



RESEARCH ARTICLE

Anti-Fertility Activity of *Pterocarpus Santalinus* Heart Wood Extracts in Female Rats

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Manuscript No: IJPRS/V3/I2/00183, Received On: 14/04/2014, Accepted On: 20/04/2014

ABSTRACT

The present study was undertaken to evaluate the anti-fertility activity of *Pterocarpus santalinus* heart wood using different experimental models such as Anti-implantation activity, Estrous cycle study, and estrogenic /Anti-estrogenic activity. Toxic symptoms and mortality was studied for both ethanol and chloroform extract of *Pterocarpus santalinus* heart wood and both the extracts were found to be well tolerated up to 2g/kg. Hence 1/4th (500mg/kg) and 1/10th (200mg/kg) of the dose of this were selected for the study. Ethynyl estradiol 0.1µg/rat, i.m. (EED) was used as standard drug. The ethanol extract of *Pterocarpus santalinus* heart wood in both doses (500mg/kg and 200mg/kg) possesses anti-implantation activity by significantly reduced the number of implantation sites. Administration of ethanol extract to immature rats at doses 500 and 200mg/kg showed increase in the ovary weight and also resulted in increase in the levels of alkaline phosphatase and glucose levels but also showed a significant increase in the cholesterol level and hence proved to be an anti-fertility agent. The chloroform extract of *Pterocarpus santalinus* heart wood in both doses (500mg/kg and 200mg/kg) also possesses anti-implantation activity by slightly reduced the number of implantation sites. In immature rats the chloroform extract results in increase in the ovary weight and slightly increase in alkaline phosphatase, cholesterol and glucose levels and showed anti-fertility activity. Hence, it is concluded that the ethanol extract of *Pterocarpus santalinus* heart wood in both doses (500mg/kg and 200mg/kg) showed more anti-fertility activity than chloroform extract of *Pterocarpus santalinus* heart wood.

KEYWORDS

Pterocarpus Santalinus Heartwood, Anti-Implantation Activity, Estrous Cycle

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INTRODUCTION

The history of birth control began with the discovery of the connection between coitus and pregnancy. Probably the oldest methods of contraception are coitus interrupts lactation, certain barrier methods, and herbal methods.

Coitus interrupts probably predates any other form of birth control. Although it is commonly believed that pre-ejaculate fluid can cause pregnancy, modern research has shown that pre-ejaculate fluid does not contain viable sperm^{1,2}.

There are historic records of Egyptian women using a pessary made of various acidic substances and lubricated with honey or oil, which may have been somewhat effective at killing sperm³. Various abortifacients have been used throughout human history in attempts to terminate undesired pregnancy. Some of them were effective, some were not; those that were most effective also had major side effects. One abortifacient reported to have low levels of side effects-Silphium was harvested to extinction around the 1st century⁴.

Since the population rising tremendously, this may affect drastically the economic growth of India. Family planning has been promoted through several methods of contraception, but due to side effect produced by the use of steroidal contraceptive and use of abortifacient drugs⁵, so there is a need of drug which is effective with lesser side effects. The investigation of plant constituents with anti-fertility properties represents a potential alternative approach to birth control from the existing available methods.

Furthermore, plants can act as anti-fertility agents. In addition there are also plants that regulate fertility in males; these include antispermatogenic plants, spermicidal, semen coagulant plants, and fertility inhibiting plants⁶. Mechanism which is intended to reduce the likelihood of the fertilization of an ovum by a spermatozoon may more specifically be referred to as contraception⁷.

A large number of indigenous plants having such activities are recorded in ayurvedic literatures. In developing countries, all over the world, 80% of population continues to use traditional medicine in primary medical problems⁸. In spite of considerable development in contraceptive technology, search for female anti-fertility agent in plants continues to be a potential area of investigation.

Pterocarpus santalinus belongs to the family Fabaceae commonly called as Red Sandal wood or Rakthachandan. It is mentioned in the literature that a combination of red sandal wood, mustard and brown sugar in equal quantity prevents conception in women⁹. Rakthachandan has number of activities which include astringent, tonic and diaphoretic. A paste of the wood is used as cooling external application for inflammations and headache. It is said to be useful in bilious affections and skin diseases. A decoction of the fruit is used as an astringent tonic in chronic dysentery. Drinking water in Rakthachandan wooden cups twice a day used for the treatment of diabetes¹⁰. A histological stain prepared from the heart wood has been found to be an excellent nuclear stain for various cells of animal and plant origin^{11,12}.

Pterocarpus santalinus contains two aurone glycosides, 6 hydroxyl 5 methyl 3', 4', 5' trimethoxy aurone 4-O- α -L rhamnopyranoside and 6,4' dihydroxy aurone 4-O-rutinoside¹³, β -sitosterol, lupeol, epicatechin³, isoflavone glucoside (4', 5-dihydroxy 7-O-methyl isoflavone 3'-O-beta-D-glucoside¹⁴, pentacyclic triterpene¹⁵.

Though it is mentioned in the ayurvedic literature that it has anti-fertility activity no published scientific data is available for this. Hence an attempt will be made to evaluate the anti-fertility activity of Red sandal wood extracts in female rats.

MATERIALS AND METHOD

Plant Material

The *Pterocarpus santalinus* heartwood was collected in Andhar Pradesh from (Tirupati), India and authenticated by Dr. Madhav Shetty and retained in our laboratory for further studies.

Preparation of Plant Extract

1kg of the heartwood powder of *Pterocarpus santalinus* was defatted by extraction with petroleum ether and successively extracted further with chloroform and ethanol in a Soxhlet

extractor. The extracts will be dried under vacuum.

