

ABORTIFACIENT EFFICACY OF *INDIGOFERA TRIFOLIATA* LEAVES EXTRACT ON FEMALE ALBINO RATS

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ABSTRACT

Objective: To study the potential abortifacient activity of the *Indigofera trifoliata* leaves extract (aqueous, alcohol, Ethyl acetate and chloroform) in female albino rats.

Methods: Pregnant rats weighing 120 to 200 gm were randomized into 13 groups. Rats were laprotomised on 10th day of pregnancy and live fetuses were observed in both the horns of the uterus. Rats in group 1 (control) were orally administered, with 0.5 ml of distilled water once daily while those in group 2 to 13 (experimental groups) were administered 100, 200 and 400 mg/kg body weight doses of aqueous, alcoholic, chloroform and ethyl acetate extract of *Indigofera trifoliata* leaves respectively. The doses were administered from day 11th to 15th of pregnancy of rats and then the animals were allowed to go full term.

Results: The phytochemical screening revealed the presence of alkaloids, flavonoids, simple phenolics, steroids, tannins and saponins. Clinical toxicity symptoms such as respiratory distress, salivation, weight loss, dull eyes, diarrhea, and change in the appearance of fur, and mortality were not observed in the animals at any period of the experiment. All the four extracts of *I. trifoliata* leaves exhibited abortifacient activity (7- 92.30%). The extracts significantly reduced the number of live fetuses, whereas the resorption index and post implantation losses increased significantly. The % of abortion was found to be highest (92.30%) with 400 mg/kg dose of alcoholic extract of *I. trifoliata* leaves. The hormonal assay shows that, there was reduction in the level of FSH and LH hormone, while level of estrogen increased however there was slight decrease in the level of progesterone hormone. The administration of extract shows significant changes in the body weight of animals, however there was slight decrease in weight of ovary and increase in the uterine weight in treated rats.

Conclusion: The study has provided evidence for the abortifacient activity of all the four extracts of *Indigofera trifoliata*. However the abortifacient properties were found to be more Pronounced at 400 mg/kg dose of aqueous extract.

Keywords: Abortifacient, Female albino rat, *Indigofera trifoliata*, Post implantation, Resorption

INTRODUCTION

Throughout the history women have tried to control or enhance their fertility with various levels of societal support. Many herbal remedies are traditionally used as contraceptive (to prevent the ovulation or fertilization), abortifacients (to prevent implantation) and emmenagogues (to prevent the uterine flow) or oxytocics (to stimulate uterine contractions, particularly to promote labour) [1]. A number of plants species have been tested for fertility regulation beginning about 50 years ago and were subsequently fortified by national and international agencies [2, 3]. Numerous herbs have been reported used historically by women to aid child delivery, stimulate menstrual flow or reduce fertility [4]. Therefore, the screening of plants with abortifacient activity and the subsequent identification and characterization of the active principle will prove to be useful guide towards the formulation of cheaper, affordable contraceptive with reduced toxicity. One plant that featured prominently from our ethanobotanical survey on herbal contraceptive is *I. trifoliata* which is also claimed to be used as "wash the uterus" by the tribal's of Dhamangaon region (20° 46' N and to 78° 8' E) of Amravati district of Maharashtra state of India.

Indigofera trifoliata L (Hindi: Diwali) belongs to family Fabaceae, which is a shrub widely distributed in various parts of India. The genus *Indigofera* comprises around 700 species that are distributed geographically in tropical regions. It is usually an erect plant about 70 cm tall, the leaves are trifoliolate, the lower surface particularly are densely covered with 2-armed or branched hairs; small circular glands are also present. Flowers are present in cluster on the leaf axils and are in red colour about 3.5 mm long, densely covered on the back with hairs and glands. The pod is square in cross-section; length varies from 8-18 mm and contains 4-8 seeds. The stem is used as an antitumor, anti-inflammation, antiviral and antimycobacterial [5]. Leaves and flower are used in skin cancer,

leprosy, cough and swelling of abdomen [6]. Leaves exclusively are used as abortifacient [7]. The plant has very specific use in psoriasis [8]. Root is chewed as a remedy for toothache [7].

The present work was undertaken to validate scientifically the abortifacient role of *I. trifoliata* leaves as acclaimed by the traditional tribal user of Dhamangaon region of Amravati district, Maharashtra. But to the best of our knowledge, there is no information in the open scientific literature that has substantiated or refuted the abortifacient claims of *I. trifoliata* leaves in the folklore medicine.

