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Efficacy of Ascorbic Acid on Anti-Infertility Effects of Alcoholic Extract of *Nicotiana Tabacum* Leaf in Male Wistar Rat

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Abstract:-

➢ ***Background:***

Tobacco intake has led to negative impacts on the neurological and endocrine systems, possibly due to the compromised immune system that is linked to tobacco use. Despite the long history of using the tobacco plant (Nicotiana tabacum L.), cigarettes dominate the global market, making up 96% of all sales of manufactured tobacco products. Despite being taken in smokeless form, reports indicate that the plant is toxic and has negatively affected several bodily cellular activities. This study looked at how an alcoholic extract made from Nicotiana tabacum leaf affected the Wistar male rats' reproductive parameters. According to estimates from the World Health Organization, tobacco usage resulted in 100 million deaths worldwide throughout the 20th century, and 5.4 million fatalities in 2004.

Except for humans, non-human primates, and guinea pigs, the livers of most mammalian species synthesis vitamin C, also known as ascorbic acid, a six-carbon lactone molecule. Scurvy, also known as scorbutus, is a life-threatening disorder caused by a shortage of vitamin C. It can only be cured via proper treatment. Therefore, humans must consume vitamin C to ensure their survival.

The testis is the primary male reproductive organ. The structure is analogous to the ovaries in females. The testes are the main endocrine and reproductive organs in the male body. They generate sex steroid hormones and mature haploid sperm. The testes are located in two pairs within the scrotum, with the epididymis situated at the back edge.

➢ ***Material and Method:***

The extract was obtained from the plant's leaf using the process of alcoholic extraction. The experiment utilized a sample of 30 male Wistar rats, which were assigned randomly to five groups: A, B, C, D, and E. Each group consisted of six rats with an average weight of 115g.

➢ ***Result:***

Changes in body weight, testis weight, semen quality, and morphology were all studied in this investigation. Furthermore, testicular histology evaluations were carried out. Overall, the alcoholic extract derived from the leaf of Nicotiana tabacum had a significant negative impact on various male reproductive indices. This study demonstrates that ascorbic acid possesses prospective and restorative effects against testicular damage caused by the alcoholic extract of Nicotiana tabacum leaf. However, it should be noted that these effects were not fully observed at the current dosage. Further investigation is necessary to fully understand the long-lasting effects on reproductive health.

Keywords:- *Testis, Semen Analysis, Inflammation.*

I. INTRODUCTION

With the potential to treat a variety of ailments such abdominal pain, constipation, urinary tract obstruction, tooth pain, gastrointestinal disorders, and dermatitis,[1] tobacco is widely studied and acknowledged as a major commercial crop.[2] The leaves of plants in the genus *Nicotiana* are used to make tobacco, an agricultural product.[3] Numerous biochemical pathways, including those involved in antibacterial, antifungal, antimicrobial, anthelmintic,[4] and anti-Alzheimer's effects, have been shown to be stimulated by *Nicotiana tabacum*.[5]

In recent times, there has been a significant rise in antibiotic-resistant strains of medically significant pathogens,[6] leading to the introduction of new bacterial strains that are resistant to multiple antibiotics.[7] Therefore, there is a requirement to search for substances from alternative sources that have confirmed antimicrobial activity.[8]

Researchers have identified tobacco smoke exposure as a significant factor in causing death.[9] Nicotine is just one of the many chemical components in tobacco smoke that can disrupt bodily systems.[10] It produces almost 4,000 chemicals.[11] The kind of tobacco used, how densely it is packed, how long the tobacco column is, the quality of the

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filter and paper, and the temperature at which the tobacco burns all affect the amount of smoke that the smoker gets.[12]

Previous research has documented a decrease in body weight and its negative impact on the formation of blood clots in the coronary arteries.[13] Research indicates that the intake of tobacco is linked to a decrease in life expectancy and has contributed to the rise in death rates.[14] An individual's blood pressure, attentiveness, and alertness are all negatively impacted by tobacco use.[15] Moreover, it raises free fatty acid levels, which may interfere with insulin-mediated glucose absorption.[16] *Nicotiana sylvestris*, *Nicotiana tomentosiformis*, and maybe *Nicotiana otophora* are thought to be its progenitor among wild *Nicotiana* species; further research is needed to confirm this.[17]

II. MATERIALS AND METHOD

A. Collection and Extraction of Plant Material

The leaf of tobacco (*Nicotiana tabacum*)[18] plant was harvested at Alaropo, beside Esinele village, Ogbomoso, Oyo State, on July 26, 2023. The plant was authenticated at the LAUTECH Herbarium and was assigned a voucher number of LHO 741.[19]

