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Full-Length Research Article

Experimental Evaluation of Sorbitol's Impact on the Testicular Axis in Male Wistar Rats

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Summary: Infertility is a growing public health concern with male factors contributing substantially; sorbitol use as a low calorie sweetener raises questions about its reproductive effects. This study evaluated regimen and dose dependent effects of oral sorbitol on the male reproductive axis in adult Wistar rats. Thirty-six male Wistar rats were randomly assigned into six groups: sub chronic low dose (ALDS; 45 mg/kg for 4 weeks), sub chronic high dose (AHDS; 90 mg/kg for 4 weeks), chronic low dose (CLDS; 45 mg/kg for 8 weeks), chronic high dose (CHDS; 90 mg/kg for 8 weeks), and their respective controls; ethical approval was obtained. Outcomes included body and testicular weight, sperm count, morphology and vitality; serum GnRH, FSH, LH and testosterone; serum and testicular lipid profiles; oxidative markers (MDA, GSH, SOD, GPX); inflammatory markers (TNF α, CRP); and histology (H&E, Masson trichrome). Body weight remained unchanged across groups. Chronic high-dose sorbitol reduced testicular weight. Subchronic low-dose exposure increased sperm count but also raised abnormal forms and reduced viability, indicating transient stimulation with compromised quality. Chronic regimens consistently lowered sperm counts. Hormonal responses varied: subchronic high dose elevated testosterone, low dose increased LH, while chronic high dose suppressed GnRH with downstream gonadotropin decline. Lipid profiling showed systemic shifts under subchronic exposure and intratesticular accumulation under chronic regimens, paralleling oxidative stress changes. Subchronic exposure reduced systemic oxidative markers and increased testicular GSH, whereas chronic high dose elevated MDA, induced GPX, and altered TNF-α/CRP signalling. Histology revealed early seminiferous epithelial disruption in subchronic groups and interstitial collagen deposition consistent with fibrosis in chronic groups. Findings suggest possible oxidative lipid dysregulation contributing to gonadal impairment, indicating short-term metabolic signals but clear long-term reproductive risks requiring mechanistic validation and interventional studies.

Keywords: male factor infertility, MDA, sorbitol, TNF-α, GnRH

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INTRODUCTION

Infertility is a major global health challenge, affecting an estimated 15% of couples worldwide, with male factors contributing to nearly half of all cases (Agarwal *et al*., 2021). Beyond its medical implications, male infertility carries profound social and psychological burdens, particularly in regions such as Africa and Southeast Asia where prevalence is rising (Burumandnia *et al*., 2022; Obeagu *et al*., 2023). Environmental, dietary, and metabolic influences are increasingly recognized as critical determinants of male reproductive health, impacting sperm quality, hormonal balance, and testicular function (Durairajanayagam, 2018).

Sorbitol, a sugar alcohol widely used as a sweetener in processed foods, pharmaceuticals, and oral hygiene products, is generally considered safe. However, its consumption has increased substantially, and emerging evidence suggests that excessive intake may produce unintended metabolic and reproductive consequences (Alabi *et al*., 2019). While endogenous sorbitol accumulation in diabetic states is well characterized, the reproductive effects of exogenous sorbitol exposure remain poorly understood. Importantly, related sugar alcohols such as xylitol and mannitol have been implicated in reproductive toxicity, raising concern that sorbitol may exert similar effects.

Mechanistically, sorbitol is generated through the polyol pathway, where excess glucose is converted by aldose reductase. Because sorbitol is poorly transported across cell membranes, it accumulates intracellularly, producing osmotic stress, oxidative damage, and impaired signaling (Srikanth and Orrick, 2022). These processes are known to disrupt testicular function and spermatogenesis, yet direct

evidence linking sorbitol intake to male reproductive outcomes in non-diabetic populations is lacking.

Accordingly, the present study was undertaken to evaluate the dose and duration dependent effects of oral sorbitol on the male reproductive axis in Wistar rats, focusing on sperm quality, hormonal variations, and testicular histology. By addressing this gap, we aim to clarify whether sorbitol intake poses reproductive risks beyond its established metabolic effects, thereby contributing to a more comprehensive understanding of dietary influences on male fertility.

MATERIALS AND METHODS

Chemicals and reagents: Analytical-grade sorbitol powder (Catalog number: 50-70-4), manufactured by Molychem Ltd., Mumbai, India**,** was procured from a certified vendor.

Animal Grouping and Treatment: Thirty-six (36) healthy male Wistar rats, weighing (150–200g) at baseline, were procured from the Department of Veterinary Medicine, University of Ibadan, Oyo State, Nigeria. Upon arrival, the animals were housed in standard laboratory cages at the Animal Research Centre, Afe Babalola University, Ado Ekiti, Ekiti State, Nigeria, and allowed to acclimatize for one week before the commencement of the experiment. They were maintained under controlled conditions of temperature, humidity, and a 12-hour light/dark cycle, with commercial pelletized rat feed and ad libitum access to clean drinking water.To minimize the impact of baseline weight variation, animals were randomly assigned into six (6) experimental groups of six (6) rats each, ensuring balanced distribution across treatments. All experimental procedures were conducted in accordance with institutional guidelines and approved by the Afe Babalola University Health Research Ethics Committee (ABUADHREC), protocol number ABUADHREC/2911/2024517.

Table 1:
Experimental Grouping and Treatment

Phase	Control (Normal Saline)	Low-Dose Sorbitol (45mg/kg)	High-Dose Sorbitol (90mg/kg)
Sub-Chronic	Group 1 (ACNS)-1mL/day	Group 3 (ALDS)-(45mg/kg)	Group 4 (AHDS)-(90mg/kg)
Chronic	Group 2 (CCNS)-1mL/day	Group 5 (CLDS)-(45mg/kg)	Group 6 (CHDS)-(90mg/kg)

Sacrifice and Collection of Samples: At the end of each experimental phase (day 28 for sub-chronic groups and day 56 for chronic groups), animals were anesthetized using an approved protocol (ketamine–xylazine combination for induction, followed by isoflurane inhalation for maintenance) in accordance with institutional animal welfare guidelines. After confirming adequate anaesthesia, dissection was performed to expose internal organs, and samples were collected. Blood was drawn from the left ventricle using a sterile syringe and transferred into plain sample bottles. The samples were allowed to clot and centrifuged at 3000rpm for 10 minutes to obtain serum, which was stored at −80°C for subsequent biochemical assays.

Preparation of Testicular Tissue Homogenates: The scrotum was dissected, and the epididymis was isolated and suspended in normal saline for sperm analysis. The right testis was fixed in 10% formaldehyde for histological evaluation.

