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COMBINED EFFECT OF AQUEOUS EXTRACTS OF *ZINGIBER OFFICINALE* AND *CURCUMA LONGA* ON MALE REPRODUCTIVE HORMONES OF MALE WISTAR RATS

Njoku-Oji, Njideka Nancy[1]; Ifegwu, Njoku Oji[2]; Nzejekwu, Sandra Nmesoma[3]; Uchefuna, Roy Chinwuba[4], Agbai, Johnson Ukwa[5]; Mbanaso, Eberechukwu Lolly[6]; Egege, Amaka Nkechukwu[7].

[2,3,4.] Department of Human Physiology, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.
[2,5.] Department of Anatomy, College of Medicine and Health Sciences, Abia State University Uturu, Abia State, Nigeria.
[6.] Department of Physiology, College of Medicine and Health Sciences, Abia State University Uturu, Abia State, Nigeria.
[7.] Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Abia State University Uturu, Abia State, Nigeria.

***Corresponding Author: Ifegwu, Njoku Oji.**
Department of Anatomy, College of Medicine and Health Sciences, Abia State University Uturu, Abia State, Nigeria.

ABSTRACT

Objective: This study was carried out to investigate on the combined effect of aqueous extracts of *zingiber officinale* (*Z. officinale*) and *Curcuma longa* (*C. longa*) on male reproductive hormones of male wistar rats.

Methodology: Thirty-five (35) male wistar rats weighing 130-180g were procured and acclimatized for two weeks, after which they were divided into seven (7) groups of five (5) rats each, and were housed in cages. The groups were designated as groups A, B, C, D, E, F and G. Group A served as control group and received only distilled water; while Groups B and C received 200mg/kg and 400mg/kg of *Z. officinale* extract respectively, Groups D and E received 200 and 400mg/kg of *C. longa* extract respectively, Group F received 100mg/kg of *Z. officinale* + 100mg/kg *C. longa* extracts, and Group G received 200mg/kg of *Z. officinale* + 200mg/kg of *C. longa* extracts for 21 days through oral route with the aid of oral gastric tube. On the 22nd day, the animals were sacrificed by chloroform inhalation, and blood samples were obtained through cardiac puncture for hormonal assays. Data obtained were analyzed using SPSS version 25 and ($P<0.05$) was considered significant.

Result: The hormonal levels of testosterone, luteinizing hormone and follicle stimulating hormone were significantly ($P<0.05$) increased in groups C, E and G which received 400mg/kg of the extracts when

compared with the control group. However, there was no significant difference on the levels of testosterone, LH and FSH levels for groups B, D and F which received 200mg/kg of the extracts compared with the control group.

Conclusion: Combined extracts of *Z. officinale* and *C. longa* are more potent when taken together for the prevention and treatment of male infertility.

Keywords: *Zingiber officinale, Curcuma longa*, testosterone, luteinizing hormone (LH) follicle, stimulating hormone (FSH).

1.0 INTRODUCTION

Research has shown that infertility affects millions of people of reproductive age worldwide, and has an impact on their families and communities [1]. A suggested estimate between 48 million couples and 186 million individuals that live with infertility globally has been made [2, 3, 4]. 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 [5]. In the male reproductive system, infertility is most commonly caused by problems in the ejection of semen [5], absence or low levels of sperm, or abnormal shape (morphology) and movement (motility) of the sperm [1]. Infertility is a condition with psychological, economic, medical implications resulting in trauma, stress, particularly in a social set-up like ours, with a strong emphasis on child-bearing [6]. According to the International Committee for Monitoring Assisted Reproductive Technology, World Health Organization (WHO), infertility is a disease of reproductive system defined by failure to achieve the clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [7]. It can also be defined as failure of couple to conceive after 12 months of regular intercourse without the use of contraception in women <35 years; and after 6 months of regular intercourse without the use of contraception in women ≥35 years [8]. One of the most serious social problems facing developed countries today is the declining birth rate, although it is generally not well recognized that the number of infertile couples is on the rise in these countries [9]. While both social (i.e., social progress for women and the resulting increase in the age at which women marry) and environmental (i.e., pollution and global warming) factors are behind part of the increase in the number of patients with infertility, infertility in the male partner contributes to approximately half of all cases [9].

