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Histopathological and Biochemical Evaluation of β-Sitosterol from Lawsonia Inermis in Aspartame-Induced Testicular Toxicity in Wistar Rats

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Abstract

Introduction: This study aims to evaluate the modulatory impact of β-sitosterol isolated from *lawsonia inermis* on Aspartame mediated testicular toxicity in Wistar rats. ***Settings and Design:*** Forty-five male Wistar rats were used for the study. The rats were divided into nine groups; each containing five rats and were treated daily for 30 days. Group 1 received 5mls of distilled water orally, group 2 received 40mg/kg of aspartame (ASP) for 20 days, while group 3 received 160mg/kg of ASP for 20 days, and group 4 was given 60mg/kg of β-sitosterol (β-sit.) for 20 days, while group 5 received 100mg/kg of β-sit. for 20 days. Group 6 received 40mg/kg of ASP for 15days followed by 60mg/kg β-sit. for the next 15 days, while group 7 had 160mg/kg ASP for 15 days, then 100mg/kg of β-sit for 15 days. Group 8 was given 60mg/kg β-sit. for 15 days, preceding 40mg/kg ASP for 15 days while group 9 received 100mg/kg β-sit. for 15 days, then 160mg/kg ASP for 15 days. ***Results:*** At the end of the experiment it was observed that there was less weight gained in group 2, 3, 6 and 8 while group 5 and 7 gained weight significantly. Histological studies using H&E showed cellular necrosis within the seminiferous tubules (ST) as well as marked edema in the intertubular spaces in groups 2 and 3 while group 4 appeared fairly normal, groups 5 and 6 showed the lumen with very few spermatozoa present. Groups 7, 8, and 9 had normal seminiferous tubules with mild edema levels in the intertubular spaces. These observations were corroborated by PAS staining technique which further revealed extensive erosion of the basement membrane and loss of connective tissue and Leydig cells in groups 2 and 3 including cellular distortion, groups 5, 6, 8, and 9 showed mild to moderate levels of edema in the intertubular spaces. An assay of GPX showed increased levels mostly in group 3 ($p<0.05$). MDA was also significantly higher in groups 2, 3 and 6 ($p<0.05$). Catalase activity decreased significantly in groups 2, 3, and 5 ($p<0.05$) with improvements in groups 6, 7, and 8. ***Conclusion:*** It was observed from the results that aspartame can induce a series of deleterious oxidative changes in the testes resulting in possible impaired testicular function, however, β-sitosterol isolated from *lawsonia inermis* may modulate the effects of aspartame when administered 15 days sequel to an initial 15 days exposure to aspartame.

Keywords: aspartame, β-sitosterol

1. Introduction

Infertility is a significant reproductive health challenge with long-term economic, social, and psychological impacts, particularly in African societies where childbirth rates are high. It is defined as the failure of a couple to achieve pregnancy after one year of regular, unprotected sexual intercourse (Leslie et al., 2020). Globally, an estimated 8–12% of couples of reproductive age experience infertility, with male factors contributing to approximately 40–50% of cases (Agarwal et al., 2021; Pandruvada et al., 2021). Historically, infertility research has focused predominantly on female reproductive health, leading to a relative lack of extensive studies on male

infertility (Petok, 2015). Societal norms and cultural beliefs have also contributed to the underestimation and underdiagnosis of male infertility.

Male infertility is often attributed to defects in spermatogenesis, with oxidative stress playing a crucial role. Approximately 30–80% of infertile men exhibit sperm damage due to reactive oxygen species (ROS) (Bui et al., 2018). Other contributing factors include environmental toxins, radiation exposure, electromagnetic waves, and various chemical substances, many of which are commonly found in processed foods, pharmaceuticals, and daily consumables (WHO, 2013).

One such chemical of concern is aspartame, an artificial sweetener widely used in food and beverages, including diet sodas, chewing gum, dairy products, breakfast cereals, and pharmaceutical formulations (EFSA, 2013). Aspartame is metabolized into phenylalanine, aspartic acid, and methanol, the latter of which is further converted into formaldehyde and subsequently into superoxide anions and hydrogen peroxide—compounds known to induce oxidative stress (EFSA, 2013). Chronic exposure to oxidative stress has been implicated in male infertility through mechanisms such as lipid peroxidation of sperm membranes, DNA fragmentation, and mitochondrial dysfunction (Anbara et al., 2020).

Several studies have linked aspartame consumption to metabolic and reproductive disorders. It has been associated with obesity, insulin resistance, and type II diabetes, conditions that indirectly impact male fertility by altering endocrine homeostasis (Almiron-Roig & Drewnowski, 2003; Sørensen et al., 2005). Furthermore, aspartame has been implicated in preterm deliveries and allergic conditions in neonates (Halldorsson et al., 2010; Maslova et al., 2013). Animal studies suggest that early exposure to aspartame may lead to metabolic dysfunction and impaired glucose tolerance in male offspring (DiPietro & Voegtline, 2017). Additionally, formaldehyde, a metabolic byproduct of aspartame, has been reported to cause DNA damage, potentially leading to genotoxicity and increased susceptibility to neurodegenerative diseases such as Parkinson's and Alzheimer's (Pontel et al., 2015; Villareal et al., 2016).

Given these concerns, there is growing interest in natural bioactive compounds with antioxidant properties that may counteract the detrimental effects of oxidative stress on male reproductive health. One such compound is β-sitosterol, a plant sterol structurally similar to cholesterol, which is abundant in rice bran, wheat germ, peanuts, corn oil, and soybeans (Rakel, 2018). β-sitosterol is known for its anti-inflammatory, anti-cancer, and analgesic properties and has been extensively studied for its role in managing benign prostatic hyperplasia (Lomenic et al., 2015). It is hypothesized that β-sitosterol may also exert protective effects on male fertility by mitigating oxidative stress.

A notable natural source of β-sitosterol is Lawsonia inermis, commonly known as henna. This subtropical and tropical plant has been widely studied for its rich content of bioactive phytochemicals with potential therapeutic applications (Yusuf, 2016). Preliminary evidence suggests that Lawsonia inermis may possess antioxidative properties that could counteract ROS-induced damage in male reproductive tissues. However, further research is necessary to elucidate its specific mechanisms of action and potential clinical applications in male infertility management.

