

## **NATURAL COMPOUNDS TO TREAT MALE INFERTILITY**

Raju B. Akondi\*, Krishna Manjari Pawar, Siva Redy Challa

Department of Pharmacology, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530002, Andhra Pradesh, INDIA.

### **Summary**

Infertility is a growing problem all over the world. About 13–18% of couple suffers from it and approximately in 39% cases, semen analyzed as abnormal. There are several causes of male infertility i.e. Hypogonadism, Drugs, alcohol, smoking, Bad semen quality, Teratospermia, Oligospermia, Azoospermia, genetic factors, Vas deference obstructions, Testicular Torsion, impotence etc. There are several therapeutic approaches to alleviate male infertility problem including Allopathy, ayurveda, Unani etc. The present review provides an overview of the prevalence, causes, treatment of male infertility using herbal medicines. Screening techniques to find the activity of drugs in male infertility is also added in this review.

**Key Words:** Male infertility, Herbal drugs,

### **#Correspondence Address:**

**Raju B. Akondi, M.Pharm**

Research Scholar

Department of Pharmacology,

University College of Pharmaceutical Sciences,

Andhra University, Visakhapatnam-530002,

Andhra Pradesh, INDIA.

E-mail: [akondi\\_r@yahoo.com](mailto:akondi_r@yahoo.com).

Phone: 09246622478

## Introduction

Infertility affects 15% of all couples. In 39% of these couples, the male generates semen analyzed as abnormal. Spermatogenic failure, including azoospermia and oligospermia, is one of the important causes of male infertility. (1) Treatments for male infertility range from surgical intervention or intrauterine insemination (IUI) to various forms of ART, such as *in vitro* fertilization (IVF) or intra cytoplasmic sperm injection (ICSI). Depending on the source of the problem, sperm can be taken from the man's ejaculate for use in assisted fertilization procedures.(2) Apart from the above procedures pharmacological intervention is needed, particularly in the cases of Oligozoospermia and idiopathic infertility to improve the quality of semen. The drugs used in modern medicine for the various abnormalities are listed below.

Various drugs are used to treat different conditions of male infertility in modern medicine i.e. Antibiotics, Antiphlogistics, kallikrein, corticosteroids, hormone preparations etc with uncertain results. In the search of new compounds it is obvious to depend on alternative systems of medicine. Alternative systems of medicine have many branches which treat patients through naturopathy. Herbal drugs from naturopathy (Ayurveda, Sidha, Unani and Chinese traditional medicine) are popular to treat male infertility despite the lack of scientific experimentation to assess its effectiveness. (3)

Over 20 new drugs launched in the market between 2000 and 2005 originating from terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates to treat various conditions(4). This number shows the importance of natural sources in drug discovery.

The purpose of this review is to name few herbal drugs which are popularly used in alternative systems of medicine and to discuss scientific experimentations conducted to prove their effectiveness in male infertility. Various standard ayurvedic text books and literature on herbal drugs quoted importance of below plants in the treatment of male infertility.

*Withania somnifera* (Solanaceae,) seeds of this plant are used to prepare medicine and it is used for psychosexual condition, for erectile dysfunction and to improve sperm count.

Ashwagandha is patented for its increasing male sperm count in USA and JAPAN (Us patent No- Us 6,866,872B1 Dated march 15, 2005). (10) Asparagus recemosus (Solanceae) is a vitaliser and regulates hormone imbalance. Clerodendrum serratum (Verbanaceae) root of this plant proved much effective in the male infertility associated with chronic chest infection like pulmonary tuberculosis. Solanum surratense (Solanceae) is proved beneficial in patients with idiopathic infertility. Tribalus terrestris (Zygophilliceae) contains saporins of the turostanole type with high content of protodioscine, which stimulates spermatogenesis and libido. This herb is beneficial alone, also used in combination with Satavari and Ashwagandha. Dioscorea bulbifera (Dioscoreaceae) is also a well known herb and its action is same as Gokshru. Nyctanthes arbartristis (Nyctantheceae) is a choice of drug for homosexual person to change their libido. Semecarpus anacardium (Anacardiaceae) is regenerative drug and helps in maturation of spermatozoa. Elettaria cardamomum (Zingiberaceae) seeds activate secretions in genital and other systems. Plumbago zeylanica (Pulbanajaceae) is a powerful stimulator of generative cells and regulate seminal fluid and prostatic secretions. (3)