In the male reproductive system, infertility may be caused by obstruction of the reproductive tract causing dysfunctionalities in the ejection of semen which can occur in the tubes that carry semen (such as ejaculatory ducts and seminal vesicles) and are commonly due to injuries or infections of the genital tract; hormonal disorders leading to abnormalities in hormones produced by the pituitary gland, hypothalamus and testicles e.g. testosterone regulate sperm production; testicular failure to produce sperm, for example due to varicoceles or medical treatments that impair sperm-producing cells (such as chemotherapy), and abnormal sperm function and quality [1]. Conditions or situations that cause abnormal shape (morphology) and movement (motility) of the sperm negatively affect fertility. For example, the use of anabolic steroids can cause abnormal semen parameters such sperm count and shape [10]. Environmental and lifestyle factors such as smoking, excessive alcohol intake and obesity can affect fertility. In addition, exposure to environmental pollutants and toxins can be directly toxic to gametes (eggs and sperm), resulting in their decreased numbers and poor quality, leading to infertility [10, 11]. This research aims at investigating on the use of herbal medicines to prevent or ameliorate the effect of infertility in men since they possess lower side effects and are also easily accessible to all level of income earners. Thus, *Zingiber officinale* and *Curcuma longa* are being researched in this study.

Z officinale also known as gimger belongs to *Zingiberaceae* family. It is a popular spice used globally especially in most of the Asian countries [12]. It contains over 400 different compounds, and the major constituents are carbohydrates (50–70%), lipids (3–8%), terpenes, and phenolic compounds [13]. Its terpene components include zingiberene, β-bisabolene, α-farnesene, β-sesquiphellandrene, and α-curcumene, while its phenolic constituents include gingerol, paradols, and shogaol, with gingerols (23–25%) and shogaol (18–25%) found in higher quantity than others. Besides these, amino acids, raw fiber, ash, protein, phytosterols, vitamins (e.g., nicotinic acid and vitamin A), and minerals are also present [14, 15].

Ginger is being used as a spice as well as medicine in India and China since ancient times. Medicinally it is also being used to prevent nausea resulting from chemotherapy, motion sickness, and surgery [16], remedy for nausea during pregnancy [14], act as an anti-inflammatory agent for joint problems [15], antiviral for treatment of cold and flu [17], flavoring agent in foods and beverages and as a fragrance in soaps and cosmetics [18], promotes ulcer healing by acting as an antioxidant and prevents gastric mucosal damage [19], acts as an antiemetic for cancer chemotherapy [20] and prevents hepatocarcinogenesis [21]. Studies have also shown that *Z. officinale* also possesses pro-fertility properties in male rats which might be a product of both its potent antioxidant properties and androgenic activities [22], enhances sperm healthy parameters [23] and improves spermatozoa characteristics and semen hormone level [24].

Curcuma longa (Turmeric) is a plant in the ginger family. It is native to Southeast Asia and is grown commercially in that region, primarily in India [25]. Its rhizome is used as a culinary spice and traditional medicine. Historically, it was used in Ayurveda and other traditional Indian medical systems, as well as Eastern Asian medical systems such as traditional Chinese medicine [25]. In India, it was traditionally used for disorders of the skin, upper respiratory tract, joints, and digestive system [25]. Today, it is promoted as a dietary supplement for a variety of conditions, including arthritis, digestive disorders, respiratory infections, allergies, liver disease, depression, and many others [25]. Turmeric is a common spice and a major ingredient in curry powder and curcumin - its major component and its activities are commonly attributed to curcuminoids (curcumin and closely related substances) [25]. Curcumin gives turmeric its yellow color. *C. longa* dietary supplements are made from the dried rhizome and typically contain a mixture of curcuminoids. It is also made into a paste for skin conditions [25].

Turmeric is a rich source of carbohydrates and fiber. Also, it contains some proteins and fats, but there is no cholesterol in it. Furthermore, it contains pyridoxine, vitamin C, potassium, calcium, magnesium, and phosphorous in appropriate amounts, making it one of the nutritionally rich natural food products [26]. Turmeric and its ingredient can be considered as multitargeted phytochemicals for cancer treatment. For example, apoptosis, autophagy, and cell cycle arrest can be affected by their use [27]. *C. longa* has anti-inflammatory properties that may be attributed to its ability to inhibit both biosynthesis of inflammatory prostaglandins from arachidonic acid, and neutrophil function during inflammatory states [28]. Curcuminoids, a major phytochemicals of the turmeric responsible for its characteristic yellow color has been revealed to be medicinally useful as antioxidant, anti-hypertensive, anti-inflammatory, anticarcinogenic, thrombus suppressive, hypoglycaemic and antiarthritic properties [29, 30, 31, 32].

