

## RESORPTION / ABORTIFACIENT AND TERATOGENIC ACTIVITY OF SELECTED INDIGENOUS MEDICINAL PLANTS 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 tuberosa* (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 resorption / abortifacient and teratogenic activity at a dose of 250 mg/kg. body weight for 10 days on the test animals. The plants *A. precatorius* (T1), *M. oleifera* (T7), *P. harmala* (T8) and *M. philippensis* (T4) showed 100, 78, 68 and 30 percent abortifacient activity. Zero percent abortifacient activity was observed in treatments of *C. deodara* (T2), *A. hexapetalus* (T3), *O. tenuiflorum* (T5) and *M. tuberosa* (T9) although *M. oleifera* (T7), *P. harmala* (T8) and *M. philippensis* (T4) recognized themselves in interstitial position regarding abortifacient and resorption activity. 100 percent abortifacient activity was seen only in one plant product *A. precatorius* (T1) out of nine selected plants. Although these plants have been listed for their use as an emmenagogue or abortifacient in ethnomedicine and Ayurvedic literature. The plant products like *O. tenuiflorum* (T5), *A. hexapetalus* (T3), *M. oleifera* (T7) *C. deodara* (T2) and *P. harmala* (T8) showed resorption capacity. The pregnancy was partially terminated in the case of *M. oleifera* (T7), *A. hexapetalus* (T3), *P. harmala* (T8), *C. deodara* (T2) and *M. philippensis* (T4) but the remarkable teratogenic effects were observed in rest of the neonates as well as on fetus. These defects mainly includes reduction anomalies like non-ossification of bones in different regions of neonates body. The details of gross anomalies are categorized as semi-solid brain, non-ossified caudal bone, non-ossified cervical bone, hip joint defect, everted claw and wrist drop. The interpretative clinical chemistry determination and role of calcium remarkably summed as that the hypo-level of calcium is a major creator to organize the anti-fertility or abortifacient or resorption percentage.

**Key words** : Ethnomedicine, resorption, abortifacient, teratogenic, emmenagogue, reduction anomalies, neonates, fetus.

### INTRODUCTION

The use of plant preparations and medicaments for pregnancy interception was known to ancient physicians of India is evident from some of the available reviews (Chopra and Chopra, 1958; Nadkarni and Nadkarni, 1976; Satyawati *et al*, 1976 and 1987). The use of plants as emmenagogues, abortifacient and as local contraceptive was well known to the ancient physicians of India. Abortion was usually achieved by insertion of irritant twigs and twigs of plants sticks coated with astringent plant juices into the uterine cervix or after oral administration of plant decoctions, ergot or quinine to initiate uterine contractions for expulsion of the fetus. The emmenagogues, which often increase the menstrual flow in the non-gravid uterus, have been extensively used to induce abortion. These agents include drastic purgatives and irritant volatile oils which are believed to induce uterine contractions secondary to intestinal irritants and violent gastro-enteritis. Even today the Northern and Eastern tribes of India are alleged to use plant contraceptives to limit their family size (Singh *et al*, 2003. Successful implantation in

mammals solves synchronized development of the egg of the endometrium under the influence of a delicate balance and interaction of estrogen and progesterone to achieve healthy embryo and well prepared endometrium for embryo-uterine interaction culminating in pregnancy. Early pregnancy is then maintained by progesterone produced by the corpus luteum. The plants that prevent implantation of the embryo by interfering with the pre and peri-implantation events of pregnancy have been presented under anti-implantation activity where as those disrupting early pregnancy are dealt with under abortifacient activity (Pakrashi and Basak, 1976; Prakash *et al*, 1978 Misra *et al*, 1979 and Kamboj, 1988). The search for an anti-fertility drug is not only a matter of great interest but of great need with uncontrolled population explosion. Many synthetic as well as indigenous drugs have been introduced but none of them is a completely satisfactory drug at present. The birth defects in neonates in the cases where pregnancy is not interrupted have not been studied so far. According to the impressive views of Sethi *et al*, 1990 that the population explosion is posing an alarming

