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Experimental Research Article

Omega-3 PUFAs counteracts high fructose diet-induced gonadal impairment via fortified antioxidant defense

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ABSTRACT

Introduction: The consumption of fructose sweeteners as an alternative to high-calorie diets has been linked to various metabolic diseases due to increased oxidative stress. This study investigated changes in testicular and ovarian functions following Omega-3 polyunsaturated fatty acid supplementation (ω-3 PUFAs) in rats fed a High Fructose Diet (HFD).

Methods: Forty Wistar rats were equally and randomly distributed into four groups. Each group was further subdivided by gender, with an equal number of animals in each sub-group. Group A assigned as control, while Groups B and C were administered HFD containing 10% and 20% v/v fructose solution, respectively. Group D rats were provided 20% v/v HFD and ω-3 PUFAs.

Results: After six weeks of consumption, HFD resulted in reduced semen quality and gonadal reproductive hormone levels, while increasing testicular and ovarian oxidative stress. ω-3 PUFAs improved semen quality, reproductive hormone concentrations, and antioxidant defense system.

Conclusion: HFD impairs gonadal function, however, dietary supplementation with ω-3 PUFAs improves gonadal functions by enhancing the antioxidant defense mechanism.

Keywords:
High-fructose diet
Infertility
Omega-3
Ovary
Oxidative stress

Introduction

The persistent consumption of refined sugars and high-calorie diets has been shown to significantly impact human health (Gómez-Crisóstomo et al., 2017; Tchernof and Després, 2013). Sweeteners such as fructose are widely accepted due to their economic benefits and perceived value as dietary alternatives for avoiding metabolic diseases, leading to a significant increase in their use (DeChristopher et al., 2015). However, clinical findings have demonstrated that chronic fructose consumption contributes to the progression of diabetes mellitus (Softic et al., 2016), Obesity (Basciano et al., 2005), dyslipidemia, high blood pressure (Pereira et al., 2017), and insulin resistance (Toop and Gentili, 2016).

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In addition, regular fructose consumption, particularly at high levels, has been associated with increased oxidative stress and inflammation in multiple organ systems (Jarukamjorn et al., 2016).

The pathophysiology of oxidative stress, a contributing factor in conditions such as diabetes, arteriosclerosis, and liver damage, also plays a significant role in reproductive health (Spahis et al., 2017; Tan et al., 2015). Current findings have shown that oxidative stress has a deleterious impact on fertility, with deficiencies in both enzymatic and non-enzymatic antioxidant systems in the gonads (Agarwal et al., 2012; Martins and Agarwal, 2019). Disruption of these antioxidant systems leads to excessive production and accumulation of reactive oxygen species (ROS), compromising the structural and functional integrity of testicular and ovarian cells (Dobrakowski et al., 2018; Wang et al., 2017).

Long-chain polyunsaturated fatty acids (PUFAs), such as Omega-3 fatty acids, are essential fatty acids (EFAs) and vital components of membrane phospholipids in organs such as the brain, retina, and spermatozoa (Weylandt et al., 2015; McGlory et al., 2019; Salem et al., 2001). The benefits of Omega-3 (ω-3) PUFAs have garnered significant attention due to their positive effects on lipid profiles, vascular tone, and blood coagulation, among other benefits (Marik and Varon, 2009; Thusgaard et al., 2009). Numerous factors influence fecundity and fertility, some of which, such as age and genetic predisposition, are unchangeable. However, modifiable factors that adversely affect reproductive tissues, including unhealthy diets, weight gain, excessive drinking, and smoking, can be addressed (Rossi et al., 2014). These modifiable factors generate ROS, leading to testicular and ovarian dysfunction and, consequently, infertility, which can fortunately be mitigated (Showell et al., 2014).

The recognition that certain dietary sweeteners and additives are linked to infertility underscores the need for further research in reproductive health and fertility. There remains a lack of information on oxidative stress and antioxidant defense mechanisms as pathological contributors to infertility following a high-fructose diet. Furthermore, it is crucial to investigate the ameliorative potential of ω-3 fatty acids in counteracting the effects of high-fructose diet-induced damage to gonadal functions. This study examines the impact of high fructose consumption on enzymatic indicators of oxidative stress and gonadal physiological and morphological features, as well as the potential protective and restorative effects of ω-3 PUFAs.

Materials and Methods

Ethics

The animals were treated in strict conformity with the National Institutes of Health guidelines for the care of laboratory animals (NIH Publications No. 8023, revised 1978). The experimental protocols were approved by the Institution's Research and Bio-Ethics Committee (FBS/RBC/19/052).

Experimental Protocols

For fourteen days, the experimental rats (10–12 weeks old) were acclimatized and provided with fresh rat food and clean water. At the start of the experiments, forty albino Wistar rats weighing 180±20 g were randomly allocated to one of three treatment groups, each with ten specimens. Each group was further divided into two equal-sized subgroups based on gender. Group A rats were fed a normal diet (Control), Groups B and C were administered a fructose solution containing 10% and 20% v/v High Fructose Diet (HFD), respectively, and Group D rats were treated with 20% v/v HFD along with 150mg/Kg B.W. ω-3 PUFAs.

