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SCREENING OF SOME INDIGENOUS MEDICINAL PLANTS FOR ANTI-IMPLANTATION / ANTI- FERTILITY ACTIVITY IN FEMALE ALBINO RATS

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ABSTRACT – Alcoholic extracts of *Abrus precatorius* (T1) Linn. (dried seeds and inflorescence), *Moringa oleifera*(T7) Lam. (dried bark), *Peganum harmala* (T8) Linn. (dried seeds) and *Momordica tuberose* (T9) (Roxb.) Cogn. (dried roots), petroleum ether extracts of *Cedrus deodara* (T2) (Roxb). Loud. (dried stem bark), *Artabotrys hexapetalus* (T3) (Linn. F. Bhandri.) (fresh leaves), *Ocimum tenuiflorum* (T5) (Linn.) (dried seeds) and *Jasminum arborescence* (T6) (Roxb.) (fresh flower buds) and extract of *Mallotus philippensis* (T4) (Muell. Arg.) (dried bark) prepared from edible mustard oil through oil emulsion process (Ayurvedic Tel pak vidhi), were screened for anti-implantation / anti- fertility activity at a dose of 250 mg/kg. body weight on the test animals. The treatment was continued up to 7th day of post-mating period. The remarkable high anti-implantation / anti-fertility activity was exhibited in plant extracts of *C. deodara* (T2), *P. harmala* (T8), *O. tenuiflorum* (T5), *A. hexapetalus* (T3), *A. precatorius* (T1) and *M. philippensis* (T4) at 100, 100, 95, 72, 63 and 47 per cent, respectively. Low anti-implantation / anti-fertility activity i.e., in between 19% to 38% was found in the treatments of *M. oleifera* (T7), *M.tuberosa* (T9) and *J. arborescens* (T6), respectively. 100% anti-implantation / anti-fertility activity was seen only in two extracts i.e., *C. deodara* (T2) and *P. harmala* (T8). The other seven extracts were also mentioned in Ayurvedic literature as an anti-fertility agents and their reference is also mentioned in the ethnomedicines as well as in folk remedies, unknowingly the fate of fetus and neonates in such failure case of anti-fertility. Findings indicate that the petroleum ether extracts of *C. deodara*, *O. tenuiflorum and A. hexapetalus*, alcoholic extracts of *P. haramala* and *A. precatorius* and emulsion oil extract of *M. philippensis* can affect the estrous cycle by blocking the biogenesis of ovarian steroids in high percentage at any intermediary stage along with the remarkable imbalance of ascorbic acid and cholesterol contents in ovary and exhibited them as significant contraceptive, antiestrogenic and antiprogestational activities. Conclusively, *C. deodara* and *P. harmala* would be worthwhile in serving as a tool in an absolute potential for birth control.

Key words : Anti-implantation, anti-fertility, ethnomedicines, antiestrogenic, antiprogestational, birth control.

INTRODUCTION

Use of plant preparations and medicaments for pregnancy interception was known to ancient Indian physicians. Plants were used as emmenagogues, abortifacients and as local contraceptives. A large number of indigenous plants having interceptive activities are recorded in ancient Indian literatures. Recently in the area of fertility regulation, more and more emphasis is being given on efforts to develop non-toxic contraceptive drugs of plant origin. In this connection a number of workers in India have screened several species of plants for their anti-fertility activity with some success (Kanungo and Patnaik, 1964; Bhakuni *et al*, 1969; Prakash and Mathur, 1976 and Aswal *et al*, 1984; Mazumder *et al*, 1992; Dhanasekaran *et al,* 1993; Misra, 1996; Gupta *et al*, 2003; Dhanpal *et al*, 2005; Yadav and Jain, 2006 and Sharma *et al*, 2007). In India the use of herbal preparations is still prevalent and popular among some tribes like, Kumbis, Gawdas, Kanjars and Dhangars (These tribes regularly use plants like *Psidium guajava, Lucas aspera*, *Moringa oleifera*, *Ocimum sanctum*, *Adathoda vasica*, *Coccinea indica*, *Andrographis paniculata* and *Mimosa pudica* etc., for treating ailments ranging from skin diseases and dog bites to bronchitis, kidney stones and contraceptive (asafoetida, *Ferula narthex*, Boiss. to induce irreversible sterility; as progeny of asafoetida fed female rats are born with gross external anomalies in neonates and have a short life span, Borker *et al*, 1996). Although, the plants also enjoy a reputation for their established medicinal properties in Ayurvedic system to cure a variety of human ailments and control of fertility (Singh, 1969; Kamboj, 1988; Warrier *et al*, 1995; Ambasta, 2000; Chatterjee, 2000 and Singh, *et al*, 2003) However, no detailed systematic clinical study have been undertaken on the anti-implantation / anti-fertility activity of selected medicinal herbs therefore, keeping this in view, the anti-implantation and anti-fertility property of selected medicinal plants which are recommended in the ancient Indian literature as female contraceptive have been investigated and determined systematically with proper clinical observations on female albino rats in the present study.