B. Preparation of Alcoholic Extract

We dried the *Nicotiana tabacum* leaves at room temperature of about 26–30°C for about two months.[20] The resulting dried leaves were then ground to powder using a blending machine,[21] which was then further weighed to give a net weight of 481.8 g, and then soaked in 3.46 liters of absolute alcohol for about 4 days.[22] We then filtered the mixture using a filter cloth and took the filtrate to the Chemistry Department for evaporation.[23] The filtrate was heated at a temperature of 40 °C until a paste-like extract was obtained.[24] We then let the remaining ethanol evaporate for a week.[25] The dried extract weighed about 400 g, and it was then mixed with an equal volume of distilled water to get the stock solution.[26]

C. Animals

Thirty (30) male Wistar rats weighing between 100 and 130g were obtained from the Animal House,[27] Department of Anatomy, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomosho, Oyo State, and were all acclimatized for a period of two weeks.[28] The rats were weighed weekly at each stage of acclimatization throughout the whole experimental process.[29] Throughout the four-week experimental period, we kept them at a room temperature and fed them standard rat feed and water ad libitum.[30] The Canadian Council of Animal Care (CCAC, 2015) has established international, national, and institutional guidelines for the use of laboratory animals in biomedical research and for the care of such animals. These guidelines were followed during the experimental procedures involving the animals and their care.[31]

D. Experimental Design

The experiment was carried out using 30 male Wistar rats which were randomly distributed into five groups: A, B, C, D, and E and each group had six rats with an average weight of 115g. All the rats were allowed to feed freely.

- GROUP A (Control A) – were fed with feed and water ad libitum for two weeks just like every other experimental group.
- GROUP B – were administered 500mg/kg per body weight of *Nicotiana tabaccum* leaf extract orally for 2 weeks.
- GROUP C – were administered 1000mg/kg per body weight of *Nicotiana tabaccum* leaf extract orally for 2 weeks.
- GROUP D – were administered with 500mg/kg per body weight of *Nicotiana tabaccum* leaf extract and 200mg/kg of vitamin C concurrently for 2 weeks.
- GROUP E – were administered 1000mg/kg per body weight of *Nicotiana tabaccum* leaf extract and 200mg/kg of vitamin C concurrently for 2 weeks.

The rats were weighted twice weekly throughout the experimental period. Also, at the last day of the experiment the body weight of each animal was recorded.[32]

E. Animal Sacrifice and Collection of Organs

Every experimental animal was fully dissected by opening it up from the abdominal cavity to the thoracic cavity, and all of the animals were killed by cervical dislocation.[33] The top of the heart was used to take blood samples.[34] Testes were located and collected. The bouin's fluid was combined with the testes.[35] Quick action was taken to avoid autolysis throughout this process.[36] To create a histology slide, tissue was prepped. For semen analysis, the epididymis head was sent straight to the laboratory.[37]

F. Collection and Analysis of Sperm Samples

Each rat was put to sleep by having its cervical disc detached, and its epididymis was meticulously removed before the sperm samples were collected and examined. To make evaluation easier, sperm samples were taken from the caudal region of the epididymis reserve and smeared onto heated glass slides.[38]

➢ *Sperm Motility*

To measure sperm motility, El-Sherbiny's et al., (2022) methodology was used. Sperm samples from each treatment group that were collected by epididymal washings were analyzed as soon as possible to determine whether or not they included increasingly motile sperm cells. A drop of the sperm sample was placed on a glass slide that had been heated beforehand. The sample was then subjectively assessed as a percentage using light microscopy at magnifications of X10 and X40. Sperm cells that moved straight ahead were the only ones included in the motility count; those that moved in circles, in reverse, or in a pendular fashion were not. Sperm motility was categorized on an individual basis using the following scale: (I) Progressive Motility (PM), (II) Non-Progressive Motility (NPM), and (III) Immotile (IM).[39]

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➢ *Sperm Viability (Live Proportion)*

Using Eosin-Nigrosin stain on a droplet of the extracted sperm sample allowed for the identification of live sperm cells. The stained slide was subjected to 30 seconds of drying, alcohol treatment to fix it, then oil immersion light microscopy examination at X100 magnification. Using a manual counter and a portable stopwatch, the percentage of viable sperm cells was manually counted. The count consisted of 300 sperm cells in total. El-Sherbiny et al., (2022) claimed that non-viable sperm cells absorbed the stain rather than living sperm cells.[40]

