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Ameliorative Effects of Pumpkin Seed Oil on Sexual-Reproductive Indices of Salt-loaded Male Rats: Involvement of the Hypothalamic-Pituitary-Gonadal Axis

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ABSTRACT

Background and Purpose: High sodium intake is a well-established contributor to hypertension, but its adverse effects on male reproductive health remain underexplored. Pumpkin seed oil (PSO) is known for its therapeutic potential, particularly in promoting reproductive health. This study aimed to evaluate the modulatory effects of PSO on the hypothalamic-pituitary-testicular (HPT) axis in adult male Wistar rats exposed to high salt-induced toxicity.

Methods: Twenty-four (24) adult male Wistar rats (9-11 weeks old; 150-180 g) were randomly assigned to four groups (n=6): Control (distilled water, 0.1 mL), High Salt (HS; 3 mL/100 g/day of 8% NaCl solution), HS + PSO (3 mL/100 g/day of 8% NaCl solution + 4 mL/kg/day of PSO), and PSO only (4 mL/kg/day). Treatments were administered orally for 14 consecutive days. Sexual behavioral parameters (mounting and intromission) were assessed. After treatment, blood and tissue samples were collected for hormonal assays, oxidative stress biomarkers, histological examination, and semen analysis.

Results: High salt exposure significantly disrupted the HPT axis, resulting in reduced sexual behavior, poor sperm quality, hormonal imbalance, oxidative stress, gonadotroph cell degeneration, and disordered spermatogenesis. In contrast, PSO administration markedly mitigated these adverse effects by restoring sexual performance, enhancing sperm quality, preserving testicular histoarchitecture, improving reproductive hormones, and boosting antioxidant enzyme activity.

Conclusion: Excessive salt intake compromises male reproductive function via oxidative damage and hormonal dysregulation. PSO exhibited significant ameliorative effects against salt-induced reproductive and neuroendocrine toxicity, likely due to its antioxidant and phytoestrogenic properties. These findings suggest that PSO could serve as a beneficial dietary intervention for safeguarding male fertility in salt-exposed populations.

INTRODUCTION

Reproduction, a fundamental process for the continuity of life, encompasses systemic physiological functions, behaviours, and anatomical structures essential for organisms' existence (Enye *et al*., 2020). In males, this process relies on the harmonious interaction of the hypothalamus, pituitary gland, and testes, collectively known as the hypothalamic-pituitary-testicular (HPT) axis. This axis serves as the major regulatory pathway governing male reproductive function, developmental processes, and the emergence of secondary sexual characteristics (Dhole and Kumar, 2017). Central to this cascade are reproductive regulatory hormones such as hypothalamic gonadotropin-releasing hormone (GnRH), pituitary luteinizing hormone (LH), follicle-stimulating hormone (FSH), and gonadal steroids (Dwyer and Quinton, 2019). The rhythmic release of GnRH from the hypothalamus initiates this physio-steroidal mechanism. Upon reaching the anterior pituitary via the hypophyseal portal circulation, GnRH binds to its receptors (GnRHR) on gonadotropes, stimulating the synthesis and release of LH and FSH. LH facilitates testosterone production by Leydig cells in the testes and enhances androgen-binding protein synthesis by Sertoli cells. FSH acts in collaboration with testosterone to promote the growth, meiosis, and maturation of germ cells by binding to Sertoli cell receptors (Bliss *et al*., 2010).

In almost half the number of infertility cases, the underlying cause is attributed exclusively to the male partner. Evidence indicates that the quality of human semen has declined by 50%–60% over the past four decades (Gauthaman *et al*., 2002). Extensive research has demonstrated that diet directly influences semen quality and that the consumption of Western processed food might cause reproductive dysfunction (Stevenson *et al*., 2007).

The Global Burden of Disease (GBD) research highlights the severe health consequences of excessive salt intake, which resulted in approximately 70 million years of healthy lives being lost and contributed to 3 million deaths globally in 2017 (Roth *et al*., 2018). In most developed nations, around 80% of salt intake comes from food products at the processing stage, leaving consumers often unaware of the substantial amounts of salt added to these foods (James *et al*., 1987; Partearroyo *et al*., 2019). In contrast, in non-industrialized nations, up to 75% of daily salt consumption is attributed to salt added during cooking, at the table, or through the use of condiments such as soy sauce and fish sauce for seasoning (Brown *et al*., 2009). Elevated sodium consumption is strongly correlated with health conditions such as cardiovascular diseases (CVDs) (Xue *et al*., 2023), and disruptions in steroidal balance, which can potentially lead to sexual dysfunction in males (Fang *et al*., 2018; Abdelnour *et al*., 2020).

Pumpkin seeds possess potent antioxidant properties, attributed to their ability to neutralize free radicals and interact directly with the iron or copper binding sites of lipids, proteins, and DNA molecules (Shaban and Sahu, 2017). Furthermore, extracts from pumpkin seeds have been employed as adjuncts in immunomodulation, reproductive health, and as therapies for diverse array of disease conditions (Stevenson *et al*., 2007). However, the impact of pumpkin seed oil on high salt-induced reproductive toxicity in males needs to be evaluated since it remains incompletely understood. Therefore, the present study aimed to investigate this relationship.

MATERIALS AND METHODS

Animals

Twenty-four (24) male Wistar rats, aged between 9 and 11 weeks and weighed approximately 150-180 g were used for this experiment. The rats were sourced from Mctemmy Animal Concept in Ogbomoso, Nigeria. They were provided with standard rat pellet diet and had access to water at all times. Six (6) animals were housed in a polypropylene cage measuring 8" × 12" × 8" with metal grill tops, and kept under controlled standard conditions. The animals were kept under natural conditions of temperature, humidity, and light. Beddings were replaced every three days to maintain appropriate housing conditions, and all experimental procedures were conducted in accordance with relevant guidelines as previously reported (Couto, 2011).

Ethics and Animal Welfare

All necessary measures were taken to minimize pain and discomfort in the animals. The Health Research Ethical Committee of Afe Babalola University, Ado-Ekiti, reviewed and approved the study protocol (ABUADHREC/06/08/2024/090).

Pumpkin Seed Oil, Salt and Chemicals

Pumpkin seed oil (PSO) was procured from Puritan's Pride, Oakdale, NY, USA. NaCl was purchased from Sigma-Aldrich Corporation, USA. Assay kits for malondialdehyde and glutathione (Biovision Inc., Milpitas, CA, USA) and hormonal assay kits were sourced from Calbiotech Inc., CA, USA).

Experimental Design

Following a week of acclimatization, the animals were randomly allocated into four (4) experimental groups, with six animals in each group (n = 6), as follows:

Group A (control) was administered 0.1 mL/day of distilled water.

Group B (high salt) was administered 3 mL/100 g/day of 8% NaCl solution (Peneme, 2023).

Group C (high salt + PSO) was administered 3 mL/100 g/day of 8% NaCl solution, concurrently with 4mL/kg/day of PSO (Rouag *et al*., 2020).

Group D (PSO) which received 4 mL/kg/day of PSO.

All dosages were related to the body weight of the animals and administration was by the oral route using orogastric tube and lasted 14 days.

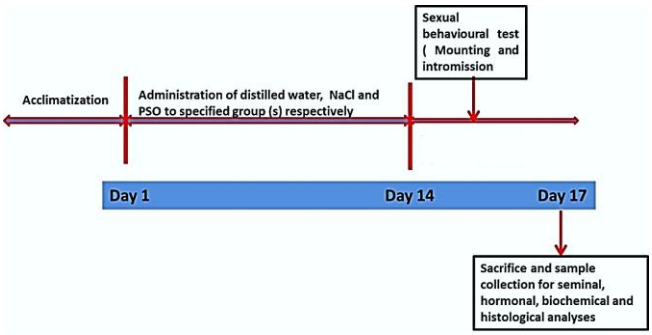

Figure 1: The experimental design.

