



# EFFECT OF SHATAVARI GHRITA (POLYHERBAL FORMULATION) ON TESTICULAR DYSFUNCTION IN STREPTOZOCIN INDUCED DIABETES IN WISTAR ALBINO RATS

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**Abstract:** Shatavari ghrita is a herbal medicine containing Shatavari (*Asparagus recemosus*) root, milk and ghee in the ratio of 1:4:16. Diabetes mellitus can damage the gonads, leads to spermatogenic cell damage. To evaluate the effect of SG on testicular dysfunction by streptozocin induced diabetes in wistar albino rats. The dose should be selected by acute toxicity study and it has no morbidity and mortality rate so it has been selected that 1/5th dose (400mg/kg) and 1/10th dose (200mg/kg). 30 wistar albino rats were divided into 5 groups (n=6) Group I (control), Group II to IV treated with single dose injection (i.p) of STZ (40mg/kg). After induction of diabetes, Group II has diabetic control, Group III treated with mucuna pruriens chooranam (200mg/kg), group IV treated with SG 200mg/kg and Group V treated with SG 400mg/kg for 60 days. On the day 61, rats were sacrificed, FBG estimated, semen collected from epididymis, serum collected for antioxidant enzymes and MDA level and histopathological examination. All the data were analysed by one way ANOVA followed by Dunnett's test. In SG 400mg/kg, there was increased in sperm count, motility and viability, increased reproductive organ weight, increased serum testosterone level and anti-oxidant activity, decreased the level of MDA and blood glucose level. Present findings provide experimental in-vitro and in-vivo evidence that the Shatavari ghrita reversed by the diabetes induced testicular dysfunction. Phytochemicals present in SG possess anti-hyperglycemic and anti-oxidant properties.

**KEYWORDS:** Shatavari ghrita, Diabetes mellitus, Testicular dysfunction, Streptozocin, Mucuna pruriens

## I. INTRODUCTION

Diabetes mellitus is a common metabolic disorder, characterized by hyperglycemia. Type 2 DM which contributes major share of total diabetic population occurs due to diminished insulin secretion or insulin resistance. There is an increasing trend of diabetic population encompassing 108 million in 1980 to 422 million in 2014.(1) Hyperglycemia is a major cause of serious macro-vascular diseases, affecting almost every system in the body. Both male and female reproductive system has been suffer with diabetes mellitus. About 90% of diabetic patients usually suffer from sexual dysfunction that includes decrease in libido, impotence, erectile dysfunction and infertility. Various studies showed that two factors such as increased oxidative stress and decrease in antioxidant capacity. Diabetes mellitus that seriously endangers in human health. It is due to the absolute or relative deficiency of insulin (INS) and insulin resistance

(INR) which leads to decrease in the insulin utilization rate. In patients with DM, long term hyperglycemia can cause diabetic vascular neuropathy. Diabetes also leads to oxidative stress, abnormal zinc metabolism and resistance syndrome, all of which affect male fertility and reproductive health.

## II. RESEARCH METHODOLOGY

### Maintenance of animals:

In this study, male Wistar Albino rats (150-250 gm) were used as test animals and were obtained from Animal Experimental Laboratory, Madras Medical College, Tamil nadu, India. The rats were maintained under temperature controlled environment at a temperature of  $25^{\circ}\text{C} \pm 20\text{C}$  with a 12-h light and 12-h dark cycle. The rats were individually housed in polypropylene cages (18"×10"×8") lined with sterilized paddy husk and provided filtered tap water and rat food ad libitum. The study was approved by the Institutional Animal Ethics Committee. All the experiments were carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India (Proposal Number : 08 / SA / IAEC / MMC / 2022)

### ACUTE TOXICITY STUDY:

**Principle and Purpose:** The main purpose of acute toxicity is to evaluate the degree of toxicity in a quantitative and qualitative manner with the purpose of comparing it with other drug substance.

**Experimental Animals:** Three healthy adult wistar albino rats weighing between 150-250gm were selected for the study. For all the three animals food, but not water was withheld overnight prior to dosing.

**Selection of dose levels and administration of dose:** Being a traditional herbal formulation, the mortality was unlikely at the highest starting dose level (2000mg/kg b.w). Hence a limit test one dose level of 2000mg/kg b.w was conducted in all the three animals as per the OECD guidelines 423.

