



Preliminary antifertility screening of two Folklore based medicinal plants used as antifertility and contraceptive agents in Eastern Himalaya

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Publication History

Received: 27 April 2015

Accepted: 04 June 2015

Published: 1 July 2015

Citation

Bora D, Babulal NM, Bora M, Nath SC. Preliminary antifertility screening of two Folklore based medicinal plants used as antifertility and contraceptive agents in Eastern Himalaya. *Discovery*, 2015, 33(146), 2-4

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General Note



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ABSTRACT

Based on the extensive field surveys conducted in various parts of North East India during 2008 to 2014, numerous folklore based medicinal plants were documented and among these only two folk-medico claims used for contraception is selected for preliminary screening of antifertility activity as reports of their corroborative biological activity is deficient. Significant anti-implantation activities were observed in corroboration to their folk claims which enhances credibility of the folk-medico claims and encourages for further research in the line of drug development.

Key words: Folklore claim, contraceptive agent, credibility, Eastern Himalaya

1. INTRODUCTION

Ancient literature mentions the use of a number of plants/preparations for fertility regulation. Some plant based contraceptive agents have also been described in various Traditional texts including Ayurveda. Experimental screening or clinical data are, however, lacking to validate folklore claims particularly. Therefore, present communication attempts to validate two folklore based medicinal plants (parts) against their use in fertility control used by some tribes of Eastern Himalaya.

Eastern Himalayan region extends from hills of Sikkim to Arunachal Pradesh in North east India. The region is full of medicinal plant resources and more than 150 indigenous tribal communities uses parts of these plants in their folklore medicinal practices (Nath and Bordoloi, 1988) which is inherited orally in their generations. Roots of *Alysicarpus vaginalis* (L.) DC. (Fabaceae) are found to be used by Lepcha tribe of Sikkim in juice form as a contraceptive herbal drug to check conception and roots of *Momordica dioica* Roxb. Ex Wild (Cucurbitaceae) are used by Khamti tribe of Arunachal Pradesh in raw form with betel leaf continuously for a period of six months to check conception forever.

Alysicarpus vaginalis (L.) DC. (Fabaceae) is an annual or perennial herbaceous legume, considered to be of paleotropical origin but introduced throughout the neotropics, which is now widespread across tropical regions around the globe, both in the wild and cultivated as a cover and green manure crop, fodder and hay crop (Duke, 1981; Lewis et al., 2005; Wagner et al., 2014). It is native to India and distribution in the study area is sporadic (Kanjilal et al., 1934-40). Presence of chemical constituents in *A. vaginalis* like lupeol, beta sitosterol and stigmasterol etc. which possesses various pharmacological activities like antiinflammatory, anticancer, estrogenic, gonadotropic, hepatoprotective etc. (Pokle et al., 2012) might responsible for antifertility activity as claimed in the folk-medico use of the tribe investigated.

Momordica dioica Roxb. Ex Wild (Cucurbitaceae) is perennial, dioecious climber found throughout the region (Kanjilal et al., 1934-40). The plant is sometimes found growing wild and often cultivated for its fruits which are used as vegetable also (Sastri, 2004). Presence of phytochemical constituents like alkaloids, lectins, sitosterol, saponin glycosides, triterpenoids, saponins, long chain aliphatic hydrocarbons, tannins etc. (Ghosh et al., 1981; Ali and Srivastava, 1998) which possesses various pharmacological activities like antiinflammatory, hepatoprotective, antimicrobial, antifungal and anti-diabetic (Reddy et al., 2006; Shreedhara and Vaidya, 2006) etc. might responsible for antifertility activity. Also some other species of genus *Momordica* is known for their anti-ovulatory, anti-implantation and abortifacient activities (Koneri et al., 2006, Koneri et al., 2007) which may be correlated with the credibility of the folk claim. Even, presence of isoflavones in *M. dioica* (Kale and Laddha, 2012) may display anti-fertility, anti-ovulatory effect (Farnsworth et al., 1975).

2. MATERIALS AND METHODS

Survey and review on folk claim- Extensive surveys related to medico-ethno-botanical claims against treatment of various disease conditions have been conducted during 2008 to 2014 in different places of North East India under different programmes. For the purpose of this communication, special emphasis was given to collect folk information for the use of medicinal plants used as Antifertility and contraceptive agents for regulation of fertility among different tribes. Though the information is not easy to gather due to introvert nature of some tribe, somehow, more than a dozen species were recorded to have such uses. On review of biological activities of reported plant species all are found to have corroboration except two new reports of contraceptive use. These two species are selected for the present study.