MATERIALS AND METHODS**Collection of plant material**

The leaves *I. trifoliata* plant were collected from Dhamangaon region of Amravati district of during the flowering period of September to December, identified and authenticated by experts from Botanical Survey of India, Pune (Accession No. DD- 3).

Procurement and rearing of experimental animal

Healthy wistar strain female albino rats of about two month old and weighing 150- 250 g were procured from Sudhakarrao Naik Institute of Pharmacy, Pusad (Maharashtra). The rats were housed in polypropylene cages and maintained under environmentally controlled room provided with a 12:12 hour light and dark cycle approximately at 25 °C. They were fed on pellets (Trimurti Lab Feeds, Nagpur) and tap water *ad libitum*. The rats were allowed to acclimatize to laboratory environment for 15 days before experimentation.

All experimental protocols were subjected to the scrutinization and approval of Institutional Animal Ethics Committee [registration number 1060/ac/07/CPCSEA (IAEC/7/2009)].

Preparation of extract

The leaves of *I. trifoliata* were collected, shade dried, powdered and subjected to soxhlet extraction successively with distilled water, ethanol, chloroform and ethyl acetate. The extract was evaporated to near dryness on a water bath, weighed and kept at 4 °C in refrigerator until further use.

Phytochemical screening

The presence of various plant constituents in the plant extract was determined by preliminary phytochemical screening as per Thimmaiah [9].

Acute toxicity study

Healthy female albino rats were starved for 3- 4 hour and subjected to acute toxicity studies as per Organization of Economic Co-operation and Development (OECD) guidelines No: 423 [10]. They were divided into 5 groups of 6 animals each and kept singly in separate cages during the experiment. Group 1 represented the control group, which received 10 ml/kg of distilled water orally. Groups 2- 5 received suspension of different extract (aqueous, alcohol, chloroform and ethyl acetate) of *I. trifoliata* leaf orally at the doses of 1000, 2000 and 4000 mg/kg daily for 7 days respectively. The rats were observed continuously for 2 hours for behavioral, neurological and autonomic profile, and for next 24 and 72 hours for any lethality or death.

Abortifacient activity

The plant extracts were tested in female albino rats for abortifacient activity as per Khanna et al [11]. The female rats in proestrous phase were caged with males of proven fertility in the ratio of 2:1, in the evening and examined the following day for the evidence of copulation. Rats exhibiting thick clump of spermatozoa in their vaginal smear were separated and that day was designated as day 1 of pregnancy. These rats were randomly distributed into 13 groups, 1 control group and 12 experimental groups of 6 animals each. On the day 10 of pregnancy animals were laprotomised under light ether anesthesia using sterile conditions. The two horns of uteri were examined to determine the implantation sites. Thereafter the abdominal wound was sutured in layers. The extract to be tested were then fed to operated pregnant rats i.e. aqueous extract, alcoholic extract, ethyl acetate extract and chloroform extract of *I. trifoliata* (leaves) at doses of 100, 200, 400 mg/kg body weight (one tenth of the highest tolerable dose) once daily by an intragastric (i. g.) soft rubber catheter from day 11 up to the 15th day of pregnancy. The animals were allowed to go full term. After delivery the pups were counted and the abortifacient activity of extract was evaluated. The following parameters were computed: number of live and dead fetuses; % survival ratio= (number of live fetus/number of live+ dead fetus) ×100; resorption index= (total number of resorption sites/total number of implantation sites) ×100; postimplantation loss= (number of implantations-number of live fetuses/ number of implantations) ×100. The anogenital distance (AGD) and crown rump length (CRL) of litters were measured by using a measuring tape. The variations in birth weight of litters and gestation period between control and experimental animal were also determined to check the abortive effect of *I. trifoliata* [12].

Effect on body weight and reproductive organ weight

The aqueous extract of *I. trifoliata* leaves was found to be most active amongst the four treatments in the abortifacient testing. Hence aqueous extract was used for detailed investigation in rats for their body weight changes as well as for their reproductive organ weight analysis. Sexually experienced female albino rats were divided into 4 groups of 6 animals each; Group 1 represented the control group, which received 10 ml/kg of distilled water orally. Group 2- 4 received suspension of the aqueous extract of *I. trifoliata* leaves orally at the dose 100, 200 and 400 mg/kg, daily for 21 days.

After 21 days of treatment, the next morning (Day 22), all the control and experimental groups of female rats were evaluated. The animals were completely anaesthetized with anesthetic ether (Narsons Pharma) and then sacrificed by cervical decapitation. The ovary and uterus were carefully removed and weighed using digital electronic balance (Adair Dutt) [13- 15].

