INTRODUCTION-

An aphrodisiac is defined as any food or drug that arouses the sexual instinct, induces venereal desire and further increases pleasure, performance. This word is derived from 'Aphrodiate' the Greek goddess of love and these substances are derived from plants, animals or minerals and since time immemorial they have been the passion of man. There are two main types of aphrodisiacs, psychophysiological stimuli (visual, tactile, olfactory and aural preparations) and internal preparations (food, alcoholic drinks and love portion). Erectile dysfunction (ED) or (male) impotence is a sexual dysfunction characterized by the inability to develop or maintain an erection of the penis. Various causes such as; cardiovascular leakage and diabetes many of which are treated. Erectile dysfunction may be caused by physiological or psychological dependence. Folk remedies have long been advocated, with some being advertised widely since the 1930s. The introduction of the first pharmacologically approved remedy for impotence, sildenafil (trade name Viagra), in the 1990s caused a wave of public attention, propelled in part by heavy advertising. (Rawat et al., 2012)

The Food and Drug Administration defines an aphrodisiac drug product as "any product that bears labeling claims that it will arouse or increase sexual desire, or that it will improve sexual performance." Presently, there are no approved medications for the treatment of lowered desire for women, and many opt for "natural" products. (West et al., 2015)

M. pruriens is a widespread fodder plant in the tropics. The whole plant is fed to animals as silage, dried hay or dried seeds. *M. pruriens* silage contains 11-23% crude protein, 35-40% crude fiber, and the dried beans 20-35% crude protein. It also has use in the countries of Benin and Vietnam as a biological control for problematic *Imperata cylindrica* grass. It was found that Male reproductive capacity to be deficient in nearly 50% of infertile couples according to a study carried out by the World Health Organization in 1987. Although further figures for this decade are still awaited, it is certain that stressful life style has enhanced the number of subject's suffering from one form of sexual dysfunction or the other. Main factors that decrease the probability of conception in the female partner are frequently congenital, immunological, iatrogenic, or endocrine cause. Oligozoospermia, sexual, and ejaculatory dysfunction are further responsible for inability to conceive in numerous cases. Although many synthetic drugs are available and/or used to treat these problems, some of the drawbacks for these drugs include them being expensive and also their ability to provoke serious adverse effects, effective natural treatments are therefore still in demand. Even if many of the plants or natural products claim to prove their effectiveness without scientific evidence, a number of them are active and possess biological activity, proven by scientific data. Moreover, there is a dearth of systematic review of scientific literature on experimental

evidence generated for medicinal plants useful in treating erectile dysfunction and there is a need for in depth pharmacological evaluation. (Chauhan et al., 2014)

ED occurs from multifaceted, complex mechanisms that can involve disruptions in neural, vascular, and hormonal signaling. Research on central neural regulation of penile erection is progressing fastly with the identification of key neurotransmitters and the association of neural structures with both spinal and supraspinal pathways that carry out sexual function. In association with parallel to advances in cardiovascular physiology, the most extensive efforts in the physiology of penile erection have focused on elucidating mechanisms that carry out the functions of the endothelium and vascular smooth muscle of the corpus cavernosum. Major health related problems such as atherosclerosis, hyperlipidemia, hypertension, diabetes, and metabolic syndrome become well integrated into the investigation of Erectile Dysfunction. (Dean et al., 2006)

Physiology of Erectile Function:

The penile corpora cavernosa are specialized with spongy vascular structures encapsulated by the envelope of the tunica albuginea. Penile erection needs sufficient relaxation of cavernous smooth muscles and dilation of penile arterioles allowing inflow, and subsequent trapping of blood, within the erectile tissue (Dean and Lue, 2005). This process is totally depend on the parasympathetic nervous system, which induces smooth muscle relaxation allowing arterial pressure blood into the corpus cavernosum via the actions of nitric oxide (NO) (Rajfer et al., 1992). NO is generated by three nitric oxide synthase (NOS) enzyme isoforms: neuronal, endothelial and inducible. The neuronal isoform observes to be the primary mediator of physiologic erection (Burnett, 1995). Neuronal NO is used to induce erections while shear stress also propagates the erectile response via endothelial NO. Regardless of source, NO modulates smooth muscle cyclic GMP to induce relaxation in a paracrine fashion. Vascular relaxation in turn allows arterial blood to fill the corpora which, by distention, creates a venous seal to maintain erection. (Kovac et al., 2014)

Anatomy of an Erection: (Evan et al., 2000)

