EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 8
619-623
Year: 2017

ANTIFERTILITY ACTIVITY OF N-BUTANOL FRACTION METHANOLIC EXTRACT OF ARISTOLOCHIA INDICA LINN ROOT IN FEMALE RATS

Kanakam Vijayabhaskar*[1], Mathukumalli Sai Laxmi[4], Naini SravanKumar[1], Dr. M. Arunadevi[3], Bairy Padma[2], Kalakota Chaitanya Prasad[5]

[1]Department of Pharmacognosy, Department of Pharmaceutics, Sahasra Institute of Pharmaceutical Sciences, Warangal, Telangana, India 506007.
[2]Department of Pharmaceutical Chemistry, University College of Pharmaceutical Sciences, Kakatiya University, Warangal-506001.
[3]Department of Chemistry, Sri Balaji College of Pharmacy, Karimnagar-505415.
[4]Department of Pharmacology, Max Institute of Pharmaceuticals Sciences, Khammam, Telangana, India-507002.
[5]Swamy Ramananda Tirtha Institute of Pharmaceutical Sciences, Nalgonda. 508002.

***Corresponding Author: Prof. Kanakam Vijayabhaskar**
Department of Pharmacognosy, Department of Pharmaceutics, Sahasra Institute of Pharmaceutical Sciences, Warangal, Telangana, India 506007.

Article Received on 06/06/2017 **Article Revised on 27/06/2017** **Article Accepted on 18/07/2017**

ABSTRACT

Background: Aristolochia indica Linn is a large plant genus with over 500 species, collectively known as birthworts, pipevines or Dutchman's pipes belong to the family Aristolochiaceae. Is a perennial climber shrub with woody base stocks. The leaves are simple, alternate, short petioled, the blade ovate or somewhat wedge shaped. It has flowers which are greenish white in colour, while fruits are round. Traditionally used in Ayurveda, Yunnan herbal medicine and has numerous therapeutic benefits. But little is known about its potentially negative effects on pregnant women. **Objective:** The aim of the present study was to evaluate the antifertility effect of n-butanol fraction of methanolic extract of Aristolochia indica root in female Wistar rats. **Materials and Methods:** Four groups of rats were administered orally n-butanol fraction of methanolic extract of Aristolochia indica root at doses of 100, 200 and 400 mg/kg body weight daily for 19 days. The control group received distilled water. On day 20 of gestation, each rat was laparatomised and number of corpora lutea of pregnancy, number of live fetuses as well as the postcoitum fertility index, weights of the foetuses and placentae were determined. **Results:** Oral administration of the extract from days 1 to 19 of gestation showed reduction ($p<0.05$) in the number of corpora lutea of pregnancy and number of live fetuses. Weights of fetuses of extract treated female rats were also smaller ($p<0.05$) compared with the control. Anti-implantation activity of the treatment groups were 49.04%, 52.13% and 58.11% for groups II to IV respectively, whereas antifertility activity of the groups was found to be 40%, 60% and 60% in the same order. **Conclusion:** N-butanol fraction of methanolic extract of Aristolochia indica root could induce negative effects on reproductive functions in female albino rats.

KEYWORDS: Albino rats, Antifertility, Aristolochia indica root, N-butanol.

INTRODUCTION

There is an increasing trend in the use of medicinal plants, botanicals or herbal preparations particularly in developing countries where these products are readily available. Several animal studies have revealed anti zygotic, blastocytotoxic, anti-implantation and abortifacient properties of water and organic solvent extracts of many commonly used medicinal plants, sometimes in dose dependent manner. In an investigation of antifertility property of a triterpenoid glycoside isolated from Dalbergia saxatilis in female rats, a decrease in maternal body weights and inhibition of conception were observed.[1] Similar observations on antifertility, antiimplantation or pregnancy interceptory properties suggestive of anovulatory, antiprogesterogenic or estrogenic effects have been made on extracts of Calotropis gigantean.[2] However, other animal studies showed none inhibitory effects of plant extracts on female reproductive functions. For example, Carapa guianensis seed oil administered orally during the period of organogenesis failed to impair implantation and induce the death of foetuses.[3] Sensitivity of experimental animals, dose of extract used, period and route of administration as well as physiological or pharmacological mechanisms are some of the factors affecting the implantation process.

