AKENTEN APPIAH-MENKA UNIVERSITY OF SKILLS TRAINING AND ENTREPRENEURIAL DEVELOPMENT

EFFECTS OF AQUEOUS GARLIC EXTRACT ON THE REPRODUCTIVE FUNCTION AND HEMATOLOGY OF MALE WISTAR RATS TREATED WITH MONOSODIUM GLUTAMATE

CHARLES NYARKO OSEI

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BY

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DECEMBER, 2023

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this university or elsewhere.

Charles Nyarko Osei

Signature: ……. Date: …..……

Supervisor's Declaration

I hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development.

Dr. Holy Kwabla Zanu

Signature: ……. Date: …..……

ABSTRACT

This study aimed to evaluate the effects of different doses of aqueous garlic extract on the reproductive function and hematology of male Wistar rats treated with monosodium glutamate. A total of 25 male Wistar rats, with an average body weight of 135 g, were randomly divided into 5 treatment groups, with 5 replicates per group. Treatment 1 (control) was administered normal saline. Treatment 2 was given 120 mg/kg BW of MSG. Treatment 3 was administered 120 mg/kg BW of MSG and 500 mg/kg BW of AGE. Treatment 4 was administered 120 mg/kg BW of MSG and 750 mg/kg BW of AGE. Treatment 5 was administered 120 mg/kg BW of MSG and 1000 mg/kg BW of AGE by oral gavage for 21 days. The study assessed the sperm count, motility, and morphology of epididymal sperm, white blood cells (WBC), granulocytes (GRA), red blood cells (RBC), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), hemoglobin (HGB), lymphocytes (LYM), platelets (PLT), platelet large cell ratio (P-LCR) counts, reproductive organ's weight, and testis histomorphometry at the end of the experiment. There was no significant difference in sperm count among the treatments ($p > 0.05$). Sperm motility increased in treatments 4 and 5 ($p < 0.05$). Immotile sperm were high in treatment 2. Treatments 3, 4, and 5 had a high level of normal sperm morphology. The weights of both the left and right testes were higher ($p < 0.05$) in treatment 5. Treatment 2 had decreased levels of WBC, RBC, and P-LCR in comparison to the control group. However, treatment 2 recorded increased levels of GRA. There were deformities in the seminiferous tubules of treatment 2, but they were considerable in treatments 3 and 4. Treatment 5 saw a reduction

in the size of the seminiferous tubules. The research findings suggest that garlic has a dose-dependent impact on mitigating the negative effects of MSG on the reproductive function and hematology of male Wister rats.

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DEDICATION

This work is dedicated to Almighty Jehovah and My family.

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ABBREVIATIONS

AGE	Aqueous Garlic Extract
MSG	Monosodium Glutamate
BW	Body weight
WBC	White Blood Cells
RBC	Red Blood Cells
P-LCR	Platelet Large Cell Ratio
HCT	Haematocrit
MCV	Mean Corpuscular Volume
MCHC	Mean Corpuscular Hemoglobin Concentration
MCH	Mean Corpuscular Hemoglobin
HGB	Hemoglobin
LYM	Lymphocytes
PLT	Platelets
GRA	Granulocytes
WHO	World Health Organization
SDG	Sustainable Development Goal
EFSA	European Food Safety Authority
ADI	Average Daily Intake
FDA	Food and Drug Administration
EFSA	European Food Safety Association
GRAS	Generally Recognized As Safe
EDTA	Ethylene Diamine Tetra Acetic Acid

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Infertility is a medical disorder that affects either the male or female system of reproduction, resulting in the inability to get pregnant after engaging in consistent, sexual activity without protection for a period of 12 months or more (Szkodziak et al., 2020). Infertility is acknowledged by the World Health Organization as a matter of public health concern (Kundu et al., 2023). The frequency of infertility among couples exhibits significant variation across different regions worldwide (Eisenberg et al., 2023). However, a consistent pattern emerges, with developing countries generally seeing a higher proportion of infertile couples compared to developed ones (Dudel & Klüsener, 2021).

In the United States, the prevalence of infertility is observed in approximately 12.5 % of couples attempting to conceive for the first time and in approximately 16.7 % of couples desiring a second child. However, it is important to note that in sub-Saharan Africa, the prevalence rates exhibit significant variation (Thoma et al., 2013). Rates in certain sub-Saharan African nations have been reported by different authors to range from 21 % to 30 % (Ombelet et al., 2008; Ochako et al., 2015). According to Laryea (2012), the current estimations put the prevalence of infertility in Ghana within the range of 11.8 % and 15.8 %. The observed disparity in the prevalence rates of infertility between developed and developing nations may be attributed, in part, to significant differences in the availability

of resources dedicated to the early detection, diagnosis, and treatment of this condition (Kamel, 2010).

Male infertility has a variety of underlying causes, such as anatomical and genetic flaws, testicular disease and injury, sperm abnormalities, hormone imbalances, ageing, and environmental and lifestyle-related variables. Bad lifestyle choices and unhealthy habits, including increased drug use, long-term drunkenness, wearing tight underwear, taking lots of hot baths, and leading sedentary lives, can impact sperm quality (D'Argenio et al., 2021). Sperm quality is influenced by sperm count (the number of sperm present in a certain volume), morphology (size and form), and motility (the sperm's capacity to move forward for an extended period of time) (Dogra et al., 2022; WHO, 2021). The primary cause of male infertility is commonly related to the inability to produce an adequate number of viable and motile sperm cells (Rahimi-Madiseh et al., 2020). As a result, the proper functioning of the testicles is identified as a crucial factor in male reproductive health. The flavor enhancer monosodium glutamate is utilized in many different dishes around the globe. Spices have been used by humans for thousands of years to enhance the taste, look, and food longevity. These spices have components that improve consumers' overall health. Spices that are grown naturally are both expensive and bulky. Due to this, the majority of customers now rely largely on artificial spices. The majority of artificial spices are known to include MSG as their active component. There is no specific recommended daily quantity for monosodium glutamate, despite its widespread promotion as a harmless food additive. MSG consumption is on the rise due to excessive MSG concentration in packaged

foods sold without labels. It has been hypothesized that using synthetic spices frequently could be hazardous to consumers' health.

Allium sativum is a natural spice and multipurpose vegetable that is commonly used to improve the flavor, taste, and aroma of a wide range of foods. According to Tudu et al. (2022), it can regulate the body's detoxification processes and is a dependable source of phytochemicals that have antioxidant properties. Many research studies have been published in the last ten years alone on the health advantages of garlic and its preparations. Many people consider it one of the best foods to eat in order to stay healthy because of its many beneficial effects. Garlic has earned a particular place in the traditions of many nations throughout the years as a potent preventative and healing medicine (Radovanović et al., 2023). Garlic has been extensively studied for its therapeutic properties in several settings, including in vitro experiments, animal and human clinical trials, and epidemiological reviews. Garlic and garlic preparations have a reputation for helping people and animals with lipid-lowering and anti-atherogenic issues (Ezeorba et al., 2022).

According to Bazaraliyeva et al. (2022), the Egyptian Cordex Ebers, which dates back to 1550 BC, mentions its use in treating tumors, headaches, and heart problems. Garlic has a long history of traditional medicinal use in various parts of the world, including the Near East, China, and India; it is also mentioned in the Quran and the Bible (Bazaraliyeva et al., 2022). This plant has caught the interest of modern medicine, specifically for its potential to treat and prevent cardiovascular disorders (Sahidur et al., 2023).

1.2 Problem Statement

Monosodium glutamate, a commonly consumed food additive, has been associated with impaired male fertility (Kayode et al., 2022). In Ghana, MSG is extensively utilized by commercial food operators, including restaurants, roadside food vendors, and local food sellers (often known as chop bars), as well as individuals, with the aim of improving the flavor of the prepared meal. Monosodium glutamate was initially brought from foreign countries into Ghana, but it has since undergone a shift in production as local companies have begun manufacturing MSG and distributing it in the open market. Within the hotel and restaurant industry, particularly among food operators, MSG is frequently used as a means to enhance flavor and taste, often without a comprehensive understanding of its potential health implications (Moldovan et al., 2021).

Dietary components and micronutrients are vital for reproductive system accurate functioning, according to research. (Ma et al., 2022). Research has demonstrated that male Wistar rats exposed to monosodium glutamate develop infertility due to its detrimental impact on sperm quality and quantity. Examples of these negative effects on the testis include hemorrhage, degeneration, and changes in the quantity and shape of sperm cells. Hajihasani et al. (2020) asserts that, having glutamate receptors in the reproductive system makes them a target for glutamate-induced harm, rendering them disposable under excessive glutamate levels (Miller et al., 2020).

Given the widespread usage of MSG as a food additive, it is crucial to understand and tackle the possible negative effects it could have on reproductive health. This highlights the need of understanding and addressing this issue for the public's overall welfare.

The reproductive system of men is vital to the continuation of the human race. The presence of any substance that has the potential to disrupt the regular functioning of a system, particularly if it is linked to infertility or other reproductive diseases, gives rise to significant concerns.

The available evidence indicates that alternative treatments have the potential to mitigate the impact of MSG. The addition of antioxidants such vitamin E, quercetin, and vitamin C to the diet of rats decreased the damage and genotoxicity caused by MSG, as reported by Farombi (2006). Waiz's (2015) findings also showed that vitamin C has a mitigating effect on the hepatotoxicity MSG causes in rats.

Due to its numerous applications in the prevention and treatment of human ailments, including cancer and cardiovascular disease, garlic earned a great deal of attention from modern medicine. Garlic may help restore testicular function and improve sexual function, according to research by Jiang et al. (2022). Research by Lim et al. (2022) indicates that, garlic has demonstrated ameliorative effects on a range of physiological processes. Garlic possesses a high concentration of organosulfur compounds, notably allicin, which demonstrate significant antioxidant attributes (Hossain et al., 2023). Free radicals, produced by oxidative stress and chemicals like MSG, can do damage to the body, but these

characteristics help mitigate that. The anti-inflammatory properties of garlic suggest it could have a role in controlling the body's inflammatory response (Shao et al., 2023). Garlic has been observed to possess immunomodulatory properties, which have the potential to augment the body's capacity to recognize and neutralize detrimental agents (Zugaro et al., 2023). The effects of different doses of aqueous garlic extract (AGE) on the reproductive function and hematology of male Wistar rats treated with MSG have not been extensively studied, although Ahmed et al. (2022) found that AGE mitigates the negative effects of MSG on Johnson's Scoring of fertility in adult Wistar rats.

1.3 Justification and Significance of the Study

Evaluating the impact of AGE on MSG could potentially lead to mitigating the deleterious effects of MSG on both the fertility and hematological well-being of males. This will go a long way to decrease the rate of infertility in men and curb the consequences that come with it.

Possible implications for human health and MSG usage in food products may arise from this study's findings. By assessing the effects of MSG on reproductive function and hematology in male Wistar rats, this study may provide insight into the potential harmful effects of MSG on human health, especially men's health. The outcomes of this study could assist researchers to better comprehend garlic's medicinal uses and its ability to regulate several bodily processes through natural processes.

Sustainable Development Goal (SDG) 3.7 aims to ensure universal access to sexual and reproductive healthcare services, including family planning, information, and education, by 2030 (Obisie-Nmehielle et al., 2022). This research could help attain this goal by providing valuable insight into the potential benefits of using AGE as a natural remedy for reproductive dysfunction. It may help in developing new treatment options for male infertility, which can ultimately contribute to improving sexual and reproductive health outcomes.

Similarly, by 2030, the goal of SDG 3.8 is to ensure that everyone has access to affordable, high-quality, safe, and effective healthcare, including protection from financial risk, and to healthcare services. (Ghebreyesus 2017). The findings of this research may help in attaining SDG 3.8 by providing evidence for the potential of using AGE as a natural and cost-effective treatment option for reproductive dysfunction in men.

1.4 Main Objective of the Study

The study primarily aimed to evaluate the effect of AGE at different doses on sperm production and hematology of male Wistar rats treated with MSG.

1.5 Specific Objectives

The specific objectives were to:

1. assess the count, motility, and morphology of epididymal sperm of male Wistar rats subjected to MSG and given varying quantities of AGE;

2. evaluate the reproductive organ weight of male Wistar rats administered with MSG and treated with different doses of AGE;
3. assess the hematological parameters of male Wistar rats treated with MSG and subjected to distinct levels of AGE;
4. evaluate the histomorphometry of the testes in male Wistar rats that were given MSG and treated with various dosages of AGE.

CHAPTER TWO

LITERATURE REVIEW

2.1 Monosodium Glutamate (MSG)

Monosodium glutamate is a commonly utilized substance that enhances the taste of food. Monosodium glutamate (MSG) is widely acknowledged for its umami taste, which was first identified as a distinctive flavor in Asian culinary traditions and then adopted by Western societies. Monosodium glutamate is a common ingredient in many meats, seafood, and vegetables (e.g., broccoli and tomatoes). MSG is one of the five basic tastes, along with bitter, sweet, sour, fish, and salt. According to Arapa et al. (2023), the presence of MSG contributes to the umami experience, hence augmenting the overall intensity of flavor and increasing the attractiveness of food.

