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Evaluation of possible effects of *Persea americana* seeds on female reproductive hormonal and toxicity profile

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ABSTRACT

Ethnopharmacological relevance: The seed of Avocado (*Persea americana*, Lauraceae), non-edible part of the fruit is used as health product. It has been reported as traditional female contraceptive and sterilizer in Peru and some Asian countries and in Nigeria as cardio-protective agent. The present study focused on the effect of hydro-methanolic seed extract of *Persea americana* on female hormones and toxicity profile using animal models.

Materials and methods: The serum follicle stimulating hormone (FSH) and progesterone (PROG) concentrations in mature non-pregnant female rats were assayed using hormonal kits. The toxicity profile was assessed using Lorke's acute toxicity model, haemato-biochemical evaluation and histopathological studies of reproductive related organs. Parameters were measured on day-30, 60 and 90. Presence of biomarker flavonoid compounds were confirmed using High Performance Liquid Chromatography.

Results: The extract at 20, 100 and 500 mg kg^{-1} altered FSH and PROG hormone profile of the treated groups. The extract initially, dose-dependently decreased FSH level in day-30 (6.95, 3.97, 2.08 IU/L respectively) compared to untreated group before a significant increase was observed for day 60 and 90. Progesterone increased dose-dependently in the treated groups throughout the 90-day treatment duration. This may be Indicating cumulative effect on the hormone. No deleterious or toxicity effect was noticed.

Conclusions: The extract of *Persea americana* seed affects female hormone activity. This may find application in various hormonal management procedures, maternal and reproductive health and fertility control/help health facilities. However, it should be used with caution in women intending to conceive.

1. Introduction

Man has always recognized and made use of plant products for food and healthcare purposes (Falodun, 2010). Information on folkloric use of medicinal plants has resulted in the development of drug templates and finished drug products. The therapeutic efficacy of medicinal plant is dependent on the presence and concentration of the different phytoconstituents it contains. The pharmacological differences or the same effects observed by the different morphological parts of a plant are also due to different parts having different or the same phytochemical constituents. Medicinal plants contain several bioactive compounds working together synergistically or catalytically towards producing pharmacological effects. These plant components can also be a source of health concern. The belief that plant drugs are of natural origin and thus will exhibit little or low side effects is a misconception that has proven to be fatal (Magee and Loiacono, 2004). The side effect of a drug can be immediate or delayed or triggered by long exposure or exposure at high doses.

Traditional medicine and herbal products are of value to over 80% of the world's population, who depend on them for their basic healthcare (Patrick-Iwuanyanwu et al., 2012; Ekor, 2014). The growing dependence on natural product is more prominent in developing countries like Nigeria due to socio-economic factors, high burden of diseases, low patient-to-medical doctor ratio, poor access to healthcare facilities, cultural beliefs, as against easy accessibility, ready availability and affordability compared to the conventional drugs (Orabueze et al., 2016;

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WHO 2019).

Maternal health, reproductive health and fertility control are of importance in developing countries such as Nigeria, where the rate of increase of the population is high and does not commensurate the available healthcare outlets as well as the socio-economic empowerment of the people. Some bio-active chemical constituents contained in some plants have been reported to affect adversely the female reproductive organs and hormonal profile (Kayode et al., 2007). Understanding of the possible pharmacological effects of such phytoconstituents may be of beneficial use in the management of various female reproductive needs. Plants containing bio-active compounds that can alter the hormonal profile of the body can cause hormonal imbalance and disruption of fertility index. Such medicinal plants can be used in fertility control, management of hormonal induced pregnancy health challenges, general management of reproductive ailments, treatment of hormonal imbalance disorders (polycystic ovary syndrome, ovarian hyperstimulation syndrome, hypogonadotropic hypogonadism) as well as menopausal complaints later in life (Lauritzen et al., 1997; Raji et al., 2005; Azadey et al., 2007; Farahbod and Soureshjani 2018).

Persea Americana Mill. (Lauraceae), commonly known as avocado pear or alligator pear is locally called "ube oyibo or ube-beke" (Igbo) and "Igba/apoka" (Yoruba) in Nigeria. It is a green-yellow fruit with a big single seed. Production of *Persea Americana* (PAA) worldwide is a huge business that runs into over three million tons of it being harvested annually (Segovia et al., 2018). It is a fruit that is widely consumed and accepted as a "super food" due to its richness in medical, nutraceutical, nutritional and cosmetic-oriented phytochemicals. The health benefits of PAA has been scientifically investigated and reported as an inhibitory agent to dengue virus replication, antimycobacterial, anti-inflammation, trypanocidal, anti-hyperglycemia, insecticidal (Deep Jyoti Bhuyan et al., 2019). However most of these investigations have been done on the pulp of *Persea Americana* more than its by-products (seed, peel and the leaf).

The seed is the underutilized, non-edible and waste product part of the fruit (Ramos-Jerz et al., 2007; Adaramola et al., 2016; Bahru et al., 2019). Ethnopharmacological uses of PAA have been reported to include: management of mycotic and parasitic infections, dysentery, diabetes, diarrhea, toothache, inflammation, intestinal parasites, and gastrointestinal irregularities. Other uses include anti-anemic, diuretic, anti-hypertensive, anti-hyperlipidemic agents and preparations for skin treatment (Dabas et al. (2013), Duke and Vasquez 1994, Ramos-Jerz et al., 2007, Asaolu et al., 2010, Dabas et al. (2013). Owolabi et al. 2014, Evbuomwan and Inetianbor 2017). Topical application of avocado seed paste has been used for treatment of arthritis, dandruff and the seed oil for various dermatological and cosmetic preparations (Nayak et al., 2008; Yang et al., 2010; Dabas et al., 2013). Owolabi et al. (2014) reported that the aqueous extract of PAA has a relaxant effect on the uterine smooth muscles contraction. The seed has been documented to have high polyphenolic content and thus good antioxidant value compared to the pulp (Deep Jyoti Bhuyan et al., 2019).

In Nigeria, the avocado seed powder is commonly used as an alternative for prevention and management of hypertension or as cardio-protective supplement (Ozolua et al., 2009; Kayode et al., 2007; Anaka et al., 2009; Imafidon and Amaechina 2010). They are sliced, dried, ground and taken as tea or mixed with fermented corn meal (commonly known as pap, ogi in Yoruba and akamu in Igbo). The seed of *Persea americana* is known as palta in Peru. The seed has been reported being taken orally by women of Peru as natural product contraceptive and for sterilization (Bussmann and Glenn, 2010). A survey conducted in Paraguay, among obstetricians, healers and medicinal plant vendors has listed the leaf of PAA prepared as decoction or cold maceration as folkloric female contraceptive and/or abortive agent (Arenas and Azorero, 1977; Hnatyszyn et al., 1974). Literature search revealed the use of hot water extracts of dried seeds, leaves and fruit for sterility in women, abortifacient and as an emmenagogue in some countries (Ross 2003). Such countries include Colombia, Costa Rica, Cuba, Ecuador, Mauritius, Mexico and South Africa (Sussman, 1980; Gonzalez and Silva 1987; Ross 2003). In Mexico, decoction of seeds and fresh leaves is taken orally for female contraception and dysmenorrhea, to enhance childbirth and as an emmenagogue (Ross, 2003).

