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Effect of ethanol extract of ginger (*Zingiber officinale*) on the histology of ciprofloxacin-induced testes of male Wistar rats

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Abstract

Objective: This research study was carried out to investigate the effect of ethanol extract of ginger (*Zingiber officinale*) on the histology of Ciprofloxacin-induced testes of male wistar rats.

Methodology: Twenty-five (25) male wistar rats weighing 110 g to 150 g were procured and acclimatized for two weeks, after which, they were divided into five (5) groups of five (5) rats each, and were housed in cages. The groups were designated as groups A - E. Group A served as the control group and was not induced with Ciprofloxacin, while Groups B – E were induced. Group A received distilled water only, Groups B - E received Ciprofloxacin only, Vitamin C, 100 mg/kg of body weight of ethanol extract of *Zingiber officinale*, and 300 mg/kg of body weight of ethanol extract of *Zingiber officinale* respectively for 14 days through oral route with the aid of oral gastric tube. On the 15th day, the animals were weighed and sacrificed via chloroform inhalation, and testes were harvested from the rats for histological study.

Results: Histopathological findings showed a testicular tissue with active seminiferous tubules that are lined with interstitial cells of the Leydig (ICL), Sertoli cell (SC), and moderately enhanced spermatogenesis (S) for animals in group A; severe degeneration with severe spermatogenic arrest (SA), severe interstitial fibrosis (IF), and severe necrotic (N) appearance of the testicular cells for animals in group B; adequate healing with moderately enhanced spermatogenesis (S) and moderate active appearance of the testicular cells for animals in group C; mild regeneration with moderate spermatogenic arrest (SA), severe interstitial fibrosis (IF) and moderate necrotic (N) appearance of the testicular cells for animals in group D; and mild regeneration with moderate spermatogenic arrest (SA), and mild necrotic (N) appearance of the testicular cells for animals in group E.

Conclusion: Ethanol extracts of *Zingiber officinale* have ameliorating effect on the histology of Ciprofloxacin-induced testes of male wistar rats, and the ameliorating effect improves with increase in the dosages of the extract

Keywords: Ciprofloxacin; Testes; *Zingiber officinale*; Infertility

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1. Introduction

Infertility is a global health issue affecting millions of people of reproductive age worldwide [1]. According to WHO [1], available data suggests that globally one in six people experience infertility in their lifetime. Infertility is a disease of the male or female reproductive system defined by the failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse [1]. It is responsible for 20–30% of infertility cases, while 20–35% are due to female infertility, and 25–40% are due to combined problems in both partners [2]. In 10–20% of cases, no cause is found [2]. Its causes include endocrine disorders (usually due to hypogonadism) at an estimated 2% to 5%, sperm transport disorders at 5%, primary testicular defects (which includes abnormal sperm parameters without any identifiable cause) at 65% to 80% and idiopathic (where an infertile male has normal sperm and semen parameters) at 10% to 20% [3]. Male infertility may also be due to retrograde ejaculation, low testosterone, functional azoospermia (in which sperm is not produced or not produced in enough numbers) and obstructive azoospermia in which the pathway for the sperm (such as the vas deferens) is obstructed [4]. In men who have the necessary reproductive organs to procreate, infertility can be caused by low sperm count due to endocrine problems, drugs, radiation, or infection [5].

Ciprofloxacin is a fluoroquinolone antibiotic used to treat a number of bacterial infections including bone and joint infections, intra-abdominal infections, certain types of infectious diarrhea, respiratory tract infections, skin infections, typhoid fever, and urinary tract infections, among others [6]. It is 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid. Its empirical formula is $C_{17}H_{18}FN_3O_3$ and its molecular weight is 331.4 g/mol. It is a faintly yellowish to light yellow crystalline substance [7]. It kills certain types of bacteria by disrupting their ability to create and repair their DNA [8]. For some infections, it is used in addition to other antibiotics and can be taken by mouth, as eye drops, as ear drops, or intravenously [6]. Its common side effects include nausea, vomiting, and diarrhea [6]. Severe side effects include an increased risk of tendon rupture, hallucinations, and nerve damage [6]. It can cause liver damage, acute renal failure relating to interstitial nephritis, and testicular function impairment [9].

