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Effect of methanolic extract of *Uvaria chamae* and *Cassythia filiformis* on reproductive hormones of female albino rats

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ABSTRACT

The high cost of treating infertility and its associated risk prompted the need to search for a plant with fertility potentials. The effect of sub-acute (28) administration of methanolic extracts of *Uvaria chamae* and *Cassythia filiformis* on reproductive hormones of female albino rat was investigated. A total of forty (40) rats were used for the experiment and divided into four groups: positive and negative control groups, with two other experimental groups. The control groups receive five animals each, whereas the other two experimental groups received 15 rats each. Fifteen female albino rats were used for each of the extract in this study. Each of these group of fifteen were further divided into three sub groups; A, B and C of five rats each. Each sub group were administered plant extracts orally by means of calibrated syringe at a daily dose of 125 mg/kg for group (A), 250 mg/kg for group (B) and 500 mg/kg for group (C) for 28 days. The negative control group were given water ad libitum and those in the positive control group were given clomiphene citrate at 10 mg/kg. The enzyme-linked immunosorbent assay (ELISA) was used to determine the levels of the estrogen, follicle stimulating hormones (FSH) Luteinizing hormone (LH) and progesterone. Phytochemical screening of the extracts revealed the presence of flavonoid, reducing sugar, saponin, tannins, and terpenoid for *Cassythia filiformis*. The same are also present in *Uvaria chamae* in addition to steroid, with the exception of flavonoid. It was found that the extract of *C. filiformis* produced significant increase in estrogen and progesterone level ($p < 0.05$), but has no significant effect on the follicle stimulating hormone, and the luteinizing hormone whereas the *U. chamae* extract generally increased the progesterone, luteinizing hormone and follicle stimulating hormone significantly ($p < 0.05$). The increase in the level of reproductive hormones occasioned by the administration of these plant extracts indicates their potential in the enhancement of fertility.

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Keywords: Infertility, hormonal assay, phytochemicals, albino rats, conception, treatment.

INTRODUCTION

The use of plants as medicines goes back to early man. For a long time, plants have been the almost exclusive therapy accessible to humans. Today, most of the population in

developing countries still relies on traditional medical practitioners and medicinal plants for primary health care (Newman et al., 2003). The plant kingdom remain an essential source of new molecules with therapeutic potentials.

During the last decade, works on natural compounds have been particularly successful in the field of fertility drug research. According world health organization (WHO, 2013) and the international committee for monitoring assisted reproduction technology, infertility is a disorder of the reproductive system which is defined by the failure to achieve a clinical pregnancy for one year or more of regular unprotected sexual intercourse. Infertility in females may be affected by many factors which include irregular menstruation, malfunctions of uterus and hormonal imbalance. This health care problem can lead to serious psychological disorders, severe stressful and depressing life for parents.

The treatment of infertility has become a major issue ranging from manufacturing and prescribing fertility hormones and other drugs; to *in-vitro* fertilization operation. In 2010, the World Health Organization estimated that about 45.5 million couples worldwide were infertile and 1.9% of women aged 20–44 who wanted a child were unable to have their first live birth. In addition, 10.5% of women who had previously given birth were unable to have another baby after five years of trying (Mascarenhas et al., 2012; Hindin et al., 2016). The infertility treatment can range from medication therapy to induced ovulation to invasive manipulation of eggs and sperm outside of the body (Hrometz and Gates, 2009). Treatment of infertility is very expensive, and the majority of Nigerians being either below or at the average class cannot afford the treatment. Hence, these people now seek alternative in herbal medicine where the plants are available, accessible, and acceptable on their culture.

A huge number of plant families have been reported to be effective in the treatment of infertility in females and males as reported by Nidal and Abdel (2019). In Imo –State, a traditional recipe combination in a single decoction of the root of *Uvaria chamae*, the

aerial part of *Cassytha filiformis*, and three other plants are used as a traditional cure against STDs and infertility. In this work, we focused on the evaluation of the effect of sub-acute administration of the extracts (28 days) on the fertility hormones of female wistar albino rats

