

Evaluation of synergistic cytotoxic potential of *Zingiber officinale* and *Allium sativum* on Wistar rats Testes¹*Joseph Oyepata Simeon, ¹Joseph Opeyemi Tosin¹Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Federal University, Oye-Ekiti, Ekiti State, Nigeria.¹Department of Pharmacology, Faculty of Pharmacy, Lead City University, Ibadan, Nigeria.**Abstract**

Plants decoction has been used since ancient times for various disease manifestations. It is usually believed that combining different plants source ensures efficacy. Garlic and ginger combination have been used in treating various illness such as cold, growth, immunity and infections. The combined effects of *Zingiber officinale* and *Allium sativum* extracts were tested on the testicles of Wistar rats by evaluating haematological, cell viability and histopathological parameters. The study was done by dividing male Wistar rats into 4 groups. Each group received different concentrations of extracts (group 1; 100 mg (ginger) + 150 mg/kg garlic, group 2. 200 mg + 300 mg/kg, group 3; and group 4; 400 mg + 450 mg). Cell isolation, cell viability and histological analysis were performed in the testicles. Compared with the control group, there was no increase in RBC ($P < 0.05$) but a decrease in WBC ($P < 0.05$) in all groups given GG extract. Compared with the control group, there was no significant difference in the level of living cells in groups 2 and 3 ($P < 0.05$), while in group 4 the level of dead cells decreased slightly compared to the control group ($P < 0.05$). No significant change in superoxide dismutase was seen in molecular tests. Histological examination showed a significant improvement in cellularity. The results show that taking garlic and ginger extract has no effect on tumor function.

keyword: garlic, ginger, rat, blood, testicle

Corresponding Author name: Joseph Oyepata Simeon

Email Address: oyepata.joseph@fuoye.edu.ng**Introduction**

In all bilateral animals, including humans, the testes or testicles (plural: testes) are the male glands or gonads. It is associated with female ovaries [1]. The testicles are responsible for producing sperm and male hormones, especially testosterone. Anterior pituitary luteinizing hormone controls testosterone release, while anterior pituitary follicle-

stimulating hormone and gonadal testosterone control sperm production. Males have two identical testicles in the scrotum, which is an extension of the abdominal wall [1]. Scrotal asymmetry is a condition in which one testicle extends further into the scrotum than the other. This is due to changes in the body's blood vessels. In 85% of men, the right testicle is lower than the left testicle [2].

Cytotoxicity refers to properties that are toxic to cells [3]. Poisons involve the body's immune system and various types of poisons. When cells are treated with cytotoxic drugs, they can become many cell types. Necrosis occurs when cells lose their membranes and die rapidly due to cell lysis. Cells may stop growing and dividing (lack of cell viability) or activate a genetic program that leads to controlled cell death (apoptosis). Necrosis causes cells to grow rapidly, loss of their membranes, lack of metabolism, and leakage of their contents into the environment. Cells that are rapidly damaged in vitro do not have sufficient time or energy to activate the apoptotic machinery and therefore lack apoptotic markers. Apoptosis is characterized by a series of cellular and molecular events, such as changes in cellular refractive index, cytoplasmic shrinkage, nuclear condensation, and fragmentation of DNA into disordered particles. Secondary necrosis occurs in cultured apoptotic cells. They stop metabolism, lose the membrane and disintegrate [4].

Medicinal plants have advantages over other plants because they contain phytochemicals. These phytochemicals are substances used to kill bacteria in the human body. Ginger (*Zingiber officinale*) is a flowering plant. It is popular as a spice or folk medicine [5]. Ginger is a perennial plant that is related to turmeric (*curcuma longa*), reaching up to one meter in height. Originally from the Indian subcontinent to the tropical rainforests of South Asia, where the ginger tree is distinct. Ginger is made from gingerol, which converts to shogaol during

