HISTOLOGICAL EFFECTS ON REPRODUCTIVE ORGANS AND CAUDA EPIDIDYMAL SPERM COUNT AND MOTILITY: EFFECT OF SOLANUM MELONGENA (GARDEN EGG) AQUEOUS LEAF EXTRACT [SMALE] IN ADULT ALBINO RATS

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Abstract

This study investigates the histological effects of Solanum melongena (garden egg) aqueous leaf extract (SMALE) on the reproductive organs, specifically focusing on sperm motility, sperm count, and histological changes in the testes and epididymis of adult male albino rats. The rats were administered varying doses of SMALE (400 mg/kg and 800 mg/kg body weight) over a period of time. Sperm motility and count were assessed, with significant improvements observed in the 400 mg/kg dose group, indicating a dose-dependent enhancement in sperm function. Histological examination revealed normal testicular architecture in the control group, with intact spermatocytes and spermatids. The 400 mg/kg SMALE treatment showed increased proliferation and maturation of spermatocytes and Leydig cells, while the 800 mg/kg dose led to signs of testicular degeneration, characterized by atrophied Leydig cells and pycrosis. These findings suggest that SMALE at moderate doses may enhance reproductive health, while higher doses could induce toxicity and impair sperm maturation. The study highlights the potential of Solanum melongena as a natural supplement for male fertility, with important implications for reproductive health and safety.

Keywords: *Solanum melongena*, Fertility, Phytochemicals, Antioxidants.

Introduction

Globally, medicinal plants have long been utilized to treat various diseases; however, some plant compounds may have toxic effects on the body. Certain plants are known to possess teratogenic and abortifacient properties, while others are thought to negatively impact the male reproductive system (Mengue et al., 2001; Dos Santos et al., 2016). The male reproductive system can be adversely affected by a range of factors, including interference with sexual maturation, sperm development and transport, sexual behavior, and overall reproductive function (Kimmel et al., 1999). These effects can result from substances that either disrupt or promote the production of testosterone, a key hormone in male reproductive health (D'Cruz et al., 2010). Some studies have shown that toxic agents can impair the function of the epididymis, affecting sperm maturation and accessory sex glands (Ahmed et al., 2011; Zenick et al., 1994). In developing countries, approximately 80% of the population uses medicinal plants and plant-based products due to their affordability, availability, and accessibility for treating common ailments (Telefo et al., 2002; Cherdshewasart et al., 2007).

Solanum melongena (commonly known as garden egg or eggplant) is a widely cultivated plant in tropical and subtropical regions, including India, Nigeria, and various parts of Europe (Veeraragavathatham et al., 2006; Westerfield, 2008). It is a popular vegetable, cultivated on over 1.7 million hectares globally, with China, India, Bangladesh, Nepal, and Sri Lanka contributing to 75% of the world's production (Jami et al., 2015). Solanum melongena has been historically used as a prokinetic agent for weight loss, asthma control, and for its potential fertility-regulating effects (Jamil et al., 2015). Despite its extensive use, research on its medicinal properties, particularly in relation to male reproductive health, remains limited. The plant contains various bioactive compounds such as alkaloids, saponins, tannins, flavonoids, proteins, and carbohydrates, which are believed to

contribute to its medicinal and nutritional value (Noda et al., 2000; Hanson et al., 2006).

Spermatogenesis, the process by which sperm cells mature, can be influenced by various factors, including hormonal regulation, environmental agents, and toxins (Griswold, 2016; Azenabor et al., 2015). Disruptions in spermatogenesis can lead to decreased sperm count and motility, ultimately affecting male fertility. Certain environmental toxins, including some pharmaceuticals and industrial chemicals, are known to disrupt spermatogenesis by interfering with Leydig cells, which are responsible for testosterone production (Zirkin and Papadopoulos, 2018). The effects of these toxic agents on the epididymis can impair sperm maturation and reduce reproductive potential (Zenick et al., 1994). Given the widespread use of medicinal plants in developing countries for fertility-related issues, there is an urgent need to evaluate their potential benefits and risks.

Solanum melongena, being a widely used plant in many regions, has been suggested to possess fertility-regulating properties. However, empirical studies on its impact on male fertility are scarce. The primary aim of this study was to investigate the histological effects of Solanum melongena aqueous leaf extract (SMALE) on the reproductive organs of adult male albino rats, with a focus on the cauda epididymal sperm count and motility. Additionally, the study determined the oral median lethal dose (LD50) of SMALE to assess its toxicity and safety for use.