Curcumin present in turmeric has great antioxidant properties that bring about significant improvement in sperm motility and sperm viability. In addition to curcumin, turmeric also contains Vitamin C that helps increase sperm count [33]. Therefore, turmeric can help improve the entire sperm profile, and in turn, have positive effects on male fertility [33]. Curcumin is also enriched with antioxidants that protect the male reproductive system from oxidative stress, and thus help maintain proper testosterone levels and sperm DNA integrity [33]. In addition to oxidative stress, turmeric also protects the sperms and male reproductive system from toxic compounds like chromium (VI), nitrate pollutants, and some toxins present in cigarettes. These compounds can damage the sperms and reduce their quality [33]. Medical experts claim curcumin to have antidepressant effects as well. Thus, turmeric also works effectively in dealing with the psychological causes that affect male fertility [33]. The rhizomes in tumeric prevent hypertension-mediated male reproductive dysfunction [34].

Therefore, this study was carried out to investigate the effect of aqueous extracts of *Z. officinale* and *C. longa* on reproductive hormones of male wistar rats as no research work has been carried out it.

2.0 MATERIALS AND METHODS

2.1 Animal procurement, care and treatment

Thirty five (35) male wistar rats weighing between 130 to 180g were procured and housed at the Animal house, Department of Human Physiology, Nnamdi Azikiwe University, Nnewi, Anambra State, with wire

gauze cages in a well-ventilated area with temperature between 25 to 28 degrees. They were fed with standard commercial pellet diet and water *ad libitum*. There were 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 and preparation of material

Z. officinale and *C. longa* rhizomes were purchased from the local market at Nkwo Nnewi, Anambra State Nigeria. They were washed, peeled and cut into smaller pieces, shade dried and milled into a coarse powder using local grinder. The powdered extracts were macerated in distilled water, poured into a beaker and sealed. The two mixtures were placed differently in a mechanical shaker for 24 hours and were sieved with filter papers into clean tubes. The filtrates were preserved in the refrigerator at 40 degree Celsius. At the time of use, 250mg of these extracts/kg body weights were dissolved in 10mls of distilled water and administered to the animals.

2.3 Experimental protocol

The animals were grouped into seven (7) groups of five (5) rats each. Different doses of the *Zingiber officinale* and *Curcuma longa* 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	200mg/kg *Zingiber officinale*
Group C	400mg/kg *Zingiber officinale*
Group D	200mg/kg *Curcuma longa*
Group E	400mg/kg *Curcuma longa*
Group F	100mg/kg *Zingiber officinale* + 100mg/kg *Curcuma longa*
Group G	200mg/kg *Zingiber officinale* + 200mg/kg *Curcuma longa*

2.4 Sample collection and analysis

The extracts were administered for twenty one (21) days. On the 22nd day, the animals were sacrificed by anaestethizing under chloroform vapour and blood samples were collected from each of the rats by cardiac puncture for hormonal assays. The obtained blood samples were spun at 2500pm for 10min using wisperfuge model 1384 centrifuge at 10-25^{0}C, serum samples were collected, refrigerated and assayed for follicle stimulating hormone, testosterone and luteinizing hormone using the microwell enzyme linked immunoassay (ELISA) technique, and analytical grade reagent (Syntron Bioresearch Inc, USA).

2.5 Statistical Analysis

All data were tabulated and statistically analyzed using SPSS version 25.0. Results were expressed as Mean ± standard error of mean (SEM). Comparative analysis amongst groups was done using one-way analysis of variance (ANOVA). A post-hoc analysis using Bonferoni multiple comparative tests was performed to identify significant groups. $P<0.05$ was taken as statistically significant.