Fructose solution and ω-3 PUFAs Administration

Absolute fructose solution and ω-3 PUFAs, manufactured by Roche Nigeria Ltd, were acquired from a reputable pharmaceutical store. The ω-3 PUFA treatment involved daily oral gavage with a suspension comprising industrially prepared 60% eicosapentaenoic acid (EPA) and 40% docosahexaenoic acid (DHA), obtained from capsules (150 mg/kg BW daily) (Kokacya et al., 2015). The desired fructose doses were freshly prepared with distilled water and administered daily via gavage, with 10 mL/kg of 10% and 20% (v/v) fructose solutions, respectively (Nyakudya et al., 2018).

Sample Collection

At the end of the four-week experimental period, the animals were euthanized via cervical dislocation. Blood samples were collected via cardiac puncture into heparinized tubes for testosterone, β-estradiol, and progesterone analyses. Identical amounts of semen were collected for seminal fluid analysis. The rats' testes and ovaries

were harvested; one pair of each was homogenized for tissue antioxidant evaluation, while the other was preserved in Bouin's solution for histological investigation.

Semen Analysis

The epididymis was incised at the caudal and caput ends, and its contents were transferred to a glass slide. After adding one milliliter (1ml) of phosphate-buffered saline (PBS), an epididymal plasma suspension was formed. Sperm parameters such as sperm count, percentage of non-motile sperm cells, and percentage of abnormal sperm morphology were then determined using a Neubauer hemocytometer. To count sperm cells, 10 microliters of the suspension were pipetted and diluted with 990 microliters of paraformaldehyde and sodium citrate solution. The diluted epididymal plasma suspension was placed in the hemocytometer counting chamber (10 microliters), and sperm cells were counted using a light microscope at x400 magnification. The sperm concentration was calculated based on the hemocytometer's dimensions and expressed in millions of sperm per milliliter (Ahmed et al., 2011). Similarly, the percentage of non-motile sperm cells was determined by collecting 5 microliters of supernatant containing epididymal plasma with a micropipette. This suspension was placed between the glass slide and cover slip, then examined using negative phase microscopy at x100 magnification. To assess abnormal sperm morphology, 20 microliters of sperm suspension were loaded onto a clean glass slide, mixed, and dispersed evenly across the surface. The smear was air-dried and then stained with Carbol Fuchsin by Ziehl-Neelsen, followed by an aqueous solution of Loeffler's methylene blue. After quickly washing the stained smear with water and air-drying it again, defective sperm cells were identified by the presence of multiple heads/tails, twisted mid-pieces, missing heads/tails, and uneven heads. The percentages of non-motile and abnormal sperm cells were then calculated (Menkveld et al., 2011).

Hormonal Assay

Plasma testosterone, β-estradiol, and progesterone concentrations were measured using the ELISA Stat Fax 303 enzyme-linked immunosorbent assay. According to the kit instructions (Randox Laboratories Limited, UK), endogenous hormones in the rats' plasma competed for binding to the coated antibody with a hormone-horseradish peroxidase conjugate. The absorbance of the test specimens was measured at 450 nm using an automated reader.

Antioxidant and Lipid Peroxidation Analysis

The testis and ovaries were homogenized in a phosphate buffer with a pH of 7.4. To analyze the gonads' oxidative stress markers, the antioxidant indices superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) activity were measured. The breakdown of nitroblue tetrazolium (NBT) by superoxide radicals produced in the xanthine-xanthine oxidase system was used to evaluate the activity of the SOD enzyme. The formazan generated after the reactions was blue and had the greatest absorbance at 560 nm (Sun et al., 1988). SOD enzyme activity was calculated as μ/mgHb. CAT activity was determined by measuring the rate of H2O2 degradation using a spectrophotometer, as H2O2 absorbs light at 240 nm (Aebi, 1984). CAT activity was calculated as μ/mgHb. GPx activity was evaluated spectrophotometrically because it catalyzes the oxidation processes of glutathione (GSSG) with glutathione disulfide reductase (GR), resulting in GSH and the concurrent oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) to NADP+. The enzymatic activity of GPx was determined by observing the disappearance of NADPH at an absorbance wavelength of 340 nm (Paglia and Valentine, 1967). Gonadal GPx activity was calculated as μ/mgHb. Lipid peroxidation in the testis and ovary was determined by assessing the concentration of the MDA marker. MDA created a pink complex with thiobarbituric acid (TBA), and the absorbance was read at 532 nm (Placer et al., 1966). The testicular and ovarian MDA content was expressed as nmol/gm tissue.

Determination of estrous Cycle

For this investigation, female rats with identical estrous cycle patterns beginning in the proestrus phase, were selected. Vaginal swabs were obtained using cotton wool dampened with physiological saline as part of a technique to investigate the different stages of the estrous cycle. The cotton wool was gently rolled on the rat's vaginal wall to collect the swab. These swabs were then repeatedly dabbed and evenly distributed on a glass slide before being stained with 400 L of Papanicolaou stain. The slide was flushed with water and examined

under a light microscope (x200 magnification). The estrous cycle of rats in the proestrus phase was confirmed using a swab that revealed more nucleated cells than epithelial cells and leucocytes (McLean et al., 2012). Blood samples were collected during this phase, as the proestrus stage in Wistar rats corresponds to the human follicular or proliferative phase, which is accompanied by a rise in plasma β-estradiol

Preparation of Tissues for Histological Analysis

Bouin's solution was used to preserve bloodstain-free samples of the testis and ovary for the histological investigation. The gonads were dehydrated in multiple progressive grades of alcohol after 24 hours of fixation. The tissues were then cleared in xylene and embedded in paraffin wax. They were sectioned with a rotary microtome, mounted on clean slides, and stained with Hematoxylin and Eosin (H&E). To reduce bias during histopathologic analysis, every structure of interest within the tissues was collected using a stereological approach.

