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Research Article

Plumeria acuminata: An Herb with Abortifacient Potential

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ABSTRACT

Lack of access to safe, timely, affordable and respectful abortion is a critical public health and human rights issue. Abortion has been a uniquely debated problem in the field of biomedical consciences. Ethnopharmacological data reveals that plant *Plumeria acuminata*, of the *Apocynaceae* family, has exhibited several activities like anti-fertility, anti-pyretic, anti-nociceptive, anti-bacterial, anti-diabetic, anti-tumor, and immune-modulatory as well as abortifacient activities. As per our knowledge, no exploratory studies have been conducted to discover the actual abortifacient potential of this plant till to date. For this purpose, this study aimed to evaluate the biological activity of *P. acuminata* leaves and roots ethanolic extracts concerning their effects in hormonal and histological regulations, eventually leading to abortion. Ethanolic extracts of harvested *P. acuminata* leaves and roots were prepared through a cold maceration process, and subjected to qualitative phytochemical analysis and acute toxicity test. Based on the LD_{50} values, 100, 200, and 400 mg/kg doses of both extracts were determined for abortifacient activity in mated adult Wistar female rats. Misoprostol was administered at 0.1 mg/kg, *p.o*, as a standard abortion-inducing drug. Standard and test-item treatments were given from gestation day (GD) 7 to 14. Morphological, hematological, hormonal, and histological examinations were performed on GD20 after euthanizing animals. Administration of extracts significantly altered the hormonal levels up to ~20-30%, i.e., decreased estrogen and progesterone and increased PGE-1 and acetylcholine. Anatomical alterations in reproductive organs were confirmed by observing cystic follicles and atrophied squamous cells during histopathological evaluation. Visual observations of uterine horns confirmed ~25% live fetus, ~28% early resorption, ~30% late resorption resulting in ~75% post-implantation loss. Obtained abortifacient results can be attributed to the presence of plumericin, sterol and lupeol triterpene groups of phytochemicals present in leaves and roots ethanolic extracts, making them a potent candidate for natural abortifacient medicines.

Introduction

In present days, the economic crisis is occurring due to the current growing population in developed and developing countries. According to the UN, this increment in the growth of population will be reached 8.1 billion in 2025 and 9.6 billion in 2050. In a country like India, the population is expected to reach around 1.40 billion by 2025, and to overcome these, necessary steps should be taken to regulate population growth.[1]

Females and teenagers are progressively finding abortifacient drugs through familiar ways, including accessible services, hospitals, pharmacies and human drug seller though this trend and the recent occurrence have not been well recognized. The part of an investigation in this area is to determine safety, efficacy, access to services, and to maximize meeting women's preferences and comfort with therapeutic abortion. Exploration gaps can be brief in three comprehensive categories: women's first choice and understanding with self use of therapeutic abortion, the spreading and setting up of therapeutic abortion information and medications, and experimental conclusions following self-use.[2]

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The abortifacients are the elements that encourage miscarriage. Miscarriage, a natural loss of an embryo or fetus is widely known as abortion that causes gestation damage. The collective sign of miscarriage is vaginal discharge or bleeding along with or without discomfort.[3] According to WHO, the utmost risky abortions can lead to problems such as partial abortion, heavy blood loss, certain types of infection, uterine damage, and injury to the genital tract and other internal organs. The serious signs and symptoms of problems needing instant attention comprise irregular vaginal blood loss, slight to moderate abdominal pain, and shock (vascular system failure).[4]

Herbs that block ovulation by modifying the secretion of luteinizing (LH) and follicle-stimulating hormones (FSH) are considered anti-fertility agents, and some alter the uterine environment suitable for implantation anti-implantation agent, and herbs that cause uterine contraction are called abortifacient agents.[5]

Although significant development has been done throughout the world by producing synthetic abortifacient drugs, the greatest puzzling hunt in reproductive health care is to explore more effective, newer, harmless, and cheap methods to be easily accessible across the world.[6] Instead of having vast knowledge, especially in traditional medicine, these pearls will be lost if it is not quickly investigated. After the thalidomide tragedy, communities like medical health professionals and the pharmaceutical industry are more careful during the gestation phase and for that several regulatory bodies are run by the respective governments. Modern therapy includes oxytocic agents, which cause uterine hyperstimulation to induce labor.[7] Surgical intervention, prostaglandins (misoprostol), and anti-progestogen (mifepristone) are currently commonly proficient methods that can cause various side effects like abdominal pain, nausea, headache, and cramping. So the hunt for an orally safe abortifacient drug is still ongoing. However, herbal medicine is given preference due to less side effects, low cost, and easy availability.[8-10]

Abortion is illegal in many countries and some countries cannot provide proper health care, this situation ties woman's hands to seek abortion themselves. An ejection of the fetus, due to uterine contractive behavior of herbs, is mediated by various receptors like PGE-1, PGE-2, oxytocin, histamine, serotonin, and muscarine.[11]

Plumeria acuminata (L.) commonly known as Frangipani (Eng.) belongs to the *Apocynaceae* family showed various activities like anti-fertility, anti-pyretic, anti-nociceptive, anti-bacterial, anti-diabetic, anti-tumor, and immune-modulatory activities.[12–21] Phyto-constituents present in leaves and roots such as plumericin, sterols, and lupeols are believed to exhibit abortifacient activity.[13,15] Plumericin, found from the roots of *P. acuminata* is a terpene lactone. Reports suggested that lactone found in the plant might be responsible for the inhibition of pregnancy and the termination of pregnancy. Lactones have a role during pregnancy and on the development of fetus. The major fears include causing abortion, reproductive hormone modulation, maternal toxicity, teratogenicity, and embryo-fetotoxicity.[22] Sterol present in leaves extract of plant is ideally sterol lipids. Ethano-medicine reports of various plants indicate that steroid, sterol lipid and saponins can cause abortion if taken for a long period.[23,24] Stigmasterol, a phytosterol, has shown anti-cancer, anti-pyretic, anti-inflammatory, and immune-modulating effects.[25-31] Lupeol, a pentacyclic triterpene, exhibits anti-cancer and anti-inflammatory properties.[32,33] Botanical report of many plants suggests that plant with β-sitosterol, lupeol may possess abortifacient properties.[34–36] Stigmasterol and lupeol have shown excellent anti-estrogenic activity.[37,38] Triterpene Lupeol has shown an antifertility effect on male rats by decreasing spermatogenesis.[39]

Moreover, none of the experiments documented the possible mode of action till date. As there are less data available on the abortifacient effect of PAL and PAR extract, in the present investigation we emphasize on abortifacient effect of *P. acuminata* leaves and roots extract in female rats that will expose the new paths to examine rich phytoconstituents for additional analysis to develop the potential herbal abortifacient agents.

