A COMPARATIVE ASSESSMENT OF TESTIS HISTOLOGY OF YOUNG AND AGED RATS TO THE EFFECT OF ATRAZINE AND CARRAGEENAN

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GOU/U22/BCH/316

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CHEMICAL SCIENCE DEPARTMENT
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A PROJECT RESEARCH SUBMITTED TO THE DEPARTMENT OF CHEMICAL SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE IN BIOCHEMISTRY

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APPROVAL PAGE

This research titled "Comparative Effects of Atrazine and Carrageenan in Young and Aged Rats: Serum Testosterone Analysis and Histological Features of the Testes" has been evaluated and authorized by Godfrey Okoye University's Department of Chemical Sciences and Faculty of Natural Sciences and Environmental Studies in Enugu, Nigeria.

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CERTIFICATION

This is to certify that this research project titled "Comparative effects of atrazine and carrageenan in young and aged rats: serum testosterone analysis and histological features of the testis's carried out by Adonu, Blessing Ogechukwu with registration number GOU/U22/BCH/316 under supervision in the Department of chemical science, Godfrey Okoye University Enugu Nigeria.

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DEDICATION

This work is dedicated to Almighty God for his goodness upon my life. I also dedicate this work to my beloved parents and family for their prayers, encouragement, sacrifices, and unwavering support.

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To Almighty God, whose infinite grace, wisdom, strength, and unfailing love made the successful completion of this research possible. All glory, honour, and thanksgiving belong to Him for His guidance, protection, and faithfulness throughout this academic journey.

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ABSTRACT

This study investigated the Comparative assessment of testis histology of young and aged rats to the effect of atrazine and carrageenan using serum testosterone concentration and testicular histology as indices of reproductive function. Thirty male rats were grouped into six experimental groups: control aged, control young, atrazine-aged, atrazine-young, atrazine plus carrageenan-aged, and atrazine plus carrageenan-young. Atrazine was administered orally at a dose of 200 mg/kg body weight 3 times per week for eight weeks, while carrageenan (0.1%) was administered on the last day of atrazine treatment in the designated groups. At the end of the experiment, blood samples were collected for serum testosterone assay using ELISA, while testes were harvested for histological examination using Hematoxylin and Eosin staining. The results showed variations in testosterone levels across the groups, with age-related differences in hormonal response. Histological findings revealed normal testicular architecture in the control groups, while atrazine-treated groups showed varying degrees of testicular distortion, including degeneration of spermatogenic cells, Leydig cell distortion, vascular congestion, and altered seminiferous tubular structure, with more severe changes observed in aged rats and in the atrazine plus carrageenan-aged group. The young rats showed relatively preserved seminiferous tubules, although interstitial alterations were still evident in the combined treatment group. These findings suggest that aging increases susceptibility to atrazine-induced testicular damage and highlight the need to minimize exposure to atrazine, particularly in vulnerable populations.

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Environmental pollution by agrochemicals and its adverse effects to biological systems have become one of the major problems worldwide. Exposure of animals to agrochemicals could lead to disturbance of hormone balance by interfering with the axis function hypothalamus-pituitary-gonad. Extensive research has been done to demonstrate the endocrine-disrupting effects of one of the most popular herbicides, atrazine on mammals' reproduction (Hayes *et al*., 2020). It has been demonstrated in previous study that the level of serum testosterone in male rodents were significantly reduced after atrazine treatment due to perturbation in steroidogenic pathway and failure of leydig cell's function. In several cases, blocking the synthesis of testosterone occurred via inhibition of 3-hydroxysteroid dehydrogenase and cytochrome P450 enzymes by atrazine (Abarikwu*et al*., 2023; Zhao *et al*., 2023). Reduced serum testosterone level can compromise sperm production and thus performance in reproductive parameters.

In addition to hormone regulation, some studies have indicated that atrazine increases oxidative stress and damaged testicular tissues due to disproportionate level of reactive oxygen species and antioxidant in cell, causing lipid peroxidation and apoptosis in testicular cell (El-Gendy *et al*., 2021; Kumar *et al*., 2024). These changes manifest as structural changes in the testes: degenerative seminiferous tubule, attenuation of germ cell layer, vacuolization of testicular cells. Histopathological studies on atrazine treated testes has shown some significant morphological alterations: seminiferous tubules desquamation, decrease in epithelial thickness, and more germ cell apoptosis in compared to non-treated (Olatunji *et al*., 2023; Chen *et al*., 2025) which

correlated directly with impairments of spermatogenesis and low sperm quality characterized by reduced motility and viability. Another fact is the age of animal, which affects the sensitivity to toxicants. A previous study hase shown that the sensitivity of animal to atrazine is correlated with their age since older animals have low capability of regenerating organs (Santos *et al*., 2024). Toxicity of toxicant involves inflammation as a primary toxicity mechanism. Carrageenan-induced inflammation is a widely used experimental model to reproduce inflammation. Co-administration of atrazine and carrageenan to animal could lead to further oxidative stress and cellular damage to reproductive systems (Kumar *et al*., 2022), but few studies focused on these effect on age related aspect. In spite of various reported harmful effect of atrazine on overall health, limited information are available in studies including assessment of both hormone profile and histology of aged and young animal under inflammatory conditions. Therefore, this research intended to study the impact of atrazine through a model of carrageenan-induced inflammation in young and aged rats considering serum testosterone level and testis histology.

1.2 Statement of the Problem

The severe deleterious impacts of atrazine to the male reproductive system (hormonal dysbalance and morphological changes) have been well-documented in the scientific literature. Many studies investigate the effect of atrazine on serum testosterone level and testis histological damage. However, effects of this pesticide under different age stages and its effect with and without inflammation are still underdeveloped and limited.

One more problem to investigate, which can enhance the deleterious effect of atrazine, is inflammation as inflammatory response leads to increased oxidative stress in the body and therefore increase toxicity. The lack of data that compare young and aged animals toward

sensitivity to atrazine effects in condition of inflammation is considered to be urgent research topic.

1.3 Aim of the Study

To evaluate the comparative assessment of testis histology of young and aged rats to the effect of atrazine and carrageenan with focus on the level of serum testosterone and histological analysis of the testes.

1.4 Objectives of the Study

The aim of this study was :

1. To investigate the effect of atrazine on serum testosterone level of young and aged rats.
2. To evaluate the histological changes in testes induced by atrazine exposure.
3. To assess the combined effect of inflammatory (induced by carrageenan) and toxicity of atrazine.
4. To ascertain the differences, if any, in the effects of atrazine with and without inflammation among young and aged rats.

1.5 Significance of the Study

This study aimed to add to the knowledge of how prolonged atrazine exposure impacts serum testosterone level and testes histopathology under inflammatory conditions, such as those induced by carrageenan. By comparing young and aged rats, this research seeks to elucidate whether age-related factors influence sensitivity to atrazine-induced toxicity and exacerbate the effects of inflammation. The findings of this research could serve as valuable baseline data for further studies on the combined impact of environmental pollutants and inflammatory states on male reproductive health, potentially contributing to the development of informed preventive

measures and public health policies for reducing exposure to harmful agrochemicals and protecting health.

1.6 Scope of the Study

This research work focused on determining the effect of atrazine alone and combined with carrageenan on serum testosterone levels and testicular histology in young and aged male rats. Other parameters and biological systems were beyond the scope of this study.

1.7 Justification of the Study

Atrazine is widely utilized in agricultural production worldwide and has been commonly identified in soil, water, and food samples. Chronic exposure to atrazine has been associated with a variety of health problems including endocrine disruption, oxidative stress, inflammation, and adverse effects on the reproductive system in both human and animal models. Despite numerous investigations on the adverse effects of atrazine, there remains a lack of comprehensive understanding regarding the synergistic effect of this environmental contaminant in the presence of inflammation and how its effects vary across different age groups. Inflammation plays a crucial role in numerous pathologies, potentially exacerbating the damaging effects of exposure to chemical agents, including those impacting reproductive health.

The use of a carrageenan-induced inflammation model offers a reproducible and effective experimental paradigm for exploring these complex interactions. Given that aging alters immune function, redox status, and reproductive capacity, studying both young and aged rats is essential for elucidating age-related differences in sensitivity to atrazine and the inflammatory insult. Assessing serum testosterone and testicular morphology can provide valuable insights into the

endocrine and structural alterations associated with such exposure. Thus, this study is well justified by its potential to fill the existing knowledge gap on the intertwined roles of age, inflammation, and atrazine exposure on male reproductive health.

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

In modern day agriculture, there has been an increase in the usage of agrochemicals due to its efficiency, effectiveness and increased yield. However, the prolonged use of agrochemicals leads to multiple hazards associated with it which impacts human and environment. The commonly

used herbicide atrazine has become a subject of major research in the past decade, due to its persistence in the environment as well as its role as an endocrine disrupting chemical (EDC). Endocrine disruptors have been described as "exogenous compounds that interfere with the production, secretion, transport, binding, action, or elimination of endogenous hormones and other signal molecules in the endocrine system" (Hayes *et al*., 2020). Studies on atrazine have reported its impact on various aspects of the male reproductive system, including the reproductive hazards it imposes on male, especially, its potential to decrease testosterone levels and causing testicular damages (Abarikwu*et al*., 2023; Zhao *et al*., 2023).