Cassytha filiformis is a leafless, climbing, twining, vine-like, parasitic seed-bearing plant in the plant family *Lauraceae*. It grows on a wide variety of coastal plants throughout Hawaii, the Pacific, and the Tropics worldwide. Indigenous to Hawaii, it is one of many higher flowering plant species that have, through evolutionary divergence, become parasitic on various organs of other higher plants. *Cassytha filiformis* clings to other, mainly woody plants for physical support, nutrition and water (Adonu et al., 2013) . *Cassytha filiformis* is believed to be effective in several traditional treatment of many diseases such as vermifuge and also in the suppression of lactation after still birth by several tribes in Nigeria (Ambi et al., 2017) . There are some other oral claims that the plant is boiled in water and administered for varying lengths of time to treat jaundice. Men were also reported to use it in the love magic while women used the extracts of the vine as a colouring agent or as a dye to provide a black color for fabric (Ambi et al., 2017). Several aporphinoid alkaloids have been isolated from the samples originating from Taiwan, Brazil, Australia and New Guinea but compositions were found to be quite variable among the different origins. Six aporphines from *C. filiformis* were shown to have *in vitro* cytotoxic properties out of which actinodaphnine, cassythine and dicentrine, also show *in vitro* anti trypanosomal properties against *Trypanosoma brucei brucei*, a very deadly disease that claims up to 70,000 lives per year (Quetin Leclercq, 2004; Oyibo et al., 2009). Aqueous and alcoholic extracts of *C. filiformis*

were tested for the diuretic activity in Wistar rats, and were found to exhibit significant diuretic activity by causing a marked increase in the Na⁺ and K⁺ excretion (Sharma et al., 2009).

Uvaria chamae is a plant that belongs to the family, Annonaceae. It is commonly called *Mmimi ohia* by the Igbo people, *Kaskaiḡi* by the Hausas, as well as *Oko-oja* by the Yorubas in Nigeria. It is a plant predominantly found in the tropical rain forest of West Africa (Okwu and Iroabuchi, 2009). It is an evergreen plant that grows about 3.6 to 4.5 m high, cultivated as well as wild (Okwu, 2004). The fruits are yellow when ripe and have a sweet pulp which is widely eaten. All parts of the plant are fragrant with wide spread medicinal use among traditional West African medical practitioner.

A new cytotoxic acetogenin chamuvarinin, containing a tetrahydropyran ring with an adjacent bis-tetrahydrofuran ring, which corresponds to a novel carbon skeleton in this series, was isolated from the root of *Uvaria chamae*, also acetogenins, squamocin, desacetylurvaricin and neoannonin (Djibril et al., 2004). *U. chamae* has been found to have anti-malaria activity (Okokon et al., 2013). The root bark is used to treat inflammation of the mucous membrane and also used in phytomedicine for the treatment of piles, menorhagia, epiostaxis, haematuria and haemalysis (Omajali et al., 2011). The juice from the roots, stems or leaves is commonly applied to wounds and sores (Omale et al., 2013). The anti-fungal and anti-bacterial inhibitory properties of the plant have been reported (Okwu and Iroabuchi, 2009). In folk medicine, extracts of the roots, barks and leaves of *U. chamae* are used to treat gastro enteritis, vomiting, diarrhea, dysentery, wounds, sore throats, inflamed gums and a number of other ailments (Okwu, 2004). Among the Igbos in South East Nigeria, *Uvaria chamae*, and *Cassytha filiformis* form part of

treatment of infertility in women. The objective of this research therefore is to carry out phytochemical screening and investigate the effect of the extracts of *U. chamae* and *C. filiformis* on the fertility hormones of female Wistar albino rats.

MATERIALS AND METHODS

Plant collection and preparation

The roots of *Uvaria chamae* and the aerial parts of *Cassytha filiformis* used for this study were collected from Urualla, Ideato-North Local Government Area of Imo State. The plants were identified and authenticated in plant biology and biotechnology Department of University of Benin, Edo State, Nigeria. The voucher specimens were deposited in the same herbarium.

The roots of plant *Uvaria chamae* and *C. filiformis* (aerial) were washed thoroughly with running tap water to remove dirt. After which root bark were detached and air-dried for five weeks. The dried root bark and *C. filiformis* were ground (separately) well in a mortar. The powdered material obtained was kept in an air tight container for subsequent use.

Extraction

The plant materials were extracted with methanol (absolute, analytical grade) using the Soxhlet apparatus. The extracts were further concentrated to dryness using rotary evaporator.