In this study, we evaluated the effect of **β-sitosterol** on aspartame-induced toxicity in male Wistar rats.

2. Materials and Method

2.1 Chemical

Aspartame powder containing 98% aspartame, manufactured by Globexia Limited was procured from World Corsica Science Limited, Benue Crescent, Makurdi.

2.2 Lawsonia Inermis Procurement and Extraction Procedure

Fresh leaves of *Lawsonia inermis* were procured from Wadata market, Makurdi. The plant was authenticated in the Department of Botany, Benue State University by the chief taxonomist. With voucher number: BS1724.

The leaves were thoroughly washed and cut into smaller pieces, then allowed to dry at room temperature of 25^{o}C for Fourteen days. Afterward, it was ground into a fine powdered form.

The extraction of plant material was carried out using the method described by Aziz et al. (2018). Briefly: Extraction of terpene Shade-dried pulverized plant material (100 g) from plant leaves was extracted by Soxhlet apparatus with hexane (700 ml), the extract was filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator. The hexane extract was analyzed for the presence of terpene using thin-layer chromatography (TLC) with spray reagent and confirmed by gas chromatography-mass spectrometry (GC-MS) and liquid chromatography (LC)-MS analysis. Isolation of sitosterol by preparative TLC Preparation of stationary phase Ready-made silica gel GF 254 plates with a layer thickness of 0.25 mm, dimension 20 cm×20 cm. The plates were reactivated by heating in the oven at 100°C for 15 min, left to cool, and used for application after allocation of the baseline and the solvent front. Preparation of solvent system Mobile phase for sterols:

(chloroform: acetone) was mixed in a conical flask and introduced in the jar. The jar was lined with filter paper, closed tightly, and left for saturation.

Application of sample: About 2 g of the sample was dissolved in absolute methanol and applied on the baseline of TLC plates Detection of separated spots. Detection was done by the spraying side of plate with vanillin-sulfuric acid reagent. The purity of each band was checked by analytical TLC until single spot on TLC plate is obtained for identification with a reference standard.

2.3 Experimental Animals

Fifty-four (54) adults male Wistar rats weighing between 150-180g were procured from the animal house of the College of Health Sciences, Benue State University, Makurdi. The animals were kept and maintained in the research laboratory at the Animal House of the College of Health Sciences, Benue State University, for the duration of the experiment. Before the commencement of the experiment, the animals were then housed in polypropylene cages and kept in a well-ventilated room where they were acclimatized over two weeks. During this period the animals were subjected to standard room atmospheric temperature (25±5˚C) and 12/12-hour light-dark cycle. They were also adequately fed with standard rat chow and allowed access to water *ad libitum*.

2.4 Animal Grouping and Treatment

The animals were randomly divided into nine (9) groups: I, II, III, IV, V, VI, VII, VIII, and IX, each consisting of five animals. The treatment for each group was as follows:

Group I received 5 ml/kg of normal saline for 30 days. Group II was administered 40 mg/kg of aspartame for 20 days. Group III was given 160 mg/kg of aspartame for 20 days. Group IV received 60 mg/kg of β-sitosterol for 20 days. Group V was treated with 100 mg/kg of β-sitosterol for 20 days. Group VI was administered 40 mg/kg of aspartame for 15 days, followed by 60 mg/kg of β-sitosterol for the next 15 days. Group VII received 160 mg/kg of aspartame for 15 days, followed by 100 mg/kg of β-sitosterol for the next 15 days. Group VIII was given 60 mg/kg of β-sitosterol for 15 days, followed by 40 mg/kg of aspartame for the next 15 days. Group IX received 100 mg/kg of β-sitosterol for 15 days, followed by 160 mg/kg of aspartame for the next 15 days.

2.5 Animal Sacrifice and Sample Collection

The rats were allowed to fast for 12 hours to: 1) synchronize the metabolic state of the animals, 2) Enhance the sensitivity of the biochemical assays, 3) Reduce food-related stress and 4) Ensure the accuracy of tissue sampling weighed before sacrifice then anesthetized by chloroform inhalation. Blood samples were collected from each rat immediately after sacrifice through cardiac puncture. The abdominal cavity was dissected through a midline incision to expose the reproductive organs. The testicular weights of each animal were evaluated with an electronic analytical precision balance, manufactured by ORMA Limited, Japan. The two tests of each rat were measured and the average value was obtained. One of the tests of each animal was fixed in Bouin's fluid for histological and morphometric analysis. The remaining testes of each animal were stored at – 25ºC for subsequent biochemical assays.

2.6 Histological Study

The preparation of tissue for histological study was carried out at the histopathology unit, at the University of Ibadan according to the method described by Slaoui M., & Fiette L. (2011). Testicular tissue was collected and immediately fixed in Bouin's fluid, these were allowed to fix for 48 hours. The tissues were dehydrated in ascending grades of ethanol for 1 hour each beginning with 70%, followed by 90%, and terminating finally in 2 changes of absolute ethanol each lasting for the same period. Following treatment with ethanol, the tissue was cleared in three changes of xylene lasting 15 minutes each. Impregnation with molten paraffin at 60ºC was done overnight before embedding in paraffin blocks. The blocks were trimmed and Sections (5µm) was taken using a semi-automatic Rotary Microtome manufactured by Leica, Germany, at room temperature. The sections were floated in a warm water bath at 28ºC and then collected on glass slides smeared with albumin and air-dried. The slides were stained with hematoxylin and eosin dye for Light microscopic observation.

2.7 PAS (Periodic Acid-Schiff)

The PAS staining technique was done following the protocols described by Mokobi (2022). Briefly, the tissue was deparaffinized then hydrated in water. 0.5% of periodic acid solution was added for oxidation for 5 minutes then rinsed to distilled water. The stained tissue was dehydrated by placing it in Schiff reagent for 15 minutes, then washed with lukewarm tap water for 5 minutes, the sample was counterstained with Mayer's hematoxylin for 1 minute, then washed in tap water for 5 minutes. The sample was dehydrated and mounted with DPX.

