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Improved Fertility Potential of Ethanolic Leaf Extracts of Petroselinum Crispum

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ABSTRACT

Background: Parsley (Petroselinum crispum) is a bright green biennial plant in temperate climates and it is an annual herb in tropical and subtropical areas. **Objectives:** This work was aimed at evaluating the effect of the ethanolic leaf extract of parsley (P. crispum) on the histology of the ovaries and the reproductive hormone (oestrogen). **Materials and Methods:** A total of fifteen (15) adult female Wistar rats were used, they were divided into three groups each comprising of five rats. The groupings were as follows: Group A (Normal Control), Group B (825mg/kg per body weight of parsley leaves ethanolic extract), Group C (1,650mg/kg per body weight of parsley leaves ethanolic extract). The extract was administered through orogastric tube daily for 28 days after which the animals were sacrificed using chloroform. The ovaries were excised and processed histologically using Hematoxylin and Eosin stains and blood was collected for oestrogen hormone assay. **Results:** There were no signs of toxicity on body weight and there were no significant changes in the cytoarchitecture of the ovary of low dose group. However, in the high dose group, administered 1,650mg/kg bw of the extract results showed a significant increase in the oestrogen level of the animals and fatty infiltration in the ovarian cytoplasm. **Conclusion:** Ethanolic leaf extracts of Petroselinum crispum has the potential of improving fertility through its action on estrogen hormone levels, at high doses.

Key words: Petroselinum crispum, Ovaries, Oestrogen

INTRODUCTION

Many aromatic plants and herbs exist worldwide, particularly originating from the Mediterranean area. Parsley *(Petroselinum crispum)* an aromatic plant, is a bright green biennial plant in temperate climates. It is an annual herb in tropical and subtropical areas[1] used as a green vegetable, for garnish, and for medicinal purposes.

Parsley has been used as carminative, gastro tonic, diuretic and antiseptic of the urinary tract. It has also been used in the treatment of amenorrhea, dysmenorrhea, gastrointestinal disorder, hypertension, cardiac disease, urinary disease, otitis media, as antidandruff, and an expectorant[1]. A mixture of seeds and root is used to promote lactation after childbirth and it also helps to contract the uterus[1]. Parsley helps treat convalescents and

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anaemia because it is also a mild laxative, diabetes and various dermal disease in traditional and folklore medicines[1].

P.*crispum* is rich in ascorbic acid, carotenoids, flavonoids, apiole, terpenoic compounds, coumarin, phenylpropanoids, phthalides, tocopherol, and furanocoumarins. The leaves hold high content of vitamins (A, C, and K), b-carotene, and also lutein, zeaxanthin, folate, choline, niacin, and pantothenic acid. Together with leaves, the roots are a good source of a range of minerals (Ca, K, Mg, B, P, Fe, Na, Zn, F, Mn, and Se). The major constituent of the P. crispum leaf oil is p-1,3,8-menthatriene,followed by b-phellandrene, myristicin, and b-myrcene, while the major root oil constituent is apiole, followed by myristicin and terpinolene[2]. Phytochemical constituents and compounds have been isolated from seeds, roots, leaves, or petioles through bioassay-guided methods. These phytochemical constituents can be grouped into the essential oils, flavonoids, carbohydrates, furocoumarins, and other miscellaneous compounds[3].

Oestrogen is the primary female sex hormone, responsible for the development and regulation of the female reproductive system and secondary sex characteristics. Oestrogen is primarily known as an ovarian steroid hormone with important roles in reproductive function[4] however, the effects of oestrogen extend far beyond the reproductive tissues. Indeed, oestrogen can be considered a pleiotropic hormone with significant functions in many non-reproductive tissues including bone[5],[6].17β-Estradiol is the main oestrogen synthesized and secreted by normal female ovaries. Its oxidized analog, estrone, is secreted to a significantly lesser degree by premenopausal ovaries[7].

Parsley has been used to stimulate menstrual flows and manage menstrual disorders[8]. However, experimental research has been scarce to support its folkloric use. Given this and the potential danger of overconsumption of the Plant due to its assumed nontoxic effect, this study was undertaken to ascertain its possible effects on the ovaries and consequently, its potential to improve fertility.

MATERIALS AND METHODS

Plant collection and identification

Parsley (*Petroselinum crispum*) was purchased at Marian market, Calabar, Cross River State. It was identified by a taxonomist of the department of Plant and Ecological Sciences, University of Calabar with the voucher number; Herb/Bot/UCC/027.

