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Reproductive potency of methanolic bi-herbal (*Zingiber officinale* and *Chrysophyllum albidum*) extract on masculine wistar rats

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ABSTRACT

The study is aimed at evaluating the fertility potential of bi-herbal methanol extract on male Wistar rats. This research finding based it fact of fertility in male Wistar rats to investigate through the phytochemicals, antioxidant assay, hormonal indexes, lipid profile, body/organ weight changes and histopathological study across the graded doses (25, 50, 100 mg/kg) of the treated groups of bi-herbal (*Zingiber officinale* and *Chrysophyllum albidum*)) methanol extract using standard procedure. Results from the phytochemical screening elicited the essential constituents (alkaloids, flavonoids phenol, tannins and saponnins). The antioxidant assay showed broad scavenging property against free radicals, which possibly is associated with one of its mechanisms of action. An increase in the level of significant ($p > 0.05$) in hormonal indexes (testosterone, progesterone, follicle stimulating hormone and luteinizing hormone) and slight increase in the level of lipid profile (cholesterol, triglyceride, HDL LDL and VLDL) activities among the treatment groups when compared with the control. There absent significant change ($p > 0.05$) in the body and testes mass ratio. No pronounce histopathological variation on the testes state in comparative to the control. The dependent from the bi-herbal extract with extreme exhibition of fertility potential as its therapeutic effect.

Keywords: Reproductive fertility, Bi-herbal, *Zingiber officinale*, *Chrysophyllum albidum*. Masculine Wistar rats.

INTRODUCTION

Natural effective products obtained from plant materials were used in the enhancement of man survival in supporting its health from antiquity [1]. Since previous decades, phytochemicals associated with plants synthesize an important pharmaceutical discovery. Active components found in plants as sources of medicine has triggered substantial scientific awareness to research on biological properties [2]. Several studies have been carried out to constrained ranges of plant types with proficient inspection scientific detailed, and the knowledge in relative to insufficient properties regarding to their prospective function to nature. Henceforth, possible execution of practical insight of natural products requires in inclusive investigations with biological properties linked with the various plants and the essential phytochemicals [1]. In a therapeutic scenery, plants with prolong historical folklore are enriched active phytoconstituents delivering therapeutic or health aids against several ailments and disorders.

Zingiber officinale Roscoe belongs to Zingiberacae family, a perennial herbaceous rhizomatous of approximately 90 cm in height beneath cultivation. Ginger is known for its medicinal importance with comprehensive health benefits majorly in Nigeria and Africa in general. The pharmacological health related benefits is found in ginger such as; rheumatism, sprains, fertility, arthritis, pains and muscular aches [3]. Ginger is associated with carminative, stimulant and aromatic isolate that possess GIT, stomachic, antispasmodic, vasodilator, appetizer, digestive, expectorant, topical, local stimulant, bronchodilator, aphrodisiac, laxative, Antitussive, analgesic and anti-flatulent. Studies on laboratory animals, showed that gingerrols upsurge gastrointestinal tract motility with antipyretic, antibacterial, analgesic and sedative activities [4].

Chrysophyllum albidum exhibited a prevailing canopy tree with varied rainforests and low-land occasionally, riverine. African star apple is broadly ingested in Southern Nigeria who's prevalent between children and women [5, 6]. Natural antioxidants constituents elicited a stimulated health benefits via scavenging free radical submerge from oxidative stress associated disorders includes; diabetes, coronary heart and cancerous diseases [7, 8].

Infertility is explant as incompetence to develop pregnancy subsequently for 12 months of steady, unsafe sex among couples or frequent meeting [9]. The impossibility to conceived children distresses couples universally and brings about responsive and mental misery among men and women. Certain factors that could be responsible for infertility such as; physiological, social, genetic and environmental enhances