Majority Marketed preparations in Ayurveda contains above herbs and their combinations. Few studies on their effectiveness were conducted on rats and humans. A study on indigenous drug *Speman* (The Himalaya drug company, Mumbai) which contains some of the above herbs showed significant effect on various sperm parameters. After three months of treatment with *Speman* the mean sperm density increased from  $14.38 \pm 0.42$  million/ml to  $62.86 \pm 1.26$  million/ml. The mean motility before treatment of *Speman* was 35.73%, which increased to 46.62% after treatment. There was an improvement from 49.13% to 62.71% in the sperm morphology. The mean testosterone level before *Speman* treatment was  $4.27 \pm 0.26$  ng/ml and increased to  $5.86 \pm 0.34$  ng/ml (5).

*French maritime bark extract* shows significant effect on sperm quality and function. 38% mean improvement is recorded in that study (6). Plant extracts *Rubus Coranus* on sperm parameters showed significant increases in the weight of the testes, Epididymal sperm count, and sperm motility compared to the control group. RF also increased the expression of CREM at both the mRNA and protein levels. These results suggest that RF may improve male fertility by enhancing spermatogenesis. (7)

A report published on the effects of *Shilajit* on spermatogenesis and ovogenesis were studied using male and female rats. *Shilajit* was administered orally to 7-week-old rats over a 6-week period. In the male rats, the number of sperms in the testes and epididymides was significant higher than in the control. A histological examination revealed an apparent increase in the number of seminiferous tubular cell layers in the testes of the treated rats. However, there were no significant differences in the weights of heart, spleen, liver, kidney, brain, testes and epididymides. In the female rats, the effect of *Shilajit* was estimated by the ovulation inducing activity. Over a 5-day, ovulation was induced in seven out of nine rats in the *Shilajit* administration group and in three out of nine rats in the control. It was estimated that *Shilajit* had both a spermiogenic and ovogenic effect in mature rats. (8)

A study on the aqueous extract of *P. guineense* at both doses (122.5 and 245 mg/kg) had a positive impact on the male reproductive function since it stimulated the secretions of the testes, epididymis and seminal vesicles. (10) A report from India suggest that administration of total alkaloids of *Mucuna pruriens* exhibited appreciable increase in the weights of testes, seminal vesicles and prostate, and the results were comparable with testosterone enanthate activity. As androgenic activity is attributed to testosterone levels in the blood, it is suggested that the alkaloids simulate the secretion of testosterone to ensure greater availability to gonads. (11)

The results of study undertaken on aqueous extract of *D. hatagirea* showed significant anabolic effect which is comparable to testosterone treatment. Genesis of steroids is one of the causes of increased body and sexual organ weights and an increase in this parameter could be regarded as a biological indicator for effectiveness of the herbal drugs in improving the genesis of steroidal hormones. (12)

The effect of *Korean ginseng (ginseng)* on spermatogenesis and cAMP-responsive element modulator (CREM) in rat testes was evaluated using sperm analysis, reverse transcription polymerase chain reaction, and Western blot analysis. The ginseng-treated rats exhibited significantly increased sperm count and motility with enhanced levels of CREM messenger RNA and protein. Ginseng appears to induce spermatogenesis via CREM activation in rat testis. (13)

Mature male albino rats were given *Hibiscus macranthus* (Malvaceae) and *Basella Alba* (Basellaceae) aqueous extracts from both fresh and dry leaves. This was to evaluate their effects on male reproductive function. Testis of treated rats showed high testosterone production in vitro (136 and 62%, respectively for treated and control after 15 days, compared to those of 3 days). Activity of prostatic acid phosphatase was high in prostate, testis and serum of treated rats in all experimental period. From these findings and observations, it was concluded that the aqueous extract of *H. macranthus* and *B. Alba* had anabolizing and virilizing effects. (14)

Various traditional Chinese medicines are reported spermatogenesis activity. *Hachimijiogan* is one of them. *Hachimijiogan* used in male infertility in which it improves spermatogenesis and helps with impotence by reducing serum prolactin levels. *Hachimijiogan* is a blend of eight herbs: *Rehmannia* root, *Comus* fruit, *Dischorea* rhizome, *Alisma* rhizome, *Hoelen*, *Moutan* bark, *Cinnamon* and *Aconite* root, which are blended, pulverized and added to a medically inert fraction. 5-10 g of this is taken daily (19). *Astragalus membranaceous* has been shown to increase sperm motility in vitro. Red clover, nettle, raspberry leaf, ladies mantle and chaste berry are also cited. *Saw palmetto* (*Serenoa serrulata*) is reputed to strengthen the male reproductive system and increase the production of testosterone.

