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Review Article

CAUSES, DIAGNOSIS AND MANAGEMENT OF MALE INFERTILITY: PHYTOCOMPOUNDS AS NATURAL SERMs-A REVIEW

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ABSTRACT

Infertility is an inability to achieve pregnancy in spite of regular, unprotected coitus in one year. Infertility affects about 15% of couples in which the malefactor contributes to 40% worldwide. There are various risk factors, causes and treatment modalities for male infertility of which Selective Estrogen receptor modulators (SERMs) are one of the promising treatments available for treating idiopathic male infertility. Idiopathic male infertility refers to an abnormality in semen parameters without an identifiable cause and diagnosis recently known. SERMs are structurally diverse non-steroidal compounds which bind to estrogen receptor (ER) to exert tissue-specific estrogen agonist and antagonist effects. Several compounds including clomiphene, tamoxifen, toremifene are currently available synthetic SERMs of which clomiphene and tamoxifen are commonly used in treating idiopathic male Infertility. While these compounds are effective in treating male infertility, their long-term use increases the risk of stroke, cataract, vision-threatening ocular toxicity, deep vein thrombosis, pulmonary embolism and even more. Aim of this review is to highlight the causes, diagnosis and management of male fertility and to reveal the unforeseen effect of long-term use of SERMs. Also, this article will review the safe use of phytocompounds as natural SERMs.

Keywords: Infertility, Idiopathic male Infertility, Phytocompounds, SERMs, Natural SERMS.

1. INTRODUCTION

Infertility rates have dramatically increased in the last two decades, especially in men. It has been reported that environmental factors and lifestyle habits affect semen quality. Among males, the commonest cause of infertility is Azoospermia (semen contains no sperms) and Oligozoospermia (Semen contains very few sperms) resulting from various pretesticular, testicular and post-testicular causes. Idiopathic male infertility refers to abnormal semen analysis without etiological factors identifiable from history, physical examination or endocrine factors.

A wide number of agents are being used to treat male infertility of which SERMs are Non-Steroidal compounds which acts as tissues specific Estrogen receptor agonists or antagonists. The anti estrogenic activity of SERMs on hypothalamus increases gonadotropins with a significant increase in testosterone while maintaining the spermatogenesis. Among SERMs, Clomiphene and Tamoxifen have successfully been used for decades to treat idiopathic oligozoospermia and azoospermia. Though the efficacy of SERMs to induce spermatogenesis is proven, the long term use of SERMs has been associated with an increase in risk for cataract, pulmonary embolism, deep vein thrombosis, stroke, vision threatening ocular toxicity etc. Because of these long-term effects of synthetic SERMs, there is a growing interest in the pharmacological evaluation of phyto-compounds as natural SERMs.

2. LITERATURE REVIEW

2.1. Defining Infertility

Infertility is the inability of a sexually active couple to achieve clinical pregnancy in 1 year of unprotected coitus (WHO) [1]. The incidence of infertility has increased worldwide in the last three decades, where 60-80 million couple worldwide and 15-20 million are in India alone [2].

Infertility is a gynaecological problem affecting about 15% of couples attempting for 1st pregnancy (Primary Infertility) and 10% of the couple trying for subsequent pregnancy (Secondary Infertility) [3].

There are various tools to study the fertility status of the male. Semen analysis is the basic and single most useful

investigations to assess the male infertility with 89.6% sensitivity [4]. This simple test provides an insight into sperm count, motility, morphology and other immunelogical factors as well [5].

Male Infertility can be possibly due to (According to WHO 2010 criteria):

- Azoospermia (no spermin the ejaculate)
- Oligozoospermia (reduced sperm count)
- Asthenozoospermia (reduced sperm motility)
- Teratozoospermia (reduced sperm morphology)
- Necrozoospermia (reduced vitality)

or combinations of these.

Various pre-testicular, testicular or post-testicular factors affect semen parameters [6]. Another group is idiopathic male infertility where the cause of Infertility is unknown.

