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ANTI-FERTILITY EFFICACY OF ISOLATED COMPOUNDS FROM *LEPTADENIA RETICULATA* AND *LAWSONIA INERMIS*

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ABSTRACT

Plant based traditional medicines for birth control has been in practice in rural populations of India, since time immemorial. Fast rise in population has caused serious issues within the economic process, resulting in impoverishment in developing countries like India. To control these issues Synthetic Abortifacient drugs were used which leads to major side effects. So the other urge towards medicinal plants have been used. Dry roots of *Lawsonia inermis* and whole plant of *Leptadenia reticulata* plant parts used traditionally by different ethnic communities in India for birth control. The present investigation is aimed to justify the scientific basis in traditional use of *Lawsonia inermis and Leptadenia reticulata* as anti-fertility agent. Compounds which is isolated from *Leptadenia reticulata* are 6-amino-3-hydroxy-4-(4'-methylphenyl)-2*H*chromen-2-on is LR S1. The compounds isolated from the *Lawsonia inermis* was 3, 7, 4', 5'-Tetrahydroxy-6 methoxyflavone is LI S1. Acute toxicity test of compounds was carried out in mice. The dose has been fixed as 200mg/kg. The abortifacient and anti-implantation activities of the compounds were investigated by Animal studies. The results revealed the effectiveness both in Anti-implantation and abortifacient activity. As a conclusion, the present study indicates that isolated flavonoid compounds shows the antifertility effect and it is safe at the effective antifertility doses used in this study.

KEYWORDS: **Anti-fertility, *Lawsonia inermis, Leptadenia reticulata,* Population, Investigations.**

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INTRODUCTION

One of the elemental areas of human life is fertility and birth control. Fast rise in population has caused serious issues within the economic process and well-rounded human development in countries like African country and India, resulting in impoverishment. Birth prevention has been promoted through many strategies of birth control and abortion like surgical intervention, medication like artificial endocrine contraceptives (e.g. abortion pill, misoprostol), prostaglandins and antiprogestins, however they're typically marked with serious aspect effects like canal issues, severe and painful female internal reproductive organ contractions, general health problem, permanent sterility or perhaps death.[1] Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being.[2] A number of investigations are dole out on historically claimed anti-fertility plants to validate the claim. Recent literature review discovered that forty eight out of seventy two historically utilized medical plants for fertility management had anti-fertility potential .[3] *Lawsonia inermis* is a vital medicative plant within the Indian system of medication. it's normally referred to as henna, that grows in heat and arid regions. The dye derived from inexperienced leaves of henna and it is employed to brighten the body with involved styles and therefore the principle coloring matter is lawsone, 2-hydroxy-1, 4- fat-soluble vitamin.[4] Root is taken into account as a potent drugs for venereal disease and herpes infection. Root is astringent could also be pulped and used for sore eyes. Pulped root may additionally be applied to the heads of youngsters forboils. Ready indigo is used as a robust abortifacient. The basis is meant to be helpful in treatment of hysteria and nervous disorders.[5-7] *Leptadenia reticulata* (jivanti) is distributed in tropical and sub-tropical elements of Asia and continent. In India, it's found in Gujarat, sub -Himalayan tracts from geographic region to countryside and Khasi hills and throughout land Republic of Republic of India, ascending up to a altitude of 900 metres. In tamilnadu it's found in several districts particularly in thirunelveli and it conjointly grows in heat and arid regions.It boosts energy of the body and it's useful for the patient for the persons from weak feebleness or absence of energy. It can increase longevity, memory improvement ,immune modulation and adoption.[8] The whole plant ameliorates 'tridoshas' (Vatta, passeriform bird and Kapha), and is of nice worth commonly feebleness, involuntary seminal discharge, as a stimulant and snake bite[9-10],abortifacient, tonic, restorative, antiseptic, antifabrifuge, wound healer and in mouth lesion.[11]

MATERIALS AND METHODS

Collection and Identification of Plant materials

Dry roots of *Lawsonia inermis* and whole plant of *Leptadenia reticulata* were collected from Thirunelveli district, Tamil Nadu, India. This root and whole plant was identified and authenticated by Dr. V. Chelladurai, Research officer - Botany (scientist C),Central council for research in Ayurveda and Siddha, Govt. of India; Thirunelveli. The voucher Specimen of *Lawsonia inermis* have been deposited at the Rapinat Herbarium (trichy) and the voucher number is RHCDBP2010.For *Leptadenia reticulata* the specimen is deposited in BSI Herbarium (Coimbatore) and voucher no is 54373.The roots and whole plant were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags till use.

