Endocrine and Metabolic Effects of Hydroethanolic Extract of *Solenostemon monostachyus* on Haloperidol Induced Hyperprolactinemia

Omoloye Adesina Adebiyi¹, Quasim Kifayat Olabisi¹, Johnson Samuel Onnolome² and Murtala Abdullahi Akanji³

1. Department of Clinical Pharmacy, Faculty of Pharmacy, Olabisi Onabanjo University Teaching Hospital, Sagamu-Ogun State 234, Nigeria
2. Department of Chemical Pathology and Immunology, Olabisi Onabanjo University Teaching Hospital, Sagamu-Ogun State 234, Nigeria
3. Department of Pharmacology, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Sagamu-Ogun State 234, Nigeria

Abstract: The rapid increase in consumption of herbal remedies worldwide has been stimulated by several factors, including the notion that all herbal products are safe and effective. Hyperprolactinemia is a major cause of infertility, and herbal remedies have been employed locally for treatment. This study was designed to investigate the effects of hydroethanolic extract of *Solenostemon monostachyus* on the reproductive hormones and metabolic parameters of haloperidol-induced hyperprolactinemic rats. Thirty six female albino rats were divided into 6 groups of 6 in each group. Groups A, B, C, D and E were given increasing doses (2, 3 and 4 mg/kg body weight in five-daily increments) of haloperidol by intramuscular injection for 15 days after which they were treated for another 15 days with either 2.5mg/kg body weight of bromocriptine (group D only) or 75, 112.5 or 225mg/kg body weight of the extract (groups A, B and C, respectively). Group F was given distilled water only. After treatment, the animals were sacrificed and blood was taken from each group for plasma analysis of the reproductive hormones and metabolic parameters. The total protein and the lipid profile (total cholesterol and HDL (high-density lipoprotein) and triglycerides were also determined. Phytochemical investigation revealed the presence of saponins, phenols, alkaloids, flavonoids, and tannins. The result of endocrine investigation showed a dose-dependent, statistically significant reduction in prolactin and testosterone (\( P < 0.05 \)) level by the extract with statistical significant increase (\( P < 0.05 \)) in the levels of the follicle stimulating hormone, LH (luteinizing hormone) and estrogen. There was also a decrease in the levels of the triglycerides and total cholesterol while HDL was increased (\( P > 0.05 \)). It can be concluded from this study that hydroethanolic extract has a prolactin reducing activity compared with Bromocriptine and exhibited a corresponding statistically significant difference in other reproductive hormones, with no detectable alteration on metabolic parameters such as: albumin, total cholesterol, and high density lipoprotein.

Key words: Hyperprolactinemia, bromocriptine, haloperidol, reproductive hormones, *Solenostemon monostachyus*.

1. Introduction

The practice of traditional medicine using medicinal plants has a long history in many cultures. This type of health care can be described as herbalism, traditional medicine or botanical medicine. The use of traditional medicine is important for the treatment and management of a number of diseases in the African continent, popular among the practice is treatment of infertility in woman through the use of herbal product. *Solenostemon* comprises some dozens of species and occurs in Africa and Asia. It is sometimes included in Plectranthus, with distinctive difference in its calyx of which the lower teeth are united at the base only, whereas the lateral teeth are more or less equal to the lower. *Solenostemon monostachyus* is variable and has been divided into 4 subspecies, but intermediate specimens occur. It is annual or perennial, slightly

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Corresponding author: Omoloye Adesina Adebiyi, B. Pharm., M. Sc., FPCPharm., clinical pharmacist and pharmacologist, research field: neuropharmacology and toxicology. E-mail: adesinaloye@gmail.com.
succulent, aromatic herb up to 100 cm tall, branched; stem erect or decumbent, 4-angled, shortly pubescent. *Solenostemon monostachyus* is an important herb that is native to West and Central Africa. The leaves have been traditionally used for various medicinal purposes [1]. This plant has significant abilities to scavenge hydrogen peroxide and hydroxyl radicals, and also significant ability to reduce ferric ions in vitro. The extract also possessed significant abilities to reduce lipid peroxidation and haemolysis in erythrocytes induced by hydrogen peroxide when compared with the ability of ascorbic acid to do the same. This is ascribed to the possession of antioxidant phytochemicals which acted in synergy. The antidiabetic and anticonvulsant activities of *Solenostemon monostachyus* have also been documented [2, 3]. However, traditional medicine healers in Africa have employed the use of this plant in the management of infertility in woman. Therefore, the aim of this study was to investigate this claim and possibly postulate mechanism of action for the ethnobotanical information.

