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Evaluation of shukral activity of sariva (*Hemidesmus indicus* R.Br) with special reference to Spermatogenic activity an experimental study

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Abstract

Introduction- Ayurveda, the life science has explained about the health definition and also the measure to be healthy. Plants play a vital role in curing the disease and maintenance of health. Due to lifestyle modification, use of pesticide sprinkled food grains, fruits and vegetables, exposure to environmental chemicals, xenobiotics, have affected physiological, mental health of reproductive system of individuals. *It is believed and proved that exposure to environmental estrogen, will end up in decreased sperm count and motility and male infertility.* According to WHO about 80% of couples are infertile among which male contribute about 30% due to lifestyles changes, food with high pesticide content and environment estrogen exposures. So the present study was intended to see the efficacy of Sariva Moola Phanta in improving sperm count and motility.

Materials and methods- Experimental study was designed for period of 30 days. 24 male adult healthy wistar albino rats were divided into 4 groups. Each group with six animals was kept in separate cages after proper labeling. Group A was received normal food and water; Group B was treated with Estradiol 500 µgm/kg body weight intramuscularly for 14 consecutive days and scarified on 15th day; Group C treated with Estradiol 500 µgm/kg body weight intramuscularly for 14 consecutive days followed by Sariva moola Phanta 8.64ml/kg body weight orally for further 15 days; and Group D was treated with Estradiol 500 µgm/kg body weight intramuscularly for 14 consecutive days and left untreated for further 15 days.

Result –Sariva has significant results in Shukral activity in terms of increasing sperm count, sperm motility and serum testosterone.

Keywords: Ayurveda, Estradiol, Sariva, spermatogenic, environmental estrogen, sperm count, sperm motility, Xenobiotics.

Introduction

Plants play a vital role in curing the disease and maintenance of health. But now a days because of lifestyle modification, use of pesticide sprinkled food grains, fruits and vegetables, and exposure to environmental chemicals, xenobiotics directly and indirectly are affecting the physiology of all the systems including reproductive system, which has affected physiological, mental health of reproductive system of individuals, which tend to cause Oligo-Asthenozoospermia which are one of the leading cause for male infertility. *It is believed and proved that exposure to environmental estrogen will end up in decreased sperm count and motility and male infertility* [1]. In the last 100 yrs the average sperm count has become 50-60 million/ml from the previous average 110-120 million/ml. Sariva (*Hemidesmus indicus* R.Br) being herbal and safe to use and it is having properties like Madhur (sweet taste) rasa, Sheeta virya (cold potency) which directly acts on Shukravaha Srotas (reproductive system). So the present study was intended to see the efficacy of Sariva Moola Phanta in improving sperm count and motility.

Materials and methods

Procurement of animals and quarantine

24 male Wistar albino rats weighing 200-250 gm were taken for study, and 7 days acclimatization was given with ambient climatic condition, and maintained throughout the

experiment. Experimental procedures were approved by the institutional ethical committee (BMK/IAEC/RES14/09/2015) held at KLE'S Shri B M Kankanwadi Ayurveda Mahavidyalaya Shahapur Belgavi Karnataka.

Procurement of Sariva and Estradiol

Drug Sariva (*Hemidesmus indicus* R.Br) was collected from college herbal garden, subjected for preliminary phytochemical and analytical tests. Sariva Moola Phanta was prepared freshly using 1:4 ratio (Sariva moola churna: portable water) Estradiol was procured from Sigma Aldrich Company USA, from Venkatesh enterprises Dharawad. Estradiol was prepared freshly daily using 1:9 ratio of ethanol and olive oil to make solution and dispensing volume was 0.1ml intramuscularly.

Location of the study- All the preliminary phytochemical test and analytical procedures and animal experiments were carried out at AYUSH approved Central Research laboratory, and animal house, Shri BMK Ayurveda Mahavidyalaya Shahapur Belagavi, Karnataka, India.

Grouping- The acclimatized rats were divided into 4 groups (6 rats in each group). Control, Disease induced, Treatment and Natural recovery. To identify animals marking has been done on 1) head, 2) neck, 3) body, 4) tail, 5) right hind limb and 6) left hind limb

Total number of animals: 24

Duration of study: 30days

Dose of Sariva (*Hemidesmus indicus* R.Br) Phanta in animals: 8.64ml/kg body weight

Dose of Estradiol: 500µgm /kg body weight (effect of *Eurycoma longifolia* jack on spermatogenesis in Estradiol treated rats.)^[4].

Blood collection: retro orbital

Organ tissue collected: Epididymis and testis (kept in 10% formalin).

Experimental protocol

Group A (Control) received normal food and water and sacrificed on 30th day to see the normal sperm parameters. Group B, C and D Estradiol 500µgm /kg body weight was administered intramuscularly for 14 days along with normal food and water. Group B was sacrificed on 15th day to see the extent of testicular toxicity. Group C was given Sariva (*Hemidesmus indicus* R.Br) Moola (root) Phanta orally at a dose of 8.64 ml /kg body weight over a period of next 15 days, after 14 days of Estradiol administration, with normal food and water and sacrificed on 30th day to see the efficacy of drug Sariva (*Hemidesmus indicus* R.Br). Group D was left untreated for further 15 days sacrificed on 30th day to see in there any natural (auto) recovery process.