**Procedure:** Animals are observed individually after dosing at least once during the first 30mins, periodically during the first 24 hours with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal.

**Observation:** The following clinical observation were made and recorded 1. Pre-terminal deaths 2. Body weight 3. Cage side observation such as the home cage activity, colour and consistency of the faeces, behavior of the animal were observed once in two days. 4. Physical examination Observations include skin and fur, eyes i.e. lacrimation, eye prominence, eye lid closure corneal reflex, lighting reflex and mucus membrane. Attention was directed to sign of tremor, convulsion, salivation, lethargy, diarrhea, sleep and coma noted. Tail elevation, static limb position, head position, righting reflex and pinna reflex were monitored.

### Induction of Diabetes

Diabetes was induced by (except 1st group) administration of single i.p. injection of Streptozocin in 0.1M citrate buffer at a dose of 400mg/kg in overnight fasted rats. Fasting blood glucose level was measured on day 2 and day 7 and individual fasting blood glucose level above 250mg/dl was considered as Diabetic.

### Grouping of animals

Totally 30 male albino rats will be used in this study. The animals are divided into 5 groups, each group containing 6 animals. Group I (control), Group II to IV treated with single dose injection (i.p) of STZ (40mg/kg). After induction of diabetes, Group II has diabetic control, Group III treated with mucuna pruriens chooranam (200mg/kg), group IV treated with SG 200mg/kg and Group V treated with SG 400mg/kg for 60 days.

### Necropsy

After completion of the experimental period (60 days), the rats were fasted overnight. On the 61st day, the rats were weighed and sacrificed by cervical dislocation. Testes and epididymis are dissected out and weighed for the nearest milligram immediately using electronic balance after clearing off the adhering tissues.

### Evaluation Parameters

#### 1. Reproductive organ index

From the weight of testes, epididymis, seminal vesicles, vas deferens and prostate. Reproductive organ indices were determined using the formula

$$\text{TSI} = [\text{weight of the tissue (g)} / \text{Body weight of the animal (g)}] \times 100$$

## 2. Sperm parameters

### Epididymal sperm analysis

To analyze the percent motile sperm, the diluted epididymal fluid is placed in Neubaur hemocytometer and total, motile and non motile sperms are counted. The number of motile and non motile sperms will be determined microscopically. For the analysis of the live and dead sperms, trypan blue reagent method was used in this study. Briefly, one drop of the diluted epididymal sperm suspension were mixed with one drop of 1% trypan blue solution and incubated at 37°C for 15mins. The mixture was placed on a microscope slide, covered with a cover slip and observed under a microscope. Sperms are considered as viable if they are unstained and considered as dead if they are stained with the trypan blue.

## 3. Estimation of lipid peroxidation in Serum

In this study, the levels of TBARS was analyzed spectrophotometrically in the serum of controls and experimental rats 0.25ml of TBA reagent was added with serum and the mixture was incubated at 95°C for 1hour. Finally an equal volume of n-butanol were added and the contents were centrifuged for 15min at 4000 rpm. The organic layer was carefully transferred into a clear tube and its absorbance were measured spectrophotometrically at 532 nm. The rate of lipid peroxidation was expressed as  $\mu$  moles of Malonaldehyde formed/ ml of serum.

## 4. Assay of anti-oxidant enzymes

### A. Super oxide dismutase

The reaction mixture contained 0.05M carbonate buffer (pH 10.2), 30mM epinephrine (freshly prepared) and the serum. Changes in absorbance are recorded at 480 nm and measured at 10s intervals for 1 min in a spectrophotometer. The enzyme activity was expressed as units pg/ml.

### B. Catalase

The reaction mixture in a volume of 2.5 ml contained 0.05M phosphate buffer (pH 7.0), 19 mM H<sub>2</sub>O<sub>2</sub> and appropriate amount of serum. Then the absorbance was read at 240nm and measured at 10s intervals for 1 min in a spectrophotometer. CAT activity was expressed as nmol/mg.

