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Short Communication

Evaluation of some indigenous plant extracts for antiimplantation activity in albino rats

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SUMMARY

In the present investigation twelve indigenous medicinal plants have been screened for their antiimplantation activity in albino rats. The plant material was subjected for soxhlation successively and separately from non-polar solvents to polar solvents i.e., petroleum ether benzene and ethanol. Out of these three extracts the petroleum ether extract of seeds of *Citrus medica*, aerial part of *Oxalis corniculata* and *Tinospora cardifolia* have showed maximum antiimplantation activity. Ethanol extract of leaves of *Cardiospermum helicacabum*, roots of *Echinops echinatus*, leaves of *Melia azedarach*, seeds of *Momordica charantia* and bark of *Terminalia bellirica* have shown maximum antiimplantation activity amongst the three extracts of each plant material screened. Though all the three extracts of seeds of *Annona squamosa* and leaves of *Zizyphus jujube* screened for antiimplantation activity, no extract has showed any loss in implantation. The details of the results obtained are discussed.

Key words: Antiimplantation; Medicinal plants; Rats

INTRODUCTION

Use of plant material for fertility regulation has a long standing history among Indian physicians. A large number of indigenous plants having such activities are recorded in Indian literature (Kirtikar and Basu, 1935; Anonymus, 1966). So far available research information on antifertility activity of indigenous medicinal plants has been reviewed by many investigators (Chaudhury, 1966; Kapoor *et al.*, 1974; Garg, 1976; Garg *et al.*, 1978; Satyavati, 1984; Patel and Patel, 2004). Recently a few investigators have reported the antiovulatory and antispermatogenic activities of some indigenous plants (Chaturvedi *et al.*, 1995; Murthy *et al.*, 1997; Sharanabasappa *et al.*, 2002; Sharanabasappa *et al.*, 2003). Antiimplantation activity is an important milestone in reducing fertility index and also a safe procedure. Therefore in this study a comprehensive programme of screening 12 indigenous medicinal plants for antiimplantation activity is undertaken. Three solvent extracts of each plant obtained after soxhlation were taken for testing the antiimplantation activity in albino rats.

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Table 1. Evaluation of some indigenous plant extracts for antiimplantation activity in albino rats

Name of the plant	Part used	Extract	Dose administration (mg/100 g body weight)	Rats having no implantation sites on day 10	% inhibition of pregnancy	Mean number of implants	% inhibition of implantation sites
Control	-	Tween-80(1%)	-	Nil	Nil	10.0 ± 0.31	Nil
Annona squamosa (Anonaceae)	Seed	Pet. ether	25 mg	Nil	Nil	9.63 ± 0.31	3.70
			50 mg	Nil	Nil	9.80 ± 0.20	2.00
		Benzene	25 mg	Nil	Nil	9.50 ± 0.22	5.00
			50 mg	Nil	Nil	9.33 ± 0.26	6.70
		Ethanol	25 mg	Nil	Nil	9.71 ± 0.30	2.90
			50 mg	Nil	Nil	9.27 ± 0.66	7.30
Cardiospermum helicacabum (Sapindaceae)	Leaves	Pet. ether	25 mg	Nil	Nil	9.80 ± 0.20	2.00
			50 mg	Nil	Nil	9.60 ± 0.24	4.00
		Benzene	25 mg	Nil	Nil	10.0 ± 0.31	Nil
			50 mg	1	16.66	8.16 ± 0.30	18.40
		Ethanol	25 mg	Nil	Nil	8.58 ± 0.21	14.20
			50 mg	1	16.66	5.66 ± 1.17***	43.40
Citrus medica (Rutaceae)	Seed	Pet. ether	25 mg	Nil	Nil	4.1 ± 0.24***	54.00
			50 mg	1	16.66	3.66 ± 0.33***	63.40
		Benzene	25 mg	Nil	Nil	9.83 ± 0.16	1.70
			50 mg	1	16.66	8.16 ± 0.30	18.40
		Ethanol	25 mg	Nil	Nil	7.75 ± 0.43*	22.50
			50 mg	Nil	Nil	6.50 ± 0.95**	35.00
Echinops echinatus (Asteraceae)	Root	Pet. ether	25 mg	Nil	Nil	10.0 ± 0.31	Nil
			50 mg	Nil	Nil	8.53 ± 0.45	14.70
		Benzene	25 mg	Nil	Nil	8.83 ± 0.42	11.70
			50 mg	1	16.66	8.75 ± 0.46	12.50
		Ethanol	25 mg	Nil	Nil	8.00 ± 0.52*	20.00
			50 mg	Nil	Nil	7.00 ± 0.36*	30.00
Gymnema sylvestre (Asclepiadaceae)	Aerial part	Pet. ether	25 mg	Nil	Nil	10.0 ± 0.31	Nil
			50 mg	Nil	Nil	9.18 ± 1.07	8.20
		Benzene	25 mg	Nil	Nil	6.62 ± 1.01**	33.80
			50 mg	1	16.66	5.16 ± 1.10**	48.40
		Ethanol	25 mg	Nil	Nil	8.81 ± 0.41	11.90
			50 mg	1	16.66	8.29 ± 0.60	17.10
Hibiscus rosa sinensis (Milaca)	Flower	Pet. ether	25 mg	1	16.66	8.29 ± 0.60	17.1
			50 mg	1	16.66	6.83 ± 0.54*	31.7
		Benzene	25 mg	1	16.66	5.33 ± 0.33**	46.70
			50 mg	2	33.32	2.25 ± 1.26***	77.50
		Ethanol	25 mg	Nil	Nil	7.16 ± 0.60*	28.40
			50 mg	Nil	Nil	6.16 ± 0.47**	38.40