Experimental Animals

Female albino wistar rats weighing between 200-250gm were used. Institutional Animal Ethics Committee approved the experimental protocol; animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control, and Supervision on Experiments on Animals (CPCSEA). The animals were procured from authorized institution Central Animal Research of NIMHANS, Bangalore, 2 weeks prior to the study, so that animals could adapt to the new environment.

Acute Oral Toxicity Study

The toxicity studies conducted as per internationally accepted protocol drawn under OECD No 420 guidelines. The concentration was adjusted in such a way that it did not exceed 1ml/kg b/w of the animal¹⁶. The female albino rats of Wistar strain (200-250g) were maintained under control standard animal house conditions with access to food and water ad libitum. The rats were acclimatized for 5 days and fasted overnight, food but not water was withheld.

Preparation of Extract

Chloroform Extract

High dose: In a clean glass mortar-pestle 500mg of chloroform extract was mixed with tween 80 (1%) with 4 ml of water.

Low dose: In a clean glass mortar-pestle 200mg of chloroform extract was mixed with Tween 80 (1%) with 2 ml of water.

Ethanol Extract

High dose: In a clean glass mortar-pestle 500mg of ethanol extract was dissolved in 4 ml of water.

Low dose: In a clean glass mortar-pestle 200mg of ethanol extract was dissolved in 2 ml of water.

Phytochemical Analysis

Preliminary phytochemical screening of the extracts was carried out using standard methods¹⁷.

Experimental Models

Anti-implantation Activity^{18, 19}

Colony bred female albino rats of wistar strain (200-250g) were maintained under controlled standard animal house conditions with access to food & water ad libitum. Vaginal smears from each rat were monitored daily. Only rats with normal estrus cycle were selected for the experiment. The female rats were caged with male rats of known fertility in the ratio of 3:1. Rats exhibiting the copulation plug or thick clumps of spermatozoa in their vaginal smears were separated and that day was designated as day 1 of pregnancy. Pregnant rats were divided into 5 groups containing 6 animals in each group.

Treatment given from day 1 to day 7 of pregnancy as follows

Group I: Received vehicle only (Tween 80, 1%) and served as control (p.o daily)

Group II: Chloroform extract low dose (200 mg/kg, p.o)

Group III: Chloroform extract high dose (500mg/kg, p.o)

Group IV: Ethanol extract low dose (200 mg/kg p.o)

Group V: Ethanol extract high dose (500mg/kg p.o)

On the 10th day of pregnancy, laparotomy will be performed under ether anesthesia and the uterine horns will be inspected for number of implants. The abdomen will be closed and allowed for delivery after full term. During the experiment animals were observed for vaginal bleeding. The number of litters was determined after the completion of one gestation period in both the control and test groups.

The percentage of anti-implantation activity and abortifacient activity was determined by following formula.

Estrous Cycle Study²⁰

A normal estrous cycle in rats was defined as 4–5 days. Three types of cells to be recognized: round and nucleated ones are epithelial cells; irregular ones without nucleus are the cornified cells; and the little round ones are the leukocytes. The proportion among them was used for the determination of the estrous cycle stages. The estrous cycle stages are (1) proestrus (mainly epithelial cells), (2) estrus (mainly cornified cells), (3) metestrus (cornified and leukocytes), and (4) diestrus (mainly leukocytes) present. Colony bred virgin female rats of wistar strain (200-250g) Showing normal estrous cycle were selected and were maintained under controlled standard house conditions with access to food and water ad libitum, divided into 5 groups containing 6 animals in each group.

Group I: Received vehicle only (Tween 80) and served as control (p.o daily), for thirty days

Group II: Chloroform extract low dose (200 mg/kg p.o), for thirty days

Group III: Chloroform extract high dose (500mg/kg p.o), for thirty days

Group IV: Ethanol extract low dose (200 mg/kg p.o), for thirty days

Group V: Ethanol extract high dose (500mg/kg p.o), for thirty days

The vaginal smear from each animal was observed every morning in all the five groups to check for any variation in proestrous, diestrous, metaestrous and frank estrous phases of the estrous cycle.

Estrogenic/Anti Estrogenic Activity¹⁸

When estrogens are administered to immature rats, measurable increase in uterus weight and size is seen. Hence the following model is selected.

Colony bred wistar strain female albino rats, 25 to 30 days old, weighing between 35 to 45grams

(immature rats) were used and treated for 8 days. Animals were randomly divided into 10 groups consisting of 6 animals in each group.

Group I: Received vehicle only (Tween 80) and served as control (p.o daily)

Group II: Chloroform extract low dose (200 mg/kg p.o)

Group III: Chloroform extract high dose (500mg/kg p.o)

Group IV: Ethanol extract low dose (200 mg/kg, p.o)

Group V: Ethanol extract high dose (500mg/kg, p.o)

Group VI: Ethynyl estradiol 0.1µg/rat, i.m. (EED)

Group VII: Chloroform extracts low dose + EED

Group VIII: Chloroform extracts high dose + EED

Group IX: Ethanol extracts low dose + EED

Group X: Ethanol extracts high dose + EED

Ethynyl estradiol in arachis oil mg/kg body weight was injected (i.m) for 7 days. On the 8th day, the rats were sacrificed by either anaesthesia and the ovaries were dissected out, surrounding tissues were removed, blotted on the filter paper and weighed quickly on a sensitive balance. The ovaries were stored then subjected to biochemical estimations. Tween-80 (1%) was administered orally to the control animals and extracts. on the 8th day of the experiment, all the animals were killed by decapitation under ether anesthesia and ovary were dissected out, cleared of their surrounding tissue, blotted on the filter paper and weighed quickly on a sensitive balance. The ovaries were stored then subjected to biochemical estimations. The ovary was homogenized with ice-cold distilled water in a pre-cooled mortar and pestle to contain 10mg of tissue/ml, the homogenate was centrifuged at 3000 rpm for 15 min and the supernatant was used for estimation of glucose²¹, glycogen, protein, cholesterol and

alkaline phosphatase²². The readings were taken in semiautomatic analyzer.

