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Anti-Fertility Activity of Methanol Extract of Crude Seed *Abrus precatorius* (Jequirity)

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ABSTRACT

The dried seeds of Abrus precatorius were powdered and they were extracted with petroleum ether, chloroform, methanol and water by Soxhelation. The extracts of four different solvent were subjected to phytochemical analysis which confirms the presence of chemical constituent like alkaloids, steroid, flavones, triterpenoids, proteins, amino acids etc. Among them steroids present in A. precatorius seeds are considered to be primarily responsible for the anti-fertility activity. The anti-fertility activity was done on adult female Wistar albino rats of weight 150-180 g. The dose of 10 mg/kg and 20 mg/kg was given to the female rats by homogenizing the extract with tween 80 and was dissolved in distilled water. The dose was prepared for all four extracts and were administered orally for 7 days and mated. Among them the methanol extract of dose 20 mg/kg showed the activity. The steroidal content present in the extracts shows the contraceptives activity which is compared with the stigmasterol and beta-sitosterol. This indicates that the basic metabolites from methanol extract would serve as potential anti-fertility activity on female rats.

Keywords: Seed, Extract, Phytochemical, Anti-fertility.

INTRODUCTION

Natural products in general and medicinal plants in particular rare believed to be an important source of new chemical substances with potential therapeutic effect. Natural ways stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. Nature has provided a complete storehouse of remedies to cure all aliments of mankind. About 80% of the world population depends on herbal based alternative system of medicines (Ayurveda, Unani and siddha, Chines traditional medicine). Herbal drugs have played a vital role in curing diseases throughout history of mankind [1,2].

Plants extract as possessing secondary metabolites have important therapeutic role in treatment of many human diseases. Natural products mainly from plant kingdom offer a wide range of biologically active compounds [3]. Despite the major advances in the modern medicine, the development of new drug from natural products is still considered as important. An estimated 70,000 plants are used medicinally. Ayurveda utilizes about 2000 plants to cure different ailments. The Chines system depends on the 5757 plants listed in the encyclopedia of traditional Chines medicinal substances. Japanese and Korean systems of medicine also include large number of medicinal herbs. Indian Material Medica includes 2000 natural products of therapeutic importance, out of which 400 are of mineral, animal origin and rest are of vegetable origin. India perhaps the largest producer of medicinal herbs and is rightly called "Botanical Garden of the World". Approximately 1250 Indian medicinal plants are used in formulating therapeutic preparation, according to Ayurveda and other traditional system of medicine [1,2].

Modern medicines have provided several preventive and corrective methods of contraceptives none of them are safe and with many serious side effect. The Synthetic or Chemical based drugs can interfere with the endocrine system and produce reproductive, neurological, developmental and metabolic effect in body. These compounds may have negative effect on the synthesis, secretion, transport and activity of natural hormones. They disturb the normal hormone level either by inhibiting the production and metabolism of hormones or by blocking the hormonal action. Due to this reason, it is necessary to developpurely herbal drugs having high efficacy and that will not have any adverse effects on the reproductive system [4].

At present global attempt has been taken to search out the effect of herbal product for contraceptive purposes. The plant products are becoming more popular than the synthetic drugs. Hence, there is a need for searching suitable product from indigenous medicinal plants that could be effectively used in the place of pills [5].

Several herbs have been shown to possess anti-fertility, abortifacient effect, in additional many native practioners are adopting several locally available herbs as contraceptive and abortifacient. An *A. precatorius* seed is one such herb, which have been adopted and used by native practioners as contraceptive. Further there are reports that the phytoconstituents namely stigmasterol and β-sitosterol present in *A. precatorius* seeds possess anti-fertility effects [6]. Upon literature review, there are reports regarding its contraceptive role.

As the herbal drugs are rich in phytoconstituents, it is essential to separate out those compounds which are responsible for therapeutic effect.

Developing new methods of standardization of compounds isolated from *A. precatorius*.

Among the traditional system of medicine *A. precatorius* is one of the important herbs commonly known as Indian Liquorice belonging to family *Fabaceae*, which are used as anti-fertility agent. As birth control, one seed completely swallowed during the menstrual period and is sufficient to prevent conception for 1 year without any side effect [7].

The reviews have specified that it acts by blocking the estrus cycle at the met estrous stage. Whereas the extract significantly reduces uterine weight and affect the estrous cycle by blocking biogenesis of ovarian steroids at an intermediate.

Therefore the present study is designed to screen for anti-fertility activity and to isolate and characterize the phytoconstituents present in *A. precatorius* seeds. By seeing the outcome of the study, will not only support the native practioner claim but also may provide a lead molecule for further study. In addition the study may be useful to exploring the natural contraceptive which may help national family planning program. Hence the study is justified and needed.

MATERIALS AND METHODS

Sample collection and preparation of extract

Dried *A. precatorius* seed was collected from ayurveda store Amruthkasar, Bengaluru and authenticated by Regional Ayurveda Research Institute for Metabolic Disorders (Central Council for research in ayurveda science, ministry of AYUSH, Govt. Of India) and justified as *A. precatorius* L. Belonging to the family *Fabaceae*. The dried seed were ground into powdered. About 100 g of powdered seed were extracted with 500 ml of methanol by Soxhelation for 24 h. The extract was collected and allowed to evaporate and weighed.

Test organism

The test organism was obtained from Drugs Testing Laboratory (DTL), Bengaluru. The test organism selected was female *Wistar albino* Rats.

