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## RESEARCH ARTICLE

### EFFECT OF *BUTEA MONOSPERMA* FLOWER EXTRACT ON MALE REPRODUCTIVE ORGAN OF ALBINO RATS - A HISTOLOGICAL STUDY

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#### ABSTRACT

Population control is a significant issue worldwide especially in developing countries like India. Population breakout has responsible for various deleterious effects on life surviving resources on the earth. Therefore fertility regulation is necessary for the conservation of life supporting resources as well as good reproductive life of both males and females. Fertility control is a significant issue of global and national public health concern. To control the menace of population explosion, many nations have enmarked various programmes of family welfare. The fertility control has become more important and urgent mainstay of all biomedical and biosocial problems facing the mankind. The need for evolving more effective means of contraception for both male and female with nil or minimum side-effect is more actually felt now and then even before. Various chemical methods of contraception are available today but these methods possess several side effects. Herbal medicinal plants have been used as safe alternatives of the chemical methods. The oral contraceptive pill for women has had significant impact on societal dynamics and socio economic benefits, while the development of male contraceptive options equivalent to female products has proven an elusive goal. The main reason for this is that while sperm production can be controlled by the administration of sex steroids, there is also a decrease in testosterone that requires "add-back" therapy. The World Health Organization suggested that practice of usage of traditional medicine for the control of fertility, instead of synthetic drugs, as cost effective management for birth control. For this World Health Organization has given great emphasis on folklore use of the anti-fertility herbs. In the recent years number of plants have been identified and evaluated for their anti-fertility activity. So, formulation of new herbal medicines has become a growing trend in modern on-going experiments which includes the use of different plant parts extracts having anti-spermatogenic activities but their exact mechanism of action is not cleared. Initiative has been taken globally to find out the efficiency of herbal products for male contraceptive.

#### INTRODUCTION

One of the important concerns today is the problem of Overpopulation. Population explosion is creating worldwide problems. The population of the world is growing faster than the supplies of food, shelter and fuel. The increase in population is becoming a comprehensive dilemma, causing much pressure on economic, social and natural assets. In the present scenario world's population has amplified at an alarming rate and is the main cause of poverty [Global food crisis looms as climate change and population growth strip fertile land, 2007] World Health Organization (WHO) noted that majority of the World's population depends on traditional medicine for primary healthcare. Population control is a significant issue worldwide especially in developing countries like India. To control the menace of population explosion, many nations have enmarked various programmes of family welfare. This has brought down the rate of population to some extent (Khanna, 1968).

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The World Health Organization suggested that practice of usage of traditional medicine for the control of fertility, instead of synthetic drugs, as cost effective management for birth control. [3] For this World Health Organization has given great emphasis on folklore use of the anti-fertility herbs. In the recent years number of plants have been identified and evaluated for their anti-fertility activity (Unny *et al.*, 2003) : So, formulation of new herbal medicines has become a growing trend in modern on-going experiments which includes the use of different plant parts extracts having anti-spermatogenic activities but their exact mechanism of action is not cleared. Initiative has been taken globally to find out the efficiency of herbal products for male contraceptive (Gupta, 2006). India is known as the "Emporium of Medicinal Plants". The country also has to its credit the well-known traditional systems of medicine like Ayurveda and Siddha. These systems of medicines derive their drugs primarily from plant origin. The World Health Organization (WHO) has also recognized the importance of the traditional systems of medicine as to achieve its aim "Health for All". India is known for its richest diversity in medicinal plants. The tribal and rural population of India is highly, dependent on the medicinal plant therapy for their health care.

Hence there is a need of raising the traditional knowledge of rural and tribal people about medicinal plant to world standard. From ancient literature it is evidence that the various parts of the plants were used in Siddha, Ayurvedic and Unani medicine for the treatment of disease of human being. In Indian Materia Medica, 2000 drugs have been extracted from 1800 plants of forest origin. The active principles found in medicinal plants are – Alkaloids, Glucosides and other complex compounds. The active ingredients are found in one or more parts of the plant in varying proportions. It may be found in root, bark, stem, leaf, fruit, flower and seeds. NathVijendra *et al.* (2010). India is known for its richest diversity in medicinal plants. The tribal and rural population of India is highly, dependent on the medicinal plant therapy for their health care. Hence there is a need of raising the traditional knowledge of rural and tribal people about medicinal plant to world standard. From ancient literature it is evidence that the various parts of the plants were used in Siddha, Ayurvedic and Unani medicine for the treatment of disease of human being.

