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Modulation of female reproductive hormones by *Zingiber officinale* and *Allium sativum* ethanol extracts administered singly and in combinations in female Wister rats

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Abstract

The reproductive system in females is responsible for producing gametes, sex hormones, and maintaining fertilized eggs as they develop. The female reproductive system is regulated by a balance of hormones. This study was aimed at analyzing the modulation of female reproductive hormones including FSH, LH and prolactin by *Zingiber officinale* and *Allium sativum* separately and in combination in female Wister rat models. A total of 40 mature female Wister rats were divided into 8 groups (n=5). Groups 1 were treated with distilled water and served as the normal control. Group 2 were treated with *Zingiber officinale* ethanol extract alone. Group 3 were treated with *Allium sativum* ethanol extract alone. Group 4-8 were treated with combined administration of *Zingiber officinale* and *Allium sativum* ethanol extracts in the ratios of ZO:AS = 2:8, 4:6, 5:5, 6:4, and 8:2 respectively.. Treatment were administered for 90 days. On the 91st day of treatment after administration of the 90th doses, blood samples were collected by ocular puncture from all the rats from each of groups 1-8 for serum hormone testing. *Zingiber officinale* monotherapy increased the serum levels of FSH and LH and decreased the level of prolactin. The reverse was the case with *Allium sativum* monotherapy. The combination of the two herbs increased FSH and LH; or decreased prolactin only when the proportions of *Zingiber officinale* were higher. In conclusion, only the monotherapy of *Zingiber officinale* enhanced the serum levels of FSH and LH and modulated prolactin level to a desired level.

Keywords: *Allium sativum;* Female reproductive hormones; Follicle stimulating hormone; Luteinizing hormone; Prolactin; *Zingiber officinale*

1. Introduction

The reproductive system in females is responsible for producing gametes (called eggs or ova), certain sex hormones, and maintaining fertilized eggs as they develop into mature fetuses and become ready for delivery [1]. The female reproductive system includes the ovaries, fallopian tubes, uterus, vagina, accessory glands, and external genital organs. The female reproductive system is regulated by a delicate balance of hormones that are produced by various glands in the body. Follicle-Stimulating Hormone (FSH) is produced by the pituitary gland in the brain and plays a crucial role in the growth and development of the follicles in the ovaries. The follicles are small sacs in the ovaries that contain the eggs. FSH stimulates the growth and maturation of the follicles, which eventually leads to the release of an egg during ovulation. Luteinizing Hormone (LH) is also produced by the pituitary gland and is responsible for triggering ovulation. During ovulation, the mature follicle bursts open and releases the egg, which then travels through the fallopian tubes and into the uterus. LH also stimulates the production of progesterone by the corpus luteum [2]. Understanding the role of these hormones is essential for maintaining reproductive health and treating infertility and other reproductive

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disorders. Another hormone that plays crucial functions in female is prolactin. Prolactin is a polypeptide hormone responsible for lactation, breast development, and hundreds of other actions needed to maintain homeostasis. In a certain study, prolactin was noted to contribute to hundreds of physiologic functions, but the two primary responsibilities are milk production and the development of mammary glands within breast tissues. Prolactin promotes the growth of mammary alveoli, which are the components of the mammary gland, where the actual production of milk occurs [3]. Hormone imbalance occurs when the human body has too much, too little or out-of-sync production of a hormone or hormones that are important for regulating bodily processes. The proper balance of hormones is essential for efficient reproductive cycles such as the ovulation process in women and the overall system of conception. Hormonal imbalances are the leading cause of infertility in women. Disorders, like polycystic ovary syndrome (PCOS) and anovulation, can be the result of a hormone imbalance in women. Some of the most common hormone-related conditions include: irregular menstruation (periods), polycystic ovary syndrome (PCOS), amenorrhea, infertility, diabetes, obesity among others. The main causes of hormonal imbalances are issues with the thyroid, stress, and eating disorders and some of the symptoms include irregular periods, low sex-drive, unexplained weight gain, and mood swings. In a particular study, infertility was defined as the failure to achieve a successful pregnancy after 12 months' sexual activity that affects 15%–17% of couples in the world and about 50% of them are related to female infertility factors. The researchers presented some evidence for role of herbal medicine in the treatment of female infertility. They showed that different parts of some plants are rich in polyphenolic compounds (isoflavones and flavonoids) and other compounds which are beneficial to in reproductive health in women. The compounds in these plants, along with regulating the female endocrine pathways, and improving symptoms of menopause, treat female reproductive disorders such as polycystic ovary syndrome (PCOS), premature ovarian failure (POF), endometriosis, hyperprolactinemia, and hypothalamic dysfunction [4]. This study is therefore intended to analyze the modulation of female reproductive hormones including FSH, LH and prolactin in female Wister rat models.

