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Research Paper

Effect of *Buteamonosperma* Flower Extract on the Male Reproductive Organs of Albino Rats

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Abstract:
Population growth in present time especially in India cause upon economic pressure, skirmishes for resources like water etc. If not checked, in coming years the population of India is expected to be 1.5 billion.
It is thus, a check is needed. The best would be contraceptive. Modern reproductive bio-medicine provides several preventive and effective method of contraception (both in male and female) yet these medicines provide side effects.
WHO suggested that practice of using traditional medicine for control of fertility, instead of synthetic drugs, as cost effective management of birth control and has given great emphasis on "folklore use of antifertility herbs". With this idea in mind, the present author has worked upon the effect of ***Buteamonosperma****(Palash) (a plant which has a reference in the ancient literature) on the reproductive phase of* ***Albino Rats*** *and has found that it has:-*

- *Substantial effect on fertility potential of rat which shows body weight to increase after methanolic extract administration whereas the weight of testis, epididymis and seminal vesicle shows decrease in weight compared to control.*
- *Decrease in Tissue Somatic Index of testis, epididymis and seminal vesicle as compared to control*
- *The author has also found that fructose in seminal vesicle, sialic acid in epididymis and cholesterol in blood decreases as compared to control.*

Keywords: ***Buteamonosperma,*** *testis, epididymis, seminal vesicle, cholesterol and sialic acid*

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I. INTRODUCTION:

Modern reproductive biomedicine has provided several preventive and effective methods of contraceptives for fertility control in male and female but none of which is very safe and without any serious side-effects.[1].

The overpopulation can be checked through biological means with special reference to modulation in the human fertility ability.Along with the advancement in the reproductive biomedicine different hormonal contraceptive pills are developing but all have side effects.

Despite the number of contraceptive methods available, unintended pregnancies still occur worldwide. There is still a worldwide unmet need for more affordable , effective and practical contraceptives, indicating that further technological advancement or innovations to existing products are required.[2].

Contraceptive pill in women is effective yet some women do not maintain the regularity in taking the pills hence can safely conceive. Therefore it is better to control the mobility of sperms and its count in males, with this idea in mind the author took up the study of ***Buteamonosperma***flower methanolic extract (made power form) safe contraceptive for males.

*Buteamonosperma*HISTORYIN VEDIC ANDLITERATURE

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 o the family Fabaceae.[3]. It is locally called palas, palasha, mutthuga, bijasneha,dhak,khakara,chichra,BastardTeak,Bengal Kino, Nourouc and it is distributed over large area in Asia, for example, in SriLanka, Burma and India. In India our state Jharkhand has a great distributation 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.[4]. 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.[5,6].

Photograph ofButeamonosperma(Linn.) Plant

Photograph ofButeamonosperma(Linn.)Flower

It holds an important place because of its medicinal and other miscellaneous uses of economic value. One of the important traditional uses of palas leaves is in making plates for serving food. The traditional healers consider the food served in these plates is best for general health and helps to develop natural resistance. The hot food served in these plates may result in some beneficial chemical reactions. Bark fibers are obtained from stem for making cordage [5].Stem bark powder is used to stupefy fishes. Young roots are used for making ropes.[7]. Green leaves are good fodder for domestic animals. Leaves are used for making platters, cups, bowls and beedi wrappers.[7,8]. Leaves are also used for making Ghongdato 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.[3]. Orange or red dye is used for colouring garments and for making skin ointment [9]. Fresh twigs are tied on horns of bullocks, on occasion of 'pola' and dry twigs are used to feed the sacred fire.[3].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. [8]

B.monospermaisextensibly 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.[10].

The flowers of ***B.monosperma***are widely used in treatment of hepatic disorders, viral hepatitis and diarrhea.[11]. The flowers have anti-convulsive, anti-hepatotoxic, anti-implantation, hypoglycaemia, astringent, diuretic aphrodisiac properties and tonic.[12,13]. The contents of flowers are butein, butrin, isobutrin, plastron, coreipsin and isocoreipsin.[14,15].

The roots are useful in treatment of night blindness, filariasis, piles,ulcers and tumors.[16].