The search for plants for male-fertility regulation is comparatively smaller as it is directed towards the inhibition of millions of sperms produced daily as against one ovum released every month in females. Attention has been given in this modern era and attempts have been made to bring out safe, effective plant preparations as ideal contraceptives for males (Zeherea *et al.*, 1998). The palash (*Butea monosperma* Lamk.Taub.) (Sharma *et al.*, 2002) belongs to Fabaceae is used for therapeutic used since ancient time. In the traditional system of medicine known as “Ayurveda”, *Butea monosperma* has been used in the treatment of a variety of ailments including liver disorders (Burlia, 2007). About 45 medicinal uses are associated with *B.monosperma* and out of these claims almost half of the numbers of claims have been reported to be associated with flowers of the plant.(Burlia and Khadeb, 2007).

It is distributed in greater parts of India, Himalaya's upto 900m and in peninsular India upto 1,200m height. *B.monosperma* is a species of *butea* native to tropical and sub-tropical parts of the Indian sub-continent and South East Asia, ranging across India, Bangladesh, Nepal, Sri Lanka, Myanmar, Thailand, Laos, Cambodia, Vietnam, Malaysia and Western Indonesia.<sup>[10]</sup> It grows through the Indian sub-continent especially in Indo-Gangetic Plains (Chopra *et al.*, 1958). In West Bengal, it is associated with spring, especially through the poems and songs of Nobel Laureate Rabindranath Tagore, who likened its bright orange flame-like flower to fire. In Shantiniketan, where Tagore lived, this flower has become an indispensable part of the celebration of spring. The plant has lent its name to the town of Palashi, famous for the historic ‘Battle of Plassey’ fought there (Davidovich *et al.*, 2008; Wagner *et al.*, 1986; Acharyya *et al.*, 2008). In the State of Jharkhand Palash is associated with the folk tradition. Many folk literary expressions describe palash as the forest fire. The beauty of dry deciduous forests of Jharkhand reaches their height when most trees have fallen their leaves and Palash is in its full bloom. Palash is also the State Flower of Jharkhand. It is one of the most beautiful tree. *B.monosperma* is extensible used in Ayurveda, Unani and Homeopathic medicine and has become a cynosure of modern medicine. The plants of this genus are well known for their colouring matters. Commonly *B.monosperma* is used as tonic, astringent, aphrodisiac and diuretics (Nadkarni, 2002).

The roots are useful in treatment of night blindness, filariasis, piles, helminthiasis, ulcers and tumors.<sup>[16]</sup> It is reported to possess antifertility, aphrodisiac and analgesic activities.<sup>[17]</sup> Flowers are useful in diarrhoea, astringent, diuretic, depurative and tonic (Bhalla *et al.*, 1999). The stem bark is useful in indigenous medicine for the treatment of dyspepsia, diarrhoea, dysentery, ulcer, sore throat and snake bite. Besides medicinal uses it is also having the economic use such as Leaves are used for making platters, cups, and bowls and beedi wrappers. [The Wealth of India –Raw Materials, 1988; The Wealth of India, A dictionary of India raw material and Industrial products, 1988; Ambasta, 1994] Leaves are also used for making Ghongdato, which protect from rains and are eaten by buffaloes and elephants. Tribal's use flowers and young fruits as vegetables. Flowers are boiled in water to obtain a dye (Patil, 2006). Orange or red dye is used for colouring garments and for making skin ointment (Agarwal, 1986) Fresh twigs are tied on horns of bullocks, on occasion of ‘pola’ and dry twigs are used to feed the sacred fire (Patil, 2006). In addition wood of the plant is mainly used for well-curbs and water scoop. It is also employed as a cheap board wood and for structural work; wood pulp is suitable for newsprint manufacturing (Ambasta, 1994; Ambasta, 1994). Bark fibers are used for making cordage (Kirtikar, 1935). Butea is also a host to the Lac insect, which produces natural lacquer (Sequeira, 1998).

## MATERIALS AND METHODS

### Preparation of Methanolic extracts of *Butea monosperma* Flowers

Fresh flowers of *B. monosperma* (Palash) were procured commercially, authenticated in the Department of Botany, University of Ranchi, India. The flowers were dried at room temperature. After drying completely fine powder was made in grinder. The powdered flowers (500g) was extracted with methane (60-80°). Methanolic extract was prepared with the help of Soxhlets apparatus and the powder was left for 20 hours in reduced pressure in rotator evaporator to obtain reddish orange powder. The extract were filtered using Whatman filter paper and fine powder was prepared after drying.