Materials and Methods

Identification of Plant and Collection of Plant Materials

The Plant specimens (Leaves and Roots) for the study were collected in the Month of Feb 2020, from the herbal botanical garden of Shree Dhanvantary Pharmaceutical Analysis and Research Centre, Surat, Gujarat (India). Geographical location coordinates for the same are 21.3929° N, 72.9247° E. Plant has been authenticated by Dr. Bimal Desai, HOD Department of Botany and Forestry, Navsari Agriculture University, Gujarat (India). Plant herbarium was prepared and certificate for the same has been raised. (This herbarium certificate can be availed from the first author, upon reasonable request). Care was taken to select healthy fully grown normal plant parts.

Preparation of Plant Materials

After thoroughly washing with deionized water, the collected plant parts were sliced into small pieces. The pats were air-dried (shade) properly at room temperature (25-30°C) for a week after that, it was grinded to a uniform powder with the help of a suitable grinder. The powder plant parts were then stored in an airtight container and kept in a cool and dry place for future analysis.

Preparation of Extracts

The ethanolic extracts were prepared based on the principle of the cold maceration method by soaking 1000 g each of dry powdered plant parts in 2000 mL of ethanol in an airtight container for about 14 days. Whatman

No.1 filter paper was used for filtration of the whole mixture. The filtrate was concentrated and evaporated to dryness using a rotary evaporator with the water bath set at 40°C. It renders a gummy (paste) concentrate of dark brown color (leaves and Roots) and light brown color (roots). The gummy concentrate was designed as a crude extract or ethanolic extract, which were then weighed. The percentage of yield was calculated using Eq.1.[12,40–42]

Extract yield (%)= (Net weight of extract (g))/(Total weight of powder used for extraction (g)) ×100

Phytochemical Analysis

The ethanolic extracts of the different parts of the plant were analyzed to identify the presence of various phytoconstituents such as carbohydrates, alkaloids, glycosides, amino acids and proteins, phenols, tannins, flavonoids, sterols, saponins, steroids, and terpenoids by using reported standard tests.[43-46] Analytical grade reagents and chemicals were consumed for this analysis.

Animal Study

Wistar rats were used as an animal model for abortifacient study.

Age, Sex, and Housing Condition of Animals

The abortifacient activity of the crude ethanolic extract was studied using adult male and female rats. The animals were housed under standard environmental conditions of temperature 22 ± 3°C, at humidity 50 ± 20%, and a 12 hours light-dark cycle. Rats were given a standard rodent pellet chow diet and water *ad libitum*.

Standard Drugs and Test Items

Misoprost-100 (Misoprostol 0.1 mg, Cipla Pharmaceutical) was purchased from the local market and used as standard drugs in the abortifacient model. peripheral arterial line (PAL) and post-anesthesia room (PAR) extracts were used as test items for this model.

Materials

Various enzyme-linked immunoassay (ELISA) kits were purchased from different sources for estimation of hormones, prostaglandins, and acetylcholine levels likes, estrogen (Abcam, Catalog No. ab108667), progesterone (Alpco, Catalog No. 55-PROMS-E01), FSH (Elabscience, Catalog No. E-EL-R0391), LH (MyBioSource, Catalog No.MBS729873), PGE-1 (MyBioSource, Catalog No. MBS268786), PGE-2 (Elabscience, Catalog No. E-EL-0034) and acetylcholine (MyBioSource, Catalog No. MBS282680). Chemical and reagents used for qualitative estimation of phytoconstituents were purchased from different sources like Merck, Thermo fisher, TCI, etc.

Dose Selection for PAL and PAR Extracts

A safety dose of the plant extracts was determined by performing acute toxicity studies as per the Organisation for Economic Co-operation and Development (OECD) 423 (Acute toxic class method).[16,47] 2000 mg/kg dose was chosen as a starting dose, and given to the first set of three adult female Wistar rats. Carefully monitored all the animals for any clinical sign after dosing every 30 mins till 4 hours. Animals were observed for further 14 days for any sign of toxicity. Fresh three animals were selected for the second set and were given the same dose as the first set. Based on mortality in both sets, the LD_{50} value was determined.

Experimental Procedure and Study Design

An experimental study was designed to explore abortifacient characteristics of PAL and PAR extracts. The LD_{50} of the plant extracts was found to be more than 2 g/kg. Three different doses of 100, 200, and 400 mg/kg of PAL and PAR extracts were determined. The doses were referred to as PAL-100, PAL-200, PAL-400, PAR-100, PAR-200, and PAR-400 for this study.