Numerous studies have reported that Chinese herbs can significantly improve the quality and quantity of sperm. Incubation of human spermatozoa with decoctions of *semencuscutae*, *rhizoma curculiginis* and *radix morindae officinalis* at various concentrations for 30 min and performed tests for sperm capillary penetration, sperm motility and hypo osmotic swelling. Sperm motility improved markedly and sperm membranes were stabilized, indicating that herbal decoctions may be beneficial in promoting sperm function for **IUI** and **IVF**. (20)

A larger, non randomized study of 202 infertile men with abnormal semen profiles was conducted for 2 months using the *shengjing* pill. Sperm density, motility and viability were significantly improved. The concentrations of serum FSH, LH and testosterone were normalized by treatment, and 78% of the 116 spouses conceived. Co administration of *bushenshenjing* decoction obtained an improvement in spermatogenesis. The serum LH and total and free testosterone concentrations increased significantly compared with the controls.

Hong *et al.* (1992) screened aqueous extracts of 18 Chinese medicinal herbs for effects on the motility of spermatozoa *in vitro* using a trans-membrane migration method. Only one herb was effective i.e. *Astragalus membranaceus*, and it stimulated a 1.4-fold increase in forward progression compared with sperm from controls. Effect of the alcoholic extract of *Lepidium meyenii* (Maca) on the spermatogenesis in male rats was evaluated. Epididymal sperm count was increased. Serum testosterone levels were not affected. It is concluded that the alcoholic extract of *Maca* activates onset and progression of spermatogenesis at 48 mg/day or 96 mg/day in rats. (23)

Aqueous extracts of *Zingiber officinale* and *Pentadiplandra brazzeana* were tested for their possible androgenic activity in male Wistar rats. The aqueous extract of *Z. officinale* significantly increased in the relative weight of the testis, the serum testosterone level, and testicular cholesterol level and epididymal  $\alpha$ -glucosidase activity. The aqueous extract of *P. brazzeana* significantly increased the weights of the testis, seminal vesicles and prostate. It also significantly increased the serum and testicular testosterone level. The fructose,  $\alpha$ -glucosidase and cholesterol levels in *P. brazzeana*-treated rats were increased by 28 %, 35 % and 114 %, respectively. The aqueous extracts of both *P. brazzeana* and *Z. officinale* have an androgenic activity, which seems to be more potent with *P. brazzeana* than with *Z. officinale*.(24)Effect of the aqueous extract of *Mondia whitei* (Periplocaceae) roots on testosterone production and fertility of male rats was evaluated. Chronic treatment for 8 days induced a significant increase in the testicular weight, the serum and testicular testosterone, the testicular protein content and the sperm density ( $P<0.05-0.01$ ). *Mondia whitei* root extract possesses an androgenic property. (25)

This study was undertaken to study the effect of Satureja khuzestanica essential oil (SKEO) in male rat fertility. SKEO significantly improved all the parameters evaluated such as potency, fecundity, fertility index, and litter size. Moreover, concentrations of FSH and testosterone were significantly increased in SKEO-treated groups. Also the weights of testes, seminal vesicles, and ventral prostate weights were increased by SKEO (225 mg/kg). Histopathological analysis showed that in male rats treated with SKEO (150, 225 mg/kg) the number of spermatogonium, spermatid cords, Leydig cells, and spermatozooids was increased. Also in these groups, the Sertoli cells were hypertrophic.(29)

**METHODS AND PROCEDURES FOR EVALUATION OF SPERMATOGENIC EFFECT:****1) On Normal Rats**

Six-week-old albino male rats weighing 200 g can be used for this experiment. The animals should be maintained under a controlled temperature ( $23\pm 1^\circ\text{C}$ ), relative humidity ( $55\pm 10\%$ ), and a 12-h light/dark cycle per day. Standard laboratory chow and tap water can be provided ad libitum. Drug can be administered after a 1-week acclimatization period. The body weight before the start of dosing, every 3 or 4 days, and on the day of the necropsy should be noted. (9) One group of rats should receive testosterone enanthate and results with control and test group can be compared with testosterone enanthate activity. As spermatogenesis is also governed by testosterone, increase in the level of testosterone by the test drug may increase seminiferous tubules either by its action on pituitary function or on Leydig's cells that store testosterone for regulation of gonadal function.