Hormonal imbalance in the reproductive system, one of the common abnormalities, involves either an increased or decreased secretion and regulation of hormones. In the male reproductive system, Leydig cells are responsible for the production of testosterone which is vital for male development, spermatogenesis and expression of male characteristics 0. Literature suggests that, exposure to atrazine may decrease the levels of the male sex hormone testosterone that may lead to infertility and reduced sperm production (El-Gendy *et al*., 2021). This often involves disruptions in the crucial enzymes involved in the steroidogenesis pathway and the hypothalamus-pituitary-gonadal axis (HPG) (Zhao *et al*., 2023).

Another crucial factor in explaining the toxicological impact of atrazine is oxidative stress, a phenomenon caused by excessive formation of ROS compared to the antioxidant defense mechanisms, resulting in the damage to the cell membranes, proteins, and DNA (El-Gendy *et al*., 2021). In testes, this leads to degenerative changes in seminiferous tubules and germ cells as well as disorders of spermatogenesis (Kumar *et al*., 2024). Histological examination of testicular tissue is an essential method to evaluate structural and functional integrity of the male reproductive system and it has been established that, exposure to atrazine leads to changes such

as alterations in the structure of seminiferous tubules, reduction in germinal epithelial tissue, vacuolations of Sertoli cells, increased apoptotic germ cells etc. (Olatunji *et al*., 2023; Chen *et al*., 2025), consequently reducing the production of quality male gametes.

In addition to the nature of toxicant, age is also an important variable determining the toxicological profile of a given substance. Younger organisms exhibit a stronger antioxidant capacity and higher capacity for regeneration than aged ones, making the latter more susceptible to certain xenobiotics, which implies a lower tolerance to the exposure to potentially toxic compounds (Santos *et al*., 2024). For these reasons, comparative assessment of effects of toxic substances on animals of different age group becomes crucial. The inflammation process also plays a crucial role in the modulation of actions of xenobiotics. In experimental settings, carrageenan has been widely used to induce acute inflammation in laboratory animals. Carrageenan administration is known to release mediators of acute inflammation such as cytokines, prostaglandins, reactive oxygen species etc., resulting in increased levels of oxidative stress in organism, that may cause additional tissue damage in the presence of such toxic substances like atrazine (Kumar *et al*., 2022).

Although a substantial amount of literature concerning atrazine-induced reproductive toxicity has been published on various organisms, one should emphasize that there is scarcity of studies that have simultaneously investigated several physiological processes, especially the impact of combined effects of atrazine and inflammation (carrageenan induced), and a comparative evaluation of their impacts on young and aged animals. Besides, research focused on the comparison of histological results with those of biochemical analyses are limited.Based on these issues, the present literature review covers mechanisms of endocrine disruption by atrazine, its effect on serum testosterone levels and testicular histopathology, its association with oxidative

stress and inflammation and finally the toxicant induced changes on physiological functions dependent on age. The gained knowledge will form a rational basis for the current investigation that will study the effect of atrazine in carrageenan induced inflamed rat models on young and old animals using biochemical tests to assess serum testosterone levels and histological techniques to evaluate testicular histopathology.

2.1.1 Atrazine: An Endocrine Disruptor

Atrazine is one of the most widely used herbicides in the world, predominantly utilized in agricultural practices to control broad-leaf and grassy weeds. Besides its excellent weed killing capabilities, atrazine has become well-known in the literature as an EDC because it can act on hormone system in various species including mammals and wildlife animals (Hayes *et al*., 2020). An EDC is a chemical compound that negatively interferes with a normally functioning hormone system and causes undesired side effects such as hormonal disruption, developmental defects, reproductive and neurological disorders, and immunological problems.

The action mechanism of atrazine as endocrine disruptor is based on its influence on the HPG (hypothalamic-pituitary-gonadal) axis that regulates hormone synthesis and release. Atrazine causes disruptions in the release of gonadotropin-releasing hormone (GnRH) synthesized in hypothalamus and the luteinizing hormone (LH) and follicle-stimulating hormone (FSH) released by pituitary gland, leading to impaired testicular function and inhibited release of testosterone by affecting Leydig cells of testis (Hayes *et al*., 2020). Furthermore, atrazine has been shown to inhibit synthesis of testosterone, the key male steroid hormone that is essential for spermatogenesis and the maintenance of male sexual functions and traits. It is documented that the substance inhibits the 3-hydroxy-steroid dehydrogenase (3-HSD) and cytochrome P450 family of enzymes responsible for testosterone biosynthesis in the testis (Abarikwu; *et al*., 2023).

Besides reducing testosterone synthesis, atrazine also increases activity of aromatase enzyme, thereby diverting more androgens to convert into estrogens which ultimately creates hormonal imbalances leading to reproductive failure in male (Hayes *et al*., 2020). This excessive amount of estrogen contributes to feminist effect in male which impairs sperm production.

Also, it should be highlighted that, the endocrine disruption activity of atrazine is often related to mechanisms of oxidative stress. Increased formation of ROS and reactive nitrogen species (RNS) generated under atrazine exposure results in damaging Leydig cells and testicular tissue, making difficult for them to produce male hormones (El-Gendy *et al*., 2021). Numerous studies confirmed the endocrine disrupting and reproductive toxic effect of atrazine through histological examination which shows degenerative alterations of the testes, such as reduction of the number of germ cells, degeneration of seminiferous tubules, increased apoptosis of germ cells and, as a consequence, a decrease in the levels of testosterone and sperm production (Olatunji *et al*., 2023; Chen *et al*., 2025). The biological response to this xenobiotic is also modulated by the dose, route, duration of exposure, age and physiological condition; atrazine has proven that its long-term exposure at sub-lethal doses could elicit potent endocrine and reproductive effects, hence the risk from an ecological point of view (Abarikwu*et al*., 2023).

All in all, atrazine exert its endocrine disrupting activity through various mechanisms including suppression of the HPG axis, steroidogenesis inhibiting effects, resulting in hormonal disruption and creation of oxidative stress and inflammation, which collectively leads to impaired testicular morphology and depressed testosterone production, creating basis for investigation in age-related study on this toxin under inflammation state.

2.1.2 Testosterone and Male Reproductive Function

Testosterone is the chief androgen in males and one important factor responsible for the regulating physical function concerned to reproduction. The majority of circulating testosterone is made by testicular Leydig cells by the action of the HPG (Hypothalamic–pituitary–gonadal) axis, i.e. Hypothalamus secretes GnRH hormone that acts on the anterior pituitary to produce luteinizing hormone, LH, which in turn stimulates the Leydig cells to produce testosterone (Abarikwu*et al*., 2023; Zhao *et al*., 2023).

Testosterone controls the mechanism of spermatogenesis which occurs in the seminiferous tubules. It functions through regulation of Sertoli cells to promote the development and differentiation of germ cells into the mature spermatozoon. So that appropriate level of testosterone is mandatory for normal spermatogenesis and thus to maintain normal motility and viability of the sperm (El-Gendy *et al*., 2021). Loss or malfunction in testosterone metabolism leads to reduced spermatogenesis and infertility. Moreover, the synthesis and development of male secondary sexual characters i.e. Male type hair growth, large muscle mass and sex drive depend upon the adequate level of testosterone.

Proper structure and functions of testes also depend on sufficient amount of testosterone. Testosterone level can reduce and may lead to the testicu5.17 atrophy, decrease in seminiferous tubules, deterioration of germinal epithelium (Olatunji *et al*., 2023). Production of testosterone involves different enzymatic reactions where cholesterol convert in to steroids, catalyzed by key enzymes such as 3-hydroxysteroid dehydrogenase (3-HSD) and cytochrome P450 enzyme like CYP17. Toxic substances interference on these enzymes' function leads to deficit in testosterone production (Abarikwu*et al*, Zhao *et al*., 2023).

The actions of the environmental toxicant such as Atrazine cause a loss of testosterone through inhibiting steroidogenesis, arresting the LH signaling pathway, increasing oxidative stress in

Leydig cells (Kumar *et al*., 2024). The Oxidative stress plays a vital role since it injures the components of cells that produces steroid hormones and cause deficiency of hormones. Reduced amount of androgen has significant effect in structure of the testicular tissue as the level of androgen decline; degeneration of seminiferous tubules, germ cells apoptosis (programmed cell death) will arise and the cellularity of the testicular tissue is decreased (Chen *et al*., 2025).

Moreover, Age plays significant role on testosterone level as in younger subject testosterone production rate will be high whereas in older age it declines along with the functionality of the testicular tissues and so they become sensitive to the hazardous substances like Atrazine and causes their more impact on reproductive health (Singh *et al*., 2024).

