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Effect of *Buteamonosperma* Flower Extract in Male Reproductive Organ of Albino Rats - A Histological Study and Biochemical 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.
Buteamonosperma *popularly known as "Flame of the Forest", Dhak, Palash or "Bastard Teak" which has immense potential.****Buteamonosperma*** *is reported to have medicinal potential in various ancient literatures. It has been used as traditional medicines for centuries. Various parts of the tree are used in the Ayurvadic System of Medicine.* ***Buteamonosperma(BM)*** *has been used for medicinal purposes since ancient times.*
The present study was designed to evaluate effect of ***Buteamonosperma*** *on male reproductive organ of male albino rats by the help of* ***Buteamonosperma flower extract(BMFE)*** *at various dose levels.*
Result: *The present result suggest that administration of methanolic flower extract of* ***Buteamonosperma*** *significantly affect the reproductive organ of male albino rats, which was treated with 50 mg/kg and 500 mg/kg body weight of* ***ButeamonospermaFlower Extract (BMFE)*** *caused the impairment of testicular, epididymis and seminal vesicle structures, which led to significant decrease in spermatogenic activity in seminiferous tubules. Depletion of Leydig cells in tubular interstitial also causes reduction in serum testosterone level. The reduced testicular and accessory sex organ weight indicate a wide spread damage, which could be reduced protein contents in these organs, which ultimately effect the male fertility. Therefore, the process of maturation of spermatogenic cells and sperm production in the organ was affected by the extract administration which may lead to infertility in treated rats.*
Conclusion: *Thus from the above study it may be concluded that BMFE supresses the male fertility.*
Key Words: *Problem of Overpopulation; Herbal products; Male contraceptive*

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I. Introduction:

One of the important concerns today is the problem of Overpopulation.Population control is a significant issue worldwide especially in developing countries like India. Population breakout is 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 thishas brought down the rate of population to some extent.[1] The fertility control has become more important and urgent mainstay of all biomedical and biosocial problems facing the mankind.[2] 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.[3]

Herbal medicinal plants have been used as safe alternatives of the chemical methods. Plants have been used as alternative medicine for about 60,000 years. Nevertheless, these are frequently employed without scientific knowledge of its chemical properties, biological activities.Despite the move towards synthetic medicines and use of sophisticated drugs, traditional plant-based remedies still play an important role in the world's medicine. Adverse effects of herbal products have often been ignored apparently due to its common use

and acceptance over allopathic. In modern society, because of the undesirable adverse health effects appearing due to the use of plant-based outcomes, it has been clearly realized that "natural" is not equal to "safe".

Medicinal plants are distributed worldwide but they are most abundant in tropical countries. [4] It is estimated that about 25% of all modern medicines are directly or indirectly derived from plants. [5] According to Bodeker.et.al. [6] 65 to 80% of the world's population, living in developing countries, depends mostly on plants for health care due to poverty and lack of modern medicine. The rural communities depend on medicinal plants as a source of primary health care due to the high cost and unavailability of synthetic drugs. [7] Traditional medicines are now widely accepted due to its cultural acceptability, compatibility with the human body, effectiveness and less side effects. [8]

Contraception is important to health, development and quality of life and has allowed couples to plan their families and safely space births. Several methods of contraception for family planning has been used over the years, however, due to adverse effect associated with synthetic contraceptives, herbal plants have been investigated for their contraceptive potentials.[9-14]

The World Health Organization (WHO) suggested that practice of usage of traditional medicine for the control of fertility, instead of synthetic drugs, as cost effective management for birth control. [15] 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. [16]. 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. [17]

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"**.

In Indian MateriaMedica, 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) [18]

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. [19]

Buteamonosperma (Linn.)Kuntze is a medicinal plant, commonly known in Ayurveda as "Palasha". ***B.monosperma*** (Lam.)is commonly known as "Flame of the Forest", belongs to the family Fabaceae.[20] It is locally called palas, palasha, mutthuga, bijasneha, dhak, khakara, chichra, Bastard Teak, Bengal Kino, Nourouc and it is distributed over large area in Asia, for example, in Sri Lanka, Burma and India. In India our state Jharkhand has a great distribution of ***B.monosperma***and also found in Madhya Pradesh, Chhattisgarh, North Maharashtra etc, it does not grow in very arid parts. It grows gregariously on open grassland and scattered in mixed forest.[21] Plantations can be raised both on irrigated and dry lands. In India, palas ranks next to Kusum (schleicheratrijuga) as a host tree for lac insect. [22, 23]

Buteamonosperma (Lamk.)Taub.[24]is a medium-sized deciduous tree growing throughout India, South Asia, Indonesia, Japan, Laos, Myanmar, Nepal, Sri Lanka, Thailand and Vietnam. It is commonly found up to an altitude of 1200m except in very arid regions. [25] Generally it grows gregariously on open grassland and scattered in mixed forest. Plantation can be raised both on irrigated and dry lands.

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, Myammar, Thailand, Laos, Combodia, Vietnam, Malaysia and Western Indonesia. [26] It grows through the Indian sub-continent especially in Indo-Gangetic Plains. [27]

It grows on a wide variety of soil including shallow, gravelly sites, black cotton soil, clay loams and even saline or waterlogged soils. Seedlings thrive best on a rich loamy soil with pH 6-7 under high temperature and relative humidity. [28, 29]

In the traditional system of medicine known as "Ayurveda", ***Buteamonosperma*** has been used in the treatment of a variety of ailments including liver disorders. [28]

Buteamonosperma popularly known as "Flame of the Forest", Dhak, Palash or "Bastard Teak" which has immense potential. ***Buteamonosperma*** is reported to have medicinal potential in various ancient literatures. It has been used as traditional medicines for centuries. Various parts of the tree are used in the Ayurvadic System of Medicine. ***Buteamonosperma*****(BM)** has been used for medicinal purposes since ancient times.

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).[28]

Besides medicinal uses it is also having the economic use such as Leaves are used for making platters, cups, and bowls and beedi wrappers. [30,31,32] 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. [33] Orange or red dye is used for colouring garments and for making skin ointment [34] Fresh twigs are tied on horns of bullocks, on occasion of 'pola' and dry twigs are used to feed the sacred fire.[33] In addition wood of the plant is manly 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. [36,37] Bark fibers are used for making cordage. [38]

Butea is also a host to the Lac insect, which produces natural lacquer. [39]

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 Shantinikitan, 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. [40-41]

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 trees.

In India it is especially found in Maharashtra(Kolhapur), Karnataka (Coorg, Chikmagulur, Mysore, Shimoga, S.Kanara), Kerala(Alapuzha, Idukki, Kasaragod, Kollam, Kozhikode, Malapuram, Palakkad), Rajasthan (Jaipur, Udaipur, Kota), Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Delhi, Dadra-Nagar-Haveli, Gujarat, Haryana, Himachal Pradesh, Jammu-Kashmir, Madhya Pradesh, Meghalaya, Orissa, Punjab, Tamil Nadu, Uttar Pradesh,West Bengal and Jharkhand. It grows through the Indian sub-continent especially in Indo-Gangetic Plains. [27]

B.monospermaisextensible 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. [42]

The roots are useful in treatment of night blindness, filariasis, piles, helminthiasis, ulcers and tumors. [43] It is reported to possess antifertility, aphrodisiac and analgesic activities. [44]

Flowers are useful in diarrhoea, astringent, diuretic, depurative and tonic. [47]

The stem bark is useful in indigenous medicine for the treatment of dyspepsia, diarrhoea, dysentery, ulcer, sore throat and snake bite.