➢ *Abnormal Sperm Proportion*

The El-Sherbiny (2024) approach was used to calculate the percentage of aberrant sperm cells. Eosin-Nigrosin was used to stain a drop of the sperm sample, which was then applied to a glass slide. The slide was examined at a lesser magnification (X40) in order to identify any aberrant primary and secondary sperm cells. El-Sherbiny et al. (2024) quantified the percentages of several anomalies, such as head, tail, and mid-piece abnormalities.[41]

G. Histological Examination

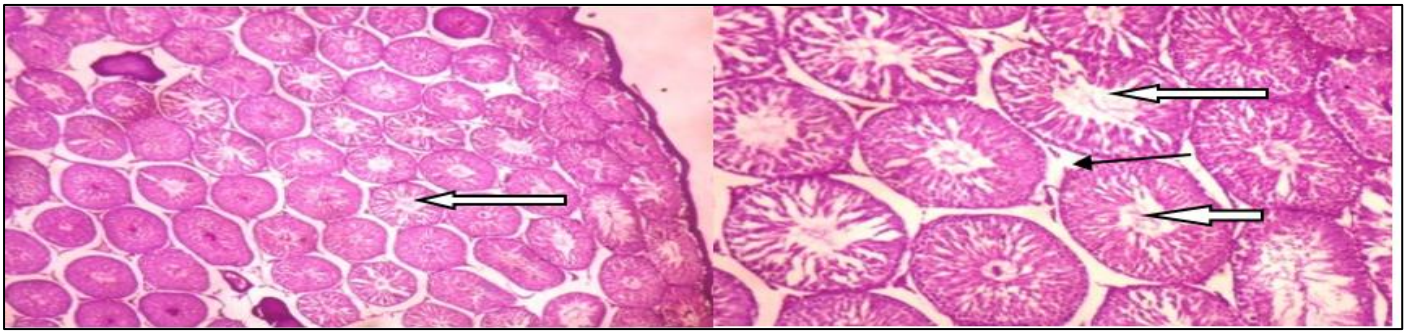
Histological examinations were performed using the methodology outlined by Mustafa and Elhanbaly (2021). Testicular tissues that were stored in a solution of 10% formalin were prepared into slides for histological investigation. Following the removal of water using a process of gradually increasing ethanol concentration, the tissue samples were placed in paraffin wax and cut into sections that were 5 µm thick using a Shandon Finesse Manual Rotary Microtome (model 325, Thermo-scientific). The sections were desiccated onto cryogenic microscope slides (Fisher Scientific, Pittsburgh, PA, USA). The slides were dewaxed and dehydrated before undergoing Hematoxylin and eosin (H&E) staining, allowing for observation under light microscopy. Microscopic images were acquired by the connection of a microscope to a computer.[42]

H. Statistical Analysis

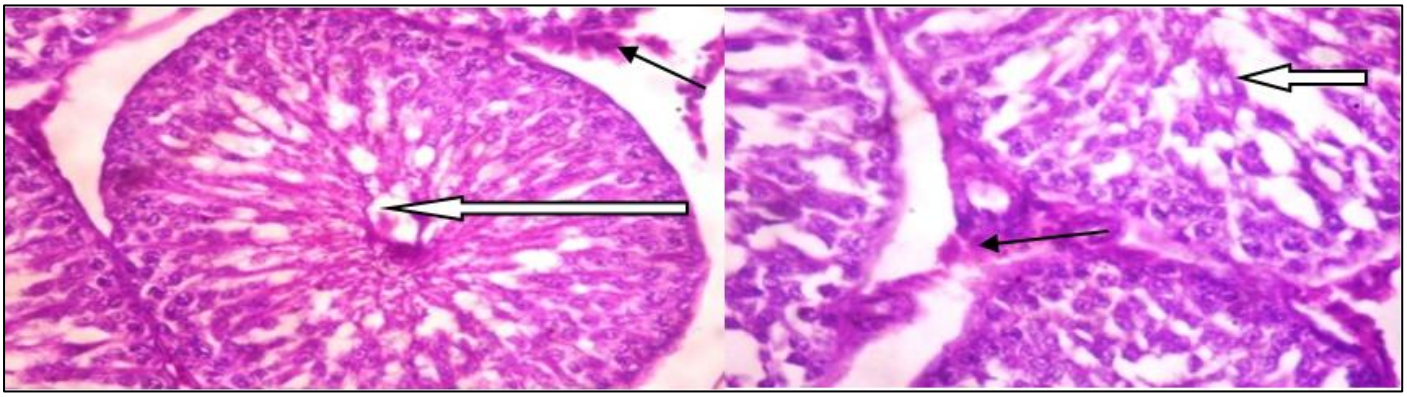
All quantitative data were analyzed using GraphPad Prism® software (version 6). One-way Analysis of Variance (ANOVA) with Significance level set at ($P<0.05$) (95% Confidence Interval) was used for the comparison of relative expression levels for different groups followed by Turkey Post Hoc test. The outcomes were represented in bar charts with error bars to show the mean and standard error of mean (M ± SEM) respectively.

