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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *COSTUS AFER* AQUEOUS LEAF EXTRACT AND ITS EFFECT ON REPRODUCTIVE FUNCTIONS PARAMETERS IN ADULT MALE WISTER RATS

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ABSTRACT

Nanotechnology is a rapidly growing area of scientific interest due to its wide applications in catalysis, solar energy, waste management, and sensing technology. Nanomaterials are efficiently used in medicine for drug delivery, diagnosis, treatment of cardiovascular diseases, wound healing, and development of antimicrobial agents. The present study aimed to evaluate the effect of *Costus afer*-AgNPs extract on Testosterone, luteinizing hormone, and Follicle stimulating hormone in male rats. In this experimental study, 18 adult male rats were used. The rats were randomly divided into six groups. The first group (control) adequately consumed compressed food and water without any restrictions during the experiment. The second, third, fourth, fifth, and sixth experimental groups respectively consumed 200mg, 400mg, 600mg, 800mg, and 1000mg *Costus afer*-AgNPs extract per kilogram body weight in a daily manner. Blood samples were taken from all groups after four weeks through anesthesia. The serum was isolated. Serum concentrations of testosterone, LH, and FSH were measured. The collected data were analyzed using SPSS, ANOVA, and LSD tests. Serum levels of testosterone, LH, and FSH significantly increased

in the experimental groups receiving 200mg, 400mg, 600mg, 800mg, and 1000mg *Costus afer*-AgNPs extract per kilogram body weight compared to the control group ($P < 0.05$). *Costus afer*-AgNPs maintained testosterone, luteinizing hormone, and follicle-stimulating hormone levels and could increase them.

KEYWORDS: Infertility, Medicinal plants, Nanotechnology, Green synthesis.

INTRODUCTION

Researchers in the past years have turned to biological systems for nanoparticle synthesis (Tsibakhashvil *et al*., 2010). Synthesis of nanoparticles by biological methods, using microorganisms, enzymes, and plant or plant extract, has been suggested as a possible eco-friendly alternative to chemical and physical methods (Nair and Pradeep, 2002; Schultz *et al*., 2000). The biosynthesis of nanoparticles by plants surpasses other biological methods by reducing the complicated process of maintaining cell culture. Plant materials contain various phytoconstituents which reduce the silver ions into silver nanoparticles (Caro et al., 2010). The formation, morphology, and topography of plant-based nanoparticles are regulated by factors such as temperature, reaction incubation period (Sun and Xia, 2015), pH, plant extract concentration, and AgNO3 concentration (Ibrahim, 2015). Quick synthesis of green silver nanoparticles can be achieved under sunlight exposure without any use of instruments (Kumar *et al*., 2016). In previous reports, plant extracts of Xanthium strumarium and Erigeron bonariensis (Kumar *et al*., 2016) were used for the synthesis of silver nanoparticles under sunlight exposure. Silver nanoparticles synthesized by using medicinal plant extracts have been utilized for various pharmaceutical applications (Thota & Crans, 2016).

Male infertility is defined as the inability of a male to impregnate a female, despite trying to, for at least one year. In couples struggling to conceive, one out of three times the root cause of infertility lies with the man. There can be multiple reasons for male infertility, which may include physical problems with the testes, blockage in the sperm-carrying ducts, genetic disorders, hormonal imbalance, and lifestyle-related factors.

There are a lot of methods for the synthesis of silver nanoparticles such as physical, chemical, and biological methods. The physical and chemical approaches for the synthesis of silver nanoparticles have some disadvantages (Ag-NPs) such as the long duration for preparation, not being economical, the need to use high temperature, pressure, and energy, and not being environmentally friendly. Nanobiotechnology and its derived products are

unique not only in their treatment methodology but also due to their uniqueness in particle size, physical, chemical, and biochemical properties, and the broad range of applications as well. This current emerging field of nanobiotechnology is at the primary stage of development due to a lack of implementation of innovative techniques on a large industrial scale and yet has to be improved with modern technologies. In Addition, infertility affects approximately 10–15% of couples, even up to 30% in some regions of the world (Inhorn & Patrizio, 2015). Although male infertility contributes to more than half of all cases of global childlessness, infertility remains a woman's social burden, as the scientific literature and other media have neglected the male component of reproduction other than its sexual nature for a long time (Cassatella *et al*., 2013; Petok, 2015). These very problems have led to the present study.