The left testis was homogenized in 10% w/v ice-chilled phosphate buffer saline (PBS) (pH 7.4, 0.1 M) and centrifuged at 3000 RPM for 10 minutes. The supernatant was separated and stored at −80°C for biochemical estimations.

Biochemical Assays: The concentrations of oxidative stress markers in serum and testicular homogenates were determined using commercial assay kits (Foortress Diagnostics Limited, United Kingdom): Malondialdehyde (MDA; Cat: E-BC-K025-M), Superoxide Dismutase (SOD; Cat: E-BC-K019-S), Glutathione (GSH; Cat: E-BC-K030-M), and Glutathione Peroxidase (GPx; Cat: BXC0321K/L/M). Assays were performed according to the manufacturer's instructions. Briefly, MDA was quantified by thiobarbituric acid reactive substances (TBARS) spectrophotometry at 532 nm; SOD activity was assessed by inhibition of nitroblue tetrazolium reduction at 560 nm; GSH was measured using Ellman's reagent at 412 nm; and GPx activity was determined by monitoring NADPH oxidation at 340 nm. Serum levels of reproductive hormones and inflammatory markers were analyzed using rat ELISA kits (Foortress Diagnostics Limited, United Kingdom): Gonadotropin-Releasing Hormone (GnRH; Cat: E-EL-0071), Follicle-Stimulating Hormone (FSH; Cat: FS046F), Luteinizing Hormone (LH; Cat: LH231F), Testosterone (Cat: TE187S), C-Reactive Protein (CRP; Cat: E-EL-R0506), and Tumor Necrosis Factor-α (TNF-α; Cat: E-EL-R2856). ELISAs were conducted following the manufacturer's protocols, with absorbance measured at 450 nm using a microplate reader.

Sperm and Histological Evaluation: The testes and epididymis were isolated by removing surrounding connective tissue. The epididymal tail was placed in a Petri dish, and several incisions were made to release sperm. The tissue was divided into smaller pieces and incubated. Sperm counting was performed by preparing a 1:20 dilution and placing 10 µL of the diluted solution onto a Neobar slide. Sperm morphology and viability were assessed under a light microscope (Rahimi *et al*., 2023). For histological evaluation, testes were fixed in 10% formalin, embedded in paraffin, and sectioned at 5 µm thickness. Sections were deparaffinized and stained with hematoxylin and eosin following standard protocols, then examined under a light microscope for structural assessment (Ghosh *et al*., 2022). Additional staining was performed using Celestine blue, Biebrich Scarlet Fuchsin, phosphotungstic-phosphomolybdic acid, and aniline blue. Collagen fibers appeared blue, nuclei black, and cytoplasmic components red. Stained slides were observed under a light microscope for detailed evaluation (Carson *et al*., 2015).

Statistical Analysis

All data are expressed as mean ± standard error of the mean (SEM). Prior to analysis, data normality was tested using the Shapiro–Wilk test. Group comparisons were performed using one-way ANOVA. When significant differences were observed, Tukey's post hoc test was applied to identify pairwise differences. Statistical analyses were conducted using GraphPad Prism software version 8.5, and differences were considered statistically significant at $p < 0.05$.

RESULTS

Evaluation of the average body and testicular weight: The mean body weight of animals in both groups increased progressively throughout the study. No statistically significant differences were observed between groups at the end of either the acute (4 weeks) or chronic (8 weeks) phases, regardless of sorbitol dose. Testicular weight remained largely unchanged across treatment groups. However, the chronic high dose sorbitol group (CHDS) showed a reduction in testicular weight compared with the chronic control group. All other sorbitol treated groups exhibited no notable differences in testicular weight relative to their respective controls (Figure 1A–D).

Sperm count, morphology and viability: Sperm count differed by treatment in a dose and regimen dependent manner. The sub-chronic low dose sorbitol group (ALDS) exhibited a marked increase in total sperm count compared with control, whereas the sub-chronic high dose group (AHDS) showed no notable change (Figure 2A). In the chronic regimen, the low dose group (CLDS) displayed a reduction in sperm count relative to control, while the chronic high dose group (CHDS) did not differ significantly (Figure 2B).

Assessment of sperm quality parameters revealed selective effects on morphology and viability. The proportion of abnormal spermatozoa was elevated in the ALDS group, with no significant morphological differences detected in AHDS, CLDS, or CHDS groups (Figure 2C–D). Sperm viability was comparable between ALDS and AHDS groups; however, both chronic treatment groups (CLDS and CHDS) showed a relative increase in viability compared with control, possibly reflecting compensatory selection for healthier sperm despite reduced overall count and testicular damage (Figures 2E–F).

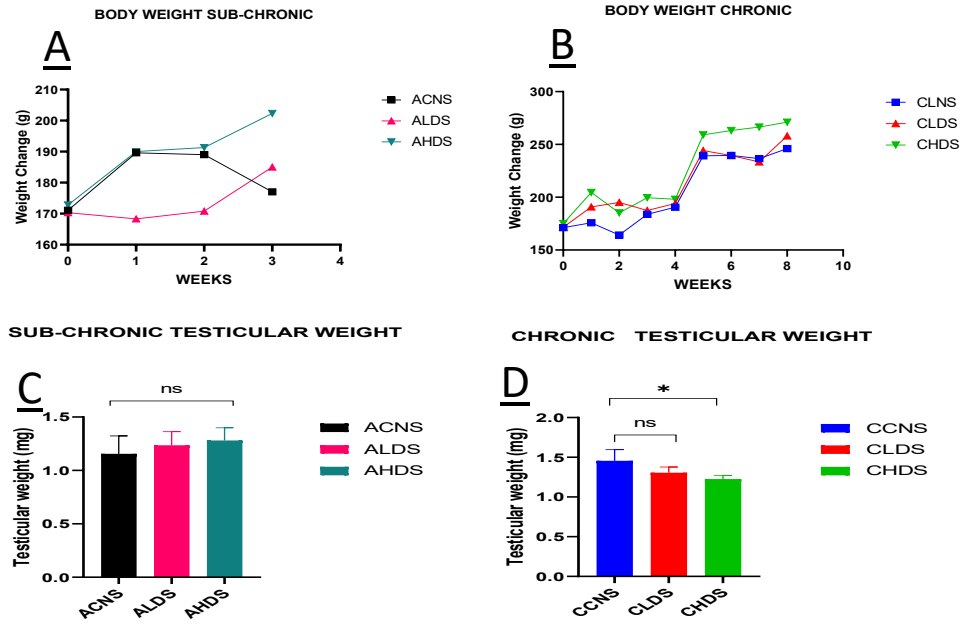


Figure 1:
Effects of sorbitol administration on average body weight during the sub-chronic (A) and chronic (B) phases, as well as on testicular weight in the acute (C) and chronic (D) phases in male Wistar rats. Statistical significance is indicated by *p < 0.05