Objective:

The present study was designed to evaluate effect of ***Buteamonosperma*** on male reproductive organ of male albino rats by the help of ***Buteamonosperma flower extract*****(BMFE)** at various dose levels.

II. Materials and Methods:

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 (60-80º).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 paperand 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 ± 2ºC, 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 (Treatment Phase)
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 ***Buteamonosperma*** flower extract. (BMFE) (Low dose).
Group III: - treated with 500 mg/kg body weight extract of ***Buteamonosperma*** flower extract. (BMFE) (High dose).

The Wister male albino rats were treated daily for 3 months.World Health Organization (WHO) noted that majority of the World's population depends on traditional medicine for primary healthcare.

Euthanization:
After administration of last scheduled dose of extract, animals were autopsied under mild ether anesthesia.

Parameters
1.1 ***Determinationof 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.

1.2*Body and Sex Organ Weight:*

The initial and final body weight of animal was recorded every fortnightly. The testes, epididymis and seminal vesicle dissected out, freed from adherent tissue and blood and weighted to the nearest milligram. Organ weights were reported as relative weights .
(Organ Weight/ Body Weight x 100).

The wet weight of testis, epididymis and seminal vesicle were recorded to calculate the Tissue Somatic Index (TSI) by using the following formula:
Tissue Somatic Index (TSI) = (Tissue Weight/Total Body Weight) x 100.

2.*Tissue Biochemistry*
Biochemical marker of reproductive organ
2.1. Fructose in Seminal Vesicle
Immediately after the Seminal vesicles was removed, Fructose concentration in the seminal vesicle was estimated by the method of Foreman *et al* **[45]** and was expressed as µg/mg tissue weight
2.2. Sialic Acid in Epididymis
Immediately after the Epididymis was removed, Sialic acid in the epididymis of both control and treated animals were determined by the method of Jourdian*et al***[46]** and was expressed as µg/mg fresh tissue weight.

2.3. Cholesterol in Blood
Serum Cholesterol was measured by Cholesterol oxidase – Peroxidase (CHOD - PAP) enzymatic method, recommended by Katterman, Jaworek and Moller was used for this purpose. The kit used for this purpose was Boehringer Mannheim, West Germany with Photometer – 4010 (auto analyser) .

3.Hormone Analysis
Radioimmunoassay of Testosterone:
Blood samples were also collected for estimations of Serum Testosterone by Radioimmunoassay (RIA) (WHO Method Manual, 1987). Serum samples were separated by standard procedures and stored at -20°C for subsequent analysis.

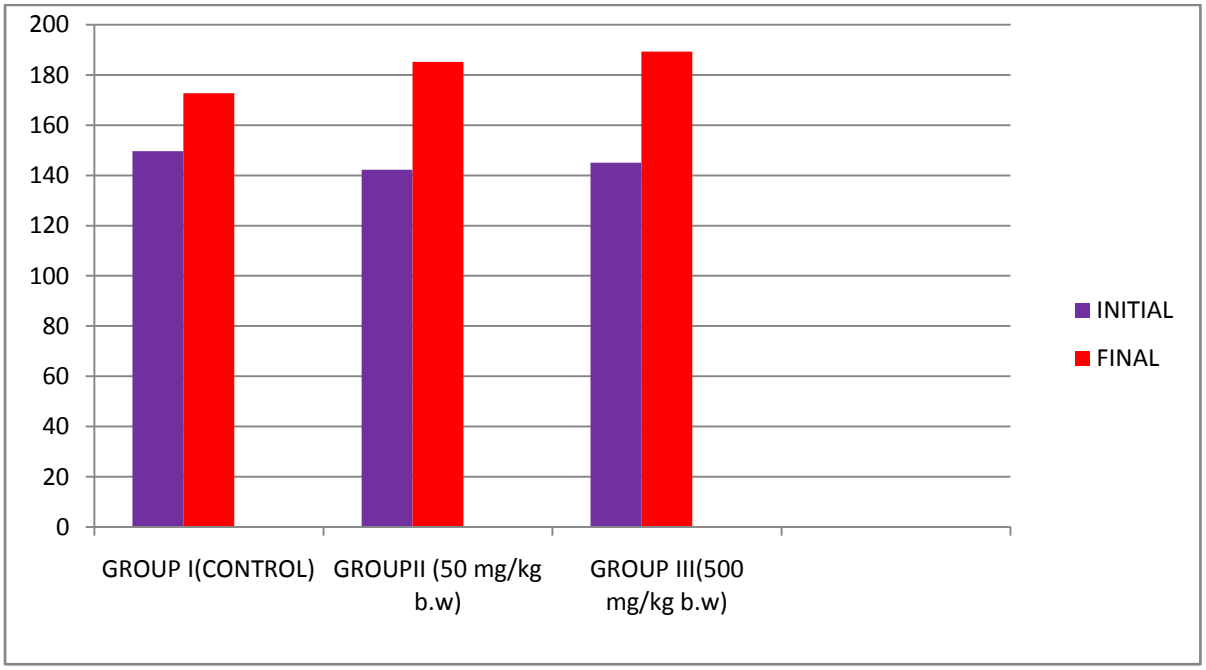
4. ***Histopathology:***
For histopathological evaluation portion of testis, epididymis, and seminal vesicle were fixed in bouin's fluid, dehydrated in ethanol, cleaned in xylene and embedded in paraffin wax.
Five micron thick sections were stained with haematoxylin and eosin and observed under light microscope.
Statistical Analysis:
All the result are expressed as mean ± SEM and significance was analysed statically
bystudents't' test and $p<0.05$ was considered as significant level.

III. Result

1.1 Body weight (in gram)

Group	Initial (g)	Final (g)
Group I (Control)	149.6±19.02	172.73±11.53
Group II (50mg/kg b.w)	142.2±26.26	185.21±9.63
Group III (500mg/kg b.w)	145±12.36	189.32±13.94

Table 1: Initial and Final body weight of the Control and Treated Animals



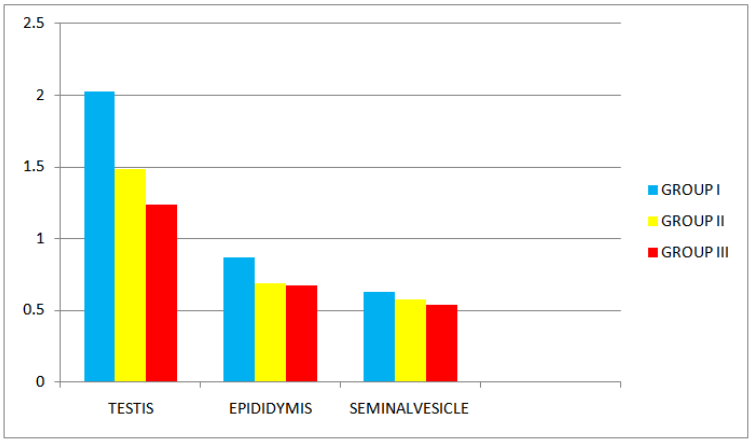
Graph 1: Group I, Group II and Group III showing Initial and Final body weight of the Control and Treated Animals

1.2 Reproductive organ weight (g /pair)

Group	Testis	Epididymis	Seminal Vesicle
Group I (Control)	2.03±0.03	0.87±0.08	0.63±0.05
Group II (50mg/kg b.w)	1.49±0.01*	0.69±0.11*	0.58±0.02
Group II (500mg/kg b.w)	1.24±0.08*	0.68±0.02*	0.54±0.03*