Phytochemical screening

The qualitative phytochemical analysis of methanolic extract of *U. chamae* and *C. filiformis* were carried out to identify the secondary metabolites following the method described by Association of Official Analytical Chemists (A.O.A.C) (2005) as described by Harowitz.

Experimental animal

A total of 40 female albino rats of wister strain weighing between 126 g and 198 g were used for this study. The animals were obtained from the animal house in the department of Pharmacology, Faculty of Pharmacy, University of Benin, Benin City.

Assay kit

The assay kits for estradiol were supplied by Abcam, London, UK, while kits for follicle stimulating hormone, luteinizing hormone and progesterone were supplied by Abcam, Boston, USA. All other reagents used were of analytical grade.

Animal grouping and extract administration

The rats were bred for two weeks in animal house to allow them acclimatize to their environment. The rats were fed with rat pellets and with water *ad libitum* throughout the experiment period. The rats were then picked at random, weighed, painted with an indelible ink at different parts of the body and divided into four groups: positive control, negative control and two other experimental groups. The control groups receive five animals each, whereas the other two experimental groups receive 15 rats each. Fifteen female albino rats were used for each of the extract in this study. Each of these group of fifteen were further divided into three sub-groups; A, B and C of five rats each. The 5 rats in each sub group were administered plant extracts orally by means of calibrated syringe at a daily dose of 125 mg/kg for group A, 250 mg/kg for group B and 500 mg/kg for group C for 28 days. The rats in the negative control group were given water *ad libitum* and those in the positive control group were given clomiphene citrate at 10 mg/kg. This study was carried out after approval has been obtained from the Ethical

Committee on the Care and Use of Experimental Animals for Research, University of Benin.

Preparation of serum

After administration of the methanolic extract for 28 days, the rats were fasted overnight. The following morning, they were sacrificed under chloroform anesthesia by being placed in a sealed cotton wool soaked chloroform inhalation jar. Blood was collected through abdominal aorta using 5 ml syringe into plain bottles for hormonal assay.

Hormonal assay

The procedure described in the hormonal assay kit was used. The appropriate serum reference and specimen (0.01 ml) was pipetted into prepared micro-plate wells. The working hormone enzyme conjugate (Estrogen, follicle stimulating hormone, Luteinizing hormone and progesterone) was added and incubated for one hour. The contents were removed from each well; washed the wells three times with dilute wash solution. Further preparations of the micro-plates were done using hormone biotin enzymes reagent which were incubated for fifteen minutes before stopping the reaction with stop solution. The absorbance in each well was read at 450 nm wavelength in a micro-plate reader.

Statistical analysis

Data were analyzed by Graph Pad Instat version 2.0.5 software (UK). Mean values from controls (positive and negative) were compared with mean values from different groups by analysis of variance (One way ANOVA) using Dunnett Multiple Comparison test. Results are expressed as mean \pm standard error of mean (SEM) and are regarded as significant at $P < 0.05$.

RESULTS

The results of qualitative phytochemical screening of *Cassythia filiformis* (aerial), and root-bark of *Uvaria chamae* extracts are as shown in Table 1. Phytochemical compounds that were found to be present in *Cassythia filiformis* are flavonoid, reducing sugar, saponin, tannin and terpenoid. While cardiac glycoside, starch polysaccharide, steroid, phlobatannin and alkaloids were not detected as shown in Table 1. *Uvaria chamae* was also found to contain reducing sugar, saponin, steroid, tannin and terpenoid, while cardiac glycoside, flavonoid, starch polysaccharide, phlobatannin and alkaloid were not detected (Table 1).

The results of hormonal assay of blood serum of albino rats fed with *Cassythia filiformis* (aerial), and root-bark of *Uvaria chamae* extracts are as shown in Figures 1 and 2. There is no significant increase in the level of estrogen across all the groups in albino rats

fed with *Uvaria chamae* extract. The comparison of all the groups with the control indicates that there is no significant increase as $P > 0.05$ in all, as shown in Figure 1. The *Uvaria chamae* extract increased the level of luteinizing hormone (LH) and progesterone in the serum of albino rats that received 250 mg/kg and 500 mg/kg of extracts as the P value for these categories is less than 0.05 (Figure 1). The level of estrogen in all groups of rats that received *Cassythia filiformis* extract increased significantly but the effect is higher in group B and C (250 mg/kg and 500 mg/kg). The *Cassythia filiformis* extract does not affect the level of the follicle stimulating hormone and luteinizing hormones of all groups of rats that received them (Figure 2). The $P > 0.05$, therefore the effect is considered as insignificant. The progesterone level is considered significant for all groups of animals.

