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Methanol leaf extract of *Abelmoschus caillei* increased the serum levels of some hormones involved in follicule maturation in female Wister rats

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Abstract

There are ethnobotanical claims that the plant *Abelmoschus caillei* A.Chev. (Malvaceae) is beneficial for child bearing and in child labour. In fact, the inhabitants of Igboora town, South-West Nigeria claim the consumption of the leaf plays a role in the high incidences of multiple births experienced in the area. The study evaluated the effects of the methanol leaf extract of *Abelmoschus caillei* (MLAC) on some reproductive hormones involved in the maturation of graffian follicles of female wistar rats. The young leaves of authenticated *Abelmoschus caillei* plant were collected, air-dried, powdered and extracted with methanol. The extract (25, 50, 100, 200 and 400 mg/kg body weight, p.o.) was evaluated for its effects on the serum concentrations of oestradiol, lutenising hormone and follicle stimulating hormone using Enzyme Linked Immunosorbent Assay (ELISA) method. Clomiphene citrate was administered as standard drug. The analysis of the result showed that serum concentrations of the three hormones were significantly ($P < 0.05$) higher at the administered doses compared with the negative control (distilled water) except at 25 mg/kg. The study concluded that *Abelmoschus caillei* A.Chev. (Malvaceae) significantly increased the serum conceentrations of LH, FSH and Oestradiol in female wistar rats and probably plays a role in the high incidences of multiple births observed in Igboora town in Nigeria.

Keywords: *Abelmoschus caillei*; Reproductive Hormone; Oestradiol; Clomiphene; Wistar rats

1. Introduction

Humans are affected by a wide range of diseases and ailments, some have been around for a long time and others are relatively recent. Humans have relied on natural medicines to solve their health problems since the dawn of time. Paracelsus, a reformer of Western medicine who lived in the 15th century, thought that there was always a treatment for every sickness in nature [1].

Infertility is described as a woman's failure to conceive after a year of regular, unprotected sexual contact. Medical examinations may be started early in couples who currently have infertility risk factors or women over the age of 35. Depression, worry, sexual anxiety/difficulty, connection problems with partner, family, and friends, and an elevated sense of self-blame and guilt are all frequent side effects of infertility [2]. Age, oligomenorrhea/amenorrhea history, known or suspected uterine/tubal disease, endometrosis or decreased ovarian reserve, and a history of infertility are all factors that affect male and female fertility [3].

According to an ethnobotanical survey carried out by [4], the crushed fruit and mucilage from the leaf and other parts of the plant are used ethnomedicinally to enhance child bearing and child labour among the Bini, Igbo, Urhobo and Isoko people. Multiple births are common in Igboora, a town in South-West Nigeria [5]. The consumption of the plant

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Abelmoschus caillei leaf is said to play a part in this phenomena, however no scientific investigation has been done to verify this claim.

Abelmoschus caillei is a robust, annual to biennial, erect plant up to 4 m tall, typically heavily branched; woody at base, glabrous or with scattered, stiff hairs, often red-blotched; branches erect to bent downwards. However, *Abelmoschus caillei* (West African okra) is only found in West and Central Africa. Its range is limited to humid and pre-humid climes in Africa, between 12°N and 12°S, most usually between 5°N and 10°N, whereas the common okra (*Abelmoschus esculentus* L.) Moench is found in the tropics, subtropics, and temperate regions. It is well adapted to local environmental conditions and pests [6].

2. Material and methods

2.1. Plant collection and processing of plant materials

The plant, *Abelmoschus caillei*, was collected in Igboora a town in Oyo State, Nigeria. Mr. G. A. Ademoriyo of the Ife Herbarium, Department of Botany, Obafemi Awolowo University, Ile – Ife, identified and authenticated the plant, which was given the voucher number IFE-17637. The leaves were dried in the open air before being ground into powder. The powdered leaf (850 g) was macerated in 5 liters of methanol for 48 hours for cold extraction. Throughout the 48-hour period, a mechanical shaker was employed to continuously agitate the extraction medium. The resultant extract was filtered, and the filtrate was dried in vacuo in a rotary evaporator set to 40°C before being weighed.

2.2. Laboratory materials

2.2.1. Drug

Clomiphene Citrate (Clomid®, Doppel Farmaceutici S.r.l., Italy), was used as the standard/reference drug for this investigation.