The stems bark posses' antifungal activity. The compounds isolated from stem bark are stigmasterol, stigmasterol-βD-glucopyranoside, nonacosanoic acid, 3α-hydxoxyeuph-25-ene and 2, 14-dihydroxy-11, 12-dimethyl-8-oxooctadec-11-encychohexane.[17]. The gum is powerful astringent.

Seed of the plant is a popular folk medicine which has been used as a contraceptive.[18,19]. And anti-helmintic.[20]. The seed extract can cause liver, lungs and spleen congestion,[21] and the seed suspension can cause teratogenic effect in rats.[22]. Further the seed extract is experimental animal,[23-24]and physostigmine like action in experimental studies.[25]. In-vitro the seed oil showed a significant bactericidal and fungicidal effect.[26]. It is also reported that butin flavones of seed is being used as an anti-fertility agent,[27] and the crude saline extract (0.9%) of seed agglutinates the erythrocytes of several animal species.[28]. The seed extract exhibited low mortality in mice on acute toxicity test.[29].

B.monospermais reported to possess anti-fertility, aphrodisiac, analgesic and anti-helmintic properties. The tubers of Buteasuperba have been found to contain estrogenic substances similar to follicular hormone. The active constituents have been identified as butin. Butin also exhibits male contraceptive properties. Anti-fertility effect of seed extract of Buteafrondosa has also been reported in mice. An extract from flowers is used in India for the treatment of liver disorder. Butin is isolated from flowers of Buteamonosperma shows potentiality of

male and female contraceptive.[30]. Much work have been done regarding female reproduction but still it is utmost important to find out efficacy of such a useful and easily available trees .Male hormone i.e. testosterone produced primarily in the gonads under the influence of Follicular Stimulating Hormone (FSH) and Luteinizing Hormone (LH). The increase in concentrations of sex hormones is known to exert positive feedback at the level of the Pituitary Gland. The hormone Prolactin (PRL) is generally only thought as a factor in female infertility. Prolactin (PRL) also plays a role in male fertility. High Prolactin (PRL) level have an adverse effect on the function of testicles and can cause increased testosterone levels or abnormal sperms.[31].
In this study methanolic extract of ***B.monosperma flower*** were evaluated to be a safe contraceptive for males.

II. MATERIALS AND METHODS

Preparation of Methanolic extracts of *Buteamonosperma*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 (60-80º).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.

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 ***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.
EUTHANIZATION:
After administration of last scheduled dose of extract, animals were autopsied under mild ether anesthesia.
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 testis, epididymis and seminal vesicle were dissected out, freed from adherent tissue and weighted.
BODY AND SEX ORGAN WEIGHT:
The initial and final body weight of animal was recorded every fortnightly. The testis, 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.
TISSUE BIOCHEMISTRY
BIOCHEMICAL MARKER OF REPRODUCTIVE ORGAN
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* **[34]** and was expressed as µg/mg tissue weight.

SIALIC ACID IN EPIDYDIMIS
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***[35]** and was expressed as µg/mg fresh tissue weight.
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) .
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.

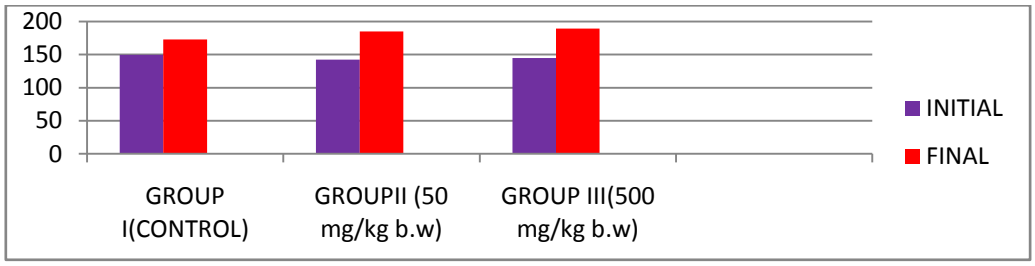
Statistical Analysis:
All the result are expressed as mean ± SEM and significance was analysed statically bystudents't' test and $p<0.05$ was considered as significant level.

