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ANTI-FERTILITY POTENTIAL OF METHANOLIC EXTRACT OF (HENNA) LAWSONIA INERMIS (LYTHRACEAE) ROOT IN FEMALE RATS

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ABSTRACT

Background: Methanolic extract of *Lawsonia Inermis* root (*L.Inermis*) has been used as an anti-fertility plant in India. **Objective:** To investigate the effects of *L.Inermis* on the fertility rate in female rats. **Material and Methods:** In this experimental study, female albino rats were divided randomly into five groups according to pharmacological screening models. Group 1 was taken for investigating the pre-implantation activity, group 2/ for investigating anti implantation activity, 3/ for investigating of biochemical parameters, 4/ for the Teratogenic activity and 5/ for the body weight and uterus weight. In these groups, the animals were divided into three groups as: 1. Control group received distilled water 2. Treated group received plant extract at dose of 100mg/kg of body weight 3. Treated group received plant extract at dose of 200 mg/kg on the 1st day pregnancy according to the model. In group 1, treated animals were euthanized at 7th days of pregnancy and number of implantation sites was counted. In group 2, treated animals maintained till delivery time and after delivery, the number and weight of neonates were investigated. **Results:** Data showed that administration of *L. Inermis* extract on days 1-5 of pregnancy significantly decreased the number of implantation sites, number and weight of neonates. **Conclusion:** The results of present experiment suggested that the methanolic extract of root of *L. Inermis* exerted a significant anti-fertility effect in female rats. The dose and efficacy of results could be extrapolated in future clinical trials.

KEYWORDS: Anti-fertility, Lawsonia Inermis.

INTRODUCTION

From several times, modern drugs have their origin in historical plant medicine. The therapeutic efficacies of many indigenous plants for several disorders have been described by practitioners of traditional herbal medicines. Natural products are a significant source of synthetic and traditional herbal medicine and are still used in primary health care system.[1]

Anti-fertility agents are the drugs that regulate fertility and are also termed as oral contraceptive. These drugs act on and are influence in the menses and ovulation in females. Estrogen and progesterone in joint form are given as birth control pills. Anti-fertility substance is deemed to be active in females when it prevents fertilization, prevents ovulation, implantation, and destroys the zygote or causes abortion.[4]

Infertility is of two types as following:

- Primary infertility is infertility in a couple who have never had a child.
- Secondary infertility is failure to conceive following a previous pregnancy.

Herbal contraceptives are in pleasing demand because they are well paid, easily available from local origin and have fewer side effects. However, herbal medicines may deteriorate fertility in male and female animals or humans. The current attempt is to review and assemble updated information on different aspects of *L. inermis*, a plant used all over the world. This plant is commonly known as Henna or Mehndi and sufficiently available in tropical and subtropical areas. Antique history of India defines its diverse use and observable role in ayurvedic or natural herbal medicines. Henna has been used as cosmetic agent and medicinally for over 9,000 years.[8]

Plant profile: *Lawsonia inermis* Linn. is commonly known as 'Henna'. It belongs to the family "*Lythraceae*". It is a fern/hedge ordinarily cultivated in the Middle East,

Leaves of henna plant

Seeds of henna plant

Along the African coast of the Mediterranean Sea and India All parts of the plant (root, stem, leaves, flower pods, and seeds) are of great medicinal importance. It is commonly used in cosmetic and as a medicinal plant.[7]

Botanical classification

Kingdom	**Plantae**
Division	Magnoliophyta
Class	Magnoliopsida
Order	Myrtales
Family	Lythraceae
Genus	*Lawsonia*
Species	*L. Inermis*
Habitat	In India, it is scarcely present in dry deciduous forests and cultivated as hedge plant.[9]

Extract of henna root

Root of henna plant

MATERIAL AND METHODS

Plant collection: The root of *Lawsonia Inermis* was collected from the local region of Bareilly District. The plant was identified as *Lawsonia Inermis* and authenticated by the botanist of Department of Plant Science, M. J. P. Rohilkhand University Bareilly U.P. The voucher specimen has been deposited in department for further reference.

