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Effect of Syzygium aromaticum on Male Reproductive Hormones and Spermatogenesis in Aluminium and Benzo[a]Pyrene-Exposed Rats

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Abstract- ***Environmental pollutants such as aluminium (Al) and benzo[a]pyrene (BaP) are recognized reproductive toxicants capable of inducing oxidative stress, endocrine dysfunction, and impaired spermatogenesis, thereby contributing to male infertility. Although Syzygium aromaticum (clove) possesses potent antioxidant and anti-inflammatory phytochemicals, its protective efficacy against combined Al and BaP-induced reproductive toxicity remains insufficiently investigated. This study evaluated the protective effects of Syzygium aromaticum extract on reproductive hormone profiles and spermatogenesis in male Wistar rats exposed to combined aluminium and benzo[a]pyrene toxicity. Fifty-four adult male Wistar rats were randomly assigned into nine experimental groups (n = 6): normal control, aluminium-treated, benzo[a]pyrene-treated, aluminium plus benzo[a]pyrene-treated, aluminium plus benzo[a]pyrene co-administered with S. aromaticum extract (1000, 1500, and 2000 mg/kg body weight), S. aromaticum extract alone, and aluminium plus benzo[a]pyrene co-administered with ascorbic acid. At the end of the treatment period, serum concentrations of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were determined using standard immunoassay techniques. Epididymal sperm count, motility, viability, and morphology were evaluated using established laboratory procedures, while testicular tissues were processed for histopathological examination. Data were analysed using one-way analysis of variance followed by an appropriate post hoc test, with statistical significance set at $p < 0.05$. Combined exposure to aluminium and benzo[a]pyrene significantly disrupted reproductive endocrine function, as evidenced by reduced serum testosterone, LH, and FSH concentrations compared with the normal control group ($p < 0.05$). Significant deterioration in sperm count, motility, viability, and normal morphology was also observed, accompanied by marked histopathological alterations in the testes, including degeneration of seminiferous tubules and impaired spermatogenesis. Treatment with S. aromaticum extract produced dose-dependent improvements in reproductive hormone levels and sperm quality while preserving normal testicular architecture. The highest dose demonstrated the greatest protective effect, with responses comparable to those observed in the ascorbic acid-treated group. Syzygium aromaticum effectively ameliorated aluminium and benzo[a]pyrene-induced reproductive dysfunction by restoring hormonal balance, improving sperm characteristics, and preserving testicular histoarchitecture. These findings suggest that S. aromaticum possesses considerable therapeutic potential as a natural antioxidant for mitigating environmentally induced male reproductive toxicity.***

Keywords: ***Syzygium Aromaticum, Aluminium, Benzo[A]Pyrene, Reproductive Toxicity, Testosterone, Spermatogenesis, Oxidative Stress, Male Infertility.***

I. INTRODUCTION

Male infertility has emerged as a significant public health concern, accounting for approximately half of all infertility cases worldwide. The World Health Organization estimates that infertility affects millions of couples globally, with male factors contributing to nearly 50% of diagnosed cases. The decline in male reproductive health over recent decades has been attributed to multiple factors, including lifestyle changes, genetic abnormalities, endocrine disorders, infections, and increasing exposure to environmental toxicants (Wang et al, 2025). Among these, environmental pollutants have attracted considerable attention because of their ability to disrupt endocrine homeostasis, induce oxidative stress, and impair spermatogenesis, ultimately compromising male fertility.

Aluminium (Al) is one of the most abundant metals in the earth's crust and has become an unavoidable

environmental contaminant due to its widespread industrial and domestic applications. Human exposure occurs through contaminated food and water, pharmaceuticals, food packaging materials, cosmetics, occupational activities, and environmental pollution. Although aluminium has no known physiological role in the human body, excessive exposure has been associated with neurotoxicity, hepatotoxicity, nephrotoxicity, and reproductive dysfunction (Rajeswari & Sailaja, 2014; Mitra et al, 2022). Experimental studies have demonstrated that aluminium readily accumulates in the testes, where it disrupts steroidogenesis, damages Sertoli and Leydig cells, alters sperm production, and interferes with the hypothalamic–pituitary–gonadal axis. These alterations are largely mediated through excessive generation of reactive oxygen species (ROS), resulting in oxidative stress, lipid peroxidation, mitochondrial dysfunction, DNA damage, and apoptosis of germ cells.

Benzo[a]pyrene (BaP), a representative polycyclic aromatic hydrocarbon (PAH), is another ubiquitous environmental pollutant generated during incomplete combustion of organic materials. Significant sources of BaP include cigarette smoke, industrial emissions, automobile exhaust, grilled foods, and biomass combustion (Jorge et al, 2021). Owing to its lipophilic nature, BaP readily penetrates biological membranes and undergoes metabolic activation by cytochrome P450 enzymes to form highly reactive intermediates capable of binding cellular macromolecules. These metabolites induce oxidative stress, inflammation, DNA adduct formation, and cellular apoptosis, thereby disrupting normal reproductive function. Numerous experimental and epidemiological studies have associated BaP exposure with reduced sperm concentration, impaired sperm motility, abnormal sperm morphology, decreased testosterone production, and degeneration of testicular architecture (Traini et al, 2023).

Although aluminium and benzo[a]pyrene have individually been implicated in male reproductive toxicity, humans are rarely exposed to a single environmental contaminant. Instead, simultaneous exposure to multiple toxicants is increasingly common because of urbanization, industrialization, environmental pollution, occupational hazards, and dietary sources. Combined exposure may produce additive or synergistic toxic effects that exceed those observed following individual exposures. Nevertheless, studies investigating the reproductive consequences of concurrent aluminium and benzo[a]pyrene exposure remain limited, despite their widespread environmental occurrence and shared mechanisms involving oxidative stress and endocrine disruption. Understanding the biological consequences of such combined exposure is therefore essential for accurately assessing environmental health risks and developing effective therapeutic interventions.

Normal male reproductive function depends on the integrity of the hypothalamic–pituitary–gonadal (HPG) axis. Gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus stimulates the anterior pituitary to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH acts on Leydig cells to promote testosterone synthesis, whereas FSH primarily regulates Sertoli cell function and supports the development and maturation of germ cells within the seminiferous tubules. Testosterone, in turn, plays a central role in maintaining spermatogenesis, sperm maturation, libido, and overall male reproductive capacity. Disruption of this tightly regulated hormonal axis by environmental toxicants often results in reduced testosterone synthesis, impaired spermatogenesis, poor semen quality, and infertility (Olanrewaju et al, 2021).

Oxidative stress is considered one of the principal mechanisms underlying toxicant-induced reproductive dysfunction. Excessive production of reactive oxygen species overwhelms endogenous antioxidant defense systems, leading to lipid peroxidation of sperm plasma membranes, oxidation of proteins and nucleic acids, mitochondrial dysfunction, and activation of apoptotic pathways (Mannucci et al, 2022). Because spermatozoa possess limited intrinsic antioxidant capacity and contain high concentrations of polyunsaturated fatty acids within their membranes, they are particularly susceptible to oxidative damage. Consequently, oxidative stress contributes to reduced sperm count,

decreased motility, impaired viability, increased morphological abnormalities, and compromised fertilizing potential.

Natural products with potent antioxidant properties have gained increasing attention as potential therapeutic agents against environmentally induced reproductive toxicity. Syzygium aromaticum (clove), a member of the family Myrtaceae, is widely used as a culinary spice and medicinal plant (Khalil & Ashour, 2021). The flower buds of S. aromaticum are rich in bioactive phytochemicals, particularly eugenol, together with eugenyl acetate, β-caryophyllene, flavonoids, tannins, phenolic acids, and other antioxidant constituents. These phytochemicals exhibit diverse pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, anti-apoptotic, and cytoprotective effects. Several experimental studies have demonstrated that clove extracts enhance endogenous antioxidant enzyme activities, reduce lipid peroxidation, preserve cellular membrane integrity, and attenuate tissue injury induced by various xenobiotics. Emerging evidence also suggests beneficial effects of S. aromaticum on reproductive function through preservation of steroidogenesis, improvement of sperm quality, and protection of testicular histoarchitecture (Taghipour et al, 2023).
Despite the well-documented antioxidant properties of S. aromaticum, evidence regarding its protective efficacy against reproductive toxicity induced by combined aluminium and benzo[a]pyrene exposure remains scarce. Most previous investigations have focused on single-toxicant models, leaving an important knowledge gap concerning the effects of mixed environmental contaminants that more closely resemble real-life exposure scenarios. Furthermore, limited information is available regarding the ability of S. aromaticum to simultaneously restore reproductive hormone homeostasis, improve spermatogenesis, and preserve testicular architecture following co-exposure to these toxicants.

Therefore, the present study investigated the protective effects of Syzygium aromaticum extract against combined aluminium- and benzo[a]pyrene-induced reproductive dysfunction in male Wistar rats. Specifically, the study evaluated changes in serum reproductive hormones (testosterone, luteinizing hormone, and follicle-stimulating hormone), sperm characteristics, and testicular histopathology following toxicant exposure and assessed the ability of S. aromaticum to ameliorate these alterations. It was hypothesized that the antioxidant and cytoprotective properties of S. aromaticum would attenuate oxidative damage, preserve endocrine function, and improve spermatogenesis, thereby mitigating the adverse reproductive effects associated with combined environmental toxicant exposure.

