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**Scientific validation of *Dhadhu virthi kuligai* for its  
spermatogenesis activity in wister albino rats by ethanol  
induced method**

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**Abstract**

Male infertility is common nowadays due to various reasons because of the changing human lifestyles. Since vedic times so many herbals were being in use to promote spermatogenesis without causing any side effects. Infertility affects peoples not only physically, cause some psychological stress also. Siddha system is uncompetable in giving treatment to male infertility while comparing other medical systems. Many herbals are indicated to treat male infertility because of their potent spermatogenesis activity. *Dhathu viruthi kuligai* is a specialised poly herbal formulation which was indicated for spermatogenesis in siddha sastric texts. This siddha drug was evaluated in wister albino rats by ethanol induced method for its spermatogenesis activity. This research study on siddha poly herbal formulation *Dhathu viruthi kuligai* confirms its promising potent spermatogenesis activity. This work will be a platform for further clinical trails on this effective drug to treat male infertility.

**Keywords:** Dhathu Viruthi, Spermatogenesis, Siddha tonic, Herbal, male infertility.

**Introduction**

Traditional system of medicine especially *Siddha* system has a remarkable role in the treatment of infertility. Many infertility patients of idiopathic origin have effectively recovered with *Siddha* medicine. A recent health survey revealed some startling facts a whopping 46% of Indians, between the ages of 31 to 40, require medical intervention to conceive as one or both partners suffer from fertility problems. While polycystic ovarian disorder was the main reason in women, poor sperm count topped the list in men. Male infertility has been attributed to a variety of causes including lifestyle factors, gonadotoxin exposure, hormonal dysfunction, chromosomal disorders, varicoceles, testicular failure, ejaculatory disorders and obstruction. *Dhadhu virthi kuligai* is a poly herbal formulation referred from *Noikalukku siddha parigaram* (part -2), The ingredients

of this *kuligai* are used for male infertility in Siddha system<sup>[1]</sup>.

**Materials and Methods**

**Procurement and rearing of experimental animal:**

Adult male Wistar rats weighing 180-210 gms were used for this study. The inbred animals were procured from the animal house of TANUVAS, Madhavaram, Chennai and the study was conducted at National Institute of Siddha, Chennai, India. They were housed six per cage under standard laboratory conditions at a room temperature at 22±2<sup>0</sup> C. The animals were subjected under standard photoperiodic condition of 12:12 hrs light:dark cycle. The animals were fed with standard rodent pellet procured from Sai meera foods Pvt Ltd,

Bangalore and water ad libitum. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. The protocol for experimentation was approved by Institutional Animal Ethics Committee (IAEC Approval No: 1248/AC/09/CPCSEA-9/DEC 2013/12) of National Institute of Siddha, Chennai, India.

### Experimental design:

Sample Size: 24 albino rats  
Experiment Duration: 60 days

### Animal grouping and interventions:

The animals were randomly selected and divided into four groups (I, II, III and IV) of six rats (n=6) each.

Individual identification of the animal was made by marking. Group I animals served as control and received only 1ml milk, p.o. for 60 days. *Group II* served as negative control (Alcohol induced), received 0.5 ml of 25% ethanol/kg/BW/day for 60 days. Experimental groups split into group III and IV served as the treated groups and received DVK which was grounded in mortar-pestle with milk. *Group III* was treated with 25% of 0.5 ml ethanol/kg/BW/day along with 100mg/kg of DVK orally once for 60 days. *Group IV* was treated with 25% of 0.5 ml ethanol/kg/BW/day along with 200mg/kg of DVK orally once for 60 days. Administration was done once a day by oral gavage in the morning.

