Investigation Of Pro-fertility Actions Of "Female Corrective" Poly-herbal Medicine Formulation Using Female Wister Rat Model

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Abstract
Plants contain numerous phytochemical compounds that may be used to cure or prevent diseases. In this study, the pro-fertility actions of "Female corrective" poly-herbal formulation were investigated using female Wister rat model. Phytochemicals, acute toxicities, body weight changes, and antioxidant and female reproductive hormonal studies were carried out. Serum levels of glutathione, superoxide dismutase and catalase were also evaluated. Micro well experiment was used to study the impact of the poly-herbal formulation on reproductive hormones. The phytochemical compounds present in the "Female corrective" were glycosides, saponins, tannins, flavonoids, steroids and terpenoids. In the median lethal dose estimation, no mortality nor obvious signs of toxicity were recorded at the dose of 88 mg/kg body weight. At a concentration of 4.4 mg/mL the body weights of the rats were increased in a dose dependent manner; 2.06, 4.11 and 8.22 mL/kg body weight gave changes in body weights of 17.13, 20.00 and 22.44% respectively after 14 days. Antioxidant effects were recorded as increase in serum concentration of glutathione, superoxide dismutase and catalase. Also, the serum levels of follicle stimulating hormone and luteinizing hormone were increased significantly ($p < 0.05$) and dose dependently; at the same administered doses, serum levels of follicle stimulating hormone were 3.06 ± 0.06, 4.61 ± 0.05 and 5.25 ± 0.09 mIU/mL respectively and that of luteinizing hormone were 1.02 ± 0.10, 1.71 ± 0.23 and 2.08 ± 0.20 mIU/mL respectively. The possible mechanisms of the herbal drug formulation might include antioxidant as well as pro-gonadotropin activities.

Keywords: Antioxidants, "Female Corrective", Gonadotropin hormones, Pro-fertility

Introduction

Humans have been using natural remedies from sources such as plants, animals, and microorganisms in traditional medicines for prophylaxis and treatment of illnesses. Plants contain abundant phytochemicals that may be used in monotherapy or in combination as to produce synergistic effects through different mechanisms for prophylaxis or cure of diseases (Liu *et al*., 2022). In Nigeria, the rate of consumption of herbal medicine has been on the high side due to its perceived higher effectiveness, accessibility, affordability and the general view that herbal medicines have

low or no risk of adverse reaction when compared to pharmaceutical medicines (Li *et al*., 2020). Herbal medicines are used to treat a varieties of conditions in Nigeria. These include: infertility, malaria, ulcers, and sexually transmitted diseases among others. Overall, infertility is defined as a condition in which a woman is not able to get pregnant after one year of unprotected sex. In trying to find out the cause of infertility, ovarian function of patients are measured. The most commonly used markers of ovarian function include serum level of follicle stimulating hormone (FSH), anti-müllerian hormone value (AMH), and antral follicle count (AFC) (Shahrokh *et al*., 2016). Other hormones that are involved in ovarian function include follicle releasing hormone (FRH), follicle stimulating hormone (FSH) and luteinizing hormone (LH). These hormones that maintain normal ovarian function are produced in hypothalamus and pituitary glands of the brain. Some of the factors that cause infertility in women include the production of excess prolactin by the pituitary gland which may inhibit ovulation in women. Many previous studies had shown that oxidative stress plays an important role in human fertility. Elevated concentrations of reactive oxygen species (ROS) may have detrimental effects on the spermatozoa, oocytes, sperm oocyte interaction and embryos both in the Fallopian tube and the peritoneal cavity leading to infertility (Mahmood *et al*., 2021). "Female corrective" is a poly-herbal medicine formulation that is used in the southeastern part of Nigeria for the treatment of women infertility. Female corrective have components from 8 herbs namely: *Lopidium meyenii, Cinnamon, Tibulus terrestrils, Vilex aganus, Ginseng, Zingiber officinale, Trifolium pretense and Piper suineense*. Each of this herbs has been confirmed to have fertility enhancing properties. Based on this, it is expected that the labelled claim of potent infertility correction property should be valid. However, because of the possibility of antagonism, this study was designed to validate the infertility correcting property of the Female corrective irrespective of the observed anti-fertility properties of the component herbs in combination. Specifically, the effects of the poly-herbal formulation on glutathione, superoxide dismutase (SOD), FSH, LH and prolactin levels in female Wister rat models were evaluated to estimate its effect on ovarian function.