2.2. Causes, diagnosis and management of male Infertility

This article presents an overview of the causes, evaluation and management of male Infertility.

2.2.1. Testicular deficiency

Testicular deficiency can be caused by either of the four

1. Congenital abnormalities including Anorchia, Cryptorchidism, testicular dysgenesis and Genetic abnormalities.
2. Acquired due to trauma, post-inflammatory like mump orchitis, exogenous factors like medication and testicular torsion.
3. Systematic diseases including varicocele, testicular atrophy, testicular tumour.
4. Primary testicular deficiency of unknown aetiology.

Evaluation of primary testicular failure from history and physical examination would reveal unilateral or bilateral cryptorchidism, testicular torsion/trauma, UTI, exposure to radiation, testicular cancer, absence of testes, absence of secondary sexual characters, gynecomastia, abnormal testicular volume and or consistency or varicocele.

In the case of testicular deficiency, semen analysis shows no sperm after centrifugation at 3000g for 10 minutes [7]. USG Scrotum with doppler would reveal conditions like rete testis, enlarged epididymis with cystic lesions or absent vas deference, testicular dysgenesis or testis tumour [8]. Endocrine factors shows an increased Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) with or without a decrease in Testosterone (T) [9,10]. Testicular biopsy can be considered as a part of ICSI if viable sperm could be retrieved.

2.2.2. Genetic disorders

The incidence of chromosomal abnormality is 5.8% [11]. Genetic disorders on infertility can be confirmed by screening genomic DNA from peripheral blood. Chromosomal abnormalities can be numerical or structural. The frequency of chromosomal abnormality increases with increase in testicular deficiency and is ten folds higher in patients with sperm count <5m/ml [12, 13].

Klinefelter syndrome is one of the most common sex chromosome abnormalities [14]. Adults with Klinefelter syndrome have relatively small testis without germ cells and other characteristics, including scanty body hair, long arm and legs, female hair distribution [15]. The endocrine evaluation shows a normal or decreased T, normal or increased Estradiol (E2) and increased FSH. PGD/amniocentesis should be considered when IVF or ICSI is planned for men with translocation [16, 17].

Management of genetic disorders, including androgen replacement therapy in patients with hypo-androgenism would be beneficial in maintaining general health too [18].

In X Linked Genetic disorders the defect will be transmitted to daughters and not sons. Some of the X Linked disorders include, but not restricted to

1. Kallmann's syndrome a condition where patients have hypogonadotropic hypogonadism and features like cleft palate, facial asymmetry, colour blindness, deafness and maldescended/undescended testes which is due to a mutation in kalig1 gene on X Chromosome [19, 20]. In this case, hormonal treatment can be the best choice to induce spermatogenesis.
2. Mild Androgen insensitivity syndrome is a condition where there is a mutation in the AR gene located on the long arm of the X chromosome. Phenotype ranges from Morris syndrome, Reiferrstein syndrome. Risk of transmission is nearly negligible since the affected male cannot have their biological children [21-24].

2.2.3. Y chromosome and Infertility

Microdeletions in the Y chromosome are one of the commonest and frequent causes of severe oligozoospermia and Azoospermia [25]. The Highest frequency is found in 8-12% of azoospermic male, followed by 3-7% in oligozoospermic men. Since Y microdeletions can be

transmitted to male children where offspring can develop turner's syndrome with chromosome mosaicism and ambiguous genitalia [26], where genetic counselling plays a major role.

2.2.4. Cystic fibrosis

Cystic fibrosis is a fatal autosomal recessive disorder caused by a mutation in chromosome 7 P which encodes membrane protein that influences the formation of the distal two-third of the epididymis, seminal vesicle, ejaculatory duct, vas deference. One of the complications of mutation in the gene 7P is Azoospermia due to congenital absence of bilateral vas deference, where ICSI can be considered.