Aristolochia indica a shrub or perennial herb, prostrate or twining belongs to the family Aristolochiaceae and well documented in Ayurveda and Unani system of medicine

to treat different ailments,[4,5] Vernacular Names Sanskrit: Ahigandha, Arkamula, Ishvara, Nakuli, Sunanda. Hindi: Isharmul English: Indian Birthwort Bengali: Isarmul Gujarati: Arkmula, Ruhimula Telugu: Dulagovela, Eswaramulli, Ettakalabanda, Govila, Isvara Tamil: Adagam, Isadesatti, Isura, Isuraver, Karudakkodi, Perumarindu Tulu : Isaraberu Malayalam: Eswaramullu, Garalavegam, Iswaramuli, Perumarunna, The plant has been used in skin diseases where there is morbidity of vata, pittaand kapha. It is used as an appetizer, aphrodisiac and anthelmintic. The fresh juice of the leaves is a popular antidote to snake poison. The leaves and bark are used in bowel complaints of children, diarrhea and in intermittent fevers.In traditional medicine, the roots of the plant are rubbed with honey and given totreat leprosy; and macerated with black pepper, it is prescribed in diarrhoea. Several phytoconstituents like aristolochic acid, ceryl alcohol, β-sitosterol, stigmast-4-en-3-one, friedelin, cycloeucalenol and rutin have been isolated from different parts of the plant. It has been recommended for the treatment of dry cough, joints pain, inflammation, biliousness, dysphoea of children, snake bite and also used as abortifacient. Most importantly, the studies have shown that the plant exhibited significant antimicrobial activity. This probably explains the use of this plant by the indigenous people against a number of infections. It has significantly decreased the fertilizing capacity in experimental animals.[6]

The aim of the present study was to evaluate the effect of n-butanol fraction of methanolic extract of Aristolochia indica root on fertility of female Wistar rats.

MATERIALS AND METHODS

Plant material

Aristolochia indica is a perennial climber shrub root was collected within the premises of Kakatiya University, Warangal and authenticated by Mr. P. Srinivas department of botany Kakatiya University, Voucher specimen was deposited.

Extract fraction

The n- butanol fractions were collected from column chromatography method by mehanolic extract of Aristolochia indica root.

Animals

Twenty white albino rats of weighing 150 g to 190 g were obtained from the mahavir enterprises, hjyderabad. The animals were kept in polypropylene cages under room temperature, with 12-hour light and 12-hour dark cycle and were allowed to acclimatize for two weeks. And clean water ad libitum. Protocols for this experiment was in accordance with the guidelines on the care and well-being of research animals.[7]

The rats were paired overnight with sexually active males in the ratio of 2:1. Successful mating was confirmed by the presence of vaginal plug and or sperm cells in the vaginal smear the following morning between 9.00 and 10.00 hours. The day sperm cells were found in the vaginal smear was considered as day 1 of pregnancy. Thereafter, the female rats were randomly divided into four groups of five rats each thus: Group 1 received by gavage distilled water (1ml/ 100g) daily for 19 days and served as control. Groups II–IV rats were given the n-butanol fraction of methanolic extract of Aristolochia indica root at doses of 100, 200 and 400 mg/kg body weight daily for 19 days respectively. The rats were weighed daily and observed for any untoward effects.

On day 20 of gestation, each rat was laparatomised under high ether anaesthesia. The uterine horns were exteriorized and incised at the greater curvature of the horns. The latter were examined for sites of implantation and resorption. Number of corpora lutea of pregnancy, number of live foetuses as well as weights of the foetuses and placentae were also determined. The postcoitum fertility index was evaluated using the following parameters according to the methods.[8]

1. Percentage of pregnant female animals in each group (PPF).
2. Mean live foetal number per pregnant female (LFN).
3. Mean day 20 foetal crown-rump length (FCRL).
4. Mean corpus luteum number per pregnant female (CLN).