III. RESULT

➢ *Histological Findings*



X40 X100



X400

Fig 1: Photomicrograph of the Normal Testicular Section Showing Normal Seminiferous Tubules Showing Lumen Containing Spermatozoa (White Arrow), Normal and Completely Developed Germinal Cells. The Interstitial Spaces Show Leydig Cells (Slender Arrow)

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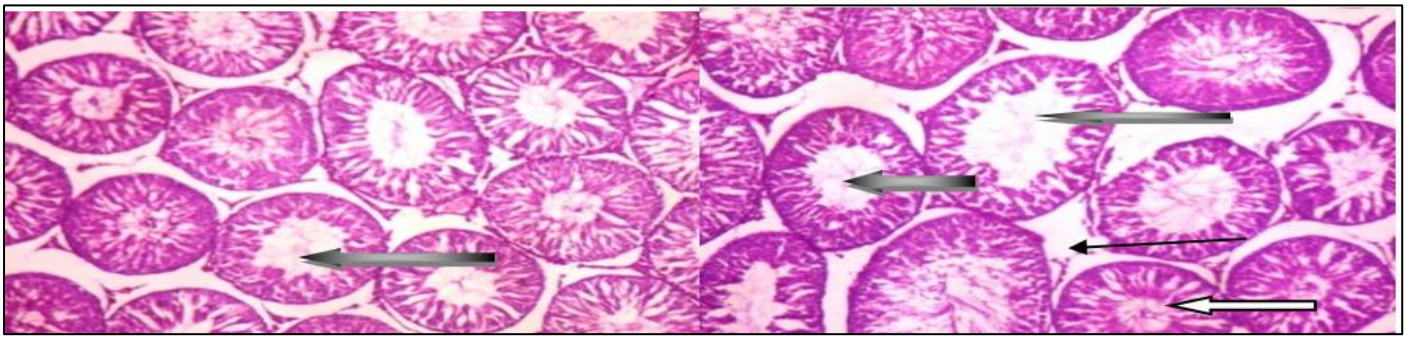

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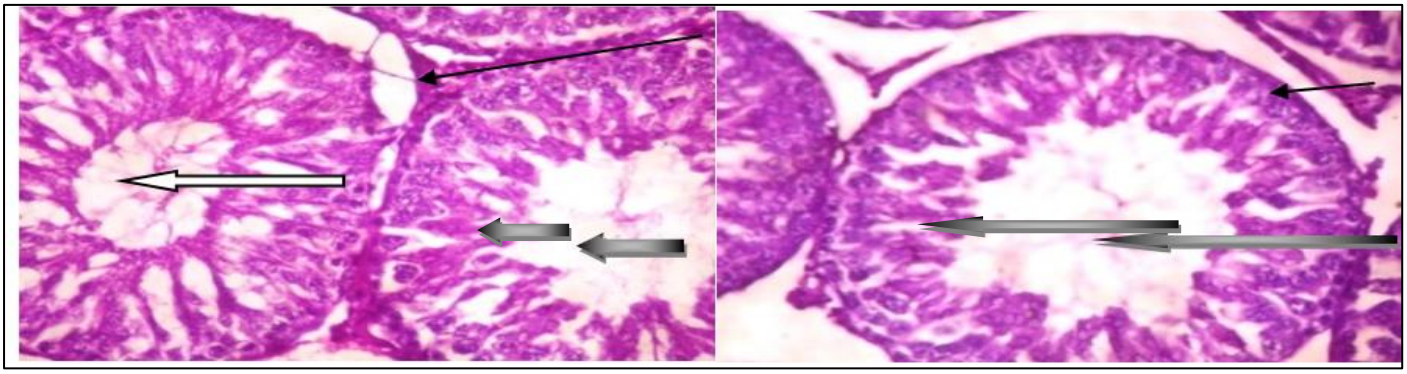