Costus afer has reportedly been known for differs therapeutic actions traditionally by researchers. *C. afer* is a useful medicinal plant that is highly valued for its ant-idiabetic, anti-inflammatory and anti-anthritic properties in South-East and South-West Nigeria (Omokhua, 2011). In Ogba community of Rivers State, the leaf and stem of *C. afer* when cut and crushed into smaller bits, boiled together with the leaf and bark of Alchornea cordiflora is used for the treatment of hunch bark and malaria. Among the Ikwerre ethnic group in Rivers State, it is applied in various ways. The leaves are reputed to be an effective remedy for fever and malaria when boiled with leaves of carica papaya (pawpaw), citrus species (orange) and bark of *Mangifera indica* (mango) (Omokhua, 2011). The stem and juice has traditional use for the treatment of cough, measles and malaria in Aluu community of Rivers State. The juice of *C. afer* is extracted and used as an instillation for eye inflammation and defects in Ogoni land, Rivers State. The young and tender leaves when chewed is believed to give strength to the weak and dehydrating patient. An infusion of the inflorescence is taken to treat stomach complaints. A stem decoction (the mashed or chewed stem or the pounded fruit) mixed with sugarcane juices are taken to treat cough, respiratory problems and sore throat (Omokhua, 2011). Screening active compounds from plants has led to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases including cancer and alzheimers disease. The biosynthesis of nanoparticles by plants surpasses other biological methods by reducing the complicated process of maintaining cell culture. Plant materials contain various phytoconstituents which reduce the silver ions into silver nanoparticles (Caro *et al*., 2010). Hence, the aim of the study was to carry out the green synthesis of silver nanoparticles using *Costus afer* leaf extract and evaluate its effect on some reproductive parameters in male wister rats.

MATERIALS AND METHODS

Plant Materials

The leaves of *Costus afer* were collected from a farm in Umuaduru in Osisioma L.G.A of Abia State Nigeria. The plant sample was identified by a Botanist, Dr. Duru, C.N. of Environmental Biology Federal Polytechnic Nekede.

Animals

Adult male rats were used for this study. These animals were purchased from a local breeder in Ihiagwa Owerri-West L.G.A of Imo State. The animals were kept in well-aerated stainless steel wire cages in the animal house of the Department of Biochemistry. The rats were given standard feed for at least two weeks after purchase to acclimatize them to the laboratory environment before use.

Chemicals and Reagents

Chemicals

All chemicals to be used in this study was of good and analytical grade.

Methods

Preparation of plant material and Extraction

Fresh and healthy leaves were collected locally and rinsed thoroughly first with tap water followed by disilled water to remove all the dust and unwanted visible particles, cut into small pieces and dried at room temperature. About 10 g of these finely incised leaves of each plant type were weighed separately and transferred into 250 mL beakers containing 100 mL distilled water and boiled for about 20 min. The extracts were then filtered thrice through Whatman No. 1 filter paper to remove particulate matter and to get clear solutions which were then refrigerated (4°C) in 250 mL Erlenmeyer flasks for further experiments. In each and every steps of the experiment, sterility conditions were maintained for the effectiveness and accuracy in results without contamination.

Synthesis of Silver Nanoparticles (Ag-np)

The green synthesis of Ag-np was prepared following the method reported in the literature (Khan *et al*., 2018). Preparation was done by reacting 10 mL of the *Costus afer* leaf extract with 90 mL AgNO3 solution (1 mM) and was agitated on the air bath magnetic stirrer for 15 minutes at room temperature. A colour change was observed from colourless to pink. The mixture was centrifuged and dried in the oven at temperature between 50°C – 60°C overnight

Experimental design

The experimental animals were randomized into 3 groups of 5 rats each and treated as follows;

Group 1: The rats in this group served as control and was given normal diet and distilled water.

Group 2: Synthesized nanoparticle of *Costus afer* mediated AgNps was administered orally at 200 mg/kg (Low Dose) to the rats in this group.

Group 3: Synthesized nanoparticle of *Costus afer* mediated AgNps was administered orally at 400 mg/kg (low Dose) to the rats in this group respectively via oral gavage daily for 28 days.

Group 4: Synthesized nanoparticle of *Costus afer* mediated AgNps was administered orally at 600 mg/kg (High Dose) to the rats in this group respectively via oral gavage daily for 28 days.