III. RESULT

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



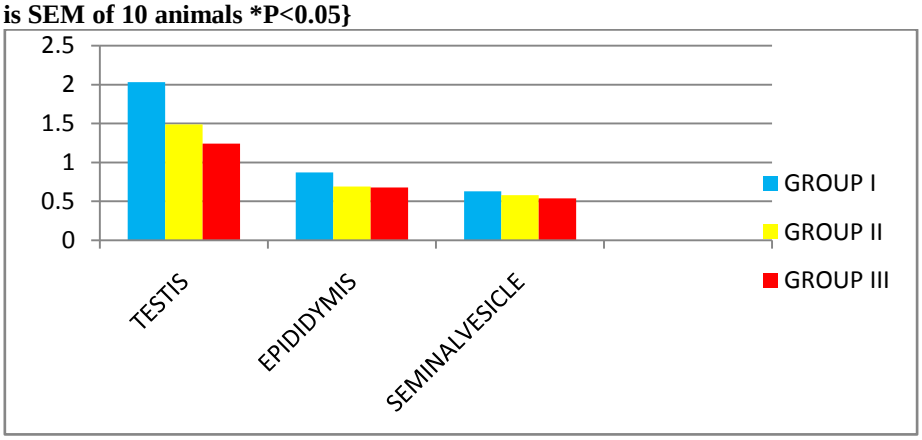
Vertical Bar 1: Group I, Group II and Group III showing Initial and Final body weight of the Control and Treated Animals

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 III(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}



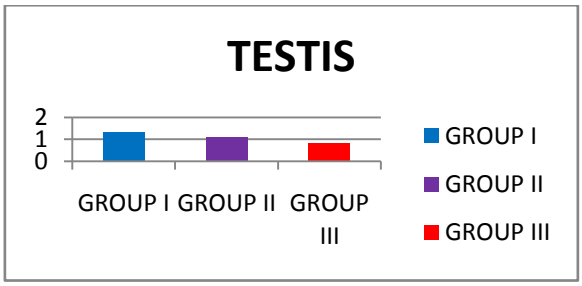
Vertical Bar 2: Reproductive organ weight of the Testis, Epididymis and Seminal Vesicle of Control and Treated Animals

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 III (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}

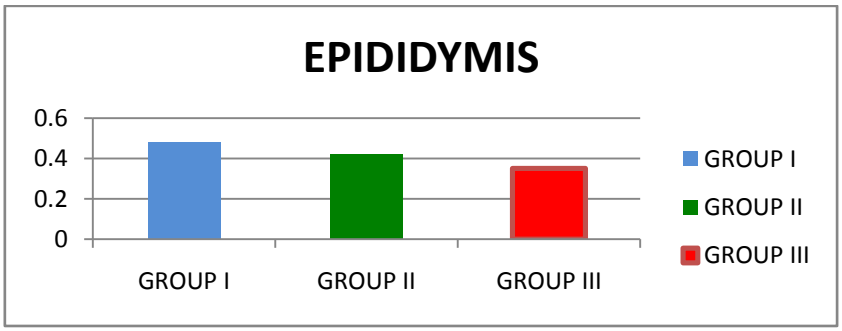


Vertical Bar 3: Tissue – Somatic Index (TSI) of the Testis of Control and Treated Animals

EPIDIDYMIS

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

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

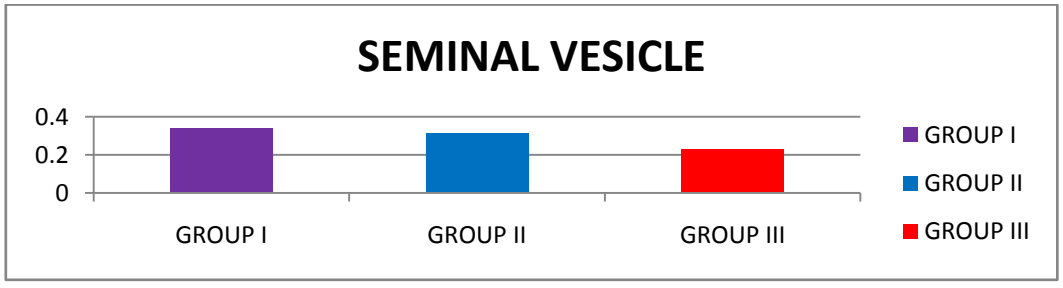


Vertical Bar 4: Tissue – Somatic Index (TSI) of the Epididymis of Control and Treated Animals