Statistical Analysis

Statistical analysis was performed using IBM SPSS version 26, and data were displayed in bar charts generated with GraphPad Prism version 8.04 (GraphPad Software, San Diego, USA). Data were expressed as mean ± standard error of the mean (SEM) and analyzed using the Shapiro-Wilk normality test. Subsequently, statistical significance was evaluated using One-Way Analysis of Variance (ANOVA), followed by the LSD post-hoc test. A p-value of less than 0.05 was considered statistically significant.

Results

Effect of HFD on Semen Quality

It was observed in Fig 1A that a HFD caused a significant (P<0.05) decrease in sperm count, which was mitigated by co-administration of ω-3 PUFAs. However, this change was also significant (P<0.05) when compared with the control. In a dose-dependent manner, both 10% HFD and 20%HFD significantly (P<0.05) increased the percentages of abnormal sperm morphology and non-progressively motile sperm cells. ω-3 PUFAs reversed the effect of 20%HFD-induced increase in the percentage of abnormal sperm cells (Fig 1B), however, the effect on the percentage of non-motile sperm cells was minimal (Fig 1C).

Effect of HFD on Gonadal Hormonal Profile

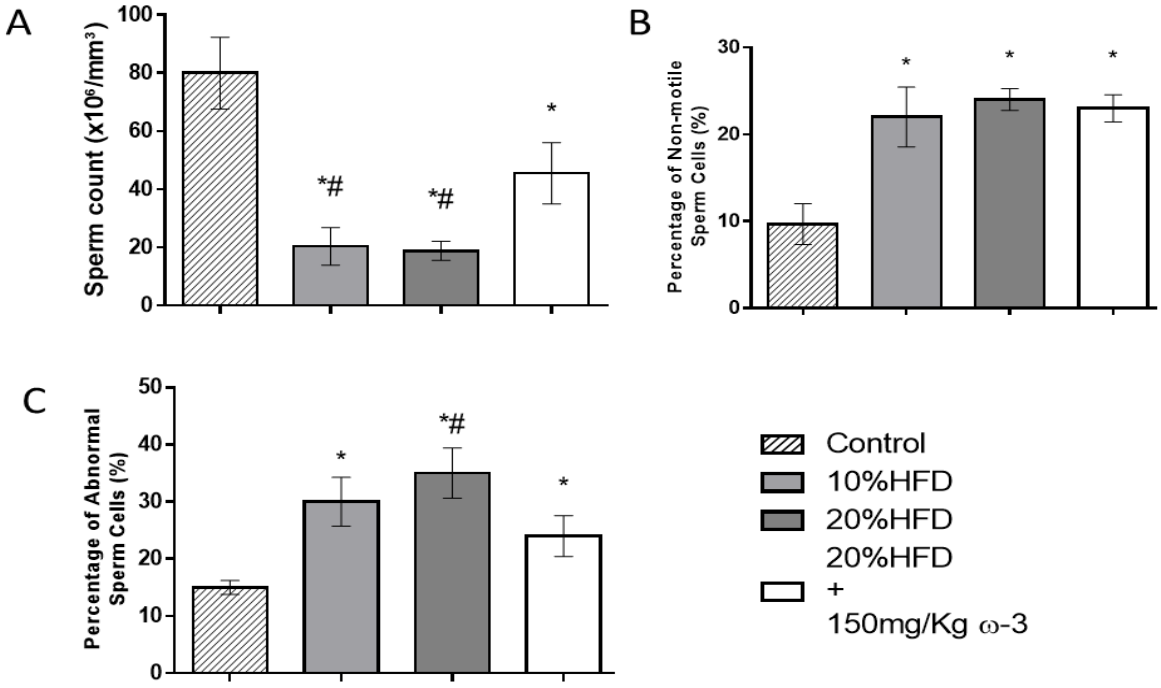

FIGURE 1. Effect of High Fructose Diet on Sperm Count (A), Percentage of Non-Motile Sperm Cells (B), and Percentage of Abnormal Sperm Cells (C). Each column represents means ± SEM. One-way ANOVA and post hoc LSD were used. $^{*}P < 0.05$, as compared to control group; $^{\#}P < 0.05$ as compared to 20%HFD + ω-3 PUFAs treated rats, n = 10