Phytochemical screening of the crude seed sample

Test for steroids and sterols

***Lieberman Burchard Test*:** A drop of extract is added to 2 ml chloroform in a dried test tube, to this mixture 10 drops of acetic anhydride and 2 drops concentrated sulphuric acid was added. Reddish ring at the junction of two layers were observed [3].

Salkowski Test: Chloroform solution of extract when shaken with concentrated sulphuric acid and on standing yields red colour [3].

Powder+concentrated sulphuric acid give reddish brown colour which confirms presence of steroids [8].

Preparation of basic metabolite

About 10 mg/animal and 20 mg/animal of methanol extract was used to prepare suspension for female rat, which was homogenised with tween 80 and dissolved in 1 ml of distilled water. For study purpose 4 female rats were used in one group, the dose was given for 7 days after 7 days female rats were mated with male rats in 2: 1 ratio. Procedure for preparation of dose for 4 female rats for 7 days is, weigh 0.56 g of methanol extract for 20 mg/animal (for 7 days) and homogenised with tween 80 and dissolved in 28 ml of distilled water it means 1 ml/day/animal for 7 days. Same procedure is repeated for 10 mg/animal with 0.28 g of methanol extract in 28 ml distilled water for 7 days.

Anti-fertility test

The anti-fertility test was done *In vitro* using female *W. albino* rats which was permitted by CPCSEA approval and ethical clearance of Government College of pharmacy, Bengaluru. The wistar rats were collected from Drug Testing Laboraties, Bengaluru, there were of 6-8 weeks old, weighing between 170-200 g were housed in standard well ventilated cages in the animal control room. They were allowed free access to laboratory chow and distilled water and *adlibitum.* The temperature range was between 26ºC-28ºC, relative humidity 50%-55% and animals were exposed to 12 h light and 12 h dark cycle. They were left to adapt to laboratory conditions for 2 weeks before initiation of the experiment and were re-weighed [9,10].

After 2 weeks the test was started by administering plant extract orally at dose 20 mg/animal. The duration of treatment was 7 days. After treatment female rats were allowed for mating in the 2: 1 ratio (2 female: 1 male) and visually absorbed for the anti-fertility activity. The day that sperm is detected in the vaginal smear is designated as day 1 of gestation. By day 13 of gestation, the abdominal enlargement is visible and mammary development and nipple enlargement can be observed on day 14 of gestation, in absence of these developments after treating the female rats for 7 days and allowed them for mating, it concludes that, the methanol extract of *A. precatorius* has contraceptive activity [11].

RESULTS

The preliminary qualitative phytochemical analysis of the crude seed extract reveals the presence of steroid and anti-fertility activity on female *albino* rats revealed that methanol extract (20 mg/animal) of *Abrus precatoris* has shown contraceptive activity.

DISCUSSION

The *A. precatorius* seed was extracted by using four different solvents such as petroleum ether, chloroform, methanol and water. All four extracts were used for anti-fertility activity at the dose of 10 mg and 20 mg per animal. The four extract collected were of different form. Petroleum ether extract was rich in resins (oil) which were in brown colour, chloroform and methanol extracts collected were in green solid mass after drying. Aqueous extract was in liquid form. All four extract were insoluble in water, so extracts were homogenized with the drops of tween 80 and then were dissolved in distilled water and were used as oral dosage form. Among them methanol extract with the dose 20 mg per animal showed anti-fertility activity.

The female rat estrous cycle is short, lasting four to five days. It occurs throughout the year, with no seasonal effect. The cycle length *A. precatorius* increases slightly with age and lasts about 6 to 7 days. The estrous cycles in the rats consist of four stages known as proestrous, estrous, metestrous and diestrous. Proestrous lasts approximately 12 h; estrous, 9 to 15 h; metestrus, 21 h; and diestrus (the longest phase), over 57 h [11].

Since the stages lasts for hours, it is difficult to identify them practically. Because of this, the female rats were randomly selected and made into 8 groups that are 4 female rats in a group. Group I, III, V, VII were administered with 10 mg/animal dose of petroleum ether, chloroform, methanol, water extract respectively for 7 days. Same as group II, IV, VI, VIII were administered with the 20 mg/animal dose of petroleum ether, chloroform, methanol, water extract respectively for 7 days. [12] Then after 7 days the treated female rats were mated with male rats in 2: 1 ratio (2 female: 1 male). Then they were detected visually for 14 days because at 13^{th} or 14^{th} day abdominal enlargement can be seen if gestation is there or else in absence of abdominal enlargement at 13^{th} or 14^{th} day it confirms that extract have shown the contraceptive activity [11].

CONCLUSION

Present investigation indicates that the role of seed extract of *A. precatorius* as a contraceptive or antifertility activity. Since population explosion is leading cause of poverty and pollution in developing countries. Several potential approaches for induction of infertility have been investigated over a long period. Herbal contraceptives offer alternatives for men and women who have problems with or lack access to modern contraceptives particularly living in the rural areas in developing nations with very high population like India. However, the search for an orally active, safe and effective plant preparation or its compound is needed for fertility regulation due to incomplete inhibition of fertility or side effects. Hence the work on crude seed *A. precatorius* has been confirmed for the contraceptive activity with no side effects. The results obtained from these studies portend the contraceptive effect, hence the extract can be useful in female fertility management. Hence this review may focus the researcher's attention for clinical studies which could be of great scientific contribution to the society.

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