Materials and Methods

Materials

Animals

Swiss female Albino rats (135 – 140 g) were used for the study. All the animals were obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria. The animals were housed in standard laboratory conditions of 12 hours light, room temperature, and 40 - 60% relative humidity and fed with rodent feed (Guinea Feeds Nigeria Ltd). They were allowed free access to food and water. 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 (Approval number: ESUT/AEC/0188/AP217). There was additional approval by the Nnamdi Azikiwe University's Ethical Committee for the use of Laboratory Animals for Research Purposes (Approval number: NAU/AREC/2023/00070).

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), prolactin tracer reagent, prolacting wash decant, prolactin working signal reagent.

Chemicals for phytochemical analysis

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).

Equipment

Rotary evaporator (Heidolph**,** Germany), Analytical Weighing Balance (Metler H30, Switzerland), Electric Oven (Gallenkamp, England), Spectrophotometer (B. Bran Scientific &Instrument Company, England), Water Bath (Techmel & Techmel, Texas, USA), National Blender (Japan), Micropippete (Finnipipette® Labsystems, Finland), Plethysmometer (Biodevices, New Delhi, India) and Intubation tubes. Precision pipettes (25, 50, 100 and 300 µl, 1,000 µL) (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); 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. Prolactin assay kit (Sigma Aldrich USA): Prolactin calibrators, prolactin tracer reagent, streptavidin coated micro-wells, wash solution concentrate, signal reagent A, signal reagent B, microplate luminometer, microplate cover.

Drugs

Female corrective poly-herbal formulation (Emma Kings Natural Healthcare Nigeria Limited, Enugu State, Nigeria), and Clomiphene (Jianxi Xier Khanghai Pharmaceutical Industry Ltd, China)

Methods

Phytochemical analysis of "Female corrective" poly-herbal medicine formulation

The qualitative phytochemical analysis of the herbal product was carried out using standard methods described by Odoh *et al*. (2019a).

Test for alkaloids

The sample (0.2 g) was heated in 20 mL of 2% acid solution (HCL) in a water bath for about 2 minutes. The resulting solution was allowed to cool and then filtered. A 5 mL of the filtrate was used for the following tests:

Dragendorff's test: A 5 mL of the filtrate was added, followed by 1 mL of Dragendorff's reagent. Formation of orange or red precipitates indicated the presence of alkaloids.

Hager's test: The filtrate (5 mL) was placed in labeled test tubes and a few drops of Hager's reagent (saturated picric acid solution) were added. Formation of yellow precipitate indicated the presence of alkaloids.
Wagner's test**:** The filtrate (5 mL) was 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 indicated the presence of alkaloids.
Mayer's test: A 5 mL of the filtrate was placed in labeled test tubes and a few drops of the Mayer's reagent (potassium mercuric iodide solution) were added. Formation of cream colored precipitate indicated the presence of alkaloids.

Test for glycoside
The sample was extracted with 1% H_2SO_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. One milliliter of Fehling's solution (equal volume of A and B) was added to the resulting solution and heated on hot water bath for 2 minutes. Brick red precipitate indicated the presence of glycosides.

Test for saponins
The herbal product (0.2 g) was extracted in water and the resulting solution used for the following test:
Frothing test**:** The solution (5 mL) was placed in test tube and 5 mL of distilled water was added and the mixture shaken vigorously. The test tubes were observed for the presence of persistent froth.

Test for tannins
The herbal product (0.2 g) was extracted in water and the resulting solution used for the following test. To 3 mL of solution a few drops of 1% Ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for flavonoids
Using ethanol, 0.2 g of the concentrate was dissolved and resulting solutions 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 sample and allowed to stand for 2 minutes. Yellow coloration at the lower ammoniacal layer indicated 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 sample 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 the sample was mixed gently with conc. sulphuric acid and observed for color change, yellowish orange color (anthocyanins), yellow to orange color (flavones), orange to crimson (flavonones).
Test for steroids and terpenoids
Salkowski test: A 0.2 g of the concentrate was dissolved 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: A 0.2 g of the concentrate was dissolved in 2 mL of chloroform and acetic anhydride (2 mL) was added, then concentrated H_2SO_4 (2 mL) was carefully added to the resulting mixture and observed for color change from violet to blue or green.