Statistical Analysis²³

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnet comparison test. The values are expressed as mean \pm SEM and $p < 0.05$ was considered significant.

RESULTS

Preparation of Plant Extract

Blackish brown residue was obtained from *Pterocarpus santalinus* heart wood. The yield of ethanol extract and chloroform extract is 150g and 100g per 1 Kg respectively.

Preliminary Phytochemical Analysis of *Pterocarpus santalinus* Heart Wood Extracts

The results of preliminary phytochemical analysis are shown in Table 1 & 2. The chloroform extract of *Pterocarpus santalinus* heart wood shows the presence of glycosides, flavonoids, phenols and sterols. The ethanol extract of *Pterocarpus santalinus* heart wood shows the presence of glycosides, flavonoids, alkaloids, tannins, phenols, saponins and sterols.

Toxicity Study

The acute and sub-acute oral toxicity study was done according to OECD 423 guide lines (Acute toxicity class method). Both the extracts of *Pterocarpus santalinus* heart wood were safe at a dose of 2000mg/kg. Hence, 1/4th and 1/10th of the dose of this was selected for the study.

Anti-Implantation Activity

The results are expressed as percentage of implants, the no of implants in the female rats were found to be significantly reduced in the chloroform extract (both low & high doses) of laprotomised group when compared to control. Hence chloroform extract was showed anti implantation activity.

The no of implants in the female rats were also found to be highly significantly reduced in the ethanol extract (both low & high doses) of laprotomised group when compared to control and showed anti implantation activity. Hence high dose of ethanol extract is more effective as anti-fertility activity then chloroform extract (Table: 3).

Effect on Late Stage of Pregnancy

The mean number of litters delivered were decreased significantly ($p < 0.05$) in lower dose of ethanol extract of *Pterocarpus santalinus* heart wood treated group & whereas it decreased more significantly in higher dose of ethanol extract of *Pterocarpus santalinus* heart wood treated groups when compared with control group. In case of chloroform extract of *Pterocarpus santalinus* heart wood the mean number of litters delivered were slightly decreased in both lower as well as high dose. Hence, the % Abortifacient activity is more in high dose of ethanol extract then in both chloroform extract of *Pterocarpus santalinus* heart wood (Table:4).

Table 1: Details of preliminary phytochemical screening of chloroform extract of *pterocarpus santalinus* heart wood

S. No.	Constituents	Test	Chloroform Extract
1.	Steroids	a) Libermann's burchard test b) 5% potassium hydroxide	Present Present
2.	Phenols	a) Ferric chloride b) 10% Sodium chloride	Present Present
3.	Glycosides	Glacial acetic acid + Ferric chloride + Con. Sulphuric acid	Present
4.	Flavanoids	a) Amyl alcohol + Sodium acetate + Ferric chloride b) Con. H ₂ SO ₄ c) Magnesium turning test	Present Present Present

Table 2: Details of preliminary phytochemical screening of ethanol extract of *pterocarpus santalinus* heart wood

S.No.	Constituents	Test	Ethanol Extract
1.	Alkaloids	a) Mayer's reagent b) Dragendorff's reagent c) Hagner's reagent d) Wagner's reagent	Present Present Present Present
2.	Steroids	a) Libermann's burchard test b) 5% potassium hydroxide	Present Present
3.	Phenols	a) Ferric chloride b) 10% Sodium chloride	Present Present
4.	Tannins	a) 10% Lead acetate solution b) 10% Sodium chloride c) Aqueous bromine solution	Present Present Present
5.	Flavanoids	a) Amyl alcohol + Sodium acetate + Ferric chloride b) Con. H ₂ SO ₄ c) Magnesium turning test	Present Present Present
6.	Glycosides	Glacial acetic acid + Ferric chloride + Con. Sulphuric acid	Present
7.	Saponins	Foam test	Present

Table 3: Data showing Anti-implantation activity of *Pterocarpus santalinus* heartwood extracts

Groups	Treatment	Chloroform Extract		Ethanol Extract	
		No of implants	Percentage of implants	No of implants	% of implants
1	Control	12.37± 2.10	100%	13.5±0.71	100%
2	Low dose (200 mg/kg p.o)	6.12 ± 1.02**	49.47%	5.8±0.30**	42.9
3	High Dose (500 mg/kg p.o)	5.45 ± 1.08**	44.78%	1.8±0.65**	13.33

Values are mean ± SEM (n=6) one way ANOVA. * represents significant at p<0.05, ** represents highly significant at p< 0.01, and *** represents very significant at p<0.001 when compared with control group

Estrous Cycle Study

Treated group showed a significant decrease in the duration of proestrous, estrous and metaestrus and significant increase ($P < 0.001$) in the duration of diestrus phase, when compared to control group. Hence both the extracts treated group proved to be anti-estrogenic in nature (Table: 5).