Groups	Intervention	No of Rats
Group I - Normal Control	1 ml of Milk	6
Group II - Negative Control	25% of ethanol (0.5 ml/kg/day)	6
Group III - Treatment group	DVK (100mg/kg/day) + 25% of ethanol (0.5 ml/kg/b.w/day)	6
Group IV - Treatment group	DVK (200mg/kg b.w/day) + 25% of ethanol (0.5 ml/kg/b.w/day)	6

### Sampling, Sacrifice and Surgical procedure:

Twenty-four (24) hours after the 60<sup>th</sup> day of treatment, following over-night fasting (12 hrs), the animals were sacrificed with i.p. (intraperitoneal) injection of thiopentone. The abdominal cavity was opened up through a midline abdominal incision to expose the genital organs. Testes, Prostate, seminal vesicle and epididymis were excised, trimmed of all fat and other tissues, mopped with tissue paper and then weighed. Left testicles were collected to monitor the spermatozoal characteristics and Right testicles to conduct testicular and epididymal histopathology. The sections were studied microscopically for changes in histo architecture or morphology. The left caudal epididymis were transferred into sterile bottles containing 2 ml of normal saline for semen analysis. Semen samples from caudal epididymis (left) were subjected to parameters such as count, motility, viability and abnormality. Counting was performed using a haemocytometer and light microscope with 100X.

### Enumeration of sperm parameters:

#### Semen analysis:

Examination of sperm count, sperm motility, viability and spermatozoal abnormalities was carried out by making small cuts in the area of the cauda epididymis close to the vas deferens and apply gentle pressure to exude epididymal contents

#### Sperm Count:

The sample was drawn in to WBC pipette and diluted to the ratio of 1:100 with the modified Krebs Ringer-bicarbonate buffer containing 0.05% collagenase (pH 7.4) followed by this 1:1000 dilution was performed with 1.8% NaCl and 2% formalin). The sperm suspension was placed in the haemocytometer with improved double Neubauer ruling was used for the counting of spermatozoa. Counts for 2-4 haemocytometer chambers were averaged. The sperm suspension was evaluated for sperm count.

Total number of sperm cells in all the four chamber = X  
X multiplied by 10,000 to obtain the number of cells (Y) per ml of diluted sample  
Y multiplied by 100 (the dilution factor) to obtain (Z) sperm cells per ml of original semen sample.

#### Sperm Motility: [2]

The sample was mixed with 20mm HEPES (4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid contains L-Glutamine with 5% BSA (Bovine serum albumin)). Final sample suspension mixed with formalin and used to assess motility. Bright field microscope magnification 100x.

$$\text{Motility (\%)} = \frac{\text{Number of motile spermatozoa}}{\text{Total number of spermatozoa (Motile + Immotile)}} \times 100$$

**Percentage viability:** <sup>[3]</sup>

Viability was assessed by eosin Y staining (5% in saline). Forty micro liter samples of the freshly sperm suspension were placed on a glass slide, mixed with 10 µL eosin and observed under a light microscope

(x400 magnification). Live sperms remained unstained following staining; whereas, those that showed any pink or red coloration were classified as dead. At least 200 sperm were counted from each sample in ten fields of vision randomly, and the percentage of live sperms was recorded

$$\text{Percentage of sperm viability} = \frac{\text{Total Viable cells (Unstained)}}{\text{Total cells (Viable +Dead]}} \times 100$$

**Sperm morphology :** <sup>[4]</sup>

**Staining :**

A suggested method for staining uses 1ml of sperm suspension which was transferred to a test tube. Two drops of 1% eosin Y were added to the test tube and mixed by gentle agitation. The above mixture was incubated at room temperature for approximately 45-60 minutes to allow for staining.

placed approximately 1cm from the frosted end of a pre-cleaned microscope slide lying on a flat surface. A second slide was held in the right hand with the slides's long edge gently touching across the width of the sperm slide and pulled across to produce a sperm smear. After drying the smears were fixed with formalin.

**Slide preparation :**

Slides should be cleaned with detergent, washed in water followed by alcohol and dried before use. One to two drops of the stained sperm suspension were

**Characterization of normal and abnormal sperm:**

Abnormality in sperms were calculated based on the following parameter like curved tail, Tail less head, Headless tail, looped tail and coiled tail etc. Normal sperm were calculated based on the appearance and absence of above mentioned parameters.

$$\text{Percentage of normal sperm} = \frac{\text{No of Normal sperm}}{\text{Total number of sperms in the filed}} \times 100$$

$$\text{Percentage of abnormal sperm} = \frac{\text{No of abnormal sperm}}{\text{Total number of sperms in the filed}} \times 100$$

**Procedure for histopathology** <sup>[5]</sup>

The rats from each group were anethetized by drugwith out any injury after lower pelvic region. The collected samples were washed with normal saline and fixed in 10% neutral formalin for 48 hrs for further histological observation. Paraffin section were taken at 5 µm thickness processed in alcohol-xylene series and was stained with Haematoxylin-eosin dye. The sections were examined microscopically for histopathological changes. The magnification for low

power was carried out at 10 X and for high power at 45 X.