2. Material and Methods

2.1 Plant Collection

Fresh leaves of *Solenostemon monostachyus* were harvested from Itunmoro area of Ikenne town, Ogun state in June 2013 at about 7:00 a.m. and was identified by Mr O. O. Oyebanji of the Department of Botany, Faculty of Science, University of Lagos, Lagos State, who kept a voucher specimen and gave herbarium No. LUH 5910.

2.2 Extract Preparation

*Solenostemon monostachyus* leaves were air dried until a constant weight was obtained and the dried material was grounded to fine powder. One hundred g of the plant material was macerated in 1000 mL of hydroethanol (1:1) for 48 h, after which the liquid was decanted and filtered twice to remove all debris. The residue from the process was re-macerated in same volume of hydroethanol to ensure exhaustive extraction (× 2). The filtrate from each extraction process was combined and evaporated to dryness at 40 °C under reduced pressure. The solid extract obtained was reconstituted in distilled water before each experimental session.

2.3 Animals

Thirty six female rats were obtained from an in-breed private colony at the Nigerian Institute of Medical Research, Yaba, Lagos State, Nigeria. They were housed in the animal handling facility of the Nigerian Institute of Medical Research, Yaba, Lagos State, Nigeria. The rats were used after an acclimatization period of 14 days to the laboratory environment. The animals were allowed free access to pellet feeds (obtained from LADOKUN feeds, Ibadan) and water. The rats were completely randomized into six groups of 6 each as follows: Groups A, B, C, D and E were given increasing doses (2, 3 and 4 mg/kg body weight in five-daily increments) of haloperidol by intramuscular injection for 15 days after which they were treated for another 15 days with either 2.5mg/kg body weight of bromocriptine (group D only) or 75, 112.5 or 225mg/kg body weight of the extract (groups A, B and C, respectively). Group F was given distilled water only. The animal experiments were performed according to the OECD (organization for economic co-operation and development) guidelines for testing of chemicals [4].

2.4 Phytochemical Analysis

Phytochemical screening of the hydroethanolic leaf extract of *Solenostemon monostachyus*, to determine the presence or absence of various phytochemicals, was carried out according to the methods of [5, 6] Extract was dissolved individually in dilute Hydrochloric acid and filtered. Filtrate was treated with Mayer’s reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates
the presence of alkaloids.

Extract was diluted with distilled water to 20 mL and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins (Froth test).

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins (Gelatin test).

Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids (Alkaline reagent test).

Powdered leaves of Solenostemon monostachyus, are boiled with dilute sulphuric acid. Filtered and cooled. The filtrate is extracted with chloroform or benzene and dilute ammonia is added to it. The ammonical layer becomes pink to red due to the presence of anthraquinones derivative (Borntrager’s test).

The extract is to be dissolved in pyridine and a few drops of 2% sodium nitroprusside together with a few drops of 20% NaOH are to be added. A deep red colour which faded to a brownish yellow indicates the presence of cardenoloides (Legal test).

2.5 Acute Toxicity Study

Mice were randomly divided into five groups of five animals per group. Graded doses of the extract (5000, 9000, 10000 and 25000 mg/kg) were administered to the animals orally ad libitum. The control group was administered 0.5 ml/kg distilled water orally. Mice were observed for 24 h post-treatment for mortality, behavioural changes (restlessness, dullness, agitation) and signs of toxicity.

2.6 Hormonal and Biochemical Assay

2.6.1 Assay Kits

The assay kits for prolactin, progesterone, estradiol, follicle stimulating and luteinizing hormones were supplied by Diagnostic Automation Inc., Calabasa, CA, USA. All other reagents used were of analytical grade and supplied by B and G Pharma Ltd. and were prepared in volumetric flask using glass-distilled water.

2.6.2 Preparation of Serum

The procedure described by Ref. [7] was employed. Briefly, under ether anaesthesia, the veins after being slightly displaced (to prevent blood contamination by interstitial fluid) were cut with a sterile scalpel blade and 5 mL of the blood was collected into clean and dry centrifuge tubes.

