

Teratogenicity of *Asparagus racemosus* Willd. root, a herbal medicine

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Received 15 September 2005; revised 20 April 2006

Asparagus racemosus (AR) is a herb used as a rasayana in Ayurveda and is considered both general and female reproductive tonic. Methanolic extract of *A. racemosus* roots (ARM; 100 mg/kg/day for 60 days) showed teratological disorders in terms of increased resorption of fetuses, gross malformations e.g. swelling in legs and intrauterine growth retardation with a small placental size in Charles Foster rats. Pups born to mother exposed to ARM for full duration of gestation showed evidence of higher rate of resorption and therefore smaller litter size. The live pup showed significant decrease in body weight and length and delay of various developmental parameters when compared to respective control groups. AR therefore, should be used in pregnancy cautiously as its exposure during that period may cause damage to the offspring.

Keywords: *Asparagus racemosus*, Herbal medicines, Satavari, Teratogenicity

Roots of *Asparagus racemosus* (AR) locally known as "Satavari" have been advocated for ailments like dyspepsia¹, lactagogue, antidiarrhoeal, antiseptic, diuretic, nutritive tonic, demulcent, aphrodisiac, antispasmodic and diseases of liver and kidney etc²⁻⁴. Juice and methanolic extract of fresh roots of AR has ulcer protective effects both in rats^{5,6} and patients of duodenal ulcers⁷ and antioxidant activity in rats⁶. *Asparagus racemosus*, a rasayana⁸ akin to modern classification of adaptogens⁹, has been reported to have potent adaptogenic activity¹⁰.

Long term administration of powder or extracts of roots of AR did not show any toxicity in animals¹¹⁻¹³. Recently, with the methanolic extract of AR (ARM), we did not observe any acute toxicity in adult mice (50-2000 mg/kg) or subacute toxicity in adult rats (28 days treatment, 100-1000 mg/kg) or in young rats (35-40 g, 60 days treatment, 1000 mg/kg) in terms of mortality, changes in physiological and biochemical parameters or gross changes in the structure, hemorrhage or necrosis in tissues of ARM treated rats¹⁴. ED₅₀ of ARM could not be calculated as it did not produce any mortality in rats even up to 5 g/kg oral

dose, a dose which was 50 times of the effective ulcer protective dose of ARM (100 mg/kg)⁶.

However, pre- and post-natal studies did indicate some teratogenic effects with ARM. There are four criteria for a drug to be called as teratogen. They are (i) foetal resorption or death, (ii) stunting in size or growth retardation, (iii) malformations - gross or microscopic, and (iv) functional disorder or behavioral changes¹⁵. The present study has been undertaken to evaluate the teratogenic evaluation of ARM in animal model (rat).

Materials and Methods

Animals — Charles-Foster (CF) strain albino rats of either sex weighing between 35-40 g were obtained from the Central animal house of the Institute. Male and female rats were kept separately in the departmental animal house at 26° ± 2°C, 44-56% RH and 10:14 hr L:D cycle. Animals were provided with standard rodent pellet diet (Pashu Ahar Kendra, Varanasi). 'Principles of laboratory animal care' (NIH publication no. 82-23, revised 1985) guidelines were followed. Approval of Institutional Ethical Committee was sought prior to the commencement of the experimental work.

Extraction of *Asparagus racemosus* — The fresh roots of *A. racemosus* (1 kg) were extracted with

methanol (1 L) by cold percolation method for 5 days. The extracts were filtered, vacuum dried and stored in a refrigerator until further use. The yield of methanolic extract of roots of *A. racemosus* (ARM) was 5.6%.

Phytochemical screening of ARM — The methanolic extract of *A. racemosus* (ARM) was taken for column chromatography over silica gel CC and was eluted with ethyl acetate, chloroform and methanol¹⁶. The TLC analysis (Silica gel G with 13% calcium sulphate as binder) of eluates obtained from ethyl acetate, chloroform and methanol solvents shows positive colour reaction to ferric chloride and benzidine sodium metaperiodate reagents, indicating the presence of flavonoids and glycosides.