At the end of the treatment period, the rats can be sacrificed with pentobarbital sodium (50 mg/kg, i.p.). The testes should be dissected, cleared of the adhering tissues, and note the weight. The epididymis is removed and can be used for sperm analysis. Testes samples can be frozen for subsequent reverse transcription polymerase chain reaction (RT-PCR) and Western blotting assays.

**2) Compromised reproductive function restoring effect:**

It has come to be known of late that endocrine disturbing chemicals (environmental hormones) existing in our living environment, such as bisphenol A, dibutyl phthalate, vinclozolin, polychlorobiphenyls, ethynylestradiol, nonylphenol, etc., not to speak of dioxins, affect the reproductive functions of animals to reduce their sexual activities either reversibly or at times irreversibly and impair male genital organs causing decreases in sperm count, in particular. These endocrine disturbing chemicals are present in the environment and act at low concentrations so that they have become a social problem. Many herbal drugs are proved to be effective in restoring the reproductive function damaged by the environmental factors. The below procedure can identify such properties. Rats aged 11 weeks (in group of 8) were orally dosed with 3 mg/kg of the endocrine disturbing chemical Ethynyl Estradiol suspended in 0.5%

CMC solution (E.estradiol 0.6mg/ml) or as control, 5ml/kg of 0.5%CMC solution once daily in the morning for 2weeks and after the administration course, the testis, epididymis, prostate and seminal vesicles were respectively weighed. It is expected that rat genital organs are atrophied due to the endocrine disturbing chemical. Then the above rats (In groups of 8) with the reproductive function compromised by the endocrine disturbing chemical were orally dosed with different doses of herbal drugs. Physical and Biochemical tests which are given below can be done for every group (control& tests) of animals.

### **3) Sperm Analysis**

Epididymal sperm count and motility can be evaluated as described by [Connolly et al. \(2005\)](#), with some modifications. To obtain the sperm count, the entire epididymis from the rats should be minced in M199 media containing 0.5%bovine serum albumin (BSA) and incubated for 5 min at 37 °C. The sperm concentration is determined by manual evaluation using a hemocytometer. For assessment of sperm motility, sperm are recovered from the excised cauda epididymidis and allowed to capacitate for 5 min in M199 media containing 0.5% BSA at 37°C. Sperm are scored as motile if any movement was detected. The total number of sperm and the number that were motile were determined.

### **4) RNA Isolation and RT-PCR**

#### **a) Total RNA Isolation from Rat Testes**

Fenozol is added to the testis tissue samples. The samples are then homogenized and incubated for 5 min at 50°C. Chloroform is added and the samples are centrifuged (12,000×g, 10 min, room temperature). Aqueous phases are transferred to fresh tubes and isopropanol was added. The supernatants were incubated for 10 min at room temperature and centrifuged (12,000×g, 15 min, and 4 °C). The RNA pellets were then washed with 70% ethanol, air dried, and redissolved in diethylpyrocarbonate(DEPC) treated water. Total RNA samples were analyzed by gel electrophoresis. The final amount of RNA was estimated by determining the optical density at 260 nm.



**b) cDNA Synthesis and PCR-Amplification:**

First-strand cDNA synthesis with total RNA is performed using reverse transcriptase. Subsequently, PCR-amplification is performed by the method described by Saiki et al. (1986). The PCR-products are separated on a 1.5% agarose gel and visualized by ethidium bromide staining.

**c) Western Blotting Assay:**

Proteins from homogenized testes are separated using a nuclear extract kit, according to the manufacturer's protocol with minor modifications (Active and Motif, USA). Fifty micrograms testicular protein is loaded. Protein concentrations are determined by the Bradford method (Bradford, 1976). The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting assays are performed as described previously (Florin et al., 2005). Protein extracts were separated in 10% Tris-glycine gels by SDS-PAGE and electrophoretically transferred to nitrocellulose membranes. Transfer is performed at a constant voltage of 120mA for 1 h. After the transfer, membranes are blocked and incubated with the primary antibody for CREM-1 (Santa Cruz, USA) over night at 4 °C. After incubation, the membranes were rinsed and incubated with secondary antibody for 1 h at room temperature. The antigens are visualized with a See Pico CBB stain kit.