According to Liu et al. (2021), MSG exhibits the highest level of umami intensity across various substances. In comparison to MSG, the umami properties of L-glutamic acid and its disodium salt analogue are not as prominent. Chemical changes, like esterification or amide synthesis, reduce the umami taste. Succinic acid, L-homocysteine acid, and L-aspartic acid are acidic analogues of glutamic acid that have been found to exhibit certain umami effects. According to Rachma and Saptawati (2021), theanine, which is a naturally occurring compound found in green tea, demonstrates umami characteristics. Moreover, it has been observed that cyclic derivatives of glutamic acids, such as pyroglutamic acid or ibotenic acid, exhibit umami characteristics when they possess a free carboxylic group (Wijayasekara & Wansapala, 2017).

It has been observed that individuals residing in European industrialized nations typically ingest an estimated quantity of 0.3-1.0 grams of MSG on a daily basis (Kayode et al., 2020). Despite the fact that most organizations concerned with food safety consider MSG to be safe, certain studies have raised concerns about its safety, especially when used for a long time. The issue arises from the recognition that endogenous glutamate is involved in important physiological processes and its connection to pathological disorders (Yudhana et al., 2022). In living things, glutamate does more than just provide energy; it also helps with protein metabolism, acts as a precursor to important metabolites, and is an excitatory neurotransmitter in the brain. Several research studies have linked high levels of glutamate to an increased likelihood of brain injury and the onset of chronic neurodegenerative diseases; they include Al-Agili (2020), Halim et al. (2020), and Rachma and Saptawati (2021).

All three of these authorities have established that MSG is safe for human consumption, so it is listed in the Generally Recognized as Safe (GRAS) category: the FDA in the United States, the EFSA in Europe, and the JECFA, the Joint FAO/WHO Expert Committee on Food Additives. Nonetheless, a number of authorities have argued that new information on toxicity tests calls for an update to the GRAS certification criteria. In 2006, the ADI for MSG and other food additives was established by the European Food Safety Authority (EFSA) using the no-observed-adverse-effect level (NOAEL) as its basis.

2.1.1 Effects of Monosodium Glutamate on Health

MSG's safety has been a subject of longstanding concern and deliberation. The purpose of this review is to conduct a comparative analysis of various studies that have examined the toxicological effects of MSG on different physiological aspects, specifically focusing on its impact on reproductive function (Bayram et al., 2023; de Paula et al., 2021; Liu et al., 2021; Mostafa et al., 2021; Okoye et al., 2016; Wijayasekara & Wansapala, 2017; Zhao et al., 2014), hematological parameters (Kolawole, 2013; Nakama et al., 2021; Nasir, 2019; Nusaiba et al., 2018; Subandiyono & Hastuti, 2022), and potential protective agents.

A number of research investigations have explored the impact of MSG on reproductive function, predominantly employing animal models. Certain physiological factors, which include sperm count, sperm morphology, sperm motility, testicular weight, and body weight, are altered by exposure to MSG, according to previous investigations (Okoye et al., 2016; Wijayasekara & Wansapala, 2017; Mostafa et al., 2021; Liu et al., 2021; Bayram et al., 2023; de Paula et al., 2021; Zhao et al., 2014).

Studies investigating the impact of MSG on hematological indices, including white blood WBC, RBC, HGB, and PLT, have yielded inconclusive findings. While certain research has shown modifications in these parameters, other investigations have failed to observe any statistically significant variations. The observed inconsistencies can be ascribed to differences in the administration of dosages, utilization of animal models, and applied research procedures (Kolawole, 2013; Nakama et al., 2021; Nasir, 2019; Nusaiba et al., 2018; Subandiyono & Hastuti, 2022).

2.1.2 The Influence of Monosodium Glutamate on Body Weight

Aghaei et al. (2021), Hazzaa et al. (2020), Nakama et al. (2021), Nasir (2019), Nurmasitoh et al. (2018), Nusaiba et al. (2018), and Subandiyono & Hastuti (2022) are among the animal studies that have examined the link between MSG consumption and weight alterations. Some of these studies have suggested that high doses of MSG may lead to weight gain in animal models (Hazzaa et al., 2020; Nurmasitoh et al., 2018). One hypothesis is that MSG intake may enhance the perception of umami taste in foods, leading to increased food intake and calorie consumption. This, in turn, can contribute to an energy surplus and subsequent weight gain over time. Another possible explanation is that MSG may affect certain brain pathways related to hunger and satiety, potentially disrupting the body's natural regulation of food intake (Nakama et al., 2021). But it is imperative to remember that human outcomes are not always directly applicable to studies done on animals. Research on a connection between MSG consumption and changes in body weight in humans has shown contradictory results. While some research has failed to find an interaction between MSG consumption and weight gain in humans, other studies have hinted at a possible link between the two.

In a study employing a randomized controlled trial (RCT) design, for 12 weeks, the researchers studied the consumption of MSG on participants' body weight. The partakers were allocated into two distinct groups: one group was administered a moderate dosage of MSG at a rate of 3 grams per day, while the other group received a placebo substance (Hazzaa et al., 2020; Subandiyono & Hastuti, 2022). The results showed no statistically significant disparities in the changes of body weight between the two groups that were

studied. The findings from this experiment suggest that healthy individuals may not be directly affected by moderate MSG use in terms of body weight. On the other hand, a longitudinal cohort research was conducted to investigate the food patterns and fluctuations in body weight among a substantial sample of individuals over an extended period of time. A positive association between higher MSG consumption and weight gain was seen in the study's subjects. The weight gain was more noticeable in individuals that took higher amounts of MSG compared to those who consumed smaller quantities. Nevertheless, the observed correlation was rather small and might potentially be affected by several confounding variables, such as food habits and personal lifestyle preferences (Kolawole, 2013).

A rodent-based experimental investigation was carried out to examine the impact of long-term intake of MSG on body weight. During the period of the experiment, rats were administered with MSG as a dietary supplement. Individuals treated with MSG gained considerably more weight than the control group. It was also observed that the rats given MSG ate more calories than the control group, suggesting that their eating habits were changed (Nasir, 2019). The outcomes of this study show that prolonged exposure to MSG in animal models may result in increased body weight due to alterations in the regulation of hunger. This study set out to determine whether there is any correlation between MSG consumption and BMI in a human population through a cross-sectional analysis. This study's findings are supported by the outcomes of Nasir (2019), Nakama et al. (2021), and Nusaiba et al. (2018).

There is no agreement among the results of the various research that have looked at how MSG affects body weight. While some studies in animals and humans have shown a link between MSG and weight gain, other studies have found no such association when looking at randomized controlled trials or cross-sectional data. The divergent outcomes may be ascribed to various reasons, including the methodology employed in the study, the magnitude of the sample, the distinctive attributes of the population under investigation, and the inherent heterogeneity in individuals' reactions to the consumption of MSG. In light of the intricate nature of the subject matter, further comprehensive investigation, encompassing extended-term studies with varied cohorts, is vital to ascertain a definitive correlation between the consumption of MSG and alterations in body weight. For a complete understanding of how MSG may affect weight, it is also important to think about how other food and lifestyle choices may have a role. Therefore, it is crucial to emphasize the importance of advocating for a well-rounded diet and a lifestyle that promotes good health in order to effectively manage weight and preserve general well-being, irrespective of the consumption of MSG.

2.1.3 Effects of Monosodium Glutamate on Sperm Count

Fertilization and the subsequent development of offspring depend on sperm, which is produced and transported via a complex network within the adult male reproductive system. The male reproductive system is made up of the testes, which are joined to the penis by means of the vas deferens and their respective epididymis. (Anbarkeh et al., 2019). The process for producing germ cells occurs within the testes, followed by their passage through the epididymis, during which they undergo maturation and acquire motility

(Airaodion, 2019). After this, during copulation, the sperm are released into the female reproductive canal as semen, where they complete their maturation process, which is called capacitation. As mentioned by Ogbuagu et al. (2019), this procedure gets the sperm ready for fertilization.

Medical conditions characterized by impaired performance of any part of the reproductive system in men are collectively known as male reproductive dysfunction. The aforementioned phenomenon can potentially provide substantial implications for an individual's holistic well-being and potentially give rise to subsequent ailments (Cláudia et al., 2015). Male reproductive dysfunction can be ascribed to a range of issues, including hormonal disorders, oxidative stress, testicular inflammation, endocrinal disturbances, sexually transmitted diseases infections, heat exposure, smoking, alcohol consumption, illnesses, injuries, chronic health conditions, exposure to heavy metals and radiation, genetic defects, way of life, and diet (Kianifard, 2016). Considerable knowledge has been acquired regarding the determinants of male reproductive failure. However, the possible effects of dietary constituents, specifically Monosodium Glutamate (MSG), on sperm count and male fertility, continue to be a subject of continuous investigation and discussion. There have been a lot of studies looking at the link between MSG consumption and sperm count alterations in humans and animal models. Comprehending this correlation is of utmost importance due to its substantial ramifications for male reproductive health and its potential to inform dietary guidelines for persons with fertility concerns (Ashaolu et al., 2011; Cx et al., 2021; Jubaidi et al., 2019; Manufacturing & Farming, n.d.; Umeh & Chinko, 2023).

Many studies involving animals have looked into the link between MSG consumption and changes in sperm count, yielding valuable insights into the potential effects on male reproductive health. In a study done by Ashaolu et al. (2011), rats were subjected to a high dosage of MSG (200 mg/kg) for 56 days. The group exposed to MSG had significantly fewer sperm than the control group, according to the results. The same is true for rabbits; one study found that oral administration of 10 mg of MSG per kg of body weight for consecutive 28 days had the same effect. In their study, Umeh and Chinko (2023) found that rabbits treated with MSG had significantly fewer sperm than the control group.

The duration of oral intake of MSG, on the other hand, varied across studies. In particular, two studies used oral dosing for 28 days and 42 days, respectively, whereas the third trial lasted just 14 days. The two aforementioned studies reported statistically significant decreases ($p < 0.05$) in sperm count when comparing the group exposed to a high dosage of MSG to the control group. Furthermore, a research investigation that utilized intraperitoneal (IP) injections as the method of MSG administration observed noteworthy ($P < 0.0001$) declines in sperm count after both 15 and 30 days. The levels of sperm count control displayed significant variability when assessed in terms of total sperm count (ranging from 216.31×10^6 to 925.56×10^6), while demonstrating relatively less variability in terms of sperm concentration values (ranging from 36.88×10^6/ml to 49.90×10^6/ml). Moreover, numerous animal research has investigated the mechanisms that underlie the observed alterations in sperm count. Kayode et al. (2020) identified that disturbances in the axis of the hypothalamic-pituitary, hormonal abnormalities, and heightened oxidative stress may be factors in the reduction of sperm production.

2.1.4 Monosodium Glutamate's Effect on Sperm Motility

The extant collection of literature examining the impact of MSG on sperm motility comprises investigations undertaken in both rodent models and human participants. While the existing body of research offers useful insights, it is necessary to acknowledge the limits and differences present in the studies, such as the variations in the dosage levels employed. According to a study conducted by Farhat (2021), there was an observed correlation between increased intake of food items containing MSG, such as fast food and processed meat, and a decrease in sperm motility. Nevertheless, the investigations did not involve the administration of precise and regulated doses of MSG. Similarly, Yang et al. (2023) found that in a broad Asian population, sperm motility was negatively correlated with greater use of snack foods containing MSG. However, it is noteworthy that the study did not delineate a precisely regulated dosage of MSG.

The administered doses of MSG exhibited variability across the animal models utilized in the investigations. For ten days in a row, adult male Wistar rats were orally dosed with 4 grams of MSG per kilogram of body weight. A study that was conducted by Cx et al. (2021), MSG was administered orally to rats at the pubertal stage. Over the course of 8 weeks, a dosage of 5 g/kg of body weight was used. In their study, Sabiu et al. (2016) administered an oral dose of MSG at a concentration of 4 mg per 100g of body weight to Wistar rats for a duration of 30 consecutive days. In addition, Rahman and colleagues (2018) administered MSG orally to mice over a period of 30 consecutive days, with a dosage of 500 mg/kg body weight. In a study conducted by Farhat (2021), rabbits were administered oral MSG at a dosage of 1.25 g/kg body weight over a period of 30

consecutive days. The aforementioned experiments conducted on animals consistently revealed an adverse impact of MSG on the movement of sperm, as observed within the prescribed dosage schedules (Akataobi, 2020; Dixit et al., 2014; Farhat, 2021; Haddad et al., 2021; Padrón-González et al., 2019; Tordoff et al., 2012).

