JOURNAL OF COMPLEMENTARY MEDICINE RESEARCH, 2018
VOL 8, NO. 1, PAGE 1–10
10.5455/jcmr.20180210021526

eJManager

Original Research

Open Access

Follicular development and post-implantation loss assessments in non-pregnant and pregnant rats orally exposed to *Polyscias fruticosa* leaf extract

Alex Boye[1,2], Desmond Omane Acheampong[3], Victor Yao Atsu Barku[4], Adamu Yussam[1], Ernest Amponsah Asiamah[3]

[1]Department of Medical Laboratory Science, School of Allied Health Sciences, College of Health and Allied Sciences, University of Cape Coast, Cape Coast, Ghana

[2]Department of Pharmacology, Institute of Natural Medicine, Anhui Medical University, Hefei, China

[3]Department of Biomedical Science, School of Allied Health Sciences, College of Health and Allied Sciences, University of Cape Coast, Cape Coast, Ghana

[4]Department of Chemistry, School of Physical Sciences, College of Agriculture and Natural Sciences, University of Cape Coast, Cape Coast, Ghana

ABSTRACT

Background: Previously, folk claims of *P. fruticosa* were ascertained pharmacologically; and its safety studied, but its effect/safety on female reproductive system remained unknown.

Objective: The study assessed *P. fruticosa* leaf extract (PFE) on follicular development in non-pregnant rats; implantation and post-implantation loss in pregnant rats.

Methods: The study used healthy adult non-pregnant and pregnant female and male Wistar rats (150–200 g). Non-pregnant rats were randomly assigned to five groups: normal saline (5 ml/kg *po*), clomiphene citrate (CL) (50 mg/kg *po*), and PFE (100, 200, and 500 mg/kg *po*) and treated once daily for 21 days. Dams were sacrificed under deep anesthesia on day 22. Enzyme-linked immunosorbent assay kit was used to measure serum estrogen, follicle stimulating hormone (FSH), and luteinizing hormone (LH). Uterus and ovary were histologically assessed. Dams were co-habited with fertile males for 1 week; confirmed day 1 pregnant rats were randomly re-assigned to five groups with misoprostol (200 mg/kg) as reference and treated once daily for 15 days. Implantation and post-implantation loss were assessed (6 and 15 gestations).

Results: PFE and CL increased follicular development at the primordial and primary follicle stages compared to control. PFE improved uterine musculature compared to control. PFE decreased serum FSH, but increased ($P < 0.05$) serum estrogen and LH compared to control. PFE increased gravid uterine weight compared to control. Total implantation sites were comparable across all groups. Misoprostol and PFE (500 mg/kg) produced post-implantation loss compared to control.

Conclusion: PFE (≤100 mg/kg) improved follicular development in non-pregnant rats, but pose risk of post-implantation loss in pregnant rats at ≥500 mg/kg.

ARTICLE HISTORY

Received February 10, 2018
Accepted April 03, 2018
Published April 10, 2018

KEYWORDS

Follicular development; *Polyscias fruticosa*; post-implantation loss

Introduction

Use of herbs as medicament to promote human health has become common globally [1,2]. Primary healthcare of most developing countries relies on the use of indigenous healing systems, mostly herbal medicine therapy as first line of treatment for common diseases [3]. In effect, several herbs are used to improve general health and specifically reproductive health [2,4]. Previously, folklore claims of *Polyscias fruticosa* for the treatment of upper respiratory disorders including asthma were ascertained pharmacologically [5–7]. The medicinal importance of *Polyscias fruticosa* in folk medicine has earned it many local names across Afro-Asian

Contact Alex Boye ✉ boye_alex@yahoo.com Department of Medical Laboratory Science, School of Allied Health Sciences, College of Health and Allied Sciences, University of Cape Coast, Cape Coast, Ghana.

regions of the world such as "Taiwan momiji" in Japanese, "cay goi ca" in Vietnamese language, "imba" in Sudanese language, and "ming aralia" in common English. Importantly, it was demonstrated that *Polyscias fruticosa* leaf extract (PFE) at 100 mg/kg improved caudal epididymal sperm count in a testosterone-independent manner [8]; however, its effect on female reproductive system particularly follicular development, implantation, and post-implantation events remained unresolved. The present study investigated PFE on follicular development in non-pregnant Wistar rats, in view of the crucial role of folliculogenesis in ovulation and overall female fertility [9,10]. Also, pregnancy rate, total implantation sites, gravid uterine weight, and post-implantation events were monitored in pregnant rats.

Materials and Methods

Chemicals and drugs

Chemicals and drugs used in this study included: absolute ethanol (PS Park Scientific Limited, Northampton, UK), clomiphene citrate (CL) (Doppel Farmaceutici S.p.a, Via delle Ande 15, 00144 Rome, Italy), normal saline (NS) (Amanta Healthcare Ltd., Gujarat, India), silica gel (VWR International bvba/spr, Haasrode, Belgium, Batch: 09B200018), phosphate buffered saline, distyrene, a plasticizer and Xylene, and chloroform (Khimprom JSC, Promyshlennaya STR 101, Russia), 10% Neutral Buffered formalin, 1% Eosin W/V (BDH Chemicals Ltd, England) and sodium hydrogen carbonate (PROLABO®, EC-EMB 45053), misoprostol (Piramal Healthcare UK Limited, Northumber, UK), chloroform (Khimprom JSC, Promyshlennaya STR 101, Russia), xylene (BDH Chemical Ltd, Poole, England), and toluidine blue (Alpha Chemika, India).

Collection, identification, and authentication of **P. fruticosa**

Fresh *P. fruticosa* leaves were obtained from the botanical gardens of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Identification and authentication were done at the Herbal Medicine Department, KNUST. A voucher specimen (KNUST/HM/13/W010) was deposited at herbaria as previously reported [6,7].