Estrogenic/Anti-estrogenic Activity

The weight of the ovary was determined after blotting. The weight of the ovary in chloroform extract of *Pterocarpus santalinus* heart wood treated animals showed significantly increased in high dose of the chloroform extract treated animals and in ethanol extract of *Pterocarpus santalinus* heart wood showed highly significant increase in weight of the ovary in high dose (Table 6).

Biochemical Estimations

Effect on Glucose, Glycogen, Protein, Cholesterol Content and Alkaline Phosphatase Activity in Immature Rat Uterus

The glucose content, glycogen content, protein content, cholesterol content and alkaline phosphatase activity was increased in both the extracts of *Pterocarpus santalinus* heart wood treated animals and EED treated rats when compared with control. The low dose of chloroform and ethanol extracts rats showed

slightly increase in glucose content, when compared with control.

The high dose of chloroform and ethanol extracts treated rats + EED treated rats showed highly significant increase in glucose content, glycogen content, protein content, cholesterol content and alkaline phosphatase activity when compared with control (Table: 6 & Table: 7).

Histological Examination

Effect on Diameter of Uterus, Thickness of Endometium, Thickness of Myometrium and Height of Endometrial Epithelium of Immature Rats

The diameter of uterus, thickness of endometium, thickness of myometrium and height of endometrial epithelium was significantly increased in high dose and slightly in low dose of ethanol extract treated rats, when compared with control whereas it increased significantly in EED treated rats when compared with control.

The chloroform extract also showed increase in diameter of uterus, thickness of endometium, thickness of myometrium and height of endometrial epithelium in high dose and slightly in low dose treated rats, when compared with control whereas it increased significantly in EED treated rats when compared with control (Table: 8).

Table 4: Effect on late stage of pregnancy in *Pterocarpus santalinus* heartwood extracts

Group	Treatment	Dose (Kg^{-1} body weight)	Mean. no. of litters delivered.	% Abortifacient activity
1	Control	2 ml	7.21 ± 1.52	100
2	Ethanol extract	Low dose 200mg/Kg	$2.14 \pm 0.15^{**}$	70.31
3	Ethanol extract	High dose 500mg/Kg	$1.06 \pm 0.04^{**}$	85.29
4	Chloroform extract	Low dose 200mg/Kg	$5.44 \pm 1.15^{**}$	24.59
5	Chloroform extract	High dose 500mg/Kg	$4.14 \pm 1.50^{**}$	42.57

Values are mean \pm SEM (n=6) one way ANOVA. * represents significant at $p < 0.05$, ** represents highly significant at $p < 0.01$, and *** represents very significant at $p < 0.001$ when compared with control group

Table 5: Effect of *Pterocarpus santalinus* heartwood extracts on estrous cycle study

Group	Treatment Dose (mg/kg body weight)	Duration of estrous cycle (Days)	Proestrous (days)	Oestrus (days)	Metaestrous (days)	Dioestrus (days)
1	Control (Tween 80) Vehicle	4.60 ± 0.10	0.88± 0.07	1.58 ± 0.12	0.87 ± 0.15	1.18 ± 0.21
2	Ethanol extract Low dose 200mg/kg	5.10± 0.52	0.49 ± 0.32**	1.33 ± 0.23**	0.50 ± 0.56**	2.60 ± 0.46**
3	Ethanol extract High dose 500mg/kg	4.90 ± 0.54	0.47 ± 0.23**	0.99± 0.19**	0.78 ± 0.16**	2.38 ± 0.25**
4	Chloroform extract Low dose 200mg/kg	4.87 ± 0.52	0.56 ± 0.13**	1.35 ± 0.48**	0.60± 0.25**	2.40± 0.45**
5	Chloroform extract High dose 500mg/kg	4.62 ± 0.42	0.86 ± 0.56**	1.25 ± 0.23**	0.86 ± 0.13**	1.85 ± 0.21**

Values are mean ± SEM (n=6) one way ANOVA. * represents significant at p<0.05, ** represents highly significant at p< 0.01, and *** represents very significant at p<0.001 when compared with control group.

Table 6: Effect of *Pterocarpus santalinus* heartwood extracts on Ovary wt, ALP and Glucose level

Groups	Treatment	Ovary weight (mg)	Alkaline phosphatase (U/L)	Glucose (mg %)
1	Control (Tween 80)	55.10± 0.17	58.27±0.21	20.38±0.15
2	Standard EED	40.20± 0.17**	160.11±0.14**	40.14±0.14**
3	Chloroform extract Low dose	49.40± 0.10**	87.87±0.14**	35.43±0.22**
4	Chloroform extract High dose	38.06± 0.10**	93.77±0.25**	50.47±0.24**
5	High dose of Chloroform extract+ EED	35.37 ± 0.14***#	164.76±0.13***#	50.59±0.23***#
6	Low dose of Chloroform extract +EED	45.62±0.15**	159.08±0.16**	45.10±5.026**
7	Ethanol extract Low dose	45.2± 0.1**	159.00±4.36**	60.22 ± 4.22**
8	Ethanol extract High dose	35.02± 1.40**	175.98±4.19**	75.56 ± 2.75**
9	Low dose of Ethanol extract + EED	35.60± 1.44**	166.45±3.59**	80.24 ± 4.19**
10	High dose of Ethanol extract High dose +EED	25.30 ± 2.34***#	179.11± 4.22***#	97.29 ± 6.21***#

Values are mean ± SEM (n=6) one way ANOVA. * represents significant at p<0.05, ** represents highly significant at p< 0.01, and *** represents very significant at p<0.001 when compared with control group, #p<0.05 is considered as significant.

