), and the left testis was homogenized in ice-cold phosphate-buffered saline (0.1 M, pH = 7.4) and centrifuged at 10,000 g at 4°C for 5 min to obtain the homogenate used for the biochemical assays. The right testis was fixed in Bouin's solution and processed for histopathology.

2.5.2 Homogenization of Tissue

The left testes were cut into pieces in 4 volumes of the ice-cold normal saline and crushed using a mortar and pestle that were surrounded by ice to maintain a cold preservative temperature. After crushing, it was then transferred back into the sample bottle. The samples were centrifuged at 10,000 g, and the collected supernatant was used for the analysis of oxidative stress and other biochemical parameters.

2.6 Histology of the Testis

The standard histological technique was followed by the method of Gurr (1959). After fixation of tissues in Bouin's solution for 24 hrs, the tissues were dehydrated in a progressive graded ethanol series (70%, 80%, and 95%) for 40 minutes and 100% (absolute alcohol) for 1 hour. The tissues were cleared using xylene as the clearing agent and then embedded in paraffin wax. Thin sections of the tissues were prepared and stained with Harris's hematoxylin and eosin for histological evaluation using a light microscope.

2.7 Biochemical Assay

2.7.1 Determination of Reduced Glutathione Concentration

Principle of Assay:

The assay method involves the oxidation of GSH by the sulfhydryl reagent 5, 5'-dithio-bis (2-nitrobenzoic acid) to form the yellow derivative 5'-thio-2-nitrobenzoic acid (TNB), measurable at 412 nm (Moron et al., 1979).

Reagents

- 0.2 M of disodium hydrogen phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) was prepared by dissolving 14.33 g of the salt in 200 ml of distilled water.
- 10 mM of 5'-dithiobis-(2-nitrobenzoic acid) DTNB was prepared by dissolving 0.0396 g of the salt in 10 ml of phosphate buffer (0.1 M, pH 7.4).

Assay Procedure

1.8 ml disodium hydrogen phosphate (0.2 M) was mixed with 80 μl DTNB and 320 μl sample. The mixture was kept in the dark for 20 minutes to allow for the development of the yellow chromophore. The absorbance was read at 412 nm against water as a blank.

2.7.2 Determination of Catalase Activity

The enzymatic performance of catalase was ascertained via the technique of Aebi (1974) with little change in the methodology.

Principle of Assay

This assay is premised on the basis of the splitting of hydrogen peroxide in the analyte by catalase. The reaction is monitored spectrophotometrically at a wavelength of 240 nm. A unit of catalase is commensurate with the amount of protein that transforms H₂O₂ per minute.

Procedure of the Assay

H₂O₂ (0.4 ml of 5.9 mM solution) was pipetted into a 1 cm quartz cuvette, and 2.5 ml of 50 mM phosphate buffer and 200 µl of the analyte were introduced. The resultant solution was rapidly turned upside down for it to properly blend, and the result was obtained spectrophotometrically at an absorbance value of 240 nm every 10 seconds for 1 minute.

2.7.3 Determination of Lipid Peroxidation.

Level of lipid peroxidation (LPO) was determined according to the method of Varshney and Kale (Varshney and Kale, 1990). The method involved the reaction between malondialdehyde (MDA; product of lipid peroxidation) and TBA to yield a stable pink chromophore with maximum absorption at 532 nm. Briefly, the reaction mixture consisted of 1.6 ml. Tris-KCl buffer, 0.4 ml. of the test sample, 0.5 ml. of 30% TCA, and 0.5 ml. of 0.75% TBA and the mixture were placed in a water bath for one hour at 95°C. This was then cooled and subjected to centrifugation at 3000 rpm. The supernatant was collected, and the absorbance was measured against a blank of distilled water at 532 nm.