**Animals:** Adult Wistar male albino rats, approximately three months old, weighting 125-150g, were used in this investigation. The animals were maintained in individual polypropylene cages with a 12:12 h light: dark schedule. The temperature in the animal house during the study period was maintained at  $23 \pm 2^\circ\text{C}$ , and the relative humidity ranged between 32 – 70%. The feeding schedule consisted of two rat pellet meals per day, and water was provided *ad libitum*. Daily intake of food and water were quantified precisely. The animals were maintained under veterinary supervision in accordance with the Guidelines for Care and Use of Animals in Scientific Research (INSA). The experimental protocol has the approval of the Institutional Animal Ethical Committee (IAEC).

**Experimental Design:** Group of five (5) animals were randomly divided into three groups. One controlled group and two different treated groups with high and low dose.

**Group I:** - Control group received water and food orally.

**Group II:** - treated with 50 mg/kg body weight of *Butea monosperma* flower extract. (BMFE) (Low dose).

**Group III:** - treated with 500 mg/kg body weight extract of *Butea monosperma* flower extract. (BMFE) (High dose).

The Wister male albino rats were treated daily for 3 months.

**Parameters**

**Determination of Body and Reproductive Organ Weight:** The initial and final body weight of animal was recorded every fortnightly. Blood sample were collected by retro orbital puncture, then the testes, epididymis and seminal vesicle were dissected out, freed from adherent tissue and weighted.

**Histopathology:** For histopathological evaluation portion of testis, epididymis, and seminal vesicle were fixed in bouin's fluid, dehydrated in ethanol, cleaned in xylene and embedded in paraffin wax. Five micron thick sections were stained with haematoxylin and eosin and observed under light microscope.

**Statistical Analysis:** All the result are expressed as mean ± SEM and significance was analysed statically by students 't' test and p<0.05 was considered as significant level.

**RESULTS**

**Histopathology**

**TESTIS (Control)**

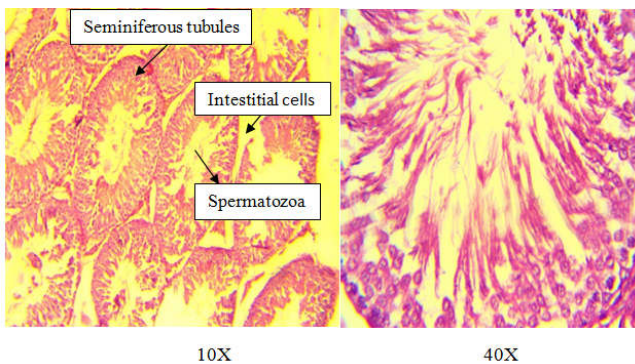


Figure 1. Testis of control animals showing well-arranged Seminiferous tubules, Interstitial cells and Spermatozoa

**TESTIS (Group II, received 50mg/kg body weight of BMFE)**

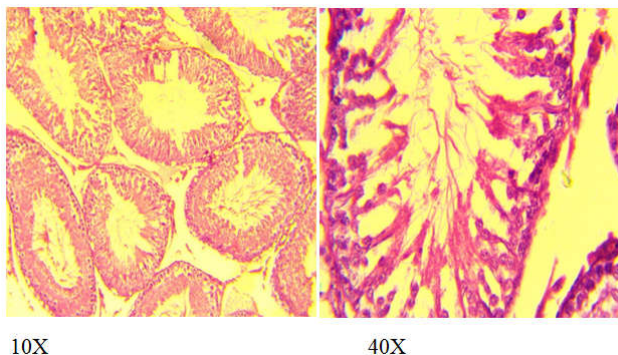


Figure 2. Here Interstitial cells are degenerating and spermatozoa are also becoming degenerated

**TESTIS (Group III, received 500mg/kg body weight of BMFE)**

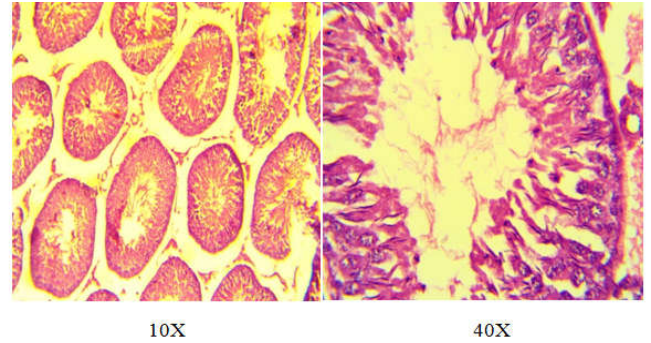


Figure 3. Here Interstitial cells are degenerating and spermatozoa are also becoming degenerated

**Epididymis (Control)**



Figure 4. Epididymis in control is normal.