Table 1. (Continued)

Name of the plant	Part used	Extract	Dose administration (mg/100 g body weight)	Rats having no implantation sites on day 10	% inhibition of pregnancy	Mean number of implants	% inhibition of implantation sites
Control	-	Tween-80(1%)	-	Nil	Nil	10.0 ± 0.31	Nil
Melia azedarach (Meliaceae)	Leaves	Pet. ether	25 mg	Nil	Nil	9.18 ± 1.07	8.20
			50 mg	1	16.66	7.40 ± 0.58	26.00
		Benzene	25 mg	Nil	Nil	9.16 ± 0.30	8.40
			50 mg	1	16.66	7.27 ± 0.91	27.30
		Ethanol	25 mg	1	16.66	7.33 ± 0.49	26.70
			50 mg	1	16.66	5.16 ± 1.10**	48.40
Momordica charantia (Cucurbitaceae)	Seed	Pet. ether	25 mg	Nil	Nil	6.33 ± 0.66**	36.70
			50 mg	Nil	Nil	5.83 ± 0.70**	41.70
		Benzene	25 mg	Nil	Nil	7.50 ± 0.42	25.00
			50 mg	1	16.66	6.55 ± 0.69**	34.50
		Ethanol	25 mg	Nil	Nil	5.87 ± 1.08**	41.30
			50 mg	Nil	Nil	2.71 ±1.90***	72.90
Oxalis corniculata (Oxalidaceae)	Aerial part	Pet. ether	25 mg	Nil	Nil	5.33 ± 0.33**	46.70
			50 mg	1	16.66	2.83 ±0.30***	71.70
		Benzene	25 mg	Nil	Nil	8.10 ± 0.79	17.30
			50 mg	1	16.66	8.27 ± 0.76	19.00
		Ethanol	25 mg	Nil	Nil	7.96 ± 0.78	20.40
			50 mg	Nil	Nil	7.64 ± 0.82	23.60
Terminalia bellirica (Combretaceae)	Bark	Pet. ether	25 mg	Nil	Nil	8.21 ± 0.73	17.90
			50 mg	Nil	Nil	8.12 ± 0.68	18.80
		Benzene	25 mg	Nil	Nil	8.23 ± 0.67	17.70
			50 mg	1	16.66	7.50 ± 0.22	25.00
		Ethanol	25 mg	Nil	Nil	6.28 ± 1.06**	37.20
			50 mg	1	16.66	2.71 ± 1.19***	72.90
Tinospora cardifolia (Menispermaceae)	Aerial part	Pet. ether	25 mg	Nil	Nil	7.75 ± 0.39	22.5
			50 mg	1	16.66	6.16 ± 1.64**	38.4
		Benzene	25 mg	Nil	Nil	9.09 ± 0.40	9.10
			50 mg	1	16.66	8.61 ± 0.48	13.90
		Ethanol	25 mg	Nil	Nil	9.04 ± 0.43	9.60
			50 mg	Nil	Nil	8.94 ± 0.48	10.60
Zizyphus jujuba (Rhamneae)	Seed	Pet. ether	25 mg	Nil	Nil	9.61 ± 0.31	3.90
			50 mg	Nil	Nil	9.16 ± 0.45	4.00
		Benzene	25 mg	Nil	Nil	8.74 ± 0.54	12.60
			50 mg	Nil	Nil	8.72 ± 0.59	12.80
		Ethanol	25 mg	Nil	Nil	10.0 ± 0.31	Nil
			50 mg	1	16.66	.98 ± 1.07	8.20

Duration: 1-7 days. Six animals were maintained in each group. Values are mean ± S.E. *P<0.05, **P<0.01, ***P< 0.001 when compared to control. Percent inhibition of implantation is calculated in relation to control.