Preparation of plant extract

The leaf samples were cleaned and air dried for 14 days after which they were pulverized (grounded into powdered form). A measured amount of 340grams of powered leaves was extracted using 2.5 liters of absolute ethanol for 48 hours with intermittent gyration of sample holder. After 48hrs of soaking, the extract solution was first double filtered with Chess cloth, then with filter paper (Whatman 1 filter paper). The filtrate (extract) was thereafter concentrated under reduced pressure at 45^{o}C in rotary evaporator to 10% volume and then to complete dryness using regulated temperature water bath yielding 71grams (about 20%) of crude extract. The crude extract obtained was stored in a refrigerator until required.

Animal Breeding

Fifteen (15) Adult Female Wistar albino rats weighing 100-150g were used for this experiment. These rats were purchased from the animal house in the College of Medical Sciences, University of Calabar, Calabar. They were then grouped and housed in cages and taken care of under standard laboratory conditions and were bred in the animal house.

The rates were fed daily with "Vital Feed Pellet" and given water daily. Sawdust used for their beddings and was obtained from Akim Timber market, Calabar. These beddings were changed daily to avoid infection and possible contamination to the animals. The rats were kept for two weeks to achieve acclimatization, prior to administration.

Table 1

Distinguishing marks were given on each rat to differentiate the individual rats as well as each group. The animals were divided into three (3) groups (A,B,C) of 5 rats each. Group A was the control group, this group received normal water and feed, Group B being low dose group received 825mg/kg per body weight and Group C being high dose group received 1,650mg/kg per body weight of *Petroselinum crispum*.

The plant extract was administered orally using orogastric tube after dissolving it in distilled water. The total period of administration was twenty-eight (28) days.

Animal Sacrifice

At the end of the 28 days, the animals were sacrificed 24 hours after administration of the last dose. The experimental animals were anaesthetized with chloroform vapour. The ovaries were harvested from the animals in the various experimental groups and fixed in 10% buffered formal saline for histological processing and blood was collected through cardiac puncture for hormonal assay.

Determination of the Weight of Animals

The weight of the experimental animals were measured using an electric weighing balance.

Determination of concentration of Estradiol (E2) in serum using assay kits from Monobind Inc (Abraham, 1981)

The desired number of coated wells was secured in a holder. Twenty-five microliter (25ul) of standards, specimens and control were dispensed into appropriate wells. Estradiol Biotin reagent (50ul) was dispensed into each well, swirled thoroughly and allowed to mix for 20-30 seconds. The mixture was allowed to incubate for 30 minutes at room temperature. Estradiol Enzyme reagent (50ul) was also dispensed into each well, swirled thoroughly and allowed to mix for 20-30 seconds. The mixture was allowed to incubate for 90 minutes at room temperature. The contents of the micro wells were discarded by decantation, then rinsed and flicked 3 times with wash buffer (350µl). Substrate solution (100ul) was dispensed to each well. The mixture was incubated at room temperature (18-22°C) for 20 minutes. The reaction was stopped by addition of stop solution (50µl) to each well and gently mixed for 15-20 seconds to ensure a complete colour change. Absorbance at 450nm (using a reference wavelength of 620-630nm to minimize well imperfection) was read within 30 minutes with a microplate reader.

Statistical Analysis

The results were expressed as Mean $\pm$ Standard Error of Mean (M$\pm$ SEM). Data obtained from biochemical studies were analyzed using one-way ANOVA for comparison between means for treated groups and control group for statistical difference using SPSS package version 25.0. The values of $p<0.05$ were considered as significant.

RESULTS

Morphological observations

At the end of the 28 day period the weight of the animals were observed as follows:

Normal Control: There was no significant increase in body weights at the end of the experiment at $p>0.05$ (Table 1)

Low Dose Group: There was a slight decrease in body weights though not significant at the end of the experiment at $p>0.05$ (Table 1)

High Dose Group: There was no significant increase in body weights at the end of the experiment at $p>0.05$ (Table 1)

Hormonal assay

Oestrogen analysis result

At the end of the twenty-eight (28) days, the oestrogen level was analyzed from each experimental group. The results showed:

Group A: Normal Control

Results showed oestrogen level to be 44.26 ± 0.91

Group B: Low Dose

There was no significant increase compared to normal control at $p<0.05$

Group C: High Dose

Results showed a significant increase in oestrogen level 49.52 $\pm$ 1.29 when compared to control at $p<0.05$

Histological observations

At the end of the 28 days research work, the processed sections of the ovary were viewed under the light microscope.

The following observations were made:

Group A (Normal Control)

Histological section showed;Presence of developing secondary follicles and corpus luteum.

- Presence of graafian follicle embedded in stroma with blood vessels.

Group B (Low Dose Group)

Histological section of the ovary showed;

- Presence of graafian follicle, embedded in stroma and blood vessel.
- Presence of vacuolated and lightly stained theca interna.

Group C (High Dose Group)

Histological sections of the ovary showed:

- Presence of graafian follicle embedded in stroma with blood vessels.
- Presence of fatty infiltration in the cytoplasm of stroma cells.