II. MATERIALS AND METHODS

Chemicals and Reagents

Aluminium chloride ($AlCl_3$) and benzo[a]pyrene (BaP) used in this study were of analytical grade and obtained from certified commercial suppliers. Ascorbic acid was used as the reference antioxidant. Commercial enzyme-linked immunosorbent assay (ELISA) kits for the determination of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were procured from reputable manufacturers and used according to the manufacturers' instructions. All other chemicals and reagents employed were of analytical grade.

Collection and Identification of Plant Material

Dried flower buds of Syzygium aromaticum (L.) Merr. & L.M. Perry were obtained from a certified herbal market in Nigeria. Botanical authentication was carried out by a qualified taxonomist in the Department of Plant Science and Biotechnology, University of Port Harcourt (UNIPORT).

Preparation of Syzygium aromaticum Extract

The dried flower buds were thoroughly cleaned, air-dried where necessary, and pulverized into a fine powder using an electric grinder. The powdered material was extracted using distilled water by maceration for 7 days with intermittent agitation. The extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at controlled temperature. The concentrated extract was further dried to obtain a stable crude extract, which was stored at 4°C until use. Fresh suspensions were prepared daily in distilled water immediately before administration.

Experimental Animals

Fifty-four healthy adult male Wistar rats weighing approximately 160grams were obtained from the Animal House of UNIPORT. Animals were housed in well-ventilated cages under standard laboratory conditions (temperature: 22 ± 2°C; relative humidity: 50–60%; 12 h light/12 h dark cycle) and allowed free access to standard commercial rat pellets and clean drinking water throughout the study. Animals were acclimatized for two weeks before commencement of the experiment.

Experimental Design

Following acclimatization, the rats were randomly assigned into nine experimental groups (n = 6 per group):

1) Group I (Normal Control): Received distilled water only.
2) Group II (Aluminium): Received aluminium chloride (100 mg/kg body weight).
3) Group III (Benzo[a]pyrene): Received benzo[a]pyrene (100 mg/kg body weight).
4) Group IV (Aluminium + Benzo[a]pyrene): Received aluminium chloride (100 mg/kg) and benzo[a]pyrene (100 mg/kg).
5) Group V (Al + BaP + SAE): Received aluminium chloride, benzo[a]pyrene, and SAE (1000 mg/kg body weight).
6) Group VI (Al + BaP + SAE): Received aluminium chloride, benzo[a]pyrene, and SAE (1500 mg/kg body weight).
7) Group VII (Al + BaP + SAE): Received aluminium chloride, benzo[a]pyrene, and SAE (2000 mg/kg body weight).
8) Group VIII (S. aromaticum only): Received SAE only (1000 mg/kg body weight).
9) Group IX (Al + BaP + Ascorbic Acid): Received aluminium chloride, benzo[a]pyrene, and ascorbic acid (10 mg/kg body weight).

All treatments were administered once daily by oral gavage for 28 days.

Sample Collection

Twenty-four hours after the final treatment, animals were fasted overnight and anaesthetized using chloroform. Blood samples were collected via cardiac puncture into plain sample bottles and allowed to clot. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at −20°C until hormonal analysis. The testes and epididymides were carefully excised, blotted dry, weighed, and processed for sperm analysis and histopathological examination.

Determination of Reproductive Hormones

Serum concentrations of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were determined using commercially available ELISA kits according to the manufacturers' protocols. Absorbance was measured using a microplate reader at the specified wavelength, and hormone concentrations were calculated from standard calibration curves.

Evaluation of Sperm Characteristics

Sperm Count

The cauda epididymis was carefully dissected and minced in physiological saline to release spermatozoa. Sperm count was determined using an improved Neubauer haemocytometer under a light microscope and expressed as millions of sperm cells per millilitre.

Sperm Motility

A drop of freshly prepared sperm suspension was placed on a clean glass slide and examined immediately under a light microscope maintained at approximately 37°C. At least 200 spermatozoa were evaluated from randomly selected fields, and the percentage of progressively motile spermatozoa was calculated.

Sperm Viability

Sperm viability was assessed using the eosin–nigrosin staining technique. Live spermatozoa excluded the stain, whereas non-viable spermatozoa absorbed the dye. A minimum of 200 sperm cells were counted, and the percentage of viable spermatozoa was determined.

Sperm Morphology

Morphological evaluation was performed using eosin–nigrosin stained smears under oil immersion

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microscopy. Approximately 200 spermatozoa per animal were examined and classified as normal or abnormal based on abnormalities involving the head, midpiece, or tail. The percentage of morphologically normal spermatozoa was subsequently calculated.

Histopathological Examination

Testicular tissues were immediately fixed in 10% neutral buffered formalin for at least 24 hours before routine histological processing. Fixed tissues were dehydrated through ascending grades of ethanol, cleared in xylene, embedded in paraffin wax, sectioned at approximately 5 µm thickness using a rotary microtome, and stained with haematoxylin and eosin (H&E). Prepared slides were examined under a light microscope by a qualified histopathologist who was blinded to the treatment groups. Photomicrographs were captured using a digital microscope imaging system to evaluate seminiferous tubular architecture, spermatogenic cell arrangement, Leydig cell morphology, and evidence of degenerative changes.

Statistical Analysis

All data were collected and expressed as Mean ± Standard Error of Mean (SEM). Statistical analysis was performed using IBM SPSS Statistics (Version 27) and GraphPad Prism (Version 10.4.2). To determine significant differences between group means, a One-way Analysis of Variance (ANOVA) was conducted. Where significant differences were observed, Tukey's Multiple Comparisons Test (Tukey's HSD) was used as the post-hoc test to separate the means. A probability value of less than 0.05 ($p < 0.05$) was considered statistically significant. The results were presented in graphical forms. A single asterisk (*) indicates an adjusted p-value ≤ 0.05; two asterisks (**) indicate $p \leq 0.01$; three asterisks (***) indicate $p \leq 0.001$; and four asterisks (****) indicate a highly significant difference at $p \leq 0.0001$.

III. RESULTS

HORMONE PROFILE

Effect on Follicle Stimulating Hormone (FSH)

Alterations in FSH and LH indicate disruption of the hypothalamic–pituitary–gonadal axis. Clove extract normalized these hormones, suggesting endocrine restoration.

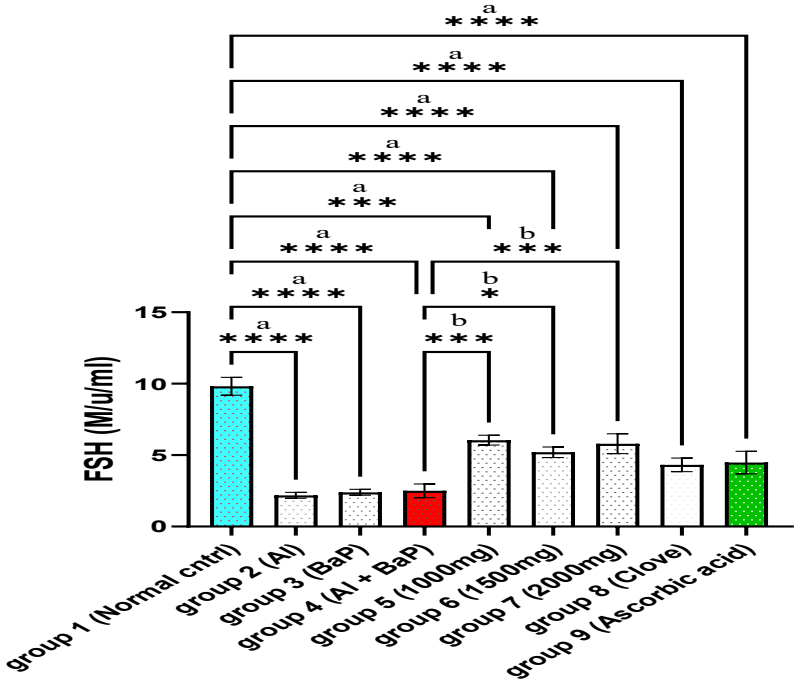

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Figure 1: Effect of Syzygium aromaticum Extract on FSH level. Data are presented as Mean ± SEM (n=6). "a" indicates significant difference ($p < 0.05$) compared to Normal Control (Group 1). "b" indicates significant difference ($p < 0.05$) compared to Negative Control (Group 4). A single asterisk (*) indicates an adjusted p-value ≤ 0.05; two asterisks (**) indicate $p \leq 0.01$; three asterisks (***) indicate $p \leq 0.001$; and four asterisks (****) indicate a highly significant difference at $p \leq 0.0001$.

Effect on Luteinizing Hormone

Alterations in FSH and LH indicate disruption of the hypothalamic–pituitary–gonadal axis. Clove extract normalized these hormones, suggesting endocrine restoration.

