EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 5
104-108
Year: 2018

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

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Article Received on 22/03/2018 **Article Revised on 15/04/2018** **Article Accepted on 06/05/2018**

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 ± 0.11ng/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±2°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 37C^{o} 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^{0} 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 ×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 ± 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 treatement 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 ± 0.25g) 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 funtions 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. Phytoochemical 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 ingrediant in PKG, which belongs to a family of phyto estrogenous plants.[15] Animal experiments with *Asparadus 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 *Phylanths embilika* contained in PKG are commonly used ingredients in traditional systems of mdicine and are repoted to strengthen all the tissues of the body, prevent cells damage, and promote life expentancy.[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 ($p<0.05$), decrese in abnomalities 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.**

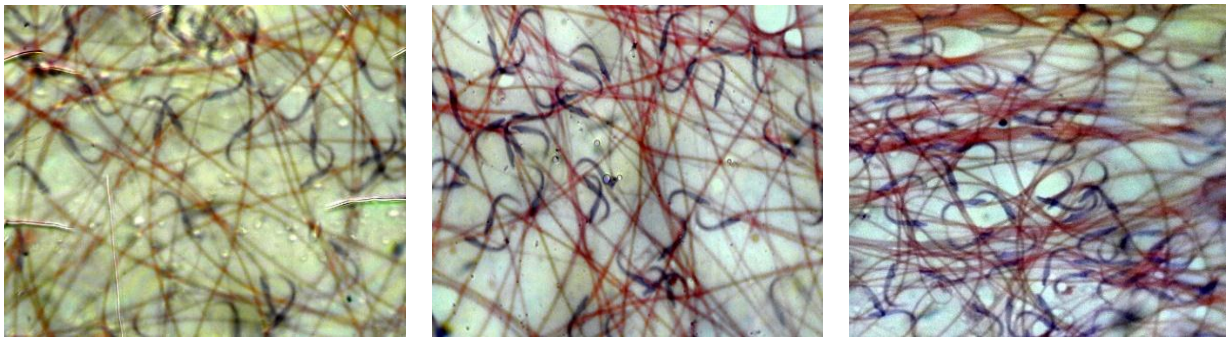
Parameter	Group I (Control)	Group II (900/ mg/kg)	Group II1 (1800 mg/kg)
Serum Testosterone level ng/ml	1.33 ± 0.11	3.46±0.69*	4.98 ± 2.1*
Sperm concentration (X $10^{6/}$ mL)	18.00±7.56.	28.67±6.13.	31.6.±4.21.
Sperm Motility %	8.83±1.82.	11.00±1.04.	15.50±1. 86.
Motile sperm concentration x$10^{6/}$mL	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 10^6 ml) compared to Group 1I, (28.67 ± 6.13 X10^6 mL) (p = 0.001) and Group I, (18.00 ± 7.56. x 10^6 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]



a)Group I **(Control). b) Group II. (PKG treated900 mg /kg)** **c) Group III. (PKG treated 1800mg/kg).**

Figure 1: Microphotographs ellustrating 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.

ACKNOLEDGEMENT

Authors would like to thank the University of Colombo and NCAS, Sri Lanka for providing the financial assistance for the study.

