Acknowledgement

At the outset, I would like to pay my tribute to our almighty respected Rashtrasant Tukadoji Maharaj because of whom thousands of students from our University every year put their step towards bright and prosperous future.

It is a privilege to express my sincere regards, deep sense of gratitude and indebtedness to my guide ***Dr. P. R. Itankar,*** *Assistant Professor (Sr. Gr.), Dept. of Pharmaceutical Sciences, R.T.M., Nagpur University, Nagpur, who stood beside me during every moment of my work and especially at the time of difficulty. His intelligent, invaluable guidance, skillful supervision, helpful suggestions and constructive ideas encouraged me to complete my research work.*

I welcome this opportunity to express my heartfelt gratitude and thankfulness to my revered co-supervisor ***Dr. S. K. Prasad*** *his stimulating suggestions and encouraging ideas helped me throughout the period of my research. I always remain indebted for his diligent efforts and professional competencies, which contributed for me to get encouraged towards the completion of the project methodically. I feel very much honored and it has been a real privilege for me to get an opportunity to work under his guidance.*

I am grateful to ***Dr. G .J. Avari*** *Head, Dept. of Pharmaceutical Sciences, R.T.M., Nagpur University, Nagpur, for providing the amicable environment and allowing me to access necessary facilities, in department during the course of this study, which made it possible to reach the destination without hindrances.*

I express my sincere thanks to ***Mrs. Nandini Bhojraj mam*** *for giving chance to me for industrial project in* ***Go-Vigyan Anusandhan Kendra Deolapar,*** *Nagpur, Maharashtra.*

I am grateful to ***Dr. Dongarwar*** *Assistant Professor, Dept. of Botany, R.T.M. Nagpur University, Nagpur, for identification and authentication of plant.*

It is my pleasure to thank all my respected teachers ***Prof. M. R. Tajne, Prof. P. K. Puranik, Prof. P. M. Sable, Dr. D. M. Kokare, Dr. P. B. Khedekar, Dr. R. B. Kakde, Dr.***

N. A. Raut, Dr. V. Belgumwar, Dr. P. S. Salve, Dr. Rajesh Ugale, Dr. Rita Wadatwar for their valuable guidance and academic support.

Most importantly, I would like to put my heartiest warm regards and eminent indebtedness to my ***Parents*** *whose love, moral support and blessings have encouraged me more and more to reach this height in my journey of present existence. A heart full of loving appreciation is reserved for my family members for whom words cannot express my wholehearted gratitude.*

I would like to express my special thanks and indebtedness to my most supportive ***Santosh, Soumen, Amol, Danish sir, Divya, Sandhya, Asawari, Jija, Minal, Leena, Payal, Bhagyashree*** *who always helped me whenever I was in need. I will never forget the practical as well as theoretical knowledge given to me by them, which will definitely play a major role in developing bright and prosperous career.*

I extend my heartfelt gratitude and indebtedness to my beloved seniors, ***Mrs. Sonal Charde Mam, Miss Komal Parmar mam and Mrs. Rupali Prasad mam, Chandu sir, Sanjay sir*** *and all Research scholars of this department for their affection, love and help.*

I pay my sincere thanks to, ***Mr. Paradkar Sir, Mrs. Nikose Mam, Mr. Meshram Sir, Mr. Chaware Sir, Mr. Madavi Sir, Mr. Gedam Sir, Mr. Nanetkar Mr. Mankar Sir*** *and all other non-teaching staff of the department who had provided me all the requirements that I needed for my research.*

And last but not the least, my unlimited thanks to number of lab ***animals*** *sacrificed for finding the solutions to the aimed questions, in this research work. I would always look out for ways to put in to practice, the '3R' principles to make it easier, for animal welfare.*

To all these benefactor and God Almighty, who made all the things possible for me till the end. I offer my thanks and dedicate the results of my efforts.

Date: ***Pavankumar P. Kawale***
Place: Nagpur

OBJECTIVE:

The herbal formulation "Vanari Gutika" is used as potent aphrodisiac agent. However, there is no any data available on its Physichochemical, Phytochemical and Preclinical profile, therefore present investigation was carried out to perform a complete Physichochemical, Phytochemical and Preclinical standardization of Vanari gutika formulation.

PLAN OF WORK

- Procurement of plant material *(M. pruriens seed)* and its authentication.
- Procurement of marketed Vanari gutika formulation.
- Extraction and enrichment of extract.
- In house preparation of Vanari gutika formulation.
- Standardization of Vanari gutika formulation.
- Physicochemical evaluation.
- Qualitative Phytochemical study.
- Quantitative Phytochemical study.
- Chromatographic evaluations i.e. TLC, HPTLC, HPLC by using suitable marker.
- Preclinical evaluation of marketed as well as in house formulation of Vanari gutika by using suitable animal model for its aphrodisiac property.
- Biochemical analysis of animal blood sample.
- Histopathology of testis of animal
- Epididymal sperm count of animal

Summarizing the effect of Vanari gutika in comparison to extract for its aphrodisiac potential.

INTRODUCTION:-

Human sexuality, general term referring to various sexually related aspect of human life including physical and psychological development, behavior, attitude and social customs associated with the individual's sense of gender, relationship, sexual activity, mate selection and reproduction. Sexuality permeates many areas of human life and culture thereby setting human apart from other members of the animal

Kingdom, in which the objective of sexuality in more often confined to reproduction (Richard, 2000).

All living thing reproduced, Reproduction – the process by which organism make more organism like themselves – is one of the thing that sets living thing apart from non-living thing. But even though the reproductive system is essential to keep a species alive, unlike other body system it's not essential to keep an individual alive.

One of the main aim of marriage is the procreation (reproduction) and more importantly for sexual fulfillment of both partners. For life to continue, an organism must reproduce itself before it dies. In *Homo sapiens,* reproduction is initiated by the mating of a male with a female in sexual intercourse which facilitates the coming together of sperm and egg for the purpose of fertilization. For there to be a normal sexual intercourse and sexual fulfillment in males, the male sexual organ (the copulatory organ, the penis) and factor relating to erection must function normally, inability to perform the function effectively is a major problem facing the reproductive process. This is known as sexual dysfunction. This condition which is of various types can be managed by the use of aphrodisiac (The Merck manuals – Online Medical Library).

Aphrodisiac:

Aphrodisiac named after *Aphrodite,* the Greek goddess of sexual love, beauty and fruitfulness identified in Roman Mythology with the goddess Venus, who was the daughter of Zeus and Dione (Yakubu et. al., 2007). However, the Greek word '*aphros*' means 'foam' and according to the tradition recounted by Hesoid, Aphrodite arouse from the foam generated when the severed genital of Uranius personification of Heavens were thrown into the sea. Several ancient authorities agreed that she was the wife of the lame blacksmith, Hephaestus (Taberner, 1985).

An aphrodisiac can therefore be described as any substance that enhances sex drive and or sexual pleasure. Aphrodisiac can also be viewed as any food, drug, scent or device that can arouse or increase sexual drive or libido. Most aphrodisiac also heighten other aspect of sensory experience such as light, touch, smell, taste and hearing; and this enhanced sensory awareness contributes to sexual arousal and pleasure (Yakubu et. al., 2007).

According to Ayurveda one of the product recommended for the management of sexual dysfunction is Vanari gutika (Yog-Ratnakar 2002). It is prepared as per the Ayurvedic protocol using *Mucuna pruriens* seed powder, cow milk, honey and sugar. Clinically, it has been found to be useful in cases of male infertility.

Causes of impotence: (Yukubu et. al., 2007)

Sexual dysfunction is more prevalent in males than in female and thus, it is conventional to focus more on male sexual dysfunction. It has been discovered that men between 17 and 96 years old could suffer sexual dysfunction as a result of psychological or physical health problem. Generally, a prevalence of about 10% occurs across all ages. Because sexual dysfunction is an inevitable process of ageing, the prevalence is over 50% in men between 50 and 70 years of age. As men age, the absolute number of leyding cells decreases by about 40%, and the vigor of pulsatile luteinizing hormone release is dampend. In association with these events, free testosterone level also decline by approximately 1.2 % per year. These have contributed in no small measure to prevalence of sexual dysfunction in the aged.

Male sexual dysfunction (MSD) could be caused by various factor. These include:

- Psychological disorder (performance anxiety, strained relationship, depression, stress, guilt and fear of sexual failure)
- Androgen deficiency (testosterone deficiency, hyperprolactinemia)
- Chronic medical condition (diabetes, hypertension, vascular insufficiency, atherosclerosis, venous leakage etc.)
- Penile disease (peyronie's, priapism, smooth muscle dysfunction)
- Pelvic surgery (to correct arterial or inflow disorder)
- Neurological disorder (Parkinson disease, stroke, cerebral trauma, Alzheimer's spinal cord or nerve injury)
- Drugs (side effect) (anti-hypertensives, central agents, psychiatric medication, antiulcer, antidepressants and anti-androgen)
- Life style (chronic alcohol abuse, cigarette smoking, narcotic addiction)
- Aging (decrease in hormonal level with age)
- Systemic disease (cardiac, hepatic, renal pulmonary, cancer, post-organ transplant)

Management of MSD: (Yukubu et. al., 2007) (Brook, 2003)

The option include psychological/behavioral (therapy with a trained counselor aimed at helping people to address feeling of anxiety, fear and guilt that may have an impact on sexual function); drug therapy (use of testosterone replacement therapy for cases of androgen insufficiency and other pharmacological agent); non surgical devices which include vacuum pump (expand the penis and reduced pressure within the cavernous sinusoidal space) and constrictive rings (external device used for managing erectile dysfunction in patients with mild to moderate venous leakage); surgical treatment which include venous legation (used to correct leakage of blood from the veins); penile prosthesis (creates adequate space within the tissue of each cavernosal body); penile implants (involves inserting a malleable or rigid substance into the penis to effect a semi-rigid state) and phytotherapy (involves the use of herbs (medicinal plant)).

Medicinal plants: (Yukubu et. al., 2007)

Plants are extensively used to relive sexual dysfunction. *Panax ginseng* for example, is an essential constituent in traditional Chinese medicine and at least 6 million Americans use the root of this slow-growing perennial. Another root, known as maca *(Lepidium meyenii)*, has traditionally been used by Peruvian inhabitants living at high altitudes as a nutrient, an energizer, as aphrodisiac and/or fertility-enhancing agent. Similarly, other authors have lead scientific credence to the use of *Fodogia agrestis* (English: black aphrodisiac, Hausa: baakin gagai) stem as an aphrodisiac by increasing the concentration of serum testosterone made possible by its saponin content.

Traditional herbs have also been a revolutionary breakthrough in the management of sexual inadequacies (sexual dysfunction) and have become known worldwide as an “instant” treatment. Some of these herbs include *Terminalia cattapa* seed (Almond fruit), root of *Garcina kola* Heckel (Yoruba: orogbo), stem bark and twig of *Carpolobia albe* (Yoruba: osunum, osun), whole plant of *Euphorbia hirta* L. (Yoruba: egele) and leaves, roots and fruits of *Musa parasidiaca* L. (plantain).

Aphrodisiac drugs (Ogah, 1999)

Testosterone

Libido is clearly linked to levels of sex hormones, particularly testosterone. Other anabolic steroids such as trenbolone which mimic the effect of testosterone may also cause increased libido in users, although side effect such as testicular atrophy are likely to decrease libido, possibly permanently, following prolonged use of these hormones.

Yohimbine

Yohimbine is the main alkaloid of yohimbe. Yohimbe, but not yohimbine, is often popularly referred to as a "weak MOA inbitor". Its main action is as alpha-adrenergic antagonist, by which yohimbine may increase genital blood flow and both sexual sensitivity and excitation in some people. Side effects can include rapid pulse, sweating, and anxiety reaction in susceptible people.

Bremelanotide

Compound that activate the melanocortin receptor MC3-R and MC4-R in the brain are the first class actually effective and selective aphrodisiac drugs. One compound from this class, bremelanotide, formerly known as PT-141, was undergoing clinical trials for the treatment of sexual arousal disorder and erectile dysfunction. It was intended for both men and women. Preliminary results proved the efficacy of this drug, but the development was discontinued due to its side effect of increasing blood pressure. The related compound PL-6983 will now be developed instead.

Melanotan II

Melanotan II, bremelanotide's precursor has been demonstrated to have aphrodisiac properties.

PEA

There is some debate in lay circles as to whether a chemical called phenylethylamine present in chocolate in an aphrodisiac. There is some evidence to support the theory that phenethylamine release in the brain may be involved in sexual attraction and arousal, but this compound is quickly degraded by the enzyme MAO

and so it is unlikely that any significant concentration would reach the brain when phenethylamine is taken orally.

Other drugs

Stimulant affecting the dopamine system such as cocaine and amphetamines (e.g. methamphetamine) are frequently associated with hyperarousal and hypersexuality, through both may impair sexual functioning particularly with long term use. A newer dopamine reuptake inhibitor MDPV has also been noted to have characteristic hypersexual effects.

Some directly acting dopamine agonist may also cause increased libido, although they can also cause various side effect. Pramipexole is the only dopamine agonist used in medicine as an aphrodisiac and is sometimes prescribed to counteract the decrease in libido associated with SSRI antidepressant drugs. The older dopamine agonist apomorphine has been used for the treatment of erectile dysfunction, but is of poor efficacy and has a tendency to cause nausea.

Drugs not considered aphrodisiac

Some psychoactive substances such as alcohol, cannabis, methaqualone, GHB and MDMA can increase libido and sexual desire. However these drugs are not aphrodisiacs in the strict sense of the definition, as they do not consistently produced aphrodisiac effect as their main action. However, these drugs are sometimes used to increase sexual pleasure and to reduce sexual inhibition.

Anti-erectile dysfunction drugs, such as Viagra and Levitra are not considered aphrodisiac because they do not have direct effect on the brain, although increased ability to attain an erection may be interpreted as increased sexual arousal by users of these drugs.

PHYTOCHEMICAL REVIEW:

***Mucuna pruriens* Linn:-**

Bell and Janzen, (1971), Dhamodharan and Ramasamy, (1937) Four alkaloids in *Mucuna pruriens* seeds were recently reported. They are:

- L- 3-caboxy- 1, 2, 3, 4-tetrahydroisoquinoline
- (-)-1-methyl-3carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline
- Dimethyl-3carboxy- 6,7-dihydroxy- 1, 2, 3, 4-tetrahydroisoquinoline
- (-)- 1- 3-carboxy- 1, 1-dimethyl- 7, 8-dihydroxy- 1, 2, 3, 4-tetrahydroisoquinoline.

Dr. Dukes, (1992) describes diversified chemical constituents in *Mucuna* seeds like**,** 5- hydroxytryptamine,5-methoxy- N, N-dimethyltryptamine -N- oxide, 5-oxyindole-3- alkyl amine, 6-methoxyharman, arahidic acid, arginine, aspartic acid, behenic acid, betacarboline, betasitosterol, bufotenine, choline, cis- 12,13-poxyctadec- trans- 9-cis-acid, cis-12, 13-epoxyoctadec- trans- 9-enoic acid, Gallic acid, glutamic acid, glutathione, indole-3-alkylamine, linoleic acid, mucunadine, mucunain, mucynine, myristic-acid, niacin, nicotine, oleic acid, palmitic acid, prurienine, riboflavin, saponins, serotonin, stearic acid, thiamine, vernolic acid. In *Mucuna* leaves the data base reveals the presence of L-dopa, 6-methoxyharman, genistein, hydroxylgenistein in minimal concentration.

Misra and Wagner, (2004) describes recently three new lipid derivatives were also reported, triactont-5, 7, 9-triene, docos-2, 4, 6-triene- 1, 8-diol and docos-5- en- 1-oic acid.

PHARMACOLOGICAL REVIEW:

Katzenschlager et al., (2004) revealed that 30 g *Mucuna* seed powder preparation has considerable faster action in treating PD patients than conventional standard drugs, namely, Levodopa or Carbidopa and suggested that natural source of L-DOPA might possess advantages over conventional drugs in long term management of PD.

Donati et al., (2005) shown that using a combination of chromatographic and NMR techniques, the presence of D-chiro-inositol and its two galacto-derivatives having antiglycaemic effect was demonstrated in *M. pruriens* seeds.

Bhaskar et al., (2008) investigated the hypoglycemic effect in normal, glucose load conditions and streptozotocin (STZ)-induced diabetic rats at dose of 100 and 200 mg/kg body weight) significantly reduced the blood glucose levels 2 hr after oral administration of seed extract.

Satheesh et al., (2010); Kumar and Muthu, (2010) investigated that *In vitro* assays of whole plant of ethyl acetate and methanolic extract of *M. pruriens*, containing large amounts of phenolic compounds, exhibited high antioxidant and free radical scavenging activities.

Meenatchisundaram and Michael, (2010) about 0.16 and 0.19 mg of *M. pruriens* seed extracts were able to completely neutralize the lethal activity of 2LD50 of cobra and krait venom, respectively, thus suggesting that aqueous extracts of *M. pruriens* seeds possess compounds, which inhibit the activity of cobra and krait venoms.

Shukla and Mahdi, (2010) demonstrated that oral administration of 5g of *Mucuna pruriens* seed powder once in a day for men with decreased sperm count and motility ameliorated psychological stress and seminal plasma liquid peroxide levels along with improved sperm count and motility.

Rayavarapu and Kaladhar, (2011) Reported that the methanolic extract showed high antibacterial activity against *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas marginalis*, *Pseudomonas acruginosa*, *Xanthomonas campestris* and high anti fungal activity against *Curvularia lunata*, *Fusarium oxysporum*, *Pencillium expansum*, *Rhizoctonia solani*, *Tiarosporella phaseolina*, *Ustilago pomaydis.*

Guerranti et al., (2002) The aqueous extract of seed of *Mucuna pruriens* has been reported to have a great antivenom activity against Echis carinatus venom (EV).