2.1.3 Oxidative Stress and Male Reproductive Toxicity

The concept of oxidative stress seems the heart of different types of toxicity due to exposure to the chemical. Oxidative stress can define as an imbalance between generation of ROS and scavenger activity of antioxidant systems to neutralize the highly reactive species (El-Gendy *et al*., 2021). An overload of the ROS generation damages biomolecules like lipids, proteins and DNA in a process called lipid peroxidation. There are many features making testicular tissues prone to oxidative stress such as abundance of highly oxidizable PUFAs, higher rate of cell division and lack of capacity of defense system for protection against a huge generation of ROS in case of poisoning (Kumar *et al*., 2024). There are numerous studies reporting increased ROS production in testicular tissue exposed to Atrazine.

It has been proved that Atrazine can disrupt the functionality of mitochondria resulting in synthesis of reactive oxygen radicals (ROS) such as super oxide anions, hydroxyl radicals, hydrogen peroxide. These active free radicals initiate lipid peroxidation of the cell membranes (El-Gendy *et al*., 2021). In addition to damage on cellular membranes, oxidative stress can result

in damage of highly sensitive cell types of the testis. For example; Leydig and Sertoli cells damage by oxidative stress would impair hormone production. Specifically, loss of the normal function of steroid producing Leydig cells cause to decrease in level of testosterone in blood plasma (Abarikwu*et al*, 2022). Sertoli cells normally support for development and growth of germ cells so damage of Sertoli cells leads to problems in spermatogenesis (El-Gendy *et al*., 2021).

The high levels of ROS promote programmed cell death or apoptosis of germ cells in the testis; consequently; there will be a reduction in sperm count and motility leading to fertility issues (Chen *et al*., 2025).

Oxidative stress can also cause histopathological changes including disorganization of testicular structure, degenerative and disordered seminiferous tubules, atrophied germinal epithelium, vacuolated cells, interstitial edema and may progress to testicular fibrosis and atrophy (Olatunji *et al*., 2023). Oxidative stress also causes impairments in normal cellular signaling pathways and could inhibit some enzyme activities such as SOD, catalase and glutathione peroxidase which function in protection against ROS (Kumar *et al*., 2024). Furthermore, oxidative stress can be potentiated by inflammatory process, as inflamed tissue itself can generate ROS; inflammatory agents such as carrageenan, release pro-inflammatory mediators and thus boost ROS production (Kumar *et al*., 2022). Old age is another risk factor for oxidative stress as older people possess reduced ability of antioxidants as compared to young age group (Singh *et al*., 2024) and therefore will exhibit more severe pathological effects from oxidative stress caused by toxicants.

So overall oxidative stress in testicular tissues due to exposure of atrazine would be important reason for the observed reproductive toxicity because of damage caused by lipid peroxidation, DNA damage, proteins damage and germ cells' and Leydig's cell apoptosis.

2.1.4 Testis Histological Features

The structure of testis tissues areHighly specialized and it makes it the functional organ for male reproduction. Testis has two important regions i.e., seminiferous tubule, which is considered as functional unit and interstitial tissue which consists of blood vessel, connective tissues, Leydig cell (Olatunji *et al*., 2023). The seminiferous tubule have layered structure named germinal epithelium which includes various stages of spermatogenic cells (spermatogonia, primary and secondary spermatocytes, spermatid and sperm) surrounded by Sertoli cell.

These cells have provided nutrients and structural and nutritional support and create suitable microenvironment to facilitate spermatogenesis. Sertoli cell also form blood testis barrier which provide protective barrier to developing germ cells from damaging agents (Chen *et al*., 2025). Leydig cell located in the interstitial, responsible to produces and secrete testosterone hormone that helps maintain the normal structure and function of testes and involved in spermatogenesis. Therefore, structural integrity of testis (seminiferous tubules and interstitial tissues) are highly essential and cannot injury. Under normal and non-toxic condition seminiferous tubules look orderly with compact arrangement, thick germinal epithelium, clear lumina, and well-organized arrangement of seminiferous epithelium that contains well-arranged of seminiferous germ cells and clear lumina filling with spermatozoa at 0.01% DMSO concentration. The basement membrane is also well maintained, and sperms are organized in a clear lumina and well-organized cellular organization in the seminiferous tubule (Olatunji *et al*., 2023).

Conversely, the application of toxic agents such as atrazine to the testis will induce histopathological changes in its histological structure. Some studies indicated that atrazine degenerates and disorganize the seminiferous tubules and caused shrinkage, flattening of the epithelium, thinning of germinal layer, loss of spermatogenic cells and vacuolated spaces in

germinal cells (Abarikwu*et al*., 2023, Mgbudom-Okah *et al.,* 2024). Vacuolation of Sertoli cells, often reported in cases of testicular toxicity, implies damage to these cells' ability to nourish developing germ cells. Other changes associated with testicular toxicity include desquamation of germ cells into the lumen of seminiferous tubules, which suggests interference with normal spermatogenesis (Olatunji *et al*., 2023). Testicular germ cell apoptosis is another hallmark of testicular toxicity; it can reduce germ cell counts, thereby decreasing sperm production (Chen *et al*., 2025). Histopathological alterations may also impact interstitial tissues, particularly Leydig cells. Exposure to atrazine seems to decrease Leydig cell number and impair their functioning, thus reducing testosterone synthesis (Abarikwu *et al*., 2024).

Interstitial oedema and fibrosis may arise from atrazine exposure, leading to impairment of the gland's physiologic capabilities. Oxidative stress mediates these changes. ROS may damage cell membranes and genetic material, resulting in the structural damage to the testicular tissue (El-Gendy *et al*., 2021). Furthermore, Carrageenan induces an inflammatory response which exacerbates tissue damage, likely via promoting inflammation and increased ROS generation (Kumar *et al*., 2022).

Additionally, age factor plays a significant role in these observed results; younger individuals generally exhibit greater resistance to structural disruptions due to more efficient cellular repair processes compared to aged individuals. Thus, the morphological characteristics of the testis are highly pertinent to the male reproductive system's functional status. Integrity of seminiferous tubules, Sertoli cells, and Leydig cells is essential for the normal functioning of spermatogenesis and hormone production. Any tissue disorganization resulting from toxic exposures or inflammation can impair these vital processes.

2.1.5 Carrageenan-Induced Inflammation

This is a physiological response against pathological stimuli within tissues. It is induced experimentally using various chemical compounds. One of the widely utilized and most consistent inducing agents is carrageenan a polysaccharide derived from red seaweeds. Its reliability and replicability in eliciting all four stages of acute inflammation in experimental animals make it an excellent model for inflammation research (Kumar *et al*., 2022).

The carrageenan-induced inflammatory response typically occurs in two phases. The initial phase, observed within the first hour post-injection, involves the release of histamine, serotonin, and bradykinin. The second phase, starting 1-6 hours later, is marked by the release of prostaglandins, nitric oxide (NO), reactive oxygen species (ROS), and pro-inflammatory cytokines such as TNF-α and interleukins (Kumar *et al*., 2022). Given its prominent contribution of neutrophils and ROS production, the second phase of carrageenan-induced inflammation is particularly relevant for toxicological investigations.

A significant aspect of carrageenan-induced inflammation is its propensity to increase ROS production. These ROS can cause significant damage to cellular macromolecules, including lipids, proteins, and nucleic acids. Consequently, inflammation models like carrageenan are valuable for evaluating the effects of oxidative stress on various types of injuries mediated by chemical agents (El-Gendy *et al*., 2021). The role of inflammation in male reproduction, as highlighted in reproductive toxicology studies, is crucial.

The unique testicular environment and high hormonal regulation render these organs highly susceptible to inflammation, which can lead to impaired Leydig and Sertoli cell function, disruption of the blood-testis barrier, and diminished hormone secretion, consequently affecting

spermatogenesis (Abarikwu *et al*., 2023). Carrageenan-induced inflammation has also been observed to exacerbate the damage caused by environmental toxins. When combined, inflammation intensifies oxidative stress, leading to more severe cellular damage and overall tissue injury. This synergy is especially relevant when studying endocrine-disrupting agents such as atrazine, where oxidative stress and hormonal imbalances play critical roles in mediating toxicity (Kumar *et al*., 2020).

Moreover, inflammation alters several physiological functions. Pro-inflammatory cytokines have been shown to impact the hypothalamic-pituitary-gonadal axis, resulting in reduced gonadotropin release and decreased testosterone synthesis (Zhao *et al*., 2023). These observations emphasize the importance of considering the role of inflammation in reproductive toxicity experiments. Furthermore, the age of the subject is a critical factor, with aged individuals being more susceptible to inflammation due to increased levels of pro-inflammatory cytokines and a diminished ability to resolve inflammatory responses.

The concept of "gaming" in older adults refers to chronic, low-grade inflammation (Santos *et al*., 2024). Therefore, the carrageenan-induced inflammation model serves as a valuable tool for studying acute inflammation responses and their interplay with various toxic substances. Specifically, its capacity to induce oxidative stress, cytokines, and tissue injury makes it ideal for exploring whether inflammation exacerbates atrazine-induced reproductive toxicity in young and aged rats.

2.1.6 Age-Associated Susceptibility to Toxicity

Due to Aging, the susceptibility of an organism to toxicants, including endocrine disruptors such as atrazine, is heavily influenced by biological factors, such as age. Interspecific

variations in the anatomy and physiology of young versus old individuals are reflected in changes to absorption, distribution, metabolism, and elimination of toxicant; ultimately this impacts toxic response (Santos *et al*., 2024). This makes older organisms susceptible to damage caused by toxicants because of diminished cellular or physiological protections against harm (Santos *et al*., 2024).

