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ANTIFERTILITY EFFECT OF HERB OF *INDIGOFERA LINNAEI ALI* IN FEMALE ALBINO RATS

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ABSTRACT

To evaluate anti-fertility activity of herb of *Indigofera linnaei Ali* by using 70% ethanolic, 95% ethanolic and aqueous extracts. The different experimental models used for the evaluation of anti-fertility activity are estrogenic activity in immature female rats was carried out by taking ethinylestrodiol as standard. Uterine weight, vaginal cornification and uterotropic potency determined and biochemical changes in the uterus was determined and compared with control and standard. Anti-implantation and early abortifacient activity was performed on female rats, the number of implants and resorbtions were compared with control. The 95% ethanolic, 70% ethanolic and aqueous extracts of herb of *Indigofera linnaei Ali* were found to possess significant estrogenic activity at the dose of 500 mg / kg and 250 mg / kg b.w. as indicated by significant increase in uterine weight, vaginal cornification and uterotropic potency like; diameter of the uterus, thickness of the endometrium and height of the endometrial epithelium and there was also a increase in glucose, cholesterol and alkaline phosphatase in compared to control, but not significantly greater than standard. Where as in case of anti-implantation and early abortifacient activity. The 70% ethanolic, aqueous and 95% ethanolic extracts were found to be highly significant when compared to control in dose dependent manner as evident by decrease in number of implants and increase in number of resorbtions. 70% ethanolic and aqueous extracts of *Indigofera linnaei Ali* were found to possess highly significant anti-fertility activity in dose dependant manner whereas 95% ethanolic extract was found to be less significant when compared to other two extracts in dose dependent manner.

KEY WORDS: The whole herb of *Indigofera linnaei Ali*, cholesterol, glucose, alkaline Phosphatise.

INTRODUCTION

The rapid growth of the world's population over the past one hundred years results from a difference between the rate of birth and the rate of death. The human population will increase by 1 billion people in the next decade. This is like adding the whole population of China to the world's population. The growth in human population around the world affects all people through its impact on the economy and environ-ment. The current rate of population growth is now a significant burden to human well-being. Understanding the factors which affect population growth patterns can help us plan for the future.

It took the entire history of humankind for the population to reach 1 billion around 1810. Just 120 years later, this doubled to 2 billion people (1930); then 4 billion in 1975 (45 years). The number of people in the world has risen from 4.4 billion people in 1980 to 5.8 billion today. And it is estimated that the population could double again to nearly 11 billion in less than 40 years. This means that more people are now being added each day than at any other time in human history (Miller TG *et al.*, 1992).

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Rich and poor countries alike are affected by population growth, though the population of industrial countries are growing more slowly than those of developing one. At the present growth rates, the population of economically developed countries would double in 120 years. The Third World, with over three quarters of the world's people, would double its numbers in about 33 years. This rapid doubling time reflects the fact that 37 percent of the developing world's population is under the age of 15 and entering their most productive childbearing years. In the Third World countries (excluding China), 40 percent of the people are under 15; in some African countries, nearly half are in this age group.

Their application as medicine dates back to prehistoric period. Considerable number of drugs used in modern medicine has figured in ancient manuscripts such as the Rigveda, the Bible, and the Quran. The Ayurveda is a part of Atharvaveda one among the four Vedas. Vedas have revealed that the herbs, shrubs and trees have got life much before the modern life science said it. The ayurveda has laid a scientific foundation for such thinking. Our ancient Philosophers and Biologists like Sushruta, Charaka and Vagbhata have made investigations on medicinal plants and enriched Ayurveda.

India is known as the "Emporium of medicinal plants". The country also has to its credit that well known traditional systems of medicine like Ayurveda and Siddha. These systems of medicine derive their drugs primarily from plant origin. The World Health Organization (WHO) has also recognized the traditional systems of medicine as one of the tools to achieve its aim "Health for all".

The whole herb of *Indigofera linnaei Ali* reported to contain phytochemical constituents such as, steroids, flavonoids, alkaloids, glycosides, tannins, carbohydrates and proteins. Keeping this in view after extensive literature survey available from all scientific sources revealed no information about the pharmacological validation of the anti-fertility activity of herb of *Indigofera linnaei Ali.* Thus the present study deals with the screening of anti-fertility efficacy by using different experimental model (Chetty KM *et al*., 2008; Rastogi RP *et al*., 1990-1994).