Sexual Behavioural Tests

Male rats were exposed to sexually receptive females three times over four days prior to the experiment, between 19:00 and 23:00 hours and under dim lighting. Female receptivity was induced by sequential subcutaneous administration of 10 µg/100 g body weight of estradiol benzoate and 0.5 mg/100 g of progesterone, 48 h and 4 h, respectively, before pairing. After a 30-minute adaptation period in a plastic cage measuring 33.0 cm × 20.5 cm × 19.0 cm, the receptive female was introduced to the male. Proceptive behaviours of the female and precopulatory behaviours of the male were observed from the side of the cage. Four rats from each group were monitored for sexual behaviour within a 30-min observation period after all administration at day 14. Experiments were conducted over two days between 19:00 and 23:00 h and under dim lighting (Figure 1).

Following the standard procedures outlined by Odetayo and Olayaki (2022) the following indices of male sexual behaviour were recorded or calculated during the observation period: mount frequency (MF), defined as the number of times the male assumed a copulatory position without achieving intromission, characterized by lifting its fore body over the female's hindquarters and clasping the female's flanks with its forepaws; and intromission frequency (IF), defined as the number of vaginal penetrations achieved by the male.

Euthanasia and Tissue Collection

At the end of the experiment, the rats were euthanized by cervical dislocation (Aguwa *et al*., 2020). Blood was collected using 2 mL syringes, stored in plain blood sample bottles, and left to clot on the bench. Caudal epididymis of each rat was excised without perfusion. Animals for histological studies were transcardially perfused with phosphate buffered saline (PBS, pH 7.4) to clear the blood vessels and prevent cell shrinkage, then with 10% neutral buffer formal saline (NBF) which served as a pre-fixative. Brain for biochemical studies were perfused with PBS only and frozen for further analyses.

Spermatological Analysis

Semen samples were collected from a 10 mm segment of the caudal epididymis, known for its role in storing mature sperm. The epididymal segment was carefully dissected, cut into several pieces, and placed into specimen bottles containing 1 mL of physiological saline. The samples were then homogenized to release the spermatozoa. The specimen bottles were maintained at 37°C for 15 min to allow sufficient diffusion of sperm into the saline. The semen was then extracted from the specimen bottles using a 1 mL pipette, with drops placed onto cleaned microscope slides and covered with cover slips. The slides were examined under a light microscope to assess sperm parameters, including count, motility, and morphology, for all experimental groups (Hashemi, 2013).

Sperm count

A counting chamber was utilized for determining sperm count. Drops of semen were placed on two edges of the chamber, covered with cover slips, and examined under a light microscope (Omax MD82EZ, South Korea) at ×100 and ×400 magnification for cell counting. This procedure was repeated five times, and the average count was calculated to ensure accuracy and minimize bias.

Sperm morphology

Cells were stained and observed under a light microscope (Omax MD82EZ, South Korea) with the oil immersion objective set at × 100 and ×400 magnification. The percentage of normal and abnormal spermatozoa were quantified and compared.

Sperm motility

Sperm motility was assessed according to the guidelines established by the World Health Organization (WHO, 1999). Specifically, a 10 µL sample of the culture-sperm mixture was placed on a microscope slide. The motility of a minimum of 200 spermatozoa per specimen was evaluated across at least five different microscopic fields. The analysis quantified the percentage of sperm exhibiting progressive motility, non-progressive motility (in situ), and immotility.

Biochemical Assays

Determination of Malondialdehyde (MDA) and Gluthatione (GSH) Levels

The plasma lipid peroxidation levels were measured according to the concentration of thiobarbituric acid

reactive species (TBARs) (Placer *et al*., 1966) and the amount malondialdehyde (MDA) produced was used as an index of lipid peroxidation. Briefly, one volume of the test sample and two volumes of stock reagent (15%, w/v trichloroacetic acid in 0.25 N HCl and 0.375% w/v thiobarbituric acid in 0.25 N HCl) were mixed in a centrifuge tube. The solution was heated in boiling water for 15 min. After cooling, the precipitate was removed by centrifugation at 1500 *g* for 10 min, and then absorbance of the supernatant was read at 532 nm against a blank containing all reagents except test sample on a spectrophotometer (Shimadzu 2R/UV-visible, Tokyo, Japan). The MDA level was expressed in nmol/mL.

The plasma levels of reduced glutathione (GSH) were determined at 412nm using Sedlak and Lindsay method (1968). Samples were treated with 50% trichloroacetic acid and centrifuged at 1000g for 5 min. The reaction mixture consisted of 0.5 mL of the supernatant, 3.0 mL of a 0.2 mol/L Tris EDTA buffer (pH 8.9), and 0.1 mL of a 0.01 mol/L solution of 5,5'-dithiobis (2-nitrobenzoic acid). The solution was left at room temperature for 5 min before being analysed at 412 nm using a spectrometer. The GSH concentrations were expressed in nmol/mL.

Hormonal Assays

Male reproductive hormones in serum (GnRH, FSH, and LH and testosterone) were analysed using standardized immunoassay ELISA kits for testosterone (Cat. No. TE187S); LH (Cat. No. LH231F); FSH (Cat. No. FS046F); and GnRH (Cat. No. E-EL-0071). All the kits were ordered from Calbiotech Inc., California, USA. The calculations were done based on the instructions and steps provided on the reagent kits and in accordance with the methods used by Wang *et al*. (2018).

Histological Analysis

The fixed tissues of the testis, pituitary gland, and hypothalamus were subjected to dehydration using a graded series of alcohols, followed by clearing in xylene, embedding in paraffin, and sectioning with a microtome at a thickness of 5 µm. The brain and testis sections were stained with haematoxylin and eosin (Cardiff *et al*., 2014) which revealed cellular information and histoarchitecture. Testis was further stained using Periodic Acid-Schiff (Shafiei *et al*., 2014) and Masson's Trichrome stains (Ozawa and Sakaue, 2020). The brain tissues (hypothalamus and pituitary gland were stained with cresyl violet (Alvarez-Buylla, *et al*., 1990).

Statistical Analysis

The Inferential statistics was done using one-way analysis of variance (ANOVA) with Tukey's post hoc test (GraphPad Prism Software 9.0.0). Data were plotted as graphs (mean ± SD) and significance between compared data was at the threshold $P<0.05$. ImageJ software (1.48 v/java 1.6.0_20) was used in histomorphometric analysis.

RESULTS

Effects of Pumpkin Seed Oil on Sexual Behaviour

Effects of pumpkin seed oil (PSO) on high salt-induced sexual dysfunction in adult male Wistar rats are shown in Figure 2. There was a significant ($P<0.0001$) reduction in number of mounts following high salt toxicity (HS) administration when compared with control. There was also a significant difference in number of mounts in HS+PSO ($P<0.001$) and PSO ($P<0.0001$) groups compared with the HS group (Figure 2A). Similarly, the analysis of intromission frequency showed significantly ($P<0.0001$) reduced intromission following high salt administration (HS group) when compared with the control group (Figure 2B). There was a significant difference in number of intromissions seen in HS+PSO ($P<0.01$) and PSO ($P<0.0001$) groups compared with the HS group (Figure 2B).

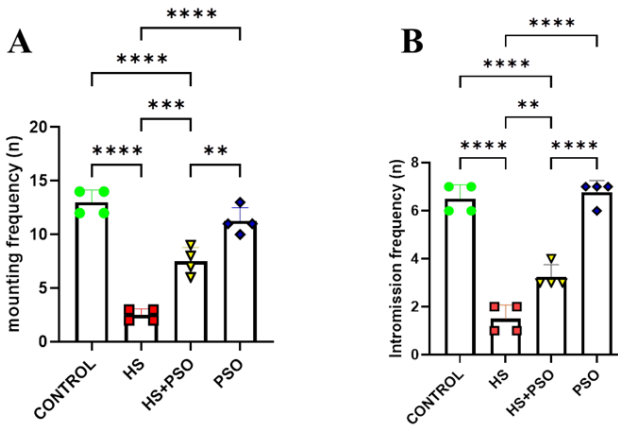

Figure 2: Effects of pumpkin seed oil on sexual behaviour by male Wistar rats following oral administration of high salt for 14 days. A: Mounting frequency; B: Intromission frequency. Data are expressed as mean ± SD; n=6, one-way ANOVA followed by Tukey's post hoc test. HS=High salt; HS+PSO=High salt with pumpkin seed oil; PSO=Pumpkin seed oil. **$P<0.01$; ***$P<0.001$, ****$P<0.0001$.

