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RESEARCH ARTICLE

Studies on the anti-fertility efficacy of Abrime and Embrelin, the compounds of plant origin on mouse testis and uterus

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ABSTRACT:
The abortifacient contraceptive drugs (Allopathic) available in the market are regularly taken by women. It may result with many side effects and damage the vital organs, genetic material leading to mutational changes which in-turn prove to be carcinogenic. Abrine and Embelin, the antifertility agents of plant orgin were tested on the uterus, testis, foetuses of mice. The results were indicated that these compounds are toxic to the foetuses, uterus and testis of mice by reducing the spermatozoid production, hypertrophic and haemorrhageic uterus and also anomalies in the morphology and skeletal system of foetuses. The present study revealed that these compounds are also inducing high mutation rate. Hence, these compounds may be used as antifertility or abortifacient agents after further analysis for the safe usage by women as well as men.

KEYWORDS: Abortifacient, Mutation rate, Hypertrophic, Skeletal system, Antifertility agents.

INTRODUCTION:
Hormonal and non- hormonal drugs used as abortifacients are flooded in the market. The wide spread use of these preparations around the time of pregnancy or just after or prior to has raised many questions about their effects on foetal development and infant heater [1]. Many herbal remedies are traditionally used to prevent the ovulation, fertilization, implantation, uterine flow or to stimulate uterine contraction in women [2]. However, such antifertility agents were not yet developed for men, either to inactive the spermatozoids, or to decrease the development of sperms. Many researches are carried out to develop allopathic antifertility drugs for men. Many herbs have been reported historically, used by women but not men, to stimulate menstrual flow or to reduce fertility [3]. The use of hormonal contraceptives has been a necessary evil in the realm of population control by women without knowing its side effects. Constant and regular use of these drugs by women may result with many genetic changes both in mothers and infants.

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There is a need to analyse these changes by considering the mutation rate as induced by the test chemicals. This warrants, to develop an antifertility agent that shall be used both by men and women. This, require the screening of the herbal compounds like abrine and embelin with abortifacient and antifertility activity will be a useful guide towards the development of a cheaper, affordable antifertility and contraceptive agents that are less harmful to the system.

MATERIALS AND METHODS:
EXPERIMENTAL MATERIAL:
Abrine, an antifertility agent, is a glucosidic alkaloid, phytolectin in nature. The compound is obtained from *Abrus precatorius* L., a species of the family fabaceae. The molecular configuration of this compound was given by Cabrill and Jackson [4].

Embelin, a benzoquinone is the pure compound of the berries of *Embelia ribes* Brun, of the family Myrsinaceae. This compound was first isolated by Hefter[5]. The test compounds were obtained from Bio-organic laboratory, Chennai.

Table – 1 Teratological assessment of foetuses born to female mice treated with antifertility compounds:

Name of the Compound	Dose mg/kg body weight	Number of Foetuses	Foetus weight examined x ± SD	Head length (cm) x± SD	Head width (cm)x ± SD
Control [Distilled water]	0	51	1.76±0.06	1.30±0.13	1.07±0.09
Solvent control [Carboxymethyl cellulose]	1%	31	1.40±0.04	1.15±0.05	1.03±0.04
Abrine	0.02	33	1.27±0.07	1.09±0.06	0.98±0.03
Embelin	50	55	1.29±0.19	1.08±0.05	0.94±0.05

Table – 1 Continued

Name of the Compound	Body length (cm)x± SD	Tail length (cm)x± SD	Hind limb length (cm)x± SD	Fore limb length (cm)x ± SD	Percentage of foetuses showing physical and skeletal abnormalities %
Control [Distilled water]	2.76±0.28	1.58±0.20	1.21±0.15	1.30±0.21	0.0
Solvent control [Carboxymethyl cellulose]	2.68±0.08	1.30±0.09	1.11±0.03	1.00±0.03	3.22
Abrine	2.60±0.11	1.17±0.11	1.09±0.06	0.98±0.06	0.0
Embelin	2.48±0.12	1.11±0.07	1.02±0.08	0.92±0.07	7.27