This research provides valuable insights into the potential fertility-enhancing properties of SMALE and offers a scientific basis for its traditional use. The findings from this study will contribute to the growing body of literature on the health benefits of indigenous plants, particularly in the context of reproductive health. As herbal remedies continue to gain popularity, it is crucial to substantiate their claims with empirical evidence. By evaluating SMALE's effects on male reproductive health, this study aimed to validate or otherwise its use as a natural supplement for improving fertility.

Moreover, this study emphasizes the importance of understanding the toxicological profile of herbal extracts. Despite the widespread use of medicinal plants, many traditional remedies lack

comprehensive safety data, which may result in unintended consequences when used improperly. Determining the LD50 of SMALE and evaluating its effects on health parameters, such as sperm motility, will help inform safer usage practices for herbal products. The results of this study have significant implications for public health policies, particularly in regions where Solanum melongena is a common dietary component. Understanding the potential health benefits and risks associated with this plant will help guide its use in promoting reproductive health.

Furthermore, this research lays the groundwork for future studies investigating the pharmacological properties of Solanum melongena. By identifying its active phytochemicals and understanding their biological effects, further research could explore the therapeutic potential of the plant in modern medicine. While Solanum melongena is widely recognized for its medicinal uses, there is a paucity of scientific studies evaluating its safety and efficacy. This research fills a critical gap by conducting a comprehensive assessment of SMALE's effects on reproductive health, providing important insights into its potential as a natural remedy.

Materials and Methods

Experimental animals

This research used a total of thirty-six (36) healthy Albino rats with a weight range of between 54 and 100 grammes. The animals were obtained from the Animal House of the Department of Physiology, University of Nigeria, Enugu Campus, and housed in the Animal House of the College of Medicine, University of Nigeria Teaching Hospital. The rats were acclimatized for two weeks and provided with ad libitum access to commercial feed and water during this period.

Ethics consideration

This study received ethical approval from the Ethics Committee of the University of Nigeria Teaching Hospital.

Plant material collection

Solanum melongena leaves were collected and authenticated from the Abor District, Udi Local Government Zone, Enugu County, by the Department of Botany, University of Nigeria, Nsukka.

Plant aqueous extraction

Solanum melongena leaves were dried at room temperature for 10 days. Using a clean, small plastic pestle and motar, the leaves were manually crushed after drying to obtain 300 g of fine powdered Solanum melongena leaves, which were dissolved in 1000 ml of steamed water, then sewn with filter paper and stored in the refrigerator until required.

Phytochemical screening

Phytochemical elements that are comprised of *S. Melongena* leaf was calculated using the method established by the Department of Pharmacognosy, Faculty of Pharmacology, University of Nigeria, Nsukka (Trease & Evans, 1989).

Proximate analysis

In order to calculate the proportion of crude protein, crude fibre, fat, carbohydrate and ash content in *S. Melongena* leaves, the method described by Pearson (1976) was used. In order to determine the mineral content of the plant material, the AOAC (1990) technique was used.

Tentative immediate toxicity studies

Using the Lorke (1983) variant technique, immediate toxicity monitoring [LD50] was accomplished with minimal adaptation. Doses of 10, 100, 1000, 2000, 5000 mg/kg body weight (b.wt) were issued to groups A, B, C, D and E of 4 rats each. Each species safely acquired a single oral dose of SMALE in its respective groups after 24 hours. In order to assess mortality or clinical exposure controls, organisational monitoring was conducted on an hourly basis for 24 hours after the medication was administered. Acute toxicity monitoring [LD50] was performed using the Lorke (1983) variation technique with limited change. Rated doses of 10, 100, 1000, 2000 mg/kg body weight (b.wt) were issued to 4 rats each in groups A, B, C, D and E. Each animal obtained a single oral dose of SMALE in the appropriate groups after 24 hours. Surgery was performed on an hourly basis for 24 hours following the administration of the drug in order to monitor death or clinical indicators of overdose.

Experimental design

A total of thirty-six (36) adult male Albino Wistar rats, each weighing between 150-200 g and aged 3-4 months, were used in this study. The rats were divided into three groups (I, II, and III), with 12 rats in each group. Each group was further subdivided into three batches (A, B, and C), with 4 rats per batch. The study was conducted over 4, 6, and 8-week periods.

The experimental protocol was as follows:

- Group I received a daily oral gavage of 400 mg/kg body weight (b.wt.).
- Group II was administered 400 mg/kg b.wt. of Solanum melongena (Garden Egg) aqueous leave extract (SMALE) treatment.
- Group III was given 800 mg/kg b.wt. of the same treatment.