concern in the world even threatening the survival of mankind. How to control the birth rate is a problem which has drawn the attention of the political leaders and Governments including the scientists of not only the thickly populated under developed/ developing countries, but also of many developed countries. Population living in countries which support the family planning needs new and effective contraceptive agents and / or methods having a minimum of side reactions and giving a maximum protective effects. In this context a large number of indigenous plants have been tested for anti-fertility activity but as only few have been found successful as a potent contraceptive agent without any side effects. The value of proper experimental schedules further, in culling out the information from ancient texts or reviews, the recommended part of the plant, the preparation used and activity mentioned seem to have been overlooked by some investigators. This may be one of the reasons for not finding activity in the plants well documented although, the botanicals do not cause a permanent disturbance in balance and interaction of oestrous / estrogen and progesterone level and degrade itself after few days of the termination of their use in spite allopathic drugs reciprocate themselves., this is one of the advantage of herbs having medicinal properties over the allopathic medicines. Keeping this in view nine indigenous medicinal plants *viz.*, *Abrus precatorius*, *Cedrus deodara*, *Artabotrys hexapetalus* *Mallotus philippensis*, *Ocimum tenuiflorum*, *Jasminum arborescence*, *Moringa oleifera*, *Peganum harmala* and *Momordica tuberosa* were tested for their resorption / abortifacient and teratogenic activity in female albino rats.

## MATERIALS AND METHODS

Proven fertile adult male and female of Charles faster strain albino rats (150-210 g) body weight were used. The rats were maintained in a well aerated spacious wooden cage at normal room temperature and natural day light and were fed with pesticide free soaked Punjabi Gram and water *ad libitum*. The vaginal smears of female rats were examined daily.

Fifty female rats were divided into two groups. The group I were again sub-divided into nine groups. In each sub-section of group I five females and three male rats were introduced. Group II served as control have five females and three males and the rats received normal food with same quantity of gum acacia mixed with the treated group. 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 to the group I animals. The plant extracts having high percentage of oil/terpenoids like *C. deodara*, *A. hexapetalus*, *O. tenuiflorum* and *J. arborescence* were either mixed with A.R. grade tween-80 or ethyl alcohol as per requirement.

The females which have perceived three successful strokes of mating was confirmed after six hours by observing the presence of sperms in the vagina. When vaginal smears were positive for sperms this day was considered as first day of pregnancy. The vaginal smear of these rats was further examined for four successive day if dioestrous stage was confirmed all the times then it was expressing a firm confirmation of the pregnancy. The doses of the test material of nine herbs were applied after 5th day of pregnancy up to 15th day of gestation period. A weekly inspection of female rats was carried out to observe any change in the body weight. An increase in weight of female rats was also related to confirm the fetal development.

One female rat from each sub group of group I was laparotomized on 20th day of pregnancy and 3-5 replications of pregnant rats were examined, as uterine weight, total implantation left and right, total resorption left and right, number of corpora lutea left and right, gross abnormalities if any and the number of dead and live litters/pups were recorded (Photo-I and II).

All nine treatments and control was just compared and then processed statistically on the basis of collected data. No gross abnormalities occurred in any of the horns of uterus or in the ovary. In the control group all the implants developed into fetus and were found to be alive and healthy. 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 plants *A. precatorius* (T1), *M. oleifera* (T7), *P. harmala* (T8) and *M. philippensis* (T4) showed 100, 78, 68 and 30 percent abortifacient activity, respectively (Table 1, Fig 1, 2 and 3). Zero percent abortifacient activity was observed in treatments of *C. deodara* (T2), *A. hexapetalus* (T3), *O. tenuiflorum* (T5) and *M. tuberosa* (T9) although *M. oleifera* (T7), *P. harmala* (T8) and *M. philippensis* (T4) recognized themselves in interstitial position regarding abortifacient and resorption activity. In laboratory nine selected plant products were tested for anti-fertility activity ; 100 percent abortifacient activity was seen only in one plant product *A. precatorius* (T1) out of nine selected plants. The nine selected plant parts have been listed for their use as an emmenagogue or abortifacient in ethnomedicine. Ayurvedic literature mostly