Abortifacient Model

Adult male and female Wistar rats weighing 200–250 g were used for this investigation. A vaginal smear was taken by flush technique daily for monitoring estrous cyclicity. Female rats that showed a normal estrous cycle were selected for the experiments. Males with known fertility were housed with female rats with proestrus phase in the ratio of 1:1 and examined the presence of sperm or evidence of copulation on the next morning. Female rats showing the presence of sperm or thick clump of spermatozoa or the copulation plug in their vaginal smears were housed individually and day zero of pregnancy (GD-0) was assigned to that female. As shown in Table 1, pregnant rats were divided into eight groups, each group containing six animals. Animals of all groups received respective treatment from day 7 (GD-7) to day 14 (GD-14) of pregnancy. On the 20th day (GD-20) blood was collected from the retro-orbital sinus and animals were euthanized by CO_2 asphyxiation. WBC, RBC, Hb, HCT, MCV, MCH, platelet, and neutrophil counts were evaluated from the blood sample using a hematology analyzer (Siemens,

Table 1: Experimental design for abortifacient study.

Group no.	*Group Name*	*Animal ID*	*Treatment from GD-7 to GD-14*
Group I	Normal Control	01-06	Normal Saline, p.o.
Group II	Standard Control	07-12	Misoprostol 0.1 mg/kg, p.o
Group III	PAL_100	13-18	PAL extract 100 mg/kg, p.o
Group IV	PAL_200	19-24	PAL extract 200 mg/kg, p.o
Group V	PAL_400	25-30	PAL extract 400 mg/kg, p.o
Group VI	PAR_100	31-36	PAR extract 100 mg/kg, p.o
Group VII	PAR_200	37-42	PAR extract 200 mg/kg, p.o
Group VIII	PAR_400	43-48	PAR extract 400 mg/kg, p.o

Advia 2120i). FSH, LH, estrogen, and progesterone levels were quantified from serum samples using ELISA methods. Ovaries, uterus, vagina, oviduct, and cervix were collected; surrounding tissue was removed, blotted on filter paper, and weighed quickly. Uterine horns were inspected for the number of implants. Uterus was dissected in two half parts, one of which was homogenized using PBS (pH 7.0), and the homogenate was subjected to estimation of prostaglandins and acetylcholine level by ELISA methods. The other part of the uterus and the other collected organs were fixed in 10% formalin buffer, dehydrated in alcohol, and embedded in paraffin for sectioning. 6μ thick sections were fixed on slides and subjected to H & E staining process. The stained sections were observed for histological changes in the organs. A number of corpora-lutea (CL) was counted from the ovary section using a digital light microscope (ZEISS, Axio Lab A1). The pre-implantation loss, post-implantation loss, live fetus, early resorption, and late resorption in the uterus was calculated using Eq.2-6.[48-51]

Pre-implantation loss (%) = (No of Corpora Lutea(CL) – No of implant)/(No of Corpora lutea)×100 (Eq.2)

Post-implantation loss (%)= (No of implant-No of live fetus)/(No of implant)×100 (Eq.3)

Live fetus (%) = (No of live fetus)/(No of implant) × 100 (Eq.4)

Early resorption (%) = (No of early resorption)/(No of implant) × 100 (Eq.5)

Late resorption (%) = (No of late resorption)/(No of implant) × 100 (Eq.6)

Table 2: Results of qualitative detection tests for phytochemical components from ethanolic PAL and PAR extracts.

Test	*Reference Result*	*Observed Result*	*PAL*	*PAR*
Test for detection of carbohydrates				
Molisch's test	Reddish violet ring at junction	Dark Reddish Violet ring present at junction	+	+
Benedict's test	Red precipitate	Light right precipitate	+	+
Fehling's test	Brick red precipitate	Red precipitate	+	+
Test for detection alkaloids				
Mayer's test	Dull white precipitate	Light white precipitate	+	+
Hager's test	Yellow precipitate	Yellow precipitate	+	+
Wagner's test	Reddish brown precipitate	Brown precipitate	+	+
Test for detection of glycosides				
Killer-Kilani test	Reddish brown color at junction	Reddish color present at junction	+	+
Salkowski's test	Development of reddish brown color	Reddish brown color	+	+
Libermann's test	violet to blue to green color	Green color	+	+
Test for detection of proteins and amino acids				
Xanthoproteic test	Orange color	No color development	-	-
Millon's test	Appearance of white precipitate, which turns red upon gentle heating	No white precipitate appears	-	-
Ninhydrin test	Blue to purple color	No color	-	-
Test for detection of Phenol				
Ferric chloride test	Formation of bluish black color	Appearance of dark blue color	+	+
Test for detection of Tannin				
Lead acetate test	Development of a white precipitate	White precipitate	-	+
Test for detection of steroids				
Libermann's test-Burchard test	Formation of dark pink color	Pink color appeared	+	+
Salkoeski test	Red color obtained in upper chloroform layer	Light red color appeared in chloroform layer	+	+
Test for detection of flavonoids				
Alkaline reagent test	Intense yellow color upon addition of base and turns colorless on addition of acid	Yellow colorization on adding NaOH and become colorless on adding HCl	+	+
Shinoda test	Red color	Red color appears	+	+
Lead acetate test	Appearance of yellow color precipitate	Yellow color appears	+	+
Test for detection of saponins and terpenoids				
Froth test	Formation of froth	1-2 cm height froth formed	+	+

+: Present was detected for component in test; -: Present was not detected for component in test.

Table 3. Effect of PAL and PAR extract on body weight during abortifacient activity.