2.8 Assay of Glutathione Peroxidase (GPX) Activity

Glutathione Peroxidase activity was measured by the method described by Saalu et al. (2011). The reaction mixture contained 2.0ml of 0.4M Tris-HCL Buffer, PH 7.0, 0.01ml of 10mM sodium azide, 0.2ml of enzyme,

0.2ml of 10mM glutathione and 0.5ml of 0.2mM H_20_2. The contents were incubated at 37^0c for 10 minutes followed by termination of the reaction by addition of 0.4ml 10% (v/v) TCA, centrifuged at 5000rmp for 5 minutes. The absorbance of the product read at 430nm and expressed as nMol/mg protein.

2.9 Estimation of Lipid Peroxidation (Malondialdehyde)

Lipid peroxidation in the supernatant was estimated colorimetrically by the thiobarbituric acid reactive substances (TBARS) method described by Tsikas (2017). Concentration was calculated using the molar absorptivity of malondialdehyde which is 1.56 x 105 M-1 cm-1 and expressed as nmol/mg protein.

2.10 Assay of Catalase (CAT) Activity

This was measured according to the method of Weydert & Cullen (2009). The rate of nm. The activity of the enzyme was expressed as U/mg protein.

3. Results

3.1 Physical Observation: Body Weight Changes

The body weight changes observed across the experimental groups are shown on Figure 1.

Statistical analysis using one-way ANOVA reveals significant variations in initial and final body weights, as well as in body weight differences, among the groups. The control group showed a mean initial body weight of 124.00 ± 19.02 g and a final body weight of 175.00 ± 17.30 g, resulting in a weight gain of 51.00 ± 2.67 g. This represents the baseline body weight changes and serves as a reference for comparison. All treatment groups demonstrated statistically significant differences in initial body weights compared to the control group ($p < 0.05$). For the final body weights, groups 4 – 8 showed a statistically significant increase in final body weight when compared to the control group. For body weight gain (body weight changes), groups 2, 3, 6, 8, and 9 showed a significantly lower body weight gain compared to the control group ($p < 0.05$).

The result in Figure 1 implies that the treatments in Groups 2–9 variably influenced body weight changes, with some groups showing reduced weight gain compared to the control (e.g., Groups 2, 3, 6, and 8), while others exhibited comparable or enhanced growth trends (e.g., Groups 5 and 7). The variations observed may be attributed to differences in treatment effects, metabolic responses, or physiological adaptations.

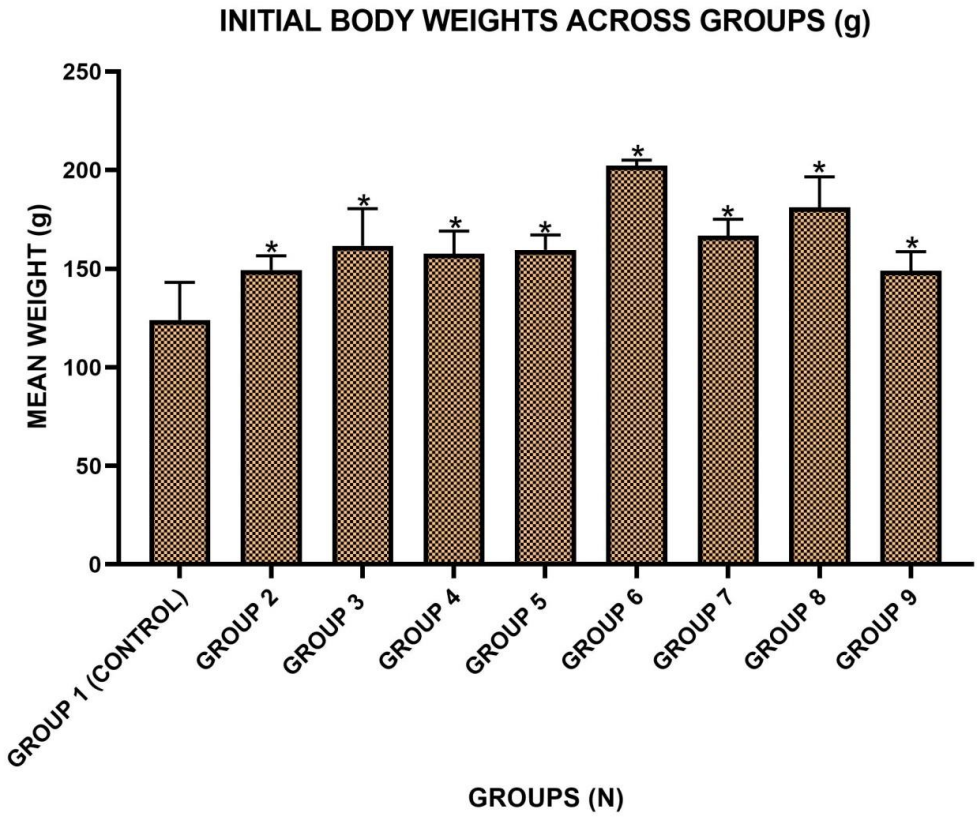


Figure 1.1 Simple Bar Chart Showing the Mean Initial Body Weights across Groups

N = 5, * = statistically significant difference in mean at $p<0.05$ compared to the control group

