Histological Changes in Ovaries of Mice Exposed to *Butea monosperma*: Preliminary Study


**SUMMARY:** In Ayurvedic practice *Butea monosperma* (Palash) is in clinical use for hundreds of years as a contraceptive. Seeds of *Butea monosperma* are also used as an anthelmitic (Ansani *et al.*, 1979) and antimicrobial (Avirutnant & Pongpan, 1983). *Butea monosperma* (Fabaceae family) locally known as Palash (Dhak) if given for 3 consecutive days acts as an antifertility agent for which it has been traditionally used since time immemorial. The objective of the present study was to search the effect of *Butea monosperma* seeds on the ovary of mice. Observations in the present study were massive degeneration of ova in almost all the follicles, irrespective of the stage of their development. The ova from treated animals showed different stages of necrotic process. Moreover, the arrangement of follicular cells was also disturbed. The Palash seeds in the form of powder when administered orally with distilled water, according to the body weight i.e. 2g/Kg, of female mice, for three consecutive days showed notable changes in ovaries. The animals were sacrificed on day next to the last day of treatment and ovaries were extirpated. Ovaries studied histologically after Haematoxylin & Eosin staining showed most of the follicle in immature state with undefined nucleus and nucleoli in the ovum. Others showed degenerative changes in the ovum. Follicles had lost their normal shape and arrangement and organization of granulosa cells. It was conspicuous to find that almost all follicles including graafian follicles of treated ovaries were undergoing degenerative changes simultaneously. The rate of apoptosis in the granulosa cells when studied was found increased in treated cases as compared with control. The study suggests that the disintegration of ova in the ovaries is a specific effect of *Butea monosperma* seed administration.

**KEY WORDS:** Palash; *Butea monosperma*; Apoptosis; Disintegration; Follicles.

INTRODUCTION

Various Ayurvedic preparations are known to interfere with the reproductive ability of women (Bhattacharya *et al.*, 1980; Chaundry, 1993; Takase *et al.*, 1995; Tandon *et al.*, 2003; Prasad *et al.*, 2006; Ganguly, 2007). These preparations are in clinical use in Ayurveda for thousands of years to prevent pregnancy. However, the mechanism of action of these preparations in interfering with the process of conception remains largely unclear.

The ancient literature of Ayurveda, *Yogachintamani: Mishradhikar-1*, mentions that a recipe for producing sterility in the prostitutes is by using seeds of Palash (*Butea monosperma*) taken with water in the form of a fine powder for three consecutive days following menstruation. In another ancient Ayurvedic literature designated as the Garudapuran, 198:27, it is stated that Palash when made to a paste by adding honey and then taken by the women during the menstrual period leads to sterility, preventing both menstruation and conception. In *Yogratna Samuchaya*, Anant Kumar, *Yogratna Samuchchaya – Part III Bandhya – Rogadhikar*, 187, Trivancore University, Trivandrum (1940) there is no mention of the prevention of conception by the intake of this recipe but only the blockage of the menstruation is described as one of its primary effect. In order to understand the mechanism of the antireproductive action of Palash (*Butea monosperma*) seeds on ovary, the present study was undertaken. To study the effect of oral administration of Palash (*Butea monosperma*) seeds on the ovarian histology.
Mice were used as an animal model. Hence a need was felt to study scientifically if such claims about Palash (Butea monosperma) are true, and this can be used effectively as a drug to prevent conception.

MATERIAL AND METHOD

Twenty seven Swiss white female mice weighing 18-34 g and age of about 50 days were used. These were handled with utmost human care in an animal laboratory having air conditioned room with temperature maintained at 75°F, daily dark and light cycle of 12 hours each with humidity at 40%. The mice were fed on commercially obtained diet pellets (Hindustan Lever, Mumbai, India) and tap water was provided ad libitum.

Mature seeds of Palash (Butea monosperma) were collected from a tree in the botanical garden of department of Dravyaguna, Faculty of Ayurveda. These were made dry and powdered in a grinder in the Ayurvedic Pharmacy of the Institute. This powder was then sieved to isolate a fine powder. The seed powder of Butea monosperma was administered orally in the dose of 2g/kg body weight, mixed with 0.5ml of distilled water through a cannula fitted to a syringe, for three consecutive days to adult female Swiss albino mice of reproductive age group (weighing 20-34g, nulliparous). The mice were sacrificed by overdose of ether anaesthesia on the day next to last day of treatment. In the control mice no drug was administered only vehicle (distilled water) was given orally. After opening the abdomen by midline incision, both uterine horns and ovaries were inspected and removed from mice and collected in 10% formalin after these were examined under dissecting microscope for external morphology. Ovaries were fixed, and cut in sections of 8 micron thickness and stained with Haematoxylin & Eosin and observed under a light microscope.