**Photo I**

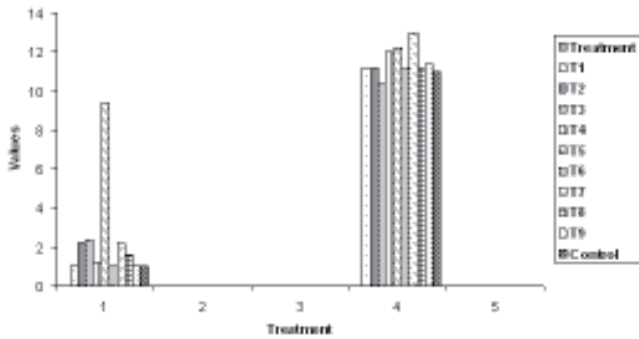


**Photo I :** The complete uterus of control mice containing partially developed litters.

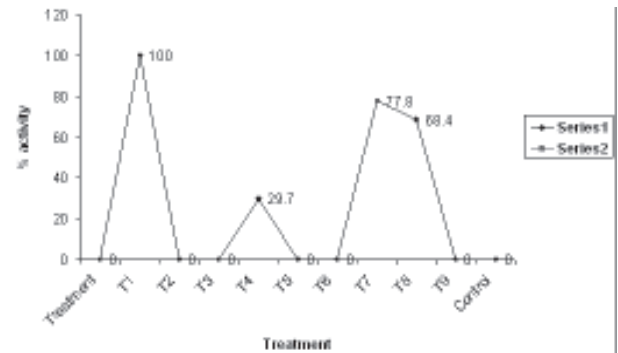
**Photo II**



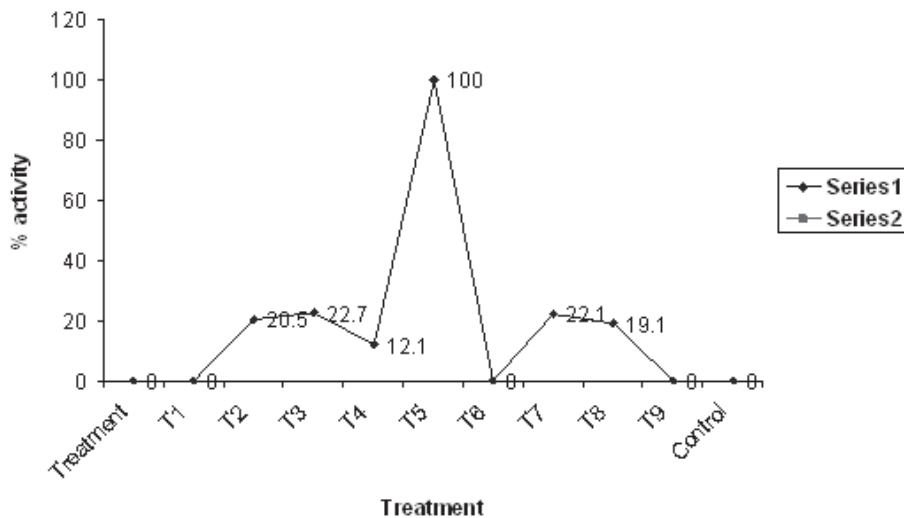
**Photo II :** Resorption activity of nine selected indigenous medicinal plant products in female albino rats: 100% resorption by *O. tenuiflorum* (T5) confirmed through withered embryos in uterus.



**Fig. 1 :** Resorbed implantation and number of Corpora lutea (L+R) effected by selected indigenous medicinal plant extracts in female albino rats.