3.0 RESULTS

Table 1: Effect of aqueous extracts of *Zinigiber officinale* and *Curcuma longa* on serum testosterone level

Groups	Dosage of aqueous extracts	Testosterone (ng/dl)
A	Control (Distilled water)	3.38 ± 0.19
B	200mg/kg *Zingiber officinale*	3.59 ± 0.22
C	400mg/kg *Zingiber officinale*	4.57 ± 0.12*
D	200mg/kg *Curcuma longa*	3.37 ± 0.30
E	400mg/kg *Curcuma longa*	4.71 ± 0.26*
F	100mg/kg *Zingiber officinale* + 100mg/kg *Curcuma longa*	3.95 ± 0.16
G	200mg/kg *Zingiber officinale* + 200mg/kg *Curcuma longa*	5.14 ± o.21*

Table 1 showed a significant increase (P<0.05) on the serum testosterone level of the animals in groups C, E and D that received 400mg/kg of *Z. officinale*, 400mg/kg of *C. longa* and 200mg/kg of *Z. officinale* + 200mg/kg of *C. longa* respectively when compared with the control group A. However, there was no significant difference (P>0.05) on the serum testosterone level of the animals in groups B, D and F that received 200mg/kg of *Z. officinale*, 200mg/kg *C. longa* and 100mg/kg of *Z. officinale* + 100mg/kg of *C. longa* respectively when compared with the control group A.

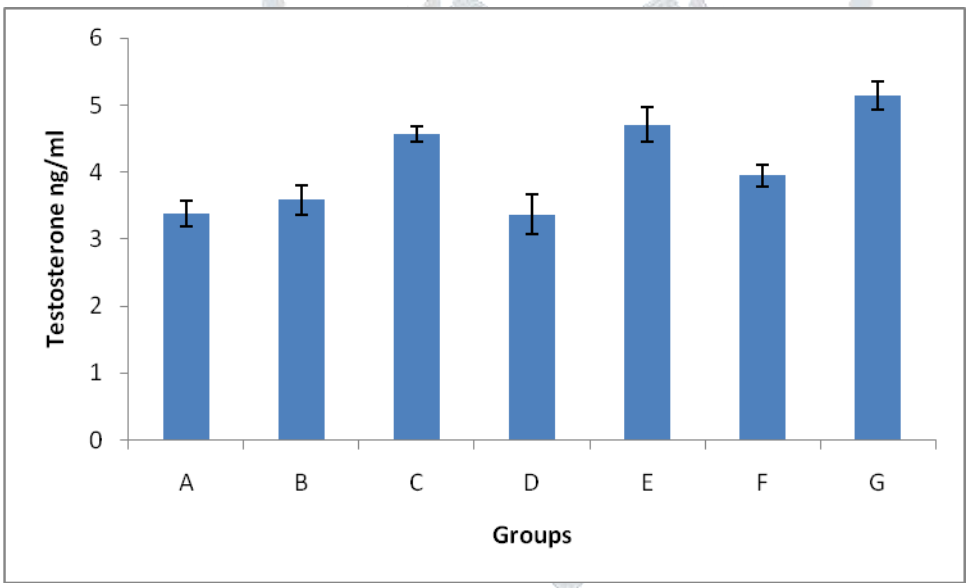

Fig 1: Effect of aqueous extracts of *Z. officinale* and *C. longa* on serum testosterone level of male wistar rats.

Table 2: Effect of aqueous extracts of *Zinigiber officinale* and *Curcuma longa* on serum luteinizing hormone (LH) level

Groups	Dosage of aqueous extracts	LH (IU/L)
A	Control (Distilled water)	3.64 ± 0.08
B	200mg/kg *Zingiber officinale*	3.76 ± 0.10
C	400mg/kg *Zingiber officinale*	4.56 ± 0.18*
D	200mg/kg *Curcuma longa*	3.37 ± 0.30
E	400mg/kg *Curcuma longa*	4.71 ± 0.26*
F	100mg/kg *Zingiber officinale* + 100mg/kg *Curcuma longa*	3.95 ± 0.16
G	200mg/kg *Zingiber officinale* + 200mg/kg *Curcuma longa*	5.14 ± 0.21*

Table 2 showed a significant increase (P<0.05) on the serum luteinizing hormone (LH) level of the animals in groups C, E and D that received 400mg/kg of *Z. officinale*, 400mg/kg of *C. longa* and 200mg/kg of *Z. officinale* + 200mg/kg of *C. longa* respectively when compared with the control group A. However, there was no significant difference (P>0.05) on the serum luteinizing hormone (LH) level of the animals in groups B, D and F that received 200mg/kg of *Z. officinale*, 200mg/kg *C. longa* and 100mg/kg of *Z. officinale* + 100mg/kg of *C. longa* respectively when compared with the control group A.

