ORIGINAL ARTICLE

Antifertility activity of ethanolic extracts of *Plumbago indica* and *Aerva lanata* on albino rats

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The ethanolic extract of roots of *Plumbago indica* and aerial parts of *Aerva lanata* were evaluated for antifertility activity using anti-implantation, abortificient, and motility of rat spermatozoa (*in-vitro*) models. The anti-implantation effect seems to be depending on the dose as well as the initiation of treatment on specific days of pregnancy. *P. indica* has showed percentage pre-implantation loss of 40% and 50% against control at the doses of 200 and 400 mg/kg b/w. Percentage pregnancy failure among treated groups was 60% and 70% at the doses of 200 and 400 mg/kg b/w, whereas *A. lanata* has shown pre-implantation loss of 20% and 30% against control at the dose of 200 and 400 mg/kg b/w, respectively. Percentage pregnancy failure among treated groups was 30% and 40% at the dose of 200 and 400 mg/kg b/w, respectively. Both *P. indica* and *A. lanata* at a concentration of 10% have shown no motility of rat spermatozoa within 60 seconds.

Key words: *Plumbago indica, Aerva lanata*, anti-implantation, abortifacient, spermatozoa

INTRODUCTION

The world population explosion has pointed out the need for new, effective, and safe contraceptive agents or methods of maximum protection. Side effects of synthetics on normal and natural human body are much more aggressive and unpredictable at prolonged use as long as one which gender expects to use them. Now time is alarming us to think of some alternative in the field of contraception. Hence, efforts are made to look back on our natural heritage.

Plumbangin and α-amyrin are well-known active constituents at one or the other folds of invention in the field of contraceptive research. Apart from this, several folkloric claims are made on Plumbago species and *Aerva lanata*. To establish scientific claims on reality these plants are taken for this work.

Plumbago indica L. (Plumbaginaceae) is commonly known as Rosy flowered lead wort (English), enlidhsitaparu (Oriya). Jain tribes of Orissa (India) have been effectively using it as an oral contraceptive for birth control.[1]

A. lanata (L) Jauss. Ex schultes (Amaranthaceae) commonly known as Aerva (English) and Gorkhabundi (Hindi) has been documented for its therapeutic effect in controlling kidney disorders, diuretic, as anti-inflammatory, and antidiabetic.[2-4]

MATERIALS AND METHODS

Plant Material

P. indica roots were collected from Udupi district and aerial parts of *A. lanata* was collected from Hubli and Dharwad regions of Karnataka, India. These plants were aunthenticated by Dr. G.R Hegde Professor of Taxonomy, Department of Botany Karnataka University Dharwad, Karnataka, India. The voucher specimens (118/03 and 119/03, respectively) were deposited in Department of Phytochemistry, Karnataka Lingayat Education Societies, College of Pharmacy Belgaum, Karnataka, India.

Animals

Albino rats (SD) of ~125–150 days with average body weight of 150–200g were used for the experiment. The animals had free access to water and food throughout the experiment.

Preparation of Extract

The shade dried roots of *P. indica* and aerial parts of *A. lanata* were coarsely powdered to a mesh size of 40# using a hammer mill and extracted with ethanol (90% v/v) using soxhlet apparatus for 18 hours. The extract was concentrated using rotavapour (Buchi make) and dried at <60º C under vacuum.

Pharmacological Methods

LD50 was determined by the up and down method as per the OECD guidelines[5] using albino rats. The emulsion of the plant extract was prepared using Tween 20 and was administered orally.

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The parameters determined for antifertility activity were anti-implantation potency, abortifacient potency,[6] and sperm motility—an *in-vitro* study.[7]

Based on LD50 studies, doses of 200 mg/kg b/w and 400 mg/kg b/w were selected for experimentation for both the plant extracts.

Anti-implantation Activity

The female albino rats of regular ester cycle were observed for vaginal smear analysis every morning (microscopically). Proven fertility animals were left overnight with proven fertile male albino rats with 1:3 ratio at an early estrous stage of the estrous cycle. Then the vaginal smear of females was observed for the presence of sperms and formation of vaginal plug. Subsequent day was selected as day I of pregnancy. Such pregnant females were grouped into three groups of six each for each extract as follows:

Group I: Served as control, received vehicle orally for 1–7 days of pregnancy.