dehydration. Ginger is the active ingredient in ginger and has been used to treat animal models of rheumatoid arthritis. It is also used to reduce body temperature in mice [6]. Ginger is cytotoxic against many types of cancer, and its activity against colon, ovarian, breast and pancreatic malignancies has been studied in vitro with promising results [7]. Garlic (*Allium sativum*) is a member of the Amaryllidaceae family. It belongs to the genus *Allium*. It has long been a popular food around the world. Its origin includes Central Asia and Northwestern Iran. Garlic was known to the ancient Egyptians, and history shows that it was used by humans as food and traditional medicine. Garlic cloves are edible, used medicinally and as a condiment or condiment. The intensity and flavor of garlic vary depending on the cooking method. It is usually eaten with ginger and onion. Garlic leaves are also eaten as a vegetable in some Asian countries. In some parts of Northern Nigeria, Garlic and ginger are usually taken as combination to treat various illness such as cold, cough, abnormal skin growth, stomach ache, appetite stimulants, immunity and infections [7]. The aim of this study was to evaluate the combined effects of *Zingiber officinale* and *Allium sativum* on the testicles of Wistar rats.

Materials and Method

Study area

This study was conducted at the University of Uyo, Akwa Ibom, State, Nigeria. Between February 10, 2022 and April 30, 2022. Uyo, Nigeria is in the Nigerian National Urban Areas

category with GPS coordinates of 5° 2' 20.2668" N and 7° 54' 34.0920" E on the day of arrival. Uyo has a summer season, a rainy

season, and a hot, dry climate; Annual temperatures range from 69°F to 87°F. [8] (Fig. 1).

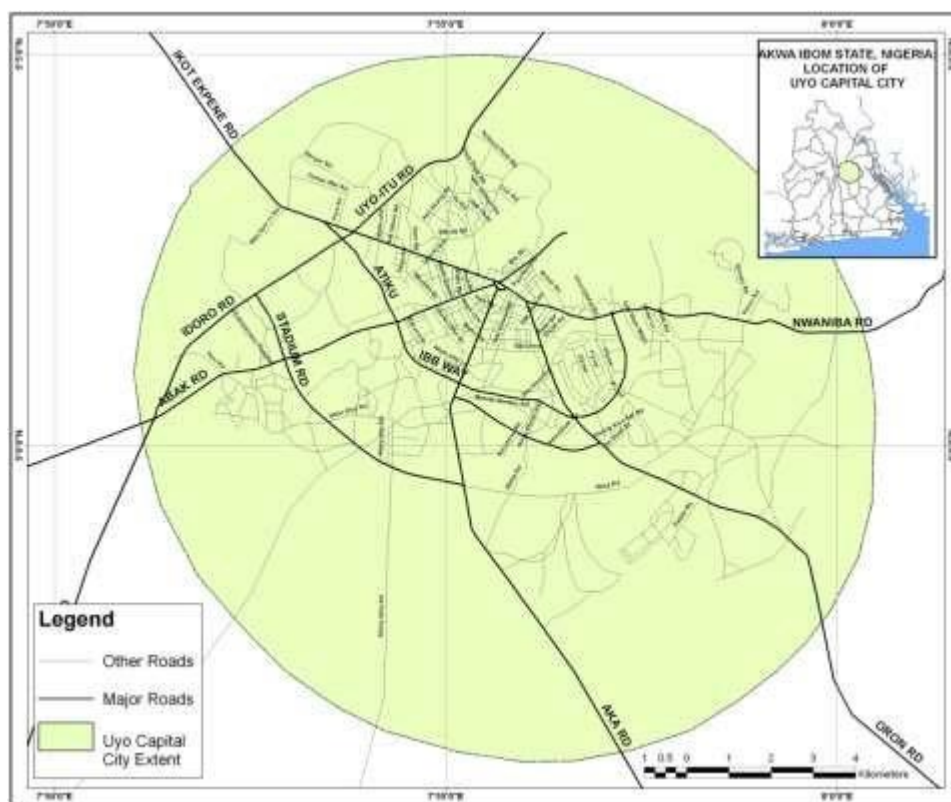


Figure 1. Map of the research area [8].

Plant collection

Zingiber officinale and *Allium sativum* were acquired in Uyo, Akwa Ibom State, Nigeria, from a grocery store. The plants were verified by the Department of Botany at the University of Uyo in Akwa Ibom State, Nigeria. The voucher specimens were stored in the herbarium department (No: 254 and 255).

Zingiber officinale (ginger)

The ginger was cut into small pieces and air-dried for 2 weeks. The dried ginger was ground to fine powder using a grinder. Ginger powder (110 g) was extracted in 400 ml of ethanol for

18 h in a soxhlet apparatus following the method of Misra *et al* [9]. The extract was dried at reduced pressure and stored at 0–4 °C until further experiments.

