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Effects of Oral Administration of *Petroselinum Crispum* (Parsley) on Serum Estrogen Concentration and Ovarian Histology of Female Albino Rats.

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ABSTRACT

*Petroselinum crispum (Parsley) is a green leafy vegetable and an aromatic herb employed in traditional medicine for the treatment of different diseases, such as hypertension, gastrointestinal disorders, cardiac disease, urinary disease, dysmenorrhea, and amenorrhea. This study was designed to investigate the effect of oral administration of the ethanol leaf-extract of P. crispum on the estrogen hormone concentration and the histology of the ovaries of female albino rats. Fresh parsley leaves were collected and prepared. Extraction was carried out by the use of Soxhlet extraction technique. Twenty (20) female albino rats with average body weight (117-228 g) were divided into four groups each comprising five animals. The ethanol leaf extract was administered orally for 14 days in varying doses of 500, 1000, 2000 mg/kg body weight (bw) to rats in Group B, C and D, respectively, while rats in Group A, were administered l ml of distilled water as control. Qualitative phytochemical analysis of the extract showed the presence of tannins, phenols, saponins, flavonoids, terpenoids, steroids, volatile oils, and alkaloids while cardiac glycosides was absent. Result from the hormonal analysis showed that there was significant increase in the estrogen hormone concentration at P <0.05 in the lowest dose of 500 mg/kg bw (2.415±0.045 ***10^{-3} μ mol/ml)** when compared with the control Group A (2.208 ± 0.03***10^{-3} μ mol/ml**). Histopathological evaluation of the ovary revealed atrophy of the ovarian tissue and poor follicular development was observed at the highest dose of the plant extract (2000 mg/kg bw) only. Conclusively, it was observed that the plant has a toxic effect at the highest dose of 2000 mg/kg bw. Therefore, its use in enhancing reproduction in female animals should be done with caution.*

***Key words**: Estrogen, Ovarian, Petroselium crispum, Parsely, Histology.*

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INTRODUCTION

Medicinal plants are important sources of treatment for many ailments in humans and animals However, some of these plants may have side effects on various systems including the reproductive system. [1]. *P. crispum* is an herb belonging to the carrot family *Apiaceae* and the genus *Petroselium*. It is a bright green biennial plant in temperate climates, an annual herb in tropical and subtropical areas; widely used as a table garnish in soups, salads, meat and vegetables. [2]. *P. crispum* has an explicitly aromatic odor because of the presence of essential oils or volatile oils which are largely confined in the seeds, leaves, and roots. [3].

P. crispum is of value in traditional and contemporary medicine in the treatment of different ailments. Traditionally the leaf, seed, and roots of *P. crispum* are used in herbal medicine as enema, orally as tea to control high blood pressure, and as tonic to strengthen the bladder. [4]. Several other traditional benefits, include its use in the treatment of inflammation, such as gastrointestinal disorders, hypertension, cardiac diseases, urinary diseases, diabetes, and various dermal diseases [4]. It is also used for the management of menstrual disorders such as dysmenorrhea, and amenorrhea, and as a carminative, spasmolytic, emmenagogue, and galactagogue [5, 6]. *P. crispum* has numerous pharmacological activities such as antifungal, antibacterial, hepatoprotective, antidiabetic, analgesic, immunosuppressant, antiplatelet, gastroprotective and estrogenic effects [6].

Research shows that apiol and myristicin found in Parsley are the essential oil compounds responsible for toxicity, although recent data indicate myristicin's potential anticancer activity [7]. It is pertinent to note that the ingestion of the leaves of *P. crispum* is toxic to many domestic animals including horses, cats, and dogs due to the action of furocoumarins, causing symptoms such as photosensitization, ulceration, dermatitis and ocular toxicity [8]. *P.crispum* has also been shown to have resorption properties by accumulation of heavy metals when grown on soil irrigated with untreated waste water thus making the plant a potential source of heavy metal toxicity [9]. Acute toxicity of *P. crispum* was evaluated in rats and no toxicological effect was observed at a dose of 1g/ kg bw [10**]**.

Estrogen is the primary female sex hormone, responsible for the development and regulation of the female reproductive system and secondary sex characteristics [11]. Estrogen is primarily known as an ovarian steroid hormone with important roles in reproductive function [12].

There is the assumption that the plant *P. crispum* is nontoxic thus its use for the treatment and management of several diseases. A recent study by the authors though yet to be published involving the exposure of rats to *P.crispum*, showed the toxicity to the liver and the kidney tissues at a concentration of 2000 mg/kg. In view of the above, this study was undertaken to evaluate and ascertain the toxic effect of *P.crispum* on estrogen hormone and the ovary, both of which are functional components of the female reproductive system.