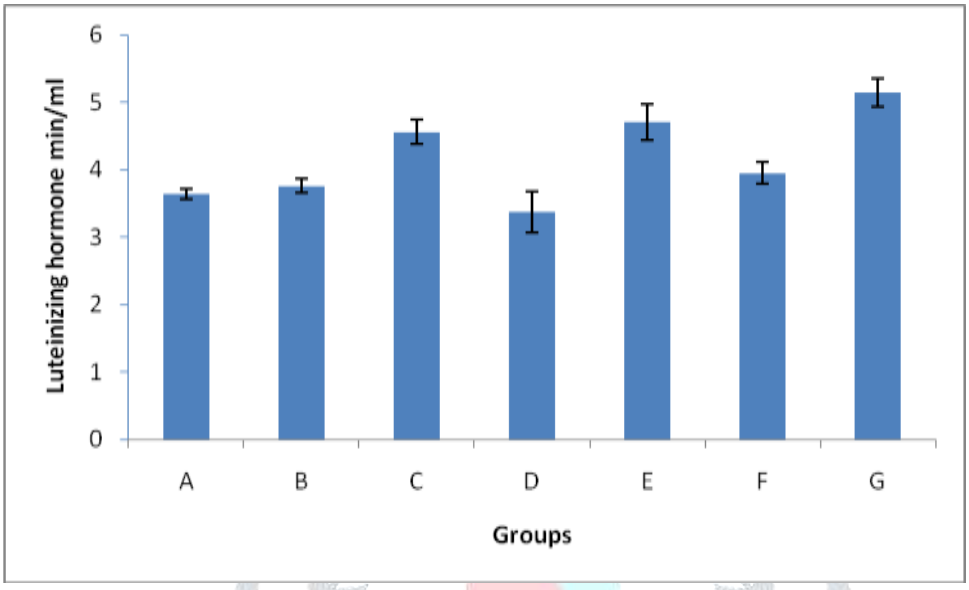

Fig 2: Effect of aqueous extracts of *Z. officinale* and *C. longa* on serum luteinizing hormone level of male wistar rats.

Table 3: Effect of aqueous extracts of *Zinigiber officinale* and *Curcuma longa* on serum follicle stimulating hormone (FSH) level

Groups	Dosage of aqueous extracts	FSH (IU/mL)
A	Control (Distilled water)	3.71 ± 0.14
B	200mg/kg *Zingiber officinale*	3.81 ± 0.13
C	400mg/kg *Zingiber officinale*	4.65 ± 0.18*
D	200mg/kg *Curcuma longa*	3.75 ± 0.12
E	400mg/kg *Curcuma longa*	4.34 ± 0.15*
F	100mg/kg *Zingiber officinale* + 100mg/kg *Curcuma longa*	3.89 ± 0.20
G	200mg/kg *Zingiber officinale* + 200mg/kg *Curcuma longa*	5.19 ± 0.17*

Table 3 showed a significant increase (P<0.05) on the serum follicle stimulating hormone (FSH) level of the animals in groups C, E and D that received 400mg/kg of *Z. officinale*, 400mg/kg of *C. longa* and 200mg/kg of *Z. officinale* + 200mg/kg of *C. longa* respectively when compared with the control group A. However, there was no significant difference (P>0.05) on the serum follicle stimulating hormone (FSH) level of the animals in groups B, D and F that received 200mg/kg of *Z. officinale*, 200mg/kg *C. longa* and 100mg/kg of *Z. officinale* + 100mg/kg of *C. longa* respectively when compared with the control group A.