The blood was then left for 10 min to clot at room temperature.

The tubes were thereafter centrifuged at 33.5 g x 15 min using Uniscope Laboratory Centrifuge (Model SM800B, Surgifriend Medicals and Essex, England). The sera were later aspirated with Pasteur pipettes into clean, dry, sample bottles and were then used within 12 h of preparation for the hormonal assay.

2.6.3 Hormonal Assay

The procedure described in the hormone assay kits was used according to the principle highlighted by Ref. [8] for prolactin, estradiol and progesterone while that of Ref. [9] was used for luteinizing and follicle stimulating hormones.

2.6.4 Biochemical Assay

For biochemical analysis, the parameters determined were, Albumin, T.CHOL (total-cholesterol), TG (triglyceride), HDL-cholesterol, and total protein using commercially available kits (RANDOX, United Kingdom) and an auto biochemical analyzer machine (Rayto).

2.7 Statistical Analysis

Results were expressed as the mean of five replicates ± S.D. except for the phytochemical screening. Means were analyzed using a one-way ANOVA and values at $P < 0.05$ were considered statistically significant. In all the Figures, bars carrying letters different from the control for each day are significantly different
(P < 0.05).

3. Results

3.1 Phytochemical Analysis

The results of the chemical tests performed in the preliminary phytochemical screening revealed the presence of flavonoids, saponins, alkaloids, (strongly present) cardenolides, anthraquinone, phenolics and tannins in the hydroethanolic leaf extract of Solenostemon monostachyus. Phlobatamins and oils were found to be absent.

3.2 Acute Toxicity Test

Solenostemon monostachyus did not cause any mortality and visible signs of toxicity when administered orally up to 22.2 g/kg and observed for fourteen days. Behavioural manifestations observed for

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>D/Ta</th>
<th>Signs of toxicity observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.5 mL (H₂O)</td>
<td>0/5</td>
<td>No toxic changes observed</td>
</tr>
<tr>
<td>B</td>
<td>5,000</td>
<td>0/5</td>
<td>No toxic changes observed</td>
</tr>
<tr>
<td>C</td>
<td>9,000</td>
<td>0/5</td>
<td>No toxic changes observed</td>
</tr>
<tr>
<td>D</td>
<td>10,000</td>
<td>0/5</td>
<td>Slight dullness was observed in 2 Animals in the first 2 h</td>
</tr>
<tr>
<td>E</td>
<td>25,000</td>
<td>0/5</td>
<td>Slight dullness was observed in 2 animals in the first 2 h</td>
</tr>
</tbody>
</table>

D/Ta—Number of mice deaths/total number of mice (n = 5).

Table 3 Effects of extract of S. monostachyus on reproductive hormones.

<table>
<thead>
<tr>
<th>Group</th>
<th>Prolactin (IU)</th>
<th>LH (IU)</th>
<th>FSH (IU)</th>
<th>Testosterone (IU)</th>
<th>Estradiol (IU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (75 mg/kg extract)</td>
<td>0.73 ± 0.06</td>
<td>2.67 ± 0.55</td>
<td>0.03 ± 0.03</td>
<td>6.38 ± 1.99</td>
<td>21.00 ± 4.03</td>
</tr>
<tr>
<td>B (112.5 mg/kg extract)</td>
<td>0.38 ± 0.05</td>
<td>4.22 ± 0.34*</td>
<td>0.04 ± 0.03</td>
<td>3.82 ± 1.77</td>
<td>30.50 ± 3.36</td>
</tr>
<tr>
<td>C (225 mg/kg extract)</td>
<td>0.17 ± 0.04</td>
<td>5.90 ± 0.46*</td>
<td>0.05 ± 0.03</td>
<td>3.75 ± 1.21</td>
<td>47.50 ± 5.72**</td>
</tr>
<tr>
<td>D (2.5 mg/kg bromocriptine)</td>
<td>0.42 ± 0.04</td>
<td>1.68 ± 0.34</td>
<td>0.02 ± 0.03</td>
<td>2.58 ± 1.61</td>
<td>15.50 ± 2.35</td>
</tr>
<tr>
<td>E (2.5 mg/kg haloperidol)</td>
<td>3.62 ± 0.47</td>
<td>1.89 ± 0.63</td>
<td>0.02 ± 0.02</td>
<td>4.22 ± 1.93</td>
<td>14.50 ± 3.70</td>
</tr>
<tr>
<td>F (0.5 mL/kg distilled water)</td>
<td>1.67 ± 0.54</td>
<td>2.37 ± 0.35</td>
<td>0.03 ± 0.03</td>
<td>6.03 ± 1.35</td>
<td>18.67 ± 3.51</td>
</tr>
</tbody>
</table>

*—P < 0.05.
**—P < 0.01.