Effect on hormonal level

The aqueous extract of *I. trifoliata* leaves was found to be most active amongst the four treatments in the abortifacient testing. Hence rats treated with aqueous extract were subjected to a detailed investigation for the study of hormonal assay. Sexually experienced female albino rats were divided into 4 groups of 6 animals each; Group 1 represented the control group, which received 10 ml/kg of distilled water orally. Group 2- 4 received suspension of the aqueous extract of *I. trifoliata* leaves orally at the dose 100, 200 and 400 mg/kg, daily for 21 days. After 21 days of treatment all the control and experimental groups of female rats sera were analyzed for estrogen, progesterone, luteinizing and follicle stimulating hormone level with AccuLite master CLIA VAST Enabled kit by Chemiluminescence immunoassay (CLIA) method with semi automated Chemiluminescence analyzer and autoplex- A processor for CLIA [16- 17].

Statistical analysis

All the data are expressed as mean ± SEM (Standard error). Statistical analysis was done by Student's t-test and one way ANOVA [18].

RESULTS AND DISCUSSION

Preliminary phytochemical screening of *Indigofera trifoliata* leaves revealed the presence of alkaloid, steroids, flavanoids, phenolics compound, saponins, and tannins respectively. The flavonoid isolated from *Striga lutea* and *Striga orabanchioides* possessed strong estrogenic and abortifacient properties [19, 20]. Flavonoids isolated from *Butea monosperma* (Lam) have been reported to possess antifertility activity [21]. Sex hormones being steroidal compounds, the plant sterols were suspected to be responsible for the antifertility effects of the leaves of *I. trifoliata* [22]. Alkaloids like constituent were reported to be possibly responsible for the suppressant effect on the uterine normal contraction and the high anti- implantation activity exhibited by the aqueous extract of *Graptophyllum pictum* [23]. These alkaloids, steroids, flavonoids, saponins present in the *I. trifoliata* extract might be responsible for its contraceptive activity.

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 behavioural, neurological and autonomic profile were observed in treated groups. The highest dose 4000 mg/kg body weight was used for abortifacient activity. This suggested that short term use for this purpose is apparently safe. Similar finding was also observed by Tajuddin, et al [24], while working on ethanolic extract of *Myristica fragrans* and Zade, et al [25] on *Moringa oleifera* in female rats.

The leaves of *I. trifoliata* have been in use by the tribals of Dhamangaon region of Amravati district as a means of abortifacient even without recourse to the scientific validity of the claim. Hence this study was carried out to validate scientifically this tribal claim. The oral administration of *I. trifoliata* leaf extract (aqueous, alcohol, ethyl acetate and chloroform) at the doses of 100, 200 and 400 mg/kg body weight produced a dose dependent adverse effect on fertility index and on number of implantation in the uterine horns of the female rats by virtue of an increase in the percentage of the post-implantation embryonic loss. All the experimental extracts when evaluated for their abortifacient activity, were found to exhibit pregnancy interceptive activity. Administration of 400 mg/kg body weight of the aqueous extract resulted in 92.30% abortion (Table 1).

Table 1: Effect of aqueous, ethanol, ethyl acetate and chloroform extract of *Indigofera trifoliata* (leaves) on fertility of rats when fed orally from day 11 to 15 of pregnancy

Treatment groups	Dose (mg/ kg body wt.)	No. of foetus individual rats on day 10	No. of rats delivered (litter size)	No. of resorption in individual rats	No. of resorption (mean± S.E)	Abortifacient activity (%)
Group- 1 Control	Vehicle	8,8,9,8,6,6	6(8,8,9,8,6,6)	0,0,0,0,0,0	0	Nil
Group- 2 to 4 Aqueous extract	100	9,3,9,6,13,8	6(7,2,7,3,11,6)	2,1,2,3,2,2	2±0.25***	28.50
	200	6,10, 9,11,7,9	6(3,8,5,7,2,5)	3,2,4,4,5,4	3.66±0.42***	44.45
	400	9,13,8,10,7,11	6(0,1,0,0,0,0)	9,12,8,10,7,11	9.5±0.76***	92.30
Group- 5 to 7 Ethanolic extract	100	7,7,10,6,11,9	6(6,5,8,5,9,7)	1,2,2,1,2,2	1.66±0.21***	20
	200	11,11,8,10,7,9	6(8,7,6,6,4,6)	3,4,2,4,3,3	3.16±0.30***	34.14
	400	5,11,9,3,7,9	6(2.5.5.1.3.4)	3,6,4,2,4,5	4±0.57*	56.39
Group- 8 to 10 Chloroform	100	6,5,11,9,7,11	6(5.5.10.8.6.11)	1,0,1,1,1,0	0.66±0.20*	10
	200	8,9,11,5,6,9	6(6,6,7,3,4,7)	2,3,4,2,2,2	2.5±0.34***	31.70
	400	10,11,9,6,7,10	6(6,4,5,3,3,4)	4,7,4,3,4,6	4.66±0.61***	52.54
Group- 11 to 13 Ethyl acetate extract	100	8, 6, 4,8,10,7	6(8,5,4,7,9,7)	0,1,0,1,1,0	0.50±0.22 ^{ns}	7
	200	9,7,9,8,10,8	6(8,6,8,6,10,7)	1,1,1,2,0,1	1±0.25**	12.63
	400	10,9,11,9,9,7	6(7,5,7,7,7,4)	3,4,4,2,2,3	1±0.36***	33