**Fig. 2 :** % aborfacient activity of nine selected indigenous medicinal plant extracts in female albino rats.



**Fig. 3 :** % resorption activity of nine selected indigenous medicinal plant extracts in female albino rats.

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*

**Table 1 : Resorption/ abortifacient and teratogenic activity of nine selected indigenous medicinal 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  $\pm$  SD of 5 Animals )**

Name of Plant	Resorption activity		Percentage activity	
	No. of resorbed implantation L+R	No. of Corpora lutea L+R	Abortifacient	Reabsorption
<i>Abrus precatorius</i> (T1)	0.00000.0000	11.2000 $\pm$ 1.4832	100.00000.0000	0.00000.0000
<i>Cedrus deodara</i> (T2)	2.2000 $\pm$ 3.1937	11.2000 $\pm$ 1.6432	0.00000.0000	20.5543 $\pm$ 28.5699
<i>Artabotrys hexapetalus</i> (T3)	2.4000 $\pm$ 2.3022	10.4000 $\pm$ 1.9494	0.00000.0000	22.7423 $\pm$ 21.4017
<i>Mallotus philippensis</i> (T4)	1.2000 $\pm$ 1.3038	12.0000 $\pm$ 0.7071	29.7560 $\pm$ 8.0305	12.1200 $\pm$ 12.5134
<i>Ocimum tenuiflorum</i> (T5)	9.4000 $\pm$ 3.2863	12.2000 $\pm$ 2.5884	0.00000.0000	100.00000.0000
<i>Jasminum arborescens</i> (T6)	0.00000.0000	11.2000 $\pm$ 1.7889	0.00000.0000	0.00000.0000
<i>Moringa oleifera</i> (T7)	2.2000 $\pm$ 0.8367	13.0000 $\pm$ 1.4142	77.8320 $\pm$ 8.0351	22.1600 $\pm$ 8.0376
<i>Peganum harmala</i> (T8)	1.6000 $\pm$ 1.1402	11.2000 $\pm$ 1.4832	68.4740 $\pm$ 40.1445	19.1800 $\pm$ 14.0870
<i>Momordica tuberosa</i> (T9)	0.00000.0000	11.4000 $\pm$ 1.8166	0.00000.0000	0.00000.0000
Control	0.00000.0000	11.0000 $\pm$ 1.2247	0.00000.0000	0.00000.0000
C.V.	91.161%	14.602%	47.791%	66.148%
Std Error	0.77460	0.74967	5.90022	5.82049
SE Difference	1.09545	1.06019	8.34418	8.23142
Probability	0.0000	0.0000	0.0000	0.0000
't'. Value	2.02107	2.02107	2.02107	2.02107
C.D.	2.21397	2.14271	16.86415	16.63626

referred the use of these plants for abortion but out of nine selected plant products some of them like *O. tenuiflorum* (T5), *A. hexapetalus* (T3), *M. oleifera* (T7) *C. deodara* (T2) and *P. harmala* (T8) have resorption capacity. The pregnancy was partially terminated in the case of *M. oleifera* (T7), *A. hexapetalus* (T3), *P. harmala* (T8), *C. deodara* (T2) and *M. philippensis* (T4) but the remarkable teratogenic effects were observed in rest of the neonates as well as on fetus. These defects mainly includes reduction anomalies like non-ossification of bones in different regions of neonates body. The details of gross anomalies are categorized as :- semi-solid brain, non-ossified caudal bone, non-ossified cervical bone, hip joint defect, everted claw and wrist drop. Calcium is present in the body in larger amount than any other mineral element. It is mainly present in teeth and bone, about 99% of the body calcium is in the skeleton. It is present in bones as deposits of calcium phosphate in a soft, fibrous matrix. It is also present in small concentration in body fluid. The ionized calcium in the body fluids play important role in blood coagulation and in maintaining the normal excitability of heart, muscles and nerves. Interpretative clinical chemistry determination conclude that if the calcium values increased from its normal standard than