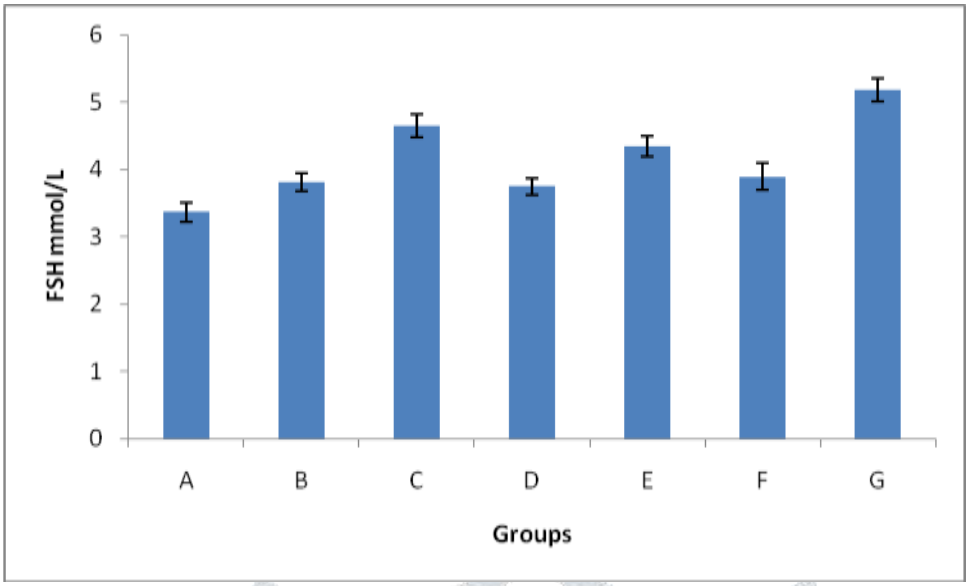

Fig 3: Effect of aqueous extracts of *Z. officinale* and *C. longa* on serum follicle stimulating hormone level of male wistar rats.

4.0 DISCUSSION

Result observed in table 1/figure 1 could be due to the fact that the curcumin in *Curcuma longa* lower blood glucose levels by reducing hepatic glucose production, thus improving insulin sensitivity. This can then lead to higher testosterone rates, as turmeric allows Leydig cells to absorb more insulin [35]. Also turmeric's antioxidant properties also have a fringe benefit as they help to burn fat at a marginally faster rate. Since testosterone heavily depends on existing body composition and fat mass levels, turmeric's properties come in handy for increasing testosterone level [35]. Studies carried out on mice showed that curcumin turmeric stops adipogenesis from taking place – the process of fat cell differentiation that allows fat cells to be stored. Turmeric destroyed fat cells through a process known as apoptosis, thus favoring testosterone production. Studies on humans have also shown that turmeric supplements lead to higher fat loss and commensurately lowered body mass index (BMI); and improved testosterone production [35]. *Z. officinale* (ginger) on the other hand enhances testosterone production by mainly increasing LH production, increasing the level of cholesterol in testes, reducing oxidative stress and lipid peroxidation in the testes, enhancing the activity of certain antioxidant enzymes, normalizing blood glucose, enhancing nitric oxide production and increasing blood flow in Leydig cells, increasing testicular weight, and recycling testosterone receptor [36]. Thus the serum testosterone level of the animals in groups C, E and D that received 400mg/kg of *Z. officinale*, 400mg/kg of *C. longa* and 200mg/kg of *Z. officinale* + 200mg/kg of *C. longa* respectively increased significantly ($P<0.05$) when compared with the control group A showing that combined dosage of the of the extracts enhances testosterone synthesis at lower dosage than when used individually.

The significant increase ($P<0.05$) on the serum luteinizing hormone (LH) level of the animals in groups C, E and D that received 400mg/kg of *Z. officinale*, 400mg/kg of *C. longa* and 200mg/kg of *Z. officinale* + 200mg/kg of *C. longa* respectively when compared with the control group A (figure 2) could be traced to the mechanisms through which ginger enhances testosterone production mainly by increasing LH production, increasing the level of cholesterol in testes, reducing oxidative stress and lipid peroxidation in the testes, enhancing the activity of certain antioxidant enzymes, normalizing blood glucose, enhancing nitric oxide production and increasing blood flow in Leydig cells, increasing testicular weight, and recycling testosterone receptors [36]. Also the protodioscin and saponins of ginger increase testosterone and

luteinizing hormone (LH) hormone levels as well as libido which can be used in traditional medicine to treat sexual dysfunctions [37]. *C. longa* improves pup viability and could promote rabbit fecundity by either promoting the production of primary ovarian follicles or stimulating the growth of follicles at all stages of folliculogenesis [38].

In figure 3, the significant increase ($P<0.05$) on the serum follicle stimulating hormone (FSH) level of the animals in groups C, E and D that received 400mg/kg of *Z. officinale*, 400mg/kg of *C. longa* and 200mg/kg of *Z. officinale* + 200mg/kg of *C. longa* respectively when compared with the control group A could be because turmeric improve semen quality and quantity, and also increase testosterone, luteinizing hormone and follicle stimulating hormone levels in serum [39]. Also the curcumin present in turmeric has great antioxidant properties that bring about significant improvement in sperm motility and sperm viability. In addition to curcumin, turmeric also contains Vitamin C that helps increase sperm count [33]. Therefore, turmeric can help improve the entire sperm profile, and in turn, have positive effects on male fertility [33]. Likewise the result also showed that *Z. officinale* possesses pro-fertility properties which might be a product of both its potent antioxidant properties and androgenic activities [22], enhances sperm healthy parameters [23] and improves spermatozoa characteristics and semen hormone level [24].