Age-related changes to antioxidants are often the cause of this increased susceptibility to toxicants, as decreased activity of potent antioxidants, like superoxide, inhibits an organism's ability to neutralize free radicals such as reactive oxygen species (ROS) (Kumar *et al*., 2024).

As this causes oxidation within the cells it results in more damaging effects from even small amounts of toxicant like atrazine since more damaging ROS can remain in the body. Age is also responsible for hormonal alterations that lead to increased sensitivity. The production of testosterone is slowed with age due to impaired gid cells and changes to communication signals within the Hypothalamic-pituitary-gonadal (HPG) axis. Reduced testosterone causes reduced sperm production and less optimal fertility which results in these processes being more susceptible to damage by a toxicant (Abarikwu *et al*., 2023; Zhao *et al*., 2023). There are a number of histological changes in testes due to aging, such as thinning of the germinal epithelium, shrinking of the seminiferous tubule diameter, reduction in number of germ cells, and proliferation of interstitial tissue in the aged animals. These changes clearly indicate reduced function of testes (Olatunji *et al*., 2023). This implies greater physical damage of the toxicant to tissues in aged animals. A further factor associated with aging is a prolonged state of low-grade inflammatory processes commonly referred to as 'gaining' characterized by significant upregulation of proinflammatory cytokines, elevated oxidative stress, and impaired inflammatory resolution pathways. Gaining makes tissue more sensitive to chemical stimulus, potentiating

detrimental consequences from toxicants like atrazine (Santos *et al*., 2024). A deficiency in metabolizing toxic compounds may further cause older organisms to experience higher risk of toxic responses. Typically, younger people have increased ability to metabolize xenobiotics in liver and kidneys than older people whose systems are unable to cope with elimination of compounds as quickly and thus have prolonged exposures (Kumar *et al*., 2024).

In the case of reproductive effects, these variations further intensify the outcomes. The aged rat testis, less able to self-repair from ROS and Inflammation damage, is therefore more prone to decrease in production of testosterone and develop greater histopathological changes when given atrazine compared to younger animals (Santos *et al*., 2024). Age plays a vital role in affecting biologic reactions to toxicants by inducing decreased activity of antioxidants, endocrine abnormalities, inflammatory responses, tissue regenerative abilities, thus making aged animals more susceptible to atrazine toxicity which affects reproduction as seen in the experiment. 2.2 Literature Review Many studies have been performed on atrazine toxicity in male rat concerning effects on reproductive system: on endocrine system, oxidative stress, testicular histoarchitecture etc. Recent studies (2020-2026) indicated that atrazine induced negative impacts on the male system due to diverse underlying mechanisms such as, disrupt of testosterone secretion, generation of oxidative stress, degeneration of the testis tissue, etc.

According to the findings of Abarikwu *et al*. (2022) a statistically significant reduction in the level of testosterone was detected in serum after atrazine treatment in male rat, attributed to altered gid cells functions and impairment in the expression of genes regulating steroidogenic pathway and inhibition of the enzymes that catalyze cholesterol into testosterone. It further confirmed the ability of atrazine to cause endocrine disruptor action as reflected by the inhibition of hypothalamic-pituitary-gonadal (HPG) axis (Hayes *et al*., 2020). A similar

hormonal alteration mechanism of atrazine was reported by Zhao *et al*. (2023), who showed atrazine inhibited the activities of steroidogenic enzymes such as 3-hydroxysteroid dehydrogenase (3-HSD) and cytochrome P450 (CYP) family enzymes that leads to impaired spermatogenesis and reduction in testosterone synthesis in male rat. Apart from disrupting hormone metabolism, a significant effect of atrazine toxicant has been the Induction of oxidative stress.

ROS generation was higher whereas antioxidant enzymes such as superoxide dismutase and catalase activity was significantly decreased in testicles of animals exposed to atrazine (El-Gendy *et al*., 2021). As well, oxidative damage is proven to be an important pathway involved in atrazine-induced nephrotoxicity via damaging the membranes lipids and proteins and apoptosis in the germ cells, ultimately leading to impairment of male fertility (Kumar *et al*., 2024). Atrazine has also demonstrated to cause histological changes in different organs. Studies conducted on different animals showed significant changes including degenerative changes in the seminiferous tubule, decreased germ cell population, reduced diameter of seminiferous tubules, and increased interstitial tissue (Olatunji *et al*., 2023).

In contrast, Chen *et al*. (2025) demonstrated a dose dependent accumulation of damage which included the generation of testicular vacuolation, germ cell apoptosis and seminiferous tubules atrophy. They further reported a disruption of the overall organization of testis and decrease in spermatogenic activity.

2.1.6 Vulnerability due to Aging susceptibility of organism to toxicants like atrazine.

Interspecific variation of anatomic and physiologic structures of young versus old animals cause change in ADME process, impacting overall toxicology process. This increases vulnerability to the damaging effect of toxicant due to loss of cell/physiological defenses (Santos *et al*., 2024).

Age decreases the capacity to eliminate and neutralize toxicants because potent antioxidants like superoxide are reduced. This leads to damaging ROS accumulation. With age, endocrine and hormonal changes also lead to sensitivity to toxicants, which is evident in male rats with slower testosterone production due to impairment in gid cells and HPG axis communication. Slow testosterone leads to a decrease in the sperm production, a less efficient reproductive system more easily damaged by toxicants (Abarikwu *et al*., 2022; Zhao *et al*., 2023). Age also causes histological alterations in male testes, including germinal epithelium thinning, seminiferous tubules shrinkage and reduced germ cells, which suggests impaired testis functionality and greater physical damage of the toxicant in aged animals (Olatunji *et al*., 2023).

Chronic inflammation, characterized by pro-inflammatory cytokines, oxidative stress and impaired resolution pathways (known as 'gaining'), makes tissues sensitive to chemical stimuli (Santos *et al*., 2024).

Reduced metabolism of toxic compounds is another age-associated vulnerability, because older organisms cannot metabolize and eliminate toxicants as quickly as younger organisms, leading to prolonged exposures (Kumar *et al*., 2024). This also intensifies reproductive outcome damage. Old rats' testis, unable to recover from ROS and Inflammation damage, is more likely to produce less testosterone and have increased damage following atrazine exposure compared to younger animals (Santos *et al*., 2024).

The impact of aging on toxicity response, and thus on reproductive toxicity of atrazine in male rats, is due to reduced antioxidants activity, endocrine changes, Inflammation and reduced repairability (Santos *et al*., 2024). 2.2 Literature Review Various research has been conducted regarding atrazine toxicity in male rat on the endocrine system, oxidative stress, and testicular histoarchitecture of the reproductive system. Research between 2020 and 2026 shows

that atrazine damages male systems through diverse mechanisms, such as reducing testosterone secretion, creating oxidative stress and causing degeneration of testicular tissue. Reduced testosterone was shown to occur due to altered gid cell functions, changes in genes of the steroidogenic pathway and inhibition of enzymes converting cholesterol to testosterone, reflecting atrazine's ability to disrupt the HPG axis (Hayes *et al*., 2020; Abarikwu *et al*., 2022).

The same hormone altering mechanism was demonstrated by Zhao *et al*. (2023), in which atrazine suppressed steroidogenic enzymes (e.g. 3-HSD, CYP), resulting in decreased testosterone production and impaired spermatogenesis. Significant induction of oxidative stress, marked by elevated ROS generation and suppressed antioxidant enzyme activities, was another prominent effect of atrazine in animal testes (El-Gendy *et al*., 2021). Damage to membranes and germ cells by oxidative stress is a known mechanism for the induction of atrazine's reproductive toxicity in male animals (Kumar *et al*., 2024). Histological changes induced by atrazine include thinning or degenerated seminiferous tubule epithelium, increased interstitial tissue, reduction in seminiferous tubule diameter and germ cell number (Olatunji *et al*., 2023).

In contrast, dose dependent damage was observed by Chen *et al*. (2025) which included testicular vacuolation, seminiferous tubule atrophy and germ cell apoptosis. They also observed disrupted tissue organization and reduced spermatogenesis in the testes of animals.

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Reagents and apparatus/equipment:

3.1.1 Apparatus used:

1. Beakers

2. Measuring cylinder

3. Stirrer

4. Dropper pipette

5. Oral gavage needles [feeding cannula]

6. Volumetric flasks

7. Disposable syringes and needles

3.1.2 Equipment used:

1. Animal cages

2. Feeding troughs and water bottles

3. Sample bottles/tubes

4. Centrifuge-BIOBASE:BK-THR16K

5. ELISA reader-BIOBASE: EL10A

6. Weighing balance-Mettler Toledo: AB204

7. PH meter-CyberScan500

8. Water bath-Jinotech: HH-W420

9. Microscope-LASEC:

10. Water distiller

3.1.3 Reagents used:

1. Atrazine

2. Carrageenan

3. Bouin's_solution

4. Hematoxylin stain

5. Eosin stain

6. Ethanol (70%, 80%, 90%, 95%, absolute)

7. Xylene

8. Paraffin wax

9. Normal saline

10. Testosterone ELISA kit

11. Distilled water

3.2 Experimental Animals

About 15 young male rats weighing 50-70g and 15 aged male rats, weighing 200-250 g were used for the experiment. They were given a week to acclimatize to the animal facility, before used in experiments. The rats were kept under standard conditions of temperature, relative humidity, and had free access to standard diet and drinking water at free will.