infertility [10]. World Health Organization proclaim infertility effect via sexual transmitted disorders or reproductive tract infections is principal in Latin America and Africa [11]. Diverse causes characteristically evaluated using semen analysis since its spare head infertility in men. Semen analysis presently is the major fertility appraisal and it recurrently define semen quality such as morphology, viability and sperm motility) and sperm count concentration [12]. Atypical semen investigation does not necessarily denote infertile, rather semen analysis supports the control of male as a causative factor to infertility [9]. The main origins of infertility associated with men such as; testicular disorder or ejaculatory role: the caused could possibly be by varicocele (testicular veins enlargement which could be as a result of over-heat (heat disrupt the quantity or form of sperm); testicular ordeal; unhealthy conducts like anabolic steroid; alcoholism, smoking, cancer chemotherapy; surgical removal of testicles; radiation, medical situation include; cystic fibrosis, diabetes, definite categories of autoimmune diseases; ; sperm antibodies; occurrence of anti-thyroid antibodies [9]. Infection: Extensive period of untreated sexual transmitted diseases e.g., *Neisseria gonorrhoeae*, *Chlamydia trachomatis* display deleterious effect on fertility.

MATERIALS AND METHOD

Collection and Identification of plant material

The leaves of *Zingiber officinale* and *Chrysophyllum albidum* were collected in University of Benin, Benin City, Nigeria. The various plants were identified and authenticated in the Herbarium unit of Plant Biology and Biotechnology, University of Benin, Benin City.

Preparation of the Crude Extract

Zingiber officinale and *Chrysophyllum* leaves were rinsed in clean water and shade dried at room temperature. The dried plant samples were pulverized into fine powder. The weight of the pulverized samples (850 gram per each) was extracted via maceration using ratio 1:1 mixture of the samples and dissolved in 3.0 L of methanol for 72 h with continuously shaking and stirring for proper extraction. The macerated bi-herbal samples were sieved and concentrated using rotary evaporator equipment. The extract was refrigerated using conducive temperature for further uses.

Animals

Male Wistar rats weighing 180 – 210 g were procured from the Animal House of Biochemistry University of Benin, Benin City, Nigeria. They were housed in a wooden cage in the Animal House of the Department of Animal and Environmental Biology, University of Benin, Benin City, Nigeria. They were allowed to acclimatize for 14 days with free access to feeds and water *ad libitum.* Animals were exposed to natural conditions of temperature and lighting. Experimental Protocols for this study were in line with ethical guidelines on animals' care and handling during research study.

Acute Toxicity Study

Methanol bi-herbal extract was used for toxicity screening on Swiss mice. Acute toxicity test was performed using Lorke's method [13]. It includes two phases namely, phases 1 and 2 respectively. Animals were randomly allotted into group of four (n=5). They were orally administered using the doses (10, 100 and 1000 mg/kg) of the extract. The mice were kept back under intimate observation for 4 hour all through 24 hours, for suitable monitoring of their physical behavioral variations also to note potential death.

Phase 2

Sixteen Swiss mice were selected into four groups (n=5). The animals were administered at higher doses of 1600, 2900 and 5000 mg/kg of bi-herbal extract and observed for 24 h (distinctive devotion was given for the first 4 h) and once every day all through 14 days for toxicological signs such as; skin color alteration, paw/licking, fur changes, nostril discharge, eye lacrimation, tremor, convulsion, salivation, diarrhea and death.

The LD_{50} was calculated using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality.

Experimental design

Twenty-five male Wistar rats were randomly selected into four groups (n=5). Group A served as the control and the rats were given distilled water (0.2 ml/kg/day, p.o.). Group B, C and D were given oral doses of 25, 50, and 100 mg/kg per day of the extract for 56 days.

Collection and separation of sera samples for hormonal analysis

Animals in their separate groups were sacrificed; blood samples were isolated via abdominal aortal vein (under mild chloroform anesthesia). Collected blood samples were centrifuge for hormonal analysis. The testes were isolated, cleared of connective tissues and weighed.

Semen characteristics analysis

The two ends of the vas deference were tightening and placed in a pre-warmed Petri dish containing 1 ml of physiological saline solution (maintained at 37 ^{0}C). It was incisions to allow sperm swim out. Semen analysis was performed instantly via the novel upgraded Neubauer'shaemocytometer counting chamber to evaluate spermatozoa concentration. Sperm motility was measured instantaneously via counting motile and spermatozoa immotile per unit area using a magnification of 40x. Sperm viability was measured with eosin-nigrosin test. Percentages of unstained (live) and stained (dead) spermatozoa were calculated by counting 200 spermatozoa per sample. The morphology form of typical and atypical spermatozoa was resolute by investigative stained smears below the oil immersion lens (x100), and the percentages were calculated [11].