Effects of Pumpkin Seed Oil on Testicular and Brain Weights of Salt-loaded Rats

There was a significant ($P<0.0001$) decrease in testicular weight following high salt (HS) administration when compared with control and there were also significant differences in testicular weight in HS+PSO ($P<0.001$) and PSO ($P<0.0001$) groups compared with the HS group (Figure 3A). The analysis of brain weight showed that there was no significant difference when HS, HS+PSO, and PSO groups were each compared with control (Figure 3B).

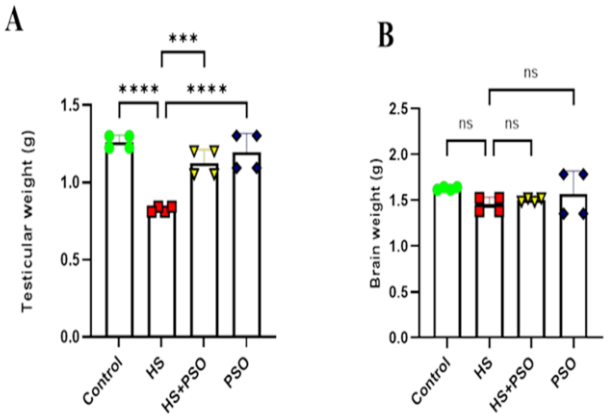

Figure 3: Effects of pumpkin seed oil on testicular and brain weights of salt-loaded Rats. A: Testis weight; B: Brain weight. Data are expressed as mean ± SD; n=6, one-way ANOVA followed by Tukey's post hoc test. HS=High salt; HS+PSO=High salt with pumpkin seed oil; PSO=Pumpkin seed oil. ***P<0.001, ****P<0.0001, ns=not significant.

Effects of Pumpkin Seed Oil on Sperm Count of Salt-loaded Rats

There was a significant (P<0.0001) decrease in percentage of sperm cells in the high salt (HS) group compared with the control group. There were also significant differences in sperm cell percentage in HS+PSO (P<0.05) and PSO (P<0.001) groups compared with the HS group (Figure 4A). High salt administration also caused the lowest percentage of normal sperm cells when compared with the control (P<0.0001), HS+PSO (P<0.001) and PSO (P<0.0001) groups (Figure 4B). In addition, the HS group exhibited the highest percentage of abnormal sperm cells when compared with the control (P<0.0001), HS+PSO (P<0.001), and PSO (P<0.0001) groups (Figure 4C).

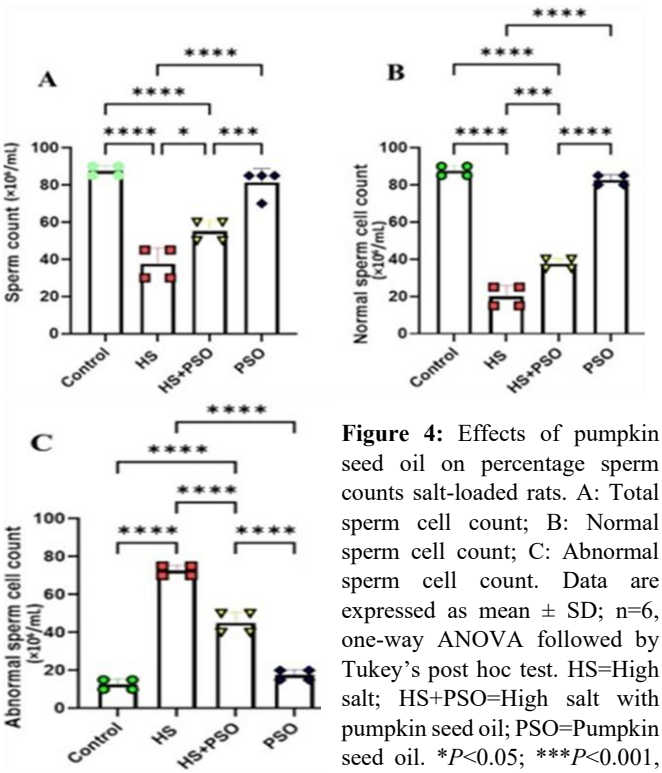

Figure 4: Effects of pumpkin seed oil on percentage sperm counts salt-loaded rats. A: Total sperm cell count; B: Normal sperm cell count; C: Abnormal sperm cell count. Data are expressed as mean ± SD; n=6, one-way ANOVA followed by Tukey's post hoc test. HS=High salt; HS+PSO=High salt with pumpkin seed oil; PSO=Pumpkin seed oil. *P<0.05; ***P<0.001, ****P<0.0001.

Effects of Pumpkin Seed Oil on Sperm Progressive Parameters of Salt-loaded Rats

There was a significantly reduced percentage of rapid progressive sperm cells in the high salt (HS) and HS+PSO groups compared with the control group (P<0.0001). A significant difference P<0.01 in rapid progressive sperm cells was also observed when comparing HS+PSO with the HS group (Figure 5A). The HS group showed an increased percentage of slow progressive sperm cells compared with the control (P<0.001) and PSO (P<0.01) groups. Significant differences were also noted when comparing HS+PSO with the control (P<0.01) and PSO (P<0.05) groups, while no significant difference was observed between HS and HS+PSO groups (Figure 5B). There were no significant differences across all groups for non-progressive sperm cells (Figure 5C).

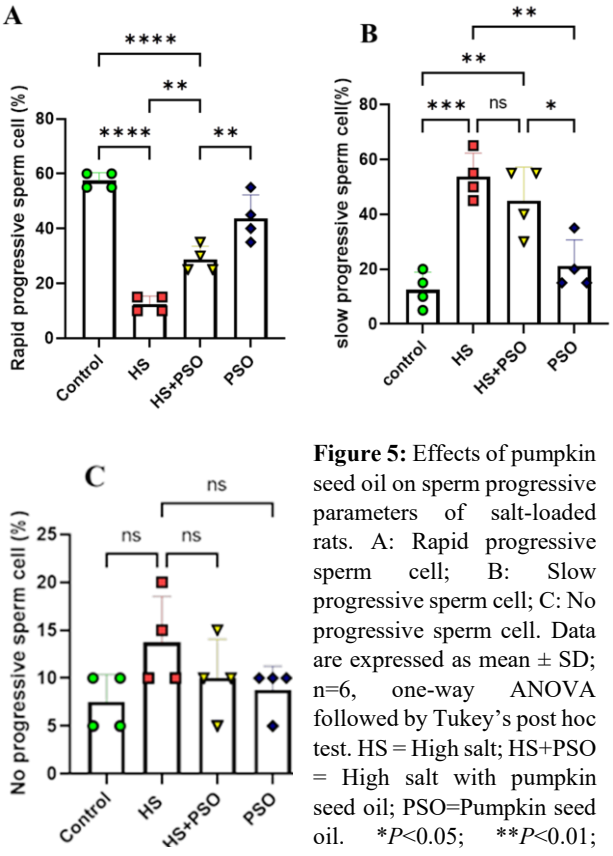

Figure 5: Effects of pumpkin seed oil on sperm progressive parameters of salt-loaded rats. A: Rapid progressive sperm cell; B: Slow progressive sperm cell; C: No progressive sperm cell. Data are expressed as mean ± SD; n=6, one-way ANOVA followed by Tukey's post hoc test. HS = High salt; HS+PSO = High salt with pumpkin seed oil; PSO=Pumpkin seed oil. *P<0.05; **P<0.01; ***P<0.001, ****P<0.0001, ns=not significant.