SEMINAL VESICLE

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

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



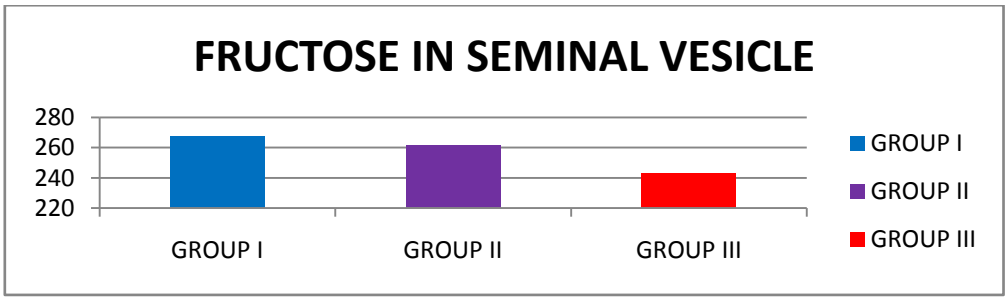
Vertical Bar 5: Tissue – Somatic Index (TSI) of the Seminal Vesicle of Control and Treated Animals

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

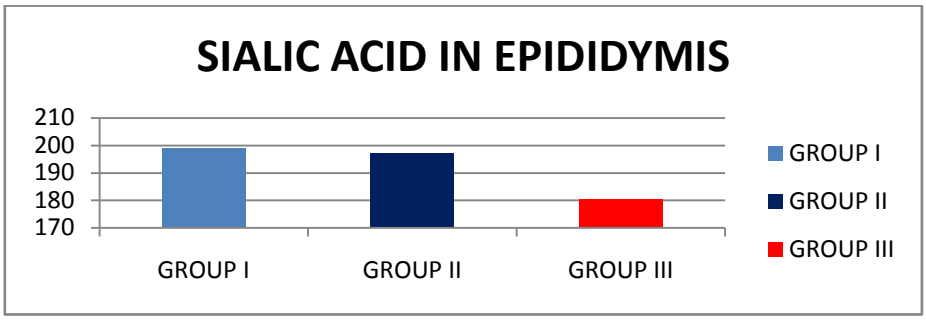


Vertical Bar 6: Fructose in control and experimental rats

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

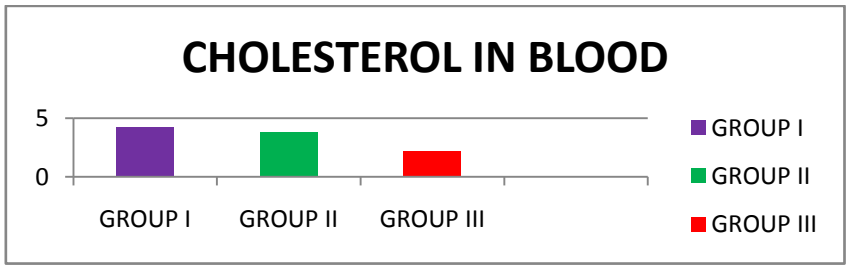


Vertical Bar 7: Sialic acid in control and experimental rats

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