Testosterone Assay Protocol

Reagent Preparation

a. All reagents should be brought to room temperature (18-25 ^{0}C) before use.

b. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.

c. Samples with expected testosterone concentrations over 18 ng/ml may be quantitated by dilution with diluent available from your vendor.

Sample Preparation

a. Serum should be used in the test.

b. No special pretreatment of sample is necessary.

c. Serum samples may be stored at 2-8 ^{0}C for up to 24 hours, and should be frozen at -20 ^{0}C or lower for longer periods. Avoid grossly hemolytic (bright red), lipemic (milky), or tubid samples.

d. Please note: Samples containing sodium azide should not be used in the assay.

Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 10 µl of standards, specimens and controls into appropriate wells.
3. Dispense 100 µl of Testosterone-HRP Conjugate Reagent into each well.
4. Dispense 50 µl of rabbit anti-Testosterone reagent to each well. Thoroughly mix for 30 seconds. It is very important to mix them completely.
5. Incubate at 37 ^{0}C for 90 minutes.
6. Remove the incubation mixture by ficking plate contents into a waste container. Rinse and flick the microtiter wells 5 times with deionized or distilled water. Do not use tap water.
7. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Dispense 100 µl of TMB Reagent into each well. Gently mix for 5 seconds.
9. Incubate at room temperature for 20 minutes.
10. Stop the reaction by adding 100 µl of Stop Solution to each well.
11. Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
12. Read absorbance at 450 nm with a microtiter well reader within 15 minutes [14].

Calculation

Calculate the mean absorbance value (OD450) for each set of reference standards, controls and samples. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculations.

Progesterone

Reagent Preparation

1. Prepare Wash buffer by diluting 1 part with 19 parts of distilled water, excess amount may be stored at 2-8 ^{0}C for couple of weeks.
2. Standard solutions if not used for more than a week, should be kept frozen at -2 ^{0}C.
3. Sample should be diluted using sample diluent, (eg: 0.05 ml sample and 0.2 ml sample diluent) and diluted samples should be kept frozen at –20 ^{0}C for further use. This dilution may be an example one should work out the range to fit the sample in the standard range. This will also help you to avoid adding a very small volume (10 ul) which will lead to errors.

Assay Procedure

1. All reagents should be allowed to reach room temperature (18-25 ^{0}C) before use.
2. Pipette 50 ul of standards (ready to use) and diluted samples into appropriate wells within 5 minutes.
3. Add 100 ul of progesterone Enzyme Conjugate Solution to each well (except those set for blanks). Mix well for 30 sec. and incubate for 60 minutes at 37 ^{0}C. You may use parafilm to cover the wells or use appropriate zip-lock bag to store the plate during the incubation.
4. Discard the contents of the wells and wash the plate 5 times with Wash Solution (250-300 ul) per well. Invert plate, tap firmly against absorbent paper to remove any residual moisture.
5. Add 100 ul (TMB) Substrate solutions to all wells. Remember to follow the pipetting order.
6. Incubate the plate at room temperature (18-28 ^{0}C) for 10 minutes without shaking.
7. Stop reaction by adding 50 ul of Stopping Solution to wells in the same sequence that the Substrate Solution was added and gently mixed.
8. Read the absorbance at 450 nm with a microwell reader.

Liuteining Hormone Assay

Reagent preparation

1. All reagents should be brought to room temperature (18-25 ^{0}C) before use.
2. To prepare the wash buffer add one part of the reagent buffer to 19 parts of distilled water. Prepare desired amount and excess solution can be stored (refrigerated) and is stable for one week.
3. Store Reference standard solutions at –2 ^{0}C if not used immediately.

4. Assay Procedure

5. Secure the desired number of coated wells in the holder.
6. Dispense 50 ul of standards, specimens, and controls into appropriate wells.
7. Dispense 100 ul of Enzyme Conjugate into each well. Mix for 30 seconds. It is very important to have completed mixing at this step.
8. Incubate at room temperature (-37 ^{0}C) for 2 hours.
9. Remove the incubation mixture by flicking the plate contents into a waste container.

