

**SPERM PARAMETERS AND TESTOSTERONE LEVELS OF SPRAGUE DAWLEY RATS WITH
PALA KALYANA GHRITA : A TRADITIONAL FORMULA**

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ABSTRACT

Pala Kalyana Ghrita (PKG) is a poly herbal formula used for enhancement of fertility by traditional medical practitioners from ancient times. However, the scientific evaluation of this natural herbal medicine is yet to be explored. The present study was under taken to evaluate pharmaceutical significance of PKG on some fertility parameters of male Sprague Dawley rats. Eighteen rats were randomly divided into three groups (n = 6 /group). The control group, (Group I) received only distilled water. Groups II and III were orally administered with 900 mg and 1800 mg of PKG / kg body weight respectively, once daily, for 12 weeks. Pretreatment mean body weight and Post treatment mean body weight, mean testicular weight and sperm parameters were measured. Although there was no significant difference in mean body weights of the rats included control and the treatment groups prior to treatment, a significantly higher mean body weight of both the treatment groups (248.83 ± 1.60 and 252.50 ± 1.63) than the control group (231.33 ± 6.80 g) ($p = 0.003$) was observed after the treatment period. . The testicular weights of both treated groups were also significantly higher than that of the control group. Similarly, serum testosterone level of both treated groups (3.46 ± 0.69 ng/mL and 4.98 ± 2.1 ng/mL) was elevated compared to the control group (1.33 ± 0.1 ng/mL) ($p = 0.001$). PKG has fertility enhancing effects as evident by increasing in mean testicular weight, testosterone level and sperm parameters of male Sprague Dawley rats in the treated groups.

KEYWORDS: Palakalyana Ghrita, Spermatogenesis, Testosterone, Testis.

1. INTRODUCTION

Pala Kalyana Ghrita (PKG) is a poly herbal formula used by traditional physicians for the treatment of infertility. The Ayurveda Pharmacopeia of Sri Lanka describes PKG as a fertility enhancing and aphrodisiac herbal remedy.^[1] PKG formula consisted of nineteen herbal ingredients, cow's milk, and cow's ghee. Roots of *Asparagus racemosus* (Willd) is the main ingredient of this formula. Other plant materials are *Terminalia chebula* (fruits), *Terminalia bellirica* (fruits), *Phyllanthus emblica* (fruits), *Picrorhiza kurroa* (rhizomes), *Curcuma domestica* (rhizomes), *Rubia cordifolia* (roots), *Vitis vinifera* (fruits), *Nymphoides cristata* (Flowers), *Santalum album* (wood), *Saussure lappa* (roots), *Glycyrrhiza glabra* (rhizomes), *Pterocarpus santalinus* (wood), *Sida cordifolia* (roots), *Ipomoea peniculata* (tubers), *Coscinium fenestratum* (stem), *Sacchrum officinarum* (stem), *Vernonina cineria* (whole plant) and *Withania somnifera* (roots).

Use of PKG as a fertility enhancing medicament is based on evidence gained by long term use of this medicine by traditional physicians. However, there is no scientific data pertaining to the reproductive efficacy of PKG, as for most other commonly used herbal remedies.^[2] As PKG is a widely used herbal remedy, evaluation of the pharmacological properties of this drug will result in a better insight to its therapeutic potential. Therefore the aim of present study was to determine the effects of orally administered PKG on reproductive parameters of male Sprague Dawley rats.

2. EXPERIMENT

Ethical clearance to carry out this research was granted by the ethical committee of the faculty of Medicine, Colombo, Sri Lanka (reference: EC-11-158).

2.1 Preparation of herbal formulae

The recipe described in Ayurveda Pharmacopeia of Sri Lanka was used to prepare PKG for the present experiment.^[1] The plant material were identified and

authenticated by the Department of Materia Medica, Institute of Indigenous Medicine, University of Colombo, Sri Lanka.

