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

Cambios Histológicos en Ovarios de Ratón Expuestos a *Butea monosperma*: Estudio Preliminar

*Neelam Gupta; **Gajendra Singh; ***S. M. Singh & ****K. R. C. Reddy

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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 anthelmintic (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 (Bhattachaya *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, Travancore 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.

* PhD Scholar, Department of Anatomy, Institute of Medical Sciences Banaras Hindu University, India.

** Professor, Department of Anatomy, Director, Institute of Medical Sciences Banaras Hindu University, India.

*** Professor School of Biotechnology, Banaras Hindu University, Varanasi-221 005, U.P. India.

**** Reader, Department of Rasashatra, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221 005, U.P. India.

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 0.01 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 ± 1.11 mg 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 ± 0.82 mg 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.

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).

Table I. Showing weight of ovary in control & treated mice.

Group	n of mice	Side	Weight of Ovary		% Decrease	Comparison between the groups
			Range (mg)	Mean ± SD		
Control	10	Left	9.1-11.49	10.35 ± 0.82	42.21	p < 0.001 t = 10.83
		Right	8.9-11.51			
Treated	17	Left	4.40-7.60	5.97± 1.11		
		Right	4.60-7.40

D. Like the fig. B & C this section also show degenerative changes in the ovum. There is shrinkage and shift of ooplasm to one side (Black arrow) with associated non visibility of nucleus and nucleolus and absence of zona pellucida (Red arrow). The area surrounding the ovum shows certain cavities (Green arrows). In the stroma of the ovary many vacant areas (Blue arrows) were seen, some of these are full of fluid (Yellow arrows). The typical appearance of a follicle has been totally lost (X400.)

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 there 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, D & 3A, B, C).

The first sign noticed during the atresia in a follicle was pyknosis

A. Photomicrograph of a section of control mice ovary showing a mature Graafian follicle with ovum and its nucleus and nucleolus (Black arrow) which is surrounded by the follicular cells the corona radiata (Red Arrow). Zone of granulosa cells are well defined with a demarcating basement membrane (Green arrow). The Graafian follicle is located towards the surface of the ovary as if ready to deliver ovum. The follicle is surrounded by theca externa (Yellow arrow) and theca interna cells (Blue arrow). The follicular cells surrounding the follicular cavity the membrana granulosa (Thin Black arrow) is well defined. The ovum is attached to membrana granulosa by cumulus oophorus (Star) (X 400.)

B. The follicle from a treated ovary showing a prominent ovum with well demarcated nucleus (Red arrow) and well defined nucleolus (Thin Black arrow) the zona pellucida is present only in a limited zone (Blue arrow) and is not present in the lower part of the photograph (Green arrow). The cells surrounding the ovum can be well defined corona radiata (Thick Black arrow), however, the granulosa cells in the region of cavity appearing in its territory, show increased incidence of apoptosis (Yellow arrows). Such apoptosis is not visible in the follicular antrum, in the periphery, in Fig. 1 A (X1000).

C. In this photomicrograph of ovary collected from treated mice with BM, the region of the ovum and follicular antrum are well defined. However, the ooplasm shows marked degenerative changes without any indication of residue of nucleus or nucleolus. The changes of the section not going through nucleus cannot be ruled out. However, the entire region of corona radiata (Black arrow) and follicular antrum show massive apoptosis (Red arrows) which can not be seen in Fig. 1 A (X 400).

and fragmentation of the inner granulosa cells (Fig. 1C). This was followed by the cloudy changes in the follicles (Valsala & Karpagaganapathy, 2002) (Fig. 3C). The resulting eosinophilic granular cell residual material passes in 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

Fig. 2. All the four photomicrograph in the plate i.e. A, B, C, D have been taken from treated mice on day next to three days of the treatment by BM seeds powder. The follicles in all the sections are devoid of healthy ova. In section 'A' one of the follicle appears to be mature but the ovum, corona radiata and cumulus oophorus are absent (star). In Fig. 'C' two mature follicles can be seen but with degenerative changes in the ovum, corona radiata and cumulus oophorus (Black arrow). The follicular cavities are well developed in both the follicle but do not show any bulging stigma on the surface and there are evidence of increased apoptosis (X400.). In all the four section more or less all the follicle show devoid of nuclei except in one follicle in section 'D' (Black arrow).

(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 oophorus, 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.

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 ooplasm shows clustering of the substance with different levels of opacities. 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 oophorus (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 ascribed to butin as an active isolate. In another study Rajdan *et al.* (1969) suggested antifertility effect of *Butea frondosa* seeds on female mice and rat.

3C). This also explains effect on all the follicles simultaneously. Damage to ovum, increased at the rate of apoptosis in granulosa cells following arrival of toxins through blood streams, explains shrinkage in size of ovary. The sequences of events are very rapid and the animals become infertile immediately. The damage however, is not permanent.

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.

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RESUMEN: En la práctica Ayurvédica *Butea monosperma* (Palash) se encuentra en uso clínico durante cientos de años como método anticonceptivo. Semillas de *Butea monosperma* también se utilizan como un antiemético y antimicrobiano. *Butea monosperma* (familia Fabaceae) conocida localmente como Palash (Dhak) si se administra durante 3 días consecutivos actúa como un agente anticonceptivo que se utiliza tradicionalmente desde tiempos inmemoriales. El objetivo del presente estudio fue buscar el efecto de las semillas de *Butea monosperma* en ovarios de ratones. Se observó degeneración masiva de los óvulos en casi todos los folículos, independientemente de la fase de su desarrollo. Los óvulos de los animales tratados mostraron las diferentes etapas del proceso necrótico. Por otra parte, la disposición de las células foliculares se mostró alterada. El polvo de semillas de Palash, cuando se administra a los ratones, por vía oral en agua destilada, i.e. 2g/Kg peso corporal, durante tres días produce cambios en los ovarios. Los animales se sacrificaron al día siguiente terminado el tratamiento y fueron extirpados los ovarios. Los ovarios se estudiaron histológicamente con HE mostrándose la mayoría de los folículos en estado inmaduro, con núcleo definido y nucléolos en el óvulo. Otros mostraron cambios degenerativos en los óvulos. Los folículos habían perdido su forma normal y la disposición y organización de células de la granulosa. Se encontró que casi todos los folículos incluyendo los folículos mostraban cambios degenerativos de manera simultánea. En los casos tratados, la tasa de apoptosis en las células de la granulosa estaba aumentada, en comparación con el grupo control. El estudio sugiere que la desintegración de los óvulos en los ovarios es un efecto específico de la administración de las semillas de *Butea monosperma*.

PALABRAS CLAVE: Palash; *Butea monosperma*; Apoptosis; Desintegración; Folículos.

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Correspondence to:
Dr. Neelam Gupta
Department of Anatomy
Institute of Medical Sciences
Banaras Hindu University
Varanasi -221005, U.P.
INDIA

Email: neelam_ims1@yahoo.co.in
directorims@satyam.net.in

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