Some literature claims that a decoction of the seed of PAA taken each month during menses has contraceptive effects (Duke and Vasquez, 1994). Duke and Vasquez (1994), listed crushed seed of PAA as being used for female contraceptive and as an abortifacient. Literature search produced no documented science based data or anecdotal evidence supporting efficacy of the seed of PAA as a fertility control or hormone altering or enhancing agent, nor has histopathologic studies on the reproductive organs of animals treated with PAA been examined. The aim of this study therefore was to investigate the claim of the seed being a reproductive hormone altering agent. Also of concern is that amongst the population using the plant seed product as a cardio-productive agent are females of child bearing age. This prompted the evaluation of *Persea americana* seed for any possible effect on female reproductive science such as the hormonal profile (functional) and reproductive organs (structural). The safety profile was also studied for short and long term usage in an attempt to provide some scientific information that can sustain or discourage the commonly daily street use of the seed as an anti-hypertensive supplement. The investigations aimed at exploiting the possible use of this waste product for therapeutic purposes.

2. Methods and materials

2.1. Reagents

Dimethyl sulphoxide acid (DMSO), methanol, ethyl acetate, chloroform and n-hexane (all of analytical grade) were procured from certified suppliers. Catechin, Rutin, apigenin, quercetin and Kaempferol were acquired from Sigma Chemical Co (USA).

2.2. Plant collection and identification

The fruits (*Persea americana*) used for this study were purchased at Idi-Oro market in Mushin, Lagos state, Nigeria on the 15th of June 2019. The seeds were extracted from the fruit pulp manually. The plant was identified and authenticated by Mr. Nwafor F. of Department of Pharmacognosy, Faculty of Pharmaceutical sciences, University of Nigeria, Nsukka, Enugu state. The herbarium specimen was prepared and deposited with voucher number PCG/UNN/0012.

2.3. Preparation and extraction of plant material

The seeds were sliced, air dried at room temperature for 7 days and oven dried at 40 °C for 2 h. The dried seed slides/chips were reduced into coarse powder using a grinding mill (Hamburg 76 West Germany). The PAA powder (1098 g) was extracted by the process of cold maceration using 80% methanol (2000 ml) for 2 days and filtered. The extraction process was repeated twice, using 2 × 800 ml of solvent. The filtrates were combined and filtered using double-layered white muslin material. The filtrate was dried in a water bath at 40 °C, in small crucibles to obtain dried crude extract and transferred to desiccator for further drying.

The dried crude extract (143 g; 13.02% w/w) was stored in the refrigerator at 4 °C until required for use.

2.4. Collection of animals

A total number of 120 female Sprague-Dawley rats (100–122 g, starting weight), that have undergone 2 successive 4 days cycle, shown to have regular and similar estrous cycle were used for the hormonal activity experiment. Male Swiss albino mice (24), weighing between 24 and 27 g were used for acute toxicity studies. The animals were obtained from the Animal Laboratory Centre, College of Medicine of the University of Lagos, and housed in the Faculty of Pharmacy Animal Room.

They were acclimatized for 7 days prior to the beginning of the experiment.

2.5. Ethical approval

This study was approved by the Health Research Ethics Committee of College of Medicine of the University of Lagos with the CMULHREC number CMUL/HREC/11/19/677.

2.6. Animal management and handling

The animals were kept in plastic cages and housed in institutional experimental animal laboratory facility, maintained at 12 h dark and 12 h light cycle at room temperature (26–29 °C) in the Faculty of Pharmacy Animal House, University of Lagos. They were maintained on standard pelleted diet and water *ad libitum*. The environment of the animals was always kept clean. All animal experiments were handled in accordance with the internationally accepted guidelines on laboratory animal use, care, and handling.

2.7. Preliminary phytochemical screening

Qualitative tests for the presence of some phytochemicals such as carbohydrates, glycosides, reducing sugars, anthraquinones, alkaloids, saponins, flavonoids, phenols and tannins were carried out on the crude hydro-methanolic extract using standard procedures as described by various authors (Sofowora, 2008).

2.8. Oral acute toxicity test

Male Swiss albino mice (average weight of 25 g) were divided into four (4) groups of six (6) animals each for the oral toxicity studies. Lorkes's method (Lorke, 1983) with modification was used in determining the median lethal dose (LD_{50}) and assessing the safety profile of the extract. The animals were fasted overnight prior to the study and had free access to water. Single oral doses of 1000, 2000 and 4000 mg kg^{-1} of the extract were given to different groups of animals. The fourth group received the vehicle, 5% DMSO. The animals were observed for behavioural and clinical signs of toxicity and mortality for the first 24 h and then over a duration of seven days. Observation was extended to the 14th day for signs of delayed toxicity.

2.9. Experimental design and collection of specimens for hormonal studies

A total of 120 female Sprague-Dawley rats were randomly divided into 6 groups of 20 rats per group. The groups T1, T2, T3 received the PAA extract at doses of 20, 100 and 500 mg kg^{-1} respectively, while T4 [(5% DMSO (0.2 ml)-vehicle)], T5 [0.15 mg kg^{-1} levonorgestrel (LNG)-positive control] and T6 (negative control-no treatment). The treatment doses used represent one-fifth of pharmacologically active dose (20 mg kg^{-1}), 100 mg kg^{-1} (pre-determined pharmacologically active dose) and 500 mg kg^{-1} (five times the pharmacologically active dose) (Ibrahim et al., 2016). All treatments were administered orally once a daily.

On day 30, 60 and 90, six (6) animals per group were randomly selected and fasted from feed for 12 h but had access to drinking water. Blood samples were collected from the 6 animals via orbital sinus using capillary tube (ocular puncture) into three different bottles, namely, plain sample bottles for hormonal assay, EDTA bottles for haematological assay and heparinized bottles for biochemical assay. After blood collection, the 6 animals/group were sacrificed by cervical dislocation under inhaled diethyl ether anaesthesia for histological studies. A deep longitudinal incision was made into the ventral surface of the abdomen and thorax of the sacrificed rats and by blunt dissection of the muscles and fasciae vital organs such as liver, kidneys, heart, brain, lungs, ovaries and uterus were exposed and organs of interest harvested. Connective tissues and fat trimmings were removed from the organs and then weighed immediately using a sensitive electronic balance. The weight of each harvested organ was standardized for the body weight of the individual rat by dividing the organ weight by body weight (b.wt) and multiplying by 100. The harvested organs were preserved in 10% buffered formalin and later embedded in paraffin.

All animals were weighed on the first day (basal weight), weekly throughout the experimental period and immediately before sacrificing of any animal.

2.10. Female hormonal studies on follicle stimulating hormone and progesterone

The collected blood samples for hormonal assay were left for 15 min to clot. The blood samples were spun at 3000 rpm for 10 min in a centrifuge (Uniscope laboratory centrifuge model SM112). Serum samples were aspirated with Pasteur pipettes into clean sample tubes and assayed for reproductive hormones (FSH and progesterone) using ELISA auto analyzer with Elisa kit (Elabscience).

2.11. Haematological analysis

Blood samples collected were analyzed for various haematological parameters using the haematological full blood count machine auto-analyzer (Mindray C3209). Parameters evaluated include red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), platelet count (PLT) and lymphocytes.

2.12. Biochemical assay

Blood samples collected for biochemical assay were centrifuged at 3000 rpm for 10 min. Serum collected per sample was separated into plain tubes and stored at −20 °C. These were later used for the evaluation of aspartate amino transferase (AST), total bilirubin (BIL-T), creatinine (CREAT), urea, alanine amino transferase (ALT), glucose (GLU), albumin (ALB), total protein (TP), high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol (TC), triglyceride (TG) and alkaline phosphatase (ALP) using a clinical chemistry auto-analyzer (Cobas C311) with Mindray reagents.