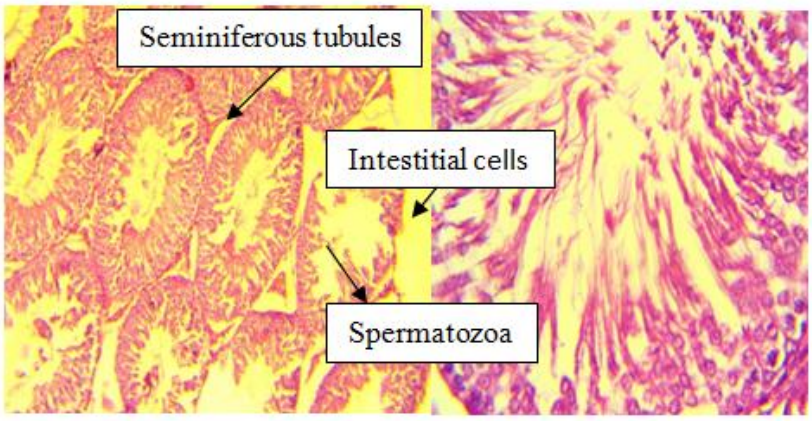


Vertical Bar 8: Cholesterol in control and experimental rats

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

Histopathology

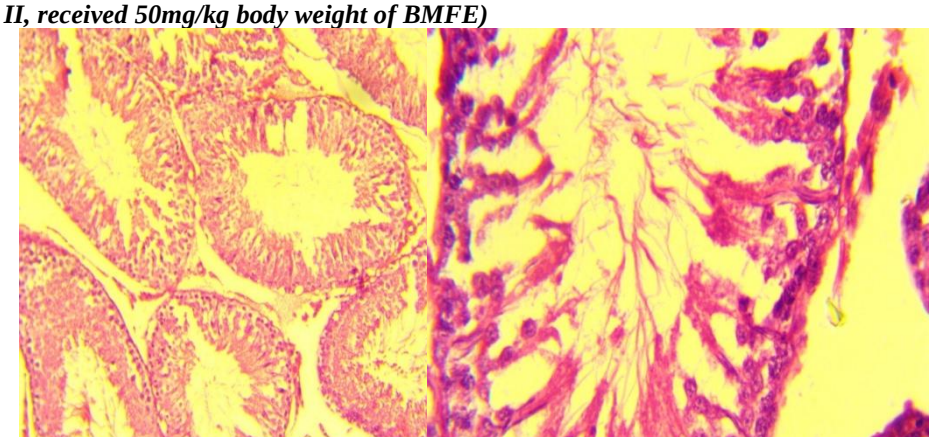
TESTIS (Control)



10X 40X

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

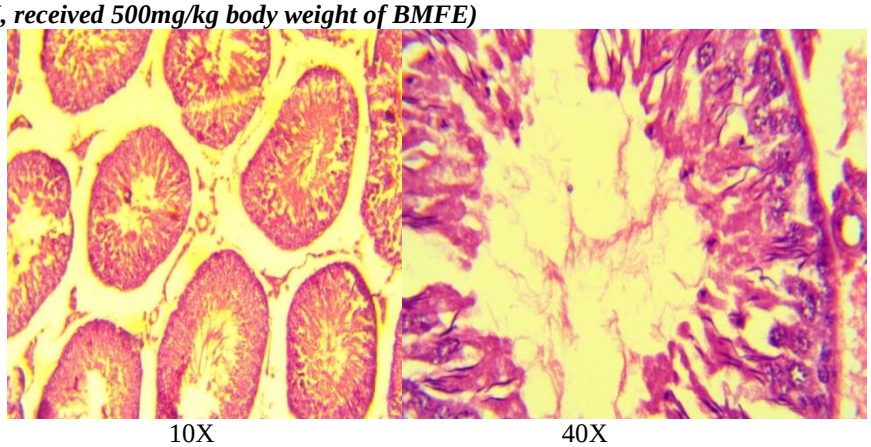
TESTIS (Group II, received 50mg/kg body weight of BMFE)



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)

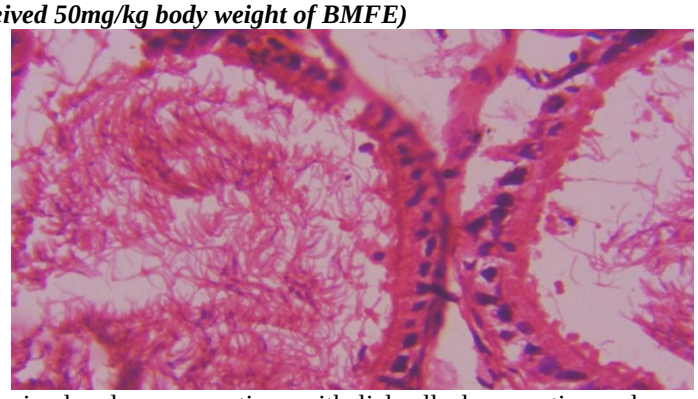


Figure 5: Epididymis showing less lumen secretion, epithelial cells degenerating and spermatozoa less.