Test for anthraquinone
Modified Borntrager's test
A 0.2 g of the concentrate was boiled with dilute H_2SO_4 and 5% $FeCl_3$ for 5 minutes. The resulting mixture was filtered while hot; then cooled and shaken with an equal volume of chloroform. The chloroform layer was separated using separating funnel. A 2 mL of

the chloroform part was shaken with 1 mL of dilute ammonium hydroxide solution (10%). Rose-pink to red color in the aqueous ammoniacal layer (Upper alkaline layer) indicated a positive test.

Acute toxicity studies (LD_{50}) of "Female corrective" poly-herbal medicine formulation

To allow for acclimatization to the laboratory conditions, the female rats were selected randomly, marked to allow individual recognition and kept in their cages for at least 5 days prior to dosing. The animals were fasted from food overnight prior to dosing but had access to water. Acute oral toxicity of the herbal preparation was performed according to the Organization of Economic Cooperation and Development (OECD) guideline 425 for testing of chemicals (Up and down method). Since the mixture is currently in use by humans and expected to have high lethal dose, a limit test at 20 mL/kg as single dose was used. This is equivalent to a solid extract dose of 88 mg/kg body weight (concentration of the poly-herbal formulation = 4.4 mg/mL). The dose was administered to the animals based on their body weight. The animals were closely observed for the first 30 minutes, then for 4 hours. Food was provided after 2 hours of dosing. After survival of the first treated animal, 4 more animals were treated with same dose at interval of 48 hours each. The control group of rats (n = 5) were administered with distilled water (vehicle used in preparing the herbal mixture) in the same volume of that of the treated group. All the groups were closely observed for 6 hours and then at a regular interval for 14 days. The animals were weighed and observed for mortality, salivation, diarrhea, asthenia, hypoactivity, hyperactivity, piloerection, hyperventilation, aggressiveness, yellowing or loss of fur, drowsiness, convulsion, tremor, dizziness and other obvious signs of toxicity.

Sub-acute toxicity studies

A total of 30 animals were grouped into 5 of 6 animals each. Group 1 served as vehicle control group and received 10 mL/kg body weight distilled water; group 2 received human equivalent dose of the herbal preparation (4.11 mL/kg body weight); while group 3 received double the human equivalent dose (8.22 mL/kg body weight); group 4 was given half the human equivalent dose (2.06 mL/kg body weight); while group 5 served as the positive control and received 10 mg/kg body weight of clomiphene (a standard pro-gonadotropin drug). Treatment was carried out twice a day in the morning by 7 am and the evening by 7 pm for 14 consecutive days. The body weights of the animals were taken before and after treatment. At the end of the treatment, blood sample was collected from the animals for evaluation of reproductive hormone and antioxidant parameters. The animals were sacrificed under general anesthesia with ketamine and xylene (80 and 10 mg/kg body weight respectively, IM) and the uterus was harvested and weighed.

Blood collection

At the end of the experiment, blood was collected from the animals through retro-orbital plexus into plain sample tube. The tube was allowed to clot for 20 minutes at room temperature and were thereafter centrifuged at 300 rpm for 10 minutes. The supernatants (serum) were collected in aliquots into labelled Eppendorf tubes.

Antioxidant assays

Serum glutathione peroxidase assay procedure

Assay for serum glutathione peroxidase concentration was carried out using sandwich Elisa kit (Bioassay technology laboratory, China) for rat glutathione peroxidase 1 (GPX1). The Elisa plate was pre-coated with rat GPX antibody. The samples (40 µL) as well as the standards were added in their respective wells. Biotinylated rat GPX1

antibody (10 µL) was added to all the wells followed by the addition of streptavidin-HRP (50 µL) and were incubated for 1h at 37°C followed by 5 times washing with washing buffer. Then 50 µL of substrate A and B were added and incubated for 10 minutes at 37°C in the dark. The optical density (OD) of each well was determined within 10 minutes after adding the acidic stop solution using a microplate reader at 450 nm. Standard calibration curve was plotted using average OD for duplicate standard concentrations prepared by serially diluting the stock solution to get concentrations ranging from 120 ng/mL – 7.5 ng/mL). OD was plotted on the Y axis while the corresponding concentration was plotted on the X-axis. Best fit curve was drawn through the points on the graph. Regression analysis was used for interpolation of the unknown GPX-1 concentration in the samples.

