



ANTIFERTILITY STUDIES OF *PHOLOGACANTHUS THYRSIFLORUS* NEES: A POTENTIAL ETHNO-MEDICINAL PLANT OF NORTH EAST INDIA

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Currently, there are number of hormonal contraceptives available in the market but they have various side effects. Keeping these views in mind, the present investigation deals with the antifertility evaluation of some traditionally using ethnomedicinal plants. *Phlogacanthus thyrsoiflorus* Nees (Family Acanthaceae) leaves extract was investigated for anti-implantation, on successive stages of embryogenesis, at the dose of 150 and 300 mg/kg body weight. The post-treatment results showed a significant inhibition site at a dose of 300 mg/kg. The observations recorded showing no change in ovulation, no change in weight of ovaries and no change in cholesterol level in the anti-ovulatory activity. Further, the acute oral toxicity test was also carried out according to the OECD guidelines; no toxic reaction or mortality on rats were observed.

Therefore, findings of the present investigations prompt us to do the detailed histological, pre-clinical as well as clinical investigation; so that, the antifertility activity of the *P. thyrsoiflorus* can be explored pharmaceutically as potential anti-implantation and abortifacient agent.

Key words: *Phlogacanthus* sp., ethno-medicinal plant, antifertility, North East, India

Population explosion is an imminent hurdle for a country's development as the natural resources are limited. The population of India is multiplying at an alarming rate and has crossed one billion. Fertility regulation has, therefore, become the major concern of people from all walks of life. In recent years, plants are more concerned with synthetic contraceptive drugs because plants are easily available, economical and less harmful side effects. A large number of plants have been reported to exhibit anti-implantation & abortifacient activity but a few have been evaluated for such effects in laboratory animals.

The genus *Phlogacanthus* is represented by 15-17 species which were found in India (Himalaya), Burma, Indo-Chinese, Malaya peninsular, Indonesia regions of the World with 10 species in sub-tropical India (Ningombam *et al.* 2014). It is represented by 5 species in Manipur (Deb 1961). The species is distributed abundantly in Manipur and its folk-medicinal uses and traditional knowledge were conserved from generation to generation through oral tradition. Many customs, myths

are related to this plant which is perpetuated from generation to generation and well dispersed into the valley districts. Some of them reflect intelligent approaches for their sustainable uses and preservation. Several works have been published on magico-religious, botanical folklore, folk-medicine and ethnobotanical aspects of the Manipuri's (Devi *et al.* 2009, Singh 1996, Singh and Singh 1996, Singh 2003, Singh *et al.* 1996, Singh and Singh 2003, Singh 2009).

The use of plant preparations and extracts for pregnancy interruption has a long-standing history among Indian physicians. Considering the side effects of hormonal contraceptives, the herbal contraceptives from indigenous plants have gained much significance in recent years. A large number of indigenous plants have such activities are recorded in ancient Indian literature. Indeed, recent reviews of Indian plants have been tested for their anti-fertility activities, the present study on *P. thyrsoiflorus* (leaves) were undertaken to explore the different extracts, to find their significant potentiality on fertility regulation in female

albino rats.

MATERIALS AND METHODS

Preparation of Extract: The leaf parts were collected from Imphal - East District, Manipur. The Leaves materials were shade dried and milled to obtain a coarse powder. The powdered materials were successively extracted with Methanol in a Soxhlet's apparatus (Soxhlet, 1879) using a continuous hot percolation method. The individual extracts were collected and concentrated by evaporation in a vacuum. Preliminary phytochemical studies showed the presence of flavonoids, tannins, phytosterols, phenol, glycosides, fatty acids, diterpene glucoside, phloganthoside, and volatile oil.

Animals: Inbred adult male Rats & female albino rats (150-200 g) were used for the experiment. The animals were maintained in a well-ventilated room at a temperature of $25\pm 2^{\circ}\text{C}$ with 12:12 hour light/dark cycle in polypropylene cages. The standard pellet feed (Hindustan lever, Bangalore) and tap water were provided ad libitum throughout the experimentation period. Animals were acclimatized to laboratory conditions 10 days before initiation of experiments.

The experiments were conducted as per the approval of the institutional animal ethical committee No. MZUIAEC/17-18/17; dt 13/07/2017.

Acute Toxicity Study: Acute toxicity study of Methanolic extract of *P. thyrsoiflorus* leaves was carried out in rats according to OECD guidelines (No, 2001). Extract at different doses up to 2000 mg/kg, p.o. was administered and animals were observed for behavioral changes, any toxicity, and mortality up to 48 h. There was no toxic reaction or mortality and found to save. Based on acute toxicity result we have selected 150 mg/kg and 300 mg/kg for anti-fertility evaluation.

Anti-Implantation Activity: The method was employed to detect the anti-implantation activity (Khanna and Chaudhury, 1968) of the methanol extract of *P. thyrsoiflorus* leaves. Female rats of the proestrus phase were kept with male rats of proven fertility in the ratio of 2:1. The female rats were examined in the following morning for evidence of copulation (Wiest *et al.* 1968). The animal which showed thick clumps of spermatozoa in vaginal smear was separated from the male partner and divided into 3 groups. Animals in the groups I have given vehicle only and serve as the control. Methanolic extract of *P. thyrsoiflorus* leaves at 150 mg/kg and 300 mg/kg were administered to group II and group III respectively from day 1 to 7 of pregnancy. The day when spermatozoa detected in vaginal smear was considered as day 1 of pregnancy. All the animals were sacrificed under light ether anesthesia and laparotomy was performed to determine the number of implantation sites on both uteri horns and the number of corpus luteum on both ovaries.