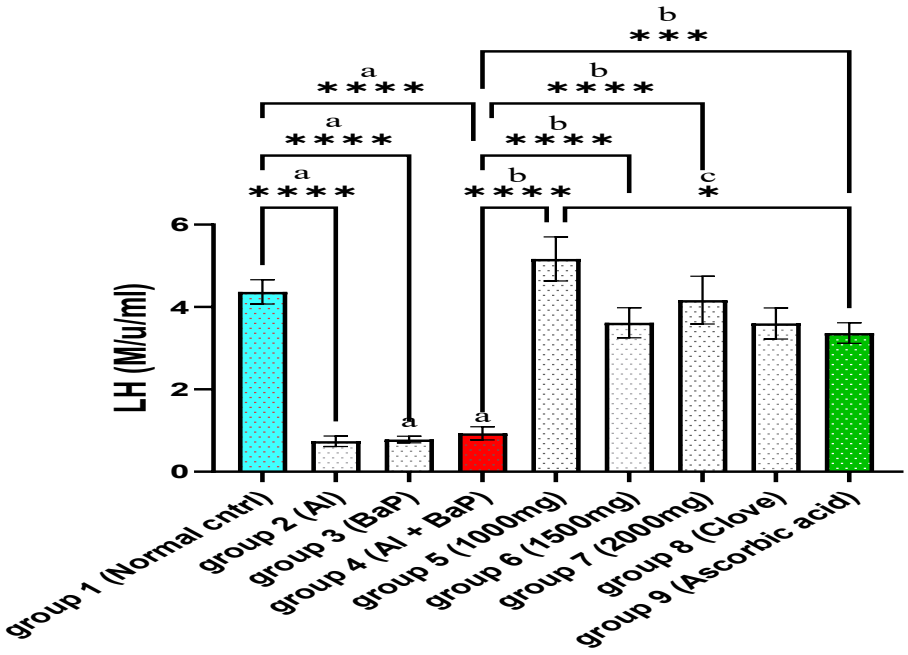

Figure 2: Effect of Syzygium aromaticum Extract on LH level.

Data are presented as Mean ± SEM (n=6). "a" indicates significant difference ($p < 0.05$) compared to Normal Control (Group 1). "b" indicates significant difference ($p < 0.05$) compared to Negative Control (Group 4). "c" indicates significant difference ($p < 0.05$) compared to the Standard drug (Group 9). A single asterisk (*) indicates an adjusted p-value ≤ 0.05; two asterisks (**) indicate $p \leq 0.01$; three asterisks (***) indicate $p \leq 0.001$; and four asterisks (****) indicate a highly significant difference at $p \leq 0.0001$.

Effect on Testosterone

Significant reduction in testosterone in toxicant groups indicates Leydig cell dysfunction. Clove extracts increased testosterone levels, indicating restoration of steroidogenesis.

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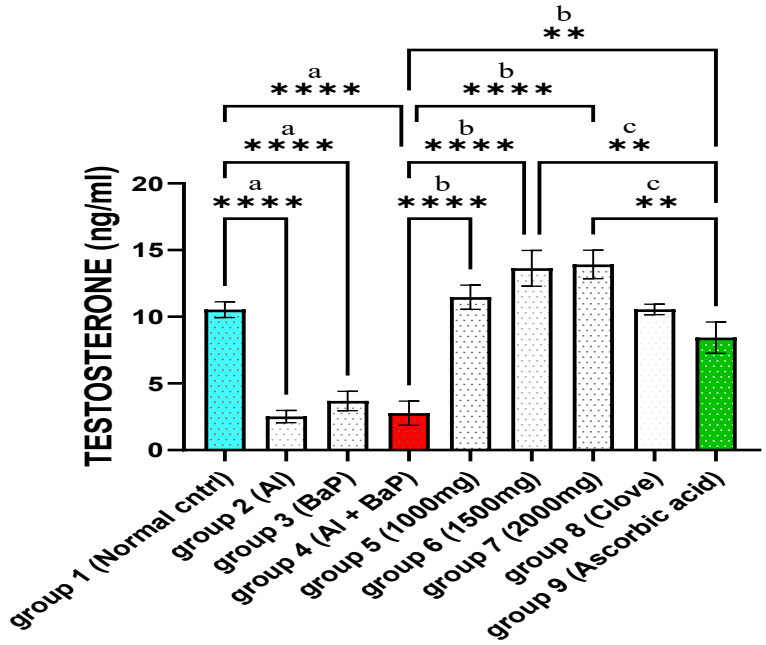

Figure 3: Effect of Syzygium aromaticum Extract on Testosterone level.

Data are presented as Mean ± SEM (n=6). "a" indicates significant difference ($p < 0.05$) compared to Normal Control (Group 1). "b" indicates significant difference ($p < 0.05$) compared to Negative Control (Group 4). A single asterisk (*) indicates an adjusted p-value ≤ 0.05; two asterisks (**) indicate $p \leq 0.01$; three asterisks (***) indicate $p \leq 0.001$; and four asterisks (****) indicate a highly significant difference at $p \leq 0.0001$.

SEMEN MORPHOLOGY

Effect on Head Defect

Toxicant exposure significantly increased sperm abnormalities, indicating impaired spermatogenesis. Clove extract reduced these defects, demonstrating protective effects on sperm structure.

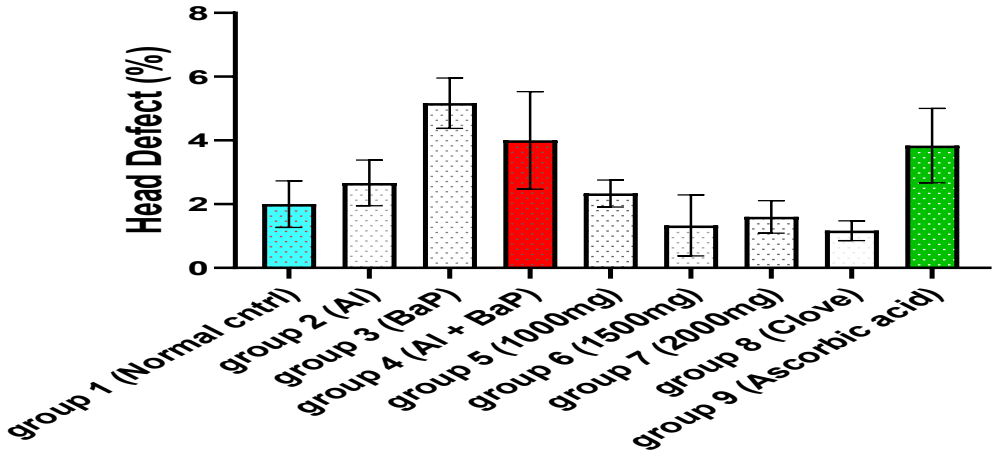

Figure 4: Effect of Syzygium aromaticum Extract on Head Defect.

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Data are presented as Mean ± SEM (n=6). "a" indicates significant difference ($p < 0.05$) compared to Normal Control (Group 1). "b" indicates significant difference ($p < 0.05$) compared to Negative Control (Group 4). A single asterisk (*) indicates an adjusted p-value ≤ 0.05; two asterisks (**) indicate $p \leq 0.01$; three asterisks (***) indicate $p \leq 0.001$; and four asterisks (****) indicate a highly significant difference at $p \leq 0.0001$.

Effect on Tail Defect

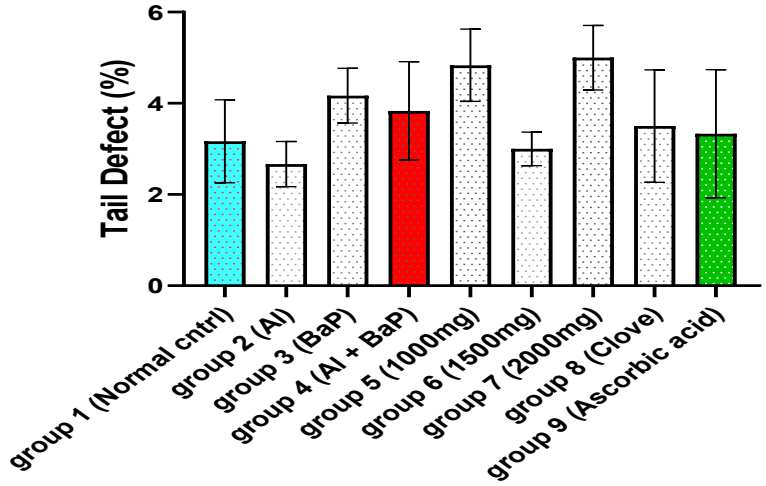

Figure 5: Effect of Syzygium aromaticum Extract on Tail Defect.

Data are presented as Mean ± SEM (n=6). "a" indicates significant difference ($p < 0.05$) compared to Normal Control (Group 1). "b" indicates significant difference ($p < 0.05$) compared to Negative Control (Group 4). A single asterisk (*) indicates an adjusted p-value ≤ 0.05; two asterisks (**) indicate $p \leq 0.01$; three asterisks (***) indicate $p \leq 0.001$; and four asterisks (****) indicate a highly significant difference at $p \leq 0.0001$.