The ovaries were weighed after dissecting them out from the mice and wiping them dry with blotting paper. The Sartorius make (MC 210 P) balance was used for this purpose which had a minimum count of 0.0l mg. Only those ovaries which could not be dissected out in toto and damaged were discarded.

RESULTS AND DISCUSSION

Reduction in weight of ovaries. Administration of Palash (Butea monosperma) seed powder, led to drastic reduction in the weight of ovary with shrinkage in size. This reduction in weight and size was bilateral.

As shown in above Table I, ovaries collected from controlled mice, had an average weight of 10.35 ± 0.82 mg, while in treated mice it was 5.97 ± 1.11mg with a range from 4.40-7.51 & 4.6- 7.49mg on the two sides. Most of the ovarian follicles in different stages of development and also the matured graafian follicles exhibited degenerative changes with different degree of severity. The difference between two values i.e. 10.35 ± 0.82mg vs. 5.97 ± 1.11 mg (control vs. treated or experimental) which was statistically highly significant (p<0.001, t= 10.83) . This loss in weight can be ascribed to degenerative changes in treated ovaries.

<table>
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<tr>
<th>Group</th>
<th>n of mice</th>
<th>Side</th>
<th>Weight of Ovary</th>
<th>% Decrease</th>
<th>Comparison between the groups</th>
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<td>Range (mg)</td>
<td>Mean ± SD</td>
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<td>Control</td>
<td>10</td>
<td>Left</td>
<td>9.1-11.49</td>
<td>10.35 ± 0.82</td>
<td>p &lt; 0.001, t = 10.83</td>
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<td>8.9-11.51</td>
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<td>Treated</td>
<td>17</td>
<td>Left</td>
<td>4.40-7.60</td>
<td>4.60-7.40</td>
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<td>5.97± 1.11</td>
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Histological changes in the ovary of adult mice. When BM (Butea monosperma) seed powder was given orally, to adult female mice for three consecutive days and animals were sacrificed on day next to the treatment i.e. on 4th day, showed the following changes in the ovaries.

In treated ovaries, the follicles were found undergoing degenerative changes and they had lost their normal shape and arrangement of granulosa cells. Cumulus oophoricus and membrana granulosa of all graafian follicles (Figs. 2 A, B, C, D) could also not be defined. There was total absence of zona pellucida. The nucleus of the ovum was also not visible in all the serial sections of the ovum. It was a conspicuous finding that all the ovarian follicles including mature graafian follicles of the treated ovaries had undergone degenerative changes (Figs. 1 C, D & 2 A, B, C, D) simultaneously. None of the follicles could be seen with intact ovum and its normal nucleus (Figs. 2 A, B, C&D).
The granulosa cells were well arranged in Graafian follicle of control ovaries, with a demarcated area between the ovum and the granulosa cells on one hand and between granulosa cell and theca interna, theca externa and stroma of the ovary on the other hand (Fig. 1A). However, in the treated ovaries the granulosa cells had lost their typical arrangement and were randomly scattered except a few of them poorly aligned around the site of ovum (Figs. 3A, B, C).

In histological study of treated ovaries the principal observation was degeneration of ova in all the follicles simultaneously, which were in different stages of their development (Figs. 2A, B, C & 3A, B, C).

The first sign noticed during the atresia in a follicle was pyknosis and fragmentation of the inner granulosa cells (Fig. 1C). This was followed by the cloudy changes in the follicles (Valsa & Karpagaganapathy, 2002) (Fig. 3C). The resulting eosinophilic granular cell residual material passes to liquor folliculi. There follows thinning of the cumulus, freeing of the oocyte into the liquor and finally sloughing of the entire follicle leaving a vacant space (Figs. 2A, B, C & D).