Concentrated water extract of *Asparagus racemosus* was prepared by boiling 600 g of root parts in 20 L water for 4-6 hours until the volume was reduced to 5 L. As per the recipe, all the other herbal ingredients of the formula were separately washed and air dried to a constant weight and powdered using an electric grinder and passed through the 10 mesh sieve. Fifteen grams (15g) of each powdered plant material, five liters of pasteurized cow's milk and 1280 g of ghee were added in to 5 L concentrated water extract of *Asparagus racemosus* and boiled again until all the water evaporated. The final herbal preparation was stored in sterilized glass bottles at room temperature. The preparation of this formulation was carried out in the pharmacy attached to the institute of Indigenous Medicine, University of Colombo.

2.2 Experimental animals

Adult male Sprague Dawley rats of about twelve weeks weighing 180 ± 20 g, were obtained from the animal house, faculty of Medicine, University of Colombo, Sri Lanka. The rats were kept in the faculty animal house in well cross ventilated room with controlled environment (temperature $27 \pm 2^\circ\text{C}$ and humidity 44-56%), light and dark cycles of 10 and 14h respectively before and during the experiments. Animals were fed with standard pellet diet and allowed water *ad libitum*.

2.3 Experimental design

Eighteen adult male rats were recruited, weighed and divided into three groups (Group I– III) each group consisting of 6 rats. The PKG was administered orally for Groups II and III in the morning around 9.00 to 10.00 am, for 12 weeks as follows;

Group I- the control group received only distilled water.

Group II - received 900 mg /kg / b.wt of PKG.

Group III - received 1800 mg / kg /b.wt of PKG.

The PKG dosage for rats was calculated as per daily dose prescribed for human, which is 10 g /50 kg /b.wt per day. The equivalent dosage when calculated for rats was 900 mg /kg /b.wt. based on the standard table of Paget and Barnes.^[3]

2.4 Serum testosterone assay

Rats were weighed after 12 weeks of treatment and blood obtained through tail vein into 2 mL syringe under light anesthesia. Once the sample was clotted, serum was separated by centrifugation at 3000 rpm for 15 minutes for Testosterone assay. Testosterone assay was performed using Electrochemiluminescence hormone/enzyme analyzer.

2.5 Semen Analysis

After withdrawal of blood, all rats were sacrificed using an overdose of ether. The entire male genital tract was carefully dissected out, placed on a petri dish and washed

with normal saline several times to remove blood and other tissue debris. The excess saline was carefully blotted out with a filter paper. The cauda- epididymis was cut lengthwise and placed in a petridis containing 10 mL of pre warmed Ferticult flushing medium. These dishes were incubated at 37°C for 30 minutes in a CO_2 incubator to allow sperm to swim out.^[4] After incubation, 1 mL of the sperm suspension was diluted (1:10) as the sperm suspension was too dense. This diluted sperm suspension was used for analysis.

2.5.1 Sperm count

10 μL of sperm suspension was placed on the Makler sperm counting chamber (Deep 1/10 LABART, Germany) to calculate the sperm concentration using light microscope described by Mohammad JA, Ja'Far Luthfi (2015).^[5]

2.5.2 Sperm motility

Sperm suspension kept at 37°C was Sperm motility was assessed in 3 replicate samples within 1 hour. A drop of sperm suspension was mixed well and a drop of the mixture was placed on a pre-warmed microscope slide and examined at $\times 200$ magnification. Approximately 200 spermatozoa per replicate was assessed for the percentage of different motile categories. Average percentage for each motility grade was calculated.^[6]

2.5.3 Sperm Morphology

A sperm suspension was smeared on a microscope slide and stained with Papanicolaou staining procedure for sperm morphology.^[7] The stained slide was examined under the microscope with oil-immersion objective. At least 200 sperm per smear were screened to assess any abnormalities for each rat. The number of normal spermatozoa was illustrated as a percentage of total number of spermatozoa.

2.6.-Statistical Analysis

Sperm parameters were represented as mean \pm SD. Data analysis was carried out using one way analysis of variance (ANOVA) by Minitab 16 version. P values < 0.05 were considered as significant.