Table 1: Phytochemical screening of *Cassythia filiformis* (aerial part) and *Uvaria chamae* root extracts.

PHYTOCHEMICALS	<i>Cassythia filiformis</i>	<i>Uvaria chamae</i>
Alkaloid	-	-
Cardiac glycoside	-	-
Flavonoids	++	-
Phlobatannins	-	-
Reducing sugar	+++	+++
Saponin	++	+
Starch Polysaccharide	-	-
Steroids	-	+++
Tannins	+	++
Terpenoid	++	++

Keys: +++ = Highly present; ++ = Much present, + = Present, - = Absent

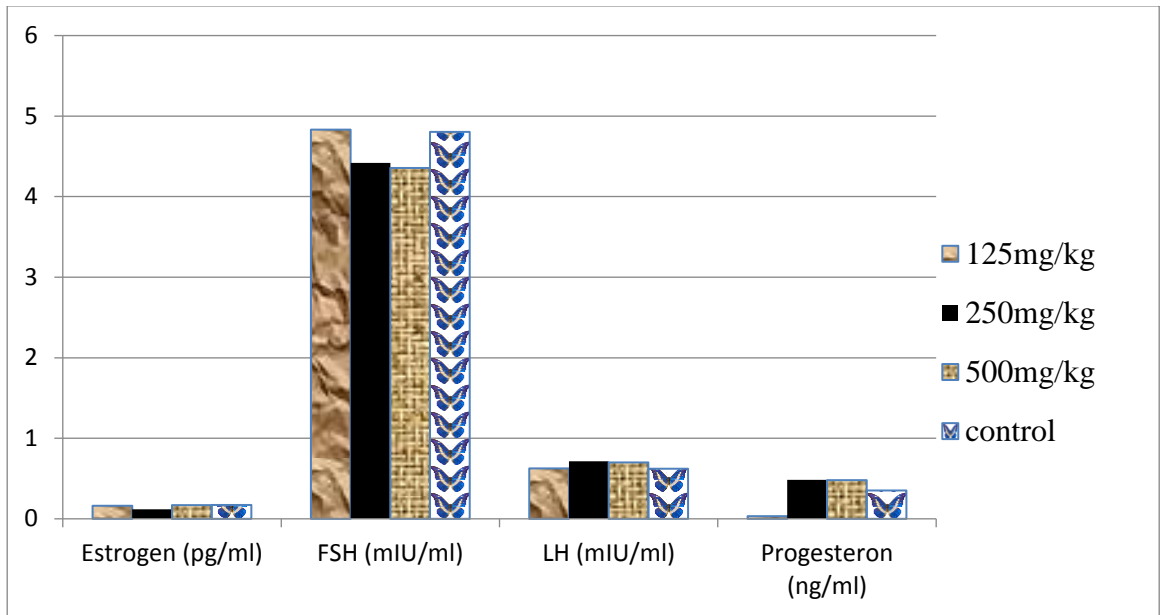


Figure 1: Effect of methanol extract of *Uvaria chamae* on fertility hormones of albino rats.

