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EVALUATION OF FERTILITY POTENTIAL OF ABHRAKA BHASMA IN OBESITY INDUCED RATES

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ABSTRACT

Aims: *This study was conducted to evaluate the fertility potential of Abhraka Bhasma in obesity induced in male wistar rats.* ***Methods:*** *The current experiment was carried out on 24 healthy young male albino wistar rats divided in to 4 groups. Sahastraputi abhraka bhasma was used as the test drug with honey as a vechicle orally. G1 group were fed with normal diet for 5 weeks and honey for next 3 weeks served as normal control. G2 group were given high fat diet for 5weeks and honey for next 3 weeks served as disease control. G3 group were given normal diet for 5 weeks and abhraka bhasma of 1.08mg/200gm of rat for next 3 weeks serves as treatment control. G4 group received high fat diet for 5 weeks and abhraka bhasma of 1.08mg/200gm of rat for next 3 weeks serves as treatment.* ***Statistical Analysis*** *Used: One –way ANOVA was used to take out the significance of the data and graph pad prism to obtain descriptive statistics.* ***Results:*** *On sacrificing animals after 50 days of experimentation it was observed that control animals (G1) had normal spermatogenesis with normal BMI. G2 group rats prevented spermatogenic profile in 50% obese rats and resulted in degenerated tubules with increase in BMI. In G3 group spermatogenic activity increased. Where as in G4 group due to the effect of test drug it contained 60% hyperactive tubules and 35% in recovery stage which are damaged due to effect of high fat diet.* ***Conclusion:*** *In conclusion, Abhraka Bhasma due to its body mass index (BMI) decreasing property has spermatogenic enhancing properties. This study confirms that abhraka bhasma is important tool in solving the problem of infertility in obese males.*

KEY WORDS

ANOVA, Sahastraputi, Hypertrophy, Sukra

INTRODUCTION

Abhraka bhasma is a herbo mineral formulation of Ayurveda constituting mica nano particles. Abhraka bhasma is like supreme ambrosia, it destroys vata(air), pitta(fire), and disease ksaya(pthisis). It is a nervine tonic and increases tone of tissue, benefits in azoospermia, erectile dysfunction, haematinic. It also acts as hepatoprotective agent. According to a new study led by Babita *et al (2012),* demonstrated that abhraka bhasma reduces testicular damage induced by heat stress in heat stroke rats. It indicates that prolonged administration will ameliorate fertility in males, especially those who are exposed to heat.

Normal BMI is necessary for normal spermatogenesis and fertility in mammals. Obesity which may be due to physical, genetic and hormonal disorders cause erectile dysfunction or altered seminal parameters which results in infertility. Corona et al, presented evidence showing that 96.5%of their s tubjects with metabolic syndrome, exhibited erectile dysfunction.

The goals of this study was set to prove that this abhraka bhasma is highly beneficial in the treatment of infertility especially in obese individuals. It brings forth that abhraka bhasma enhances the fertility in high fat diet fed obese rats by altering the body mass index and obviously improving the spermatogenic activity.

MATERIALS AND METHODS

Animals: The experiment was carried out on healthy 24 Male albino wistar rats (45 – 60gm) of 4 weeks old were obtained from Teena Biolabs Pvt ltd., Reg no 189/42/CPCSEA, Bachupally, Hyderabad. Animals are housed in cages in an air conditioned laboratory of the animal house. Animals were maintained under controlled standard conditions, with free access to pellet diet, high fat diet and water ad libtium. Before conducting the experiment, the animal ethical clearance was obtained from Institutional Animals Ethics Committee (IAEC) approved by Committee for the purpose of control and supervision of experiment on animals (CPCSEA).

Abhraka Bhasma(Test drug): Sahasra puti abhraka bhasma was used as the test drug. This is marketed preparation obtained from ayurvedic medical pharmacy. The dose was calculated by extrapolating the therapeutic dose of humans to rats on the basis of BSA ratio (conversion factor 0.018 for rats) by referring to the table of **(Paget and Barnes 1964).**

Therapeutic dose of Sahastraputi Abhraka Bhasma: 15 - 60 mg

Selected human dose: 60 mg/kg b.w.

For rats

Human dose X 0.018 = X g/200mg of rat

Vehicle = Honey is used as vehicle.

Experimental design

The animals were divided into four groups of 6 animals each.

