https://doi.org/10.33472/AFJBS.6.6.2024.522-530

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Effect of Hydro Alcoholic Extract of *Baccaurea courtallensis* (Wight) Müll. Arg. on Fertility in Male Rats

Karuppiah Nandhini[1*], Perumal Ravichandran[2], Natarajan Chidambaranathan[3]

[1*, 2] Department of Plant Science, Manonmaniam Sundaranar University, Abishekapatti - 627 012, Tirunelveli, Tamil Nadu, India.
[3]Department of Pharmacology, K. M. College of Pharmacy, Madurai – 625 107, Tamil Nadu, India.
Corresponding Email: 1*nandhuwin19@gmail.com

Article Info

Volume 6, Issue 6, May 2024

Received: 12 March 2024

Accepted: 03 April 2024

Published: 01 May 2024

doi: 10.33472/AFJBS.6.6.2024.522-530

ABSTRACT:

Infertility is considered as one of the global health problems in human reproduction. In the last few decades, research on the use of plant medicine to treat infertility and related illnesses have advanced significantly, leading to the development of novel, safe, and effective compounds for use as alternative medicines. Several plant species have tremendous potential to treat infertility and associated reproductive illness. *Baccaurea courtallensis* is an endemic tree producing wild edible fruits, used by various ethnic communities and local folks of the southern Western Ghats. Fruits of this tree have been used to treat infertility problems in men and women. The aim of study was to investigate the effects of hydro alcoholic extract of *B. courtallensis* on fertility of male rats. Hydro alcoholic extract of *B. courtallensis* (HAEBC) contains several secondary metabolites leading to testosterone metabolism and consequently to the improvement of spermatogenesis. The Hydro alcoholic extract of *B. courtallensis* (HAEBC) improves the male reproductive functions in male rats.

Keywords: wild edible fruits, ethnic communities, fertility, hydro alcoholic extract, spermatogenesis

1. Introduction

Millions of people who are of reproductive age are affected by infertility, which is recognized by the WHO as a worldwide health concern. Infertility has been a recurring problem among male and female individuals. Today, orthodox medicine has almost exceeded its limits in resolving problems of infertility. This is why the use of phytomedicine is becoming a main study in the treatment of infertility. It has been reported that alternative medicines have proven efficacious in the treatment of female infertility (Latif et al., 2008; Gaware et al., 2009). Locally grown plants have been used worldwide as supplements and therapies for several ailments. In recent times, extracts of Nigella sativa, Lophira lanceolata, Cochlospermum planchonii, Kaempferia parviflora, among others, have been reported to enhance fertility (Chaturapanich et al., 2008; Al-Sa'aidi et al., 2009; Etuk and Muhammad, 2009; Abu et al., 2012). More than 90% of male infertility cases are due to low sperm counts, poor semen quality or both. The causes of 30–40% of the cases of sperm abnormalities cannot be accounted (Lindheim et al., 1996). The active principles such as phenols, alkaloids, saponins, and most especially flavonoids are known to have estrogenic (Das et al., 2004) and androgenic (Yousef et al., 2004) activity.

Baccaurea courtallensis is a moderately sized evergreen tree of the Phyllanthaceae family, distributed from the South Konkan to South Kerala and adjoining Western parts of Tamil Nadu in the evergreen forests up to an altitude of 700 to 1000 m. *B. courtallensis* is one of the lesser – known wild edible fruit yielding tree, used by various ethnic communities and local folks of the Western Ghats, especially Muthuvan, Kani and Kadar tribes. Fruits are used to induce fertility in men and women (Daniel *et al*., 2005). The objective of the present study is to investigate the fertility enhancing effects of hydro alcoholic extract of *B. courtallensis* through the evaluation of sperm number, testicular weight, and morphology in male rats.