2.2.5. Obstructive Azoospermia

It refers to the absence of sperm and spermatogenic cells in the ejaculate due to obstruction. In this condition, endocrine factors show normal FSH. Physical examination reveals normal testes with epididymal enlargement.

Obstruction can be in either of the listed including

1. Congenital Intratesticular obstruction in 15% men [27] or acquired (post-traumatic or inflammatory)
2. Epididymal obstruction affects 30 to 67% of azoospermic men [28]. It can be congenital (young's syndrome) or acquired (secondary to gonococcal infection) or epididymitis or trauma [29, 30].
3. Obstruction in vas can be congenital due to Congenital Bilateral Absence of Vas Deferens (CBAVD) or agenesis or acquired post hernia repair orvasectomy reversal [31]
4. Ejaculatory duct obstruction in 1 to 3% of oligoasthenozoospermia can be congenital due to cyst in Mullerian duct or urogenital sinus or Acquired post-surgical or post inflammation [32] which decreases semen volume with decreased or absent fructose and acidic pH.

In obstructive Azoospermia, clinical examination reveals dilated epididymis with nodules in epididymis or vas, partial atresia or absence of vas deferens. Confirmatory tests for obstructive oligo asthenozoospermia include semen analysis having very few, or no sperm in the ejaculate, normal FSH and inhibin B. Testicular biopsy should be performed to exclude testicular causes.

Obstructive Azoospermia can be managed by MESA (Microsurgical epididymal sperm aspiration) in men with CBAVD, Testicular Sperm Extraction (TESE) in case of intratesticular obstruction, vasovasostomy in proximal vas Obstruction, microsurgical tabulovasostomy, Testicular Sperm Aspiration (TESA) or MESA or proximal vas deferens sperm aspiration in distal vas obstruction [33], Trans Urethral Resection Of Ejaculatory Ducts (TURED) in case of large post-inflammatory ejaculatory duct obstruction followed by ICSI. MESA/TESA can also be an alternative to TURED.

2.2.6. Varicocele

Varicocele is one of the conditions for male subfertility. Most of the times varicocele can be diagnosed by clinical examination and can be confirmed by USG and graded as Sub Clinical (shown by USG Doppler but not palpable), Grade 1 (Palpable during Valsalva-manoeuver), Grade 2 (Palpable at rest), Grade 3 (Visible and palpable at rest)

Microsurgical varicocelectomy would improve semen parameters in men with non-obstructive Azoospermia caused by varicocele.

2.2.7. Hypogonadism

Hypogonadism is one of the causes for the impaired testicular function which affects spermatogenesis and or steroidogenesis.

Male hypogonadism can be

1. Primary due to hyper gonadotropic hypogonadism as a result of testicular failure.
2. Secondary hyper gonadotropic hypogonadism as a result of insufficient Gonadotropin-releasing Hormone (GnRH) and or FSH, LH
3. Endorgan resistance
4. Idiopathic hyper gonadotropic hypogonadism characterized by a low level of FSH, LH and normal hypothalamic-pituitary-gonadal axis [34].

In case of hypogonadotropic hypogonadism of hypothalamic origin spermatogenesis can be induced by treatment with Human Chorionic Gonadotropin (HCG), Recombinant FSH (rFSH), Human Menopausal Gonadotropin (HMG) [35, 36] or pulsatile GnRH [37]. Anti-estrogen and aromatase inhibitors may help in treating hyper gonadotropic hypogonadism [38].

2.2.8. Cryptorchidism

Cryptorchidism is a most common congenital abnormality and is so-called testicular dysgenesis. 42% of patients with cryptorchidism are azoospermic, and 31% are Oligozoospermic. Surgical treatment followed by GnRH therapy would be beneficial in managing Infertility due to cryptorchidism.