METHADOLOGY

Plant Material

The whole herb of *Indigofera linnaei Ali* was collected in the month of December and February from the fields of Harapanahalli. The authentication was done by Prof. K. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli. A voucher specimen has been deposited at the museum of our college.

Preparation of Extracts

The whole herb of *Indigofera linnaei Ali* was collected and shade dried. The dried aerial parts were coarse powdered and the powder was packed in to soxhlet column and extracted successively with petroleum ether (60-80°c), 95% ethanol (64.5-65.5°c), 70% ethanol (75°c) and distilled water. The extracts were concentrated by using rotary flash evaporator under reduced pressure. The dried extracts were stored in airtight container in refrigerator below 10°c.The solution of 95% ethanolic, 70% ethanolic and aqueous extracts were prepared using distilled water.

Preliminary phytochemical screening

The preliminary phytochemical screening was carried out on petroleum ether, 70% ethanol, 95% ethanol and aqueous extracts of herb of *Indigofera linnaei Ali* for the detection of various phytochemical tests (Khandelwal KR, 2000) for common phytochemical were carried out by standard methods.

Animals used

Female and male albino rats (wistar strain) weighing 150-200gms, immature female rats of 21-23 days old (wistar strain) weighing 40-60gms and albino mice weighing 20-25gms of either sex were used in this study.

Determination of acute toxicity (LD_{50})

The acute toxicity for 95% ethanol, 70% ethanol and aqueous extracts of herb of *Indigofera linnaei Ali* were determined in albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment, fixed dose method was adopted as per OECD Guideline No. 420; (Annexure-2d) of CPCSEA.

III) Evaluation of Anti-fertility Activity.

Estrogenic activity on immature female rats

Immature female rats of wistar strain 21-23 days old weighing 40-60gms were used. They were divided in to eight groups of six animals each. Vagina and the vaginal smears were examined in all the animals in the treated groups for 7 days of treatment. 24 hrs of last treatment all the animals were sacrificed by decapitation and uteri were dissected out, cleared off the adhesive tissue, blotted on filter paper and weighted quickly on a sensitive balance. The tissues were fixed in Bouin's fixative for 24 hrs. Dehydrated in alcohol and embedded in paraffin. The paraffin blocks were sectioned at 6µ and stained with haemotoxylene-eosin solution (H & E Stain) for histological observations. The diameter of the uterus,

thickness of endometrium, and the height of endometrial epithelium were measured in 10 randomly selected sections using a calibrated ocular micrometer **(**Padmasali B *et al*., 2006).

Anti-implantation and early abortifacient activity in rats

The method was adopted with the modification for the anti-implantation and early abortifacient activities of 95% ethanol, 70% ethanol and aqueous extracts of herb of *Indigofera linnaei Ali* Female albino rats (Wistar strain) weighing 150-200gms were used to assess anti-implantation and early abortifacient activity. All the animals were maintained under controlled standard animal house condition with access to food and water ad libitum. A vaginal smear from each rat was monitored daily. Only the rats with normal oestrous cycles were selected for the experiment.

Female rats of proestrus phase were kept with male rats of proven fertility for mating in a ratio of 2:1. The females were examined, the following morning for evidence of copulation. The animals exhibiting thick clumps of spermatozoa in vaginal smears were separated from male partner. That day when spermatozoa were detected in the vaginal smear was considered as day one of gestation **(**John D *et al*., 1990; Khanna V *et al*., 1968).

The separated pregnant rats were divided into seven groups of six rats each. The extracts were administered orally from first day to seventh day of gestation. The control animals received only vehicle. On the tenth day laparotomy was carried out under light ether anesthesia in semisterile condition. The uteri were examined to determine the number of implantation sites. The numbers of corpora lutea in ovaries were also recorded. The abdomen was sutured and animals left in cages. The drugs were administered orally again for 3 days (day 14 to 16). On the eighteenth day laparotomy was carried out once again under light ether anesthesia for the abortifacient study.