***Lawsonia Inermis* preparation of root extract**

a) Roots of the plant (*L. Inermis*) were collected, air dried and triturate into powder form.

b) The powdered roots (250 g) were macerated in methanol (80%) and drowned for 7 days; decanted and filtered.

c) The filtrate was vaporized using water bath and dehydrated in room temperature.

d) It was then weighed and stored in sterile bottles prior to use.[13]

Animal preparation: All Experiments were performed on female wistar rats. The animals were housed in polypropylene cages. As bedding material, the Paddy husk was provided and which was changed every day. They were fed with standard pellet diet and purified water. They were kept in a well-aerated room and a 12 hour light and dark cycle was maintained. The room temperature was maintained at 25 ± 2o C. The study was conducted in the Department of Pharmacy, M. J. P. Rohilkhand University, Bareilly. Experiments were performed according to the guidelines for care and use of laboratory animals.

Acute oral toxicity: The doses of *L. inermis* root extract were selected according to acute oral toxicity test of organization for Economic and Cultural Development (OECD) Guideline no 423. Animals were kept under controlled environment 12 h. dark/light cycles (temp. 22±3ºC) and had free access to food and purified water *ad libitum* for 5 days. Three rats (overnight fast) were used for each fixed dose level 5, 50, 300 and 2000 mg/kg. 3 rats were

found dead at 300 mg/kg body weight during 4 – 24 h. From the above investigation dose selected as 100 and 200 mg/kg body weight.

Screening Models

1.) Pre-implantation model: One set of experimental pregnant rats were divided into three groups (n = 6). Group-I served as control which treated with distilled water, group-II and III were treated with *Lawsonia Inermis* (100 mg/kg and 200 mg/kg respectively) as test groups. The rats were sacrificed on 10th day of pregnancy, uteri were removed and the number of prominent corpora lutea gravidities and no. of implants were observed. The frequency of pre-implantation losses was calculated by dividing the missing number of implants (corpora lutea implants) by number of corpora lutea multiplied by 100.[5]

2.) Anti Implantation model: One set of experimental pregnant rats were divided into three groups (n=6). Group I (control) animals received distilled water 1ml/kg only. Group II and III received test drug (*L. inermis* root extract) at the dose levels of 100 and 200 mg/kg respectively. All the extract doses and vehicle were administered orally to the animals once daily throughout 7th Day of pregnancy. On 10th day of pregnancy, the animals were laparotomized under light ether anesthesia using sterile conditions and numbers of implants present in both the uterine horns were determined and the % anti implantation activity was calculated.[6]

3.) Biological parameters: Biochemical parameters were observed of 18th day of pregnant and treated female rats. The sample was collected by the ovary of various animals. The blood sample was centrifuged for collect serum.

I. Total protein

To 20 µl of sample, 1ml of total protein reagent was added and mixed. The mixture was incubated at 37ºC for 15 minutes and the absorbance was measured at 546 nm using a colorimeter.

The protein content was calculated by using the following formula and expressed as total protein in g/dL.

$$\text{Total protein} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times 200$$

II. Total cholesterol

To 10 µl of sample, 1000ml of reagent was added and mixed, incubated for 5 min at 37°C and estimated at 630 nm using a Colorimeter. The cholesterol level was determined by the following formula and expressed as total cholesterol in g/dL.

$$\text{Cholesterol level} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times 200$$

4.) Teratogenic activity: All fetuses were examined as soon as possible after delivery. The following parameters were observed of each litter.

Litter size on the day of parturition, the number of live and dead pups, and the sex and weight of each pup were observed.

Visible physical abnormalities or manner changes in the fetus were recorded.

All pups which were culled were euthanized and given an external and internal gross examination.

III. Alkaline phosphatase

20 µl of sample was mixed with 1000 µl of mixed reagent and estimated in kinetic mode using a colorimeter.