Preparation of **Polyscias fruticosa** *leaf extract*

PFE was prepared as previously described [6–8,11] with some modifications. Briefly, leaves of *Polyscias fruticosa* were washed, shade-dried in a well-ventilated area for 3 weeks and then powdered using a mechanical blender. A 1.8 kg quantity of the powder was soaked in 4.8 L of absolute ethanol in a flat bottom flask and shaken manually on daily basis for 7 days. The infusion was filtered five times using a funnel and a cotton wool. Absolute ethanol was retrieved using a rotary evaporator (Buchi Oilbath B-485, Switzerland) with a water bath set at 40°C. The residue obtained was dried with the aid of activated silica gel in a desiccator. The dried residue weighed 62.4 g with a percentage yield of 3.4%. The extract was labeled PFE and stored at 4°C n a refrigerator until use.

Qualitative phytochemical analysis of PFE

PFE was subjected to standard qualitative methods [12,13] to ascertain its phytochemical constituents. Also, thin layer chromatography (TLC) and gas chromatography coupled with mass spectrometry (GC–MS) analysis were conducted on PFE as previously reported [8].

Animal husbandry

Healthy 8–10 weeks old male and female Wistar rats weighing 150–200 g were purchased from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana and transported to the animal house of the Department of Biomedical Sciences, University of Cape Coast. The rats were allowed a period of 2 weeks to acclimatize with laboratory conditions before the start of all experiments. The rats were kept in aluminium cages (40 × 35 × 15 cm) with saw dust as bedding. The beddings of rats were regularly changed. Rats were kept under 12 hours light/dark cycle, normal ambient temperature, and humidity. Rats were fed on standard pellet diet (Essaar grower mash, Essaar Agro-West Africa Ltd, Ghana) and had access to water *ad libitum*. Rats were humanely handled and treated in accordance with standard guidelines as enshrined in the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) as well as specific national and institutional requirements regarding the use of animals in scientific studies.

Dose selection and route of administration

The selection of doses and the route of administration of drugs were based on previous studies on *Polyscias futicosa* [6,7,11]. Body weights were

measured daily and doses were adjusted to reflect body weight changes.

Experimental design

Non-pregnant adult (12 weeks old) female Wistar rats (150–200 g), with confirmed stage of estrous cycle, were randomly assigned to five groups (control group, NS: 5 ml/kg *po*, *n* = 5; CL: 50 mg/kg *po*, *n* = 5; and PFE, 100, 200, and 500 mg/kg *po*, *n* = 5) and treated daily with the respective drugs by oral gavage for 21 days. On the 22nd day, rats were sacrificed under general anesthesia. After carefully isolating Y-shaped uterus and oviduct, bilateral oophorectomy was carried out as previously described [14] with modification. Briefly, left (L) and right (R) ovaries were carefully removed and preserved in 10% formaldehyde in buffered saline for 72 hours. Isolated tissues were appropriately labeled. Sections were prepared according to standard methods and stained with hematoxylin and eosin (H&E) as previously described [15]. Prepared slides were thoroughly studied microscopically by three independent researchers. Final descriptions of observations were made by consensus. In a separate experiment, after 2 weeks of pre-treatment of female Wistar rats (12 weeks old) with PFE, female rats were co-habited with confirmed fertile males (12 weeks) for a week. After confirmation of pregnancy [16,17], dams of comparable body weights were randomly re-assigned to one of five groups [control group (NS: 5 ml/kg *po*; *n* = 5), model group (misoprostol: 200 mg/kg *po*, *n* = 5), and PFE (100, 200, and 500 mg/kg *po*, *n* = 5)] for implantation, resorption, and post-implantation loss assessments at gestational days 6 and 15, respectively.

Assessment of implantation, number of embryos, and post-implantation loss

Assessment of implantation and post-implantation loss were done by following previously described methods [16,18,19] with some modifications. Briefly, at gestational days 6 and 15, number of implantation sites, number of embryos, and post-implantation loss were, respectively, assessed at the specified gestational days for each treatment group. To assess the number of implantation sites, xylene was applied on the tails of dams to induce dilation of the tail vein making the veins more prominent. Each dam was then injected with toluidine blue (0.1 ml of 1%) through the tail vein. The dye was allowed 30–50 minutes of time to react with the uterine endometrium specifically at implantation sites, where molecules of the toluidine blue biochemically react with the implantation site making it not only prominent but also easy to be visually identified. After a period of 50 minutes, dams were sacrificed by deep anesthesia using chloroform (99.8%). The uteri of sacrificed dams were isolated and wet weight of gravid uteri measured. The Y-shaped uteri were cut opened and the number of implantation sites determined per uteri/four dams/group. The whole experiment continued to gestational day 15 with the remaining dams, but this time the number of implantation and the number of embryos were determined for each treatment group by randomly selecting four dams from each group. Dams were sacrificed and their gravid uteri isolated and weighed. Uteri were cut open and the number of implantation and embryos determined. % post-implantation loss was estimated for each group by using a previously described formula [20] as shown below:

$$\%\ \text{post-implantation loss} = \frac{\text{total number of resorption sites}}{\text{total number of implantation sites}} * 100$$

Measurement of serum sex hormone levels

Serum gonadotropins [follicle stimulating hormone (FSH) and luteinizing hormone (LH)] and ovarian estrogen were determined using rat-specific enzyme-linked immunosorbent assay (ELISA) kits (Sangon Biotech, Shanghai, Co., Ltd, 698 XiangMin Road, SongJiang District, Shanghai, China) by strict adherence to manufacturer's instructions. After preparation of sera, it was stored at −20°C until use. Absorbance of controls (calibrators) and specimens (sera from dams in each treatment group) were determined using an ELISA microplate reader (Multiskan Ascent plate reader, MTX Lab Systems, Inc., Brandenton, FL). The sensitivity of hormone detection was 0.005 ng/ml. All the samples were analyzed in a single assay to avoid inter-assay errors.