**EPIDYDIMIS (Group II, received 50mg/kg body weight of BMFE)**

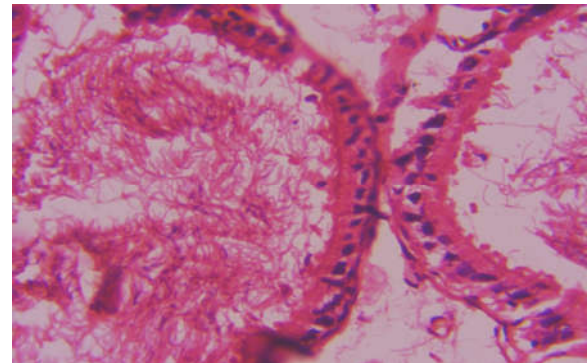


Figure 5. Epididymis showing less lumen secretion, epithelial cells degenerating and spermatozoa less

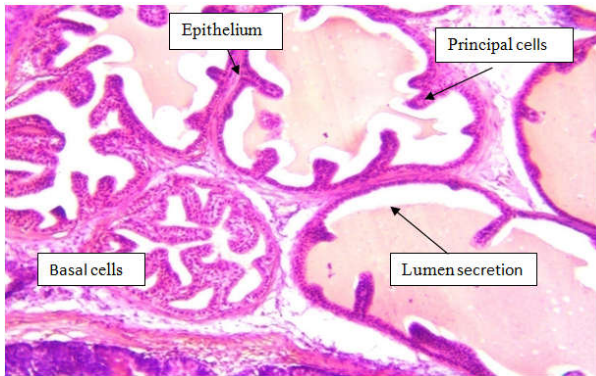
**EPIDYDIMIS (Group III, received 500mg/kg body weight of BMFE)**



Figure 6. Epididymis showing very less lumen secretion, epithelial cells degenerating and spermatozoa very less

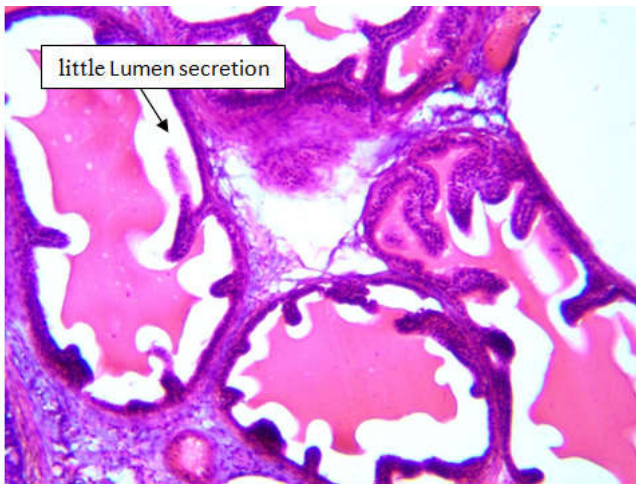
**Seminal Vesicle**

**Seminal Vesicle (Control)**



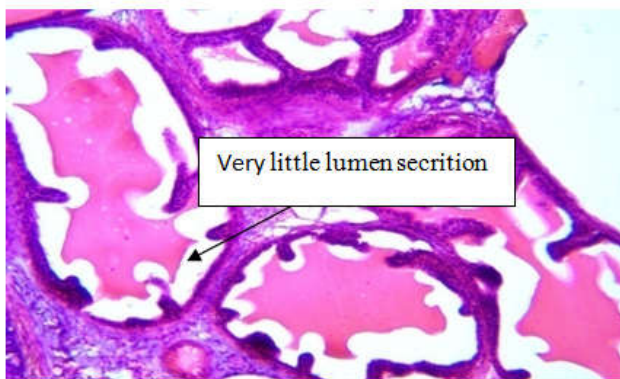
**Figure 7. Seminal Vesicle in control normal**