2. Materials and methods

2.1. Materials

2.1.1. Animals

Mature female Wister rats weighing 180 ± 10 g and aged (2 - 3 months) were procured from the Animal house of the faculty of Pharmaceutical sciences, Nnamdi Azikiwe University Awka, Agulu Campus. The animals were acclimatized for 14 days under standard conditions of temperature and illumination (12 hours dark: 12 hours light) cycle. The rats were fed commercially available rat's pellets and given access to drinking water ad libitum. Ethical approval was obtained from the Nnamdi Azikiwe University Animal Research and Experiment Committee (Approval number: NAU/AREC/2023/00021).

2.1.2. Plant materials

The plants used in this researched were *Zingiber officinale* rhizome and *Allium sativum* bulb*.* These plants were procured in a market in Enugu state of Nigeria.

2.1.3. Chemicals and Reagents

Mouse Anti-FSH Antibody-Horseradish Peroxidase (HRP) Conjugate (St John's laboratory UK), FSH Calibrators (Roche diagnostics USA), FSH (control) (Lee Biosolutions USA), Wash Buffer Concentrate (Sigma Aldrich Germany), Assay Buffer (Alpco USA), TMB Substrate (Cayman Chemical USA), Stop Solution (Cayman Chemical USA), Mouse Anti-hLH Antibody-Horseradish Peroxidase (HRP) Conjugate Concentrate (St John's laboratory UK), LH Calibrators (Monobind USA), LH control (Lee Biosolutions USA). Hydrochloric acid (Prime laboratories, India); Dragendoff reagent (Sigma Aldrich, United States of America); Ammonia (Shackti Industrial Gases, India), sodium hydroxide (Treveni Chemical Pvt., India); Ferric chloride (AkashPurochem. Pvt., India); Fehling's solution (Lab care Diagnostics, India); Million reagent (Interlab Chemical Pvt., India): Ethanol (TAJ Pharmaceutical Ltd., India); Acetic anhydride (Ashok Organics Industries, India); Concentrated sulfuric acid (Navin Chemical Pvt., India), Acetic acid (Kayla Africa Suppliers, South Africa); Molisch reagent (Interlab Chemical Pvt., India); alcoholic alpha naphatol (Prat Industry Corcopation, India).

2.1.4. Equipment

Precision pipettes (25, 50, 100 and 300 µl, 1,000 µL) (Labcompare USA); Disposable pipette tips (Labcompare USA); Distilled or deionized water (SnowPure Water Technologies USA); Plate shaker (Biocompare USA); Microwell plate reader (BioTek India); Centrifuge (Sharplex Filters Pvt., India); MouseAnti-FSH Antibody Coated Microwell Plate (Biocompare USA); Mouse Anti-hLH Antibody Coated Microwell Plate (Novus Biologicals USA); Vortex mixer (Bionics

Scientific Technologies (P) LTD, India); Microplate mixer (United Technology Trade Corp. USA); Prolactin Elisa kit (LabScience USA); Graduated cylinder for 500 ml (Boenmed Healthcare Co. Ltd, Hong Kong); Stop watch (Avi Scientific India); EDTA containers (Sure Care Corporation), heparinized capillary tube (Thomas Scientific, USA), disposable hand gloves (Supermax Malaysia), toilet tissue.