**Statistical analysis:**

Data collected in the study were expressed as the mean ± SEM and statistical analysis was carried out using Dunnett test. P value less than 0.05 was considered to be statistically significant. All data were summarized in tabular form Table (18-20).

## Results

### Semen analysis of wister albino rats

#### Sperm counting (By Neubauer Counting Chamber)



**Sperm morphology**



**Sperm viability**(unstained sperm cells are viable)



**Fig.No.4. Showing semen analysis of DVK treated wister albino rats.**

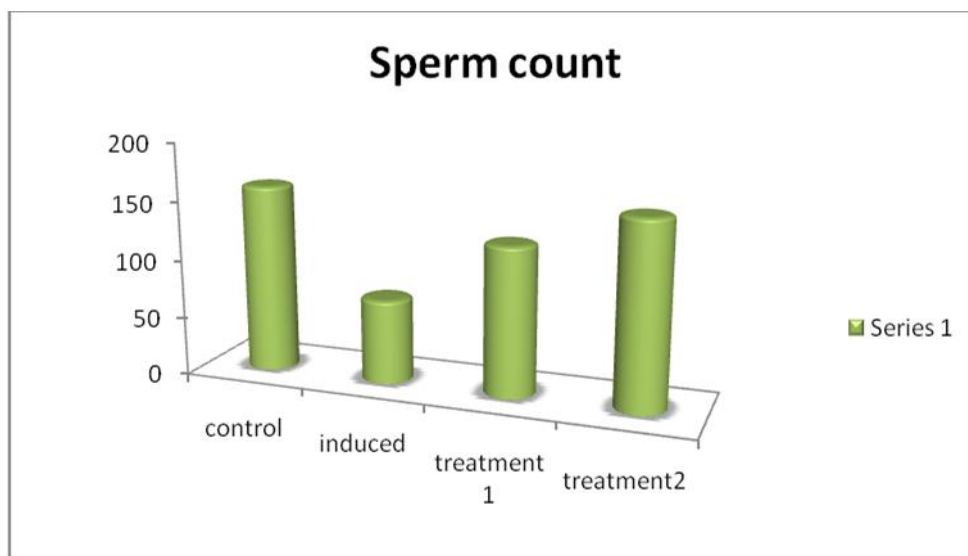
**Table 1 : Effect of *Dhadhu virthi kuligai* on sperm count and motility.**

Groups	Intervention	Sperm Count	Sperm Motility(%)
Group I [C]	1 ml of Milk	161.70±7.43	73.71±5.02
Group II [ IG]	25% of ethanol ( 0.5 ml/kg/day)	72.23±8.47 <sup>a*</sup>	26.17±4.18 <sup>a*</sup>
Group III [TG-1]	DVK ( 100mg / kg / day) + 25% of ethanol ( 0.5 ml/kg/day)	127.54±7.99 <sup>b*</sup>	65.42±3.91 <sup>b*</sup>
Group IV [TG-2]	DVK ( 200mg / kg / day) + 25% of ethanol ( 0.5 ml/kg/day)	158.70±6.22 <sup>b*</sup>	69.17±3.75 <sup>b*</sup>

Values were expressed as mean ± SEM; n=6; followed by Dunnett test.

a-Group I vs. GroupII; b-Group II vs. Group III and IV, \*P < 0.01, NS- Non-Significant.