### C. Glutathione peroxidase

The reaction mixture contained 1.59ml of 100mM phosphate buffer (pH 7.6), 0.1 ml of 10 mM EDTA, 0.1ml of 10mM sodium azide, 0.1ml of glutathione reduced, 0.01 ml of 0.2 mM hydrogen peroxide and 0.1 ml of serum. Immediately, the contents was read at 340 nm against blank, at 10s intervals for 3mins on a spectrophotometer. The activity of GPx was expressed as pg/ml.

## 5. Estimation of serum Testosterone level

Serum testosterone level was assayed using ECLIA (Electro chemiluminescence immunoassay) method based on the principle of competitive binding.

## 6. Histopathological analysis

A single testes and epididymis was isolated from each rat in all groups and then fixed in 10% neutral buffered formalin for 24 hours. The fixer was washed with running tap water overnight. The tissues were cleaned with methyl benzoate after drying using a graded series of alcohols and embedded in wax with paraffin. At 6  $\mu$ m thickness, testes and epididymis sections were cut stained with hematoxylin and eosin dissolved in 95% ethanol used to stain the counter. Testes and epididymis sections were observed under a microscope after dehydration and clearing.

### Statistical analysis

Data are presented as mean  $\pm$  SEM and the values of  $P < 0.01$  were considered statistically significant. Statistical analysis between the control and experimental groups was analyzed using one way analysis of variance followed by Dunnett's multiple comparison test using Graph Pad Prism, version (8.0.2).

### III. RESULTS AND DISCUSSION

#### Results of diabetes induced testicular dysfunction

table 1: changes in bw, sperm parameters, lipid peroxidation and serum testosterone level.

Groups	Sperm count ( $\times 10^8$ )	Sperm motility (%)	Sperm viability (%)	Lipid peroxidation (n mol/ml)	Testosterone (ng/dl)	Body weight change (%)
Normal control	130 $\pm$ 1.45	78.1 $\pm$ 0.8	58.2 $\pm$ 1.4	138 $\pm$ 3.46	10.33 $\pm$ 0.33	12.73
Disease control	58 $\pm$ 5.13	29.1 $\pm$ 1.2	27.5 $\pm$ 2.3	1031 $\pm$ 20.79	8.6 $\pm$ 0.16	-14.93
Standard control	142 $\pm$ 2.64	74.3 $\pm$ 2.93	57.7 $\pm$ 0.5	230 $\pm$ 23.18	12 $\pm$ 0.28	6.17
Treatment control I	127 $\pm$ 2.3	69.5 $\pm$ 0.35	52.2 $\pm$ 2.1	448 $\pm$ 23.33	9.6 $\pm$ 0.44	6.96
Treatment control II	139 $\pm$ 0.88	80.4 $\pm$ 0.43	60.3 $\pm$ 1.7	264 $\pm$ 15.71	11.8 $\pm$ 0.16	7.29

**ACUTE TOXICITY STUDY:** During the experiment, there is no signs of toxicity were noticed such as pre-terminal deaths, body weight, cage side observation and physical examination and no mortality occurred in all the exposed animals.

**IN-VIVO STUDY:** The differences in body and sexual organ weights were taken as a valuable index for reproductive health and toxicological studies. To identify the toxic effects of STZ and the potential protective role of Shatavari ghrita on rat reproductive system, sperm parameters, oxidative stress parameters, changes in the body and testicular weights were evaluated. STZ is targeting effects are known as oxidative stress.

Since oxidative stress can affect the steroidogenic potential of Leydig cells in the testis, as well as the germinal epithelium's ability to identify normal spermatozoa. administration of STZ and Shatavari ghrita significantly decreases the serum SOD level when compared to STZ alone exposed rats. It is noteworthy to mention that 200mg/kg of Shatavari ghrita exposed rats, the SOD level (204.66  $\pm$  2.02 pg/mg) is significantly decreased when compared to normal value (321.66  $\pm$  3.28 pg/mg) and 400mg/kg dose group showed marginally more than normal value (247.66  $\pm$  2.02 pg/mg).

These harmful effects of the STZ on sperm quality were related to the damage of leydig cells in the testicular tissue, which affected the Sertoli cell group resulting in low sperm formation and decreased sperm count. Another possible mechanism may be the inhibition of mitochondrial ATP output by uncoupling oxidative phosphorylation leading to ROS generation. This inhibition can affect the mitochondrial enzyme activities, suppressing the cell's ability to preserve their ATP levels and then damages the sperm microtubules' structure and disrupts their normal functions. In seminiferous tubules, testosterone is essential for the continual development of distinct germ cell generation. Thus, the decrease in testosterone levels found in this study may contribute to the sloughing of germ cells from the seminiferous epithelium as well as the reduction in sperm characteristics.