3. Results and Discussion

3.1 Results

3.1.1 Weight of animals

Table 1: A variable table showing the mean standard deviation of the initial body weight and final body weight of the rats.

| Groups | Testes weight | Final body weight |
|----------------|----------------|-------------------|
| Young control | 87.857±18.907 | 177.143±25.096 |
| Aged control | 216.571±31.442 | 242.143±25.327 |
| 50 mg/kg FPSE | 218.429±38.957 | 261.600±39.412 |
| 100 mg/kg FPSE | 219.429±40.282 | 281.750±33.866 |
| 200 mg/kg FPSE | 218.429±33.817 | 275.750±52.955 |
| Testosterone | 220.571±30.604 | 271.333±30.402 |

3.1.2 Effect of FPSE (fluted pumpkin seed extract) on glutathione in testes of aged Wistar rats.

GSH is tested as a marker of oxidative stress in the present study. Figure 1 shows the level of GSH in control and treated rats. The level of GSH increased in the aged control group but decreased statistically in 50 mg FPS, 100 mg, 200 mg FPS, and testosterone when compared to the negative aged control.

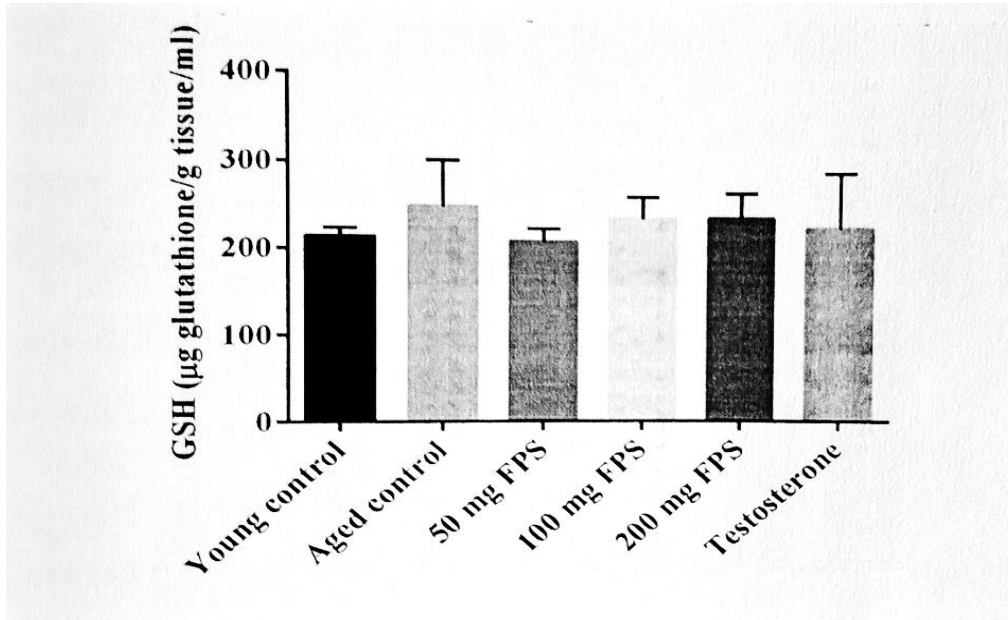


Figure 1. The level of glutathione in testes of young and aged rats in comparison to rats exposed to various concentrations of FPSE and testosterone. Data are mean \pm SD ($p \geq 0.05$).

3.1.3 Effect of FPSE (fluted pumpkin seed extract) on catalase in testes of aged Wistar rats.

CAT is tested as a marker of oxidative stress in the present study. Figure 2 shows the level of CAT in control and treated rats. The level of CAT increased in the aged group when compared to the young control group but decreased statistically in 50 mg FPS, 100 mg, 200 mg FPS, and testosterone when compared to the negative aged control.

