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Anti-Oogenic Evaluation of Seed Extract of *abrus Precatorius* L. in Swiss Albino Mice

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Abstract

Ethno-botanical reports from Rajasthan reveals utilization of Abrus precatorius L. seeds to check ova formation. So to evaluate its potential ovarian histo-architectural studies were carried out on swiss albino mice treated by body weight dependent seed extract. Ovarian histological observation of test drug treated animals show follicles in different stages of atresia and deviated oestrous cycle revealing its potential as antifertility source. This effect diminishes with time and proceeding restoration of folliculogenesis can be observed after fifteen days of the drug ingestion. Hence the present study embodies Abrus to be prolonged oral fertility regulator.

Keywords: *Abrus precatorius*, anti-oogenic, folliculogenesis, graffian follicle.

Introduction

In remote forest areas of south-east Rajasthan, tribal's use various plants for fertility regulation in which *Abrus precatorius* L. is most prominent. It was interesting to note that its seed powder is used as a powerful antifertility activator and ecbolic despite of the prevailing myth of its toxicity[1,2]. As documented it is used along with other herbs twice a week initially for fifteen days and latter on reduced to only one dose per week.

The seeds of *Abrus precatorius* are laxative and its higher quantity is an acrid poison, giving rise to symptoms resembling those of cholera[3,4]. The seed powder disturbs the uterine functions and prevents conception, when taken internally by women. Oil and crystalline steroidal fraction from the seeds of *Abrus* has been reported to possess significant antifertility activity[5-7]. The leaves and roots of this plants contain glycyrrhizen the active principle constituent of liquorice. Seeds contain both water soluble -albumin and insoluble –globulin proteins. The principle poisonous constituent of the seeds is ABRIN, a toxalbumin which has latter been shown to consists of globulin and albumose proteins. The seeds contain a fat splitting enzyme haemagglutinin and urease. The seeds also contains Abraline glucoside ($C_{13}H_{14}O_7$), Abrussic acid and fatty oil (6%) [8]. Numerous data accumulated by a variety of technique suggests that the intricate female reproductive processes are sequential each depending on the completion of the preceding one [9,10]. The orderly development of follicles from a pool of primordial follicles has led to devising a vernacular terminology to explain the structural components of the ovary[11]. Such a nomenclature facilitates comparison of histological observations relating in the developmental phases of the ovary and follicular phases under control as well as test drug experimental conditions. Such parameters include: i. Shape of the granulosa cells and the number of layers surrounding the oocyte[12], and ii. Large diameter or volume of the follicle, and combination of the number of cell layers and follicle diameter[13].

When normal phase is deviated through synthetic or natural agents, biochemical moiety triggers positively or negatively resulting in fertility enhancement or inhibition. In case of *Abrus*, the active component ruptures histological frame due to which a large number of degenerative enzymes results in follicular atresia[14-16]. After revealing its importance as an antifertility agent, an attempt was made to investigate alteration in ovarian histo-architecture directed by *Abrus* for fertility regulation and control on Swiss Albino mice.

Material and Methods

Test Animal: Sexually mature adult swiss albino mice weighing 25.0 ± 5.0 gm were selected as model for the present study to investigate the effect of seed extract of *Abrus precatorius* L. They were maintained on standard rodent chow and had *ad libitum* access to clear sterilized water. They were kept in mice cages at 26^0 C ± 2^0 C, in 10 to 12 hrs day light and were acclimatized for a month in the animal house.

Experimental Design: The female swiss albino mice were divided into 10 groups with 3 mice in each group. The group I served as control i.e. no drug was administered. The control group of animals were used for the comparative study and were sacrificed along with treated groups. Ethanolic seed extract of *Abrus precatorius* (20 mg/kg body weight) was administered to the mices of other groups (II to X). Treated mice were sacrificed on different time intervals i.e. after 1, 2, 3, 4, 5, 6, 7, 10 and 15 days.

Test drug: Mature dried seeds of *Abrus* were collected from various localities of south- east Rajasthan and were authenticated through standard herbarium sheets. The seeds were powdered and mixed with 70% ethanol. The contents were soxhlet distilled and the extract was filtered for experimental protocol.

Histo-pathological Observations: Mice from control and experimental groups were sacrificed and paired ovaries were surgically dissected out under aseptic conditions, freed of excess fascia and blood clots and were washed in chilled physiological saline. Each ovary was fixed into bouins fixative for 18 hrs. After fixation, the tissues were washed in running water to remove excess fixative, dehydrated in graded EtOH series, infiltered and embedded in the paraffin wax (mp 58^0C). Serial sections (5 µm) were stained in Ehrlichs-haematoxylin and counter stained in alcoholic eosin. The sections were then cleared in xylene and mounted in DPX. Every alternate serial section was visually appraised and appropriate areas were micro-photographed at various magnifications to record the tissue and cellular pathologies.