Table 4 Effects of extract of S. monostachyus on metabolic parameters.

<table>
<thead>
<tr>
<th>Group</th>
<th>Albumin (mg/dl)</th>
<th>Total protein (mg/dl)</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>HDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (75 mg/kg extract)</td>
<td>3.48 ± 0.29</td>
<td>7.92 ± 0.26</td>
<td>47.00 ± 2.12</td>
<td>67.67 ± 9.56</td>
<td>75.83 ± 0.20</td>
</tr>
<tr>
<td>B (112.5 mg/kg extract)</td>
<td>3.30 ± 0.78</td>
<td>8.05 ± 0.45</td>
<td>53.17 ± 5.06</td>
<td>106.67 ± 26.81</td>
<td>85.00 ± 0.25</td>
</tr>
<tr>
<td>C (225 mg/kg extract)</td>
<td>3.73 ± 0.18*</td>
<td>8.55 ± 0.43</td>
<td>40.83 ± 5.53</td>
<td>71.17 ± 8.34</td>
<td>89.67 ± 0.17*</td>
</tr>
<tr>
<td>D (2.5 mg/kg bromocriptine)</td>
<td>3.60 ± 0.27</td>
<td>7.82 ± 0.10</td>
<td>61.00 ± 6.40</td>
<td>87.17 ± 5.24</td>
<td>66.83 ± 2.20</td>
</tr>
<tr>
<td>E (2.5 mg/kg haloperidol)</td>
<td>2.88 ± 0.50</td>
<td>7.05 ± 0.24</td>
<td>42.50 ± 4.92</td>
<td>55.67 ± 2.96</td>
<td>51.17 ± 0.20</td>
</tr>
<tr>
<td>F (0.5 mL/kg distilled water)</td>
<td>3.08 ± 0.45</td>
<td>6.78 ± 0.43</td>
<td>49.17 ± 5.93</td>
<td>78.33 ± 13.09</td>
<td>86.00 ± 0.24</td>
</tr>
</tbody>
</table>

*—P < 0.05.
**—P < 0.01.
2 h post-oral treatment included reduced locomotion and calmness. The LD50 of the extract administered orally was estimated to be 22,500 mg/kg.

4. Discussion

Maturation of pre-ovulatory follicles and ovulation are under the combined and balanced influences of ovarian and extra ovarian hormones. Imbalances or alterations in these hormones lead to irregularity in the ovarian functions and duration of estrous cycle [10]. These hormonal imbalances may be caused by numerous chemical agents contained in plant extracts. Phytochemical screening has revealed many bioactive as well as toxic agents of plant extracts that can affect the regulation of oestrous cycle, conception and reproduction [7, 11]. Alkaloids and flavonoids have been shown to have effect on plasma concentrations of LH, estradiol and FSH [12, 13]. Therefore, the presence of these phytochemicals may account for the alterations in the levels of the circulating hormone observed in this study.

Prolactin helps to initiate breast development by inducing lobuloalveolar growth of the mammary gland. It also stimulates lactogenesis. Dopamine serves as the major-inhibiting factor or break on prolactin secretion [14]. The reduced level of prolactin observed in this study may be attributed to the effect of the extract probably acting as a dopamine agonist. High prolactin levels tend to suppress the ovulatory cycle by inhibiting the secretion of both follicle-stimulating and GnRH (gonadotropic releasing hormones) [14], which are necessary for ovulation thereby leading to reduced fertility. On the other hand, a reduction in prolactin levels such as that shown by the extract leads to improved fertility. The reduced level of prolactin in this study justifies the folkloric use of the plant in infertility treatment.