The percentages of anti-implantation and early abortifacient activities were calculated by using following formula.

$$\%\text{of anti-implantation activity} = 100 - \frac{\text{No. of implantation}}{\text{No. of Corpora lutea}} \times 100$$

$$\%\text{ of abortifacient activity} = \frac{\text{No. of resorbtions}}{\text{No. of Corpora lute}} \times 100$$

Anti-implantation and early abortifacient activity

A dose dependent anti-implantation and early abortifacient activity of the 95% ethanolic, 70% ethanolic and aqueous extracts was evident by significant decrease in number of implantation sites and increase in number of resorbtions, when compared with the control group. The percentage of anti-fertility activity of the 95% ethanolic extract at dose of 250 mg/kg b.w. and 500 mg/kg b.w. were found to be 26.9% and 53.89% respectively in dose dependent manner, when compared to control, where as percentage of anti-fertility activity of 70% ethanolic extract at dose of 250 mg/kg b.w. and 500 mg/kg b.w. were found to be 44.85% and 64.2% respectively, when compared to control.

However, the percentage of anti-fertility activity of aqueous extract at dose of 250mg/kg b.w and 500mg/kg b.w. were found to be 35.87% and 60.46% respectively, when compared to control the results are shown in the Table No.3

RESULTS

Table 1. Estrogenic activity of various extracts of whole herb of *Indigofera linnaei Ali* in immature female rats

S. No.	Treatment Extracts / Drugs	Dose mg/kg	Uterine wt.in mg Mean ± SEM	Vaginal Status	Vaginal cornification
1	Control (Vehicle)	5 ml/kg	31.70 ± 1.075	Not opened	Nil
2	Ethinyl estradiol	1 µg/ rat/day	129.6 ± 1.012***	Opened	+++
3	70% ethanolic extract	500	77.29 ± 1.976***	Opened	+++
4	70% ethanolic extract	250	64.12 ± 3.158**	Not opened	+ to ++
5	Aqueous Extract	500	70.65 ±1.819***	Opened	+++
6	Aqueous Extract	250	55.27±1.707**	Not opened	+ to ++
7	95% ethanolic extract	500	60.68 ± 2.879***	Opened	+ to ++
8	95% ethanolic extract	250	52.98 ± 0.972**	Not opened	+ to ++

Values are the Mean ± S.E.M. of six rats / treatment
*Significance **P<0.01 (n=6) ***P<0.001 (vs. Control).*
+ = nucleated epithelial cells
++ = Nucleated epithelial cells & cornified cells
+++ = Cornified cells

Table 2. Micrometric changes in the uterus due to administration of various extracts of *Indigofera linnaei Ali*