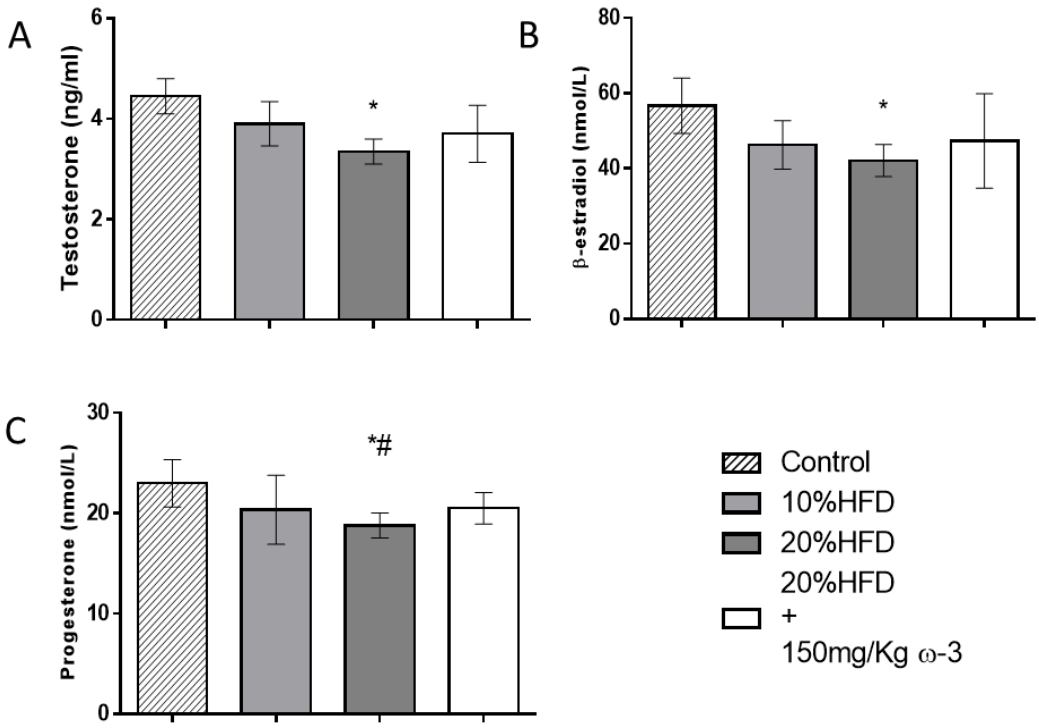

FIGURE 2. Effect of High Fructose Diet on plasma levels of testosterone (A), β-estradiol (B), and Progesterone (C). Each column represents means ± SEM. One-way ANOVA and post hoc LSD were used. $^{*}P < 0.05$, as compared to control group; $^{\#}P < 0.05$ as compared to 20%HFD + ω-3 PUFAs treated rats, n = 10

Fig 2 shows that 10%HFD and 20%HFD induced a graded decrease in plasma concentrations of testosterone in male rats. and β-estradiol and progesterone in female rats, as seen in Fig 2A and Fig 2B, respectively. This reduction in gonadal hormones was significant ($P<0.05$) in rats fed 20%HFD compared to control rats. ω-3 PUFAs reversed the effect of 20%HFD on the plasma levels of testosterone, β-estradiol, and progesterone, as no significant difference was observed when compared to the control

Effect of HFD on Oxidative Stress Markers in Testis and Ovary

In Fig 3A, 3B, and 3C, a dose-dependent reduction in SOD, CAT, and GPx activity was observed in both the testis and ovary following HFD treatment; nevertheless, the decrease in the gonadal antioxidant concentration of 20% HFD-treated rats was significant ($p<0.05$) when compared to control and 20% HFD + ω-3 PUFAs. Furthermore, 10% HFD caused a significant ($p<0.05$) decrease in ovarian GPx activity. Following administration of 10% and 20% HFD, graded tissue lipid peroxidation of the testis and ovary was observed, which was confirmed by the increasing levels of MDA in Fig 3D. ω-3 PUFAs appear to have minimal antagonistic effects against the 20% HFD in gonadal MDA concentrations, as observed in Fig. 3D. The effect of ω-3 PUFAs did not cause significant change.

Effect of HFD on Histology of Testis and Ovary

In control rats, normal architecture of seminiferous tubules with distinct phases of spermatogenic cells and characteristic interstitial cells of Leydig was seen (Fig 4A). HFD also caused dose-dependent changes in the histoarchitecture of the testis. This was confirmed in Fig 4B and 4C, which show derangement in the seminiferous tubules and interstitial Leydig cells in rats treated with a 10% HFD. Observations indicate that a 20% HFD caused similar but more severe testicular damage, accompanied by spermatogenic cell arrest within the seminiferous tubules. The photomicrographs of testes from rats co-administered with 20% HFD and ω-3 PUFAs displayed features similar to those of control rats.