2.2. Methods

2.2.1. Extraction of the active components

The plant sources, fresh *Zingiber officinale* rhizome and *Allium sativum* bulb, after being purchased from the market were washed and dried. After drying they were pulverized separately. 200 g of each pulverized plant parts was macerated in one liter of ethanol for 48 hours. The filtrates was collected by sieving through a muslin cloth. The filtrates were concentrated in a water bath at 50 °C and stored in the refrigerator until used.

2.2.2. Phytochemical analysis of Zingiber officinale and Alliun sativum separately

The qualitative phytochemical analysis of the extract and fractions were carried out using standard methods described by Odoh *et al*., (2019) [5].

2.2.3. Test for alkaloids

The plant extract and fractions (0.2 g) was heat in 20 mL of 2% acid solution (HCL) individually in a water bath for about 2 minutes. The resulting solutions were allowed to cool and then filtered then 5 mL of the filtrates used for the following tests:

- **Dragendorff's test**: To each labeled test tube, 5 mL of the sample was added, followed by 1 mL of Dragendorff's reagent. Formation of orange or red precipitates indicates the presence of alkaloids.
- **Hager's test**: The samples (5 mL) were placed in labeled test tubes and a few drops of Hager's reagent (saturated picric acid solution) were added. Formation of yellow precipitate indicates the presence of alkaloids.
- **Wagner's test**: The samples (5 mL) were placed in labeled test tubes and a few drops of Wagner's reagent (solution of iodine and potassium iodide) were added. A reddish brown precipitate indicates the presence of alkaloids.
- **Mayer's test**: A quantity of 5 mL of each of the samples was placed in labeled test tubes and a few drops of the Mayer's reagent (potassium mercuric iodide solution) were added. Formation of cream color precipitate indicates the presence of alkaloids.

2.2.4. Test for glycosides

The samples were extract with 1% H 2 SO 4 solution in hot water bath for about 2 minutes. The resulting solution was filtered and made distinctly alkaline by adding 4 drops of 20% KOH (confirmed with litmus paper). One milliliter of Fehling's solution (equal volume of A and B) was added to the filtrates and heat on hot water bath for 2 minutes. Brick red precipitate indicates the presence of glycosides.

2.2.5. Test for saponins

The plant extracts and fractions (0.2 g) were dissolved in methanol individually and the resulting solutions were used for the following test:

- **Frothing test**: The samples (5 mL) were placed in labeled test tubes and 5 mL of distilled water was added and the mixtures and shaken vigorously. The test tubes were observed for the presence of persistent froth.

2.2.6. Test for tannins

The plant extracts and fractions (0.2 g) were dissolved in methanol individually and the resulting solutions were used for the test. To 3 mL of each of the samples a few drops of 1% Ferric chloride was added and observed for brownish green or a blue-black coloration.

2.2.7. Test for flavonoids

Using methanol, 0.2 g of the plant extracts and fractions were dissolved individually and resulting solutions were used for the following test:

- **Ammonium hydroxide test**: A quantity of 2 mL of 10% ammonia solution was added to a portion of each of the samples and allowed to stand for 2 minutes. Yellow coloration at the lower ammoniacal layer indicates the presence of flavonoid.
- **Sodium hydroxide solution test**: A quantity of 10 mL of 10% sodium hydroxide solution was\ added to a portion of each of the samples and observed for color changes in the lower alkaline layer. Yellow color (flavones), Blue to violet color (anthocyanins), yellow to orange color (flavonones).
- **Concentrated sulphuric acid test**: A portion of each of the samples were mixed gently with conc. Sulphuric acid and observed for color change, yellowish orange color (anthocyanins), yellow to orange color (flavones), orange to crimson (flavonones).

2.2.8. Test for steroids and terpenoids

- **Salkowski test**: The plant extracts and fractions were dissolved in methanol individually and the resulting solutions were used for the test. A 5 mL of each of the samples was mixed in 2 mL of chloroform and concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration at the interface indicates a positive test.
- **Liebermann-Burchard test**: Acetic anhydride (2 mL) was added to 0.5 g of each of the fractions and methanol extracts. Concentrated H_2SO_4 (2 mL) was carefully added to the resulting mixture and observed for color change from violet to blue or green.