2.13. Histopathology

The histological analysis of the kidneys, livers, brains and ovaries excised from the animals were done in the histopathology laboratory of the Lagos University Teaching Hospital (LUTH), Idi-Araba, Lagos, Nigeria. The preserved organs were processed through the histokinette, sectioned (3 μm), placed on glass microscope slides, and stained with haematoxylin and eosin (H.E.). The stained slides were viewed under the microscope with magnification of × 400. Photomicrographs of the various organs were obtained for possible histological changes.

2.14. High Performance Liquid Chromatography (HPLC) analysis

HPLC analysis of hydro-methanolic extract of PAA was carried out using Agilent Technologies HPLC 1200 series. A RP-18 column (150 mm × 4.6 mm, 5 μm particle size, Merck) was used. Mobile phase was purified water containing (0.1% formic acid) and acetonitrile HPLC grade in the ratio (50:50) v/v, employing isocratic elution method and 10 min run time. The reference standards and PAA extract solutions were prepared in methanol, at a concentration of 200 μg/ml and 10 mg/5 ml respectively. The extract was analyzed at 280 nm wavelength using Agilent UV detector. All chromatographic operations were carried out at 20–25 °C temperature.

The peaks were identified by comparison of their retention time with that of the standards and UV absorption spectrum of authentic reference

standards. The reference samples used where gallic acid, catechin, rutin, quercetin, kamferol and naringenin.

2.15. Statistics

All results are presented as mean ± SEM and statically analyzed using One Way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test at $p < 0.05$. GraphPad Prism for Windows Version 7.5 (GraphPad Software, San Diego, USA) was used for all statistical analyses.

3. Results

3.1. Preliminary phytochemical screening results

The phytochemical screening of the hydro-methanolic crude extract of PAA indicated the absence of alkaloids, glycosides, reducing sugars, phlobatannins, starch while phenols, flavonoids, saponins, tannins, terpenoids and anthraquinone tested positive.

3.2. Oral acute toxicity test

The acute toxicity study showed that the extract caused no mortality at any of the doses given within the first 24 h and the following 7 days. There was no physical or behavioural changes observed in the experimental mice. No visible sign(s) of distress or overt toxicity like loss of appetite, hair, sleep, respiratory distress, stretching of the entire body, salivation and poorly formed fecal pellet was observed in the animals. This suggests that LD_{50} of the extract is greater than 4000 mg kg^{-1}.

3.3. Female hormonal activity study result

Fig. 1 (Supple. Table 1) shows the effect of hydro-methanolic extract of PAA on the follicle stimulating hormone (FSH) and progesterone (PROG) on the female albino rats. The extract exhibited a significant ($p < 0.0001$) dose dependent decrease in FSH level in the first month compared to the negative control which received no treatment (T6), likewise the group that received levonorgestrel 0.15 mg kg^{-1} (T5). The extract treated groups T1, T2 and T3 had FSH levels of 6.95, 3.97, 2.08 IU/L respectively. No significant difference was observed between T1 and T6. The positive control (T5) had a significantly low FSH level (1.92 IU/L) compared to the negative control, T6 (5.57 IU/L). In the second and third months, there was a significant ($p < 0.0001$) non-dose dependent increase in FSH level compared to T6 (negative control).

A non-dose dependent increase in progesterone was observed in the first month compared to T6 across the extract PAA treated groups and T5. The medium dose, 100 mg-kg^{-1} (T2) had the highest progesterone level (142.15 nmol/L) compared to other treatment groups. The T5 (42.93 ± 1.98) had a significantly higher progesterone level compared to T6 (22.9 ± 1.04).

A dose dependent increase in progesterone levels was observed in the second and third months compared to T6. The progesterone level values for T1, T2 and T3 for the second month were 41.94, 126.92 and 157.63 nmol/L respectively while the T5 and T6 had 27.18 and 23.80 nmol/L

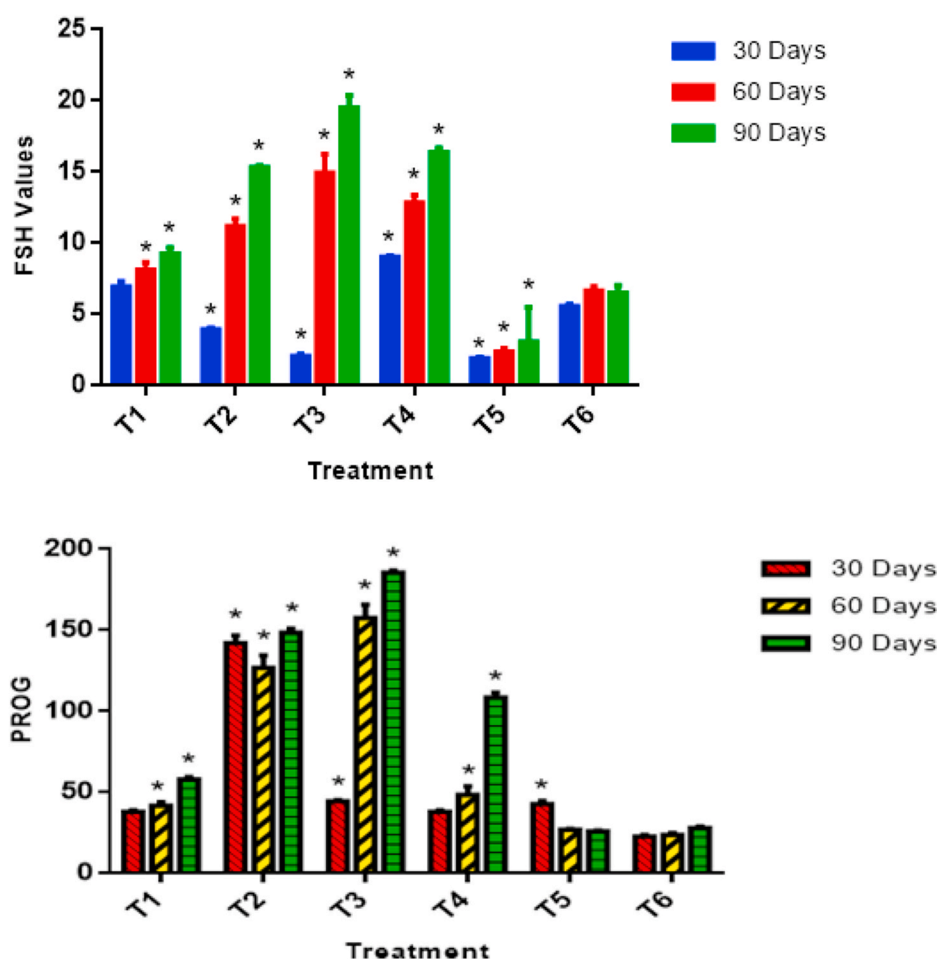


Fig. 1. Effect of hydro-methanolic extract of PAA seed on (a) Follicle Stimulating Hormone and (b) Progesterone in Female Rats in the 90 day study p <0.0001
T1: PAA extract 20 mg kg^{-1}, T2: PAA extract 100 mg kg^{-1}, T3: PAA extract 500 mg kg^{-1}, T4: 5% DMSO, T5: positive control, T6: negative control-no treatment.

Table 1
Effect of hydro-methanolic extract of *Persea americana* seed on the organ/body weight percentage ratio in female rats.