Testes are pair structures that are located within the scrotum, with the epididymis situated on the posterolateral aspect of each testicle [10]. Commonly, the left testicle lies lower than the right. They are suspended from the abdomen by the spermatic cord – collection of vessels, nerves and ducts that supply the testes [10]. Originally, the testes are located on the posterior abdominal wall. During embryonic development they descend down the abdomen, and through the inguinal canal to reach the scrotum [10]. The testes consist primarily of the seminiferous tubules, the site of sperm cell formation and maturation. Interspersed among those tubules are so-called interstitial cells, including a cell type called Leydig cells, which produce the "male" sex hormone testosterone. In addition to its function in reproduction, testosterone helps to regulate many diverse body functions, including bone and muscle development; red blood cell turnover; and development and maintenance of male sexual characteristics, such as sexual drive (i.e., libido), growth of facial and body hair, and deepening of the voice during puberty. Consequently, an insult to the hormonal system controlling testosterone production can result not only in infertility but also in other deleterious consequences, such as accelerated bone loss (i.e. osteoporosis), decreased muscle function, and lower than normal numbers of red blood cells (i.e. anemia). A second important testicular cell type is the Sertoli cells which are embedded in the inner walls of the seminiferous tubules, and play a critical role in sperm development by supporting and nourishing the sperm cells during their maturation [10].

Medicinal plants are plants that are being used to attempt to maintain health, administered for a specific condition, or both, whether in modern medicine or in traditional medicine [11, 12]. One of such plant is Ginger (*Zingiber officinale Roscoe*) - a flowering plant whose rhizome, ginger root or ginger, is widely used as a spice and a folk medicine [13]. It originates in Maritime Southeast Asia and was likely domesticated first by the Austronesian peoples. It is a herbaceous perennial plant that grows annual pseudostems (false stems made of the rolled bases of leaves) about one meter tall, bearing narrow leaf blades. Its inflorescences bear flowers having pale yellow petals with purple edges, and arise directly from the rhizome on separate shoots [14]. It belongs to the family *Zingiberaceae*, which also includes turmeric (*Curcuma longa*), cardamom (*Elettaria cardamomum*), and galangal. Its rhizomes and the leaves are used to flavour food or eaten directly. The leaves are also used to weave mats. Aside from these uses, ginger had religious significance among Austronesians, being used in rituals for healing and for asking protection from spirits. Raw ginger is 79% water, 18% carbohydrates, 2% protein, and 1% fat (table). In a reference amount of 100 g (3.5 oz), raw ginger supplies 333 kilojoules (80 kilocalories) of food energy and moderate amounts of potassium (14% of the Daily Value, DV), magnesium (10% DV) and manganese (10% DV), but otherwise is low in micronutrient content. It contains over 100 active compounds, including gingerols, shogaols, and paradols, which are thought to be responsible for its health benefits. It also contains anti-inflammatory substances, including the phenolic compounds shogaols, gingerols, and zingerone. These compounds work by inhibiting certain proinflammatory pathways in the body, like the nuclear factor-κB (NF-κB) signaling pathway, and decreasing levels of inflammatory proteins such as tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) [15].

When taken as a supplement, ginger is being associated with a number of health benefits, including reducing inflammation and improving outcomes in people with certain diseases, like type 2 diabetes [16]; and are effective for reducing symptoms of some inflammatory diseases such as arthritis [17]. It helps to reduce markers of oxidative stress, a condition that occurs when molecules called reactive oxygen species (ROS) overwhelm the body's antioxidant defenses, which leads to cellular damage [18]. Its root has been shown to be used for culinary and medicinal purposes for thousands of years. It can be consumed raw or cooked. Its warm, spicy flavor makes it a popular ingredient in dishes like curries and soups, and teas [16]. In addition to its culinary uses, ginger root has been consumed for health purposes since ancient times. It is widely used in traditional Asian medicine systems as Traditional Chinese Medicine (TCM) to treat a variety of ailments, from colds to arthritis and contains over 100 active compounds, including gingerols, shogaols, and paradols, which are thought to be responsible for its health benefits [16].