In the control rat, the ovary histology shows typical

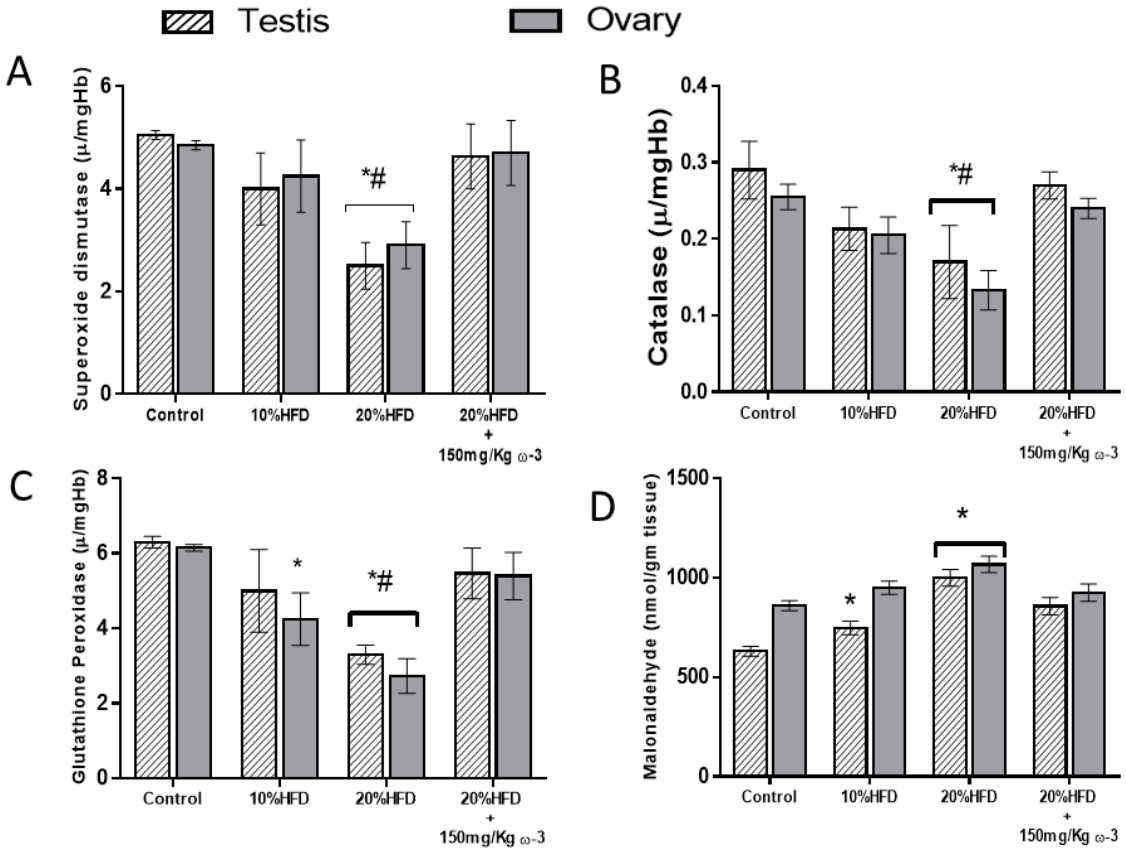

FIGURE 3. Effect of High Fructose Diet on Testicular and Ovarian Superoxide dismutase - SOD (A), Catalase - CAT (B), Glutathione peroxidase -GPx (C), and Malonaldehyde - MDA (D). Each column represents ± SEM. One-way ANOVA and post hoc LSD were used. $^{*}P < 0.05$, as compared to control group; $^{\#}P < 0.05$ as compared to 20%HFD + ω-3 PUFAs treated rats, n = 10

follicular development with visible granulosa cells and prominent oocytes (Fig 5A). However, slightly arrested but visible follicular development was observed following 10%HFD treatment (Fig 5B), making the oocytes appear almost normal, with noticeable mild hyalinosis of the arteriola. In Fig 5C, ovarian modifications resulting from a 20% HFD exposure exhibited evident cystic degeneration around the follicle, many of which were atretic, along with apparent fibrotic changes in the stroma (degenerated stromal tissue) and arteriolar hyalinosis. ω-3 PUFAs ameliorated the destructive effect of 20%HFD, as the oocytes appear almost normal with slight follicular development (Fig 5D).

Discussion

A typical diet supplies essential nutrients as well as oxidant/antioxidant components to the tissues of living organisms (de Roos and Duthie, 2015). The association of excessive fructose consumption and multi-organ oxidative stress increase has been comprehensively addressed by data obtained from various research, however, this relationship in the gonads has received less attention.

Fructose consumption decreased gonadal SOD, CAT, and GPx activities in a dose-dependent manner, and this reduction in antioxidant enzyme markers was consistent with earlier findings (Datta et al., 2000; Srividhya and Anuradha, 2002). Superoxide ions build as a result of SOD decline, restricting CAT production in the process, as demonstrated by testicular and ovarian CAT reductions (Almaghrabi, 2015). It is also understood that CAT deficiency promotes hydrogen peroxide (H2O2) accumulation in tissues or organs. As a powerful oxidant, H2O2 exacerbates oxidation damage in the testes and ovaries (Karuppanapandian et al., 2011). MDA is produced through the assault on highly polyunsaturated fatty acids in plasma membranes by reactive oxygen species (ROS). The testis and ovary, in particular, have large polyunsaturated membrane lipid contents, making