**Fig 1: Effect of *Dhadhu virthi kuligai* on sperm count:**



**Fig 2: Effect of *Dhadhu virthi kuligai* on sperm motility:**

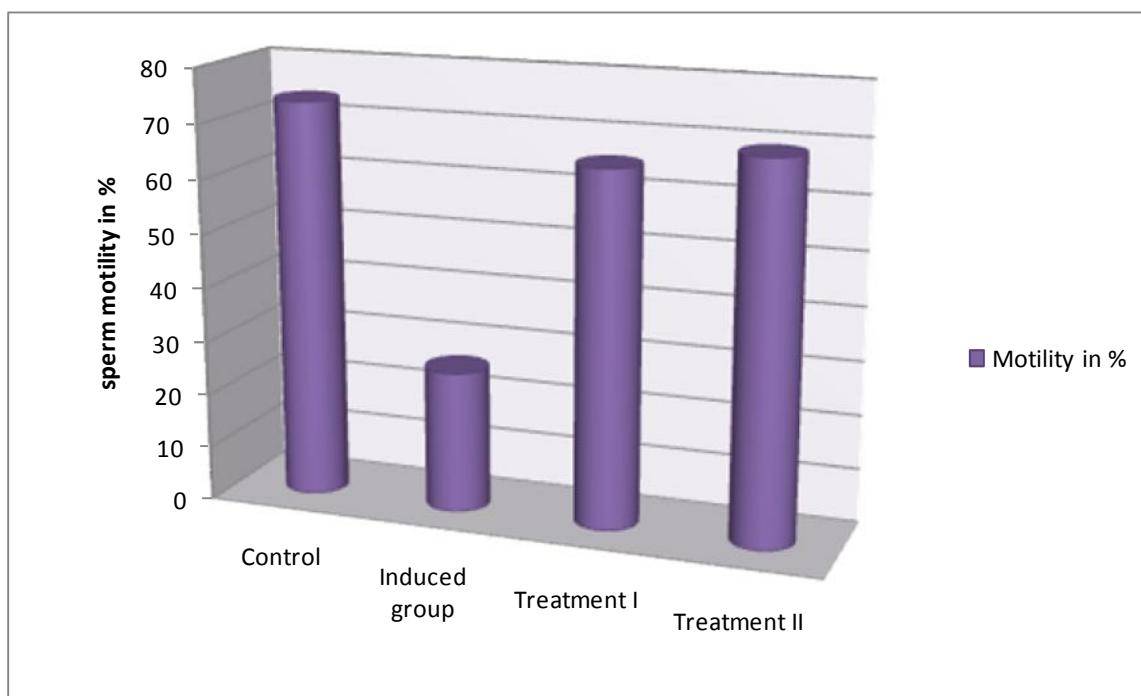


Table 2: Effect of *Dhadhu virthi kuligai* on sperm morphology and viability:

Group and intervention	Morphology		Viability
	Normal	Abnormal	
Group I [C]	78.74±5.86	21.36±5.97	70.69±2.91
Group II [IG]	30.43±2.76 <sup>a*</sup>	71.39±1.90 <sup>a*</sup>	47.49±8.27 <sup>a*</sup>
Group III [TG-1]	71.49±11.48 <sup>b*</sup>	25.67±11.54 <sup>b*</sup>	64.23±7.61 <sup>b*</sup>
Group IV [TG-2]	77.23±5.62 <sup>b*</sup>	21.08±5.86 <sup>b*</sup>	67.37±4.93 <sup>b*</sup>

Values are expressed as mean ± SEM; n=6; followed by Dunnett test.

a-Group I vs Group II; b- Group II vs Group III and IV, \*P < 0.01, NS- Non-Significant.