2. Materials and Methods

Plant Material

The collection of fruit samples of *B. courtallensis* were done in the month of May-June, at Courtallam hills, Tenkasi, Tamil Nadu. Early June was the ideal time for the collection of medicinal plants since the plants are enriched with phyto constituents during that time. Voucher specimen (No. KMCP/BC/124) was prepared and deposited in the Department of Pharmacognosy, K. M. College of Pharmacy, Madurai for future reference.

Drying

The fruits of *B. courtallensis* were washed and dried under shade for 15 days on fresh cotton cloth. After 15 days the dry weight was measured.

Extraction procedure

Cleaned mixer was used for grinding the dried fruits. After proper grinding, the weight of the powder was obtained. The powder was used for hot extraction.

Solvents order and temperature

Petroleum ether : 60º-80º C
Chloroform : 60º-62º C
Ethyl acetate : 74º-78º C
Hydro alcohol : 100º C

Preparation of Extracts of B. courtallensis by Hot Continuous Percolation Method

The flask with the given solvent was heated to a particular temperature. The vapour produced passed through the siphon tube into the thimble kept above where it was condensed and tickles down into the flask again through the thimble dissolving the active constituents in it. The method was described as the continuous extraction. The process was continued until all the soluble constituents were separated. The extract at the bottom was collected and dried under reduced temperature and pressure. Each time, before the extraction with other solvents, the powdered substance was air dried.

About 500 gm of dried powder was properly packed in Whatman filter paper (grade no.1) and kept in thimble and the soxhlet apparatus was set up. The extraction of powder was done with different solvents with solvents of increasing polarities like petroleum ether (60-80º C), chloroform, and hydro alcohol. During this process the temperature was maintenance based on the solvents used for extraction. The solvents were removed under reduced pressure using rotary evaporator and stored in desiccators. The consistency of the extract was semi solid (this method was repeated until the desired extract was obtained).

Animals

A total of 24 sexually experienced Wistar rats, aged at least 3 months and weighing between 190g and 220g, were housed in clean metabolic cages (6 per cage) contained in well ventilated standard housing conditions (temperature: 28–31°C; photoperiod: 12 hours natural light and 12 hours dark). The experiments were performed to minimize animal suffering in accordance with the CPCSEA guidelines.

The animals were acclimatized for one week under laboratory conditions before the beginning of the experiment. The rats were completely randomized into four groups (6 each) and orally treated with 1 mL/kg distilled water (control), 100, 200 and 400 mg/kg body weight (BW) per day of B. courtallensis for 21 days. During the treatment period the changes in the body weight was recorded. One day after the last treatment, the animals were sacrificed and blood sample was collected for biochemical analysis and androgen assay. Testis, seminal vesicles, epididymis and ventral prostate were removed and cleared of attached fat and connective tissue and weighed. The left testis and epididymis were used for sperm count.

Sperm Count from Testis (DSP)

Daily sperm production, spermatid number count and sperm transit

After removal of the tunica albuginea, the testes were minced and homogenized in 10 mL of 0.9% NaCl containing 0.5% Triton X-100 at medium speed in a FISATOM (720) tissue mixer for 1 min. After dilution, the number of homogenization-resistant spermatids was counted with a hemocytometer in replicate calculations. The number of spermatids per animal was also counted and this value was divided by 6.1 days, to convert them to daily sperm production (Robb et al., 1978).

Similarly, the cauda epididymis was cut into small pieces, minced and homogenized then and spermatozoa were counted as described above. The epididymal sperm transit rate was calculated by dividing the epididymal sperm number by the daily sperm production (Amann et al., 1976).

Sperm count in epididymis

The spermatozoa in epididymis were counted by method described by Gonzales *et al*., (2004) with modification which consisted to evaluate separately the number of spermatozoa in the cauda epididymis and caput/corpus. 5 mL of saline (NaCl 0.9%) was performed for homogenization of resistant epididymal sperm. After 24h in a fridge at 4°C, 10 mL of the

refrigerated homogenate was added to 70 mL of eosin (2%), and a sample was placed in a Neubauer chamber. Head sperms were counted in 25 squares for four times. The average sperm count of each rat was multiplied by 0.06 (sperm × 106/mL) and then by 5 mL (sperm × 106 per caput/corpus or cauda). Data are referred as sperm per caput/corpus or cauda epididymis.