For each batch (A, B, and C) within the three groups, the treatments were delivered daily by oral gavage using an oral cannula for 4, 6, and 8 weeks. At the end of each treatment period, the rats were euthanized under chloroform anesthesia. The testes were then excised, preserved in 10% formal saline, and prepared for further histological examination.

Epididymal sperm count and motility

The cauda epididymis was carefully excised and subjected to multiple incisions (approximately 1 mm in length) before being suspended in 1 ml of Ham F-10 solution (Sigma Aldrich Chemical Co., USA). Sperm concentration and motility were assessed after a 10-minute incubation at 37°C using a hemocytometer, following the guidelines outlined by the World Health Organization (WHO, 1999).

Histological processing

Tissue samples were cut into smaller sections (approximately 3 mm thick) and processed using an Automatic Tissue Processor. The sections were then embedded and sliced to a thickness of 5 μm using a Rotary Microtome. Staining was performed using the Hematoxylin and Eosin technique as described by Baker et al. (1985).

Microscopy and Photomicrography

The stained tissue sections were examined under an Olympus binocular microscope with an integrated illumination system. Photographs were

taken using a Hewlett-Packard® (HP) optical microscope camera attached to the eyepiece of the Olympus microscope.

Statistical analysis

Data were analyzed using SPSS software. Results are expressed as the mean ± standard error of the mean (SEM). The significance of differences between means was determined using the Student's t-test and one-way analysis of variance (ANOVA). A p-value of less than 0.05 ($p<0.05$) was considered statistically significant.

Result

Cauda epididymal sperm count and motility

The results from the sperm motility and sperm count assessments revealed a significant reduction in both motility and sperm count in groups B and C compared to the control group. Specifically, the sperm motility and count were notably lower in the higher dose (800 mg/kg) group (group C), with statistical significance observed in group B (400 mg/kg). These results suggest a dose-dependent effect on sperm motility and count following administration of *Solanum melongena* aqueous leaf extract (SMALE).

Histological finding

Histological analysis of the testes revealed distinct changes across the treatment groups. In group II (400 mg/kg body weight of SMALE), the testicular tissue displayed intact spermatocytes and spermatids with mild infiltration, indicating moderate effects on the testicular structure. In contrast, group III (800 mg/kg) exhibited significant testicular degeneration, including atrophied and pycrotic Leydig cells, and disrupted tubular architecture, suggesting that higher doses of SMALE may cause testicular damage and impair spermatogenesis. Furthermore, in group III, mild lymphocytic infiltration was observed, indicative of potential inflammation or immune response.

The histological findings suggest that while moderate doses of SMALE (400 mg/kg) may promote spermatocyte maturation, higher doses may lead to detrimental effects, including testicular degeneration.

Table 1: Cauda epididymal sperm count and motility

Group	Dose of SMALE (mg/kg body weight)	Motility (%)	Sperm count (x10^6/ml)
I (Control)	0	43.25 ± 2.32	38.25 ± 6.26
II (400 mg/kg)	400	58.75 ± 2.95*	32.20 ± 10.00*
III (800 mg/kg)	800	49.50 ± 3.30	37.00 ± 8.51

*Note: $p < 0.05$ indicates statistical significance compared to the control group.

Discussion

This study investigated the reproductive effects of Solanum melongena aqueous leaf extract (SMALE) in adult male albino rats, focusing on sperm motility, sperm count, and histological changes in the testes and epididymis. The preliminary acute toxicity test indicated that the oral median lethal dose (LD50) of SMALE exceeded 5000 mg/kg body weight, suggesting that the extract is relatively safe for consumption at low to moderate doses, according to the OECD guidelines (OECD, 1981).

Phytochemical analysis of SMALE revealed the presence of moderate amounts of tannins and proteins, and trace amounts of flavonoids, alkaloids, and saponins. These bioactive compounds are likely responsible for the observed effects on reproductive health. Specifically, flavonoids and alkaloids have been shown to possess antioxidant properties that can influence testosterone production and spermatogenesis (Saalu et al., 2007).

While no significant changes in body weight or epididymal weight were observed in the animals treated with SMALE, a slight dose-dependent decrease in epididymal weight was noted. This may suggest mild effects on reproductive organ function, but no direct correlation with impaired fertility was observed in this study.