Thus, this study will help to educate the public on the ameliorating effect of ethanol extracts of *Zingiber officinale* on the histology of Ciprofloxacin-induced testes of male wistar rats, thereby encourages its consumption especially by patients who are placed on Ciprofloxacin treatment in other to prevent toxicity of body tissues due to the drug intake.

2. Material and methods

2.1. Animal procurement, care and treatment

Twenty-five (25) male wistar rats weighing between 110 g to150 g were procured and housed at the Animal house of Anatomy Department, Abia State University; Uturu with wire gauze cages in a well-ventilated area, were maintained under standard laboratory conditions of temperature (22+2^{0}C), relative humidity (55-65%) and 12 hours light/dark cycle. They were fed with standard commercial pellet diet and water *ad libitum* and were also acclimatized for two weeks before the experiment. Their health statuses were closely monitored before and during the experiment. All procedures were carried out in strict accordance with the Institutional guidelines on the care and use of experimental animals.

2.2. Collection, identification and preparation of plant material

Zingiber officinale were purchased from a local market in Uturu in Abia State, and were authenticated at Herbarium unit, Botany Department, Abia State University, Uturu, Abia State with the Herbarium number ABSU/ANA/HERB/25/001. The *Zingiber officinale* were washed, peeled and crushed using laboratory blender to obtain fresh juice. Extractions were done using ethanol. The crude ethanol extracts were kept in an air-tight container and stored in a refrigerator at 4 ^{0}C until time of use. At the time of use, the ethanol extracts were filtered into a stainless basin with a white cloth and placed in a water bath so as to dry up the ethanol. 250 mg of these extracts /kg body weights were dissolved in 10 mls of distilled water and were administered to the animals.

2.3. Induction of Ciprofloxacin

Ciprofloxacin was purchased at pharmaceutical shop at Okigwe in Imo State. According to research study, the oral LD_{50} of Ciprofloxacin is >2000 mg/kg body weight for Wistar rats (Pfizer: Ciprofloxacin MSDS), thus, 250 mg/kg of the Ciprofloxacin were dissolved in water and 50 mls of the solution were used to induced the rats daily for fourteen (14) days.

2.4. Experimental protocol

The animals were grouped into five (5) groups of five (5) rats each. Different doses of the leaf extracts were administered via oral route with the aid of oral gastric tube as shown below:

- Group A: The control group + distilled water.
- Group B: Ciprofloxacin only.
- Group C: Ciprofloxacin + Vitamin C.
- Group D: Ciprofloxacin + 100 mg/kg of body weight of ethanol extract of *Zingiber officinale*.
- Group E: Ciprofloxacin + 300 mg/kg of body weight of ethanol extract of *Zingiber officinale*.

2.5. Sample collection and analysis

The extracts were administered for fourteen (14) days. On the 15th day, the animals were sacrificed by anaestethizing under chloroform vapour and dissected. Testes harvested from the wistar rats, weighed, and were fixed in Bouin's fluid

for 72 hours, after which they were transferred to 10% buffered formalin. This was followed by histological and histochemical methods of tissue processing.

3. Results

3.1. Histopathological findings

Micrograph 1 is the result of the microscopic examination of the testes of the animals in group A (GPA) (x400) (H/E) showing normal testicular architecture with active seminiferous tubules that are lined with interstitial cells of the Leydig (ICL), Sertoli cell (SC) and moderately enhanced spermatogenesis (S).

Micrograph 2 is the result of the histology of the testes of the animals in group B (GPB) induced with Ciprofloxacin only (x400) (H/E) showing severe degeneration with severe spermatogenic arrest (SA), severe interstitial fibrosis (IF), and severe necrotic (N) appearance of the testicular cell.