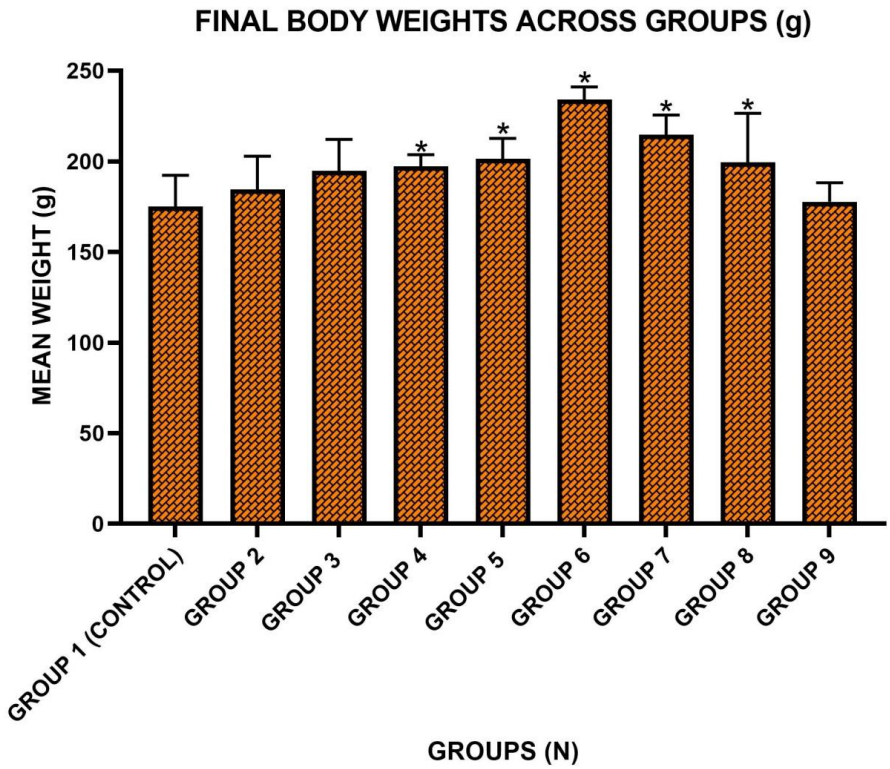


Figure 1.2. Simple Bar Chart Showing the Mean Final Body Weights across Groups

N = 5, * = statistically significant difference in mean at $p<0.05$ compared to the control group

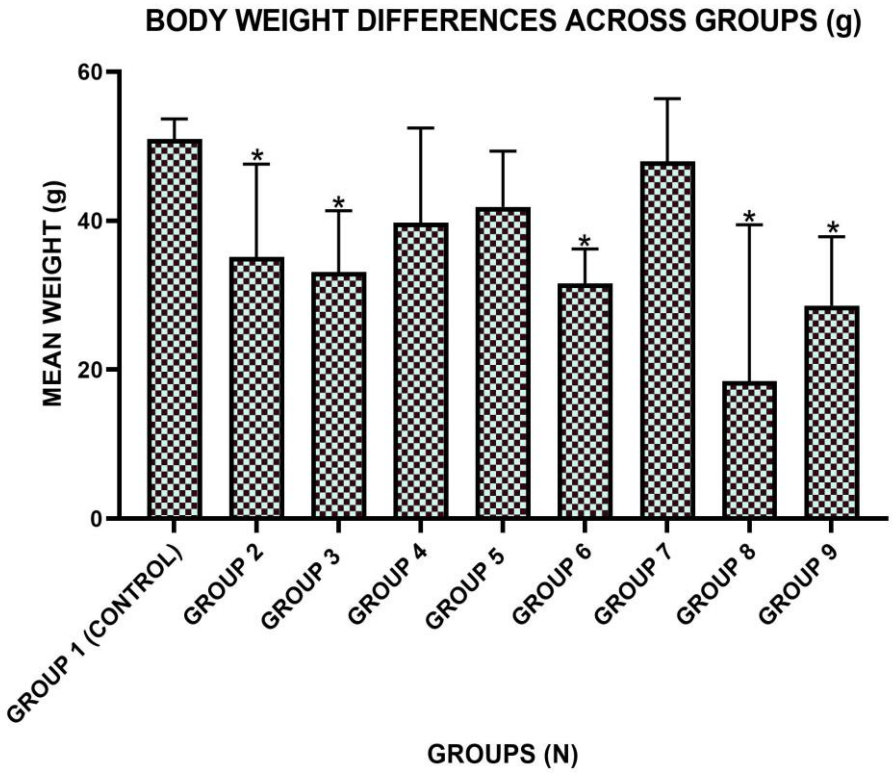


Figure 1.3. Simple Bar Chart Showing the Mean Body Weight Changes across Groups

N = 5, * = statistically significant difference in mean at $p<0.05$ compared to the control group

3.2 Histological Profile

3.2.1 H&E

The photomicrographs of the testis from group 1 (Control group) displayed the normal histological architecture of the testis which showed the seminiferous tubules, lined by spermatogenic cells surrounding a lumen, containing spermatocytes. Inter-tubular spaces containing connective tissues and interstitial cells of Leydig are also present (Plate 1.1). Slides from group 2 showed that there was cellular necrosis in the seminiferous tubules with spermatozoa present within the lumen. A marked level of edema was observed within the inter-tubular spaces and the interstitial connective tissue contained few Leydig cells (Plate 1.2). The slides from group 3 displayed; a high level of cellular necrosis in the seminiferous tubules, the presence of sperm cells within the lumen, there was also edema in the inter-tubular spaces with the interstitial connective tissue containing few Leydig cells (Plate 1.3). Group 4 slides showed normal seminiferous tubules, with a lumen containing spermatocytes and surrounding spermatogenic cells, inter-tubular spaces containing connective tissues, and Leydig cells (Plate 1.4). In groups 5 and 6, the seminiferous tubules were normal with the lumen containing spermatocytes, though there appeared to be a reduction in active spermatogenesis in some tubules. The inter-tubular spaces contained connective tissues and many Leydig cells as well (Plates 1.5 & 6.6). Groups 7, 8, and 9 had normal seminiferous tubules with lumen containing spermatocytes and surrounding spermatogenic cells (Plates 1.7, 1.8 & 1.9). The level of edema in the intertubular spaces was however mild relative to the observations in groups 2, 3, and 6.

3.2.2 P.A.S

Histopathological studies using the PAS staining technique corroborated the H&E results. Group 1 showed normal seminiferous tubules lined by spermatogenic cells enclosing a lumen containing spermatocytes. Inter-tubular spaces containing connective tissues and Leydig cells are also observed (Plate 2.1). In group 2, the seminiferous tubules were normal with spermatogenic cells surrounding the lumen containing spermatocytes; they however appeared shrunken with marked edema, there was extensive erosion of the basement membrane in some tubules with loss of connective tissues and Leydig cells in the inter-tubular spaces (Plate 2.2). The slides from group 3 showed slight tubular distortion, in addition to marked edema, erosion of the basement membrane, and loss of interstitial connective tissues with Leydig cells (Plate 2.3). The results from group 4 were normal while group 5 displayed some level of edema in the inter-tubular spaces with fewer interstitial cells and connective tissue observed (Plates 2.4 & 2.5). Groups 6, 7, 8, and 9 also showed mild to moderate levels of edema in the inter-tubular spaces (Plates 2.6, 2.7, 2.8 & 2.9).