A substantial elevation in the lipid peroxidation (MDA) levels (**Table 1**) has been obtained in serum after the STZ treatment. In rats which were treated with Shatavari ghrita and STZ, there was a significant reduction in the Malondialdehyde levels where high dose group shows more reduction than low dose group.

The sperm quality is a critical feature measurement for predicting sperm fertility and a sensitive index for studying the impact of various chemical and physical factors on reproductive cells. In the present study, STZ affect markedly sperm characteristics through a disruptive effect on sperm count (Figure 1 and 2) and decrease the percentage of sperm motility, viability, (**Table 1**) and normality, which may cause infertility. However, administration of Streptozocin and Shtavari ghrita and Mucuna pruriens chooranam restored the reduced fertility parameters such as reproductive organ weights (Table 2) especially testes, epididymis, sperm counts, sperm motility and sperm viability as compared to STZ treated rats.

These testicular damages were significantly mitigated by administration of Shatavari ghrita and Mucuna pruriens chooranam, indicating that Shatavari ghrita protects the testicular tissue from the histological damage (Figure 1 to 5) mediated by STZ. The protective role of Shatavari ghrita against STZ induced male reproductive toxicity may be due to presence of zinc which has very significant role in maintaining normal male reproductive function.

**Table 2** Shows reproductive organ weight and serum anti-oxidant enzymes.

Groups	Testes	Seminal vesicles	Prostate gland	Epididymis	Vas deferens	Serum SOD (pg/mg)	Serum CAT (mol/mg)	Serum GPX (pg/ml)
Normal control	3.80±0.006	1.06 ± 0.02	1.24±0.01	0.65 ± 0.02	0.28±0.01	256.66±4.05	313.01±4.16	57.33 ± 2.33
Disease control	1.37 ± 0.02	0.52 ± 0.008	0.37±0.002	0.4 ± 0.01	0.14±0.01	114.05±3.78	210.33±10.86	36.66 ± 2.96
Standard control	3.95 ± 0.06	0.93 ± 0.01	1.13 ± 0.05	0.68 ± 0.01	0.24±0.02	242.05 ± 5.5	323.01 ± 5.56	60.33 ± 0.33
Treatment control I	3.15 ± 0.06	0.81 ± 0.01	0.71 ± 0.02	0.56 ± 0.02	0.21±0.02	204.66±2.02	302.33 ± 2.96	49.33 ± 0.88
Treatment control II	3.77 ± 0.04	1.00 ± 0.01	0.98 ± 0.01	0.69 ± 0.006	0.29±0.003	247.66±2.02	325.66 ± 4.09	63.01 ± 0.99

## Figures

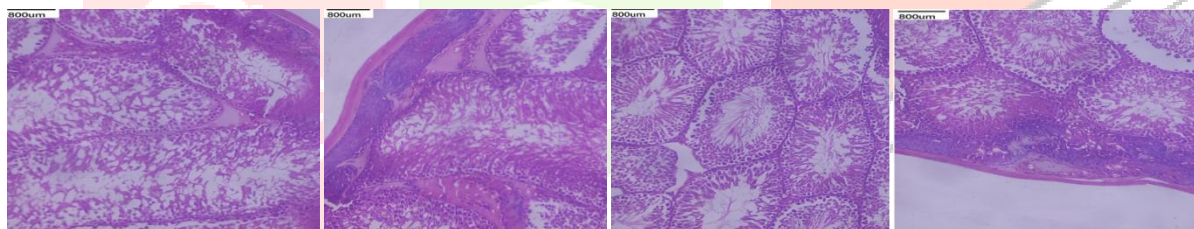


FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

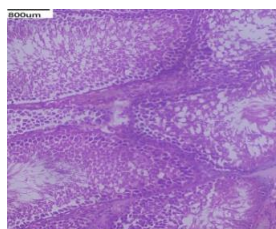


FIGURE 5

Figure 1 shows multilayered epithelium with most mature cells towards lumina, Figure 2 shows more vacuolation in the seminiferous epithelium, figure 3 shows serial arrangement of spermatogenic cells, figure 4 shows partial loss of germinal cells and decrease in number of vacuoles and figure 5 shows recovery of germinal cells and luminal spermatozoa