2.2.9. Idiopathic male infertility

About 44% of patients suffer from Idiopathic Male Infertility, where the cause for infertility is unknown. A number of molecules have successfully been used as empirical treatment for idiopathic male Infertility, including gonadotropins (HMG/rFSH/HPFSH) [39, 40] with oral antioxidant. SERMs have long been used as a successful treatment modality for idiopathic male Infertility.

3. SERMs

SERMs are non-steroidal compounds that function as ligands for estrogen receptors and acts as either estrogen agonists or antagonists depending on specific target tissue [41]. SERMs have long been used as a promising treatment to treat idiopathic male Infertility. At present clomiphene and tamoxifen are most commonly used SERMs. These compounds block negative feedback at the level of hypothalamus and pituitary thus increases FSH, LH from anterior pituitary which further increases T and thus aiding spermatogenesis. The selective effect of SERMs are as a result of differential expression of ER (Estrogen Receptor) genes, *i.e.* ERα and β. ERα and β exert a different effect on growth and differentiation of tissue including uterus, liver, bone, colon, brain, mammary gland, testes etc [42-48].

In testes, both ERα and β has different cellular expression, ERα in nuclei of Leydig cells, while ERβ in germ cells, Sertoli cells and fetal Leydig cells [49, 50].

According to a study, tamoxifen administration led to a two-fold increase in sperm concentration. Based on various studies, tamoxifen has been proposed by WHO as first-line treatment for idiopathic oligozoospermia [51].

A study from India conducted on 25 men with severe oligozoospermia had an increase in sperm count from 3 M/ml to 8.2 M/ml after three months of clomiphene therapy and in the same study 40 men with oligozoospermia had an increase in counts from 13M/ml to 24 M/ml [52].

A study on 183 patients grouped into tamoxifen only, tamoxifen and COQ 10 and COQ 10 showed an increase in motility and morphology in tamoxifen and COQ 10 and COQ 10 but not in tamoxifen only [53].

From the above studies, though it is proved that SERMs are best suited for empiric treatment of idiopathic male Infertility, some side effects have been reported on long term use including headache, nausea, pulmonary embolism, palpitation, seizure etc.

Since there is no long term safety data on these medications, the research on pharmacological effects of phytocompounds on SERMs is of greater interest in recent days.

The most common adverse effect is generally reversible visual disturbances in less than 2% of patients due to vascular sludging leading to ischemic optic neuropathy. High dose of tamoxifen is a hepato-carcinogen in rats and have been shown to induce cataracts in rats. In osteoporotic women, the comparative risk for DVT & pulmonary embolism was 3.1 times higher than placebo. Retinopathy has been reported in patients on high doses of tamoxifen; vision-threatening ocular toxicity has been rarely observed. Other common adverse effects include nausea, dizziness, oedema, vomiting, development of corneal opacification etc.

4. MEDICINAL PLANTS ON MALE INFERTILITY

Medicinal plants have been the basis of treatment for a long period in Asian and African folk medicine. They are the natural resources of modern medicine since they are the potential source of phytochemical compounds like steroids, terpenoids, glycosides, glycoprotein, proteins, alkaloids, flavonoids etc [54].

There is emerging information regarding phytoestrogens that can be regarded as natural SERMs which possess antiestrogenic activities including isoflavones like genistein, daidzein, flavones like coumestans, lignans, mycoestrogens such as zearalenone etc.

Medicinal plants can be used as an extensive source to treat various diseases [55]. Phytochemicals have vastly been studied for their antioxidant, antitumor, immunomodulatory, antimicrobial properties [56]. Many plants have been studied for their male fertility-enhancing properties.

Eurycoma longifolia jack referred to as Tongkat, a native of East Asia has found to be rich in various phytochemicals and have been indicated for a wide range of activities such as antimalarial, anticancer and antibacterial besides treating male infertility [57]. The effect of ethanolic extract was studied on both androgenic and proliferative activity and was found to overcome the late-onset of hypogonadism by biosynthesis of various androgens [58].