The animals were divided into two age categories:

1. Young rats (3-4 weeks old at the commencement of the study)
2. Aged rats (12-18 months old)

3.3 Experimental Grouping

The animals were randomly assigned into six experimental groups.

Group I: Control Aged- received distilled water and animal feed orally throughout the study period

Group II: Control Young-received distilled water and animal feed orally throughout the study

period.

Group III: ATZ Aged-received atrazine at a dose of 200 mg/kg body weigh by oral gavage 3 times per week - for eight weeks.

Group IV: ATZ Young-received atrazine at a dose of 200 mg/kg body weight daily by oral gavage 3 times per week for eight weeks.

Group V: ATZ + CGN Aged-received atrazine at a dose of 200 mg/kg body weight 3 times per week for eight weeks followed by carrageenan (0.1%) administration on the final day of treatment.

Group VI: ATZ + CGN Young-received atrazine at a dose of 200 mg/kg body weight 3 times per week for eight weeks followed by carrageenan (0.1%) administration on the final day of treatment.

3.4 Preparation and Administration of Atrazine

Atrazine was prepared by dissolving the required quantity in a ml of Dimethyl sulfoxide to give a finial concentration of 0.01% of DMSO. The appropriate dose of 200 mg/kg body weight was administered orally using a gavage tube 3 times per week for eight weeks. The volume administered was adjusted according to the body weight of each animal.

3.5 Preparation and Administration of Carrageenan

Carrageenan solution (0.1%) was prepared by dissolving carrageenan powder in DMSO. The solution was administered to the designated groups [Group 5 and 6] on the last day of atrazine treatment to induce an acute inflammatory response.

3.6 Sample Collection

Body weight was recorded once a week. After 56 treatment days, the animals were anesthetized using chloroform before being killed through cervical dislocation, the testis was

removed aseptically, rinsed and weighed. The relative weight of the organs of each rat was calculated as organ weight (mg)/body weight (g) and kept for analysis. Blood samples were collected through cardiac puncture into plain sample tubes and allowed to clot at room temperature. The blood samples were centrifuged at 3000 rpm for 10 minutes to obtain serum. The serum was carefully separated and stored at 20C until hormonal analysis.

3.7 Determination of Serum Testosterone Concentration

Serum testosterone levels were determined using a commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit according to the manufacturer's protocol. The serum testosterone concentration was determined by a Microplate Enzyme Immunoassay in rat serum and other biological fluids.

Principle of the Assay

Rat testosterone ELISA kit is an enzyme-linked immunosorbent assay based on the principle of double-antibody sandwich enzyme-linked immunosorbent binding. An unknown amount of testosterone present in the sample and a defined amount of testosterone conjugated to horseradish peroxidase compete for the binding sites of testosterone antiserum coated to the wells of a microplate. After incubation and a wash step, a substrate solution is added, and the concentration of testosterone is inversely proportional to the optical density measured.

Procedure

All reagents, serum and control were brought at room temperature (20-27C) before proceeding with the assay. The microplate wells were formatted for each sample. About 0.010 ml (10 l) of

the sample was pipetted into the assigned wells. Then 0.050 ml (50 l) of the working testosterone enzyme reagent was added to all the wells. The microplate was swirled gently for 20-30 seconds so as to allow it to mix. About 0.050 ml (50 l) of the testosterone biotin reagent was added to all the wells. The microplate was swirled gently again for 20 - 30 seconds to mix. Then the wells were covered and incubated for 60 minutes at room temperature.

The contents of the microplate were discarded by decantation after which the plate was blotted dry with absorbent paper. About 350 l of wash buffer was added to all the wells which was later decanted and the plate blotted dry. This step was repeated twice. About 0.100 ml (100 l) of working substrate solution was added to all wells. Then it was incubated at room temperature for fifteen (15) minutes. Then 0.050 ml (50 l) of stop solution was added to each well and gently mixed for 15-20 seconds. The absorbance was read at 450 nm (using a reference wavelength of 620-630 nm to minimize wells imperfections) in a microplate reader.

Table 3.1: Standard for Testosterone determination

CONC (mg/ml)	ABS
0	0.2
20	0.173
40	0.311
80	0J.329
160	0.587
320	0.76

3.8 Histology of the testis

The standard histological technique was followed by the method of Gurr (1959). The influence of the atrazine and carrageenan on the histopathology of testis was investigated. At the end of the treatment (56 days), testis from each sacrificed rat were dissected out, trimmed of excess fat, tissues and weighed. Then, the testis fixed in bouins solution and prepared for

histological examination. After fixation with bouins solution, the tissue was dehydrated, processed, and embedded in paraffin. Serial sections (5 m) were prepared and stained with Harris's hematoxylin and eosin (H&E) for histological evaluation by light microscope. Photomicrographs were captured using a digital microscope camera.

3.9 Statistical analysis

Values of the measured parameters were expressed as mean standard deviation (S.D). All data sets were found to be normally distributed around the mean when tested for normality using the Shapiro-Wilk test. Statistical analyses of the acquired data were done using GraphPad Prism version 6.0 software (GraphPad Software, Inc., San Diego, CA, USA).

For comparison between the different groups, one way analysis of variance followed by Tukey multiple comparisons test was used. The results were considered statistically significant when P-value was less than 0.05.

CHAPTER FOUR

RESULTS

4.1 Result of serum testosterone concentration

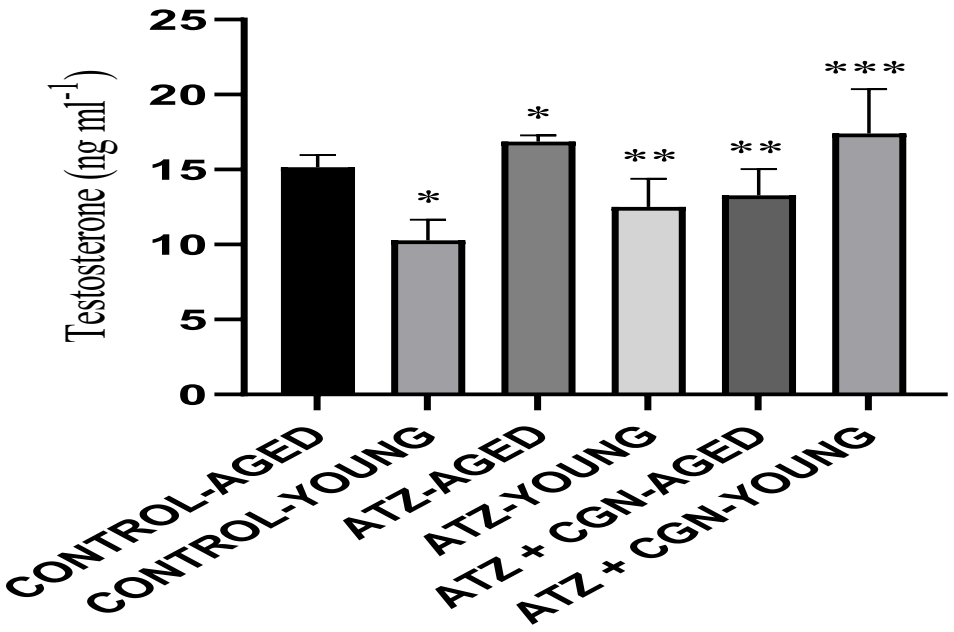

Figure 4.1 Effect of atrazine and carrageenan on serum testosterone. Data are represented as the mean ± SD (N = 5). *Versus control Aged, **Versus ATZ-Aged, ***Versus ATZ+ CGN-Aged ($p< 0.05$). CONTROL-AGED= Normal control in Aged rats; CONTROL-YOUNG= Normal control in young rats; ATZ-AGED= Atrazine in Aged rats; ATZ-YOUNG= Atrazine in Young rats; ATZ + CGN-AGED= Atrazine and Carrageenan in Aged rats; ATZ + CGN-YOUNG= Atrazine and carrageenan in young rats. ATZ (200 mg/kg), CGN (0.1%).

The results demonstrated significant differences among treatment groups, particularly between the control and atrazine-treated groups, indicating that atrazine exposure adversely affected testicular structure. The combination of atrazine and carrageenan produced more pronounced histopathological alterations, especially in adult rats, compared with atrazine treatment alone.Carrageenan increases the testosterone level in young rats and reduces the testosterone level in aged rats.

4.2 Histological Assessment of Testicular Tissues

Histological examination of the testes was carried out using Hematoxylin and Eosin (H&E) staining at ×400 magnification to evaluate the effects of atrazine (ATZ) and atrazine combined with carrageenan (atrazine + carrageenan) on testicular architecture in adult and young rats.