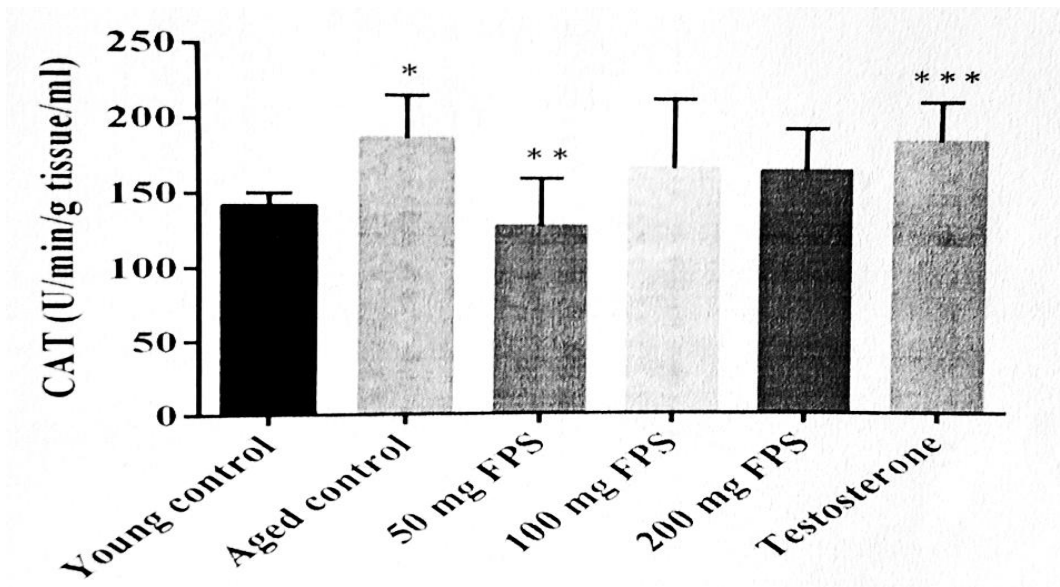


Figure 2. The level of catalase in testes of young and aged rats in comparison to rats exposed to various concentrations of FPSE and testosterone. Data are mean \pm SD ($p < 0.05$)

3.1.4 Effect of FPSE (fluted pumpkin seed extract) on lipid peroxidase (LPO) in testes of aged Wistar rats.

LPO is tested as a marker of oxidative stress in the present study. Figure 3 shows the level of LPO in control and treated rats. The level of LPO increased in the aged group when compared to the young control group but decreased statistically in 50 mg FPS, 100 mg, 200 mg FPS, and testosterone when compared to the negative aged control.

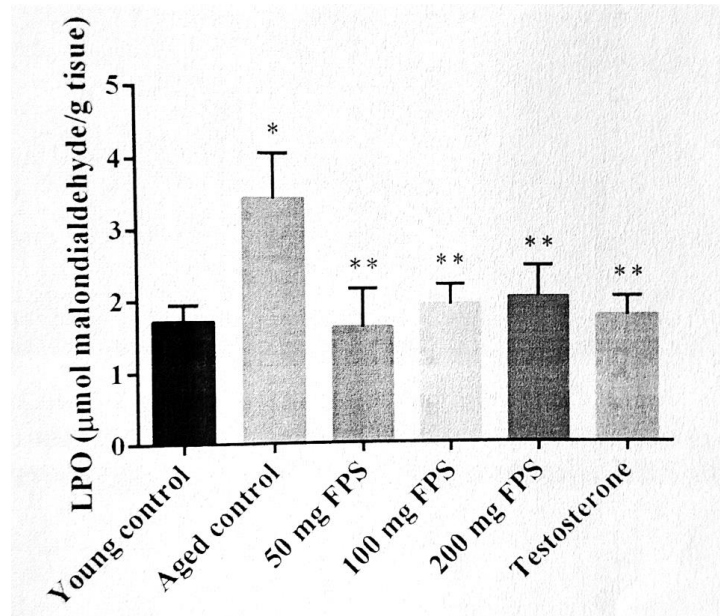


Figure 3. The level of Lipid Peroxidase (LPO) in testes of young and aged rats in comparison to rats exposed to various concentrations of FPSE and Testosterone. Data are mean \pm SD ($p < 0.05$)