MATERIAL AND METHODS

250 mg/kg of body weight of each plant extract in the form of dry powder or in semi-solid stage was mixed with A.R. grade of gum acacia along with satisfactory quantity of distilled water and thoroughly macerated in sterilized mortar, which was administered orally. The plant extracts like *C. deodara*, *A. hexapetalus*, *O. tenuiflorum* and *J. arborescence* having high percentage of oil/ terpenoids were either mixed with A.R. grade tween-80 or ethyl alcohol as per requirement.

Mature healthy adult female albino rats of Charles foster strain, 10 to 12 weeks old, weighing 150-210 gm. obtained from Central Drug Research Institute, Lucknow, directly from their animal breeding house and were kept in a wooden aerated cage on a approximate minimum temperature 22±1^{0}C under approximately uniform and maintained husbandry conditions with free asses to standard food and water *ad libitum.* First of all, properly balanced oestrous cycle was checked daily by observing the vaginal smears. As and when females attended the proestrous phase except control group the rest individual were provided the respective treatment of extract in a group of five rats for each treatment. Proestrous females were caged with males of same strain in a ratio of 1F : 3M, age and weight of males were approximately matched to the females; three to four successful copulation strokes on the female were also observed and at last the occurrence of copulation was evaluated in the next day at 8.00 A.M., by checking the presence of sperms in the

Table 1 : Anti-implantation/ anti-fertility activity of nine selected indigenous plant extracts in albino rats ; Five replication in each treatment. Dose 250 mg. /kg. body weight, vehicle-Gum-acacia or tween-80 or ethyl alcohol (Values are mean ± SD of 5 Animals).

Name of Plant	Anti-implantation activity		Body weight of female in grams		Percentage activity	
	No. of implantation L+R	No. of Corpora lutea L+R	After 1st day of pregnancy	After 10th day of pregnancy	Fertility percentage	Anti- fertility percentage
Abrus precatorius (TI)	4.6000 ±4.2190	11.8000 ±1.6432	174.1260 ±14.7351	195.1320 ±19.5956	38.7400 ±35.7938	63.2540 ±34.2938
Cedrus deodara (T2)	0.0000 0.0000	9.4000 ±1.1402	177.8940 ±9.1005	181.4780 ±8.8255	0.0000 0.0000	100.0000 0.0000
Artabotrys hexapetalus (T3)	3.2000 ±4.4385	11.2000 ±1.3038	186.0840 ±16.5072	201.3320 ±20.6540	27.8460 ±38.1307	72.1520 ±38.1334
Mallotus philippensis (T4)	7.0000 ±4.3012	13.2000 ±0.8367	169.9980 ±16.9756	182.0460 ±48.8023	52.4320 ±30.9420	47.5600 ±30.9462
Ocimum tenuiflorum (T5)	0.6000 ±1.3416	11.0000 ±1.5811	170.2340 ±13.3634	174.8440 ±14.6650	4.6140 ±10.3172	95.3840 ±10.3217
Jasminum arborescens (T6)	8.6000 ±4.9800	13.4000 ±2.3022	177.8920 ±19.6846	210.2480 ±34.3800	61.7700 ±35.3085	38.2220 ±35.3129
Moringa oleifera (T7)	10.0000 ±1.0000	12.4000 ±1.1402	177.7000 ±17.5844	241.4020 ±32.0445	80.6740 ±3.9004	19.3280 ±3.8914
Peganum harmala (T8)	0.0000 0.0000	7.8000 ±1.0954	177.9880 ±15.6773	182.0620 ±15.2315	0.0000 0.0000	100.0000 0.0000
Momordica tuberosa (T9)	9.4000 ±1.1402	12.0000 ±1.0000	183.4840 ±17.6208	225.5080 ±20.9961	78.6180 ±9.6240	21.3720 ±9.6240
Control	9.2000 ±1.0954	11.0000 ±0.7071	173.3540 ±14.7194	207.6980 ±15.1685	83.2740 ±7.1743	16.7260 ±7.1743
C.V.	55.785%	11.918%	8.968%	12.831%	53.309%	39.346%
Std Error	1.31225	0.60332	7.09351	11.48647	10.20293	10.10016
SE Difference	1.85580	0.85323	10.03174	16.24432	14.42912	14.28378
Probability	0.0000	0.0000	0.8376	0.0027	0.0000	0.0000
't'. Value	2.02107	2.02107	2.02107	2.02107	2.02107	2.02107
C.D.	3.75070	1.72443	20.27483	32.83088	29.16224	28.86849