Follicle stimulating hormone is the central hormone of mammalian reproduction, essential for gonadal development and maturation at puberty as well as gamete production during the fertile phase of life [15]. It stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulosa cells. The increase in the levels of FSH by the extract may improve folliculogenesis and maturation of the follicle in the pre-ovulatory phase. It is possible that the extract might have exerted its effect on the anterior pituitary or the hypothalamus since the secretion of FSH is regulated by the gonadotropic releasing hormone secreted by the hypothalamus. This increase in the levels of the hormone will therefore enhance conception in the female animals.

Luteinizing hormone stimulates secretion of sex steroids from the gonads. In females, ovulation of mature follicles in the ovary is induced by a large surge of LH secretion during the pre-ovulatory periods. Several authors have demonstrated that LH release surges at the proestrous stage are responsible for ovulation [16]. Any substance capable of enhancing this release could promote ovulation by increasing the number of mature follicles [11]. Therefore, the increase in the serum LH levels may be explained by an excitatory effect of the extract on the release of LH which may enhance of ovulation. This may result in enhancement of oestrous cycle; promote conception and normal reproduction in the females.

Our findings agreed with that of Ref. [17] where triterpenoid glycoside (saponins) in methanolic and lipophilic extracts of Cimicifua racemosa (Black cohosh-English) was responsible for the increase in LH concentration. Therefore, it is possible that Solenostemon monostachyus contains progonadotropic substance(s) which may have positive effect on the oestrous cycle and enhance reproduction in females.

Estradiol stimulates the growth of the uterine lining, causing it to thicken during the preovulatory phase of the cycle. It is well established that estradiol is directly responsible for the growth and development of reproductive organs. In synergy with FSH, estradiol stimulates granulose cell proliferation during follicular development [18]. Estrogens are steroid hormones which, together with other hormones, control the
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Ovulatory cycle in the female mammal. Estrogen acts in a feedback mechanism, influencing the production of FSH (follicle stimulating hormones) from the pituitary gland. It is known that the FSH in turn promotes the development of the immature ovarian follicles, which increases the production of estrogen from the ovary. Present findings indicate that the administration of the extracts showed significant increase in the estrogen level of FSH and LH resulting increased tendency to ovulate. This can result from hypersecretion of gonadotropic hormones, in which case the intensity of the hormonal stimuli simply is not sufficient to cause ovulation, or it can result from sensitization of ovaries to ovulate.

Plants with estrogenic property can directly influence pituitary action by peripheral of LH and FSH, decreasing secretion of these hormones and blocking ovulation [7, 19]. Thus, the increase in the serum concentration of estradiol observed in this study may be attributed to a process initiated by aromatase activity or substrate supplementation during estrogen synthesis [20]. Hence, increase in estradiol levels will influence zygote implantation, facilitates ovulation and sustains pregnancy state [21]. It was earlier reported that herbal formulation containing Shakuyaku (*Paeoniae radix*), Keihi (*Cinnamomi cortex*) and Botanpi (*Moutan cortex*) have effect on aromatase activity in primate granulose cells. The researchers [22, 23] reported that several plant metabolites can have effects on aromatase activity, thus altering the potential for steroid production and reproductive performance. Therefore, the metabolites in the extract may be responsible for the increased level of estradiol probably by inhibiting aromatase activity. Thus, it is possible that the extract of *Solenostomer monostachyus* contain biologically active phytochemicals which may be endocrine altering. Our findings in this study have important implications for female fertility enhancement. Plant products as fertility enhancing agents will be more acceptable for economic reasons and side effects that are less than chemical agents.

Testosterone belongs to a class of male hormones called Androgens, but women also have testosterone. The ovaries produce both testosterone and estrogen. Relatively small quantities of testosterone are released into the bloodstream by the ovaries and adrenal glands. Testosterone is involved in the growth, maintenance, and repair of reproductive tissues. It also influences other body tissues and bone mass [24].