Effect on Mild Piece

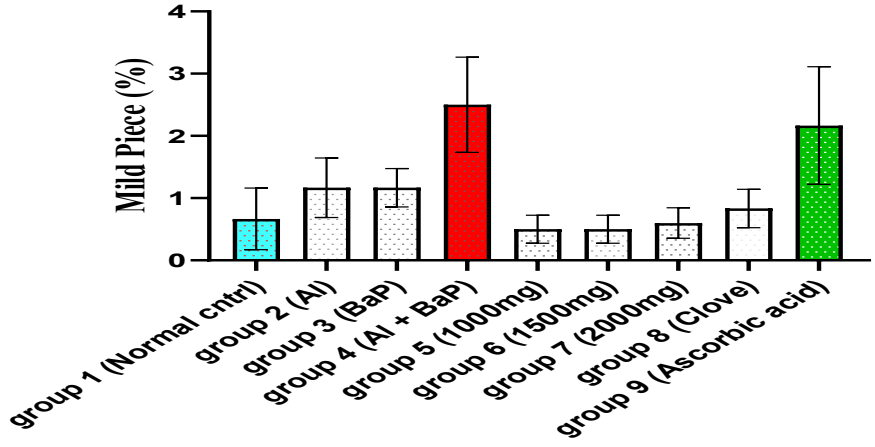

Figure 6: Effect of Syzygium aromaticum Extract on Mild Piece.

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Data are presented as Mean ± SEM (n=6). "a" indicates significant difference ($p < 0.05$) compared to Normal Control (Group 1). "b" indicates significant difference ($p < 0.05$) compared to Negative Control (Group 4). A single asterisk (*) indicates an adjusted p-value ≤ 0.05; two asterisks (**) indicate $p \leq 0.01$; three asterisks (***) indicate $p \leq 0.001$; and four asterisks (****) indicate a highly significant difference at $p \leq 0.0001$.

Viable Sperm Cells

Toxicant groups showed reduced viability and increased non-viable sperm. Treatment improved viability, indicating enhanced sperm integrity.

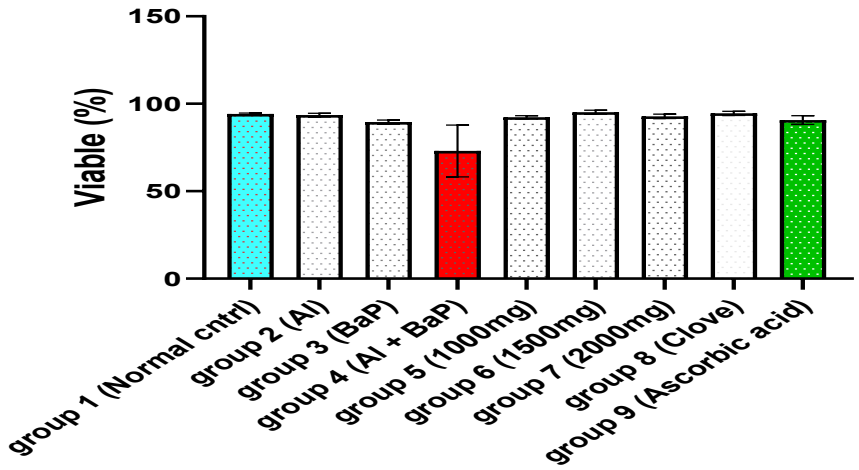

Figure 7: Effect of Syzygium aromaticum Extract on the Level of Viable Sperm Cells.

Data are presented as Mean ± SEM (n=6). "a" indicates significant difference ($p < 0.05$) compared to Normal Control (Group 1). "b" indicates significant difference ($p < 0.05$) compared to Negative Control (Group 4). A single asterisk (*) indicates an adjusted p-value ≤ 0.05; two asterisks (**) indicate $p \leq 0.01$; three asterisks (***) indicate $p \leq 0.001$; and four asterisks (****) indicate a highly significant difference at $p \leq 0.0001$.

Effect on Level of Non-viable sperm cells

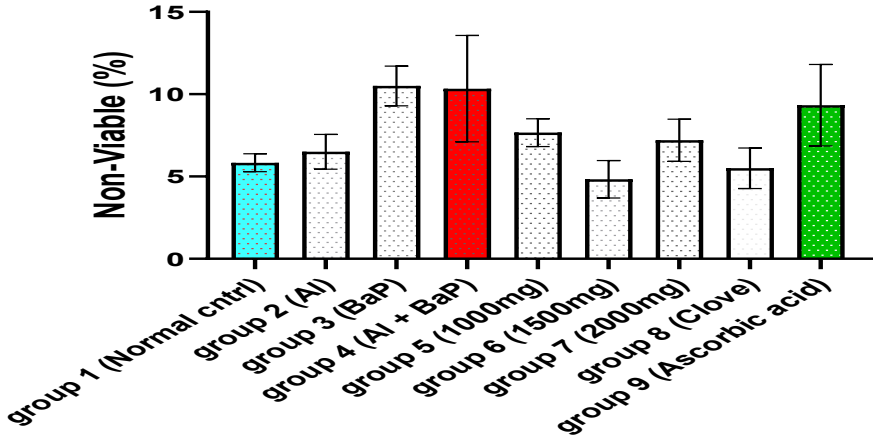

Figure 8: Effect of Syzygium aromaticum Extract on Level of non-viable Sperm cells.

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Data are presented as Mean ± SEM (n=6). “a” indicates significant difference ($p < 0.05$) compared to Normal Control (Group 1). “b” indicates significant difference ($p < 0.05$) compared to Negative Control (Group 4). A single asterisk (*) indicates an adjusted p-value ≤ 0.05; two asterisks (**) indicate $p \leq 0.01$; three asterisks (***) indicate $p \leq 0.001$; and four asterisks (****) indicate a highly significant difference at $p \leq 0.0001$.

SEMEN ANALYSIS

Effect on Motile Cells

Significant reduction in motility in toxicant groups indicates impaired sperm function. Clove extracts improved motility, suggesting enhanced mitochondrial function and energy metabolism.

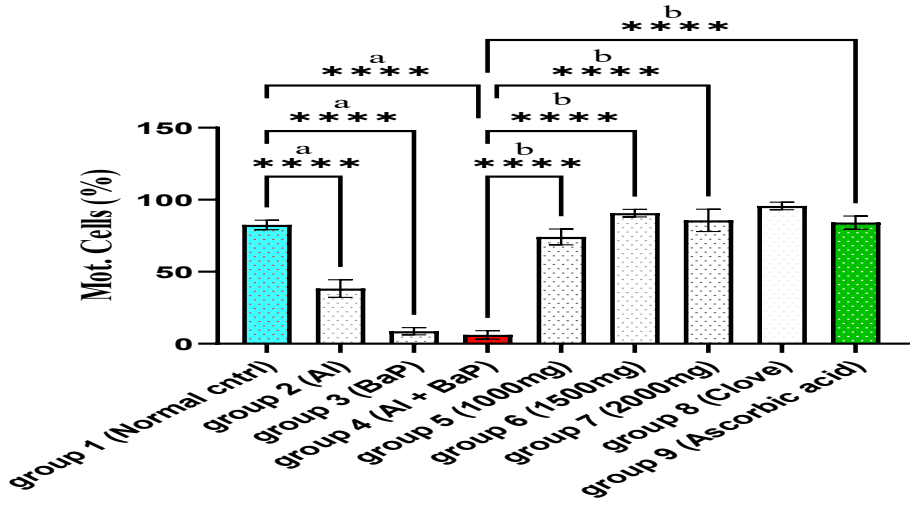

Figure 9: Effect of Syzygium aromaticum Extract on Motile Cells level.

Data are presented as Mean ± SEM (n=6). “a” indicates significant difference ($p < 0.05$) compared to Normal Control (Group 1). “b” indicates significant difference ($p < 0.05$) compared to Negative Control (Group 4). A single asterisk (*) indicates an adjusted p-value ≤ 0.05; two asterisks (**) indicate $p \leq 0.01$; three asterisks (***) indicate $p \leq 0.001$; and four asterisks (****) indicate a highly significant difference at $p \leq 0.0001$.

Effect on Active Cells

Significant reduction in motility in toxicant groups indicates impaired sperm function. Clove extracts improved motility, suggesting enhanced mitochondrial function and energy metabolism.

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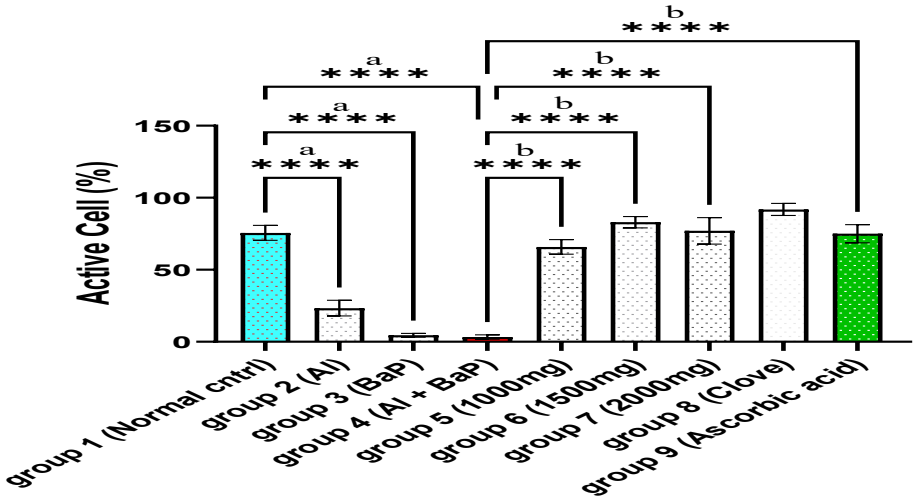

Figure 10: Effect of Syzygium aromaticum Extract on Active Cell level.