Fig 3: Effect of *Dhadhu virthi kuligai* on morphology:

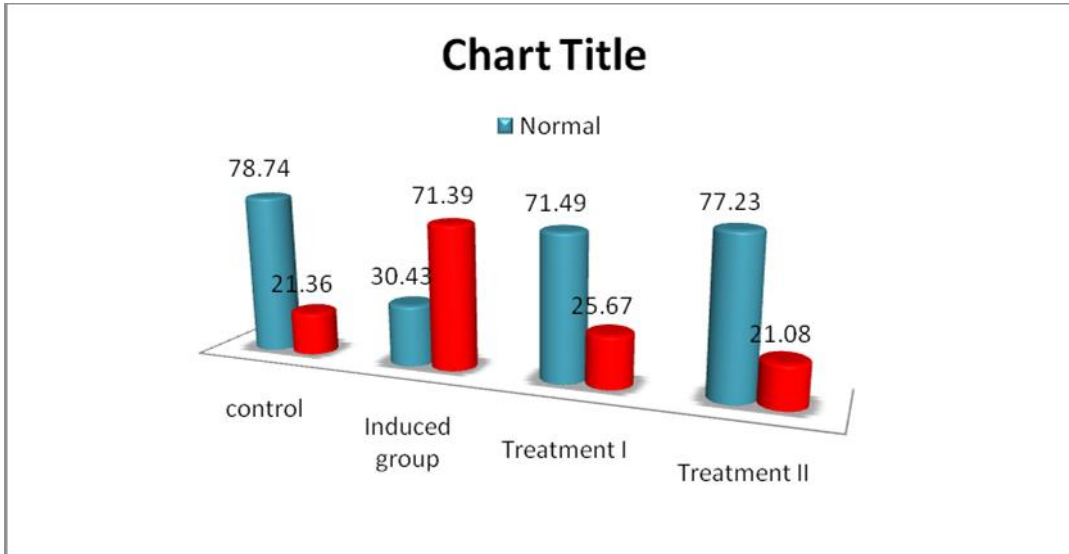


Fig 4: Effect of *Dhadhu virthi kuligai* on sperm viability:

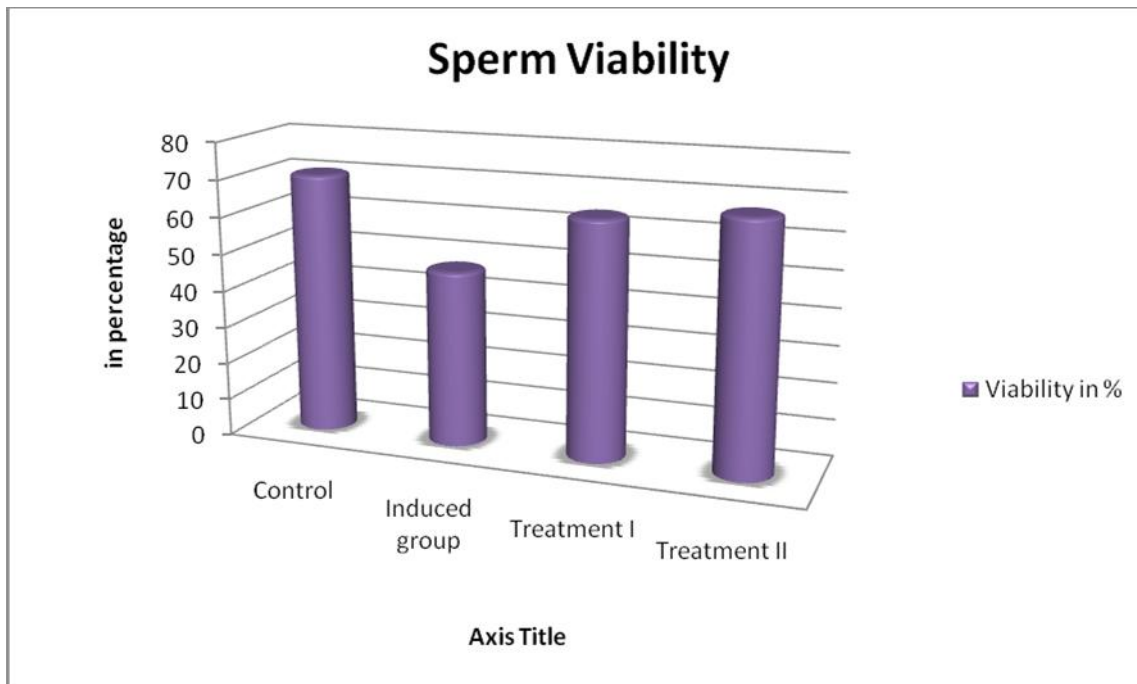
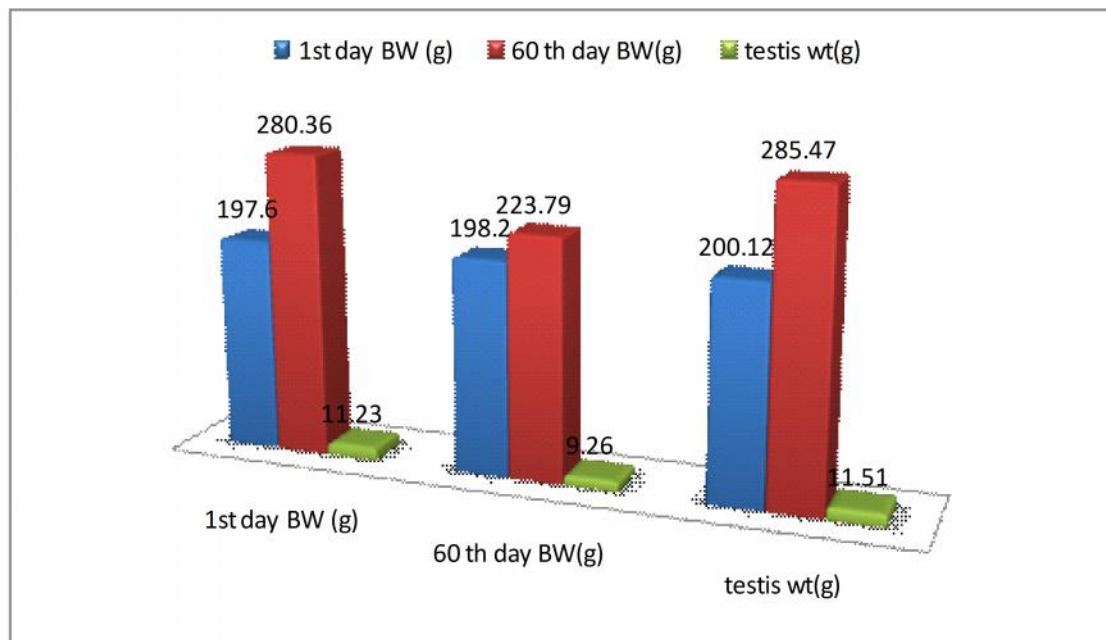