The available evidence from both human research and animal models indicates potential adverse impacts of MSG on sperm motility. However, it is crucial to acknowledge the inherent constraints of these investigations. The measurement of MSG intake in human research was primarily based on self-reported dietary information, a method that is susceptible to biases and mistakes. Furthermore, the observed discrepancies in the dosage levels employed in animal research underscore the necessity of establishing standardized and controlled doses in order to accurately evaluate the effects of MSG on sperm motility. Based on the existing body of research encompassing both human studies and animal models, the collective data indicates a plausible detrimental effect of MSG on the motility of sperm. The notion is supported by the discovery of diminished sperm motility in animal tests conducted using certain dosage regimens. In order to further our understanding of the impact of MSG on human sperm motility, it is recommended that forthcoming studies focus on utilizing regulated quantities of pure MSG and implementing rigorous procedures to reliably evaluate sperm motility.

2.1.5 Monosodium Glutamate's Impact on Sperm Morphology

The examination of sperm morphology holds great importance in assessing male fertility, since any deviations in the structure of sperm can have a substantial impact on reproductive

outcomes. There has been growing apprehension regarding the potential health hazards associated with MSG, a widely utilized taste enhancer, with specific attention given to its impact on reproductive health. Numerous animal studies consistently indicate an inverse correlation between the use of MSG and the morphology of sperm. The consumption of elevated levels of MSG has been associated with the occurrence of atypical sperm morphology, characterized by modifications in the form of the sperm head or tail, increased incidence of abnormalities, and diminished rates of fertility (Dong & Robbins, 2015; Iamsaard et al., 2014; Nosseir et al., 2012; Sakr & Badawy, 2013).

The possible effect of MSG on sperm morphology has been a focus of a great deal of animal research. Due to the diverse range of methodologies and dosages used in this field's study, evaluating their outcomes has proven to be quite complex. Dong and Robbins (2015) conducted a study wherein male mice were orally treated high dosages of MSG. The investigators conducted observations on atypical sperm morphology, which was distinguished by deviations in the structure of the head or tail. Furthermore, it was noted that the reproductive rates of the mice exposed to MSG were lower than usual, which could indicate a link between MSG consumption and impaired sperm function. Male rats were given a 5 % MSG solution in their water to consume in the research by Nosseir et al. (2012). The experimental study revealed a higher prevalence of atypical sperm morphology, indicating a possible interference with the process of spermatogenesis as a consequence of MSG intake. In their study, Sakr and Badawy (2013) conducted an investigation to study the influence of different amounts of MSG on male rabbits. The chemical was administered to the rabbits through oral gavage. The study conducted by the researchers revealed a

positive correlation between the dosage administered and the occurrence of sperm abnormalities, specifically pertaining to flaws observed in the head and tail regions. The aforementioned findings offer more evidence to substantiate the potential influence of MSG on the morphology of sperm. Iamsaard et al. (2014) performed an independent study in which male mice were given 250 mg/kg of MSG orally. The researchers noted a notable decline in the typical form of sperm and an elevation in atypical sperm exhibiting anomalies in their head and tail, indicating that MSG could have an adverse impact on the quality and structure of sperm. In a study conducted by Alalwani (2014), The male rats were given 4 grams of MSG per kilogram by mouth. Their research concluded that there were obvious distinctions in the shape and structure of sperm, particularly in the areas of the head and tail. These differences imply possible modifications in the sperm maturation process and its functional competence.

There is a negative association between MSG consumption and sperm morphology, according to the current evidence from animal research. Elevated levels of MSG have been observed to interfere with the process of spermatogenesis, resulting to significant modifications in the shape of sperm heads and tails. These abnormalities have the potential to adversely impact fertility rates by reducing the ability to conceive. Nevertheless, it is crucial to recognize the inherent constraints associated with animal studies, including disparities within species, fluctuations in doses, and variances in assessment methodologies. Hence, it is advisable to use caution when generalizing these findings to the realm of human reproductive health. Additional investigation is required in order to get a more comprehensive comprehension of the influence of MSG on the morphology of

human sperm. In order to establish more conclusive findings, it is imperative to conduct meticulously constructed human studies that incorporate uniform dosing, thorough evaluations of sperm morphology, and comprehensive long-term follow-ups.

2.1.6 Testicular Weight Changes Induced by Monosodium Glutamate

The potential negative impact of MSG, a frequently employed dietary additive, on testicular health has generated significant concern. The present analysis examined and compared the outcomes of five distinct research papers that investigated the potential influence of MSG on testicular weight. This review centers its attention on the dosage levels, length of treatment application, and observed effects in each study.

The reviewed studies consistently reveal a decrease in testicular weight caused by MSG across several animal models. The administration of higher dosages of MSG and the extension of treatment durations consistently yielded a more notable reduction in the size of the testes. The present comparative investigation highlights the correlation between the dosage of MSG intake and its influence on the health of the testes. In their study, Ashaolu et al. (2011) tested at how MSG affected the reproductive system of male rats. The study encompassed a protocol wherein male Sprague-Dawley rats were administered MSG orally on a daily basis within a 28-day timeframe. The study's results suggested that the addition of MSG in the diet contributed to a drop in testis weight, potentially due to a reduction in the density of germ cells and spermatids abundance. Furthermore, a decline in the weight of the epididymis was seen, which can be attributed to a drop in sperm storage resulting from a reduction in sperm production. Disturbances in reproductive hormones, namely

testosterone, may potentially be associated with the degradation of germinal cells in the tubule lining (Ashaolu et al., 2011).

Jubaidi et al. (2019) studied the effects of oral administration of MSG at a dosage of 150 mg/kg/day for 21 days in Sprague-Dawley rats. Testicles in the MSG-treated group of rats were significantly smaller than those in the control group, suggesting that MSG reduced testicular size. The effects of oral administration of 300 mg/kg/day of MSG for 30 days in male Wistar rats were explored by Kianifard et al. (2019). Kianifard et al. (2019) found that testicular weight was significantly reduced, lending credence to the idea that long-term exposure to MSG negatively impacts testicular dimensions.

A research investigation conducted by Ismail (2012) involved the use of Wister mice as subjects. For fourteen days, the mice were given various doses of MSG orally, namely 100, 200, and 300 mg/kg/day. The investigators found that the amount of weight loss in the testicles was dose-dependent. This finding highlights the considerable influence of even minimal amounts of MSG on the size of the testes in this particular strain of mice.

Abdollahzadeh et al. conducted an inquiry in 2017 to examine the performance of Sprague-Dawley rats after they were administered 200 mg/kg/day of MSG orally. The rats were exposed to MSG for several durations, specifically 7, 14, 21, and 28 days. The study discovered that prolonged exposure to MSG had a cumulative impact on the size of the testicles, as it was seen that the weight of the testicles fell more severely with longer treatment durations. In their study, Kianifard et al. (2019) conducted research on guinea

pigs, where they orally administered a substantial dosage of 500 mg/kg/day of MSG for a duration of 60 days. The results showed that after prolonged exposure to MSG, the testes' weight greatly reduced (Kianifard et al., 2019). The study emphasized that the combination of higher dosages and longer treatment durations had the most pronounced effect on the size of the testes in guinea pigs.

2.1.7 MSG's Effect on Hematological Parameters

The impact of administering MSG on hematological parameters has been the focus of inquiry in numerous research investigations. Nwanneka et al. (2020) undertook a study to examine the impact of MSG exposure on Wistar rats. The findings indicated that compared to the untreated group, rats given MSG alone had lower amounts of WBC, RBC, HGB, MCHC. Particularly, it is important that the MSG-treated group exhibited elevated levels of LYM and PLT. Nevertheless, there were no notable disparities detected in the average MCV and MCH between the Wistar rats that received just MSG and the remaining experimental groups. Al-Mousawi conducted a separate trial in 2017 and discovered that administering MSG led to a decline in the counts of RBC, HGB, MCHC in the trial animals, compared to the group that served as control. In contrast to the findings of Ghadhban, Al-Mousawi observed an increase in MCH and MCV in rats that were administered MSG.

In contrast, a study conducted by Ghadhban in 2018 presented divergent results. This experiment included the long-term dosing of MSG to Wistar rats. The results obtained indicated a notable elevation in WBC, RBC, and LYM levels. While the control group

showed no change in GRA, MCH, or MCHC, the rats administered MSG showed a significant decrease.

The aforementioned studies offer evidence that MSG's effects on hematology are complex and time-dependent, depending on both the sample and the specific blood parameters studied. The observed variations in the outcomes indicate that the administration of MSG may have diverse effects on distinct parts of hematology in animal models.

2.1.8 Changes in Testis Histomorphometry After MSG Administration

In their study, Jubaidi et al. (2019) conducted an assessment of the effects of daily MSG oral dosing on male reproductive performance in a rat study. For a duration of 28 days, a dosage of 60 mg/kg BW and 120 mg/kg BW of MSG were given. No discernible alterations were observed in the testicular morphology of rats given 60 mg/kg BW of MSG as compared to those in the control group. However, lessening of spermatid number was noted within the seminiferous tubules. At 120 mg/kg body weight of MSG, the researchers found that spermatids were missing from a significant portion of the seminiferous tubules in the testes of the rats. It was also observed that these rats had thinner germinal epithelial linings when contrasted with the control group. The male reproductive system was shown to be significantly impaired after being administered 120 mg of MSG per kg of body weight (kg BW). Over the period of 30 days, Anbarkeh et al. (2019) examined the effects of MSG induction on male Wistar rats. At the end of the study, the histological examination of the spermatogenic cells in the seminiferous tubules revealed a normal structure in the control group. The seminiferous tubules showed a significant number of spermatogenic cells in the

group that received a high dose of MSG, but they produced very few sperm. Additionally, these tubules displayed a predominance of spermatogonia and a reduced level of sperm formation compared to the other groups. In their study, Rahayu et al. (2021) conducted an experiment to inquire the potential ameliorative outcome of an ethanolic extract obtained from Marsilea crenata on the deleterious impact of MSG on testis histopathology in male rats. Upon the conclusion of the 30-day trial, the researchers made an observation that the germinal epithelium in the rats' seminiferous tubules that had been exposed to MSG shown a notable drop relative to the control group. The investigators noticed that compared to the control group, the MSG group had significantly less spermatogonia, spermatocytes, and spermatids on average. In addition, compared to the control group, the MSG group had a significantly lower number of Leydig cells.

2.2 Garlic

Garlic belongs to the subgenus *Allium* under the Alliaceae family, which was previously classified under the Liliaceae family and subsequently the Amaryllidaceae family. The subterranean stem that is suitable for consumption is a compound bulb consisting of multiple smaller bulbs known as cloves (Gupta & Gupta, 2016). The medicinal qualities of this vegetable have been recognized by humanity for numerous generations. The origin of garlic may be traced back to central Asia, where it is believed to have originated. Over time, it has become a significant agricultural crop in the Mediterranean region and has been widely used as a flavoring in other continents, including Africa and Europe. According to Sasi et al. (2021), India has the second position in terms of garlic production, with China occupying the first position.

According to Van Wyk and Wink (2018), garlic is classified as a perennial herbaceous plant characterized by a bulb composed of several cloves, enveloped by a thin, papery outer layer. It exhibits the growth of a vertically elongated inflorescence, characterized by little white or pink flowers, with elongated, slender, and flattened foliage. According to Rosen et al. (2023), garlic cultivation is feasible across diverse soil compositions and climatic conditions, making it a comparatively uncomplicated crop to cultivate. The propagation of this plant is commonly achieved through vegetative means, wherein individual cloves are planted and subsequently give rise to new bulbs. According to Ayisha (2022), the optimal time to harvest garlic is when its leaves exhibit a yellow coloration and start to undergo desiccation, typically occurring approximately six months after the initial planting.

Table 2.1: Garlic products and their daily dose

Garlic product	Dose/Day
Fresh raw garlic	2-5 g
Dried garlic powder	0.4-1.2 g
Garlic oil	2-5 mg
Garlic extract (solid)	300 mg-1,000 mg
Aged garlic extract (liquid)	2,400 mg

Source: WHO 1999; Verma et al., 2023

2.2.1 Pharmacological Properties of Garlic

Garlic has garnered significant recognition among medicinal plant species, establishing a prominent standing in human history. Numerous accounts substantiate its efficacy as a potent therapeutic agent, as documented by various human civilizations (Hayat et al., 2022). Hayat argues that garlic is acknowledged as plant with possible medicinal properties for a significant portion of human civilization. The recent advancements in the powerful

chemicals found in garlic and their active biological capabilities have significantly expanded our awareness and recognition of the possible applications of garlic in human life. Garlic is traditionally utilized for the treatment of infections, the common cold, diabetes, and cardiovascular problems. The utilization of garlic in ancient Egyptian society encompassed both gastronomic and medicinal applications. The use of garlic as a "performance enhancer" in competitive athletics during the early Olympics in Greece and its ingestion by the Egyptian people who engaged in hard labor during the pyramids' building are both well-documented. Additionally, the Romans recognized garlic's potential to purify the arteries (Govindaraj, 2019).

There exist numerous organic food products that provide useful attributes. Garlic is considered to be a significant component of a daily dietary regimen, as it possesses useful food properties. Foods that are typically consumed as part of a regular dietary pattern and manifest a positive impact on one or more physiological functions in the body, surpassing their role as mere sources of essential nutrients, are recognized by the European Food Safety Authority (EFSA). According to Lambré et al. (2021), the EFSA asserts that in order for a product to be classified as functional, there must be scientific evidence supporting its beneficial effect.