Effects of Pumpkin Seed Oil on Antioxidant Parameters of Salt-loaded Rats

There was a significant (P<0.0001) increase in malondialdehyde (MDA) levels in the high salt (HS) group compared with the control. Significant differences in MDA levels were also observed in HS+PSO (P<0.01) and PSO (P<0.0001) groups compared with the HS group (Figure 6A). Glutathione (GSH) levels was significantly reduced in

the HS group compared with the control (P<0.05) and HS+PSO (P<0.05) groups. There was no significant difference when HS was compared with PSO group (Figure 6B).

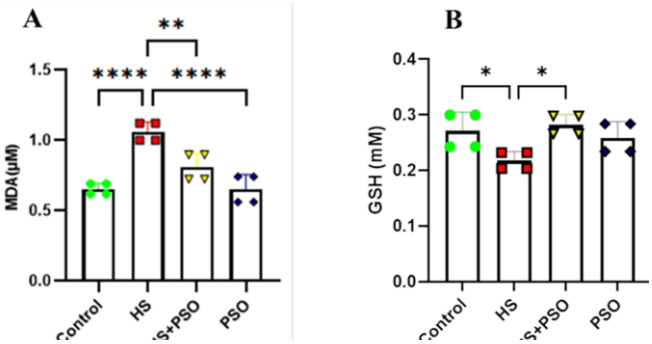

Figure 6: Figure 6: Effects of pumpkin seed oil on antioxidant parameters of salt-loaded rats. A: Malondialdehyde (MDA); B: Glutathione (GSH). Data are expressed as mean ± SD; n=6, one-way ANOVA followed by Tukey's post hoc test. HS=High salt; HS+PSO=High salt with pumpkin seed oil; PSO=Pumpkin seed oil. *P<0.05; **P<0.01; ****P<0.0001.

Effects of Pumpkin Seed Oil on Sex Hormones of Salt-loaded Rats

There was a significant (P<0.0001) decrease in plasma GnRH levels in the high salt (HS) group compared with the control and there was also a significant difference in plasma GnRH levels in HS+PSO (P<0.001) and PSO (P<0.0001) groups compared with the HS group. Similarly, significant differences were observed when HS+PSO (P<0.01) and PSO (P<0.05) groups were compared with the control (Figure 7A).

There was a significant (P<0.0001) reduction in plasma LH and FSH levels in the HS group compared with the control but there was also a significant increase in LH and FSH levels in HS+PSO (P<0.001) and PSO (P<0.0001) groups compared with the HS group (Figure 7B). Similarly, significant differences were observed when HS+PSO (P<0.0001 for LH; P<0.05 for FSH) and PSO (P<0.05 for FSH) groups were compared with control (Figures 7B and 7C).

In Figure 7D, there was a significant (P<0.0001) reduction in plasma testosterone levels in the HS group when compared with the control but this was significantly ameliorated in HS+PSO (P<0.05) and PSO (P<0.0001) groups compared with the HS group.

Effects of Pumpkin Seed Oil on Testicular Histology of Male Salt-loaded Rats

Figure 8 shows the photomicrographs of testes of the rats loaded with salt. Panels A, B, C, and D represent the various groups and panel E is the graph of testicular cell counts in the groups. While the control group (panel A) had normal histology, the HS group (panel B) had reduced testicular cellularity with abnormal histology which was markedly improved in the HS+PSO group (panel C). The PSO group (panel D) had similar histology like the control (A). In terms of testicular cell count (panel E) there was a significant (P<0.0001) reduction in testicular cell population in the HS group compared with the control and there was also a significant difference in testicular cell count in HS+PSO (P<0.01) compared with the control. Similarly, a significant increase in testicular cells was observed in HS+PSO (P<0.05) and PSO (P<0.0001) groups compared with the HS group.

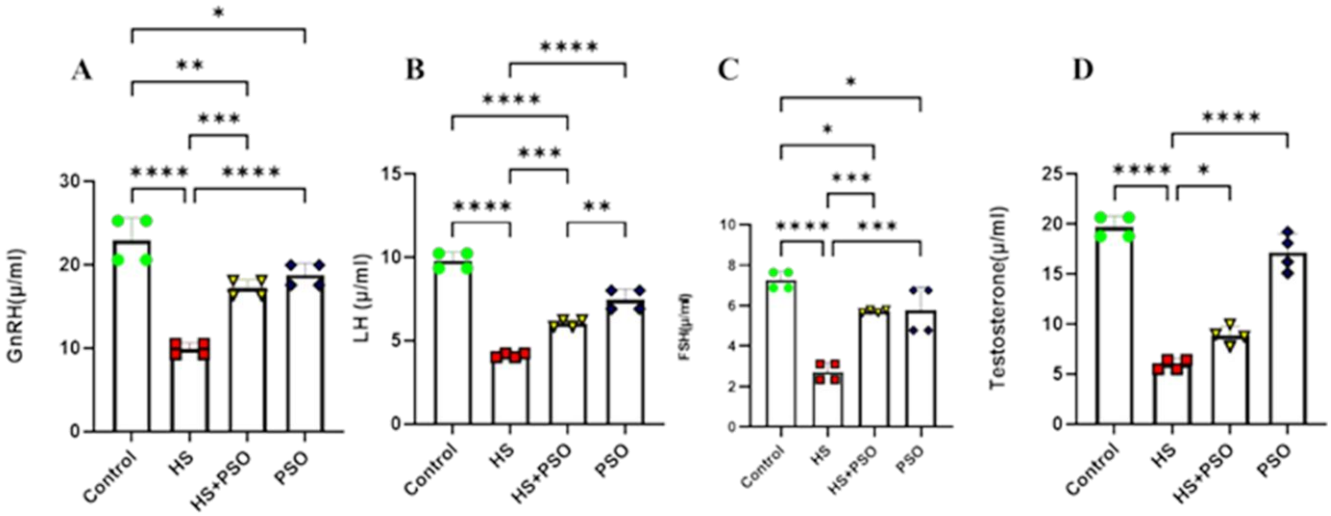

Figure 7: Effects of pumpkin seed oil on male sex hormones following oral administration of high salt for 14 days. A: Gonadotropic releasing hormone (GnRH); B: Luteinizing hormone (LH); Follicle stimulating hormone (FSH); and D: Testosterone. Data are expressed as ± SD; n=6 and analysed by one-way ANOVA followed by Tukey Post hoc test. HS=High salt; HS+PSO=High salt with pumpkin seed oil; PSO=Pumpkin seed oil. *P<0.05; **P<0.01; ***P<0.001, ****P<0.0001).

