



Effect Of Suvarna Bhasma (Gold Calx) On Reproductive System Of Male Albino Rats

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Abstract: “Suvarna Bhasma” (gold calx) (SB) prepared as per Ayurveda, a practicing medical system, is prescribed as a medicine in several ailments including conditions like oligozoospermia and asthenozoospermia in male infertility. SB is prepared from pure metal gold by one of the processing methods detailed in Ayurveda classics. Gold along with other elements was reported in normal human seminal plasma. In human pathological semen samples, the level of gold was less than normal. Gold may have a role in physiological activity of sperm as shown in case of other elements. The present study supports the hypothesis that the presence of gold is essential for sperm motility. Here we studied the effect of SB on male reproductive organs and semen from epididymis of rat. Present study was performed using Charles foster strain albino rats. Rats were divided into two groups of ten each namely, Group 1 (control) and Group 2 (study). Animals in study group were orally given a fixed amount of SB for 15 days. At the end of the study treated animals showed increase in body weight (<0.05) and testicular weight. In treated animals total sperm count (<0.05) and percentage of sperm motility were increased in epididymal fluid. Histological study showed increase in interstitial area of testes (<0.001), proliferation and branching of the epithelial layer of seminal vesicle. In the Study (gold treated) Group, the increased gold level in genital system may be responsible for the increase in sperm production and motility.

Keywords: Ayurveda, Suvarna bhasma (gold calx), male infertility, animal study, Spermatogenesis

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1. INTRODUCTION

Infertility is considered as a serious issue by medical professionals. World Health Organization¹ defines infertility as “a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (and there is no other reason, such as breastfeeding or postpartum amenorrhoea). Primary infertility in a couple is failure in achieving pregnancy even after unprotected sexual intercourse. Secondary infertility is failure to conceive following a previous pregnancy. Infertility may be caused by infection in the husband or wife, but often there is no obvious underlying cause”¹, genetic disorders, vitamin deficiency, thyroid disorders, sexually transmitted diseases, diabetes, obesity, cardiovascular diseases, stress, smoking and other environmental factors.² When a childless couple reports to a clinician for treatment both husband and wife are advised for investigations like hormone and genetic testing. In husband semen analysis is advised if necessary testicular biopsy. The knowledge on male responsible factors is not fully known. The high rate of male infertility reported from world over supports this³⁻⁵. Despite advances in the understanding of male infertility, idiopathic sperm abnormalities still account for about 30% of male infertility⁶ Limited knowledge of semen is the major reason. Different groups of workers showed some constituents in terms of organic and inorganic substances present in sperm and plasma. Inorganic elements like Calcium, Chloride, Citrate, Fructose, Glucose, Lactic acid, Magnesium, Potassium, Sodium, Zinc secured large attention⁷⁻¹². The presence of gold in normal human semen was reported in 1981^{13, 14, 15, 16}. Gold in semen was studied under Instrumental Neutron Activation Analysis¹³. In seminal plasma of normozoospermia level of gold observed was 0.37 ± 0.09 mg% and in spermatozoa which was 0.23 ± 0.07 mg%¹⁷. In all pathological samples studied which was less¹⁷. Gold was shown as present in testes and caput epididymis of rats^{18, 19}. All above studies permit us to presume gold as essential for functioning of sperm. Ayurveda, the oldest system of Medicine, established in India, practices remedies for all illnesses. In Male infertility “Suvarna Bhasma” (SB) (gold calx), is one of the medicines preferred. Rasaratna Samuchchaya mentioned gold as Agriyam (best) in aphrodisiac. A drug with gold in it is the choice of many clinicians for treating male infertile patients²⁰. Recently it was reported that, gold, copper and zinc are present in semen in a complex form²¹ so chances are there to contain such complex forms of gold in Swarna *bhasma* also. Limited studies are conducted in laboratory animals to find out the effect of gold nanoparticles. Nazer et al. (2016)²² observed sperm motility and sperm morphology were reduced in animals which depended on the dose and duration of gold nanoparticles. Four days of treatment with gold nanoparticles did not cause any disturbance in the structure of spermatogenic epithelium but affect the chromosomes of early spermatocytes²³. Liu et al. (2020)²⁴ reported after the treatment with gold nanoparticles, a significant increase in the rate of sperm malformation but without affecting fertility. Injected gold nanoparticles accumulate in the connective tissue sheath of testes and an insignificant amount was also seen in convoluted seminiferous tubules²⁵. In summary nanoparticles cross the blood test barrier and accumulate in the testes causing adverse effects on spermatogenesis²⁶.

The aim of the present study was to find out the effect of SB in albino rats, on the cytoarchitecture of testes, seminal vesicles, ventral prostate, total sperm count and percentage of sperm motility.