Table 2: Reproductive organ weight of the Testis, Epididymis and Seminal Vesicle of Control and Treated Animals

{Each value is SEM of 10 animals *P<0.05}



Graph 2: Reproductive organ weight of the Testis, Epididymis and Seminal Vesicle of Control and Treated Animals

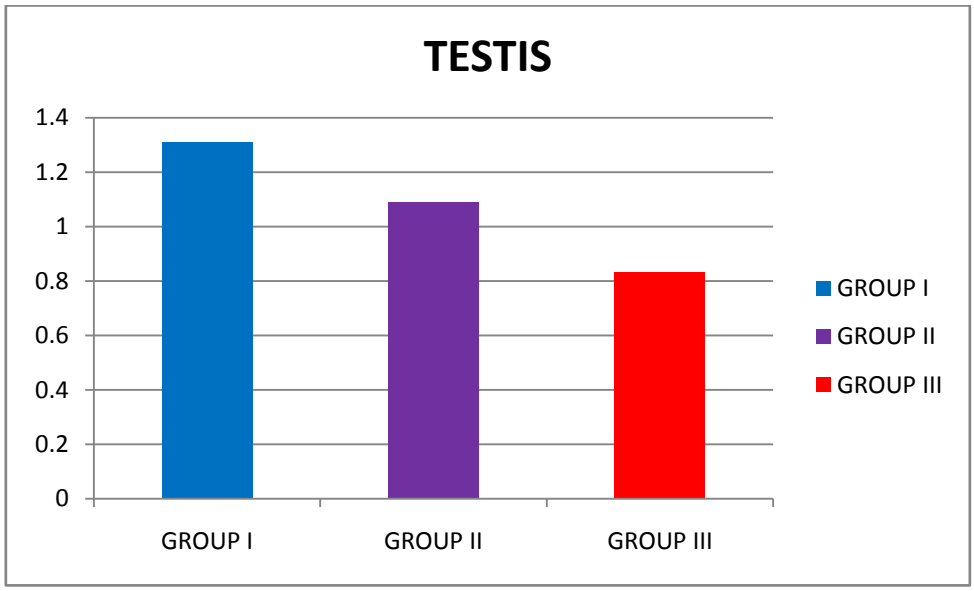
3. *Tissue –Somatic Index (TSI)*

TESTIS

Group	Testis
Group I (Control)	1.31±0.07
Group II (50mg/kg b.w)	1.09±0.24
Group II (500mg/kg b.w)	0.83±0.11

Table 3: Tissue – Somatic Index (TSI) of the Testis of Control and Treated Animals

{Each value is SEM of 10 animals}

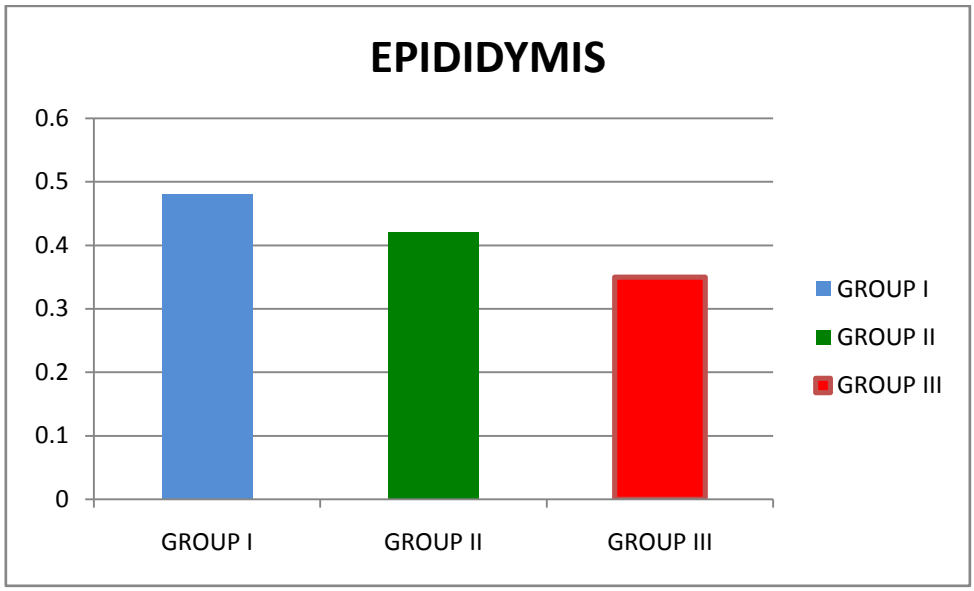


Graph 3: Tissue – Somatic Index (TSI) of the Testis of Control and Treated Animals

EPIDIDYMIS

Group	Epididymis
Group I (Control)	0.48±0.07
Group II (50mg/kg b.w)	0.42±0.07
Group II (500mg/kg b.w)	0.35±0.04*

Table 4: Tissue – Somatic Index (TSI) of the Epididymis of Control and Treated Animals

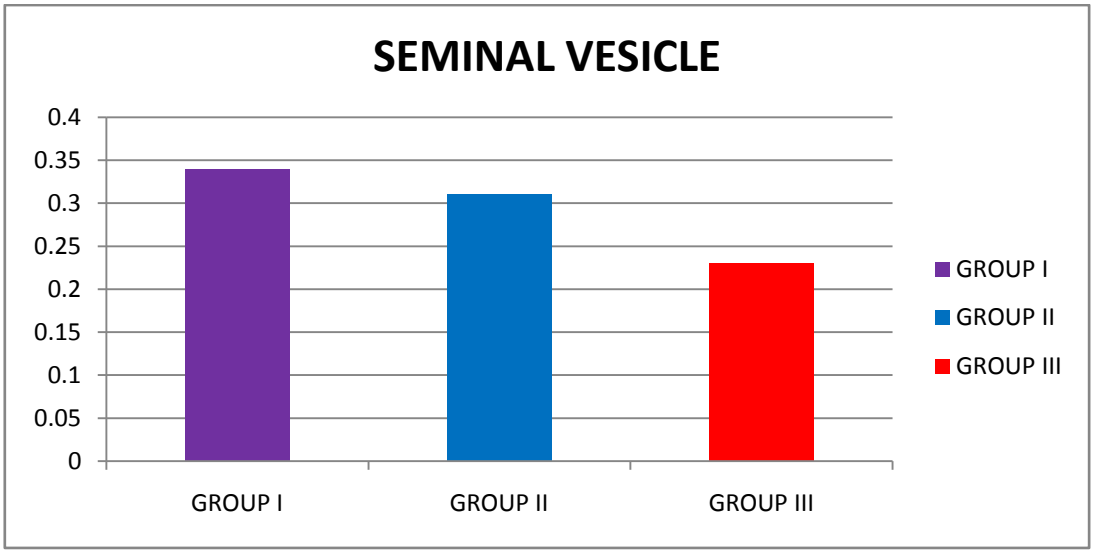


Graph 4: Tissue – Somatic Index (TSI) of the Epididymis of Control and Treated Animals

SEMINAL VESICLE

Group	Seminal Vesicle
Group I (Control)	0.34±0.06
Group II (50mg/kg b.w)	0.31±0.10
Group II (500mg/kg b.w)	0.23±0.05

Table 5: Tissue – Somatic Index (TSI) of the Seminal Vesicle of Control and Treated Animals