Preparation of extracts and phytochemical screening

The dried roots and the whole plant were extracted with Ethanol by using soxhlet extractor. The extract that is obtained is concentrated with rotary evaporator until dry powder was obtained. The preliminary chemical science investigation was finished and the identification of chemical constituents is consistent.[12]

Isolation of compounds

Isolation was done by coloumn chromatography. The compounds were isolated from both the plants of *Leptadenia reticulata* and *Lawsonia inermis* Compounds which is isolated from *Leptadenia reticulata* are 6-amino-3-hydroxy-4-(4'-methylphenyl)-2*H*chromen-2-on is LR S1.The compounds isolated from the *Lawsonia inermis* was 3, 7, 4', 5'-Tetrahydroxy-6 methoxyflavone is LI S1.[13-14]

Experimental Animals

The acute toxicity study was done by female (8-week old) young Swiss mice (20-25) g weight. For Anti-implantation and Abortifacient activity Wistar male and feminine rats were used. All the experimental animals were placed in properly numbered huge propene cages with stainless-steel high grill having facilities for pelleted food. The animals were maintained in twelve hours light-weight and unbroken darkly at 28 ^{0} C ± 2 ^{0} C in an extremely sensible maintained animal house in huge propene cages. These animals were acclimatizing to habitual workplace conditions for 10 days before the initiation of the experiment. The pelleted diet is given to the animals as a food that is purchased from Coimbatore (AVM Foods). Husk bedding is created by paddy. The animals got, unlimited of filtered drink. More animal studies, done by following moral tips suggested by the institutional animal committee (IAEC) of KMCH faculty of Pharmacy Coimbatore, (KMCRET/Ph.D/12/2014-2015). The current study was approved by Institutional of animal committee (Ethical) of KMCH college of Pharmacy Coimbatore, (KMCRET/Ph.D/12/2014-2015). The ethical number is 12.

EXPERIMENTAL PROCEDURES

Acute toxicity studies

To determine the dose of isolated compound of *Lawsonia inermis* and *Leptadenia reticulata,* acute-toxicity studies were applied. Every cluster contains three animals and can be housed singly. The test compounds had been given as one dose, whereas giving the following dose of the check medication, food ought to be withheld for one unit of time in mice. Animals are determined for behavioural modification, toxicity checking for a period of time up to 24hrs and observations are done for fourteen days after acute toxicity dose. The compounds are given at the dose of levels 5, 50, 300 and 2000 mg/kg weight. If the I st cluster(group) animals survived, the next cluster(group)

animals can receive a better dose as per OECD guidelines 423.

Vaginal smear method

Vaginal smear cytology is determined the estrous cycle stages of feminine rat. The stages of estrous cycle were determined by getting vaginal smears. For this, initial skinny cotton bud was taken that was swayback into traditional saline (0.9 % w/v). The rat was command loosely on left side, whereas the vaginal margins were separated, the cotton bud turned within dextral doubly with associate degree angle of 450. The fabric cotton obtained from the pledget was transferred to a clean slide. The slide was stained with stain(methylene blue) (0.05% w/v) for seven min. The slide was gently washed with plain water to get rid of excess stain and therefore the stained slide was left in a very temperature for ten min. The slide was later examined for the varied stages of estrous cycle beneath the magnifier. The stage of estrous cycle was classified per the cell kind determined beneath magnifier. The animals that showed the regular estrous cycle of 4-5 days were elite for more pharmacologic study.[15]

Anti-implantation activity

Vaginal smear from every rats are monitored daily and solely rats with usual oestrous cycle are choosen for the study. Proestrus phase rats are caged with males of evidenced fertility, within the relation of 3:1 ratio and examined the subsequent morning for proof of copulation. Rats exhibiting thick clumps of spermatozoa in their duct smears are separated and that day are choosed as day one of gestation and those rats divided into 3 groups, six rats in every cluster. Generally six male rats are used for mating functions for each Anti-implantation and Abortifacient activity. The selected rats were treated with group 1(control at the dose of 1% tween 80 p.o) group 2 (LR S1 at the dose of 20mg/kg) and group 3(LI S1 at the dose of 20mg/kg). These doses were given from 1st day to 7th day of pregnancy.The animals were laparotomised on day 10 of the pregnancy under Ketamine anesthesia and uteri were examined to determine the number of implantation sites.Then the animals were sacrificed under cervical dislocation method .[15] At the end of this experiment all animals are sacrificed and the blood was collected through cardiac puncture for studying haematological parameters and serum was separated for studying biochemical parameters. The uteri were dissected out and surrounding tissues removed and were blotted on filter papers. The tissues from the uteri were removed for histopathological studies.