2.2.9. Acute toxicity studies (LD_{50}) of Zingiber officinale ethanol extract

The actual median lethal dose (LD_{50}) estimation of the *Zingiber officinale* and *Allium sativum* ethanol extracts was conducted with the method described by Lorke, (1983) [6] with modifications in accordance with the description by Peter *et al*., 2023 [7].

2.3. Reproductive serum hormone assays

2.3.1. Experimental design

A total of 40 mature female Wister rats were divided into 8 groups of 5 rats per group. Groups 1 rats were treated with distilled water and served as the normal control. Group 2 were treated with *Zingiber officinale* ethanol extract alone. Group 3 were treated with *Allium sativum* ethanol extract alone. Group 4 were treated with combined administration of *Zingiber officinale* ethanol extract and *Allium sativum* ethanol extract (ratio = 2:8). Group 5 were treated with combined administration of *Zingiber officinale* ethanol extract and *Allium sativum* ethanol extract (ratio = 4:6). Group 6 were treated with combined administration of *Zingiber officinale* ethanol extract and *Allium sativum* ethanol extract (ratio = 5:5). Group 7 were treated with combined administration of *Zingiber officinale* ethanol extract and *Allium sativum* ethanol extract (ratio = 6:4). Group 8 were treated with combined administration of *Zingiber officinale* ethanol extract and *Allium sativum* ethanol extract (ratio = 8:2). Treatment were administered for 3 months (90 days). Doses were selected based on the results of the acute toxicity studies. On the 90^{th} day of treatment after administration of the 90^{th} doses, blood samples were collected by ocular puncture from all the rats from each of groups 1-8 for sub-chronic studies of the reproductive hormones notably FSH, LH, and prolactin (PRL).

2.3.2. Follicle stimulating hormone assay procedure

Assay was performed using Bybiosource assay kit (Catalog No: MBS263261, USA) following manufacturers instruction. The micro ELISA plate provided in the kit has been pre-coated with Rat FSH and uses double antibody sandwish technique. First wells for diluted standard, blank and sample were determined on the pre-coated plate respectively, and their positions recorded. Then, 100ul of Standard, Blank, or Sample were added to their respective wells while sample/standard dilution buffer was added to the blank well, the plate was sealed with adhesive tape strip and was incubated for 90 min at 37 °C. Thereafter, the plate was washed 2 times with wash buffer. Immediately, freshly prepared Biotin-labeled Antibody Working Solution (100 µl) was added into each well, the plate was covered, and the content mixed thoroughly by gentle taping and then incubated for 60 minutes at 37 °C. Thereafter, the cover of the plate was removed and the plate washed 3 times with Wash Buffer. After the last wash, remaining Wash Buffer was removed by aspirating or decanting. HRP-Streptavidin Conjugate (SABC) 100 ul Working Solution was then added into each well, and the plate was covered with a new Plate sealer, incubated for 30 minutes at 37 °C and washed 5 times with wash buffer. Then 100 ul of color reagent was then added into each well, the plate covered and incubated at 37 °C in dark for 20 minutes. The reaction was terminated by addition of 100 ul Stop Solution into each well. The color change was measured at 450 nm in Microplate Reader immediately after adding the stop solution. The OD value of blank was subtracted from each sample and standard OD. A standard curve was plotted with concentrations of the standard on

the x-axis and OD reading on the Y-axis. The FSH concentration in each sample was interpolated from this standard curve.