Group II: Received 200 mg/kg b/w emulsion of plant extract for 1–7 days of pregnancy.

Group III: Received 400 mg/kg b/w emulsion of plant extract for 1–7 days of pregnancy.

On the 10th day of pregnancy, rats of all groups were laparotimized under anesthetic ether to know the presence of implantation sites in the uterine horns to detect the anti-fertility activity. After laparotomy, the animals were maintained to check further pregnancy conditions.

Abortifacient Activity

The female albino rats which were used for the anti-implantation activity were used here for abortificient studies. Further continued with below mentioned dose upto 18th day of pregnancy.

For each plant extract:

Group I: served as control, received vehicle orally for 10–18 days of pregnancy.

Group II: received 200 mg/kg b/w emulsion of plant extract for 10–18 days of pregnancy.

Group III: received 400 mg/kg BW, emulsion of plant extract for 10–18 days of pregnancy.

Second leprotomy was performed 3 days after the completion of treatment, i.e., on 21st day of pregnancy. The number of implantation sites were counted and compared with the initial number of implantation on the 10th day of pregnancy. The percentage of pregnancy failure among treated groups was calculated.

Sperm Motility—An *In-vitro* Study

Six healthy adult male albino rats were used for this study. Animals were anesthetized using anesthetic ether. The skin of the scrotum was cut to open *tunica vaginalis*. The small incision was made to make a vent so that epididymis were removed and flushed into 1 ml of previously warmed buffer saline of pH 7.0 at 37°C to make the suspension of spermatozoa. The testis was returned back to the scrotal sac and the wound was sutured properly, then disinfectant care was taken. At least 80% initial normal fertility and normal sperm motility and 2.5×10^7/cc sperm count was considered for the selection of samples. Both 10 µl of sperm suspension and 10 µl of different plant extract (1:1) at different concentrations of 0, 1, 2.5, and 10% were placed on clean and dry glass cavity slides. Then this fluid was examined under the binocular microscope at 10× and 15× magnifications. The motility of sperm was observed at various time intervals up to 150 seconds. A 10 µl of saline buffer solution was used as control.

Statistical Analysis

Data were analyzed using the Student unpaired *t*-test.

RESULTS

Effect of the Extracts for Anti-implantation Activity

Table 1 shows the effect of extracts on percentage preimplantation loss in pregnant rats. *P. indica* ethanolic extract of roots and *A. lanata* ethanolic extract of aerial parts have shown 50% and 30% activity at 400 mg/kg b/w, respectively.

Effect of the Extracts for Abortifacient Activity

Table 2 shows the effect of extracts on percentage abortion in pregnant rats. *P. indica* ethanolic extract of roots and *A. lanata* ethanolic extract of aerial parts have shown 70% and 40% activity at 400 mg/kg b/w, respectively.

Table 1: Anti-implantation activity of *Plumbago indica* ethanolic extract of roots and *Aerva lanata* ethanolic extract of aerial parts

Group	Treatment Mg/kg b/w	No. of rats Pregnant	*Plumbago indica*		*Aerva lanata*	
			No. of implantations Mean	% Pre implantation loss	No. of implantations Mean	% Pre implantation loss
I	Control	06/06	10.0 ± 0.29	00.00	10.0 ± 0.29	00.00
II	200	06/06	6.0 ± 0.10	40.00	8.0 ± 0.13	20.00
III	400	06/06	5.0 ± 0.20	50.00	7.0 ± 0.05	30.00

Table 2: Abortificient activity of *Plumbago indica* ethanolic extract of roots and *Aerva lanata* ethanolic extract of aerial parts