Plate 1.1: A micrograph of the testis from group 1 showing; Normal seminiferous tubules (S) The Lumen (L) containing spermatocytes surrounded by spermatogenic cells (S). Intertubular spaces containing connective tissues and Leydig cells (I). **(H&E) X400**

Plate 1.2: A micrograph of the testis from group 2 showing; cellular necrosis in the Seminiferous Tubules (S), presence of sperm cells within the Lumen (L), Erosion of the basement membrane, Edema in the Intertubular Spaces, interstitial connective tissue contains few Leydig Cells (I). **(H&E)X400**

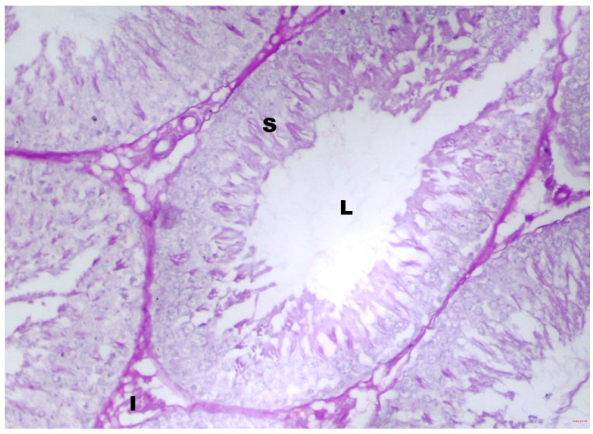
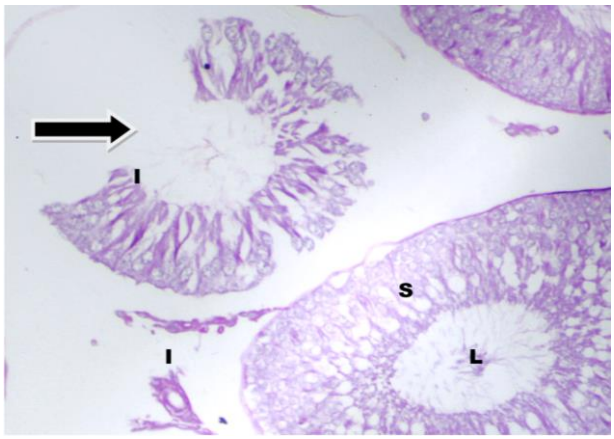


Plate 1.1



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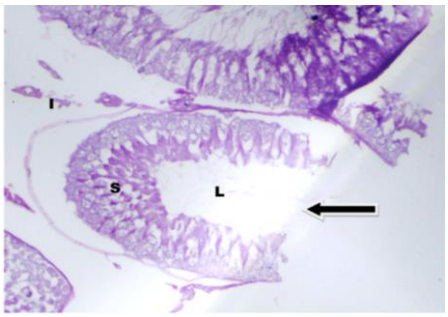

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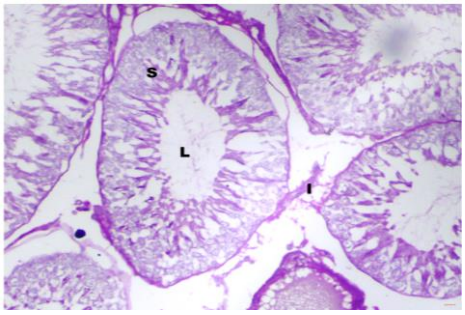

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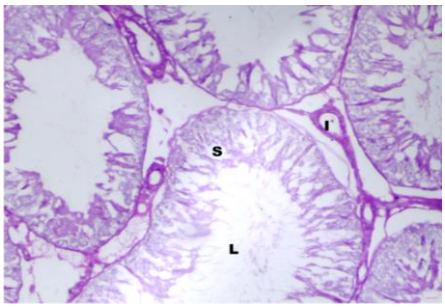

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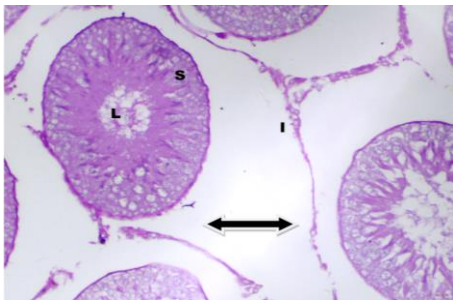

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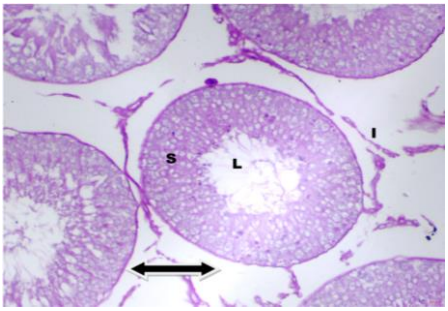

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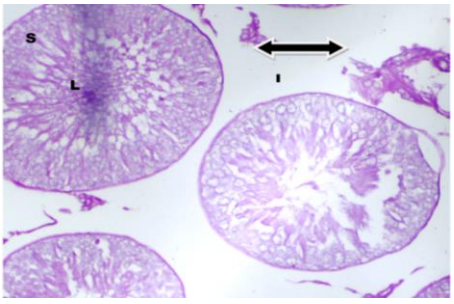

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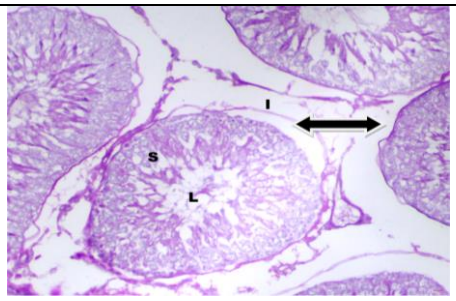

Plate 1.9

The micrographs (Plates 1.1–1.9) show normal seminiferous tubules (S) with spermatocytes and spermatogenic cells in most groups, while groups 2 and 3 exhibit cellular necrosis, basement membrane erosion, intertubular edema, and reduced Leydig cells. Group 5 shows reduced spermatogenesis, and mild to moderate edema is present in groups 6–9. (H&E) X400.