Data are presented as Mean ± SEM (n=6). "a" indicates significant difference ($p < 0.05$) compared to Normal Control (Group 1). "b" indicates significant difference ($p < 0.05$) compared to Negative Control (Group 4). A single asterisk (*) indicates an adjusted p-value ≤ 0.05; two asterisks (**) indicate $p \leq 0.01$; three asterisks (***) indicate $p \leq 0.001$; and four asterisks (****) indicate a highly significant difference at $p \leq 0.0001$.

Effect on Sluggish sperms

Increased sluggish and dead sperm in toxicant groups reflects cellular damage. Treatment reduced these populations significantly.

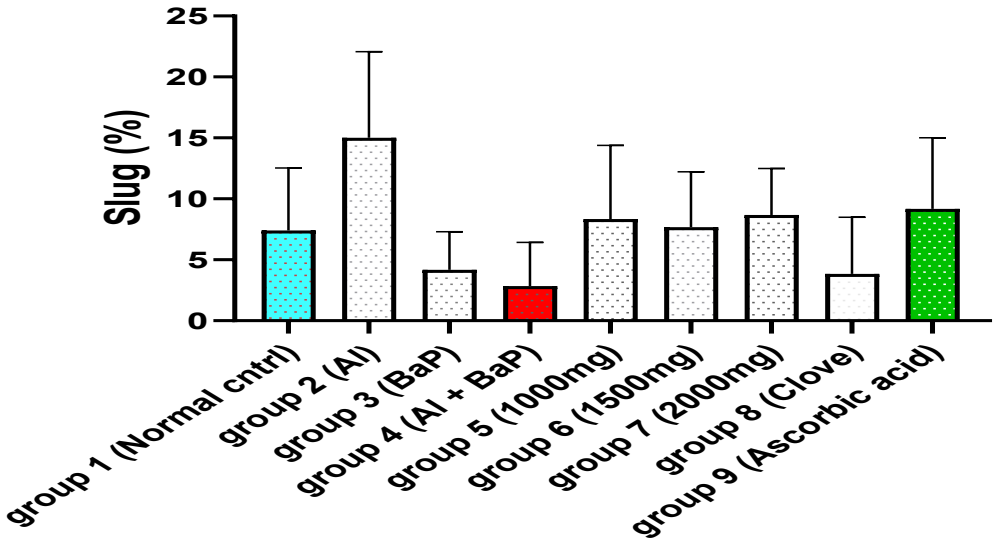

Figure 11: Effect of Syzygium aromaticum Extract on Sluggish sperms.

Data are presented as Mean ± SEM (n=6). "a" indicates significant difference ($p < 0.05$) compared

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to Normal Control (Group 1). “b” indicates significant difference ($p < 0.05$) compared to Negative Control (Group 4). A single asterisk (*) indicates an adjusted p-value ≤ 0.05; two asterisks (**) indicate $p \leq 0.01$; three asterisks (***) indicate $p \leq 0.001$; and four asterisks (****) indicate a highly significant difference at $p \leq 0.0001$.

Dead Sperm Cells

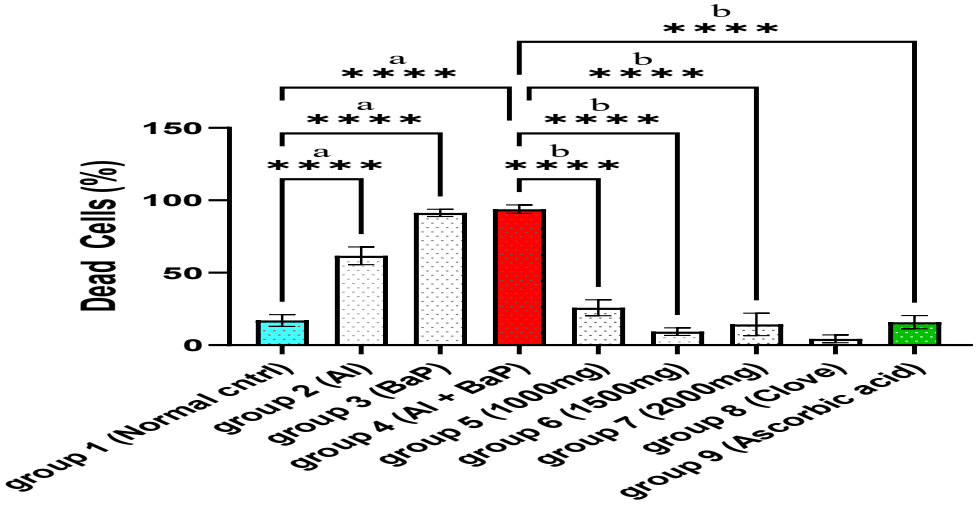

Figure 12: Effect of Syzygium aromaticum Extract on level of Dead Cells.

Data are presented as Mean ± SEM (n=6). “a” indicates significant difference ($p < 0.05$) compared to Normal Control (Group 1). “b” indicates significant difference ($p < 0.05$) compared to Negative Control (Group 4). A single asterisk (*) indicates an adjusted p-value ≤ 0.05; two asterisks (**) indicate $p \leq 0.01$; three asterisks (***) indicate $p \leq 0.001$; and four asterisks (****) indicate a highly significant difference at $p \leq 0.0001$.

Total Sperm Count

A marked reduction in sperm count was observed in toxicant groups. Clove extract restored sperm count, indicating improved spermatogenesis

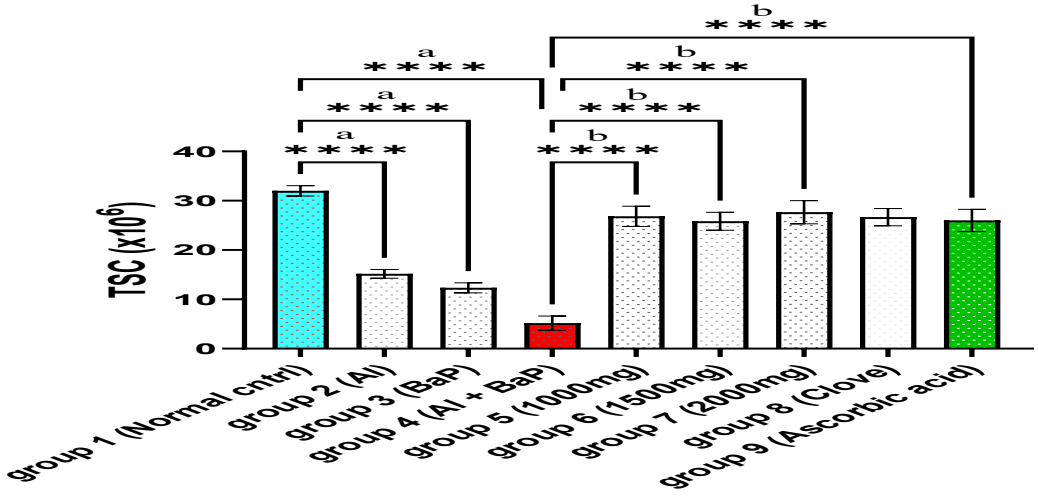

Figure 13: Effect of Syzygium aromaticum Extract on Total Sperm Count.