**Seminal Vesicle (Group II, received 50mg/kg body weight of BMFE)**



**Figure 8. Seminal Vesicle showing less lumen secretion, epithelial cells degenerating and spermatozoa less**

**Seminal Vesicle (Group III, received 500 mg/kg body weight of BMFE)**



**Figure 9. Seminal Vesicle showing very little lumen secretion, epithelial cells degenerating and spermatozoa less.**

**DISCUSSION**

**Population Explosion** is creating so many obstructions worldwide day by day.

This overpopulation can be checked through biological means with special reference to modulation in the human fertility ability. Along with the advancement in the reproduction biomedicine different hormonal contraceptive pills are developed but all have side effects. The search for plants for male-fertility regulation is comparatively smaller as it is directed towards the inhibition of millions of sperms produced daily as against one ovum released every month in females. Attention has been given in this modern era and attempts have been made to bring out safe, effective plant preparations as ideal contraceptives for males (Zeherea *et al.*, 1998) Microphotograph of control rat testis showed different stages of spermatogenesis. The lumen of seminiferous tubules was packed with spermatogenesis; inter-tubular space was filled with connective tissue and interstitial cells. The treated group showed alteration in histology: intercellular space decreased, seminiferous tubular diameters and thickness of germinal epithelium reduced drastically. Number of germ cells also decreased. Marked decline in number of sperms were clearly visible, lumen filled with cellular debris and interstitial cells were absent. Microphotograph of control rat epididymis showed that the epididymis is lined by pseudostratified columnar epithelium with stereo cilia. Large number of sperms within the lumen present in the entire length of the epididymis. Basal, principal (nuclei are at different levels), are observed with variable numbers on the epididymis. The treated group showed alteration in histology: intercellular space decreased. Number of germ cells also decreased. Marked decline in number of sperms were clearly visible, lumen filled with cellular debris and interstitial cells were absent. Less Lumen secretion and degenerating Epithelial cells was observed.

Microphotograph of control rat seminal vesical showed that the epididymis of this gland lies on the surface of interconnecting mucosal folds that extend into the lumen from the muscular wall. The sparse connective tissue within the folds constitutes the lamina propria of this mucosa. The epithelium, which may be either simple columnar or pseudostratified columnar, produces a secretion (including fructose, ascorbic acid and other components) which is expelled from the gland by contraction of the muscular wall during ejaculation, constituting about 50-80% of the semen. The treated group showed alteration in histology: intercellular space decreased. Number of germ cells also decreased. Marked decline in number of sperms were clearly visible, lumen filled with cellular debris and interstitial cells were absent. Lumen secretion less. Epithelial cells degenerating. Methanolic extract of *Butea monosperma* flower extract significantly effect the reproductive organ weight and histology of male albino rat. The negative impact of *Butea monosperma* on the male structural and functional integrity of testicular tissues was evidenced by the histopathological data, highlighting the reduction in size of seminiferous tubules. The vacuolization was observed in the sertoli cells, spermatogonia and spermatocytes. Germ cell proliferations beyond the level of the spermatocytes were affected. The lumen contained sloughed debris and few germ cells, which may be due to wide spread cellular damage and androgen deprivation.(Gupta *et.al.*,2006).<sup>[26]</sup>Reduced testicular and epididymal protein content could be correlated with absence of spermatozoa in the lumen.(Zhen *et.al.*,1995)<sup>[1]</sup> The weight reduction of the reproductive organs of treated male rats clearly indicate that the drug caused structural and functional alteration in testis, epididymis and seminal vesicle and also lowered the testosterone as these organs are androgen – dependent (Dohle,

2003). It can be concluded that, the oral administration of 50% methanolic extract of *Butea monosperma* flower to male rats has adverse effect on reproduction. The effect may have an inhibitory influence on gonadotrophin released which may be responsible for the decline in testosterone production, leading to change in spermatogenesis. *Butea monosperma* Flower Extract (BMFE) caused antispermatogenic effect evidenced by reduction in number of spermatogenic cells and spermatozoa, reduction in sperm density in epididymis which may be due to changes in the androgen metabolism. The principal cells of epididymis synthesize protein, which have important role in maturation of spermatozoa (Kasturi *et al.*, 1995). *Butea monosperma* flower extract feeding caused impairment of Leydig cell function which was evidenced by reduced Leydig cell area and nuclear dimensions and fewer number of mature Leydig Cells. Thus from the above study it may be concluded that *Butea monosperma* Flower Extract (BMFE) suppresses male fertility.

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