REFERENCES

1. Ayurveda Pharmacopeia, Department of Ayurveda Colombo, 1975; 1(1): 63.
2. Gurib-Fakim. A Medicinal plants traditions of yesterday and drugs of tomorrow. Mol Aspects Med J, 2006; (27): 1-93.
3. Paget EG, Bernes MJ. Estimation of drug activities in Pharmacometrics. New York: Academic press, 1964; 1: 161.
4. Hinting A. Methods of semen analysis. In: Assessment of human sperm fertilizing ability. Ph D thesis, Michigan State University, USA, 1989.
5. Mohammad JA, Ja'Far Luthfi. A simple and practical method for rat epididymal sperm count (*Rattus norrvegicus)*. Biology, Medicine & natural product chemistry, 2015; 4 1): 1-3.
6. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction.1999: 4th ed. Cambridge University Press, New York.
7. Narayana K, D'Souza UJ, Seetharama Rao KP. Ribavirin-induced sperm shape abnormalities in Wistar rat. Mutat Res., 2002; 513(1-2): 193–196.
8. Mooradian AD, Morley JE, Korenman SG. Biological actions of androgens. February; *Endocr. Rev*, 1987: 8(1): 1–28.
9. Thakur M. Dicxit VK, Mehrotra SA. Comparative study on aphrodisiac activity of some ayurvedic herbs in male albino rats, 2009: Arch Sex behave.
10. Al-Sa'aidi JAA, Al-Khuzai ALD, Al-Zobaydi NFH. Effect of alcoholic extract of Nigella sativa on fertility in male rats. Iraqi J Vet Sci, 2009; 23: 123–128.
11. Gautam M, Diwany S, Gairola S, Sinde Y. Immuno adjuvant potential of *Asparagus racemosus* aqueous extract in experimental system. Eth. J., 2009; 91: 251-5.
12. Rosen RC, Ashton AK. Prosexual drugs: Empirical status of the "new aphrodisiac" Arch Sex Behav, 1993; 22: 521–543.
13. Sohrabi D, Alipour M, Mellati Awsat A. Effect of Metronidazole on spermatogenesis, plasma gonadotropins and testosterone in male rats. Iran J Pharm Res., 2007; 6: 279–283.
14. Hassan AA. Effect of royal jelly on the sexual efficiency in adult male rats. Iraqi J Vet Sci., 2009; 23: 155–160.
15. Boonson T, Waranuch N, Ingkaninan K. Molecular Analysis of the genus Asparagus based on mark sequences and its application to Identify. A racemosus a medicinally phyto estrogenic species. Fitoterapia, 2012; 83(5): 947-53.
16. Thakur M, Thomson D, Collnnellan P, Desco MA, Morris C, Dicxit VK. Improvement of penile erection, sperm count and seminal fructose levels in vivo and nitric oxide release in vitro by Ayurvedic herbe .Andrologia, 2011.
17. Koumanov F, Bozadjieva E, Andreeva M, Platonva E, Ankov V. Clinical trial of Tribestan. In Experiment medicine 2nd ed. London: Academic press, 1982: 2–4.

18. Rege NN, Thatte UM, Dahanukar SA. 1999: Adaptogenic properties of six rasayanas.
19. Mamidi P,Thakar AB.Efficacy of Aswaganda (*Withania somnifera*) in the management of psychogenic erectile dysfunction..Int.qua. J of research in Ayurveda, 2011; 32(3): 322-328.
20. 20. Weiner MA, Weiner J. Aswaganda (Indian ginseng) in herbs that heals. Quantum books, Mill valley, Calif, USA, 1994; 70-72.
21. Thakur RS, Puri HS, Husain A. Major Medicinal Plants of India. 1992: Central Institute of Medicinal and Aromatic Plants, *Lucknow*, Uttar Pradesh.
22. Sakamoto H, Yajima T, Nagata M, Okumura T, Suzuki K, Ogawa Y. Relationship between testicular size by ultrasonography and testicular function. Measurement of testicular length, width and depth in patient with infertility. Int J Urol, 2008; 15: 529–533.
23. Handelsman DJ, Conway AJ, Boylan ML. Suppression of human spermatogenesis by testosterone implants of clin. Endro. & metabolism, 1992: 75(5): 1326-1332.
24. Bassil N, Alkaade S, Morley JE. The benefits and risks of testosterone replacement therapy: a review. Ther Clin Risk Manag, 2009; 5(3): 427–48.
25. Jayaprakasam B, Strasburg A, Nair MG. Potent lipid peroxidation inhibitors from Withania somnifera fruits. Tetrahedron, 2004; 60(13): 3109-3121.
26. Khaki A, Fathiazad F, Nouri M, Khaki AA, Jabarikh H, Hammadeh M. Evaluation of Androgenic Activity of Allium cepa on Spermatogenesis in Rat. Folia Morphol (Warsz), 2009: 68: 45-51.
27. Tuck SP, Francis RM (2009). "Testosterone, bone and osteoporosis". Front Horm Res. Frontiers of Hormone Research, 2009; 37: 123–32.