Ogundadare and Olorunfemi, (2007) The flavonoid and phenols present in methanolic extract of leaves of *M. prupiens* have been contributed in potential antimicrobial activity of plant

Mishra and Wagner, (2007) n-propranolol extract of seed of *M. pruriens* have reported to have potent neuroprotective activity which may be due to the presence of L-dopa and which was found to be effective in treatment of Parkinson disease.

Ujowundu et al., (2010) Methanolic extract have also been reported to have *in-vitro* and *in-vivo* antioxidant activity which may be attributed to presence of higher quantities of flavonoids.

Lampariello et al., (2011) The methanolic extract have also been reported to have a positive influence on human at have found to play a significant role in treatment of skin infection and pigmentation.

Suresh S, et al., (2009) Dose-and time-dependent effect of ethanolic extract of *M. pruriens* seed on sexual behavior of normal male rat.

Sahoo et al., (2014) Aphrodisiac activity of polyherbal formulation in experimental models on male rats. (*M. pruriens* as one of the herb in above formulation)

Mohammad et. al., (2008) Effect of *Mucuna pruriens* on semen profile and biochemical parameters in seminal plasma of infertile men.

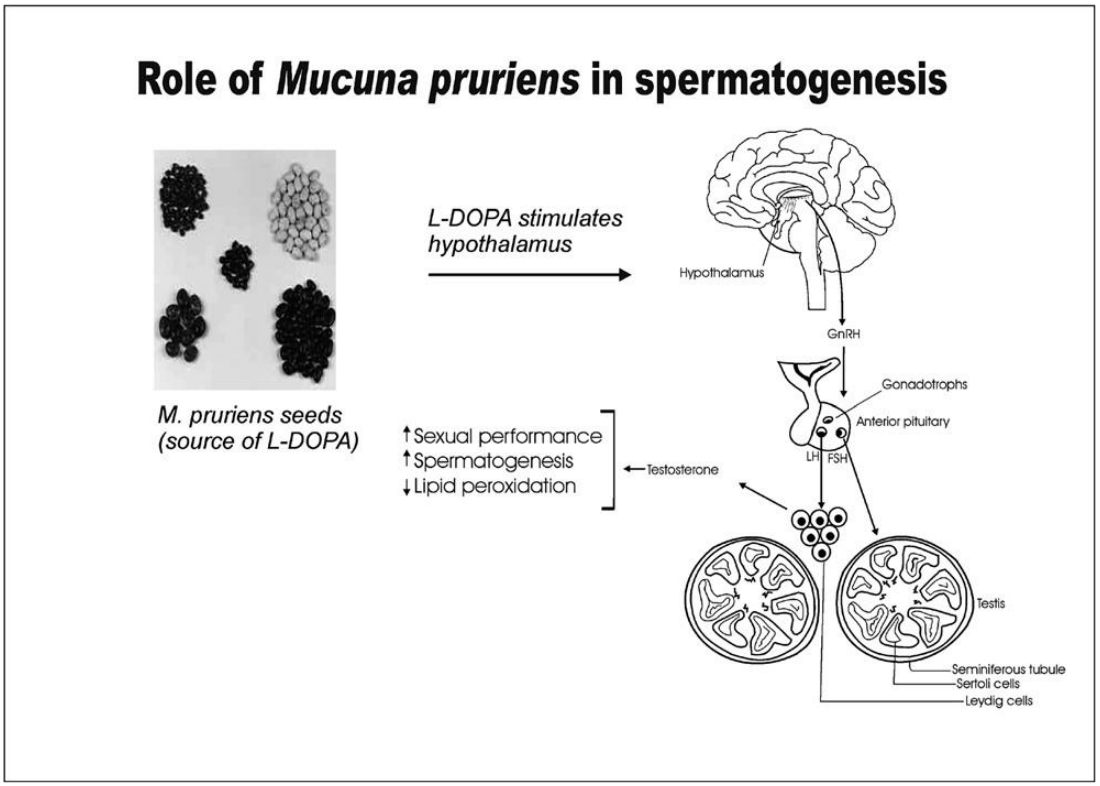


Fig 1: Role of *Mucuna pruriens* in spermatogenesis

\Physiology of a Normal Penile Erection: (Robert et. al., 2005, Joseph et. al., 2002)

A normal penile erection is the final common pathway of the integrative synchronized action of psychological, neuronal, hormonal, vascular and cavernous smooth muscle system. The patient must also be psychologically receptive to sexual stimuli. Any abnormality involving these systems, whether from medication or disease has a significant impact on the ability to develop and sustain an erection, ejaculate and experience orgasm. Tumescence, the vascular filling of the cavernous bodies, relies on neural and hormonal, mechanism operating at various level of the neural axis. This is unique among visceral function because it required central neurological input.

Vascular system

The penis comprises two corpora cavemosa on the dorsal side and one corpus spongiosum on the ventral side. The corpus spongiosum surrounded the urethra and forms the glans penis. The corpora are composed of multiple interconnected sinuses, which can fill with blood to produce an erection. The corpora are encased by the tunica albuginea, a fibrous tissue membrane, which has limited distensibility. In the flaccid state, arterial flow into and venous outflow from the corpora are balanced. During the erectile phase, arterial blood flow increase and blood fills the sinusoids within the corpora, which cause penile swelling and elongation. The erection is prolong by a decrease in venous outflow from the corpora, which is caused by compression of subtunicalvenules by swollen corpora (fig. 1)

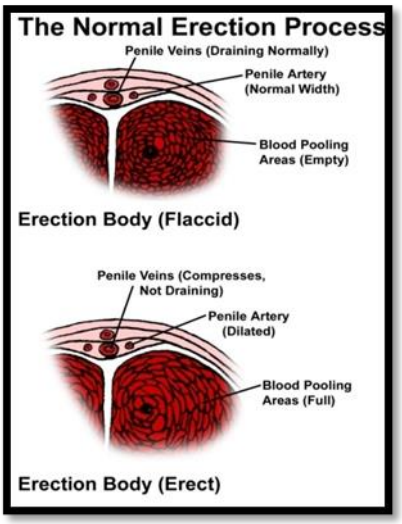


Figure 2: Microanatomy of and vascular changes in the penis in flaccid and erect state.

Arterial flow into the corpora is enhanced by acetylcholine mediated vasodilatation. Acetylcholine does not directly enhanced arterial flow to the corpora or increase sinusoidal filling to the corporal tissue. Rather, acetylcholine is a co-neurotransmitter, which works along with other nonpeptidnergic intracellular neurotransmitter including cyclic guanosine monophosphate (cGMP), cyclic adenosine monophosphate (cAMP), or vasoactive intestinal polypeptide to produce vasodilatation.

Acetylcholine probably works through two different pathways to produce an erection. In one pathway, acetylcholine, in the presence of sexual stimulation to genital tissue, enhances the production of nitric oxide by endothelial cells and noradrenergic and non-cholinergic (NANC) neurons. Nitric oxide enhances the activity of guanylate cyclase enzyme, which increases the conversion of cyclic guanosine triphosphate to cGMP. cGMP decrease intacelluler calcium concentration in smooth muscle cells of penile arteries and cavernosal sinuses. As a result, smooth muscle relaxation occure, which enhances arterial blood flow to and blood filling of the corpora an erection occur.

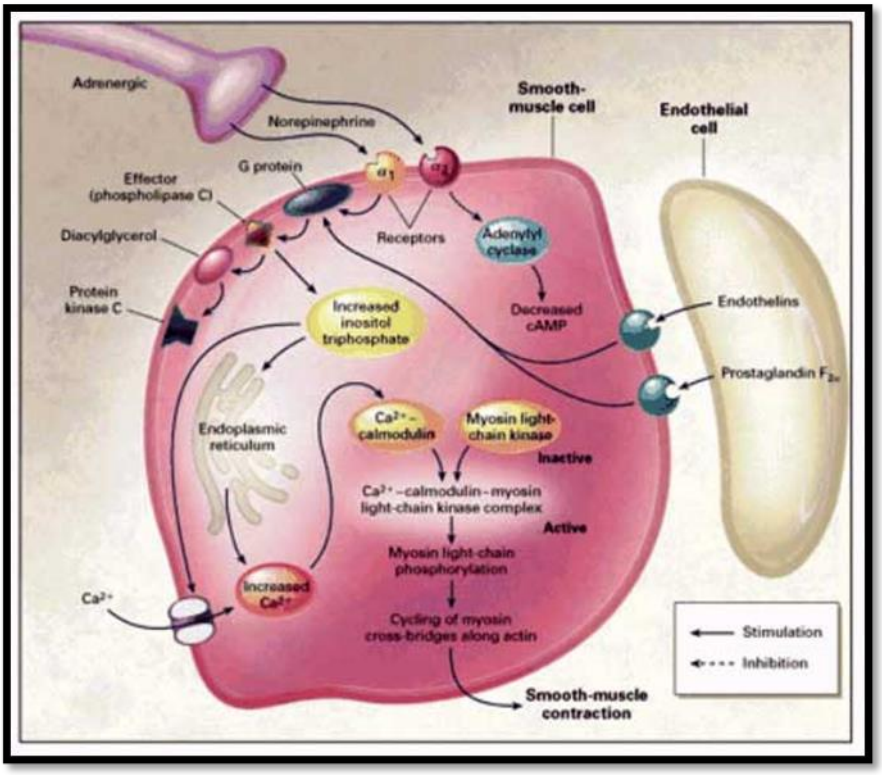


Figure 3: molecular mechanism of penile smooth muscle contraction.

(Nor epinephrine from sympathetic nerve ending and endothelins and PGF2a from the endothelium activate receptors on smooth muscle cells to initiate the cascade of reaction that eventually result in elevation of intacellular calcium concentraction and smooth muscle contraction. Protein kinase C is a regulatory component of the Ca2+-independent, sustained phase of agonist-induced contractile responses.)

Nervous system and psychogenic stimuli: (Joseph et. al., 2002)

Some erection are mediated by a sacral nerve reflux are (e.g. erection can occur while the patient is sleeping). However, in the presence of sensory sexual stimulation erection are mediated by nervous system. That is, when a patient sees an attractive partner, hears sweet words, smells a particular sent, taste or touches a pleasant object, this can result in the erection. In this case, the patient's brain processes this information and the nervous impulse is carried down the spinal cord to peripheral cholinergic nerves, which innervate the vascular supply to the corpora, resulting in an erection.

The medial preoptic area of the hypothalamus through to be that portion of the brain responsible for integrating external stimuli. Here dopamine exert a preerectogenic effect. Whereas, α-adrenergic stimulation causes the penis to become and/ or remain flaccid. Nerve impulses, after moving down the spinal cord, travel to the penis by efferent peripheral nerves, including inhibitory sympathetic neurons (T_{11}-L_2), prorectogenic parasympathetic neurons (S_2-S_4).

Detumescence, or the progression of an erect penis to a flaccid state, result from the action of norepinerphrine, which contracts vascular smooth muscle to decrease arterial inflow to the corpora and contraction of sinusoidal tissue in the corpora. As a result, venous outflow from the corpora increases.

Hormonal system: (Joseph et. al., 2002)

Testosterone stimulates libido and sexual drive in male. Within the normal psychological serum concentration range (normal, 300-1,100ng/dl) sexual drive is normal. Patient with serum testosterone level below the normal range may complain of loss of energy, loss of muscle strength, depressive mood and decrease libido.

When libido is decreased, a patient may not develop erection, but the relationship with testosterone level is a complicated one. Approximately one-third of men older than 50 years of age have hypogonadism. But patient with normal testosterone level may have erectile dysfunction, and patient with subnormal serum testosterone level may have normal sexual function.

Male sexual dysfunction: (Yukubu et. al., 2007, Miller et. al., 1999, Merck et. al., 1999)

Sex disorders of the male are classified into disorder of sexual function, sexual orientation and sexual behavior. In general, several factors must work in harmony to maintain normal sexual function. Such factors include neural activity, vascular events, intracavernosal nitric oxide system and androgen. Thus, malfunctioning of at least one of these could lead to sexual dysfunction in any kind. Sexual dysfunction in men refers to repeated inability to achived normal sexual intercourse. It can also be viewed as disorder that interferes with a full sexual response cycle. These disorders make it difficult for a person to enjoy or to have sexual intercourse. While sexual dysfunction rarely threatens physical health, it can take a heavy psychological toll, bringing on depression, anxiety and debilitating feeling of inadequacy. Unfortunately, it is a problem often neglected by the health care team who strive more with technical and more medically manageable aspect of the patient's illness.

Male sexual dysfunction can be categorized as disorder of desire, disorder of orgasm, erectile dysfunction, disorder of ejaculation and failure of detumescence.

A. **Disorder of desire:**

Disorder of desire can involve either a deficient or compulsive desire for sexual activity. Dysfunctions that can occur during the desire phase include:

a) Hypoactive sexual desire (HSD), defined as persistently or recurrently deficient (or absent) sexual fantasy and desire for sexual activity leading to marked distress or interpersonal difficulty. It results in a complete or almost complete lack of desire to have any type of sexual relation.

b) Compulsive sexual behaviors (CSBs) constitute a wide range of complex sexual behaviors that have strikingly repetitive, compelling or driven qualities. They usually manifest as obsessive-compulsive sexuality (e.g. excessive masturbation and promiscuity), excessive sex-seeking in association with

affective disorder (e.g. major depression and mood disorder), addictive sexuality (e.g. attachment to another person, objector senssetion for sexual gratification to the exclusion of everything else), and sexual impulsivity (failure to resist an impulse or temptation for sexual behavior that is harmful to self or others such as exhibitionism, rape or child molestation).

B. Erectile dysfunction (ED):

This is a problem with sexual arousal. ED can be defined as the difficulty in achieving or maintaining an erection sufficient for sexual activity or penetration, at least 50% of the time, for a period of six months. It results in significant psychological, social and physical morbidity and annihilated his essence of masculinity.

C. Disorder of ejaculation :

There exists a spectrum of disorder of ejaculation ranging from mild premature to severely retard or absent ejaculation. These include:

i. Premature ejaculation which is the most common male sexual dysfunction and can be any of the following:
   a. Persistent or recurrent ejaculation with minimum sexual stimulation that occurs before, upon, or shortly after penetration and before the person wishes it;
   b. Marked distress or interpersonal difficulty; and
   c. The condition does not arise as a direct effect of substance abuse. Premature ejaculation and sexual desire disorders were the frequent reported problem in young adult males with adverse familial relationship.

ii. Painful ejaculation which result from side effect of tricyclic antidepressant is a persistent and recurrent pain in the genital organs during ejaculation or immediately afterwards.

iii. Inhibited or retarded ejaculation: This is when ejaculation does not occur at all.

iv. Retrograde ejaculation: This is when ejaculation is forced back into the bladder rather than though the urethra and out of the end of the penis at orgasm.

D. Disorder of orgasm:

Male orgasmic disorder is defined as a persistent or recurrent delay in or absence of orgasm after a normal sexual excitement phase during sexual activity.

E. Failure of detumscence:

This is a prolong erection usually lasting for between 4 hr or greater. It is painful and always unaccompanied by sexual desire despite the fact that it is often preceded by usual sexual stimuli. Diagnostic option for male sexual dysfunction include: patient's history which embodies (evaluating historical events like chronic disease, pharmacological agents, endocrine disorder, surgeries and trauma), psychological history (assessing individual's upbringing relationship, early sexual experiences, inadequate sexual information and general psychological health), sexual history (to ascertain the time and manner of onset, current status, and associated medical or psychological problems), physical examination (entails general and systemic evaluation, assessment of gonadal function, vascular competence, neurological integrity and genital organ normalcy) diagnosis testing (include blood tests, vascular assessment, sensory testing and nocturnal penile tumescence and rigidity testing).

Erectile dysfunction: (Miller et. al., 1999, Merck et. al., 1999)

Erectile dysfunction, sometimes called "impotence", is the repeated to get or keep an erection firm enough for sexual intercourse. The word "impotence" may also be used to describe other problem that interfere with sexual intercourse and reproduction, such as lack of sexual desire and problem with ejaculation or orgasm.

Under normal circumstances, when a man is sexually stimulated, his brain sends a massage down the spinal cord and into the nerve ending of the penis. The nerve ending in the penis release chemical messenger called neurotransmitters that signal the corpora cavernosa to relax and fill with blood. As they expand, the corpora cavernosa close off other veins that would normally drain blood from the penis. As the penis becomes engorged with blood, it enlarges and stiffens, causing an erection. Problem with blood vessels, nerves or tissue of the penis can interfere with an erection.

Impotence in the male is not simply the inability to achieve and retain erection of the penis (erectile dysfunction): under this heading are commonly subsumed

premature ejaculation (precipitate emission of semen before the sexual act is fully completed), and retarded ejaculation, in which the man is either unable to achieve orgasm and ejaculation, or else finds that this take an exceedingly long time. The same individual may well experience different forms of sexual dysfunction at different times and on different occasion.

Classification (Robert and Tom 2005)

Many classifications have been proposed for ED. Some are based on the cause and some on the neurovascular mechanism of the erectile process. A classification recommended by International Society of Impotence Research is shown in Table 1.

classification of Male Erectile Dysfunction:

- **Organic:**
    1. Vasculogenic
        A. Arteriogenic
        B. Cavernosal
        C. Mixed
    2. Neurogenic
    3. Anatomic
    4. Endrocrinologic
- **Psychogenic:**
    I. Generalized
        A. Generalized unresponsiveness
            1. Primary lack of sexual arousability
            2. Aging – related decline in sexual arousability
        B. Generalized inhibition
            1. Chronic disorder of sexual intimacy
    II. Situational
        A. Partner related
            1. Lack of arousability in specific relationship
            2. Lack of arousability owing to sexual object preference
            3. High central inhibition owing to partner conflict to threat
        B. Performance related
            1. Associated with other sexual dysfunction (e.g. rapid ejaculation)

2. Situational performance anxiety (e.g. fear of failure)

C. Psychological distress or adjustment related

1. Associated with negative mood state (e.g. depression) or major life stress (e.g. death of partner)

Prevalence of erectile dysfunction: (Feldman et. al., 1994, Quinlan et. al., 1991)

Over the last years, a number of publications have addressed the incidence and prevalence of erectile and sexual dysfunction in the general population. More recent studies have examined these issues in more specialized populations.