Group 5: Synthesized nanoparticle of *Costus afer* mediated AgNps was administered orally at 800 mg/kg (High Dose) to the rats in this group respectively via oral gavage daily for 28 days.

Group 6: Synthesized nanoparticle of *Costus afer* mediated AgNps was administered orally at 1000 mg/kg (High Dose) to the rats in this group respectively via oral gavage daily for 28 days.

Body weight and organ weight measurements

The body weights of the rats were taken weekly while their organ weights were taken at the end of the experiment (after sacrifice) using a Top loader weighing balance.

Sacrifice of animals

At the end of 28 days, a transverse incision was made through the ventral wall of the abdomen of each rat under slight chloroform anaesthesia. Blood samples was also obtained from the descending abdominal aorta and homogenized in a plain bottle for hormonal assay estimation.

Haematological Assay

All the haematological indices were assayed by the method outlined by Dacie and Lewis (2001).

Determination Of Hormone Profile of The Experimental Rats

Sera obtained from the collected blood will be assayed for their hormone according to the method used by Hossein et al. (2016). Testosterone, follicle stimulating hormone and luteinizing hormone was assayed using enzyme immune assay kits according to the

procedures outlined by the commercial kit producer, SyndroBiosearch Inc., California, USA

RESULTS

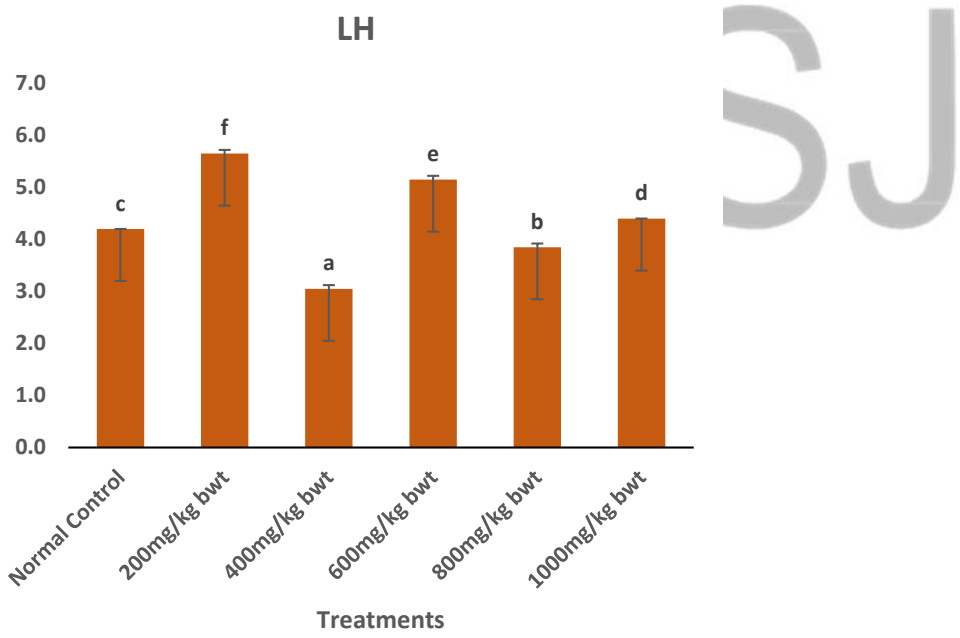

Figure 1: Graph showing the effect of *Costus afers*-AgNps on the Luteinizing hormone level of experimental rats. Results are expressed in bar chart with the different letters as data labels across bars are considered significant ($p < 0.05$) while bars with the same letters as data labels across bars are considered non-significant ($p > 0.05$). Results in figure 1 reveals that the AgNps mediated with *Costus afer* extract significantly ($p < 0.05$) raised the

LH levels when compared to control especially at doses of 200mg, 800mg and 1000mg/kg b.wt.