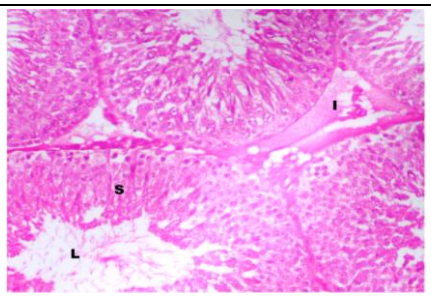

Plate 2.1

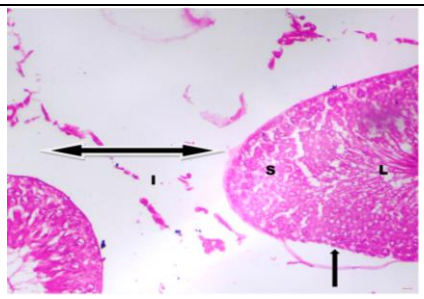

Plate 2.2

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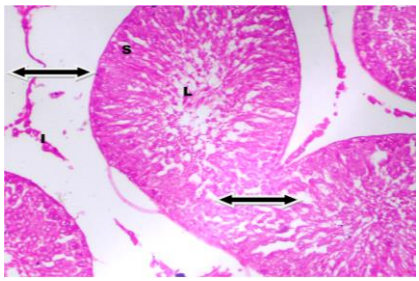
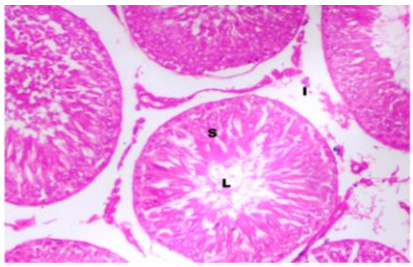
<tr><td> 
Plate 2.3</td><td> 
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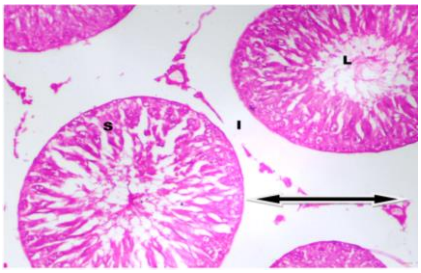
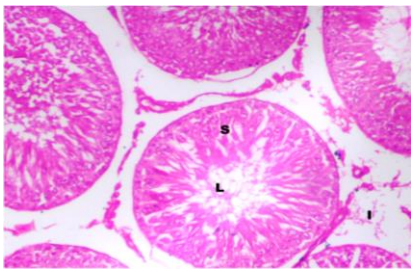
<tr><td> 
Plate 2.5</td><td> 
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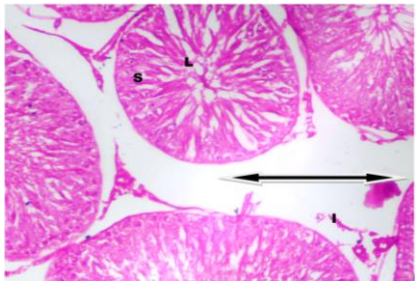
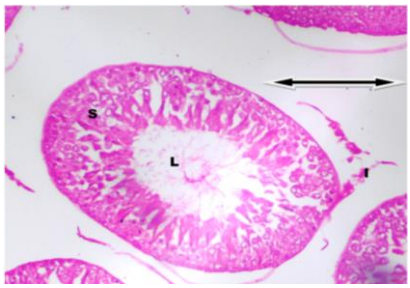
<tr><td> 
Plate 2.7</td><td> 
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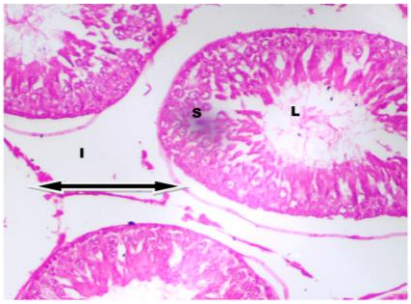
<tr><td> 
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3.3 Results from Oxidative Stress Markers

The oxidative stress markers and antioxidant enzyme activities varied across experimental groups, indicating a complex interplay between oxidative damage and defense mechanisms. Glutathione peroxidase (GPx) activity was slightly elevated in some groups, with Group 3 showing a significant increase (1.90±0.18 U/mg protein, $p<0.05$) compared to the control (1.10±0.24 U/mg protein), suggesting an adaptive antioxidant response. Malondialdehyde (MDA) levels were significantly higher in Groups 2, 3, and 6 (2.05±0.56, 2.15±0.69, and 1.95±0.56 nmol/mg protein, respectively, $p<0.05$), indicating increased lipid peroxidation and oxidative stress, while other groups maintained MDA levels closer to the control.

Catalase (CAT) activity was significantly reduced in Groups 2, 3, and 5 (15.30±1.79, 14.95±1.44, and 16.90±3.12 U/mg protein, respectively, $p<0.05$), reflecting impaired hydrogen peroxide breakdown. However, partial restoration was observed in Groups 6, 7, and 8, with Group 6 showing the most significant improvement (22.10±1.38 U/mg protein, $p<0.05$). Overall, Groups 2 and 3 exhibited pronounced oxidative stress, marked by

elevated MDA and reduced CAT activity, suggesting significant oxidative damage. Conversely, Group 7 appeared to mount a compensatory antioxidant response, with moderate CAT levels potentially mitigating oxidative stress more effectively.