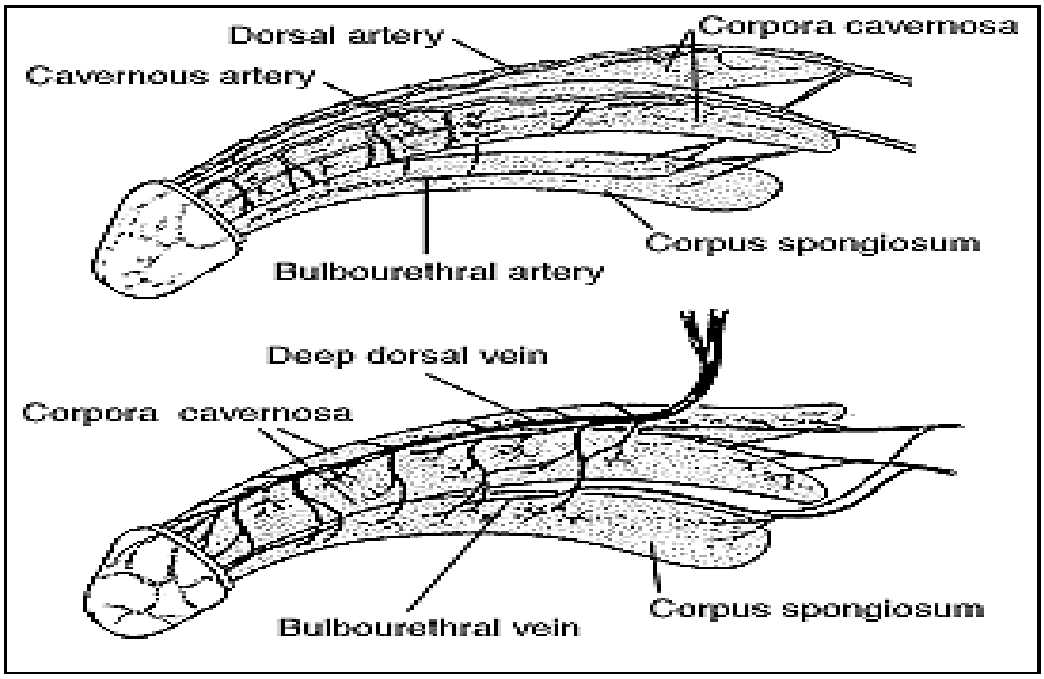


Figure 1: Anatomy of Erection

Phytochemical Review:

Mucuna pruriens Linn:

Krishnaveni and Hariharan (2017) reported the knowledge on the chemicals define the value of folk medicine. So, *Mucuna pruriens* and *Hyoscyamus niger* seeds were analyzed for its phytochemicals quantitatively, qualitatively, for its nutrient content, secondary metabolites, antioxidant, antimicrobial activities. The observed FTIR result shows *Mucuna pruriens* tannin and saponin was found to be higher, while in Hyoscyamus niger seeds, the tannin and phenol was higher. Among the nutrients studied, protein was rich in both the seeds studied. Likewise, the flavonoid content was rich in both the seeds thereby, inducing reducing power and total antioxidant activity in both the seeds. Antibacterial activity was higher in Salmonella typhi with *Mucuna pruriens* while the other sample showed no positive result with bacteria. So, it is concluded, that both the seeds are therapeutically more important emphasizing further scientific research to validate its potency.

Steroids, alkaloids, tannins, carbohydrates, amino acid and resins were present in methanolic extract of seeds of *Mucuna pruriens*. The medicinal values of the seeds may be related to their constituent phytochemicals. According to Varadarajan et al., (2008) the secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value. For example, saponins are glycosides of both triterpene and steroids having hypotensive and cardiodepressant properties, while anthraquinones posses' astringent, purgative, anti-inflammatory, moderate antitumor, and bactericidal effects (Olaleye, 2007 and Muzychkina, 1998). *Mucuna pruriens* indicates presence of both carbohydrates and starch this indicate they are polysaccharides. Presence of proteins in *Mucuna pruriens* has been described by Fathima et al. (2010) *Mucuna pruriens* contain higher crude protein when compared with commonly consumed pulse crops such as black gram, green gram, pigeon pea, chick pea and cow pea. **(Nagmain et al., 2012)**

Bell and Janzen, (1971) followed by **Dhamodharan and Ramasamy, (1937)** reported four alkaloids in *Mucuna pruriens* seeds. 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.