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#### V. REFERENCES

1. Panigrahy Ankita, Behera Jeyanthi Prav, Priyadarshi Kunal et.al. Effect of Pedalium murex dry seed extract on testicular dysfunction in streptozocin induced diabetes in wistar albino rats: data from Biomedical and Pharmacology journal. DOI: <https://dx.doi.org/10.13005/bpj/126>
2. Zubin he, Guocha yin, Qingdi Quentin et.al. Diabetes mellitus causes male reproductive dysfunction. A review of the evidence and mechanisms: data from in vivo 35:2503-2511 (2021).
3. Saurabh S.Talathi, Ryan Zimmerman et.al. Anatomy of pancreas: data from National Library of medicine.
4. Collingwood et.al. Insulin and insulin resistance. The clinical biochemist reviews. Data obtained from 2005 May 26(2) 19-39.
5. <https://training.seer.cancer.gov/anatomy/reproductive/male/>
6. Chindambara priya Dharshini kottaisamy et.al. Experimental animal models for diabetes and its related complications – a review. A national library of medicine. Data obtained from 2021 August 24 doi: 10.1186/s42826-021-00101-4.
7. C.Ronald Khan et.al. Joslin's diabetes mellitus,14th edition, 3rd Indian reprint , New Delhi: wolters kwluwer (India) pvt.Ltd 2012 p.464
8. Agarwal A, Mulgund A, Hamada A, Chyatte M R. A unique view on male infertility around the globe. *Reprod BiolEndocrinol.* 2015;13:37.
9. Cocuzza M, Alvarenga C, Pagani R et al. The epidemiology and etiology of azoospermia. *Clinics.* 2013;68(S1):15-26.
10. Anusha Bhaskar, V.G.Vidhya, M.Ramya et.al Hypoglycemic effect of Mucuna pruriens seed extract on normal and streptozotocin -diabetic rats: data from *Fitoterapia* 79(2008) 539-543.
11. Jung A, Schuppe HC. Influence of genital heat stress on semen quality in humans. *Andrologia.* 2006;39(6):203-215
12. Kolesnikova L I, Kolesnikov S I, Kurashova N A, Bairova T A. Causes and factors of male infertility. *Vestn Ross Akad Med Nauk.* 2015;5:579-584.
13. Kidd SA, Eskenazi B, Wyrobek AJ. Effects of male age on semen quality and fertility: a review of the literature. *Fertil Steril.* 2001;75(2):237-248.

14. Harris ID, Aaron KS, John CP. Ultrasonographer experience does not impact outcomes following ultrasound-guided embryo transfer. *Fertil Steril*. 2009;92(3):918- 922.
15. Vikas Sharma and V.K.Dixit et.al. A comparative study of erhanolic extract of pedaliu murex fruits and Sildenafil citrate on sexual behaviors and serum testosterone level in male rats during and after treatment. Data from *Journal of Ethnopharmacology* volume 143, issue 1. 30 august 2012, pages 201-206.
16. Kamala Kant Shukla and Abbas Ali Mahdi et.al. *Mucuna pruriens* improves male fertility by its action on the hypothalamus-pituitary-gonadal axis: data from *Fertility and Sterility* 92 (6) December 2009.
17. Reshma Saokar et.al. A review of Shatavari ghrita: data from *International Ayurveda Publications* in June 2016.
18. Momoh AR, Idonije BO, Nwoke EO, Osifo UC, Okhai O, Omoroguiwa A, Momoh AA. Pathogenic bacteria-a probable cause of primary infertility among couples in Ekpoma. *J Microbio Biotech Res*. 2017;1(3):66-71.
19. Moretti E, Capitani S, Figura N, Pammolli A et.al. The presence of bacteria species in semen and sperm quality. *J Assist Reprod Genet*. 2009;26(1):47-56.
20. Junior W B, Shiratsu R, Pinto V. Approach in sexually transmitted diseases. *An Bras Dermatol*. 2009;84(2):151-159.