The fertility index (FI) of each group was calculated as

$$FI=LFN\times FCRL\times PPFCLN$$

The anti-implantation and anti-fertility activities of the extract were calculated as follows.[9]

Anti-implantation activity= number of implants in control minus number of implants in test group divided by number of implants in control group multiplied by 100. Anti-fertility activity= number of rats showing no implantation divided by total number of animals multiplied by 100.

Statistical analysis

Statistical evaluation of data was done using one–way analysis of variance (ANOVA). Means found to be significantly different at $p<0.05$ were separated using Duncan multiple range test. The results were expressed as mean± S.E.M. using Graph Pad Prism Version 3.0 for Windows (Graph Pad Software, San Diego, California).

RESULTS

The linear increases in maternal weights and weights gained (table.I) were higher in the control compared with the treatment groups. Oral administration of the extract from days 1 to 19 of gestation showed decrease ($p< 0.05$) in the number of corpora lutea of pregnancy and number of live foetuses (table.II).

Table I: Effect of n-butanol fraction of methanolic extract of Aristolochia indica root on maternal and foetal weights. Values are expressed as means± S.E.M.; N= 5.

Parameters	Control	100 mg/kg	200 mg/kg	400 mg/kg
Maternal weights (g)				
Day 1	150.40± 0.90	161.20±0.16	158.10±0.84	151.00±1.01
Day 7	172.33±1.21^{a}	169.12±0.98^{b}	159.90±1.77^{b}	154.13±1.12^{b}
Day 14	190.10±1.42^{a}	174.10±1.11^{ab}	161.01±1.14^{b}	156.15±1.16^{b}
Day 19	208.15±1.18^{a}	190.20±1.20^{ab}	169.10±1.11^{b}	164.17±1.04^{b}
Maternal weights (g)				
Day 0 – 5	10.10± 0.90^{a}	4. 00 ± 0.31^{b}	2.10 ± 0.19^{b}	4.99 ± 0.14^{ab}
Day 6 – 14	09.10± 1.12^{a}	7. 99 ± 1.10^{ab}	8.00 ± 1.10^{ab}	6.99 ± 0.16^{b}
Day 15 – 19	16.20± 0. 41^{a}	16.27 ± 1.19^{a}	11.20±1.10^{ab}	7. 00 ± 0. 89^{b}
Day 0 – 19	38.00± 1.76^{a}	36.01±1.11^{ab}	23.05±13.01^{b}	16.10± 1.01^{b}
Foetal weight (g)	1.99 ± 0.11^{a}	1. 91 ± 0. 20^{ab}	1. 96 ± 0. 10^{b}	1. 95 ± 0. 12^{b}
Placental weight (g)	0. 37 ± 0.12^{a}	0. 28 ± 0. 02^{ab}	0. 12 ± 0. 02^{b}	0. 18 ± 0. 02^{b}

Table II: Effect of n-butanol fraction of methanolic extract of Aristolochia indica root on foetal score. Values are expressed as means ± S.E.M; "N" = 5.

Groups	No. of live foetuses(LFN)	No. of dead foetuses	REN	FCRL (cm)	CLN	PPL (%)	F.I.
Control	6. 99 ± 0. 51^{a}	0.00 ± 0. 00	0.00± 0.00	3.26 ± 0. 15^{a}	8. 40 ± 1. 03^{a}	100^{a}	910. 01
100 mg/kg	5. 07 ± 0.11^{b}	0.00 ± 0. 00	0.00± 0.00	3.10 ± 0. 34^{ab}	5. 80 ± 1. 02^{ab}	60^{b}	516. 02
200 mg/kg	3. 80 ± 0. 53^{b}	0.00 ± 0. 00	0.00±0. 00	2.10 ± 0. 15^{b}	5. 40 ±1. 34^{ab}	40^{c}	196. 00
400 mg/kg	2. 90 ± 0.54^{b}	0.00 ± 0.S 00	0.00 ± 0. 00	1.95 ± 0. 41^{b}	4. 80 ± 1. 39^{b}	40^{c}	161. 12