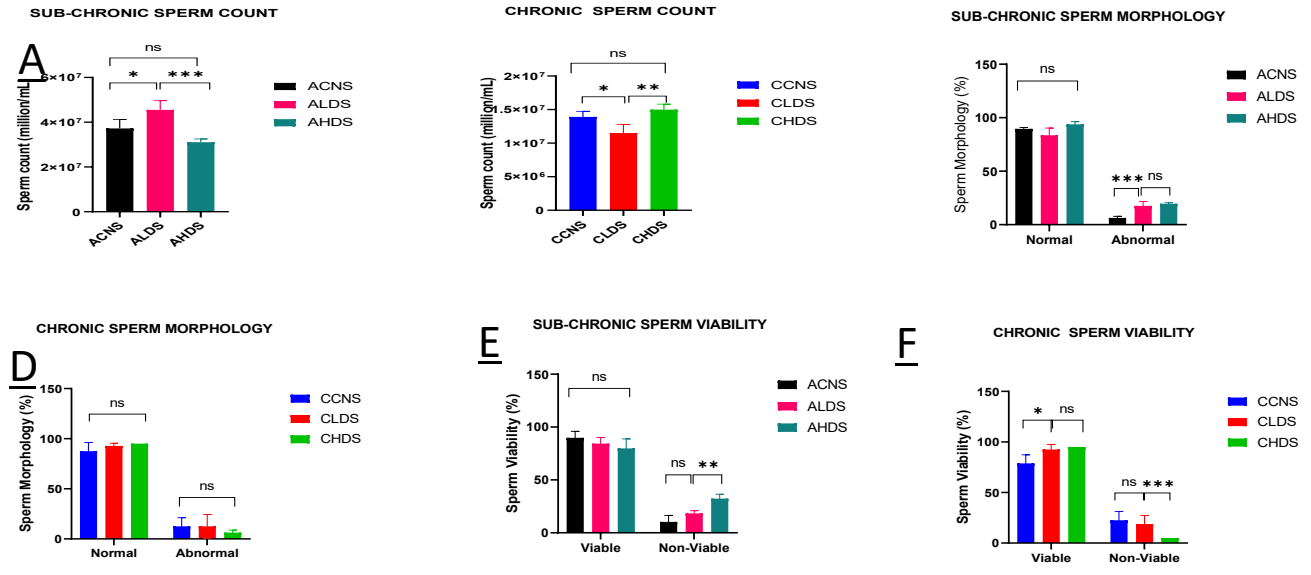


Figure 2:
Effects of sorbitol administration on sperm parameters in male Wistar rats: sperm count during the sub-chronic (A) and chronic (B) phases, sperm morphology during the sub-chronic (C) and chronic (D) phases, and sperm vitality during the sub-chronic (E) and chronic (F) phases. Statistical significance is indicated as *p < 0.05, **p < 0.01, and ***p < 0.001

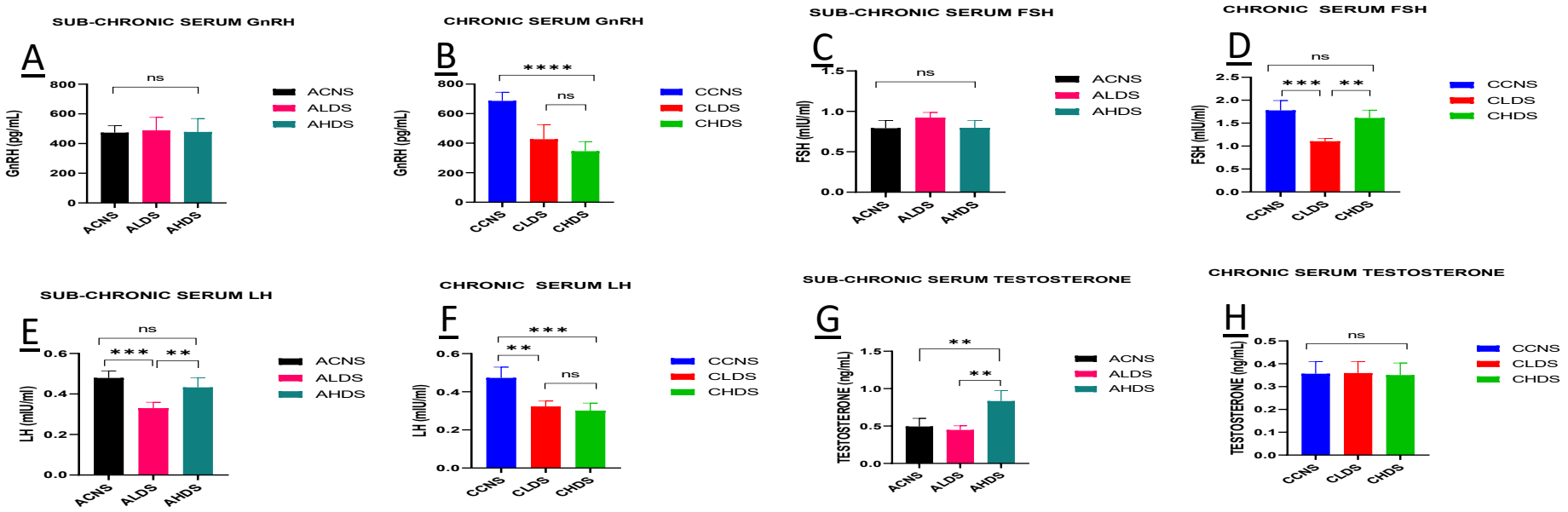


Figure 3:
Effects of sorbitol administration on the concentrations of GnRH, FSH, LH, and testosterone during the sub-chronic and chronic phases in male Wistar rats. Statistical significance is indicated as *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001