Plate 1

Plate 2

Plate 3

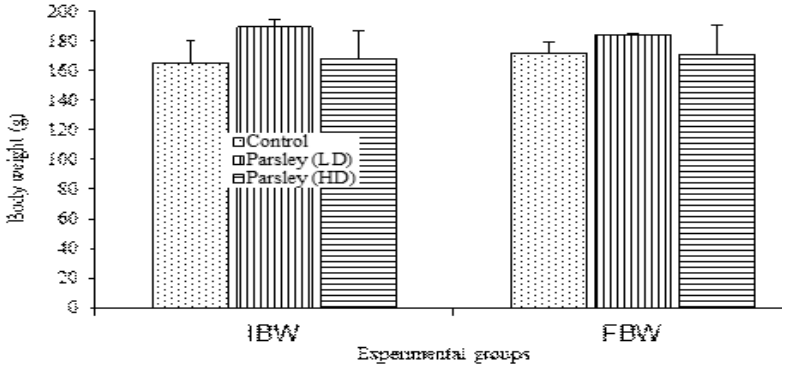

Fig. 1: Initial and final body weights of the different experimental groups

Values are expressed as mean +SEM, n = 3
No significant difference among groups

Table 2: Body weight of Normal control and experimental animals

Group	Initial weight (g)	Final weight (g)	Final Body weight (g)
A	165.00 $\pm$ 14.74	172.00 $\pm$ 7.64	7.00 $\pm$ 8.89
B	190.33 ± 4.18	184.00 ± 1.15	-6.33 ± 5.24
C	168.00 ± 19.16	171.33 ± 19.20	3.33 ± 8.21

Values are expressed as mean ± standard error of mean (S.E.M)
There was no significant difference (p>0.05) in the weight of the animals compared with Normal control group

Table 1: Experimental Design

Groups	Dosages (mg/kg)	Duration
Group A (Normal control group)	Normal feed & water	28 days
Group B (low dose)	825mg/kg (*P. crispum)*	28 days
Group C (high dose)	1,650mg/kg (*P.crispum)*	28 days

Table 3: Estradiol levels of different experimental groups

Groups	Mean (pg/ml) ± SEM
Control	44.26±0.91
Low Dose	46.95±0.73
High Dose	49.52±1.29*

Showing comparison of serum estradiol concentrations in the different experimental groups * = significantly different from control at $p<0.05$.

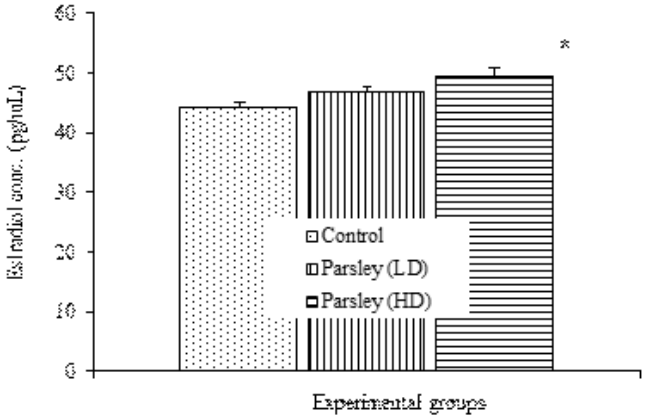

Fig. 2: Estradiol concentration of the different experimental groups.

Values are expressed as mean +SEM, n = 3.
* = significantly different from control at p<0.05

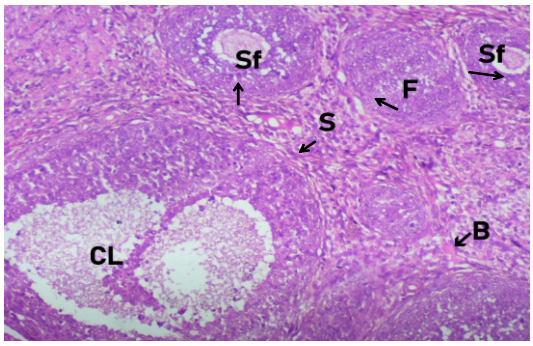

Plate 1: Photomicrograph of the Ovary of Normal Control animals. H & E Stain X100.
Ovary showing developing secondary follicles (Sf), corpus luteum (CL), graafian follicle (F), embedded in stroma (S) with blood vessel (B)

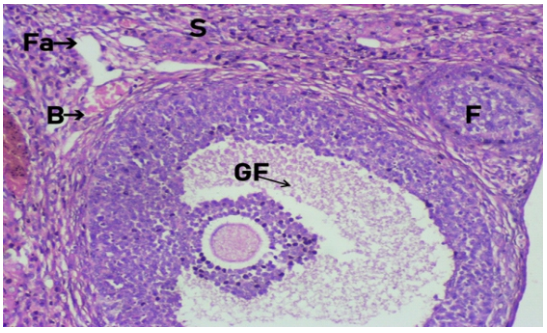

Plate 2: Photomicrograph of Ovary of low dose animals administered with 825mg/kg of ethanolic leaf extract of parsley *(Petroselinum crispum)* H & E Stain X100
Ovary showing Graafian follicle (GF), embedded in stroma (S) with oocyte surrounded by zona pellucida and granulosa cell (F), vacuolated and lightly stained theca interna (Fa) and blood vessel (B).