El-Saber Batiha et al. (2020) undertook a study that revealed that garlic holds a variety of chemicals that have the capacity to impact the immune system. Garlic and its complex components have recently been scrutinized for their possible role in immune system enhancement. The immunoregulatory activities of isolated garlic extracts and components

were thoroughly studied. It has been reported that the consumption of this functional food may have prospective benefits in the prevention and management of a variety of pathologies, including obesity, metabolic syndrome, cardiovascular disorders, gastric ulcers, and cancer. It is widely acknowledged that immune dysfunction is a significant factor in the development and progression of a variety of diseases (Sánchez-Sánchez et al., 2020). The researchers assert that garlic is involved in the regulation of cytokine release, which could provide a means of action for a lot of its medicinal uses. The production of cytokines that promote inflammation has been decreased by the principal organosulfur ingredient in garlic, alliin.

According to Okoro et al. (2023), the chemical makeup of garlic and its related compounds indicates the presence of various compounds garlic in its raw, dried, and cooked forms, as well as in its processed form. The principal bioactive components of garlic include several different organosulfur compounds, namely allyl sulfides, thiosulfinates, diallyl disulfides, and ajoene. These chemicals primarily regulate the antioxidant capacity of garlic. Vuković et al. (2023) have established a robust association between organosulfur compounds and pyruvate, as well as between garlic's phenolics and antioxidant capacity.

According to a study conducted by Kaur and Gupta (2021), several garlic formulations, including a water-soluble organo-sulfur molecule, affect the bioavailability of sulfur-containing groups compared to raw garlic. Studies on cancer cell lines have shown that garlic's antioxidant characteristics can reduce cell proliferation and increase apoptosis.

The organosulfur compounds found in garlic, whether it is fresh or dried, include γ-Glutamylcysteine and alliin, also known as S-Allylcysteine sulfoxide. The Sulphur compounds produced by preparing and boiling garlic are biosynthetically linked to these molecules (Yoshimoto & Saito, 2017). Cutting, crushing, or cowing garlic cloves physically disrupts tissue, which facilitates the enzymatic conversion of alliin to allicin. Alliinase enzymes, mostly present in vascular bundle membrane cells, are released as a result of this rupture. Both the Charron et al. (2016) and Yoshimoto & Saito (2017) experiments showed that the conversion happens quickly, requiring only a few seconds. The Sulphur compounds that give garlic its distinctive smell are made up of allicin, the principal bioactive component of garlic, which accounts for about 70 to 80 % of these compounds (Salehi et al., 2019). Having said that, allicin is quite sensitive to changes in temperature, oxygen levels, pH, and organic solvents, among other things. Its fast breakdown produces other stable organosulfur compounds because of this. The main compounds in this class are 2-vinyl-4H-1,3-dithiin and 3-vinyl-4H-1,2-dithiin, although there are also diallyl sulphides, diallyl disulphides, diallyl trisulfides, ajoenes, and vinyldithiins (Oosthuizen et al., 2018; Rouf et al., 2020).

Garlic is known to contain a wide range of bioactive compounds, including organosulfur compounds such as alliin, allicin, ajoene, allyl propyl disulfide, diallyl polysulfides, vinyldithiins, diallyl sulfide, diallyl disulfide, diallyl trisulfide, allyl methyl trisulfide, and diallyl tetrasulfide. It also contains saponins, specifically proto-eruboside B and eruboside B, as well as phenolic compounds like caffeic, ferulic, vanillic, p-hydroxybenzoic, p-coumaric, and sinapic acids (Kuete, 2017; Martins et al., 2016; Rouf et al., 2020). The

biological activities of garlic are attributed to various compounds, which encompass antioxidant, cardiovascular protective, anticancer, anti-inflammatory, immunomodulatory, anti-diabetic, anti-obesity, and antibacterial properties. Research by Rouf et al. (2020) and Martins et al. (2016) established that the chemicals had immunomodulatory, antibacterial, antifungal, anti-inflammatory, and antioxidant activities.

Table 2.2: The functional activities associated to some bioactive substances found in garlic

Bioactive compound	Functional activity	Reference
Allicin	Antimicrobial, Antioxidant, Anti-inflammatory	Ankri & Mirelman, 1999
Diallyl sulfide (DAS)	Anticancer, Antioxidant, Anti-inflammatory	Fleischauer & Arab, 2001
Diallyl disulfide (DADS)	Anticancer, Antioxidant, Anti-inflammatory	Fleischauer & Arab, 2001
Diallyl trisulfide (DATS)	Anticancer, Antioxidant, Anti-inflammatory	Nasr & Alshali, 2020
E/Z-ajoene	Antifungal, Anticancer, Antiplatelet	Ankri & Mirelman, 1999
S-allyl-cysteine (SAC)	Cardiovascular support, Anticancer, Antioxidant	Rahman & Lowe 2006
S-allyl-cysteine sulfoxide	Antioxidant, anti-inflammatory	Rahman & Lowe 2006

Source: Sahidur et al., 2023

2.2.2 Effects of Aqueous Garlic Extract on Body Weight

Several studies have been undertaken to examine the effects of aqueous garlic extract on the body weight of Wistar rats induced by MSG. The primary objective of these experiments was to investigate the long-term metabolic consequences of orally, intubation, or injectable administration of MSG in experimental animals. In a study conducted by Ahmed et al. (2019), to investigate the impact of MSG administration on body weight. The

researchers conducted a 14-day study to examine the possibility of moderating effects of orally consumed garlic on the decrease in body weight caused by MSG. MSG was delivered intraperitoneally. The study's findings indicated that the administration of aqueous garlic extract has a beneficial impact on the reduction of body weight in adult Wistar rats induced by monosodium glutamate. Although the findings could not demonstrate statistical significance across several groups, the administration of aqueous garlic extract alongside MSG was observed to contribute to weight stabilization.

According to the research by Djankpa et al. (2012), rats administered an aqueous garlic extract had a 46.5 % ($p < 0.05$) lower average body weight than rats in the control group that did not receive the extract. In contrast, there was a substantial rise of 46.2 % ($p < 0.05$) in the average mean weight of the experimental groups of rats who were not given the aqueous garlic extract. The researchers reached the conclusion that garlic exhibits a potential for inducing weight loss in rats with obesity.

Siddique et al. (2015) performed a 30-day study with 500 and 1000 mg/kg of garlic extract, but the results were contradictory. Researchers found that compared to the untreated group, those who consumed 500 mg/kg and 1000 mg/kg of garlic showed a steady increase in body weight after ingestion.

2.2.3 Effects of Aqueous Garlic Extract Sperm Count

The term "sperm count" refers to the relative concentration of sperm cells within a given volume of semen. The conventional unit of measurement is the quantification of sperm

cells per milliliter (ml) of seminal fluid. The assessment of male fertility and reproductive health necessitates the consideration of sperm count as a pivotal determinant.

The goal of the study by Emokpae and Olaode (2021) was to determine the effect of an aqueous garlic extract on sperm count in male Wistar rats as a function of dose. Various doses of the extract (500 mg/kg, 750 mg/kg, and 1000 mg/kg) were administered in the study. The results showed no statistically noteworthy distinction in the average sperm count across the groups.

Lotfi et al. (2021) researched into how garlic affected diabetic rats' testicular tissue damage. Less severe testicular tissue injury was observed due to the hypoglycemic, antioxidant, and anti-inflammatory effects of garlic, as indicated in the study. Also, the rats that received garlic supplementation exhibited increased sperm count and improved sperm quality compared to the experimental units that did not receive garlic treatment. Similarly, Mbegbu et al. (2021) studied whether an aqueous garlic extract may alleviate the effects of cadmium chloride on male rats. The results revealed that rats treated with cadmium chloride had significant enhancements in spermatogenesis, as measured by an increase in sperm count, after being given an aqueous garlic extract. This effect was observed when comparing the experimental group receiving both cadmium chloride and aqueous garlic extract to both the control group and the group alone exposed to cadmium chloride without the addition of aqueous garlic extract. The 2018 Ifeoma et al. study sought to find whether aqueous garlic extract would have a protective impact on testicular and spermatogenic changes in male rats with diabetes caused by glibenclamide. The outcome of the study

revealed that in male rats with diabetes caused by alloxan, the combined treatment of glibenclamide and aqueous garlic extract produced the most effective hypoglycemic activity and defensive capability against spermatogenic alterations.

2.2.4 Aqueous Garlic Extract's Effect Sperm Motility

The correlation between progressive sperm motility and conception rates has been established according to a study by Vogiatzi et al. (2022). The biological importance of the total number of progressively motile spermatozoa in the ejaculate has been noted by Víquez et al. (2020). The capacity of sperm cells to navigate and fertilize eggs is directly influenced by sperm motility, making it a key variable in male fertility.

Emokpae and Olaode (2021) discovered that 500 mg/kg of Allium sativum extract did not significantly alter the progressive motility of rats. However, rats supplied with 750 mg/kg of *Allium sativum* exhibited a non-significant increase in progressive motility, while those administered with 1000 mg/kg of *Allium sativum* showed a considerably higher level of progressive motility ($p < 0.05$). The presence of immobile sperm cells exhibited a statistically significant increase ($p < 0.002$) in rats subjected to a dosage of 500 mg/kg. Conversely, a statistically substantial drop ($p < 0.05$) in immobile sperm cells was seen in rats provided with dosages of 750 mg/kg and 1000 mg/kg ($p < 0.05$). Lotfi et al. (2021) demonstrated that the experimental groups receiving garlic treatment exhibited significantly improved progressive mobility. The groups who did not get garlic had a significant decrease in motility. Results from the research of Ifeoma et al. (2018) suggest that, glibenclamide and garlic extract, when given together, had a protective effect on the

level of sperm motility and the most effective hypoglycemic action in diabetic male rats. In their study, AL-Chalabi et al. (2014) observed a notable enhancement in sperm motility when a mixture of aqueous and alcohol garlic extract was administered alongside lead acetate, in comparison to the experimental group that received alone lead acetate.

2.2.5 Influence of Aqueous Garlic Extract Sperm Morphology

The importance of sperm morphology, including the dimensions, configuration, and structural soundness of spermatozoa, is paramount in relation to male reproductive capability (He et al., 2023). The precise structural composition of sperm plays a crucial role in facilitating successful fertilization and subsequent embryonic development. The presence of normal sperm morphology is essential for promoting effective motility, which in turn facilitates the successful traversal of the female reproductive canal and increases the likelihood of reaching and fertilizing the oocyte (Kodithuwakku et al., 2023).

Spermatozoa in the control group had normal heads, bodies, and tails, according to Emokpae and Olaode (2021). Teratozoospermia, which is defined as the presence of sperm cells without tails, bowed necks, and abnormally shaped heads, was observed in rats given 500 mg/kg and 750 mg/kg doses of *Allium sativum* respectively. In conclusion, animals that were fed a dosage of 1000 mg/kg *Allium sativum* exhibited clear indications of significant teratozoospermia, characterized by the presence of poorly developed heads and a bent body structure, along with the presence of immature sperm cells. In a study conducted by Hosseini and Khaki (2014) indicated that, the animals given AGE had a decreased number of spermatozoa that were morphologically normal when compared to

the control. The researchers also investigated the impact of this extract on various semen parameters and erythrocyte superoxide dismutase levels in Wistar rats.

In contrast, the investigation by Obidike et al. (2012) examined the influence of AGE on the morphology and function of the testes in albino rats treated with lead nitrate (Pb $(NO_3)_2$. After considering the negative effects of Pb $(NO_3)_2$ on sperm production, the researchers concluded that male rats treated with AGE may show improvement in sperm production. Garlic can enhance spermatogenesis in male albino rats, as shown in the study.

2.2.6 Impact of AGE on Testicular Weight

The determination of testicular weight mostly relies on the existence of various structures and tissues within the testes (Seven et al., 2020). The testes serve as the primary male reproductive organs, responsible for the synthesis of sperm and testosterone, the principal androgen hormone. The elements that make up testicular weight include seminiferous tubules, sperm cells, Leydig cells, blood vessels, and connective tissues, as elucidated by Kobir et al. (2023). Following the administration of $CdCl_2$ to male rats, Mbegbu et al. (2021) investigated the potential therapeutic advantages of an aqueous garlic extract. The researchers reached the conclusion that the administration of $CdCl_2$ resulted in a decrease in the average weight of the testes as compared to the group that alone received an AGE. No notable disparity was observed in the average testicular weight between the rats subjected to $CdCl_2$ treatment alone and those treated with both $CdCl_2$ and AGE (300 mg/kg). The study conducted by Memudu et al. (2015) examined the impact of administering AGE on the testicular cellular integrity of rats, both in the short-term and

long-term. The study's findings revealed a general augmentation in testicular weights across all experimental groups in comparison to the control group. The researchers additionally observed that the cohort subjected to prolonged administration of aqueous garlic extract exhibited a significant increase in testicular weight in comparison to the cohort subjected to short-term administration of AGE. The study has provided confirmation that garlic plays a regulating role in the weight of organs, specifically the testes.