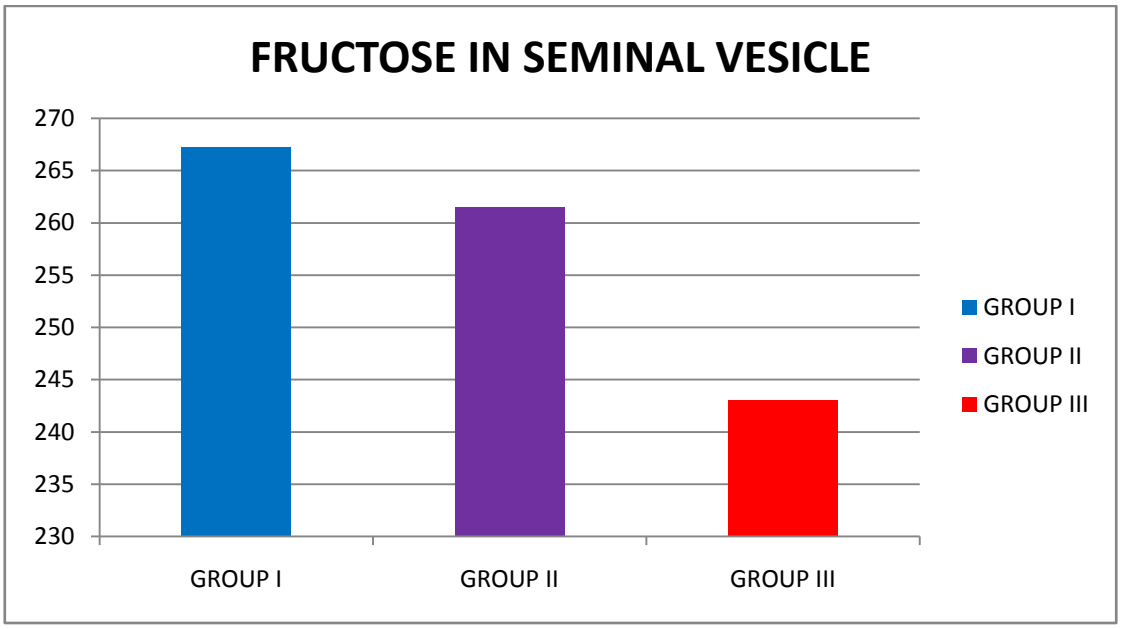
Graph 5: Tissue – Somatic Index (TSI) of the Seminal Vesicle of Control and Treated Animals

4.5 ***Tissue Biochemistry***

4.5.1 ***Fructose in Seminal vesicle***

Group	Fructose
Group I (Control)	267.27±16.30
Group II (50 mg/kg body weight)	261.55±15.67
Group III (500 mg/kg body weight)	243.07±12.40

Table 6: Fructose in control and experimental rats

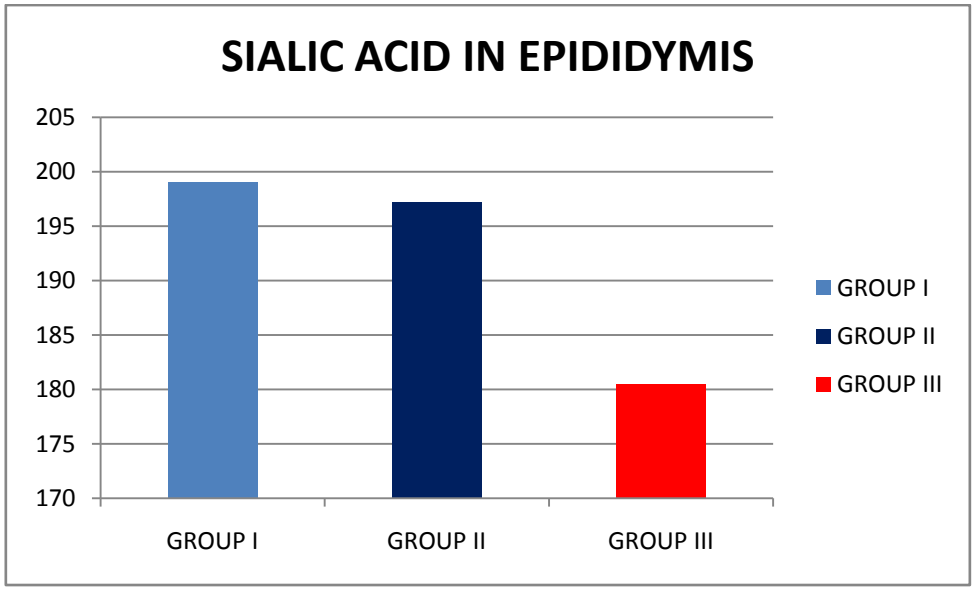


Graph 6: Fructose in control and experimental rats

4.5.2 *Sialic acid in Epididymis*

Group	Sialic acid
Group I (Control)	199±45.40
Group II (50 mg/kg body weight)	197.23±43.85
Group III (500 mg/kg body weight)	180.5±25.20

Table 7: Sialic acid in control and experimental rats

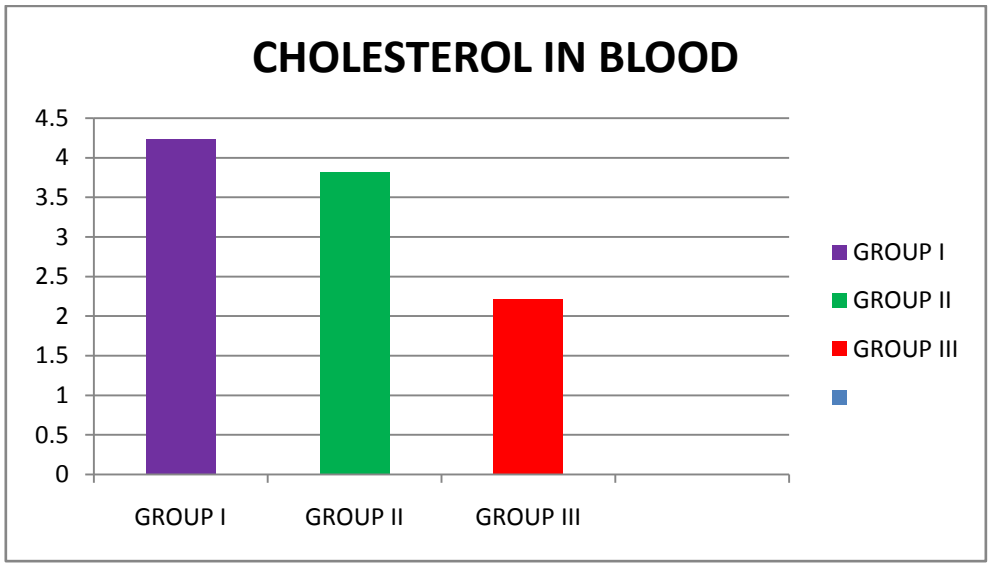


Graph 7: Sialic acid in control and experimental rats

4.5.3 *Cholesterol in Blood*

Group	Cholesterol
Group I (Control)	234.25±28
Group II (50 mg/kg body weight)	190.50±30.15
Group III (500 mg/kg body weight)	178.53±35.20

