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Anti-fertility and abortifacient potential of hydroalcoholic leaves extract of *Alstonia scholaris* in female rats: An ethnomedicine used by Papua women in New Guinea ☆

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ABSTRACT

Aim of the study: *Alstonia scholaris* Linn. (Family: Apocynaceae) commonly known as Devil's tree or Saptaparna tree, is traditionally used by the women of Papua New Guinea to induce abortion. Aim of the present study is to investigate the anti-fertility potential of hydro-alcoholic leaves extract of *Alstonia scholaris* Linn. (HAAS) in female rats.

Materials and methods: The anti-fertility activity of HAAS administered. at 250 and 500 mg/kg doses was evaluated in three animal models. In abortifacient activity, the extract was administered to female rats from 1 to 7 days of pregnancy and on 10th day, laparotomy was performed to count the number of implants. For estrogenic/anti-estrogenic activity, ovariectomized female rats were administered with the HAAS alone as well as with 17α-ethinyl estradiol (EE) (1 μ/rat/day) for 7 consecutive days. On the 8th day, all animals were sacrificed and blood serum was further processed for the estimation of biochemical parameters.

Results: The HAAS showed 41.67% and 61.91% abortifacient activities at 250 and 500 mg/kg doses respectively. In addition, HAAS also produced an irregular pattern of estrous cycle in all the treated rats. The extract also exhibited estrogenic activity as evidenced by increase in body weight, uterine weight, increased thickness and height of endometrium at higher dose when administered alone as well as along with EE as compare to control.

Conclusions: HAAS possesses anti-fertility effect due to the inhibition of implantation and estrogenic effect which in turn might be due to the presence of active phytoconstituents in the plant.

1. Introduction

The population explosion is one of the major problems of the world in present era. The increment of population hoists so many difficulties like lack of food, water, energy and raw material supply before mankind. Now scientists have started to tackle this serious problem by developing the effective contraceptives. Contraceptives are those chemical substances that inhibit either the sperm production, sperm motility in males or prevent the ovulation and fertilization in females. But synthetic contraceptives have various adverse effects like increased blood transaminase, cholesterol levels, indigestion, weight gain, headache, depression, fatigue, hyper-menorrhea, inter-menorrheal bleeding and disturb the metabolism of lipid, protein, carbohydrates, enzymes and vitamins [1].

One of the most challenging pursuits is the search for newer and more potent herbal drugs with no or little toxic effects, less expensive and completely reversible. During the latter part of this century, the practice of herbalism has become main stream throughout the world. This is due in part to the recognition of the value of traditional medical systems, particularly of Asian origin, and the identification of medicinal plants from indigenous pharmacopeias [2].

A. scholaris is an evergreen, tropical tree of Apocyanaceae family commonly known as Saptaparni or Devil's tree and native to the Indian subcontinent and Southeast Asia [3]. The plant is widely cultivated throughout India and found in sub-Himalayan tract

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from the Jumna eastward ascending to 3000 feet. It is abundantly found in Bengal and South India [4]. All parts of the plant are being utilized traditionally in treatment of various diseases like debility [5,6], arthritis [7], wounds and earache [8], asthma [9,10], leucorrhoea [11], dog bite [12], fever [13], antidote [14], abortifacient [15], thermogenic, tonic, anthelmintic, antiperiodic and febrifuge, cardiotonic, stomachic and expectorant, in cattle fever [16,17]. The diverse pharmacological observations are supposed to be due to the presence of various chemical constituents like alkaloids, flavonoids and phenolic acids [6]. Scientifically anti-fertility activity of plant has been already proved in male rats [18] but evidence justifying the role of this plant in fertility regulation of female is not yet available till date. Therefore, the present study was performed to investigate the anti-fertility effect of hydro-alcoholic leaves extract of *Alstonia scholaris* L. on female rats.

2. Material and methods

2.1. Plant collection and extraction

Alstonia scholaris Linn leaves were collected from Kurukshetra University, Kurukshetra, Haryana, India in the month of October 2012 and sample was authenticated by Dr. B. D. Vashistha, Chairman, Botany Department of Kurukshetra University, Kurukshetra where a voucher specimen (No. KUK/BOT/IPS-03) has been deposited for future references. After drying in shade for two weeks, 580 g of dried grinded leaves were extracted with hydro-alcohol (30:70) using Soxhlet at a temperature of 50 °C for 72 h. The extract was concentrated to semi-solid mass using rotary evaporator (Heidolph 4011, USA) and then lyophilized. The crude yield of the lyophilized material was approximately 27.75% w/w. The extract was then preserved in refrigerator at 4 °C.