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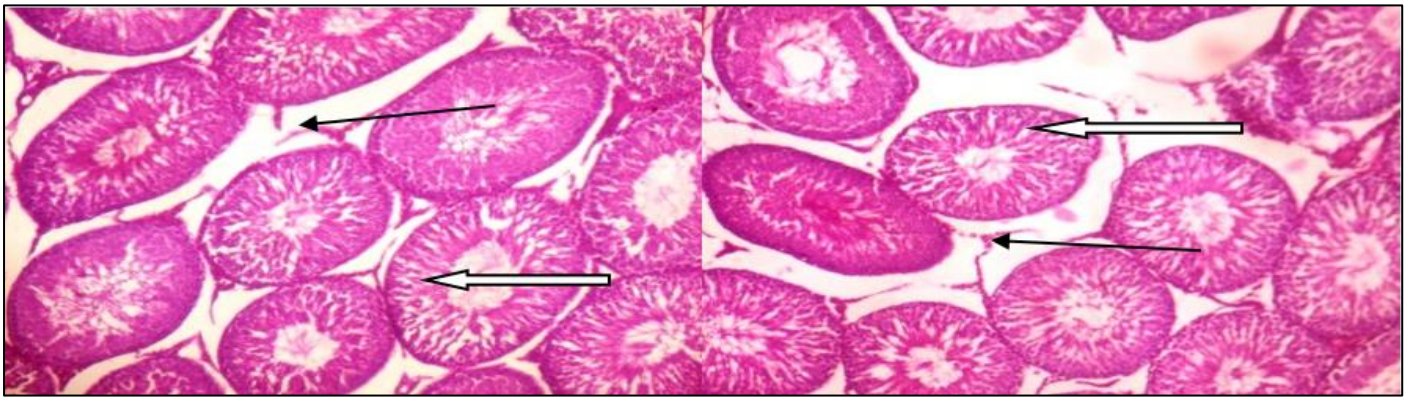
Fig 2: Photomicrograph of the Normal Testicular Section Showing Normal Seminiferous Tubules Showing Lumen Containing Spermatozoa (White Arrow), Normal and completely Developed Germinal Cells. Some Seminiferous Tubule Shows Maturation Arrest (Black Arrow). The Interstitial Spaces Show Leydig Cells (Slender Arrow)



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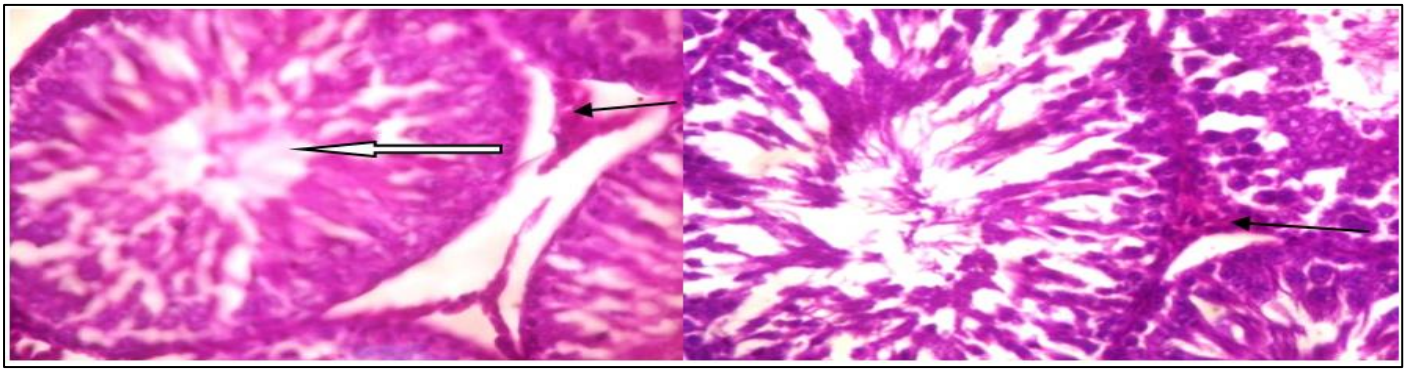

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Fig 3: Photomicrograph of the Normal Testicular Section Showing Normal Seminiferous Tubules Showing Lumen Containing Spermatozoa (White Arrow), Normal and Completely Developed Germinal Cells. The Interstitial Spaces Show Leydig Cells (Slender Arrow)