3.1.5 Histological findings

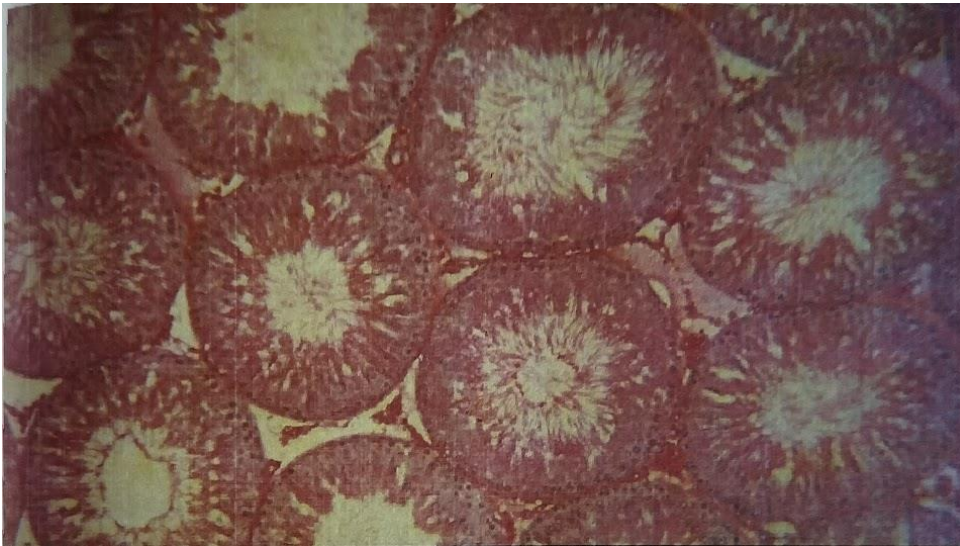


Plate 1 Photomicrograph of the testes of animals in the young control group showing normal seminiferous tubules and well-organized germ cells. H&E x100.

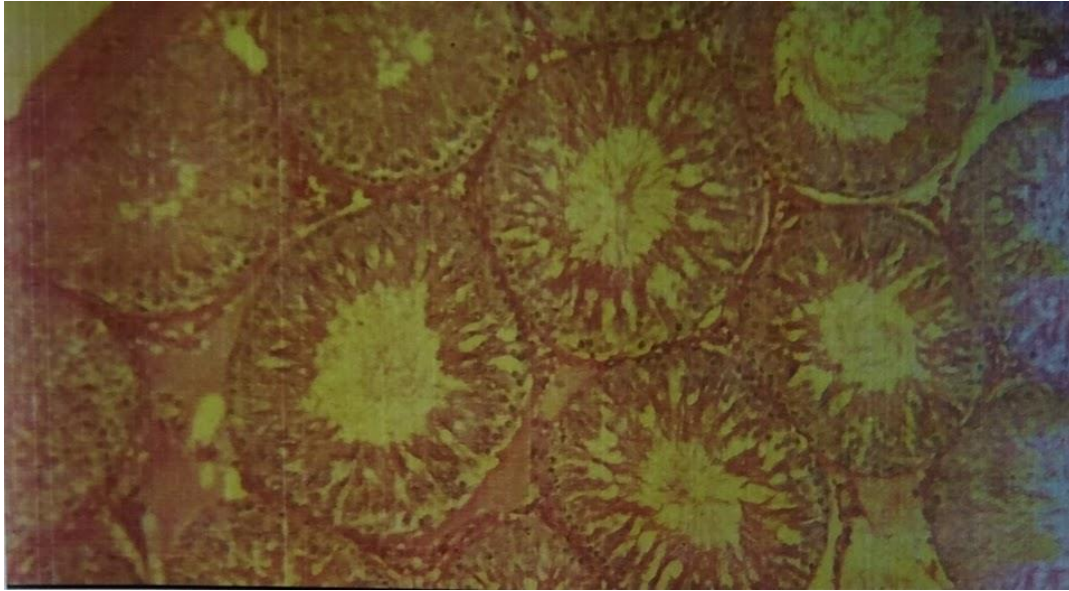


Plate 2 Photomicrograph of the testes of animals in the adult control group showing damaged epithelium, vacuolated tubules, and few numbers of germ cells. The tubules are also smaller in size, and most of the cells in the interstitial spaces have lost their morphology.

H&E x100.