Data analysis

Data were analysed by using GraphPad Prism Version 6 software for Windows (Graph Pad Software, San Diego, CA). Data were presented in tables, and line and bar graphs as mean ± standard deviation (SD). Mean comparison between groups was done by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. $P \leq 0.05$ was considered statistically significant in all analyzes.

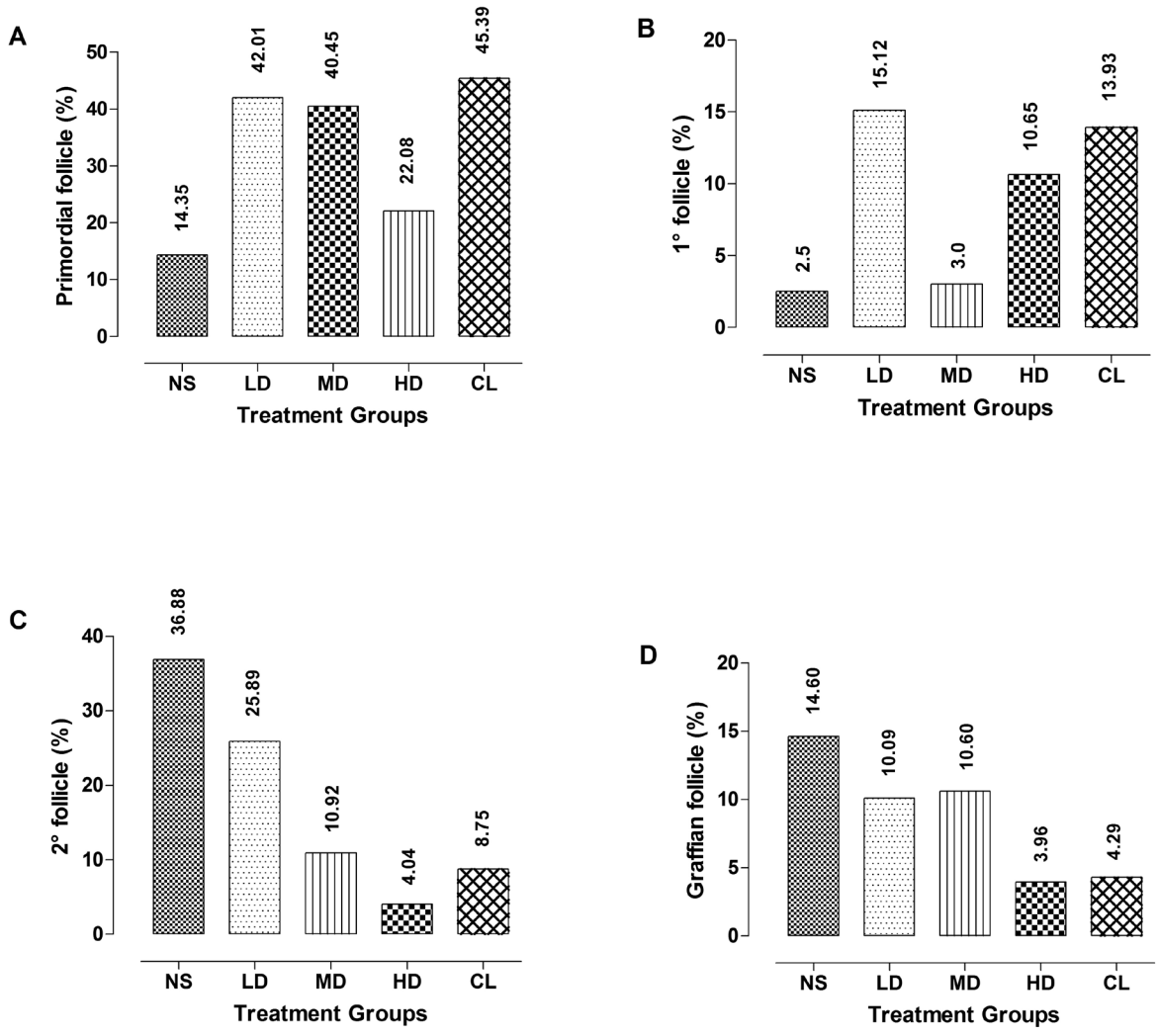

Figure 1. Effect of treatments on follicular development. (A) Primodial follicle, (B) primary follicle, (C) secondary follicle, (D) graffian follicle; NS = normal saline (5 ml/kg), LD = low dose (100 mg/kg) of PFE, MD = medium dose (200 mg/kg) of PFE, HD = high dose (500 mg/kg) of PFE, and CL = clomiphene citrate (50 mg/kg). 1° = primary and 2° = secondary.

Results

Phytochemical analysis on PFE

Qualitative phytochemical analysis on PFE showed the presence of alkaloids, saponins, cyanogenic glycosides, and sterols. TLC analysis showed four spots, while GC–MS analysis produced 12 peaks out of which eight matched library compounds (unpublished data).

PFE produced stage-specific effects on follicular development in non-pregnant rats

The number of primordial follicles of both left and right ovaries combined expressed in percentage increased in PFE-treated rats, particularly in low- and medium-dose PFE (100 and 200 mg/kg) and CL groups relative to control. Average number of primary follicles increased in all treatment groups relative to control, except PFE (200 mg/kg) group. Except PFE (100 mg), all other treatments groups did not improve the average number of secondary follicles compared to control. Average number of Graffian follicles improved in low- and medium-PFE (100 and 200 mg/kg) groups though lower compared to control (Fig. 1).