Table 7: Effect of *Pterocarpus santalinus* heartwood extracts on Glycogen content, Protein content and Cholesterol level

Groups	Treatment	Glycogen content (mg/g of ovary)	Protein content (mg/g of ovary)	Cholesterol (mg/g of ovary)
1	Control (Tween 80)	43.12±4.16	52.17±3.19	30.02±0.18
2	Standard EED	62.49±1.79**	66.44±2.68**	47.12±0.18**
3	Chloroform extract Low dose	46.14±1.79**	58.59±3.38*	48.19±0.22**
4	Chloroform extract High dose	59.55±3.51**	64.02±1.1**	51.15±0.12**
5	High dose of Chloroform extract+ EED	61.17±4.52**#	67.03 ±3.09**#	46.07±0.24**#
6	Low dose of Chloroform extract +EED	67.19±5.43**	65.5±2.09**	35.35±1.6**
7	Ethanol extract Low dose	55.24±0.17**	70.67±2.36**	64.13 ± 3.94**
8	Ethanol extract High dose	70.84±0.10**	83.69±2.11**#	75.18±5.75**#
9	High dose of Ethanol extract + EED	69.06±0.10**#	80.87±2.67**#	71.36 ± 5.23**#
10	Low dose of Ethanol extract +EED	65.62±0.15**	70.54 ± 1.19**	68.23±3.23**

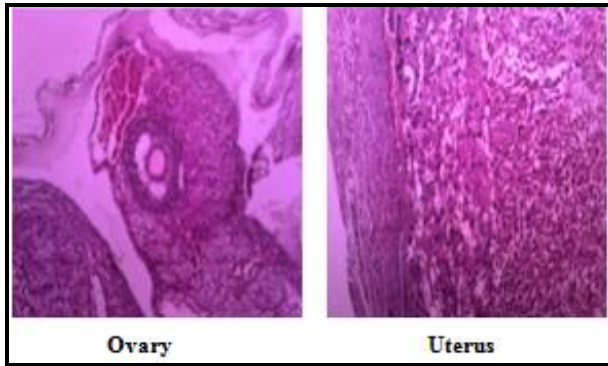
Values are mean ± SEM (n=6) one way ANOVA. * represents significant at p<0.05, ** represents highly significant at p< 0.01, and *** represents very significant at p<0.001 when compared with control group, #p<0.05 is considered as significant.

Table 8: Histological study of *Pterocarpus santalinus* heartwood extracts on Diameter of uterus, Thickness of Endometrium, Height of endometrial epithelium and Thickness of myometrium

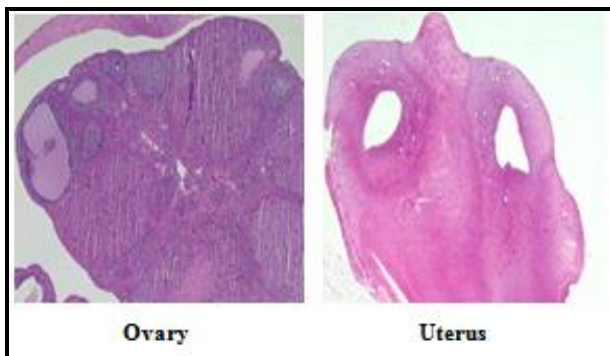
Groups	Treatment	Diameter of uterus in mm/mg t. w	Thickness of endometrium in µm/mg t. w	Height of endometrial epithelium in µm/mg	Thickness of myometrium in µm/mg t. w
1	Control (Tween 80)	2.32±0.14	82.42±2.58	15.27±0.72	43.42±1.98
2	Standard EED	5.16±0.32**	116.56±4.78**	30.12±1.37**	74.56±1.14**
3	Chloroform extract Low dose	2.31±0.71**	85.66±4.13**	18.43±2.52**	44.11±4.13**
4	Chloroform extract High dose	3.83±0.36**	98.56±3.55**	20.04±0.73**	47.49±3.13**
5	Ethanol extract Low dose	4.16±1.32**	92.56±4.78**	23.52±0.37**	54.56±1.14**
6	Ethanol extract High dose	5.31±0.31**	110.26±4.13**	30.33±2.52**	66.11±2.23**

Values are mean ± SEM (n=6) one way ANOVA. * represents significant at p<0.05, ** represents highly significant at p< 0.01, and *** represents very significant at p<0.001 when compared with control group, #p<0.05 is considered as significant.

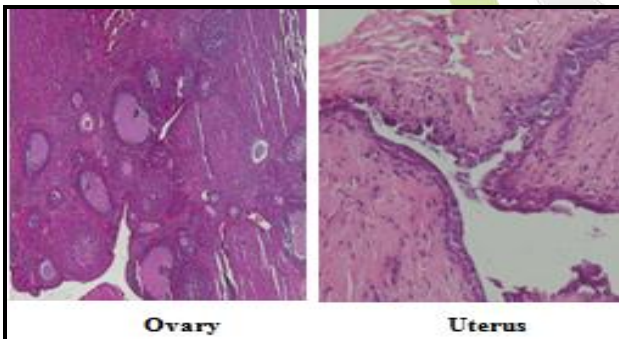
Control Group



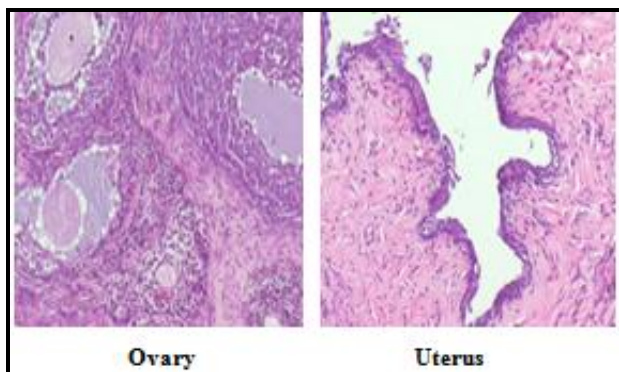
Chloroform Extracts of *Pterocarpus santalinus* heartwood - Low Dose