Cardiospermum halicacabum is a popular herb of Srilanka commonly referred to as balloon vine. Various doses (200, 400 and 800 mg/kg, twice daily for ten days) have found to increase caput and epididymal sperm count in rats due to its rich phytocompounds especially

flavonoids [59].

Grape seed extract has shown to increase semen parameters including sperm count, viability and motility in a study on aluminium chloride-induced testicular dysfunction in rats and has been reported to reduce the germ cell apoptosis induced by testicular torsion [60].

Syzygium aromaticum, a native of Indonesia, commonly referred to as clove, has traditionally been used as a cure for sexual dysfunction and libido [61].

Streptozotocin-induced diabetic rats were treated with 0.5ml/day for two months with *Nigella sativa,* which are rich in more than hundred phytocompounds and has shown to increase testosterone [62].

Normal and hemicastrated rats were treated for 14 and 21 days with 10 mg/kg of *Lycium barbarum* a Chinese herb which has long been used as an aphrodisiac and has proved to attenuate the testicular DNA damage and also by increasing testosterone [63].

Tribulus terrestis a perennial creeping herb has been identified as a cure for treating male infertility in Asia and Europe. A study of *Tribulus terrestis* on sodium valproate treated male rats showed a dose-dependent increase in the level of testosterone, FSH and LH [64].

Asteracantha longifolia have potentially been used as an aphrodisiac and androgenic agent for centuries. Ethanolic extract of *Polycarpea corymbosa* a local herb of Tamil Nadu increased the level of T and LH [65].

A study conducted on mouse Leydig cells reported the steroidogenic effect of *Taraxacum officinale* extract [66].

Rosa damascena, an ancient holy herb, was studied for its androgenic effect on male Wistar rats. 400mg/kg of the extract for 21 days found to increase FSH, LH and T [67].

5. CONCLUSION

Infertility is a condition which results in stress, trauma and drastic effect with a strong emphasis on childbearing. There are various causes and risk factors for male infertility, including but not limited to environmental and lifestyle factors. Knowledge regarding male infertility is very limited, and most of the treatments for male infertility are long term with an ineffective and also undesirable outcome as mentioned. There are studies which show the antiestrogenic effect of SERMs, which increase gonadotrophin level and therefore a significant increase in testosterone while maintaining spermatogenesis. Although SERMs are effective for treating male infertility, long term use of SERMs increases risk on general health. Because of these long term effects of synthetic SERMs, there is greater research which focuses on the use of phyto-compounds as natural SERMs. Many plant-based compounds have been reviewed in this article for its effectiveness in enhancing male fertility.

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Conflict of interest

There is no conflict of interest.

7. REFERENCES

1. WHO manual for the standardized Investigation and diagnosis of Infertile couple. Cambridge University Press; 2000.
2. Anju M, Katiyar DK, Anju A, Vineeta D, Pant KK. *Biomed Research,* 2013; **24(3)**:347-352.
3. Jimoh AAG, Olawuyi TS, Omotoso GO, Oyewopo AO, Dare JK. *JBasic ApplSci,* 2010; **8**:110-113.
4. Butt F, Akram N.*J Pak Med Assoc,* 2013; **63(5):**558-562.
5. Fisch H. *Urol Clin North Am,* 2008; **35(2):**137-146.
6. Kumar N, Singh AK. *J Hum Reprod Sci,* 2015; **8(4):**191-196.
7. WHO, WHO Laboratory manual for the Examination and Processing of Human Semen, 5th ed; 2000.
8. Lotti F, Maggi M. *Hum Reprod Update*, 2015; **21(1):**56-83.
9. Ron MD, Peter D, Smith T, Southwick GJ, Kretser D. *Fertil steril,*1995; **63(3):**631-636.
10. Pan RC, Bischof P. *Hum Reprod,* 1995; **10(8):**1940-1945.
11. Johnson MD. *Fertil Steril,* 1998; **70(3):**397-411.
12. Clementini E, Palka C, Iezzi I, Stuppia L, Guanciali Franchi P, Tiboni GM. *Hum Reprod*, 2005; **20(2):**437-442.
13. Vincent MC, Daudin M, De MP, Massat G, Mieusset R, Pontonnier F et al. *J Androl*, 2002; **23(1):**18-22.
14. Garza SA, Patrizio P. *Curr Opin Obstet Gynecol*, 2013; **25(3):**229-246.
15. Wang C, Baker HWG, Krester DM, Hudson B. 1975; **4(4):**399-411.
16. Nguyen MH, Morel F, Pennamen P, Parent P, Guilbert N, Bris MJ et al. *Andrologia*,. 2014; **47(2):** 178-185. *Mol Hum Reprod*, 1997; **3(10):**847-851.
17. Groth KA, Skakkebak A, Host C, Gravholt CH, Bojesen A. *J Clin Endocrinol Metab,* 2013; **98(1):**2012-2382.