The number of primordial follicles in left ovary (LO) was higher than that of the right ovary (RO) in low- and medium-dose PFE (100 and 200 mg/kg) group compared to control group, while this trend was also observed in misoprostol group relative to control. For all treatment groups, the number of primary follicles in the left ovary was higher than that of the RO compared to that of control. Although the number of secondary follicles in both left and right ovaries across all treatment groups was lower compared to control, the trend was similar to that

Table 1. Effect of 21 days drug treatments on follicular development in non-pregnant rats.

Group	Primodial follicle (LO) (%)	Primodial follicle (RO) (%)	1° follicle (LO) (%)	1° follicle (RO) (%)	2° follicle (LO) (%)	2° follicle (RO) (%)	Graffian follicle (LO) (%)	Graffian follicle (RO) (%)
NS	15.00	13.69	0.00	5.00	27.50	46.27	7.50	21.71
CL	50.01	40.78	19.84	8.03	9.284	8.21	6.65	1.93
PFE (mg/kg)								
100	56.67	24.24	4.00	2.00	11.50	10.33	7.78	13.42
200	48.07	35.96	18.74	11.49	22.43	29.34	16.97	3.21
500	16.22	27.93	10.89	10.40	1.428	6.64	1.46	6.46

1° = primary, 2° = secondary, NS = normal saline (5 ml/kg); CL = clomiphene citrate (50 mg/kg).

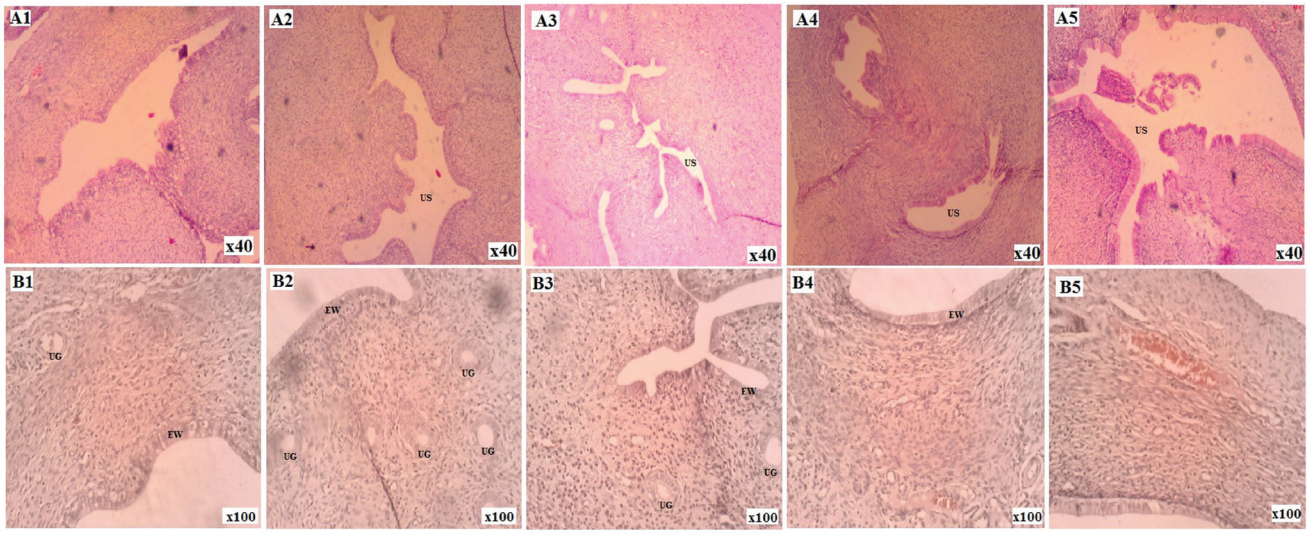

Figure 2. H&E stained sections of rat uterus (A1–A5) and rat uterine endometrial layer (B1–B5) after 21 days of drug treatments. Generally all sections showed columnar epithelium with microvilli. However, there was infiltration of lymphocytes and macrophages in intermediate endometrial layer and collagenous endometrium, especially in B4 and B5. There was presence of uterine glands in B2 and B3 compared to B1. A1 and B1 = NS (5 ml/kg), A2 and B2 = low-dose PFE (100 mg/kg), A3 and B3 = medium-dose PFE (200 mg/kg), A4 and B4 = high-dose PFE (500 mg/kg), and A5 and B5 = clomiphene citrate (50 mg/kg). US = uterine space; UG = uterine gland; EW = endometrial wall.

observed with respect to primary follicles (above) except in medium- and high-dose PFE (200 and 500 mg/kg) group. All but low- and medium-PFE (100 and 200 mg/kg) increased the number of Graffian follicles in LO relative to control. The number of Graffian follicles in RO across all treatment groups was lower compared to that of control (Table 1).

Effect of treatments on uterine musculature and ovarian structure in non-pregnant rats

Generally all sections showed columnar epithelium with microvilli. However, there was infiltration of lymphocytes and macrophages in intermediate endometrial layer and collagenous endometrium, especially in B4 and B5. There was presence of uterine glands in B2 and B3 compared to B1 (Fig. 2). Generally fimbrae with atypical pseudostratified ciliated columnar epithelium, clumped microvilli, and dilated blood vessels (DBVs) were observed in B3, B4, and B5 (Fig. 3). There were marked dilatation of blood vessels near growing follicles and corpus luteum, infiltration of macrophages and lymphocytes in ovarian stroma (ST) and corpus luteum, especially in C, D, and E (Fig. 4).