Drug treatment — The test group was treated with ARM in dose of 1000 mg/kg once a day orally for 60 days. The animals were then mixed in ratio of 1 male and 3 female for mating in both the test and control groups and treatment was continued till confirmation of pregnancy. Vaginal smear of each female rat was examined daily for the presence of sperms in the morning between 0900 – 1000 hrs and day one of pregnancy of each female rat was thus found. Treatment were then continued in pregnant female rats either up to 18 days in case of pre-natal study or continued till term (21-22 days) for post-natal study. Control group received 1% carboxy methylcellulose (CMC) in distilled water for oral administration.

Pre-natal study (intrauterine development of fetuses) — The female rats were anaesthetized for 18 days of gestation with ether in all the groups. The fetuses in each litter were taken out and studied for number of fetuses per dame, fetal resorption, abnormality in touch reflex, crown rump (C-R), card and tail lengths. Total weight (fetal + placental), fetal weight and placental weight were measured in each fetus. Attachment of cord and its position on placenta was also noted.

Post-natal study — The treatment was continued in females till delivery for post-natal study. The number of rat pups was noted. The development of rat pups was further noted up to 28 days of their post-natal life for various land mark. The number of rat pups per delivery, any abnormalities in their eyes, ears, mouth, limbs or any other defect was noted at the time of delivery. They were also observed for various other developmental parameters like body weight, body, arm, tail and leg length from day 1 to day 28 of their life. The days of ear opening, appearance of hair and descent of testis were also noted.

Statistical analysis — Statistical analysis for comparing before and after treatment was done by using unpaired Student's *t* test.

Results

Pre-natal study (intrauterine development of fetuses) — Methanolic extract of *Asparagus racemosus* roots (ARM) treated group caused an increase in resorption of fetuses, swelling of legs and slow growth of foetal body and placental parts (Table 1). ARM group showed central cord attachment in all the fetuses studied.

Post-natal study — ARM treated group showed decrease number of pups per litter and increased mortality of pups and delayed the various developmental parameters when compared to control group (Table 2). ARM showed significant decrease in body parts development as mentioned above compared with control group (Tables 3 and 4).

Hence, ARM seems to have teratogenic effects in rats.

Table 1— Prenatal (intrauterine) development of rat fetuses at 18 days of gestation

[Values are mean \pm SE from 8 animals in each group]

Observation	Control	ARM
C-R length (cm)	3.57 \pm 0.05	2.35 \pm 0.05 ^b
Tail length (cm)	1.28 \pm 0.04	0.70 \pm 0.07 ^b
Total length (cm)	4.85 \pm 0.06	3.05 \pm 0.09 ^b
Cord length (cm)	2.61 \pm 0.05	2.0 \pm 0.04 ^b
Placental weight (g)	0.62 \pm 0.02	0.53 \pm 0.02 ^a

P values: ^a <0.01, ^b < 0.001 as compared with respective control group

Table 2—Postnatal development of rat pups at 28 days of their life

[Values are mean \pm SE of pups of 4 dames]

Observation	Control	ARM
Pups per litter (No. of dames)	38 (n=4)	21 (n=4)
Pups per litter	9.37 \pm 1.61	5.30 \pm 1.50 ^a
Number of pups died	Nil	8
Appearance of hair	7-8 th day	8-10 th day
Congenital abnormality	Nil	Weak
Opening of eyes	14-16 th day	15-16 th day
Opening of ears (pinna)	14-15 th day	15-17 th day
Testis descent (in males)	18-21 st day	19-23 rd day

P value: ^a < 0.001 as compared with control group

Table 3 — Postnatal development (body weight and length) of rat pups up to 28 days of their life in control and ARM-treated group
[Values are mean \pm SE of 18 pups in each group]