Weights of foetuses of extract treated female rats were also smaller ($p<0.05$) compared with the control. However, no gross morphological abnormalities were observed. All the females in the control group became pregnant and on laparatomy, were all found to have live foetuses. The extract did not cause any abortion or vaginal bleeding. The pregnant animals did not show signs of toxicity. All rats survived till the termination day. There were no foetal resorptions. Significant differences ($p<0.05$) in the mean foetal number per pregnant female (LFN), foetal crown-rump length (FCRL) 3. 26 ± 0. 15^{a}, 3.10 ± 0. 34^{ab}, 2. 10 ± 0. 15^{b}, 1. 95 ± 0. 41^{b} and mean corpus luteum number per pregnant female (CLN) were observed in all the treated groups relative to the control Table-II. Anti-implantation activity of the treatment groups were 49.04%, 52.13% and 58.11% for groups II to IV respectively, whereas antifertility activity of the groups was found to be 40%, 60% and 60% in the same order (Table-III).

Table III: Anti-fertility and anti-implantation activities of n-butanol fraction of methanolic extract of Aristolochia indica root.

Groups	No. pregnant/ No. tested	No. of dead rats	No. of rats showing implantations	Total No. of implantations	Anti-implantation activity (%)	Anti-fertility activity (%)
Control	5/5	0	5	29	Nil	Nil
100 mg/kg	5/3	0	3	17	49.0 4	40. 0
200 mg/kg	5/2	0	2	9	52.13	60. 0
400 mg/kg	5/2	0	2	7	58.11	60. 0

DISCUSSION

Administration of n-butanol fraction Aristolochia indica at the test doses from days 1 to 19 of pregnancy resulted in strong antiimplantation and antifertility activities (Table-III). At necropsy, no evidence of embryo resorption was found in the non-pregnant rats. Several reports indicate antifertility activity of crude extracts,[11,12] and active compounds in animal models.[12] Although there were neither deaths nor clinically

Observable, treatment-related inhibitory effects of Aristolochia indica N-Butanol fraction on the pregnant rats, changes in maternal body weights Table-I provide a good index of the integrity of maternal homeostasis.[13] In the present study, significant decreases ($p< 0.05$) in maternal body weights were observed when n-butanol fraction of methanolic extract of Aristolochia indica root.

The low body weight gain in pregnant rats given the extract might suggest nil or few number of implantations. It is well established that the implantation index correlates with the number of corpora lutea and indicates blastocyst implantation in the endometrium.[14] as well as normal reproductive capacity.[15] N-butanol fraction of methanolic extract of Aristolochia indica root. Significantly reduced the number of implantations at the

test doses Table-III. The mechanism of antiimplantation activity of Monecha ciliatum has been explained by its strong uterotonic property.[16] Similarly, Musanga cecropioides, a common Nigerian medicinal plant used for its oxytocic effect has been reported to increase uterine contraction in a dose - dependent manner.[17] In a pilot study, the n-butanol fraction of methanolic extract of Aristolochia indica root. Elicited contractile effect on the isolated uterine muscle tissue (unpublished observation). However, the mechanism of antiimplantation activity of the extract requires further investigation.

The rat endometrium is sensitive to blastocyst signals in the morning of day 5.[18] but can experience failure of blastocyst implantation due to hostile and incompetent uterine environment.[19] antizygotic or blastocytotoxic property.[20] and expulsion of embryo.[21] Previous reports on antifertility effects of medicinal plants which showed resorptions of embryos and abortions.[22] are not in agreement with the present study where we observed neither resorption sites nor gross malformations of the foetuses. N-butanol fraction of methanolic extract of Aristolochia indica root perhaps did not act as an abortifacient since there was no vaginal bleeding. However, there were reductions in foetal and placental weights of the treatment groups. The endometrial environment might not be conducive for implantation.[23] as revealed by the smaller litter size. In addition to the endometrial microenvironment, hyper motility of the myometrium can also prevent implantation, especially in view of the effect of the extract on the isolated tissue (data not shown).The reduction in foetal crown- rump length (FCRL) which is a parameter for foetal growth agrees with previous findings of impaired placental formation or placental insufficiency and foetal development.[25] A reduction in weights of foetuses of pregnant rats as observed in the present study had also been reported when Acanthus montanus leaves extract was administered to pregnant rats during gestation.[26] However, other investigators showed that most pure compounds of n-butanol fraction of methanolic extract of Aristolochia indica root were of relatively low reproductive toxicity compared with the crude seed oil.[27] Similarly, aqueous extract of Garcinia kola administered to pregnant rats did not affect the number and weights of foetuses as well as implantation sites.[28]