PFE decreased serum sex hormones in non-pregnant rats

Serum level of FSH decreased in PFE (100, 200, and 500 mg/kg)-treated rats compared to control. Except PFE (100 mg/kg)-treated rats, which had increased serum estrogen compared to control, all other treatment (PFE, 200 and 500 mg/kg and CL, 50 mg/kg) groups had significant decrease in serum estrogen levels compared to control. Serum levels of LH increased significantly in PFE

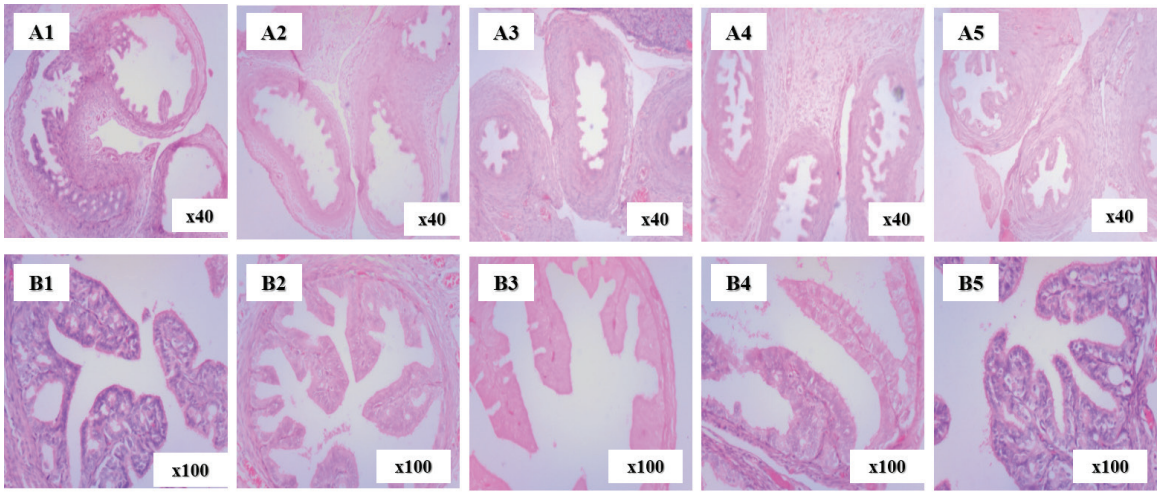

Figure 3. H&E stained sections of oviduct (A1–A5) and fimbriae (B1–B5) after 21 days of drug treatments. Generally fimbrae with atypical pseudostratified ciliated columnar epithelium, clumped microvilli, and DBVs in B3, B4, and B5. A1 and B1 = NS (5 ml/kg), A2 and B2 = low-dose PFE (100 mg/kg), A3 and B3 = medium-dose PFE (200 mg/kg), A4 and B4 = high-dose PFE (500 mg/kg), and A5 and B5 = clomiphene citrate (50 mg/kg).

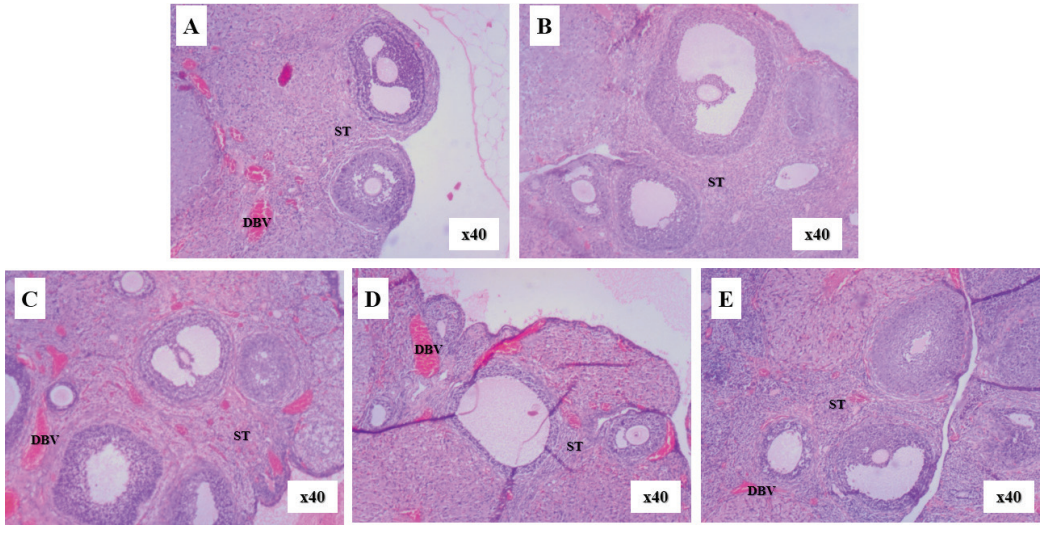

Figure 4. H&E stained sections of ovary after 21 days of drug treatments. There were marked dilatation of blood vessels near growing follicles and corpus luteum, infiltration of macrophages and lymphocytes in ovarian ST and corpus luteum, especially in C, D, and E. Normal saline (5 ml/kg) (A), low-dose PFE (100 mg/kg) (B), medium-dose PFE (200 mg/kg) (C), high-dose PFE (500 mg/kg) (D), and clomiphene citrate (50 mg/kg). ST = stroma; DBV = dilated blood vessel.

Table 2. Effect of 21-day drug treatments on serum levels of female sex hormones in non-pregnant rats.