18. Tempest H, Martin R. *Curr Opin Obstet Gyn*, 2009; **21(3)**:223-227.
19. Baccetti B, Collodel G, Marzella R, Moretti E, Piomboni P, Scapigliati G et al. *Hum Reprod,* 2005; **20(8):**2295-2300.
20. Ferlin A, Vinanzi C, Garolla A, Selice R, Zuccarello D, Cazzadore C et al. *Clin Endocrinol,* 2006; **65(5):**606-610.
21. Gottlieb B, Lombroso R, Beitel LK, Trifiro MA. *Reprod Biomed Online*, 2005; **10(1):**42-48.
22. Rajender S, Singh L, Thangaraj K. *Asian J Androl,* 2007; **9(2):**147-179.
23. Tincello DG, Saunders PTK, Hargreave TB. *MolHumReprod*, 1997; **3(11)**:941-943.
24. Krausz C, Casamonti E. *Human Genet,* 2017; **136(5):**637-655.
25. Patsalis PC, Sismani C, Murci L, Bekkouche F, Krausz C, Elreavey K. *Lancet*, 2002; **360(9341):**1222-1224.
26. Hendry W. Springer Verlag: Berlin, 1997.
27. Hendry WF, Parslow JM, Stedronska J. *Br J Urol*, 1983; **55(6):**785-791.
28. Jarvi K, Zini A, Buckspan MB, Asch M, Ginzburg B, Margolis M. *J Urol,* 1998; **160(4):**1410-1412.
29. Matthews GJ, Schlegel PN, Goldstein M.*J Urol*, 1995; **154(6):**2070-2073.
30. Schoysman R. *Acta Eur Fertil,* 1990; **21(5):**239-245.
31. Printzen IM, Ludwig M, Kohn F, Weidner W. *Hum Reprod,* 2000; **15(6):**1364-1368.
32. Sarquella J, Pomerol MD, Romero J. *Andrologia*, 1994; **26(2):**119-120.
33. Bianco SD, Kaiser UB. *Nat Rev Endocrinol*, 2009; **5(10):**569-576.
34. Dwyer AA, Raivio T, Pitteloud N.*Best Pract Res Clin Endocrinol Metab*, 2015; **29(1):**91-103.
35. Rastrelli G, Corona G, Mannucci E, Maggi M. *J Androl,* 2014; **2(6):**794-808.
36. Schopohl J, Mehltretter G, Zumbusch R, Eversmann T, Werder K. *Fertil Steri*, 1991; **56(6):**1143-1150.
37. Krausz C. *Eur Urol Rev,* 2009, 3.
38. Santi D, Granata ARM, Simoni M. *Endocr Connect,* 2015; **4(3):**46-58.
39. Kumalic S, Pinter B. Article ID 426951, 2014.
40. Plouffe L, Siddhanti S. *Ann N Y Acad Sci,* 2001; **949:**251-258.
41. Somjen D, Katzburg S, Sharon O, Cohen M, Knoll E, Stern N.*J Cell Biochem*, 2011; **112(2):**625-632.
42. Zhang B, Wu ZY.*Eur J Gastroenterol Hepatol*, 2013; **25(3):**263-270.
43. Rossi V, Bellastella G, Rosa C, Abbondanza C, Visconti D, Maione L et al. *J Cell Physiol,* 2011; **226(5):**1334-1339.