Months	Groups	Brain (%)	Heart (%)	Kidney (%)	Liver (%)	Lung (%)	Ovaries (%)	Uterus (%)
30 days	**T1**	0.99	0.62	0.71	5.60	0.69	0.18	0.24
	T2	1.01	0.48	1.05	3.64	0.78	0.084	0.20
	T3	1.04	0.40	0.64	3.77	0.84	0.045	0.20
	T4	1.01	0.37	0.66	3.88	0.73	0.12	0.29
	T5	1.007	0.4	0.63	3.87	0.76	0.12	0.33
	T6	1.01	0.42	0.59	4.51	0.79	0.086	0.17
60 days	**T1**	0.87	0.32	0.64	3.41	0.79	0.14	0.30
	T2	0.87	0.87	0.64	3.46	0.80	0.12	0.30
	T3	1.00	0.42	0.75	3.25	0.81	0.10	0.36
	T4	1.01	0.41	0.75	3.29	0.81	0.13	0.35
	T5	0.59	0.43	0.69	4.16	0.78	0.13	0.09
	T6	0.96	0.37	0.64	3.64	0.79	0.15	0.40
90 days	**T1**	0.94	0.33	0.69	3.67	0.85	0.16	0.37
	T2	0.96	0.45	0.74	4.27	0.72	0.12	0.49
	T3	0.59	0.43	0.69	4.11	0.78	0.15	0.09
	T4	1.11	0.38	0.58	3.36	0.80	0.04	0.24
	T5	0.95	0.31	0.66	4.08	0.63	0.18	0.41
	T6	1.01	0.40	0.75	3.28	1.15	0.04	0.36

n = 2.
KEY.
T1: 20 mg kg^{-1}.
T2: 100 mg kg^{-1}.
T3: 500 mg kg^{-1}.
T4: 5% DMSO Water.
T5: Positive Control (Levonorgestrel 0.15 mg kg^{-1}).
T6: Negative Control (No treatment).

progesterone respectively.

3.4. Sub-chronic toxicity result

No abnormal weight gains or loss was observed compared to the controls. There was no significant difference in the weight of the treated groups compared to the controls. The changes in the percentage organ/body weight ratio were not statistically significant in comparison to the two (2) controls.

3.5. Haemato-biochemical analyses result

Haematological and biochemical parameters are presented in Tables 2–4. The white blood cell (WBC) values for the PAA treated groups (T1, T2 and T3) were significantly indifferent compared to the no treatment group T6 and within the normal range (Tchamadeua et al., 2011). The animals showed no signs of being stressed.

In the RBC count, a significant decrease ($p < 0.0001$) in the values was observed for T1 and T2 compared with T6 at end of day 30. This dose dependent decease was also observed in haematocrit (HCT) values,

Table 2
Effect of PAA on red blood cell parameters (Haematological) of female rats for 90 days.

		RBC x10^6/µl	Hbg g/dL	HCt (PCV)%	MCV FL	Mch Pg	Mchc g/dL
30 days	**T1**	4.12 ± 0.48*	8.2 ± 0.17*	23.4 ± 0.81*	56.9 ± 0.81	13.57 ± 5.51*	35.0 ± 2.42
	T2	5.10 ± 0.69*	10.5 ± 0.46*	29.9 ± 0.75*	58.7 ± 0.35	20.5 ± 0.75	35.1 ± 0.69
	T3	7.48 ± 0.35	15.4 ± 1.27	44.6 ± 1.10	59.7 ± 1.79	20.5 ± 1.33	34.5 ± 1.33
	T4	7.48 ± 0.25	14.8 ± 0.46	43.9 ± 0.75	58.7 ± 6.0	19.7 ± 1.04	33.7 ± 0.98
	T5	8.25 ± 0.79	16.2 ± 1.5	48.2 ± 1.96	58.5 ± 2.37	19.6 ± 1.27	33.6 ± 3.0
	T6	7.7 ± 0.70	16.0 ± 1.04	45.8 ± 1.50	59.6 ± 1.39	20.7 ± 1.56	34.9 ± 1.10
N/range	–	6.4–9.5	13–18	31–54	48.5–55.0	17.5–22.0	32.0–43.0
60 days	**T1**	8.12 ± 0.55	14.3 ± 0.46	38.3 ± 2.51	50.67 ± 0.55	16.77 ± 0.27	26.5 ± 0.31
	T2	7.47 ± 0.28	15.4 ± 0.29	42.83 ± 0.50	51.63 ± 0.19	17.97 ± 0.12	28.07 ± 0.43
	T3	8.2 ± 0.18	15.5 ± 0.36	44.33 ± 0.08	54.97 ± 0.18	18.3 ± 0.12	30.43 ± 0.38
	T4	8.36 ± 0.03	14.03 ± 0.08	41.47 ± 0.43	49.53 ± 0.46	16.53 ± 0.52	24.37 ± 0.34
	T5	7.74 ± 0.23	14.2 ± 0.06	44.87 ± 0.43	61.3 ± 0.26	18.73 ± 0.29	36.3 ± 0.17
	T6	8.11 ± 0.16	15.0 ± 0.35	40.13 ± 0.79	48.17 ± 0.99	16.13 ± 0.80	23.73 ± 0.79
N/range	–	6.4–9.5	13–18	31–54	48.5–55.0	17.5–22.0	32.0–43.0
90 days	**T1**	8.83 ± 0.22	13.8 ± 0.05	48.6 ± 0.05	55.6 ± 0.25	18.3 ± 0.04	34.3 ± 1.01
	T2	7.61 ± 0.52	14.8 ± 0.12	47.9 ± 0.03	60.8 ± 0.21	18.6 ± 0.34	27.1 ± 0.64
	T3	7.96 ± 0.14	13.6 ± 0.43	49.2 ± 0.26	62.3 ± 0.11	19.4 ± 0.22	29.5 ± 0.08
	T4	8.75 ± 0.20	14.3 ± 0.20	50.3 ± 0.18	63.6 ± 0.02	17.8 ± 0.27	32.4 ± 0.14
	T5	8.59 ± 0.13	13.9 ± 0.17	49.3 ± 0.02	54.3 ± 0.54	16.9 ± 0.03	28.4 ± 0.42
	T6	8.83 ± 0.08	14.6 ± 0.26	61.7 ± 0.43	52.4 ± 0.43	15.8 ± 0.81	28.7 ± 0.02
N/range	–	6.4–9.5	13–18	31–54	48.5–55.0	17.5–22.0	32.0–43.0

N/range = Normal range Values are expressed as Mean ± SEM. $p < 0.05$ relative to no treatment control (T6).
T1: 20 mg kg^{-1}; T2: 100 mg kg^{-1}; T3: 500 mg kg^{-1}; T4: 5% DMSO Water; T5: Positive control (Levonorgestrel 0.15 mg-kg^{-1}); T6: No treatment control.

Table 3
Effect of PAA on white blood cell parameters and platelet count (Haematological) of female rats for 90 days.