Allium sativum

Maceration was used to create an extract of *Allium sativum* (garlic). The garlic was cut into smaller bits. In 70% ethanol, the material was macerated. Using a rotary evaporator, the liquid filtrates were concentrated and evaporated to dryness at 40°C under vacuum. The ethanol extract was kept at 4°C until it was used.

Animals

Male Wistar rats were obtained from the University of Uyo Animal House. They were fed animal pellets purchased from Grand Cereals Limited and given unlimited water. Permission and approval for the animal study was granted by the Animal Ethics Committee of the Faculty of Health, University of Uyo (UU/2021/115). Rats ($n = 6$) were placed in different treatment groups. Animal studies were conducted under similar conditions. Care and handling of animals in accordance with public health guidelines in the Guide for the Care and Use of Animals (2011).

Study Design

The animal house at Derindam Research Institute of Biotechnology in Uyo, Akwa Ibom State, Nigeria, provided 24 albino wistar rats weighing 100-230g. The method and doses used in this work followed a modification of Peteret *al* [10]. Male wistar rats were divided into four groups, each with six rats. The first group received normal saline, the second group received 100mg (ginger) plus 150 mg/kg garlic (GG1), the third group received 200mg+ 300 mg/kg (GG2), and the fourth group received 400mg+ 450 mg/kg (GG3). Concurrent administration of extracts was performed.

Sample Collection

The ginger and garlic (GG) extracts were concurrently given to the animals for 21 days to evaluate on blood and testes of Wistar rats, after they were deprived of feed for 24 hours to clear their bowels and stabilize the levels of biochemical markers before been euthanized.

The animals were then anesthetized in the desiccator using chloroform saturated cotton wool. Blood was obtained from the dissected rat's heart through cardia puncture and placed in EDTA and plain bottles. Each rat's testes were immediately extracted, weighed, and fixed in Bouin's solution, with the sample bottles clearly labelled.

Cell Isolation test

After harvesting the testes, 100mg of the testis was sliced up and fixed in cell dissociating fluid before usage. In a new sample vial, a fresh dissociating fluid was placed; 50mg of the testis was sliced and minced inside the dissociating fluid to release the cells. The cell was centrifuged for 30 minutes, and the supernatant was decanted. A second time, 2 ml of dissociating fluids was added, and the cell was centrifuged for 5 minutes, and the supernatant was decanted. A third time, the washing was repeated for 5 minutes, and the supernatant was decanted. The cells obtained were employed in an isolation test.

Cell viability test

Using microtube A. The cells were mixed well, centrifuged, and the supernatant was decanted. Micropipette was used to mix the cells thoroughly after which 50 μ l of cells were collected in a clean tube (Microtube C). Microtube C was filled with 100 μ l of trypan blue solution. The solution was properly mixed and the counting chamber filled with the sample containing trypan blue.

Superoxide Dismutase (SOD) Assay

About 500µl antibody was mixed thoroughly with 5ml layer buffer in the tank. 50 µl of the initial mixture were put into each well of microwell plate, The prepared samples were placed into the first two wells and the lid was closed tightly. It was left in the dark for 60 minutes. After incubation at temperature of about 30°C, the first compound from the well was aspirated. Each Well were rinsed 3 times with detergent and then dry with a brush. The second solution was used for the superoxide dismutase (SOD) assay to investigate the antioxidant potential of the combination.

Histopathological evaluations

Testes were removed and stored in buffered formalin for 12 h before histological examination. Hematoxylin and eosin staining technique [7] was used in this study. Histopathological examination was performed using a light microscope. A histopathologist was blind to the coded treatment group and reviewed the slides to observe histological changes in tissue characteristics and features.

Data analysis

The data was expressed using the mean and standard error of the mean (SEM). Before Dunnett's post hoc test for multiple comparisons between the control and treated groups, one-way ANOVA was performed to statistically examine the data. P values of less than 0.05 were considered significant.

Result

Effect of *Zingiber officinale* and *Allium sativum* on change in body weight

There was significant ($P<0.05$) increase in change in bodyweight in group 3 and 4 when compared to the control group. There was no difference in weight gain on group 2 when compared to the control group (table 1).