Serum Catalase assay procedure

Assay was performed using Bybiosource assay kit (USA) following manufacturers instruction. The kit applies the competitive enzyme immunoassay technique utilizing a polyclonal anti-CAT antibody and a CAT-HRP conjugate. The serum (100 µL) sample and buffer were incubated together with CAT-HRP conjugate in pre-coated plate for one hour. Serial fold dilutions of the reference standard (0 – 50 ng/mL) were also prepared and added to their respective wells. After one hour incubation period at 37°C, the wells were decanted and washed five times using wash buffer. The wells were then incubated with a substrate for HRP enzyme (50 µL) for 20 minutes at 37°C. The product of the enzyme-substrate reaction formed a blue colored complex. Finally, a stop solution (50 µL) was added to stop the reaction, which then turned the solution yellow. The intensity of color was measured spectrophotometrically at 450 nm in a microplate reader. The intensity of the color was inversely proportional to the CAT concentration. A standard curve was plotted relating the intensity of the color (O.D.) to the concentration of standards. The CAT concentration in each sample was interpolated from this standard curve.

Serum Superoxide dismutase assay procedure

Assay was performed using ElabScience kit (USA) following manufacturers instruction. The ELISA kit uses the Competitive-ELISA principle. The micro ELISA plate provided in the kit had been pre-coated with Rat SOD1. First wells for diluted standard, blank and sample were determined. Then 50 µL each dilution of standard, blank and samples were added into the appropriate wells. Thereafter 50 µL of Biotinylated Detection Ab working solution was added to each well immediately. The plate was covered with the sealer provided in the kit and incubated for 45 min at 37℃. Solution from each well was decanted followed by addition of 350 µL of wash buffer to each well. Excess conjugate and unbound sample or standard were washed from the plate three times, and 100 µL Avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated for 30 minutes at 37°C. The solution from each well was decanted and the washing process repeated for 5 times. Then 90 µL of substrate reagent was added to each well, covered and incubated for 15 minutes at 37°C in the dark. The enzyme-substrate reaction was terminated by the addition of 50 µL of Stop Solution to each well and the color change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of Rat SOD1 in the samples was then determined by comparing the OD of the samples to the standard curve.

Reproductive hormone assays

Serum Luteinizing hormone (LH) assay procedure

Assay was performed using Bybiosource assay kit (Catalog No: MBS764675, USA) following manufacturers instruction as was described by Oraekei *et al*., (2020). The micro ELISA plate provided in the kit had 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 two 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 and the plate covered. The contents were 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 three times with Wash Buffer. After the last wash, remaining Wash Buffer was removed by decanting. HRP-Streptavidin Conjugate (SABC) 100 uL Working Solution was then added into each well, and the plates were covered with a new Plate sealer, incubated for 30 minutes at 37°C and washed five times with wash buffer. TMB Substrate 90 uL was then added into each well and 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.

Follicle stimulating hormone (FSH) assay procedure

Assay was performed using Bybiosource assay kit (Catalog No: MBS263261, USA) following manufacturers instruction. The micro ELISA plate provided in the kit had 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, 100 uL 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 minutes at 37°C. Thereafter, the plate was washed two 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 three 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 five 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.

Statistical analysis

Results were presented as Mean ± Standard Error of Mean (S.E.M). Means were analyzed using one way analyses of variance (ANOVA) followed by post hoc Turkey's test for multiple comparisons. $P < 0.05$ was set to be statistically significant. Results analysis was conducted using Statistical Package for Social Science, SPSS- version 20.

Results

Table 1: results of the phytochemical analysis of "Female Corrective" poly-herbal medicine

Phytocompounds	**Availability**
Alkaloids	-
Glycosides	+
Saponins	+
Tannins	+
Flavonoids	+
Steroids and terpenoids	+
Anthraquinone	-

Key: *+ = Present; - = Absent*

Results of acute toxicity study

No mortality was observed and obvious signs of toxicity were not recorded throughout the observational periods.