Micrograph 3 is the result of the histology of the testes of the animals in group C (GPC) induced with Ciprofloxacin (x400) (H/E) treated with Vitamin C showing moderate healing with moderately enhanced spermatogenesis (S) and moderate active (N) appearance of the testicular cell.

Micrograph 4 is the result of the histology of the testes of the animals in group D (GPD) induced with Ciprofloxacin (x400) (H/E) treated with 100 mg/kg of body weight of ethanol extract of *Zingiber officinale* showing mild regeneration with moderate spermatogenic arrest (SA) severe interstitial fibrosis (IF) and moderate necrotic (N) appearance of the testicular cell.

Micrograph 5 is the result of the histology of the testes of the animals in group E (GPE) induced with Ciprofloxacin (x400) (H/E) treated with 300 mg/kg of body weight of ethanol extract of *Zingiber officinale* showing mild regeneration with moderate spermatogenic arrest (SA), and mild necrotic (N) appearance of the testicular cells.

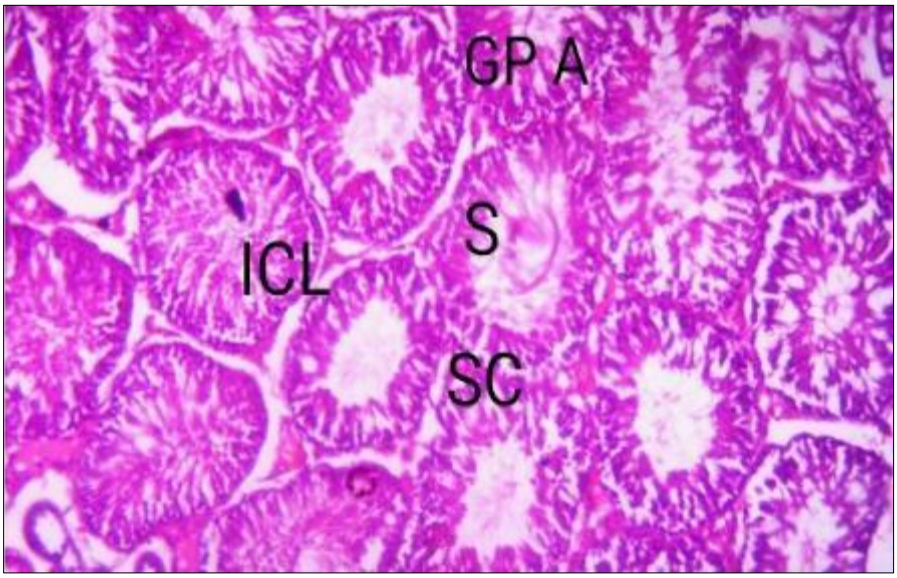

Figure 1 Micrograph 1 showing normal testicular architecture with active seminiferous tubules that are lined with interstitial cells of the Leydig (ICL), Sertoli cell (SC) and moderately enhanced spermatogenesis (S)

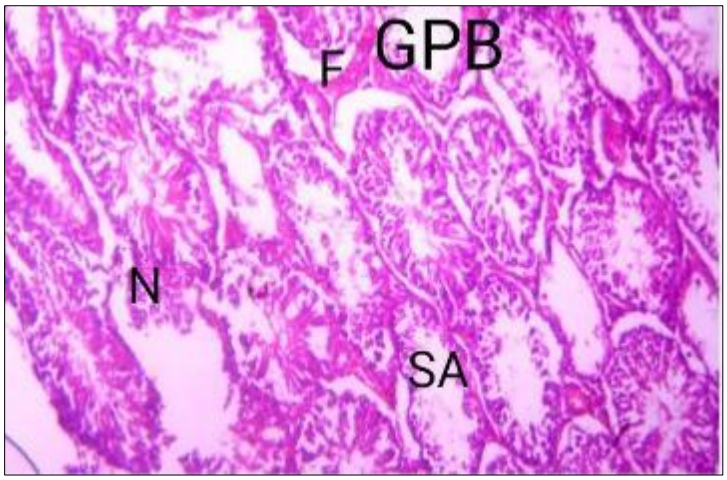

Figure 2 Micrograph 2 is showing severe degeneration with severe spermatogenic arrest (SA), severe interstitial fibrosis (IF), and severe necrotic (N) appearance of the testicular cell