An international survey (The Global Study of Sexual Attitude and Behaviors) was designed and conducted to investigate behaviors, attitude, beliefs and satisfaction regarding sex, intimacy and relationships among men and women aged 40-80 years. The survey involved 27,500 men and women in 30 countries representing all world regions. The western group (United States, Canada, Australia and New Zealand) comprised 4507 subject, whose data were used for this presentation. Overall, 82% of men and 68% of women were sexually active in the last 12 months. Lack of interest in sex was reported by almost twice as many women (34%) as men (18%), and lack of pleasure from sex was more common in women (19%) than in men (11%). Erectile difficulties were reported by 21% of men, with an increasing prevalence from 13% in men aged 40-49 years to 36% in men aged 70-80 years. In summary, the majority of mature people are still sexually active and sexual dysfunction is common and tends to increase with age (Brook et. al., 2003).

The difficulties associated with ejaculatory disorder (EjD) are often neglected in aging males. A worldwide survey (MSAM-7) that studied the incidence of lower urinary tract symptoms (LUTS) and sexual function was carried out in 14,254 men aged 50-80 years from 7 countries (United state, United kingdom, france, Germany, Italy, Spain and the Netherland). Validated questionnaires included the International Prostate Symptom Score (I-PSS), the Danish Prostate Symptom Score (Dan-PSS) and the International Index of Erectile Function (IIEF). Sexual disorders were noted to be highly prevalent in this population, with ED in 50% and EjD in 47%. Both ED and EjD were strongly correlated to LUTS, independent of age and other co morbidities. Hence, LUTS is an independent risk factor for sexual dysfunction, with both ED and

EjD being equally common and both are some in aging males with LUTS (Rosen et. al., 2003).

Erectile dysfunction affected millions of men, and although it may not mean a total loss of sexual satisfaction, it often creates a mental stress that affects the men's quality of life. Erectile dysfunction goes hand in hand with aging. The prevalence of complete ED is estimated to be approximately 5% among 40 years old, 10% among men in their 60s, 15% in men in their 70s and 30-40% in men their 80s.

Oligospermia:-

Terms **oligospermia** and **oligozoospermia** refer to semen with a low concentration of spermand is a common finding in male infertility. Often semen with a decreased sperm concentration may also show significant abnormalities in sperm morphology and motility (technically **oligoasthenoteratozoospermia**). There has been interest in replacing the descriptive terms used in semen analysis with more quantitative information (Grimes and Lopez 2007)

The diagnosis of oligozoospermia is based on one low count in a semen analysis performed on two occasions. For many decade sperm concentrations of less than 20 millions sperm/ml were considered low or oligospermic, recently, however, the WHO reassessed sperm criteria and established a lower reference point, less than 15 million sperm/ml, consistent with the 5th percentile for fertile men (Cooper et. al., 2010). Sperm concentrations fluctuate and oligospermia may be temporary or permanent.

Table 1: Levels of Low Sperm Count in various oligospermia state. (Padubidri Daftary 2011)

Sr no.	Descriptor	Sperm concentration
1	Mild oligospermia	10 million to 20 million sperm/mL
2	Moderate Oligospermia	5 million to 10 million sperm/mL
3	Severe Oligospermia	0 to 5 million sperm/mL
4	Cryptoozospermia	0-rare sperm
5	Azoospermia	0 sperm

PLANT PROFILES:

Geographical distribution:

The plant *M. pruriens*, widely known as "velvet bean," is a vigorous annual climbing legume originally from southern China and eastern India, where it was at one time widely cultivated as a green vegetable crop (Duke, 1981). Approximately 120 species have been reported from worldwide and 15 species from India. Among the various under-utilized wild legumes, the velvet bean *Mucuna pruriens* is widespread in tropical and sub-tropical regions of the world. The velvet bean has been traditionally used as a food source by certain ethnic groups in a number of countries. It is cultivated in Asia, America, Africa, and the Pacific Islands, where its pods are used as a vegetable for human consumption, and its young leaves are used as animal fodder.

Taxonomical description:

Domain	*Eukaryota*
Kingdom	*Plantae*
Subkingdom	*Viridaeplantae*
Phylum	*Magnoliophyta*
Subphylum	*Spermatophytina*
Infraphylum	*Angiospermae*
Class	*Magnoliopsida*
Subclass	*Rosidae*
Superorder	*Fabanae*
Order	*Fabales*
Family	*Fabaceae*
Subfamily	*Faboideae*
Tribe	*Phaseoleae*
Genus	*Mucuna*

Botanical description: (Kokate et al., 2010)

Synonyms	: velvet bean, cow-age, cowitch.
Botanical nam	: *Mucuna pruriens*
Family	: Fabaceae/Leguminosae (pea Family)

Subfamily : Papilionaceae

Morphological description

Mucuna pruriens is an erect branching shrub that attains a height of between 30 and 150 cm, covered in a woolly pubescence.

Flowers : Dark purple, lavender (6-30), pea like but larger, with distinctive curved petals and occur in drooping recemes. .

Leaflets : Membranous, terminal leaflets are smaller, lateral very unequal sized.

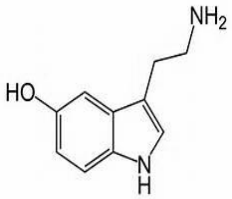
Fruits : longitudinal pods are curved, 4-6 seeded and about 10 cm long.

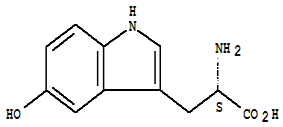
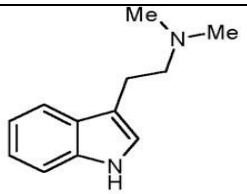
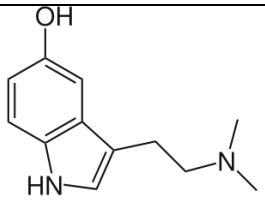
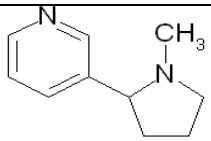
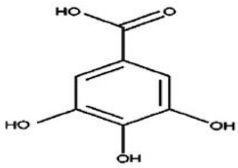
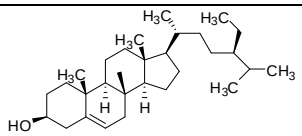
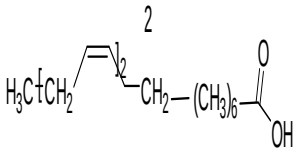
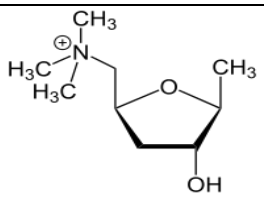
Seeds : Shiny black or brown, ovoid and 10mm long.

(a) (b) (c) (d)

Figure 4: Morphology of *Mucuna prurience* plant (a) whole plant (b) black seed (c) white seed (d) legumes

Table 2: Structure of chemical constituent of *Mucuna pruriens* seed

S. No.	Constituents	Structure	References
1.	L-Dopa	 Levodopa	(Dhamodharan and Ramasamy 1937)
2.	Serotonin		(Kavitha and Thangamani 2014)

3.	Oxitriptan		(Kavitha and Thangamani 2014)
4.	N,N-DMT		(Kavitha and Thangamani,2014)
5.	Bufotenine		(Dr. Duke Phytochemical database)
6.	Nicotine		(Dr. Duke Phytochemical database)
7.	Gallic acid		(Anonymous, 1996))
8.	β-Sitosterol		(Anonymous, 1996)
9.	Linoleic acid		(Anonymous, 1996)
10.	Mucunadine		(Dr. Duke Phytochemical database)

Traditional uses: (Natarajan et al., 2012).

The seeds are traditionally used as nervine tonic, emmenagogue, astringent, aphrodisiac, leucorrhoea and paralysis. The hairs of the pods are vermifuge and treated for round worm infections. *Mucuna monosperma* is used as an expectorant and sedative given in cough and asthma (Khory and Katrat 1999). Bark powder mixed with dry ginger is used for rubbing over painful rheumatic joints. The roots are bitter, thermogenic, emollient, stimulant, purgative, aphrodisiac, diuretic, emmenagogue, anthelmintic, febrifuge, diuretic and tonic. In Ayurveda they are useful in vitiated conditions of *vata* and *pitta*, constipation, nephropathy, strangury, dysmenorrhoea, amenorrhoea, elephantiasis, dropsy, neuropathy, ulcers, helminthiasis, fever, delirium and for treating Parkinson's disease. The leaves are aphrodisiac, anthelmintic and tonic and are useful in ulcers, inflammation, helminthiasis, cephalalgia and general debility. The seeds are astringent, laxative, anthelmintic, aphrodisiac and tonic. They are useful in gonorrhoea, sterility, vitiated conditions of *vata*, and general debility (Anonymous, 2002). The seeds are restorative and are sometimes consumed as a vegetable. Seed diet produced hypoglycaemic effect in normal rats (Anonymous, 2006).

Pharmacological uses: (Kavitha and Thangamani, 2014)

Anti-Parkinson's activity

According to "Bhasava rajyam", the Parkinsonism was treated by the administration of powdered seed of *M. pruriens* containing 4 to 6% of levodopa (Ovallath and Deepa, 2013). Hussian and Manyam (1997) indicated that for the dose, *M. pruriens* showed twice the anti-Parkinsonian activity of synthetic L-DOPA. In a clinical study, Nagashayana et al. (2000) revealed the contribution of L-DOPA in the recovery of PD followed by Ayurveda medication. Katzenschlager et al. (2004) revealed that 30 g *Mucuna* seed powder preparation has considerable faster action in treating PD patients than conventional standard drugs, namely, Levodopa or Carbidopa and suggested that natural source of L-DOPA might possess advantages over conventional drugs in long term management of PD.

Antiglycaemic effect

Using a combination of chromatographic and NMR techniques, the presence of D-chiro-inositol and its two galacto-derivatives having antiglycaemic effect was demonstrated in *M. pruriens* seeds (Donati et al., 2005).

Hypoglycemic activity

The hypoglycemic effect of the aqueous extract of the seeds of *M. pruriens* was investigated in normal, glucose load conditions and streptozotocin (STZ)-induced diabetic rats. In normal and STZ diabetic rats, the aqueous extract of the seeds of *M. pruriens* (100 and 200 mg/kg body weight) significantly reduced the blood glucose levels 2 hr after oral administration of seed extract. It also significantly lowered the blood glucose in STZ diabetic rats after 21 days of daily oral administration of the extract. Thus, it was clearly depicted that *M. pruriens* could be a source of hypoglycemic compounds (Bhaskar et al., 2008).

Antioxidant activity

In vitro assays indicated that a whole plant of ethyl acetate and methanolic extract of *M. pruriens*, containing large amounts of phenolic compounds, exhibited high antioxidant and free radical scavenging activities. These plant extracts served as a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses (Satheesh et al., 2010; Kumar and Muthu, 2010).

Antivenom activity

Research on its effects against *Naja* species (Tan et al., 2009) has shown it has potential use in the prophylactic treatment of snakebites. Aqueous extracts of *M. pruriens* seeds were tested for their activity on various pharmacological effects like lethality, phospholipase activity, edema forming activity, fibrinolytic activity and haemorrhagic activity of cobra and krait venoms. About 0.16 and 0.19 mg of *M. pruriens* seed extracts were able to completely neutralize the lethal activity of 2LD50 of cobra and krait venom, respectively, thus suggesting that aqueous extracts of *M. pruriens* seeds possess compounds, which inhibit the activity of cobra and krait venoms (Meenatchisundaram and Michael, 2010). According to Fung and Tan (2012), rats pretreated with *M. pruriens* seed extract showed protective effect against the lethal and cardiovascular depressant effects of *Naja sputatrix* venoms by neutralization neutralization of the venom toxins.

Aphrodisiac activity

Shukla and Mahdi (2010) demonstrated that oral administration of 5g of *Mucuna* seed powder once in a day for men with decreased sperm count and motility

ameliorated psychological stress and seminal plasma liquid peroxide levels along with improved sperm count and motility. The study also concluded that *M. pruriens* not only reactivates the anti oxidant defense mechanism, but also helps in the management of stress and improves semen quality.

Antimicrobial activity

M. pruriens is also used for antimicrobial properties for extracting plant metabolites against plant pathogenic bacteria and fungi. The methanolic extract showed high antibacterial activity against *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas marginalis*, *Pseudomonas acruginosa*, *Xanthomonas campestris* and high anti fungal activity against *Curvularia lunata*, *Fusarium oxysporum*, *Pencillium expansum*, *Rhizoctonia solani*, *Tiarosporella phaseolina* and *Ustilago pomaydis* (Rayavarapu and Kaladhar, 2011).

MATERIALS AND METHODS:

1. **FORMULATION:**
   a. **Procurement:** procurement of raw material and vanari gutika marketed formulation from Go-Vigyan Anusandhan Kendra Deolapar.
   b. **Authentication:** Authentication of raw material i.e. *M. pruriens* seed was done by Dr. Dongarwar sir, Department of Botany (Specimen voucher no. 10009).
   c. **Preparation:** In-house formulation is prepared by using the following formula.

Table 3: Vanari gutika composition (for 2 kg by yogratnakar, 2002)

Sr.no.	Ingredient	Weight
1	Kevanch seed	1 kg.
2	Milk	4 lit.
3	Sugar	1 kg
4	Honey	500 gm.
5	Ghee	Q.S.

d. Method of preparation of vanari gutika formulation:

Approximately1 kg of *Mucuna pruriens* seed were taken, dried & clean it and boiled in about 4 liter of fresh cow milk, concentrate it until milk get viscous. then remove the container from flame and allow to cool, collect all boiled seed and remove its coating and were triturated with concentrated viscous milk obtained after separating of boiled seed until it not get converted in to a lump mass. After that make small sphere shaped vatika (i.e. Tablet having weight App. 300mg) from the lump mass. And allow it to dry, after that fry it in fresh cow ghee until it get brownish red. And then vatika were coated with the help of sugar syrup and stored it in honey.

(A) (B) (C) (D)

Fig A: process of making a lump mass from boiled seed.
Fig B: manually formation of Vatika.
Fig C: process of frying a Vatika
Fig D: final Vanarigutika (reddish brown in color).

2. EVALUATION OF PHARMACEUTICAL PARAMETERS

(Lachman et al., 1987)

Tablets were evaluated for parameters like hardness, weight variation, and disintegration time.

Hardness test

Tablets require a certain amount of strength or hardness and resistance to withstand mechanical shock of handling in manufacture, packing and shipping. To perform this test tablets were placed between two anvils, force to the anvils and the crushing strength that just causes the tablets to break was recorded. Monsanto

hardness tester was used to measure the hardness of tablets. The results were expressed in kg/cm^2.

Weight variation test

Twenty tablets were selected randomly from each formulation batch and weighed individually. The average weight and % weight variation was calculated. As per USP, not more than two of individual weight should deviate percentage limit and none deviate more than twice that percentage limit.

Table 4: Weight variation limits for tablets as per USP

Sr. No.	Average Weight of Tablet(mg)	Maximum % Difference Allowed
1	130 or less	± 10
2	130-324	± 7.5
3	More than 324	± 5

Disintegration test (IP, 2007)

Use water as the liquid. Add a disc to each tube. Operate the apparatus for 60 minutes, unless otherwise stated in the individual monograph. Examine the state of the tablets. If any of the tablet has not disintegrate, repeat the test on a further 6 tablets, replacing water with 0.1 M hydrochloric acid. The tablets comply with the test if all 6 tablets have disintegrated in the acid medium.

pH of gutika (Pathak et al., 2015)

The pH measurement was carried out by using a calibrated digital type pH meter by dipping the glass electrode and the reference electrode completely into the 1 %w/v and 10 %w/v of water soluble portions of formulation.

Diameter of gutika (Karan et. al., 2011)

Average diameter of ten tablets was carried out by using calibrated Vernier caliper.

PHYSICOCHEMICAL EVALUATION OF SEED POWDER AND FORMULATION

Loss on Drying at 110^0C (Kokate et al., 2009)

5gof material was taken and heated at 110^0C in hot air oven. It was taken out and weighed again and again at regular interval till the consistent weight was achieved. The percentage of difference before and after subjecting the sample to heat was considered as loss on drying at that particular temperature.

Determination of Ash and Extractive Values (Khandelwal, 2007)

A. Ash Value

The ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of plant drugs results in an ash residue, which is composed of an inorganic mixture of metallic salts and silica. Unwanted parts of drugs sometimes possess a character, which will raise the ash value, for example the cork on liquorice, which is not required in the powder of the peeled drug. More direct contamination, such as sand or earth, is immediately detected by the ash value.

a. Total ash

1 g powdered drug was taken in a tarred silica dish previously dried and weighed. It was ignited in a furnace until free from carbon. The ash obtained was weighed**.**

b. Acid insoluble ash

To the crucible containing the total ash, 25 ml of dilute hydrochloric acid was added, covered with a watch glass and boiled gently for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water until the filtrate is neutral. It was dried on a hot plate and ignited to constant weight. The residue was allowed to cool in suitable desiccators for 30 minutes, and then weighed without delay.

c. Water-soluble ash

To the crucible containing the total ash, 25 ml of water was added and boiled for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, and ignited in a crucible for 5 minutes. The weight of this residue was subtracted from the weight of total ash.

B. Extractive value

This method determines the amount of active constituents in a given amount of medicinal plant material when extracted with solvents. It is employed for materials for which as yet no suitable chemical or biological assay exists.

a. Water-soluble extractive value is applied to drug that contains water-soluble active constituents of crude drugs such as tannins, sugars, plant acids, mucilage, glycosides, etc.

b. Alcohol-soluble extractive method is frequently employed to determine the approximate resin content of drug.

About 2 g of accurately weighed homogenized drugs were placed in a glass Stoppard conical flask. It was macerated with 100 ml of solvent for 6 hours, shaking frequently and then was allowed to stand for 18 hours. Extract was filtered rapidly taking care not to lose any solvent. 25 ml of the filtrate was transferred to a tarred flat–bottom dish and evaporated to dryness on a water bath. The residue was dried at 105^0 C till its weight became constant, cooled in a desiccator for 30 minutes and weighed without delay.