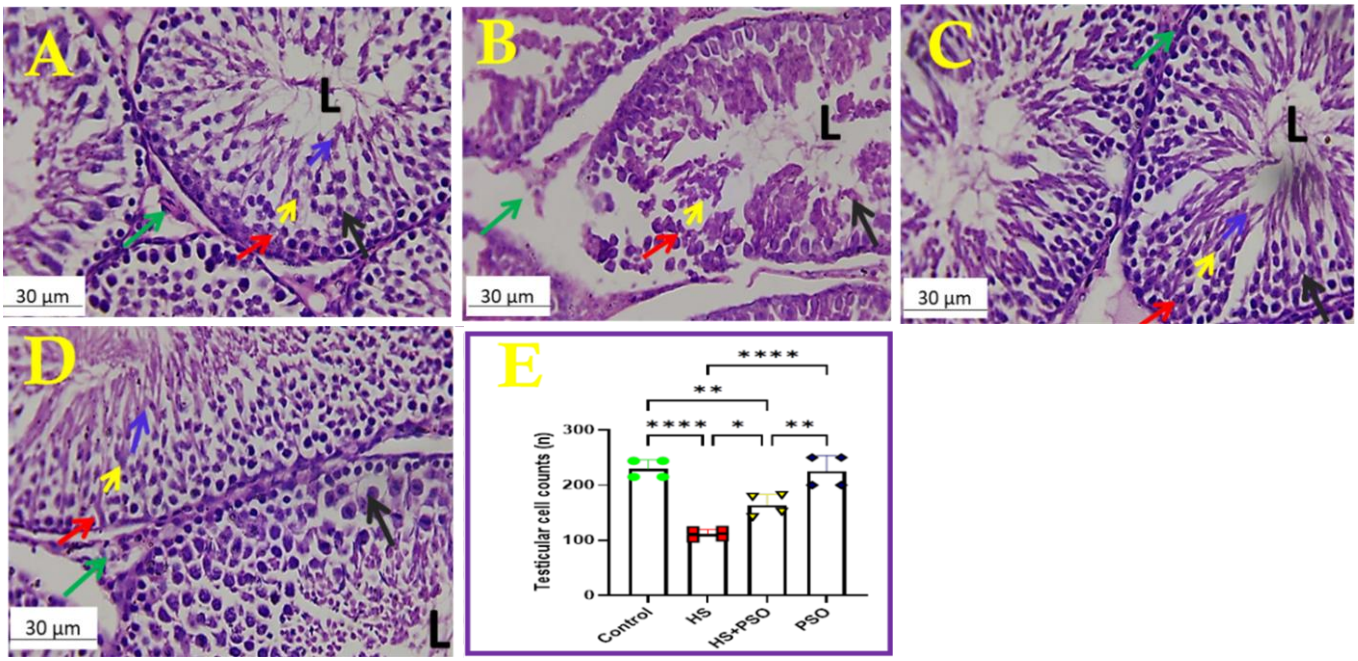

Figure 8: Photomicrographs of the testis of adult male Wistar rats treated with pumpkin seed oil following high salt administration. Group A=Control, with a significant abundance of healthy testicular cells and other components; Group B=High salt (HS) showing reduced testicular cellularity with sloughed germinal cells, Leydig cell loss, enlarged seminiferous lumina devoid of mature spermatozoa, and disrupted basal lamina and Sertoli cells; Group C=High salt concurrently administered with pumpkin seed oil (HS+PSO) showing increased viable testicular cells with restored germinal organization, intact basal lamina, and improved cellular integrity; Group D=Pumpkin seed oil (PSO) similar to the control group, showing numerous high abundance of normal testicular cells and components. Red, yellow and blue arrows (array of germinal cells); Green arrow (Leydig cells in interstitial space); L (lumen of seminiferous tubules); and E (graph of testicular cell counts, **P*<0.05; ***P*<0.01; ****P*<0.001, *****P*<0.0001). × 800 (H&E).

Effect of Pumpkin Seed Oil on Hypothalamic Histomorphology of Salt-loaded Rats

The photomicrographs of the hypothalamus (Figure 9) show normal histology in the control group (panel A). In the HS group (panel B), there was substantial reduction in the number of viable cells which were improved in the HS+PSO group (panel C). The histology of the PSO group (panel D) was essentially the same as that of the control group. Panel E shows that there was a significant (P<0.0001) reduction in viable hypothalamic cells in the HS group compared with the control. There was also a significant reduction in the HS+PSO group (P<0.05) compared with the control. Similarly, a significant increase in viable hypothalamic cells was observed in HS+PSO (P<0.05) and PSO (P<0.001) groups compared with the HS group.

Effects of Pumpkin Seed Oil on Anterior Pituitary Histomorphology of Salt-loaded Rats

Figure 10 shows that the pituitary histomorphology of pituitary was normal in the control group (panel A). In the HS group (panel B), there was a reduction in the number of viable gonadotropic cells which was improved in the HS+PSO group (panel C). The micrograph of the PSO group (panel D) was like that of the control. In panel E, there was a significant (P<0.0001) reduction in viable gonadotrophs in the HS group compared with the control. There was also a significant reduction in the HS+PSO group (P<0.001) compared with the control. Similarly, a significant difference in viable gonadotrophs was observed when HS (P<0.0001) and HS+PSO (P<0.001) groups were compared with the PSO group. However, there was no significant difference between the HS and HS+PSO groups.

Effects of Pumpkin Seed Oil on Testicular Microanatomy by MT Staining of Salt-loaded Rats

In Figure 11, the microscopic examination of testes using Masson's Trichrome (MT) staining technique revealed a relatively depleted collagen fibre of the tunica albuginea (red arrow) and congested blood vessel (blue arrow) in the high salt (HS) exposed rats (panel B). Treatment with HS+PSO resulted in mild deposition and regeneration of collagen fibre (red arrow) in the connective tissue capsule of the seminiferous tubules (panel 11C). Panels A (control group) and D (PSO group) are similar.

Effects of Pumpkin Seed Oil on Hypothalamic Nissl substance by CFV Staining of Salt-loaded Rats

Photomicrographs of hypothalamic Nissl substance after staining with CFV are shown in Figure 12. The control group (panel A) shows abundant Nissl substance in the neurons of arcuate nucleus of hypothalamus. In the HS group (panel B), there was a reduction in Nissl substance, and the neurons were noticeably degenerated, but in the HS+PSO group (panel C), these abnormalities were improved. The PSO (panel D) had similar histoarchitecture

as the control. Histomorphometry revealed that there was a significant (P<0.001) reduction in Nissl substance in the HS group compared with the control and PSO groups. A significant reduction was also observed in the HS+PSO group compared with the control (P<0.01) and PSO (P<0.001) groups. However, no significant difference was observed between the HS and HS+PSO groups.