Biochemical analysis

Total protein levels were determined in the serum and sexual organ (testis and epididymis) using colorimetric methods described by Gornall *et al*., 1949 and Bradford, 1976 respectively. The cholesterol levels in the testis were determined using the colorimetric method described by Forbes in 1930.

Hormonal Assay

Serum testosterone levels were determined by ELISA method using a commercial kit (Diagnostic Products Co., Los Angeles, CA, USA).

Statistical Analysis

All statistical analysis was conducted using the GraphPad software (version 5.0). Results were expressed as mean ± SEM (standard error of the mean). Differences between groups were assessed by one-way analysis of variance (ANOVA). Differences between pair of means were assessed by Newman-Keuls multiple range tests. A value of $P < 0.05$ was considered as statistically significant.

3. Results

Effect of Hydro alcoholic extract of *B. courtallensis* (HAEBC) on body and reproductive organs weight

There was no significant difference on body weight of treated animals, however, at dose of 100 mg/kg .200mg/kg and 400 mg/kg, the testis ($P < 0.05$; $P < 0.01$) and weight of accessory organs (prostate) $P< 0.01$; seminal vesicle $P < 0.05$) significantly increased when compared to the control (Table 1).

Table 1: Effect of hydroalcoholic extract of *B. courtallensis* on body weight (g) and reproductive organs weight (g)

Treatment Groups (mg / kg)	Organs Weight (g)				
	Body	**Testis**	**Epididymis**	**Prostate**	**Seminal vesicle**
G1- Normal Control	18.20 ± 1.65	0.53 ± 0.28	0.174 ± 0.12	0.072 ± 0.02	0.325 ± 0.07
G2 - HAEBC 100 mg/kg	17.45 ± 1.55	0.72 ± 0.43*	0.187 ± 0.24*	0.094 ± 0.09*	0.385 ± 0.14*
G3 - HAEBC 200 mg/kg	16.75 ± 1.30	0.77 ± 0.47*	0.190 ± 0.26*	0.107 ± 0.14*	0.412 ± 0.19*
G4 – HAEBC 400 mg/kg	17.85 ± 1.60	0.83 ± 0.54*	0.192 ± 0.29*	0.115 ± 0.17*	0.424 ± 0.25*

*Values are significantly different from normal control at P<0.01

Effect of Hydro alcoholic extract of *B. courtallensis* (HAEBC) on sperm count

21 days after treatment of Hydro alcoholic extract of *Baccaurea courtallensis* (HAEBC), no significant differences were observed in sperm count in epididymis and its transit in cauda. The daily sperm production significantly increased at dose of 100 mg/kg, 200mg/kg and 400 mg/kg with P<0.001(Table 2).

Table 2: Effect of hydro alcoholic extract of *B. courtallensis* on sperm count (x106)

Parameters	Treatment Groups (mg / kg) HAEBC			
	Control	100	200	400
DSP	15.15 ± 0.70	21.45 ± 1.08*	22.54 ± 1.40*	23.18 ± 0.41*
DSP / W Testis	12.30 ± 0.63	19.56 ± 1.04*	21.60 ± 1.32*	22.75 ± 0.33*
Epididymis	67.15 ± 1.55	75.10 ± 1.60*	80.45 ± 1.75*	82.85 ± 2.18*
Sperm transit in cauda	4.54 ± 0.52	3.64 ± 0.42*	3.85 ± 0.48*	4.05 ± 0.53*

*Values are significantly different from normal control at P<0.01

Effect of Hydro alcoholic extract of *B. courtallensis* (HAEBC) on biochemical parameters

After treatment with the Hydro alcoholic extract of *B. courtallensis* (HAEBC), we observed a significant reduction of total cholesterol levels ($P < 0.05$) at dose of 100, 200 and 400 mg/kg and total proteins ($P < 0.01$) at dose of 100, 200 and 400 mg/kg.