SD = Standard Deviation; X = Mean

Table – 2. Teratological assessment of foetuses born to male mice treated with test compounds

Name of the Compound	Dose mg/kg body weight	Number of Foetuses	Foetus weight examined x ± SD	Head length (cm) x± SD	Head width (cm)x ± SD
Control [Distilled water]	0	51	1.76±0.06	1.30±0.13	1.07±0.09
Solvent control [Carboxymethyl cellulose]	1%	21	1.39±0.04	1.00±0.07	1.93±0.04
Abrine	0.02	29	1.41±0.15	1.10±0.42	0.95±0.06
Embelin	50	41	1.44±0.14	1.08±0.09	0.87±0.15

Table – 2. Continued

Name of the Compound	Body length (cm)x± SD	Tail length (cm)x± SD	Hind limb length (cm)x± SD	Fore limb length (cm)x ± SD	Percentage of foetuses showing physical and skeletal abnormalities %
Control [Distilled water]	2.76±0.28	1.58±0.20	1.21±0.15	1.30±0.21	0.0
Solvent control [Carboxymethyl cellulose]	2.67±0.21	1.12±0.07	1.05±0.05	0.98±0.06	0.0
Abrine	2.49±0.10	1.12±0.10	1.02±0.06	0.95±0.10	3.4
Embelin	2.53±0.18	1.17±0.11	1.04±0.12	0.90±0.19	17.1

SD = Standard Deviation; X = Mean

Experimental Animal:

Swiss albino mice (*Mus musculus*) weighing, 25gr to 27gr were used as the experimental animal. They were procured from the central animal facility, Indian Institute of Science, Bangalore. The experiments were carried out at controlled temperature (25 □C) under pathogen free condition. Pellet diet and water was provided *ad libitum*.

Parameters Employed:

In order to evaluate the histopathological changes in the treated animals and the teratological changes in the foetuses, born to the treated parental animals and the mutational rate as the result of genetic changes (Dominant lethal) were followed in the present study.

I. Histological Study:

The female and male parents were treated for 15 days and 30 days respectively. They were sacrificed immediately after exposure by cervical dislocation. The uteral tissue and testis were fixed in Bowin's solution. The tissues were sectioned at 6 hr thickness and stained in haemotoxylin and counter stained by eosin after passing through a series of alcohol.

II. Teratological assessment:

The foetuses born out of the treated mothers and fathers were studied as detailed by Wilson [6] and Gupta et al., [7]. The litters were killed in 60% ethonal and fixed in 10% formalin for a week. The foetuses were cleared in 3% KOH until the bones become transparent. They were stained with Alizarin Red S [8]. The stained specimens were cleared by passing through grades of glycerine and 3% KOH until the skeletal bones are clearely visible.

III. Mutation rate and Dominant Lethal:

The mutation rate was assigned by considering the induced post implantation and dominant lethal by adopting the formula as given by Edward and Seale [9].

Experimental Design:

The animals were grouped as A, B, C, D, E and F groups. The group 'A' formed the distilled water control, group 'B' formed the solvent (Carboxy methyl cellulose –1%) negative control. Group 'C' formed the male animals treated with abrine and embelin separately. Group 'D' constituted vergin females treated with the test compounds. Group 'E' formed the tested males received the test compounds (0.5 ml), at a single dose of 0.02 mg/kg of abrine and 50 mg/kg of embelin for 30 days alternatively were mated with vergin females. The treated females at the same dose for 15 days formed the group 'F'. The untreated animals were separated after absorbing the 6 animals, three of them sacrificed immediately by cervical dislocation and the others were

left for mating. The foetus born out of treated mothers examined teratologically and by counting the corporalutea, living and dead implants, for analysing the mutation rate.