Group and treatment	*Mean Body weight (g)*					
	Day 0	*Day 3*	*Day 6*	*Day 9*	*Day 12*	*Day 15*
Normal Control	232.60 ± 4.49	236.03 ± 4.46	242.42 ± 4.58	267.23 ± 6.84	283.67 ± 3.86	296.88 ± 5.68
Standard Control	233.87 ± 5.50	238.47 ± 6.73	244.38 ± 5.72	248.92 ± 5.72***	253.15 ± 5.37***	256.97 ± 5.17***
PAL_100	230.85 ± 5.33	235.80 ± 5.65	244.02 ± 6.01	264.17 ± 5.41	279.13 ± 5.46	291.13 ± 6.33
PAL_200	231.83 ± 5.62	237.17 ± 6.08	243.42 ± 5.98	257.00 ± 6.43*	269.63 ± 6.81***	279.45 ± 5.20***
PAL_400	231.32 ± 4.32	234.92 ± 5.20	242.27 ± 4.71	248.68 ± 4.55***	256.65 ± 3.68***	263.78 ± 3.51***
PAR_100	232.97 ± 4.37	237.68 ± 4.58	244.68 ± 4.49	262.18 ± 3.33	275.17 ± 5.04	286.53 ± 4.04**
PAR_200	231.70 ± 5.10	235.58 ± 4.79	241.97 ± 5.07	253.93 ± 4.29**	263.78 ± 3.70***	271.83 ± 3.25***
PAR_400	231.27 ± 4.78	235.52 ± 4.95	242.10 ± 4.86	248.03 ± 5.05***	256.30 ± 5.33***	261.98 ± 5.48***

Values are expressed in Mean ± SEM for n=6, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs Normal control

Statistical Analysis

All the data are expressed as mean ± standard error of mean (SEM) for n=6. Statistical analysis was performed with one-way ANOVA followed by Dunnett's post-test at a confidence level of 0.05 (95% confidence interval) using graph pad prism version 5.03. For comparison with normal control, differences were considered to be statistically significant when * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. With decreasing the p values, the difference between the two groups is more significant and there is a lesser chance that this difference is due to an error or noise.

Results

Extract Yield

The percentage yield from leaves and roots of the *P. acuminata* was calculated using Eq.1 and the obtained values for PAL and PAR extracts were 15.3 and 13.5%, respectively.

Qualitative Phytochemical Screening

A qualitative analysis result of the phytochemical compounds from both extracts is shown in Table 2. Carbohydrates, alkaloids, phenolic, sterols, flavonoids, saponins, and terpenoids were confirmed by performing the respective test in both extracts. Proteins and amino acids were absent in both extracts, whereas tannins were detected from the root but absent from leaves extract.

Acute Toxicity Test for Dose Selection

No mortality and no female showed any sign or symptoms of toxicity but survived in healthy condition when observed for 14 post-dosing days. Hence the median lethal dose (LD_{50}) was determined to be higher than the highest dose tested, i.e. >2000 mg/kg. Hence $1/10^{th}$ of this dose, i.e., 200 mg/kg body weight was fixed as mid-dose. One lower dose and one higher dose, i.e., 100 and 400 mg/kg body weight, were fixed based on the mid-dose.

Abortifacient Model

Effect on Body Weight

The effect of the administration of different extracts on body weight is shown in Table 3. Compared to the normal control group, a statistically significant decrease in body weight was observed on day 9, day 12, and day 15 in the standard, PAL-200, PAL-400, PAR-200, and PAR-400 extract-treated group. On day 15 body weight was also noted significantly decrease in PAR-100 extract-treated group. Mean body weight among all the groups was comparable during the initial implantation phase, i.e., day 0, day 3, and day 6.

Effect on Reproductive Organs:

Organ weight data are summarised in Table 4. Compared to the normal control group, no significant visible changes were observed in the ovaries and oviduct, but significant changes were observed in the case of the uterus for all treatments as seen from Fig. 1. This observation is supported by the organ weight data (Table 4). Comparison of Group I and II unveiled a statistically significant decrease in the weight of the uterus due to post-implantation loss in a later one. Similarly, as the concentration of PAL and PAR extract increased in treatments, the uterus weight decreased, probably due to increase in the percentage of post-implantation loss.

Pictorial representation of one representative animal from diverse treatment groups. Respective treatment given to an animal is mentioned on the top row of each column.

Effect on Pre-natal Parameters

After the sacrifice of pregnant rats on GD-20, the effect of different treatments on the number of implantation loss, live fetus, and resorption are shown in Table 5 and Fig. 1. No significant changes were observed in pre-implantation loss when compared to the normal control group. In a post-implantation loss in the standard group, PAL and PAR extract-treated groups i.e., ~81%, ~25-65%, and ~48-75%, respectively, were found to be statistically significant when compared to the normal control group. Statistically

significant increase in early resorption in standard group ~38% whereas PAL and PAR extract represent ~6-15% and ~9-21% increase. Late resorption was found to have a statistically significant increase in standard, PAL, and PAR extract-treated group with the range of ~35%, ~11-25%, and ~17-24, respectively. Percentage of the live fetus was decreased in standard, PAL, and PAR extract-treated groups with the range of ~18%, ~34-74%, and ~24-51%, respectively.

Effect on Hematological Parameters

The blood analysis result is represented in Table 6. No statistically significant changes were observed in the mean value of WBC, HCT, MCV, MCH, and neutrophil compared to the normal control group. Notable decrease in dose-dependency was observed in RBC and platelet value and found statistically significant in standard and PAR-400 extract-treated groups. Mean Hb value was significantly decreased in standard, PAL-400 and PAR-400 extract treated groups.