hyperparathyroidism, metastatic-carcinoma, myeloma, sarcoidosis and thyrotoxicosis will be occurred, if the values represent them in decreasing order then hypoparathyroidism, hypoalbuminemia, chronic renal diseases, acute pancreatitis, mal-nutrition, rickets and osteomalacia will be occurred. The final common pathway of blood clotting, initiated by factor Xa, produced by either intrinsic or extrinsic pathway activates prothrombin to thrombin. Thrombin converts fibrinogen to fibrin. In short Fibrinogen-Thrombin— Fibrin. The activation of prothrombin occurs on any surface of activated platelets and require platelets anionic phospholipids, and sufficient quantity of calcium ( $Ca^{2++}$ ) molecules in any combination and factors of extrinsic or intrinsic pathway (Godkar and Godkar, 2003).The aforesaid interpretative chemistry and role of calcium remarkably summed as that the hypo-level of calcium is a major creator to organize the anti-fertility or abortifacient or resorption percentage. The present amelioration ruled out the hover tendency in selection and screening of an anti-fertility herb, conclusively those herbs marked themselves for hypo-level of calcium would be anti-fertility, abortifacient or resorption agents. In the present finding this is a final emancipation and restrict ourselves in future

from any perplexity in selecting a anti-fertility, abortifacient or resorption herb from Ayurvedic or ethno and folk medicines importance.

It may be added that although the plant have been listed for their use as emmenogues and abortifacient in ancient literature and encouraging results are obtained in the screening of few plants as abortifacient, relatively little attention have been given for the testing of plants responsible for resorption / abortifacient and teratogenic activity or mensus regulator. In identifying plants for this activity, documented literature viewed by Chopra *et al*, (1958) Nadkarni and Nadkarni, (1976) and Satyavati *et al* (1976) and the original texts need to be carefully scanned and plant used locally as irritants or orally as strong purgatives leading to abortion should be excluded from testing. Among published literature by Indian investigators, the review of Kamboj and Dhawan (1981 & 82), Chowdhury and Haq (1982), Chowdhury *et al* (1982) and Patnaik and Dhawan (1984) provided valuable reference information on extracts/compounds and testing schedules, activity profiles, hormonal profile and toxicity data of fertility regulating plant and merit consultation before identifying a plant for further investigations.

In summary, fertility regulation through plants require an appropriate plan in selection of a plant with special reference to its capability of inducing physiological functions such as ; (i) to imbalance the ascorbic acid and cholesterol level, (ii) oestrous cycle/estrogenicity, (iii) altered dietary protein status and able to induce (iv) hypo-level of calcium with due consideration of normal, hematology and biochemical parameters and psycho-behavior of the test animal used then should be processed for clinical studies. Pertinently the important role of ascorbic acid and cholesterol in the total process of anti-fertility was also established by ( Deane, 1952; Dey *et al*, 1972; Krum *et al*, 1964; Kanungo and Patnaik, 1964; Mazumder *et al*, 1992; Misra, 1996; Gupta *et al*, 2003; Dhanapal *et al*, 2005 and Johri *et al*, 2009). The more systematic approach as summarized above one may hope for useful leads in identification of plants with reproducible results for optimal chances to obtain creditable contraceptive of plant origin.

The present workers also agreed with the views of Kamboj (1988), that a few promising leads have been identified by studies on anti-fertility plants in India, but not pursued further seriously, particularly with a view of developing and interceptive contraceptive. In some cases, this appears to be lack of facilities, while in others the investigators do not seem to have realized that identification of such leads is the beginning and not the final step in the contraceptive development. The review

also suggest that most of the investigators do not seem to aware of the importance of use of properly authenticated (botanically identified) plant samples, appropriate extraction procedure and the value of proper experimental schedules, including the minimum numbers of animals to be used to get reproducible results. Further, in culling out information from ancient texts or reviews, the recommended part of plant, the preparation used and activity mentioned seem to have been over looked by some investigators. This may be one of the reasons for not finding activity even in plants well documented. Further, none of the investigators have tested the plants during different seasons of year. Thirdly, as one is not sure about dosage or formulation employed, the low dosage of crude extract listed in testing may have contributed to inconsistent interceptive activity. Moreover, the investigators have tested solvent extracts and not the compound preparation as indicated in texts. The published data dosages suggest, however, that all the interceptive plants or those parts showing activity of plants mention in the literature have not been reported in ancient texts ; some of them are of course, exotic-accordingly careful random screening of plants may be useful. In order to generate reproducible data, the approach advocated by WHO and recently by ICMR task force on plants for fertility regulation has been followed.

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