Statistical analysis were carried out by following the procedure as detailed by Zar [10].

RESULTS:

The results of the present research work is presented in tables 1 to 5 and figures 1 to 4.

I. Histological assessment:

The histological analysis of testis of the control mice showed the occurrence of spermatogonia forming a lining wall of the seminiferous tubules. The interstitial cells were located between the seminiferous tubule and the sertoli cells in between the spermatogonial cells. Numerous spermatozoids were observed in the lumen of the seminiferous tubules. The walls of the tubules consisted of two or three layers arranged uniformly and the spermatids were in different stages of development (Fig. 1A).

The testis of male mice exposed to abrine showed a great reduction in the number of spermatozoids in the lumen of the seminiferous tubules (Fig. 1 B and C). Numerous colloidal material and less number of spermatids irregularly arranged in the seminiferous tubule (Fig. 1 D).

The tissue organisation in the uterus of the control mice showed a normal endometrial and myometrial cells (Fig. 2A). The uterus of abrine treated animals showed many abnormalities (Fig . 2 B – D). The wall of the uterus was inflated and many blood clots (haemorrahage) were observed in the centre of the uterus. Cojested overies and rich myometrium and proliferating mucous membrane were recorded in the uterus of the treated animals (Fig. 2 B – D).

The embelin treated testis of mice showed similar abnormalities as that of abrine. The testis of male animals showed irregular arrangement of recrossed tubules with hypertrophy and the disintegration of sperms (Fig. 1 C).

The myometrial layer of the uterus exposed to embelin showed less proliferation with highly folded mucous layer. The ovary was hypertrophied with heavy haemorrhage (Fig. 2 D).

II. Teratological assessment:

The results of the teratological assessment of the foetuses born to abrine and embelin treated parents are summarised in the tables 1 to 5 and Fig. 3 & 4. The incidence of dead foetuses and resorptions induced by embelin was highly significant in treated females mated with vergin males. However, the live implants were found to be greater in the number (Table 3). Similarly trend of results were observed in the embelin treated males mated with untreated females (Table 4). Morpholigical measurements such as head length and width, body length, tail length, length of the fore and hind limbs were found to be not varying in the foetuses born to treated animals as compared to the control group (Table 1-2). The rib anomalies such as forked ribs, absence of ribs, shortening of ribs, bent ribs and stunted fore head were found to be of greater frequency in the foetuses born to treated parents (Fig. 3 A to L). The morphological features of the uterus of abrine treated mice showed resorption sites and thickening of uterus wall was observed in embelin treated female mice (Fig. 4 A-F).

III. Mutation rate / Dominant lethality:

The implantation activity was found to be highly significant in embelin treated males mated with untreated females as compared to that of other male animals exposed to abrine. A significant reduction in the foetal weight was abrine. A significant reduction in the foetal weight was observed ($P < 0.05$) in the foetuses born to mice treated with abrine and embelin prior to mating. The mean values of the number of corpora lutea in female animals treated with embelin are higher. Dead implants were observed to be greater in embelin treated animals (Table 5). The frequency of pre – implantation loss of eggs (PRE) and dead implants (D I) were significantly higher in animals treated with abrine than with embelin (Table 5). Lethal hits per gamete were found to be greater in embelin – treated animals (641×10^{3}) than the mice exposed to abrine . However, a lower mutation rat were observed in abrine treated animals (1.64×10^{-3}) than the others.