Group 1: Receives normal diet for 5 weeks and honey for next 3 weeks serves as normal control.

Group 2: Receives high fat diet for 5 weeks and honey for next 3 weeks serves as disease control.

Group 3: Receives normal diet for 5 weeks and abhraka bhasma of 1.08 mg/200 g of rat for next 3 weeks. (Treatment control)

Group 4: Receives high fat diet for 5 weeks and abhraka bhasma of 1.08 mg/200 g of rat for next 3 weeks. (Treatment)

Sampling and Analysis

In brief, after 21 days of treatment, three animals from each group were sacrificed by a rapid decapitation method to dissect out testes for histopathological studies.on day 21, following the overnight fast blood smples of six animals from each group were collected by retro orbital plexus. Testis were removed and cut into small pieces, fixed in formalin fixative, and dehydrated with varying percentage of ethanol for histological studies. Sections were cut (6µm), stained with eosin, and analyzed microscopically.

PARAMETERS

Body weight: The body weights of the animals were recorded on 0^{th} week after treatment (5^{th} week before treatment) and 3^{rd} week after treatment with abhraka bhasma. The results are depicted in the table no. 2 and fig no1.

Body mass index: Body mass index (BMI) of rats were recorded on 0^{th} week after treatment (5^{th}week before treatment) and 3^{rd} week after treatment with abhraka bhasma was measured by using formulas, the results are depicted in the Table No.3 And Fig No.2.

BMI = Body weight in gm**/ (**Height in cm) ^{2}

Organ weight and Fat pad weights: The testes, liver, kidney, spleen and ventral heart were dissected out, freed from adhering tissue, blotted on a filter paper and weighed on a sensitive balance to the nearest milligram. At the same time fat pads are dissected from the mesentery, epididymis and retroperitonium and weighed. The results are depicted in the Table No.4 And Fig No.3.

Sperm count: The cauda epididymis was chopped in 10ml normal saline, the aliquots of sperm suspension was filled up to 0.5 mark in WBC pipette and diluted with saline up to 11 marks. Sperm count was done in Neuber's chamber in WBC squares. Sperm count/ml was calculated as follows.

X x20 x 104/cm^2/ml/epididymis. Where 'X' is the average mean of spermatozoa of` all 4 squares

BIOCHEMICAL ESTIMATION

Estimation of cholesterol

Procedure: Pipette in a clean dry test tube labeled as Blank (B) Standard (S), and Test (T)

	B	S	T
Enzyme Reagent	1ml	1ml	1ml
Deionized water	0.01ml	-	-
Standard	-	0.01ml	-
Serum/Plasma	-	-	0.01ml

Mix and read the optical density (0D) at 500nm against blank after 5-minute incubation (37^{O}C). The final colour is stable for at least for 1 hour.

Calculation: Cholesterol Conc. In mg% = A of (T)/A of (S) X 200 (std .conc)

Estimation of triglycerides

Procedure: Pipette in a clean dry test tube labeled as Blank (B) Standard (S), and Test (T)

	B	S	T
Enzyme Reagent	1ml	1ml	1ml
Deionized water	-	0.01ml	-
Standard	-	0.01ml	-
Serum/Plasma	-	-	0.01ml

Mix and read the optical density (0D) at 546nm against blank after 10 minute incubation (37^{O}C). The final colour is stable for at least for 1 hour.

Calculation: Triglycerides Conc. in mg% = A of (T)/A of (S) X 200 (std.conc)

RESULTS

OBESITY PARAMETERS

Table 1: Initial and final obesity parameters of 4 groups during fat diet and after treatmentwith abhraka bhasma.

Parameters	Before Treatment				After Treatment			
Initial body weight (gm)	57.5±10.37	52.5±6.12	55±8.94	55.83±7.36	141.67±4.71	290±14.14	139.17±11.14	296.67±15.06
Final body weight (gm)	141.67±14.71	290±14.14	139.17±11.14	296.67±15.06	156.66±16.93	324.17±3.76	149.17±9.48	300.33±13.88
Body length (cm)	15.1±1.12	18.O7±0.42	14.88±0.240	18.07±0.42	15.75±0.93	18.18±0.29	15±0.22	19.47±0.37
Abdominal circumference(cm)	10.4±0.63	13.5±0.34	10.43±0.46	13.73±0.47	10.9±0.65	10.71±0.46	14.6±0.27	12.9±0.28
BMI (gm/cm^2)	0.623±0.03	O.89±0.02	0.892±0.02	0.91±0.012	0.63±.88	0.98±0.02	0.66±0.03	0.791±0.008