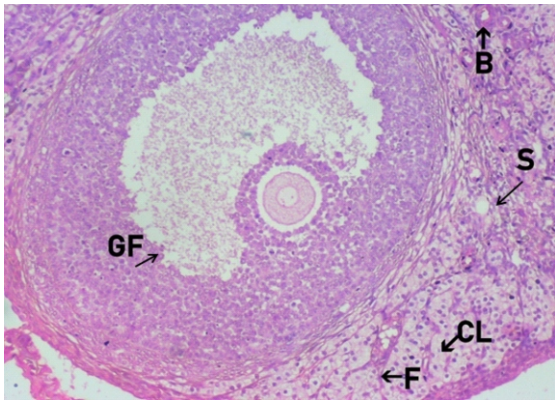

Plate 3: Photomicrograph of the histological section of Group C (High dose) administered with 1,650mg/kg of ethanolic leaf extract of parsley *(Petroselinum crispum)* H & E Stain X100
Ovary showing Graafian follicle (GF) embedded in stroma (S) with marked fatty infiltration in the cytoplasm of stroma cells formed as clusters (CL) enclosed by connective tissue fibres (F) and blood vessel (B).

DISCUSSION

Over the years, medicinal plants have been used for the treatment of various ailments. Parsley *(Petroselinum crispum)* is one of such plants which has been used in the management of amenorrhea and dysmenorrhea. Parsley contains two substances,

myristicin and apiol which influence the uterus and incite monthly cycle[9].

In this study, findings provided evidence of oestrogenic effects of this plant's extract. After 4 weeks of treatment of mature female albino wistar rats with oral administration of ethanolic leaf extract of *Petroselinum crispum*, the results showed a significant increase in the estrogenic levels of the high dose group. Therefore, this report agrees with the research by[10] whose findings showed a significant increase in serum estradiol following administration of extracts of *Petroselinum sativum* at dose of 1000 mg/kg[11] and its polyphenols at 220 mg/kg compared to the negative control[12],[13]. Similarly, several reported data have demonstrated the effect of polyphenols and flavonoids in *Petroselinum crispum* as active substances having oestrogenic effects[14],[15]. Hence, its use in decreasing menstrual issues and inconveniences[9]. Oestrogens, estradiol-17β in particular, are essential for fertility in mammals. They are known to act at key points in the reproductive process in females, such as: Development of the ovulatory follicle(s), Triggering the midcycle, preovulatory surge of gonadotropins, Altering the consistency of cervical mucus to facilitate sperm transport, Preparing the endometrial lining of the uterus for implantation. Alterations to the production and or actions of oestrogen can disrupt these processes leading to infertility[16].

Plants belonging to the Apiaceae family are also known to reduce the intensity of uterine contractions by inhibiting circulating oxytocin and prostaglandins[16].

Results obtained during the research work also showed no signs of toxicity on body weight and the histological investigation showed that the ovary of animals in low dose group had no significant changes in the cytoarchitecture as the stroma, graafian follicles and blood essels were normal. However, the high dose group showed fatty infiltration in the cytoplasm of stroma cells after 28 days of treatment on female rats. Parsley is a known plant used for weight reduction which is low in calories and accounts for the non-significant weight difference from control at $p>0.05$ [16].

Strengths and limitations of the study: The strength of the study was the co-operation of the research team which included a Professor of Anatomy. Everyone brought in their wealth of knowledge for the successful accomplishment of the study. There was no limitation of the study as the scope and objectives of the study were fully covered.

CONCLUSION

Consequent upon the increased estrogenic levels in the animals administered with high doses (1,650mg/kg b.w) of P.crispum, ethanolic leaf extracts of *Petroselinum crispum*, has the potential to improve fertility in experimental animals.

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Author Contributions: MIA and KE conceptualized and designed the study. All authors contributed to the implementation of the study. KE carried out the research under the supervision of MIA. AOI also provided supervision for the work and proof read the manuscript. The authors read and approved the final manuscript and agreed to be accountable for all aspects of the work

Data Availability: The data used to support the findings of this study are available from the corresponding author upon reasonable request

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Conflict of interest: None declared

Ethical approval: Ethical clearance was obtained from the Faculty of Basic Medical animal research ethics committee, with an issued number - 099ANA1521.

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