Pharmacological Review:

Kasture et al, (2013) stated *Mucuna pruriens* is extensively used in Ayurveda to treat kampavat (Parkinson's disease in modern medicine), a disease characterized by excess of Vata. Clinical and preclinical studies have substantiated claims on its efficacy and safety in PD and there are indications that it is more effective than the levodopa in reducing dyskinesias.

Yadav et al, (2017) stated The plant *Mucuna pruriens* (Fabaceae) is an established herbal drug, widely known as "velvet bean," 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. It has been shown that its seeds are potentially of substantial medicinal importance. The ancient Indian medical system, Ayurveda, traditionally used *M. pruriens*, even to treat such things as Parkinson's disease. *M. pruriens* has been shown to have antiparkinson and neuroprotective effects, which may be related to its antioxidant activity and used for the management of male infertility, nervous disorders,
and also as an aphrodisiac.

Saha et al, (2013) reported that All parts of *M. Pruriens* are generally used to treat impotence, diabetes mellitus and cancer whereas the seeds have muti-diversefied functions like several free radical mediated diseases management, rheumatoid arthritis, diabetes, atherosclerosis, nervous disorders, analgesic, antipyretic activity and in the management of Parkinsonism.

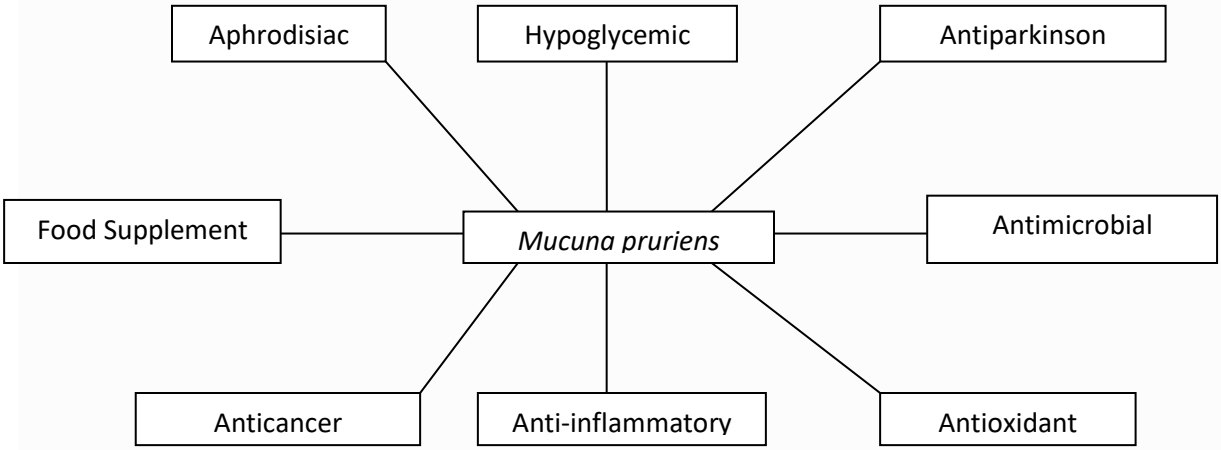


Figure 2: *Mucuna pruriens* activity **(Saha et al., 2013)**

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.

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.

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.

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

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

Kavita and Thangami, (2014) *M. pruriens* seed is a natural source of the amino acid L-3,4-dihydroxy phenyl alanine (L-DOPA), the direct precursor to the neuro transmitter dopamine which is used widely in

the treatment of Parkinson's disease (PD). Serotonin, oxitriptan, nicotine, N,N-DMT, and bufotenine are the other chemicals found in *M. pruriens* in addition to L-DOPA.

Gitanjali Deokar, Pratiksha Anil Deore, Sanjay Kshirsagar, (2016) stated *Mucuna pruriens* (L.) DC belongs to the family Fabaceae is commonly known as Velvet bean, Cowitch, Cowhage in English and Kawaanchin Hindi. *M. pruriens* has been of keen interest in phytochemical and Ayurvedic research due to its excellent medicinal values. *M. pruriens* had been evaluated and concluded as a potential medicinal herb in terms of anti cholestrolemic, antiparkinson, antidiabetic, aphrodisiac, anti-inflammatory and antimicrobial. The plant exhibits wide array of phytoconstituents like alkaloids, flavonoids, tannins and phenolic compounds which are responsible for varied potent physiological and pharmacological activities.

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)

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.