The observed increase in sperm motility and count in group II (400 mg/kg) suggests that SMALE may have a positive effect on sperm function. This is consistent with previous studies showing that antioxidant-rich compounds, such as those found in Solanum melongena, can improve spermatogenesis by scavenging free radicals and enhancing testosterone production by Leydig cells (Tiwari et al., 2009). However, the 800 mg/kg dose resulted in a reduction in sperm motility and count, likely due to the toxic effects on testicular tissue, including Leydig cell degeneration and testicular atrophy.

The histological findings provide further evidence of the dose-dependent nature of SMALE's effects. At a moderate dose of 400 mg/kg, SMALE appears to promote spermatogenesis, promote spermatocyte maturation, and maintain the integrity of the testicular structure /improve testicular architecture. These effects are likely due to the antioxidant activity of compounds such as flavonoids and alkaloids, which have been shown to support Leydig cell function and testosterone production, both essential for healthy sperm production (Saalu et al., 2007a; b).

However, at higher doses (800 mg/kg), SMALE induces noticeable testicular degeneration (which may hinder fertility), including atrophy and pycrosis of Leydig cells, which are indicative of testicular dysfunction. These findings suggest that while SMALE may have beneficial effects on male fertility at lower doses, excessive intake could lead to reproductive toxicity, impairing spermatogenesis and overall fertility.

The mild lymphocytic infiltration observed at the higher dose suggests a subtle inflammatory response, which may further compromise testicular health if exposure to the high dose is prolonged. These histological changes are consistent with findings from other studies, which indicate that high doses of certain medicinal plants can have toxic effects on the male reproductive system (Vidal & Whitney, 2014). In addition, previous research by Vidal & Whitney (2014) suggesting that while moderate doses of Solanum melongena may enhance reproductive health, higher doses may result in adverse effects. The dose-dependent effects observed in this study highlight the

importance of dosage when using medicinal plants for reproductive health.

Conclusion

The administration of *Solanum melongena* aqueous leaf extract (SMALE) in adult male albino rats demonstrates both potential pro-fertility benefits at moderate doses (400 mg/kg) and harmful effects at higher doses (800 mg/kg). While moderate doses improved sperm motility and facilitated spermatocyte maturation, higher doses resulted in testicular degeneration and decreased sperm motility and count. These findings suggest that SMALE may have therapeutic potential for treating male fertility issues, but caution is necessary when determining the appropriate dosage.

Recommendations

1. **Moderation in use**: Based on the findings, it is recommended that *Solanum melongena* aqueous leaf extract be used in moderation, particularly in individuals with fertility issues. The positive effects observed at 400 mg/kg suggest potential benefits for male fertility, but higher doses may cause harm to reproductive organs.
2. **moderate dosage for Fertility Benefits:** The results of this study suggest that moderate doses of SMALE (400 mg/kg) may be beneficial for male fertility, promoting spermatogenesis and enhancing sperm quality. However, higher doses should be avoided to prevent potential reproductive toxicity.
3. **Further research**: Additional studies are required to identify the specific bioactive compounds responsible for the observed reproductive effects of SMALE. Further research should also explore the long-term effects of SMALE on male reproductive health and its potential for use in clinical settings.
4. **Safety and dosage**: Future research should establish optimal dosages of SMALE to maximize its beneficial effects while minimizing potential toxicity. Clinical studies evaluating the

safety and efficacy of SMALE in humans are needed before it can be recommended as a fertility-enhancing supplement.

5. **Public health implications**: Given the widespread use of medicinal plants in traditional medicine, especially in developing countries, this study emphasizes the importance of understanding the safety profile of such plants. Public health initiatives should promote informed use of herbal remedies, providing guidance on safe dosages to maximize benefits and minimize risks.

Detailed histological analysis (Plates 1–5)

- Plate 2 (Group B – 400 mg/kg): The light photomicrograph revealed intact spermatocytes (S1) and spermatids (S3) within the seminiferous tubules, with mild lymphocytic infiltration (MI) noted in the interstitial spaces. This suggests that SMALE at this dose promotes spermatogenesis and does not cause significant damage to the testicular architecture.
- Plate 3 (Group C – 800 mg/kg): The photomicrograph of the testis from this group displayed signs of degeneration, with atrophic Leydig cells (At) and pycrotic cells (P) in the interstitial tissue. These changes indicate that a higher dose of SMALE may disrupt testicular function, leading to potential long-term fertility issues.
- Plate 4 (Group B – 400 mg/kg): The seminiferous tubules displayed increased proliferation and maturation of spermatocytes, along with well-formed Leydig cells (Lc) and well-organized tubular arrangements (Ta). This observation suggests that SMALE at this dose may have a stimulatory effect on spermatogenesis.
- Plate 5 (Group C – 800 mg/kg): At this higher dose, mild proliferation of Leydig cells (PLc) was noted, along with very mild lymphocytic infiltration (Min), which may signal the initiation of a minor inflammatory response. While not severe, these changes indicate that high doses of SMALE can lead to subtle inflammatory and degenerative changes in the testes.