Values are expressed as mean ± S.E. for six albino rats in each group. P values: *<0.05, **<0.01, ***<0.001, When compared between group, ns= non significant

This was evident from decrease in the percentage of live fetuses. The 400 mg/kg body weight ethanolic extract showed 56.39% fetus abortion. Whereas the same dose of chloroform and ethyl acetate extract showed 52.54 and 33% live fetuses respectively. The percent resorption index increased from zero in the control animals to 92.30% in 400 mg/kg body weight aqueous extract treated animals. Our result were corroborated with the findings of ethanolic extract of root powder of *Cassia occidentalis*, *Derris brevipes* variety *Brevipes* and *Justica simplex* showed 100% abortifacient activity at 600 mg/kg body weight [26]. Alcoholic extract of *Plumeria rubra* at a dose of 200 mg/kg resulted in 100% abortifacient effect in female albino rats [27]. The present investigation revealed that the ethanol, chloroform and ethyl acetate extract exhibit abortifacient activity ranging from 7% to 56.39%. Our result also corroborate with antifertility activity of methanolic extract of three varieties of *Ricinus communis* Linn [22] and Similar finding were reported by Yakubu, et al [28] using *Senna alata* leaves.

There was a decrease in litter size with increase in the dose of the plant *I. trifoliata* extract in all the treatment groups. The litter size of control group rats was the highest (7.5±0.50). The litter body weight

recorded in animals administered with alcoholic, aqueous and ethyl acetate extract of *Indigofera trifoliata* were not significantly different from control. The AGD to CRL ratio of the litter of rats dosed with various plant extract at doses of 100, 200 and 400 mg/kg body weight were similar to that of control group. Similarly, the total body length of litters at day 1 of birth also did not vary significantly from that of control. When the sex ratios of litter were determined it was found that the female sex was dominant to male sex. The gestation period did not show any variation in extract treated group of animals as compared to control group (Table 2). Further, the dose dependent increase in the resorption index due to the administration of the extract, in the present study is an indication of failure in the development of the embryo. Such occurrences of foetal resorption suggest that interruption of pregnancy occurred after implantation of the foetus [29]. The results of the present study also revealed that the plant extract was relatively non-embryotoxic as judged by the data on foetal body size and AGD/CRL ratio and the absence of any observable treatment related morphologic defects in the live fetuses, corroborating with the finding of Chukwuka, et al [30] and Abdulazeez, et al [12] on *Spondias* and *Carrica* respectively.

Table 2: Effect of aqueous, ethanol, ethyl acetate and chloroform extract of *Indigofera trifoliata* (leaves) on fertility of female albino rats.