Plant material- Roots of *Alysicarpus vaginalis* and *Momordica dioica* were collected during survey tour from East Sikkim district of Sikkim and Tezu area of Lohit district of Arunachal Pradesh respectively and sundried. The samples have been authenticated by North Eastern India Ayurveda Research Institute, Guwahati, Assam, India where voucher specimens were deposited (Acc: 2594 and Acc: 2418) in the Herbarium. Extraction was done by maceration process for 72 h using ethanol. The percentage yield was found to be 2.8% and 4.1% w/w respectively. These extracts are represented by 'AR' and 'MR' respectively in this communication for further convenience.

Animal- Wistar rats (160-200 g) of either sex were used for the study. They were housed under standard condition of temperature ($24\pm 10^{\circ}\text{C}$), relative humidity ($65\pm 10\%$), light and dark cycle in 14:10 h ratio and fed with recognized standard pellet food and water *ad libitum*. The initial body weight of each animal was recorded. The vaginal smear of the female rats was studied microscopically for estrus cycle every morning at 8.30-9.30 am. Only female rats with normal estrus cycle were selected for the anti-implantation activity evaluation. Experimental procedures were carried out in strict compliance with the CPCSEA guidelines approved by the AEC of the Institute.

Acute toxicity study- Acute toxicity study of ethanolic extract of AR and MR were carried out according to OECD guidelines. Extract at different doses up to 2000 mg/kg, p.o. was administered and experimental animals were observed for behavioral changes, any toxicity and mortality up to 48 h. There was no toxic reaction or mortality and found to be safe. Based on acute toxicity result we have selected 150 mg/kg and 300 mg/kg for anti-fertility evaluation.

Anti-implantation activity- As both the drugs have claimed to be contraceptive, anti-implantation activity was planned as per the method prescribed by Khanna and Chaudhari (1968). In the first day of starting the experiment female Wistar rats of proestrus phase were kept with male rats of proven fertility in the ratio of 2:1. The female rats were examined in the following morning for evidence

of copulation by observing vaginal smear under Microscope of 40X. Female animals were separated from the male partner which showed thick clumps of spermatozoa in vaginal smear and divided into 3 groups (n=6). Animals in the group 1 (G1 and g1), serve as control, were given vehicle only. Ethanolic extract of AR and MR at 150 mg/kg and 300 mg/kg were administered to group 2 (G2 and g2) and group 3 (G3 and g3) respectively from day 1 to day 7 of pregnancy. For the purpose, day 1 of pregnancy was considered when spermatozoa were detected in vaginal smear under microscope. All the animals were sacrificed under light ether anesthesia and laprotomy was performed to determine the number of corpora lutea on the both ovaries and number of implantation sites on the both uteri horn.

Statistical analysis- The values of the experiment are expressed as mean \pm SEM. ANOVA was performed to determine the differences between means and $p < 0.05$ considered as statistically significant.

3. RESULTS

Both doses of extract showed significant inhibition of number of implant site. Extract at the dose of 300 mg/kg was showed 49.98% and 58.00% inhibition of implants respectively in uterine horns when compared with vehicle treated group (Table 1.)

Table 1

Effect of ethanolic extracts of AR and MR on implantation sites after oral administration for 7 days in female rats

Treatment and dose (mg/kg p.o.)	Gum acacia (1 %, 1ml/kg)	Ethanolic extract of AR (150)	Ethanolic extract of AR (300)	Ethanolic extract of MR (150)	Ethanolic extract of MR (300)
Experimental groups	G1	G2	g2	G3	g3
Days of administration	1-7	1-7	1-7	1-7	1-7
Number of rats without implant on day 10	0	1	3	1	4
Number of implantation sites	8.4 \pm 0.51	5.4 \pm 0.8*	1.2 \pm 0.8**	4.8 \pm 0.5*	1.1 \pm 0.5**
Rats without implantation site (in %)	0	16.66	49.98	14.50	58.00

* $P < 0.05$, ** $P < 0.01$ compared with vehicle treated control group

4. DISCUSSION AND CONCLUSION

In the present study ethanolic extract of roots of two plants were evaluated for its anti-implantation activity. It is well known that for implantation of zygote, exact equilibrium of estrogen and progesterone is essential and any disturbance in level of these hormones causes infertility (Sukhdev et al., 2011). Loss of implantation caused by both the extracts may be due to antizygotic or blastocytotoxic activity. The present experimental findings suggest that, the ethanolic extract of AR and MR have anti-implantation actions in the uterus of the experimental animal which imply anti-fertility activity of the drugs. Further detailed study using different animal species is required to establish their anti-fertility activities and also to understand underlying cellular mechanism of action.

ACKNOWLEDGEMENT

The authors are thankful to the folk healers of North east India, particularly from Sikkim and Arunachal Pradesh who have responded to the survey conducted among them for cross validation of folk-medico claims.

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