2.2. Phytochemical screening [19]

HAAS was subjected to phytochemical screening as per reported chemical methods.

2.3. Animals

Colony-bred healthy fertile male and female rats (Wistar strain) in the weight range of 200–250 g were selected for the study. The animals were purchased from National Institute of Pharmaceutical Sciences and Education Research (NIPER), Mohali, Punjab (India) and were kept in Animal House of Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra and maintained under laboratory condition of temperature (20.0 ± 22 °C), humidity (60 ± 1%) and 12 h light/dark cycle. They were allowed free access to food and water ad libitum. The experimental protocol (IPS/AH/223) and procedure used in the study were approved by the Institutional Animal Ethical Committee (Reg. No. 562/GO/02/a/CPCSEA) of Kurukshetra University, Kurukshetra, and conformed to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment, Govt. of India, New Delhi.

2.4. Acute toxicity study of the extract

Adult wistar rats (200–250 g) were divided into six groups each containing six rats. Group I, II, III, IV and V animals were administered various doses of HAAS i.e. 125, 250, 500, 1000 and 2000 mg/kg orally. Group VI received Tween 80 only. The animals were observed for mortality or any sign of clinical abnormality for 72 h by housing them individually in polypropylene cages.

2.5. Anti-fertility study

2.5.1. Abortifacient activity

The female rats in proestrous phase were caged with males of proven fertility in the ratio of 2:1, in the evening and examined the following day for the evidence of copulation. Rats exhibiting thick clump of spermatozoa in their vaginal smear were separated and that day was designated as day 1 of pregnancy. Selected animals were divided into three groups, consisting of six rats in each group. The group I served as control and received vehicle only (Tween-80), II and III group received HAAS (250 and 500 mg/kg) respectively from day 8th to 14th. On the day 10 of pregnancy animals were laparotomies under light ether anesthesia using sterile conditions. The two horns of uteri were examined to determine the implantation sites. Thereafter the abdominal wound was sutured in layers. During the experiment animals were observed for vaginal bleeding. The animals were allowed to go full term. After delivery the pups were counted and the abortifacient activity of extract was evaluated by computing parameters like litter size and resorption index [20].

2.5.2. Estrous cycle study

Female rats (200–250 g) showing normal estrous cycle were selected and divided into three groups of six animals each. Group I served as control and received vehicle orally for thirty days. The II and III group received 250 and 500 mg/kg doses of HAAS respectively by oral route daily for same period. The vaginal smears were observed every morning in all the three groups of animals to check any variation in proestrous, estrous, metaestrous and diestrous phase [21].

2.5.3. Estrogenic/anti-estrogenic activity

Female Wistar rats (200–250 g) were bilaterally ovariectomised under light ether anesthesia and semi-sterile conditions. After one week, they were divided into six groups consisting of 6 animals each. Group I (control) was administered with vehicle (Tween-80, 5% v/v). Group II (standard) received standard drug EE (1 μ/rat/day, s.c) suspended in olive oil. Groups III and IV received only extract HAAS at doses of 250 and 500 mg/kg, respectively. Groups V and VI received HAAS at doses of 250 and 500 mg/kg along with EE (1 μ/rat/day, s.c) for 7 consecutive days. On the 8th day final body weight of all animals was measured. Then all animals were sacrificed under light anesthesia. Uterine weight, vaginal opening and cornification of all the animals were observed and blood serum was further processed for the estimation of biochemical parameters [22,23].

2.6. Statistical analyses

All data is expressed as mean ± SEM. The data of each experimental group was analysed using one-way analysis of variance (ANOVA) followed by Dunnett's test. The levels of significance were taken at $^{*}p < 0.05$ and $^{**}p < 0.01$ in comparison to control.

3. Results

3.1. Phytochemical screening

Preliminary phytochemical screening of HAAS reveals the presence of alkaloids, glycosides, flavonoids, steroids and carbohydrates (Table 1).

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