5. Conclusion

This study has shown that combined aqueous extracts of *Z. officinale* and *C. longa* has pro-fertility effect on the serum levels of testosterone, luteinizing hormone and follicle stimulating hormone when taken together at lower dosage than when taken differently. This research therefore support combined intake of the extracts to improve male fertility.

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Ethical Approval: Approved by Institutional ethical approval.

REFERENCES

1. World Health Organization. “Infertility”. 14 September, 2021. https://www.who.int/news-room/fact-sheets/detail/infertility
2. Mascarenhas MN, Flaxman SR, Boerma T, *et al.* National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. *PLoS Med* 2012; 9(12):e1001356.
3. Boivin J, Bunting L, Collins JA, *et al*. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Human reproduction (Oxford, England) 2007; 22(6):1506-12.
4. Rutstein SO, Shah IH. Infecundity infertility and childlessness in developing countries. Geneva: World Health Organization 2004.
5. World Health Organization (WHO). International Classification of Diseases, 11th Revision (ICD-11) Geneva: WHO 2018.
6. Naina Kumar and Amit Kant Singh. Trends of male factor infertility, an important cause of infertility: A review of literature. *J Hum Reprod Sci.* 2015 Oct-Dec; 8(4): 191–196.
7. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, et al. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. *Fertil Steril.* 2009; 92:1520–4.
8. Practice Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss. *Fertil Steril.* 2008; 90(5 Suppl):S60.

9. Toshinobu M., Akira T., Yasushi M., Eitetus K., Mikio N., Kazuo S. "Male infertility and its causes in human". *Advances in Urology*, vol 2012, Article ID 384520, 7 pages, 2012. https://doi.org./10.1155/2012/384520
10. Gore AC, Chappell VA, Fenton SE, *et al.* EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. *Endocrine Reviews* 2015; 36(6):E1-E150.
11. Segal TR and Giudice LC. Before the beginning: environmental exposures and reproductive and obstetrical outcomes. *Fertility and Sterility* 2019; 112(4):613-21.
12. Demin G. and Yingying, Z "Comparative antibacterial activities of crude polysaccharides and flavonoids from *Zingiber officinale* and their extraction," *American Journal of Tropical Medicine*, 2010; (5). 235–238.
13. Grzanna R, Lindmark L, Frondoza CG. "Ginger—an herbal medicinal product with broad anti-inflammatory actions," *Journal of Medicinal Food*, 2005; 8: (2) 125–132.
14. Langner E, Greifenberg S, Gruenwald J. "Ginger: history and use,*" Advances in Therapy*, 1998; 15: (1) 25–44.
15. Shukla, Y and Singh, M. "Cancer preventive properties of ginger: a brief review," *Food and Chemical Toxicology*, 2007; 45: (5) 683–690.
16. Holtmann S, Clarke AH, Scherer H, Hohn M."The anti-motion sickness mechanism of ginger. A comparative study with placebo and dimenhydrinate," *Acta Oto-Laryngologica*, 1989; 108 (3) 4:168–174.
17. Qidwai W, Alim SR, Dhanani RH, Jehangir S, Nasrullah A, Raza A. "Use of folk remedies among patients in Karachi Pakistan," *Journal of Ayub Medical College, Abbottabad*, 2003; 15 (2): 31–33.
18. Alam, P. "Densitometric HPTLC analysis of 8-gingerol in Zingiber officinale extract and ginger-containing dietary supplements, teas and commercial creams," *Asian Pacific Journal of Tropical Biomedicine*, 2013; 3(8): 634–638.
19. Ko KJ and Leung CC. "Ginger extract and polaprezinc exert gastroprotective actions by anti-oxidant and growth factor modulating effects in rats," *Journal of Gastroenterology and Hepatology (Australia)*, 2010; 25 (12): 1861–1869.
20. Sharma SS and Gupta KY. "Reversal of cisplatin-induced delay in gastric emptying in rats by ginger (*Zingiber officinale*)," *Journal of Ethnopharmacology*, 1998; 62 (1) 49–55.
21. Yusof YAM, Ahmad N, Das S., Sulaiman S, Murad NA. "Chemopreventive efficacy of ginger (Zingiber officinale) in ethionine induced rat hepatocarcinogenesis," *African Journal of Traditional, Complementary and Alternative Medicines*, 2009; 6 (1): 87–93.
22. Morakinyo AO, Adeniyi OS, Arikawe AP. Effects of Zingiber Officinale on Reproductive Functions in the Male Rat. *African Journal of Biomedical Research*, 2008; (11): 329 – 334.
23. Khaki A, Fatemeh F, Nouri M, Khaki A, Ozanci C, Novin M, Hammadeh ME. (2009). The effects of Ginger on spermatogenesis and sperm parameters of rat. *Iranian Journal of Reproductive Medicine.* 2009; (7): 7-12.
24. Ali A. and Jamshid GG. Effect of ginger, Zingiber offi cinale on sex hormones and certain biochemical parameters of male Wistar rats. *Biosci. Biotech. Res. Comm.* 2018; 11(1): 181-186.
25. National Center for Complementary and Integrative Health (NCCIH), "Turmeric". *National Institute of health.* May 2020. https://www.nccih.nih.gov/health/turmeric
26. Pradeep, KU, Geervani, P, Eggum, BO. "Common Indian spices: nutrient composition, consumption and contribution to dietary value," *Plant Foods for Human Nutrition*, 1993; 44 (2): 137–148.
27. Zhu Z. and Bu, S. "Curcumin induces autophagy, apoptosis, and cell cycle arrest in human pancreatic cancer cells," *Evidence-Based Complementary and Alternative Medicine*, 2017; 2017.
28. Cronin JR. Curcumin: Old spice is a new medicine. *Journal of Alternative & Complementary Therapies*. 2003; 9(1):34-8.
29. Arun N and Nalini N. Efficacy of turmeric on blood sugar and polyol pathway in diabetic albino rats. *Plant Foods Hum. Nutr.*, 2002; (57) : 41-52.
30. Kowluru RA and Kanwar, M. Effects of curcumin on retinal oxidative stress and inflammation in diabetes. *Nutr. Metab.*, 2007; 4 (1): 8.