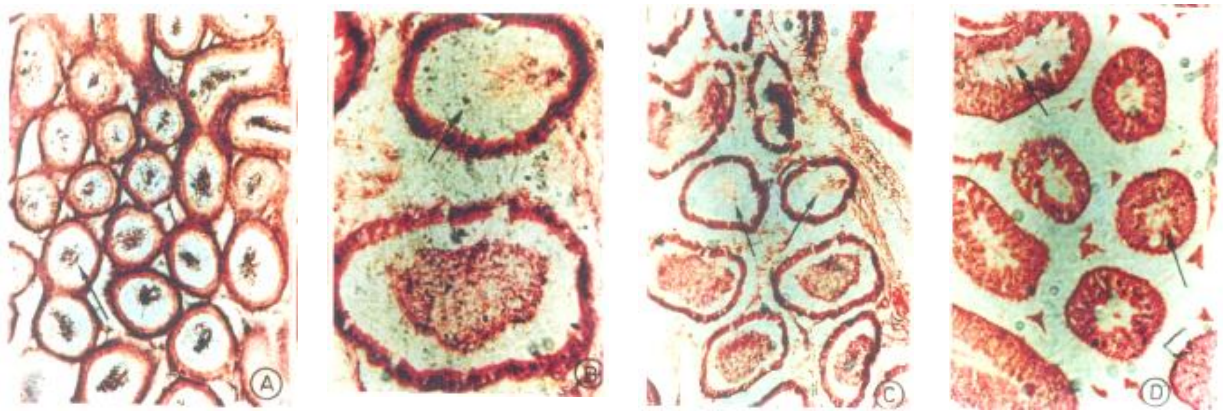

Figure 1:Mouse testis, Transverse section showing different abnormalities in the production of sperms – cax 100.
A. Testis of control mice showing the occurrence of numerous sperms in the seminiferous tubules.
B. T.S. of testis of mice treated with Abrine for 30 days alternatively, showing total disintegration of sperms in one of the seminiferous tubule.
C. Cross section of testis of Embelin treated mice for 15 days, showing the absence and full disintegration of spermatozoids in some of the seminiferous tubule.
D. Some of the seminiferous tubules show thick wall layers with greater reduction of sperms in Embelin treated mice

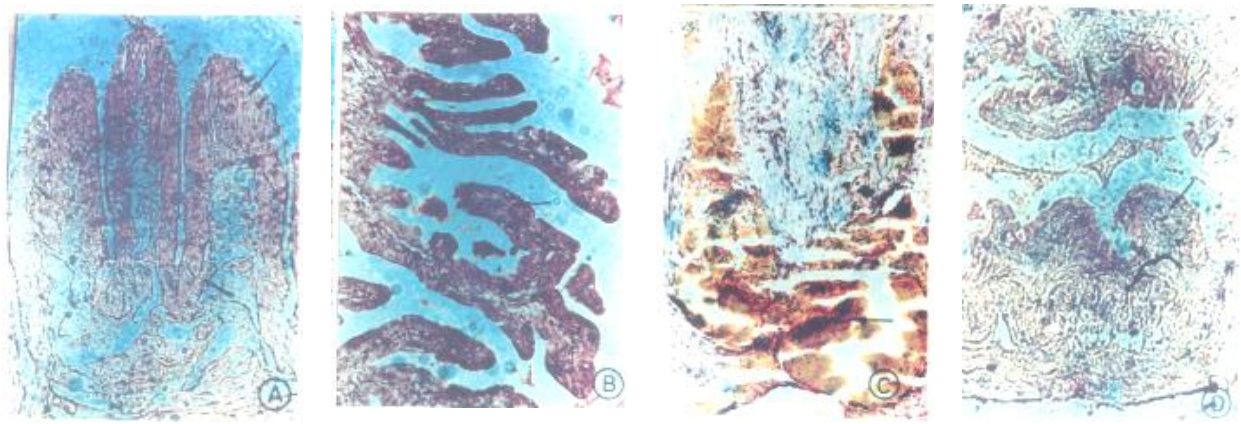

Figure 2: Transverse section of uterus of treated and control mice – cax 100.
A. Uterus with normal; myometrium of a control mice.
B. Abrine treated mice for 15 days showing the presence of rich myometrium with inflammation.
C. Uterus showing implantation sites with unorganised embryo of the mice treated with Abrine for 15 days.
D. Uterus showed less proliferation of myometrium in Embelin treated mice.