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

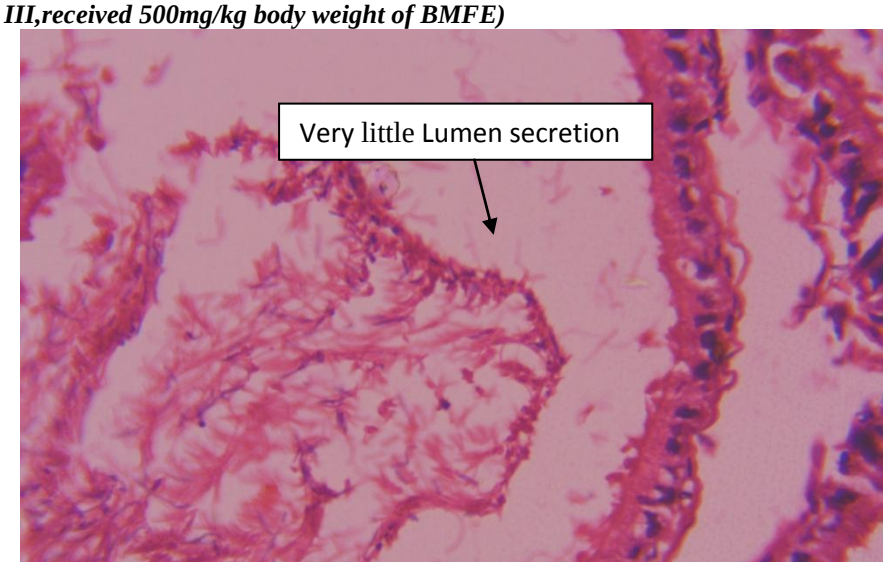


Figure 6: Epididymis showing very less lumen secretion, epithelial cells degenerating and spermatozoa very less.

SEMINAL VESICLE
Seminal Vesicle (Control)