MATERIALS AND METHODS

Plant Collection and Extraction

Fresh leaves of *P. crispum* were obtained from a local market in Abuja Municipal Area Council of the Federal Capital Territory (FCT) Nigeria. The leaves were identified by a Botanist in the Herbarium and Ethnobotany Unit, Department of Medicinal Plant Research (MPR) of the National Institute for Pharmaceutical Research

and Traditional Medicine (NIPRD) Idu, Abuja; having a specimen Voucher specimen number of NIPRD/H/7281.

The plant leaves were washed, air dried and then pulverized into a powdered form with a commercial warring blender. The powdered leaves (1kg) were soaked in 1500 ml of 95% ethanol for 18 h in the Soxhlet extractor machine. The leaf extract collected was concentrated in a vacuum using a rotary evaporator at 40°C to dryness and then stored at 4°C until used. The ethanol leaf extract was redissolved in distilled water before administration.

The percentage yield was calculated as

$$\frac{\text{Weight of extract}}{\text{Weight of Pulverized plant material}} \times \frac{100}{1}$$

Phytochemical analysis

The phytochemical analysis was carried out using standard protocols [13] for the detection of alkaloids cardiac glycosides, phlobatannins tannins, saponin, steroid, terpenoid, resins and flavonoid.

Experimental Animals

Twenty (20) female albino rats weighing between 117 g – 228 g were used for the study. The animals were housed in appropriately designed cages; fed with standard pelleted diet and clean water *ad libitum.* The animals were maintained in 12 hours light and dark cycle at room temperature in a well-ventilated animal house under natural conditions; then acclimatized for 14days prior to the commencement of the experiment. Experiment was carried out according to the guidelines set by the Organization of Economic Cooperation and Development (OECD) and the Animal Ethics Committee of the University of Abuja (UAE CAU/2024/001).

Acute toxicity Study of the *P.crispum*

The acute toxicity of the ethanolic leaf extract was conducted by a modified method of Lorke [14]. This was conducted in two phases; . In the first phase, six rats of two rats each in each group were administered single doses 10, 100, 1000 mg/kg bw of the ethanolic leaf extract ***P.crispum*** to establish the range of dose producing any toxic effect. In the second phase, the extract was administered to three (3) other rats sequentially at 1600, 2900 and 5000 mg/kg bw, and observed for 24 hours for acute signs of toxicity including behavioral symptoms and death, . The LD_{50} value was then determined using:

$LD50 = \sqrt{}(D\,0 \times D\,100)$. Where D_0 = Highest dose that gave no mortality and D_{100} = Lowest dose. The LD_{50} was calculated to be greater than 2000 mg/kg bw.

Experimental Design

The rats were randomly divided into four groups of 5 rats per group. The crude leaf extract was re dissolved in distilled water and administered orally at doses of 500, 1000, and 2000 mg/kg bw for 14 days to groups B, C and D respectively while group A(control) was given 1ml of distilled water. Rats were observed for 14 days for clinical signs.

Body Weight

The body weight of each rat was assessed using bench top digital scale, during acclimatization period, before the commencement of dosing, weekly, during the dosing period and on day 14.

Hormonal Assay

Whole Blood Sample was also collected into plain bottles and allowed to coagulate at room temperature and then centrifuged at 3000 r. p. m. for 20 min. The clear, non-haemolysed supernatant sera were quickly removed and kept

at −20 °C till used for the hormonal assay. Serum concentration of estrogen was determined using enzyme linked immunosorbent assay (ELISA kit) as described by [15].

Determination of Serum Estrogen concentration using Elisa kit as described by [15]

The desired number of coated wells (25) was secured in a holder. Twenty-five microliter (25 µl) of standards, specimens and control were dispensed into appropriate wells. Estradiol Biotin reagent (50 µl) 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 (50 µl) 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 (350 µl). Substrate solution (100 µl) 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 450 nm (using a reference wavelength of 620-630 nm to minimize well imperfection) was read within 30 minutes with a microplate reader.

Histopathological Examination of the Ovaries

The animals were sacrificed on the 14th day of the experiment and the uterus, the ovary was esterized, harvested and fixed, in buffered formalin 48 h for histopathological evaluations. The ovarian tissue was then dehydrated to an ascending grade of (alcohol, ethanol 70%) cleared in xylene and embedded in paraffin wax. Serial sections were stained with hematoxylin and eosin and allowed to dry [16]. The stained slides were then examined, and micrographs taken using bright field microscopy (Leica DM 300).

Statistical Analysis

The results are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed by one-way analysis of variance (ANOVA), followed by the analysis of level of significance between different groups based on ANOVA. Differences among means were analyzed by least significant difference (LSD) at 5% level ($p<0.05$).