Comparison of body weight before drug treatment and after drug treatment: Table 2: Effect of high fat diet and (AB) on body weights measured on 0,1st and 3rdweek of drug treatment

Time (weeks)	Body weight (g)			
	Normal control	Disease control	Treatment control	Treatment
0	141.66 ±14.71***	290 ± 14.142	139.17 ±11.14***	296.67±15.06
1	148.79±12.93***	318.19±15.21	146.83±7.94***	298.12±16.43
3	156.67±16.93***	324.17±3.76	149.17±9.47***	300.33±13.88

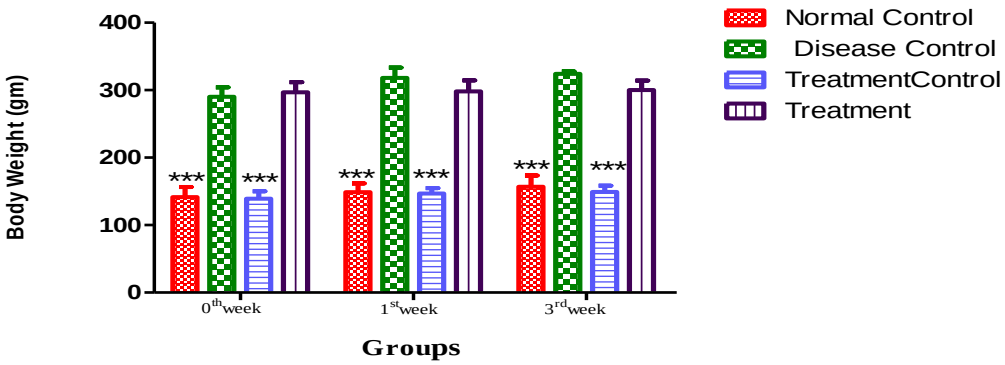

Fig1: Effect of high fat diet and (AB) on body weights measured on 0 week and 3rdweek of drug treatment.

All the values are expressed in mean ± SD of Body weights (n=6). *P<0.05, **P<0.01, *** P<0.001, ns-non-significant. Data was analyzed by one-way ANOVA followed by Dunnet's test. Comparisons are done between disease control and the remaining groups.

BODY MASS INDEX

Table 3: Effect of (AB) on body mass index measured on 0 week and 3rdweek of drug treatment

Time (weeks)	Body mass index (g/cm^2)			
	Normal control	Disease control	Treatment control	Treatment
0	0.62±0.03***	0.89±0.02	0.63±0.05***	0.91±0.01*
1	0.63±0.02***	0.93±0.01	0.65±0.01***	0.88±0.02*
3	0.63±0.02***	0.98±0.02	0.66±0.03***	0.79±0.08*

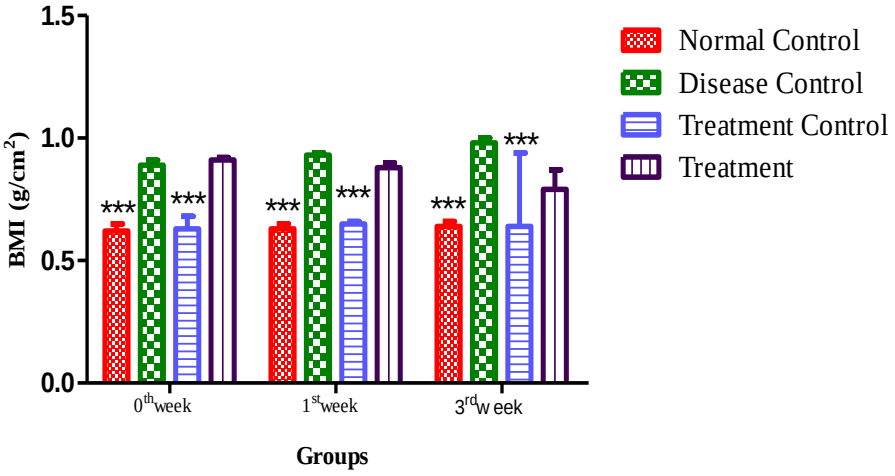

Fig 2: Effect of abhraka bhasma on body mass index measured on 0 week, 1st and 3rdweek of drug treatment.