C. Foaming index (WHO, 1998)

Many medicinal plant materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of a foaming index.

Recommended procedure: - Reduce about 1 g of the plant material to a coarse powder (sieve size no. 1250), weigh accurately and transfer to a 500 ml conical flask containing 100 ml of boiling water. Maintain at moderate boiling for 30 minutes. Cool and filter into a 100 ml volumetric flask and add sufficient water through the filter to dilute to volume. Pour the decoction into 10 Stoppard test-tubes (height 16cm,

diameter 16mm) in successive portions of 1 ml, 2 ml, 3 ml, etc. up to 10 ml, and adjust the volume of the liquid in each tube with water to 10ml. Stopper the tubes and shake them in a lengthwise motion for 15 seconds, two shakes per second. Allow to stand for 15 minutes and measure the height of the foam. The results are assessed as follows.

- If the height of the foam in every tube is less than 1 cm, the foaming index is less than 100.
- If a height of foam of 1 cm is measured in any tube, the volume of the plant material decoction in this tube (a) is used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.
- If the height of the foam is more than 1 cm in every tube, the foaming index is over 1000. In this case repeat the determination using a new series of dilutions of the decoction in order to obtain a result.

Calculate the foaming index using the following formula:

$$\frac{1000}{a}$$

Where a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed.

D. Swelling index (WHO, 1998)

The swelling index is the volume in ml taken up by the swelling of 1 g of plant material under specified conditions. Its determination is based on the addition of water or a swelling agent as specified in the test procedure for each individual plant material (either whole, cut or pulverized). Using a glass-Stoppard measuring cylinder, the material is shaken repeatedly for 1 hour and then allowed to stand for a required period of time. The volume of the mixture (in ml) is then read.

Recommended procedure: - Carry out simultaneously no fewer than three determinations for any given material. Introduce the specified quantity of the plant material concerned, previously reduced to the required fineness and accurately weighed, into a 25-ml glass-Stoppard measuring cylinder. The internal diameter of the cylinder should be about 16mm, the length of the graduated portion about 125 mm,

marked in 0.2-ml divisions from 0 to 25 ml in an upwards direction. Unless otherwise indicated in the test procedure, add 25 ml of water and shake the mixture thoroughly every 10 minutes for 1 hour. Allow to stand for 3 hours at room temperature, or as specified. Measure the volume in ml occupied by the plant material, including any sticky mucilage. Calculate the mean value of the individual determinations, related to 1 g of plant material.

3. EXTRACTION (MACERATION): (Sekar et. al., 2009)

The collected parts (seeds) of the medicinal plant were cleaned and dried under shade. The dried plant materials were then ground well to fine powder. Powdered plant materials were successively extracted with alcohol using cold extraction method i.e. maceration. The extraction was continued for three days. The, alcohol and extract were then filtered and kept at water bath to evaporate the solvent from it. Greenish black residues were obtained.

4. **PHYTOCHEMICAL SCREENING OF EXTRACT AND FORMULATION** (Khandelwal, 2007)

   Phytochemical screening of extracts and formulation were done for the presence of phytoconstituents such as alkaloids, proteins, flavonoid, saponin, steroids, glycosides, tannins, phenols and carbohydrates by the use of various reagents.

Test for Sterols

1) Salkowski test

Few mg of extract of each extract was taken in 2 ml of chloroform and in it 2 ml of concentrated sulphuric acid was added from the side of the test tube. The test tube was shaken for few minutes. The development of red colour in the chloroform layer indicates the presence of sterols.

2) Liebermann-Burchard test

Few mg of extract was dissolved in chloroform. To this, few ml of acetic anhydride was added. Boil for few seconds and cool. Then two drops of concentrated sulphuricacid were added from the side of the test tube. Brown ring indicates the presence of sterols.

Test for Alkaloid

Few mg of the residue of each extract was taken separately in 5 ml of 1.5 % v/v hydrochloric acid and filtered. These filtrates were then used for alkaloid detection.

1) Dragendroff's reagent

It was prepared by mixing solution A (17 g of bismuth sub nitrate + 200 g of tartaric acid + 800 ml distilled water) and solution B (160 g potassium iodide + 400 ml distilled water). Above Dragendroff's reagent was sprayed on Whatmann No. 1 filter paper then the paper was dried. The test filtrate after basification with dilute ammonia was extracted with chloroform and this extract was applied on the filter paper, impregnated with dragendroff"s reagent, with the help of capillary tube. Development of an orange red colour on the paper indicates the presence of alkaloids.

2) Mayer's Reagent

1.36 g of mercuric chloride was dissolved in 60 ml water and 5 g of potassium iodide dissolved in 10 ml of distilled water, solution were mixed and diluted to make up volume 100 ml. To a little of each extract taken in dilute hydrochloric acid in a

watch glass, few drops of the reagent was added, formation of cream colored precipitate shows the presence of alkaloids.

3) Wagner's Reagent:

1.27 g of iodine and 2 g of potassium iodide were dissolved in 5 ml of water and the solution was diluted to 100 ml with water. When few drops of this reagent were added to the test filtrate, a brown colour precipitate was formed indicating the presence of alkaloids.

4) Hager's Reagent

A saturated aqueous solution of picric acid was employed for this test. When the test filtrate was treated with this reagent, yellow precipitate was formed indicating the presence of alkaloids.

Test for Saponins (Foam test)

A few mg of the test residue was taken in a test tube and shaken vigorously with small amount of sodium bicarbonate and water. If stable, characteristic honeycomb like froth was obtained, saponins are present.

Test for Tannins

The test residue of each extract was taken separately in water, warmed and filtered. Tests were carried out with the filtrate using following reagent:

1) Ferric chloride test

A 5 % solution of ferric chloride in 90 % alcohol was prepared. Few drops of this solution was added to a little of the above filtrate. If dark green or deep blue color was obtained, tannins are present.

2) Lead acetate test

A 10 % w/v solution of basic lead acetate in distilled water was added to the test filtrate. If precipitate is obtained, tannins are present.

3) Potassium dichromate test

On an addition of a solution of potassium dichromate in test filtrate, dark colour was developed, tannins are present.

Test for Flavonoid (Shinoda test)

A small quantity of test residue was dissolved in 5 ml of ethanol (95 % v/v) and treated with few drops of concentrated hydrochloric acid and 0.5 g of magnesium metal. The pink, crimson or magenta colour was developed within a minute or two, if Flavonoids are present.

Test for Proteins

1) Biuret test

A few mg of the residue was taken in water and 1 ml of 4 % copper sulphate was added to it. Violet or pink colour was formed, if proteins are present.

2) Xanthoproteic test

A little residue was taken with 2 ml of water and 0.5 ml of concentrated nitric acid was added to it. Yellow color was obtained, if proteins are present.

Test for Amino acid (Ninhydrin test)

The ninhydrin reagent is 0.1 % w/v solution of ninhydrin in n-butanol. A little of this reagent was added to the test extract. A violet or purple colour was developed, if amino acids are present.

Test for Sugars

Molisch's test

This reagent was prepared by dissolving 10 g of alpha- napthol in 100 ml of 95 % alcohol. A few mg of the test extract was placed in a test tube containing 0.5 ml of water, and it was mixed with two drops of Molisch's reagent. To this solution, was added about 1 ml of concentrated sulphuric acid from the side of the inclined test tube, so that the acid formed a layer beneath the aqueous solution, without mixing with it. If a red brown ring appears at the common surface of the liquids, sugars are present.

5. QUANTITATIVE PHYTOCHEMICAL DTERMINATION:

DETERMINATION OF TOTAL PHENOLIC CONTENT (Hagerman et al., 1998)

Phenolic compounds were reported to be active, quenching oxygen-derived free radicals by donating hydrogen atom or an electron to the free radicals

(Wanasundara and Shahidi, 1996). Also phenolic compounds of plant materials have been shown to neutralize free radicals in various in-vitro models (Ruch et al 1999).

Principle:

It is based on the principle that FolinCiocalteau reagent causes the reduction of total phenolic Content in the sample forming blue colour which gets detected at 765nm.

Reagents:

Sodium carbonate: 20%

FolinCiocalteau reagent (FCR): 2N

Triple distilled water (TDW).

Procedure:

Accurately weighed 100 mg of the sample was dissolved in 100 ml of TDW to make the stock solution. This was further diluted 5 times and 1ml of this resultant solution was transferred to a test tube. To this 8 ml of TWD and 0.5 ml of 2N Folin Ciocalteau reagent (FCR) were added to the test tube followed by a vigorous mixing. After 5 minutes, about 1.5 ml of 20% sodium carbonate was added to each of the test tube following proper mixing. Then, finally the whole prepared solution was kept aside for 2 hours, after which the absorbance was recorded at 765 nm against blank using UV spectrometer and the total phenolic contents were expressed in terms of Gallic acid equivalent (mg g^{-1} of dry mass). Standard curve was prepared by using Gallic acid as standard with different concentration i.e. (1-10microgram per ml).

DETERMINATION OF FLAVONOID CONTENT (Chang et al., 2002)

Flavonoids comprise a large group of secondary plant metabolites. Presently more than 5000 individual compounds are known, which are based on very few core structures. Their multitude derives mainly from the various hydroxylation patterns (up to six hydroxy groups) and ether substitution by simple methylation or diverse mono- and di-saccharides. Their function in plants themselves most likely involves screening of UV light, in situ radical scavenging, anti-feeding effects (astringency), etc. Flavonols are a major group of flavonoids, which occur mainly in the form of glycosides in plants. The most common aglycons are quercetin, myricetin and kaempferol.

Principle

The method is based on the principle that aluminum trichloride forms a flavonoid-aluminum complex having the absorbtivity maximum at 435 nm.

Materials

- Quercetin (SigmaAldrich)
- Aluminum chloride Hexahydrate (SDFCL, Mumbai),
- 95 % alcohol (Oasis, Mumbai),
- Sodium acetate (Rankem, Mumbai)

Procedure

The total Flavonoid content was determined as, 0.1 ml of 10 % aluminumtrichloride hexahydrate ($AlCl_3\ 6H_2O$) in methanol was mixed with about 0.5 ml test solution,1.5ml 95 % alcohol and 0.1 ml 1 M sodium acetate. Make up to 5 ml with water and incubate the resultant mixture at 20°C for 40 min. Absorption readings at 435 nm were taken against a blank sample. The total Flavonoid content was determined using a standard curve with Quercetin (2-10 µg/ml) as the standard. The mean of three readings was used and expressed as mg Quercetin of equivalents (QE)/100 g of extract.

DETERMINATION OF TOTAL SAPONIN CONTENT (Desai et al., 2011)

Materials

- 80% Methanol,
- 8% Vanillin,
- Ethanol,
- 72% H_2SO_4,
- Diosgenin

Procedure

The Saponin quantification was carried out in accordance with Helaly method with slight modification. The extract was dissolved in 0.5 mL Methanol 80%. The following were added to the solution: around 0.5 mL of 8% vanillin in ethanol and 5 ml of 72% H_2SO_4 in water. The mixing of the reagents was carried out in a thermostat ice bath at 0º C. The mixture was then set in a thermostat at 60º C for 20 min and at 0º

C for 5 min and then measured at a wavelength of 544 nm. A calibration curve was constructed using purified Saponin as standard Saponin which was also treated in a similar manner.

ESTIMATION OF TANNIN (Sadashivam and Manickam., 1996)

Vanillin hydrochloride method

Principle – The vanillin reagent will react with any phenol that has an unsubstituted resorcinol/ phloroglucinol nucleus and forms a coloured substituted product which is measured at 500nm.

Materials

Vanillin HCl reagent – mix equal volume of 8 % HCl in methanol and 4 % vanillin in methanol. The solution was mixed just before use and avoids using even if it is slightly coloured.

Catechin- 1 mg/ml of methanol.

Working standard – the above stock solution was diluted 10 times. 10 ml to 100 ml (100 µg/ml).

Preparation of extract – 1g of sample was ground in 50ml of methanol, with occasionally mixing by swirling. After 20-25 hours it was centrifuged and the supernatant was collected.

PROCEDURE

- Around 1 ml of the supernatant was pipette out.
- About 5ml of vanillin HCl reagent was added quickly.
- The reading was taken in spectrophotometer at 500nm after 20 min.
- The blank was prepared with vanillin HCl reagent alone.
- The graph was prepared of standard with 20- 100 µg Catechin using the diluted stock solution**.**

ALKALOID CONTENT ESTIMATION (Wagner and Bladt, 1996)

The estimation of the total alkaloid content was done as per the gravimetric method. 5 gm powdered drug is extracted repeatedly using 0.1N $H2SO_4$ in an ultrasonic bath. The solution is filtered; the mixed acid solution is washed with 4 successive quantities of 25 ml chloroform. The chloroform washing rejected, acid solution is basified with dilute ammonia solution and extracted with diethyl ether. The

combined diethyl ether extracts are washed with 5 ml distilled water and ether is evaporated to dryness in a weighed beaker on a water bath. Residue is dried to constant weight at 105^0C.

ESTIMATION OF TOTAL CARBOHYDRATES BY PHENOL SULFURIC ACID METHOD (Sadashivam and Manickam., 1996)

Principle:

In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This forms a green colored product with phenol and absorption maximum at 490 nm.

Materials

- **Phenol 5 %:** Redistilled (reagent grade) phenol (50 g) dissolved in water and diluted to one liter.
- **Sulfuric acid 96 %:** (reagent grade).
- **Standard glucose: Stock solution**- 100 mg in 100 ml of water.
- **Working standard**: 10 ml of stock solution diluted to 100 ml with distilled water.

Procedure

Plant material (100 mg) was hydrolyzed with 5 ml of 2.5 N HCl for three hours on water bath and cooled at room temperature and filtered. The solution was neutralized with sodium carbonate until the effervescence ceased and the volume was made up to 100 ml with water. The solution was centrifuged and supernatant was collected. Of the working standard 0.2, 0.4, 0.6, 0.8 and 1 ml was pipette out in series of test tubes. Similarly of the sample solution 0.1 and 0.2 ml was pipette out in two separate test tubes. The volume was made up to 1 ml with water and 1ml of water was set as a blank. Phenol solution (1 ml) and 96 % sulfuric acid (5 ml) was added to each test tube. After 10 min the contents in the test tubes were shaken and placed in water bath at 25-30 ^{0}C for 20 min. and color was read at 490 nm. The amount of carbohydrates was calculated as a Dextrose equivalent from the calibration curve of Dextrose standard solutions, and expressed as mg of Dextrose / g of plant material.

6. CHROMATOGRAPHIC EVALUATION:

A. Thin Layer Chromatography: (Stahl, 1969, Ketan et. al., 2008) The extracts and formulation were subjected to thin layer chromatographic studies, to find out the probable number of compounds present in them. The details of the procedure are as follows:

Preparation of the plates

The adsorbent/stationary phase used for thin layer chromatography was silica gel G. About 25 g of silica gel G was taken in a glass mortar and about sufficient water was added to it. The mixture was stirred with glass rod until it became homogeneous and allowed to swell for 15 minutes. Then additional water was added to it with stirring. This suspension was then uniformly spread immediately on plates.

Drying and storage of plates

The freshly coated plates were then air dried and stacked in a drying rack and were heated in an oven for 30 minutes at 110° C. Activated plates were kept in a desiccator, till required for further use.

Sample and Standard preparation

Sample and Standard stock solution where prepared in Methanol.

Application of the sample

The test samples were applied in the form of a band, with the help of fine capillaries.

Table 5: SOLVENT SYSTEM FOR TLC

Sr. no.	Solvent system	Spraying reagent
1	n-butanol – water – glacial acetic acid	0.5%ninhydrine in ethanol

Development of TLC plates

Chromatographic rectangular glass chamber was used in the experiments. To avoid insufficient chamber saturation. Different mobile phase where tried but the satisfactory resolution was obtained in the solvent systems mentioned in Table 5. After development of plates, they were air-dried and numbers of bands were noted &R_f value (Retention Factor) were calculated.

B. High Performance Thin Layer Chromatography: (Ketan et. al., 2008)

Preparation of Standard L-Dopa A stock solution of L-dopa (1000m g/ml) was prepared by dissolving an accurately weighed 10 mg of Ldopa standard in 5 ml of anhydrous formic acid and volume was made up to 10 ml with methanol in a

volumetric flask. Standard working solutions were prepared by diluting stock solution with methanol in the concentration 100µg/ml.

Estimation of L-Dopa in Herbal Extract To determine the content of L-dopa in herbal extracts, an accurately weighed 100 mg of dry aqueous extract was transferred into 10 ml volumetric flask and 5 ml of anhydrous formic acid was added, sonicated for 10 min and volume was made up to 10 ml with methanol. The extract was filtered on a Whatman no. 1 filter paper, from which 1.0 ml of the solution was diluted to 10 ml with methanol in volumetric flask. An aliquot of sample 10m l was applied on the TLC plate.

Estimation of L-Dopa in Marketed Herbal Formulations To determine the content of L-dopa in market formulations, an accurately weighed 100 mg of powder was transferred into 10 ml volumetric flask and 5 ml of anhydrous formic acid was added, sonicated for 10 min and volume was made up to 10 ml with methanol. The extract was filtered on a Whatman no. 1 filter paper, from that 1.0 ml of the solution was diluted to 10 ml with methanol in volumetric flask. An aliquot of sample 20m l was applied on the TLC plate.

HPTLC Instrument The samples were spotted in the form of bands with a Camagmicrolitre syringe on a precoated silica gel plates 60 F254 (10 cm_10 cm with 0.2 mm thickness, E. Merck, Darmstadt, Germany) using a CamagLinomat V Automatic Sample Spotter (Muttenz, Switzerland). The plates were prewashed by methanol and activated at 60 °C for 5 min prior to chromatography. The plate was developed in a solvent system (6.0 ml) of *n*-butanol–acetic acid–water (4.0 : 1.0 : 1.0) in a CAMAG glass twin-through chamber (10_10 cm) previously saturated with the solvent for 30 min (temperature 25_2 °C, relative humidity 40%). The development distance was 8 cm. Subsequent to the scanning, TLC plates were air dried and scanning was performed on a Camag TLC scanner III in absorbance mode at 280 nm and operated by WinCats software 4.03 version. Evaluation was *via* peak areas with linear regression.