Groups	FSH (IU/L)	Estrogen (pg/ml)	LH (IU/L)	FSH/LH ratio
NS	0.55 ± 0.50	10.63 ± 0.87	1.67 ± 0.53	0.29 ± 0.20
CL	0.59 ± 0.55	4.56 ± 0.78	0.34 ± 0.09*	1.88 ± 0.80*
PFE (mg/kg)				
100	0.41 ± 0.05	14.63 ± 0.42	48.99 ± 1.10*#	0.11 ± 0.01#
200	0.15 ± 0.03	0.08 ± 0.01*#	0.65 ± 0.09*	0.10 ± 0.04#
500	0.16 ± 0.02	2.31 ± 0.87*#	0.28 ± 0.08*	0.65 ± 0.02#

Each value is the mean ± SD, $N = 3$; $P < 0.005$ was considered statistically significant in all analysis; *compared with NS = normal saline (5 ml/kg), #compared with CL = clomiphene citrate (50 mg/kg).

Table 3. Effect of drug treatments on uterine weight and implantation at gestational day 6.

Treatment groups	No. of pregnant rats	Pregnancy rate[a]	Weight of uterus (g)[b]	Total implantation sites[b]
NS (5 ml/kg)	2	20	0.430 ± 0.042	9.50 ± 0.121
Mis (200 mg/kg)	3	30	0.267 ± 0.047[ns]	0.67 ± 0.077*
PFE (mg/kg)				
100	4	40	0.553 ± 0.048[ns]	4.25 ± 0.708*
200	3	30	0.833 ± 0.021[ns]	6.33 ± 0.055[ns]
500	4	40	0.295 ± 0.054[ns]	0.75 ± 0.057*

[a]Number of pregnant rats/10 * 100/2 weeks pre-treatment; [b]determined from only the pregnant rats in each group. Each value is expressed as mean ± SD (*n* = number of pregnant rats in the respective groups). The level of significance was determined using one-way ANOVA followed by Bonferroni's multiple comparison tests. $P \leq 0.05$ was considered statistically significant in all analyses; ns = not significant; * compared with NS; Mis = Misoprostol (Cytotec).

Table 4. Effect of drug treatments on uterine weight, implantation, and post-implantation loss at gestational day 15.

Treatment groups	No. of pregnant rats	Wet weight of uterus (g)	Total implantation sites	No of embryos	Resorption[a]	% Post-implantation loss[b]
NS (5 ml/kg)	4	13.56 ± 0.16	8.50 ± 0.03	8.50 ± 0.03	0.00 ± 0.00	0.00 ± 0.00
Mis (200 mg/kg)	2	9.72 ± 0.11[ns]	6.50 ± 0.71[ns]	4.50 ± 0.71*	2.00 ± 0.71*	30.76 ± 1.42*
PFE (mg/kg)						
100	4	15.76 ± 0.67[ns]	7.75 ± 0.26[ns]	7.75 ± 0.26[ns]	0.00 ± 0.00[ns]	00 ± 0.00[ns]
200	3	19.64 ± 0.17[ns]	7.33 ± 0.06[ns]	7.33 ± 0.06[ns]	0.00 ± 0.00[ns]	0.00 ± 0.00[ns]
500	2	8.85 ± 0.72[ns]	6.50 ± 0.56[ns]	1.50 ± 0.05*	5.00 ± 0.83[a]	76.9 ± 0.07*

Values are expressed as mean ± SD (*n* = 4). The level of significance was determined using one-way ANOVA followed by Bonferroni's multiple comparison test. $P \leq 0.05$ was considered statistically significant in all analyzes; ns = not statistically significant ($P \geq 0.05$) when compared to NS group (control); *$P \leq 0.05$ Treatments *vs*. NS; [a]Total implantation sites—No of embryos; [b]resorption/total implantation sites * 100; NS = normal saline (control) and Mis = misoprostol (Cytotec).

(100 mg/kg)-treated rats compared to control. PFE (200 and 500 mg/kg) and CL groups had low serum LH levels compared to control. Low-dose PFE (100 mg/kg) had significant increase in LH compared to control (Table 2).

PFE produced dose-related effects on pregnancy rate, implantation, and post-implantation loss

Two weeks pre-treatment of rats with PFE and misoprostol showed that PFE (100 and 500 mg/kg) improved pregnancy rate compared to control. Subsequently, post-pregnancy treatment for the first 6 days produced significant ($P < 0.05$) decrease in the number of implantation sites particularly in PFE (500 mg/kg)-treated dams relative to control. Mean wet uterine weight was comparable across groups although there were differences. PFE (100 and 200 mg/kg)-treated dams had a comparable though lower number of implantation sites relative to control but a significantly ($P < 0.05$) higher number of implantation sites compared to misoprostol-treated dams (Table 3). After 15 days of gestational exposure, PFE (500 mg/kg) and misoprostol (200 mg/kg) had significantly ($P < 0.05$) lower mean number of embryos but higher resorption and % post-implantation loss relative to control group (Table 4). However, low-dose PFE (100 and 200 mg/kg) produced a decrease in both resorption and % post-implantation loss comparable to control group. There were no significant ($P > 0.05$) differences in mean gravid uterine weight amongst groups relative to control group (Table 4).

Discussion

This study investigated follicular development in non-pregnant rats, and implantation and post-implantation loss in pregnant rats after exposure of animals to three increasing dose levels of PFE. Quite recently, PFE (100 mg/kg) was shown to improve caudal sperm count in male rats [8], and also, no observed adverse effect level in rodents was established to be over 1,000 mg/kg [6,7]. Conclusions from those two studies were that it should be avoided in pregnancy, since its long term effect in pregnancy cannot be predicted easily. Presently, we showed that PFE (100 mg/kg) significantly ($P < 0.05$) improved follicular development, specifically at the primordial and primary follicle stages and that observation correlated with increase in