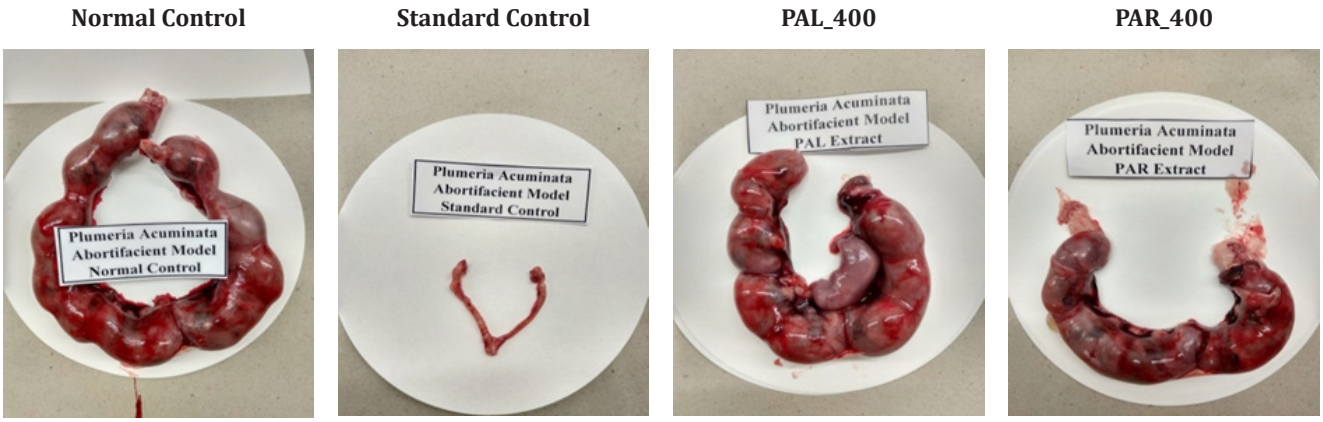

Fig. 1: Effect of PAL and PAR extract on uterus during abortifacient activity.

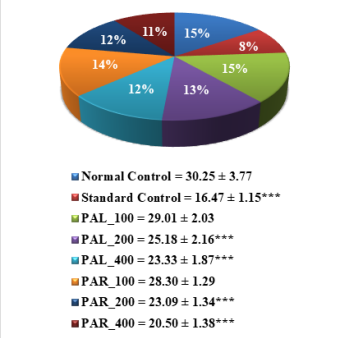

Fig. 2 a: Effect of PAL and PAR extracts on estrogen level

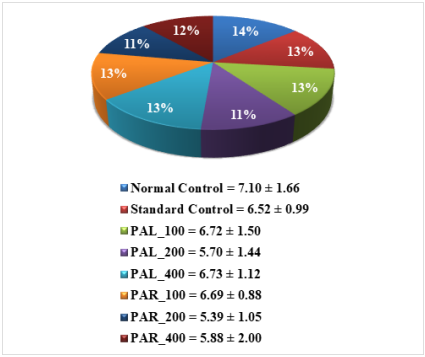

Fig. 2 b: Effect of PAL and PAR extracts on FSH level

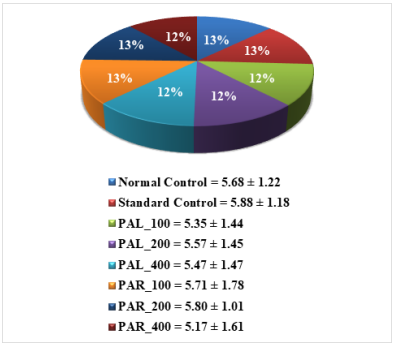

Fig. 2 c: Effect of PAL and PAR extracts on LH level

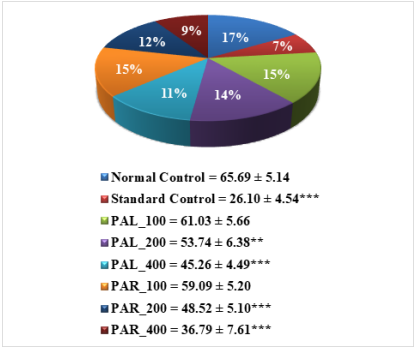

Fig. 2 d: Effect of PAL and PAR extracts on progesterone level

Fig. 2: Effect of PAL and PAR extracts on hormonal levels during abortifacient activity.
(2a) Estrogen, (2b) FSH, (2c) LH, and (2d) Progesterone. Values are expressed as Mean ± SEM for n=6, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for Test-items v/s Normal control;

Table 4: Effect of PAL and PAR extract on organs weight during abortifacient activity in adult female rats.

Group and treatment	*Mean organ weight (mg)*		
	Ovary (L+R)	*Uterus*	*Oviduct (L+R)*
Normal Control	162.83 ± 8.53	64.50 ± 9.55	39.13 ± 4.63
Standard Control	161.83 ± 13.32	25.01 ± 6.86***	37.33 ± 4.50
PAL_100	158.50 ± 12.20	58.03 ± 7.72	38.93 ± 2.72
PAL_200	160.33 ± 6.87	51.74 ± 6.64	37.42 ± 4.97
PAL_400	165.00 ± 11.06	40.68 ± 7.54***	38.60 ± 3.87
PAR_100	163.67 ± 7.43	52.07 ± 7.24	37.65 ± 5.51
PAR_200	161.33 ± 14.16	41.30 ± 7.75***	39.55 ± 3.84
PAR_400	159.33 ± 8.98	29.96 ± 8.03***	38.00 ± 5.66

Values are expressed in Mean ± SEM for n=6, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs Normal control

Table 5. Effect of PAL and PAR extract on pre-natal parameters during abortifacient activity in adult female rats.

Group and treatment	*Pre-natal parameters*				
	Pre-implantation loss (%)	*Post-Implantation loss (%)*	*Live fetus (%)*	*Early resorption (%)*	*Late resorption (%)*
Normal Control	5.14 ± 5.78	5.90 ± 6.61	94.10 ± 6.61	1.67 ± 4.08	4.23 ± 6.63
Standard Control	8.77 ± 11.93	81.92 ± 4.69***	18.08 ± 4.69***	38.95 ± 18.47***	35.65 ± 12.05***
PAL_100	4.75 ± 5.46	25.77 ± 23.37	74.23 ± 23.37	6.67 ± 7.71	11.03 ± 9.43
PAL_200	2.38 ± 5.83	53.54 ± 7.11***	46.46 ± 7.11***	7.55 ± 9.46	19.47 ± 8.45
PAL_400	3.18 ± 4.94	65.84 ± 12.41***	34.16 ± 12.41***	15.36 ± 5.58	25.13 ± 9.84*
PAR_100	6.71 ± 5.44	48.33 ± 11.16***	51.67 ± 11.16***	9.43 ± 9.99	17.48 ± 13.43
PAR_200	2.22 ± 5.44	67.25 ± 4.77***	32.75 ± 4.77***	17.37 ± 6.67	27.47 ± 9.42**
PAR_400	1.67 ± 4.08	75.51 ± 8.18***	24.49 ± 8.18***	21.76 ± 13.81**	34.55 ± 5.59***

Values are expressed in Mean ± SEM for n=6, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs Normal control

Effect on Hormonal Levels

Changes in the hormonal levels of estrogen, progesterone, FSH, and LH for different study groups are indicated in Fig. 2(a-d). A statistically significant decrease in estrogen and progesterone level was noted in standard, PAL-200, PAL-400, PAR-200, and PAR-400 extract treated groups. The standard drug, PAL, and PAR extract did not show a remarkable effect on the mean level of FSH and LH and remained unaltered.