10. Rinse and flick the microtiter wells five (5) times with wash buffer.

11. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.

12. Dispense 100 ul of TMB solution into each well. Gently mix for 10 seconds.

13. Incubate at room temperature for 20 minutes, in the dark.

14. Stop reaction by adding 50 ul (one drop) of 2N HCl to each well.

15. Gently mix for 30 seconds. It is important to observe a color change from blue to yellow.

16. Read optical density at 450 nm with a micro titer well reader.

Important note: The wash steps are very critical. Insufficient washing will result in poor precision and falsely elevated absorbency readings [15].

CALCULATION

Calculate the mean absorbency value (A450) for each set of reference standards, specimens, controls and patient samples. Construct a standard curve by plotting the mean absorbency to determine the corresponding concentration of BOVINE LH in ng/ml from the standard curve.

Follicle Stimulating Hormone

Assay Procedure

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents and samples as directed in the previous sections.

2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4 ^{0}C.

3. Set a Blank well without any solution.

4. Add 50 µl of Standard or Sample per well. Standard need test in duplicate.

5. Add 50 µl of HRP-conjugate to each well (not to Blank well), then 50 µl Antibody to each well. Mix well and then incubate for 60 minutes at 37 ^{0}C.

6. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (200 µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or auto-washer and let it stand for 10 seconds, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

7. Add 50 µl of Substrate A and 50 µl of Substrate B to each well, mix well. Incubate for 15 minutes at 37 ^{0}C. Keeping the plate away from drafts and other temperature fluctuations in the dark.

8. Add 50 µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing.

9. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm [15].

Lipid Profile

Total cholesterol was determined using enzymatic method using wet reagents diagnostic kits. This uses a modified method of Trinder [16]. 1000 µl reagent was pipette into three distinct test tube A, B and C. Distilled water, a standard solution (standard) and plasma (sample) of 10 µl each was pipetted into same test tubes A, B and C respectively. After mixing, the test tubes were left to stand for 10 minutes at room temperature to give room for colour change., after which the absorbance of the blank, sample and standard were read at 500 nm using a UV spectrophotometer. Cholesterol value was calculated as shown below:

Cholesterol (mg/dl) = Absorbance of sample/Absorbance of standard x concentration of standard (mg/dl).

Total triglycerides were determined using enzymatic method with wet reagent diagnostic kits. This is a modification of the method of Tietz [17]. The reagent (1000 µl) was pipette into three different test tubes A, B and C of 10 µl each of standard solution (standard) and plasma sample was pipetted into B and C test tubes while test A contained only the reagent (reagent blank). After mixing, the test tubes were left to stand for 10 minutes at room temperature (25 deg. Celsius) to allow for color change. The absorbance of the blank, sample and standard were read at 500 nm using UV spectrophotometer. This was repeated for all the plasma samples. The concentration of the triglyceride in the sample was calculated and expressed as thus:

Total triglyceride (mg/dl) = Absorbance of sample/Absorbance of standard x concentration of standard (mg/dl).

High density lipoprotein was determined using the enzymatic method. A precipitating agent consisting of 0.55 mmol/l phosphotungstic acid and 25 mmol/l magnesium chloride [18] were used. This was pre-diluted via ratio 4:1 with distilled water. 500 µl of the reagent pipette into two different test tubes A and B. A standard solution (standard) and plasma sample of 200 µl each were pipette into the same test tube A and B respectively. After mixing, the test tubes were allowed to stand for 10 minutes at room temperature to allow for color change, after which the standard and sample absorbance were measured at 500 nm using the UV spectrophotometer. HDL was calculated below:

HDL (mg/dl) = Absorbance of sample/ Absorbance of standard x concentration of standard (mg/dl).

Low density lipoprotein was calculated as thus:

LDL (mg/dl) = Total cholesterol – Triglycerides/5 - HDL [18].

Histology

Testes were isolated and fixed in Bouin's fluid. They were sectioned into 10 % formalin prior to histological examination. Further preparations of the slides were carried out by a Morbid Anatomist. The

slides were stained with hematoxylin and eosin for light microscopy. Micrograph of the slide preparation was taken.