2.2.2. Laboratory animals

The animals were kept in cages that were kept at ambient temperature and exposed to natural light. As bedding, wood shavings were utilized and changed every other day. They had unlimited access to Vital® Feeds and water. The animal care procedure was based on the eighth edition of the "Guide for the Care and Use of Laboratory Animals.

2.2.3. Experimental Procedures

Median Lethal Dose (LD_{50}) determination of *A. caillei* leaf extract [7] approach was used to determine the extract's median lethal dose (LD_{50}). There are two stages to the method's testing. In the first phase, doses of methanol leaf extract of A. caillei of 10 mg/kg, 100 mg/kg, and 1000 mg/kg were given to three different animal groups, each with three animals. Within 24 hours of treatment, the animals were monitored for symptoms of toxicity and mortality. In Phase 2, animals were given dosages of 1000 mg/kg (repeated), 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg, with one animal per group. The LD_{50} was calculated using the results obtained.

The positive control was Clomiphene citrate, and the dose of clomiphene citrate given to the animals was estimated using the formula:

Conversion Factor: Animal dose (mg/kg) = Human Equivalent Dose [8]

2.2.4. Determination of Rat Oestrous cycle stage

The rats' oestrous cycle stage was determined using the method given by [9]. By putting the tip of a plastic pipette with a few drops of normal saline (NaCl, 0.9 percent v/v) into the rat vagina, vaginal discharge was collected. The vaginal fluid was placed on glass slides that were labeled differently for each animal. To create a thin layer, the fluid was smeared on the slides. After allowing 5 minutes for the smears to dry, Leishmania stain was added and let to sit for 5 minutes to allow the cells to absorb the stain. Each animal was represented by a distinct glass slide. The slides were examined under a light microscope, and three types of cells were identified: round and nucleated epithelial cells, irregular cells without a nucleus (cornified cells), and small round cells (the leukocytes). The estrous cycle phases were determined using the typical cell kinds and proportions of the cells.

2.2.5. Administration of the Extract and the Control Agents

In this investigation, 35 female (non-pregnant) rats were used. The rats were divided into seven groups, each with five rodents. Each of the animal groups received a graduated dose of *Abelmoschus caillei* leaf extract (25, 50, 100, 200, and 400 mg/kg). The extract was started when the woman was in the proestrous stage. The positive control, Clomiphene citrate, was given at 4.4 mg/kg for seven cycles at the diestrus stage. Negative control group was given distilled water. The administrations lasted 28 days in a row.

The following is how the dose of Clomiphene citrate given to the animals was calculated:

Conversion Factor: Animal Dose (mg/kg) = Human Equivalent Dose

Rat conversion factor = Human Km= 37 /Animal Km 6

Human Equivalent Dose = 50 mg = 0.71 mg/kg (with 70 kg as the average human weight and 50 mg as the daily dose of Clomiphene citrate in humans). Animal dosage (mg/kg) for 70 kg = 0.71 x 6.17 = 4.4 mg/kg

2.2.6. Collection of blood samples

After the treatment period, the animals were tested twice daily for 28 days to determine their oestrous stage. Under diethylether anaesthesia, the animals were slaughtered humanely during the diestrus stage. Cardiac puncture was used to collect blood samples. The blood samples were placed in simple bottles (one for each animal) and left to coagulate. On a Gallenkamp Centrifuge machine, the clotted blood was centrifuged at 3000 revolutions per minute for 15 minutes. The serums were kept at -20°C until they were analyzed.

2.2.7. Analysis of Serum

The serums were tested for Luteinizing hormone, Follicle stimulating hormone, and Oestradiol using Accu-Bind® Enzyme - Linked Immunosorbent Assay (ELISA) kits, following the instructions in the kits' manuals.

2.2.8. Calculation of Results

From the standard curves of their respective standards, the concentrations of oestradiol, FSH, and LH in the serum specimen were determined.

2.3. Statistical Analysis

The mean and standard error of the mean (S.E.M.) were used to express the findings. Graph pad, prism 5 was used to analyze the results (version 5.01). A one-way analysis of variance was used to examine the difference between the treatment and control groups, followed by a Student – Newman – Keuls post-hoc test. Statistical significance was defined as a P value of less than 0.05.