the levels of estrogen and LH (Tables 1 and 2) even more than CL, a known ovulation inducer [21]. This observation indicates that PFE at 100 mg/kg may promote female sexual development. The hypothalamic–pituitary–gonadal axis of the endocrine system plays crucial role in sexual reproduction [22]. LH, one of the sex hormones crucial in this axis, physiologically acts on the gonads (testis and ovary) to enhance their development and function [23]. Serum levels of LH fluctuate to reflect the phase (follicular phase, mid-cycle, luteal phase, and post-menopausal phase) of normal menstrual cycle [24]. In particular, during the first phase of the menstrual cycle, LH stimulates the morphological transformation of follicles to Graaf's follicle [25]. Importantly, estrogen is produced in both males and females [26]. In females, it is produced by follicles upon stimulation by FSH. Estrogen is highly bound when in circulation, therefore, its physiological effect is attributed to the free portion which is normally about 2% [27]. In non-pregnant animals, it is secreted in a biphasic manner, for example, high levels are detected prior to ovulation in humans [28,29]. In this study, it was observed that PFE improved primordial and primary follicular development but not secondary and Graaf's follicles. For instance, the number of primordial and primary follicles in both right and left ovaries increased in low-dose PFE (100 mg/kg) group and correlated with increased estrogen and LH levels suggesting that the surge in LH and estrogen levels may have played a crucial role, and that at low doses (≤100 mg/kg), PFE may improve follicular development in non-pregnant mammals. Although the number of primordial follicles increased in CL and medium-dose PFE (200 mg/kg) groups; however, these observations negatively correlated with estrogen and LH levels, perhaps indicating that follicular development, specifically at the primordial stage may not be entirely under hormonal regulation but could also involve other unknown factors including non-hormone factors. Although PFE (100 mg/kg) treatment in non-pregnant rats improved uterine musculature, particularly thickness of the endometrial layer, increased uterine glands, and also improved ovarian microstructures such as the fimbriae, high-dose PFE (200 and 500 mg/kg) produced not only collagenous endometrial layer but also DBVs in the vicinity of developing follicles relative to control but these changes have no toxicological relevance.

To ascertain whether PFE may improve pregnancy rate, implantation, and post-implantation events after gestational exposure for 15 days, it was observed that at gestational day 6, PFE (100 mg) improved pregnancy rate which correlated positively with weight of gravid uterus but negatively correlated with the number of implantation sites. This observation perhaps corroborate an earlier observation where PFE (100 mg/kg) improved folliculogenesis, a crucial step for ovulation [10]. At gestational day 15, PFE (100 and 200 mg/kg) improved post-implantation loss, but the high-dose PFE (500 mg) group was associated with significant ($P < 0.05$) post-implantation loss compared to control and misoprostol, a known abortifacient agent [30,31]. The present study could have benefited from investigating the effect of PFE on pregnant rats up to delivery and post-delivery events such as risk of congenital malformations (teratogenicity) in pubs and post-partum maternal toxicity, notwithstanding our results provide the rationale for further reproductive studies on PFE in view of the fact that it is been used in folk medicine for many indications [5–7]. The observed effects of PFE in non-pregnant and pregnant rats are attributable to the phytochemical composition of PFE. Interestingly, alkaloids, saponins, cyanogenic glycosides, and sterols were detected in PFE corroborating an earlier study on *P. fruticosa* [8]. As indicated earlier, the observed effects of PFE are attributable to its phyto-constituents as reported elsewhere [8].

Conclusion

Put together, PFE at 100 mg/kg improved follicular development at the primordial and primary follicle stages in non-pregnant rats, improved post-implantation loss in pregnant rats, but gestational exposure of PFE > 100 mg/kg should be avoided in pregnancy despite the need to pursue translational application of PFE.

Acknowledgments

We are grateful to Ghana Standards Authority for helping us with the GC–MS analyses. Also, we thank Dr. Kafui Akakpo (School of Medical Sciences, University of Cape Coast, Cape Coast, Ghana) and Mr. Edem Hope Agbodzakey (Parliament Clinic, MLS, Accra, Ghana) for their technical support.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Bussmann RW. Ethnobotany of the Samburu of Mt. Nyiru, South Turkana, Kenya. J Ethnobiol Ethnomed 2006; 2:35.

[2] Njamen D, Mvondo MA, Djiogue S, Ketcha Wanda GJ, Magne Nde CB, Vollmer G. Phytotherapy and women's reproductive health: the Cameroonian perspective. Planta Med 2013; 79(7):600–11.

[3] Wangensteen H, Diallo D, Paulsen BS. Medicinal plants from Mali: chemistry and biology. J Ethnopharmacol 2015; 176:429–37.

[4] Abdillahi HS, Van Staden J. Application of medicinal plants in maternal healthcare and infertility: a South African perspective. Planta Med 2013; 79(7):591–9.

[5] Asumeng Koffuor G, Boye A, Kyei S, Ofori-Amoah J, Akomanin Asiamah E, Barku A, et al. Anti-asthmatic property and possible mode of activity of an ethanol leaf extract of *Polyscias fruticosa*. Pharm Biol 2016; 54(8):1354–63.

[6] Koffuor GA, Boye A, Ofori-Amoah J, Kyei S, Abokyi S, Nyarko RA, et al. Antiinflammatory and safety assessment of *Polyscias fruticosa* (L.) Harms (Araliaceae) leaf extract in ovalbumin-induced asthma. JPHYTO 2014; 3(5):337–42.

[7] Koffuor GA, Boye A, Ofori-Amoah J, Kyei S, Nouoma CK, Debrah AP. Evaluating muco-suppressant, anti-tussive and safety profile of *Polyscias fruticosa* (L.) Harms (Araliaceae) in asthma management. Br J Med Med Res 2015; 10:1–11.

[8] Boye A, Osei-Owusu AK, Koffuor GA, Barku VYA, Asiamah EA, Asante E. Assessment of *Polyscias fruticosa* (L.) Harm (Araliaceae) leaf extract on male fertility in male Wistar rats. J Intercult Ethnopharmacol 2018; 7(1):45–56.