All the values are expressed in mean ± SD of Body mass index (n=6). *P<0.05, **P<0.01, *** P<0.001, ns-non-significant. Data was analyzed by one way ANOVA followed by Dunnet's test. Comparisons are done between disease control and the remaining groups.

MEASURMENT OF ORGAN WEIGHTS:

Table 4: Measurement of organ weights on last day:

Organs	Organ weight (g)			
	Normal control	Disease control	Treatment control	Treatment
Heart	0.68	0.97	0.63	0.78
Liver	9.64	12.98	9.09	10.85
Kidney	1.56	1.97	1.41	1.85
Testis	4.06	2.94	3.56	3.27
Spleen	0.52	0.5	0.69	0.63

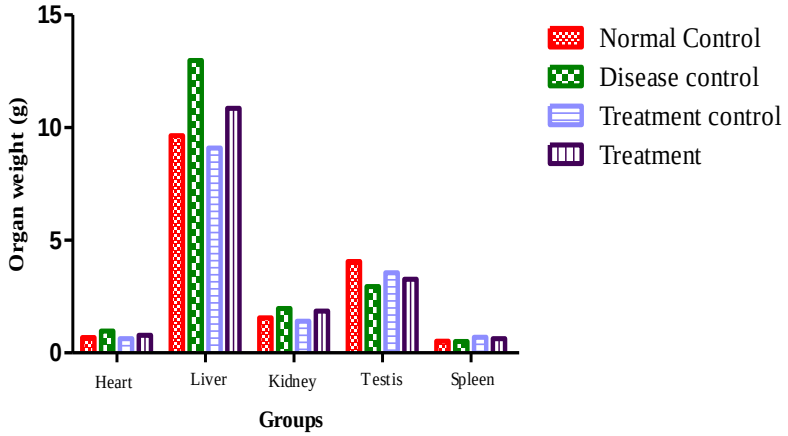

Fig 3: Measurement of organ weights on last day of the treatment.

All the values are expressed in mean ± SD of organ weights*P<0.05, **P<0.01, ***P<0.001, ns-non-significant. Comparisons are done between disease control and the remaining groups.

Table 5: Measurement of body fat pads on last day:

Fat pads	Fat pad weight (g)			
	Normal control	Disease control	Treatment control	Treatment
Mesenteric	1.15	1.95	0.92	1.21
Epididymal	1.42	3.51	1.39	2.06
Retroperitoneal	1.19	3.78	1.03	1.70

Table 5: Measurement of sperm count on last day:

Groups	Sperm count(millions/mm^3)
Normal Control	49± 3.8
Disease Control	27± 2.5
Treatment Control	69.5± 6.7
Treatment	54.25± 2.3

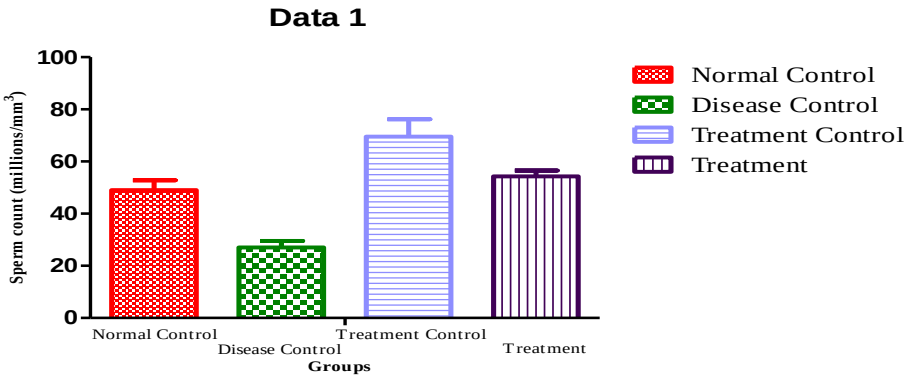

Fig4: Measurement of sperm count on last day

All the values are expressed in mean ± SD of sperm counts *P<0.0*, **P<0.01, ***P<0.001, ns-non-significant. Comparisons are done between disease control and the remaining groups.