Table 8: Cholesterol in control and experimental rats



Graph 8: Cholesterol in control and experimental rats

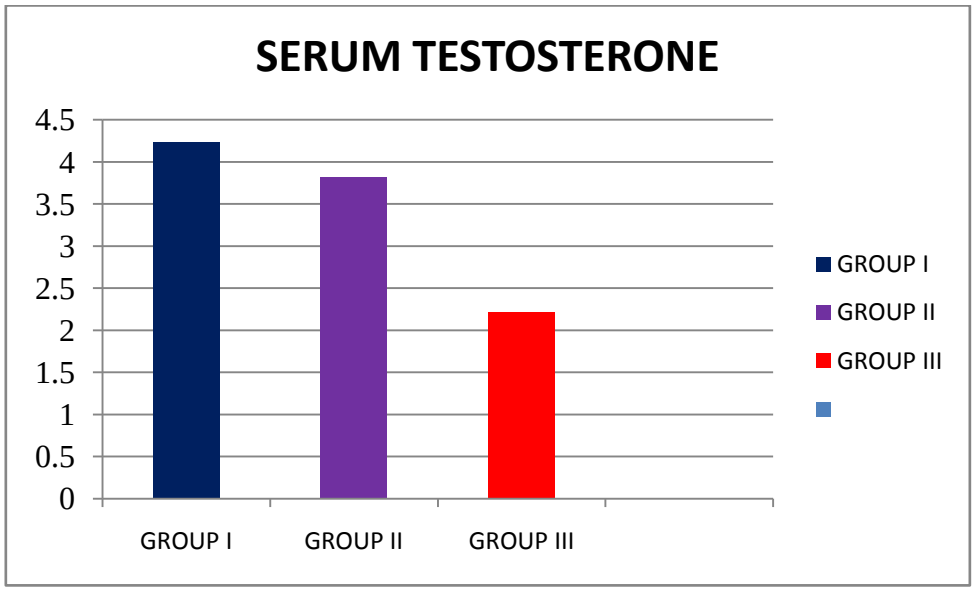
In the animals of Group II and Group III the Cholesterol value shows a statistically significant decrease at the significance level of ($P \leq 0.01$) respectively.

4.7 Hormone Analysis

Serum Testosterone

Group	Serum Testosterone level (mg/ml)
Group I (Control)	4.23±0.41
Group II (50 mg/kg body weight)	3.82±0.8
Group III (500 mg/kg body weight)	2.21±0.21

Table 9: Serum Testosterone in control and experimental rats



Graph 9: Serum Testosterone in control and experimental rats

Radioimmunoassay of testosterone :

Significant decline in serum testosterone level was observed in treated group when compared with group I (P≤0.001) (Table 9)

Histopathology

TESTIS (Control)

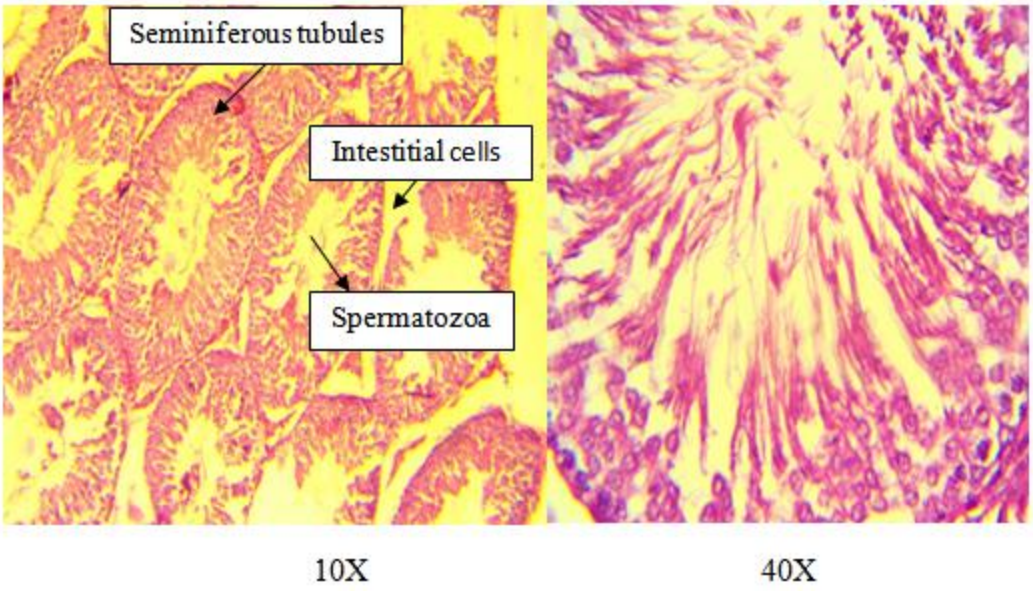
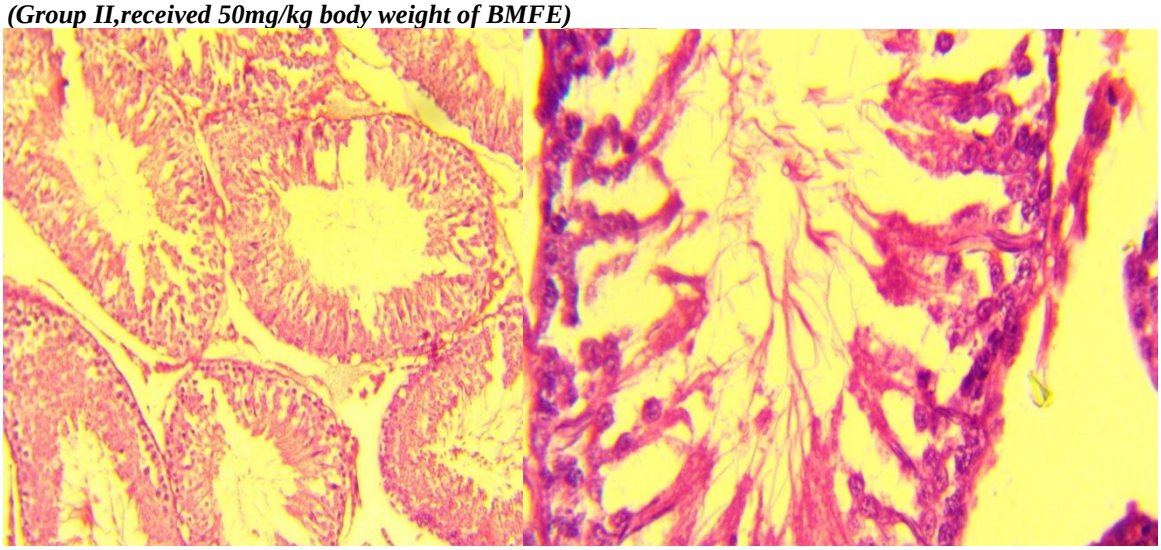


Figure 1: Testis of control animals showing well-arranged Seminiferous tubules, Interstitial cells and Spermatozoa