2.3.3. Serum Luteinizing hormone assay procedure

Assay was performed using Bybiosource assay kit (Catalog No: MBS764675, USA) following manufacturers instruction. The micro ELISA plate provided in the kit has been pre-coated with Rat LH. First wells for diluted standard, blank and sample were determined on the pre-coated plate respectively, and their positions recorded. The plate was washed 2 times with wash buffer before adding standard, sample and control (blank). Then, 50 ul of Standard, Blank, or Sample were added to their respective wells while sample/standard dilution buffer was added to the blank well. Immediately, 50 ul Biotin-labeled Antibody Working Solution was added into each well, the plate was covered, and the content mixed thoroughly by gentle taping and then incubated for 45 minutes at 37 °C. Thereafter, the cover of the plate was removed and the plate washed 3 times with Wash Buffer. After the last wash, remaining Wash Buffer was removed by aspirating or decanting. HRP-Streptavidin Conjugate (SABC) 100ul Working Solution was then added into each well, and the plate was covered with a new Plate sealer, incubated for 30 minutes at 37 °C and washed 5 times with wash buffer. TMB Substrate 90 ul TMB was then added into each well, the plate covered and incubated at 37 °C in dark for 20 minutes. The reaction was terminated by addition of 50 ul Stop Solution (sulphuric acid) into each well. The color change was measured at 450 nm in Microplate Reader immediately after adding the stop solution. A standard curve was plotted relating the intensity of the color (O.D.) to the concentration of standards. The LH concentration in each sample was interpolated from this standard curve.

2.3.4. Serum prolactin assay

The assay was carried out using rat specific prolactin Elisa kit (ElabScience, USA. Catalog no: E-EL-R3006). To the standard, blank and sample designated wells, 100 ul of the reference standards, diluent and serum samples were added and incubated for 90 minutes at 37 ºC. At timed interval, the micro well contents were decanted and biotinylated detection antibody (100 ul) was added to all well and the well incubated again for 1 h at 37 ºC. The content of the micro well were decanted and washed 3 times with wash buffer. HRP conjugate working solution (100 ul) was then added to each well and the well incubated for 30 minutes at 37 ºC followed by decantation and washing for 5 times. Substrate reagent (90 ul) was added to each well and incubated for 15 min at 37 ºC. This was followed immediately by addition of 50 ul of the stop solution and the determination of optical density of color change at 450 nm. A standard curve was plotted relating the intensity of the color (O.D.) to the concentration of standards. The serum prolactin concentrations were derived from this standard curve.

3. Result

3.1. Results of phytochemical analysis of *Zingiber officinale* and *Allium sativum* ethanol leaf extracts

Phytocompounds in *Zingiber officinale* were: Alkaloids, Tannins, Flavonoids, Steroids and terpenoids while those in *Allium sativum* were Alkaloids, Saponins, Flavonoids, and Glycosides [7].

3.2. Results of acute toxicity studies

The actual lethal doses of *Zingiber officinale, Allium sativum* and combination of the two were 8,660, 4,472, and 5,477 mg/kg body weight respectively [7].

3.3. Results of reproductive hormone assays

Table 1 Results of 91st day FSH concentration assay

Groups	Treatments given/kg body weight	Mean FSH ± SEM (mIU/ml)	P-Value
1	Distilled water 10 ml	3.53 ± 0.16	-
2	*Zingiber officinale* 530 mg	4.68 ± 0.18	0.000172
3	*Allium sativum* 530 mg	3.24 ± 0.14	0.107697
4	Ratio of Z.:A. 2:8 (106 mg:424 mg)	3.59 ± 0.14	0.707582
5	Ratio of Z.:A. 4:6 (212 mg:318 mg)	3.69 ± 0.13	0.309314
6	Ratio of Z.:A. 5:5 (265 mg:265 mg)	3.89 ± 0.11	0.033267

7	Ratio of Z.:A. 6:4 (318 mg:212 mg)	3.98 ± 0.11	0.012691
8	Ratio of Z.:A. 8:2 (424 mg:106 mg)	4.20 ± 0.05	0.000326

Table 2 Results of 91st day LH concentration assay

Groups	Treatments given/kg body weight	Mean LH ± SEM (mIU/ml)	P-Value
1	Distilled water 10 ml	0.82 ± 0.09	-
2	*Zingiber officinale* 530 mg	1.36 ± 0.38	0.010059
3	*Allium sativum* 530 mg	0.71 ± 0.10	0.040744
4	Ratio of Z.:A. 2:8 (106 mg:424 mg)	0.81 ± 0.08	0.758876
5	Ratio of Z.:A. 4:6 (212 mg:318 mg)	0.90 ± 0.04	0.064609
6	Ratio of Z.:A. 5:5 (265 mg:265 mg)	1.08 ± 0.15	0.00621
7	Ratio of Z.:A. 6:4 (318 mg:212 mg)	1.15 ± 0.09	0.000172
8	Ratio of Z.:A. 8:2 (424 mg:106 mg)	1.25 ± 0.06	2.27E-06