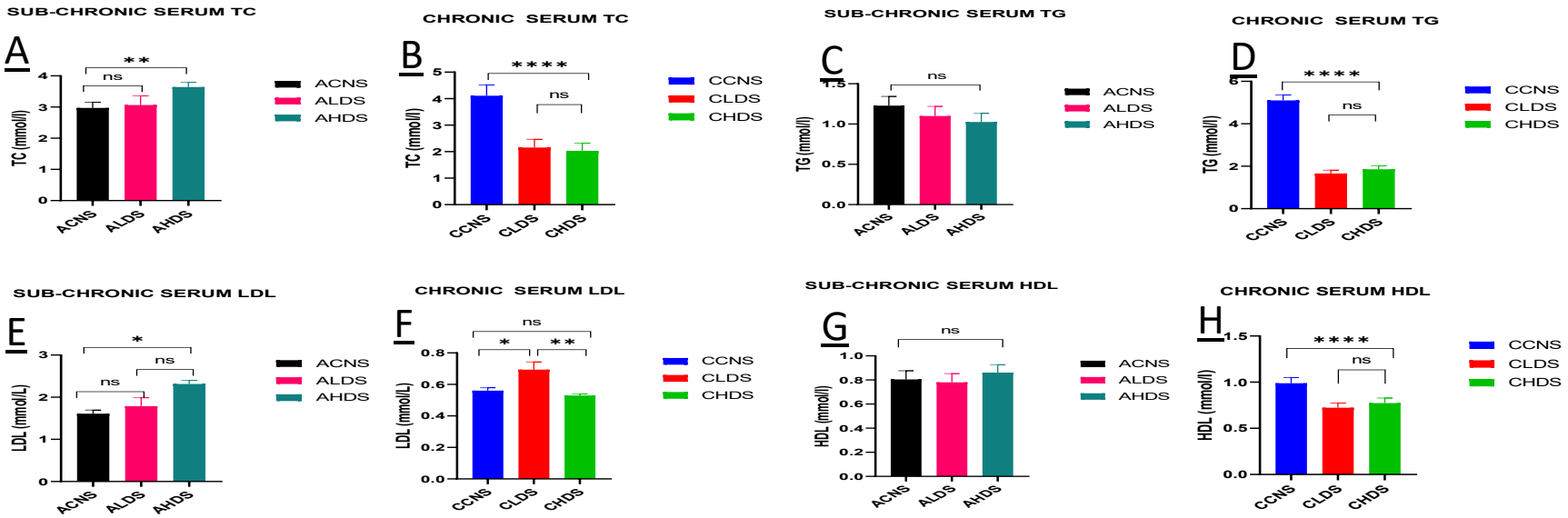


Figure 4:
Effects of sorbitol administration on serum and tissue homogenate lipid profiles during the sub-chronic (A, C, E, G) and chronic (B, D, F, H) phases in male Wistar rats. Statistical significance is indicated as *p < 0.05, **p < 0.01, and ****p < 0.0001

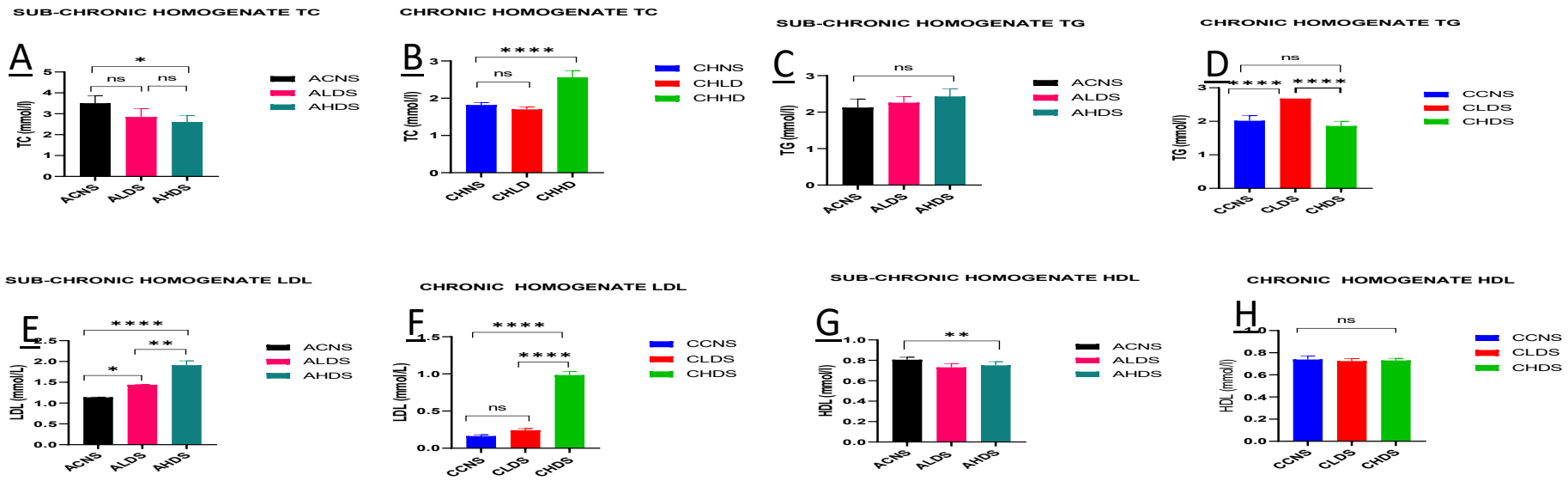


Figure 5:
Effects of sorbitol administration on serum and tissue homogenate lipid profiles during the sub-chronic (A, C, E, G) and chronic (B, D, F, H) phases in male Wistar rats. Statistical significance is indicated as *p < 0.05, **p < 0.01, and ****p < 0.0001