Both male and female libido is in large part driven by testosterone. In the man, hypotestosteronism causes impotence and a lack of sex drive, while in the female, the production and the local release of testosterone by the ovaries also profoundly influences female libido. Neither ovarian follicle growth and development nor the production of estrogen could occur without the availability of the body’s own testosterone. The hormone is produced by the connective tissue (stroma) surrounding follicles from which it is delivered in a “bucket brigade” fashion to cells that line the inside of the follicle (granulosa cells). There, enzymatic digestion triggered by FSH (follicle stimulating hormone) converts testosterone to estrogen (mainly estradiol). This causes granulosa cells to proliferate, follicles to grow in size, and eggs housed in such follicles to undergo development and differentiation. At the same time, blood estrogen levels rise progressively. Thus, without access to ovarian testosterone, human reproduction would come to a halt. However, it is also true that too much testosterone delivered to follicles (as commonly occurs in older women who have diminished ovarian reserve and women with polycystic ovarian syndrome or PCOS), can lead to exhaustion of granulosa cells, compromised egg development and poor egg and embryo quality. It is all about a delicate balance that involves regulation of ovarian testosterone production. Since this is regulated by LH (luteinizing hormone), it follows that when it comes to ovarian stimulation with fertility drugs, it is important to properly control (down regulate) the amount of LH administered and or produced immediately prior to and during stimulation. Between
primary follicle and small pre-antral follicle stages, granulosa cells (cells that surround the egg) carry androgen receptors. At those stages, androgens work in synergy with follicle stimulating hormone, and are absolutely essential to normal development of follicles/eggs, egg number and egg quality. In men, androgens stimulate growth of prostatic cancer and a reduction in androgen actions is used for palliative treatment. Therefore hydroethanolic extract of *S. monostachyus* might find use in this regard [25].

Albumin helps move many small molecules through the blood, including bilirubin, calcium, progesterone, and medications. It plays an important role in keeping the fluid from the blood from leaking out into the tissues. Albumin functions primarily as a carrier protein for steroids, fatty acids, and thyroid hormones in the blood and plays a major role in stabilizing extracellular fluid volume by contributing to oncotic pressure (known also as colloid osmotic pressure) of plasma. The increase in synthesis of steroid hormones in the presence of increased albumin is corroborated by the result of this experiment as all hormones, except testosterone was significantly increased. Albumin plays an important role in maintaining homeostasis within the body and depends on the cell membrane and the transport mechanism, including diffusion, osmosis, filtration, and active transport. The dissolved proteins, which are the only substances that do not penetrate the pores of the capillary membrane, are responsible for the oncotic pressure of the capillary membrane. Approximately 75% of the total colloid osmotic pressure is related to albumin. Lipoprotein, an independent risk factor for atherosclerotic cardiovascular disease in the general population, is known to be elevated in patients hypoalbuminemia such as nephrotic syndrome and end-stage renal disease. Hence, the extract may find use in the prevention of such conditions [26].

The total protein test measures the total amount of two classes of proteins found in the fluid portion of your blood. These are albumin and globulin. A total serum protein test measures the total amount of protein in the blood. It also measures the amounts of two major groups of proteins in the blood: albumin and globulin. Cholesterol is required to build and maintain membranes; it modulates membrane fluidity over the range of physiological temperatures. The hydroxyl group on cholesterol interacts with the polar head groups of the membrane phospholipids and sphingolipids, while the bulky steroid and the hydrocarbon chain are embedded in the membrane, alongside the nonpolar fatty-acid chain of the other lipids. Through the interaction with the phospholipid fatty-acid chains, cholesterol increases membrane packing, which reduces membrane fluidity. The increase in cholesterol seen in Group B indicates that the extract can help in enhancing membrane integrity.

A decrease in serum cholesterol, such as that seen with the extract at 75 mg/kg and 225 mg/kg is beneficial people over 40 years, people with certain disease conditions such as atherosclerosis and other cardiovascular disorders, diabetes mellitus, dyslipidemia and so on.

Triglycerides are very important to human health as they are the main form of fat in the body and determine the health of the heart. Too high level of triglycerides in the body can predispose to atherosclerosis and other lipid related conditions. Hence, the extract which brought about a decrease in two of the three doses used can be considered for reducing a level of triglycerides that is too high [27].

### 5. Conclusion

It can be deduced in this study that the hydroethanolic extract has a prolactin reducing activity greater than bromocriptine which is clinically used in humans. The results further showed a corresponding statistical significant increase in reproductive hormones with no statistical significant changes on biochemical parameters.

### Reference


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of Albumin for Motility Stimulation.” *Fertility and Sterility* 59 (6):1266-75.