4.2.1 Control Aged Group

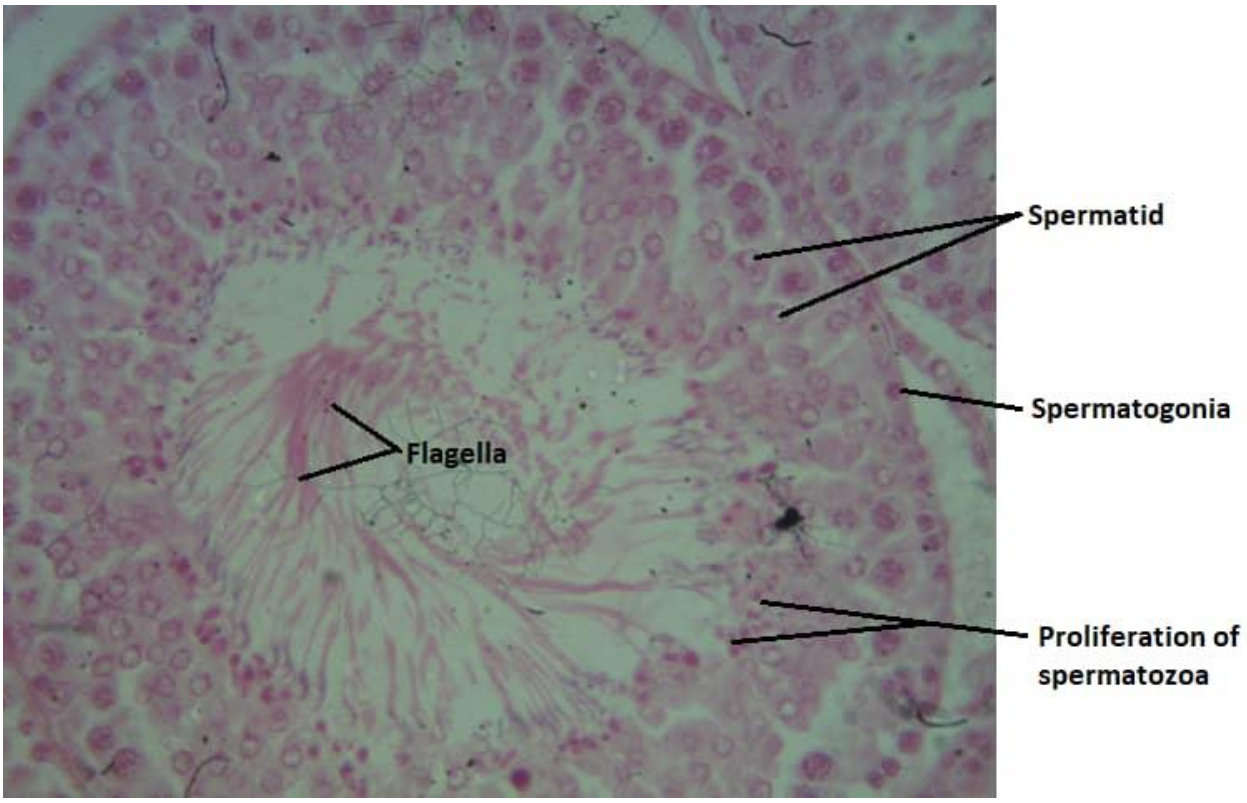

Figure 4.2 Control Aged Group

The photomicrograph of the testes from the adult control group showed normal histological architecture. The seminiferous tubules contained well-organized spermatogonia, spermatids, and numerous spermatozoa with intact flagella projecting into the lumen. The interstitial spaces contained well-defined Leydig cells with no evidence of cellular degeneration or vascular congestion. Overall, normal spermatogenesis and tissue integrity were observed.

4.2.2 Control Young Group

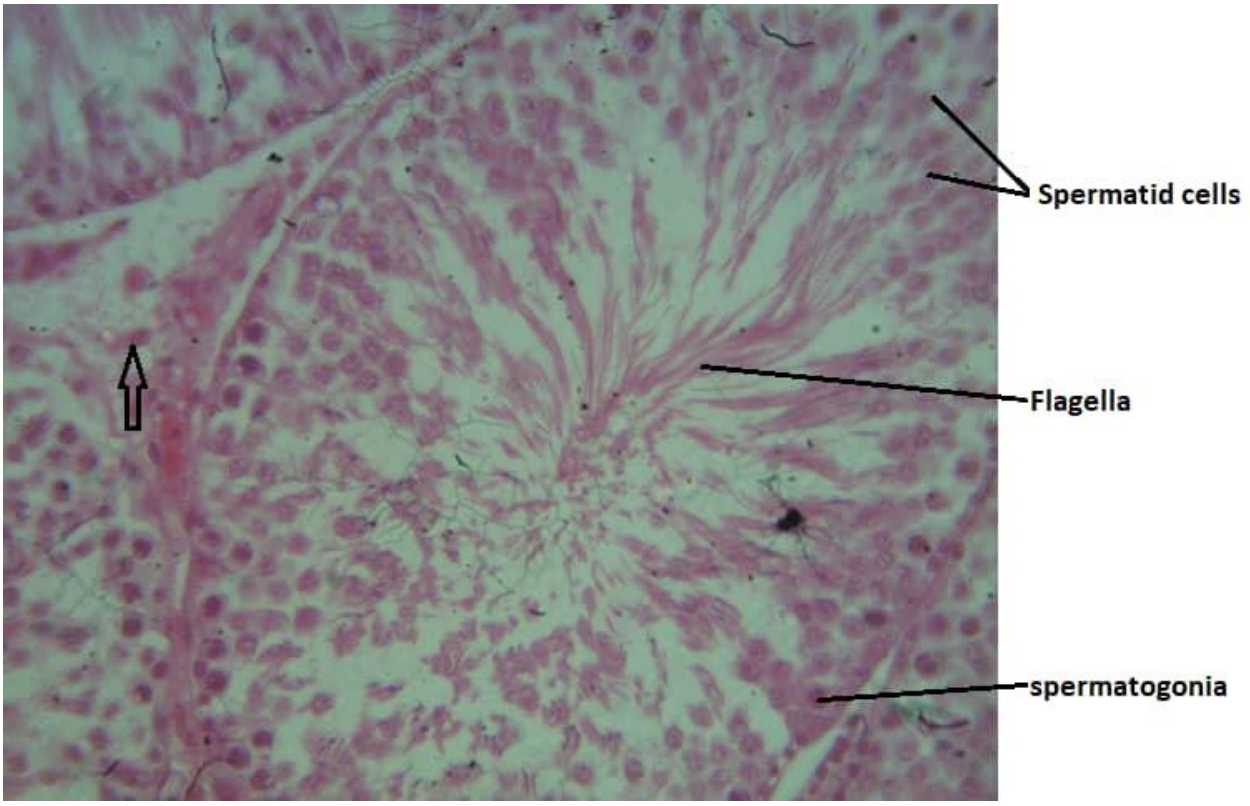

Figure 4.3 Control Young Group

The testes of young control rats exhibited normal tissue morphology characterized by numerous spermatozoa with flagella within the lumen of the seminiferous tubules. Distinct layers of spermatogonia and spermatids were observed. Most spermatid cells possessed active nuclei without visible nucleoli, indicating normal cellular activity. No histopathological alterations were detected.