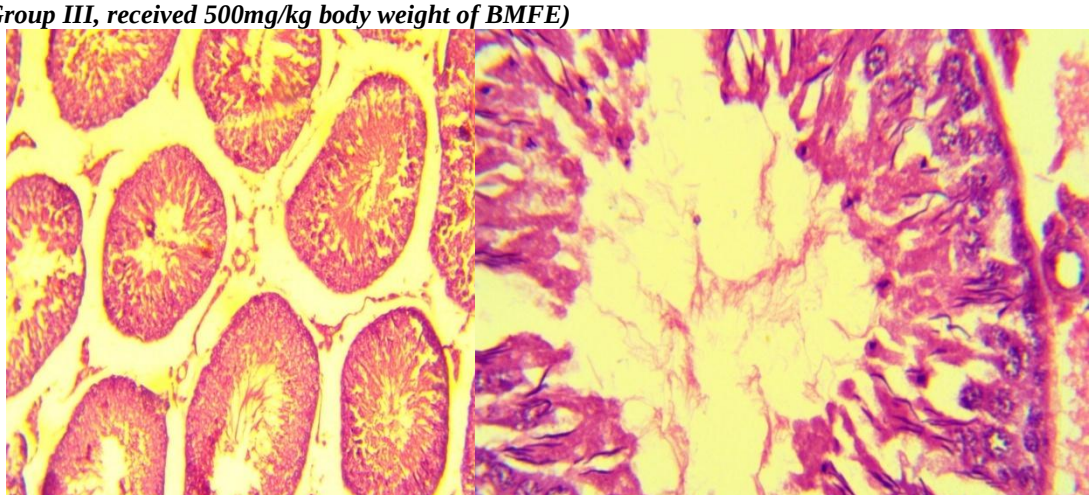
TESTIS (Group II,received 50mg/kg body weight of BMFE)



10X 40X

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

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



10X 40X

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

EPIDYDIMIS (Control)

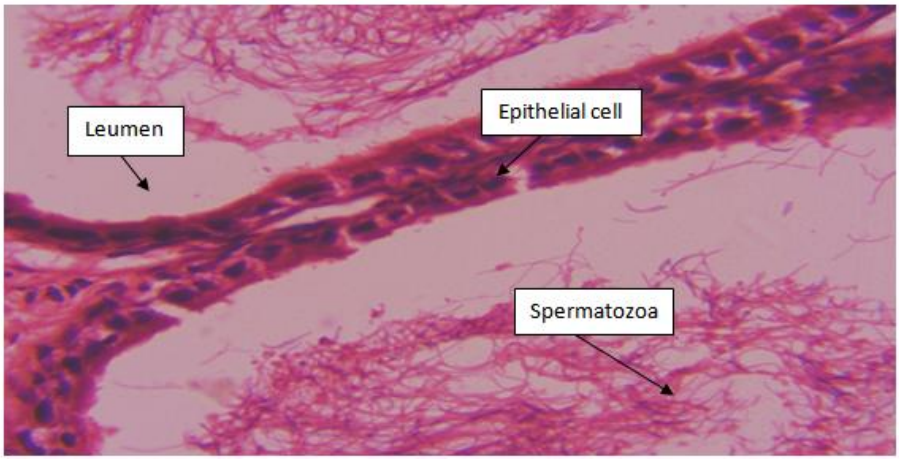


Figure 4: Epididymis in control is normal.

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

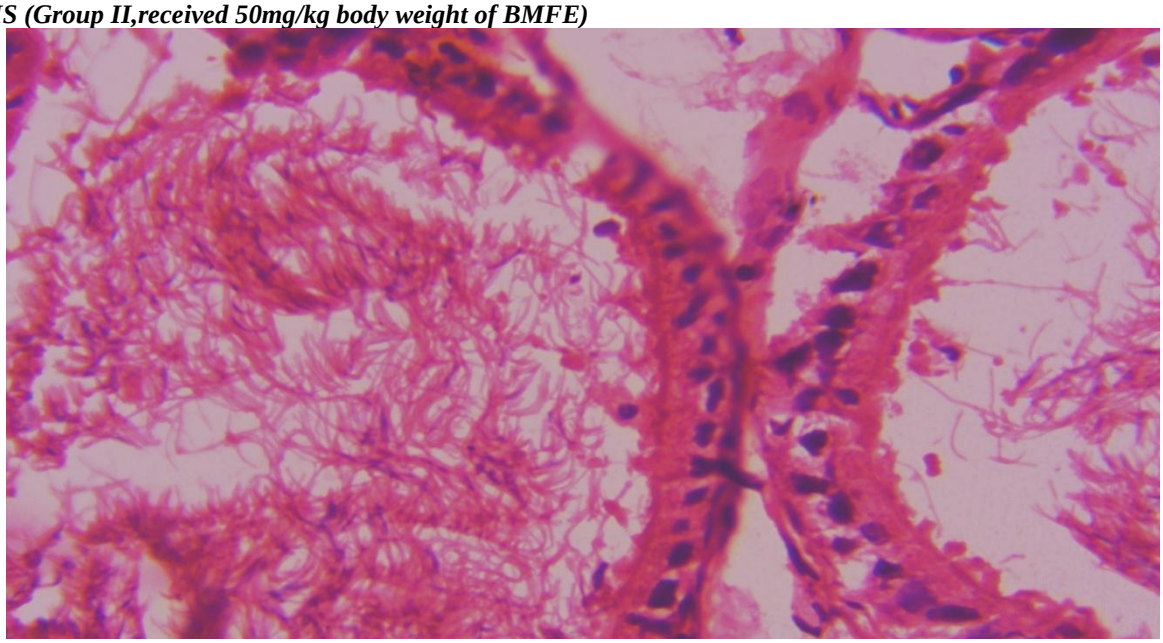


Figure 5: Epididymis showing less lumen secretion, epithelial cells degenerating and spermatozoa less.

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

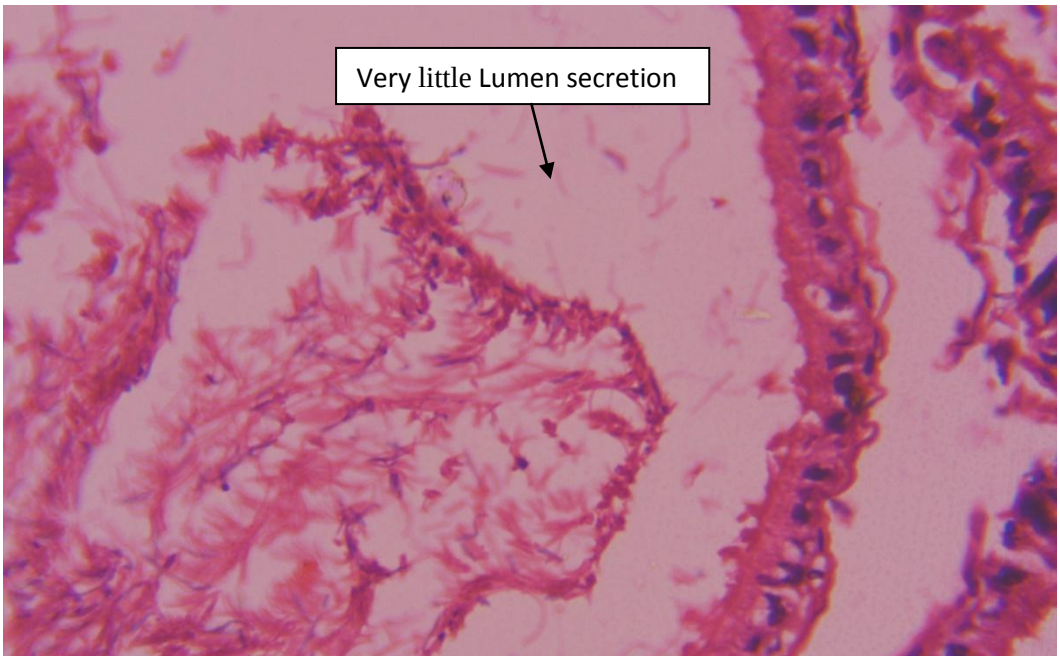


Figure 6: Epididymis showing very less lumen secretion, epithelial cells degenerating and spermatozoa very less.

