ANTIFERTILITY ACTIVITY OF (+)GOSSYPOL

R. S. R. MURTHY AND D. K. BASU
College of Pharmacy, New Delhi 110 012
AND
V. V. S. MURTI
Department of Chemistry, Delhi University, Delhi 110 007

ABSTRACT

(+)Gossypol, isolated from *Thespesia populnea* (Malvaceae), was screened for post-coital anti-implantation activity on fertile, female albino rats. Inhibition of implantation (100%) was observed at a dose level of less than 10 mg/kg body weight, by the intraperitonial route. The compound seems to be non-toxic at the effective concentration level. The LD_{50} was calculated to be 35 mg/kg as the intraperitonial dose. A possible mechanism of anti-implantation activity seems to be due to the inhibition of histamine release by (+)gossypol.

A LARGE number of Indian medicinal plants have been screened for antifertility activity in an attempt to replace hormonal oral contraceptives, and some of them have shown promising activity[1,2]. Chinese investigators have reported[3] recemic gossypol (isolated from cotton seeds) to be an effective male antifertility agent. The present communication pertains to the study of antifertility and some pharmacological activities of dextro-gossypol isolated from *Thespesia populnea*[4].

MATERIALS AND METHODS

Experiments on antifertility activity were carried out on female albino rats (150–200 g). Other pharmacodynamic studies were carried out on guinea pigs, dogs and rabbits. Toxicity studies were carried out on mice and rats.

Noracycline (M/s. CIBA-GEIGY of India Ltd.) was used as a positive standard for antifertility studies.

Anti-implantation Activity Studies

Rats, which cycled normally (identified by testing their vaginal smears), were included in the study. Female rats in proestrus or oestrus stage were left overnight with males of proven fertility. Next morning, their vaginal smears were checked for spermatozoa. The mated rats were housed in separate cages and labelled with the date, which was counted as the first day of pregnancy.

Six rats were used as one group for the study. (+)Gossypol was administered in one dose by the route selected from the first day to the fifth day of pregnancy. The intraperitoneal injection was given as sterile solution in groundnut oil and the oral dose was given as suspension in the mucilage of methyl cellulose.

On the tenth day of pregnancy, laparotomy was performed, under nembutol anaesthesia (60 mg/kg). The number of implants on both the horns of the uterus was counted. The incision was closed and the animal was kept in the cage to go to full term. After delivery, the number of litters was counted and each one examined for evidence of teratogenicity.

Pharmacodynamic Activities of (+)Gossypol

Solution of (+)gossypol in 10% ethyl alcohol was used in all pharmacological studies. Control experiments were carried out with 10% ethyl alcohol.

(*a*) *Action on isolated guinea pig ileum*[5]: (+)Gossypol was tested on isolated guinea pig ileum at the dose level of 100 μg. Tests were also carried out on the effect of (+)gossypol on the action of autacoids, *viz.*, histamine (50 ng), 5-hydroxy tryptamine (250 mg), bradykinin (5 mg) and acetylcholine (500 ng). Results are reported as percentage blockade of activity of the autacoid under the influence of (+)gossypol.

(*b*) *Effect on the release of histamine from isolated mast cells*: The inhibitory effect of (+)gossypol on histamine release induced by compound 48/80 was studied by the method of Uvnas[6].

(*c*) *Action on isolated rabbit heart*[5]: 100 μg of (+)gossypol was administered and the change in the ventricular contraction rate of isolated rabbit heart was recorded.

(*d*) *Action on blood pressure and respiration in dogs*[7]: Anaesthetised dogs (sodium pentobarbitone 35 mg/kg) were administered with 10 mg/kg body weight dose of (+)gossypol and change in the normal blood pressure and respiration rate were recorded.

(*e*) *Action on oestrogenised rat uterus*[5]: (+)Gossypol was tested on isolated oestrogenised rat uterus at the dose levels of 50–200 μg. Tests were also carried

TABLE I

Antifertility activity of (+)*gossypol in female albino rats*

Post-coital anti-implantation activity of (+)*gossypol in female albino rats*

Drug	Route/dose mg/kg	Average No. of implants	No. of rats showing no implants/ no. showing implants	Average No. of litters delivered	Inhibition of implantation compared with the control (%)	Inhibition of pregnancy compared with the control (+)
(+) Gossypol	I.P./10	0·83	4/2	0·00	88·0	100·0
	I.P./8	3·00	2/4	3·00	56·0	54·0
	I.P./5	5·83	1/5	5·70	16·6	13·0
	Oral/100	1·66	1/5	1·66	79·0	79·0
	Oral/30	3·00	3/3	3·00	63·0	61·0
	Oral/10	5·30	2/3	5·17	33·0	33·0
Noracycline (std.)	I.P./1·5	0·00	6/0	0·00	100·0	100·0
	Oral/1·5	0·00	6/0	0·00	100·0	100·0
Groundnut oil (control)	I.P.	6·83	0/6	6·50	..	..
2% Methylcellulose mucilage (control)	Oral	8·00	0/6	6·70	..	..

Note : Litters delivered showed no abnormalities.

out on the effect of (+)gossypol on the action of oxytocin (0·05 I.U.).

Toxicity Study of (+)*Gossypol*

Acute toxicity of (+)gossypol was studied in groups of male and female mice (six mice per group), at the dose levels of 20, 25, 30, 50 and 100 mg/kg body weight, by the intraperitoneal route. The animals were observed for 24 hours after injection for number of deaths and any abnormal behaviour. LD_{50} and feducial limit of error were calculated. Toxicity study by oral route was carried out on rats up to the maximum dose of 200 mg/kg body weight.