RESULTS

Extraction Plant Material

The ethanol leaf extract of *P. crispum* was greenish black and sticky in appearance with a pungent odour. The percentage yield was 6.6% w/w.

Phytochemical analysis: The Phytochemical qualitative analysis revealed the presence of tannins, saponin, phenol, steroid, alkaloid, terpenoid, flavonoids, volatile oils and the absence of cardiac glycosides, as shown in **Table 1**.

Toxicity : There were no abnormal signs or changes in behaviour with no mortality after 14 days of administration at the dose range of 500-2000 mg/kg bw. The highest dose of 2000 mg/kg bw did not cause any mortality or signs of toxicity in the treated rats during the study.

The body weight increased in the lowest dose of 500 mg/kg however there was weight loss observed in groups 3 and 4 administered 1000 and 2000 mg/kg bw **(Table 2).**

Hormonal Assay

There was significant increase of the estrogen hormone concentration at P <0.05 in the lowest dose of 500 mg/kg bw when compared with the control group A. Although there was no significant changes in the two higher doses when compared with the control group A **(Table 3).**

Table 1: Phytochemical analysis of ethanolic leaf extract of *P.crispum*.

Phytochemicals	Results
Tannins	+
Phenols	+
Steroids	+
Alkaloids	+
Flavonoid	+
Phlobatannins	+
Volatile oils	+
Terpenoids	+
Cardiac glycosides	-

Present = + Absent = -

Table 2: Effect of Oral Administration of varying doses of ethanol leaf extract of *P. crispum* on the body weight of rats

Treatment G Groups mg/kg bw	Days 0	7	14	%Weight loss	% afr	Weight gain% after14days
Control (A)	197.2 ±25.58	239.5 ±18.13	255.0 ±19.5			29.4
500 (B)	199.6± 10.13	204.6 ±13.53	212.0 ±14.3			6.5
1000 (C)	222.5 ±14.9	219.2 ±14.69	214.4±10.44	-5.4		
2000 (D)	200.0± 26.1	197.2 ±25.58	169.8± 23.66	-15.5		

Values are Mean ± SEM n =5, * means significantly different

Table 3: Effect of oral administration of varying doses of ethanol extract of *P. crispum* on serum estradiol concentration of Wistar rats

Treatment Groups (Conc. of Extract mg/kg bw)	Estradiol Concentration (μ mol/ml *10^{-3})
Group B (500)	*2.415± 0.045
Group C (1000)	2.310 ± 0.06
Group D (2000)	2.370± 0.028
Group A (Control)	2.208 ±0.03

Values are Mean ± SEM n= 5 * significantly different from control at $p<0.05$

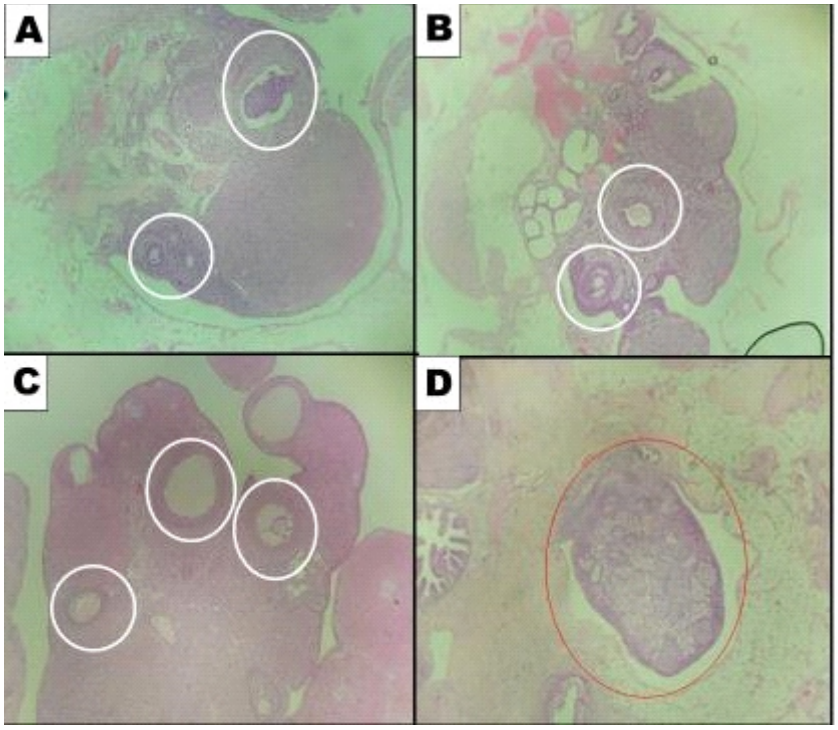

Plate 1: Photomicrographs of Ovaries of rats from groups A (control), B (500 mg/kg bw), C (1000 mg/kg bw) and D (2000 mg/kg bw), respectively (H and E stain ×100 magnifications).
Note the presence of follicles at different stages of development in A, B and C (white circle) while the ovary of the rat from group D was atrophied (red circle) making the follicles inconspicuous (H and E stain ×100 magnifications).