Effect of Hydro alcoholic extract of *B. courtallensis* (HAEBC) on testosterone level.

The serum testosterone concentration significantly increased at dose of 100 mg/kg, 200mg/kg and 400mg/kg with ($P < 0.01$) following 21 days of treatment with the Hydro alcoholic extract of B. courtallensis (HAEBC) (Table 3).

Table 3: Effect of hydroalcoholic extract of *B. courtallensis* on biochemical parameters

Groups	Treatments	Cholesterol mg / Dl	Total Proteins g / L	Testosterone ng / ml
G1	Normal Control 10 ml /kg Normal Saline	8.7 ± 0.52	5.3 ± 0.20	0.45 ± 0.08
G2	G2 - HAEBC 100 mg/kg	6.0 ± 0.28*	3.6 ± 0.14*	1.08 ± 0.20*
G3	G3 - HAEBC 200 mg/kg	5.4 ± 0.22*	3.1 ± 0.10*	1.32 ± 0.28*
G4	G4 – HAEBC 400 mg/kg	5.0 ± 0.17*	3.0 ± 0.08*	1.43 ± 0.35*

*Values are significantly different from normal control at P<0.01

4. Discussion

The development of additional methods and the active pursuit of research into new approaches to fertility control could provide enormous benefits in the area of social and public health. In the present work, the administration of the Hydro alcoholic extract of B.

courtallensis (HAEBC) did not significantly alter the body weight of the treated animals. However, a significant increase in the relative weight of the androgenic dependent organs was observed (prostate and seminal vesicles) in rats receiving 100 mg/kg, 200mg/kg and 400 mg/kg of Hydro alcoholic extract of B. courtallensis (HAEBC). These results are comparable to those reported by Varsha et al., (2013).

These results were derived from the increase in the rate of testosterone generated by the Hydro alcoholic extract of B. courtallensis (HAEBC). Indeed, it has been reported that accessory sexual organs are developed under the action of testosterone (seminal vesicles) and dihydro testosterone (prostate). By fixing to their specific receptors, those hormones activate the transcription of the genes necessary for the cellular proliferation of androgenic dependent organs and consequently increase their size (Hazard et al., 1989).

This study also shows that the significant increase in testicular weight observed after administration of the hydro alcoholic extract of B. courtallensis (HAEBC) at doses 100 mg/kg, 200mg/kg and 400 mg/kg is parallel to the significant increase in daily sperm production per testis at the same doses. The increase in the relative weight of the testis may be due to the increase in spermatogenesis (Lembè, et al., 2013) which is a consequence of the activity of testosterone and the presence of flavonoids in the preparations of hydro alcoholic extract of B. courtallensis (HAEBC) (Baril et al., 1993).

It is well known that the regulation of spermatogenesis is under the control of testosterone and androgen receptors (AR) of Sertoli cells. Testosterone deprivation studies performed in rodents have established that testosterone is required for germ cells to progress beyond meiosis and that testosterone is required for the release of mature spermatids during stage VIII in rats (Sharpe, 1994). Then, the withdrawal of testosterone or knock out of AR in Sertoli cells results in three major impairments to fertility. First, it exposes post meiotic germ cells to autoimmune attack and cytotoxic factors (Willems et al., 2010; Meng et al., 2011). Second, it causes the premature detachment of round spermatids from Sertoli cells (Holdcraft and Braun, 2004; O'Donnell et al., 1994; O'Donnell et al., 1996).