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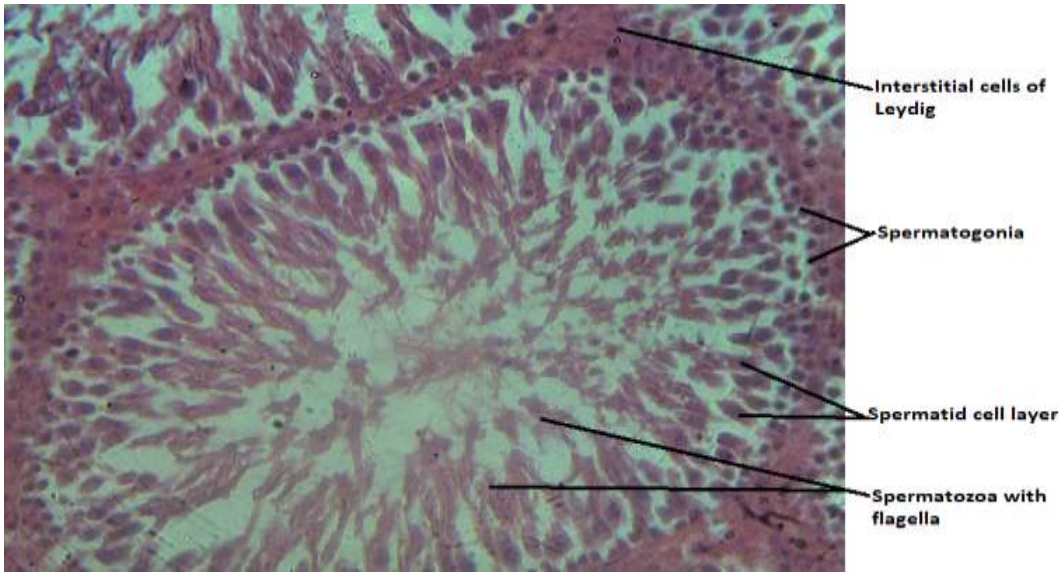
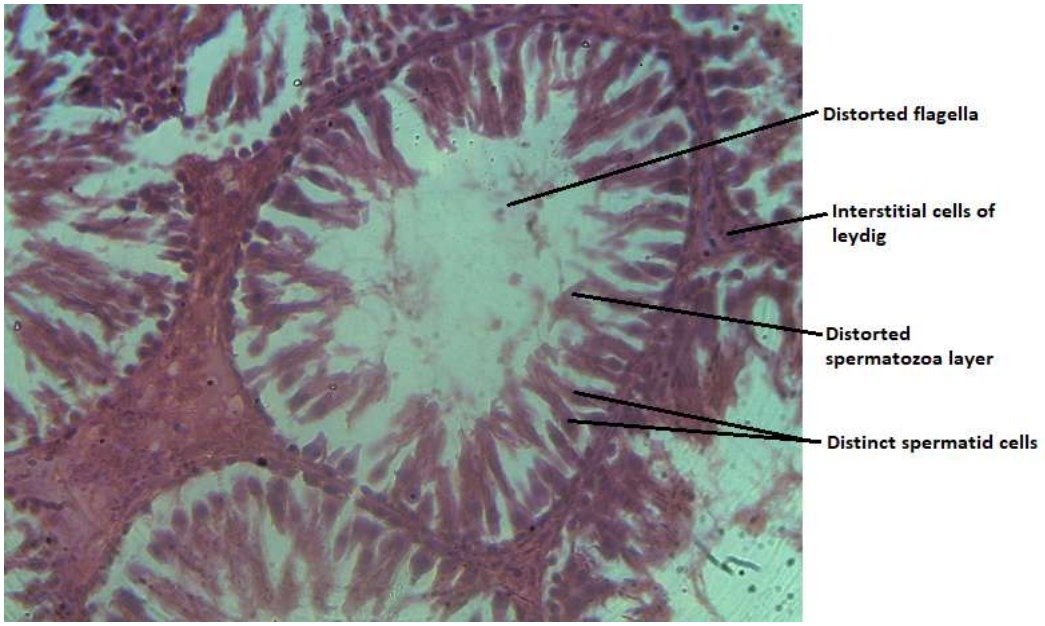
Data are presented as Mean ± SEM (n=6). “a” indicates significant difference ($p < 0.05$) compared to Normal Control (Group 1). “b” indicates significant difference ($p < 0.05$) compared to Negative Control (Group 4). A single asterisk (*) indicates an adjusted p-value ≤ 0.05; two asterisks (**) indicate $p \leq 0.01$; three asterisks (***) indicate $p \leq 0.001$; and four asterisks (****) indicate a highly significant difference at $p \leq 0.0001$.

Photomicrograph of Testes Tissues Obtained From The Experimental Animal Subjects from Groups 1 – 9



Slide 1: Photomicrograph of the testis tissue showing distinct spermatid and spermatogonia cells. Flagellated lumen of seminiferous tubule also observed. Normal tissue microstructure is indicated. H & E, X400



Slide 2: Photomicrograph of the testis tissue showing distorted spermatozoa with detached flagella (deflagellation) in lumen of seminiferous tubule. Distinct spermatid with pockets of distorted are also observed and distorted interstitial cells of Leydig. H & E, X400

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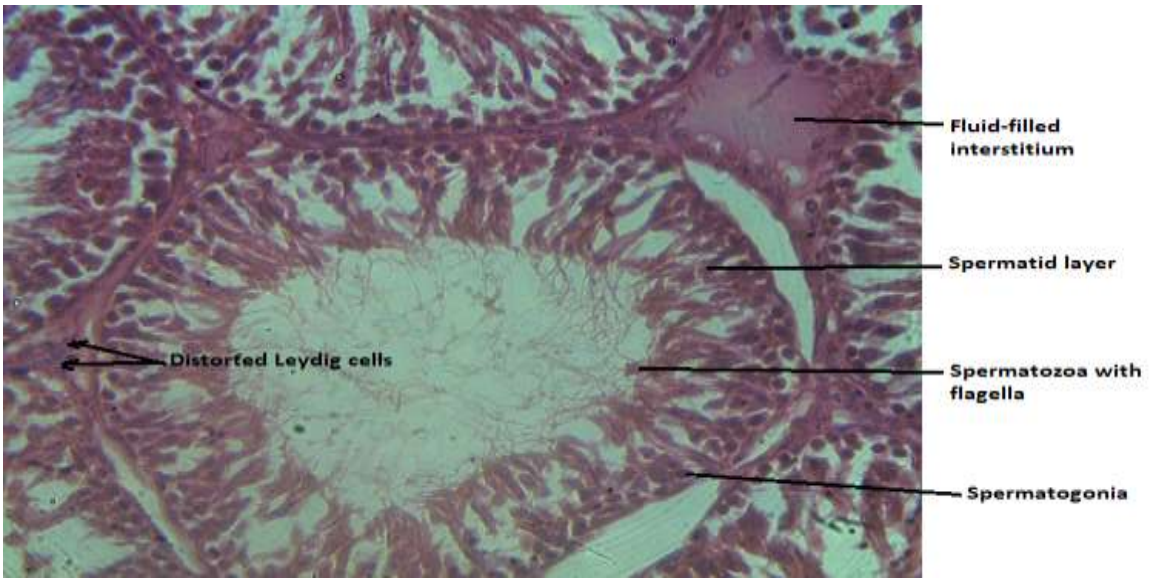
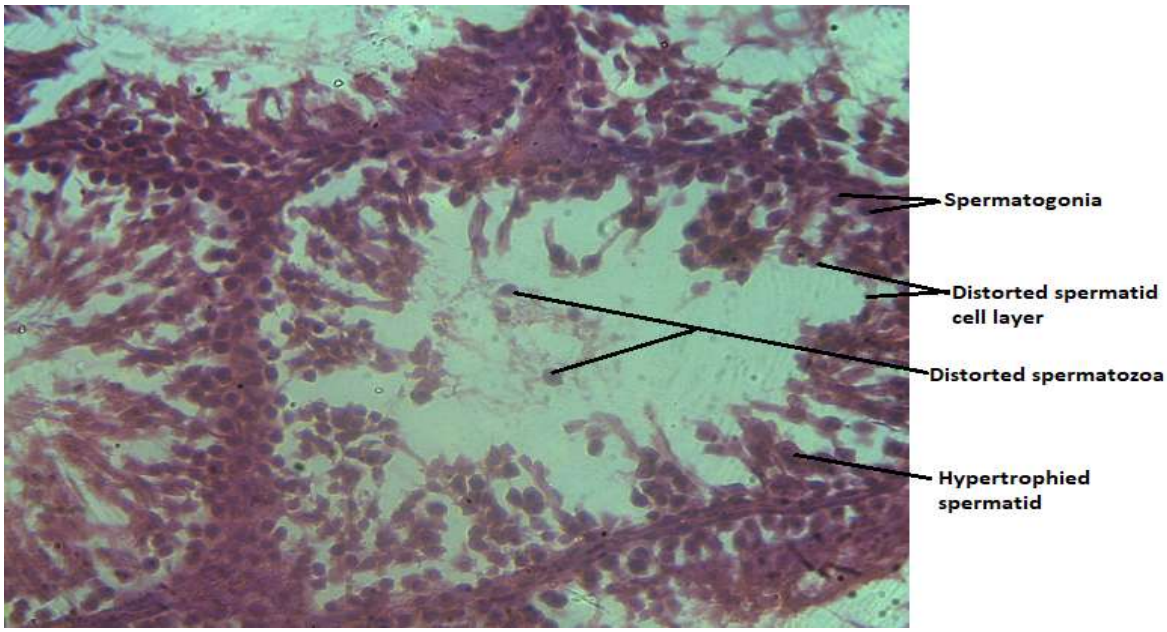


Slide 3: Photomicrograph of the testis tissue showing distorted leydig cells and fluid-filled interstitium (edematous). The spermatid and spermatogonia cell layers show normal microstructural appearance. H & E, X400



Slide 4: Photomicrograph of the testis tissue of seminiferous tubules showing distorted spermatid cell layers, distorted spermatogonia and hypertrophied spermatid cell layer. Distortion of tissue microstructure is indicated . H & E, X400