Effect on Endometrium Prostaglandins and Acetylcholine

The effect of PAL and PAR extract on endometrium prostaglandins and acetylcholine are represented in Fig. 3(a-c). Compared to the normal control group, a dose-dependent increase in PGE-1 level was observed in standard, PAL, and PAR extract-treated groups and noted as statistically significant. No remarkable changes were noted in PGE-2 levels in all treated groups. Standard and both extracts treated groups showed a significant increase in acetylcholine level and were found statistically significant at a higher dose.

Effect on Histology

In histopathology of the ovary (Fig. 4., Row 1), Group I showed normal morphology of many corpora lutea surrounding with lots of blood vessels. Whereas misoprostol, PAL, and PAR extract groups exhibited a large number of cystic follicle with less blood vessels. In the case of the oviduct (Fig. 4., Row 2), misoprostol, PAL, and PAR extract decreased the ciliated and peg cell quantity on a mucosal portion of the ampulla region when compared to the normal control group. In uterus sections (Fig. 4., Row 3), the control group showed normal morphology of uterus, including stratum compactum, stratum spongiosum, and myometrium region. In contrast, standard and both extracts treated group exhibited less thickness of the endometrium, decidualization of stromal cell, dilated blood vessels, and glandular and surface epithelium atrophy. In the cervical section (Fig. 4., Row 4), when compared to the normal control group, less proliferation in metaplastic squamous cells, a thin lining of epithelium cells, and less number of endo-cervical glands were noted in standard as well as both extracts treated groups. Similarly, in the case of vaginal sections (Fig. 4., Row 5), the height and thickness

Table 6. Effect of PAL and PAR extract on hematological parameters during abortifacient activity in adult female rats.

Group and treatment	*Haematological Analysis*							
	WBC (109/L)	*RBC (1012/L)*	*Hb (g/dL)*	*HCT (%)*	*MCV (fL)*	*MCH (pg)*	*Platelet (109/L)*	*Neutrophil (%)*
Normal Control	7.83 ± 1.12	7.33 ± 0.86	12.88 ± 1.06	35.67 ± 4.35	52.33 ± 4.04	16.47 ± 1.36	1057.67 ± 131.32	37.00 ± 4.76
Standard Control	7.30 ± 1.30	5.17 ± 0.55***	10. 07 ± 1.03***	34.05 ± 3.12	51.70 ± 4.19	16.17 ± 1.39	850.50 ± 91.18*	38.17 ± 4.49
PAL_100	7.22 ± 1.13	7.15 ± 0.82	12.07 ± 0.92	36.83 ± 3.73	51.47 ± 4.44	15.98 ± 1.25	1027.67 ± 103.87	35.83 ± 3.80
PAL_200	7.15 ± 1.03	6.83 ± 0.89	12.12 ± 1.12	35.53 ± 5.79	53.82 ± 3.98	18.68 ± 1.00	1013.83 ± 72.11	34.00 ± 4.83
PAL_400	8.15 ± 1.17	6.17 ± 0.72	11.07 ± 1.21	35.50 ± 4.76	52.40 ± 3.75	16.72 ± 1.77	923.33 ± 93.57	38.00 ± 4.28
PAR_100	7.30 ± 0.90	7.73 ± 0.60	12.15 ± 0.83	36.43 ± 4.57	51.18 ± 4.27	17.08 ± 1.56	1041.50 ± 108.32	35.33 ± 4.11
PAR_200	7.08 ± 1.22	6.22 ± 0.37	12.33 ± 0.81	36.08 ± 3.77	51.72 ± 4.30	17.00 ± 1.46	1009.67 ± 135.78	34.33 ± 4.15
PAR_400	8.27 ± 1.03	5.48 ± 0.63**	10.28 ± 0.79**	34.07 ± 2.60	54.30 ± 3.19	17.77 ± 1.11	803.33 ± 90.79**	34.17 ± 4.45

Values are expressed in Mean ± SEM for n=6, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs Normal control