Third, fully mature spermatozoa cannot be released from Sertoli cells and the germ cells are phagocytized by the Sertoli cells (Griswold, 1998). It is then clear that Sertoli cells are thought to be the major cellular target for the testosterone signaling that is required to support male germ cell development and survival (Griswold, 2005; Dawson, 1990). On the other hand, flavonoids stimulate testicular androgenesis and spermatogenesis by acting on LH receptors and FSH, which in turn activate respectively the biosynthesis of testosterone by Leydig cells and spermatogenesis by Sertoli cells (Salem et al., 2001; Ghosh et al., 2002). Flavonoids can also activate the Nrf2 transcription pathway, which acts as mediator of the induction of NFE2-related factors genes and play a major role in defending testicular tissues against oxidative stress that leads to testicular alterations (Kojo, 2004).

We can therefore presume that the hydro alcoholic extract of B. courtallensis (HAEBC) could improve spermatogenesis in rats and has no substances that can produce toxic effects on intra-testicular structures. As it was not expected, but a significant decrease of serum cholesterol at the high dose (400 mg/kg) was noticed while at the same dose the level of serum testosterone increased. This contrast could be explained as follows: According to Rommerts (1998), cholesterol required for the synthesis of testosterone arrives at the testis and the adrenal gland by two pathways: the main or direct route is to extract cholesterol fixed to plasma lipoproteins including the low density fraction and the indirect route or local synthesis pathway consists of producing cellular cholesterol from acetate, necessary for the synthesis of testosterone.

It should therefore be thought that, the elevated testosterone level found in higher dose was related to the tissue cholesterol used for the synthesis of androgen. This testosterone would

come from the indirect synthesis by both testes and/or the adrenal gland. On the other hand, sterols and Flavonoids found in the plant could interfere for androgen synthesis.
Indeed, sterol are lipid substances that could be used as a substrate for the synthesis of testosterone or for the synthesis of one of these precursors (Pazzucconi et al., 1995) while Flavonoids are substances that could inhibit the synthesis of cholesterol by interacting with 3-hydroxy-3 methylglutaryl CoA (HMG-CoA) reductase, enzyme catalyzing the conversion of HMG-CoA to mevalonic acid . Based on these findings it probable that hydro alcoholic extract of B. courtallensis (HAEBC) contains several secondary metabolites leading to testosterone metabolism and consequently to the improvement of spermatogenesis.
Similarly, the administration of the hydro alcoholic extract of B. courtallensis (HAEBC) resulted in a significant decrease of the total serum protein level at dose of 100 mg/kg, 200mg/kg and 400 mg/kg in animals. The presence of sitosterol in the plant may be the cause of this significant decline since sitosterol would act by inhibiting the synthesis of proteins in tissues (Malini and Vanithakumari, 1991).

5. Conclusion

Based on the above results, it may be concluded that the hydro alcoholic extract of *B. courtallensis* (HAEBC) could improve the male reproductive functions, but information regarding exact mechanism of action requires further investigations.

6. References

1. Abu, A. (2012). Aqueous ethanolic extract of Cochlospermum planchonii rhizome enhances spermatogenesis in male albino rats. African journal of biotechnology, 11. doi:10.5897/AJB12.225
2. Alphonse, N., Marie, N. N., Hubert, K., Landry, K. B., & Lembe, M. (2017). Evaluation of the fertility activity of the aqueous leaves extract of Zanthoxylum macrophylla (Rutaceae) on male rats. The Journal of Phytopharmacology, 6(5), 277-281.
3. Al-Sa'aidi, J. A. A., Al-Khuzai, A. L. D., & Al-Zobaydi, N. F. H. (2009). Effect of alcoholic extract of Nigella sativa on fertility in male rats. Iraqi journal of Veterinary Sciences, 23.
4. Amann, R. P., Johnson, L., Thompson, D. L., Jr., & Pickett, B. W. (1976). Daily spermatozoal production, epididymal spermatozoal reserves and transit time of spermatozoa through the epididymis of the rhesus monkey. Biol Reprod, 15(5), 586-592. doi:10.1095/biolreprod15.5.586
5. Baril, G., Chemineau, P., Cognie, Y. (1993). Manuel de formation pour l'insémination artificielle chez les ovins et caprins, Etude FAO production et santé animales 83. Station de la physiologie de la reproduction, Institut Nationale de la Recherche Agronomique (INRA), Nouzilly, 37380 Monnaie, France.
6. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 72, 248-254. doi:10.1006/abio.1976.9999.
7. Chaturapanich, G., Chaiyakul, S., Verawatnapakul, V., & Pholpramool, C. (2008). Effects of Kaempferia parviflora extracts on reproductive parameters and spermatic blood flow in male rats. Reproduction, 136(4), 515-522. doi:10.1530/rep-08-0069
8. Daniel, P., Murthy G. V. S., & Venu, P. (2005). The flora of Kerala. Botanical Survey of India. Vol 1, 96 pp.