		WBC x10^3/µl	Plt (x10^3/µl)	Neu#/GRAN#	Neu%/Gran%	Lymph%
30 days	**T1**	8.4 ± 0.46	426 ± 1.98*	1.6 ± 0.12	29.8 ± 0.52	51.9 ± 1.04
	T2	8.0 ± 0.06	524 ± 17.9	2.7 ± 0.23	33.4 ± 3.0	43.2 ± 2.08
	T3	9.3 ± 0.29	519 ± 22.71	2.8 ± 0.08	30.3 ± 1.96	54.3 ± 1.91
	T4	9.6 ± 0.12	571 ± 8.67	3.6 ± 0.17	37.1 ± 1.21	53.7 ± 2.25
	T5	11.6 ± 0.69	516 ± 6.09	3.9 ± 0.17	33.2 ± 0.64	46.6 ± 0.17
	T6	10.3 ± 0.92	582 ± 12.63	3.0 ± 0.64	29.4 ± 1.91	62.2 ± 3.41
N/range	–	6.5–11.5	450–750	–	–	–
60 days	**T1**	7.8 ± 0.17	386 ± 5.51	2.2 ± 0.05	36.37 ± 0.41	33.00 ± 0.21
	T2	8.13 ± 0.15	506.33 ± 11.46*	2.4 ± 0.12	40.7 ± 0.12	37.03 ± 0.41
	T3	8.27 ± 0.20	547.33 ± 7.53*	3.1 ± 0.17	42.73 ± 0.26	38.27 ± 0.23
	T4	8.07 ± 0.15	312 ± 8.14	1.73 ± 0.08	31.6 ± 0.51	31.67 ± 0.43
	T5	8.93 ± 0.24	574.67 ± 3.48*	3.4 ± 0.12	46.9 ± 0.21	41.87 ± 0.22
	T6	8.33 ± 0.19	258.67 ± 10.04	1.6 ± 0.08	28.63 ± 0.49	30.97 ± 0.38
N/range	–	6.5–11.5	450–750	–	–	–
90 days	**T1**	8.7 ± 0.12	366 ± 2.07	3.0 ± 0.17	33.6 ± 0.10	38.3 ± 0.98
	T2	8.9 ± 0.07	413 ± 4.20	3.1 ± 0.24	36.2 ± 0.09	41.8 ± 1.02
	T3	9.3 ± 0.23	436 ± 3.33	3.3 ± 0.51	38.3 ± 0.20	47.2 ± 0.41
	T4	9.5 ± 0.28	479 ± 6.43	3.7 ± 0.03	40.9 ± 0.18	49.4 ± 0.12
	T5	7.9 ± 0.12	416 ± 6.27	2.9 ± 0.20	32.9 ± 0.54	36.4 ± 0.44
	T6	7.7 ± 0.32	384 ± 3.40	2.7 ± 0.42	32.7 ± 0.20	85.9 ± 0.23
N/range	–	6.5–11.5	450–750	–	–	–

N/range = Normal range Values are expressed as Mean ± SEM. $p < 0.0001$ relative to no treatment control (T6).
T1: 20 mg kg^{-1}; T2: 100 mg kg^{-1}; T3: 500 mg kg^{-1}; T4: 5% DMSO Water; T5: Positive control (Levonorgestrel 0.15 mg-kg^{-1}); T6: No treatment control.

platelet count and heamoglobin (Hbg). The second (60-day) and third months (90-day) showed no significant difference in these parameters compared to T6 and values were within the normal range (Atsamoa et al., 2011; Tchamadeua et al., 2011; Ekperikpe et al., 2019).

A dose dependent decrease also occurred in the level of neutrophil in the first month while an increase occurred in the second month compared to the controls. The group that received 5% DMSO water (T4) exhibited no significant difference in the parameters compared to the T6 and the normal range.

PAA did not cause any significant difference in serum HDL, LDL, albumin and glucose. As shown in Table 4, there was no significant changes in liver enzymes (AST, ALT and ALP), triglyceride, total cholesterol, and total protein. Blood concentration of total bilirubin, creatinine and urea, showed significantly increased ($P < 0.0001$) compared to untreated group T6.

3.6. Histopathology

The lowest dose 20 mg kg-1 showed no structural damage across the 90-day study. However, 100 and 500 mg kg^{-1} effects were inconsistent. At 100 mg kg^{-1}, vascular congestion of the kidneys and liver were seen at 60 and 90-day assays. The group that received 500 mg kg^{-1} showed vascular congestion at the end of the 30- and 90-day assay but not at the end of the 60-day assay. The group T4 showed vascular congestion 30 to 60-days but no abnormality was seen at the end of 90 day assay. Reversibility is not claimed to have occurred because at a much lower dose of 100 mg kg^{-1} the abnormality was also reported. The group that received no treatment, T6's kidney histology showed both normal and vascular congested kidney and liver at inconsistent varying times during the 90-day experiment and the ovary showed vascular congestion on 90 day only.

3.7. High performance liquid chromatography

The marker flavonoid compounds identified by the peaks and comparison with the standards are gallic acid, catechin, rutin, quercetin, naringenin and kamferol (Fig. 2).

4. Discussion

It is the desire of most women to be in control of their reproductive life, to conceive as at when desired, to deliver healthy children at full term and to have good maternal life. Alteration or imbalance in the female sex hormonal profile directly disrupts the reproductive health of a woman and may have maternal health implications. Some reproductive health challenges can be managed by selective alteration of hormonal profile.

In vivo evaluation of female hormonal activity of PAA extract was carried out by estimation of the sex hormones: follicle stimulating hormone (FSH) and progesterone (PROG). The result (Fig. 1 and Suppl. Table 1) of the hormonal assay indicated that PAA extract caused an alteration in the FSH and progesterone concentration in the animals. Thus, the regularity of reproductive functions of female animals (including human) may be affected by consistent intake of PAA extract.

The hormone, FSH, is responsible for the growth and development of the ovarian follicles and this study has shown that its production may be reduced or increased depending on the dose and the duration of intake of PAA. Initial decrease in the concentration of FSH at the end of 30-day dose of PAA may be an indication of disturbance in the estrous cycle profile and ovulation (Ganguly et al., 2007).

Steroidal contraceptives are known to cause suppression of the hypothalmo-hyphophyseal system, thus affecting release of gonadotropin releasing hormone (GnRH) that secretes FSH and prevent ovulation (Bala et al., 2014). The data for second and third months showed a significant dose-dependent increase ($p < 0.0001$) in FSH concentrations in the test animals. This observation may be due to continuous cumulative effect of PAA extract in the body of the animals. Techniques such as intracytoplasmic sperm injection (ICSI) and *in vitro* fertilization (IVF) are employed in fertility clinics by using FSH to induce multiple follicles production and harvesting of multiple eggs for fertilization (Barbieri, 2014). Rising serum FSH levels is one of the earliest signs of human female reproductive aging, and declining female fertility even with continuation of ovulation (McTavish et al., 2007). High serum FSH in female indicates low ovarian reserve and often present with symptoms like those of menopause and difficulty in getting pregnant.

Significant increase ($p < 0.0001$) in progesterone concentration was observed in the PAA treated groups, across the three months when

Table 4
Biochemical Parameters in Female Rats Treated Orally for 90 days.