4.2.3 Aged Atrazine -Treated Group

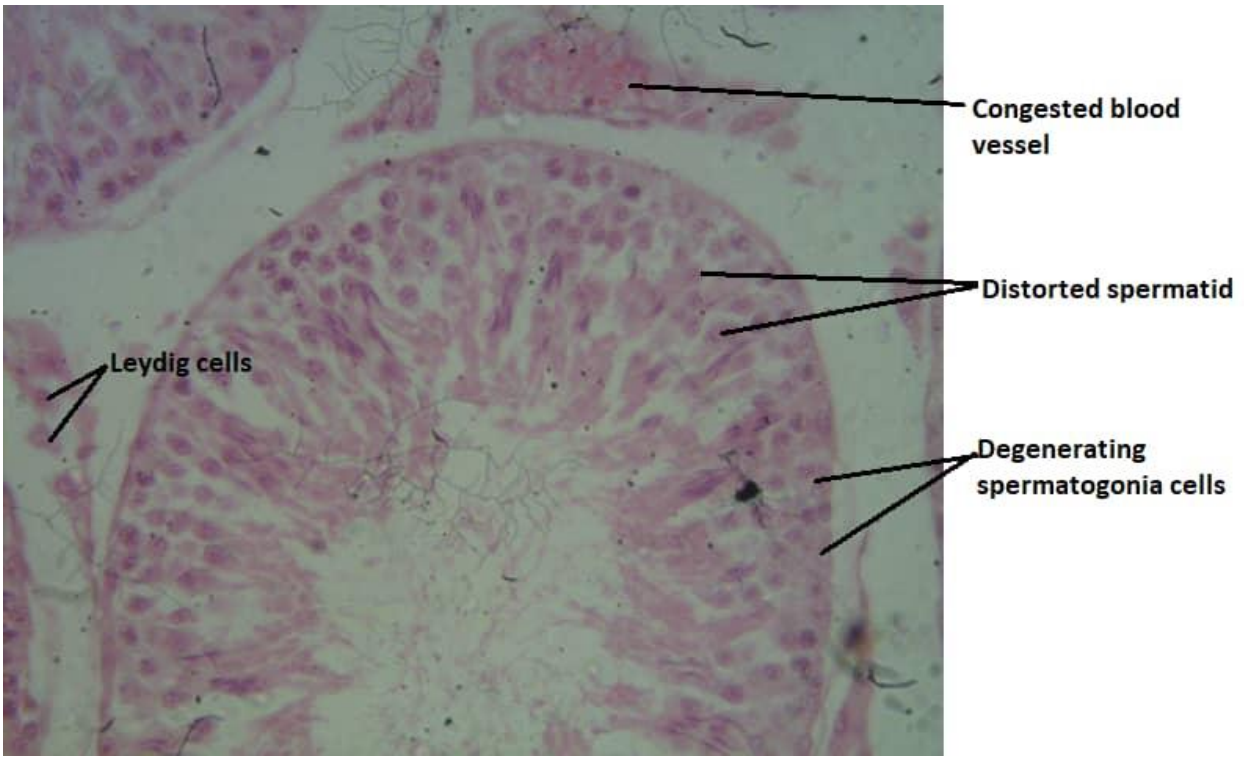

Figure 4.4 Aged Atrazine -Treated Group

Histological examination of the adult atrazine-treated group revealed marked pathological alterations. Distortion of the spermatogonia and spermatid cell layers was evident. Leydig cells within the interstitial appeared distorted, and congested blood vessels were observed. The seminiferous tubules exhibited disrupted architecture, indicating impaired spermatogenesis and testicular damage following atrazine exposure.

4.2.4 Young Atrazine -Treated Group

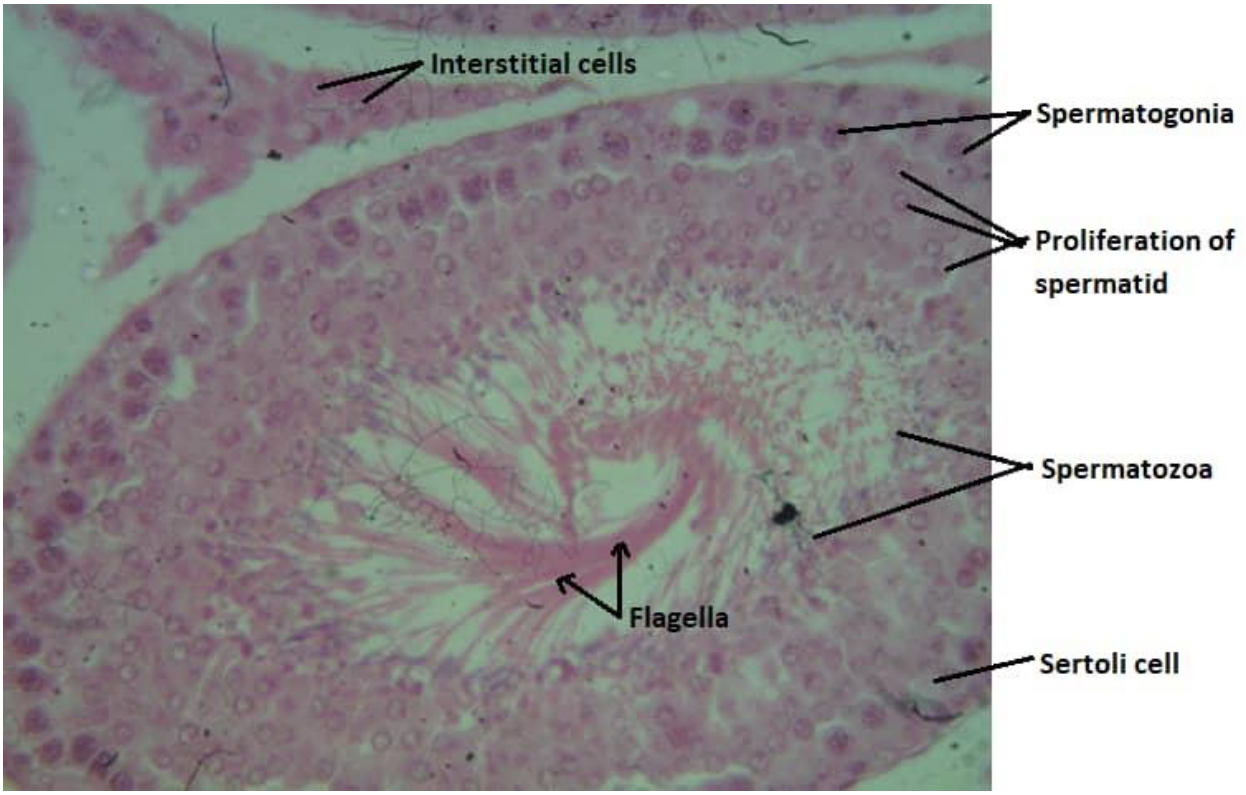

Figure 4.5 Young Atrazine -Treated Group

The testes of young rats exposed to atrazine showed relatively preserved histological features. Numerous spermatozoa with intact flagella were present within the lumen. Spermatid and spermatogonia cell layers appeared proliferative, while Sertoli cells were clearly observed between the germ cell layers. Leydig cells maintained a distinct appearance, suggesting that young animals were less susceptible to atrazine-induced damage than adult rats.

4.2.5 Aged Atrazine Plus carrageenan Group

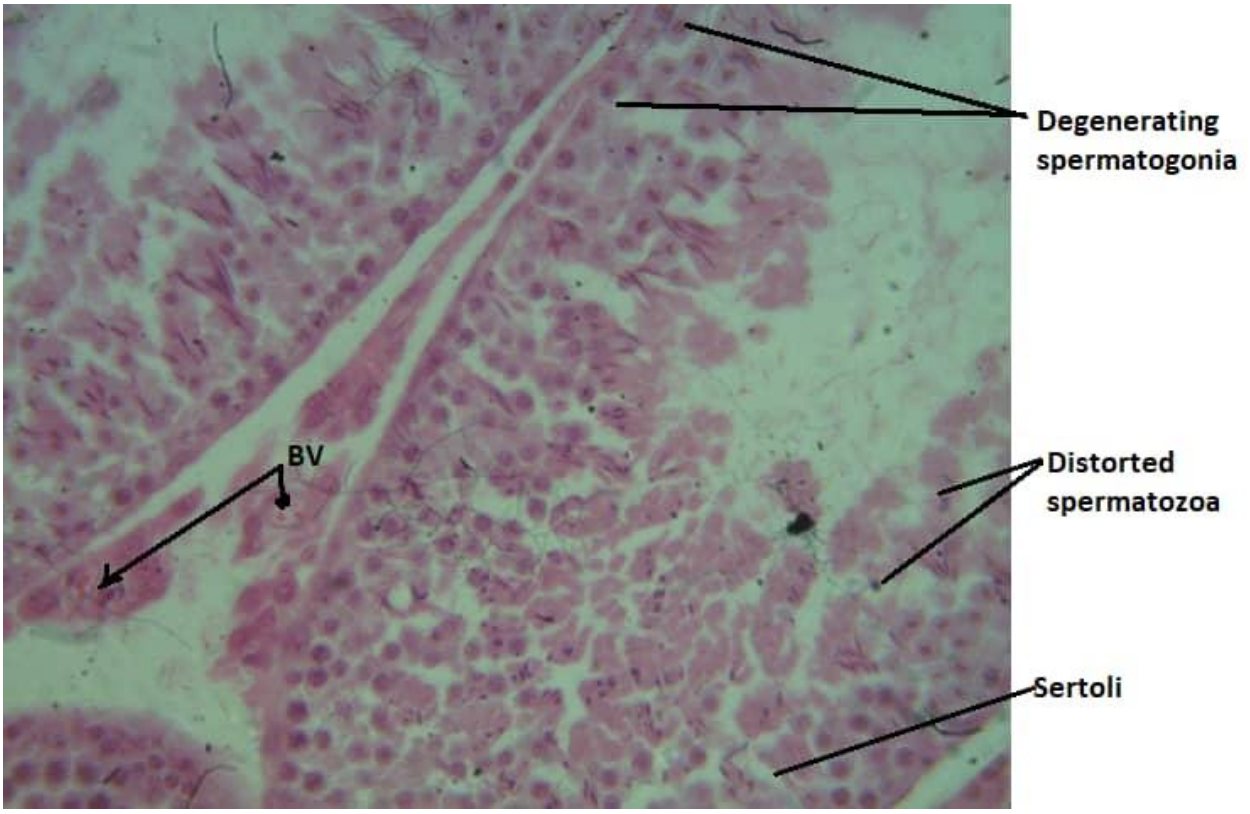

Figure 4.6 Aged Atrazine Plus carrageenan Group

The adult rats treated with atrazine and carrageenan showed severe histopathological changes. Distorted spermatozoa with detached flagella were observed within the seminiferous tubules. Degeneration of spermatogonia and spermatid cells was evident. Congested blood vessels were present within the interstitial spaces, indicating inflammatory and degenerative changes. The tissue architecture was markedly disrupted.