Table 3 Results of 91st day prolactin concentration assay

Groups	Treatments given/kg body weight	Mean PRL ± SEM (mIU/ml)	P-Value
1	Distilled water 10 ml	12.02 ± 0.82	-
2	*Zingiber officinale* 530 mg	11.18 ± 0.27	0.483025
3	*Allium sativum* 530 mg	13.43 ± 0.47	0.305069
4	Ratio of Z.:A. 2:8 (106 mg:424 mg)	12.10 ± 0.42	0.947991
5	Ratio of Z.:A. 4:6 (212 mg:318 mg)	12.07 ± 0.37	0.967449
6	Ratio of Z.:A. 5:5 (265 mg:265 mg)	12.63 ± 0.80	0.692816
7	Ratio of Z.:A. 6:4 (318 mg:212 mg)	12.65 ± 0.34	0.607863
8	Ratio of Z.:A. 8:2 (424 mg:106 mg)	11.90 ± 0.35	0.924373

4. Discussion

Generally, *Zingiber officinale* alone exhibited the best and desirable effects on the tested reproductive hormones notably FSH, LH and PRL than either of *Allium sativum* alone or the combinations of the two herbs given in different proportions. *Zingiber officinale* administered alone increased the FSH and LH significantly ($P < 0.05$) but reduced the serum level of prolactin in none significant manner ($P > 0.05$) when compared with the control group 1. In the case of FSH, the group 1 rats recorded a mean serum FSH level of 3.35 ± 0.16 mIU/ml. This was increased by *Zingiber officinale* alone to a level of 4.68 ± 0.16 mIU/ml ($P = 0.000172$). *Allium sativum* alone on the other hand increased the serum level of FSH to 3.24 ± 0.14 mIU/ml in none significant way ($P = 0.107697$) and not as much as *Zingiber officinale* monotherapy. When the two herbs were combined together in all tested proportions, the increment shown were more than that noted for *Allium sativum* alone but less than the increment observed in *Zingiber officinale* given singly. The serum levels of FSH increased progressively as the proportion of *Zingiber officinale* in the combination increased up to the Z;A = 8:2 in which the FSH was 4.20 ± 0.05 mIU/ml ($P = 0.000326$). This suggested an antagonistic interaction between the two herbs which was however competitive in nature since the antagonist (*Allium sativum*) is overcome by increased concentration of *Zingiber officinale*. In a certain study, it was shown that herbs have the potentials to either increase or decrease female reproductive hormones. The researchers discussed the role of different herbs in polycystic ovarian syndrome (PCOS) by referring to the Scopus, PubMed, Google Scholar, Crossref and Hinari databases in a thorough literature search and data mining was performed pertaining to the effectiveness of herbal remedies against PCOS. The study concluded that

herbs such as *Foeniculum vulgare*, *Panax ginseng* and *Cimicifuga racemosa* have the ability to elevate the levels of FSH and reduce the concentrations of LH making them beneficial in the treatment of PCOS [8]. In another review, the role and mechanism of medicinal plants or their products in reducing or increasing the levels of GnRH, FSH and LH in females was evaluated. Plants and plant derivatives that affect fertility disorders and mainly increase GnRH, include *Vitex agnus-castus, Thuja occidentalis L., Cimicifuga racemosa, Yucca schidigera*, isoflavones and some Chinese herbal compounds. In some cases, plants such as Emilia coccinea decrease the fertility of the fetus by lowering the FSH and LH hormones [9].