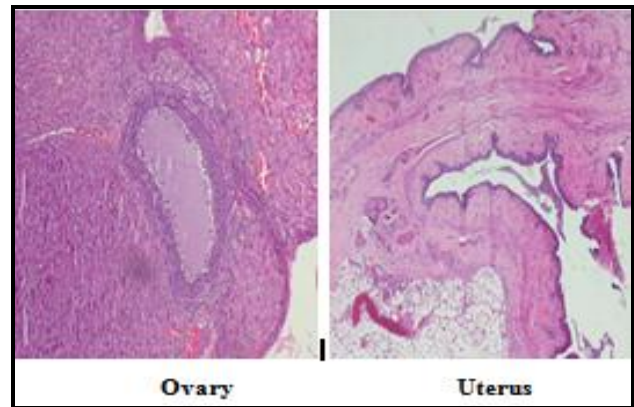
Chloroform Extracts of *Pterocarpus santalinus* heartwood – High Dose



Ethanol Extracts of *Pterocarpus santalinus* heartwood – Low Dose



Ethanol Extracts of *Pterocarpus santalinus* heartwood – High Dose



Ethynyl estradiol 0.1µg/rat, i.m. (EED)

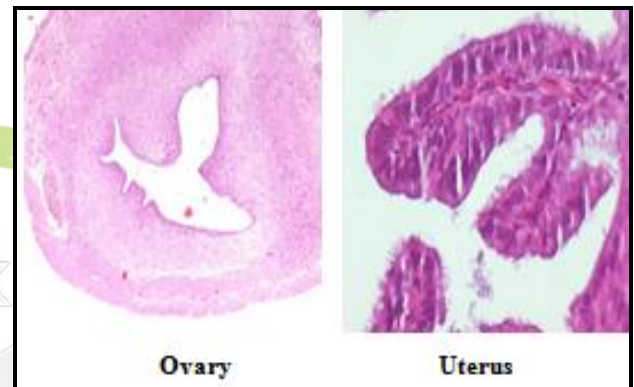


Figure 1: Histopathological study ovary and uterus for Estrogenic /Anti estrogenic activity (100X)

DISCUSSION

In the present study, the heart wood of *Pterocarpus santalinus* was tested for their anti-fertility activity in female rats. Estrogen and progesterone are ovarian hormones which plays important role in pregnancy and its production is stimulated by Follicle stimulating hormone and Leutinizing hormone. Excess exogenous estrogen inhibits FSH output and LH thus prevents ovulation. For implantation and sustenance of pregnancy, exact equilibrium of secretion of estrogen and progesterone is necessary. Any imbalance in the levels of these hormones can cause anti-implantation or can induce abortion. Estrogen is known to increase the cholesterol, glucose in the uterus, thereby changing the uterine milieu and creating non receptive conditions in the uterus⁸⁸.

In the toxicity study, toxic symptoms and mortality was studied for both ethanol and chloroform extract of *Pterocarpus santalinus* heart wood and both the extracts were found to be well tolerated up to 2g/kg. Hence 1/4th (500mg/kg) and 1/10th (200mg/kg) of the dose of this were selected for the study. Ethynyl estradiol 0.1µg/rat, i.m. (EED) was used as standard drug.

Oral administration of ethanol extract of *Pterocarpus santalinus* heart wood at both higher (500mg/kg b.w/p.o) and lower doses (200mg/kg b.w/p.o) reduced number of implants when compared with control and posses anti-implantation activity. The lower dose treated rats possessed 42.9 Percentage of implants while higher dose showed only 13.33 Percentage of implants, whereas administration of chloroform extract of *Pterocarpus santalinus* heart wood at both higher (500mg/kg b.w/p.o) and lower doses (200mg/kg b.w/p.o) also showed anti-implantation activity by reduced number of implants when compared with control. The lower dose treated rats possessed 49.47 Percentage of implants while higher dose showed only 44.78 Percentage of implants. The anti-implantation effect of this extract might be due to the disturbance of endocrine-endometrial synchrony which is dependent on estrogen and progesterone balance⁸⁹. Endometrial changes by hormonal imbalance provide the non receptive conditions which prevent the implantation of blastocyst. Nivrsarkar et al reported that extract of the leaves of *Hibiscus rosa-sinensis* caused endometrial alteration and resulted in blastocyst implantation failure in mice⁹⁰.

Vaginal bleeding of some of extract treated rats between 13-15th days of pregnancy which indicates the termination of pregnancy. This result is in agreement with Al-Dissi, et al., who reported that administration of aqueous extract of the leaves of *Inula viscosa* to rat showed abortifacient activity, accompanied with vaginal bleeding in some of the rats⁹¹.

Administration of ethanol extract of *Pterocarpus santalinus* heart wood at higher (500mg/kg b.w/p.o) showed highly significant

reduction in number of litters delivered. The ethanol extract at high dose posses 85.29 % abortifacient activity and lower dose posses 70.31% abortifacient activity and administration of chloroform extract of *Pterocarpus santalinus* heart wood at higher (500mg/kg b.w/p.o) showed significant reduction in number of litters delivered. The chloroform extracts at high dose posses 42.57 % abortifacient activity and lower dose posses 24.59% abortifacient activity. Hence both *Pterocarpus santalinus* heart wood extracts demonstrated both anti-implantation and abortifacient properties. This result agrees with the finding of Badami et al., who reported that oral administration of ethanol extract of the powdered root of *Derris brevipipes* variety *coriacea* showed both abortifacient and anti implantation effect in rats⁹².