- Plate 1: Light photomicrograph of the control group (A) showing the normal histoarchitecture of the testis, including type A spermatogonia (SA), type B spermatogonia (SB), primary spermatozoa (S1), secondary spermatozoa (S3), and mature spermatozoa (S4) (H&E: Magnification x100).

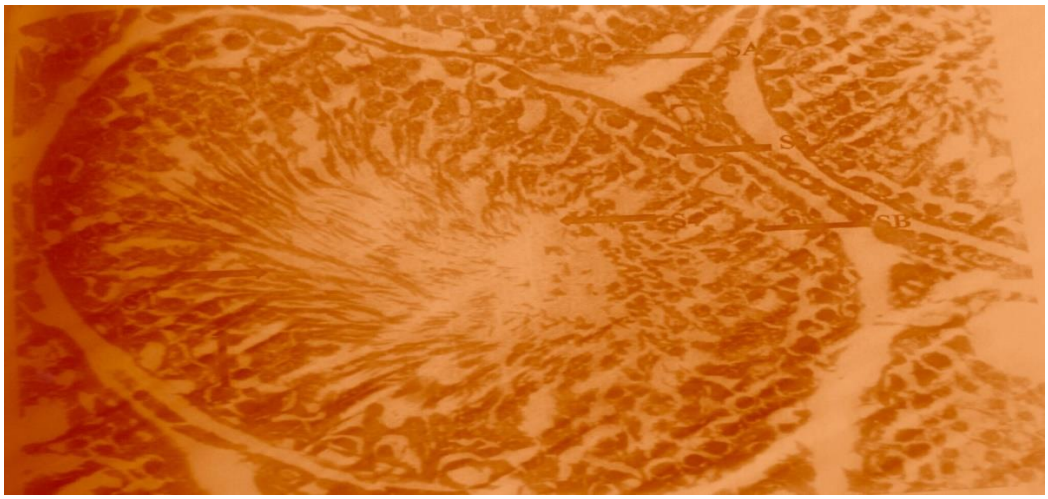

Plate 1.

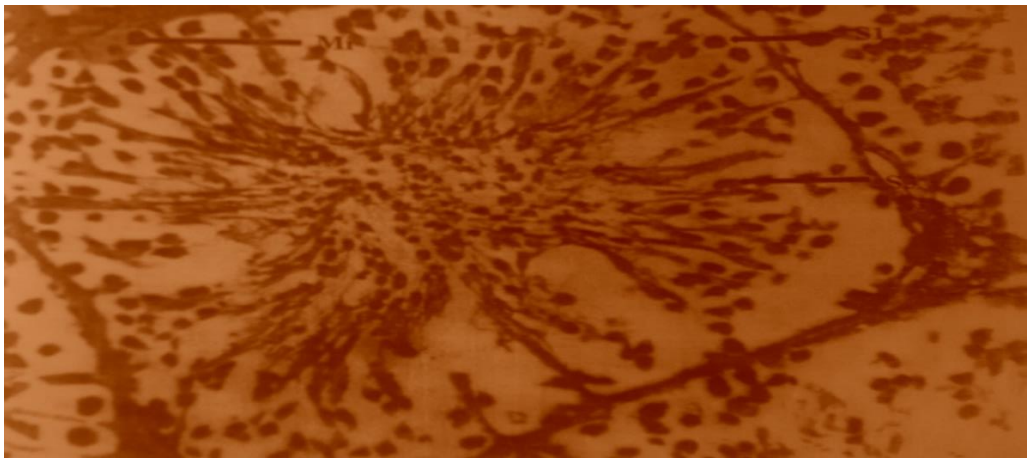

Plate 2.