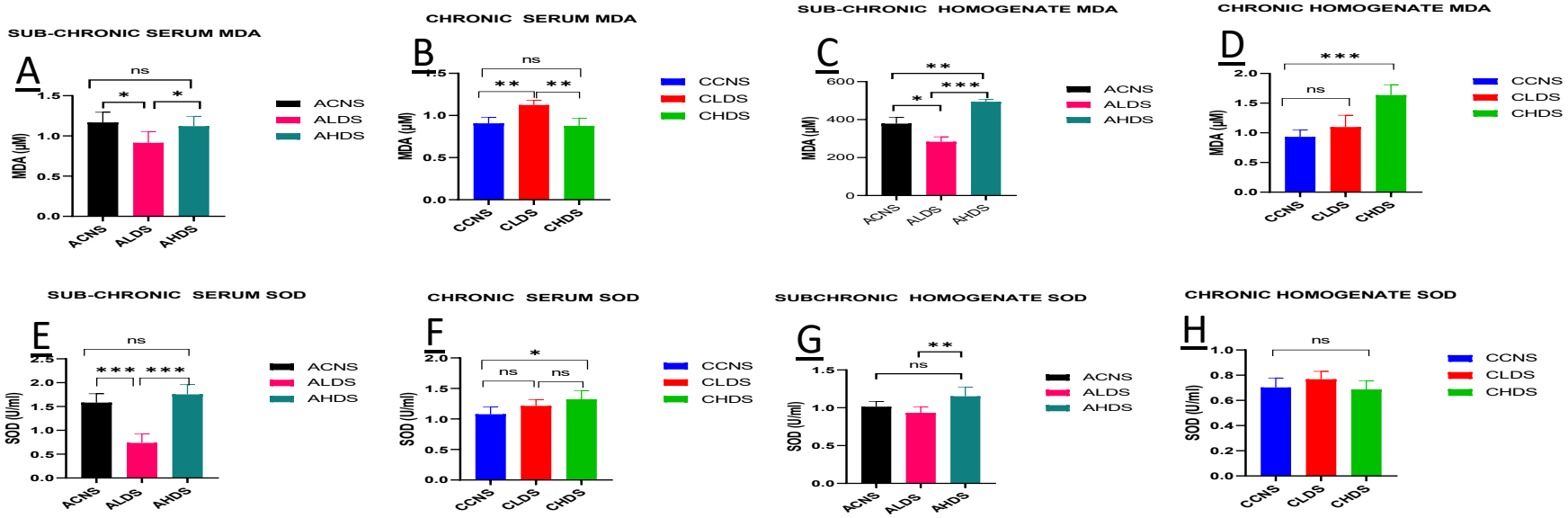


Figure 6:
Effects of sorbitol administration on malondialdehyde (MDA) and superoxide dismutase (SOD) concentrations in serum and tissue homogenates during the sub-chronic and chronic phases in male Wistar rats. Statistical significance is indicated as *p < 0.05, **p < 0.01, and ***p < 0.001

Hypothalamic–pituitary hormones and serum testosterone: Serum GnRH was unchanged in the sub-chronic exposure groups (ALDS, AHDS) but showed a reduction in the chronic high dose group (CHDS) relative to chronic control (Figure 3A–B). Follicle stimulating hormone (FSH) remained stable following sub-chronic exposure (ALDS, AHDS) but was lower in the chronic low dose group (CLDS) compared with chronic control (Figure 3C–D). Luteinizing hormone (LH) increased in the sub-chronic low dose group (ALDS) and was reduced in both chronic treatment groups (CLDS and CHDS) relative to chronic control (Figure 3E–F). Serum testosterone was unaffected by sub-chronic low dose exposure (ALDS) but increased in the sub-chronic high dose group (AHDS) (Figure 3G). No differences in testosterone were detected in the chronic low or high dose groups (CLDS, CHDS) compared with chronic control (Figure 3H).

Serum lipid profile: Serum total cholesterol increased in the sub-chronic high dose group (AHDS) compared with acute control, while both chronic low and high dose groups (CLDS, CHDS) showed reductions relative to chronic control (Figures 4A–B). Serum triglycerides remained unchanged after sub-chronic exposure (ALDS, AHDS) but were lowered in both chronic treatment groups (CLDS, CHDS; Figures 4C–D). Low density lipoprotein (LDL) concentration was elevated in AHDS and CLDS but decreased in CHDS (Figures 4E–F). High density lipoprotein (HDL) was reduced in both chronic groups (CLDS, CHDS; Figures 4G–H).

Testicular homogenate lipid markers: Tissue lipid measures showed a pattern distinct from circulating lipids. Homogenate total cholesterol was lower in the sub-chronic high dose group (AHDS) but elevated in the chronic high dose group (CHDS) (Figures 5A–B). Homogenate triglycerides were increased in the chronic low dose group (CLDS) and reduced in CHDS (Figures 5C–D). Homogenate LDL was elevated in ALDS, AHDS, and CHDS (Figures 5E–F). In contrast, homogenate HDL was reduced in both sub-chronic groups (ALDS, AHDS) (Figures 5G–H).

Evaluation of the MDA and SOD in the serum and testicular homogenate: Serum malondialdehyde (MDA) varied with regimen and dose. The sub-chronic low dose group (ALDS) exhibited a reduction in serum MDA compared with acute control, whereas the sub-chronic high dose group (AHDS) showed no change (Figure 6A). Under the chronic regimen, the low dose group (CLDS) displayed an increase in serum MDA, while the chronic high dose group (CHDS) did not differ from chronic control (Figure 6B). Testicular homogenate MDA was unchanged in ALDS, AHDS, and CLDS groups but was elevated in CHDS (Figures 6C–D). Serum superoxide dismutase (SOD) activity was decreased in ALDS, with no alteration in AHDS (Figure 6E). In the chronic regimen, CHDS animals showed a modest reduction in serum SOD, whereas CLDS did not differ from chronic control (Figure 6F). Testicular homogenate SOD activity did not differ across treatment groups (Figures 6G–H).

Evaluation of the GSH and GPX in the serum and testicular homogenate: Serum glutathione (GSH) was reduced in both sub-chronic treatment groups (ALDS, AHDS) compared with sub-chronic control, while neither chronic group (CLDS, CHDS) differed from chronic control (Figures 7A–B). In testicular homogenate, GSH was elevated in both sub-chronic groups (ALDS, AHDS) relative to sub-chronic control, whereas CLDS and CHDS were similar to chronic control (Figure 7C–D). Serum glutathione peroxidase (GPX) activity was unchanged in ALDS, but increased in AHDS compared with sub-chronic control. GPX activity was also elevated in CHDS relative to chronic control (Figures 7E–F). In testicular homogenate, GPX rose in the sub-chronic high dose group (AHDS) and showed a marked increase in CHDS (Figures 7G–H).