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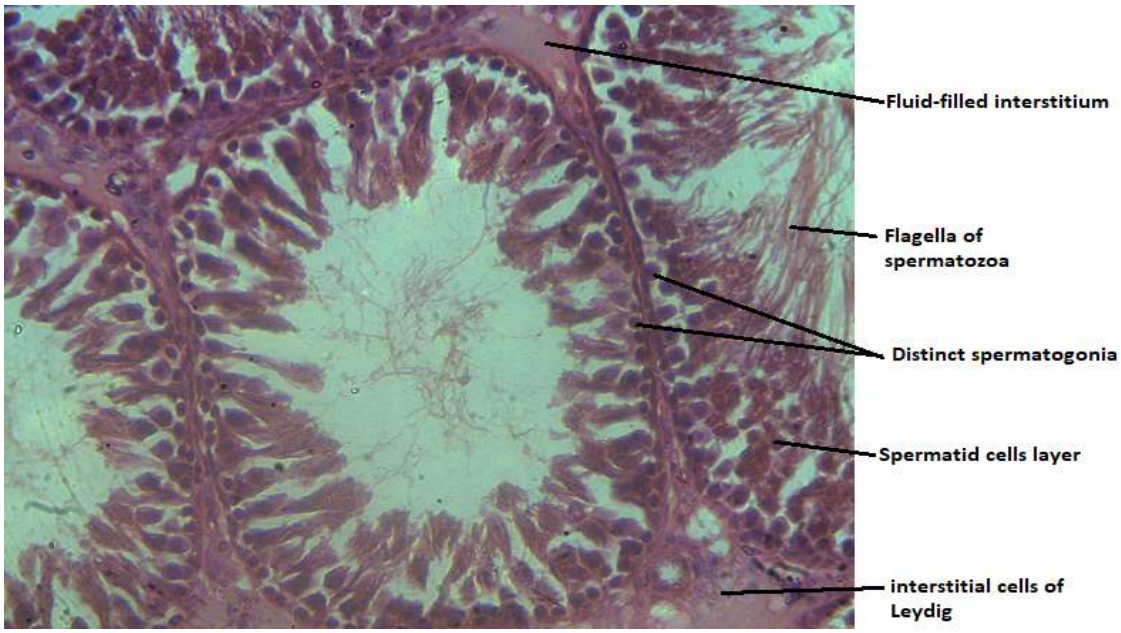
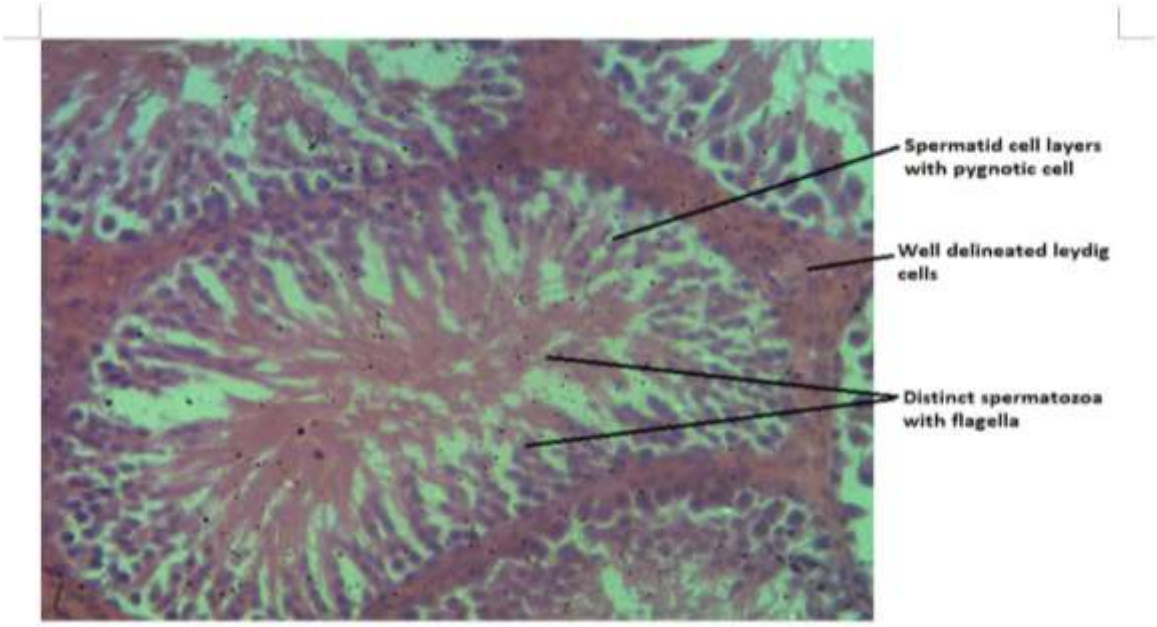


Slide 5: Photomicrograph of the testis tissue showing distinct spermatogonia with flagella in the lumen, fluid-filled interstitium (edematous). Tissue shows distortion. H & E, x400



Slide 6: Photomicrograph of the testis tissue showing well-delineated spermatid cell layers (with pockets of cellular pyknosis), distinct spermatozoa with flagella in lumen of seminiferous tubules. Tissues shows normal microstructure. H & E, X400

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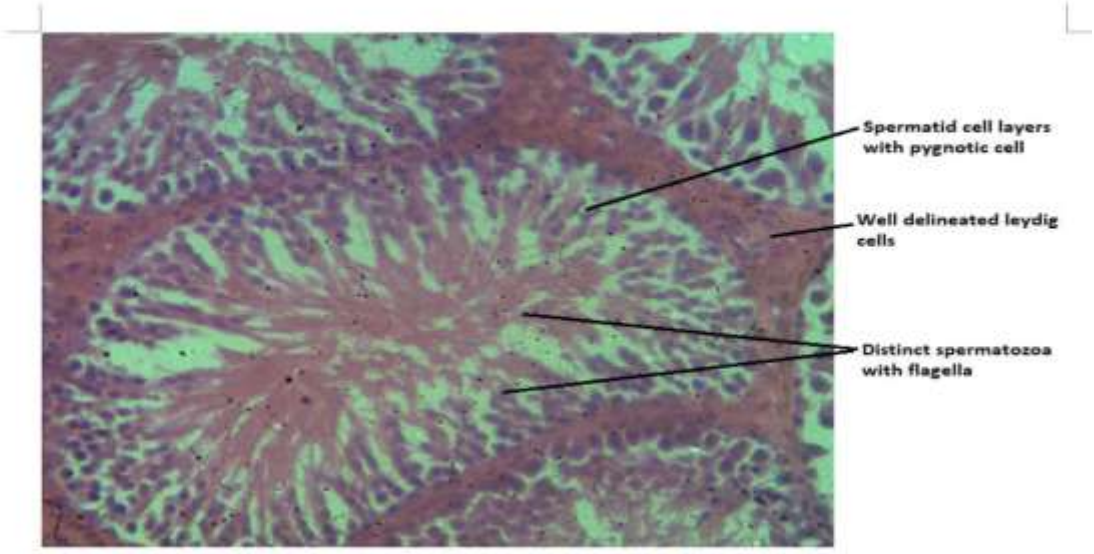
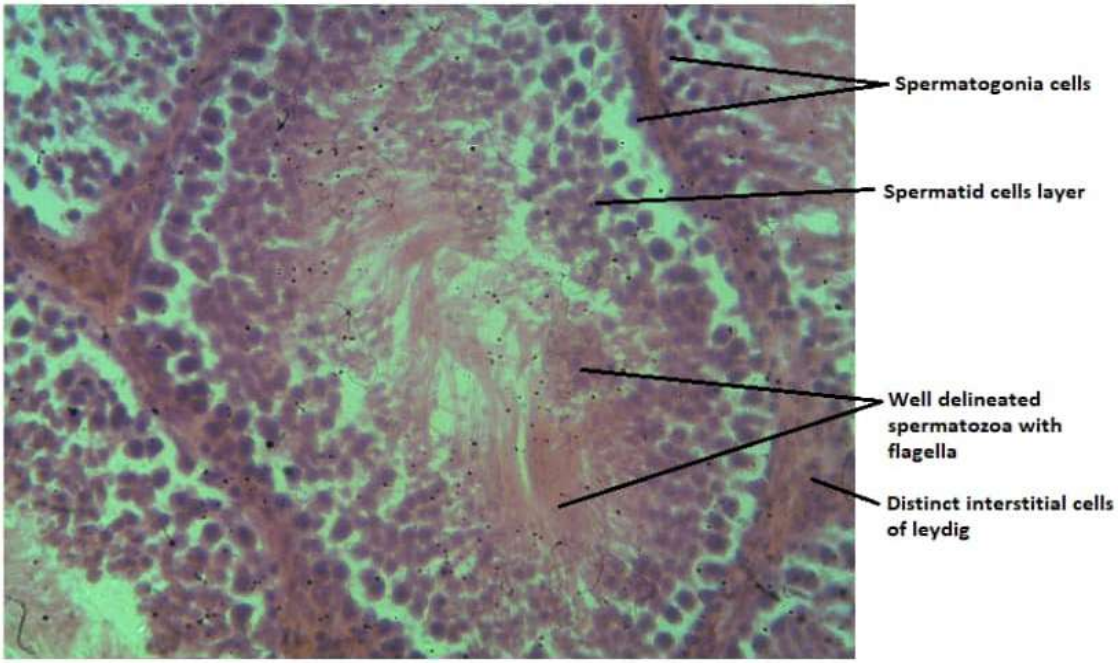


Slide 7: Photomicrograph of the testicular tissue showing largely preserved tissue architecture, with mild distortion of some interstitial cells of Leydig. Occasional distorted spermatozoa are observed within the seminiferous tubule lumen, indicating minimal residual microstructural alteration. H & E, ×400.



Slide 8: Photomicrograph of testis tissue showing well delineated spermatozoa with flagella in the lumen of seminiferous tubule. Distinct spermatid cell layer and lining the internal wall of the tubule is the spermatogonia cells. Normal tissue microstructural appearance indicated.