2. MATERIALS AND METHODS

The study was conducted at the Institute for Post Graduate Teaching and Research in Ayurveda under Gujarat Ayurveda University, Jamnagar. The details of animal study showing the type, number and sex and the experimental procedures were presented to Animal Ethical Committee of the Institute for their approval. The study was carried out in the institute after getting the approval (GAU/AEC/02/18). Charles foster strain male albino rats were used in this study. They were bred and maintained in the animal house of the Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurveda University. Inside animal house temperature was maintained between 20 and 22°C by using air conditioners. They were fed rat pellets. Tap water was given ad libitum. This study is the first of its kind using SB in animal experiments. The total study period was 15 days.

The Suvarna Bhasma (Gold Calx) used in this study was purchased from Ayurveda Vikasa Mandala Pharmacy, Junagadh. Twenty male albino rats weighing 140-150g were chosen for the study. They were divided into two groups of ten each. In Group 1 (Control), Animals were given 1ml of Lactose (1 mg/ml) to which one or two drops of honey was added²⁷. In Group 2 (Study), Animals were given SB (0.800 mg/ml)²⁷, Lactose and distilled water. One or two drops of honey was added to SB to make it a suspension. On 15th day of study, under general anaesthesia (chloral hydrate, 3.6% - 1 ml/100gm body weight) incision was made in the inguinal region and about 100mg of cauda epididymal tissue was excised, transferred to physiological saline (0.5ml) and teased with forceps to liberate spermatozoa. The incision was closed in layers. Cauda epididymis suspension was examined for sperm count in million/ml, percentage of motility and morphology²⁷. The standard decimal system was adopted to calculate the percentage.

3. STATISTICAL ANALYSIS

Simple statistical study was employed by using Graphpad Instat to find out if any significant difference was present when values of two groups were compared. (<https://www.graphpad.com/scientific-software/instat/>)

4. RESULTS

Data showing the effect of SB on rats is presented in Table 1. In Study Group, after the treatment a significant (<0.05) increase in body weight (20g) absolute weight of testes was observed (Table 1). In Study Group a significant (<0.05) increase in caudal sperm count (627.8) was seen. There was a noticeable increase in the rapid linear progressive movement of spermatozoa in Study Group. Microscopic examination of prostate, seminal vesicle did not show any noticeable change. In study group tubular volume in testis has decreased permitting more space for interstitial area (Figure 1). In study group an increase in the size of alveoli, marked proliferation and branching of the epithelial layer of seminal vesicle was seen.

Table 1. Showing the results of the total study

	Control (n=10)		Study(n=20)	
	Before Treatment	After Treatment	Before Treatment	After Treatment
Body weight (g)	141	148	139.8	160 (<0.05)*
Sperm count (million/ml)	43	271	45.6	573.4 (<0.05)*
Motility (%)	64	79	65	85.2
Weight of testis (g)	2.08		2.16	
Weight of seminal vesicles (g)	0.677		0.730	
Ratio of relative weight of testes and body weight	0.014±0.0013		0.013 ± 0.001	
Area of Interstitial tissue	822.8 ±60.8		2425 ±299.3 (<0.001)*	
Number of seminiferous tubules per field	28.08 ±4.1		38.81 ±3.5	
Diameter of seminiferous tubules	13.00 ±1.00		11.50±0.63	