3. RESULTS AND DISCUSSION

This study was conducted to assess the effect of Palakalyana Ghrita (PKG) on some selected fertility parameters of male Sprague Dawley rats. The findings illustrated that treatment with PKG resulted in enhanced the body weight, and the testicular weight (Table 01). After 12 weeks of the treatment, mean body weight of the treated group II (248.83 ± 1.60 g) and group III (252.50 ± 1.63 g) animals were significantly higher than Control group. (201.33 ± 6.80 g) ($p = 0.003$) (Table 01). Mean testicular weight in treated group III (3.87 ± 0.19 g) and group II (3.91 ± 0.25 g) were also significantly higher than the control group (2.82 ± 0.24 g) ($p = 0.001$) (Table 01). However, there was no significant different of mean body weight and mean testicular weight between treated groups.

The testes weight is one of the markers of possible alteration in androgen status. Mooradian & Korenman (1987)^[8] stated that the size, weight and secretory functions of testes, epididymides and seminal vesicles are closely regulated by Androgen levels.^[9] The increase in body weight and testes weight in treated groups of rats than the untreated control group could be due to stimulation of androgens or due to androgenic properties of the PKG, since androgens possess anabolic activity. Another research carried out by Al-Sa'aidi *et al.*^[10] reports that use of alcoholic extract of *Nigella sativa* resulted in an increase in the testes size. Gauthaman *et al.* (2002)^[11] in their research on plant medicine have demonstrated that saponin component of plants have stimulatory effects on androgen production. Phytochemical analysis of PKG performed as a part of this research (unpublished data) has demonstrated higher concentrations of saponins and vitamins as vitamin A, B12 and E, which have stimulatory effects on androgen production.^[12] Although there is an increase in the body weight and testes weight of all treatment groups, these values are within the normal range as per documented literature.^[13,14]

Most of the plants included in PKG are aphrodisiac and possess plant androgens. *Asparagus resemosus* is the

main ingredient in PKG, which belongs to a family of phyto estrogenous plants.^[15] Animal experiments with *Asparagus resemosus* have reported that doses upto 2500 mg / kg of the water extract,^[16] and 2000 mg / kg of the ethonolic extract are well tolerated by rats.^[17] *Terminalia chebula*, *Terminalia balarica* and *Phylanthus embilika* contained in PKG are commonly used ingredients in traditional systems of medicine and are reported to strengthen all the tissues of the body, prevent cells damage, and promote life expectancy.^[18] *Withania somnifer*, yet another ingredient in PKG formulae is a medicinal plant that has been used in ayurveda and traditional system of medicine for over 5000 years. It is used for centuries as an aphrodisiac medicine.^[19] When the ingredients in formula of PKG is considered, our results are in agreement with many published data with regard to the improvement of sperm parameters.^[20,21] In treated groups, the sperm quality of rats were better than control group as an increase in sperm count and motility ($p < 0.05$), decrease in abnormalities reveals the fertility potential of male rats due to PKG. The enhancement of the sperm concentration was mainly due to the increase in testosterone levels in the testicular tissue with the main hormone responsible for spermatogenesis and spermiogenesis in seminiferous tubules.^[22,23]

Table 1: Effect of PKG on Body and Testicular weight.

Parameter	Body weight (g)		Testicular weight (g)
	Before treatment	After treatment	
Group I (Control)	157.67± 2.88	201.33 ± 6.80	2.82 ± 0.24
Group II (PKG 900mg/Kg/bw)	158.50±1.52	248.83 ± 1.60	3.87± 0.19
GroupIII(PKG 1800 mg/Kg/bw)	157.33±5.50	252.50 ± 1.63	3.91 ± 0.25

Results are expressed as mean ± standard deviation (n=6), $P < 0.05$, compared to respective controls, using one way analysis of variance (ANOVA).

Table 2: Effect of PKG on sperm parameters and serum testosterone levels.