Abortifacient activity

The female rats are to be caged with male rats of well-known fertility within the ratio of 2:1 within the evening of proestrous stage. Animals are going to be examined in the following morning for the presence of sperms. Rats exhibiting thick clumps of spermatozoa within the cytologic smear are separated, and that day has selected as day one of maternity. The pregnant rats are going to be divided into three groups containing six rats in every cluster. The group1 receive the vehicle(dose of 1% tween 80 p.o). The same dose will be given for LR S1 (Group 2) and LI S1 (Group 3) as determined in anti-implantation activity.The Compounds will be administered in oral route by gastric gavages during 6th to the 15th day of pregnancy (period of organogenesis). The animals will be laparotomised under ketamine anesthesia on the 19th day of pregnancy. Both horns of the uterus will be observed for the number of implantation sites, resorptions, and dead and alive fetuses .[16]At the end of this experiment all animals are sacrificed and the blood was collected through cardiac puncture for studying haematological parameters and serum was separated for studying biochemical parameters. The uteri were dissected out and surrounding tissues removed and were blotted on filter papers. The tissues from the uteri were removed for histopathological studies.

Statistical evaluation

Data obtained which is expressed as Mean ± SEM (Standard error mean).The stastistical analysis were carried out by ANOVA method followed by Dunnett's test and P ≤ 0.05was considered constant.

RESULTS

Preliminary phytochemical screening

The extract was subjected to preliminary phytochemical screening for the detection of two plant constituents like Alkaloids, Carbohydrates, Glycosides, Phytosterols, coumarins, Flavonoids, Tannins and phenolic compounds, Proteins and amino acids, Saponins, Fixed oils. The positive results showed in two plants are Carbohydrates, Glycosides, Flavonoids, Saponins Tannins and terpenes. The compounds which are used in animal studies are the flavonoid constituents.

Table 1

Anti-implantation activity of isolated compounds in female rats

Group	Treatment	No.of pregnancies/no. of treated	No. of implantation sites in individual rats	Total no of litters delivered by individual rats	Avg. Total no litters delivered	Avg. no. of implantation	Anti - implantation activity (%)
I	Control (0.5ml tween 80 1% p.o)	6/6	8, 8, 9, 8, 10, 9	52	8.1	8.6 ± 0.4	0
II	LR S1	6/6	0,0,0,0,0,0	-	0	0	100
III	LI S1	6/6	0,0,0,0,0,0	-	0	0	100

Values are Mean ± SEM (n = 6 in each group).

Histopathological studies of uterus of animals treated with LR S1 and LI S1 for antiimplantation activity