Table – 3. Induction of dominant lethal in females after treatment with a single dose of abortifacient compounds

Chemicals treated	Dose mg/kg body weight	No. of females treated	No. of fertile females	Sterility %	No. of Corporalutea		
					Total number	Mean	Standard Deviation
Control (Distilled Water)	0	13	13	0.0	160	12.30	0.48
Carboxy methyl cellulose (C.M.C)	1%	5	4	20.00	42	10.50	1.00
Abrine	0.02	9	7	22.22	62	8.86	0.90
Embelin	50	10	10	0.0	103	10.30	1.16

Table – 3. Continued

Chemicals treated	No.of live implants			No. of dead implants [Resorption and dead]		
	Total number	Mean	Standard deviation	Total number	Mean	Standard deviation
Control (Distilled Water)	151	11.61	1.04	7	1.75	0.50
Carboxy methyl cellulose (C.M.C)	40	10.00	0.82	2	0.5	0.58
Abrine	58	8.29	1.98	3	0.43	1.13
Embelin	92	9.20	1.47	11	1.10	1.28

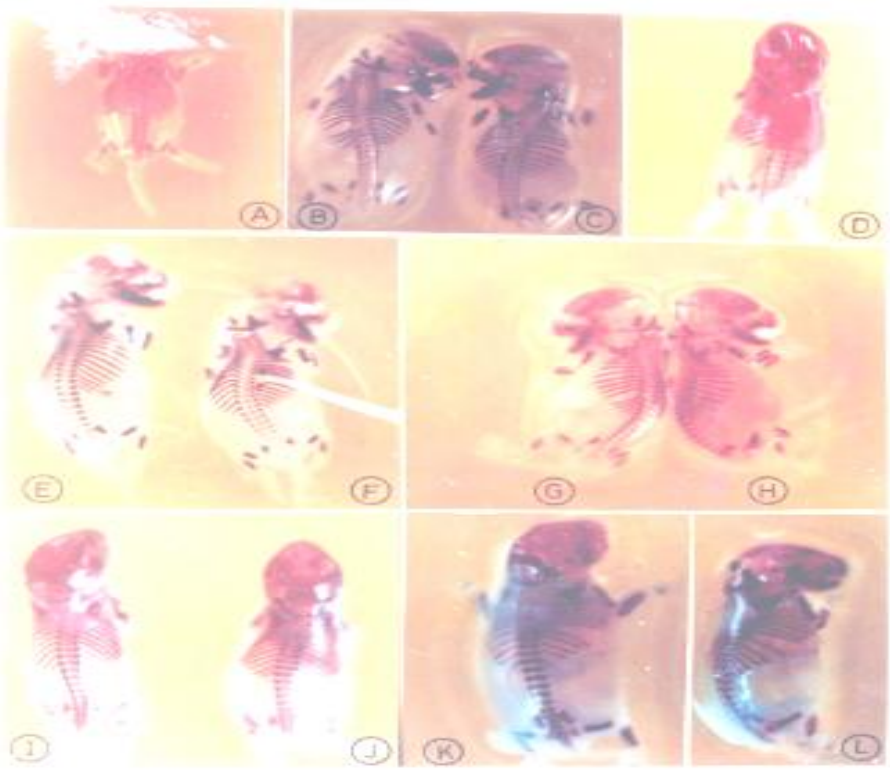

Figure 3: Skeletal malformation in the foetuses born to treated mothers.
A.Normal foetus from control mice
B. and C. Absence of 8th rib bone
D. Stunted last rib
E. Last two ribs are stunted.
F. Foetus showing the absence of two ribs
G and H. Absence of 8th rib with other stunted rib bones.
I. Foetus showing the absence of last two ribs on one side
J. Foetus born out of Embelin treated fathers showing bent fore limbs with the absence of last two ribs on one side.
K. Foetus with forked and stunted ribs.
L. Foetus showing stunted fore head and forked ribs.