9. Das, S., Parveen, S., Kundra, C. P., & Pereira, B. M. (2004). Reproduction in male rats is vulnerable to treatment with the flavonoid-rich seed extracts of Vitex negundo. Phytother Res, 18(1), 8-13. doi:10.1002/ptr.1352.
10. Dawson, E. B., Harris, W. A., & Powell, L. C. (1990). Relationship between ascorbic acid and male fertility. World Rev Nutr Diet, 62, 1-26. doi:10.1159/000417532.
11. Etuk, E. U., & Muhammad, A. A. (2009). Fertility enhancing effects of aqueous stem bark extract of Lophira lanceolata in male Spargue dawley rats.
12. Forbes, J. C. (1930). Dosage du cholesterol. Journal of Laboratory Medicine, 16:520.
13. Gaware.V., M., Parjane.S., K., MerekarAbhijit, N., PattanS., R., DigheN., S., & Kuchekar.B.S.GodgeRahul, K. (2009). Female infertility and its treatment by alternative medicine: A review. Journal of chemical and pharmaceutical research, 1.
14. Ghosh, D., Das, U. B., & Misro, M. (2002). Protective role of alpha-tocopherol-succinate (provitamin-E) in cyclophosphamide induced testicular gametogenic and steroidogenic disorders: a correlative approach to oxidative stress. Free Radic Res, 36(11), 1209-1218. doi:10.1080/1071576021000016472.
15. Gonzales, G. F., Gasco, M., Córdova, A., Chung, A., Rubio, J., & Villegas, L. (2004). Effect of Lepidium meyenii (Maca) on spermatogenesis in male rats acutely exposed to high altitude (4340 m). J Endocrinol, 180(1), 87-95. doi:10.1677/joe.0.1800087
16. Gornall, A. G., Bardawill, C. J., & David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. J Biol Chem, 177(2), 751-766.
17. Griswold, M. (2005). Perspective on the Function of Sertoli Cells (pp. 15-18).
18. Griswold, M. D. (1998). The central role of Sertoli cells in spermatogenesis. Semin Cell Dev Biol, 9(4), 411-416. doi:10.1006/scdb.1998.0203.
19. Holdcraft, R. W., & Braun, R. E. (2004). Androgen receptor function is required in Sertoli cells for the terminal differentiation of haploid spermatids. Development, 131(2), 459-467. doi:10.1242/dev.00957
20. Kojo, S. (2004). Vitamin C: basic metabolism and its function as an index of oxidative stress. Curr Med Chem, 11(8), 1041-1064. doi:10.2174/0929867043455567.
21. Latif, R., Lodhi, G. M., & Aslam, M. (2008). Effects of amlodipine on serum testosterone, testicular weight and gonado-somatic index in adult rats. J Ayub Med Coll Abbottabad, 20(4), 8-10.
22. Lembè, D. M., Koloko, B. L., Bend, E. F., Domkam, J., Oundoum, P. C. O., Njila, M. I. N., . . . Gonzales, G. F. (2014). Fertility enhancing effects of aqueous extract of Rauvolfia vomitoria on reproductive functions of male rats. Journal of Experimental and Integrative Medicine, 4, 43-49.
23. Lindheim, S. R., Barad, D. H., Zinger, M., Witt, B., Amin, H., Cohen, B., Barg, P. (1996). Abnormal sperm morphology is highly predictive of pregnancy outcome during controlled ovarian hyperstimulation and intrauterine insemination. J Assist Reprod Genet, 13(7), 569-572. doi:10.1007/bf02066610
24. Malini, T., & Vanithakumari, G. (1991). Antifertility effects of beta-sitosterol in male albino rats. J Ethnopharmacol, 35(2), 149-153. doi:10.1016/0378-8741(91)90066-m
25. Meng, J., Greenlee, A. R., Taub, C. J., & Braun, R. E. (2011). Sertoli cell-specific deletion of the androgen receptor compromises testicular immune privilege in mice. Biol Reprod, 85(2), 254-260. doi:10.1095/biolreprod.110.090621.
26. O'Donnell, L., McLachlan, R. I., Wreford, N. G., & Robertson, D. M. (1994). Testosterone promotes the conversion of round spermatids between stages VII and VIII of the rat spermatogenic cycle. Endocrinology, 135(6), 2608-2614. doi:10.1210/endo.135.6.7988449
27. O'Donnell, L., McLachlan, R. I., Wreford, N. G., de Kretser, D. M., & Robertson, D. M. (1996). Testosterone withdrawal promotes stage-specific detachment of round