As both *Pterocarpus santalinus* heart wood extracts at both the doses exhibited significant anti-implantation and abortifacient activity, it was evaluated for its estrogenic activity.

In estrous cycle, the treated group showed a significant decrease in the duration of proestrous, estrous and metaestrous and significant increase in the duration of diestrus phase, when compared to control group. Hence both the extracts treated group proved to be anti-estrogenic in nature (Table: 5).

It is known that administration of high levels of exogenous estrogenic substances to mice causes implantation failure. In mice and humans estrogen also plays a pivotal role in implantation because it participates in estrogen/progesterone balance and, therefore can affect the uterine receptivity to the Embryo⁹³. In review of Velle W detailed that the mechanism by which estrogens induces contraception and abortion in mammals. The transportation of the ova to the uterus takes a relatively short time in rats, so estrogens in dosages of 20mcg counteract the progesterone produced by the corpora lutea, speeding up the ovum's entry into the uterus before it is prepared to receive the ovum⁹⁴.

Estrogenic activity was assessed mainly by uterotrophic assay, vaginal cytology, biochemical estimations of glucose, glycogen,

protein, cholesterol, alkaline phosphatase activity and histological changes of uterus gave supportive results in both *Pterocarpus santalinus* heart wood extracts treated immature female rats. Anti-estrogenic activity was assessed in both the extracts along with Ethynyl estradiol treated immature female rats.

Administration of ethanol extract of *Pterocarpus santalinus* heart wood at doses of 500mg/kg b.w/p.o and 200mg/kg b.w/p.o showed significant increase in ovary weight when compared with control. Simultaneous administration of Ethynyl estradiol and ethanol extract of *Pterocarpus santalinus* heart wood at a dose of 500mg/kg b.w/p.o significantly increased ovary weight, when compared with Ethynyl estradiol treated animal. Estrogen is known to increase the uterus weight in immature or ovariectomised rats. This effect is due to competitive inhibition of binding of [3H]estradiol to uterus in vivo. Decrease ovary growth is due to Aplasia and hypotrophy of ovary tissue compartment and also by decreasing volume of endometrial microvasculature^{95,96}. Measure of estrogen potency and Presence of cornified cells in vaginal smear also indicates estrogen activity⁹⁷. Administration of chloroform extract of *Pterocarpus santalinus* heart wood at doses of 500mg/kg b.w/p.o and 200mg/kg b.w/p.o did showed significant increase in ovary weight when compared with vehicle control. The OECD has proposed uterine weight change as an end point of estrogenic activity⁹⁸. The results of our study strongly suggest the estrogenic activity of both *Pterocarpus santalinus* heart wood extracts increase ovary weight when compare with control. Which is comparable with report of Presa, JD et al that *Sida cordifolia* appeared to contain oestrogen-like activity which could have induced an increase in uterine weight⁹⁹.

Administration of ethanol extract of *Pterocarpus santalinus* heart wood at a dose of 500mg/kg b.w/p.o showed significant increase in glucose content and glycogen content, when compared with vehicle control. Simultaneous administration of with Ethynyl estradiol and

Ethanol extract at a dose of 500mg/kg b.w/p.o significantly increase the glucose content, when compared with Ethynyl estradiol treated animal. Estrogen controls the synthesis of some rate-limiting component responsible for transport or phosphorylation of glucose in the uterus and also accelerates the glycogen synthesis. The present finding supports the fact by increasing glucose and glycogen content of uterus¹⁰⁰. Administration of ethanol extract of *Pterocarpus santalinus* heart wood at a dose of 200mg/kg b.w/p.o showed significant increase in glucose and glycogen content of uterus when compared with vehicle control whereas administration of chloroform extract of *Pterocarpus santalinus* heart wood at doses of 500mg/kg b.w/p.o and 200mg/kg b.w/p.o showed slightly increase in glucose content and glycogen content, when compared with vehicle control.

Estrogen and progesterone alone or in combination form increases protein contents of uterus thereby increase the uterus weight. Administration of ethanol extract of *Pterocarpus santalinus* heart wood at a dose of 500mg/kg b.w/p.o showed significant increase in protein content when compared with vehicle control. Simultaneous administration of Ethynyl estradiol and ethanol extract of *Pterocarpus santalinus* heart wood at a dose of 500mg/kg b.w/p.o significantly increase protein content of uterus, when compared with Ethynyl estradiol treated animal. Administration of ethanol extract of *Pterocarpus santalinus* heart wood at a dose of 200mg/kg b.w/p.o showed slightly increase protein content when compared with vehicle control and administration of chloroform extract of *Pterocarpus santalinus* heart wood at a dose of 500mg/kg b.w/p.o and 200mg/kg b.w/p.o showed significant increase in protein content when compared with vehicle control. These results are comparable with findings of Prakash and. Jonathan that the hexane extracts of *Ferula jaeschkeana* Vatke increase in the weight of uterus and amount of total proteins, glycogen¹⁰¹.