Table 1: Showing Study Protocol

Group	Drug	Dose	Route of administration	Duration	Sacrifice	Purpose
Group A	Normal food and water		Oral	30 days	30 th day	Normal parameter
Group B	Estradiol	500µgm /kg body weight	IM	14 days	15 th day	Extent of toxicity
Group C	Estradiol and Sariva	500µgm /kg body weight 8.64 ml /kg body weight	IM Oral	14 days 15 days	30 th day	Protecting action
Group D	Estradiol	500µgm /kg body weight	IM	14days	30 th day	Natural recovery

Parameters of experimental study

- General observation- Animals of all groups were observed for their food and water intake, general toxicity, body weight throughout the experimental study.
- Biochemical investigations- Blood was collected in plain vial and subjected for performing biochemical parameter like serum Testosterone.

On 15th day group B anesthetized using high dose of di-ethyle ether and blood was drawn through retro-orbital region by pricking micro capillary tube number 100 mm (Borocilicate glass with both end open) and collected in labeled plain vials. Blood was sent to Jeevan diagnostic center Belagavi Karnataka, for biochemical investigations. Similarly group A, C and D were anesthetized and blood was collected on 30th day.

Subjective parameters-Weight of testis, sperm count and motility

Histopathological investigations-

Testis was collected immediately after anesthetized by diethyl ether then taken for dissection. Cleaned off extraneous tissue and transferred to 10% formalin solution in separate labeled containers and sent to Jeevan diagnostic center Belagavi for

histopathological study.

Method for epididymal sperm count and motility-

The epididymis was dissected out for all animals, caudal part of it was cut and tubules were dispersed into media distilled water in Petri dish that was labeled with corresponding animal code. Petri dish was kept for 10 min in room temperature to allow the sperm to disperse. The supernatant containing sperm was collected 10µl was put on clean slide which was mounted on, analyzed for motility analysis. Sperm count was performed manually by using haemocytometer.

Results

Phytochemical and analytical study of Sariva root has shown results are within the normal standard limits.

The observations made in this animal experimental study were as follows –

Weight of animals: - Body weight of all animal was recorded for every 15th day with the help of electronic weighing machine and expressed in terms of grams.

Table 2: Illustrate body weight of animal

Statistical analysis - All the values were analyzed using student't' test. The mean and standard deviation value are illustrated in following tables.

Group	Day 1	Day 15	Day 30
Group A (Control)	264.3±21.71	264.8±20.25	283.5±16.74
Group B (Disease induced)	236.3±5.9	222.1±5.19	-
Group C (Treated with Sariva Moola)	251.6±15.16	228.3±13.79	260.6±11.06
Group D (Auto recovery)	219.5±16.7	205.3±10.87	246.3±16.6s

Sperm count: - Sperm count was done on the last day of experiment as it was necessary to scarify the animals. There was after dissecting the animal epididymis was separated from testes and epididymal sperm were collected in media 99 which was specially prepared and pH was maintained between 7 to 7.5 for viability of sperms.

Sperm motility: - Sperm motility was calculated at the end of

experiment after sacrificing animals by collecting epididymal sperms. In sperm motility RLP, SLP, NP and Immotile was observed.

Sr. Testosterone

Sr. Testosterone was done at the end of the experiment before sacrificing animal, blood was collected from retro-orbital plexus.

Table 3: Illustrating results

Parameter	Normal (Control)	Estradiol (Diseased)	Estradiol + Sariva(Treated)	Estradiol (Auto recovery)
Wt of testis in gms	Rt- 1.54±0.05	0.61±0.13	0.5 ± 0.22*	0.57±0.17
	Lt- 1.54±0.05	0.6±0.17	0.62±0.20*	0.54±0.3
Wt of epididymis in gms	Rt- 0.27±0.07	0.08±0.02	0.1±0.02*	0.11±0.06
	Lt- 0.28±0.07	0.08±0.02	0.22±0.3*	0.23±0.19
Sperm count	50.3±37.4	28.6±19.5	1.6±0.7*	NIL
Rapid linear progress	9.5±13.7	6.6±23.9	2.6±1.3*	NIL
Sluggish linear progress	6.8±8.1	9.8±9.2	1.8±1.22*	NIL
Non progressive	13±10.1	22.33±18.73	46.3±36.08	NIL
Immotile	54±33.85	59.5±37.6	49.16±38.11	NIL
Serum testosterone in ng/dl	5.8±4.6	0.6±0.12	1.8±0.7**	0.14±0.04

*Significance at the level of 0.05

** Highly significant at the level of 0.001

Histopathology of testes

Testes were removed after sacrificing animals and were processed for histopathology.