4.2.6 Young Atrazine Plus carrageenan Group

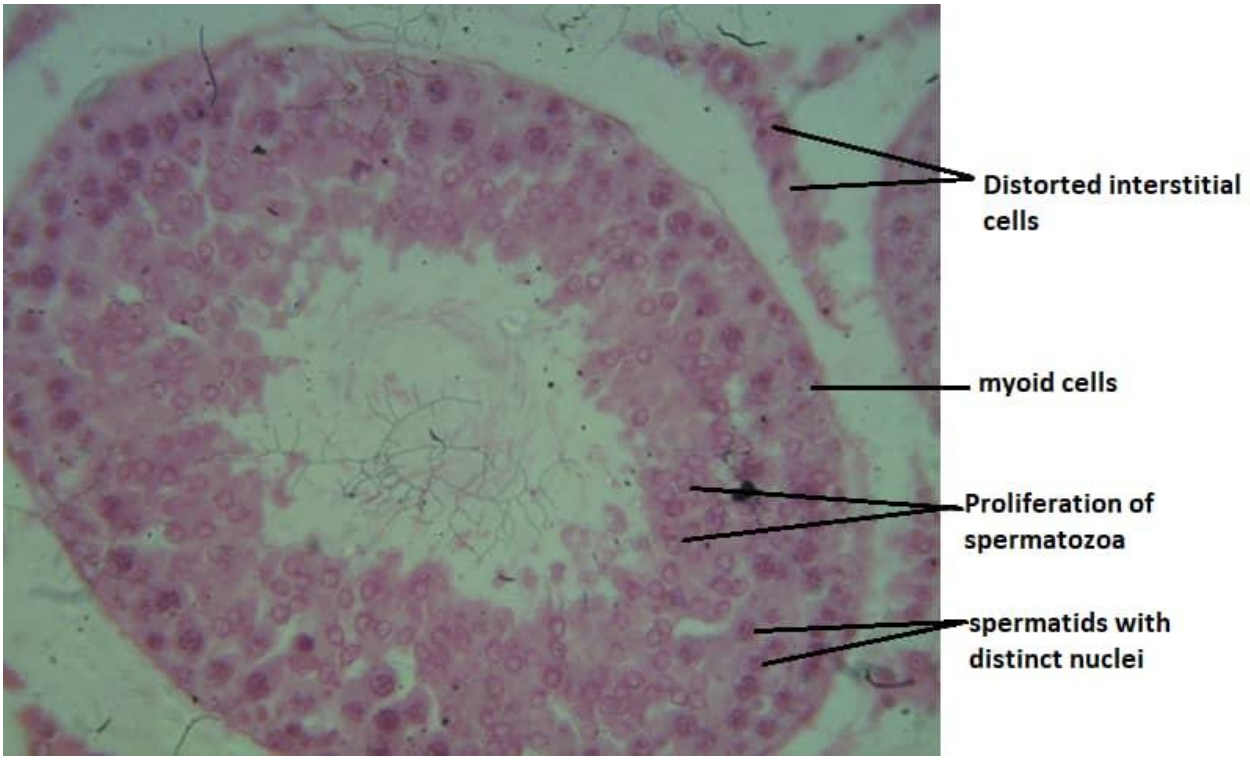

Figure 4.7 YoungAtrazine Plus carrageenan Group

Histological examination of the testes from young rats treated with atrazine and carrageenan revealed numerous spermatocytes, spermatids, and spermatogonia with distinct nuclei. However, the Leydig cells appeared distorted and exhibited pyknotic nuclei, suggesting cellular degeneration. Despite the relatively preserved seminiferous epithelium, evidence of interstitial damage was observed.

CHAPTER FIVE

DISCUSSION CONCLUSION AND RECOMMENDAT

5.1 Discussion

One of the objectives of this research was to evaluate the differential effects of atrazine alone or in combination with carrageenan on the testis histology of aged and young rats. Observations in this study revealed a differential damage pattern in response to atrazine exposure between the aged and young male rats, with the former having higher degree of alterations. Normal histological morphology was obtained in aged and young control rats in terms of well organized

seminiferous tubules, prominent germinal epithelium, abundant spermatozoa and normalLeydig cells, which are an indication of the health status of the animal's tissues.

Atrazine had significant impact on the testes of the aged rats, evidenced by the disorder of spermatogenic cells' arrangement, abnormal Leydig cells and congestion of blood vessels in the interstitial compartment, indicative of impacts on spermatogenesis and steridogenesis.

Distortions of spermatogonia and spermatids might have resulted in decreased production of spermatozoa and subsequent reduction in fertilization capacity. Congestion of blood vessels within the interstitial part of the seminiferous tubule might have indicated increased activity in tissues due to the damaging effect of atrazine and it also suggested inflammatory response and injury of the tissue. Contrary to aged ones, young rats from the atrazine treated group showed relatively normal and intact testes with numerous spermatozoa and distinguishable Sertoli cells as well as regular organization of the germinal layers. The study, based on data from both testosterone concentration and testicular histomorphology, supports the evidence that atrazine and atrazine in presence of carrageenan, influence the hormones level and testicular morphology of young and old male rats in a time-, age-, and dose-dependently manner.

With respect to the hormonal change, young control group exhibited lowest testosterone levels among the other young groups while the testosterone in young rats treated with Atrazine + Carrageenan was the highest among the groups in the study and, also, a significant decrease in testosterone was detected in young control groups as compare to control group of aged male rats. Also Atrazine treated aged group exhibit a significant increase in serum testosterone levels when compared to Atrazine + Carrageenan treated young groups. These findings were similar in pattern but differs from other studies. Many studies indicated that atrazine induced significant changes in serum and testicular testosterone concentration via suppression of genes expression

coding for some key enzyme including; STAR (steroidogenic acute regulatory protein), androgen binding protein, and steroidogenesis in the testis (Singh *et al*.,2020; Sharma *et al*., 2021; Zhang *et al*.,2022).

Contrary, the rise in serum testosterone concentration in aged atrazine treated groups was an unusual outcome compared to widely accepted atrazine effects that have been widely proven and may suggest a compensatory response in aging animals with alterations in the normal hormonal feedback mechanism. Similarly, the elevated testosterone level in Atrazine + Carrageenan young groups indicates an unusual response to the combination and suggests that even inflammatory conditions may impact the endocrine response in a contrary manner in young male rats. Similar trends to these hormonal changes were observed in some studies (Guimares-Ervilha *et al*., 2024), whereas the observed histomorphological differences were closely comparable with some previous studies that reported damage to male reproductive tract after exposure to various chemicals (Cooper *et al*., 2025).

The aged atrazine treated and Atrazine + Carrageenan group exhibited severe and higher damage to the seminiferous tubules with notable abnormalities of the germinal and spermatogenic cell layers, the Leydig cells' pyknosis and rupture, degeneration of the spermatozoa and vascular congestion, in comparison with aged and young control rats. Similar findings were previously described by many scientists (Zhu *et al*.,2022; Takalani *et al*., 2025). Also Leydig cells and other interstitial components like vasculature in aged atrazine treated animals were affected, indicating potential for atrazine exposure at the interstitial level of testes. However, in young male rats atrazine treatment result in significantly less disturbed seminiferous tubule structure than the aged rats even though they received the same amount of treatment, they still demonstrated some damage such as degeneration of seminiferous tubules, some distorted seminiferous epithelial

arrangement and disarrangement of germinal epithelium with prominent germ cell apoptosis in Atrazine + Carrageenan groups. This difference in age may attributed to the development of germinal epithelium and relatively low rate of steroid production in young animals. This age may not represent a vulnerable period for germinal damage in rodents.

Although, young rats' Leydig cells showed some abnormality in shape and cellular nuclei morphology, with less numerous interstitial compartment. Thus, overall findings strongly support the general conception that atrazine have adverse effects on male reproduction that impacts spermatogenesis and the architecture of testes (Takalani *et al*., 2025). Notwithstanding the reduction in male hormones and consequent damage on testis and related biological function due to the administration of chemicals on male reproductive system have already been published by many scientist (Song *et al*.,2014; Zhu *et al*., 2021; Cooper *et al*., 2025; Guimares-Ervilha *et al*., 2024; Takalani *et al*., 2025)

5.2 Conclusion

This present study has shown that atrazine induces histopathological changes on testes of adult male animals compared to young Male rats, whereby aged ones has shown more destructive changes in comparison with young male rats with the more severe effects observed in aged rats treated with combination of atrazine and carrageenan.

5.3 Recommendations

1. It is recommended that further studies should be carried out to assess mechanism behind reproductive toxicity of atrazine and carrageenan on the testes of male animals at different ages.
2. Study of oxidative stress parameters and hormone levels in future is recommended in assessing the reproductive performance.

3. It will be worth to perform long-term studies for evaluating chronic reproductive impact.

4. The study recommends that general awareness about potential adverse effect of indiscriminate usage of herbicides like Atrazine should be created in the general public.

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