Statistical analysis and data presentation

Results are expressed as mean ± SEM (standard error of the mean). Data for the groups were compared using one-way analysis of variance with Dunnett test. Differences between data were considered significant at $P < 0.05$.

RESULTS

Table 1: Preliminary qualitative Phytochemicals

Phytochemicals	Bi-herbal
Flavonoids	+
Saponins	+
Terpenoids	-
Phenols	+
Alkaloids	+
Tannins	+

KEY: + present, - Not detected

Table 2: Preliminary quantitative Phytochemicals

Phytochemicals	Bi-herbal (%)
Alkaloids	2.94±0.24
Flavonoids	481.00±1.02
Phenol	642.99±2.07
Saponins	6.62±0.00
Tannins	155.38±0.31

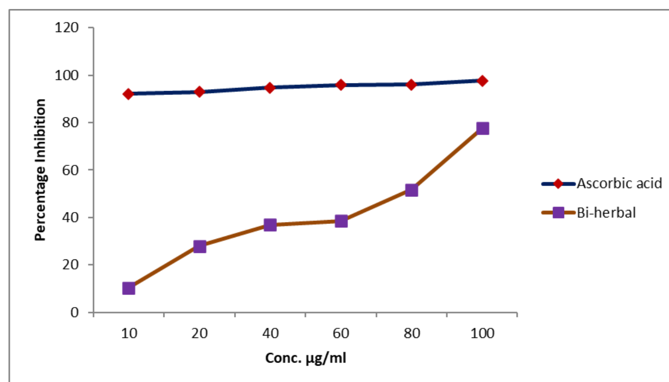


Figure 1: Effect of Bi-herbal on DPPH antioxidant potential.

Acute Toxicity Test

No mortality was recorded at the maximum dose of 5000 mg/kg after fourteen days of observation. No sign of toxicity was observed during the fourteen days observation period (Table 3.5).

Table 3: Acute toxicity effect of Bi-herbal leaves extract

Groups	Doses (mg/kg)	No of lethality	% mortality
Control	DW	0/5	Absent mortality
Bi-herbal	10	0/5	Absent mortality
Bi-herbal	100	0/5	Absent mortality
Bi-herbal	1000	0/5	Absent mortality
Bi-herbal	1600	0/5	Absent mortality
Bi-herbal	2900	0/5	Absent mortality
Bi-herbal	5000	0/5	Absent mortality

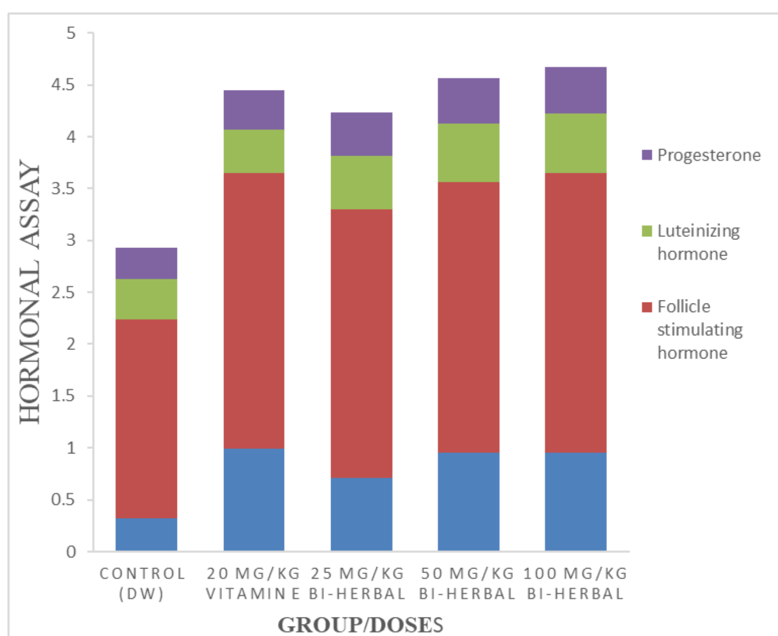


Figure 2: Effect of Bi-herbal extract on testosterone level

Table 4: Effect of Bi-herbal extract on sperm cells indices in male fertility study.