Effect of *Zingiber officinale* and *Allium sativum* on haematological parameters

There was no significant ($P<0.05$) change in the level of RBA and WBC in all group administered with combine extract when compared to the control group. There was significant ($P<0.05$) decrease in the level of neutrophils and basophils at GG2 dose when compared to the control while was no significant ($P<0.05$) change in the of PCV, eosinophils, lymphocytes and monocytes in all doses administered when compared to the control group (Table 1 and 2).

Effect of *Zingiber officinale* and *Allium sativum* on cell viability

There was no significant ($P<0.05$) decrease in the level of live cells at GG1 dose when compared to the control group while there was no significant increase in level of dead cells at GG2 and GG3 doses when compared to control. There was no significant change in level of dead cells in GG1, GG2 and GG3 doses when compared to control group (table 3 and figure 2).

Effect of *Zingiber officinale* and *Allium sativum* on Molecular Assay using Superoxide Dismutase

There was significant ($P < 0.05$) increase in the level SOD in group two when compared to the control. There was no significant ($P < 0.05$) difference in group 3 and 4 when compared to the control group (table 4 and figure 3).

Effect of *Zingiber officinale* and *Allium sativum* on rat histology

Histological examination from the study revealed a significant improvement in histological features in all groups that received *Zingiber officinale* and *Allium sativum* when compared to the group that received normal saline (figure 4).

Table 1: body weight, organ weight, red blood cell and White blood cell of the animal

Groups	CWG	OW (TESTES)	RBC	WBC
1	37.50±10.0	0.8±0.1972	1.4±0.3	81400±2.49
2	26.67±4.0	0.8±0.09	2.3±0.5	57900±9.91
3	17.8±5.8*	0.9±0.1014	2.8±0.5	51633±1.6
4	16.2±7.2*	1.3±0.08913*	3.7±1.4*	53867±9.6

Values are Mean ± SEM of 6 rats in a group.

*significantly different compared to control group ($P < 0.05$)

(Keys: IBW- Initial body weight; FBW-Final body weight; CBW- Change in body weight; OW- Organ weight; RBC- Red blood cell; WBC- White blood cell)

Table 2: Hematological parameters

Group	PCV	Platelet	Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes
1	32.3±6.5	4.8±2.1	9.50±2.8	1.8±1.1	7.04±0.2	39.83±1.6	33.5±1.2

2	39.3±3.2	1.50±0.4*	10.50±2.2	1.8±0.6	8.50±0.2	45.3±7.4	39.3±3.3
3	39.5±1.8	2.5±0.5*	6.7±1.4*	1.0±0.00	8.04±0.3	52.83±2.5	33.3±0.5
4	39.7±1.2	2.3±0.6	11.5±3.2	1.8±0.4	8.3±0.2	39.83±6.1	38.3±0.8

Table 3: Showing the Cell Viability (Live Cells, Dead Cells and SOD)

Groups	Live Cell	Dead Cell	SOD
1	1.7±0.2	3.0±0.5	2.7±0.3
2	1.6±0.2	3.3±0.7	3.3±3.3*
3	0.8±0.2	2.8±0.4	2.1±0.26
4	0.8±0.2*	3.5±0.6	2.2±0.1

Keys: SOD-Superoxide Dismutase.