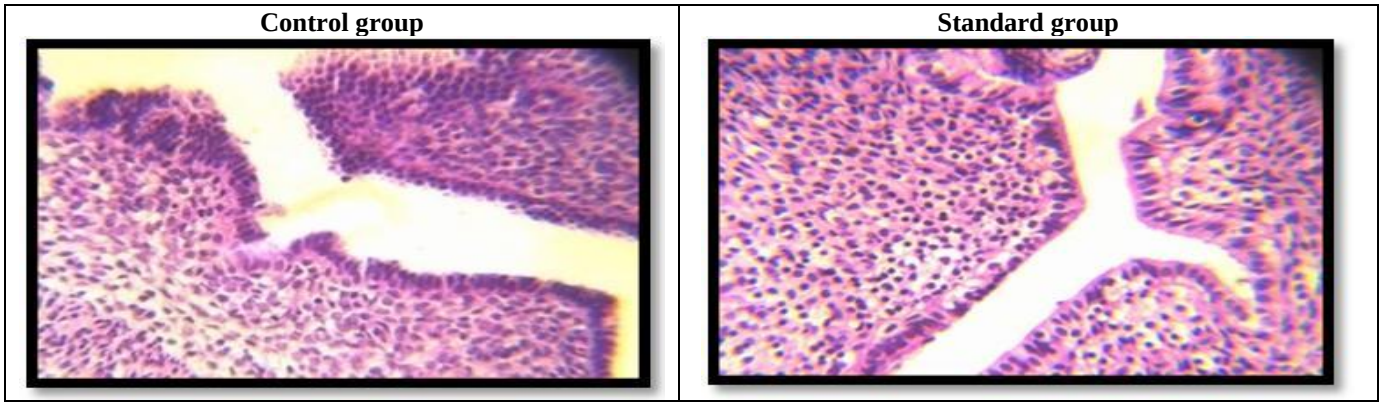
S. No.	Treatment Extracts / Drugs	Dose mg/kg	Diameter of Uterus (μm)	Thickness of Endometrium (μm)	Epithelial cell height (μm)
1	Control (Vehicle)	Tween 80 (1%, 5 ml/kg)	600.0 ± 5.77	296.7 ± 2.108	9.833 ± 0.166
2	Ethinyl estradiol (Estradial valerate)	1 μg/rat/day	1810 ± 7.30***	645.0 ± 4.282***	17.58 ± 0.153***
3	70% ethanolic extract	500 mg	1008.00 ± 10.14***	523.3 ± 6.146***	14.83 ± 0.166***
4	70% ethanolic extract	250 mg	780.00 ± 5.77**	340.0 ± 2.582**	12.58 ± 0.083**
5	Aqueous Extract	500 mg	928.3 ± 8.33***	486.7 ± 3.333***	13.92 ± 0.083***
6	Aqueous Extract	250 mg	716.7 ±9.54**	333.3 ± 1.054**	11.17 ± 0.166**
7	95% ethanolic extract	500 mg	850.00± 13.17***	400 ± 5.774***	13.25 ± 0.111***
8	95% ethanolic extract	250 mg	648.3 ± 3.07**	316.7 ± 1.667*	9.833 ± 0.166^{ns}

*Significance **P<0.01 (n=6) ***P<0.001 (vs. Control).* ns = Not Significance.

Table 3. Biochemical changes in the uterus due to administration of various extracts of *Indigofera linnaei Ali*

Sl. No.	Treatment Extracts / Drugs	Dose mg/kg	Glucose mg/dl	Alkaline phosphatase Iu/dl	Cholesterol mg/dl
1	Control (Vehicle)	Tween 80 (1%, 5 ml/kg)	0.595 ± 0.007	0.458 ±0.014	5.10 ±0.081
2	Ethinyl estradial (Estradial valerate)	1 μg/rat/day	1.418 ± 0.008***	0.933 ±0.013***	8.498 ±0.010***
3	70% ethanolic extract	500 mg	1.020 ±0.012***	0.706 ±0.005***	7.333 ±0.033***
4	70% ethanolic extract	250 mg	0.746 ± 0.016**	0.546 ±0.019**	6.267 ±0.042**
5	Aqueous Extract	500 mg	0.908 ± 0.006***	0.646 ±0.012***	6.917 ±0.030***
6	Aqueous Extract	250 mg	0.728 ± 0.007**	0.530 ±0.005**	5.883 ±0.030**
7	95% ethanolic extract	500 mg	0.823 ± 0.009***	0.60 ±0.005***	6.60 ±0.036***
8	95% ethanolic extract	250 mg	0.656 ± 0.012**	0.495 ±0.003^{NS}	5.80 ±0.036**

Values are the Mean ± S.E.M. of six rats / treatment.
*Significance **P<0.01 (n=6) ***P<0.001 (vs. Control).*
ns – Not significant