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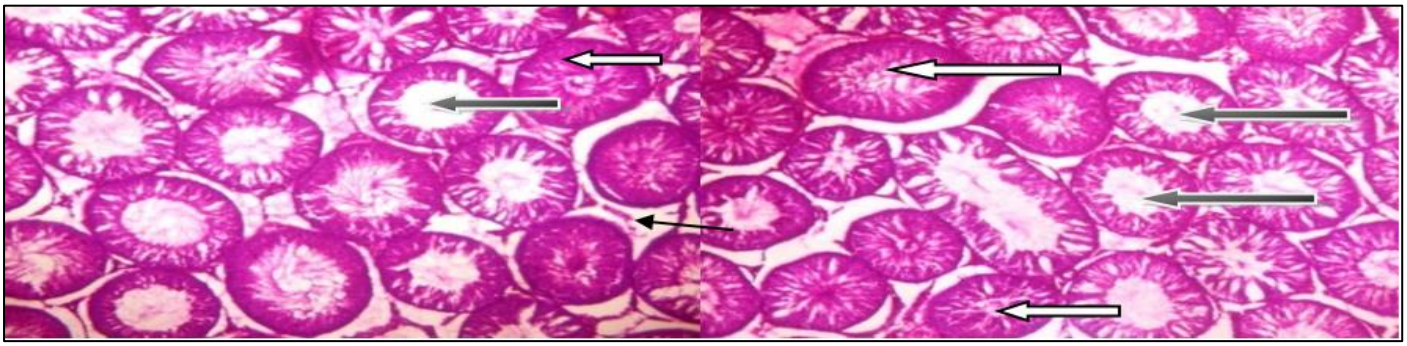

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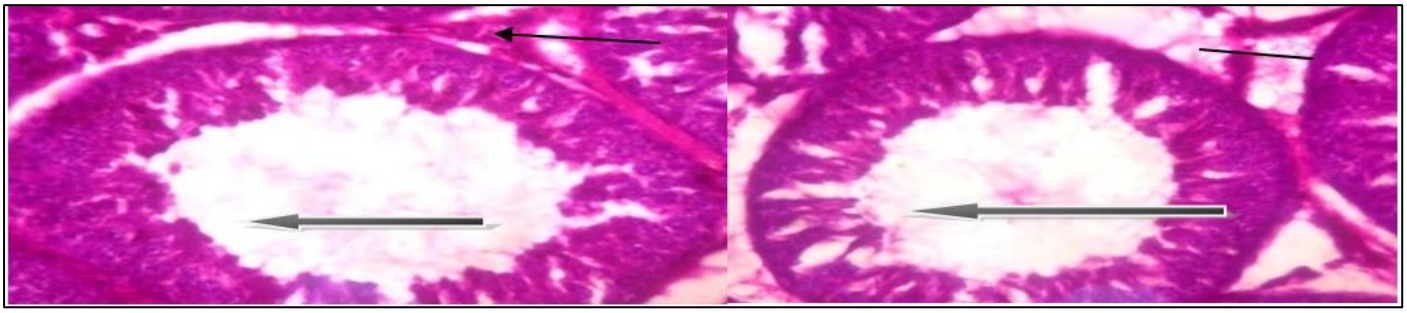

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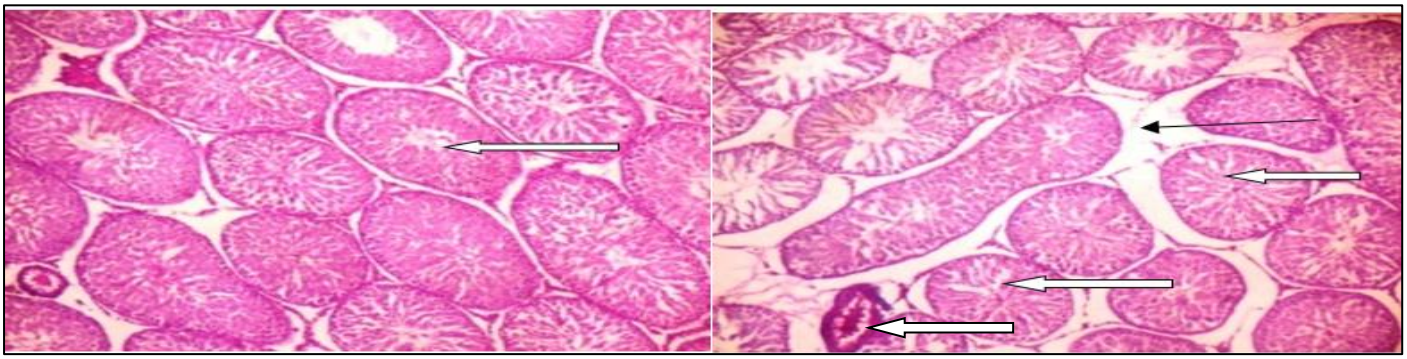
Fig 4: Photomicrograph of the Normal Testicular Section Showing Few Normal Seminiferous Tubules Showing Lumen Containing Spermatozoa (White Arrow), there are Several Seminiferous Tubules Showing Maturation Arrest, there is Incomplete Development of Germinal Cells Which Stopped at Secondary Spermatocytes Leaving a Clear Wide Lumen (Black Arrow). The Interstitial Spaces Show Leydig Cells (Slender Arrow)