C. High Performance Liquid Chromatography: (Bhumika G. Rathod et al.,)

Preparation of Standard solution

A stock solution of 100µg/ml was prepared by dissolving 10.0 mg of L-Dopa in 10 ml of 0.1 M HCl and diluted to 100.0 ml with HPLC grade methanol.

Preparation of Sample solution

Accurately weighed one gram of powders and extracts of *Mucunapruriens*were refluxed with a mixture of methanol and 0.1 M HCl (70:30) for 30.0 minutes and filtered. The extracts were evaporated to dryness. The residue was redissolved in methanol, filtered through 0.45 μm membrane filter and used for HPLC analysis.

Experimental condition

The analytical HPLC experiments were performed with a Shimadzu Carporation Kyoto Japan compact LC equipped with variable wavelength detector operating at 280 nm. Separation was carried out with C18 (5 μm) column with Water: Methanol: Acetonitrile (100:60:40) containing 0.2 % Triethylamine, pH adjusted to 3.3 as an eluent at a flow rate of 1.0 ml/minute. Validation of quantitative method was performed with samples for five injections of 20 μl each.

7. ANIMAL STUDIES:

Twelve weeks old male and female Swiss Albino mice of weighing 25-35 g were obtained from the Animal House (Reg. No 92/1999/CPCSEA Dated - 28/04/1999) (Reg.No IAEC/UDPS/2016/24), Department of Pharmaceutical Sciences, RTMNU, Nagpur. They were housed singly in separate standard cages and maintained under standard laboratory condition (temperature 24-28℃, relative humidity 60-70%, 12 hr light-dark cycle) with free access to solid pellet diet (gold mohar, lipton India) and water *ad libitum* throughout the study except during the experiment(Milind and Anupam 2009). The experimental protocol was approved by Central Animal Ethical Committee of RTMNU Nagpur University (dated 09/08/2016).

Selection of sexually active male mice

Sexually active male mice were screened and selected by mating a male with a receptive female for two consecutive weeks. Each male mice was allowed 30 minutes exposed to a stimulus female and tested three times over 10 days period for copulatory behavior. Animal showing ejaculation latency shorter than 15 minutes were selected and considered sexually experienced.

Experimental design

Sexually active male mice were divide into seven group (n=6) and placed individually in separate propylene cages during the experiment. Group 1 served as control and received 0.5ml/kg of distilled water orally. Group 3rd received suspension of the extract orally at the doses of 200 mg/kg once daily for 7 consecutive days (Sekar et. al., 2009). Group 4 and 5 received suspension of the medium dose of formulation. Group 6 and 7 received suspension of the high dose of formulation. Group 2nd served as standard group and given suspension of standard drug (sildenafil citrate) orally at the dose of 5 mg/kg, 1 hr prior to commencement of experiment (Tajuddin et. al., 2003). Since the male animal should not be tested in unfamiliar circumstances the animal were brought to the laboratory and exposed to dim light (1 w fluorescent tube in a laboratory of 14' × 14') at the stipulated time of daily testing for 6 days before the experiment.

Drug preparation

Vanari gutika formulation (17.14 mg/ml for high dose group and 8.56 mg/ml for medium dose group. The medium and high dose of formulation was calculated on the basis of prescription dose) were triturated and suspended in distilled water using CMC (0.5%) for oral administration. Similarly *Mucunapruriens* seed extract, sildenafil citrate and oestradiol were also suspended in distilled water using CMC (0.5%) separately, for oral used. Progesterone was dissolved in olive oil for subcutaneous injection. All the drug solution was prepared just before administration.

Table 6: Grouping of Animal

Sr no.	Group	No. of Mice animal	
		Male mice	Female mice
1	Control	6	6
2	Standard	6	6
3	Extract	6	6

4	Medium dose marketed formulation (MMF)	6	6
5	Medium dose in-house formulation (MIF)	6	6
6	High dose marketed formulation (HMF)	6	6
7	High dose in-house formulation (HIF)	6	6

A. Mounting behaviour test (Tajuddin et. al., 2003)

Mount is operationally defined as the male assuming the copulatory position but failing to achieve intromission. To quantify mounting behaviour, non-oestrous femalemice were paired with males treated with single dose of the drugs (500 mg/kg; p.o.). Animals were observed for 3 hrs and their behaviours were scored as described. Males were placed individually in a glass cage. After 15 minutes of acclimatization, a non-oestrous female was introduced into the arena. The numbers of mounts wererecorded during a 15 minutes observation period at the start of 1st hr. Then the female was separated for 105 minutes. Again the female was introduced and the number ofmounts was observed for 15 minutes as before at 3rd hr. All the experiments were performed between 09.00 to 12.00 hrs during day time at room temperature 26–27°C.

B. Test for libido (Milind and Anupam 2009)

The test was carried out by the method of Davidson (Davidson J.M. 1981), modified by Amin et al. (Amin, K.M.Y. 1996) sexually experienced male albino mice were divided in to 7 groups of 6 animal each and kept singly in a separate cage during the experiment. Group 1 represents the control group, which received 10 ml/kg of distilled water orally. Group 3rd received the suspension of the extract orally at the dose of 200 mg/kg, group 4 and 5 received the suspension of medium dose of marketed and in-house formulation, group 6 and 7 received the suspension of high dose of marketed and in-house formulation, respectively once a day in the evening (3:00 PM) for 7 days. Group 2nd served as standard and given suspension of sildenafil citrate orally at the dose of 5mg/kg, 1hr prior to the commencement of the experiment. The ethanolic extract of test drug was suspended in distilled water using CMC 0.5% for oral administration. The female mice were made receptive by

hormonal treatment, 10 µg of oestradiol benzoate orally 48 hr prior to the experiment and 500 µg of progesterone subcutaneously 7 hr prior to the experiment (David, 1970). The animals were observed for the mounting frequency (MF) on the evening of 7^{th} day at 10:00 AM. Each animal was placed individually in a cage. The number of mounting was noted. The animals were also observed for intromission and ejaculation. The MF in control test and standard animal reading was statistically analyzed by employing one-way analysis of variance (ANOVA) method.

The test was terminated if the male failed to evince sexual interest or if the female did not show receptivity it was replaced by another artificially warmed female. The occurrence of events and phases of mating were called out to be recorded on a video camera as soon as they appeared. Later, the frequencies and phases were determined from cassette transcription. The sexual behavior parameter analyzed were

Mounting frequency (MF): Number of mount without intromission from the time of introduction of female until ejaculation. Mounting is the climbing of one animal by another from the posterior end with intention of introducing one organ into another position but failing to achieve intromission.

Intromission frequency (IF): Number of intromission from the time of introduction of female until ejaculation. Intromission is the introduction of one organ or parts into another e.g. the penis into vagina.

Mounting latency (ML): Time interval between the introduction of female and the first the first mount by the male.

Intromission latency (IL): Time interval between times of introduction of female to the first intromission by the male. This is characterized by pelvic thrusting and springing dismount.

Ejaculatory latency (EL): Time interval between the first intromission of a series and ejaculation. This is usually characterized by longer, deeper pelvic thrusting and slow dismount by a period of inactivity. Ejaculation is the act of ejecting semen brought about by a reflex action that occurs as the result of sexual atimulation.

Post-ejaculatory interval (PEI): Time interval between ejaculation and the first intromission by the male of the following series.

C. Biochemical estimations (Turk, 2010)

All the groups of male mice were sacrificed at the end of treatment on day 7 under ether anaesthesia in lethal chamber and the blood samples were collected. The samples were centrifuged and serum was separated and used for the estimation of testosterone, LH and FSH using respective standard Electro Chemiluminescence Method. The entire hormones were analyzed by J.M. pathology lab, sitaburdi Nagpur.

D. Histopathological examination (Turk, 2010)

Testes were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer. Tissues were removed and dehydrated through upgraded ethanol, then cleared with xylene, and finally embedded in paraffin. Sectioning was done by using microtome (7 μm thickness). Tissues were counterstained with haematoxylin in eosin, then examined and photographed under a Leica DM 2500 microscope.

E. Sperm count (WHO Laboratory manual 2000)

Here the dilution of semen used in 1:200 having a concentration of 100mil/ml. Take 9.3 cc of semen normal saline solution in a beaker, to this solution add 0.5 cc of semen and mix it thoroughly. Take 9 cc of 0.5 cc eosin solution in another beaker and transfer 1 cc of mixed semen first beaker in to second beaker. Mix well and keep the second beaker in ice boxes or in cool temp for 5 min. to kill the sperms. Keep the coverslip over Neubaur'shaemocytometer chamber and put the proceed solution in Neubaur's slide Observe under LEICA DM 2500 microscope.The Neubaur's haemocytometer is marked into square having 1 sq. mm area. The central square is divided into 16 small square at 1/40 sq.mm area. Height of chamber 1/10 mm after putting the coverslip so that volume of each small square is 1/100 cu. mm. Count the sperm and note the observation.

Statistical analysis:

The all experimental results were expressed as mean ± SEM, with six animals in each group followed by one-way analysis of variance (ANOVA). Newman-Keuls test for multiple comparisons was applied for determining the statistical significance between different groups. Except mounting behavior group, in which experimental results were expressed as mean ± SEM, with six animals in each group followed by two-way ANOVA Bonferroni multiple comparision test. Graph Pad Prism, version 5

software, was used for all statistical analysis. P values <0.05 were considered to be significant.

RESULTS

Table 7. Organoleptic properties of Vanari gutika formulation

Sr no.	Properties	Observation
1	Colour	Dark brownish
2	Odour	Characteristics
3	Taste	Sweet
4	Shape	Sphere

Table 8. Pharmaceutical parameter of Vanari gutika formulation

Sr no.	Parameter	Observation Marketed formulation	Observation In-house formulation
1	Weight variation	Pass	Pass
2	Hardness (kg/cm)	2.9 ± 0.05	3.0 ± 0.05
3	Diameter (mm)	8.1 ± 0.66	8.3 ± 0.66
4	pH	4.79	4.94

Values are mean ± SD from observations

Table 9. Physichochemical properties of *M. Pruriens* seed powder

Sr no.	Parameter	Observation
1	LOD (%w/w)	9.5
2	Swelling index (ml)	1.7
3	Foaming index	12.5
4	Total Ash (%w/w)	3.715
5	Acid insoluble Ash (%w/w)	0.149
6	Water soluble Ash value (%w/w)	1.895

7	alcohol extractive value (%w/w)	13.2
8	Aqueous soluble extractive value (%w/w)	25.5
9	Ether extractive value (%w/w)	6.1

Table 10. Physicochemical properties of vanari gutika formulation

Sr no.	Parameter	Marketed formulation (%w/w)	In-house formulation (%w/w)
1	Total Ash	1.0	0.6
2	Acid insoluble Ash	0.3	0.05
3	Water soluble Ash value	0.9	0.65
4	Alcohol extractive value	7.8	19
5	Aqueous soluble extractive value	29.9	30.6
6	Ether extractive value	5.8	2

Table 11. Phytochemical screening

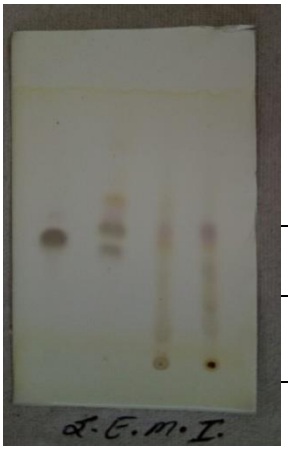
Plant constituents	Test reagents	Alcoholic extract	Marketed formulation	In – house fiormulation
Steroids	Salkowaski reaction	+	+	+
Tri-terpenoids	Liebermann-Burchard test	+	+	+
Alkaloids	Dragendorff's reagent	+	+	+
	Mayer's reagent	+	+	+
	Hager's reagent	+	+	+
	Wagner's Reagent	+	+	+
Tannins	Bromine water Test	+	+	+
	10% Lead acetate test	+	+	+
Flavonoids	Shinoda test	-	+	+
Carbohydrates	Molish's test	+	+	+
Proteins	Biuret test	+	+	+
Saponins	Foam test	+	-	-
Phenolic content	Ferric chloride test	-	+	+
Amino acid	Ninhydrin solution	+	+	+

+ indicates presence – indicates absence

Table 12. Quantitative estimations of Extract and formulation

Sr no.	Parameter	Extract	Marketed formulation	In-house formulation
1	Total alkaloids (%w/w)	0.34	0.56	0.60
2	Total Phenol Content (mg/g) Eq Gallic acid	0.75	1.05	1.39
4	Saponin content (mg/g) Eq Diosgenin	0.46	-	-
5	Tannin Content(mg/g) Eq Catechin	4.07	2.30	1.95
6	Carbohydrade content (mg/g) Eq dextrose	0.28	1.36	1.52

Thin Layer Chromatography (TLC)



Mobile phase	Spraying reagent	Rf value
n-butanol – water – glacial acetic acid (4:1:1) v/v/v	0.5%ninhydrine in ethanol	0.46

Figure 5:- TLC of std., extract, marketed formulation and In-house Formulation

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

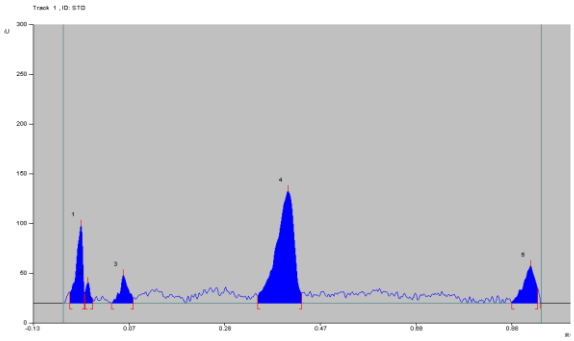


Fig.6: HPTLC fingerprint of Standard L-DOPA

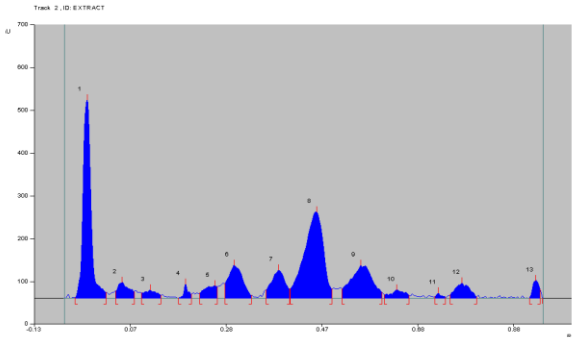


Fig.7: HPTLC fingerprint of Extract

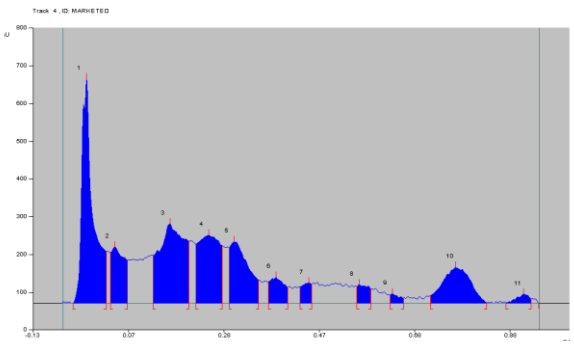


Fig.8: HPTLC fingerprint of Marketed Formulation

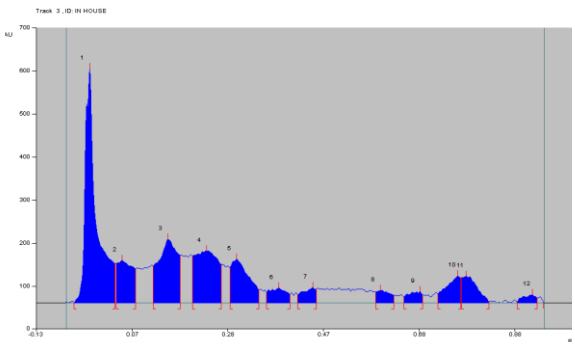


Fig.9: HPTLC fingerprint of In-house formulation

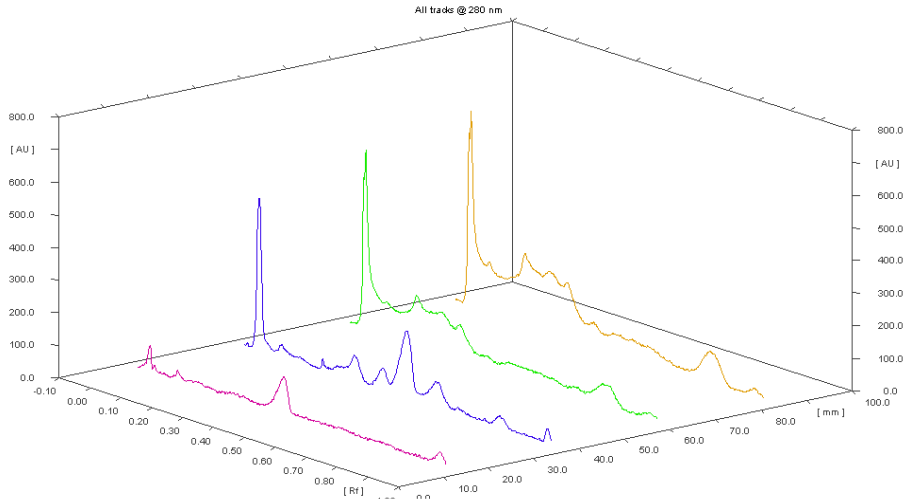


Figure 10: Densitogram of std L-dopa, Extract, Marketed and In-house formulation.