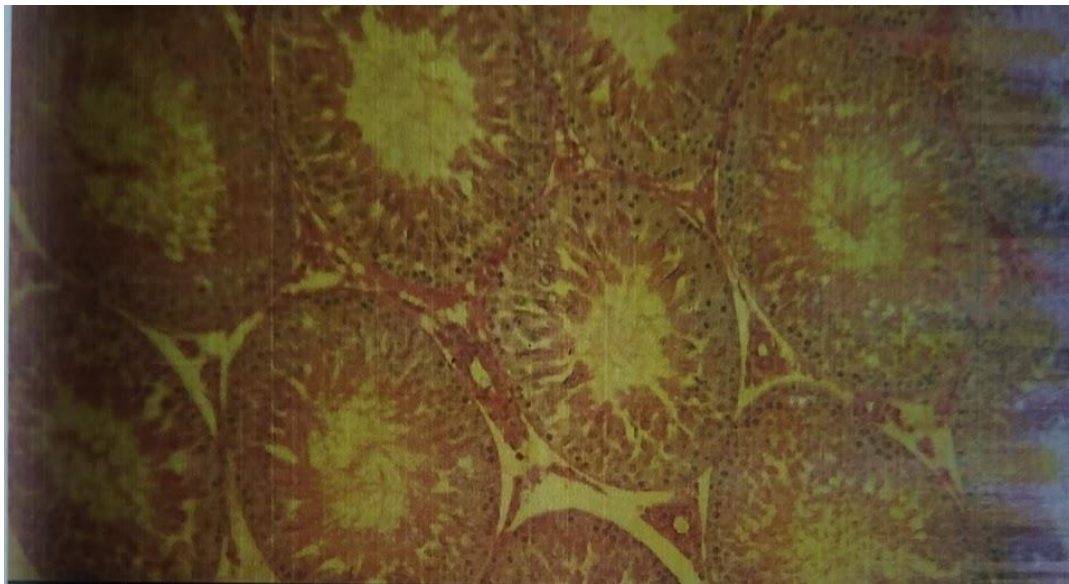


Plate 3 Photomicrograph of the testes of animals treated with 50 mg/kg b.w. FPSE showing the recovery of the germ cells in the epithelium of most of the tubules. Although the population of the germ cells was still small in some tubules, and they had few numbers of vacuoles. The interstitial space also appeared normal and similar to the control animals. **H&E x100.**