Table 3: Effect of *Dhadhu virthi kuligai* on body and testis weights

Group and intervention	Body weight(g)		Testis weight (g)
	1 st day	60 th day	
Group I [C]	197.6±3.46	280.36±5.97	11.23±0.91
Group II [IG]	198.2±2.91 <sup>a*</sup>	223.79±1.90 <sup>a*</sup>	9.26±1.27 <sup>a*</sup>
Group III [TG-1]	200.12±6.48 <sup>b*</sup>	285.47±3.54 <sup>b*</sup>	11.51±1.61 <sup>b*</sup>
Group IV [TG-2]	198.58±5.62 <sup>b*</sup>	297.75±4.86 <sup>b*</sup>	13.26±0.93 <sup>b*</sup>

Values are expressed as mean ± SEM; n=6; followed by Dunnett test. a- Group I vs. Group II; b-Group II vs. Group III and IV, \*P < 0.01, NS- Non-Significant.

Fig 5 :Effect of Dhadhu virthii kuligai on body and testis weights



## Discussion

Decrease in sperm count observed after administration of ethanol was reversed by treating with the study drug Dhadhu virthii kuligai. The parameters like Sperm count, Motility, Viability and Morphology of DVK 200 mg /kg treated group shown significant increase than ethanol alone treated group (negative control). In testis histopathological of negative control (group II) animals showed germinal damage and treated group shows significant restoration. From these results it is obvious that *Dhadhu virthii kuligai* has good spermatogenic activity.

## Conclusion

Herbal medicines are always best in their therapeutic action without causing any serious adverse events. This research work confirms the promising spermatogenesis activity of siddha medicine *Dhadhu virthii kuligai*. If clinical trial will be followed based on this research work, it contributes a high beneficial to the society.

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