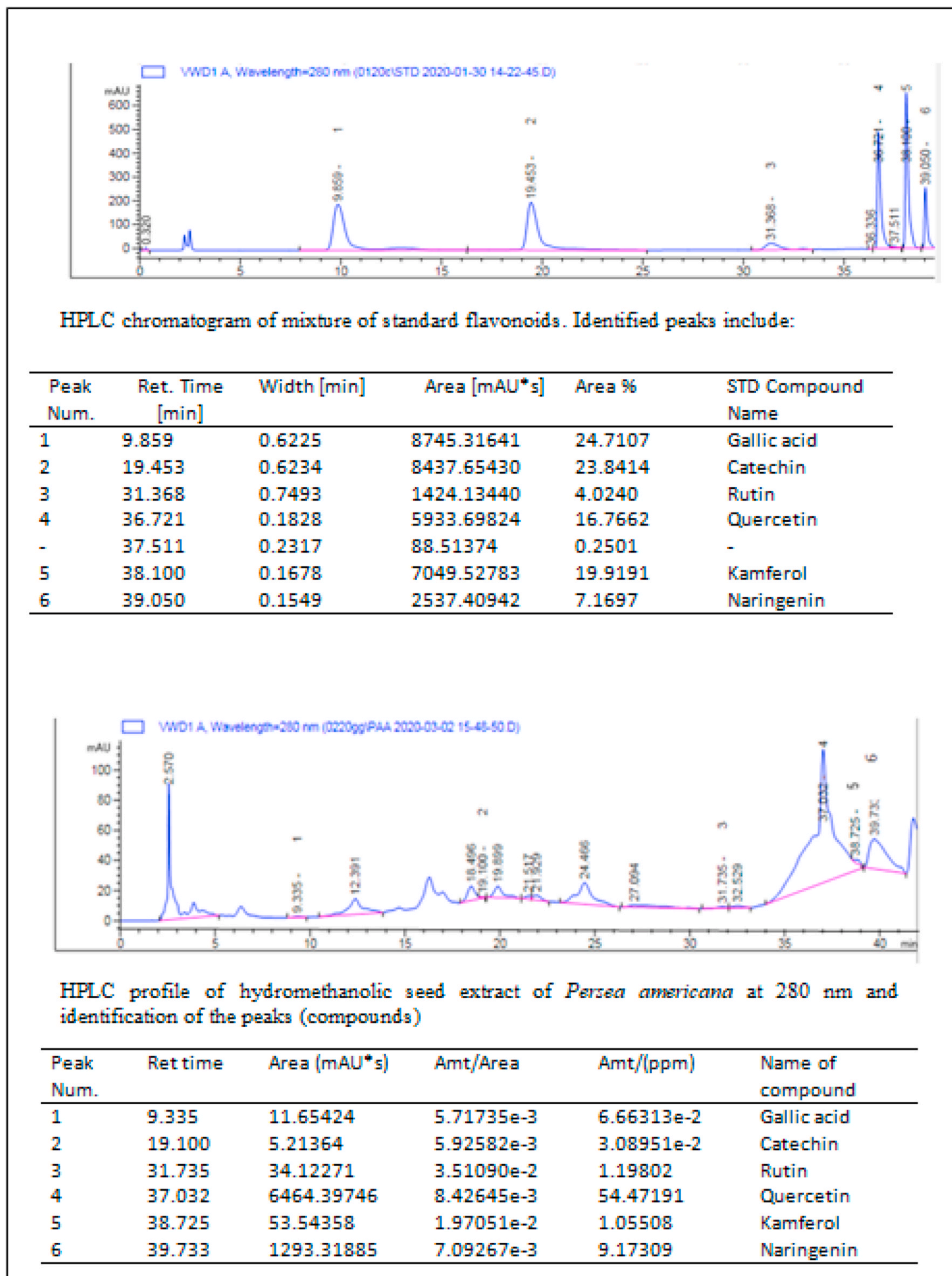
	T1	T2	T3	T4	T5	T6
30 days						
AST μ/l	87.4 ± 0.01	89.8 ± 0.20	92.3 ± 0.40	85.8 ± 0.02	84.1 ± 0.41	76.3 ± 0.13
ALT μ/l	41.3 ± 0.02	41.8 ± 0.02	42.9 ± 0.03	38.9 ± 0.02	41.5 ± 0.01	35.3 ± 0.41
ALP μ/l	21.8 ± 0.10	123.9 ± 0.02	126.1 ± 0.05	118.3 ± 0.16	120.7 ± 0.02	88.5 ± 0.05
HDL Mmol/L	1.22 ± 0.02	1.27 ± 0.0.4	1.39 ± 0.10	0.93 ± 0.03	0.92 ± 0.21	0.96 ± 0.09
LDL Mmol/L	0.37 ± 0.03	0.54 ± 0.11	0.41 ± 0.02	0.37 ± 0.03	0.39 ± 0.07	0.48 ± 0.01
CHOL Mmol/L	1.42 ± 0.01	1.46 ± 0.02	1.57 ± 0.07	1.43 ± 0.01	1.50 ± 0.15	1.42 ± 0.07
TG Mmol/L	0.86 ± 0.11	0.61 ± 0.08	0.44 ± 0.12	0.70 ± 0.04	0.45 ± 0.02	0.73 ± 0.02
ALB g/l	41.7 ± 0.10	43.4 ± 0.02	44.9 ± 0.23	38.7 ± 0.02	40.3 ± 0.02	39.8 ± 0.03
TP g/l	66.02 ± 0.04	67.19 ± 0.01	70.32 ± 0.11	62.63 ± 0.13	64.27 ± 0.02	63.61 ± 0.01
BIL-T μmol	1.4 ± 0.02	1.5 ± 0.02	1.8 ± 0.18	1.4 ± 0.07	1.3 ± 0.04	0.8 ± 0.04
CREAT μmol	1.1 ± 0.02	1.2 ± 0.05	1.4 ± 0.22	1.1 ± 0.03	0.9 ± 0.02	0.5 ± 0.07
UREA Mmol/L	6.7 ± 0.06	6.9 ± 0.02	7.7 ± 0.04	6.2 ± 0.02	6.9 ± 0.10	5.6 ± 0.02
GLU Mmol/L	4.9 ± 0.02	4.6 ± 0.01	4.7 ± 0.03	5.0 ± 0.23	5.1 ± 0.03	4.9 ± 0.13
60 days						
AST μ/l	86.1 ± 0.02	87.9 ± 0.05	89.2 ± 0.03	85.4 ± 0.02	84.5 ± 0.05	76.1 ± 0.13
ALT μ/l	40.7 ± 0.09	41.2 ± 0.01	41.8 ± 0.01	38.6 ± 0.16	41.9 ± 0.01	35.1 ± 0.07
ALP μ/l	122.6 ± 0.06	124.9 ± 0.01	126.8 ± 0.02	117.2 ± 0.01	120.9 ± 0.02	87.6 ± 0.03
HDL Mmol/L	1.39 ± 0.01	1.48 ± 0.04	1.52 ± 0.02	0.96 ± 0.03	1.09 ± 0.01	0.98 ± 0.02
LDL Mmol/L	0.41 ± 0.03	0.32 ± 0.02	0.43 ± 0.03	0.39 ± 0.12	0.36 ± 0.01	0.52 ± 0.02
CHOL Mmol/L	1.52 ± 0.05	1.61 ± 0.13	1.69 ± 0.07	1.47 ± 0.03	1.51 ± 0.03	1.45 ± 0.05
TG Mmol/L	0.46 ± 0.02	0.54 ± 0.09	0.49 ± 0.02	0.68 ± 0.02	0.49 ± 0.03	0.81 ± 0.06
ALB g/l	40.8 ± 0.02	41.6 ± 0.04	41.9 ± 0.02	39.6 ± 0.05	40.6 ± 0.01	40.1 ± 0.01
TP g/l	61.19 ± 0.14	62.61 ± 0.02	63.46 ± 0.03	62.72 ± 0.01	63.18 ± 0.06	64.26 ± 0.02
BIL-T μmol	1.4 ± 0.08	1.4 ± 0.01	1.6 ± 0.02	1.3 ± 0.07	1.4 ± 0.05	0.8 ± 0.12
CREAT μmol	1.0 ± 0.11	1.1 ± 0.01	1.2 ± 0.02	1.0 ± 0.01	1.0 ± 0.03	0.5 ± 0.03
UREA Mmol/L	6.3 ± 0.02	6.5 ± 0.02	7.1 ± 0.08	6.1 ± 0.03	7.0 ± 0.07	5.5 ± 0.02
GLU Mmol/L	4.7 ± 0.04	4.8 ± 0.16	4.6 ± 0.03	4.8 ± 0.04	5.1 ± 0.01	4.7 ± 0.02
90 days						
AST μ/l	88.2 ± 0.08	91.6 ± 0.13	94.9 ± 0.11	85.6 ± 0.03	84.8 ± 0.02	75.9 ± 0.11
ALT μ/l	43.1 ± 0.11	45.6 ± 0.01	48.2 ± 0.02	38.2 ± 0.01	42.6 ± 0.02	34.9 ± 0.03
ALP μ/l	125.3 ± 0.02	125.7 ± 0.04	127.9 ± 0.05	116.8 ± 0.14	122.5 ± 0.03	87.9 ± 0.01
HDL Mmol/L	1.16 ± 0.09	1.19 ± 0.03	1.25 ± 0.02	0.98 ± 0.10	1.22 ± 0.02	0.91 ± 0.18
LDL Mmol/L	0.42 ± 0.02	0.36 ± 0.08	0.32 ± 0.02	0.43 ± 0.02	0.36 ± 0.04	0.46 ± 0.12
CHOL Mmol/L	1.51 ± 0.01	1.32 ± 0.02	1.18 ± 0.02	1.50 ± 0.13	1.46 ± 0.12	1.40 ± 0.03
TG Mmol/L	0.75 ± 0.01	0.56 ± 0.07	0.43 ± 0.05	0.79 ± 0.02	0.61 ± 0.01	0.76 ± 0.02
ALB g/l	40.9 ± 0.01	42.7 ± 0.01	43.4 ± 0.02	37.8 ± 0.02	39.4 ± 0.01	38.9 ± 0.02
TP g/l	64.67 ± 0.06	65.93 ± 0.07	66.81 ± 0.02	61.94 ± 0.01	62.76 ± 0.03	63.82 ± 0.01
BIL-T μmol	1.5 ± 0.02	1.6 ± 0.02	1.8 ± 0.09	1.3 ± 0.11	1.4 ± 0.02	0.9 ± 0.01
CREAT μmol	1.2 ± 0.02	1.3 ± 0.03	1.5 ± 0.01	1.0 ± 0.04	1.0 ± 0.12	0.6 ± 0.02
UREA Mmol/L	6.8 ± 0.01	6.9 ± 0.05	7.8 ± 0.17	6.0 ± 0.02	7.1 ± 0.02	5.4 ± 0.03
GLU Mmol/L	4.8 ± 0.02	4.6 ± 0.13	4.8 ± 0.01	4.9 ± 0.02	5.0 ± 0.02	4.6 ± 0.04