HISTOPATHOLOGICAL STUDY OF TESTES

Testes of Normal control (normal diet + Vehicle)

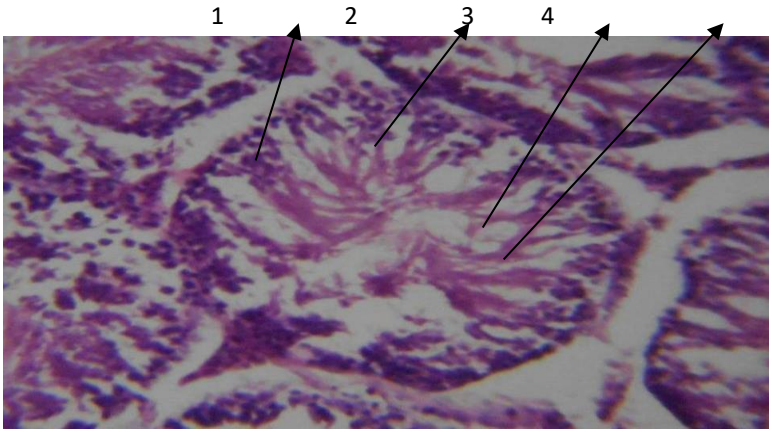

Fig :5(a) Rat Testes of Normal control Group (G_1) showing normal spermatogenesis after 8 weeks of experimentation. (1) Spermatogonia, (2) PrimarySpermatocyte, (3) Spermatozoa, (4) Tubular lumen.

Testes of Disease control (High fat diet + Vehicle)

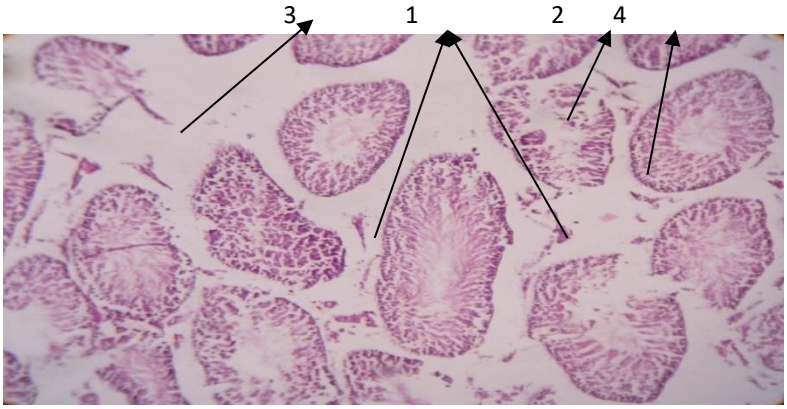

Fig : 5 (b) Rat Testes of Disease control group (G_2) after 8weeks of experimentation (1) Remains of interstitial cells, (2) disintegrated tubules, (3) Interstitial cells absent, (4) Normal tubule Showing normal Spermatogenesis.

Testes of Treatment Control (G_3) (Normal diet + Abhraka Bhasma)

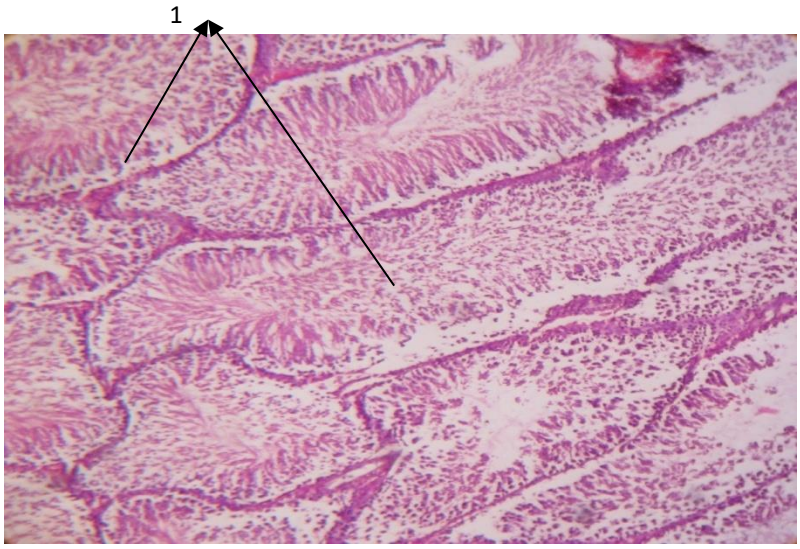

Fig 5 (c) Rat Testes of treatment control after 8 weeks of experimentation
1) Hyperactive tubules showing greater spermatogenesis.