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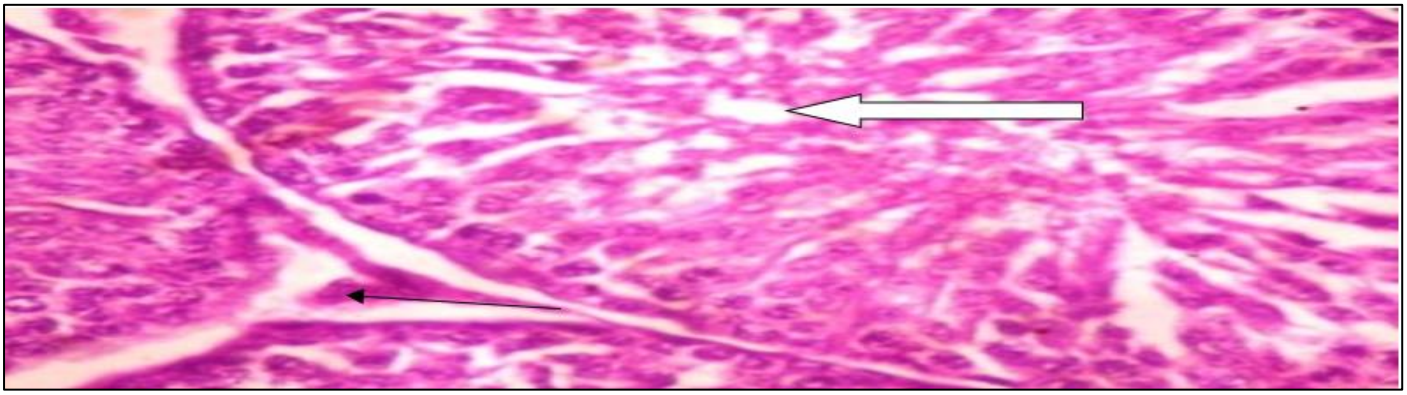

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Fig 5: Photomicrograph of the Normal Testicular Section Showing Normal Seminiferous Tubules Showing Lumen Containing Spermatozoa (White Arrow), Normal and Completely Developed Germinal Cells. The interstitial Spaces Show Leydig Cells (Slender Arrow).

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IV. DISCUSSION

The male reproductive system plays a pivotal role in the perpetuation of species and the transmission of genetic information. Its proper functioning is essential for human and animal populations' reproductive success and overall health (Gurung *et al.,* 2023). Over the years, there has been growing concern about the potential adverse effects of environmental factors, including exposure to various plant-derived substances, on male reproductive parameters. One such plant, *Nicotiana tabacum*, commonly known for its toxic properties (Soni *et al.,* 2012). It has been traditionally used for medicinal and psychoactive purposes in different cultures. However, its potential impact on male reproductive health has raised significant scientific interest and concern. Investigation from this study on the effects of *Nicotiana tabacum* leaf extract and ascorbic acid (as antioxidant) on male reproductive parameters would provide valuable insights to the effect of *Nicotiana tabacum* leave extract on the male reproductive system.

This study employed statistical analysis to examine the body weights, mean weights, and relative mean weights of the testis in male Wistar rats. Tobacco leaf includes nicotine, a potent and intricate substance that interacts with excitable cells in several regions of the body, including the testis (Rao *et al.,* 2024 and Sharma *et al.,* 2023).

Vitamin C has demonstrated strong antioxidant properties. It can have a direct impact by interacting with peroxyl radicals in water, as well as an indirect impact by restoring the antioxidant properties of vitamin E, which is soluble in fat. These activities' antioxidant properties aid in regulating lipid oxidation in cellular membranes, particularly those that enclose intracellular organelles. Vitamin C can potentially decrease the impact of intracellular free radicals on non-lipid nuclear components (Bendich *et al.,* 1986).