[9] Vigil P, Lyon C, Flores B, Rioseco H, Serrano F. Ovulation, a sign of health. Linacre Q 2017; 84(4):343–55.

[10] Morotti F, Moretti R, Dos Santos GMG, Silva-Santos KC, Ramos Cerqueira PH, Seneda MM. Ovarian follicular dynamics and conception rate in Bos indicus cows with different antral follicle counts subjected to timed artificial insemination. Anim Reprod Sci 2018; 188:170–7.

[11] Asumeng Koffuor G, Boye A, Kyei S, Ofori-Amoah J, Akomanin Asiamah E, Barku A, et al. Anti-asthmatic property and possible mode of activity of an ethanol leaf extract of *Polyscias fruticosa*. Pharm Biol 2015; 54:1–10.

[12] Cheruth AJ, Al Baloushi SA, Karthishwaran K, Maqsood S, Kurup SS, Sakkir S. Medicinally active principles analysis of Tephrosia apollinea (Delile) DC. growing in the United Arab Emirates. BMC Res Notes 2017; 10(1):61.

[13] Kumar S, Yadav A, Yadav M, Yadav JP. Effect of climate change on phytochemical diversity, total phenolic content and in vitro antioxidant activity of *Aloe vera* (L.) Burm.f. BMC Res Notes 2017; 10(1):60.

[14] Tian FM, Li SY, Yang K, Luo Y, Dai MW, Liu GY, et al. Orally administered simvastatin partially preserves lumbar vertebral bone mass but not integrity of intervertebral discs in ovariectomized rats. Exp Ther Med 2017; 13(3):877–84.

[15] Fischer AH, Jacobson KA, Rose J, Zeller R. Hematoxylin and eosin staining of tissue and cell sections. CSH Protoc 2008; 2008(5):pdb. prot4986.

[16] Boye A, Boampong VA, Takyi N, Martey O. Assessment of an aqueous seed extract of *Parkia clappertoniana* on reproductive performance and toxicity in rodents. J Ethnopharmacol 2016; 185:155–61.

[17] Lee KY, DeMayo FJ. Animal models of implantation. Reproduction 2004; 128(6):679–95.

[18] Yakubu MT, Musa IF. Effects of post-coital administration of alkaloids from *Senna alata* (Linn. Roxb) leaves on some fetal and maternal outcomes of pregnant rats. J Reprod Infertil 2012; 13(4):211–7.

[19] Shaikh MV, Kala M, Ravat N, Nivsarkar M. Abortifacient potential of methanolic extract of *Anthocephalus cadamba* stem bark in mice. J Ethnopharmacol 2015; 173:313–7.

[20] Dabhadkar D, Zade V, Thakare V. Antifertility effect of aqueous extract of *Indigofera trifoliata* leaves on reproductive abilities of female Albino rats. Int J Pharm Sci Res 2013; 4(9):3645.

[21] Zhao Y, Ruan X, Mueck AO. Letrozole combined with low dose highly purified HMG for ovulation induction in clomiphene citrate-resistant infertile Chinese women with polycystic ovary syndrome: a prospective study. Gynecol Endocrinol 2017; 33(6):462–6.

[22] Skowronski MT, Mlotkowska P, Tanski D, Lepiarczyk E, Oklinski MK, Nielsen S, et al. Pituitary gonadotropins, prolactin and growth hormone differentially regulate AQP1 expression in the porcine ovarian follicular cells. Int J Mol Sci 2017; 19(1):1–16.

[23] Lunenfeld B, Buhler K. The neuro control of the ovarain cycle—a hypothesis. Gynecol Endocrinol 2017; 34:1–5.

[24] Hackbart KS, Bender RW, Carvalho PD, Vieira LM, Dresch AR, Guenther JN, et al. Effects of propylene glycol or elevated luteinizing hormone during follicle development on ovulation, fertilization, and early embryo development. Biol Reprod 2017; 97(4):550–63.

[25] Kranc W, Budna J, Kahan R, Chachula A, Bryja A, Ciesiolka S, et al. Molecular basis of growth, proliferation, and differentiation of mammalian follicular granulosa cells. J Biol Regul Homeost Agents 2017; 31(1):1–8.

[26] Gore-Langton RE. Cyclosporine differentially affects estrogen and progestin synthesis by rat granulosa cells in vitro. Mol Cell Endocrinol 1988; 57(3):187–98.
[27] Siiteri PK, Murai JT, Hammond GL, Nisker JA, Raymoure WJ, Kuhn RW. The serum transport of steroid hormones. Recent Prog Horm Res 1982; 38:457–510.
[28] Francois CM, Petit F, Giton F, Gougeon A, Ravel C, Magre S, et al. A novel action of follicle-stimulating hormone in the ovary promotes estradiol production without inducing excessive follicular growth before puberty. Sci Rep 2017; 7:46222.
[29] Liu W, Xin Q, Wang X, Wang S, Wang H, Zhang W, et al. Estrogen receptors in granulosa cells govern meiotic resumption of pre-ovulatory oocytes in mammals. Cell Death Dis 2017; 8(3):e2662.
[30] Wax JR, Conroy K, Pinette MG, Litton C, Cartin A. Clinical pitfalls in misoprostol-based medical management of first-trimester induced and presumed spontaneous abortion. J Clin Ultrasound 2017; doi:10.1002/jcu.22573.
[31] Beucher G, Dolley P, Carles G, Salaun F, Asselin I, Dreyfus M. Misoprostol: off-label use in the first trimester of pregnancy (spontaneous abortion, and voluntary medical termination of pregnancy). J Gynecol Obstet Biol Reprod 2014; 43(2):123–45.