Table 13: HPTLC data (Track 1: Standard L-dopa, Track 2: Extract, track 3: Marketed formulation, Track 4: In-house formulatin

VANRIGUTIKA_HPTLC.cna* winCATS

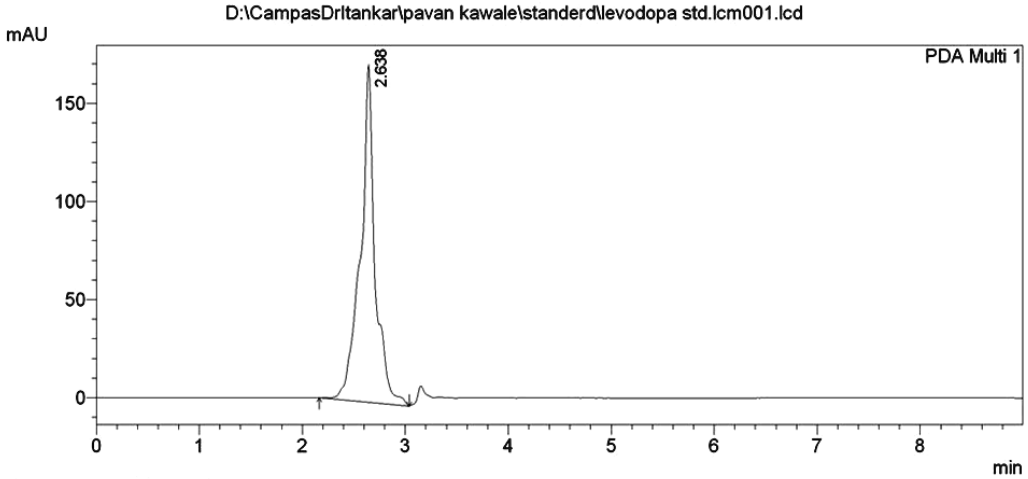
Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	1	0.34 Rf	5.6 AU	0.41 Rf	10.5 AU	00.00 %	0.44 Rf	7.2 AU	719.7 AU	00.00 %	unknown *
2	1	0.17 Rf	0.0 AU	0.19 Rf	33.5 AU	7.14 %	0.20 Rf	3.0 AU	281.3 AU	1.99 %	unknown *
2	2	0.22 Rf	1.8 AU	0.24 Rf	27.0 AU	5.76 %	0.24 Rf	3.0 AU	393.3 AU	2.78 %	unknown *
2	3	0.27 Rf	3.3 AU	0.29 Rf	76.0 AU	16.22 %	0.33 Rf	5.9 AU	063.1 AU	14.56 %	unknown *
2	4	0.36 Rf	7.2 AU	0.39 Rf	63.3 AU	13.51 %	0.41 Rf	3.2 AU	346.3 AU	11.62 %	unknown *
2	5	0.41 Rf	9.5 AU	0.47 Rf	98.2 AU	42.29 %	0.50 Rf	7.3 AU	220.8 AU	50.96 %	unknown *
2	6	0.53 Rf	7.5 AU	0.56 Rf	70.7 AU	15.08 %	0.60 Rf	3.8 AU	564.6 AU	18.10 %	unknown *
3	1	0.13 Rf	5.9 AU	0.15 Rf	67.4 AU	28.73 %	0.18 Rf	7.6 AU	369.9 AU	24.56 %	unknown *
3	2	0.20 Rf	3.0 AU	0.24 Rf	62.9 AU	26.83 %	0.26 Rf	3.0 AU	526.4 AU	37.16 %	unknown *
3	3	0.28 Rf	6.9 AU	0.30 Rf	59.8 AU	25.47 %	0.34 Rf	0.3 AU	390.8 AU	24.87 %	unknown *
3	4	0.35 Rf	0.4 AU	0.38 Rf	12.0 AU	5.12 %	0.41 Rf	1.3 AU	268.9 AU	3.96 %	unknown *
3	5	0.42 Rf	1.4 AU	0.45 Rf	18.1 AU	7.70 %	0.46 Rf	5.0 AU	329.8 AU	4.85 %	unknown *
3	6	0.58 Rf	0.2 AU	0.59 Rf	14.4 AU	6.15 %	0.62 Rf	3.0 AU	313.2 AU	4.61 %	unknown *
4	1	0.14 Rf	5.4 AU	0.16 Rf	96.5 AU	30.49 %	0.19 Rf	5.0 AU	529.3 AU	27.08 %	unknown *
4	2	0.22 Rf	9.1 AU	0.24 Rf	90.7 AU	28.65 %	0.27 Rf	1.5 AU	576.9 AU	38.29 %	unknown *
4	3	0.29 Rf	1.3 AU	0.30 Rf	90.1 AU	28.46 %	0.35 Rf	3.8 AU	575.0 AU	27.57 %	unknown *
4	4	0.37 Rf	4.8 AU	0.39 Rf	20.4 AU	6.45 %	0.40 Rf	5.7 AU	363.9 AU	3.90 %	unknown *
4	5	0.44 Rf	6.1 AU	0.45 Rf	18.8 AU	5.95 %	0.46 Rf	5.9 AU	296.0 AU	3.17 %	unknown *

Table 14: % of L-dopa found in various samples by HPTLC

Track	Rf value	Area	Concentration %w/w
Standard L-DOPA	0.41	719.7	-
Extract	0.47	220.8	1.53
Marketed formulation	0.45	296.0	2.05

In-house formulation	0.45	329.8	2.28

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

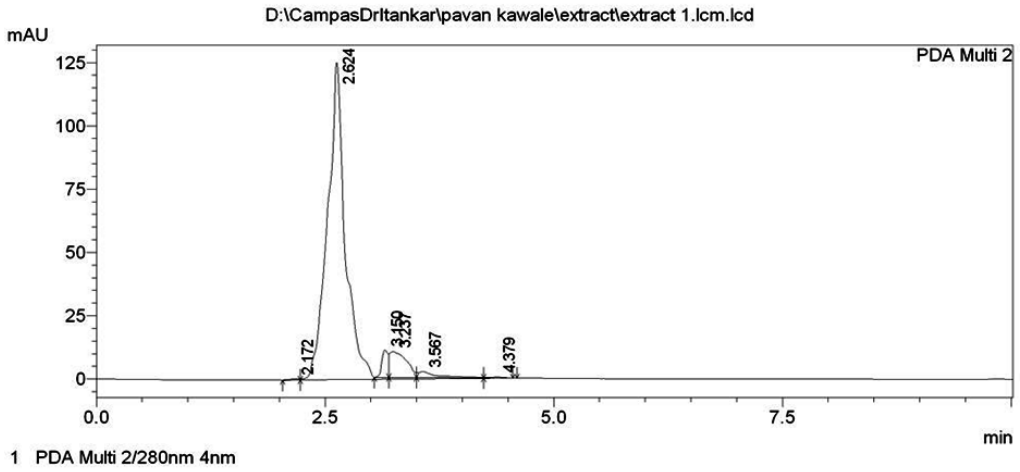


PeakTable

PDA Ch1 280nm 4nm

Peak#	Ret. Time	Area	Height	k'	Tailing Factor
1	2.638	1704008	172027	0.000	0.968
Total		1704008	172027		

Fig. 11: HPLC fingerprint of standard levodopa

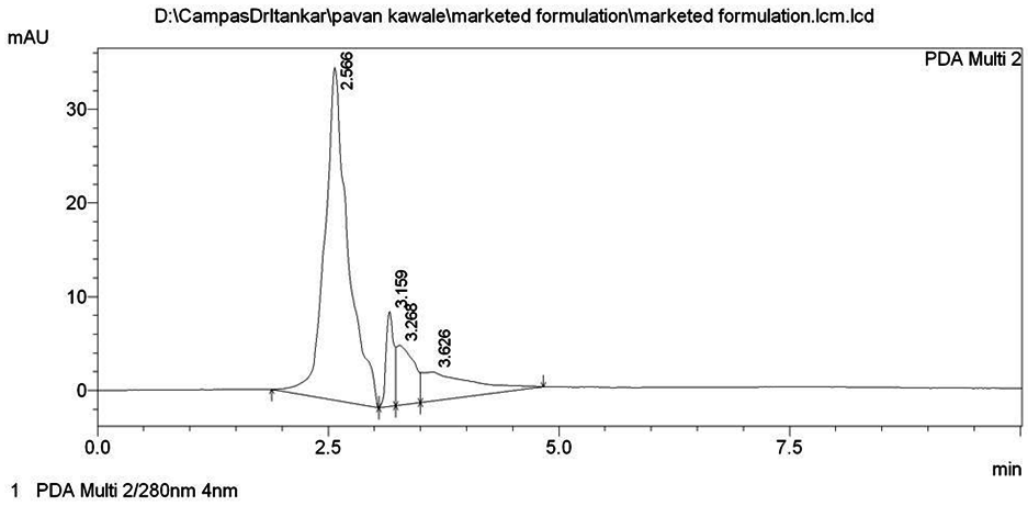


PeakTable

PDA Ch2 280nm 4nm

Peak#	Ret. Time	Area	Height	k'	Tailing Factor
1	2.172	3569	418	0.000	0.000
2	2.624	1719359	125078	0.208	1.134
3	3.150	55321	10917	0.450	0.000
4	3.237	128045	10233	0.491	0.000
5	3.567	35676	2340	0.642	0.000
6	4.379	3139	279	1.016	0.000
Total		1945108	149264		

Fig 12: HPLC fingerprint of Extract

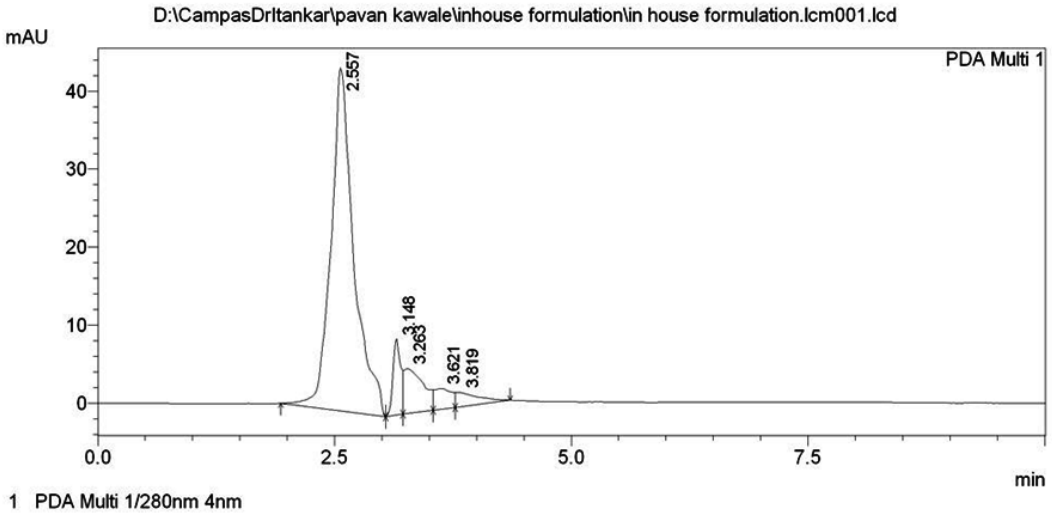


PeakTable

PDA Ch2 280nm 4nm

Peak#	Ret. Time	Area	Height	k'	Tailing Factor
1	2.566	623104	35495	0.000	1.250
2	3.159	59262	10100	0.231	0.000
3	3.268	81530	6364	0.274	0.000
4	3.626	110745	3099	0.413	0.000
Total		874641	55058		

Fig 13: HPLC fingerprint of Marketed formulation



PeakTable

PDA Ch1 280nm 4nm

Peak#	Ret. Time	Area	Height	k'	Tailing Factor
1	2.557	735462	43935	0.000	1.375
2	3.148	56353	9722	0.231	0.000
3	3.263	82135	5749	0.276	0.000
4	3.621	33264	2654	0.416	0.000
5	3.819	29526	1843	0.493	0.000
Total		936740	63904		

Fig 14: HPLC of fingerprint of In-house formulation

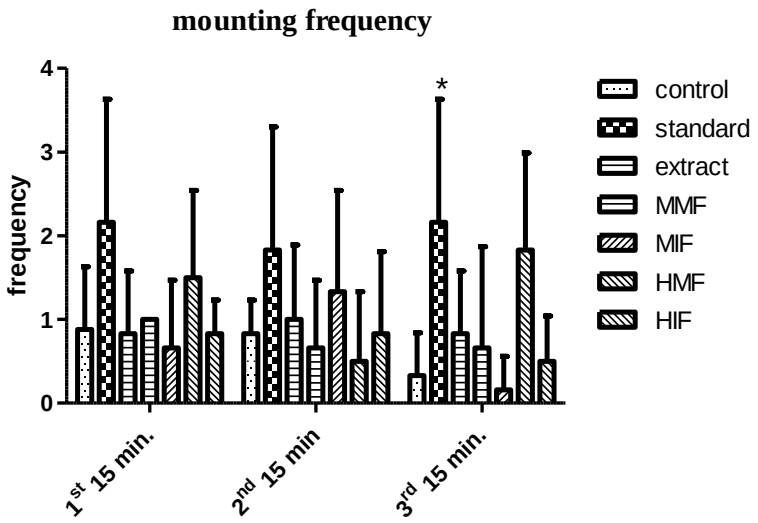
Table 15: % of L-dopa found in various samples by HPLC

Track	concentration	Ret. Time	Area	Concentration %w/w
Standard L-dopa	100µg/ml	2.63	1704008	-
Extract	500µg/ml	2.62	1719359	5.04
Marketed formulation	500µg/ml	2.56	623104	1.82
In-house formulation	500µg/ml	2.55	735462	2.15

Table 16: Mounting behaviour:

Sr n.	Group	No. of mounts		
		1st 15 min.	2nd 15 min.	3rd 15 min.
1	Control	0.83±0.30	0.83±0.16	0.33±0.21
2	Standard	2.16±0.60*	1.83±0.60*	2.16±0.60*
3	Extract	0.83±0.30	1±0.36	0.83±0.30
4	Medium dose marketed formulation	1±0	0.66±0.33	0.66±0.49
5	Medium dose in-house formulation	0.66±0.33	1.3±0.49	0.16±0.16
6	High dose marketed formulation	1.5±0.42*	0.5±0.34	1.8±0.47*
7	High dose in-house formulation	0.83±0.16	0.83±0.40	0.5±0.22

Value are expressed as mean ± S.E.M. n=6



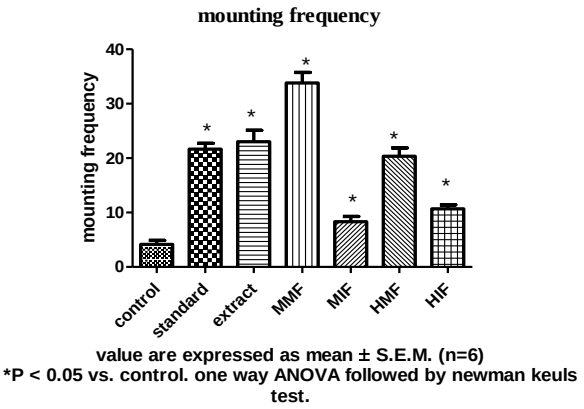
value are expressed as mean ± S.E.M. (n=6) *P≤0.05 vs. control. Two way ANOVA followed by Bonferroni multiple comparision test.

Test on libido:

Table 17: Mounting and Intromission frequency: -

Sr no.	Group	No. of mount	No. of Intromission
1	Control	4.16±0.70	3.33±0.84
2	Standard	21.66±1.0*	4.83±0.94
3	Extract	23±2.12*	8.33±1.96*
4	Medium dose marketed formulation (MMF)	33.83±1.90*	19.16±1.13*
5	Medium dose in-house formulation (MIF)	8.33±0.95*	11.5±2.48*
6	High dose marketed formulation (HMF)	20.33±1.56*	4±1.41
7	High dose in-house formulation (HIF)	10.66±0.76*	1.16±0.80

Value are expressed as mean ± S.E.M.; n=6;



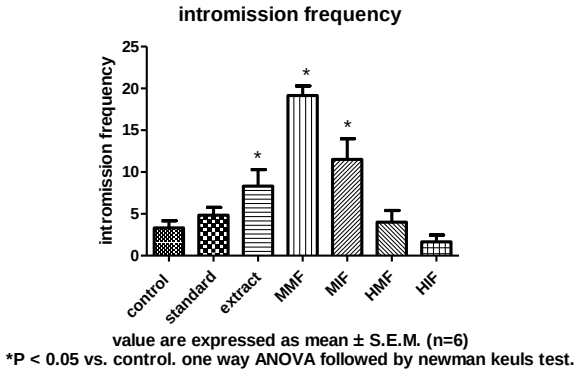
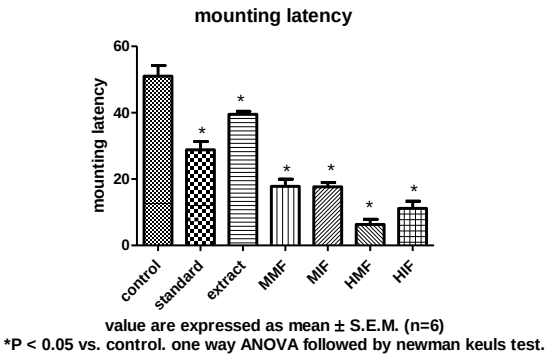


Table 18: Mounting and intromission latency

Sr no.	Group	Mounting latency	Intromission latency
1	Control	51±3.22	129.33±8.22
2	Standard	28.83±2.48	49.83±8.82
3	Extract	39.5±0.92	40.5±4.24
4	Medium dose marketed formulation (MMF)	17.83±2.15	19.83±3.59
5	Medium dose in-house formulation (MIF)	17.66±1.30	26.5±3.78
6	High dose marketed formulation (HMF)	6.33±1.52	10.5±2.76
7	High dose in-house formulation (HIF)	11.16±2.16	39.66±4.79

Value are expressed as mean ± S.E.M.; n=6;



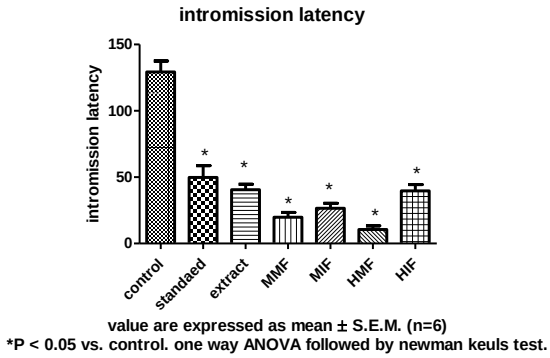


Table 19: Ejaculation frequency

Sr. no	Group	Ejaculation frequency

1	Control	0
2	Standard	0.5±0.34
3	Extract	0
4	Medium dose marketed formulation	0.5±0.22
5	Medium dose in-house formulation	0.33±0.21
6	High dose marketed formulation	0.33±0.21
7	High dose in-house formulation	0.16±0.16

Value are expressed as mean ± S.E.M., n=6

Table 20: Epididymal sperm count

Sr. no	Group	Sperm count ($\times 10^6$ Cu/mm)
1	Control	143.66±7.46
2	Standard	807.5±9.53**
3	Extract	440.66±10.94**
4	Medium dose marketed formulation	263.16±2.67
5	Medium dose in-house formulation	224.16±2.57
6	High dose marketed formulation	584.66±50.50**
7	High dose in-house formulation	710.83±43.15**

Value are expressed as mean ± S.E.M.; n=6; *P<0.05 vs. Control. One way ANOVA followed by Newman keuls test.