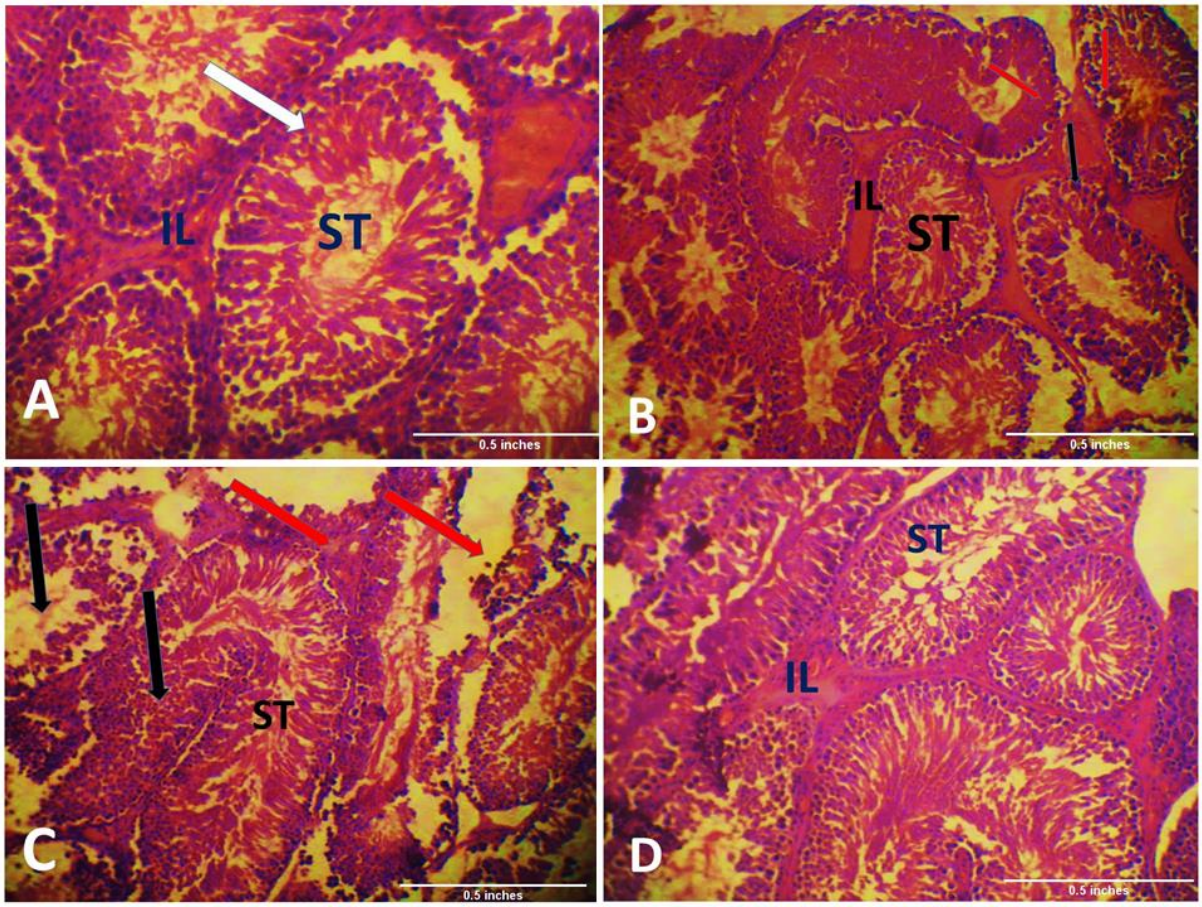

FIGURE 4. Photomicrographs of Rat's testis x100, n = 10 (A) control, (B) 10% High Fructose Diet, (C) 20% High-Fructose Diet, (D) 20% High-Fructose Diet + ω-3 PUFAs. Hematoxylin & Eosin stain. Group A (Control) shows normal histo-architecture with organized layers of different spermatogenic cells at different stages of maturation and no pathological changes in the ST or IL. The testis of rats in Group B shows distortion in both the seminiferous tubules (black arrow) and interstitial cells (red arrow). Testis of Group C rats showed further derangement of spermatogenic cells in the ST (black arrow) and IL (red arrow). Group D revealed notable restoration of the ST with visible spermatozoa and normal arrangement of IL.
ST; seminiferous tubule, IL; interstitial cells of Leydig

them vulnerable to oxidative stress (Al-chalabi et al., 2016; Asadi et al., 2017; Sahoo et al., 2007). In our investigation, fructose dramatically elevated testicular and ovarian MDA activity, consistent with previous research (Chandramohan and Pari, 2013). Lipid peroxidation in the gonads is demonstrated by the rise in MDA levels, further suggesting tissue damage induced by elevated oxidative stress.

HFD treatment exhibited detrimental impacts on sperm functional characteristics, significantly lowering sperm count while also increasing non-motile sperm and sperm with morphological abnormalities. The decrease in sperm count signals HFD interference in spermatogenic processes via enhanced ROS production (Mupfiga et al., 2013). The spermatotoxic effects of HFD can be attributed to reduced antioxidant enzyme defense (Vijaya Bharathi et al., 2015). According to Aitken et al. (2016), Turner and Lysiak (2008), and Arafa et al. (2009), superoxide ions and H2O2 generated during antioxidant enzyme insufficiency impair sperm fertilization potential by reducing the percentage of progressively motile sperm and normal sperm morphology. the disruption of spermatogenesis leading to the decline of semen quality examined in this study strongly indicates genetic damage; therefore, HFD can also induce testicular and ovarian DNA damage (Sarabia et al., 2009).