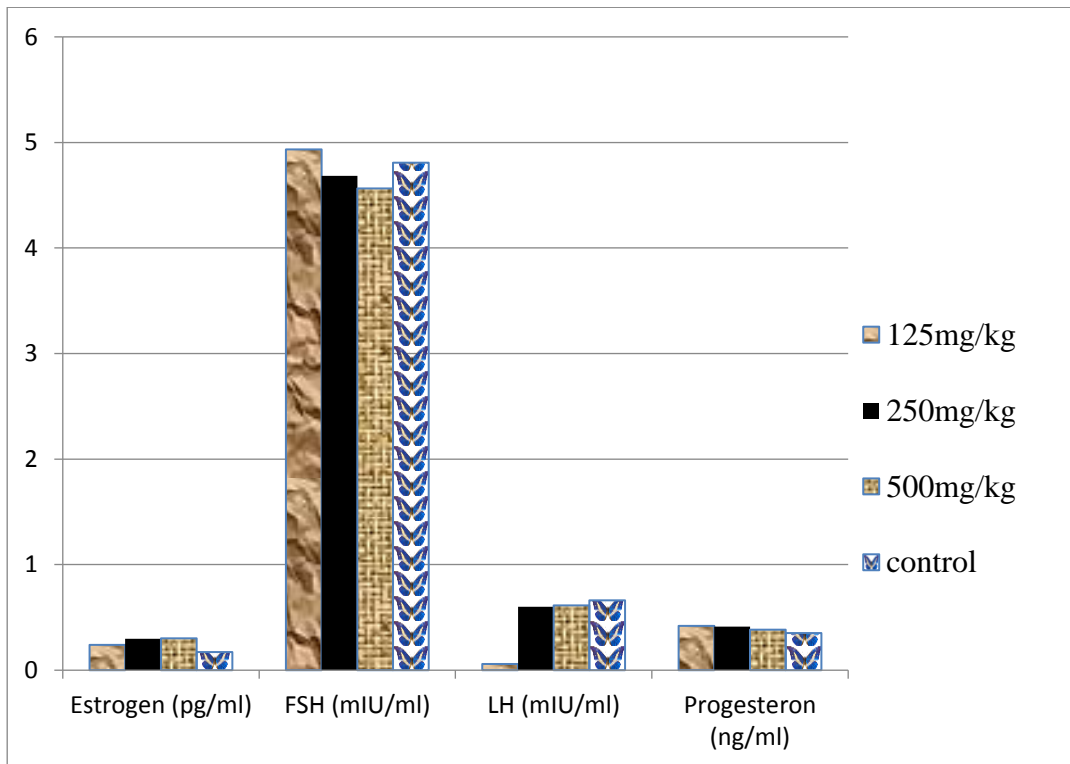


Figure 2: Effect of methanol extract of *Cassytha filiformis* on fertility hormones of albino rats.

DISCUSSION

The increase in some of the female reproductive hormones in the treatment groups is an indication that these plants may likely enhance fertility, especially those that increased estrogen and gonadotropins. In humans, these four hormones control the menstrual cycle by initiating and ending a series of stepwise phase (Van-Itel, 2016). Estrogen are a group of biologically active steroid hormones. As signaling molecules, they bind to receptor molecules in cells to signal specific changes to occur within the body. Estrogen stimulates growth of the uterine lining, causing it to thicken during the pre-ovulatory phase of cycle. It is well established that estrogen is directly responsible for growth and development of reproductive organs (Van-Itel, 2016). In synergy with Follicle stimulating hormone (FSH) estrogen stimulates granulosa cell proliferation during follicular development (Levin and Hammes, 2011). FSH is also central in mammalian reproduction, essential for gonadal development and maturation at puberty as well as gamete production during the fertile phase of life. It stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulosa cells. The increase in the level of FSH by the extract (s) may enhance folliculogenesis and maturation of follicle in the pre-ovulatory phase (Mbemya et al., 2017). The luteinising hormone (LH) stimulates secretion of sex steroid from the gonads. In females, ovulation of mature follicle in the ovary is induced by large surge of LH during the pre-ovulatory period. Many studies have confirmed that the surge in LH at pre-oestrous stage are responsible for ovulation. The progesterone also helps to regulate menstrual cycle, prepare the body for conception and pregnancy and as well as stimulate sexual desire (Van-Itel, 2016). The increase in levels of estrogen and progesterone also causes the milk ducts in the breasts to dilate and become larger, resulting in swelling and possible breast soreness prior to the onset of menstruation.

Conclusion

The increase in some of the female rat reproductive hormones by these extracts are

indications of favorable effect on stimulating ovulation and balancing hormone. Consequently, the extract may enhance fertility and conception. This further justifies the use of these drugs in synergy, as a traditional remedy for curing infertility.

COMPETING INTERESTS

The authors declare they have no competing interests.

AUTHOURS' CONTRIBUTIONS

FEO designed the study and contributed to the critical revision of the manuscript. NJU conducted the study, extracted and analysed data, search the literature and drafted the manuscript, and is the corresponding author. OHU supervised/monitored the animal experiment.

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