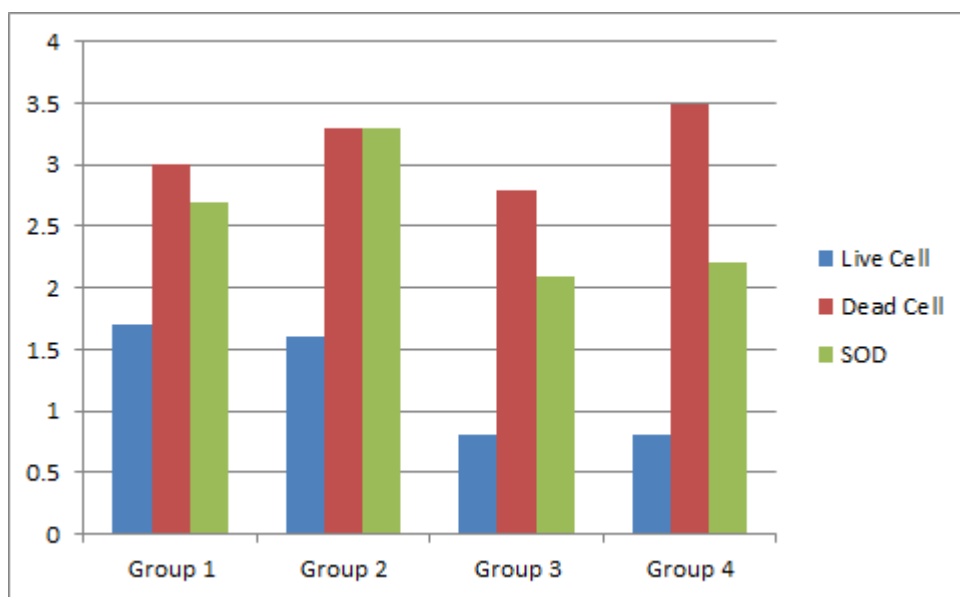


Figure 2: Showing the Cell Viability (Live Cells, Dead Cells and SOD)