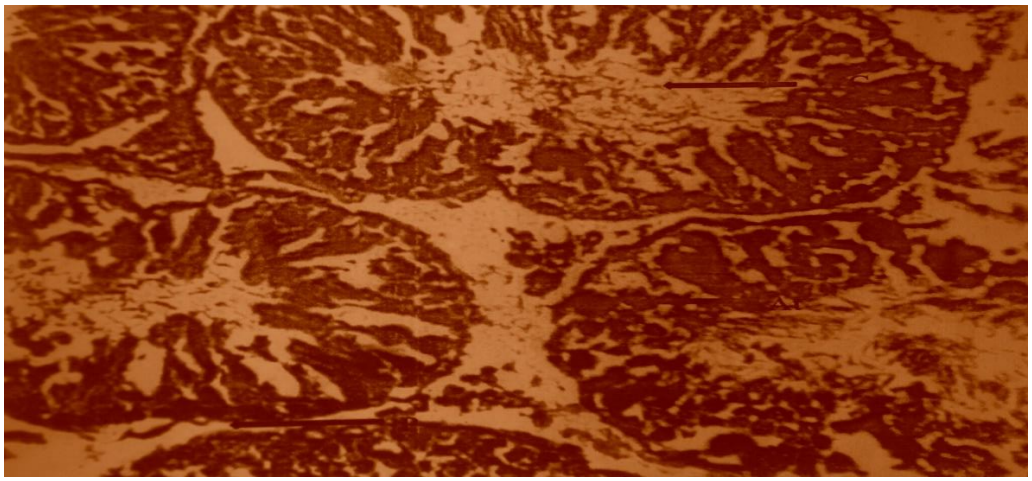

Plate 3.

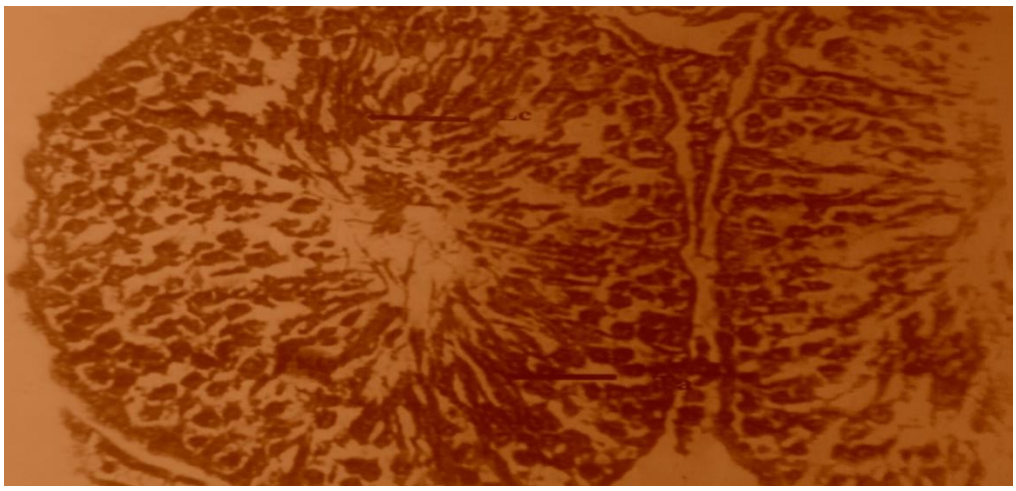

Plate 4.

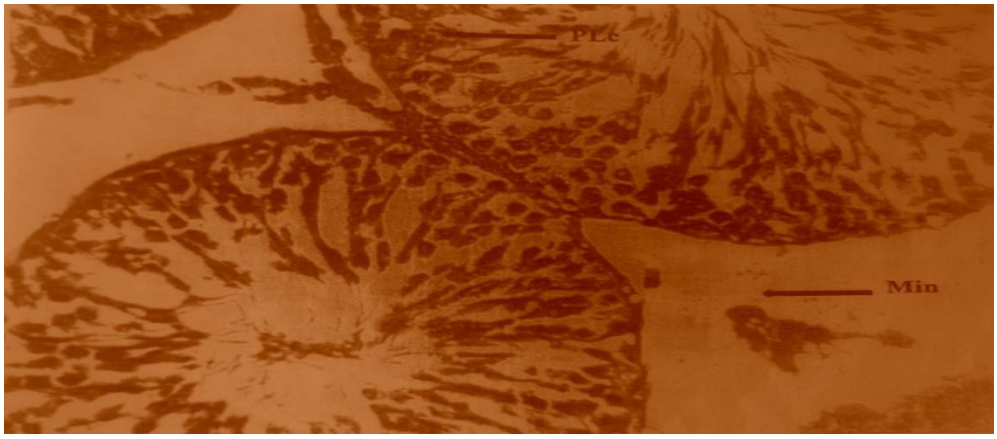

Plate 5.

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