**d) CREM Studies:**

The transcription factor cAMP-responsive element modulator (CREM) plays an essential role in primate spermatid maturation. Male mice lacking a functional CREM gene are infertile. Infertile men with arrested round spermatid maturation exhibit substantially reduced levels of CREM protein and CREM messenger (m) RNA or none at all. Combinations of genetic changes in the human CREM gene can explain some forms of male infertility. Korean ginseng (ginseng), the root of *Panax ginseng* and some other herbs are worked on this. CREM expression in rat testes can be done using sperm analysis and a molecular biologic method.

### Conclusion

The causes of male infertility include semen or sperm abnormalities, sperm transport disorders, aspermia, improper spermatogenesis and decreased libido. This results from disorder of the testis function in producing normal sperm. Many modern scientific studies on various drugs from Ayurveda, Chinese herbal medicine and other alternative methods are screened for their effectiveness to treat sperm abnormalities. Some of them showed significant activity both in pre clinically and clinically. Herbs like Ashwagandha, shilajit, Korean ginseng are few examples using extensively as formulations. The studies conducted on the above drugs reveals that they are effectively increases sexual desire associated with spermatogenesis and suggested that they could be recommended in male sexual disorders like decreased libido, oligospermia. However, further research will be needed to identify the active components of these herbal drugs. There is need to evaluavate parameters like cAMP-responsive element modulator (CREM) in rat testes. Enhanced levels of CREM messenger RNA and protein indicates induction of spermatogenesis via CREM activation in rat testis. It is necessary to conduct sperm function tests using reverse transcription polymerase chain reaction, and Western blot analysis. Some of the methods described above including compromised reproductive function restoring effect are important to follow where ever necessary.

### References

1. Wan Su Park, Dong Youp Shin Do Rim Kim, Woong Mo Yang, Mun Seog Chang, Seong Kyu Park, Korean ginseng induces spermatogenesis in rats through the activation of cAMP-responsive element modulator (CREM), *Fertil Steril* 2007;88:1000–2.
2. Papolu Rama Devi, Vijaya Laxmi, Chatterjee Charulata, Adusumilli Rajyalakshmi. “Alternative medicine” a right choice for male infertility management, *International Congress Series* 1271 (2004) 67– 7.
3. Young-Won Chin, Marcy J. Balunas, Hee Byung Chai , and A. Douglas King horn *Drug Discovery From Natural Sources*, *The AAPS Journal* 2006; 8 (2) Article 28
4. Rang and Dale *Pharmacology Text Book*. Fifth edition, page nos747-749
5. Krishna Mukherjee, Evaluation of the Efficacy of Speman in the Management of Male Sub fertility, *Indian Journal of Clinical Practice* (2003): 13(11), 29-3.