Previous reports indicate the roots contain aristolindiquinone, aristolide, 2-hydroxy-1-methoxy-4Hdibenzo quinolone-4,5-(6H)-dione, cephradione, aristolactam IIa, stigmastenones II and III, methylaristolate, β-sitosterol-β-D-glucoside aristolactam glycoside I, ishwarol, ishwarone, methylaristolate and aristolochene.[29,30] A new naphthoquinone Aristolindiquinone.[31] Aristolochic acids and Aristolactams was reported from A.indica. The plant contain methyl ester of 12-nonacosenoic acid,[32] besides n-heptadecane, n-triacontane, palmitic acid, hexacosanoic acid, stigmast-4-en-3-one, friedelin, cycloeucalenol and rutin . A cytotoxic lignin, savinin has been isolated from the roots of the plant. in medicinal plants with contraceptive or pregnancy interceptory effects. Fertility of rats in groups treated with the extract was significantly different ($p<0.05$) from the control as reflected by the reduced pregnancy and fertility index Table-II. A similar observation on reduced fertility was made when pregnant rats were treated with Dalbergia saxatilis.

The results suggest that n-butanol fraction of methanolic extract of Aristolochia indica root could induce inhibitory effects on reproductive functions in female albino rat.

ACKNOWLEDGEMENTS

The authors are thankful to the Managements Dr.T.Ramesh and Dr.T.Dheeraj of Sahasra institute of pharmaceutical sciences, Warangal, Telangana, India for their support.

REFERENCES

1. Uchendu CN, Kamalu TN, Asuzu IU. A preliminary evaluation of antifertility activity of a triterpenoid glycoside from Dalbergia saxatilis in female Wistar rats. Pharmacol Res, 2000; 41: 521–525.
2. Srivastava S, Keshri G, Bhargevan B, Singh C, Singh MM. Pregnancy interceptive activity of the roots of Calotropis gigantea Linn in rats. Contraception, 2007; 75: 318–322.
3. Costa-Silva JH, Lima CR, Silva EJ, Araujo AV, Fraga MC, Ribeiro E Ribeiro A, et al. Acute and sub-acute toxicity of the Carapa guianensis Aublet (Meliaceae) seed oil. J Ethnopharmacol, 2008; 116: 495–500.
4. Rastogi RP, Mehrotra BNP. Compendium of Indian medicinal plants Vol II. CDRI and National Institute of Sciences Communication, New Delhi, 2001; 660.
5. The Wealth of India: Dictionary of India Raw materials and industrial products-Raw material series, Publications and information Directorate, Council of Scientific & Industrial search, New Delhi, I: 88.
6. Hemlata Sati1, Phytochemical and Pharmacological Potential of Aristolochia indica: October – December 2011 RJPBCS. 2(4).
7. National Institute of Health (N.I.H) Guide for the care and use of laboratory animals. Bethesda; U.S.A.: DHEW Publication, Office of Science and Health Reports; 1985.
8. Tafessel G, Mekonnen Y, Makonnen E. In vivo and in vitro antifertility and antiimplantation properties of Leonotis ocymifolia in rats. Afr J Trad CAM, 2005; 2: 103–112.
9. Uchendu CN, Isek T. Antifertility activity of aqueous ethanolic leaf extract of Spondias mombin (Anacardiaceae) in rats. Afr Health Sci, 2008; 8: 163–167.
10. Yadav R, Jain GC. Antifertility effect and hormonal profile of petroleum ether extract of seeds of Cassia