MATERIALS AND METHODS

Plant material

Table 1 depicts the details of the plants and their parts used for antiimplantation activity. Plant materials were collected from Hyderabad-Karnataka region and were authenticated in the Department of Botany, Gulbarga University, Gulbarga, India, where voucher specimens were deposited.

Extract preparation

The plant materials were shade dried, powdered and subjected to soxhlet extraction successively and separately with petroleum ether, benzene and ethanol. The decoctions were evaporated under reduced pressure below 45°C. The residual extracts thus obtained were suspended in tween-80 (1%) for making homogenous solution and then screened for their antiimplantation activity in albino rats.

Animals

Adult, healthy and virgin Wistar strain female albino rats, 60 - 70 days old and 150 - 200 g body weights were selected for experimentation. These rats were maintained under standard animal house conditions with a balanced food prescribed by CFTRI (Central Food and Technological Research Institute), Mysore, India and water *ad libitum*.

Treatment

The pregnant animals were divided into seven groups of six animals each and each plant extract was administered orally as follows for seven days by intragastric catheter.

Group 1: received vehicle tween-80 (1%) and served as control

Group 2: received 25 mg/100 g body weight of petroleum ether extract

Group 3: received 50 mg/100 g body weight of petroleum ether extract

Group 4: received 25 mg/100 g body weight of benzene extract

Group 5: received 50 mg/100 g body weight of benzene extract

Group 6: received 25 mg/100 g body weight of ethanol extract

Group 7: received 50 mg/100 g body weight of ethanol extract

The above said groups and dose levels were fixed for all the screening plants separately.

Pregnancy detection and antiimplantation activity

Female albino rats of proven fertility with normal estrous cycle (Hariharan, 1980) were selected for experiments. The animals showing proestrous smear were left with males for mating overnight in the ratio 2 : 1. Mating was confirmed by appearance of sperms in vaginal smear next morning (Wiest *et al.*, 1964; Hariharan, 1980). Rats exhibited thick clumps of spermatozoa in their vaginal smear were selected for the experiments and that day was designated as day 1 pregnancy. The animals were laparotomized under light ether anesthesia on day 10 and both the horns were examined for implantation sites. The abdominal wound was sutured in layers and the animals were allowed to go to term.

Statistical calculation

The statistical calculation like Arithmatic mean and standard error (SE) was made as described by Fisher (1936) and Snedecor (1946). Students '*t*' test was applied.

RESULTS

The results of the various extracts of twelve plants and the dose levels of the extract administered have been summarized in Table 1. Number of rats having no implantation sites on day ten, percentage inhibition of pregnancy, mean number of implants and percentage inhibition of implantation sites are presented in detail.

The findings of the present investigation reveal that alcohol extract of *C. halicacabum, E. echinatus, M. azedarach, M. charantia, T. bellirica* posses potential antiimplantation activity. Among these plants *M.*

charantia, T. bellirica have showed 70 - 90% inhibition of implantation sites.

Benzene extract of *H. rosa sinensis, G. sylvestre* have shown 80 - 90% and 40 - 50% antiimplantation activity respectively. Petroleum ether extract of *C. medica, O. carniculata, T. cardifolia* was also shown antiimplantation activity respectively.

DISCUSSION

In the present investigation the petroleum ether extract of *C. medica, O. carniculata* and *T. cardifolia,* the benzene extract of *H. rosa sinensis* and *G. sylvestre* and ethanol extract of *C. halicacabum, M. charantia, E. echinatus, M. azedarach* and *T. bellirica* shown antiimplantation activity in albino rats. The antiimplantation activity of the above plant extracts may be due to the failure of the egg to develop up to the required blastocystic stage for implantation. These must have caused the imbalance in the required hormonal microenvironment for endometrial interaction. Hafez (1970) has described this type of property as antizygotic or blastotoxic effect of plant extract. Anderson (1972) has described failure of implantation process due to administration of plant extracts is because of imbalanced hormonal environment. This is also possible that the administration of the extracts might have caused the contractions of uterine smooth muscles, which has resulted in the expulsion of ova from the uterus.

In the present investigation twelve plant extracts have been screened for antiimplantation activity and each extract differ in its degree of activity. Therefore it is planed to take up maximum effective extract for detail study like identifying its nature, type of action and its chemical property.

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