6. Scott J. Roseff, M.D. Journal of reproductive medicine, Improvement in sperm quality and function with French maritime pine tree bark extract. vol 47, no 10, October 2002
7. Myung Sook Oh, Woong Mo Yang, Mun Seo Changa, Wansu Park, Do Rim Kima, Hyun Kyung Lee, Won Nam Kima, Seong Kyu Park, Effects of Rubus coreanus on sperm parameters and cAMP-responsive element modulator (CREM) expression in rat testes
8. Jeong-Sook Park, Gee-Young Kimb, Kun Hana, The spermatogenic and ovogenic effects of chronically administered Shilajit to rats, Journal of ethno pharmacology, 2006, vol. 107, n3, pp. 349-353.
9. Hironori Tomi, Shiga, Masaru Yoshida, Kyoto, Kouichi Kishida, Kyoto Withania somnifera dunal extracts for increasing male sperm count, Patent No-US6866872b1 (March 15, 2005)
10. F. G. Y. Mbongue, P. Kamtchouing, O. J. L. Essame, P. M. Yewah, T. Dimo, D. Lontsi. Effect of the aqueous extract of dry fruits of Piper guineense on the reproductive function of adult male rats.
11. Nadkarni. K.M, Indian Materia Medica, Vol I, 3rd ed. Popular Prakashan Pvt. Ltd. Bombay, 1976, p. 818.
12. Mayank Thakur and V. K. Dixit Aphrodisiac Activity of Dactylorhiza hatagirea (D. Don) Soo in Male Albino Rats Evid Based Complement Alternat Med. 2007 September 4(Suppl 1): 29–31
13. W. Park, D. Shin, D. Kim, W. Yang, M. Chang, S. Park, Korean ginseng induces spermatogenesis in rats through the activation of cAMP-responsive element modulator (CREM) Fertil Steril 2007; 88: 1000–2. 2007.
14. F.P. Moundipa, P. Kamtchouing, N. Koueta, Justine Tantchou, N.P.R. Foyang, Fe'licite, T. Mbiapo Effects of aqueous extracts of Hibiscus macranthus and Basella alba in mature rat testis function. Journal of ethnopharmacology, 1999, vol. 65, pp. 133-139
15. Lowana Veal, Complementary therapy and infertility: an Icelandic perspective, Complement Ther Nurs Midwifery. 1998 Feb; 4(1):3-6.
16. A. O. Arowojolu, O. Akinloye and O. B. Shittu Serum and seminal plasma prolactin levels in male attenders of an infertility clinic in Ibadan, Journal of Obstetrics and Gynaecology (April 2004) Vol. 24, No. 3, 306.
17. Krishna Mukherjee, Amita Tripathi, Dr. Kala Suhas Kulkarni. Evaluation of the Efficacy of Speman in the Management of Male Sub fertility Indian Journal of Clinical Practice (2003): 13(11), 29-31.
18. Scott J. Roseff, Improvement in sperm quality and function with French maritime pine tree bark extract, Journal of reproductive medicine, vol 47, no 10, October 2002.

19. Patki,P.S, Role Of Indigenous Drugs In The Management Of Male Sexual Disorders Probe,1988,1,25-28.
- 20.Xian Xu, Hang Yin, Daiyi Tang Li Zhang and Roger G. Gosden. Application of traditional Chinese medicine in the treatment of infertility,Human Fertility (2003) 6, 161–168
21. Nikoloas V.Sofikitis Endocrinological Biophysical and Biochemical Parameters Of Semen Collected Via Masturbation Versus Sexual Intercourse, Journal Of Andrology, Vol-14,No5, Sep/Oct,1993.
22. A. O. Arowojolu, O. Akinloye, Serum and seminal plasma prolactin levels in male attenders of an infertility clinic in Ibadan, Journal of Obstetrics and Gynecology (April 2004) Vol. 24, No. 3, 306
- 23.Gustavo F. Gonzales, Julio Rubio, Arturo Chung, Manuel Gasco, Leon Peru,Effect of alcoholic extract of *Lepidium meyenii* (Maca) on testicular function in male rats, , Asian J Androl 2003 Dec; 5: 349-352 .
- 24 .Pierre Kamtchouing Gisele Yolande Mbongue Fandio ,Théophile Dimo; Hermine Boukeng Jatsa, Evaluation of androgenic activity of *Zingiber officinale* and *Pentadiplandra brazzeana* in male rats, , Asian J Androl 2002 Dec; 4: 299-301 .
25. Pierre Watcho, Pierre Kamtchouing, Selestin D. Sokeng, Paul F. Moundipa, Justine Tanchou, Jean L. Essame, Noussithe Koueta Androgenic effect of *Mondia whitei* roots in male rats Asian J Androl 2004 Sep; 6: 269-272.
- 26.Gerhard Haidl, Frank-Michael Köhn, Wolf-Bernhard Schill,Drug treatment of male fertility disorders, Asian J Androl 2000 Jun; 2: 81-85.
27. jLiu , P. Liang , C. Yin , T. Wang , H. Li , Y. Li and Z. Ye, Effects of several Chinese herbal aqueous extracts on human sperm motility in vitro. *Andrologia*, Volume 36 Issue 2, Pages 78 – 83.
28. Saiki et al." A Novel Method for the Detection of Polymorphic Restriction Sites by Cleavage of Oligonucleotide Probes: Application to Sickle-Cell Anemia "*BioTechnology* 3(11):1008-1012.
29. Sulmaz Haeri, Bagher Minaie, Gholamreza Amin, Shekoufeh Nikfar,Reza Khorasani, Hadi Esmaily, Alinazar Salehnia, Mohammad Abdollah, Effect of *Satureja khuzestanica* essential oil on male rat fertility, *Fitoterapia* 77 (2006) 495–499.