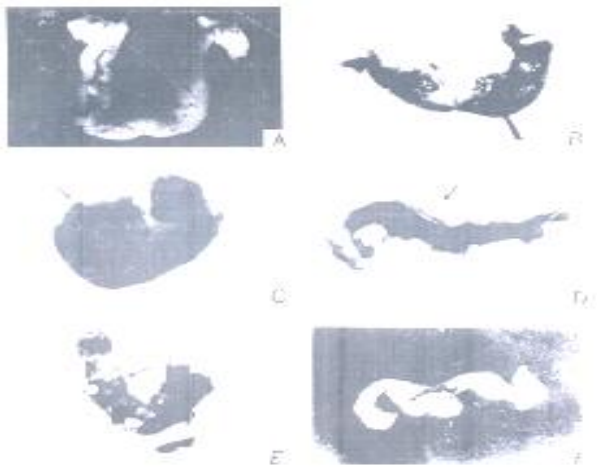

Figure 4: Morphology of Uteri of mice treated with Abrine and Embelin for varing periods.
A. Uterus showing normal features of an untreated control mice (Distilled water).
B. Uterus of Abrine treated mice showing resorption site.
C. Uterus of Embelin treated mice (mothers) showing resorptions.
D. Uterus of untreated female mice mated with Abrine treated males showing resorbed foetus at many sites.
E. Uterus of Embelin treated female mice mated with untreated male animals showing thickening of uterus with resorptions.
F. Uterus of mice received solvent (CMC) showing normal features.

Table – 4. Induction of dominant lethals in males treated with abortifacient compounds

Experimental Chemical	Dose mg/kg body weight	No. of males treated	No. of females treated	No. of fertile females	No. of Corporalutea		
					Total number	Mean	Standard Deviation
Control (Distilled Water)	0	13	13	13	160	12.30	0.48
Carboxy methyl cellulose (C.M.C)	1%	4	6	4	46	11.50	1.00
Abrine	0.02	5	6	5	49	9.80	3.63
Embelin	50	7	8	8	75	9.38	1.60

Table – 4. continued

Experimental Chemical	No. of live implants			No. of dead implants [Resorption and dead foetus]		
	Total number	Mean	Standard deviation	Total number	Mean	Standard deviation
Control (Distilled Water)	151	11.61	1.04	7	0.54	0.88
Carboxy methyl cellulose (C.M.C)	44	11.00	0.82	2	0.50	0.58
Abrine	49	9.80	3.63	0.0	0.00	0.0
Embelin	68	8.50	2.72	7	1.88	1.36

Table – 5 Table showing dominant lethal effects and mutation rate of abrine and embelin

Name of the Chemical	No. of females	Number of Corporalutea	No. of implants	% PRE	No.of implants per female	No.of dead implants[DI]
Control [Distilled water]	13	160	158	1.25	12.20	7
Solvent control [Carboxymethyl cellulose]	4	49*	42	14.28	10.50	2
Abrine	7	86*	61	29.10	8.70	3
Embelin	10	123*	103	16.26	10.30	11

Table – 5. continued

Name of the Chemical	Percentage of dead implants[DI]	Percentage of PRE + DI	Dead implants per female	No.of live implants [LI]	Mutation rate [μ]
Control [Distilled water]	4.40	5.60	0.54	11.62	0
Solvent control [Carboxymethyl cellulose]	4.08	18.36	0.50	10.00	0
Abrine	4.90	32.60	0.43	8.29	1.64×10^{-3}
Embelin	8.94	25.20	1.10	9.20	1.5×10^{3}

*=Calculated according to the controls; PRE = Preimplantation loss of Eggs; DI = Dead implants; LI = Live implants; μ=Mutation rate (lethal hits per gamete)