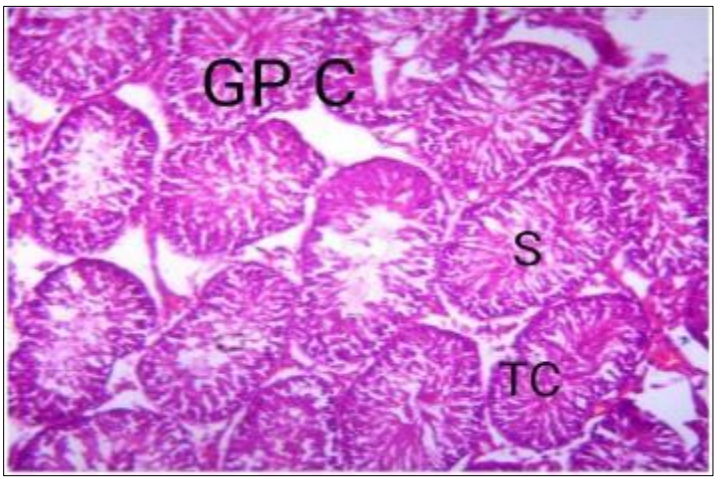

Figure 3 Micrograph 3 is showing moderate healing with moderately enhanced spermatogenesis (S) and moderate active (N) appearance of the testicular cell

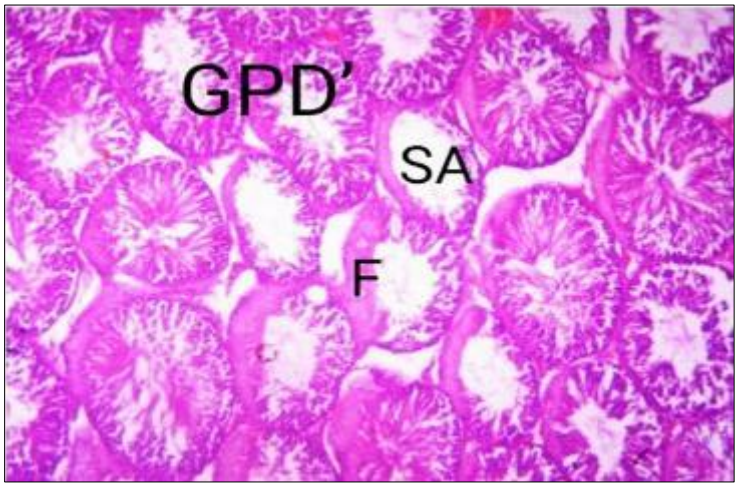

Figure 4 Micrograph 4 is showing mild regeneration with moderate spermatogenic arrest (SA) severe interstitial fibrosis (IF) and moderate necrotic (N) appearance of the testicular cell

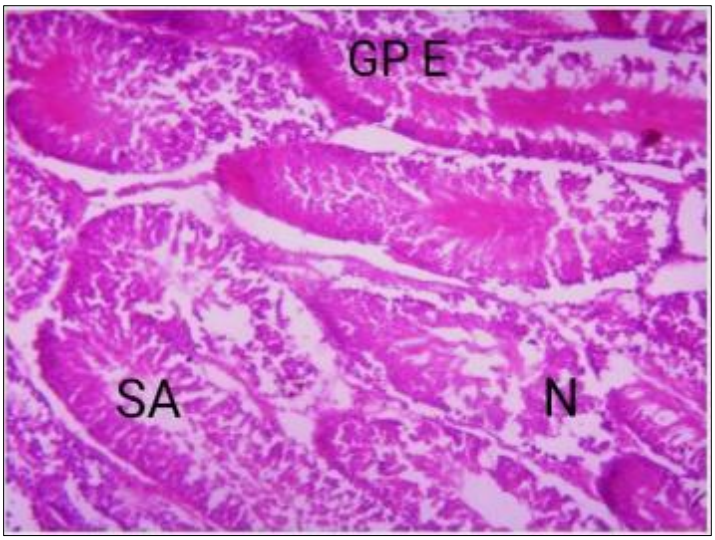

Figure 5 Micrograph 5 is showing mild regeneration with moderate spermatogenic arrest (SA), and mild necrotic (N) appearance of the testicular cells