Treatment groups	Dose (mg/ kg body wt.)	Gestation period (days)	Litter size (No.)	Litter body weight (gm)	AGD/CRL (mm)	Total body length of litter at 1 st day of birth (mm)	Sex ratio of live fetuses (male/female)	Viable fetuses (%)	Fetuses resorption (Resorption index) (%)
Group- 1 Control	Vehicle	22.16±0.30	7.5±0.50	4.46±0.06	1.33±0.03	62.3±0.05	24/21	100	0
Group- 2 to 4 Aqueous extract	100	22.67±0.21*	6±1.32***	4.79±0.24 ^{ns}	1.38±0.02 ^{ns}	64 ±0.16***	15/21	71.50	28.50
	200	22.83±0.16***	5±0.42 ^{ns}	4.82±0.33*	1.64±0.04**	63.8±0.18*	14/16	55.55	44.45
	400	0	0	0	0	0	0	7.7	92.30
Group- 5 to 7 Ethanolic extract	100	22.53±0.30***	6.66±0.61 ^{ns}	5.84±0.05***	1.57±0.02**	62.2±0.29*	17/23	80	20
	200	22.92±0.40**	6.16±0.54*	6.19±0.02**	1.42±0.03*	61.7±0.04 ^{ns}	18/19	65.86	34.14
	400	22.78±0.56*	3.33±0.66**	6.48±0.03***	1.59±0.04*	63.5±0.37*	9/11	43.61	56.39
Group- 8 to 10 Chloroform extract	100	22.66±0.33 ^{ns}	7.5±1.06 ^{ns}	5.23±0.26**	1.14±0.03*	64.7±0.40***	21/24	90	10
	200	21.33±0.21 ^{ns}	5.5±0.67 ^{ns}	5.69±0.11**	1.27±0.02**	65.5±0.20***	19/14	68.3	31.70
	400	22.50±0.54***	4.16±0.70*	5.76±0.16***	1.21±0.02**	65.2±***	13/12	47.46	52.54
Group- 11 to 13 Ethyl acetate extract	100	22.80±0.33*	6.66±0.76 ^{ns}	4.14±0.02**	1.31±0.02 ^{ns}	58.2±0.17***	19/21	93	7
	200	22.90±0.40**	8.5±0.42*	5.05±0.01**	1.53±0.08*	59.2±0.17***	21/24	87.37	12.63
	400	22.86±0.56*	9.16±0.54**	5.59±0.02***	1.56±0.02**	61.6±0.23 ^{ns}	17/20	67	33

Values are expressed as mean ± S.E. for six albino rats in each group. P values: *<0.05, **<0.01, ***<0.001, When compared with control, ns= non significant

Table 3: Effect of aqueous extract of *Indigofera trifoliata* leaves on body weight, reproductive organ weight in female albino rats Values are

Treatment groups	Dose (mg/ kg body wt.)	Body weight (gm)		Reproductive organ weight (mg)	
		Initial	Final	Ovary	Uterus
Control	Group-I Vehicle	154.83±1.80	163.16±1.32 ^{ns}	55±3.66	118.83±2.79
Aqueous leaves extract of <i>I. trifoliata</i>	Group-II (100)	165.48±1.48	178.50±0.78**	54.32±0.21 ^{ns}	147.50±2.04***
	Group-III (200)	155.60±0.66	179±0.78**	54.50±2.42*	152±1.58***
	Group-IV (400)	180±2.14	166.33±2.39***	50±0.68**	169.56±3.46***

expressed as mean ± S.E. for six albino rats in each group. P values: *<0.05, **<0.01, ***<0.001, When compared with control, ns= non significant

Table 4: Effect of aqueous extract of *Indigofera trifoliata* leaves on hormonal profile of female albino rats Values are expressed as mean ± S.E. for six albino rats in each group.

Parameters	Control (Vehicle)	Aqueous leaves extract of <i>Indigofera trifoliata</i>		
	Group- I	(mg/kg body weight)		
		Group- II -100	Group- III -200	Group- IV -400
FSH (mIU/ml)	0.167±0.01	0.145±0.01*	0.128±0.05**	0.105±0.02***
LH (mIU/ml)	0.13±0.005	0.12±0.03 ^{ns}	0.10±0.01*	0.07±0.001***
Estrogens (pg/ml)	70.32±0.80	63.23±1.36*	72.33±0.88 ^{ns}	86.64±2.78***
Progesterone (ng/ml)	43.61±0.96	43.08±0.16**	38.56±1.56**	32.16±0.98***

P values: *<0.05, **<0.01, ***<0.001, When compared with control, ns= non significant

The doses 100, 200 and 400 mg/kg body weight of aqueous of *I. trifoliata* leaves extract shows a significant increases in the body weight and uterine weight but there was a slight decrease in the weight of ovary in the in treated rats compared to the control rats (Table 3). These results suggest that aqueous leaves extract of *I. trifoliata* possesses estrogenic activity which might be responsible for fetus resorption (abortifacient). Our result also coincide with the findings of Pradeepa, et al [31], while working on *Indigofera linnaei* ali in female albino rats.

CONCLUSION

The abortifacient activity lends support to the claims for its traditional usage of *Indigofera trifoliata* as an abortive medicine. Thus, this study may prove to be an effective and safe alternative remedy for contraception. Further studies to identify the bioactive principle of abortifacient activity of the extract are in progress.

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