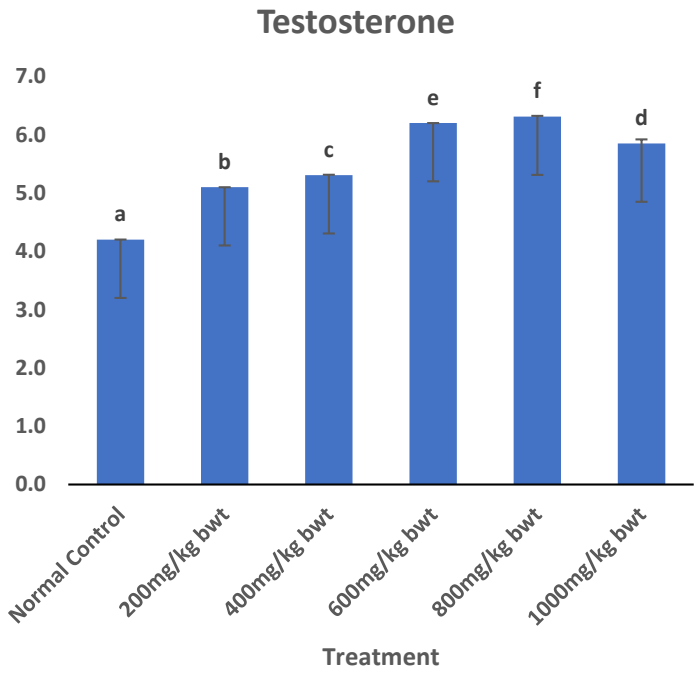

Figure 2: Graph showing the effect of *Costus afers*-AgNps on the Testesterone level of experimental rats. Results are expressed in bar chart with the different letters as data labels across bars are considered significant ($p < 0.05$) while bars with the same letters as data labels across bars are considered non-significant ($p > 0.05$). Results in figure 2 reveals that the AgNps mediated with *Costus afer* extract significantly ($p < 0.05$) raised the testesterone levels of experimental rats when compared to control especially at doses of 200mg, 400mg, 600mg, 800mg and 1000mg/kg b.wt.

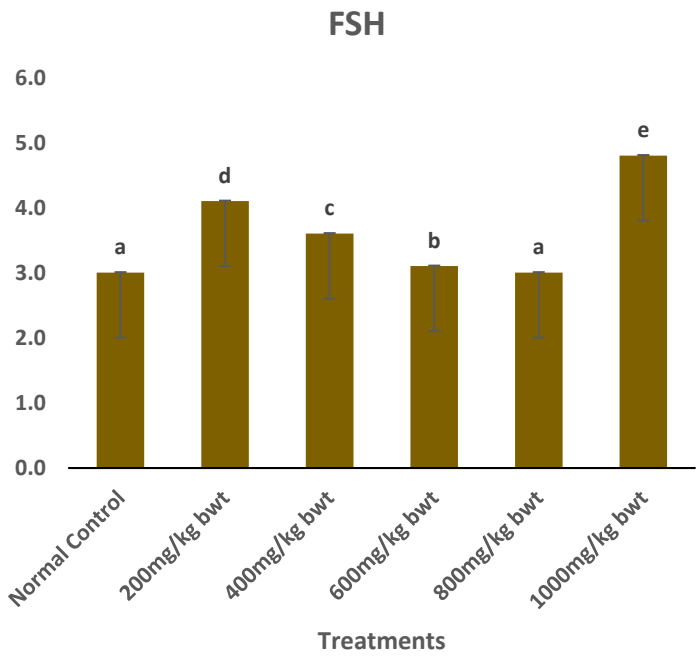

Figure 3: Graph showing the effect of *Costus afers*-AgNps on the Follicle stimulating hormone level of experimental rats. Results are expressed in bar chart with the different letters as data labels across bars are considered significant ($p < 0.05$) while bars with the same letters as data labels across bars are considered non-significant ($p > 0.05$). Results in figure 3 reveals that the AgNps mediated with *Costus afer* extract significantly ($P<0.05$) raised the FSH levels at doses of 200mg, 400mg, 600mg, and 1000mg/kg b.wt. However, FSH level of control group and test group treated with 800mg/kg of extract were non-significantly different ($p>0.05$) from each other.

DISCUSSION

Medicinal plants and other ethnobotanical can stimulate reproductive hormones due to the activity of the phytonutrients in the herbal therapies or by either binding to hormone receptors, which result in conformational changes that will enhance the physiological function of the hormones, or bind to enzymes that are involved in the synthesis of such reproductive hormones.