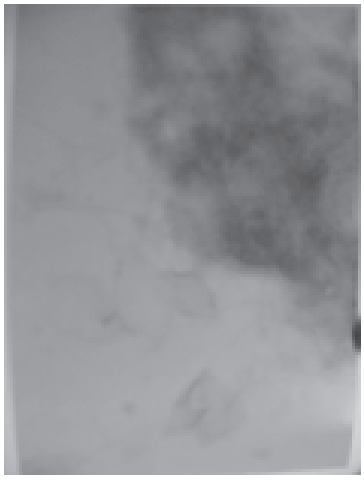


Photo I : Successful pregnancy confirmed through vaginal smear, showing the presence of sperms with cells of oestrous phase.

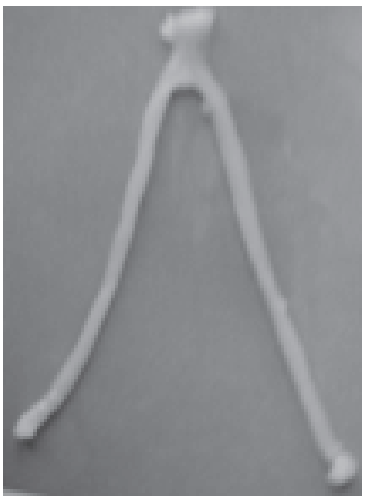
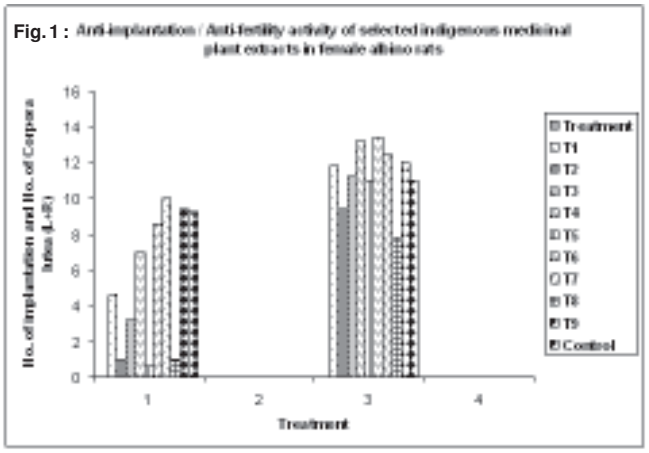
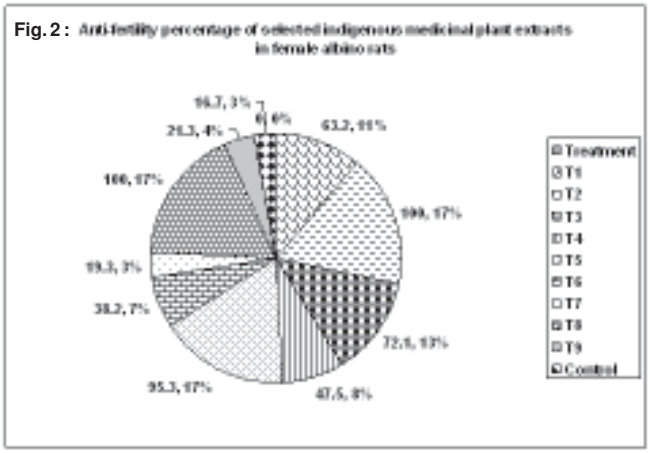


Photo II : Anti-implantation/anti-fertility activity of nine selected indigenous medicinal plant products in female albino rats: 100% interceptive action of two plant product (*P. harmala* (T8) and *C. deodara* (T2) showing normal uterus condition.