44. Helguero LA, Faulds MH, Gustafsson JA, Haldosén LA.*Oncogene*, 2005; **24(44)**:6605-6616.
45. Hiraike OW, Imamov O, Hiraike H, Hultenby K, Schwend T, Omoto Y et al. *Proc Natl Acad Sci USA,* 2006;**1 03(8):**2959-2964.
46. Hiraike OW, Hiraike H, Okinaga H, Imamov O, Rodrigo PA, Andrea M. *Proc Natl Acad Sci USA*, 2006;**103(48)**:18350-18355.
47. Clark JA, Alves S, Gundlah C, Rocha B, Birzin ET, Cai SJ et al. *Neuropharmocol*, 2012; **63(6):**1051-1063.
48. Buvat J, Ardaens K, Lemaire A, Gauthier A, Gasnault JP, Herbaut MB. *Fertil Steril*, 1983; **39(5)**:700-703.
49. Kotoulas IG, Cardamakis E, Michopoulos J, Mitropoulos D, Dounis A.*Fertil Steril*, 1994; **61(5):**911-914.
50. Rowe PJ, Comhaire FH, Hargreave TB, Mellows HJ. Cambridge Univ press, 2000 37-60.
51. Patankar SS, Kaore SB, Sawane MV, Mishra NV, Deshkar AM. *Indian J Physiol Pharmacol*, 2007; **51(2):**195-198.
52. Tang KF, Xing Y, Wu CY, Liu RZ, Wang XY, Xing JP.*Zhounghua Nan Ke Xue,* 2011; **17(7):**615-618.
53. Santhi K, Sengottuvel R. *Int J Of Curr Microbiol Appl Sci*, 2016; **5(1)**:633-640.
54. Ashokkumar P, Kanimozhi M. *Middle East J Sci Res*, 2010; **5(6):**477-482.
55. Ukpo G, Ogbonnia S, Ethianeta T, Bashir S. *Int J pharm pharm Sci*, 2013; **5(2)**:569-572.
56. Sholikhah EN, Wijayanti MA, Nurani LH, Mustofa. *Med J Malaysia*, 2008; **63(1):**98-99.
57. Tambi MI, Imran MK, Henkel RR. *J Andrologia,* 2012; **44(1):**226-230.
58. Peiris LD, Dhanushka MA, Jayathilake TA.*Biomed Res Int*, 2015; 175726.
59. Bayatli F, Akkus D, Kilic E, Saraymen R, Sonmez MF.*World J Urol,* 2013; **31(3):**615-622.
60. Fakim A. *Mol Aspects Med*, 2006; **27(1):**1-93.
61. Haseena S, Manjunath A, Kusal K, Saheb SH. *Int J Pharm Sci Res*, 2015; **7(8)**:514-517.
62. Luo Q, Li Z, Huang X, Yan J, Zhang S, Cai YZ. *Life Sci,* 2006; **79(7):**613-621.
63. Shalaby MA, Hammouda AA. *J Intercult Ethnopharmacol*, 2014; **3(3):**113-118.
64. Mohan VR, Balamurugan K, Shakthidevi G. *Asian j Pharm Clin Res*, 2013; **6(5)**:151-155.
65. Chung HY, Noh Y, Kim MS, Jang A,Lee CE, Myung SC. *Anim Cells Syst*., 2018; **22(6):**407-414.
66. Jahromi HK, Jashni HK, Dialemeh S. *Int J Med ResHealth Sci*, 2016; **5(5)**:267-271.