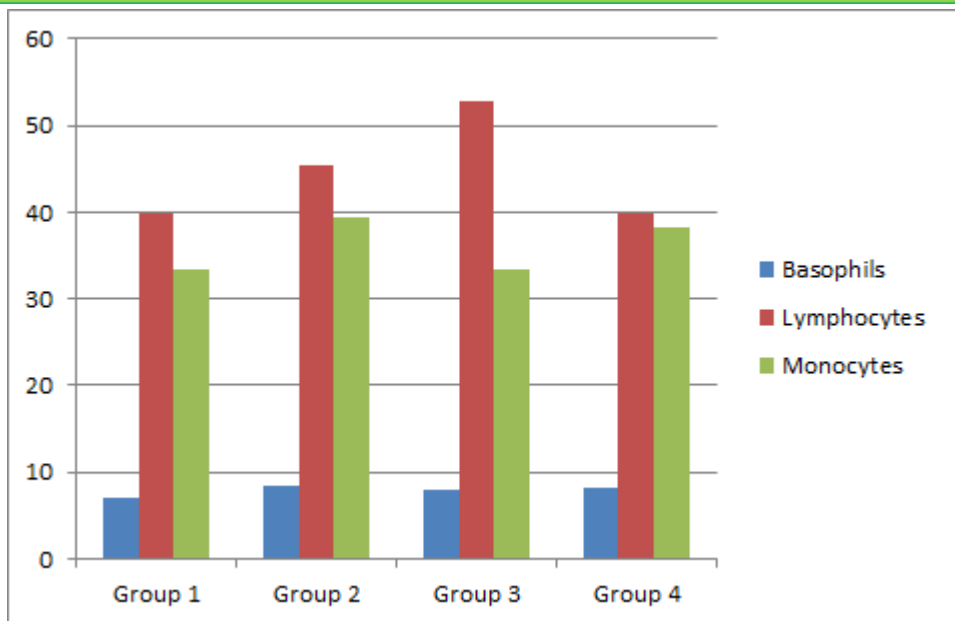


Figure 3: Showing Basophils, Lymphocytes and Monocytes

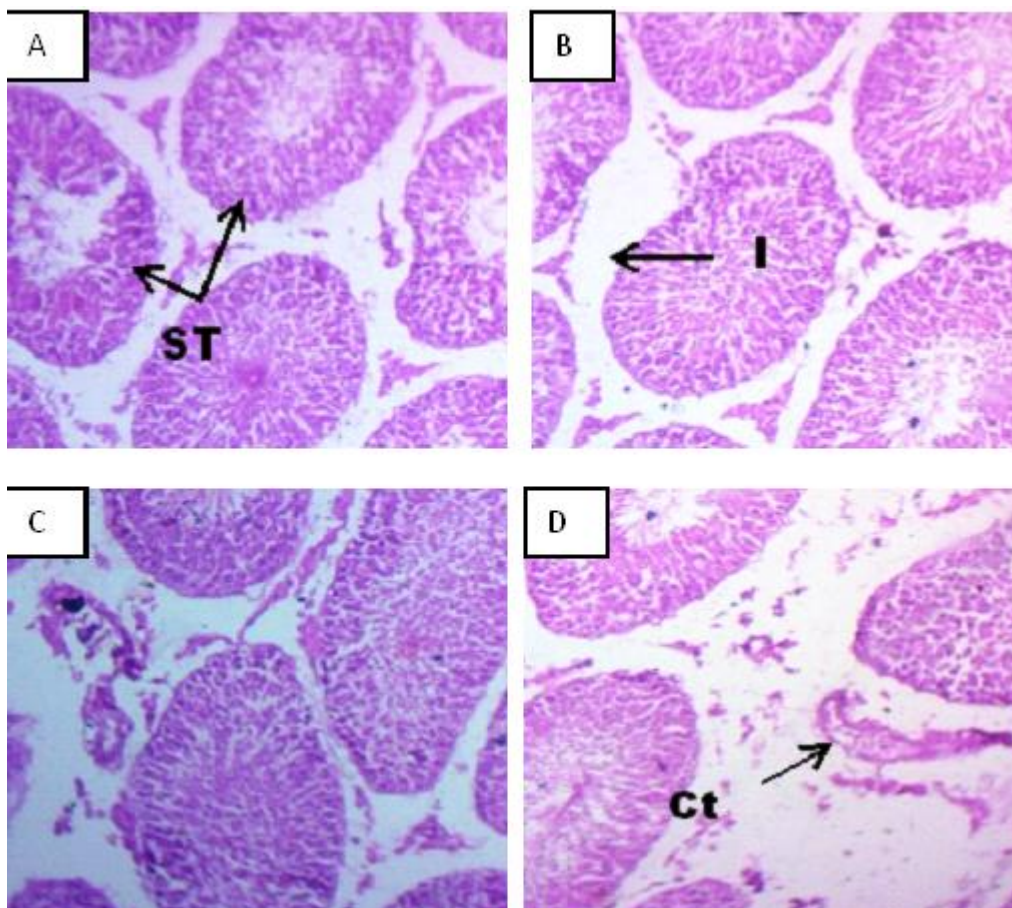


Figure 4: Histologic section of the Testes treated with section A, normal saline; Section B 100mg (ginger) + 150 mg/kg garlic (GG1); Section C 200mg+ 300 mg/kg (GG2), and Section D, 400mg+ 450 mg/kg (GG3). H&E staining techniques at magnification C(x100) and D(x400) revealed

normal cellular profile of significantly enhanced Testicular cyto-architecture in all groups when compared to control.

Discussion

There was no significant difference in the levels of hematological parameters in the group given the combined extract compared to the control group. Changes in these parameters in different animals are considered anaemic. Blood is widely used as a promising source of physiological alterations and alterations in toxicological research and environmental monitoring [10]. The data showed that simultaneous administration of ginger and garlic did not cause a decrease in red blood cells, hemoglobin, and erythrocyte count, resulting in the formation of red blood cells in mice. Importantly, this means that GG does not affect the oxygen-carrying capacity of the blood. With respect to the immune system, changes in leukocytes and their indicators (lymphocytes, neutrophils, eosinophils, and monocytes) are thought to be a potent factor in the stress response in many vertebrates, including mice. Various types of leukocytes are mainly involved in regulating immunity against foreign bodies and infections [11].

Superoxide dismutase (SOD) is the first line of defence against free radicals and is the main enzyme that catalyses the removal of superoxide free radicals [12]. It is increasingly recognized that regulation of SOD activity in growth, metabolism and oxidative stress response is important for tumor progression and survival [13,14]. Studies have shown that SOD is associated with colon cancer

[15, 16], but a clear relationship has not yet been established. Solomon *et al.* [4] found that SOD detection was associated with cancer and claimed that this finding provided some insight into the molecular basis of cancer development. The reduction in SOD levels in mice showed that ginger and garlic extracts did not cause or promote the growth of cancer cells.

In this study, while there was no decrease in live cells compared to the control group at the GG1 dose, there was a decrease in the number of dead cells compared to the control group at the GG2 and GG3 doses. A slight increase in the number of dead cells was seen when doses of GG1, GG2 and GG3 were compared with the control. The results showed that ginger and garlic extracts had cytotoxic effects on HeLa cells. Cells in culture are often used in routine cytotoxicity assays; where cells are exposed to a test substance and after a certain incubation period the markers are examined to influence the number of cells associated with the presence of good (toxin) and bad (toxin, vehicle) control process. In addition to determining live cells, it can also be used to measure the number of dead cells accumulated during the experiment and can distinguish between cytotoxicity and cell arrest or growth [16]. In some cases, estimating the number of dead cells may be more meaningful than measuring the decrease in markers used to estimate the number of living cells. In the presence of malignant cells, organosulfur