B. Ankita et al, (2017) Many plants are essential in human health care, both in self-medication and in national services. *Mucuna pruriens* (L.) DC belongs to the family Fabaceae is commonly known as Velvet bean, Cowitch, Cowhage in English and Kawaanchin Hindi. *M. pruriens* has been of keen interest in phytochemical and Ayurvedic research due to its excellent medicinal values. *M. pruriens* had been evaluated and concluded as a potential medicinal herb in terms of antiparkinson, antidiabetic, anti-inflammatory and antimicrobial. The plant exhibits wide array of phytoconstituents like alkaloids, flavonoids, tannins and phenolic compounds which are responsible for varied potent physiological and pharmacological activities.

Yog Ratnakar single herbal formulation "Vanari gutika"

Contain:- *Mucuna pruriens* seed, Milk, Ghrita, Sugar, Honey

Milk:-

- *M. pruriens* seed powder give synergistic effect with milk.(Medicinal plant of the world by Ivan A. Ross Vol. 1)
- Increase in sperm count reported by Panchgavya ayurvedic chikitsa by Go Vidhnyan Anusandhan Kendra, Devlapar- Nagpur.

LITERATURE REVIEW ON DISEASE:-

Partners' perspective of erectile dysfunction

It is a problem which men may or may not share with their partners for a variety of reasons. This literature review of 26 articles revealed the partners' perspective of men with erectile dysfunction. Partners fell into four groups: supportive partner/acceptable to patient (the optimum category); supportive partner/unacceptable to patient; non-supportive partner/acceptable to patient; and non-supportive partner/unacceptable to patient. With comprehensive education and counseling by urology nurses, the couples could move into the optimal category. There is increasing recognition that the partner should be involved in the assessment, diagnosis, patient education, counseling and choice of treatment for long-term treatment to be successful, unless the informed patient is unwilling. (Dorey G., 2001)

Erectile dysfunction is common, and its prevalence increases with age owing to age-related diseases of vascular, hormonal, neurogenic and psychogenic factors. A literature review was undertaken to explore the impact of smoking on erectile dysfunction. The literature review of 18 studies revealed the detrimental effect of smoking on erectile function. Smokers were 1.5 times more likely to suffer erectile dysfunction than non-smokers. Men may be unaware of the effect of tobacco on erectile function. This literature review presents strong reasons for stopping smoking, and highlights the need for education within a comprehensive smoking cessation programme. All men should be offered smoking cessation treatment which includes nicotine replacement therapy and continued support. Randomized controlled trials are needed to explore the effect of smoking and smoking cessation on erectile dysfunction. (Dorey G., 2001)

Sexual stimulation triggers release of neurotransmitters from the cavernous nerve terminals. This results in relaxation of these smooth muscles and the following events:

1. Dilatation of the arterioles and arteries by improved blood flow in both the diastolic and the systolic phases
2. Traps incoming blood by the expanding sinusoids

3. Compression of the subtunical venular plexuses between the tunica albuginea and the peripheral sinusoids, reducing the venous outflow
4. Stretching of the tunica to its capacity, which occludes the emissary veins between the inner circular and the outer longitudinal layers and further lowers the venous outflow to a minimum
5. An increase in PO2 (to about 90 mmHg) and intracavernous pressure (around 100 mm Hg), which raises the penis from the dependent position to the erect state (the full-erection phase)
6. A further pressure increase (to several hundred millimeters of mercury) with contraction of the ischiocavernosus muscles (rigid-erection phase)

The angle of the erect penis is determined by its size and its attachment to the puboischial rami (the crura) and the anterior surface of the pubic bone (the suspensory and funiform ligaments). In men with a long heavy penis or a loose suspensory ligament, the angle usually will not more than 90 degrees, even with full rigidity.

Three phases of detumescence have been reported in preclinical study. The first entails a transient intracorporeal pressure increase, indicates the beginning of smooth muscle contraction against a closed venous system. The second phase shows a slow pressure decrease, suggesting a slow reopening of the venous channels with resumption of the basal level of arterial flow. The third phase shows a fast pressure lowers with fully restored venous outflow capacity.