fistula in female rats. International Journal of Pharmacology and Technology Research, 2009; 1: 438–444.
11. Hikim AP, Lue YH, Wang C, Reutrakul V, Sangsuwan R, Swerdloff RS. Post testicular antifertility action of triptolide in the male rat: evidence for severe impairment of cauda epididymal sperm ultra-structure. J Androl, 2000; 21: 431–437.
12. Almeida FC, Lemonica IP. The toxic effects of Coleus barbatus on the different periods of pregnancy in rats. J Ethnopharmacol, 2000; 73: 53–60.
13. Tafessel G, Mekonnen Y, Makonnen E. Antifertility effect of aqueous and ethanol extracts of the leaves and roots of Asparagus africanus in rats. Afr Health Sci., 2006; 6: 81–85.
14. Chang CV, Felicio AC, Reis JE, Guerra MdE O, Peters VM. Fetal toxicity of Solanum lycocarpum (Solanaceae) in rats. J Ethnopharmacol, 2008; 81: 205–209.
15. Uguru MO, Okwuasaba FK, Ekwenchi EE, Uguru VE. Uterotonic properties of the methanolic extract of Monechma ciliatum. J Ethnopharmacol, 1998; 62: 203–208.
16. Ayinde BA, Onwukaeme DN, Nworgu ZAM. Oxytocic effects of the water extract of Musanga cecropioides R Brown (Moraceae) stem bark. . Afr J Biotechnol, 2006; 5: 1350–1354.
17. Singh MM, Kamboj VP. Fetal resorption in rats treated with an antiestrogen in relation to luteal phase nidatory estrogen secretion. Acta Endocrinol (Copenh), 1992; 26: 444–450.
18. Emiliani S, Delbaere A, Devreker F, Englert Y. Embryo-maternal interactive factors regulating the implantation process: Implications in assisted reproduction. Reprod Biomed Online, 2005; 10: 527–540.
19. Hafez B, Hafez ESE. Reproduction in Farm Animals. 7th Ed. Lippincott; Williams and Wilkins, 2000; 3–95.
20. Orihuela PA, Rios M, Croxatto HB. Disparate effects of estradiol on egg transport and oviductal protein synthesis in mated and cyclic rats. Biol Reprod, 2001; 65: 1232–1237.
21. Vasudeva N, Sharma SK. Estrogenic and pregnancy interceptory effects of Ach yranthes aspera Linn root. Afr J Trad, Complement Altern Med, 2007; 4: 7–11.
22. Hazarika A, Sarma HN. Effects of crude root extract of Polygonum hydropiper on estrous cycle and induction of reversible sterility in female albino rat. Journal of Endocrinology and Reproduction, 2007; 11: 36–40.
23. Rasheed RA, Bashir AK, Ali BH, Padmanabhan R. Effect of Rhazya stricta on the developing rat fetus. Reprod Toxicol, 1997; 11: 191–199.
24. Schwarz A, Pinto E, Haraguchi M, de Oliveira CA, Bernardi MM, Spinosa HS. Phytochemical study of Solanum lycocarpum unripe fruit and its effects on rat gestation. Phytotherapy Research, 2007; 21: 1025–1028.
25. Nana P, Asongalem ER, Foyet HS, Folefoe GN, Dimo T, Kamtchouing P. Maternal and developmental toxicity evaluation of Acanthus montanus leaves extract administered orally to Wistar pregnant rats during organogenesis. J Ethnopharmacol, 2008; 116: 228–233.
26. Boeke SJ, Boersma MG, Alink GM, Van loon JJA, VanHuis A, Dicke M, et al. Safety evaluation of neem (Azadirchta indica) derived pesticides. J Ethnopharmacol, 2004; 94: 25–41.
27. Iranloye B, Owokunle B. Effect of Garcinia kola seed extract on female reproductive functions in rats. Int J Pharmacol, 2008; 4: 276–281.
28. Achari B, Chakrabarty S, Bandyopadhyay S, Pakrashi SC. Heterocycles, 1982; 19: 1203-1206.
29. Achari B, Chakrabarty S, Bandyopadhyay S, Pakrashi SC. Heterocycles, 1983; 20: 771-774.
30. Che CT, Cordell GA, Fong HHS, Evans CA. Tetrahedron Lett. 1983; 24: 1333-1336.
31. Mix DB, Guinaudeau H, Shamma M. J Nat Prod, 1982; 45: 657-666.
32. Mahesh VK, Bhaumik HL. Ind J Chem, 1987; 26: 86.