Group	Treatment Mg/kg b/w	No. of rats Pregnant	Plumbago indica			*Aerva lanata*		
			No. of implantations 10th day	No. of Implantations 18th day	% of Abortion	No. of implantations 10th day	No. of Implantations 18th day	% of Abortion
I	Control	06/06	10.0 ± 0.29	10.0 ± 0.29	00.00	10.0 ± 0.29	10.0 ± 0.29	00.00
II	200	06/06	6.0 ± 0.14	4.0 ± 0.04	60.00	8.0 ± 0.13	7.0 ± 0.06	30.00
III	400	06/06	5.0 ± 0.06	3.0 ± 0.06	70.00	7.0 ± 0.05	6.0 ± 0.10	40.00

Table 3: Effect of Ethanol extract of *Plumbago Indica* on motility of rat spermatozoa *in vitro* Percent Motility of Spermatozoa at different duration (Sec)

Treatment	0 sec	15 sec	30 sec	60 sec	90 sec	120 sec	150 sec
Control	82.0±1.40	82.0±1.40	81.8±1.59	81.8±1.59	81.6±1.30	81.6±1.30	80.9±1.18
P.Indica 1%	82.0±1.40	70.0±1.97*	62.0±1.95*	48.5±1.40*	31.7±2.40*	20.6±1.10*	Motility nil
P.Indica 2.5%	82.0±1.40	50.2±2.80*	40.8±2.06*	28.7±2.00*	06.2±1.80*	Motility nil	
P.Indica 5%	82.0±1.40	36.8±2.50*	17.8±2.06*	05.8±1.80*	Motility nil		
P.Indica 10%	82.0±1.40	20.7±1.90*	08.2±1.20*	Motility nil			

Data were analyzed by ANOVA followed by Dunnett's test. Values are represented as mean ± S.E.M. (n=6); *P< 0.01 was considered as significant.

Table 4: Effect of ethanol extract of *Aerva lanata* on motility of rat spermatozoa *in vitro* Percent Motility of Spermatozoa at different duration (Sec)

Treatment	0 sec	15 sec	30 sec	60 sec	90 sec	120 sec	150 sec
Control	82.0±1.59	82.0±1.59	82.0±1.59	81.6±2.50	81.0±2.25	80.6±2.00	80.0±1.6
A.lanata 1%	82.0±1.59	60.0±1.97*	58.5±2.48*	46.7±2.48*	36.6±2.48*	12.6±1.14*	Motility nil
A.lanata 2.5%	82.0±1.59	50.0±2.86*	39.8±2.05*	28.7±2.56*	6.2±1.80*	Motility nil	
A.lanata5%	82.0±1.59	36.6±1.68*	17.6±2.19*	6.4±1.22 *	Motility nil		
A.lanata 10%	82.0±1.59	15.7±1.59*	8.2±1.12 *	Motility nil			

Data were analyzed by ANOVA followed by Dunnett's test. Values are represented as mean ± S.E.M. (n=6); *P< 0.01 was considered as significant

Effect of the Extracts for *In-vitro* Motility of Rat Spermatozoa
Table 3 shows the effect of extracts on the percentage motility of rat spermatozoa at different duration in seconds. *P. indica* extract has shown nil motility at 60 seconds.

The effect of *A. lanata* extract on the percentage motility of rat spermatozoa has been shown in Table 4. Motility nil was observed at 90 seconds at 10% concentration.

DISCUSSION

Oral administration of *P. indica* ethanolic root extract at two different doses (200 and 400 mg/kg b/w) has shown most significant activity in all the three modes of antifertility studies. Preimplantation losses of 40% and 50% were observed in the anti-implantation model, whereas 60% and 70% of pregnancy failure were observed in abortifacient activity.

The presence of α-amyrin in *A. lanata* (Chandra, 1997) and its use as antifertility in male albino rats[8] made us to take up this work. The study was carried out at two different doses of 200 and 400 mg/kg b/w. Corresponds to anti-implantation activity at a dose of 200 mg/kg b/w that shows only 20%, whereas 400 mg/kg b/w shows 30% preimplantation loss. Similarly, in the abortifacient model, 200 and 400 mg/kg b/w show pregnancy failure of 30% and 40%, respectively.

Among the different concentrations of extracts of *P. indica* and *A. lanata* used for motility of rat spermatozoa, an *in-vitro* study showed a good response at lower time duration of 60 seconds and no motility by 90 seconds at a concentration of 10% and 5%, respectively.

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