3. Results

3.1. Median Lethal dose (LD_{50}) is > 5000 mg/kg

No death or any sign of toxicity was observed at the 10 mg/kg, 100 mg/kg, 1000 mg/kg, 1600 mg/kg, 2900 mg/kg and 5000 mg/kg doses administered to the rats.

3.2. Effects of MLAC on lutenising hormone, follicle stimulating hormone and oestradiol

3.2.1. Effect of Methanol leaf extract of A. caillei on the concentration of serum LH in female wistar rat

The doses (50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg) of the Methanol leaf extract of *A. caillei* administered produced significantly higher concentrations of serum LH when compared with distilled water except at 25 mg/kg dose. Higher doses (50 - 400 mg/kg) of the extract, all produced significantly increased LH serum levels at $P < 0.001$. When compared with the standard drug (CC), the 25 mg/kg dose caused an increase in serum LH levels at $P < 0.05$, while the other administered doses (50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg) increased serum LH level significantly at $P < 0.001$. The CC administered increased serum LH level significantly at $p<0.05$ when compared with the distilled water group. This result is shown in Figure 1.

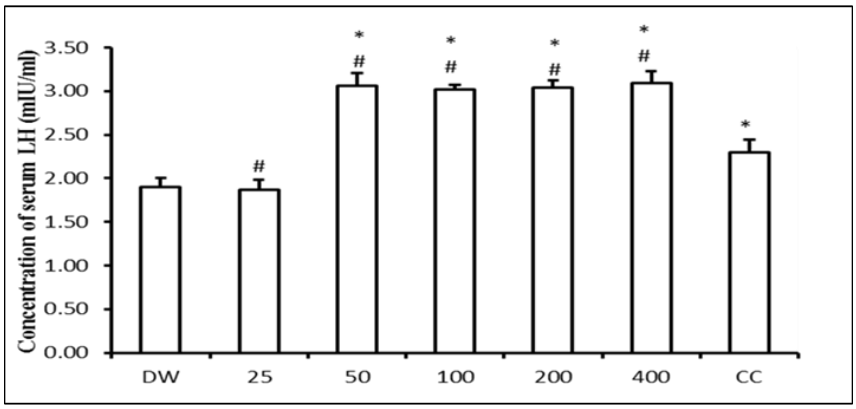

Figure 1 Effect of methanol leaf extract of A. caillei on the serum concentration of Lutenising Hormone in female wistar rats

Each bar represents the mean ± SEM (n = 5)
* P < 0.05 when compared with distilled water, # P < 0.05 when compared with CC. DW –Distilled water (p.o.), CC – Clomiphene Citrate (4.4 mg/kg, p.o)

3.2.2. Effect of Methanol leaf extract of A. caillei on the concentration of serum FSH in female wistar rat

The doses (50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg) of the Methanol leaf extract of *A. caillei* administered produced significantly (P<0.05) higher concentrations of serum FSH when compared with distilled water except at 25 mg/kg dose. The 50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg all produced significantly increased FSH serum levels at P < 0.05. When compared with the standard drug (CC), the administered doses (50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg) increased serum LH level at P<0.05. There was no significant difference in the effects produced by CC, DW. Methanol leaf extract of *Abelmoschus caillei* (25 mg/kg). This result is shown in Figure 2.