Increased ROS formation in the testis and ovaries reportedly alters tissue function and histo-architecture substantially, compromising reproductive capacity (Devine et al., 2012; Naeimi et al., 2017). Testicular and ovarian histo-architecture were adversely altered in animals given increasing doses of HFD. Spermatogenic arrest

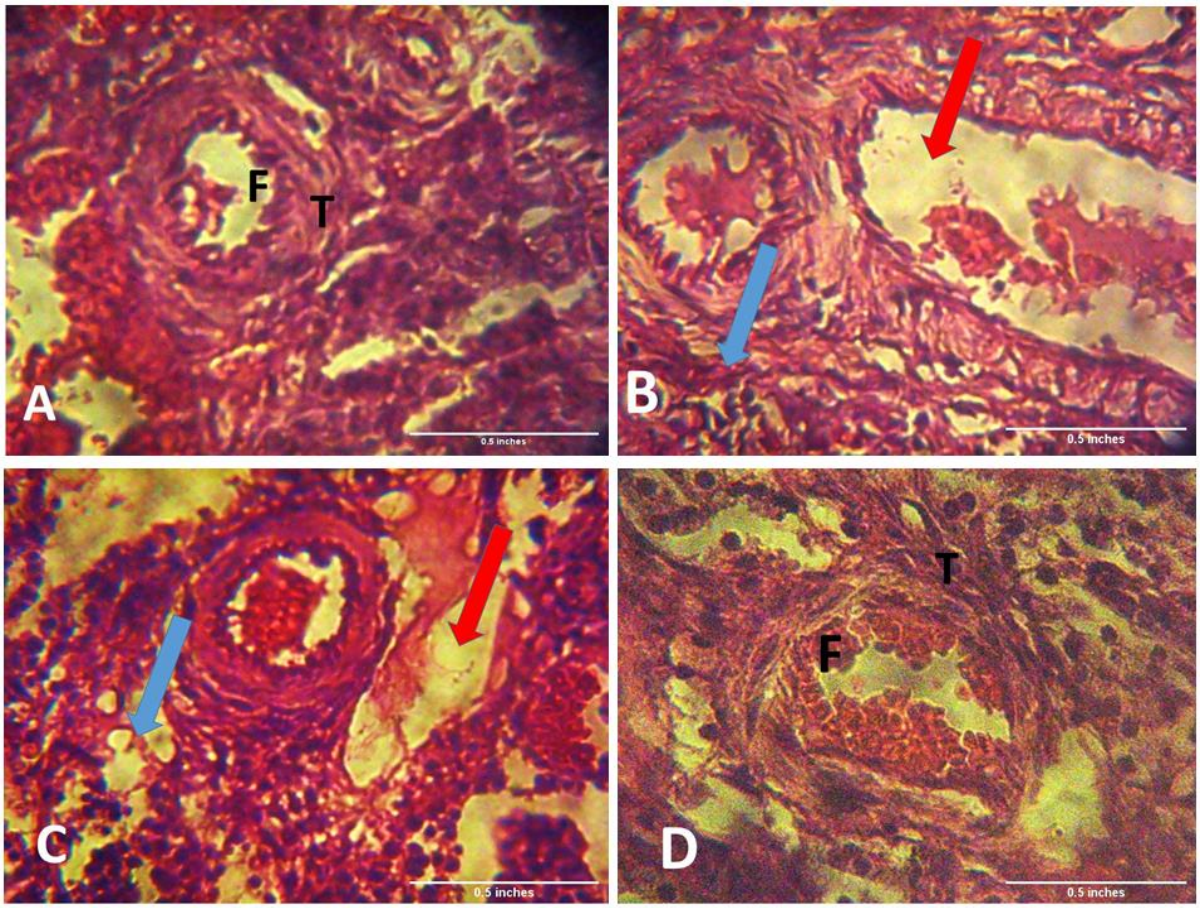

FIGURE 5. Photomicrographs of Rat's Ovary x100, n = 10 (A) control, (B) 10% high-fructose diet, (C) 20% high-fructose diet, (D) 20% high-fructose diet + ω-3 PUFAs. Hematoxylin & Eosin stain. Group A (Control) showed prominent oocytes with normal follicular and granulosa cells. Group B oocytes show cysts surrounding the follicles (blue arrow) and visible atresia. Fibrotic changes and degeneration are seen in stromal tissue (red arrow). Group C also exhibited similar deteriorating changes in follicular cells and ovarian tissue stroma. Group D shows that the ovary shares similar histological features with the control. Normal follicular and granulosa cells were observed.

within germ cell lines, seminiferous tubule (ST) malformations, and Leydig cell shrinkage reported in HFD rats demonstrate that HFD has a deleterious influence on gonadal histological architecture, subsequently impairing the spermatogenic process. Lipid peroxidation caused by HFD- induced MDA elevation is believed to result in Leydig cell shrinkage and ST degeneration (Farombi et al., 2013; Leong et al., 2013). HFD caused similar ovarian histology abnormalities with visible cystic degeneration of the ovarian follicles, possibly due to the susceptibility of the unsaturated fatty acids in the ovarian membrane to damage from lipid peroxidation and oxidative stress.