When the assay of LH was considered, similar trend was observed in which *Zingiber officinale* alone increased the serum level of LH significantly ($P < 0.05$) while *Allium sativum* alone decreased it significantly ($P < 0.05$). The combination therapy had significant increment only when the proportion of *Zingiber officinale* is much higher than that of *Allium sativum*. This was in accordance with the insight obtainable form the assay of FSH which suggested an antagonistic interaction between the two herbs. In the case of LH too, the interaction is competitive because the antagonist was overcome by increased concentration of *Zingiber officinale*. This was evident in group 8 in which the proportion of the herbs was Z:A = 8:2 and which recorded LH level of 1.25 ± 0.06 mIU/ml ($P = 2.27E-06$) when compared with the control group 1 that had LH level of 0.82 ± 0.09 mIU/ml. In an earlier study that evaluated the effects of Super7 – a polyherbal antimalarial drug - on female Wister rats' gonadotropin hormones. The test animals were randomly allocated into six groups I-VI (n=7). Group I – III received 507.3 mg/kg, 1,014.6 mg/kg and 2,029.2 mg/kg body weight of Super7 respectively. Group IV, V and VI rats served as the general, positive and negative controls and received 5 ml/kg body weight of distilled water, 0.3 mg/kg body weight of Levonorgestrel, 50 mg/kg body weight of Clomiphene respectively. Treatments were administered daily for 30 days. Both pretreatment and post-treatment gonadotropin hormonal assays were conducted. The test drug showed positive gonadotropin properties and good antioxidant potentials by increasing post treatment LH levels and mean superoxide dismuthase enzyme units respectively [10]. LH spurs ovulation and helps with the hormone production needed to support pregnancy. LH has various functions, which differ between women and men. In women, LH triggers the creation of steroid hormones from the ovaries. Additionally, LH helps to regulate the length and order of the menstrual cycle in females by playing roles in both ovulation and implantation of an egg in the uterus [11].

The reverse was the case with respect to serum levels of prolactin; this however was in line with the pro-gonadotropin with a consequent pro-fertility effects demonstrated by *Zingiber officinale* monotherapy by its virtue of enhancing the serum levels of FSH and LH. This is based on the fact that excess prolactin in females can lead to infertility. Expectedly, *Zingiber officinale* reduced PRL level while *Allium sativum* increased it when compared with control group 1. Specifically, group 1 rats recorded mean serum level of PRL of 12.02 ± 0.82 mIU/ml. This was decreased to 11.18 ± 0.27 mIU/ml in group 2 which were treated with *Zingiber officinale* alone ($P = 0.483025$) and increased in group 3 which were treated with *Allium sativum* alone to 13.48 ± 0.47 mIU/ml ($P = 0.305069$). The combination of the two herbs in various proportions showed PRL levels that were always between the levels recorded for *Zingiber officinale* and *Allium sativum.* These once again suggested an antagonistic interactions which was also dose dependent. Prolactin was shown in a study to have antifertility effects in females. In the study, a 29-year-old woman was referred for management of infertility. Evaluation revealed hyperprolactinemia with serum prolactin of 90 ng/ml. Other pituitary functions tested were normal. Therapy was initiated with bromocriptine, but it was poorly tolerated. Prolactin levels declined to the 30s–40s, but she was never able to tolerate the medication sufficiently to attain normal prolactin levels. She and her husband had not conceived despite regular unprotected intercourse [12].

5. Conclusion

In conclusion, *Zingiber officinale* alone showed a reputable potentials in promotion of the tested female reproductive hormones while *Allium sativum* alone had anti-reproductive hormone activities. The combination of the two herbs had pro-hormone effects that is dependent on the proportions of *Zingiber officinale* and *Allium sativum.* Increased doses of *Zingiber officinale* was able to counter the antagonistic effects of *Allium sativum*.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest

Statement of ethical approval

Maintenance and care of all animals were carried out in accordance with EU Directive 2010/63/EU for animal experiments. Guide for the care and use of Laboratory Animals, DHHS Publ. # (NIH 86-123) were strictly adhered to. Ethical approval was obtained from the Animal Ethical Committee of the Enugu State University of Science and Technology. There was additional approval by the Nnamdi Azikiwe University's Ethical Committee for the use of Laboratory Animals for Research Purposes; (Approval number is NAU/AREC/2023/00021).

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