Drugs	Doses (mg/kg)	Visciosity	PH	Mortility %	Morphology (%) abnormal	Morphology (%) normal	Viability (%) viable	Total sperm count (10^6/ml)
Control	1 ml	Low	6.9	31.58	91.17	21.16[a]	29.33[a]	12.90±0.11[a]
Vitamin E	20	Moderate	7.2	89.99	23.63	67.03[c]	88.16[c]	40.14±1.05[c]
Bi-herbal	25	Moderate	7.1	45.79	31.07	71.21[c]	69.09[b]	38.32±0.41[c]
Bi-herbal	50	Moderate	7.3	60.01	38.52	69.77[c]	70.52[b]	39.18±1.24[c]
Bi-herbal	100	Moderate	7.2	63.04	30.33	73.12[c]	79.98[c]	41.30±1.10[c]

P-value = a---p< 0.1, b--- p<0.01 n=5

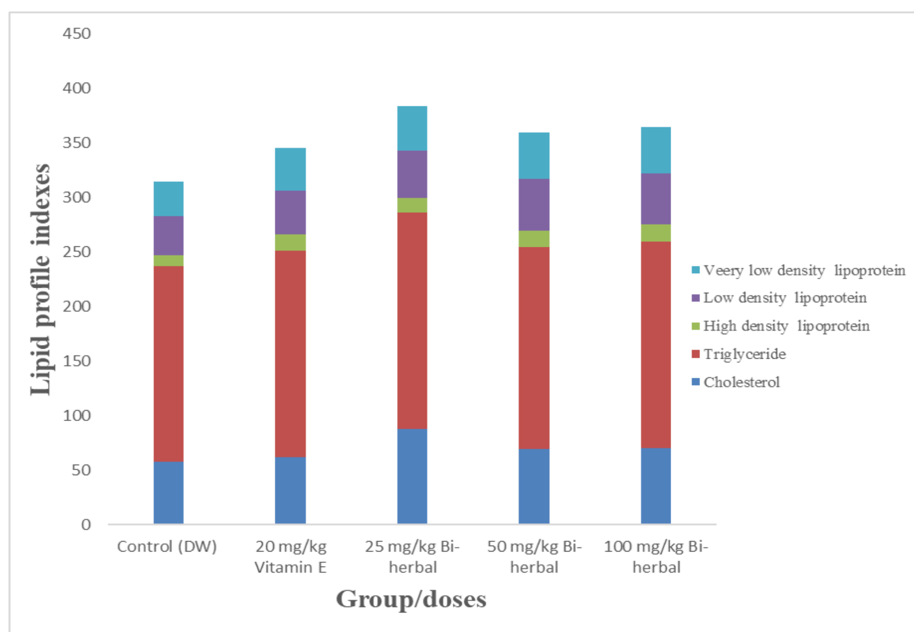
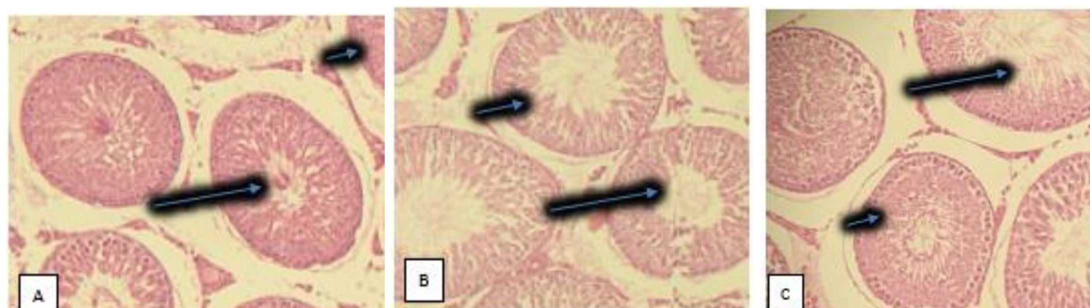