spermatids from the rat seminiferous epithelium. Biol Reprod, 55(4), 895-901. doi:10.1095/biolreprod55.4.895

28. Pazzucconi, F., Dorigotti, F., Gianfranceschi, G., Campagnoli, G., Sirtori, M., Franceschini, G., & Sirtori, C. R. (1995). Therapy with HMG CoA reductase inhibitors: characteristics of the long-term permanence of hypocholesterolemic activity. Atherosclerosis, 117(2), 189-198. doi:10.1016/0021-9150(95)05571-d.
29. Robb, G. W., Amann, R. P., & Killian, G. J. (1978). Daily sperm production and epididymal sperm reserves of pubertal and adult rats. J Reprod Fertil, 54(1), 103-107. doi:10.1530/jrf.0.0540103.
30. Rommerts, FFG. (1998). Testosterone; an overview of biosynthesis, transport, metabolism and nongenomic actions. In E. Nieschlag, & H. M. Behre (Eds.), Testosterone: Action - Deficiency - Substitution (pp. 1-31). Springer-Verlag.
31. Salem, M. H., Kamel, K. I., Yousef, M. I., Hassan, G. A., EI-Nouty, F. D. (2001). Chemical, pharmacological and botanical studies on Pelalium merex. International Journal of Medicinal Aromatic Plants. 26(3):64-96.
32. Sharpe, R.M. (1988) Regulation of Spermatogenesis. In: Knobil, E. and Neill, J.D., Eds., The Physiology of Reproduction, Raven Press, New York, 1363-1434.
33. WHO, Research Guidelines for evaluating the safety and efficacy of herbal medicines, World Health Organizations, Geneva, 1998; 30-33.
34. Willems, A., Batlouni, S. R., Esnal, A., Swinnen, J. V., Saunders, P. T., Sharpe, R. M., . . . Verhoeven, G. (2010). Selective ablation of the androgen receptor in mouse sertoli cells affects sertoli cell maturation, barrier formation and cytoskeletal development. PLoS One, 5(11), e14168. doi:10.1371/journal.pone.0014168.
35. Yousef, M. I., Esmail, A. M., & Baghdadi, H. H. (2004). Effect of isoflavones on reproductive performance, testosterone levels, lipid peroxidation, and seminal plasma biochemistry of male rabbits. J Environ Sci Health B, 39(5-6), 819-833.