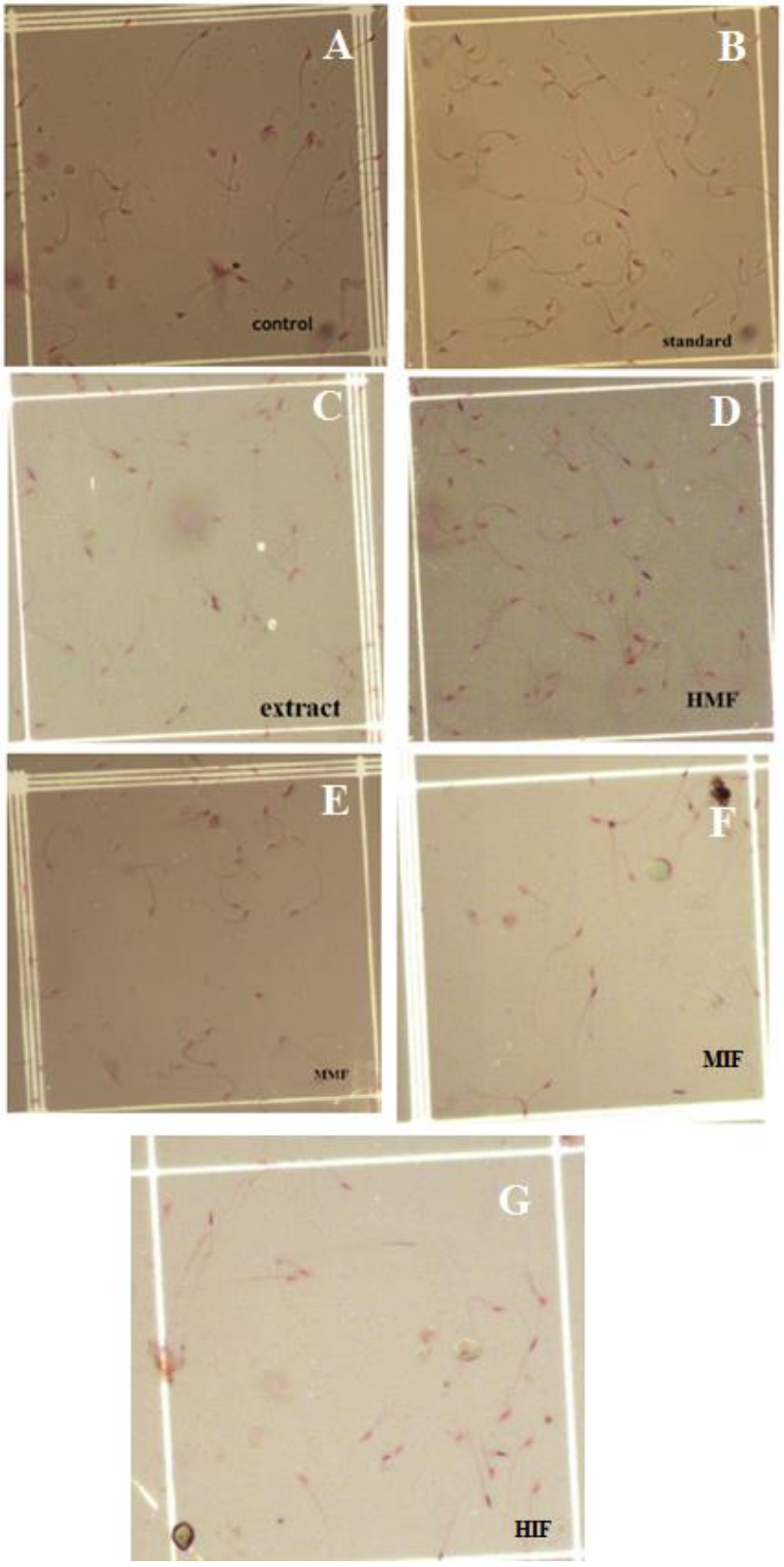
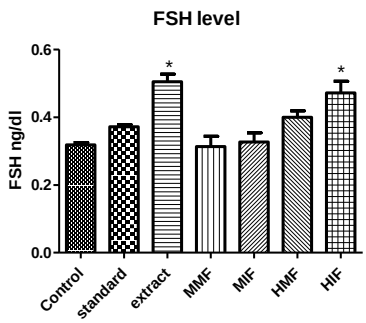


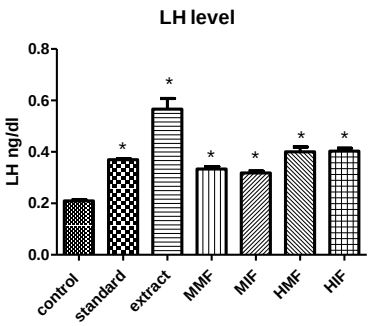
Fig.15: Epididymal sperm count at 40x. (A): Control group, (B): Standard group, (C): Extract group, (D): HMF group, (E): MMF group, (F): MIF group, (G): HIF group

Table 21: Biochemical analysis

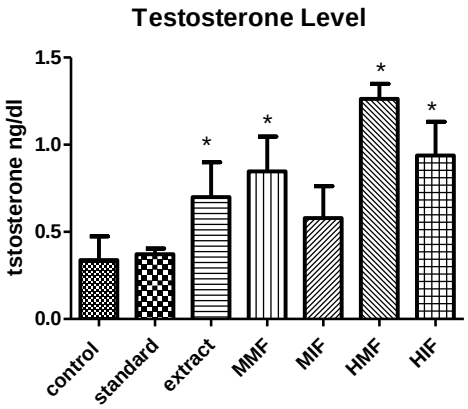
Sr no.	Group	FSH	LH	Testosterone
1	Control	0.31±0.007	0.21±0.003	0.33±0.13
2	Standard	0.37±0.006	0.37±0.003*	0.37±0.03
3	Extract	0.50±0.02*	0.56±0.04*	0.7±0.19*
4	Medium dose marketed formulation	0.31±0.03	0.33±0.009*	0.84±0.19*
5	Medium dose in-house formulation	0.32±0.02	0.31±0.007*	0.63±0.17
6	High dose marketed formulation	0.4±0.01	0.4±0.01*	1.26±0.08*
7	High dose in-house formulation	0.47±0.03*	0.40±0.01*	0.93±0.19*



value are expressed as mean ± S.E.M. (n=6)
*P<0.05 vs. control. one way ANOVA followed by newman keuls test.



value are expressed as mean ± S.E.M. (n=6)
*P<0.05 vs. control. one way ANOVA followed by newman keuls test.



value are expressed as mean ± S.E.M. (n=6)
***P<0.05 vs. control. one way ANOVA followed by newman keuls test.**

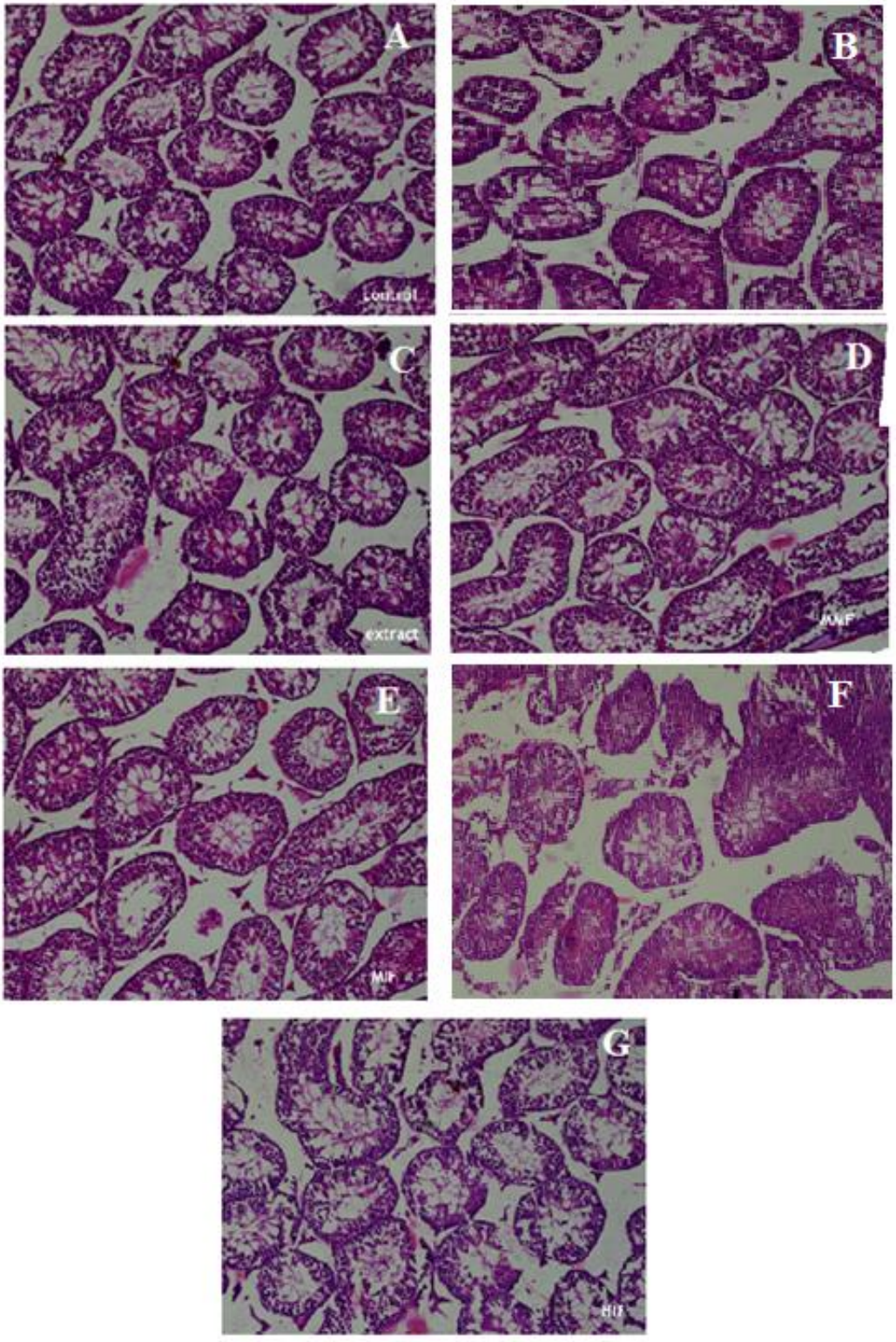


Fig.16: T.S. of testis at 10x (A): Control group, (B): Standard group, (C): Extract group, (D): MMF group, (E): MIF group, (F): HMF group, (G): HIF group

Histopathology of Testis:

The presence of thick collagenous connective tissue and vascular loose connective tissue has been present around the testicular cells. The seminiferous tubules embedded in interstitial connective tissue. The leydig cells and sertoli cells have been observed. Spermatogenetic cells forming a stratified epithelial and sperms are often found in clusters embedded in cytoplasm of sertoli cells. The acidophilic cells known, as leydig cells are occasionally found in each seminiferous tubule spermatogenesis also observed. While T.S. of testis treated mice with high dose

Summary and Discussion:

Sexual dysfunction is a major health problem, the consequence of which can affect both the man and his family. There has been worldwide increase in the incidence of ED, probably due to aging population and other risk factor such as the presence of chronic illnesses e.g. heart disease, hypertension and diabetes mellitus (Paul et. al., 2012), smoking, stress, alcohol, drug abuse and sedentary lifestyle (Taymour, 2010).

Many synthetic drugs are available to treat ED but, they are expensive and can provoke fatal adverse effect. Since many people are now relying on herbal medicines for health care, so there should be a renewed interest towards the search of traditional herbs. Which are being constantly claimed for treatment of this dysfunction.

According to Ayurveda one of the product recommended for the management of sexual dysfunction is Vanari gutika (Yog-Ratnakar 2002). It is prepared as per the Ayurvedic protocol using *Mucuna pruriens* seed powder, cow milk, honey and sugar. Clinically, it has been found to be useful in cases of male infertility.

In present research work, attempts were made for the developing a quality control profiling of Vanari gutika formulation along with evaluation of its preclinical aphrodisiac potential. The present study also included the pharmacognostical and pharmacological evaluation of seeds of *mucuna pruriens* (Kevanch seed) (Family: Fabaceae) for its Aphrodisiac activity. Kevanch seed is one of the major component

of the Vanari gutika and having a potent aphrodisiac potential (Sekar et. el., 2009). But, vanari gutika has not been scientifically reported for its activity against Sexual dysfunction and oligospermia therefore, was the basis of the selection.

Estimation of various qualitative and quantitative parameters was carried out which will help us in setting standards for a particular drug; these standards might prove beneficial for identification and characterization of that particular drug/formulation. With the help of these standards one can maintain quality and purity of that particular drug and its formulation and prevent it from being adulterated by drug of same genus or other species having low potency (Shanbhag, 2008).

Various pharmaceutical parameters of Vanari gutika were performed. The pharmaceutical parameters include organoleptic characteristic, weight variation, hardness, disintegration test, pH, and diameter, and were found to be within the limits stated by Indian Pharmacopoeia.

In case of disintegration test, it was observed that the disintegration time of the vanari gutika was found to be 6 hr in case of in-house preparation and 8 hr in case of marketed formulation. The result obtained in the present study is a contrasting one. As the disintegration time for sugar coated tablet in our study is reported to be 60 min. maximum. Thus in our case the disintegration is very high which may be attribute to the presence of cow milk which is highly reach in carbohydrate that may act as natural binder which kept the gutika intact for a longer period of time. Further the preparation procedure of vatika include frying of gutika in ghee, which itself provide an oil/fat coating on the gutika and may result in extended disintegration time for the gutika. Further it has been reviewed in the literature that the gutika are consumed in the form of chewable tablet. Thus, our result so obtained truly support these statement as the chewable tablet should have a prolong effect and thus must have a longer disintegration time observed in our case.

The physicochemical parameters such as LOD, total ash, acid insoluble ash, extractive values were also evaluated (Khandelwal 2007). The results showed the presence of negligible moisture content. It is very essential to control the moisture content, since higher moisture content in plant material may lead to its deterioration and may therefore result in percentage variation of active constituents.

There was higher extractive value in aqueous medium as compared to alcohol and petroleum ether in *M. pruriens* seed powder and formulation. The amount of active chemical constituents present in plant material depends on the extractive values extracted through different solvents for which as yet no suitable chemical or biological assay exist. The chemical nature of the active constituents present in the plant material can be identified by performing preliminary phytochemical screening of that plant material.

The observations from phytochemical screening showed the presence of steroids, alkaloids, tannins, flavonoids, carbohydrate, proteins, phenol and amino acid in vanari gutika formulation however the saponins was absent. Further the phytochemical screening of alcoholic extract showed the absence of flavonoids and phenol. The vanari gutika formulation and alcoholic extract were subjected to quantitative estimations such as total polyphenol, total flavonoid content, saponin content, tannin content and alkaloidal content and it was found that marketed and in-house formulation showed higher amount of alkaloid, phenol, and carbohydrate while the tannin content was higher in extract.

With the help of TLC one can easily determine purity of sample, examination of reaction, identification of compounds in a mixture, separation of multicomponent in pharmaceutical formulation and in cosmetic industries for the separation and identification of colour, preservatives and sweetening agents (Kasture et al., 2004). In the present study, TLC was done for the determination of different components present in the formulations and extract. The TLC of formulation and extract confirm the presence of L-dopa, having the same Rf value 0.46 as that of standard L-dopa.

The vanari gutika formulations and extract were then subjected to HPTLC and HPLC quantitative analysis.

Aphrodisiac potential:-

The present study designed in two parts, first is short term effect (on 1st day) and second is long term effect (for seven days). The aphrodisiac potential can be predicted on the basis of mating behavior parameter like Mounting frequency (MF), Mounting latency (ML), Intromission frequency (IF), Intromission latency (IL) and Ejaculation frequency (EF).

The study exhibits a marked change in sexual behavior in male mice that were treated with standard drug or with 200 mg/kg dose of alcoholic extract of *M. pruriens* or with high/medium dose of vanari gutika formulation. All the parameter of mating behavior test was observed on 1st day and after 7 day.

In the short term effect i.e. on 1st day, the mounting frequency was observed after 30 min. of administration of test drug and standard. The MF was observed at least three time within 3 hr. (i.e. 15 min. observation period and 45 min. is resting period). The MF involved the determination of number of mounts without intromission from the time of introduction of female until ejaculation by male mice. The result revealed that standard drug at the dose of 5 mg/kg significantly increase mounting frequency as compare to control, also the high dose marketed vanari gutika formulation show the increasing in mounting frequency as compare to extract treated group. While the medium dose formulation group did not show any significant effect on 1st day.