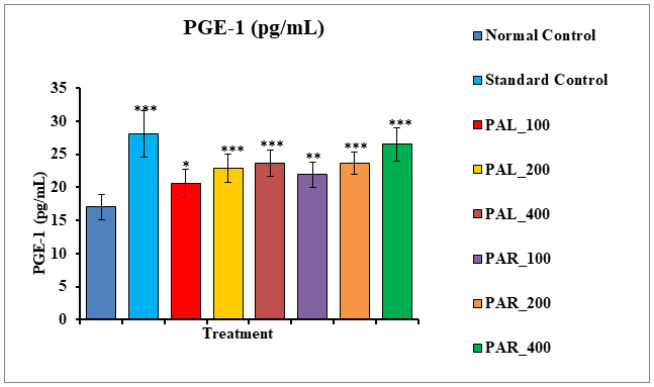

Fig. 3a: Effect of PAL and PAR extracts on PGE-1 level

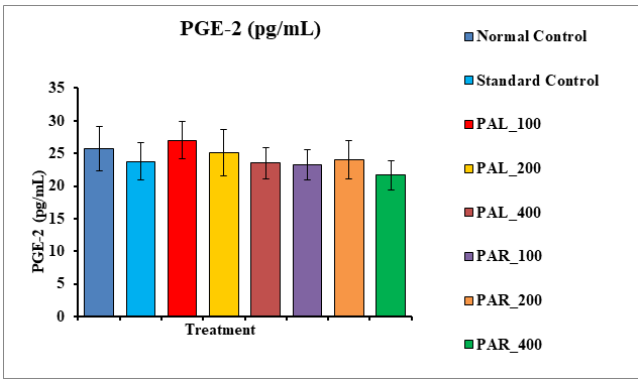

Fig. 3b: Effect of PAL and PAR extracts on PGE-2 level

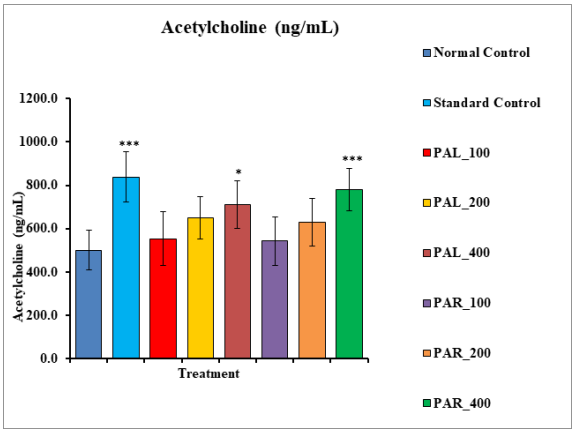

Fig. 3c: Effect of PAL and PAR extracts on acetylcholine level

Fig. 3: Effect of PAL and PAR extracts on prostaglandins and acetylcholine levels during abortifacient activity.
(3a) PGE-1, (3b) PGE-2, (3c) Acetylcholine. Values are expressed as Mean ± SEM for n=6, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for Test-items v/s Normal control;