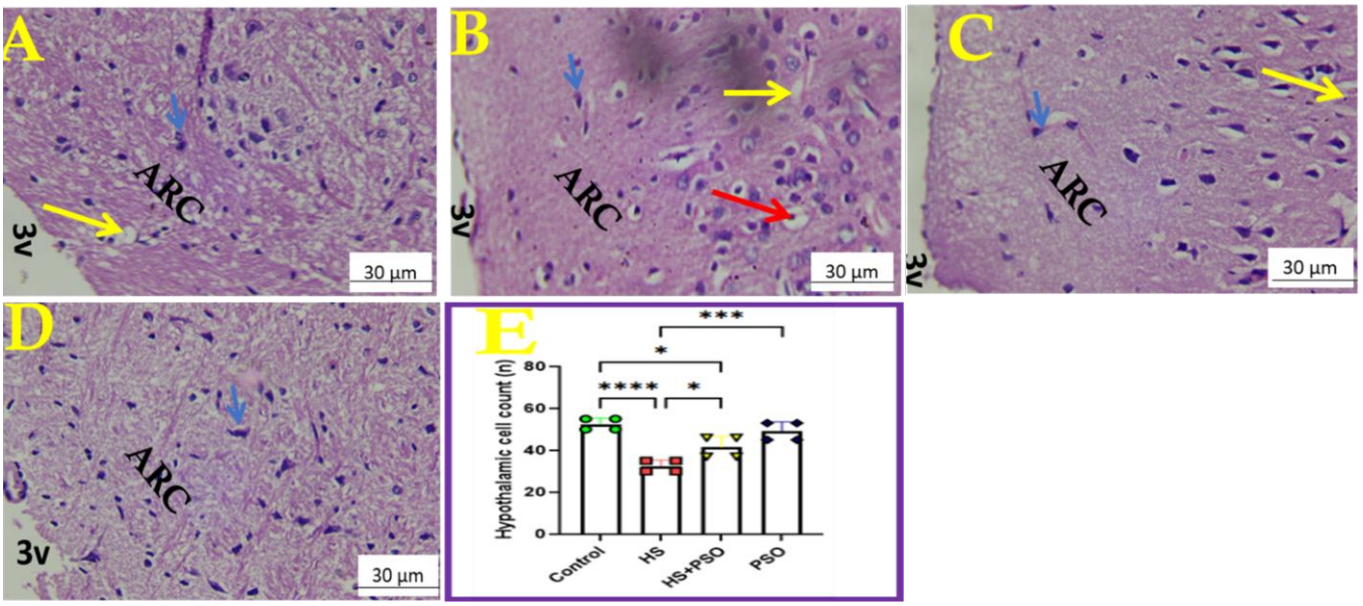

Figure 9: Photomicrographs of hypothalamus of adult male Wistar rats treated with pumpkin seed oil following high salt administration. Group A=Control with a significant abundance viable cells and normal cellular structure; Group B=High salt administration (HS) with a substantial reduction in viable cells, pyknotic and degenerated cells are notable; Group C=High salt concurrently administered with pumpkin seed (HS+PSO) with improved viable cells number and regeneration of sloughed hypothalamic cells; Group D=Pumpkin seed oil treatment (PSO), similar to control group, with high abundance of normal hypothalamic cells. Red (pyknotic cell); blue arrow (normal cells); yellow arrow (vacuolation); ARC (arcuate nuclei); 3V (third ventricle); and E (graph of hypothalamic cell counts, *P<0.05; **P<0.01; ***P<0.001, ****P<0.0001). ×800 (H&E).

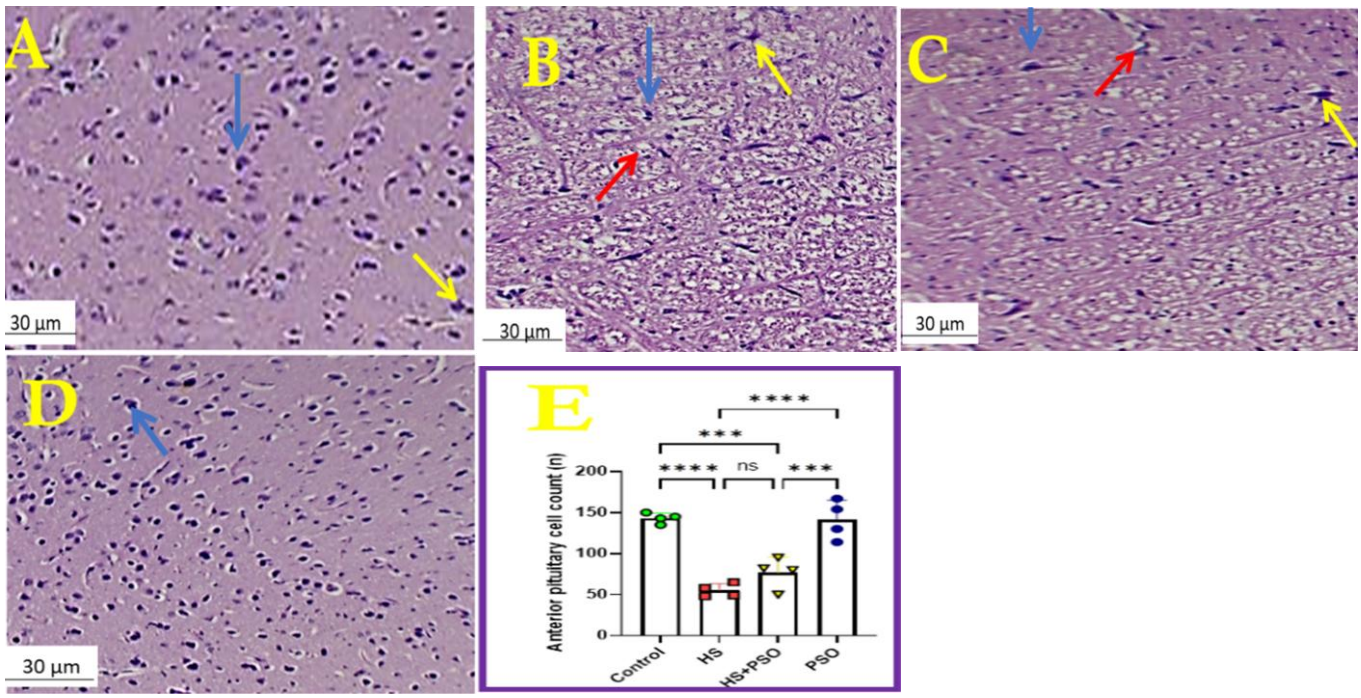

Figure 10: Photomicrographs of anterior pituitary gland of salt-loaded rats administered pumpkin seed oil. Group A=Control - with an abundance gonadotropic cells; Group B=High salt administration (HS) with a substantial reduction in viable cells, pyknotic cells and shrunken gonadotrophs' cytoplasm are notable; Group C=High salt concurrently administered with pumpkin seed oil (HS+PSO) with improved cellular integrity; Group D=Pumpkin seed oil (PSO) similar to control group by showing numerous high abundance of cells. Red (pyknotic cell); blue arrow (gonadotrophs); yellow arrow (pituicytes); and E (graph of anterior pituitary cell counts, *P<0.05; **P<0.01; ***P<0.001, ****P<0.0001). ×800 (H&E).

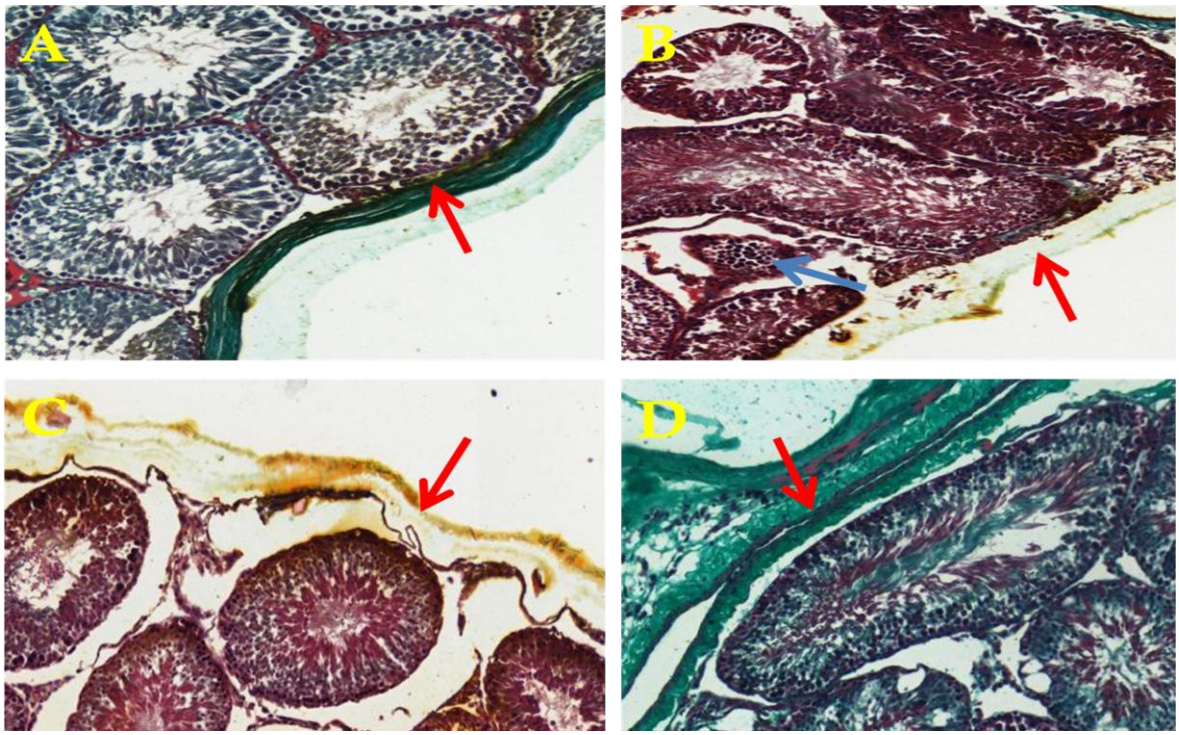

Figure 11: Photomicrographs of testis of adult male Wistar rats treated with pumpkin seed oil following high salt administration. Group A= Control with connective tissue capsule (tunica albuginea) of seminiferous tubules lined by fine structured collagen fibre; Group B= High salt administration (HS) with depleted and minimum collagen fibres are present in the connective tissue capsule of seminiferous tubules, congested seminiferous tubules with thickened blood vessels; Group C= High salt concurrently administered with pumpkin seed oil (HS+PSO) showing mild and improved collagen fibres and shaped seminiferous tubules; Group D= Pumpkin seed oil treatment (PSO) with seminiferous tubules outlined by normal and fine collagen fibres as seen in control group. Red arrow (collagen fibres), blue arrow (blood vessel) ×800 (MT).