31. Aggarwal BB and Harikumar KB. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplasic diseases. *Int. J. Biochem. Cell*, 2009; (41): 40-59.
32. Akinyemi AJ, Ademiluyi AO, Oboh G. Inhibition of angiotensin-1-converting enzyme activity in high cholesterol fed diet in rats by two varieties of ginger (*Zingiber officinale*), *J. Med. Food*, 2014; 17 (3) : 317-323.
33. Pristyn Care Team. How Effective Is Turmeric For Male Fertility? *Pristyn Care*, Updated on 23rd August 2021. https://www.pristyncare.com/blog/turmeric-for-male-fertility-pc0441/
34. Akinyemi AJ, Adedar IA, Thome GR, Morsch VM, Rovani MT, Mujica LKS, Duarte T, Duarte M, Oboh G, Schetinger MRC. Dietary supplementation of ginger and turmeric improves reproductive function in hypertensive male rats. *Elsevier*, 2015; (2): 1357-1366.
35. Gary Kawesch. "Turmeric and Testosterone". Renew vitality testosterone clinic. February 26, 2019. https://www.vitalityhrt.com/blog/turmeric-and-testosterone/
36. Banihani, Saleem. Ginger and Testosterone. *Biomolecules.* 2018; 8(4): 119.
37. Morakinyo A, Adeniyi O, Arikawe A. 2008 Effects of *Zingiber officinale* on Reproductive Functions in the Male Rat. *Afr. J. Biomed. Res.* 11: 329 – 34.
38. Sirotkin AV, Kadasi A, Stochmalova A, Balazi A, Földesiová M, Makovicky P, Makovicky P, Chrenek P, Harrath AH. Effect of turmeric on the viability, ovarian folliculogenesis, fecundity, ovarian hormones and response to luteinizing hormone of rabbits. *Animal*, 2018; 12 (6): 1242–1249.
39. Haider Salih Jaffat. Improvement role of *Curcuma longa* against oxidative stress by lithium carbonate in the male rat. *Research Journal of Pharmaceutical, Biological and Chemical Sciences,* 2017; 8(2): 19-25.