Morphometric analysis of different developmental stages of the ovarian cells were undertaken to determine the vulnerability of the cell types in various stages of growth, cell-division and differentiations.

Results and Discussion

Varying degrees of morphological, structural and histological alterations were observed in the ovaries of sexually mature Swiss albino mice exposed to ethanolic seed extract of *Abrus precatorius*. (Plate AP-F)

Control: The ovaries were divisible into the broad cortex and the medulla. In stroma, varying stages of developing follicular elements were present. The primordial follicle consists of a control germ cell or ovum encircled by a flattened or low embodial layer of epithelium or membrane granulosa. Polyovular follicles were also recognized. The follicles showed a central cavity or antrum. Graffian follicles were visualized with both theca externa and theca interna. Interna was vascularised with blood vessels and the germ cells area consisted of cumulus oophorus or discus and corona radiata (figure 1(C)).

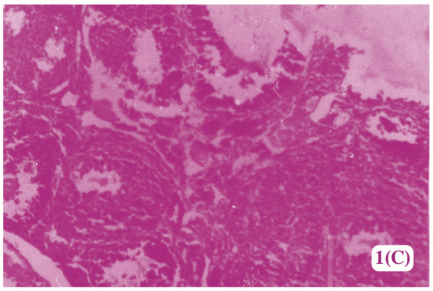

Figure-1
Stroma showing varying stages of developing follicular elements revealing normal oogenesis

After one and two days of treatment: A major shift in histo-architecture was observed in large number of follicles. Folliculogenesis was suspended and no evidence of post partum graffian follicle or ovulation was observed but there was no significant change in vascular theca interna and theca externa (figure 2 and 3).

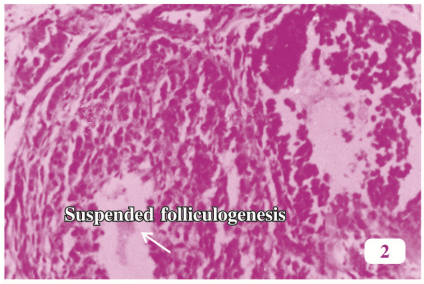

Figure-2
Alteration in ovarian epithelium after 1 day of treatment

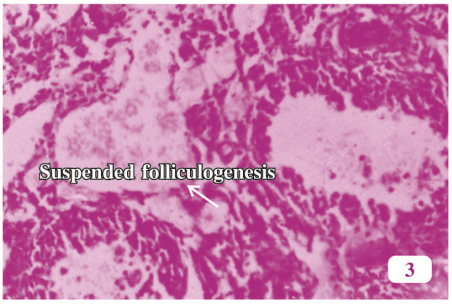

Figure-3
Alteration in ovarian epithelium after 2 day of treatment

After three, four and five days of treatment: The prominent effects observed after 24 and 48 hrs also persisted after three, four and five days of treatment and major histological deviation were observed in theca interna and theca externa. After 3rd day, theca interna was totally ruptured and only traces were observed whereas on 4th and 5th day, theca interna and theca externa both showed degeneration. Upto this stage no mature or developing follicle was observed (figure 4, 5 and 6).

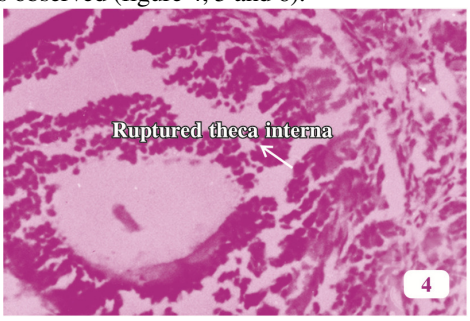

Figure-4
Alteration in ovarian epithelium after 3 day of treatment

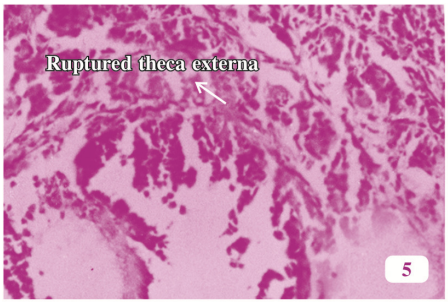

Figure-5
Alteration in ovarian epithelium after 4 day of treatment

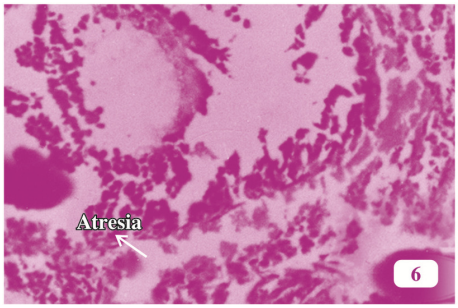

Figure-6
Alteration in ovarian epithelium after 5 day of treatment

After six and seven days of treatment: After 6th day no folliculogenesis was observed and atretic condition prevailed, but after 7th day a slight regeneration towards folliculogenesis was visualized , as some of the parts of ovary showed a traces of primary follicles, while major moiety was in degenerative phase (figure 7 and 8).