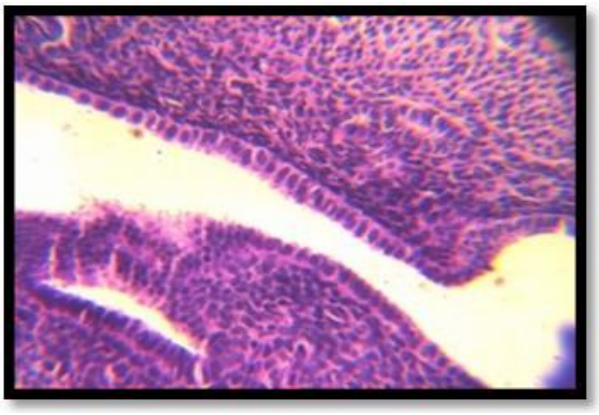
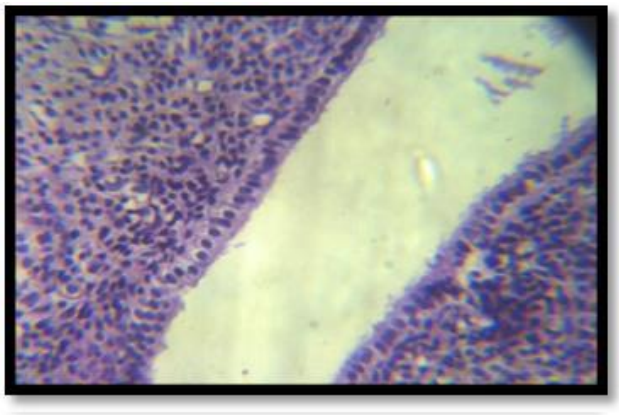
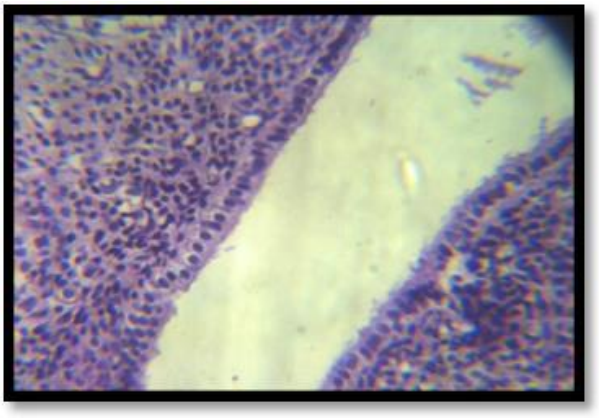
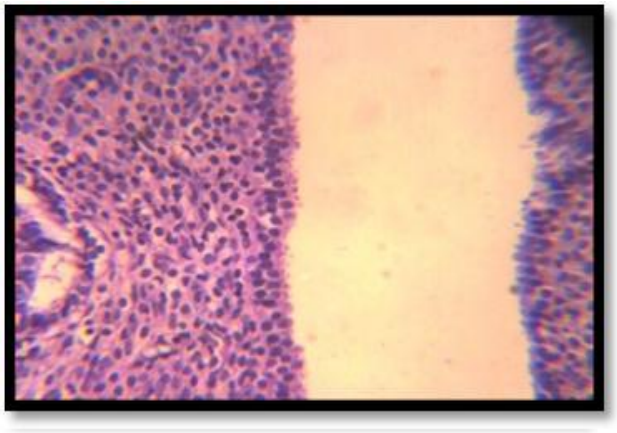
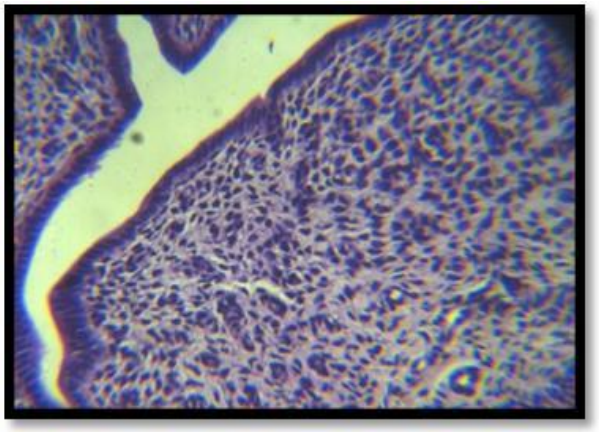
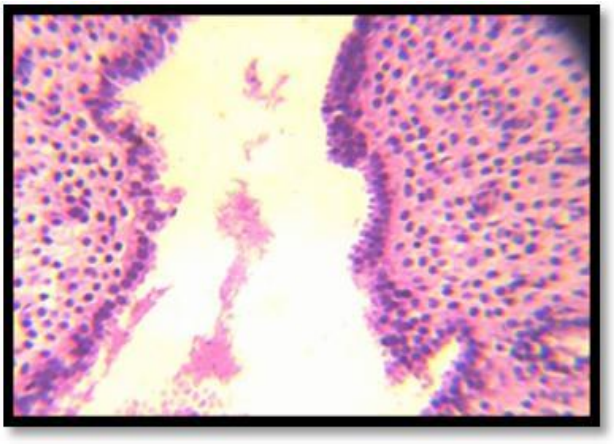
70% ethanolic extract 	**70% ethanolic extract** 
500mg/kg aqueous extract 500 mg/kg 	**250mg/kg aqueous extract 250 mg/kg** 
95% ethanolic extract 500 mg/kg 	**95% ethanolic extract 250 mg/kg** 