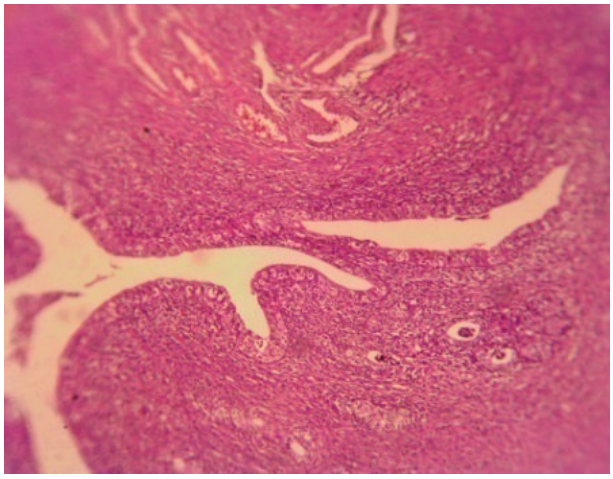

Figure 1
Normal control rats treated with tween 80 p.o

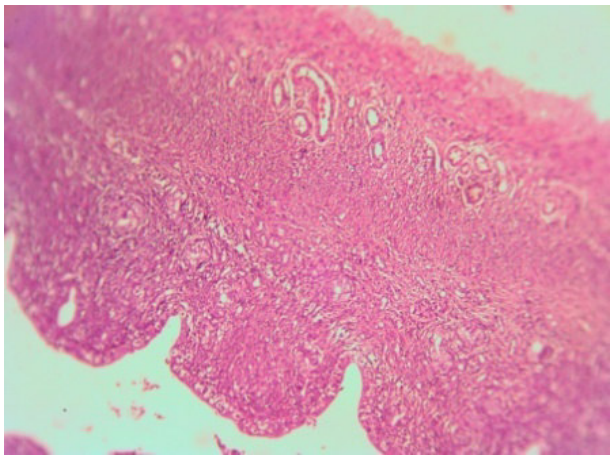

Figure 2
Rats treated with LR S1 at 20 mg/kg/b.w

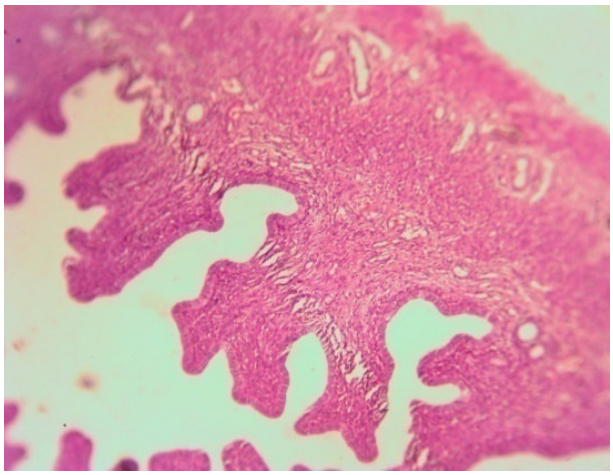

Figure 3
Rats treated with LI S1 at 20 mg/kg/b.w

Table 2
Hematological parameters of isolated compounds (Anti-implantation activity)

Parameters	Normal values	Control	LR S1	LI S1
RBC (mill/mcl)	7-10	9.04 ± 0.10	9.16 ± 0.13	9.18 ± 0.14
WBC (thous/mcl)	6-18	12.40 ± 0.20	12.82 ± 0.14	12.98± 0.21
Haemoglobin (g/dl)	11-19.2	13.04 ± 0.15	13.72± 0.15	13.64 ± 0.29
HT (%)	35-48	41.20 ± 0.86	41.60 ± 0.92	42.20 ± 0.86

Values are Mean ± SEM (n = 6 in each group).

Table 3

Biochemical parameters of isolated compounds (Anti-implantation activity)

S. No.	Parameters	Normal values	Groups		
			Control	LR S1	LI S1
1.	Blood glucose(mg/dL)	75-140	136± 4.19	126.40± 2.01	134.20± 1.88
2.	Cholesterol (mg/dL)	20-80	72.40± 2.01	61.20± 3.70*	87.40± 3.31**
3.	Triglycerides(mg/dL)	124-156	144.80±4.87	107.60±4.20***	168.80±5.16**
4.	LactateDehydrogenate (LDH) (IU/L)	120-220	174.20±2.76	168.20±2.87	164.60±2.73
5.	Total Protein (g/dL)	5.3-7.5	6.52± 0.13	7.40± 0.12**	5.78± 0.19*
6.	Acid Phosphatase (IU/L)	20-35	25.40± 0.92	23.40± 1.32	21.60± 0.92
7.	Alkaline Phosphatase (IU/L)	90-170	134.20±1.24	129.20± 1.68	206.80± 28***
8.	SGOT (IU/L)	42-98	77.40± 2.01	80.80± 2.05	83.60±3.01
9.	SGPT (IU/L)	12-67	53.60± 1.80	47.20± 2.26	50.40± 2.06
10.	GGT (IU/L)	22-50	38.40±2.13	36.40±1.72	33.60±1.63

Values are Mean ± SEM (n = 6 in each group).

****P< 0.05 when compared with the control; **P< 0.01 when compared with the control.***

Table 4

Abortifacient effects of isolated compounds in female rats

Group	Treatment	No. of foetus in individual rat	No of resorptions l rat	AveragNo. fetuses	Foetus weight (g)	No. of Rats aborted	Abortion (%)
I	Control (1% Tween80 1%)	9,10,8,10,11,8	0,0,0,0,0,0	9.33 ± 1.05	1.46 ± 0.05	0/6	0
II	LR S1	0,0,0,0,0,0	-	-	0	6/6	100
III	LI S1	0,0,0,0,0,0	-	-	0	6/6	100

Values are Mean ± SEM (n = 6 in each group).