Testes of Treatment Group (G_4) (High fat diet + Abhraka bhasma)

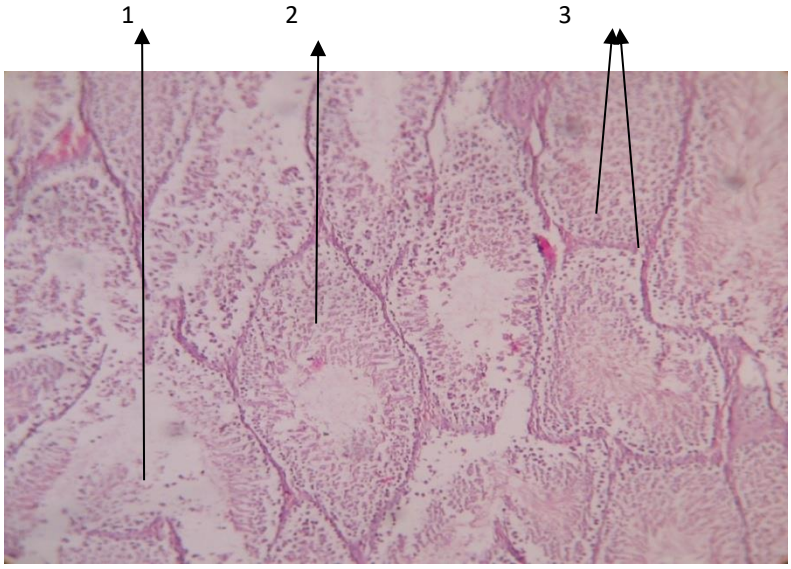

Fig: 5(d) Rat Testes of treatment Group showing cells in different stages of degeneration, recovery and fully recovered hyperactive germ cells after inducing obesity and then treatment with abhraka bhasma.
(1) Dead tubule, (2) Tubule showing normal spermatogenesis, (3) Hyper active tubules.

DISCUSSION

In this study, Abhraka Bhasma has been evaluated for fertility potential in obesity induced rats.

Anti-obesity activity in male rats

Body weights and fat pad weights in male rats:

The antiobesity effect in male rats decreases body weight and fat pad weights. In the present study after administration of Abhraka Bhasma with dose of 1.08mg/200g with honey showed no significant increase in body weight of obesity induced (high fat diet) rats. Whereas, in disease control group (only fat diet) there is a large increase in the body weight when compared with the normal group.

The fat pad weights are greater in disease control and slightly more in treatment group when compared to normal group.

Body mass index in male rats:

The antiobesity effect in male rats decreases the body mass index. This study showed that there is decrease in body mass index in (obesity induced Abhraka bhasma treated rats) treatment group. Whereas, there is great increase in the body mass index in the disease control group compared to normal and treatment control groups.

Biochemical estimation

From the results we observed that decrease in cholesterol and triglycerides levels in the treatment group which further increased fertility potential with the treatment of abhraka bhasma. There is an increased cholesterol and triglycerides levels in disease group which caused decreased fertility activity when compared to normal and treatment control group.

Fertility activity in male rats

Spermatogenic activity in male rats

The spermatogenic effect results in the great increase in the spermatogenesis. It is indicated by the increase in the sperm count. The results in the present study after administration of Abhraka Bhasma with dose of 1.08mg/200g with honey in obesity induced rats indicates that there is significant increase in the sperm count values when compared to disease control (only fat diet).

Histopathological study of testes

In Group 1 results showed that there is no significant change on microscopic examination, the testes appeared pink, firm, uniform in size and did not reveal anything abnormal. Gross examination of group 2 rat testes revealed that the overall spermatogenic profile in some tubules (50%) were considerably prevented in obese rats. It resulted in degenerated tubules. In group 3 testis contained numerous highly coiled seminiferous tubules and evidenced by highly granulated cytoplasm. About 60% hyperactive tubules and about 35% are in recovery stage and 5% are in degenerative stage in group 4 testis.

CONCLUSION

From the results it could be concluded that abhraka bhasma showed fertility activity in obese rats which was not in ancient literature.

The overall increase in the spermatogenic process and histometry of the testis is due to spermatogenic enhancing properties of bhasma by reducing BMI. Abhraka bhasma reduces infertility induced by obesity in high fat diet treated rats. This study confirms that Abhraka Bhasma is important tool in solving the problem of infertility in obese males due spermatogenic activity.

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