N/range = Normal range, Values are expressed as Mean ± SEM. $p < 0.0001$ relative to no treatment control (T6).
T1: 20 mg kg^{-1}; T2: 100 mg kg^{-1}; T3: 500 mg kg^{-1}; T4: 5% DMSO Water; T5: Positive control (Levonorgestrel 0.15 mg-kg^{-1}); T6: No treatment control.

compared with T6 group. High levels of progesterone as seen in some synthetic oral contraceptive pills (Levonorgestrel, T5) works by inhibiting development of follicle and ovulation from a negative feedback mechanism on hypothalamus leading to decreased secretion of FSH and LH (Baird and Glasier, 1993; Cooper and Mahdy, 2019). Progesterone plays an important role in regulating the monthly menstrual cycle of a woman, getting the uterus ready for conception, maintenance of established pregnancy, getting the milk producing glands of the breast ready to function when delivery takes place and stimulating sexual desire (Anasti et al., 2004; Obasi et al., 2016). Plants with the ability to induce increase of progesterone production may be useful in management of some reproductive health issues due to low progesterone that could occur during menstruation and pregnancy. Some plants have been reported in literature to significantly increase progesterone serum concentrations in experimental treated animals (Sharaibi and Afolayan, 2017). The group that received 5% DMSO water exhibited an increase in the hormonal levels compared to the T6 (negative control) indicating a possible activity of its own with effect on hormonal profile.

The phytochemical analysis revealed that the hydro-methanolic extract of PAA contains flavonoids, saponins, tannins, terpenoids, anthraquinone, phenols and volatile oil which are active biological plant constituents. Alkaloids absence is in contrast with the study done by Evbuomwan and Inetianbor (2017) where it was reported that alkaloid and cardiac glycosides were present in ethanolic and aqueous extracts of the seed. The disparity in constituent might be due to the difference in the solvent used for extract and environmental factors (Evbuomwan and Inetianbor, 2017).

High Performance Liquid Chromatography (HPLC) is a sensitive, specific and accurate technique used for the separation and identification of phenolic compounds in plants (Obafemi et al., 2017). The result showed the high content of quercetin, a compound that has been associated with many health benefits. Other marker flavonoid compounds with known body wellbeing benefits were also identified. The phytochemical assay indicated the presence of flavonoids in the crude extract. This was done as a preliminary standardization of the crude extract for future aid in identification of *P. americana* seed. However, literature has



HPLC chromatogram of mixture of standard flavonoids. Identified peaks include:

Peak Num.	Ret. Time [min]	Width [min]	Area [mAU*s]	Area %	STD Compound Name
1	9.859	0.6225	8745.31641	24.7107	Gallic acid
2	19.453	0.6234	8437.65430	23.8414	Catechin
3	31.368	0.7493	1424.13440	4.0240	Rutin
4	36.721	0.1828	5933.69824	16.7662	Quercetin
-	37.511	0.2317	88.51374	0.2501	-
5	38.100	0.1678	7049.52783	19.9191	Kamferol
6	39.050	0.1549	2537.40942	7.1697	Naringenin

HPLC profile of hydromethanolic seed extract of *Persea americana* at 280 nm and identification of the peaks (compounds)

Peak Num.	Ret time	Area (mAU*s)	Amt/Area	Amt/(ppm)	Name of compound
1	9.335	11.65424	5.71735e-3	6.66313e-2	Gallic acid
2	19.100	5.21364	5.92582e-3	3.08951e-2	Catechin
3	31.735	34.12271	3.51090e-2	1.19802	Rutin
4	37.032	6464.39746	8.42645e-3	54.47191	Quercetin
5	38.725	53.54358	1.97051e-2	1.05508	Kamferol
6	39.733	1293.31885	7.09267e-3	9.17309	Naringenin

Fig. 2. HPLC Chromatograms of mixed standard flavonoid compounds and those from 80% methanol crude seed extract of *Persea americana* at 280 nm.

cited the possible effects of flavonoids and phenols in increasing serum reproductive hormone levels by inhibiting cyclo-oxygenase-2 and multifunctional endocrine-disrupting activities following oral consumption (Liang et al., 1999; Nordeen et al., 2013; Sorelle et al., 2019). Generally, polyphenols have been known to directly or indirectly influence fertility and sexual development (Ly et al., 2015). Liu et al. (2020) reported in his study that the total flavonoid contents of diet may have a distinctive effect in regulating reproductive hormonal profile and reproductive success of animals. These Phytoestrogens binds to oestrogen receptors and trigger oestrogen-like effects on humans and animals (Dusza et al., 2006; Mlynarczuk and Kotwica, 2006; Wang et al., 2013).