The experimental group, Group A, has exhibited a significant increase in body weight compared to their starting body weight. Group B, which received a dosage of 500 mg/kg of the alcoholic extract of *Nicotiana tabacum* leaf alone, exhibited a noticeable rise in body weight in comparison to their initial weight. Group C, which received a dosage of 1000 mg/kg of the alcoholic extract of *Nicotiana tabacum* leaf only, exhibited a noticeable rise in body weight from the beginning to the end of the study. Group D, which received a dosage of 500 mg/kg of the alcoholic extract of *Nicotiana tabacum* leaf and 200 mg/kg of ascorbic acid, exhibited a significant rise in body weight from the beginning to the end of the study. Group E, which received a dosage of 1000 mg/kg of the alcoholic extract of *Nicotiana tabacum* leaf and 200 mg/kg of ascorbic acid, had a significant rise in body weight from the beginning to the end of the study. Hence, the consumption of an alcoholic extract derived from *Nicotiana tabacum* leaves affects the weight of animals due to the presence of protein, fat, and other necessary vital components, as indicated by the earlier research conducted by Iranloye and Bolarinwa in 2009.

The sperm analysis revealed that Groups B and C, which were administered 500 mg/kg and 1000 mg/kg per body weight of an alcoholic extract of *Nicotiana tabacum* leaf, respectively, had a reduced sperm count compared to the control group. Group D, which was administered a dosage of 500 mg/kg of alcoholic extract of *Nicotiana tabacum* leaf and 200 mg/kg of vitamin C, and Group E, which was administered a dosage of 1000 mg/kg of alcoholic extract of *Nicotiana tabacum* leaf and 200 mg/kg of vitamin C, exhibited a higher sperm count compared to Groups B and C. However, their sperm count was still lower than that of the control group. The histological analysis revealed that group A, which served as the control, exhibited a typical testicular structure characterized by intact seminiferous tubules, a lumen holding spermatozoa, and fully developed germinal cells. Additionally, it indicates the presence of Leydig cells within the interstitial gaps. Group B, which received a dosage of 500 mg/kg of the alcoholic extract of *Nicotiana tabacum* leaf, exhibited a normal testicular section with intact seminiferous tubules, a lumen containing spermatozoa, and fully developed germinal cells. Additionally, it displays the current Leydig cells in the interstitial areas. Nevertheless, certain seminiferous tubules exhibited maturation arrest, providing additional evidence that aligns with the prior findings of Iranloye and Bolarinwa in 2009. Their research concluded that the alcoholic extract of *Nicotiana tabacum* has a negligible impact on certain testis organelles. Group C, which received a dosage of 1000 mg/kg of the alcoholic extract of *Nicotiana tabacum* leaf, exhibited a normal testicular section with intact seminiferous tubules, a lumen containing spermatozoa, and normal, fully developed germinal cells. Additionally, it demonstrates the current presence of Leydig cells in the interstitial gaps. This is identical to the control. This suggests that at this particular dosage, the observed impact does not have enough statistical evidence to be considered significant. Group D, which received a dosage of 500 mg/kg of the alcoholic extract of *Nicotiana tabacum* leaf and 200 mg/kg of Vitamin C, exhibited a normal testicular section with a few normal seminiferous tubules. The tubules contained spermatozoa, but a few tubules showed maturation arrest. Additionally, there was incomplete development of germinal cells, which halted at the secondary spermatocyte stage, resulting in a wide lumen that was clear. The interstitial gaps display Leydig cells. Ascorbic acid suggests the potential to undo the damage. Group E, which received a dosage of 1000 mg/kg of the alcoholic extract of *Nicotiana tabacum* leaf and 200 mg/kg of Vitamin C, exhibited normal testicular sections with intact seminiferous tubules, a lumen containing spermatozoa, and normally developed germinal cells. The interstitial gaps display Leydig cells. It suggests that ascorbic acid has the ability to improve or alleviate the harm.

V. CONCLUSION

This study demonstrates the potential therapeutic role of ascorbic acid in mitigating against testicular damage in adult male Wistar rats induced by alcoholic extract of *Nicotiana tabacum* leaf. Therefore, ascorbic acid is recommended as an effective antioxidant for testicular damage induced by the alcoholic extract of *Nicotiana tabacum* leaf.

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- **Ethics Approval:** The Animal Usage Committee of the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomosho, Oyo State, approved the use of all the animals.
- **Availability of Information and Resources:** Upon request, the corresponding author can supply the datasets used in and/or analysed during the current study.
- **Competing Interests:** The authors declare that none of their known financial conflicts of interest or intimate relationships could have influenced the research described in this study.
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- **Authors' Contributions:** The present study's conceptualization, design, implementation, interpretation of research findings, and article writing were all equally contributed to by the authors of this paper.

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