Table 2: 'Female corrective' (FC) 14 days oral administration (bd) body weight changes (g)

Grps	Treatments given	Pre-treatment Mean ± SEM	Day 7 Mean ± SEM	Day 14 Mean ± SEM
1	Distilled water	133.33 ± 0.88	145.33 ± 0.88	157.33 ± 0.67
2	4.11 mL/kg BW FC	133.00 ± 1.00	144.67 ± 0.88	159.67 ± 1.45
3	8.22 mL/kg BW FC	133.67 ± 1.20	145.00 ± 1.53	163.67 ± 1.45
4	2.06 mL/kg BW FC	132.33 ± 1.45	143.67 ± 1.45	155.00 ± 1.73
5	Clomiphene 10mg/kg BW	133.00 ± 1.00	151.33 ± 1.76	175.00 ± 1.16

*BW = Body weight

Table 2 shows the body weight measurements of all the rats following the pre-, 7th and 14th days of treatment; recorded as Mean ± standard error of mean. The body weight depended on both the dose of the treatment and the number of days prior to measurement.

Table 3: 'Female corrective' (FC) percentage change in mean body weight (%)

Grps	Treatments given	Day 7 % change in Mean BW	Day 14 % change in Mean BW
1	Distilled water	9.00	18.00
2	4.11 mL/kg BW FC	8.77	20.00
3	8.22 mL/kg BW FC	8.48	22.44
4	2.06 mL/kg BW FC	8.57	17.13
5	Clomiphene 10 mg/kg	13.78	31.58

	BW		

*BW = Body weight

Table 3 shows the percentage change of the mean body weight. This gave the best comparison between the different groups since each group had a different basal value.

Table 4: results of antioxidant (SOD) assay

Groups	Treatment	Mean post treatment conc. ± SEM (ng/mL)
1	10 mL/kg body weight DW	1.94 ± 0.12
2	4.11 mL/kg BW Female corrective	7.53 ± 1.45
3	8.22 mL/kg BW Female corrective	10.65 ± 1.47
4	2.06 mL/kg BW Female corrective	4.37 ± 0.60
5	10 mg/kg body weight clomiphene	2.18 ± 0.15

*DW = Distilled water; *BW = Body weight

According to figure 4, the superoxide dismutase concentration also increased dose dependently and more than both positive and negative controls.

Table 5: results of antioxidant (glutathione) assay

Groups	Treatment	Mean post treatment conc. ± SEM (U/L)
1	10 mL/kg body weight DW	33.35 ± 0.70
2	4.11 mL/kg BW Female corrective	53.40 ± 2.32
3	8.22 mL/kg BW Female corrective	62.40 ± 2.07
4	2.06 mL/kg BW Female corrective	42.28 ± 1.45
5	10 mg/kg body weight clomiphene	34.87 ± 1.47

*DW = Distilled water; *BW = Body weight

Table 5 indicated the dose dependent increase in glutathione levels which is also greater than those of the positive and negative controls.

Table 6: results of catalase assay

Groups	Treatment	Mean post treatment conc. ± SEM (ng/mg)
1	10 mL/kg body weight DW	13.07 ± 0.60
2	4.11 mL/kg BW Female corrective	19.80 ± 0.50
3	8.22 mL/kg BW Female corrective	25.63 ± 1.02
4	2.06 mL/kg BW Female corrective	15.44 ± 0.34
5	10 mg/kg body weight clomiphene	13.64 ± 0.33

*DW = Distilled water; *BW = Body weight

From table 6, "Female Corrective increased catalase levels dose dependently. This implies that it pro-fertility effects do not involve activity on catalase level.

Table 7: results of FSH assay

Groups	Treatment	Mean post treatment conc. ± SEM (mIU/mL)
1	10 mL/kg body weight DW	3.30 ± 0.04
2	4.11 mL/kg BW Female corrective	4.61 ± 0.05
3	8.22 mL/kg BW Female corrective	5.25 ± 0.09
4	2.06 mL/kg BW Female corrective	3.60 ± 0.06
5	10 mg/kg body weight clomiphene	11.35 ± 0.46

*DW = Distilled water; *BW = Body weight

Table 7 showed a dose dependent increase in follicle stimulating hormone; though not as much as in the positive control.

Table 8: results of LH assay

Groups	Treatment	Mean post treatment conc. ± SEM (mIU/mL)
1	10 mL/kg body weight DW	0.79 ± 0.01
2	4.11 mL/kg BW Female corrective	1.71 ± 0.23
3	8.22 mL/kg BW Female corrective	2.08 ± 0.20
4	2.06 mL/kg BW Female corrective	1.02 ± 0.10
5	10 mg/kg body weight clomiphene	4.09 ± 0.23

*DW = Distilled water; *BW = Body weight

In table 8, luteinizing hormone was noted to increase dose dependently; more than the increase in the negative control, but less than that of the positive control.