In treated group ovaries, it was observed that the sizes of almost all follicles
(at any stage of development they were) appeared smaller or even degenerated nucleus and nucleoli were difficult to define (Figs. 2A, B, C) with rare exception (Fig. 2D). Nucleus and nucleolus could be seen in certain treated ova (Fig. 1B & 2D) and the general pattern of follicular granulosa cells appeared in well arranged follicle but the ovum showed shrinkage in size, getting detached from periphery. Cell wall of the ovum showed folding and zona pellucida present only on undetached side. The granulosa cells showed very massive apoptosis. Throwing its residue into the follicular antrum (Fig: 1B, D & 3A).

The study was suggestive that the powder of Palash (*Butea monosperma*) seeds, damages ovaries selectively. The follicles at any stage of development showed the signs of degeneration.

The ovum showed two types of degenerative changes.

1. The majority of them showed shrinkage in size and folding of the cell wall of ovum with presence of degenerating nucleus but mostly its total absence.
2. In other cases the ovum showed cloudy degeneration maintaining its size.

As reported by Takase *et al*., shrinkage of the ooplasm due to severe loss of granulosa cells by increased apoptosis, followed by detachment of ova from the periphery from granulosa cells is supposed to be because of pyknosis and fragmentation of the inner granulosa cells. The detachment is shown from the whole periphery in a circular zone but in the present case the detachment was only on one side of the ooplasm zona pellucida was at times intact on undetached side. These were followed by thinning of cumulus oophoricus, freeing of the oocyte into the liquor and finally sloughing of the ovum and entire granulosa at times giving appearance of vacant area. In the second type of change it appears that the ooplasm had undergone cloudy degeneration following agglutination because of percolation of various toxins present in Palash (*Butea monosperma*) seeds through wall of ovum. The toxins must be entering the ovum through the granulosa cells getting it poisoned and the ooplasm did not show any shrinkage. Ooplasm agglutination makes the ovum physiologically dead. This is suggestive of effect of toxins first on granulosa cells through blood supply followed by permeation to the ovum causing agglutination of ooplasm (Fig. 2).
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Fig. 3. A. Photomicrograph of a cross section of ovary from treated mice with BM seed powder orally for 3 consecutive days. The treatment appears to have made its assault when this Graafian follicle was fully matured and was near the surface of the ovary. The ooplasm and the zona pellucida are well demarcated. However the granulosa cells have started undergoing degenerative changes and increased apoptosis (Black arrow). Some of these cells have entered in the zone of follicular antrum. Periphery of these cells cannot be defined. The nucleus and nucleolus are not visible, probably due to plain of sectioning at a different level (X400).

B. Photomicrograph of a section of an ovary of mice treated, with BM seed powder for three consecutive days, four follicles at different stages of development can easily be seen. In the Graafian follicle 1 - the ovum and its nucleus can be faintly demarcated with degenerative changes in the ooplasm. The corona radiata cells and membrana granulosa layer, lining the follicular antrum have lost their natural arrangement are showing increased activity of apoptosis. Follicles marked 2, 3 and 4 also show degenerative changes. Increased apoptosis is seen in ovarian stromal cells (X400).

C. Photomicrograph of the section of treated ovary, the ovum does not show the nucleus & nucleolus and its ooplasm is not homogenous in appearance. The zona pellucida (Black arrows) is not seen as the surrounding cells have failed to secrete it. The corona radiata cells are forming a very thin layer (Red arrow). A cavity, the follicular antrum is surrounding the ovum throughout the periphery except at a small zone, the narrow cumulus oophoricus (Green arrow). The outlines of the granulosa cells cannot be well demarcated. Granulosa cells have degenerated leaving the free space (Blue arrow) (X1000).

It can be inferred that the disintegration of ova is a specific effect of the drug *Butea monosperma* Bhargava (1986) while working on *Butea monosperma* administered to adult female rats orally described butin as the active ingredient mediating the antireproductive action of the herbal preparation. In the present study also the drug was administered orally and the ovary can be described to butin as an active isolate. In another study Rajdan et al. (1969) suggested antifertility effect of Butea frondosa seeds ion female mice and rat.

The study suggests that the disintegration of ova in the ovaries is a specific effect of *Butea monosperma* seeds. These effects of the drug on the ovaries causing destruction of the follicles are being reported for the first time.
Palabras clave: Palash; Butea monosperma; Apoptosis; Desintegración; Folículos.

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