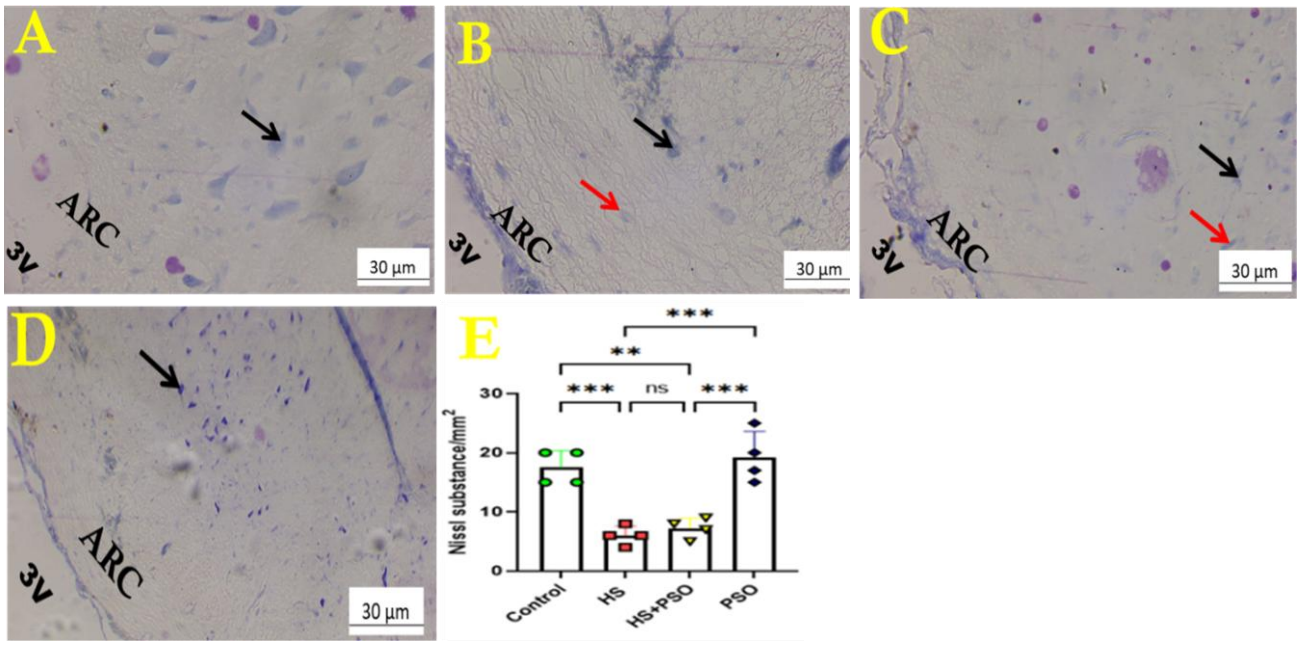

Figure 12: Photomicrographs of hypothalamus of adult male Wistar rats treated with pumpkin seed oil following high salt administration. Group A= control with a significant abundance Nissl substance in the neuron of arcuate nucleus of hypothalamus; Group B=High salt exposed (HS) with a substantial reduction in Nissl substance at the arcuate region of hypothalamus, pyknotic and degenerated neurons are notable; Group C=High salt concurrently administered with pumpkin seed oil (HS+PSO) with improved neurons and cellular structure but slightly much Nissl substance than group B; and Group D=Pumpkin seed oil treated (PSO) similar to control group, showing notable Nissl substance in hypothalamic arcuate cells. Red (pyknotic cell); black arrow (neuron with adequate Nissl substance); ARC (arcuate nuclei); 3V (third ventricle); and E (graph of hypothalamic Nissl substance area, **P*<0.05; ***P*<0.01; ****P*<0.001, *****P*<0.0001, ns=not significant. ×800 (CFV).

Effects of Pumpkin Seed Oil on Anterior Pituitary Nissl Substance by CFV Staining of Salt-loaded Rats

Figure 13 shows CFV-stained Nissl substance in the anterior pituitary of the rats. There was substantial reduction of Nissl substance in gonadotropic neurons in the HS group (panel B) when compared with the control (panel A) in which they were in abundance. In the HS+PSO group (panel C), there was an increase in the Nissl substance in the HS group. The micrograph of the PSO group (panel D) is similar to that of the control (panel A). Histomorphometric plot (panel E) showed a significant (P<0.001) reduction in Nissl substance in the HS group compared with the control. A significant reduction was also observed in the HS group compared with the HS+PSO group (P<0.01). Similarly, a significant difference was observed when comparing the HS+PSO group with the PSO group (P<0.001).

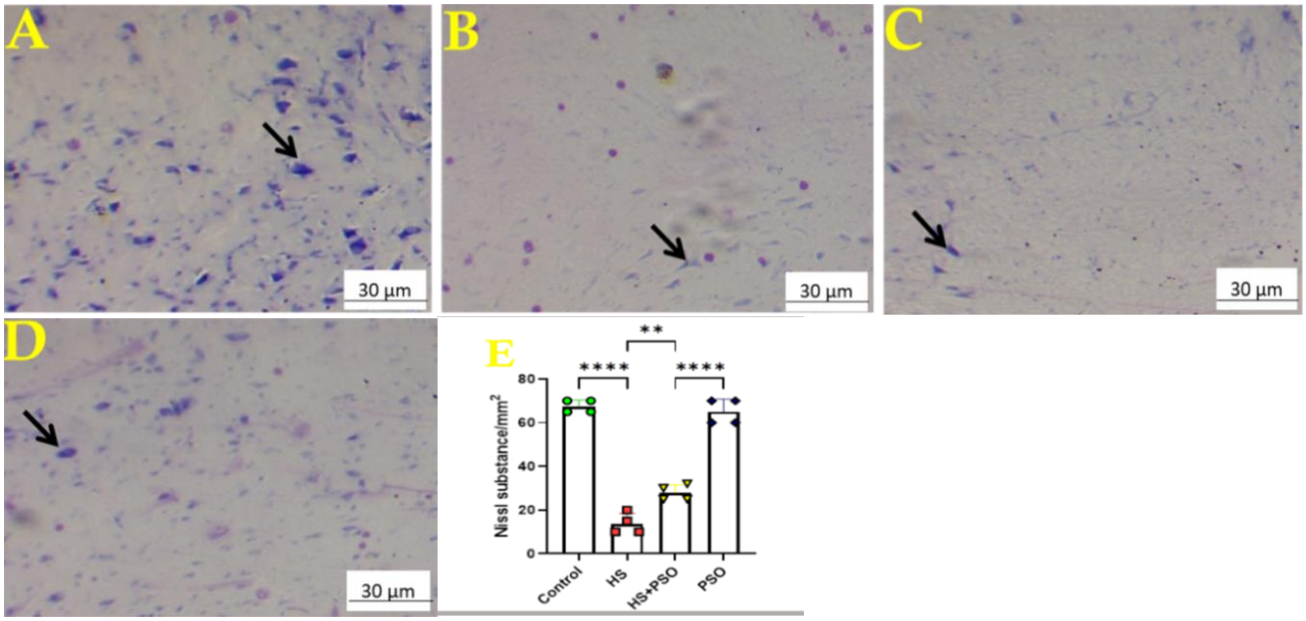

Figure 13: Photomicrographs of anterior pituitary of adult male Wistar rats treated with pumpkin seed oil following high salt administration. Group A= control with an abundance Nissl substance in gonadotropic neurons; Group B=High salt (HS) with a substantial reduction of Nissl substance; Group C=High salt concurrently administered with pumpkin seed oil (HS+PSO) ameliorating anterior pituitary histological structure; and Group D=Pumpkin seed oil treatment (PSO) similar to control group, showing numerous high abundance Nissl substance in gonadotropic cells. Black arrow (gonadotrophs with Nissl substance) and E (anterior pituitary Nissl substance area, *P<0.05; **P<0.01; ***P<0.001, ****P<0.0001). ×800 (CFV).