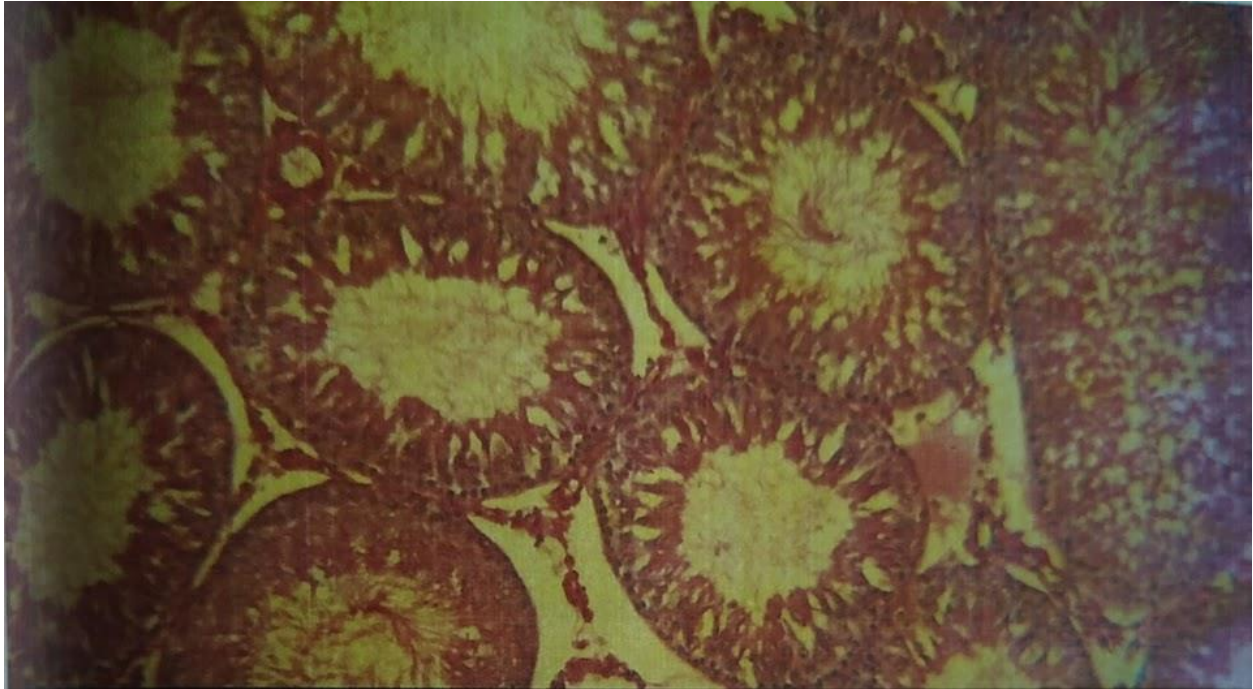


Plate 4 Photomicrograph of the testes of animals treated with 100 mg/kg b.w. FPSE showing normal and well-organized germ cells in the epithelium. It is similar to the control animals. H&E x100.

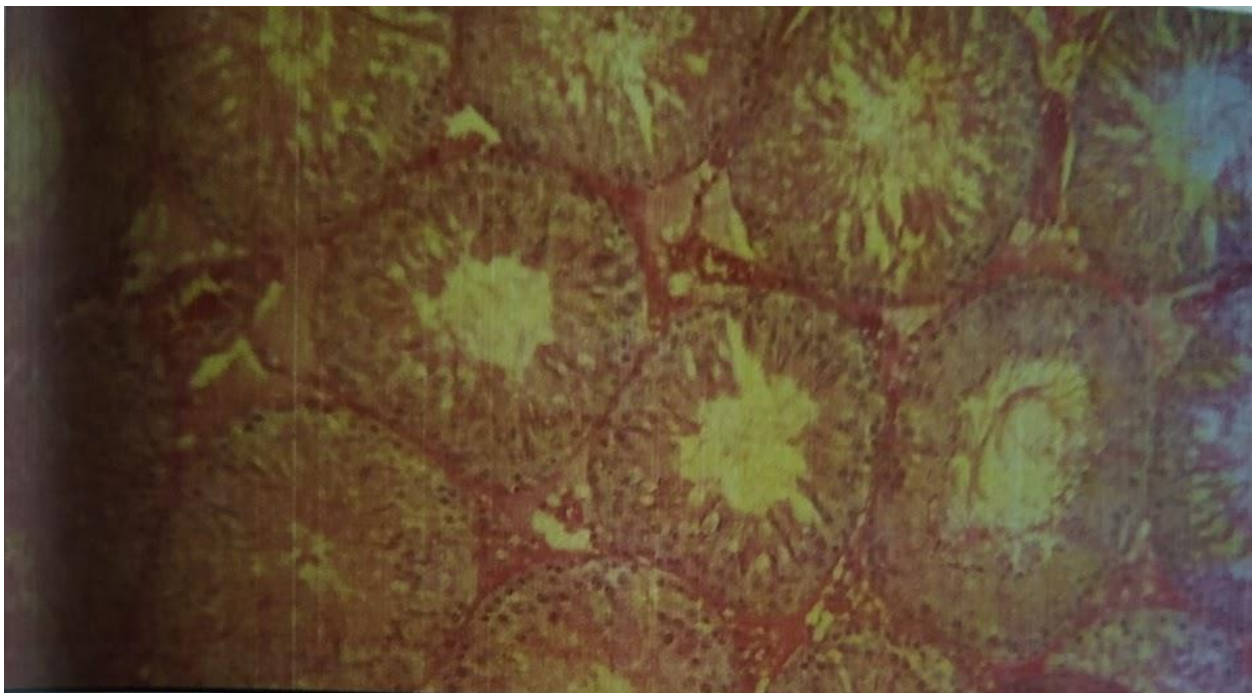


Plate 5 Photomicrograph of the testes of animals treated with 200 mg/kg b.w. FPSE showing normal and well-organized germ cells in the epithelium. It is similar to the control animals. H&E x100.