The Alkaline phosphatase level was determined by this formula:

ALP concentration (U/l)

$$= \Delta A/\text{min} \times V_t \times 10^6 \, E \times L \times V_s$$

Where,

Molar absorbance (E) of NADH at 405 nm is 18450

L - Light path 1cm

Vt-Total reaction volume is 1.02 at 37°C

Vs - Sample volume is 0.02 at 37°C

Nursling of all litters was done for 3 weeks after delivery.[10]

5.) Body weight and uterus weight: One set of the pregnant female animals were divided in three groups (n=6). The first group was received distilled water, second and third group were

received the extract 100 and 200mg/kg of body weight for 10 days by oral route respectively. On the 11th day, all the animals were weighed and sacrificed under diethyl ether anesthesia. The ovaries and uteri were dissected out, freed from surrounding tissues, blotted on filter paper and weighed quickly on a balance sensitive to 0.0001 gm. The ovary and uterine ratios were then calculated by dividing the ovary and uterine weight in mg by body weight in grams.[3]

Statistical Analysis: All statistical analysis was conducted using XLSTAT. Results were expressed as mean ± S. E. M. (Standard Error Mean). Differences between groups were analyzed using One way analysis of variance (ANOVA). The *P* value was considered to be significant as $P<0.05$.

RESULT

1) **Effect of methanolic root extract of *L. inermis* on Pre-implantation activity:** On the 10th day, the no. of implants was observed by the no. of corpora lutea. The formula of the frequency of pre-implantation activity was given below. *L. inermis* at both test 1 and test 2 (100 mg/kg and 200 mg/kg) showed significant increase in the frequency of pre-implantation compared to control group.

Formula for pre-implantation activity

$$\textbf{Frequency of Pre-implantation} = \frac{\text{Total no. of Corpora lutea} - \text{Total no. of implants} \times 100}{\text{Total no. of corpora lutea}}$$

Table 1: Pre-implantation activity of Methanolic extract of *L. Inermis* root.

Groups	Treatment	Mated Females	No. of Corpora Lutea/female	No. of Implants/ female	% Frequency of Pre-implantation losses
I.	Control (Distilled water 1ml/kg)	6	8.33 ± 0.65	6.33 ± 0.65	24.20 ± 2.20
II.	Test 1 (*L. Inermis* 100 mg/kg)	6	6.83 ± 0.60	4.66 ± 0.37	31.60 ± 2.98*
III.	Test 2 (*L. Inermis* 200 mg/kg)	6	6.66 ± 0.65	3.50 ± 0.43	47.61 ± 2.95*

Effect of methanolic root extract of *L. inermis* on Anti implantation activity: Results are described as per the anti implantation on day 10th of pregnancy.

The % anti-implantation activity was calculated as per the above given formula. *L. inermis* at both doses test 1 and test 2 (100 mg/kg and 200 mg/kg) showed significantly increase in the

% anti implantation activity by the no. of implants. Number of implants in group II and III was found to be reduced when compared to control group.

The % Anti implantation activity was calculated by the formula:

$$\% \text{ Anti implantation} = \frac{\text{No. of implants in control} - \text{No. of implants in test}}{\text{No. of implants in control}} \times 100$$

Table 2: Anti implantation activity of *L. inermis* root

Group	Treatment	No. of Implants	Anti implantation activity (%)
I.	Control (Distilled water 1ml/kg)	9.16 ± 0.60	00
II.	Test 1 (*L. Inermis* 100 mg/kg)	6.33 ± 0.41*	30.81 ± 2.62*
III.	Test 2 (*L. Inermis* 200 mg/kg)	5.66 ± 0.41*	39.80 ± 5.22*

Biochemical effect of Methanolic extract of *L. Inermis* root:

On the 18th day of pregnancy and treatment, various biochemical parameters were observed. The Total protein level, Cholesterol estimation and alkaline phosphatase level were observed.

The response of the ovary for the level of Protein, Cholesterol and Alkaline phosphatase at dose (100 mg/kg and 200 mg/kg) were significantly decreased when compared with control group.

Table 3: Level of Total protein, Cholesterol, and Alkaline phosphatase/

Group	Treatment	Cholesterol level (mg/dL)	Total Protein level (mg/dL)	Alkaline Phosphatase level (U/L)
I.	Control (Distilled water 1ml/kg)	57.95 ± 1.75	5.24 ± 0.01	9.42 ± 0.19
II.	Test 1 (*L.Inermis* 100mg/kg)	46.95 ± 1.99*	4.72 ± 0.16*	8.13 ± 0.10*
III.	Test 2 (*L.Inermis* 200mg/kg)	40.58 ± 1.92*	3.97 ± 0.07*	7.40 ± 0.10*

Teratogenic effect of methanolic extract of *L. inermis* root: After delivery the litters were observed for quality and appearance.