DISCUSSION:

The present analysis on the effects of abrine and embelin showed many anomalies in the histoarchitechture of testis and uterus, teratological changes in the foetuses of mice and high mutation rate might be the resultants of damages in the genetic material. This is in accordance with the earlier reports on the genotoxic effects of embelin [11]. These abnormalities, induced by the test compounds might lead to the infertility in the organisms. The reduction of spermatozoids as induced by abrine is akin to the reports made on the exposure of male mice to the ethanolic extract of *Abrus precatorius* [12]. The incidence on the induction of total sterility by the test compounds in mice is in accordance with the reports made on the similar effects by the oily extract of *Abrus* seeds, abridine a crystalline fraction of the seeds and an another glucoside extract of *Trigonella willifordii* on male and female rats [13]. The inflammation of the uterus of the abrine treated female mice is akin to the reports made in guine – pig treated with methanol extract of *Abrus* [14]. The reduction in the number of foetuses born to the treated animals is comparable to the reports made on the *in vivo* experiments on the exposure of gossypol acetic acid on the human system [15]. The antifertility effect, inhibition of oocytes and abortifacient activity were observed in male and female mice. Similar trend of results were reported in albino rats treated with dihydropyridazinone [16], gangetin of Desmodium and phasdus [17].

The antifertility effect of embelin in female rats [18], anti-implantation efficacy [19], and antiestrogenic property in rats [20], and contraception of embelin in rats [21 & 22], such effects were also observed in the present investigation. Physical, skeletal and mutation rates induced by embelin is higher when compared to abrine. These results are akin to the late pregnancy effect observed in rats [23]. The antifertility effect of embelin and abrine is comparable to the effects of flavonoids isolated from Srtiga [24] and Butea [25]. The aqueous extracts of *Graptophyllum pietunum* showed a similar trend of results when administered to rats [26]. The present study on the antifertility and abortifacient effect is similar to the leaf extracts of *Indigofera trifoliate* administered to female albino rats [27]. The teratological changes observed in the present study is similar to the induction of such changes by *Plumera rulra* pad extract in female albino rats [28].

The resorption index as induced by abrine and embelin in the present work 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 foetuses [29]. The endometrial changes observed in the uterus treated with the test compound is in agreement with the uterus of rat treated with the extract of *Plumera rubra* [28].

In conclusion, all the two test compounds are inducing antifertility and abortifacient effect with minimal damage to the system and hence these compounds may also be used as male contraceptive. Further, studies are needed to confirm and identity the bio-active principles and pharmaceutical technology to introduce them as male and women contraceptive drug.

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REFERENCES:

1. WHO report – on effect of female sex hormones on foetal development and infant health. WHO 1981.Geneva.
2. Ritehie HE. The safety of herbal medicine use during pregnancy. Frontier in foetal health. 3; 2001: 259 – 266.
3. Farnsworth NR, Bingel AS, Cordel GA, Crane FA and Fong H.H, potential value of plants as source of new antifertility agents. J. Pharma Sci. 1975; 64: 535 – 598.
4. Cahill, Jackson, J . Bio. Chem. 1938; 1261 29.
5. Hefter F. Arch. Pharm. 1900; 238:15.
6. Wilson JC. Embryological Considerations in teratology. Methods for administering agents and detecting malformations in experimental animals. In Teratology – Principles and techniques. University Chicago Press, Chicago. 1965; pp. 251-277.
7. Gupta SK, Satyachandra V, and Saxena D.K. Teratogenic and embryotoxic effects of endosulfan in rats. Acta. Phar. Et. Tax. 1978; 42 (2): 150-152.
8. Staples RE and Schnell VL. Refinement in rapid clearing technique in KOH Alizarim Red S method for foetal bone. Stain Tech. 1914; 39: 61-63.
9. Edward RG and Searle AC. Genetic radiosensitivity of specific post-dietyate stages in mouse oocytes. Genet. Res. 1963; 4: 389-398.
10. Zar J.H. Biostatical analysis. Prentice-Hall Inc., Englewood Cliff NJ. 1974.
11. Vivekanandan OS. Genotoxic and foetotoxic effects of embelin on mouse system. J. Cytol. Genet. 2002; 3(NS) 191-195.
12. Jodan A and Mathur R. Effects of *Abrus precatorius L* seed extracts on biochemical constituents of male mice. J. Jiwaji univ. 1981; 9(1): 100-103.
13. Vivekanandan OS. Mutagenic and teratogenic studies of plant abortifacient compounds-Abrine and Embelin on plant and animal systems. Ph.D. Thesis. University of Madras. 1987; 99-100.
14. N wodo ORC and Botting J.H. uterotoxic activity of extracts of the seeds of *Abrus precatorius L* Planta Med. 1988; 47 (4): 230-233.
15. Rastula K, Hankkamma M, Wichman K and Luukkainar T. Vaginal contraction with gossypol- A critical study. Contraception. 1983; 27 (6): 571-576.
16. Tyler JPP, Matson PL, Collins WP and Duke M. Dissociation of oocyte maturation and ovulation in mice pre-treated with a derivative of dihydropyridozinone. J. Reprod. Fert. 1981; 62: 455-458.
17. Sun CQ, Wang SR, Hu ZY, Zou ZW and chen Q.H. studies on anti-fertility action of *Phaseolus vulgaris*. Acta. Pharm. Sci. 1983; 18 (2): 81-85.
18. Bhargawa SK, Dixit VP and khanna P. Antifertility effects of embelin in female rats. Fitoterepia. 1984; 55 (5): 302-304.
19. Radhakrishnan N and Alum M. Antifertility activity of embelin in albino rats. Indian J of Exp. Biol. 1975; 13: 70-71.
20. Reddy MK, Kokate EK, Rao KN and Chari N. Antifertility activity of crude drug combinations in albino rats. Indian Drugs. 1984; 21 (12): 533-535.
21. Prakash AO, Saxena V, Chand GK and Mathur R. Antifertility investigation on embelin oral contraceptive of plant origin. II. Effect on uterine biochemical constituents of ovaricctonize albino rats. Com. Physical. Ecol. 1983; 8 (4): 271-275.
22. Mohana K and Purushoththaman K.K. Antifertility properties of *Embelia ribes*. Indian J. Exp. Biol. 1980; 18: 1359-1360.
23. Prakash AO. Effects of E. Ribes extracts on the uterus of rat. Probe. 1979; 18 (3): 178-184.
24. Hiremath SP and Hammantha R.S. Antifertility efficacy of the plant *Srtiga lutka* on rats. Contraception. 1990; 42: 466-477.
25. Khanna U and Chandhary RR. Antifertility screening of plants. Part I. Investigations on *Butea monosperma L*. Nig. J. Pharma.Sci.2007; 6: 78-83.
26. Stella OOD, Grace EU, Herbert ABC and Sammuel AD. Oxytoeic acid and anti-implantation activities of the leaf extract of *Gratophyllum pictum*. Afr. J. Biotech. 2009; 8: 5979-5984.
27. Dabhadkar Dinesh and Zade Varsha. Abortifacient efficacy of *Indigofera trifoliata* leaves extract on female albino rats. Asian J. Pharm. Clin. Res. 2013; 6 (3): 75-79.
28. Dinesh Dabhadkar and Varsha zade. Abortifacient activity of *Plumeria rubra* pod extract in female albino rats, Indian J Exp. Biol. 2012; 702-707.
29. Elbeticha A, Oran A, Alkofahi A, Dhamani H and Raies AM. Foetotoxic potential of *Globularia Arabica* and *Globularia alypus* in rats. J. Ethnopharmacol. 2000; 72: 215.