Administration of ethanol extract of *Pterocarpus santalinus* heart wood at a dose of 500mg/kg b.w/p.o showed significant increase in

cholesterol content when compared with control. Simultaneous administration of Ethynyl estradiol and ethanol extract at a dose of 500mg/kg b.w showed significantly increases the cholesterol content, when compared with Ethynyl estradiol. Administration of chloroform extract of *Pterocarpus santalinus* heart wood at a dose of 500mg/kg b.w/p.o and of 200mg/kg b.w/p.o also showed increase in cholesterol content when compared with control. Estrogens are well-known stimulators of hepatic low density lipoprotein (LDL) catabolism and delayed the metabolism of chylomicron cholesterol by marked reduction in the apoE content of chylomicrons thereby increase the cholesterol content^{102,103,104}.

Administration of ethanol extract of *Pterocarpus santalinus* heart wood at dose of 500mg/kg b.w/p.o showed significant increase in alkaline phosphatase activity when compared with vehicle control. Simultaneous administration of Ethynyl estradiol and ethanol extract of *Pterocarpus santalinus* heart wood at a dose of 500mg/kg b.w showed highly significantly increase the alkaline phosphatase activity, when compared with Ethynyl estradiol. Alkaline phosphatase is believed to involve in growth and secretory function of the tissue cells, metabolism of carbohydrate and lipids, nucleic acid and increase in cell permeability. Administration of chloroform extract of *Pterocarpus santalinus* heart wood at a dose of 500mg/kg b.w/p.o and of 200mg/kg b.w/p.o showed dose increase in alkaline phosphatase activity when compared with vehicle control. Estradiol and several other estrogens greatly enhance the activity of alkaline phosphatase, an enzyme known to be regulated by ovarian hormones in the non pregnant and pregnant rodent and monkey uterus indicates estrogenic activity¹⁰⁵. Increase in alkaline phosphatase activity may result in alterations of secretory functions by influencing cell permeability, thereby changing uterine milieu which is directly involved in implantation of eggs. These results agree with findings of Badami et. al that *Derris brevipes* variety *coriacea* exhibited estrogenic activity as shown by the significant

increase in increase in uterine content of glucose, cholesterol and alkaline phosphatase⁹².

Morphological changes of the epithelial lining of reproductive tract tissues provide another indicator of estrogenic action. Administration of ethanol extract and chloroform extract of *Pterocarpus santalinus* heart wood at a two doses 500mg/kg b.w/p.o and 200mg/kg b.w/p.o respectively produced histological changes such as increase in diameter of uterus, thickness of endometrium, thickness of myometrium and height of endometrial epithelium when compared with vehicle control. These responses are similar with J. A. Ford Jr. Et.al reports that Genistein increases the epithelial cell height in the uterine lumen and uterine glands, as well as the width of uterine glands^{106,107,108}.

Administration of ethanol extract of *Pterocarpus santalinus* heart wood at a dose of 500mg/kg b.w/p.o produce significant estrogenic activity and administration of chloroform extract of *Pterocarpus santalinus* heart wood at two doses of 500mg/kg b.w/p.o and 200mg/kg b.w/p.o also posses estrogenic activity. These suggest that both the extracts of *Pterocarpus santalinus* heart wood may possess significant anti estrogenic activity.

Plant products exhibiting estrogenic activity and producing anti-fertility effects are known in the literature. Chukwuka N. et. al reported that *Spondias mombin* leaf extract possessed abortifacient and estrogenic activity in rats¹⁰⁹. Neeru Vasudeva et. al reported that the ethanol extract of *Hibiscus rosa-sinensis* root possesses anti-implantation activity, and the estrogenic property of the extract may be responsible for this anticonceptive effect¹¹⁰. Hiremath et al., reported that flavanoids isolated from *Striga lutea* and *Striga orobanchioides* possessed strong estrogenic and antifertility properties¹¹¹. The ethanolic extract of *Striga lutea* and *Striga orobanchioides* exhibiting estrogenic activity reduced the number of viable fetuses and increased the number of resorptions in female pregnant rats¹¹². Atal CK. isolated the vasicine from *Adathoda vasica* showed potent abortifacient and uterotonic effects in guinea

pigs¹¹³. Padmashali reported that the abortifacient activity of *Balnites Roxburghii* may be due to mild estrogenic activity of the extracts causing expulsion or resorption of the implants by the uterus¹¹⁴. Ravichandran et.al reported that *Ailanthus excelsa* posse's mild estrogenic activity at its contraceptive dose¹¹⁵. Badami et. al reported that antifertility activity of *Derris brevipes* variety *coriacea* may be mediated by mild estrogenic nature of the plant⁹². Flavanoids have been reported to possess' antifertility and estrogenic properties. Hiremath et al. reported that flavanoids isolated from *Striga lutea* and *Striga orobanchioides* possessed strong estrogenic and antifertility properties¹¹¹.

Preliminary phytochemical analysis of both the extracts shows rich possession of phytochemical such as flavones, flavanoids which belongs to phytoestrogens which exert their effects primarily through binding to the estrogen receptor (ER) and most are reported for estrogenic activity¹¹⁶ might be responsible for anti-fertility.

CONCLUSION

It is concluded that ethanol and chloroform extract of *Pterocarpus santalinus* heart wood in both doses possess anti-implantation activity and abortifacient properties. Prolonged diestrous phase and reduced the duration of Proestrous, estrous and metaestrous phase inboth the extracts. Administration of extracts to immature rats showed increase in the ovary weight and increase in the levels of glucose, glycogen, protein, cholesterol content and alkaline phosphatase activity and showed increase in diameter of uterus, thickness of endometium, thickness of myometrium and height of endometrial epithelium. Hence, it is concluded that *Pterocarpus santalinus* heart wood extracts proved to possess' anti-fertility agent.

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