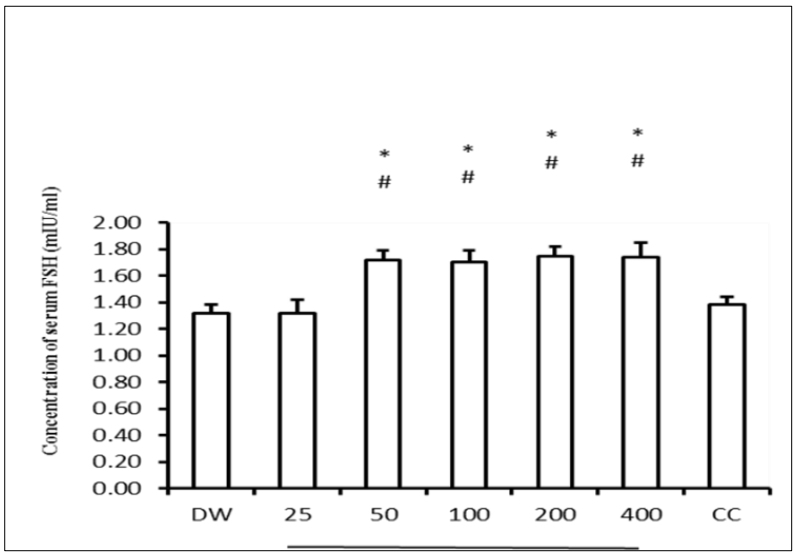

Figure 2 Effect of methanol leaf extract of A. caillei on the serum concentration Follicle Stimulating Hormone in female wistar rats

Each bar represents the mean ± SEM (n = 5)
* P < 0.05 when compared with distilled water, # P < 0.05 when compared with CC.
DW –Distilled water (p.o.), CC – Clomiphene Citrate (4.4 mg/kg, p.o.)

3.2.3. Effect of Methanol leaf extract of A. caillei on the concentration of Oestradiol in female wistar rat.

The doses (50 - 400 mg/kg) of the Methanol leaf extract of *A. caillei* administered produced significantly (P< 0.05) higher concentrations of serum oestradiol when compared with distilled water except at 25 mg/kg dose. For the 50 mg/kg and 200 mg/kg doses, the differences were significant at P < 0.001; while for 100 mg/kg, 400 mg/kg, doses P < 0.01. When compared with the standard drug (CC), with the exception of 25 mg/kg dose, the doses of the Methanol leaf extract of *A. caillei* administered produced significantly higher concentration of oestradiol at P < 0.001 for 50 mg/kg, 200 mg/kg and 400 mg/kg and P < 0.01 for 100 mg/kg. This result is shown in Figure 3.

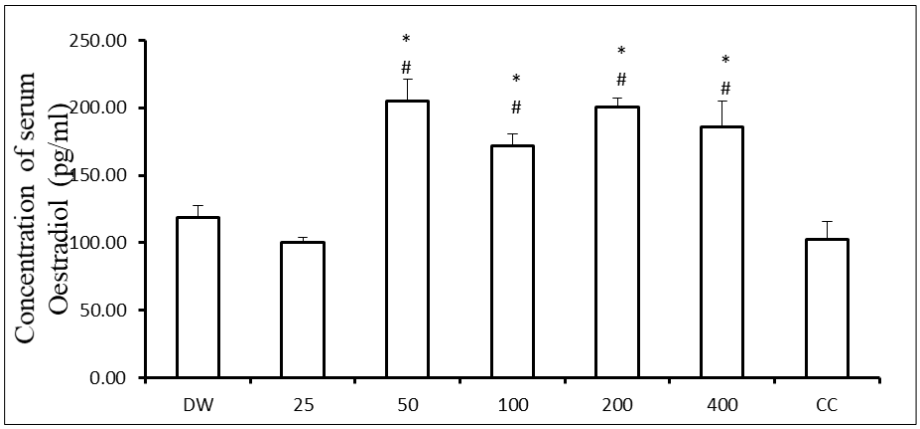

Figure 3 Effect of methanol leaf extract of A. caillei on the concentration of Oestradiol in female wistar rats

Each bar represents the mean ± SEM (n = 5)
* P < 0.05 when compared with distilled water, # P < 0.05 when compared with CC.
DW –Distilled water (p.o.), CC – Clomiphene Citrate (4.4 mg/kg, p.o.)