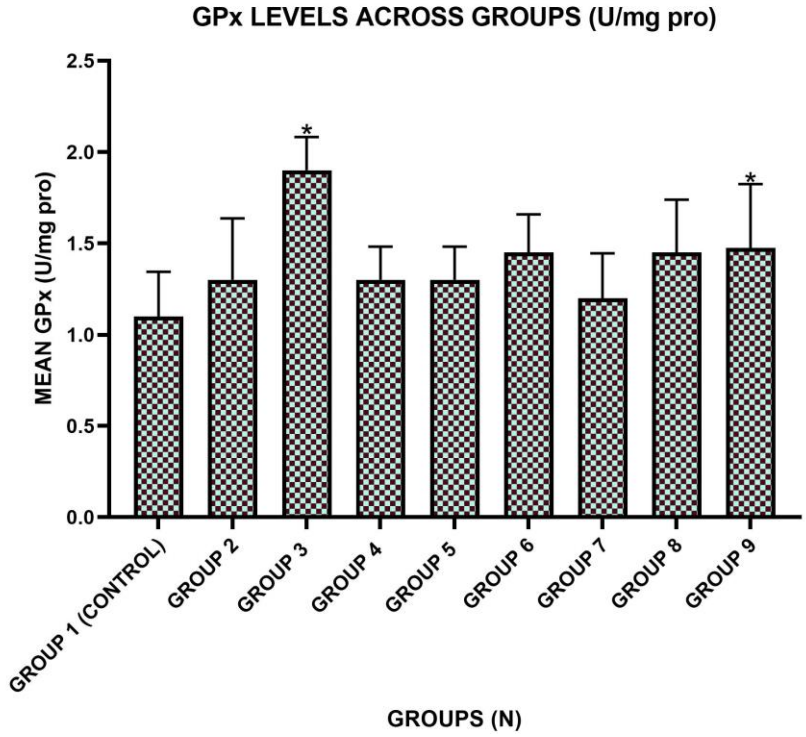

Figure 2.1. Simple Bar Chart Showing the Mean GPx Levels across Groups

N = 5; * = Statistically Significant Difference in Mean at $p<0.05$ Compared to the Control Group

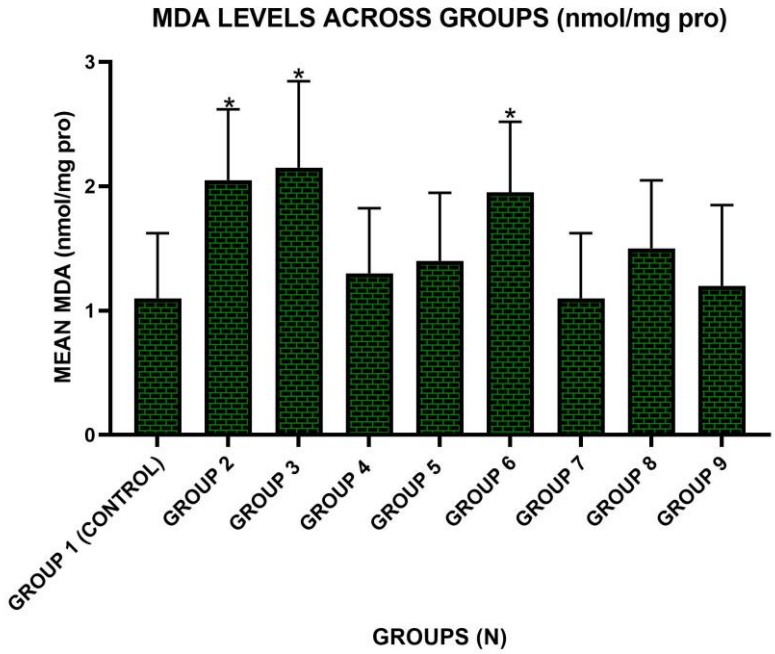

Figure 2.2. Simple Bar Chart Showing the Mean MDA Levels across Groups

N = 5; * = Statistically Significant Difference in Mean at $p<0.05$ Compared to the Control Group

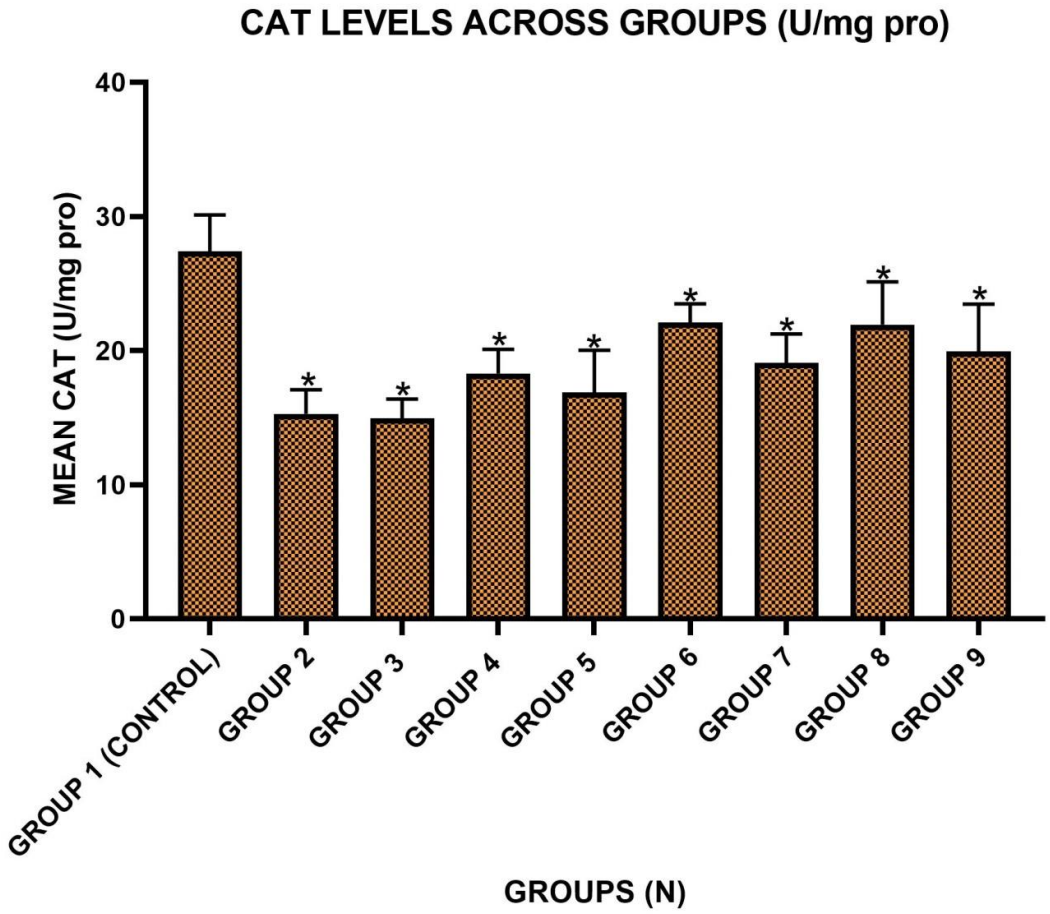

Figure 2.3. Simple Bar Chart Showing the Mean CAT Levels across Groups

N = 5; * = Statistically Significant Difference in Mean at $p<0.05$ Compared to the Control Group