2.2.7 Effects of Aqueous Garlic Extract on Hematological Parameters

In recent times, there has been an increasing inclination towards exploring the potential impacts of garlic extract on diverse physiological processes, encompassing hematological markers. The possible impact of AGE on hematological markers has been the subject of investigation, with various experimental investigations undertaken to examine its effects. Research by Elkelawy et al. (2020) looked at how male Bouscat rabbits' hematological parameters were affected by garlic administration. The researchers observed a significant rise in the total count of WBC and RBC, as well as the HGB levels in rats that were administered varying dosages of garlic (3, 9, or 27 mg/kg body weight) once a week for a duration of 8 weeks. This increase was shown to be dose-dependent, indicating that higher doses of garlic resulted in greater increments in these blood parameters. The researchers, in their conclusion, indicated that the administration of a smaller dose of garlic can be deemed safe, while cautioning against the potential adverse effects associated with a higher dosage. Ahmad et al. (2023) conducted a study with the objective of investigating the impact of AGE on hematobiochemical activities in rabbits induced with paracetamol over a period of 21 days. The researchers determined that mice subjected to AGE treatment with

doses of 100 mg/kg body weight and 200 mg/kg body weight exhibited elevated levels of white blood cell concentration. Nevertheless, the intervention failed to significantly enhance the HCT and MCHC. Compared to the control, the animals undergoing treatment with AGE did not exhibit any major alterations in HGB, RBC, and PLT levels. In their study, Shokrollahi et al. (2016) conducted an evaluation of the impact of varying concentrations of garlic extract, when added to milk, on the hematology of newly born goat kids. Aqueous garlic extract was given for 42 days at dosages of 125 and 250 mg per kg of live body weight. The researchers observed elevated levels of HGB, HCT, RBC, LYM, and WBC in mice that received supplementation of garlic extract, in comparison to the control group. The research findings indicated that the addition of AGE to milk resulted in enhanced hematological parameters, specifically in relation to immunity. Yunita et al. (2023) conducted a study to investigate the impact of orally administering a single-bulb extract on the hematological profile of male Sprague Dawley rats induced by E-cigarettes. The investigators administered different dosages of 75 mg/kg BW, 100 mg/kg BW, and 125 mg/kg BW for a period of 14 days. Studies found that the lowest white blood cell count was observed in the group that received the highest dose of garlic extract (125 mg/kg body weight) in the trial. The mean HGB was highest in the experimental group that received the highest quantity of garlic extract, followed by the groups that received the medium and low doses. Among the groups of animals fed with the garlic extract, the group that received a low dose (75 mg/kg BW) had the highest mean PLT, followed by the group that received the highest dose. No statistically significant variation in HCT levels was seen across the animal groups that were administered varying doses of garlic extract in comparison to the control groups. A considerable increase in red blood cell count was observed in the mice

that were provided with a medium dose of garlic extract, followed by the highest dose, and finally the low dose. The maximum dose of garlic extract resulted in a considerable increase in LYM counts. The research's conclusions showed that administering dosages of garlic extract orally, specifically from a single bulb, had a discernible impact on the hematological profile of male rats exposed to e-cigarettes. This impact was observed through an increase in RBC, HGB, and LYM counts.

2.2.8 Testicular Histomorphometry

The method of assessing testicular histomorphometry entails the study of diverse histological parameters inside the tissue of the testes. This study offers significant contributions to our understanding of the anatomical arrangement and physiological processes occurring within the testes, shedding light on the functional mechanisms involved in spermatogenesis and the overall well-being of the testicular system.

Emokpae and Olaode (2021) presented a photomicrograph illustrating the impact of delivering a dosage of 500 mg/kg of *Allium sativum* on rats in their research. The photomicrograph displayed circular outlines of the seminiferous tubules at various phases of growth, along with pyknotic Sertoli cells, Leydig cells, and other cells participating in the process of spermatogenesis. Additionally, there was obstruction found in the intercellular vessels. Rats at a dosage of 750 mg/kg show circular portions of the seminiferous tubules in their testes, which are characterized by a little reduction in size and a slightly thicker outline. The histological examination revealed the existence of pyknotic reactive Sertoli cells and other cells involved in spermatogenesis, although the presence of

Leydig cells was not conspicuous. Rats that were given a dosage of 1000 mg/kg of *Allium sativum* showed seminiferous tubules with a mostly circular shape, although some mild deformations were noted at various phases of development. In addition, there were pyknotic Sertoli cells, Leydig cells, and other cells that play a role in the production of sperm. There was also congestion found in the interstitial arteries. The researchers suggested that the injection of *Allium sativum* extract has a negative effect on the procreative capabilities of rats in a dose-dependent manner.

In their study, Obidike et al. (2012) examined sections of the testes obtained from a group of untreated rats, referred to as the control group. The observed testicular morphology was found to be within the normal range, characterized by the presence of seminiferous tubules lined with spermatogenic and Sertoli cells, which are characteristic components of the testes. The testicular tissue sections obtained from rats at AGE dosage of 300 mg/kg body weight exhibited a significant augmentation in spermatogenic activity, characterized by hyperplasia of the spermatogenic cells. Severe hemorrhages were noticed in the interstitial area and the inner cavity of the seminiferous tubules in histological examination of testes samples taken from rats that were administered a dosage of 2 mg/kg body weight of Pb $(NO_3)_2$. These observations potentially indicate compromised spermatogenic activity, as evidenced by the scarcity of spermatogenic cells in close proximity to the lumen found in the seminiferous tubules. Rats given a combination of 2 mg/kg body weight of Pb $(NO_3)_2$ and 300 mg/kg body weight of AGE showed a significant increase in the number of spermatogenic cells in their testes. A large number of spermatogonia and spermatids were found in the seminiferous tubule's basal epithelium and lumen, respectively. In summary,

the research findings indicate that the use of AGE on male rats has the potential to mitigate the adverse impact of Pb $(NO_3)_2$ on spermatogenesis.

In their investigation, Mbegbu et al. (2021) provided histological pictures of the testes, demonstrating that Group A rats (Control) exhibited preserved testicular interstitium and seminiferous tubules characterized by healthy germinal epithelial layers and a lumen that was abundantly populated with sperm cells. Testicular interstitium and seminiferous tubules of rats given 300 mg/kg AGE had normal shape, with a germinal epithelium that was healthy and a lumen that was full of sperm. Rats that were administered a dosage of 2 mg/kg $CdCl_2$ exhibited deficient testicular interstitium, seminiferous tubules with a limited amount of germinal epithelium, and a lumen that was inadequately filled. In order to reduce the harmful effects of CdCl2 on testes tissues, the study found that 2 mg/kg of CdCl2 was administered first, followed by a dosage of 300 mg/kg of AGE given two hours later.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area and Duration of Study

The study was conducted at the Animal Science Department of the College of Agriculture Education of the Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development (Mampong-Ashanti Campus).

The Mampong Municipality is one of the Municipal Assemblies within the Ashanti Region of Ghana. This designation was established by the division of the previous Sekyere West District into two separate entities: The Mampong Municipal and the Sekyere Central District. This division was officially enacted through the implementation of Legislative Instrument (L.I.) 1908. The administrative center of the Municipality is Mampong. The location's geographic coordinates lie between longitudes $0^0$05 W and $1^0$30 W, and latitudes $6^0$55 N and 7 ^{0}30 N.

The geographical extent of the Municipality encompasses around 23.9 square kilometers. The Municipality is made up of several prominent towns, such as Mampong, Krobo, Dadease, Asaam, Kofiase, and Adidwan.

3.2 Experimental Rats

In this study, a total of 25 sexually mature male Wistar rats aged 6-8 weeks with an average body weight of 128 g to 138 g were obtained from a local breeder located in Asante Mampong, Ashanti part of Ghana. The animals were transferred to the Animal Experimental Farm located inside the Animal Science Department of the College of Agriculture Education at the Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development (Mampong-Ashanti Campus), where the experiment was conducted. The animals had a 21-day acclimatization period.

3.3 Research Design

A complete randomized design (CRD) was used to allocate the experimental animals to five treatments, with each treatment having five replicates. The treatments were assigned as follows: Treatment 1 (Control) received a normal saline solution, Treatment 2 received 120 mg/kg BW of MSG, Treatment 3 received 120 mg/kg BW of MSG along with 500 mg/kg BW of AGE, Treatment 4 received 120 mg/kg BW of MSG along with 750 mg/kg BW of AGE, and Treatment 5 received 120 mg/kg BW of MSG along with 1000 mg/kg BW of AGE.

3.4 Treatment Administration

The administration of all treatments was given orally by oral gavage in a continuous manner for a duration of 21 days.

3.5 Ethical Approval

The Institutional Review Board of the University of Development Studies, Tamale, examined and approved all experimental protocols (ethical clearance number UDS/RB/176/23).

3.6 Housing and Feeding

The male Wistar rats utilized in this investigation were accommodated within an aluminum cage that was equipped with a wire mesh covering. The cage was partitioned into five parts, each measuring 70 cm × 60 cm × 40 cm. The enclosure offered adequate airflow and effectively hindered any attempts of escape. The utilization of wire mesh effectively assured the containment of the rats, while also facilitating the movement of air. A sufficient amount of space was allocated to facilitate the rats' unrestricted movement within their environment. The self-cleaning feature of the cage contributed to the maintenance of hygiene and resulted in a decreased need for manual cleaning. Gates were strategically located at the uppermost section of every compartment to facilitate entry into the interior without causing substantial disturbance to the rats or their environment. This approach ensures improved care by reducing stress during activities such as feeding, as well as during the removal of water and cleaning of feeding troughs.

Throughout the trial, the Wistar rats had unrestricted access to rodent food and water.

3.7 Aqueous Garlic Extract Preparation

The garlic bulbs utilized in this research were from Asante Mampong main market, Ghana. The recognition and authentication of the garlic was done by Mr. Isaac Ntekor, an agronomist at Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development. Subsequently, the desiccated external layers of the garlic were removed prior to the division of the bulbs into smaller units known as cloves. The cloves were washed with distilled water. Subsequently, the garlic cloves, weighing 200 g, were processed using a sterile silver crest blender. Afterward, the pulverized garlic was combined with 200 ml of cold and sterilized physiological saline solution. The solution was filtered multiple times using a muslin cloth in order to get the filtrate, which was identified as the aqueous garlic extract.

The AGE that had been filtered was kept in a functioning refrigerator at a temperature of 4 °C until it was used.

3.8 Preparation of Monosodium Glutamate

Monosodium seasoning was purchased from Mampong municipal main market, Mampong, Ghana. 50 g of the MSG granules were dissolved in 100 ml of sterile distilled water. Until it was needed, the solution was kept in a functioning fridge at 4 °C.

3.9 Dose Calculation and Administration

The average body weight (grams) of each treatment group was converted to kilograms by dividing the average body weight (grams) by 1000. The results of the average body weight

in kilograms were multiplied by each treatment dosage per kilogram of body weight. The answer was divided by the concentration of the treatments.

Doses were freshly prepared each morning during the experimental period and were administered by oral gavage. The rats were carefully held wearing surgical gloves and the treatments were administered orally.

Before administering doses of MSG and AGE to the rats in the experiment, the solutions were left to warm up to room temperature for a few hours.

3.9.1 Parameters Measured

Body weight of experimental animals was measured weekly. Count, motility, the morphology of epididymal sperm, reproductive organs weight, hematology, and testis histomorphometry were analyzed after the experimental period.

3.9.2 Body Weight

Individual body weight (g), was recorded from each treatment of the experimental rats. The body weights were measured using a Camry top loading sensitive scale that was made in China by a company called Jadever Company Limited with a readability or sensitivity of 0.1 g. The weighing was done by first weighing an empty container, the weighing scale is then tared to 0. The rats were then put in the container individually and their body weights were recorded when they become stable in the container. The container was used to prevent the escape of rats during weighing.

3.9.3 Dissection

Upon completion of the experiment, the animals underwent macroscopic examination, were measured for weight, and were euthanized using a mild chloroform anesthesia. Following the rat's two-minute anesthesia, the rat was supinely positioned on a dissection board, pinned down to prevent movement during the dissection, and an abdominal incision was made through the middle line on the ventral surface of the rat with a sterilized surgical blade, scissors, pins, and forceps to expose the internal organs of the rat. Rats were dissected in the Biology Laboratory of Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development-Mampong Campus.

3.9.4 Evaluation of Sperm Indices

The epididymis was minced by anatomic scissors inside a sterile petri dish of 10 ml phosphate buffer saline and allowed to incubate at 37 °C for about 10 minutes. The stock sperm solution was well mixed by swirling it manually for 20 seconds. 50 µl of the stock sperm solution was mixed with 950 µl fixative prepared from a one-liter volume of an aqueous solution that contains 50 g Sodium bicarbonate ($NaHCO_3$), 10 ml of 38 % formaldehyde solution. The dilution with fixative immobilized spermatozoa and ensured accurate count.

A hemocytometer with improved Neubauer ruling with CAT. NO. 1103 which was made in China was prepared by placing coverslip on the two surfaces of the counting chambers. The dilution was thoroughly mixed for 60 seconds to avoid sedimentation. An aliquot of 10 µl was immediately removed by a pipette to fill the entire area of the counting chambers.