compounds (OSCs) in garlic can inhibit cell proliferation, causing apoptosis and cell cycle arrest [17]. Arivazhagan *et al.* [18] investigated the preventive effect of garlic extract on the growth of human breast cancer. According to Niles *et al.* [19], due to the presence of vitamin E, garlic has antioxidant properties that protect against hydrogen peroxide. Results from cell growth and cytotoxicity tests indicate that the antioxidant properties of garlic can reduce tumor toxicity of hazardous drugs while increasing spermatogenesis and fertility in male Wistar rats. In another study, garlic extract-mediated silver nanoparticles (Ag-S2) synergistically reduced the cell viability of MCF-7 cancer cells [20, 21]. However, it was observed that gold nanoparticles (G-AuNPs) produced from garlic extract did not harm MCF-7 cells. Histopathological examination revealed a significant increase in lymph nodes and normal cellularity in all groups compared to the control group. This is the same with other things, the combination of ginger and garlic does not disrupt the cell structure, on the contrary, it preserves its integrity.

Conclusion

The results of the study indicate that the combination of ginger and garlic extracts may exhibit mild cytotoxicity to some cells. Further research is needed to determine the extent and mechanism of the cytotoxic process.

Contribution of the authors

JOS and JOT designed the protocol for the research. They were also responsible for

interpreting of results and writing of the manuscript.

Conflict of Interest

The authors declare that there are not any potential conflicts of interest

Acknowledgement

The authors will like to thank everyone who has assisted in the successful outcome of this work

References

- [1] Steger, K., and Weidner, W (2011). "Anatomy of the Male Reproductive System". Practical Urology: Essential Principles and Practice. Springer Science & Business Media. pp. 57–59. ISBN 978-1-84-882034-0. Archived from the original on 2023-06-29. Retrieved 2022-06-01.
- [2] Lao, M., Smith, S., and Gilbert, B.R. (2020). "Male Reproductive Ultrasound". Practical Urological Ultrasound. Springer Nature. p. 298. ISBN 978-3-03-052309-1. Archived from the original on 2023-06-29. Retrieved 2022-07-05.
- [3] Rhoades, R.A. and Bell, D.R. (2012). Medical Physiology: Principles for Clinical Medicine. Lippincott Williams & Wilkins. p. 681. ISBN 978-1-60-913427-3. Archived from the original on 2023-06-29. Retrieved 2022-07-05.
- [4] Solomon, I.P., Oyebadejo, S.A., Ukpo E.M., and Joseph, O.S. (2015). Changes in

- serum electrolyte, creatinine and urea of fresh Citrus limon juice administered to growing rabbits (*Oryctolagus cuniculus*). International Journal of Agricultural Science Research. Vol. 4(8), pp. 180-183.
- [5] Condorelli, R., Calogero, A.E., La Vignera, S. (2013). "Relationship between Testicular Volume and Conventional or Nonconventional Sperm Parameters". International Journal of Endocrinology. 2013: 1–6. doi:10.1155/2013/145792. PMC 3780703. PMID 24089610.
- [6] Cho, S and Bae, J.H. (2017). "Penis and Testis". Clinical Regenerative Medicine in Urology. Springer. p. 281. ISBN 978-9-81-102723-9. Archived from the original on 2023-06-29. Retrieved 2022-06-01.
- [7] Simeon, J.O., and Tosin J.O. (2024). Toxicological Evaluation of Sub-Chronic Administration of Ethanol Leaf Extract of *Ocimumcanum* on Lipid Parameters, Heart Histology and Feed Consumption of Wistar Rats. Caliphate Journal of Science and Technology Journal/Caliphate Journal of Science and Technology/Vol. 6 No. 1. Pp. 70-76.
- [8] Weather for Uyo, Akwa Ibom, Nigeria". www.timeanddate.com. Retrieved 2023-08-27.
- [9] Misra, D.S., Rajkumar, M., Saradindu, B., Koushik, D., Ghosh, D. (2005) Protective effects of composite extract of *Withaniasomnifera*, *Ocimum sanctum* and *Zingiber officinale* on swimming in male rat. *Iranian J Pharma Thera.*;4:110–117.
- [10] Peter, I.A., Olumide Samuel F., Bamidele S.A., Adebola O.A., Olubukola S.O., (2021). Combined ginger and garlic extract improves serum lipid profile, oxidative stress markers and reduced IL-6 in diet induced obese rats, *Obesity Medicine*, 23:341–352.
- [11] Huhtaniemi, I. (2018). Encyclopedia of Endocrine Diseases. Academic Press. p. 667. ISBN 978-0-12-812200-6. Archived from the original on 2023-06-29. Retrieved 2022-06-02.
- [12] Schlegel, P.N.; Katzovitz, M.A. (2020). "Male Reproductive Physiology". Urologic Principles and Practice. Springer Nature. p. 50. ISBN 978-3-03-028599-9. Archived from the original on 2023-06-29. Retrieved 2022-06-02.
- [13] Riss, T.L., Moravec, R.A., Niles, A.L., Duellman, S., Benink, H.A., Worzella, T.J., et al. (2004). Cell Viability Assays. In: Sittampalam GS, Coussens NP, Brimacombe K, Grossman A, Arkin M, Auld D, et al., editors. Assay Guidance Manual. Bethesda (MD).
- [14] Korzeniewski, C., and Callewaert, D.M. (1983). An enzyme-release assay for natural cytotoxicity. *J Immunol Methods*. 64(3):313–20. Epub 1983/11/25. [PubMed]
- [15] Joseph, O. S., Joseph, O. T., El-Gazzar, A. M., Mahmoud, M. H., Omoirri, M. A.,