Table 4. Effect of various extracts of *Indigofera linnaei Ali* on anti-implantation and early abortifacient activity

Group and Treatment	Animals Used	Anti-implantation		Early Abortifacient	% of Anti-implantation activity	% of Early Abortifacient activity	% of Anti-fertility activity
		No. of Implantation	No. of Corpora-lutea	No. of Resorbed Implantation			
I. Control (vehicle)	6	13 13 12 14 12 13	14 15 16 14 13 15	- - - - - -	15.3	0.00	15.3
70% ethanolic extract (500mg/kg)	6	5 6 8 7 4 6	11 13 14 12 14 13	2 1 1 2 1 1	53.9	10.30	64.2
70% ethanolic extract (250mg/kg)	6	9 6 8 10 8 8	13 10 13 11 12 13	1 1 1 - 1 1	38.47	6.38	44.85
IV. Aqueous Extract (500mg/kg)	6	8 6 6 5 7 6	14 13 13 14 13 10	1 - 1 1 2 2	51.54	8.92	60.46
V. Aqueous Extract (250mg/kg)	6	10 11 8 9 9 8	13 14 13 12 13 12	- - 1 1 1 1	30.8	5.07	35.87
95% ethanolic extract (500mg/kg)	6	8 5 7 8 7 8	14 11 12 14 12 14	1 1 1 1 - 2	46.2	7.69	53.89
95% ethanolic extract (250mg/kg)	6	10 8 11 10 11 9	12 12 14 13 14 13	- 1 - 1 - 1	23.1	3.8	26.9

DISCUSSION

Research on fertility regulating plants has been given priority by Central Drug Research Institute (CDRI) Lucknow and Indian Council of Medical Research (ICMR) New Delhi, in recent years, but so far not a single plant product is marketed, which can be used as anti-fertility agent in this direction the efforts have been made on the anti-fertility activity of *Indigofera linnaei Ali*. The data obtained in the present study indicates that 70% ethanolic, aqueous and 95% ethanolic extract of herb of *Indigofera linnaei Ali* exhibited more significant anti-fertility activity in dose dependent manner. 95% ethanolic, 70% ethanolic and aqueous extracts at dose of 500 mg/kg b.w., 250 mg/kg b.w. were found to posses highly significant estrogenic activity as indicated by increase in uterine weight, vaginal cornification and uterotropic responses. In immature female rats, when compared to control, but not significantly greater than standard in dose dependent manner.

In the present study 70% ethanolic and aqueous extracts at the dose of 250 mg/kg b.w. and 500 mg/kg b.w. tested for anti-implantation and abortifacient activity exhibited a significant decrease in number of implantation sites and increase in number of resorbtions in a dose dependent manner, where as 95% ethanolic extract exhibited less significant anti-implantation and abortifacient activity, when compared to other extracts in dose dependent manner.

Estrogenic activity is shared by many steroidal and non-steroidal compounds. The three principal native forms of known endogenous estrogens are 17-β estradiol estrone and estriol. The most potent biologic form is 17-β estradiol, which is used as a component of oral contraceptives for inhibiting gonadotropin secretion.

One of the first non-steroidal estrogens is diethylstilbestrol, which is structurally similar to estradiol. The non-steroidal compounds with estrogenic activity including flavonoids (flavones, flavonones and isoflavonoids) alkaloids, phenolics, occur in variety of plants are well documented as anti-fertility agents (Anderson LL *et al*., 1972; Heeshma Khushalani *et al*., 2006). It has been observed that 70% ethanolic, aqueous and 95% ethanolic extracts of herb of *Indigofera linnaei Ali* at dose of 250 mg/kg b.w. and 500 mg/kg b.w. provoked significant increase in the uterine weight, induces vaginal opening and cornification of vaginal epithelial cells and increases the uterotropic potency in dose dependent manner.