4. Discussion

The results of this study demonstrate significant alterations in body weight, testicular histology, and oxidative stress markers across the experimental groups following aspartame and β-sitosterol treatments. These findings suggest that aspartame consumption may adversely affect reproductive health, while β-sitosterol exhibits potential protective and restorative effects, albeit with some inconsistencies. Body weight analysis revealed significant differences in initial and final body weights across the groups. The control group exhibited expected weight gain, while groups exposed to aspartame (Groups 2 and 3) showed significantly lower weight gain. This observation aligns with previous reports indicating that aspartame consumption can alter metabolic function and food intake regulation, possibly through its effects on hypothalamic pathways and gut microbiota (Fernandes et al., 2020; Choudhary & Lee, 2018). The lower weight gain in these groups may be attributed to metabolic disruptions induced by aspartame metabolism, leading to reduced energy efficiency or altered appetite regulation. Interestingly, groups co-treated with β-sitosterol (Groups 6 and 8) also exhibited lower weight gain compared to the control, suggesting that β-sitosterol might exert additional metabolic effects. While β-sitosterol has been recognized for its cholesterol-lowering properties (Chauhan et al., 2021), its role in weight regulation remains less understood. Notably, groups pre-treated with β-sitosterol before aspartame exposure (Groups 5 and 7) showed comparable or enhanced weight gain, suggesting a protective metabolic effect when administered before exposure to aspartame toxicity.

Histopathological analysis using H&E staining revealed that aspartame exposure (Groups 2 and 3) led to notable testicular damage, characterized by cellular necrosis, edema in inter-tubular spaces, and reduced Leydig cell populations. These findings corroborate previous reports demonstrating aspartame-induced reproductive toxicity via oxidative stress and inflammatory pathways (Ibrahim et al., 2018; Abdallah, 2016). The observed Leydig cell reduction suggests impaired testosterone synthesis, a key factor in spermatogenesis and male fertility. Conversely, groups treated with β-sitosterol (Groups 4, 5, and 7) exhibited relatively normal seminiferous tubules, with mild edema in some cases. The ability of β-sitosterol to mitigate aspartame-induced damage aligns

with its established antioxidative and anti-inflammatory properties (Baskar et al., 2020). Interestingly, groups receiving β-sitosterol post-aspartame exposure (Groups 8 and 9) still showed mild inter-tubular edema, indicating that while β-sitosterol provides some degree of protection, its efficacy is greater as a pre-treatment rather than as a post-treatment intervention.

The PAS staining technique further confirmed aspartame-induced testicular toxicity. In Groups 2 and 3, seminiferous tubules displayed extensive basement membrane erosion, marked edema, and loss of interstitial cells, indicating structural damage. These findings support prior evidence that aspartame metabolites, particularly formaldehyde, can induce oxidative stress, leading to cellular degeneration (Ashok et al., 2017). Groups treated with β-sitosterol exhibited improved basement membrane integrity, with Groups 4 and 5 showing near-normal histology. However, groups that received β-sitosterol post-aspartame exposure (Groups 8 and 9) still displayed mild to moderate edema, suggesting that while β-sitosterol offers protective effects, its ability to reverse aspartame-induced damage is limited.

Oxidative stress markers provided biochemical validation of the histopathological findings. Increased malondialdehyde (MDA) levels in Groups 2, 3, and 6 suggest enhanced lipid peroxidation and cellular damage, consistent with previous studies on aspartame-induced oxidative stress (Ibrahim et al., 2018). The concomitant reduction in catalase (CAT) activity in these groups further supports the notion that aspartame exposure impairs the testicular antioxidant defense system. Notably, glutathione peroxidase (GPx) activity was significantly increased in Group 3, suggesting a compensatory antioxidant response to oxidative stress. However, the increase in GPx was not sufficient to counteract the damaging effects of aspartame, as evident from the histological results. Groups treated with β-sitosterol (Groups 5 and 7) showed partial restoration of CAT activity, indicating its role in enhancing antioxidant defenses. This aligns with studies suggesting that β-sitosterol can upregulate antioxidant enzymes and reduce lipid peroxidation (Baskar et al., 2020).

The findings suggest that aspartame exposure induces testicular toxicity primarily through oxidative stress mechanisms, leading to histological and biochemical alterations. The protective effects of β-sitosterol may be attributed to its antioxidant, anti-inflammatory, and membrane-stabilizing properties (Chauhan et al., 2021). However, the observation that β-sitosterol was more effective as a pre-treatment than as a post-treatment suggests that its primary mode of action may be preventive rather than curative. Aspartame exposure resulted in significant testicular histopathological changes, oxidative stress, and metabolic disruptions. The administration of β-sitosterol exhibited a dose-dependent protective effect, with greater efficacy when administered before aspartame exposure. However, its ability to reverse established damage remains limited. These findings underscore the potential reproductive risks associated with prolonged aspartame consumption and highlight the need for further research into natural compounds such as β-sitosterol as potential therapeutic agents.

Future studies should extend the duration of treatment to cover a full spermatogenic cycle (approximately 62 days in rodents) to determine whether observed effects persist over longer periods. Additionally, investigations in higher animal models (e.g., primates) and clinical studies in humans are necessary to validate these findings and assess their translational relevance.

Conflict of Interest

We hereby declare that there was no conflict of interest in the course of this research.

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