The weight of litters were increased in test 1, and decreased in test 2 when compared with control group. The crown rump (C-R) length was increased when compared with control group. The litter size were decrease of test 1 and test 2 group when compared with the control group.

Table 4: Effect on Litter quality and appearance.

Groups	Treatment	Appearance	Litter quality		Litter size (pups/dam) Mean ± SEM
			Weight (g)	C-R length(cm)	
I	Control (Distilled water 1ml/kg)	Pink	4.50 ± 0.48	3.05 ± 0.40	6.50 ± 1.07
II	Test 1 (*L. Inermis*100mg/kg)	Pink	5.23 ± 0.23	4.11 ± 0.30	5.56 ± 0.56
III	Test 2 (*L. Inermis* 200mg/kg)	Pink	4.01 ± 0.21	4.23 ± 0.31	4.23 ± 0.52

Effect on Body weight and uterus weight: On 11th day of pregnancy, the animals were observed for the body weight and uterus weight variation.

Table 5: Effect on body weight and uterus weight.

Groups	Treatment	Body weight (g)		Uterus weight (mg)
		Initial wt.	Final wt.	
I	Control (Distilled water 1ml/kg)	181.05 ± 0.13	184.06 ± 0.14	180.80 ± 0.09
II	Test 1 (*L. inermis*100mg/kg)	178.90 ± 1.53	180.01 ± 0.13*	191.50 ± 0.11*
III	Test 2 (*L. inermis*200mg/kg)	180.11 ± 0.09	181.05 ± 0.08*	195.60 ± 0.08*

DISCUSSION: The higher dose (200 mg/kg) of *Lawsonia inermis* was used for acute oral toxicity studies. Clinical toxicity symptoms such as respiratory distress, salivation, weight loss and change in appearance of hair as well as maternal mortality were not observed at any period of the experiment. Similarly no mortality and changes in the behavioral, neurological and autonomic profile were observed in treated groups. This suggested that short term use of the drug (*L. inermis*) for this purpose is apparently safe.[11]

In the present study oral administration of methanolic extract of *Lawsonia Inermis* root at the doses 100 and 200mg/kg orally, produced a dose dependent potential on frequency of pre-implantation losses. The number of implantations in uterine horns of the female rats showed an increase in the percentage of the pre-implantation embryonic loss.[12]

In the present study, we evaluated female rats by assessing their anti-implantation, effect on estrus cycle. Normally, the implantation of concepts occurs on gestation days 4–5 in rodents. Chemical insult prior to completion of the implantation process should result in pre-implantation embryonic loss. During this period, a series of changes occur in uterine wall due to synchronized balanced of estrogen and progesterone concentration before implantation of

fertilized ovum. It is well known that for implantation exact equilibrium of estrogen and progesterone is essential, and any disturbance in the level of these hormones may cause infertility. In our study, the *Lawsonia inermis* has shown more anti-implantation potential as significant number of female rats not succeeded to show implantation sites. The test drug significantly decreased the number of implants and litter born as compared to control group.[5]

The total protein level was significantly found to be elevated by the *L. inermis*. The increased protein level might be due to the presence of steroids. The accumulation of cholesterol and alkaline phosphatase was significantly increased as the dose and duration of the extract increases.[2]

The Teratogenic activity was determined by the appearance of the litter. In which the litter size, weight and color of pups were recorded after delivery. At 100/200 mg/kg of *L. inermis* dose showed significantly decreased in the weight and size of the litter and increased in the C-R (Crown Rump) length of litter as compared to the control group.

The body weight and uterus weight of the female rats after 10^{th} day of pregnancy were observed**.** The *L. inermis* showed increased in the body weight and decreased in the uterus weight as compared to the control group.

CONCLUSION: From the above analysis of results, it can be concluded that *Lawsonia Inermis* extract shows significant anti fertility activity. This activity is additively acts to reach a common goal of finding drug having anti fertility activity. As drug is given orally, the main objective to find an alternative orally active herbal drug with lesser side effect can be achieved. The extract also exhibit biochemical parameters which can be useful. Further studies are required to understand the exact mechanism of action and to isolate the active compounds responsible for the anti fertility action. The results of this study conclude that the methanolic extract of *L. inermis* root at higher dose and prolonged duration, it inhibits the ovarian function, change the uterine alteration and prevent the implantation thus control the fertility in female rats.

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