Anti-ovulatory Activity: The method was employed to detect the anti-ovulatory activity (Koneri *et al.*, 2006) in female rats. The animal used in these methods were female rats divided into 3 groups (n=6) fasted overnight and allow free access to water *ad libitum*. Different groups of female rats were treated with the test drugs at 150mg/kg and 300 mg/kg, p.o, vaginal smear from each rat was examined daily for 15 days and those rats exhibited three regular cycles (Evans and Sack, 1973) were used. Drugs and vehicles were started in the estrous phase and administered orally, daily for 15 days. Group I received vehicle only (1% gum acacia, p.o daily) and served as control. Group II and III received a methanolic extract of *P. thyrsoiflorus* at 150 and 300 mg/kg, respectively. The 15-day treatment was to cover three regular estrous cycles. Vaginal smear from each animal was observed every morning between 9-10 A.M. On the 16th day, 24 hrs after the last treatment, the rats from each group were anesthetized and sacrificed.

Table-1: Effect of Methanolic extracts of *P. thyriflorus* leaves on implantation site after oral administration for 7 days in female rats

Treatment	Dose	No. of Implants	No. of corpus leuteum	% Anti-implantation activity
control (saline)	1 ml	15.6± 0.50	17.4±0.74	10.57
Methanolic extract	150 mg/kg	9.8±0.66**	15.2±1.06**	36.84
Methanolic extract	300 mg/kg	5.6±0.50**	14.1±0.80**	60.56

Table-2: Effect of Methanolic extracts of *P.thyriflorus* leaves on ovarian weight and cholesterol level

Treatment and dose (mg/kg p.o)	Ovarian weight in mg/kg body weight	Cholesterol level in ovary (mg/50mg)
Gum acacia (1%, 1ml/kg)	37.46 ± 0.68	0.37 ± 0.06
Methanolic extract of <i>P.thyriflorus</i> leaves (150)	39.59 ± 0.83	0.42 ± 0.10
Methanolic extract of <i>P.thyriflorus</i> leaves (300)	36.24 ± 1.23	0.41 ± 0.09

*P<0.05, **P<0.01 compared with vehicle treated control group

Ovaries and uteri were dissected out, freed from extra deposition, and weighed on a sensitive balance. One ovary from each animal was processed for biochemical analysis of cholesterol.

Statistical Analysis: Statistical analysis was carried out using Analysis of variance (ANOVA) and is presented as the mean ± standard error of the mean (SEM).

RESULTS

Anti-Implantation Activity: Both doses of the extract showed significant inhibition of the number of the implant site. Extract at the dose of 150 mg/kg and 300 mg/kg shows inhibition of implants in uterine horns when compared with the vehicle-treated group.

Anti-ovulatory Activity

Methanolic extracts of *P. thyriflorus* leaves at 150 mg/kg and 300 mg/kg body wt. showed no change in the weight of ovaries and cholesterol level, when compared with the vehicle-treated group (**Table 2**).

DISCUSSION

Literature reveals that the number of researches has already been made on the antifertility activity of plants. *Aloe barbadensis* Mill. with zinc acetate is used as a vaginal contraceptive (Fahim and Wang, 1996). Ripened and unripe fruits and fresh leaves of *Ananas comosus* (L.) Merr. as fertility antidote has been reported by different field survey at West Bengal, Andhra Pradesh and Kerala (Tarafeder 1983, Pandranga *et al.* 2011, Ajesh *et al.* 2012)]. However, on the other hand, *in vivo* study with unripe fruit juice of *Ananas comosus* in pregnant Wistar rats also reported the same conclusion as above (Prakash 1976). Further, the use of *Azadirachta indica* A. Juss. (flower extract, neem seed oil) as potential antifertility drug have also been reported by many authors (Sinha *et al.* 1984, Upadhyay *et al.* 1993, Deshpande *et al.* 1980), Gbotlorun *et al.* 2008, Siddiqui and Hassan 2009, Mukherjee *et al.* 1996, Bhargava 2010).

Similarly, the findings of the current

investigation also revealed that the methanolic extract of *P. thyrsoiflorus* leaves has the potential anti-fertility activity. The observations show that there is no change in cholesterol level and ovarian weight, in the treated group of animals. However, it is known that for implantation exact equilibrium of estrogen and progesterone is essential for any disturbance in level of these hormones causes infertility (Psychoyos A 1966).

The anti-implantation activity of methanolic extract of *P. thyrsoiflorus* leaves was recorded 36.84% and 60.56% respectively, at a dose of 150 and 300 mg/kg body wt. Moreover, the anti-implantation activity might be possible due to the presence of chemical constituents like flavonoids, tannins, phytosterols, phenol, glycosides, fatty acids, diterpene glucoside, phloganthoside. Thus, to popularize the antifertility activity of the methanolic extracts of *P. thyrsoiflorus*; detailed *in vivo* investigations, using different animal models as well as clinical trials are required.

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