Discussion

The phytochemical compounds present in the "Female Corrective poly-herbal product included: glycosides, saponins, tannins, flavonoids, steroids and terpenoids; while alkaloids and anthraquinones were absent. These phytochemicals might have been the reason for the pharmacological activities of "Female Corrective" including the increase in body weight, among others. In a certain study that assessed the toxicity of lutein-rich purple sweet potato leaf (PSPL) extract in male Sprague–Dawley rats, it was shown that phytochemicals had potentials to increase body weight of rats (Safiyyu'd-din *et al*., 2023). In another study that evaluated the effects of Super7 – a polyherbal antimalarial drug - on female Wister rats' gonadotropin hormones, it was shown that herbal products due to the phytochemicals in them have the potentials to change the body weights of laboratory animals (Oraekei *et al*., 2020). Results of toxicity investigations showed that "Female Corrective" poly-herbal product had wide margin of safety because no mortality was observed and obvious signs of toxicity were not recorded throughout the observational periods. Herbal products have also been shown to inhibit and/or induce drug-metabolizing enzymes. This implied that despite the level of safety as indicated by the LD_{50}, traditional medicines should also be used with caution (Ekor *et al*., 2014). In this study, it was observed that "Female Corrective" poly-herbal product had a reputable anti-oxidant effects that is statistically significant ($p < 0.05$) when compared with the normal and the positive

controls. Specifically, the SOD activities were very much higher than that of the control groups and groups 2, 3, and 4 treated with 2.06, 4.11 and 8.22 ml/kg body weight had SOD activities of 4.37 ± 0.60, 7.53 ± 1.45, and 10.65 ± 1.47 ng/mL respectively as against 1.94 ± 0.12 and 2.18 ± 0.15 ng/mL recorded for normal control (group 1 - distilled water) and positive control (group 5 - clomiphene) respectively. Glutathione which is the most prominent of all antioxidants was also elevated significantly ($p < 0.05$) and in a dose dependent manner. These intense antioxidant activities of "Female Corrective" poly-herbal drug formulation might be one of the herbal drugs' mechanisms of pro-fertility effects. Its ability to improve the levels of natural anti-oxidant molecules such as SOD, glutathione suggests that it will affect the accumulation of ROS negatively which a stress reducing effect. The herbal product potently increased, the concentrations of FSH and LH significantly ($p < 0.05$) when compared with the normal control group. However, its ability to elevate prolactin was an anti-fertility activity which could be insignificant against the combined antioxidant and pro-gonadotropin effects of the herbal product. Ginger extract at higher doses has been reported to have better effects in improving PCOS which is a condition that causes gonadotropin hormone deficiency (Shekoufeh *et al*., 2017). Other studies had indicated that a Chinese herbal preparation containing *Lycium barbarum*, regulates the levels of FSH and LH and it has been used to enhance male fertility for thousands of years (Zhou *et al*., 2019). Furthermore, the researchers reported that Ginsenosides were able to stimulate cultured anterior pituitary cells to secrete FSH and LH in vitro. Additionally, the extracts of the seed of *Cuscuta japonica* Choisy (*Semen cuscutae* extracts) significantly improved FSH and LH levels in adenine-induced model rats (Shao *et al*., 2019). These reports support the observed dose-dependent increase in the levels of FSH and LH by "Female Corrective" poly-herbal drug. The compounds in these plants, along with regulating the female endocrine pathways, could be used to treat female reproductive disorders such as polycystic ovary syndrome (PCOS), premature ovarian failure (POF), endometriosis, hyperprolactinemia, and hypothalamic dysfunction; moreover, because of their antioxidant among other properties (Mohsen *et al*., 2021).

Conclusion

In conclusion, "Female Corrective" poly-herbal medicine formulation demonstrated significant anti-oxidant activities and pro-gonadotropin effects in rats. The pro-fertility actions of "Female Corrective" poly-herbal drug formulation could be attributed among other factors to its anti-oxidative and pro-gonadotropin potentials. These therefore justify the use of "Female corrective" as a fertility enhancing herbal medicine formulation.

Recommendation

We recommend that "Female corrective" should be applied in cases of female infertility especially when the problem is connected to hypogonadism.

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Conflict of interest

The authors declare no conflict of interest.

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