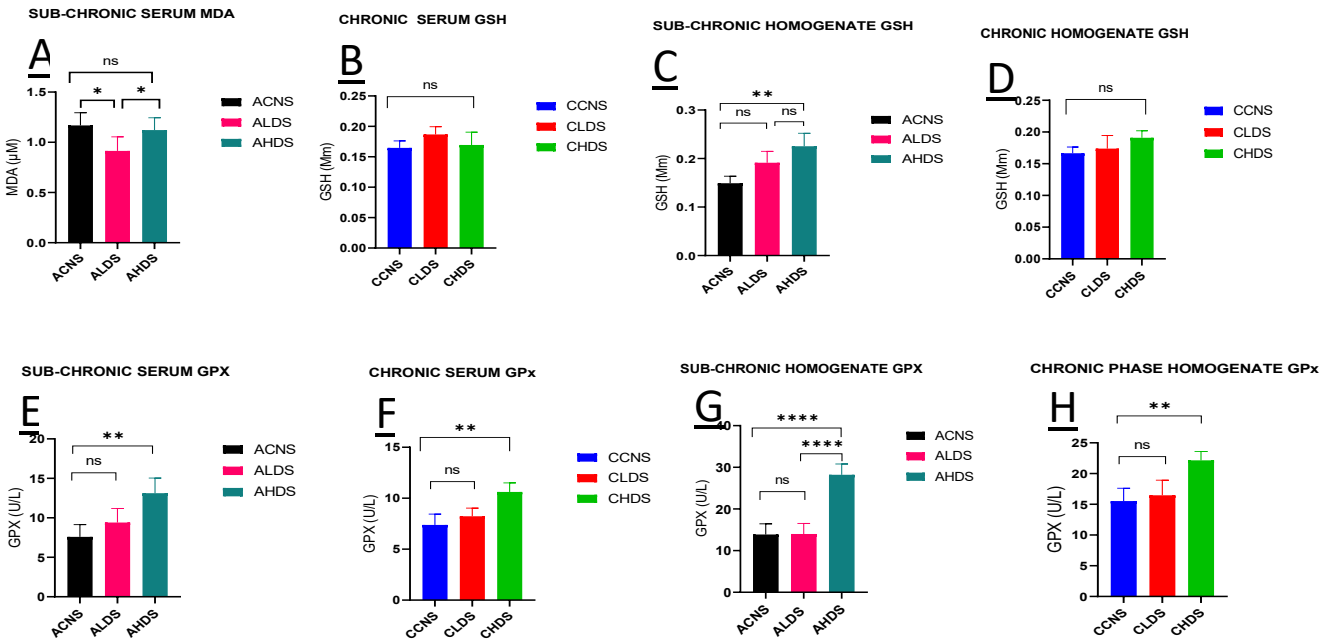


Figure 7:
Effects of sorbitol administration on glutathione (GSH) and glutathione peroxidase (GPx) concentrations in serum and tissue homogenates during the sub-chronic and chronic phases in male Wistar rats. Statistical significance is indicated as *p < 0.05, **p < 0.01, and ***p < 0.001

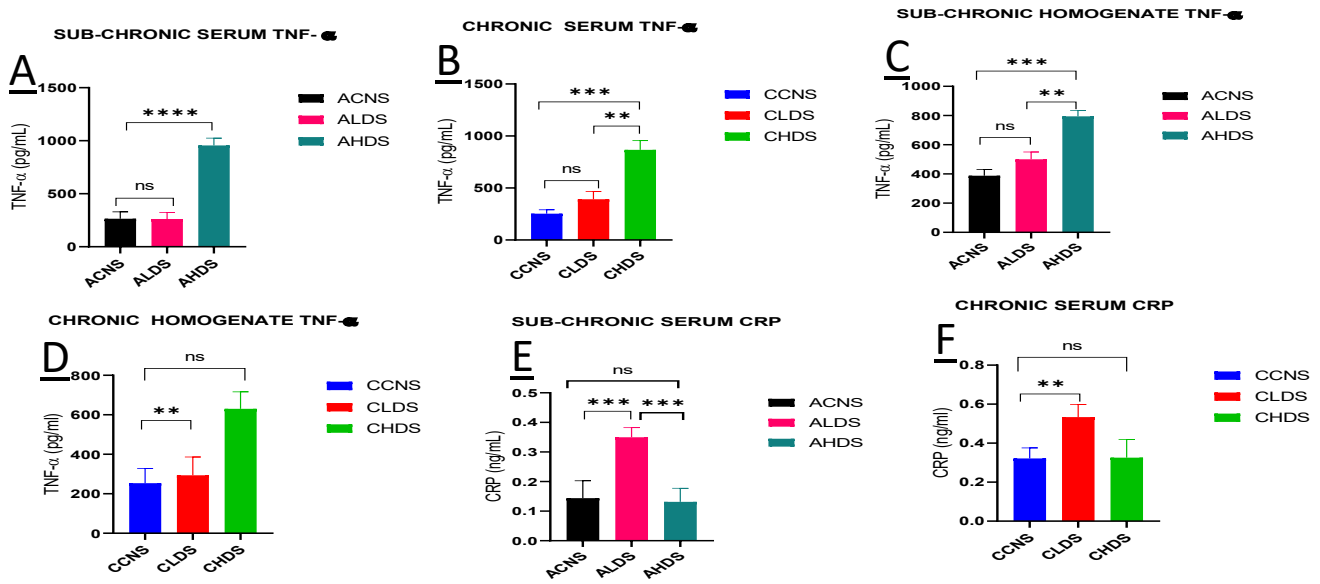
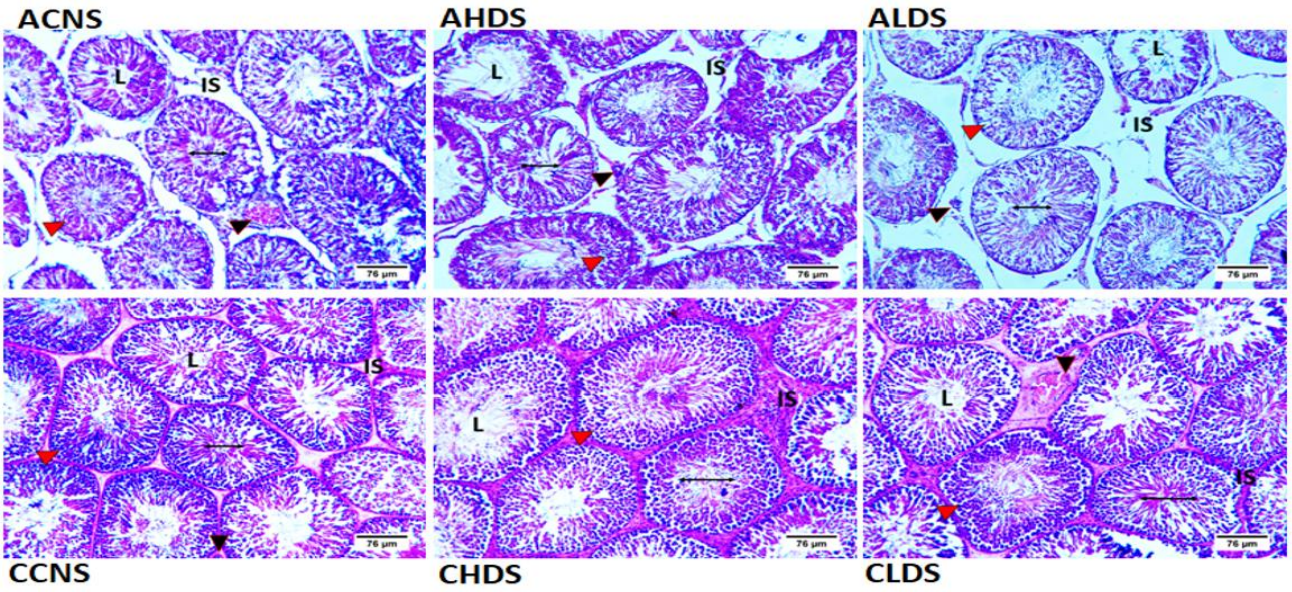