Many cases of male infertility remain idiopathic, revealing the current inability to define the underlying causes. In these cases, a rational pharmacological treatment does not exist, and clinicians attempt to stimulate spermatogenesis with various and not univocal results. Estrogen receptor modulators (SERM), such as tamoxifen and clomiphene citrate, are used off-label in some countries for treating male infertility (Patel *et al.,* 2016). Many drugs consumed have been associated with some side effects that could affect fertility in males. In this traditional system of medicine, plant preparations in the forms of decoctions, concoctions, macerations, or infusions are used to treat a wide range of diseases. Some of these plants are used in connection with human reproductive health problems, which are an important public health and social problem the world over (Diame, 2010). In developing countries, particularly in Sub–Saharan Africa, the latter afflictions pose a major burden (Tsobou *et al*., 2016). Kamatenesi-Mugisha and Oryem-Origa (2007) have argued that reproductive healthcare is the second most prevalent healthcare problem in Africa. The dominant use of medicinal plant decoctions for various ailments associated with the reproductive health system might be related to their proven effectiveness over many years of trial and indigenous knowledge accumulated on the efficacy of such preparations. On the other hand, the frequency of this method of preparation by a majority of respondents may be due to the fact that boiling the ingredients will kill some unwanted microbes that are present in the plant material used (Tsobou *et al*., 2016). Also, heat facilitates the extraction of active compounds from the plant part that is an ingredient in the remedy. Decoction also preserves the prepared medicine longer (Tsobou *et al*., 2016).

Luteinizing hormone (LH) is a glycoprotein hormone that is co-secreted along with follicle-stimulating hormone by the gonadotrophin cells in the adenohypophysis (anterior pituitary). Luteinizing hormone is a part of a neurological pathway comprised of the hypothalamus, the pituitary gland, and gonads. In this pathway, LH release is stimulated by gonadotropin-releasing hormone (GnRH) and inhibited by estrogen in females and testosterone in males. LH has various functions, which differ between women and men (Illahi and Illahi, 2022). LH stimulates the production of testosterone from Leydig cells in males. Decreased secretion of LH can result in the breakdown of gonadal function (hypogonadism). In males, this disorder is usually manifested as failure in the development of normal numbers of sperm. In females, amenorrhoea is commonly observed. FSH and LH are collectively responsible for the development and maintenance of secondary sexual character in males (Dasofunjo *et al*., 2020). From this work, an increase in serum LH in all

experimental groups suggests that the extract aid gametogenesis, via the hypothalamic axis (Dasofunjo *et al*., 2020). FSH stimulates testosterone spermatozoa development and promotes seminiferous tubule formation. FSH is responsible for the development, growth, pubertal maturation, and reproductive processes of the human body (Dasofunjo *et al*., 2020). Excess secretion of FSH is responsible for early puberty, whereas deficiency causes infertility and underdevelopment of gonads. In this present research work, the observed increase in LH levels which was observed at doses of 200mg, 800mg and 1000mg/kg and were significantly different from the control ($p<0.05$) group which suggests that the extract possesses a sex-enhancing potential due to the presence of phytoconstituent such as flavonoids and alkaloids or saponin that may likely be present in the mediated synthesized extract with *Costus afer*. From this study, the observed consistent level of serum FSH between the control and various test groups indicates that the extract significantly ($p<0.05$) improved secondary sexual characteristics, and sexual health with libido.

Testosterone is a male hormone with a significant impact on spermatogenesis (Amanatkar *et al*., 2014). Leydig cells of the testicles secrete testosterone, the adrenals, and ovaries, and is the most important androgen secreted into the blood (Mansoureh *et al*., 2016; Gauthaan *et al*., 2002). Testosterone, deficiency is presented with delayed puberty or regression of previously established male characteristics that depend on testosterone, such as hair distribution, potency, and libido. An elevated level of testosterone has been associated with a moderate but significant increase in sexual desire and penile function (Dasofunjo *et al*., 2020). Clinical data on testosterone show that a slightly increased testosterone level in adult males results in enhanced sexual desire and arousability (Dasofunjo *et al*., 2020). The level of testosterone has been reported to be related to LH and FSH such that an increase in the levels of gonadotropins results in a corresponding increase in testosterone (Dasofunjo *et al*., 2020). However, the findings of the study suggest that *Costus afer* leaf mediated with synthesized silver particles could be used for reproductive function or related problems as regards infertility.

CONCLUSION

The compounds contained in extracts of *Costus afer* leaf mediated with synthesized silver particles effectively maintained the activity of the male reproductive tract with increase in the secretion of testosterone, Luteinizing hormone and follicle stimulating hormone. Conclusively, the AgNps mediated with *Costus afer* could enhance sexual health and libido in males. It could be possibly be used in the management of erectile-related dysfunction.

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