This was done by gently touching the pipette tip to the edge of the chamber and the coverslip. The pipette's plunger was gradually depressed, allowing the chamber to fill by capillary action. The coverslip remained stationary during the filling process, and the chamber was filled to an appropriate level without exceeding or falling short of the desired volume. The hemocytometer was placed in a horizontal position for a duration of 10 minutes to facilitate the full settling of spermatozoa within the 100 µm deep chamber. The procedure was carried out at room temperature in a humid chamber that included a petri dish covered with paper tissue that was wet with water. To keep the sample from drying out, a humid environment had to be maintained.

The quantification of sperm was done in both chambers of the hemocytometer under a light microscope at a magnification of ×400. In each replication, a minimum of 200 spermatozoa were assessed and their count was recorded using a laboratory counter. The sum and the difference of the two chambers were calculated for each sample and the difference between the two replicates was compared to the acceptable difference provided by the World Health Organization (WHO).

The sperm count was determined in accordance with the standards set forth by the World Health Organization in 2021.

With the goal to assess the motility of epididymal sperm, the stock sperm solution, microscope slides, and coverslips were pre-warmed to a temperature of 37 °C. A 10 µl portion of the prewarmed stock sperm solution was carefully dispensed onto a prewarmed

microscope slide using a pipette, and afterwards covered with a prewarmed coverslip. Systematic counting was conducted using a light microscope at a magnification of ×400. The counting process commenced with progressive spermatozoa, followed by non-progressive spermatozoa, and concluded with immotile spermatozoa.

Two hundred (200) spermatozoa were approximately assessed per replication; every replication was a separate, fresh wet preparation per sample. The replicate values were compared to check if they were acceptably close before the calculation of each category (progressive motility, non-progressive motility, and immotility) was done. The most common category was used to find the acceptable difference from World Health Organization (2021) criteria.

The determination of sperm morphology involved the utilization of a pipette to combine 10 µl of a 50 µl stock sperm solution with 950 µl of fixative. This mixture was then carefully deposited on a clean frosted microscope slide. A secondary slide was employed to facilitate the movement of the semen droplet across the surface of the slide. The slide was given some time to air dry. A light microscope with a 1000x magnification was used to examine the sample. A minimum of 200 spermatozoa were reviewed by the examination of deformities in the head, midpiece, and tail regions, afterwards being categorized as either normal or abnormal. The calculation was performed for each category per replicate in accordance with the recommendations outlined in the manual on semen analysis provided by the World Health Organization (2021).

The morphology, motility, and count of epididymal sperm were evaluated by Freddy Opoku Boateng, a laboratory technologist at the Science laboratory at Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development, Mampong Campus.

3.9.5 Reproductive Organ Weight

The testes, which are the male reproductive organs, were removed from the scrotal sac during the dissection procedure. The testes were carefully dissected to remove any surrounding tissues and afterwards measured using a precise top-loading scale. The initial mass of a sterile petri dish was measured, followed by the individual placement and subsequent weighing of the testes on the petri dish. The weight of the petri dish with the testis within was subtracted from the weight of the dish when it was empty to get the testicular weight measurement. The weights of the left and right testicles of each animal were measured.

3.9.6 Hematological Parameters

Blood was collected by cardiac puncture, using a sterile syringe with a needle manufactured by Jiangsu Shenli Medical Production Company Limited, China, and transferred into tubes containing Ethylene-Diamine-Tetra-Acetic Acid (EDTA) as anticoagulant for hematological assays.

The white blood cell (WBC), GRA, RBC, HCT, MCV, MCHC, MCH, HGB, LYM, PLT, and P-LCR were determined using a hematology auto-analyzer (Automatic Hematology

Analyzer, with a model number Rayto RT-7600s made in Guangzhou, China) at Ashanti Mampong Government Hospital.

3.9.7 Testis Histomorphometry

The reproductive organs (testes) were washed with buffer saline prior to fixation in freshly prepared 10 % formalin. The specimens underwent dehydration using various ethanol percentages, followed by xylene immersion, and then fixed in paraffin. The tissues were subsequently sliced to a thickness of 5 μm and affixed onto glass slides. These slides were then subjected to staining with hematoxylin and eosin. The resulting samples were viewed under a light microscope to observe the seminiferous tubules, germinal epithelium, interstitium, and lumen (Jubaidi et al., 2019).

3.9.8 Data Analysis

Data analysis was done by using Minitab statistical software (Version 20.0). One-way ANOVA and post hoc Tukey's test were executed to identify the remarkable difference between groups. All data collected were presented as plates, tables, and as mean ± SEM. Values were considered statistically significant if $p < 0.05$.

CHAPTER FOUR

RESULTS

4.1 Effects of Monosodium Glutamate and Varied Doses of Aqueous Garlic Extract on the Body Weight of Wistar Rats

The effects of MSG and Varied Doses of AGE on the body weight of Wistar rats are presented in Table 4.1.

Table 4.1: Effects of MSG and Varied Doses of AGE on the body weight of Wistar rats

Body weight (g)	Control	MSG (120 mg/kg BW)	MSG + AGE (500 mg/kg BW)	MSG + AGE (750 mg/kg BW)	MSG + AGE (1000 mg/kg BW)	SEM	p-value
Day 0	136.8	135.2	137.8	136.4	128.4	5.29	0.986
Day 7	157.6	145.4	143.4	146.6	160.0	4.93	0.817
Day 14	161.6	136.6	142.8	140.2	171.7	6.45	0.438
Day 21	155.6	155.5	171.7	133.0	174.3	6.22	0.338

MSG (Monosodium glutamate 120 mg/kg BW), AGE (Aqueous garlic extract 500 mg/kg BW, 750 mg/kg BW and 1000 mg/kg BW respectively), BW (Body weight)

In comparison with the control group, no differences ($p > 0.05$) were observed in the body weight among the treatment groups throughout the experimental period (Table 4.1).

4.2 Effects of Monosodium Glutamate and Varied Doses of Aqueous Garlic Extract on Sperm Parameters of Wistar Rats, d 21 of the Experiment

The effects of Monosodium glutamate (MSG) and Varied Doses of Aqueous Garlic Extract (AGE) on sperm parameters of Wistar rats are shown in Table 4.2.

Table 4.2: Effects of MSG and Varied Doses of AGE on sperm parameters of male Wistar rats, d 21 of the experiment

Sperm Parameters	Control	MSG (120 mg/kg BW)	MSG + AGE (500 mg/kg BW)	MSG + AGE (750 mg/kg BW)	MSG + AGE (1000 mg/kg BW)	SEM	p-value
Sperm Count (10^6 sperm/ml)	420.00	409.67	426.00	427.33	422.67	2.34	0.097
Progressive motility (%)	78.67^{b}	77.00^{b}	80.67^{b}	83.67^{ab}	88.33^{a}	1.23	0.004
Non-Progressive motility (%)	18.00^{a}	18.00^{a}	16.33^{a}	13.67^{ab}	9.67^{b}	0.99	0.006
Immotility (%)	4.67^{bc}	6.67^{a}	5.00^{ab}	4.00^{bc}	3.00^{c}	0.36	0.001
Normal morphology (%)	78.67^{b}	68.67^{b}	76.33^{ab}	81.00^{a}	82.33^{a}	1.61	0.026
Abnormal morphology (%)	21.33^{a}	31.67^{a}	23.67^{ab}	19.00^{b}	17.67^{b}	1.63	0.021

^{abc} Means in the same row with different superscripts are significantly different ($p < 0.05$)

MSG (Monosodium glutamate 120 mg/kg BW), AGE (Aqueous garlic extract 500 mg/kg BW, 750 mg/kg BW and 1000 mg/kg BW respectively), BW (Body weight)

Table 4.2 shows that no treatment effect ($p > 0.05$) was detected on the total sperm count across the groups, according to the data on sperm parameters.

Rats treated with 120 mg/kg BW of MSG + 750 mg/kg BW of AGE or 120 mg/kg BW of MSG + 1000 mg/kg BW of AGE exhibited higher progressive motility ($p < 0.01$). The rats in the control group showed a similar and lower progressive response, as did the rats given 120 mg/kg BW of MSG alone and the rats given 120 mg/kg BW of MSG + 500 mg/kg BW of AGE ($p > 0.05$).

Rats assigned to 120 mg/kg BW of MSG + 1000 mg/kg BW of AGE had a lower non-motile progression rate ($p < 0.01$) compared to the control group, the group given 120 mg/kg BW of MSG alone, the group given 120 mg/kg BW of MSG + 500 mg/kg BW of AGE, and the group given 120 mg/kg BW of MSG + 750 mg/kg BW of AGE.

The groups treated with 120 mg/kg BW of MSG and 120 mg/kg BW of MSG + 500 mg/kg BW of AGE exhibited the highest immotile sperm count ($p < 0.01$), followed by the control group and the 120 mg/kg BW of MSG + 750 mg/kg BW of AGE group. The immotile sperm count was lowest in the group that received 120 mg/kg BW of MSG and 1000 mg/kg BW of AGE.

The groups of rats treated with 120 mg/kg BW of MSG plus AGE beyond 500 mg/kg BW (750 mg/kg BW and 1000 mg/kg BW of AGE) had a higher number of sperm cells with

normal morphology ($p < 0.01$) than the control and 120 mg/kg BW of MSG alone, which were similar.

In comparison to the groups treated with 120 mg/kg BW of MSG + 750 mg/kg BW of AGE and 120 mg/kg BW of MSG + 1000 mg/kg BW of AGE, the control group, 120 mg/kg BW of MSG only, and 120 mg/kg BW of MSG + 500 mg/kg BW of AGE all showed a higher level of aberrant sperm cell morphology ($p < 0.01$).

4.3 Effects of Monosodium Glutamate and Varied Doses of Aqueous Garlic Extract on Testicles Weight of Wistar rats, d 21 of the Experiment

The effects of Monosodium glutamate (MSG) and Varied Doses of Aqueous Garlic Extract (AGE) on Testicles Weight of Wistar rats are presented in Table 4.3.

Table 4.3: Effects of MSG and Varied Doses of AGE on Testicles Weight of Male Wistar Rats, d 21 of the Experiment

Testicles weight (g)	Control	MSG (120 mg/kg BW)	MSG + AGE (500 mg/kg BW)	MSG + AGE (750 mg/kg BW)	MSG + AGE (1000 mg/kg BW)	SEM	p-value
Left Testis	0.42^{b}	0.37^{b}	0.51^{b}	0.43^{b}	1.00^{a}	0.07	0.001
Right Testis	0.41^{b}	0.36^{b}	0.54^{b}	0.41^{b}	1.06^{a}	0.07	0.001

^{abc} Means in the same row with different superscripts are significantly different ($p < 0.05$)

MSG (Monosodium glutamate 120 mg/kg BW), AGE (Aqueous garlic extract 500 mg/kg BW, 750 mg/kg BW and 1000 mg/kg BW respectively), BW (Body weight)

The weights of both the left and right testes were higher ($p < 0.01$) in rats administered with 120 mg/kg BW of MSG + 1000 mg/kg BW of AGE (Treatment 5) compared to treatments 1, 2, 3 and 4. Treatments 1, 2, 3 and 4 had similar left and right testes weight (Table 4.3).

4.4 Effects of Monosodium Glutamate and Varied Doses of Aqueous Garlic Extract on Hematological Parameters of Wistar rats, d 21 of the Experiment

The effects of Monosodium glutamate (MSG) and Varied Doses of Aqueous Garlic Extract (AGE) on Hematological Parameters of Wistar rats are indicated in Table 4.4.

Table 4.4: Effects of MSG and Varied Doses of AGE on hematological parameters of male Wistar rats, d 21 of the experiment

Hematological Parameters	Control	MSG (120 mg/kg BW)	MSG + AGE (500 mg/kg BW)	MSG + AGE (750 mg/kg BW)	MSG + AGE (1000 mg/kg BW)	SEM	p-value	Reference Range *
WBC 10^9/L	4.04^{ab}	2.35^{b}	4.41^{a}	4.35^{a}	4.71^{a}	0.26	0.008	4.03-9.50
GRA 10^9/L	2.09^{ab}	2.10^{a}	2.05^{b}	2.06^{ab}	2.06^{ab}	0.01	0.029	1.5-3.5
RBC 10^12/L	7.44^{a}	5.94^{b}	8.13^{a}	7.44^{a}	7.93^{a}	0.24	0.005	6.73-8.57
HCT %	42.07	40.27	43.50	38.60	41.20	0.81	0.420	40.24-49.38
MCV fL	56.47	52.93	53.60	54.60	52.00	0.66	0.269	51.84-63.96
MCHC g/dL	35.10	35.00	35.90	35.23	35.90	0.27	0.770	29.76-34.00
MCH pg	28.17	25.97	26.87	26.87	26.20	0.32	0.229	17.05-20.15
HGB g/dL	16.00	15.20	16.97	14.73	16.00	0.31	0.162	12.7-15.38
LYM 10^9/L	2.52	1.16	2.27	2.51	2.16	0.26	0.534	58.40-88.15
PLT 10^9/L	539.00	456.70	488.70	478.40	577.70	25.5	0.630	463.95-947.4
P-LCR %	13.73^{ab}	11.63^{c}	12.67^{bc}	14.63^{a}	13.40^{ab}	0.30	0.001	15-20

^{abc} Means in the same row with different superscripts are significantly different ($p < 0.05$)

* Patel et al. (2024)

MSG (Monosodium glutamate 120 mg/kg BW), AGE (Aqueous garlic extract 500 mg/kg BW, 750 mg/kg BW and 1000 mg/kg BW respectively), BW (Body weight), WBC (White blood cell), GRA (Granulocyte), RBC (Red blood cell), HCT (Haematocrit), MCV (Mean corpuscular volume), MCHC (Mean corpuscular hemoglobin concentration), MCH (Mean corpuscular hemoglobin), HGB (Hemoglobin), LYM (Lymphocyte), PLT (Platelet), and P-LCR (Platelet large cell ratio).