RESULTS AND DISCUSSIONS

Anti-implantation Activity Studies

The anti-implantation activity of (+)gossypol on female albino rats is reported in Table I, in terms of percentage inhibition of implantation and percentage inhibition of pregnancy as compared with the control run using groundnut oil (intraperitoneal) and 2% methyl cellulose mucilage (oral). (+)Gossypol at the dose of 10 mg/kg body weight (intraperitoneal) showed 100% anti-implantation activity. However, the activity of (+)gossypol by oral route is comparatively low.

Pharmacodynamic Activity

(+)Gossypol showed no effect on isolated guinea pig ileum, but it inhibited the action of autacoids especially bradykinin. At the dose of 100 μg, (+)gossypol inhibited the action of bradykinin (100%), 5-hydroxy tryptamine (54 $\pm$ 3·76%), histamine (30 $\pm$ 3·23%) and acetylcholine (50 $\pm$ 2%). Mast cell degranulation studies showed that (+)gossypol inhibits the histamine release from mast cells upto 60 $\pm$ 7·16%. On the basis of the above results it may be suggested that mechanism of anti-implantation action could be by antagonising histamine, an essential mediator in decidulaization during midation. (+)Gossypol did not show any action on isolated oestrogenized rat uterus but potentiated (10%) the action of oxytocin on the tissue, which reveals that (+)gossypol may have slight oestrogenic activity.

(+)Gossypol did not show any marked effect on the heart rate of the isolated rabbit heart. However, slight decrease in the blood pressure was observed with doses more than 15 mg/kg body weight.

Toxicity Studies

The acute toxicity studies in mice showed the LD_{50} to be 34·5 $\pm$ 5·63 mg/kg body weight (intraperitoneal). Toxicity studies conducted on groups of rats by oral administration did not show any death even at the

dose levels of 200 mg/kg body weight. No major abnormalities were observed in animals during toxicity studies.

Conclusion

(+)Gossypol shows promising post-coital anti-implantation activity in fertile female albino rats at a dose level less than 10 mg/kg body weight by intraperitoneal route. By oral route, the compound is active at the dose of 30 mg/kg body weight.

The effect of (+)gossypol on autacoid induced spasm and on the release of histamine from mast cells suggest that the anti-implantation activity of (+)gossypol may be due to the inhibition of decidualization by blocking histamine release or by competitive binding with histamine receptors. (+)Gossypol may also possess slight oestrogenic activity.

(+)Gossypol seems to be non-toxic in the effective dose level now tested.

Acknowledgement

The authors are grateful to the University Grants Commission for the financial assistance to carry out a part of this work.

1. Pakrashi, A., Chakrabarty, B. and Dasgupta, A., *Experientia*, 1976, **32**, 394 and earlier references.
2. Garg, S. K., *Indian J. Pharmacology*, 1973, **5**, 272 and earlier references.
3. National Co-ordination group on male antifertility agents, *Chinese Medical Journal*, 1978, **4**, 417.
4. Datta, S. C., Murti, V. V. S. and Seshadri, T. R., *Indian J. Chem.*, 1972, **10**, 263.
5. Burn, *Practical Pharmacology*, Blackwell Scientific Publications, Oxford, London, 1952.
6. Uvnas, B. and Inga-Lisathon, *Exper. Cell Res.*, 1959, **18**, 512.
7. Ghosh, M. N., *Fundamentals of Experimental Pharmacology*, Scientific Book Agency, Calcutta, 1971, p. 70.
8. Sheleshnyak, M. C., *Endocrinology*, 1954, **54**, 396.

SYNTHESIS AND CNS ACTIVITY OF 2-ARYL-3-(SUBSTITUTED-PHENOXYACETYL-HYDRAZONO)-METHYLENYL-INDOLES

RAJESH AGARWAL, R. K. SATSANGI, SHOBHA MISHRA AND S. S. TIWARI
Department of Chemistry, Lucknow University, Lucknow 226007

Abstract

Twelve of the title compounds have been synthesised by the reaction of 2-aryl-indol-3-aldehydes with substituted phenoxy acetyl hydrazines under mild acidic conditions of hydrazone's synthesis. The structures of these compounds have been confirmed by elemental analysis and the I.R. spectra. The compounds are non-toxic and psychotropics. The compounds have shown a marked effect on body temperature.

DIFFERENT indole derivatives impart a variety of reactions on the CNS such as depressant, anticonvulsant[1,2], analgesic,[3] antidepressant[4] and anti-Parkinsonian[5] activities. The indolyl chemical neurotransmitter, serotonin, itself has been found to exhibit variable CNS activities. According to Koelle[6], the effects and responses of serotonin on the CNS differ not only between the different species of animals but also between the individuals of the same species and even in different tests on the same animal. This has controversy regarding the effects of serotonin on the CNS. Furthermore, the hydrazines, hydrazides and hydrazones have been reported to be the inhibitors of monoamine oxidase[7,8] an important enzyme affecting the concentration of adrenergic neurotransmitters. In view of these valid observations, the authors have synthesised twelve "2-aryl-3-(substituted-phenoxyacetyl-hydrazono)-methylenyl-indoles" to observe the gross effects of them on the CNS of mice.

2-Aryl-indoles and 2-aryl-indol-3-aldehydes

These were prepared by the methods of 'Blades and Wilds'[9] and Weisbach *et al.*[10]

Substituted-phenoxyacetyl hydrazines

The method of Conti[11] was used for the synthesis of these compounds.

2-(4'-Chloro-phenyl)-3-(p-methyl-phenoxyacetyl hydrazono)-methylenyl-indole

It was synthesised by mixing 2-(4'-chloro-phenyl) indol-3-aldehyde (0·0025 mole) and 4-methyl-phenoxy-acetyl hydrazine (0·0025 mole) in ethanol (40 ml) containing 2 drops of glacial acetic acid. The solution