Leydig cell derangement is known to impede testosterone synthesis, which eventually leads to disturbances in spermatogenesis (Rajendar et al., 2011). The decreased testosterone levels in HFD rats compared to controls in our study confirm recent findings by Tkachenko et al (2020). HFD caused a decrease in serum concentrations of β-estradiol and progesterone, which is not surprising given the fructose-induced destruction of ovarian cells observed in this study, potentially jeopardizing ovarian gametogenesis and steroidogenesis, as well as uterine secretory and proliferative roles. According to Matsuda et al. (2012), ovarian follicle and granulosa lutein insufficiency can result during periods of amplified oxidative stress, in this instance mediated by HFD, resulting in β-estradiol and progesterone reductions. Studies from Gunzel-Apel et al. (2012) and Robeck et al. (2012), further confirm our observation.

Treatment with ω-3 PUFAs significantly reduced the lipid peroxidation damage caused by HFD administration. Reproductive dysfunction has been associated with inadequate antioxidant capacity in gonadal tissue, as ob-

served in several studies (Agarwal et al., 2012; Martins and Agarwal, 2019).

However, the results of the study indicated that ω-3 PUFAs increased the concentrations of SOD, CAT, and GPx in the testes and ovaries of rats on a HFD.

In addition, ω-3 PUFAs reduced MDA levels in the gonads. Although studies about ω-3 PUFA's protection against HFD-induced oxidative stress in male and female gonads are not available, several studies have shown that the ω-3 PUFAs protect the reproductive system from oxidant-induced toxicity (Wakefield et al., 2008). Meital et al. (2019) reported that ω-3 PUFAs exert enhanced antioxidant enzyme defense and radical scavenging to showcase their antioxidant properties. ω-3 PUFAs possess ROS neutralizing ability, which is important in their reported protection against intrinsic and extrinsic factors destruction to macromolecules and tissue (Kamaraj et al., 2011; Pradeep et al., 2012).

Damage to the lipid matrix of the male germ cell membrane will eventually lead to decreased sperm concentration, impaired sperm motility, incomplete sperm development, complete suppression of the sperm maturation process, and various morphological abnormalities (Aydos et al., 2015). Following ω-3 PUFAs treatment, the negative effects of HFD on sperm parameters were mitigated by increasing sperm count, and decreasing the percentage of defective sperm cells. As a result, ω-3 PUFAs improved testicular function in rats while adversely affecting those on a HFD, possibly due to their inherent antioxidant properties. Progressive sperm motility was marginally improved in rats on a 20% HFD by ω-3 PUFAs, although the improvement was not statistically significant. According to Zalata et al. (1998), the proportion of motile sperm and ω-3 PUFA concentrations are strongly positively correlated. Cooray et al. (2022) demonstrated a comparable ω-3 PUFA impact on sperm motility in an earlier investigation. Although there is evidence that several fatty acids might alter sperm motility, the data from the current study are not sufficient to determine why ω-3 PUFAs had such a little effect on 20%HFD rats. We assume that ω-3 PUFAs, however, hindered spermatozoa capacitation. This argument is further validated by Liu (2007), who reported that arachidonic acid (an example of which is ω-3 PUFAs), inhibits the mobilization of calcium ions required for sperm capacitation and motility through calcium ion channels.

Consumption of ω-3 PUFAs resulted in considerable recovery from HFD-induced testicular and ovarian damage, preserving normal gonadal histo-architectures. Soliman et al. (2017) and Stoffel et al. (2020) reported similar findings. These improvements were attributed to the powerful antioxidant properties of the ω-3 PUFAs (Olutope et al., 2014; Spencer et al., 2009). Antioxidant supplementation, such as melatonin (Mohd Mutalip et al., 2018) and resveratrol (Lian et al., 2013), reduces inflammatory damage from oxidative stress in the ovaries by enhancing the number of oocytes. ω-3 PUFAs ameliorated the deteriorating effects of HFD by improving the ovarian enzymatic antioxidant defense system. This action led to increased plasma concentrations of β-estradiol and progesterone in the rats.

Limitation

The study's main drawback is that we did not look into the optimal treatment dose of ω-3 PUFAs in this model of impaired fertility. To compare various doses and administration durations, this crucial problem requires well-designed research.

Conclusion

In conclusion, HFD consumption resulted in gonadal toxicity, as evidenced by increased gonadal lipid peroxidation and disruption of the pro-and anti-oxidant balance. These adverse effects were accompanied by reduced semen quality, altered reproductive hormone levels, and histological complications in the testis and ovary. However, due to their enhanced antioxidant defense properties, ω-3 PUFAs improved all indices of HFD-induced gonadal damage. As a result, they show remarkable therapeutic potential in guarding against testicular and ovarian toxicity caused by HFD exposure.

Acknowledgment

We acknowledge the sacrifice, dedication, and commitment of the staff, and personnel in our research laboratory, Department of Physiology, Delta State University, Nigeria.

Conflict of interest

The authors declare that there is no conflict of interest

Ethical Approval

The experimental protocol was approved by the

Animal Ethics Committee of Faculty of Basic Medical Sciences, Delta State University, Nigeria (FBS/RBC/19/052). The animals were handled in strict conformity with the requirement of the National Institutes of Health guide for the care of laboratory animals (NIH Publications No. 8023, revised 1978).

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