DISCUSSION

Salts, particularly sodium chloride, are essential nutrients for human survival. However, contemporary dietary habits often result in excessive salt intake, which presents significant health risks at both individual and population levels. There is substantial evidence linking high salt consumption to cardiovascular diseases (Baldo *et al*., 2015) and neurological disorders (Li *et al*., 2012; Abdoli, 2017), but the connection between high salt intake and reproductive dysfunction remains under-explored and inadequately evidenced.

Pumpkin seed oil (PSO) offers a promising intervention due to its health benefits, largely attributed to its rich fatty acid profile and phytoestrogenic compounds (Basir *et al*., 2025), which may positively influence reproductive health. Investigating these properties could provide valuable insights into its efficacy in addressing reproductive dysfunction associated with high salt consumption. The significant decrease in testicular weight in the high salt group in this study, which improved upon PSO administration. Wube *et al*. (2009) reported that 3.5% or 5% NaCl over 6–8 weeks reduced spermatogenesis and testicular weight in mice. Similarly, Abdelnour *et al*., (2020) found that a high salt diet decreased testis weights in rats, with changes in testicular mass potentially affecting seminiferous tubules. Given the hypothalamus and pituitary gland's roles in male reproduction, we examined brain weight and found no significant differences across groups, thereby corroborating Verbalis (2010) report that noted the brain's compensatory mechanisms to maintain sodium balance. This study also investigated the role of PSO in enhancing sexual function in high salt-administered rats. Results showed significant improvements in mounts and intromissions. Kishimoto *et al.,* (2020) observed that a high-salt diet increased blood pressure and impaired erectile function in Dahl-S rats.

High salt ingestion has been linked to oxidative damage of cells and DNA (Krajina *et al.,* 2022). Cellular energy creation, largely reliant on oxidative phosphorylation within mitochondria, produces reactive oxygen species (ROS) under oxidative stress. These ROS impair

biomolecular functions, affecting proteins, lipids, amino acids, and DNA (Juan *et al*., 2021). Our findings indicate that impaired antioxidant defenses following high salt administration, evidenced by increased MDA levels and reduced GSH levels, were mitigated by administration of PSO. Iranloye *et al.* (2013) noted increased MDA levels and decreased antioxidant status in high salt diet-fed rats, indicating oxidative stress. Our findings are strengthened by those of Ekpono *et al*. (2024) that pumpkin seeds possess anti-oxidative properties which may account for their health benefits.

Sperm cells quality and male reproductive functions are regulated by complex hormonal interactions (Dutta *et al*., 2019). The global decline in semen quality over recent decades is a major concern, necessitating exploration of underlying mechanisms. Endocrine disruptions in male reproductive functions significantly affect semen quality (Sengupta, 2014). The hypothalamo-pituitary-gonadal (HPG) axis controls spermatogenesis, with the hypothalamus stimulating anterior pituitary gonadotropin secretion via pulsatile GnRH release (Darbandi *et al*., 2018). Steady high intratesticular testosterone levels, mediated by Leydig cells upon LH stimulation, maintain spermatogenesis. The low levels of male sexual hormones (GnRH, LH, FSH, and testosterone) in high salt administered group were ameliorated by treatment with PSO which demonstrates its phytoestrogenic properties and potential to treat hormonal imbalances induced by high salt intake. This aligns with Fang *et al*. (2018), who found that excessive salt intake reduces sex hormone levels and reproductive performance. Abed and Alkalby (2018) also reported that PSO extract normalized male sex hormone levels after chlorpyrifos-induced reproductive toxicity.

Sperm parameters, such as count, motility, and morphology, are crucial fertility indicators. Decreased sperm count, motility, and increased abnormal sperm cells often lead to reduced fertility rates (Irvine, 1996). In this study, high salt-fed rats had low sperm counts, increased abnormal sperm cells, and slow progressive motility. These were all improved with PSO demonstrating its effectiveness in improving sperm parameters. Studies have linked semen quality to lifestyle factors, including diet (Gabrielsen and Tanrikut, 2016; Shirani *et al*., 2020). Highly processed foods, known for high salt content, contribute to current high salt intakes (Gibson *et al*., 2000). Our findings is supported by that of Aghaei *et al*. (2014) that pumpkin seed extract significantly improved sperm parameters and minimized cyclophosphamide toxic effects.

Histological changes such as decreased and sloughed germ cells, deformed seminiferous tubules, widened interstitium with Leydig cell necrosis, absence of cells in seminiferous tubule lumens, and loss of collagen fibres observed in the high salt group are consistent with the histoarchitectural alterations reported by other workers (Alalwani, 2014; Anderson *et al*., 2020). It has been suggested that these histological changes may result from direct chemical effects or hormonal imbalances (Dalsenter, 2006). Exfoliated spermatids and vacuolar degeneration indicate testicular toxicity and cell degeneration (El-Deeb *et al*., 2000). Alawani (2014) linked pyknosis of cell nuclei to functional inefficiency. Treatment with PSO increased viable germ cells and improved testicular histoarchitecture which agrees with the finding by Mousa *et al*. (2023) that PSO alleviated lead acetate-induced testicular pathology.

Reduced gonadotrophs with cell deterioration and death evidenced by pyknotic cells and loss of Nissl substance in the hypothalamus and anterior pituitary gland of high salt-fed rats were ameliorated by PSO administration and aligns with the findings of Anderson *et al*. (2020).

CONCLUSION

Taken together, the findings from this study highlight the detrimental effects of high salt intake on male reproductive health. Specifically, high salt consumption adversely affects sexual function, hormonal balance, semen parameters, oxidative stress, inflammation, and structural integrity of the male reproductive axis. This includes pronounced atrophy and degeneration of germinal cells, gonadotropic cells in the anterior pituitary (adenohypophysis) and neuronal loss in the arcuate nucleus of the hypothalamus. Furthermore, this study highlights the therapeutic potential of pumpkin seed oil in mitigating these adverse effects. The administration of pumpkin seed oil following high salt exposure resulted significant amelioration of testicular cell atrophy, restoration of degenerated gonadotropic cells, and regeneration of hypothalamic neurons. Additionally, there was a notable improvement in sexual function, levels of male sex hormones and semen quality in adult male Wistar rats exposed to high salt toxicity. Exploring interactions with key cellular processes involved in spermatogenesis, such as mitochondrial function and apoptotic pathways, may uncover novel targets for intervention in male reproductive disorders exacerbated by dietary factors.

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AUTHORS' CONTRIBUTION

TAA & MAA: validation, investigation, formal analysis, data curation, writing of draft. LAE & EEE: conceptualization, project supervision, writing, reviewing and editing. OSS, SOA, OIA & EEE: investigation, formal analysis, data curation, conceptualization, methodology, project administration, writing, reviewing and editing.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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This research received no grant from any funding agency.

AUTHORS' DECLARATION

The authors declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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