SEMINAL VESICLE
Seminal Vesicle (Control)

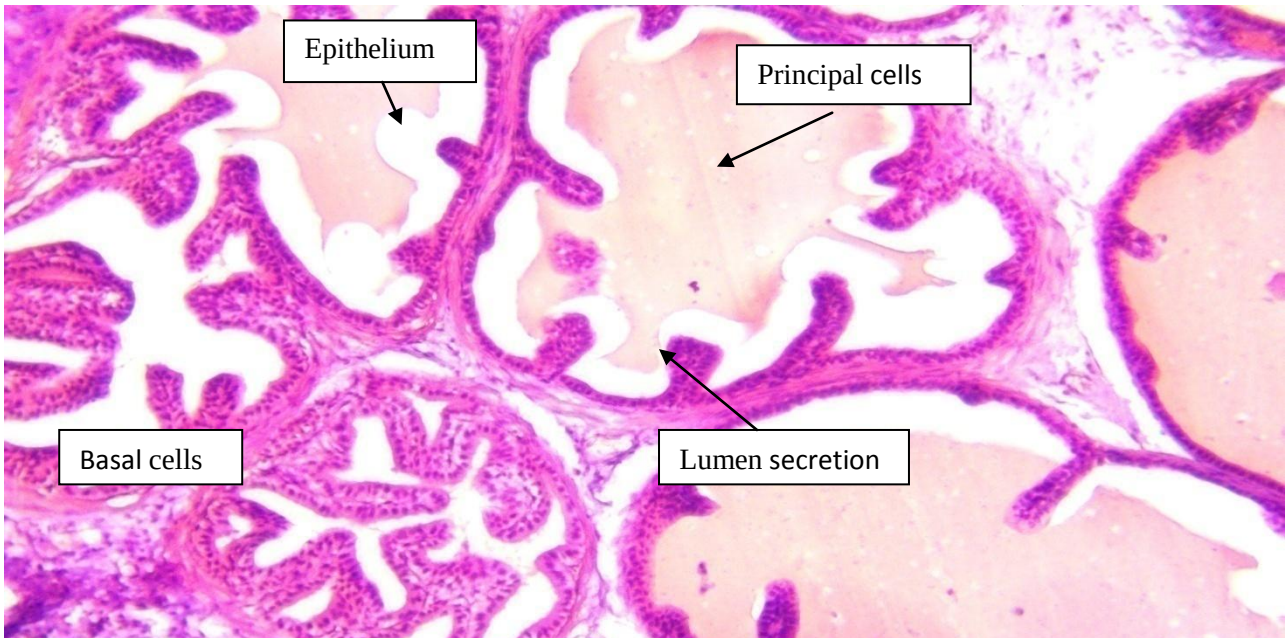


Figure 7: Seminal Vesicle in control normal

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

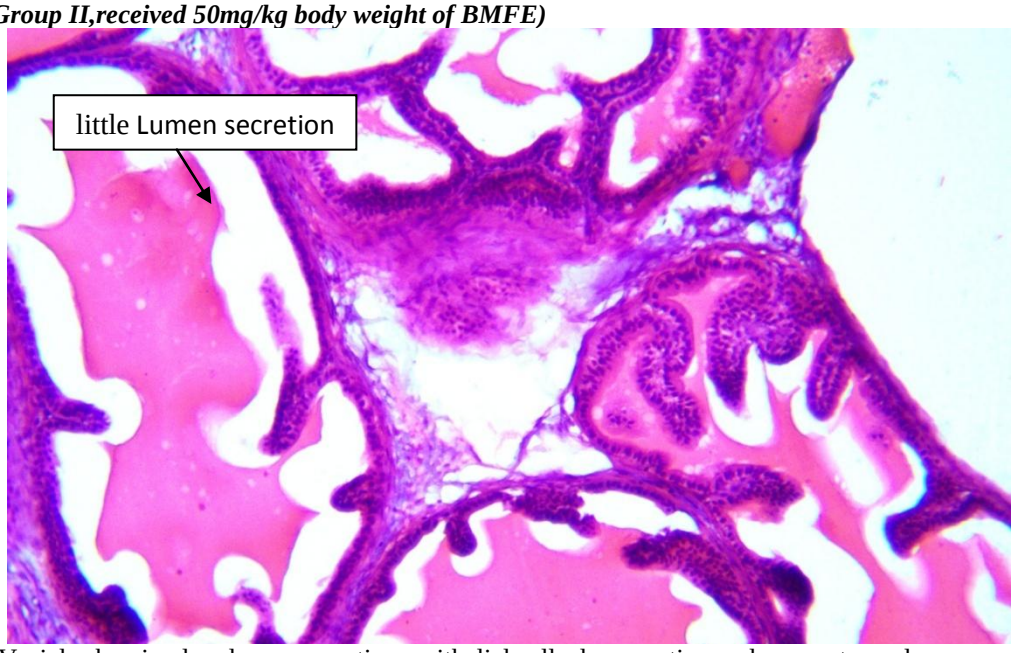


Figure 8: SeminalVesicle showing less lumen secretion, epithelial cells degenerating and spermatozoa less.

SeminalVesicle(GroupIII,received500 mg/kg body weight of BMFE)

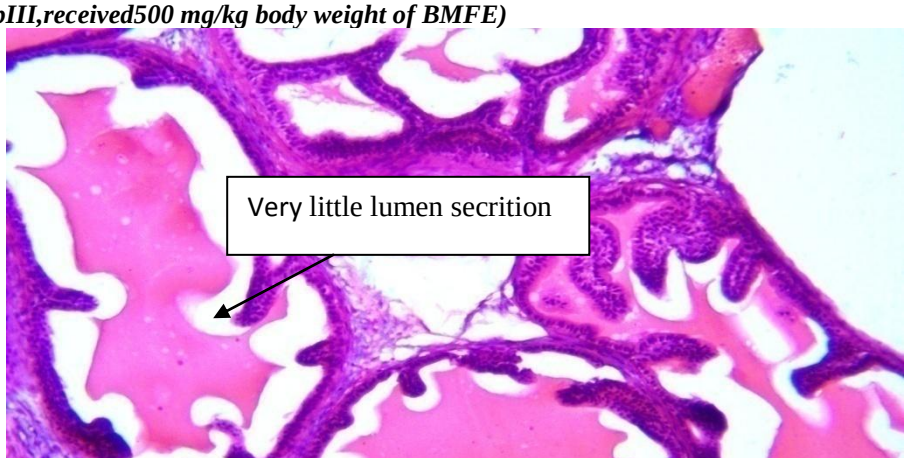


Figure 9: Seminal Vesicle showing very little lumen secretion, epithelial cells degenerating and spermatozoa less.

IV. Discussion

Medicinal plants play a major role in health care irrespective of advances in modern medicine. These plants are distributed worldwide, although more abundant in tropical regions. Pharmaceutical companies have demonstrated interest in the investigation of higher plants as source for new lead structures and for development of phytotherapeutic agents with proven efficiency, safety and quality.[47-49]

Population explosion is one of the biggest challenges prevalent in third world countries The herbal medicines are being used by up to 80% of the population in developing countries. Majority of the population dwell in rural areas without any approach to modern methods of family planning thereby relying on herbal medicines to control population growth rate, such as inducing abortion, prevent conception and sterilization of either the couple. Literature abounds on research carried out on medicinal plants with antifertility effects. [50-59] Despite widespread use, few scientific studies have been undertaken to ascertain the safety and efficacy of traditional remedies. The present study was undertaken to evaluate the male reproductive toxicity of the methanolic extract of ***Buteamonosperma*** flower extract , which is a herbal medicine. The impact of the extract on the reproductive system, particularly in males has not previously been investigated in details.