- & Gaber, E. S. B. (2024). Toxicological evaluation of ethanol leaf extract of *Pterocarpus santalinus* on lungs, stomach, brain and hematological parameters of Wistar rats. *Cogent Food & Agriculture*, 10(1), 2303828. Link: <https://www.tandfonline.com/doi/full/10.1080/23311932.2024.2303828>
- [16] Olsson, T., Gulliksson, H., Palmeborn, M., Bergstrom, K. (1983). Thore A. Leakage of adenylate kinase from stored blood cells. *J Appl Biochem*. 1983;5(6):437–45. Epub. [PubMed].
- [17] Squirrell, D., and Murphy, J. (1997). Rapid detection of very low numbers of micro-organisms using adenylate kinase as a cell marker. A practical guide to industrial uses of ATP luminescence in rapid microbiology. 107-13.
- [18] Arivazhagan, S., Kavitha, K., Nagini, S. (2007). Erythrocyte lipid peroxidation and antioxidants in gastric cancer patients. *Cell BiochemFunct*. 15(1):15–18.
- [19] Niles, A.L., Moravec, R.A., Eric,Hesselberth, P., Scurria, M.A., Daily, WJ., Riss, T.L. A (2007). homogeneous assay to measure live and dead cells in the same sample by detecting different protease markers. *Anal Biochem*. 366(2):197–206. Epub2007/05/22. [PubMed] [CrossRef]
- [20] Oyepata, J.S., Opeyemi, J.T., Adekunle, A.T., and Moses, O.A. (2024). Effect of *Pterocarpus santalinus* Ethanol Leaf Extract on Haematological, Histopathological and Lipid Profile Indices in Wistar Rats. *Trop J Nat Prod Res*. 2024; 8(3):6681-6685. <https://doi.org/10.26538/tjnpr/v8i3.30>
- [21] Yatsuya, H., Toyoshima, H., Tamakoshi, K, et al. (2005). Serum levels of insulin-like growth factor I, II, and binding protein 3, transforming growth factor beta-1, soluble fas ligand and superoxide dismutase activity in stomach cancer cases and their controls in the JACC Study. *J Epidemiol*. 15(2): S120–25.
- [22] Oyepata, J.S. and Opeyemi, J.T. (2024). Evaluation of Proliferative Potential of *Musa Paradisiaca* Stem Juice on the Pancreatic Cells of Wistar Rats. *Trop J Nat Prod Res*. 2024; 8(1):6035-6038. <http://www.doi.org/10.26538/tjnpr/v8i1.46>
- [23] Oyepata, J.S. and Adekunle, A.T. (2024). Haematological, Renal and Gastric Effects of Ethanol Leaf Extract of *Bauhinia variegata* Linn. *Trop J Phytochem Pharm. Sci*. 2024; 3(2):174-178. <http://www.doi.org/10.26538/tjpps/v3i2.3>