Scale 1 = 0



T1 = *A. precatorius*, T2 = *C. deodara*, T3 = *A. hexapetalus*, T4 = *M. philippensis*, T5 = *O. tenuiflorum*, T6 = *J. arborescens*, T7 = *M. oleifera*, T8 = *P. harmala*, T9 = *M. tuberosa*

vaginal smears (Photo-I).

Assuming the copulation time between 9.00 P.M. and mid-night, this day was announced as first day of pregnancy. The treatment was continued up to 7th day of post-mating period. 5 females were used in each group of treatment, means 45 females were used for the treatment of nine plant extracts. In the control group of 5 rats, the normal food was mixed with same quantity of gum acacia as was used in the treated groups.

One female from each group of treatment including control was laparatomized on 10th day of post-mating period under anaesthesia and caesarean of uterus was performed. All the females/mothers were checked for anti/implantation sites were recognized (Photo-II).

All the nine treatments were compared with the control and then processed statistically on the basis of collected data. The research adhered to the principles of Laboratory Animal Care NIH Publication No. # 85-23, revised 1996) were strictly followed throughout the studies.

RESULTS AND DISCUSSION

The plant extracts of *C. deodara* (T2), *P. harmala* (T8), *O. tenuiflorum* (T5), *A. hexapetalus* (T3), *A. precatorius* (T1) and *M. philippensis* (T4) showed 100, 100, 95, 72, 63 and 47 per cent anti-fertility activity, respectively (Table 1). Low anti-fertility activity i.e., in between 19% to 38% was found in the treatments of *M. oleifera* (T7), *M.tuberosa* (T9) and *J. arborescens* (T6), respectively.

In the laboratory nine plant extracts treatment were tested for anti-implantation / anti-fertility activity ; 100% anti-fertility activity was seen only in two extracts i.e., *C. deodara* (T2) and *P. harmala* (T8) out of nine plant materials. The other seven were also mentioned in

Ayurvedic literature as an anti-fertility agents and their reference is also mentioned in the ethnomedicines as well as in folk remedies, unknowingly the fate of the fetus and neonates in such failure case of anti-fertility.

It is extremely important to know the effect of nine selected botanicals as anti-implantation / anti-fertility agent in an innocent subject to the developing fetus. However, the complete reports are still not available in previous work where teratogenic activity of the different Ayurvedic herbs are described. Thus, it is necessary to screen and evaluate teratological activity of the selected anti-fertility agents referred in Ayurvedic literature, for this very purpose further screening is required to know the fate of neonates. In this context three plant extracts *viz*., *M. olefera* (T7), *M. tuberosa* (T9) and *J. arborescens* (T6) which did not show much high anti-fertility activity and might be encircled them for remarkable defects in neonates. Findings indicate that the petroleum ether extracts of *C. deodara*, *O. tenuiflorum and A. hexapetalus*, alcoholic extracts of *P. haramala* and *A. precatorius* and emulsion oil extract of *M. philippensis* can affect the estrous cycle by blocking the biogenesis of ovarian steroids in high percentage at any intermediary stage along with the remarkable imbalance of ascorbic acid and cholesterol contents in ovary and exhibited them as significant contraceptive, antiestrogenic and antiprogestational activities. Such facts have already been established in the three previous amelioration (Johri *et al*, 2009). The aforesaid pleading and findings of present workers exclusively on *Abrus precatorius* could only allowed to be compared in an agreement with previous findings of Desai and Rupawala, 1967 and Agrwal *et al*, 1970 while rest can not be compared for want of proper clinical observations in reference to selected medicinal plants.

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