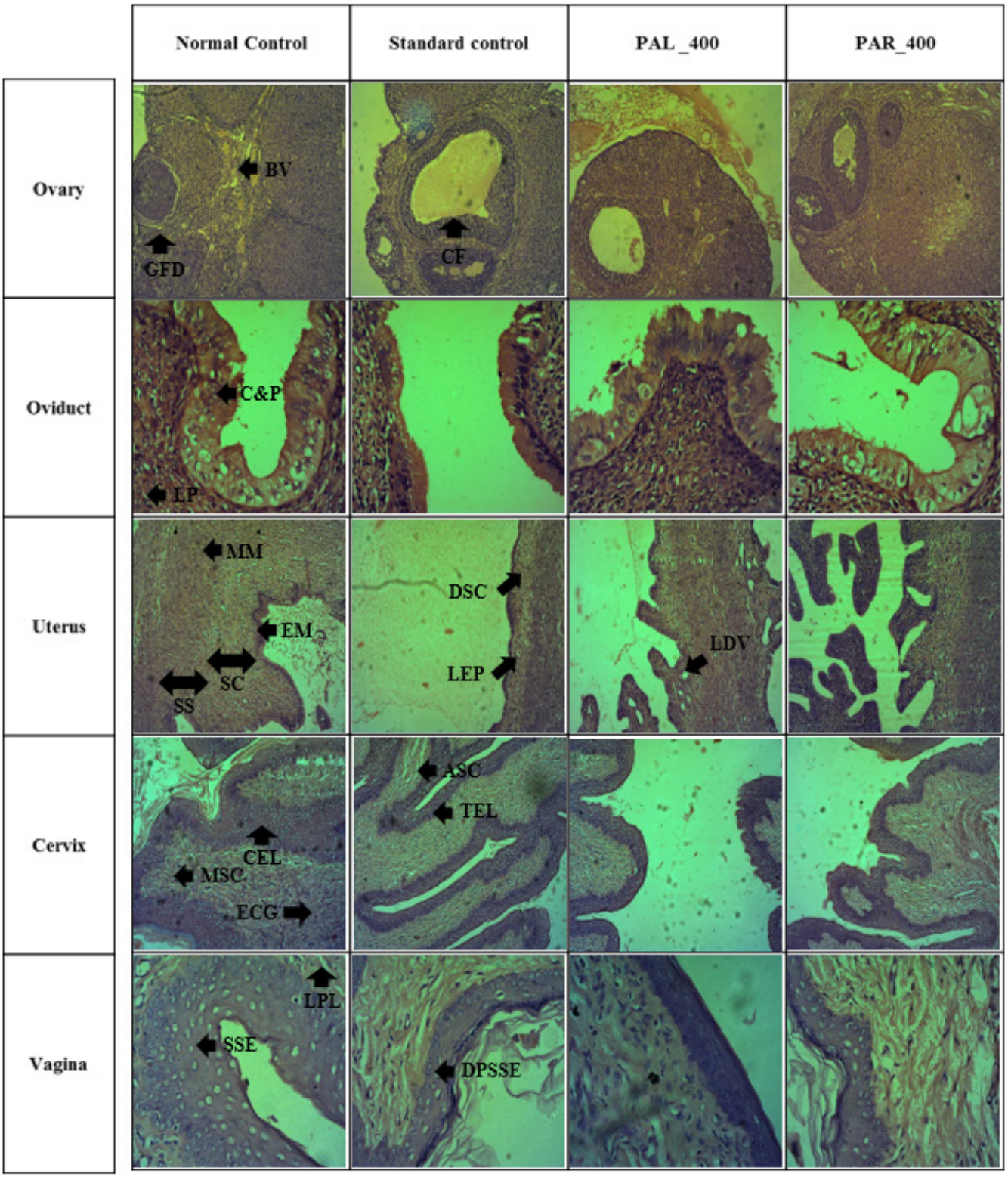

Fig. 4: Effect of PAL and PAR extracts on histology of reproductive organs during abortifacient activity.
Representative H & E stained photomicrographs of the (Row 1-5) ovaries, oviduct, uterus, cervix, and vagina, respectively from diverse treatment groups. Respective treatment given to animals is mentioned at the top of each column. Ovary: Graafian follicle degeneration (GFD), Blood vessels (BV), Cystic follicle (CF) visualized at 10x; Oviduct: Ciliated and peg cell (C&P), Lamina propria (LP) visualized at 40x; Uterus: Stratum compactum (SC), Stratum spongiosum layer (SS), Endometrium (EM) and Myometrium (MM), Decidualisation of stratum cell (DSC), Less endometrium proliferation (LEP), Large dilated vessels (LDV) visualized at 10x; Cervix: Metaplastic squamous cells (MSC), Columnar epithelium lining (CEL), Endo-cervical glands (ECG), Atrophied squamous cell (ASC), Thin epithelium lining (TEL) visualized at 10x; Vagina: Stratified squamous epithelium (SSE), lamina propria layer (LPL) and Decreased proliferation of SSE (DPSSE) visualized at 40x.

of the epithelium layer were decreased in misoprostol, PAL, and PAR treated groups due to a decrease in proliferation of stratified squamous epithelium layer when compared to the normal control group.

Discussion

According to ethnobotanical data, a wide number of naturally occurring moiety from plant origin has been used as an anti-fertility drug by eliciting different activities in the uterine environment. Stimulating labor and assisting easy delivery is the generally prime concept of most abortifacient agents.[52]

With the help of modern technology, a plant-derived *in -vivo* experiment has made noteworthy improvements in the development of new antibiotics, and remedies for cancer and still contributing to the exploration of the reproductive area where they can be beneficial in terms of safe abortion, pre-natal complication, and pre-and post-coital contraceptives. *Carica papaya* seeds, *Garcina kola* seeds, *Bambusa vulgaris* leaves, etc., have been validated in rats as an abortifacient drug.[53]

During pregnancy, an elevated level of estrogen plays a significant role in creating new blood vessels in the region of the uterus and placenta. Progesterone is necessary for ovulation, implantation, maintenance of uterus lining, maturation of the fertilized egg and stopping the release of an egg from the ovaries until gestation has been finished. [10] Prostaglandins belong to the family of eicosanoid proteins. They are one type of lipid mediator secreted by the placenta and uterus that can cause uterine contraction. [54] The contractility of the uterus is regulated by various factors such as phospholipase C, inositol triphosphate, protein kinase C and A.[55] Prostaglandins encourage Ca^{2+} release from myometrium cells. It has been reported that PGE-1 and PGE-2 play a crucial role in contracting the myometrium, ripening of the cervix, and commencement of parturition in humans and animals. Research scholars noted that prostaglandins also affect hormonal synthesis and directly or indirectly alter uterine sensitivity.[56–58] Plumericin, sterol and lupeol already have been reported to possess inflammatory and anti-inflammatory activities by increasing prostaglandin synthesis as well as by interrupting natural action of PGE-1 and PGE-2 receptors [27] Reports also indicate that with sterol treatment, the release of prostaglandins PGE2 and PGF-1α has been significantly increased which are the well-known mediators of inflammation and myometrium contraction. [59] Stigmasterol also takes part in prostaglandins, nitric oxide (NO) and capsaicin-sensitive sensory neurons (CPSN) regulatory functions.[60] Lupeol also regulates various enzymes, namely Na^{+}, K^{+}-ATPase, Ca^{2+}-ATPase (responsible for myometrium contraction), and Mg^{2} as well as their release and functions.[61] Thus, PAL and PAR extracts containing diverse phytochemicals as shown in Table 2 were considered effective for authentication of their abortifacient activity and their results from animal models are discussed further. After performing acute toxicity, doses were selected based on no mortality and no adverse clinical sign noted in all animals.

Numerous parameters estimated in this model, are beneficial to judge the capacity of a plant as an abortifacient agent and its possible mechanism of action. Maternal body weight is directly proportional to the number of implants growing in the uterus and decreases in the maternal body weight, suggesting less number of the implant with more post-implantation loss.

Animals lost a substantial amount of blood during an abortion, which was the reason for decreasing the level of RBC and Hb in standard, PAL, and PAR extract treating groups. The fall in red blood cell (RBC) and homo globin (Hb) is associated with metrorrhagia, pre-clinically and clinically reported in several animal and human studies. [50,62-64]

Low levels of estrogen and progesterone observed in standard, PAL, and PAR extract could be due to extracting's effect on ovarian hormone synthesis or release or binding to their respective receptors. The consistent steadiness of both estrogen and progesterone is required and any kind of disturbances may lead to infertility or abortion.[65] Various *in vivo* pharmacological studies reveal that mean levels of estrogen and progesterone, in the case of abortion, were found significantly lower compared to normal pregnant rats.[66] A low level of both E and P in the body fluids could be correlated to pregnancy failure.[5]

PAL and PAR extract did not have a significant effect on the weight of ovaries and oviducts on the other hand uterus weight was dose-dependently reduced due to thin lining of squamous epithelium, lack of hormonal support, due to uterine bleeding, and less number of growing fetuses. Previously experimented studies clarify that proper regulation of estrogen and progesterone is necessary for maintaining uterine weight and its growth.[67]

When animal behavior was observed, no sign of discomfort, pain, or stress was noted. PAL and PAR extracts caused vaginal bleeding that could be due to a low level of progesterone on different days of gestation which confirmed the abortifacient potential of both extracts in rats.

A key component of female reproductive physiology, especially in the ovary, is folliculogenesis.[68] Atresia is the condition where most of the ovarian follicles, above 99.9% go through a deteriorating phase at different stages of their development, and out of these only a few follicles touch the ovulatory phase.[69] Alteration of estrogen and FSH could be the reason for developing large cystic follicles in the ovary. *In-vivo* rat studies suggests that estrogen and FSH might alter the pre-ovulatory follicle development greater than 600 μ in diameter and the same may increase with the age.[70,71] The fallopian tube is furnished with