*p value significant, ± SD

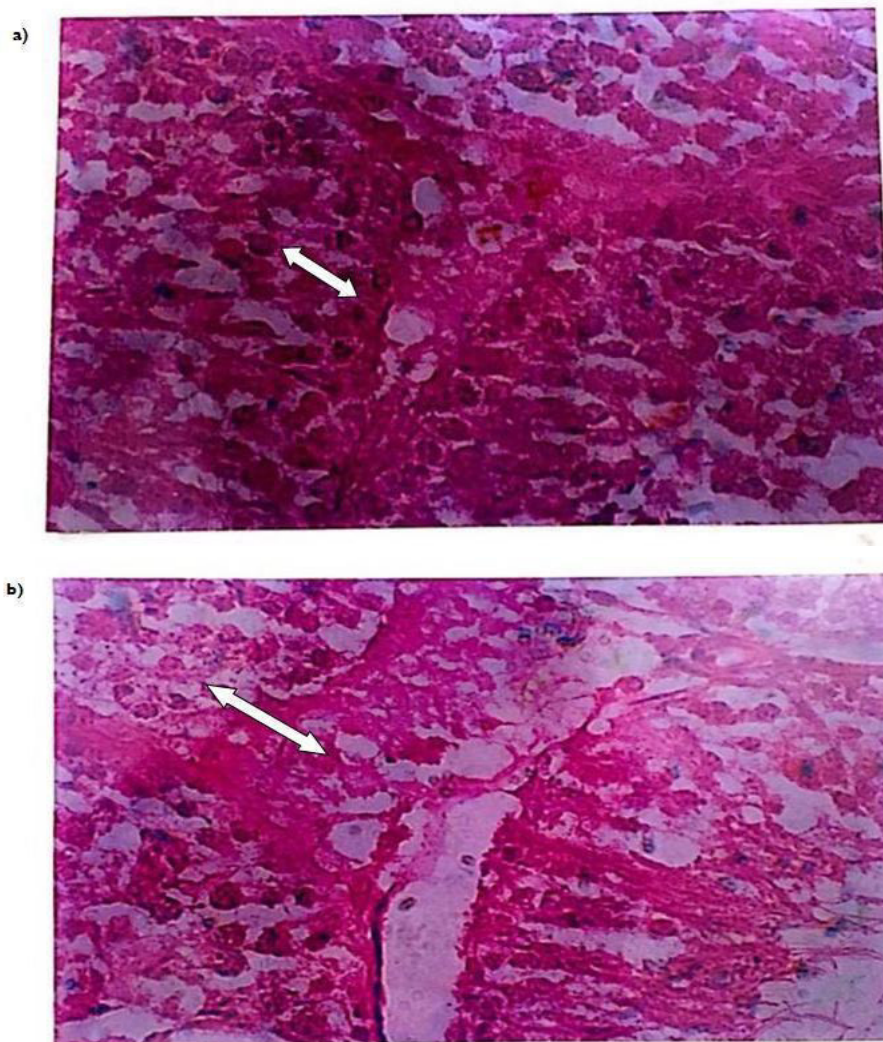


Fig. 1Photographs showing interstitial tissue per field of a) control and b) suvarna bhasma treated rats (magnification X 400)

5. DISCUSSION

In the present study SB was administered to animals in study group. SB is prepared from pure metal gold by one of the processing methods detailed in Ayurveda classics²⁸. SB is prescribed to patients in some clinical conditions including male infertility. Results of different studies in seminal plasma of normal and pathological samples showed gold as an essential element for fertility.^{13, 21} In this study, we observed weight gain of animals in study group (SB) which may be due to increased androgen level (Table 1). The ratio of the relative weight of testes with body weight was almost identical in both groups. Reduction observed in tubular volume in study group was probably to accommodate extra growth of interstitial space (Fig. 1). Decrease in the diameter of seminiferous tubules seen in study group may be due to contraction of interstitial tissue (Table 1). The

interstitial tissue consists of an irregular meshwork of loose connective tissue and Leydig cells. The endocrine role of Leydig cells is known since 1905²⁹. Fawcett and co-workers³⁰ studied interstitial tissue in various mammals. Christenssen³¹ observed in man and rat a corresponding increase in volume of interstitial tissue and Leydig cells, its size and daily production of testosterone. The daily output of testosterone in rat was reported as 0.44pg³² which diffuses into the interstitial tissue and seminiferous tubules either through lymph vessel or myoid cell layer^{33, 34}. Mori et al.³² observed environmental changes influence number and size of Leydig cell population. The seasonal variation of Leydig cells in rats is known^{30, 33, 34}. Given above two reasons are not applicable in case of the present study, as 1. the total study period was a short span of 15 days and 2. no change in atmosphere was present during study period to influence spermatogenesis³³ and number of Leydig cell³⁰. This

explains the basis of increase in testosterone level. The endocrine product of Leydig cells promotes spermatogenesis. The time period of spermatogenesis in rats is 48 days³³. Fifteen days is a short span of time to increase sperm count. Our present experimental study showed SB increased stroma and Leydig cell population in turn which increased production of testosterone. Action of SB may be leading to increase the contractile mechanism of seminiferous tubules, rete testis, caput epididymis and corpus epididymis which push out stored spermatozoa from these organs. SB also may be increasing the activity of cilia present to bring out more sperm³⁶. The movement of spermatozoa is assisted by contraction of myoid elements present around seminiferous tubules^{34, 35} and by contraction of the testicular capsule. Inside the tubule, testosterone promotes spermatogenesis^{37, 38}, enhancing and maintaining the motility of spermatozoa. Gold present in Suvarna Bhasma (Gold Calx) is the likely responsible factor for increase in sperm motility²⁸. The other possibility of the action of SB is as follows. Gold was shown to remain present in all normozoospermic semen samples.^{12, 18, 19, 39, 40}. The locations of entry are likely to be caput epididymis and prostate gland¹⁴. Histochemically gold particles were shown on the rim of seminiferous tubule²¹. Results of all studies lead to the conclusion that gold is present in the fluid medium of male genital system and is essential for sperm motility. Studies showed gold level was less or absent in semen where sperm motility was poor^{12, 19}. In the present study, probably the gold level was high in genital system on gold treated animals and which was responsible for the increase in the

quality of motility. The present study is showing gold is important for sperm production and its motility which is supported by our earlier clinical study³⁹ and experimental study⁴⁰.

6. CONCLUSION

Albino rats treated with Suvarna Bhasma (SB) (0.800mg per day) for 15 days showed significant ($p < 0.05$) increase in body weight, Total Sperm Count and Percentage of Sperm Motility. In treated animals increase in area of interstitial tissue was seen which was statistically significant (< 0.001). Study shows gold directly acts on male reproductive system. In conclusion, SB prescribed to male infertile patients is beneficial for increasing total sperm count and percentage of sperm motility.

7. AUTHORS CONTRIBUTION STATEMENT

Godatwar P, Prasad BS, Skandhan KP, Mehra BL, Singh G, and Mounika B contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript. All the authors read and approved the final version of the manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none

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