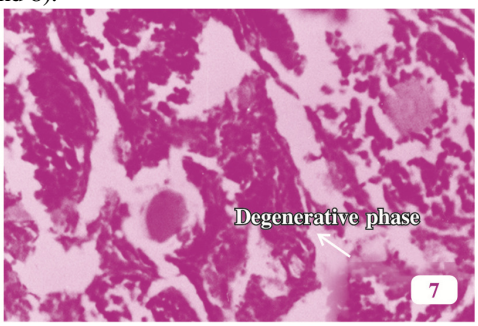

Figure-7
Alteration in ovarian epithelium after 6 day of treatment

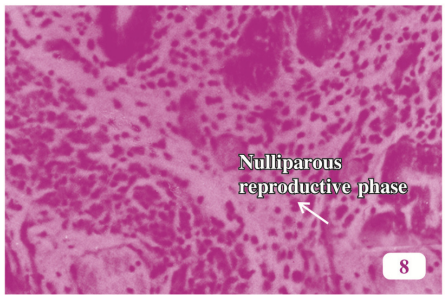

Figure-8
Alteration in ovarian epithelium after 7 day of treatment

After ten and fifteen days of treatment: Active folliculogenesis appeared after ten and fifteen days of treatment, showing the neutral responses toward treatment. Nulliparous reproductive state was characterized by normal pre-antral and atretic follicles. Various phases of follicular development was attained but theca interna and theca externa showed no significant change from that of the control one (figure 9 and 10).

Discussion: Female mice are mono-oestrous animals and during their breeding season, if they are kept separated from males for about one month, they automatically acquire their normal oestrous cycle. This phase was confirmed by the examination of the vaginal smeas. The administration of the experimental dose disturbs the normal cycle and degenerative follicular phases were observed. The present study embodies the results of a time-mediated approach to assess the effects of seed extract of *Abrus precatorius* L. on germ cells and their metabolic status. Ovarian histoarchitecture of test drug treated mices showed significant differences with respect to different time periods over control group. Follicular atresia was one of the prominent feature during folliculogenesis of sexually active females. The stages in atresia comprised desquammation of granulosa cells, fragmentation of the nuclei, disruption of cell membrane and formation of resultant cell debris. Hemorrhagic spots were also seen in some of the follicles of these ovaries. Concornitant degenerative perturbations also occurred in the ovum nucleus and its constituents. Effect was also pronounced on theca interna and theca externa. The active principle might had interfered with the cyclic rythm directly by altering the metabolic pool of the ovaries or by altering the plasma moeityof gonadotropins indirectly by knocking the release factors from pituitary glands. Hemorrhagic spots in the ovaries and disintegrating follicles reveal that it is a very potential drug but the anti-oogenic effect can be amplified only if the time mediated dose is administered.

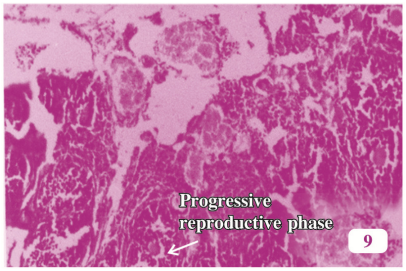

Figure-9
Alteration in ovarian epithelium after 10 day of treatment

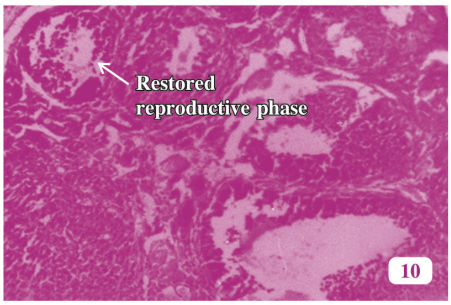

Figure-10
Alteration in ovarian epithelium after 15 day of treatment

Conclusion

Abrus seeds can serve as potential drug for a prolonged time mediated atretic contraceptive. As its fatal character cannot be overlooked so its dosage has to be managed with respect to age, ovarian milieu and body weight. Further with add-on of these additive parameters *Abrus* can be recommended for the novel anti-oogenic drug.

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