The White Blood Cell count was reduced ($p < 0.01$) only in the group that was treated with 120 mg/kg BW of MSG only. The WBC recorded in all the other groups was similar.

The Granulocyte was higher ($p < 0.01$) in rats treated with 120 mg/kg BW of MSG only (Treatment 2) and it was similar to those in treatments 4 and 5. Wistar rats treated with 120 mg/kg BW of MSG + 500 mg/kg BW of AGE recorded lower GRA count.

The RBC was only reduced ($p < 0.01$) in the group that was administered with 120 mg/kg BW of MSG only. The RBC recorded in the other groups was similar.

The rats exposed to 120 mg/kg BW of MSG + 750 mg/kg BW of AGE recorded higher ($p < 0.01$) P-LCR followed by the control and 120 mg/kg BW of MSG + 1000 mg/kg BW of AGE. The groups that received 120 mg/kg BW of MSG alone or 120 mg/kg BW of MSG with 500 mg/kg BW of AGE had the lowest P-LCR.

None of the treatments had a substantial impact on the HCT, MCV, MCHC, MCH, HGB, LYM, or PLT ($p > 0.05$).

The effects of Monosodium glutamate (MSG) and Varied Doses of Aqueous Garlic Extract (AGE) on testis histomorphometry of Wistar rats are presented in Plate 4.1.

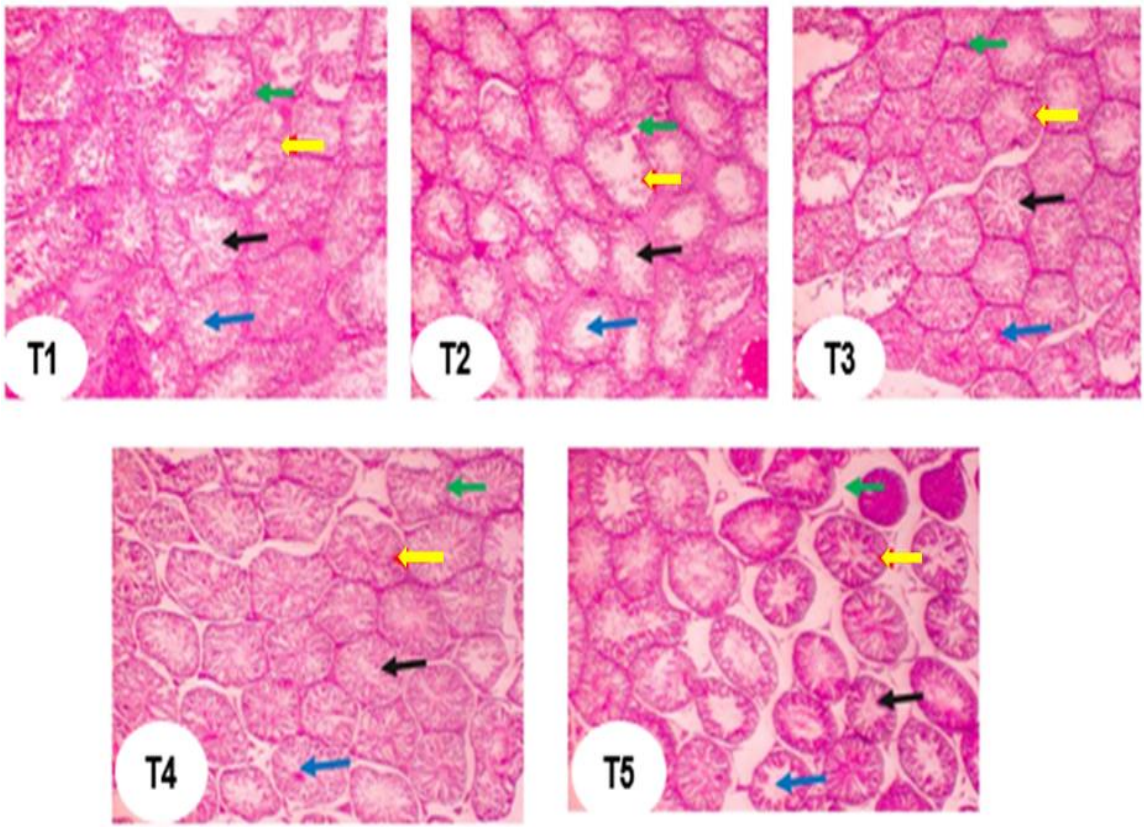

Plate 4.1: Representative photomicrographs of the testicular cross-section of male Wistar rats, d 21 of the experiment

T1 (Treatment 1/Control): normal saline; T2 (Treatment 2): 120 mg/kg BW of MSG only; T3 (Treatment 3): 120 mg/kg BW of MSG + 500 mg/kg BW of AGE (low dose); T4 (Treatment 4): 120 mg/kg BW of MSG + 750 mg/kg BW of AGE (medium dose); T5 (Treatment 5): 120 mg/kg BW of MSG + 1000 mg/kg BW of AGE (high dose).

Treatment 1 (Control) shows normal testicular seminiferous tubules (black arrow) and interstitium (green arrow) with healthy germinal epithelium (red arrow) richly filled with lumen (blue arrow). Treatment 2 shows slightly depleted seminiferous tubules (black arrow) and interstitium (green arrow) with scanty germinal epithelium (yellow

arrow) and slightly disturbed lumen (blue arrow). Treatment 3 shows slightly distorted seminiferous tubules (black arrow) and interstitium (green arrow) with moderately rich germinal epithelium (yellow arrow) and a filled lumen (blue arrow). Treatment 4 shows improved testicular seminiferous tubules (black arrow) and slightly dispersed interstitium (green arrow) with better germinal epithelium (yellow arrow) and richly filled lumen compared to Treatment 3. Treatment 5 shows deformation of the seminiferous tubules (black arrow) and distorted epithelium layer (yellow arrow) with highly depleted interstitium (green arrow) and poorly filled lumen (blue arrow) compared to Treatments 1, 2, 3 and 4.

CHAPTER FIVE

DISCUSSION

5.1 Effects of MSG and AGE on Body Weight

The outcome on the effects of MSG and different doses of AGE on body weight agrees with the conclusions by Jubaidi et al. (2019) who recorded no marked differences between the body weights of all groups of experimental animals after administering 60 mg/kg body weight of MSG and 120 mg/kg body weight for 28 days. Similar findings were made by Hazzaa et al., (2020) and Subandiyono & Hastuti, 2022 who also recorded no significant difference in body weight between experimental units after the administration of MSG. In contrast, Kolawale, 2013 reported a positive correlation between MSG consumption and weight gain. This report was made in reference to high dose of MSG intake. Nasir, 2019 reported a notable increase in the body weight of rats that were given MSG through food. Ahmed et al. (2019) reported significant weight loss in rats administered with 4 gm/kg of MSG for 14 days.

The results from the research indicated that different doses of AGE displayed no ameliorative impact on the body weight of MSG-induced rats throughout the experimental period. Contrary to what Ahmed et al. (2019) found, when they administered 200 mg/kg body weight of AGE intraperitoneally to MSG-induced rats for 14 days, these researchers found that an aqueous garlic extract enhanced the negative effects of MSG on the weight of Wistar rats. Siddique et al. (2015) reported gradual weight gain in experimental animals that were treated with 500 mg/kg and 1000 mg/kg body weight of AGE through orogastric tube for 30 days. Djankpa et al. (2012)

on the other hand concluded that garlic has a weight loss effect in obese mice after 44 days feeding experiment.

Variations in garlic extract formulation, AGE and MSG administration methods, and treatment duration can account for the discrepancies in the results. The mode of administration plays a crucial role, as the route through which MSG and AGE are introduced to the subjects can affect absorption rates, bioavailability, and distribution within the body. Variations in the preparation methods of MSG and AGE is also a factor, with differences in chemical structures and concentrations potentially impacting the observed outcomes. The duration of the experiment is a critical consideration, as short-term versus long-term exposure to these substances may reveal distinct physiological responses, influencing the overall interpretation of the study's findings (Li et al., 2017).

5.2 Effects of Monosodium Glutamate and Aqueous Garlic Extract on Sperm Count

The total sperm count did not change among groups as a result of the treatment (MSG), according to the data. The results are in conformity with those of Kadir et al. (2021), who studied the impact of MSG on various characteristics of semen. The researchers recorded no significant difference in sperm count after administering monosodium glutamate orally at different doses for 14 days. Earlier studies reported reduction in sperm count in MSG-treated rats (Ashaolu et al., 2011). Umeh & Chinko, 2023 also recorded decrease in sperm count in MSG-exposed rabbits. The differences in the findings could be attributed to the period of administration of MSG.

Varied doses of aqueous garlic extract showed no marked variation in sperm count among the experimental groups. This outcome is in line with Emokpae & Olaode (2021) who recorded no differences in sperm count among groups of experimental animals after administering 500 mg/kg body weight, 750 mg/kg body weight, and 1000 mg/kg body weight of AGE on male rats to determine dose dependent influence of AGE on sperm variables. Lotfi et al. (2021) found that garlic increased sperm count and quality in rats compared to the rats that were not treated with garlic. This was because garlic lessened the severity of damage in the testicular tissues of diabetic rats. Similarly, according to Mbegbu et al. (2021), AGE increased sperm count in rats given cadmium chloride ($CdCl_2$) unlike the control group and the group that only received cadmium chloride without the addition of AGE, enhancing spermatogenesis. The differences could be attributed to the variability in experimental animals and their response to the treatment. The levels of AGE administered might not have been sufficient to effect change in sperm count in the period of the of the study.

5.3 Effects of Monosodium Glutamate and Aqueous Garlic Extract on Sperm Motility

The results on sperm motility showed that the combination of MSG (120 mg/kg BW) with specific doses of AGE has differential effect on sperm motility. Monosodium glutamate (120 mg/kg BW) only, MSG + 500 mg/kg BW of AGE and MSG + 750 mg/kg BW of AGE resulted in lower sperm progressive motility, higher non-progressive sperm motility and higher immotile sperm cells compared to the control group. However, the administration of MSG (120 mg/kg BW) + 1000 mg/kg BW of AGE demonstrated improved progressive sperm motility, decrease in sperm immotility and reduced non-progressive sperm motility, indicating ameliorative effect of higher

dose of AGE on MSG-induced sperm motility. This results is in harmony with Farhat, (2021) who established that increased consumption of foods containing MSG, such as fast food and processed meat, was associated with lower sperm motility. Similar findings were made by Yang et al. (2023), who discovered a link between lower sperm motility and increased use of MSG-containing snack foods in the overall Asian population.

The results from the study indicated that the experimental group that received higher dose of AGE (1000 mg/kg BW) recorded higher percentage of progressive sperm motility. In agreement with their finding, progressive motility of rats was found to be lower in rats that were administered 500 mg/kg BW of AGE and 750 mg/kg BW of AGE, and it was higher ($p < 0.05$) in rats that were subjected to 1000 mg/kg BW of AGE, according to Emokpae and Olaode (2021). Similarly, Lotfi et al. (2021) established that experimental groups treated with garlic were associated with notably better progressive sperm movement as compared to those that were not treated with garlic. According to Ifeoma et al. (2018), the best hypoglycemic action and a protective impact on the percentage of sperm motility were observed in diabetic male rats when glibenclamide and garlic extract were administered together. Report by AL-Chalabi et al. (2014) supports the current finding. They recorded a significant increase in sperm motility after giving aqueous and alcoholic garlic extracts combined with lead acetate compared to the experimental animals treated with only lead acetate.

The increase in the percentage sperm motility in the experimental group that received higher dose (1000 mg/kg BW) of AGE could be attributed to the antioxidant properties such as allicin, diallyl disulfide and s-allyl cysteine at dose dependent response which

could be optimal for producing the observable redeeming effects by neutralizing free radicals and reactive oxygen species produced by MSG (Mukherjee et al., 2023) in order to protect sperm cells and improve motility.