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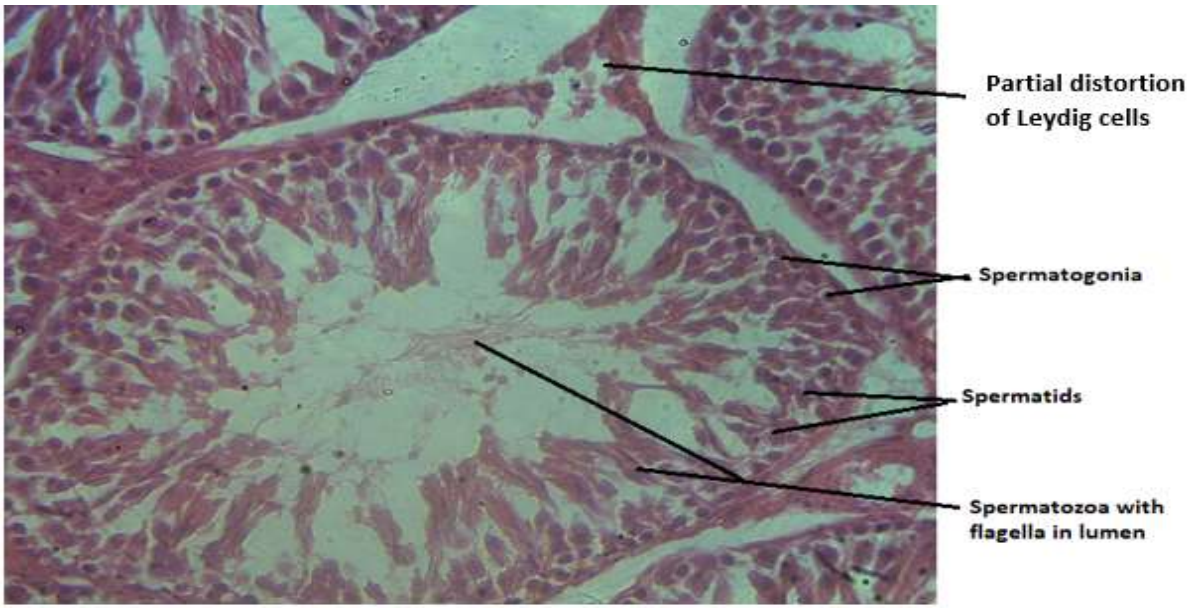

Slide 9: Photomicrograph of the testicular tissue showing partially distorted interstitial cells of Leydig, with relatively preserved and well-delineated spermatogonia and spermatid cell layers. Focal areas of abnormal and distorted spermatozoa are observed within the lumen of the seminiferous tubules. H & E, ×400.

Overall, Aluminium and Benzo[a]pyrene induced multi-organ toxicity characterized by oxidative stress, endocrine disruption, and impaired spermatogenesis, while Syzygium aromaticum demonstrated significant dose-dependent protective effects across biochemical, hormonal, and histological indices.

IV. DISCUSSION

Medicinal plants remain central to health management in Nigeria and other developing regions, and Syzygium aromaticum (clove) has emerged as a plant of considerable biomedical relevance due to its dense phytochemical composition and demonstrated protective role in reproductive, hepatic, renal, and haematological function. Its phytochemical profile is characterized by abundant flavonoids, alkaloids, tannins, saponins, and phenolic compounds, with eugenol playing a dominant role in its antioxidant, anti-inflammatory, antimicrobial, and cytoprotective activity (Khalil & Ashour, 2021; Taghipour et al, 2023).

Toxicological evidence indicates that S. aromaticum is relatively safe at low to moderate doses, consistent with the finding of Ogbonna et al. (2025) that a 2500 mg/kg dose effectively ameliorated lipid and haematological profiles in rats. However, dose optimization remains important, since very high or chronic dosing can overwhelm hepatic and renal detoxification pathways (Falade et al., 2022; Taghipour et al., 2023).

Aluminium promotes reactive oxygen species (ROS) generation and disrupts antioxidant enzyme systems, while benzo[a]pyrene undergoes metabolic activation to reactive intermediates that further amplify oxidative injury (Jorge et al., 2021; Fares et al., 2024). Co-administration of these toxicants likely creates a more hostile intracellular environment in the testes, overwhelming antioxidant defences and damaging lipids, proteins, and DNA by compromising germ cell integrity, steroidogenic efficiency, and sperm maturation. Clove extract significantly attenuated these alterations, consistent with restoration of redox balance and reinforcement of endogenous antioxidant systems (Taghipour et al., 2023).

Global data show male infertility now affects roughly one in six couples worldwide, with male factors implicated in about half of all cases (Wang et al., 2025). Given that synthetic fertility drugs are often costly and carry side effects, plant-derived

antioxidants represent an increasingly attractive alternative. In the present study, clove produced a clear dose-dependent amelioration of sperm count, motility, viability, and morphology in Groups 5–7, most plausibly through its high flavonoid and eugenol content. Similar protective effects of plant-derived phenolics and flavonoids on sperm quality have been reported elsewhere, including with clove itself co-administered against reproductive toxicants (Akinwande et al., 2019; Taghipour et al., 2023).

The reduction in sperm count, motility, viability, and active sperm cells observed in the toxicant groups reflects oxidative injury to the seminiferous epithelium and sperm membranes, which are particularly vulnerable given their high polyunsaturated fatty acid content. The combined aluminium and benzo[a]pyrene group showed more pronounced damage than either single-toxicant group, indicating that co-exposure intensifies reproductive injury (Jorge et al., 2021; Fares et al., 2024). This is likely because aluminium promotes oxidative stress and enzyme disruption while benzo[a]pyrene's reactive metabolites directly damage DNA and membranes. Treatment with S. aromaticum significantly improved these parameters, an effect closely tied to restoration of testosterone, LH, and FSH, and consistent with protection of Leydig and Sertoli cells from oxidative damage (Oduwole, Huhtaniemi, & Misrahi, 2021; Sharma et al., 2019).
The observed decline in testosterone, LH, and FSH in toxicant-treated groups suggests disruption of the hypothalamic–pituitary–gonadal (HPG) axis. Since LH stimulates Leydig cells to synthesize testosterone and FSH supports Sertoli cell function, suppression of these hormones directly explains the poor sperm indices in Groups 2–4, and is consistent with oxidative injury to steroidogenic enzymes such as 3β- and 17β-hydroxysteroid dehydrogenase (Olanrewaju et al., 2021; Traini et al., 2023).

Co-administration of S. aromaticum extract improved the reproductive hormone profile in a dose-dependent manner (Groups 5–7), with values approaching those of the normal control. This is likely attributable to clove's phytochemical constituents, chiefly eugenol, eugenyl acetate, and β-caryophyllene, which scavenge free radicals, reduce lipid peroxidation, and help preserve testicular structural integrity and steroidogenic enzyme function (Taghipour et al., 2023).

Histological findings corroborate the biochemical and hormonal data. Disruption of seminiferous tubules, germ cell degeneration, and Leydig cell damage in the toxicant groups provide structural confirmation of reduced sperm count and testosterone, since intact tubular architecture and functional Leydig cells are essential for normal fertility. The more severe lesions in the combined toxicant group suggest sustained oxidative stress and possible apoptotic cell death, while clove-treated groups showed restoration of tubular architecture, indicating both protective and regenerative capacity.

Overall, these findings support the hypothesis that combined aluminium and benzo[a]pyrene exposure produces synergistic reproductive toxicity through oxidative stress and HPG axis disruption, and that S. aromaticum's antioxidant and cytoprotective phytochemicals meaningfully counteract this damage in a dose-dependent manner, with the 2000 mg/kg dose offering the strongest overall protection in this model.

V. CONCLUSION

The present study demonstrates that combined exposure to aluminium and benzo[a]pyrene exerts profound adverse effects on male reproductive function through disruption of reproductive hormone homeostasis, impairment of sperm quality, and degeneration of testicular architecture. These alterations are indicative of compromised spermatogenesis and endocrine dysfunction, highlighting the detrimental reproductive consequences of concurrent exposure to these ubiquitous environmental toxicants.

Administration of Syzygium aromaticum extract significantly ameliorated these toxic effects in a dose-dependent manner. Treatment restored serum testosterone, luteinizing hormone, and follicle-stimulating hormone levels towards normal, improved sperm count, motility, viability, and morphology, and preserved the structural integrity of

the seminiferous tubules and interstitial tissues of the testes. The protective effects observed are likely attributable to the rich phytochemical composition of S. aromaticum, particularly its antioxidant and cytoprotective constituents, which mitigate oxidative stress-induced cellular damage and preserve the functional integrity of the hypothalamic–pituitary–gonadal axis.

Overall, these findings provide experimental evidence that Syzygium aromaticum possesses considerable therapeutic potential as a natural protective agent against environmentally induced male reproductive toxicity. Given the increasing human exposure to multiple environmental contaminants, the use of plant-derived antioxidants may represent a promising complementary strategy for preserving male reproductive health. Nevertheless, further investigations involving molecular mechanisms, gene and protein expression analyses, pharmacokinetic evaluation, and well-designed clinical studies are required to validate these findings and establish the translational potential of S. aromaticum in the prevention and management of toxicant-induced male reproductive dysfunction.

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