Figure 3: Effect of Bi-herbal extract on lipid profile assay

Table 5: Effect of Bi-herbal extract on the body/organ mass indexes

Treatment/ Days/Organ	Untreated	20 mg/kg Vitamin E	25 mg/kg Bi-herbal	50 mg/kg Bi-herbal	100 mg/kg Bi-herbal
1	165±0.13	180±0.05	196±2.27	165±2.01	165±2.11
7	203±1.01	205±1.23	205±0.88	196±0.24	196±1.53
14	208±0.21	210±0.96	205±6.08	196±1.53	196±1.44
21	216±1.04	216±2.11	213±2.52	227±4.15	227±5.15
28	213±1.00	223±2.20	217±3.13	238±3.20	238±4.06
35	217±0.25	229±1.33	223±2.92	244±3.06	264±3.13
42	223±1.05	237±1.00	228±3.25	249±4.09	258±2.92
49	228±1.14	243±2.44	236±5.06	255±4.02	263±4.92
56	235±0.84	251±3.17	247±4.04	269±3.20	269±6.12
Testes	1.16±0.53[a]	1.01±0.22[a]	1.05±0.09[a]	1.10±0.29[a]	1.11±0.31[a]



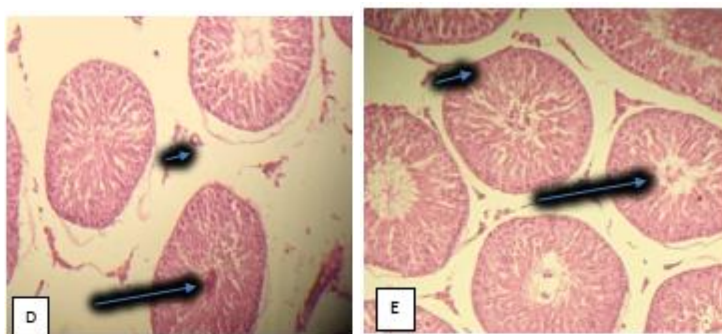


Plate 1: Effect of Bi-herbal extract on male Testes A. CONTROL 0.5 ml/kg TESTIS: showed impartially spherical Seminoferous tubules [long arrow] with mild spermatogonia, spermatids and sertoli cells [short arrow]. B. 20 mg/kg Vitamin E TESTIS: showed impartially spherical Seminoferous tubules [long arrow] with pronounce spermatogonia, spermatids and sertoli cells [short arrow]. C. 25 mg/kg Bi-herbal extract TESTIS: showed impartially spherical Seminoferous tubules [long arrow] with pronounce spermatogonia, spermatids and sertoli cells [short arrow]. D. 50 mg/kg Bi-herbal extract TESTIS: showed impartially spherical Seminoferous tubules [long arrow] with pronounce spermatogonia, spermatids and sertoli cells [short arrow]. E. 100 mg/kg Bi-herbal extract TESTIS: showed impartially spherical Seminoferous tubules [long arrow] with pronounce spermatogonia, spermatids and sertoli cells [short arrow].

DISCUSSION

Antioxidant property and phytochemical constituents includes; polyphenols, tannins, alkaloids and flavonoids for long have been standardized due to their reproduction effects in animals [19], and act indirectly on androgen discharge via triggering or inhibiting pituitary glands and hypothalamus thus impelling spermatogenesis and other function associated with other glands [20]. Findings have revealed saponins being responsible for intermediary character in androgens synthesis by facilitating the pituitary gland to secretion luteinizing hormone (LH) enhancing testosterone synthesis via Leydig cells of the testis [21]. Flavonoids are concerned in maintaining the production of androgens via impeding 17β-estradiol aromatase, an enzyme complex in converting testosterone into estrogen. The present of alkaloids have shown its aphrodisiac, anti-fertility and fertility properties [22]. Flavonoids and polyphenols display promising effect on female and male fertility owing to their sturdy antioxidant activities [23]. Findings by Silberstein *et al.* [24] exhibited antioxidants level connects with sperm concentration and motility. The presence of antioxidant and phytochemicals in *bi-herbal* extracts exhibited what its own the capacity to scavenge surplus reactive oxygen species (ROS), spermatozoa is liable for any oxidative impairment done to sperm cells. This is probably the significant increase noted in sperm feature in rats (Figure 1) as shown across treatment groups. Preliminary qualitative and quantitative phytochemicals screening of bi-herbal extract showed the presence of; alkaloids, tannins, saponnins, flavonoids and phenol as shown in Tables 1 and 2.