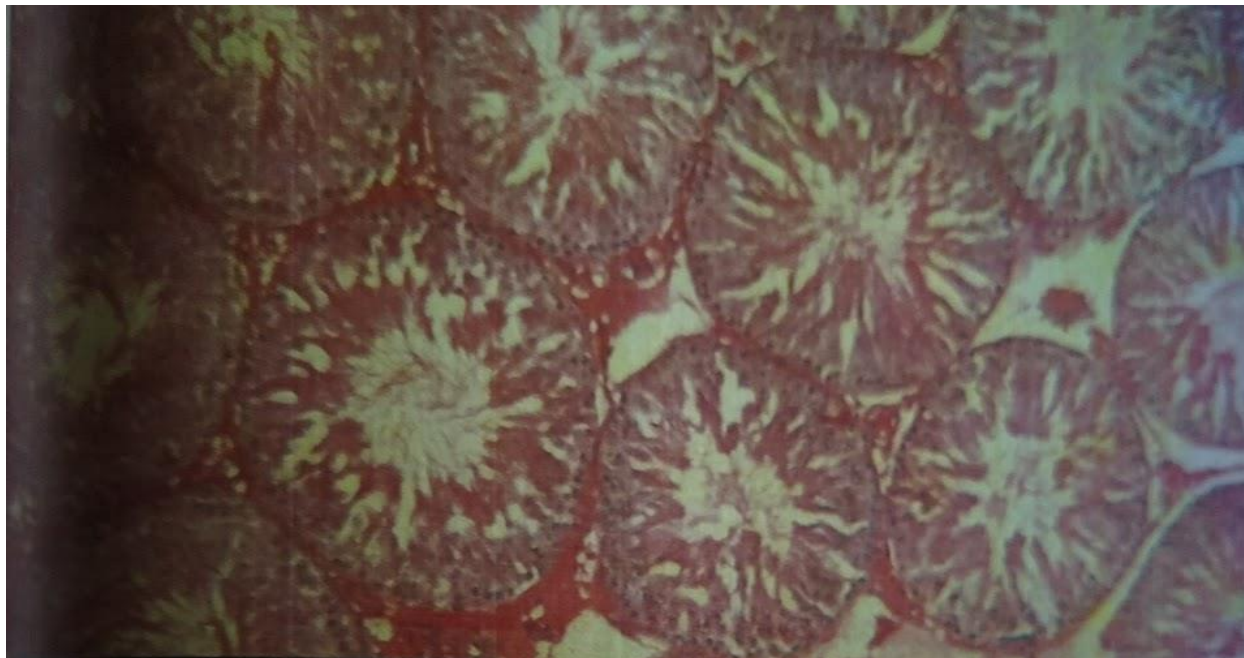


Plate 6 Photomicrograph of the testes of animals treated with testosterone showing normal and well-organized germ cells in the epithelium. H&E x100.

3.2 Discussion

Testicular oxidative stress results from an imbalance between the body's production of free radicals and its response to antioxidants (Abarikwu, 2013). Testicular function generally declines with aging in animals, impacting not just their ability to reproduce but also their general health and quality of life (Pataky et al., 2021). The increased apoptotic events that have been observed in the human testis with aging (Jiang et al., 2014) may also be, at least in part, explained by the accumulating ROS in aged testes due to reactive oxygen species (ROS) and the consequent oxidative stress.