In the present study the histological evidence of the uterus treated with 95% ethanolic, 70% ethanolic and aqueous extracts clearly supports an unfavorable uterine milieus, showing obliterated lumen with loose stroma, increased height of luminal epithelium and stimulated uterine gland in respective extracts, therefore from the present findings it can safely be said that all the extracts possesses estrogenic activity in dose dependent manner.

Anti-implantation and early abortifacient activity.

Implantation in rat depends on the completion of basic sequence of events occurring both in fertilized egg and endometrium. The fertilization takes place in the fallopian tube and then the developing ovum following the above pattern enters the uterus different extracts of *Indigofera linnaei Ali* administered orally from day 1 to 7 of pregnancy in rats exhibited highly significant loss of implants suggesting anti-implantation activity exhibiting the highest loss of implants with 70% ethanolic extract at dose of 500 mg/kg b.w. this effect may be due to the imbalance in the estrogen-progesterone environment. Pituitary hormones are essential for first 11 days of pregnancy, progesterone a pregnancy hormone secreted by corpora lutea is sustained by reduction with FSH through day 1-7, LH becomes the important luteotropic hormone from day 8-12 of pregnancy to maintain the progesterone secretion of corpora lutea and thereafter placenta will take over the function. The level of estrogen secretion during pregnancy is comparatively lower, compared to progesterone as the former is in the range of nanograms and later in micrograms, throughout pregnancy except near term (Padmashali B *et al*., 2006; Ashok Rao *et al*., 2006).

Thus progesterone is the main hormone to maintain pregnancy, the synergistic action of estrogen and progesterone during gestation is necessary for maintenance of pregnancy successfully. In several species including many non-humans progesterone and estrogen synergistic action is essential for blastocyst implantation and for maintenance of pregnancy in all phases.

In the present study the anti-implantation and abortifacient activity of the extract is mainly due to its confirmed estrogenic activity, high dose of estrogen improportionate to progesterone leads to resorbtion of fetuses (Vasudeva Neeru B *et al*., 2006; Geremew Tafesse *et al*., 2006). The foetal loss in the present study is mainly due to the resorbtion of embryos because of absence of vaginal bleeding. The persistence of placentomas in the uterus observed on 18^{th} day of pregnancy also supports that foetal loss is mainly due to resorbtion, it is evident from the above facts that the 95% ethanolic, 70% ethanolic and aqueous extracts contains the compounds which are anti-implantation and abortifacient (Geetha M B *et al*., 2005; *et al*., Amira Kassem 2006).

Thus the data obtained form phytochemical and pharmacological evaluations of whole herb of *Indigofera linnaei Ali* tend to suggest that 70% ethanolic and aqueous extract possess significant and 95% ethanolic extract possess less significant estrogenic, anti-implantation and early abortifacient activity in a dose dependent manner.

CONCLUSION

The present study of anti-fertility activity was carried out on 70% ethanolic, 95% ethanolic and aqueous extracts of *Indigofera linnaei Ali* by using estrogenic anti-implantation and abortifacient models. The 70% ethanolic and aqueous extracts of *Indigofera linnaei Ali* were found to possess highly significant and 95% ethanolic extract found to possess less significant anti-fertility activity against estrogenic anti-implantation and abortifacient experimental models in dose dependent manner. The estrogenic activity of 70% ethanolic, aqueous extracts and 95% ethanolic extract at the dose 500 mg/kg b.w. were found to possess significant estrogenic activity as indicated by increase in uterine weight, vaginal carnification and uterotropic potency in immature female rats when compared to control, but not significantly greater than the standard. The biochemical changes like con. of glucose, cholesterol and alkaline phosphatase is also increase in treated group with plant extract when compare with control group but significantly less than standard group.

The anti-implantation and early abortifacient activity of 70% ethanolic and aqueous extracts of *Indigofera linnaei Ali* at a dose of 500 mg/kg b.w. were found to be more significant when compared with other doses of the extracts. Whereas 95% ethanolic extract showed less significant anti-implantation and early abortifacient activity when compared to other two extracts in a dose dependent manner.

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