Treatment (mg/kg, x days)	Body weight (g/rat)			Body length (cm/rat)		
	1 st day	14 th day	28 th day	1 st day	14 th day	28 th day
Control (1% CMC)	5.11 \pm 0.35	14.33 \pm 0.93	39.43 \pm 2.81	5.13 \pm 0.03	7.84 \pm 0.02	10.21 \pm 0.02
ARM 1000	3.71 \pm 0.52 ^a	8.93 \pm 0.82 ^b	18.69 \pm 1.72 ^b	3.94 \pm 0.05 ^b	6.67 \pm 0.04 ^b	8.08 \pm 0.02 ^b

P values: ^a < 0.05, ^b < 0.001 compared with respective control group

Table 4—Postnatal development (tail, leg and arm length) of rat pups up to 28 days of their life in control and ARM treated groups
[Values are mean \pm SE of 18 pups in each group]

Treatment (mg/kg, x days)	Tail length (cm/rat)			Leg length (cm/rat)			Arm length (cm/rat)		
	1 st day	14 th day	28 th day	1 st day	14 th day	28 th day	1 st day	14 th day	28 th day
Control (1%CMC)	1.81 \pm 0.04	4.13 \pm 0.2	8.33 \pm 0.3	1.31 \pm 0.05	2.34 \pm 0.05	3.15 \pm 0.07	1.13 \pm 0.02	1.57 \pm 0.02	2.13 \pm 0.04
ARM 1000	0.93 \pm 0.04 ^c	3.13 \pm 0.3 ^c	6.11 \pm 0.7 ^a	0.89 \pm 0.03 ^c	2.01 \pm 0.07 ^b	2.62 \pm 0.09 ^c	0.73 \pm 0.05 ^c	1.17 \pm 0.05 ^c	1.41 \pm 0.04 ^c

P values: ^a < 0.05, ^b < 0.01 and ^c < 0.001 compared with respective control group

Discussion

In Ayurveda, root of *A. racemosus* (AR) has been considered and described as absolutely safe for long term use, even during pregnancy and lactation^{2,3}. Systemic administration of higher doses of its extracts did not produce any abnormality in behavioral pattern of mice and rats¹⁷. LD₅₀ of the product lactare containing *A. racemosus* roots could not be assessed since it did not produce mortality even up to the oral dosage of 64 g/kg¹⁸. No adverse reactions have been reported in the doses of root powder used clinically¹⁹. *Asparagus* genus like, *Asparagus adscendens* seeds had shown to have an abortifacient effect in 28% of rats tested and in instances where the pregnancy proceeded, marked malformations were seen in neonates. *Asparagus* root extract has been reported to inhibit fetal implantation in animals and significantly change the weight and length of the fetuses²⁰.

ARM administration manifested as delay of various pre- and post-natal developmental disorders. Prenatal study satisfies first 3 criteria of teratogenicity i.e. fetal resorption and stunting in size, malformations manifested only on gross examination. Post-natal study confirmed the fourth criterion i.e. functional or behavioral disorders¹⁵. ARM though safe in young or adult rats, had produced teratological effect in pre-natal and post-natal study.

AR has been reported to contain major chemical constituents like steroidal glycosides, shatavarin 1-4

(refs 22,23), racemosol¹⁶, terpenoids and saponins²⁴ and isoflavones²⁵. We also observed the presence of flavonoids and glycosides in ARM. However, It is difficult at this stage to pin point any one of these constituents to produce teratogenicity.

The German Commission E and the American Herbal product Association list no contraindication to the use of *Asparagus* during pregnancy and lactation in humans²⁴. However in our country where Ayurvedic medicines are preferred over allopathic modern medicines even during pregnancy, drug safety profile of ARM must be explored in detail. Finally to conclude, ARM should be given with caution during pregnancy.

Acknowledgement

The author (RKG) is thankful to University Grants Commission, New Delhi for grant-in aid.

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