Acute toxicity study of the extract in mice indicated that oral administration of extract up to 4000 mg kg^{-1} induced no untoward effect or mortality. There was no noticeable physical sign of distress. The maximum dose (4000 mg kg^{-1}) used in this study for LD_{50} determination

is assumed to be within the safety margin of the plant. A higher dose of 10 g kg^{-1} aqueous extract of PAA has been reported as not exhibiting any toxicological negative feedback for acute toxicity test (Ozolua et al., 2009). In two other different studies by two different research teams, ethanolic and aqueous extracts of PAA seed were reported presenting LD_{50} value of 1200.75 mg kg^{-1} and 1767 mg kg^{-1} (Paha-Ramos et al., 2012; Eduardo Padilla-Camberos et al., 2013). This observed discrepancy may be due to the difference in solvents and extraction methods used. And the seed possibly has a wide safety margin. The doses used in this experiment are therefore within therapeutic range.

The chronic toxicity study was done to evaluate possible health risk that could be associated with long term treatment with PAA. Also of interest was observation of cumulative adverse effects on target internal organs and general body system. A non-significant progressive increase in body weight was observed across the twelve weeks in all the groups (T1 to T6) as also reported by the study of Imafidon and Amaechina (2010) on the same seed's polar extract. There was no significant difference in the mean weight of the body weight or the internal organs in the treatment groups compared to the negative control (T6) after oral administration of the extract for 90 days. Reduction in body weight and/or internal organ weights would have been an indication of possible toxicity following prolonged and high dosed exposure to this drug agent, PAA (Dongmo et al., 2019). At the end of 90 days in this study, death occurred in the T6 and T1 only and no general clinical sign toxicity was seen.

The haematological parameters of the animals assessed as part of safety profile evaluation showed no significant abnormalities at the end of the study (90 days). However, some variable effects were noticed at the first 30 days of the study, which normalized by the second (60 days) and third (90 days) months. A significant decrease ($p < 0.0001$) in red blood cells (RBC), haemoglobin, haematocrit levels and platelet count occurred compared to T6 and normal range in the first 30-day but no significant difference was observed in the second and third months. The same thing was observed with the neutrophil count. Decreased RBC, haemoglobin and haematocrit level can lead to anaemia. The extract might have initial immunosuppressive or haemolytic effect which later reversed off after continuous use. It is also possible that erythropoietin in the kidney was slightly suppressed leading to depressed erythropoiesis. The drug extract may have induced temporary stress on the animals which their body systems were able to adapt to or adjust to as the duration of the drug administration continued into the second and third month. Thus, no significant alteration of the blood parameters can be attributed to PAA. The ability of PAA to slightly increase the platelet count dose-dependently may be a useful trend for blood-clothing as reported by Neboh et al. (2015). Another possible cause of raised platelet is thrombosis which is a predisposing risk factor to stroke or heart attack (Okon et al., 2011; Traesel et al., 2016). However, no death can be attributed to this effect.

The biochemical test showed no abnormalities in the parameters compared to the untreated group T6, however, all the treated group (T1, T2, T3, T4, and T5) showed increased levels of total bilirubin, creatinine and urea. This was observed through the 90-day toxicity test duration. The increase in these three biochemical parameters may be an indication of possible impaired liver and kidney functions but this study results showed that relevant liver enzymes (AST, ALT and ALP) and serum total protein were not elevated. Bilirubin on the contrary appears to increase in some phyto-therapy experiments using animal models. This may be explained by the recent discovery of some plant producing bilirubin (Dwarka et al., 2017). Furthermore, slight increase in bilirubin has been found to be beneficial due to its antioxidant potential (Thomas et al., 2008; Pirone et al., 2009). Because of the normality of the liver enzymes and proteins (Total protein and ALB) one can safely say that there was no hepatotoxicity (Olagunju et al., 2000; Atsamoa et al., 2011). The result gives credence to previous report of PAA not being hepatotoxic (Ozolua et al. (2009)).

The glucose level (GLU) which showed no changes compared to the untreated group T6 indicates the PAA does not cause hyperglycemia. Indeed, the hypoglycemic effect of *P. americana* has been demonstrated (Ezejiofor et al., 2013).

Creatinine and urea determinations serve as markers of kidney dysfunction (Emil den Bakker et al., 2018). Thus an increase in theses parameters (creatinine and urea) compared to the values of the untreated group T6, can be indicative of kidney compromise caused by PAA administration (Wasan et al., 2001). Gross histopathological examination of the kidney showed various degrees of congestion. This however is inconclusive since the congestions observed was not dose dependent and the untreated group T6 on 90-day had the same presentation. This could be as a result of distress caused by restriction due to caging.

Histological analysis revealed no changes in the brain tissues apart from the third month of 500 mg kg^{-1} group where mild vascular congestion occurred.

Apart from 20 mg kg^{-1} which showed no abnormality through the 90-day experiment, normal to mild vascular congestions were seen in the microphotographs of the kidney and liver in the PAA treated groups. These were not dose dependent, not consistent or duration dependent between the 30–60 – 90-day periods. Since vascular congestions were also noticed in negative control group (T6) and 5% DMSO (T4) treated groups, this rules out the abnormality effect being PAA induced (Tchamadeua et al., 2011) Thus no significant negative effects from the toxicological perspective of structural changes in the histopathological assays occurred.

Histologic sections of the ovarian tissue of all the PAA treated groups showed normal ovary and follicles at varying stages of development and corpora lutea. Progesterone secretion is increased during corpus luteum and it prepares the endometrium for implantation of possible conceptus. When progesterone production is constantly high (Fig. 1) the possibility of ovulation is reduced and the uterus preparedness for conception and implantation reduced (Liang et al., 2018).

5. Conclusion

In vivo study on the effect of PAA on reproductive hormones, FSH and progesterone indicated that the extract may contain some bioactive constituents which could cause alteration in female hormonal profile and hence can distort reproductive functions. The results suggest that the PAA may be useful in management of female reproductive hormonal oriented health issues, depending on dosage, age and period of estrous cycle the drug is given. Its possible dose-dependent use in management of spontaneous abortion, as contraceptive, hormonal imbalance and fertility agent may be subjected to further studies. The dose range administered within this study showed no observed toxicity or remarkable adverse effect or lethality. However, the observed slight increase in kidney function test may be further investigated if the drug is intended for daily and long term use as contraceptive or antihypertensive use. HPLC analysis showed that PAA contains high concentration of quercetin and other flavonoids.

Contribution

This work was carried out with collaboration among all authors. Authors ORABUEZE and ASARE designed the study and managed the literature searches, supervised the work and wrote the protocol. Authors ORABUEZE, BABALOLA, AZUONWU, and OKOKO, carried out all experimental and data collection procedures. All the authors were involved in results evaluation, manuscript preparation and editing. Final approval of manuscript was done and accepted by all the authors.

Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this research paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2021.113870.

Abbreviations used

HPLC	High Performance Liquid Chromatography
UV	Ultra violet
FSH	Follicle stimulating hormone
PAA	*Persea Americana*
AST	Aspartate amino transferase
ALT	Alanine amino transferase
ALP	Alkaline phosphatase
ALB	Albumin
BIL-T	Total bilirubin
MCH	Mean corpuscular haemoglobin
MCV	Mean corpuscular volume
HDL	High density lipoprotein
LDL	Low-density lipoprotein
RBC	Red blood cell
LH	Luteinizing hormone
PROG	Progesterone
LNG	Levonorgestrel
GnRH	Gonadotropin releasing hormone
IVF	*in vitro* fertilization
ICSI	Intracytoplasmic sperm injection
Hbg	Heamoglobin
WBC	White blood cell
HCT	Haematocrit
EDTA	Ethylene-Diamine-Tetra-Acetic acid
ANOVA	One Way Analysis of Variance
DMSO	Dimethyl sulphoxide acid

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