Figure 8:
Effects of sorbitol administration on tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP) concentrations in serum and tissue homogenates during the sub-chronic and chronic phases in male Wistar rats. Statistical significance is indicated as *p < 0.05, **p < 0.01, and ***p < 0.001



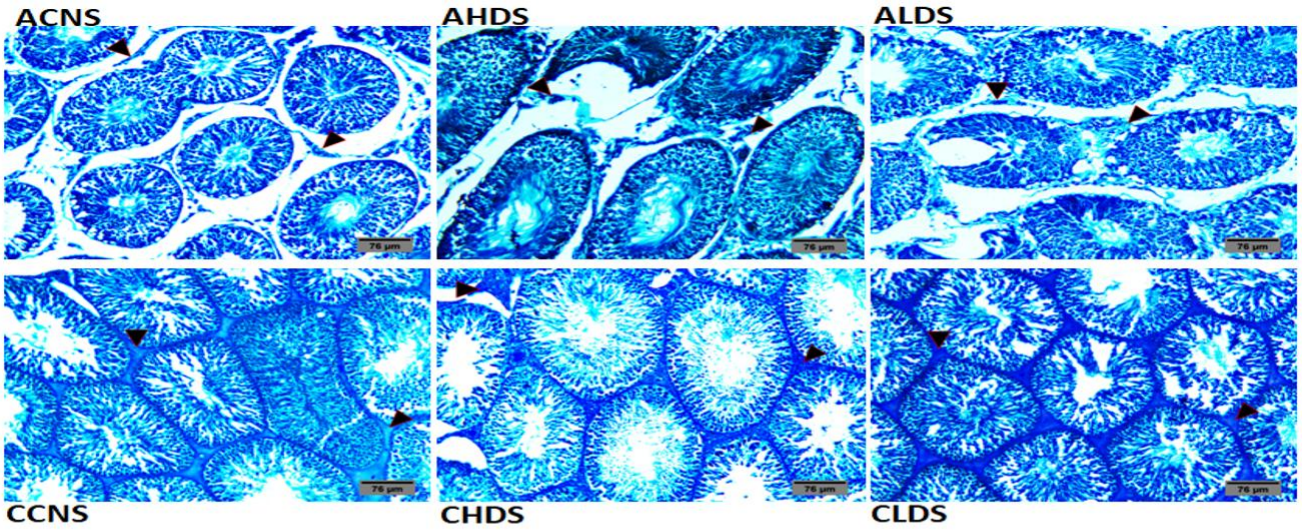
SEMINIFEROUS TUBULES DIAMETER

ACNS	AHDS	ALDS	CCNS	CHDS	CLDS
163.30	165.12	186.28	181.50	172.30	221.10
184.32	158.43	185.28	182.42	188.39	225.63

Plate 1
Photomicrograph of the histology of paraffin embedded rat testis, showing the seminiferous tubules diameter. (Red arrow-head) and Sertoli cells resting on the basement membrane. (IS: Interstitial space; L: Lumen; Black arrow-head: blood vessel within the connective tissues: Red arrow-head: seminiferous tissue cells: Arrow: spermatids within the Lumen). H&E (Mag. x800: Scale bar: 76µm).

TNF-α (serum and testicular homogenate): Serum TNFα was unchanged in the sub-chronic low dose group (ALDS) compared with sub-chronic control, but elevated in the sub-chronic high dose group (AHDS) (Figure 8A). Under the chronic regimen, both CLDS and CHDS showed reductions in serum TNFα relative to chronic control (Figure 8B). Testicular homogenate TNFα mirrored the serum pattern in the sub-chronic groups: no change in ALDS, but an increase in AHDS (Figure 8C). In the chronic groups, CLDS exhibited a reduction in homogenate TNFα, while CHDS did not differ significantly from chronic control (Figure 8D).

C reactive protein (CRP): Systemic CRP was elevated in the sub-chronic low dose group (ALDS) compared with sub-chronic control (Figure 8E). The chronic low dose group (CLDS) also showed an increase in serum CRP relative to chronic control (Figure 8F). In contrast, no differences in CRP were observed in the sub-chronic high dose (AHDS) or chronic high dose (CHDS) groups compared with their respective controls (Figures 8E–F).



ACNS	AHDS	ALDS	CCNS	CHDS	CLDS
168.792	166.906	164.492	168.512	170.533	142.198
155.290	166.906	164.492	168.512	170.533	142.198

Plate 2
Photomicrograph of the histological structure of paraffin embedded rat testis, showing the distribution of collagen fibers within the interstitial space. Black arrow-head: Collagen fibers. Stained with Masson Trichrome Stains (Mag. x800: Scale bar: 76µm).

Seminiferous tubule morphology: Mean seminiferous tubule diameter was unchanged in the sub-chronic low dose (ALDS), sub-chronic high dose (AHDS), and chronic low dose (CLDS) groups compared with their respective controls. In contrast, the chronic high dose group (CHDS) exhibited an increase in tubular diameter (Figure 9). This change was consistent across sections and was accompanied by focal widening of luminal spaces and thinning of the germinal epithelium on representative micrographs.

Staining intensity: Semi quantitative assessment of staining intensity revealed no appreciable changes in ALDS, AHDS, or CLDS compared with their respective controls. In contrast, the chronic high dose group (CHDS) demonstrated an increase in staining intensity relative to chronic control (Figure 10). This elevated intensity was spatially concordant with areas of increased tubular diameter and germ cell alteration observed in representative micrographs.

DISCUSSION

The global prevalence of infertility is rising, with male factor infertility contributing substantially in regions such as Southeast Asia and Africa (Burumandnia *et al*., 2022; Obeagu *et al*., 2023). Environmental and lifestyle factors, including increased consumption of sweeteners such as sorbitol, may play a role (Meldgaard *et al*., 2022). While endogenous sorbitol accumulation in hyperglycaemic states is well characterized (Khalid *et al*., 2022), the reproductive effects of exogenous sorbitol—particularly the dose and duration dependent impacts on the hypothalamic–pituitary–testicular axis—remain inadequately investigated. The present study demonstrates that oral sorbitol induces regimen and dose dependent alterations in reproductive physiology, with distinct sub-chronic versus chronic outcomes.

Across four weeks (sub-chronic) and eight weeks (chronic) of oral sorbitol administration, mean body weight increased without intergroup differences, consistent with Mohammed *et al*. (2024). These null systemic effects contrast with reports of weight loss under different dosing regimens (Al Salih and Abbas, 2021; Alabi *et al*., 2019), highlighting the importance of dose, duration, and strain. In contrast, chronic high dose sorbitol reduced testicular weight, suggesting a targeted gonadotoxic effect rather than generalized toxicity. This may reflect intratesticular sorbitol accumulation causing osmotic stress and tissue dehydration (Srikanth and Orrick, 2022).