Acute toxicity study revealed the bi-herbal methanol extract which relatively exhibited no toxicity with LD_{50} higher than 5000 mg/kg (Table 1). Absent toxicological signs like weight loss, salivation, respiratory ache, variations in fur and skin coloration, mortality and paw licking were observed in the animals during the period of observation. This recommends that the extract potency might be unharmed with the tested doses. [13] Categorized constituents as somewhat toxic if the LD_{50} varies from 100 to 1000 mg/kg. Clarke and Clarke [25] as well stated that substances are affirm low or absent toxicity if the LD_{50} is equal to 1000 mg/kg.

Conventional forms of semen analysis can be considered the primary choice for the assessment of fertility state and it usually explain semen quality (morphology, viability and sperm motility) and sperm count concentration [26]. Observable sperm count increase and percentage motility increase, morphology and viability with reduction in percentage abnormality in sperm cells as noted in the treated rats when compared with the control, indicated that bi-herbal extract has fertility improving effect as shown in Table 4.

Testosterone, the main androgenic hormone liable for sexual appeal to displays an essential role in spermatogenesis. Findings showed that it triggered sexual zeal and maintain penis tissues that facilitated erection [27]. Studies from Yakubu *et al* [28], showed that male sexual dysfunction is related with several factors such as; androgen insufficiency. Testosterone agents (hormonal replace therapy) exhibited to develop sexual role and libido. Some plant extracts that promote an increase in testosterone level, possess aphrodisiac and fertility effects [28]. A significantly increase in testosterone level is relatively dose dependent as indicated by bi-herbal extract across treated groups when compared with the control, also increase in follicle stimulating hormone, luietenizing hormone and progesterone stimulate the release of testosterone indicating the bi-herbal extract possess fertility enhancing potentials as shown in Figure 2. Increased in the level of testosterone level could as well be associated with the observed increase in weights of the testis with in normal ranges as means of enlargement to enhance androgen dependent.

Hyperlipidemia is as a result of sudden increase in blood lipids that is triggered by certain plant types to increase or decrease the concentration. Lipids comprises of cholesterol, lipoprotein, triglycerides and phospholipids. They can be transferred in larger particles typically called lipoproteins [29]. Humans cholesterol is a metabolic steroid hormone include, aldosterone, estrogens, androgens and cortisol) that standardize massive physiologic characters. Increased cholesterol concentration (above 200 mg/dl) serves as a risk factor to triggered coronary heart disorders. Bi-herbal methanol extract at graded doses (25, 50 and 100 mg/kg) regulate TAG, TC and LDL level within standard ranges, with insignificant increased across the concentrations when compared with the control (Figures 1 & 2). The control of TC, TAG and LDL concentration in typical quantity proposed its capability to improve steroidal hormones necessary for fertility. Data acquired from this study exhibited significant increase with dose dose-dependent in testicular weights across the treated groups of the extract. Increase in testicular weights enhanced androgen (testosterone) increase [30]. Increased in the level of androgen enhanced Leydig cells to stimulate luteinizing hormone resultant with an increased in spermatogenesis and increase epididymal sperm. Findings by Choudhary and Steinberger [31] shown that androgens control the weight, size and secretory role of epididymis, testes and auxiliary organs. Modifications in the circulating

level of androgen may distort the basic and efficient capability of reproductive tissues subsequent with an initiate on spermatogenesis [32]. Alteration in reproductive organ indexes uses a marker to improved circulating concentration of androgen, since reproductive glands can be particularly complex to androgen levels.

Histopathological analysis of male reproductive organ (testes) exhibited standard anatomical structure of the testes when compared with the control Plate 1). Treated organs at 25, 50 and 100 mg/kg of the extracts exhibited improved architecture when compared with the control. This recommends that, the bi-herbal extract promotes physiological architechture of the testes in contrast with the biochemical results of significant increase ($P < 0.05$). These remarks might be endorsed to antioxidant property and possible phytoconstituents.

CONCLUSION

Bi-herbal extracts contains several bioactive agents with antioxidant properties that are possibly responsible to improve semen indexes to further potentiated fertility by promoting sperm number, quality, and improved serum testosterone concentration. This study hence advances confidence of this extract as a potent male fertility enhancer in line with folklore reports.

Conflict of interest

The authors declare no conflict of interests

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