4. Discussion

The histopathological finding of this present study of the testes of the animals in group A (GPA) (x400) (H/E) of Micrograph 1 showed normal testicular architecture with active seminiferous tubules that are lined with interstitial cells of the Leydig (ICL), Sertoli cell (SC) and moderately enhanced spermatogenesis (S) due to non-exposure to Ciprofloxacin. This is in line with the publication of Jana [20] that the histology of a normal testes is made up of tubules surrounded by the connective tissue stromal cells which contain testosterone secreting Leydig (interstitial) cells. The tubules are lined with a layer of seminiferous epithelium, which contains supporting Sertoli (sustentacular) cells, and spermatogenic cells.

While, the histopathological result of the histology of the testes of the animals in group B (GPB) induced with Ciprofloxacin only (x400) (H/E) of Micrograph 2 showed severe degeneration with severe spermatogenic arrest (SA), severe interstitial fibrosis (IF), and severe necrotic (N) appearance of the testicular cell. This could be due to the toxic nature of Ciprofloxacin as study has shown that Ciprofloxacin causes liver damage, acute renal failure relating to interstitial nephritis, and testicular function impairment [9].

Micrograph 3 of the histology of the testes of the animals in group C (GPC) induced with Ciprofloxacin (x400) (H/E) and treated with Vitamin C showed moderate healing with moderately enhanced spermatogenesis (S) and moderate active (N) appearance of the testicular cell. This could be due to the ameliorating property of Vitamin C to the induced drug. Research has shown that Vitamin C may play a pivotal role in alleviating acetaminophen-induced liver and kidney damage across different dosage regimens, potentially serving as a therapeutic intervention for preventing or treating drug-induced organ injuries [21]. Also, according to Adikwu and Deo, [22] Vitamin C is a major water-soluble antioxidant believed to decrease lipid peroxidation either directly or indirectly by regenerating vitamin E; and is an important free radical scavenger in extracellular fluids, trapping radicals and protecting biomembranes from peroxide damage. It can effectively scavenge singlet oxygen, superoxide, hydroxyl, water soluble peroxyl radical and hypochlorous acid [22]. Also, Vitamin C is an essential co-factor involved in many biochemical functions and acts as an electron donor or reducing agent.

The result of the histology of the testes of the animals in group D (GPD) induced with Ciprofloxacin (x400) (H/E) and treated with 100 mg/kg of body weight of ethanol extract of *Zingiber officinale* of Micrograph 4 showed mild regeneration with moderate spermatogenic arrest (SA) severe interstitial fibrosis (IF) and moderate necrotic (N) appearance of the testicular cell; While in Micrograph 5, the result of the histology of the testes of the animals in group E (GPE) induced with Ciprofloxacin (x400) (H/E) and treated with 300 mg/kg of body weight of ethanol extract of *Zingiber officinale* showed mild regeneration with moderate spermatogenic arrest (SA), and mild necrotic (N) appearance of the testicular cells. These could be due to the ameliorating effect of *Zingiber officinale* which increases with the increase in extract dosage. The positive effect observed on the histology of the testes in Micrographs 4 and 5 could be due to the ability of *Zingiber officinale* to reduce markers of oxidative stress, a condition that occurs when

molecules called reactive oxygen species (ROS) overwhelm the body's antioxidant defenses leading to cellular damage [18].

5. Conclusion

Ethanol extracts of *Zingiber officinale* have ameliorating effect on the histology of Ciprofloxacin-induced testes of male wistar rats, and the ameliorating effect is dose-dependent, and improves better with increase in dosages of the extract.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

This research work was approved by the Ethical Approval Committee, Basic Medical Sciences, Abia State University, Uturu, Abia State, Nigeria.

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