The finding of the present study showed that methanolic extracts of ***Buteamonosperma*** significantly altered the fertility potential of male rats. There waslittle changes (increase) in the body weight.

The significant decrease in the organ weight of the treated animals is indicative of the toxic effect of the extract. Decrease in organ weight after administration of a chemical agent has been reported by Simmons *et al*[60] to be an indicator of toxicity. The high significant decrease observed in the weights of testis, epididymis and seminal vesical of treated animals at 500 mg/kg may be due to loss of spermatogenic elements in the testis and the absence of sperm in the epididymis. Several reports have shown degenerative changes in Seminiferous tubules without a significant change in organ weight [61] The present data show that the administration of ***Buteamonosperma*** brought about a highly significant loss in testis and sex organ weights, which are known to be mostly related to the number of spermatides and spermatozoa in this tissue. The decreasing weight of the reproductive organs in the extract-treated male rats clearly indicated that the extract caused structural and functional alteration in the testes, epididymis and seminal vesicle.(Sarkar*et.al*,2000) [62] Reduction in the weight of testis and other sex organ might be due to low level of androgen, which was not enough to maintain the weight of gonads and accessories.(Singh and Singh, 2009).[63] **.** Low level of androgen, not only suppress spermatogenesis, leading to low sperm concentration but alters the epididymis milieu also, which renders its hostile for maturation andsurvival of the spermatozoa. [64,65]Decreasedtesticular weight causedalteration in seminiferous tubules that reflect a wide spread cellular damage and its consequence is androgen deprivation and arrest in the process of spermatogenesis.[66] At the testicular level, the absence of stimulation by LH would cause leydig cell dysfunction, there by resulting in testosterone depletion, which is responsible for diminished spermatogenesis and hence, reduction in Sperm Count.[67,68] Decreased number of spermatozoa or reduced androgens production may affect the level of Sialic acid in testis. The reduced sialic acid content might alter the structural integrity of acrosomal membrane, ultimately affects the metabolism, motility and fertilizing capacity of spermatozoa.[72] which could not penetrate the cervical mucus and thus failed to fertilize the ova.[69,70]

In the present study in Tissue Biochemistry, the levels of Fructose in Seminal Vesicle also decrease. Any change in the biochemical composition of semen may lead to change in sperm activity such as reduction in sperm motility [71]. Fructose is an important source of energy for the sperm. It is the principle source of the sperm motility under anaerobic conditions [72]. Low fructose concentration may be another cause of reduction in

sperm motility as seminal fructose provides energy for sperm motility. Lower intensity of fructose oxidation in gamete mitochondria leads to accumulation of lactate and inhibition of dehydrogenases activity [73]. This sugar has been studied extensively, because it is considered as a marker for seminal vesicle function [74]. A low level of seminal fructose may coincide with other symptoms of hormonal malfunction and poor quality of spermatozoa. The function of seminal vesicle is important for fertility. Low level of seminal fructose has been observed in hypo function of the seminal vesicles and has been related to male infertility.

A significant reduction in the Sialic Acid content in epididymis has been observed in the present study. Reduced androgen production was reflected in low levels of sialic acid in epididymis. Sialic acid is an important constituent of mucopolysaccharides and sialomucoproteins which are essential for the maturation of spermatozoa in epididymis.[75]**.**Reduced androgen production may affect the level of sialic acid in epididymis. The reduced sialic acid content might alter the structural integrity of acrosomal membrane which ultimately affects the metabolism, motility and fertilizing capacity of spermatozoa.[76]

A significant reduction in the Cholesterol level in blood was observed in the present study. Cholesterol helps build cell membranes; contributes to rescue work of blood vessels; protects nerve fibers and aids the production of Vitamin D, bile acids and hormones that are essential to fertility. "Cholesterol is a precursor for steroid hormones, such as estrogen and testosterone, which are very important for men and women". Cholesterol combines with protein in the blood stream to create High – Density Lipoprotein (HDL) and Low – Density Lipoprotein (LDL) particles. LDL delivers cholesterol to body tissues and although it is considered the "bad cholesterol", LDL has been shown to aid in muscle building. The "good cholesterol" HDL, delivers cholesterol to the adrenals and ovaries for hormone production. The blood cholesterol might be related to fertility as the body uses cholesterol to manufacture sex hormones like testosterone and estrogen.

Significant decrease was recorded in serum testosterone level in the treatment groups especially in high dose group compared to control. Testosterone is produced by Leydig cells in the testes and decreased number of Leydig cells and their nuclear diameter diminishes the production of testosterone.(Bhatt *et.al*., 2007).[77] Testosterone level is depleted in serum of treated animals. The number and nuclei diameter of Leydig cells were reduced in ***Buteamonosperma***treated rats. This may be due to the deleterious effect on leydig cell that may consequently be responsible for testicular and epididymal dysfunction as a result of androgen deprivation. This may affect the process of sperm production and maturation in both organs leading to loss of fertility in treated rats.

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: interacellular 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:interacellular 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 degeneratingEpithelial 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:interacellular 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.

V. Result:

Methanolic extract of ***Buteamonosperma*** flower extract significantly effect the reproductive organ weight and histology of male albino rat. The negative impact of ***Buteamonosperma*** 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).[78]Reduced testicular and epididymal protein content could be correlated with

absence of spermatozoa in the lumen.(Zhen *et.al*.,1995).[79] 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.[80]
The reduced sperm count and motility in epididymis is of importance with regard to fertilization (Bedford, 1983; Raji*et.al*., 2006).[81] Low level of sialic acid in testis, epididymis may be correlated with loss of androgen.(Gupta *et.al*.,2001).[82]

Cholesterol is the major substrate responsible for the anabolic effect of testosterone in males (Carreau, 1996;[83]Bhasin*et al*, 1998).[84]A significant decrease in the concentration of cholesterol was observed in rat treated with ***B.monosprma*Flower Extract (BMFE)** suggesting inhibition of spermatogenesis. This result is in corroborative with decrease in testosterone level which is also observed in the present study. The present finding is in agreement with (Bargatell, 1996and Bremner, 1996 [85], Kamtchouing*et al*, 2002;[86]Vijaykumar*et al*, 2004)**.**[87]

In the present study, the reduced testicular and sex organ weights, and sperm count indicate a wide spread damage, which could be due to reduced protein contents in these organs, which ultimately effect the male fertility.

It can be concluded that, the oral administration of 50% methanolic extract of ***Buteamonosperma***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. ***Buteamonosperma*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.[88]***Buteamonosperma*** 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 ***Buteamonosperma*Flower Extract (BMFE)**supresses male fertility .

VI. Conclusion:

The present result suggest that administration of methanolic flower extract of ***Buteamonosperma***significantly effect the reproductive organ of male albino rats, which was treated with 50 mg/kg and 500 mg/kg body weight of ***Buteamonosperma*Flower Extract (BMFE)**caused the impairment of testicular, epididymis and seminal vesicle structures, which led to significant decrease in spermatogenic activity in seminiferous tubules. Depletion of Leydig cells in tubular interstitial also causes reduction in serum testosterone level. The reduced testicular and accessory sex organ weight indicate a wide spread damage, which could be reduced protein contents in these organs, which ultimately effect the male fertility. Therefore, the process of maturation of spermatogenic cells and sperm production in the organ was affected by the extract administration which may lead to infertility in treated rats. Thus from the above study it may be concluded that BMFE supresses the male fertility.

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