4. Discussion

LH has been found in studies to play a crucial function in the development of follicles in women. When LH levels fell below a certain level, follicles ceased developing, according to [10]. It works by stimulating alterations in gene expression in the theca cells, allowing for the creation of androgens (androstendione and testosterone) [11]. Androgens are also employed as substrates for oestrogen (estrone and oestradiol) synthesis [12]. Similarly, recombinant LH given during the second half of the follicular phase enhanced the number of preovulatory follicles in ovulatory women [13]. Ovulation is aided by the hormone LH. Following the commencement of the LH surge, a number of events occur, culminating in the follicular rupture [14]. The increased blood LH levels identified in this study have ramifications for ovulation (greater possibilities of repeated ovulation), follicular maturation, and aromatisation processes. The methanol leaf extract of *A. caille*i significantly increased the serum concentration of LH in female rats at four out of the five doses used in this study, as shown in figure1. As a result, *A. caillei's* methanol leaf extract may be useful in treating infertility caused by low LH serum concentrations in the body. The concentration of LH in *Abelmoschus caillei* administered dosages was significantly higher than in the CC group. Under the influence of Gonadotrophin Releasing Hormone, the pituitary secretes FSH, a gonadotrophin. FSH has been identified as the primary hormone that promotes follicle maturation, particularly at later stages of development. The higher serum FSH levels in the groups treated with the aforementioned doses of *A. caillei* methanol leaf extract may provide an enabling environment for the development and maturation of more follicles. The high rate of multiple births among the Igboora people of Ibarapa Central Local Government, Oyo State, Nigeria, could be explained or contributed to by this.

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The high rate of multiple births observed in the area has been attributed to the consumption of *A. caillei* (West-African Okra) leaf soup (ilasa). FSH aids in the conversion of testosterone to oestrogen, and it has been observed that follicles with a high concentration of oestradiol in their environment are more likely to be recruited for further development than those with a low concentration. When FSH secretion is enhanced, the aromatisation process is expected to be more efficient, resulting in a higher concentration of oestradiol generated. This could be extremely beneficial in cases of infertility caused by low gonadotrophin levels in the blood.

In infertility cases, any drug that can boost the blood levels of the involved hormones is likely to help. In female rats, the methanol leaf extract of *A. caillei* significantly increased the serum concentration of FSH at four of the five doses used in this study, as shown in figure 2 LH concentrations in *Abelmoschus caillei* dosages of 50 mg/kg to 400 mg/kg were substantially greater than in the CC group.

The presence of estrogen receptors α– and –β in the ovaries [15] suggests that oestradiol has a local impact. In rats, oestradiol works in concert with FSH to stimulate granulosa cell proliferation while also increasing FSH receptors and aromatase synthesis, avoiding atresia [16]. The β- subtype appears to play a more critical role in the ovary than the α-form in terms of oestrogen receptors. Estrogen receptor knockout female mice exhibit an increased number of atretic follicles with no progression from early to late antral stage, as well as decreased oestradiol production and a lower ovulation rate, according to a study [17] estrogen receptor-β knockout female mice have an increased number of atretic follicles with no progression from early to late antral stage and show decreased production of oestradiol and a reduced ovulation rate. Also in the same animals, in the absence of oestrogen receptors-β, preovulatory follicles are also able to aromatise androgens to oestrogens to a limited level in the absence of oestrogen receptors, and demonstrate an inadequate response to FSH in terms of differentiation and activation of LH receptors, as well as a lower rate of follicle rupture in the same animals [18]. The research provided support oestrogen's critical function in follicular maturation and ovulation. The higher oestradiol serum concentration found in this study is likely to improve follicle maturation and ovulation processes, resulting in enhanced fertility. In female rats, the methanol leaf extract of *A. caillei* increased the serum concentration of oestradiol at four of the five doses used in this study, as shown in figure 3.3. Furthermore, because of its modulation of serum oestradiol level, the constituents of the *A. caillei* leaf may be useful in alleviating the symptoms caused by reduced oestrogen production in postmenopausal and early onset menopausal women, as shown by the results of this experiment.

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The serum concentrations of FSH, LH, and oestradiol in male rats were significantly increased in a study using a methanol extract of the seeds of *Abelmoschus esculentus* (the same genus as the *A. caillei* utilized in this work) [20]. The *Abelmoschus esculentus* and *Abelmoschus caillei* utilized in this study are members of the same genus, which could explain the similar effects that had on the reproductive hormones of rats.

5. Conclusion

The methanol leaf extract of *A. caillei* leaf has low acute toxicity profile and this corroborates the consumption of the leaf by humans. In female wistar rats, the methanol leaf extract of *A. caillei* increased serum concentrations of LH, FSH, and oestradiol implying that it contains constituents that may promote superovulation and multiple gestation. This finding coorroborates its use ethnomedicinally for child bearing.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

The animal studies followed an approved procedure with the number: PHP14/15/H/0215 by the Postgraduate College on behalf of Obafemi Awolowo University, Ile-Ife and Research Committee.

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