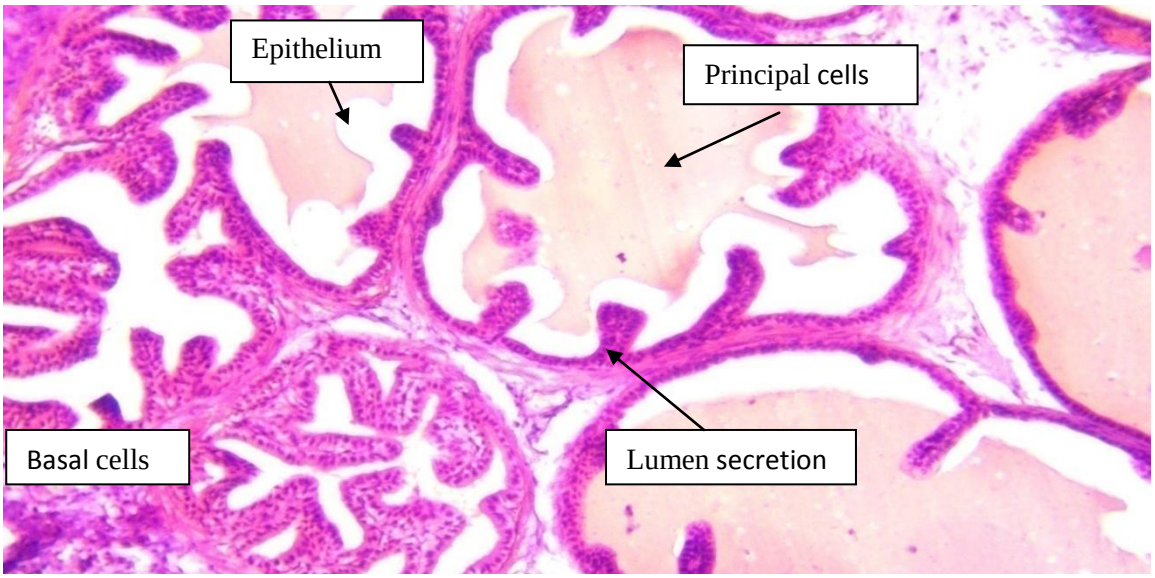


Figure 7: Seminal Vesicle in control normal

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

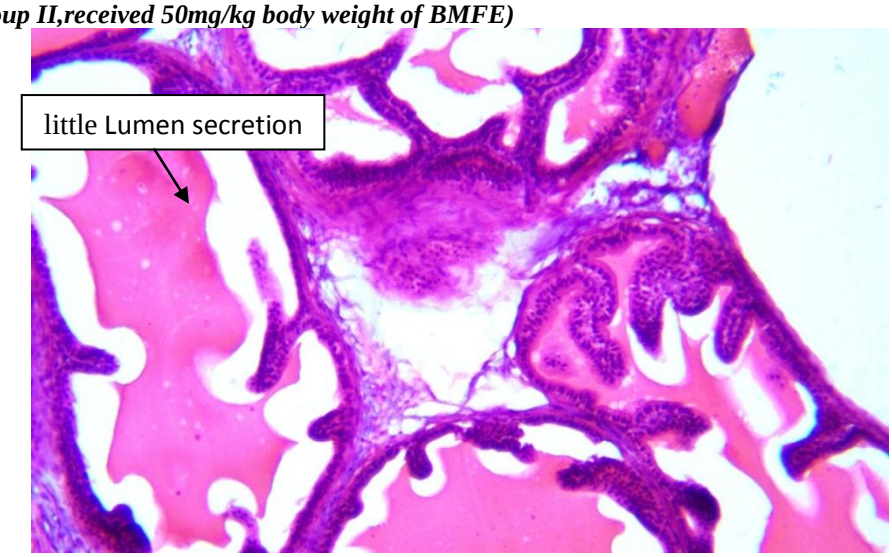


Figure 8: SeminalVesicle showing less lumen secretion, epithelial cells degenerating and spermatozoa less.

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

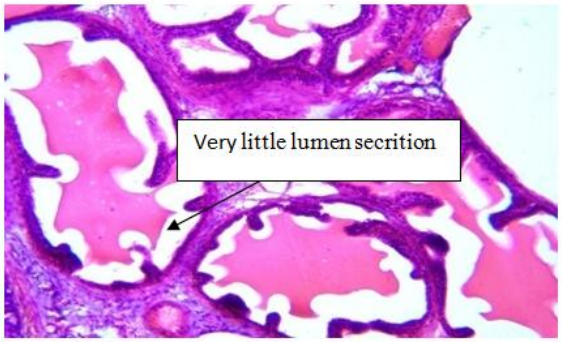


Figure 9: Seminal Vesicle showing very little lumen secretion, epithelial cells degenerating and spermatozoa less.

IV. OBSERVATION AND DISCUSSION

It was observed that:

1. Body Weight increased in treated rats.
2. Testis, Epididymis and Seminal Vesicle decreased in treated rats(Group II &III) compared to control.
3. Tissue Somatic Index (TSI) decreased in Testis, Epididymis and Seminal Vesicle decreased in treated rats(Group II &III) compared to control.
4. Fructose in Seminal Vesicle, Sialic acid in Epididymis, Cholesterol in Blood too decreased in treated Group II & IIIrats compared to control.
5. i) Testis of controlled rats showedwell-arranged seminiferous tubules, interstitial cells and spermatozoa but in Group II & III treated rats, the interstitial cells and spermatozoa degenerates.

ii) Epididymis in treated Group II & III rats showed degeneration in epithelial cells & loss of Spermatozoa.

iii) The Seminal Vesicles also show degeneration in epithelial cells & loss of Spermatozoa.

Possibly, the ingredients in the flower of ***Buteamonosperma*** help in reducing the sperm count & therefore its utility in male fertility control cannot be denied.

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