DISCUSSION

This study investigated the effect of the oral administration of the ethanolic leaf extract of *Petroselinum crispum* on serum estrogen and the microarchitecture of the ovaries of female albino rats.

The ethanolic leaf extract of *P. crispum* gave a percentage yield of 6.6% w/w and this was observed to be higher when compared with the report of [17]; which gave a yield of 4.35%w/w. This difference in percentage yield could be because of differences in the age, the specie or the geographical region of its cultivation, the plant part used, method of extraction employed and the season of extraction.

The qualitative phytochemical analysis revealed the presence of tannins, phenols, saponins, steroids alkaloids, flavonoids, volatile oils and terpenoids which agrees with the findings of [17]. Phytochemically, the leaves and seeds of *P. crispum* has been shown to contain high levels of essential oil known as apiole, while the tender buds contain psoralen and related compounds that can induce photosensitivity [18].

Results obtained during this research work showed no signs of toxicity on body weight since there were no significant changes in body weight. Parsley is a plant known and used for weight reduction due to low calories by the mechanism of inhibition of gluconeogenesis and direct stimulation of glycolysis [19, 20].

This study showed the estrogenic effects of the plant's extract as a result of the presence of flavonoids, phenols, steroids and volatile oils upon the oral administration of ethanolic leaf extract of *Petroselinum crispum, as observed from the* significant increase in estrogen concentration of the lowest dosed group of 500 mg/kg bw (Group B); 2.415$\pm$ 0.045 μ **mol/ml** $*10^{-3}$ at $P \leq 0.05$) ; this result is similar to a study on another specie of the same plant as reported by [21]. whose findings showed a significant increase in serum estradiol following administration of extracts of *Petroselinum sativum Hoffm* at hydro-ethanolic extract though at a higher dose of 1000 mg/kg and its polyphenols at 220 mg/kg compared to the negative control. This indicating the potential presence of secondary metabolites such as genistein, daidzein, and lignans which act on the mammalian reproduction pathways [21, 27]. A twenty-eight days study in the South Eastern part of Nigeria also showed that ethanolic leaf extract of *P.crispum* caused an increase in serum estrogen concentration at the dose of 1650 mg/kg bw after 28 days oral administration and this shows an estrogenic effect of parsley though at a higher dose [22].

Similarly, several reported data have demonstrated the effect of polyphenols and flavonoids as active substances having estrogenic effects [23, 20, 24]. From this study, it was also observed that there was no significant change in the estrogen concentration of the rats administered the higher doses of 1000 and 2000 mg/kg bw when compared with the control.

Over the years, medicinal plants have been used for the treatment of various ailments. Parsley from the Apaiceae family, is one of such plants which has been used in the management of amenorrhea and dysmenorrhea; it has been shown to contain two substances called myristicin and apiol, which might almost certainly influence the uterus and incite monthly cycle [25, 20]. Indeed, other studies in other countries revealed the traditional use of Parsley in the treatment of female infertility [26].

The histological investigation showed that the ovaries of animals in the group with the lowest dose of 500 mg/kg bw, had no significant changes in the cytoarchitecture as the stroma, graafian follicles and blood vessels were normal. However, the highest dosed group of 2000 mg/kg bw showed atrophied ovaries

making the follicles inconspicuous after 14 days of administration of the leaf extract; This further implies a decrease in ovarian tissue and indicative of ovarian injury thus showing that it is toxic at this concentration. A study by Montesano *et al*., [27] show that the aerial parts of *P.crispum* are used as an abortifacient.

CONCLUSION

The result of this study indicated that daily administration of ethanolic leaf extract of *Petroselinum crispum* (Parsley) resulted in no significant changes in the cytoarchitecture of the ovaries of the rats as well as the observed increase in estrogenic effect in the lowest dosed group of 500 mg/kg bw. This could be suggestive in ethnomedicine or therapeutics for amenorrhea or to stimulate menstruation. However, the highest dose of 2000 mg/kg bw was shown to be toxic to the ovaries as observed from the study; It is thus advisable that caution should be taken when Parsely is being employed in ethnomedicine, especially at the higher concentration of 2000 mg/kgbw dosage, to avoid damage to the ovaries of the female animals.

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