Antioxidant activity declines with aging, and ROS levels rise (Weir & Robaire, 2007). It is well known that oxidative stress is caused by an imbalance between ROS and antioxidants in the male reproductive system (Jervis & Robaire, 2004) and in the spermatozoa of elderly rodents. Advanced paternal age (APA) is related to a poor impact on the quality of male germ cells, despite the fact that spermatozoa are constantly produced with APA. (Lawson & Fletcher, 2014)

Glutathione can exist in two different states: reduced (GSH) and oxidized (GSSG). According to Lu (2013), an elevated GSSG:GSH ratio is a sign of increasing oxidative stress. The ratio of reduced glutathione to oxidized glutathione within cells is a measure of oxidative stress in the cell. According to the current investigation, Cd poisoning increased GSH. In response to the toxicity of Cd, there was a rise in GSH concentration (Ogasawara et al., 2014). According to Lan et al. (2008), rising Cd concentrations caused the loss of cellular GSH content. From this research, the level of GSH increased in the aged control group but decreased statistically in 50 mg FPS, 100 mg, 200 mg FPS, and testosterone when compared to the negative aged control.

Almost all living things, including bacteria, plants, and animals that use oxygen, include the enzyme catalase. This enzyme essentially guards the cell against oxidative damage brought on by reactive oxygen species (ROS) by catalyzing the breakdown of hydrogen peroxide into water and oxygen (Chelikani et al., 2004). Increased catalase activity in 4-week studies points to increased oxidative stress caused by damaged spermatozoa in the epididymis and phagocytotic activity of Sertoli cells in the testes (Bedwal et al., 2009). From this present study, the level of CAT increased in the aged group when compared to the young control group. This is as a result of oxidative stress experienced in the testes of aged animals but decreased statistically in 100 mg FPS, 200 mg FPS, and testosterone, with 50 mg FPS showing the highest reduction when compared to the negative aged control.

Lipid peroxidation is the series of oxidative lipid breakdown processes. It is a process that occurs spontaneously in the body in modest levels, primarily as a result of the interaction of numerous reactive oxygen species (Feng et al., 1997). The oxidation's chemical byproducts are referred to as lipid peroxides (Huang et al., 2002). Reactive aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE), the latter of which is referred to as the "second messenger of free radicals" and a key bioactive marker of lipid peroxidation, are the end products of lipid peroxidation (Ostrea et al., 1985). Lipid peroxidation has recently come to light as a critical stage in the etiology of a number of disorders in both adult and newborn patients (Mohammad et al., 2014). From this study the level of LPO increased in the aged group when compared to the young control group but decreased statistically in 50 mg FPS, 100 mg FPS and testosterone when compared to the negative aged control.

In the histopathology results, there was loss of germ cells and cellular debris in the lumen of the aged animals. The group that was administered FPS extract of the various doses had regenerating germ cells, with the group that received 50 mg/kg b. w of FPSE with the most mitigation of damage. The testosterone group also had regenerated germ cells. However, the result showed that testosterone mitigated the damage caused in aged rat testes as a result of oxidative stress. Recent studies demonstrated that aging can cause testicular damage and reduced testicular germinal epithelium (Dong et al., 2022). This result demonstrated that aging affected the epithelium and the tubular diameter of the testes; it also depleted the germ cells.

4. Conclusion

In this study, oxidative stress caused by aging was inhibited, demonstrating the mitigating ability of FPSE. However, the majority of the noteworthy outcomes were observed in the testicles of animals who had received a lower dose of FPSE treatment. Accordingly, the findings suggested that a modest intake of FPSE would be advantageous in lowering oxidative stress in older men, thereby minimizing the effect of aging on male fertility.

The information presented in this study suggests that FPSE consumption may be advantageous in reducing the harm done to the reproductive organs of old male rats as a result of aging by oxidative stress. The decrease in oxidative stress indicators in the testes of rats given different dosages of FPSE served as proof of this. Overall, administering a lower dose of fluted pumpkin seed extract was more effective at reducing the effects of oxidative stress on the testis than administering a greater amount.

The addition of fluted pumpkin seeds in a diet plan for the treatment of infertility issues in older men is advised by health and nutritional organizations in light of the study's findings. The impact of the extract on the gonads of older female rats should be the subject of more research. This present work has provided additional information on the ameliorating effect of fluted pumpkin seed extract on oxidative-induced testes of aged male rats.

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