Histopatological studies of Uterus of LR S1 and LI S1 treated animals in abortifacient activity

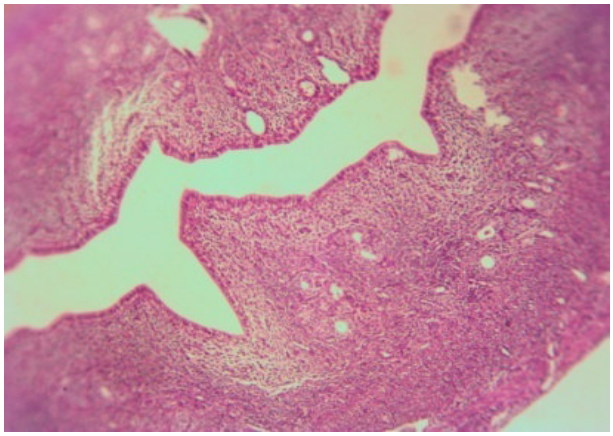

Figure 4

Section of the uterus in normal Control rats treated with tween80

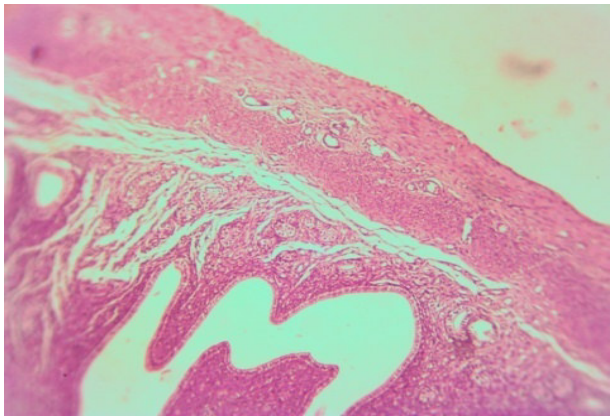

Figure 5

Section of the uterus in rats treated with LR S1 at 20mg/kg/b.w

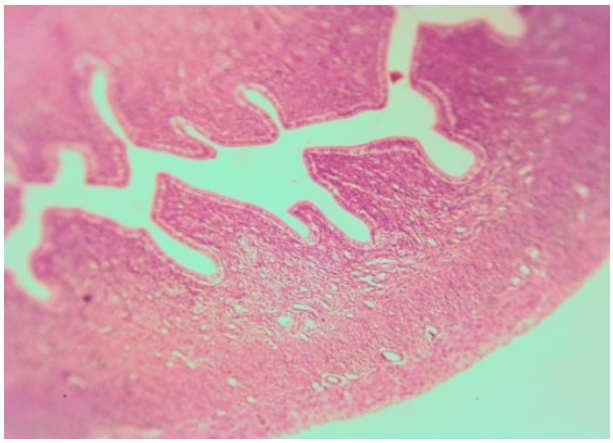

Figure 6
Section of the uterus in rats treated with LI S1 at 20mg /kg

Table 5
Hematological parameters of isolated compounds (Abortifacient Activity)

Parameters	Control (Tween 80 p.o)	LR S1	LI S1
RBC (mill/mcl)	8.04 ± 0.31	8.52 ± 0.13	8.64 ± 0.12
WBC (thous/mcl)	12.64 ± 0.25	13.04 ± 0.09	13.22 ± 0.13
Haemoglobin (g/dl)	14.46 ± 0.37	15.30 ± 0.18	15.02 ± 0.11
HT (%)	40.20 ± 0.39	39.24± 0.21	39.50 ± 0.26

Values are Mean ± SEM (n = 6 in each group)

Table 6
Biochemical parameters of isolated compounds (Abortifacient activity)

S. No	Parameters	Normal values	Groups		
			Control (Tween 80.p.o)	LR S1	LI S1
1.	Blood glucose (mg/dL)	75-140	127.30± 1.05	125.50± 0.76	130.30 ± 1.89
2.	Cholesterol (mg/dL)	20-80	74.67± 1.47	79.67± 0.80*	124.50± .43***
3.	Triglycerides (mg/dL)	124-156	143.80± 1.66	114.00±1.65***	157.30±1.59***
4.	Lactate Dehydrogenase (LDH) (IU/L)	120-220	170.80±1.37	164.00±1.31	163.50±4.01
5.	Total Protein (g/dL)	5.3-7.5	6.78± 0.08	10.27± 0.19***	5.45± 0.17***
6.	Acid Phosphatase (IU/L)	20-35	26.80± 0.58	28.80± 0.86	29.20± 1.06
7.	Alkaline Phosphatase (IU/L)	90-170	122.80± 1.24	125.80± 2.00	179.50± 2.57***
8.	SGOT (IU/L)	42-98	79.40± 1.72	84.60±1.50	80.60± 2.13
9.	SGPT (IU/L)	12-67	50.40± 1.80	54.80± 2.59	52.40± 2.06
10.	GGT (IU/L)	22-50	38.20±1.20	38.80±2.15	34.80±2.72