The sub-chronic low dose sorbitol (ALDS) group increased total sperm count but also elevated abnormal morphology, indicating quantitatively enhanced but qualitatively compromised spermatogenesis. This discordant pattern suggests accelerated but dysregulated sperm production, potentially linked to sorbitol derived fructose fueling spermatogenesis without preserving structural integrity (Srikanth and Orrick, 2022). Chronic exposure, by contrast, produced genotoxic outcomes consistent with osmotic and oxidative stress impairing germ cell development. These findings reconcile contradictory reports (Alabi *et al*., 2019) by emphasizing timing of exposure: sub-chronic metabolic stimulation may coexist with early genotoxic signals that evolve into net reproductive harm with sustained exposure.

The sub-chronic exposure elevated LH without GnRH suppression, consistent with short term pituitary stimulation. Chronic high dose exposure suppressed GnRH, LH, and FSH, indicating central hypogonadotropic dysfunction. These results partially contrast with Al Moussawi *et al*. (2024), likely due to methodological differences. A plausible mechanism is polyol accumulation in hypothalamic and

pituitary neurons, inducing osmotic and oxidative stress that impairs GnRH synthesis and release (Silva and Caron, 2021). This transition from the sub-chronic stimulation to chronic suppression implicates central disruption as a mediator of reproductive deficits.

The sub-chronic high dose exposure transiently increased testosterone, suggesting short term Leydig cell stimulation or altered steroid metabolism. This effect may reflect enhanced substrate availability (sorbitol → fructose) supporting Leydig cell energy metabolism (Hsia *et al*., 2022). Chronic exposure, however, eliminated this gain, consistent with oxidative stress impairing Leydig cell steroidogenesis (Dutta *et al*., 2021). Pituitary modulation also contributed: sub-chronic LH elevation stimulated Leydig cells, whereas chronic GnRH suppression limited gonadotropin driven testosterone production. These findings emphasize the importance of timing and regimen in interpreting sorbitol's effects on steroidogenesis.

Serum and testicular lipid changes diverged, indicating altered trafficking rather than simple systemic dyslipidemia. The sub-chronic high dose exposure raised serum cholesterol but lowered testicular cholesterol, consistent with redistribution or rapid use for transient testosterone synthesis. Chronic high dose exposure reduced serum cholesterol but increased intratesticular cholesterol, suggesting sequestration and impaired efflux. Elevated testicular cholesterol and triglycerides provided substrates for lipid peroxidation, matching increased MDA in chronic groups (Dutta *et al*., 2021). This local lipid accumulation predisposes germ cells and Leydig cells to oxidative damage, impairing spermatogenesis and endocrine function. Paradoxical increases in sperm viability despite injury may reflect transient metabolic support, but lipid peroxidation ultimately undermines sperm quality (O'Flaherty and Scarlata, 2022).

Serum and testicular MDA results revealed a biphasic, exposure dependent effect of oral sorbitol. The sub-chronic low dose exposure reduced systemic lipid peroxidation, whereas chronic exposure—particularly chronic high dose—produced increased lipid peroxidation at the tissue level. These findings support short term antioxidant effects of sorbitol followed by progressive peroxidative damage with sustained exposure, consistent with prior reports (Khaeruddin *et al*., 2024; Alabi *et al*., 2019). Elevated homogenate MDA in CHDS may reflect intratesticular lipid accumulation, providing substrates for peroxidation and promoting germ cell injury (Dutta *et al*., 2021).

Systemic antioxidant indices fell during the sub-chronic phase (serum GSH and SOD reductions), while testicular homogenate GSH increased in both the sub-chronic groups and GPX activity rose only in CHDS. This dissociation suggests compartmentalized redox responses, with rapid mobilization of local buffering in the testis during early exposure and compensatory enzyme induction under chronic oxidative burden. The absence of consistent homogenate SOD changes despite serum declines underscores tissue specific regulation and reconciles divergent literature findings (Alabi *et al*., 2019; Zhang *et al*., 2021).

Inflammatory responses showed a complex temporal pattern. The sub-chronic high dose exposure increased TNFα in both serum and homogenate, while chronic groups exhibited profound TNFα suppression. CRP increased in ALDS and CLDS but not in AHDS or CHDS. These results suggest an early pro inflammatory response that may amplify oxidative stress, followed by chronic immunomodulation or down regulation of pro inflammatory signaling. The divergent TNFα and CRP trajectories imply that sorbitol's chronic immunomodulatory effects are not uniformly reflected across the sub-chronic phase reactants (Yokoi *et al*., 2024).

Taken together, lipid, oxidative, and inflammatory findings support a sequence in which the sub-chronic sorbitol exposure transiently perturbs systemic redox and inflammatory markers while upregulating local testicular antioxidant buffering. Chronic exposure, by contrast, leads to lipid sequestration, overwhelmed antioxidant defenses, increased lipid peroxidation, and reprogrammed inflammatory signaling. This sequence links biochemical changes to histological degeneration, hormonal disturbances, and sperm abnormalities, identifying intratesticular lipid supply as a central mediator of oxidative injury and reproductive dysfunction (Dutta *et al*., 2021).

Histological analysis revealed exposure dependent trajectories. Haematoxylin and eosin staining showed mild degeneration of seminiferous tubules, moderate spermatid loss, and focal interstitial loosening in the sub-chronic groups, while the chronic groups retained overall tubule shape. Masson trichrome staining demonstrated reduced interstitial collagen in the sub-chronic groups but increased collagen deposition in chronic groups. This pattern of early epithelial damage followed by chronic fibrotic change reconciles reports of both short term protective effects and long term harm (Khaeruddin *et al*., 2024; Alabi *et al*., 2019). The histological profile provides a structural correlate for the biochemical, endocrine, and sperm abnormalities observed (Srikanth and Orrick, 2022; Dutta *et al*., 2021).

In summary, oral sorbitol induces dose and duration dependent disturbances of the male reproductive axis in Wistar rats. The sub-chronic exposure transiently enhances pituitary and Leydig activation, alters redox signals, and increases sperm output, albeit with compromised morphology. Chronic high dose exposure suppresses hypothalamic–pituitary signaling, induces selective testicular atrophy with intratesticular lipid accumulation, elevates lipid peroxidation, and promotes fibrotic remodelling. These findings highlight clear reproductive risks associated with prolonged high sorbitol intake and underscore the need for longitudinal, mechanistic, and translational studies to determine causality and relevance to human health.

Data availability

The authors declare that data supporting the findings of this study will be made available upon reasonable request

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