In the long term effect, that includes the administration of test drug and standard to the male mice for 7 day at evening 3 pm. On the 8 day the mating behavior parameter i.e. MF, IF, ML, IL, and EF are observed at morning 9 am to 12 am. The mounting frequency was found significantly increased in all groups as compared to control group. Also the medium dose formulation group significantly increases MF as compare to extract and standard group. Further, the intromission frequency was significantly increased in extract and medium dose of formulation group.

Also the mounting and intromission latency was found significantly decreased in all groups as compared to the control group. Which suggest that extract, standard and formulation group have intensified the sexual activity. The medium dose formulation group was found to have promising sexual behavior improving effect than high dose formulation and extract group.

Further the ejaculation frequency is shows the positive effect in standard and medium dose formulation group as compared to control, extract and high dose group.

With regard to the mechanism of action of the test drug, it is very difficult to explain the exact mechanism responsible for improving sexual function. The drug

induced changes in neurotransmitter level or their action at cellular level could change the sexual behavior.

The epididymal sperm count was significantly increased in extract, standard and high dose formulation group as compared to control group. But the medium dose formulation group also showed the increase in sperm count as compared to control group. The increase in sperm count revealed that the formulation was also useful in oligospermia condition.

The biochemical estimation of FSH, LH and Testosterone was done. And the results showed significant increase in LH hormones level in all group as compared to control group but in case of testosterone it showed significant increase in high dose group. Also, the testosterone level was found increased in medium dose formulation and extract group as compared to control group.

The Histopathology of testis treated mice with high dose of formulation revealed the ruptured seminiferous tubules. While in control, extract, standard and medium dose group, it showed continue seminiferous tubules embedded in interstitial connective tissue and the leydig cells and sertoli cells have been observed.

The HPTLC quantification of L-dopa was done in n-butanol – water – glacial acetic acid (4:1:1) v/v/v as a solvent system with standard L-dopa having a concentration 100 µg/ml and it showed that marketed, in-house vanari gutika formulation and extract contained 4.11, 4.58 and 3.06 %w/w content of L-dopa respectively.

Also the HPLC quantification analysis of vanari gutika formulation and extract was done in water **:** Methanol: Acetonitrile (100:60:40) as a solvent system with standard L-dopa having a concentration 100 µg/ml and it showed that marketed, In-house vanari gutika formulation and extract contained 1.82, 2.15 and 5.04 %w/w content of L-dopa respectively.

The seeds of M. pruriens, because of the presence of L-3, 4-dihydroxyphenylalanine (L-DOPA), a neurotransmitter precursor, have been used as an effective drug for relief in aphrodisiac. It is reported to be prophylactic against oligospermia and is useful in increasing sperm count as well as ovulation in women

(Mohammad et. al., 2008). In view of the above considerations, the present study was undertaken to investigate the impact of vanari gutika on aphrodisiac potential.

CONCLUSION

- In the present study, attempts were made for the quality control profiling and preclinical evaluation of herbal formulation Vanari gutika for its aphrodisiac potential.
- The physicochemical and pharmaceutical parameters were evaluated and were found to be within the prescribed limits as per standard values mentioned in API.
- The study also includes the pharmacognostical and pharmacological activity of seed of *Mucuna pruriens* which is one of the components of Vanari gutika. The physicochemical parameter of *Mucuna pruriens* seeds were evaluated and were found to be in prescribed range as per standard values.
- Phytochemical study revealed the presence of mainly steroids, alkaloids, flavonoids, tannins, phenol and carbohydrates which were found to be in considerable amount in Gutika formulation where as in extract the flavonoids and phenol were found to be absent.
- The TLC analysis of standard L-dopa, extract, vanari gutika marketed and in-house formulation show the single spot at same Rf value 0.46
- The quantified presence of L-dopa in Vanari gutika marketed formulation, Vanari gutika In-house formulation and extract, by HPTLC method was found to be 4.11, 4.58 and 3.06 % w/w respectively, whereas from HPLC analysis was found to be 5.04, 1.82 and 2.15 %w/w respectively.
- In the pharmacological evaluation we have successfully evaluate the aphrodisiac property of vanari gutika and *M. Pruriens* extract using short term as well as long term animal models.
- From the overall observation it was found that the medium dose of marketed vanari gutika formulation shows maximum aphrodisiac potential as compare to other group. However the high dose formulation shows maximum number of sperm count as compare to other group.
- Thus, in the present study we have successfully justified the traditional claims of the plant *Mucuna pruriens* as a potent aphrodisiac agent. Further, we have also

scientifically justified the traditional use of vanari gutika in ayurvedic system of medicine for its use as potent aphrodisiac agent.

- Further, more studies on molecular level are still required to know the exact mode of action through which the vanari gutika in a medium dose level as found to be more effective than the other group. Anonymous, the Wealth of India, Publication and Information Directorate, CSIR, New Delhi, 947-949. 1996.

REFERENCES

- Anonymous. Indian Medicinal Plants (A Compendium of 500 species). Orient Long Man Publisher: 68-72. 2002.
- Anonymous. The Wealth of India Raw Materials, NISCAIR Publishing New Delhi, L-M, Vol VI: 439-444. 2005.
- Bell EA and Janzen DH. Medical and ecological considerations of L-Dopa and 5-HTP in seeds. Nature. 136-137, 1971.
- Bhaskar A, Vidhya VG, Ramya M "Hypoglycemic effect of *Mucunaprurienss*seed extract on normal and streptozotocin-diabetic rats" Fitoterapia 79(7-8):539-543, 2008.
- Bhumika G. Rathod and Natvarlal M. Patel, *Development of validated RP-HPLC method for the estimation of L-Dopa from Mucunapruriens, its extracts and in Aphrodisiac formulation*, International Journal of Pharma Sciences and Research (IJPSR), 508-513, 2014.
- Brook G, Laumann E, Glasser DB, Nicolosi A, Gingell C, King R. Prevalence of sexual dysfunction among mature men and women in USA, Canada, Australia and New Zealand, Program and abstract from the American Urological Association 9^{8th} Annual Meeting; Chicago, Illinois, Abstract 1365, April 26 – may 1, 2003.
- **C**hang C.C.; Yang M.H.; Wen H.M. and Chern J.C.; Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis; 178-182, 2002.
- Citing: Dorland's Medical Dictionary for Health Consumers, 2007 by Saunders; The American Heritage Medical Dictionary 2007, 2004 by Houghton Mifflin Company; Mosby's Medical Dictionary, 8th edition 2009; McGraw-Hill Concise Dictionary of Modern Medicine, 2002 by The McGraw-Hill Companies

- *Cooper TG, Noonan E, von Eckardstein S, et al. "World Health Organization reference values for human semen characteristics". Hum. Reprod. Update. 231–45, (2010).*
- David A. Edwards "Induction of Estrous in Female Mice: Estrogen-Progesterone interaction" Department of psychology, Emory University, Atlanta, Georgia 30322, Hormones and behavior 1, 299-304, 1970.
- **D**esai J.V., Thomas R., Kamat S. D. and Kamat D. V.; Microwave Assisted Extraction of Saponins from *Centella asiatica*& It's In Vitro Anti-Inflammatory Study; Asian Journal of Biochemical and Pharmaceutical Research Issue 4; 331, 2011.
- Dhamodharan M and Ramasamy R. Isolation of 1-3, 4 dihydroxy phenylalanine from the seeds of *Mucunapruriens.* Biochem J.; 2149-52, 1937.
- Donati D, Lampariello LR, Pagani R, Guerranti R, Cinci G, Marinello E "Antidiabeticoligocyclitols in seeds of *Mucuna pruriens"*, Phytother. Res., 1057-1560, 2005.
- Dr. Duke Phytochemical database 1992, Available at, http://www raintree.com/db/Mucunapruriens- phytochem.html.
- Feldman H.A., Goldstein I, Hatzichristou DG, Krane RJ &Mckinlay JB "Impotence and its medicinal and psychological correlated result of the Massachusetts male aging study", J Urol 151:pp 54-61, 1994.
- G. Wagner, Erection: physiology and endocrinology. In: Wagner, G. and Green, R. (eds) Impotence: Physiological, Psychological, Surgical Diagnosis and Treatment. Plenum Press, NY, pp 25-36, 1981.
- Govier F. E., Asase D., Hefty T.R., McClure R.D.,Pritchett T.R. and Weissman R. M., "timing of penile color flow duplex ultrasonography using a triple drug mixture". J. Urol., 153: pp. 1472-1475, 1995.
- Grimes DA & Lopez LM "*Fertility and Sterility"* 88(6) 1491-94, 2007.
- **H**agerman, A.E., Riedl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeld, P.W., High molecular weight plant polyphenolics (tannins) as biological antioxidants.Journal of Agricultural and Food Chemistry 46, 1887–1892, 1998.
- Impotency Information from the NHI National Kidney and Urologic Diseases Clearinghouse, Part of the US National Institute of Health.

- Indian pharmacopoeia, volume Ⅱ, Government of India, Ministry of Health and Family Welfare, Published by the Indian Pharmacopoeia Commission Gaziyabad, 2007.
- Joseph T. D., Talbert R. L., Yee G. C., Matzke G. R., Barbara B. G. and Possey L. M., "Pharmacotheraphy- A Pathophysiologic Approch", 5th Edition, McGraw Hill Publication, New York, pp 1511-1530, 2002.
- Karan malik, Gurpreetarora, Inderbirsingh, "Locust bean Gum as Superdisintigrant-formulation and evaluation of nimesulideorodispersible tablet" Polimery W Medicynie 2011.
- **K**asture, A. V., Wadodkar, S. G., Mahadik, K. R., More, H. N., Pharmaceutical Analysis. Volume 2. Instrumental methods. 26 -27, 2004.
- Katzenschlager R, Evans A, Manson A "*Mucunaprurien*sin Parkinson's disease: a double blind clinical and pharmacological study" J. Neurol. Neurosurg. Psychiatry 75:1672-1677, 2004.
- Kavitha C. &Thangamani C.,HC & RI,TNAU ,Review on Amazing bean *(Mucunapruriens) A comprehensive review* ,J.medicinal plant research vol8(2)pp 138-143,10 jan 2014.
- KetanPravinbhai Modi, NatvarlalManilal Patel and Ramesh Kishorilal Goyal, "Estimation of L-Dopa from Mucunapruriens linn and formulation containing M. pruriens by HPTLC Method" Chem. Pharm. Bull. 56(3) 357-359 (2008).
- **K**handelwal, K.R., Practical Pharmacognosy Techniques and Experiments,149-146, 2007.
- Khory RN and Katrat NN. MateriaMedica of India and their Therapeutics. KomalPrakashan, Delhi: 218-219. 1999.
- **K**okate C.K, Purohit A.P and Gokhale S.B; Pharmacognosy, NiraliPrakashan, 35th edition; (2006) 258-259;262;351-352;395.
- Kokate CK, Purohit AP, Gokhale SB. "Textbook of pharmacognosy" Niraliprakashan; (2); pg. no. 3.79-3.81 (2010).
- Kolodny R. C., Masters W. H. and Johnson V. E., "Sexual Anatomy and Physiology" Textbook of Sexual Medicine. Little, Brown and Co., Boston MA. (1979) pp 1-28.
- **L**achman L, Lieberman H. A, Kanig J. L, "The Theory and Practice of Industrial Pharmacy", 3rd Edition,Varghese Publishing House, (1987) 296-311.

- Meenatchisundaram S, Michael A (2010). Antitoxin activity *of Mucunapruriens*aqueous extracts against Cobra and Krait venom by *in vivo* and *in vitro* methods. Int. J. Pharm. Technol Res. 2(1):870-874.
- Merck H. Beers, MD, and Robert Berkow, MD "Erectile Dysfunction" Section 17, Chapter 220 in *The Merck Manual of Diagnosis and Therapy*, Whitehouse Station, NJ: Merck Research Laboratories, 1999.
- Milind pande and Anupampathak "Aphrodisiac activity of root of *Mimosa pudika* linn. Ethanolic extract in mice" international journal of Pharmaceutical sciences and nanotechnology, volume two, issue 1, April-June 2009.
- Miller, Lucinda G., and Wallace J, Murray, eds*Herbal Medicinal:* A Cliniclan's Guide. Binghamton, N.Y.: Haworth press, 1999.
- Misra L and Wagner H. "Alkaloidal constituents of *Mucuna pruriens* seeds" Phytochemistry; 2565-7, 2004.
- Misra L and Wagner H. Lipid derivatives from *Mucunapruriens*seeds. Indian Journal of Chemistry. 45(3); 801-804, 2006.
- Mohammad Kaleem Ahmad, Abbas Ali Mahdi, Kamla Kant Shukla, Najmul Islam, et. al., "Effect of Mucuna pruriens on semen profile and biochemical parameters in seminal plasma of infertile men" American Society for Reproductive Medicine, Published by Elsevier Inc., 627-635, 2008.
- Natarajan K 1*, Narayanan N 2 and Ravichandran N 1,*review on "mucuna" - the wonder plant,*. Int. J. Pharm. Sci. Rev. Res., 86-93, 2012.
- Ogah G., Impotence, Nig. J. Gen. Pract., pp 36-41, 1999.
- Ovallath S, Deepa P "The history of parkinsonism: descriptions in ancient Indian medical literature" MovDisord. 28(5):566-568, 2013.
- Padubidri Daftary "*Shaw's Textbook of Gynaecology"*, p. 204, 2011.
- Pathak A.V., Kawtikwar P.S., Sakarkar D.M., "pharmacognostical and phytochemical standardization of TriphalaGugguluVati: An official Ayurvedic Formulation" www.jipbs.com 2015
- Patient Fact Sheet, Sexual Dysunction and Infertility American Society for Reproductive Medicine. www.asrm.org
- Paul R. Brezina, Fahd N. Yunus, Yulian Zhao, "Effects of Pharmaceutical Medication on Male Fertility" journal of reprod infertile, 3-11, 2012.

- Quality control method for medicinal plant materials "World Health Organization Geneva", 1998.
- Quinlan DM, Epstein JI, Carter BS & Walsh PC "Sexual function following radical prostatectomy: influence of preservation of neurovascular bondles". J Urol 145:pp 998-1002, 1991.
- **R**ichard F. S., Sexual Health of Men, The Complete Guide, Electronic Journal of Human Sexuality, Vol.3, August 25, 2000. www.ejhs.org/vol.3/book8,htm
- Robert C. Dean, Tom F. Lue, Physiology of Penile Erection and Pathophysiology of Erectile Dysfunction. *UrolClin North Am*. 2005 November, 32(4): pp-379.
- Rosen R, OLeary M, Munchen JA, et al. Ejaculatory disorder are frequent and bothersome in ageing males with LUTS: a worldwide survey (MSAM-7), Program and abstract from the American Urological Association 98th Annual Meeting; April 26 – May 1, 2003; Chicago, Illinois, Abstract 1365.
- **R**uch, R. J., Cheng, S. J. Klaunig, J. E., Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechin isolated from Chinese green tea. Carcinogenesis(1999) 10, 1003-1008.
- **S**adashivam S., Manickam A., "Biochemical method", New Age International (P) Limited, Publisher(1996) 2,195.
- Satheesh KD, Kottai MA, Anton SA, Manavalan R (2010) *In vitro* antioxidant activity of various extract of whole plant of *M. pruriens.Linn.*
- Sekarsuresh, ElumalaiPrithiviraj, Seppan Prakash, "Dose and time dependent effect of ethanolic extract of mucunapruriens linn. Seed on sexual behavior of normal male rats", Journal or Ethnopharmacology 122 (2009) 497-501.
- **S**hanbhag, D. A., Jayaraman, S., Research Article Application of HPTLC in standardization of Homoeopathic Mother Tincture,PHCOG MAG (2008).
- Shukla KK, Mahdi AA (2010). *Mucunapruriens*reduces stress and improves the quality of semen in infertile men. Advance Access Publication 7(1):137-144.
- Stahl E. "Thin Layer Chromatography, A Laboratory Handbook", 2nd Ed., Academic Press, New York, 52-105, 1969.
- Taberner P.V., Aphrodisiac – The Science and the Myth. University of Pennsylvania Press, NY (1985).
- Taymour mostafa, "Cigarette smoking and male infertility" Cairo university, Journal of Advanced Research, 179-186, 2010.

- The Kashi Sanskrit Series "YOGRATNAKAR" by vadyalakshmipatisastri edited by BhisagratnaBrahmasankarSastri, chaukhambha Sanskrit sansthan publisher and distributer of oriental cultural literature post box no 1139, K. 37/116, gopalmandir lane (Golghar Near Maidagin) Varanasi – 221001 (INDIA), Pg no. 482, 2002.
- The Merck Manuals- Online Medical Library, the Merck Manuals of Medical information, Home Edition, "Men's Health Issues" Published by Merck Research Laboratory.
- Toone B. K., Wheeler M., Nanjee M., Fenwick P. and Grant R., "Sex hormones, sexual activity and plasma anticonvulsant levels in male epileptics". J. Neurol. Neurosurg. Psychiatry, 46: (1983) pp 824-826.
- **W**agner H and Bladt S, Plant Drug Analysis, 2Nd edition, Springer, Berlin, (1996)230-231.
- Walton A.H., Aphrodisiac and Love Stimulant, New York: Lyle Stuart, Inc. (1966).
- **W**anasundara, U. N., Shahidi, F., 1996. Antioxidant and pro-oxidant activity of green tea extracts in marine oils. Food Chemistry, 63, 335-342.
- WHO Laboratory Manual for the "Examination of human semen & sperm-cervical mucus interaction" 4th edition published on behalf of the WHO by CAMBRIDGE university press. Pg no. 14-15, 4th edition published by Cambridge University press 1999 reprinted 2000.
- Yakubu, M. T., Akanji, M. A. and Oladiji A. T., "Male Sexual Dysfunction and Method used in Assessing Medicinal Plants with Aphrodisiac Potential" Phcog Rev.: Review Article, Vol. 1, Jan-May, 2007, pp.44-56