Values are Mean ± SEM (n = 6 in each group).
Statistically significant at *P< 0.05 when compared with the control **P< 0.01 when compared with the control

Acute toxicity studies

The results shows isolated compounds from *Leptadenia reticulata* -LR S1-(6-amino-3-hydroxy-4-(4'-methylphenyl)-2*H*chromen-2-on) and *Lawsonia inermis* –LI S1-(3, 7, 4', 5'-Tetrahydroxy-6- methoxyflavone*)* does not cause any harmful toxic effects upto 50mg/kg, but at the dose of 300mg/kg mortality and behaviourial changes were observed and LD_{50} value which is calculated as 200mg/kg, so the final dose was calculated as 20mg/kg and the oral route is selected for administration of drugs.

Anti-implantation and abortifacient activity

Table 1 and Table 4 represents the Anti-implantation and Abortifacient effects of isolated compounds in female rats. The LR S1-(6-amino-3-hydroxy-4-(4'-methylphenyl)-2*H*chromen-2-on) and LI S1- (3, 7, 4', 5'-Tetrahydroxy-6- methoxyflavone*)* shows 100% of anti-implantation activity because there is no litters delivered by individual rats and in abortifacient activity the total number (6) of rats are aborted.

Hematological parameters

Table 2 and table 5 represents the Anti-Implantation and abortifacient effects of Hematological parameters of both isolated compounds and there is no significance seen in RBC, WBC, Haemoglobin and HT % when compared to control.

Biochemical studies

Table 3 and Table 6 explained the biochemical parameters of isolated compounds for Anti-implantation and Abortifacient activity. The biochemical study shows the significance response in cholesterol, Triglyceries, Alkaline phosphatase and protein when compared to control for both Anti-implantation studies and abortifacient activity.

Histopathological studies

Histopathological studies of uterus of normal control rats (fig 1,4) showed normal uterus and normal epithelial lining. And that of LR S1(fig 2,5) and LI S1(fig 3,6) treated animals showed Anti-implantation and abortifacient activity as of disrupted epithelial cells.

DISCUSSION

The compounds which are used in this study are flavonoid compounds and it exerts the antifertility activity. In biochemical studies the significance shows in cholesterol, triglycerides, total protein and alkaline phosphatase. When the significant values are lesser than control the complete utilization take place, when it is higher than control non-utilisation takes place. In case of cholesterol, triglycerides, total protein these utilization or non utilization slighty disturbs the level of harmones like oestrogen and sometimes it inhibits the oestrogen production.[17-19] The precise role of alkaline phosphatases in the process of implantation still needs proper understanding although, it would be premature to correlate the changes in the uterine phosphatases and anti-implantation and abortifacient effect of test substances but the drop in the activity of the uterine phosphatases could conjecturally be playing a role in the prevention of pregnancy.[20] The experimental animals showed the anti-implantation and abortificent activity activity in contraceptive study. The number of litters born as a result of this treatment was significantly less (nill) in comparison to controls. This proves the anti-implantation and abortifacient nature of flavonoid compounds. This experiment clearly shows that the flavonoid compounds are most effective before and once the implantations occurred. Abortion refers to the premature expulsion of the merchandise of conception from the uterus. Abortion might even be as a result of maternal exposure to chemicals, which might disrupt maternity and cause detachment of the embroyo .[21] The Compounds LR S1 and LI S1 and B shows 100% abortifacient activity when compared with control. In the experiment oestrogen is a major reason for changes in female internal reproductive organ. It has been known that for implantation the precise equilibrium of oestrogen and Progesterone is important, and if any disturbance occur between two harmones it leads to Infertility. The compound with secretion activity usually disturbs the secretion surroundings at intervals in the feminine internal organ relates to Anti-fertility in experimental animals. Throughout this study, the anti-implantation activity might even be relating to oestrogenic activity, inflicting the expulsion of the ova from the tube, disrupting the luteotrophic activity of the blastosphere[21]

CONCLUSION

The present study indicates that isolated flavonoid compounds shows the antifertility effect and it is safe at the effective antifertility doses used in this study. Thus the herbal compounds shows the anti fertility effects equally to synthetic drugs and these herbal compounds have no side effects when compared to synthetic drugs. In future there will be more thirsty towards these herbal abortifacient drugs and their significance.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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