In the control group testis section showed normal histological texture. Diameter of seminiferous tubules varied within range. Leydig cells are normal in size germinal epithelium appears to be normal with normal epithelial height, interstitial connective tissue and no germ cell death. Spermatogenesis was normal and spermatogenic index 6.

In Estradiol treated groups leydig cells appear to be normal. There was mild to moderate distortion and congestion of testis, germinal epithelium, with decreased epithelial height. Reduction in seminiferous tubule diameter. There also noted occasional presence of multinucleated germ cells and also germ cell death with normal interstitial connective tissue. Decrease in spermatogenesis and spermatogenic index was noted.

In Sariva treated group there was mild changes in tube diameter, mild congestion in testis architecture, germinal epithelium as a result of Estradiol treatment, but spermatogenesis was increased and spermatogenic index was 4(presence of spermatogonia, spermatocytes and early

spermatid present in normal number with fewer than 5 late spermatid per tubule) [6]

Table 4: Illustrating Histopathology of testis

Photographs



TLC of Sariva

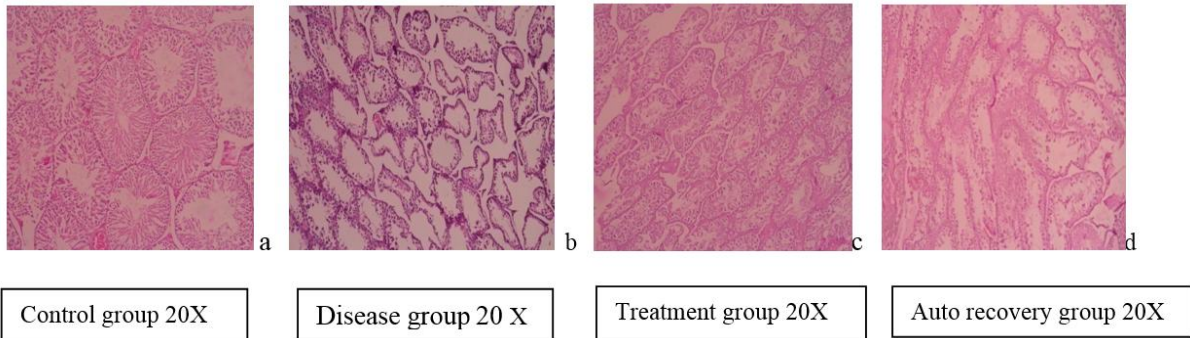


Visible light

Short wave

Long wave

Histopathology of testes



Discussion

Infertility is burning issue in present period in spite of several advancement in the field of advanced medical inventions and interventions. About 30% of the burden is contributed by male causes. The leading causes are food habits, lifestyle modification, and exposure to environmental hazards which leads to Oligo-Asthenozoospermia, which is increasing at an alarming rate. For these condition synthetic and hormonal correction treatments are themselves effective in managing the condition but not without setbacks. Estradiol is the most potent estrogen hormone which is derived after aromatization of testosterone. This induces testicular toxicity by means of increased apoptosis leading to hampered spermatogenesis thus resulting in Oligo-Asthenozoospermia (Effect of *Eurycoma longifolia* in Estradiol treated spermatogenesis) [4]. Estrogen hormone which is widely used in non-vegetarian units to increase muscle bulk of chicken and sheep. And some of the food items like skimmed milk, fish meal, soya meal, bone meal which contains phytoestrogens which are likely to cause testicular toxicity in high dose and prolonged period (Adult only exposure to phytoestrogens will disrupts germ cell) [5].

Discussion on results

The histopathological study revealed that Sariva (*Hemidesmus indicus* R.Br) treated group has shown presence of sperm cells after 30 days, where as the auto recovery group showed no sperm cells after inducing toxicity. Spermatogenic index was calculated for the assessment of the stage of spermatogenesis. And the result was statistically significant in improving sperm cells ($p < 0.05$) after inducing toxicity.

Sariva treated group has shown significantly increase in sperm count (mean score 1.6 ± 0.7), good improvement in rapid linear progress (RLP) (mean score 2.6 ± 1.3), sluggish linear progress (SLP) (mean score 1.8 ± 1.22), non progressive (NP) (mean score 46.3 ± 36.08), immotile sperm cells (mean score 49.16 ± 38.11) after 30 days, where as auto recovery group showed NIL results after inducing toxicity.

Serum testosterone in Sariva treated group has shown statistically significant result the mean score, compared with control group 5.8 ± 4.6 , was decreased to 0.6 ± 0.1 in diseased group and in auto recovery group 0.14 ± 0.04 , but increased with 1.08 ± 0.7 in Sariva treated group.

There was no significant changes occurred in body weight and Weight of testis of the animals during the experimental study and results are statistically insignificant. Weight of epididymis of treatment group was increased in Sariva treated group (group C) and it was statistically significant.

Conclusion

The present experimental study revealed that, Sariva has got significant effect on improving sperm count, sperm motility

and increasing testosterone level as compared to auto recovery group, thus proved Shukral activity.

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