5.4 Effects of Monosodium Glutamate and Aqueous Garlic Extract on Sperm Morphology

The research observed an increased proportion of normal sperm cell morphology in the experimental groups that received a dosage of 120 mg/kg BW of MSG in combination with 750 mg/kg BW AGE (referred to as the medium dose) and 120 mg/kg BW of MSG in combination with 1000 mg/kg BW of AGE (referred to as the higher dose). The incidence of aberrant sperm morphology was higher in the groups that received 120 mg/kg BW of MSG alone or 120 mg/kg BW of MSG combined with 500 mg/kg BW of AGE. According to research done by Dong and Robbins (2015), mice exposed to MSG exhibited anomalous sperm morphology, which was defined by deviations in the structure of the head or tail. Moreover, it was shown that the mice exposed to MSG had decreased reproductive rates, suggesting a plausible association between the consumption of MSG and compromised sperm functionality. In a similar vein, Nosseir et al. (2012) observed a higher prevalence of atypical sperm structure, suggesting that the use of MSG may have interfered with the process of sperm development. In their study, Iamsaard et al. (2014) also documented a noteworthy decline in the proportion of normal sperm morphology, accompanied by an elevation in the occurrence of aberrant sperm exhibiting head and tail irregularities. These findings imply that MSG may have an adverse impact on both the quality and structure of sperm. According to Alalwani (2014), there were observed modifications in the structure and form of sperm, such as abnormalities in the head and tail regions. These findings imply that the

administration of MSG to experimental animals may lead to potential disruptions in the development and functioning of sperm. All of these investigations are consistent with the present research results. The presence of MSG as a sole factor resulted in an increase in the occurrence of aberrant sperm morphology.

In the current investigation, the administration of AGE at doses of 750 mg/kg BW and 1000 mg/kg BW shown a mitigating impact on the sperm cell abnormalities induced by MSG. This confirms what was found in a prior study by Obidike et al. (2012), who used albino rats treated with lead nitrate (Pb $(NO_3)_2$ to look at how an AGE affected the shape and function of sperm. The researchers reached the conclusion that the treatment of AGE to male rats has the potential to mitigate the antispermatogenic impact caused by Pb $(NO_3)_2$. In contrast, Mild to moderate teratozoospermia, defined as the absence of tails in sperm cells, bent necks, and poorly formed heads, was observed in rats given 750 mg/kg *Allium sativum* and experimental animals given 500 mg/kg *Allium sativum*, according to Emokpae and Olaode (2021). In addition, rats that received 1000 mg/kg of *Allium sativum* showed severe teratozoospermia, including elongated bodies, underdeveloped heads, and premature sperm cells. The disparity in the results can be ascribed to the administration of MSG prior to the administration of AGE in the current investigation. In the previous trial, the researchers exclusively provided an AGE without MSG, which may have resulted in potential variations.

5.5 Effects of Monosodium Glutamate and Aqueous Garlic Extract on Testicles Weight

The statistical analysis revealed a substantial impact of MSG and varying concentrations of AGE on the weight of the testes. No notable differences were found

in the testicular weight of Wistar rats upon receiving 120 mg/kg BW of MSG alone, 120 mg/kg BW of MSG and 500 mg/kg BW of AGE at a low dose, or 120 mg/kg BW of MSG and 750 mg/kg BW of AGE at a medium dose. The findings indicate that an increased dosage of AGE (1000 mg/kg BW) had a notable impact on the weight of the testes. This observation supports the findings of Memudu et al. (2015). The researchers found that testicular weight in rats increased after continuous AGE treatment. The effect of the high garlic dosage on the testicular interstitium may explain the observed increase in testicular weight among rats given a combination of 120 mg/kg BW of MSG and a high dose of AGE (1000 mg/kg BW). According to Emokpae and Olaode (2021), it has been proposed that the administration of a high dosage of garlic may lead to the development of congestions within the interstitial blood vessels, potentially indicating the presence of inflammation. In cases of testicular inflammation, there is a potential for swelling and enlargement of the testis, resulting from the buildup of inflammatory cells, fluid, and other chemicals (Wiig & Swartz, 2012). This claim is supported by the histomorphometry of the rats that were given high dose of garlic extract.

5.6 Effects of Monosodium Glutamate and Aqueous Garlic Extract on Hematological Parameters

The examination of blood, known as hematological investigations, plays a vital role in assessing the degree of damage to the blood and identifying the impacts resulting from nutritional, environmental, and pathological causes (Ahmed et al., 2020). The blood profile of an organism may undergo significant alterations in response to stress, infections, and exposure to toxins.

Unlike the control group, rats administered 120 mg/kg body weight of MSG exhibited a significant reduction ($P < 0.01$) in white blood cell count, red blood cell count, and P-LCR. The P-LCR values for all treatments were lower than the reference range of 15-20, with treatment 2 having the lowest count. The study also noted an increase in granulocytes (GRA) within the reference range of 1.5-3.5 in the group that was exclusively administered MSG.

These findings emphasize the influence of MSG on blood parameters, indicating possible hematological strain. The reference ranges provided by Patel et al. (2024) for interpreting these findings, namely WBCs (4.03–9.50), RBCs (6.73–8.57), and P-LCR (15–20), play a crucial role in assessing the health state of the Wistar rats. Abnormal reductions in RBC and WBC, as well as a decrease in P-LCR below the normal levels, may signal the possibility of anemia and weakened immune function. This implies that MSG might be influencing the generation or lifespan of these cells.

Administering 500 mg/kg body weight of AGE seemed to mitigate certain adverse effects of MSG, specifically in GRA levels, indicating a protective function of AGE. This finding aligns with previous research; for instance, Elkelawly et al. (2020) reported raised levels of RBC and WBC after the injection of garlic. Uroko et al. (2021) discovered notable reductions in RBC and WBC counts in rats that were solely administered monosodium glutamate (MSG), which further supports concerns over MSG's influence on blood cells.

Ahmad et al. (2023) reported a notable rise in WBC levels after the administration of AGE, whereas there was no significant alteration in RBC counts. Shokrllahi et al.

(2016) determined that the administration of AGE resulted in improved hematological indicators, providing additional evidence for the possible therapeutic benefits of AGE in reducing the negative effects of MSG.

Nevertheless, inconsistencies exist in the results obtained from several investigations, possibly due to differences in the quantity of substance administered, the methods of administration, and the length of the experimental timeframe. For example, Nwaneka et al. (2020) found that mice exposed to MSG had higher platelet counts and lower levels of WBC and RBC. Similarly, Al-Mousawi (2017) showed a decrease in RBC counts in rats treated with MSG. Ghadhban (2018) observed that MSG-treated rats exhibited elevated white blood cell and red blood cell counts, whereas there was a notable reduction in granulocyte levels. These findings indicate varying reactions depending on the specific conditions of the experiment.

The observed reductions in WBC, RBC, and P-LCR in rats primarily fed with 120 mg/kg MSG may be attributed to the expedited death of these cells by MSG, potentially resulting in anemia (Asmerom et al., 2023). This phenomenon could also be ascribed to the influence of MSG on reducing the duration of red blood cells' existence, possibly because of the detrimental impact of sodium on hematopoietic stem cells in the bone marrow, as proposed by Al-Mousawi (2017). According to Uroko et al. (2021), MSG's oxidative effects may additionally lead to a decrease in blood cell counts. These findings emphasize the significance of reference ranges in evaluating the health effects of drugs such as MSG. Aberrations from the typical ranges of WBC, RBC, and P-LCR indicate that monosodium glutamate (MSG) may have notable physiological

consequences that could influence the overall health and welfare of organisms exposed to it.

5.7 Effects of Monosodium Glutamate and Aqueous Garlic Extract on Testis Histomorphometry

Histological examinations revealed a small reduction in tissue integrity when rats were given 120 mg/kg BW of MSG only. The results are in alignment with those of Jubaidi et al. (2019), who found that rats given 120 mg/kg of MSG had spermatids missing from most seminiferous tubules. Compared to the control group, the researchers found that the germinal epithelial linings were thinner. In a similar vein, Anbarkeh et al. (2019) observed a limited occurrence of spermatogenesis within the seminiferous tubules of rats subjected to MSG treatment. In their study, Rahaytu et al. (2021) found that mice subjected to MSG treatment exhibited a reduction in the thickness of the germinal epithelium of the seminiferous tubules as compared to the control group. A reduced quantity of Leydig cells was seen in the group treated with MSG.

Numerous scientific studies have demonstrated the significant cellular protection conferred by the administration of garlic against the detrimental impact of free radicals. The findings of this study indicate that the administration of AGE at specific dosages can have detrimental or beneficial effects on many aspects of male reproductive functioning. The findings of the present investigation demonstrated that AGE had a dose-dependent impact on MSG-induced testicular deformation. The experimental group of animals, which received a dosage of 750 mg/kg body weight of MSG, exhibited enhanced testicular seminiferous tubules characterized by improved germinal epithelium and a lumen that was more densely populated compared to the control group.

The administration of a low dosage of AGE at a rate of 500 mg per kilogram of body weight resulted in the observation of mildly altered seminiferous tubules and interstitium, characterized by a reduced presence of germinal epithelium. The experimental group administered a high dose of AGE (1000 mg/kg BW) had observable abnormalities in the seminiferous tubules, including deformation and distortion of the epithelial layer. Additionally, the interstitium seemed significantly depleted, and the lumen was inadequately filled, in comparison with the control group. The findings are consistent with the observations made by Emokpae and Olaode (2021), who recorded that giving 500 mg/kg BW of AGE resulted in the presence of circular seminiferous tubules containing Sertoli cells in animals. The investigators observed slight alterations in the histoarchitecture of the rats administered with a quantity of 750 mg/kg body weight of AGE. The study documented that; animals subjected to a dosage of 1000 mg/kg body weight exhibited abnormal seminiferous tubules accompanied by congested interstitial arteries. On the contrary, alternative research outcomes yielded inconsistent findings. In a study conducted by Obidike et al. (2012), it was shown that the administration of garlic to rats resulted in an augmentation of spermatogenic activity and the maintenance of normal testicular morphology. In their study, Mbegu et al. (2021) observed the presence of intact germinal epithelial layers and a lumen that exhibited a high concentration of sperm cells. The animals exhibited typical histological characteristics in their testicular interstitium. The disparity in the results may be ascribed to variations in the dosage and research methodology employed.

This study's findings imply that using MSG in combination with a high garlic intake may impair testicular function. The potential causative factor for the reduction in seminiferous tubules could be attributed to the use of a high dosage of garlic, as

suggested by Emokpae and Olaode (2021). According to a prior study conducted by Abdelmalik (2011), it was found that garlic has the potential to induce congestion in the interstitial blood vessels. The potential negative impact of a high dosage of garlic may be attributable to two factors: its inhibitory action on steroidogenesis, resulting in a reduction in testosterone levels due to depleted Leydig cells, and its phytoestrogenic activity (El Arab et al., 2021). According to Abdelmalik (2011), it is suggested that garlic may have a direct estrogen-like impact on the testes of adult male rats, perhaps leading to disturbances in testes histomorphometry.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary and Conclusion

- The 120 mg/kg BW of MSG had adverse effect on testicular function and hematology (WBC, RBC and P-LCR). This implies that testicular function and some blood parameters are negatively affected by 120 mg/kg body weight of MSG. Specifically, it affects the WBC, RBC, and P-LCR, indicating potential damage or disruption to reproductive health and hematological health.

- The 1000 mg/kg BW of AGE increased progressive sperm motility. This suggests that AGE at a dosage of 1000 mg/kg BW may improve progressive sperm motility. This suggests that AGE could enhance male fertility by promoting better sperm movement, which is crucial for successful fertilization.

- The 750 and 1000 mg/kg BW of AGE improved normal sperm morphology. This implies that administering doses of 750 and 1000 mg per kilogram of body weight (BW) of aqueous garlic extract (AGE) may lead to an improvement in normal sperm morphology. This suggests that AGE may enhance the structural quality of sperm, which is important for fertility and the ability to successfully fertilize an egg.

- The 1000 mg/kg BW of AGE increased testicular weight. This suggests that a 1000 mg/kg BW dose of aqueous garlic extract (AGE) may cause testicular

weight increase, which may be an indication of inflammation. This suggests that at this high dosage, AGE may cause inflammatory responses in the testes, leading to swelling and increased organ weight.

- The 500, 750 and 1000 mg/kg BW of AGE improved WBC, RBC, and P-LCR counts. This implies that administering doses of 500, 750, and 1000 mg/kg BW AGE could lead to improvements in WBC, RBC, and P-LCR counts. This suggests that AGE has a beneficial impact on hematological health, enhancing immune function, oxygen transport, and blood clotting mechanisms.

6.2 Recommendations

The following are worth recommending:

- The consumption of MSG should be minimized because it may negatively affect hematological (WBC, RBC and P-LCR) and reproductive function,
- Aqueous garlic extract must be consumed with caution because high dose may be detrimental to male reproductive function,
- Further research should be carried out to investigate the effects of AGE on the hormone secretion of male Wistar rats treated with MSG,
- A longer period of investigation is required to offer a more thorough understanding of the mechanisms behind reproductive and hematological dysfunction caused by MSG.

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