Parameter	Group I (Control)	Group II (900/ mg/kg)	Group III (1800 mg/kg)
Serum Testosterone level ng/ml	1.33 ± 0.11	3.46±0.69*	4.98 ± 2.1*
Sperm concentration (X 10 ⁶ mL)	18.00±7.56.	28.67±6.13.	31.6±4.21.
Sperm Motility %	8.83±1.82.	11.00±1.04.	15.50±1.86.
Motile sperm concentration x10 ⁶ mL	158.94± 11.20.	315.37±12.50.	489.8±21.35.
Morphology%	39 ±2.64.	58±2.71.	59±2.35.

Each value represent the mean ± standard deviation (n=6), values are statistically different from control at $P < 0.05$ one way analysis of variance (ANOVA).

Mean level of serum testosterone was significantly elevated in both treated group II (3.46 ± 0.67ng/mL) and group III (4.98 ± 2.10 ng/mL) with different dosages compared to the group I, the control group (1.33 ± 0.11ng/mL) ($p = 0.001$). (Table 02). Oral administration of different concentrations of PKG showed a marked improvement in the sperm count and sperm motility. The mean of sperm concentration of Group III rats, was significant higher (31.6.1±4.21 x 10⁶ ml) compared to Group II, (28.67 ± 6.13 X10⁶ mL) ($p = 0.001$) and Group

I, (18.00 ± 7.56. x 10⁶ ml) ($p = 0.001$) rats. The sperm motility percentage of PKG treated Group III was significantly increased (15.50± 1.86 %) than Group II (11.00±1.04%) ($P = 0.001$) and Group I, control Group (8.83± 1.82%) ($p = 0.003$) animals.

Documentary evidence reveals that high level of saponins in plant has the potency to enhance level of testosterone.^[24] That higher level of testosterone may be the apparent reason for the enhancement of sperm

concentration, motility and morphology. Testosterone is the primary anabolic steroid that maintains male fertility, libido, mental and physical energy levels, muscle strength, general wellbeing and bone maturation.^[25] Decreased testosterone levels in blood leads to infertility,

lack of libido, erectile dysfunction, osteoporosis etc.^[26] Furthermore, available evidence reveal that plant testosterone are safer than artificial forms of testosterone.^[27]

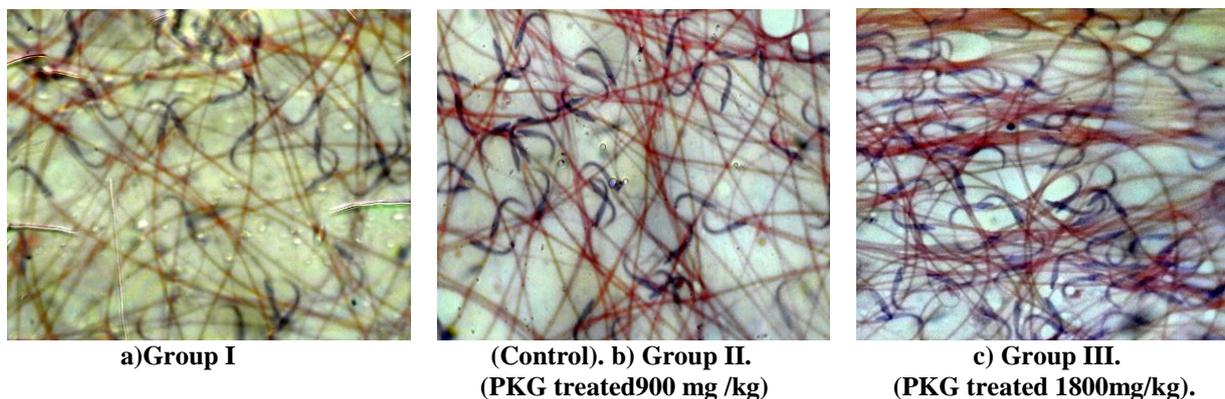


Figure 1: Microphotographs illustrating morphology of sperms in control and treated groups of rats. Showing a gross spermatozoa cluster.(x 400).

CONCLUSION

The finding of the present study illustrated that PKG possess fertility enhancing properties in male rats as evidenced by an increase in sperm count, morphology, the serum level of testosterone. The results may have some clinical implication in the management of male infertility.

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