Smooth muscle contraction and relaxation is regulated by cytosolic (sarcoplasmic) free Ca^{2+}. Norepinephrine from nerve endings and endothelins and prostaglandin F2α from endothelium activate receptors on smooth muscle cells to increase inositol triphosphate and diacylglycerol resulting in release of calcium from intracellular stores such as sarcoplasmic reticulum and/or opening of calcium channels on the smooth muscle cell membrane leading to an influx of calcium from extracellular space. This triggers a transient increase in cytosolic free Ca2+ from a resting level of 120 to 270 to 500 to 700 nM. At the elevated level, Ca2+ binds to calmodulin and changes the latter's conformation to expose sites of interaction with myosin light-chain kinase. The resultant activation catalyzes phosphorylation of myosin light chains and triggers cycling of myosin crossbridges (heads) along actin filaments and the development of force. In addition, phosphorylation of the light chain also activates myosin ATPase, which hydrolyzes ATP to provide energy for muscle contraction. (Dean et al., 2005)

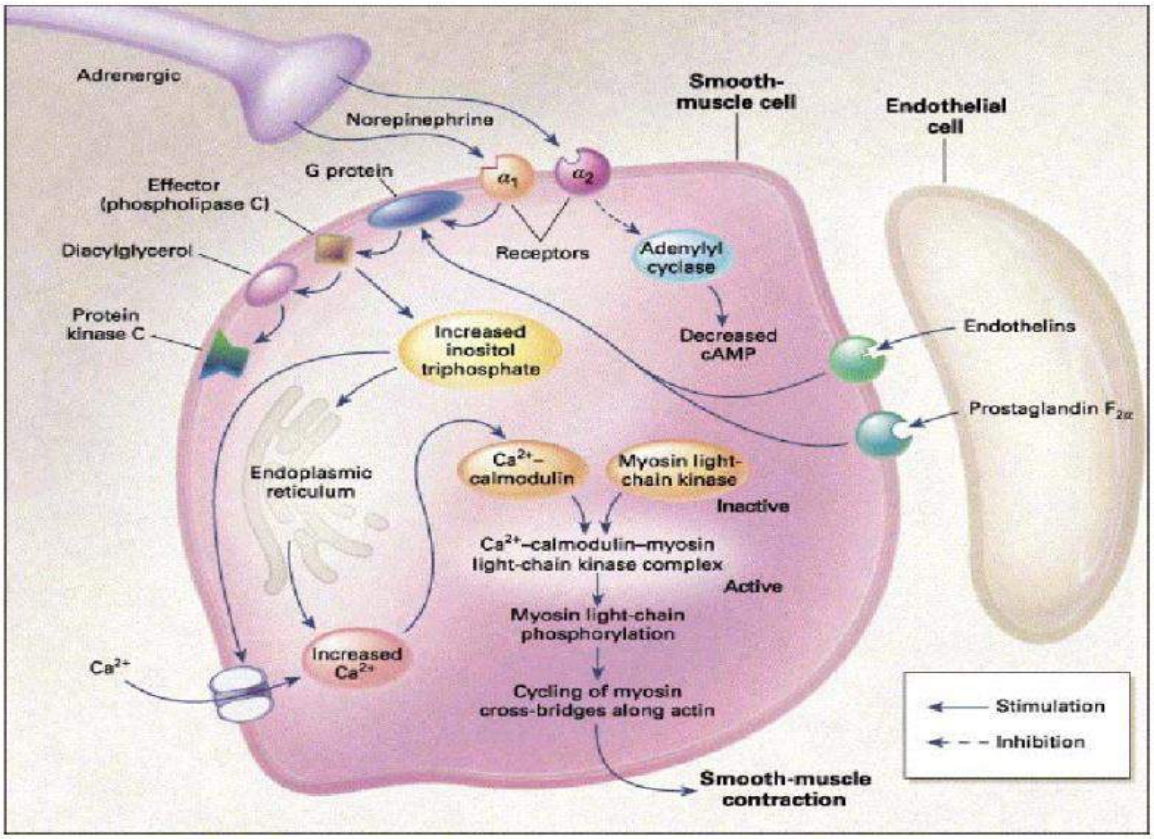


Figure 3: molecular mechanism of penile smooth muscle contraction. (Dean et al., 2005)

Once the cytosolic Ca^{2+} returns the basal levels, the calcium- sensitizing pathways take over. One such mechanism is via activation of excitatory receptors coupled to G-proteins which can also cause contraction by increasing calcium sensitivity without any change in cytosolic Ca^{2+}. This pathway involves RhoA, a small, monomeric G protein that activates Rho-kinase. Activated Rho-kinase phosphorylates and thereby inhibits the regulatory subunit of smooth muscle myosin phosphatase preventing dephosphorylation of myofilaments thus maintain contractile tone.

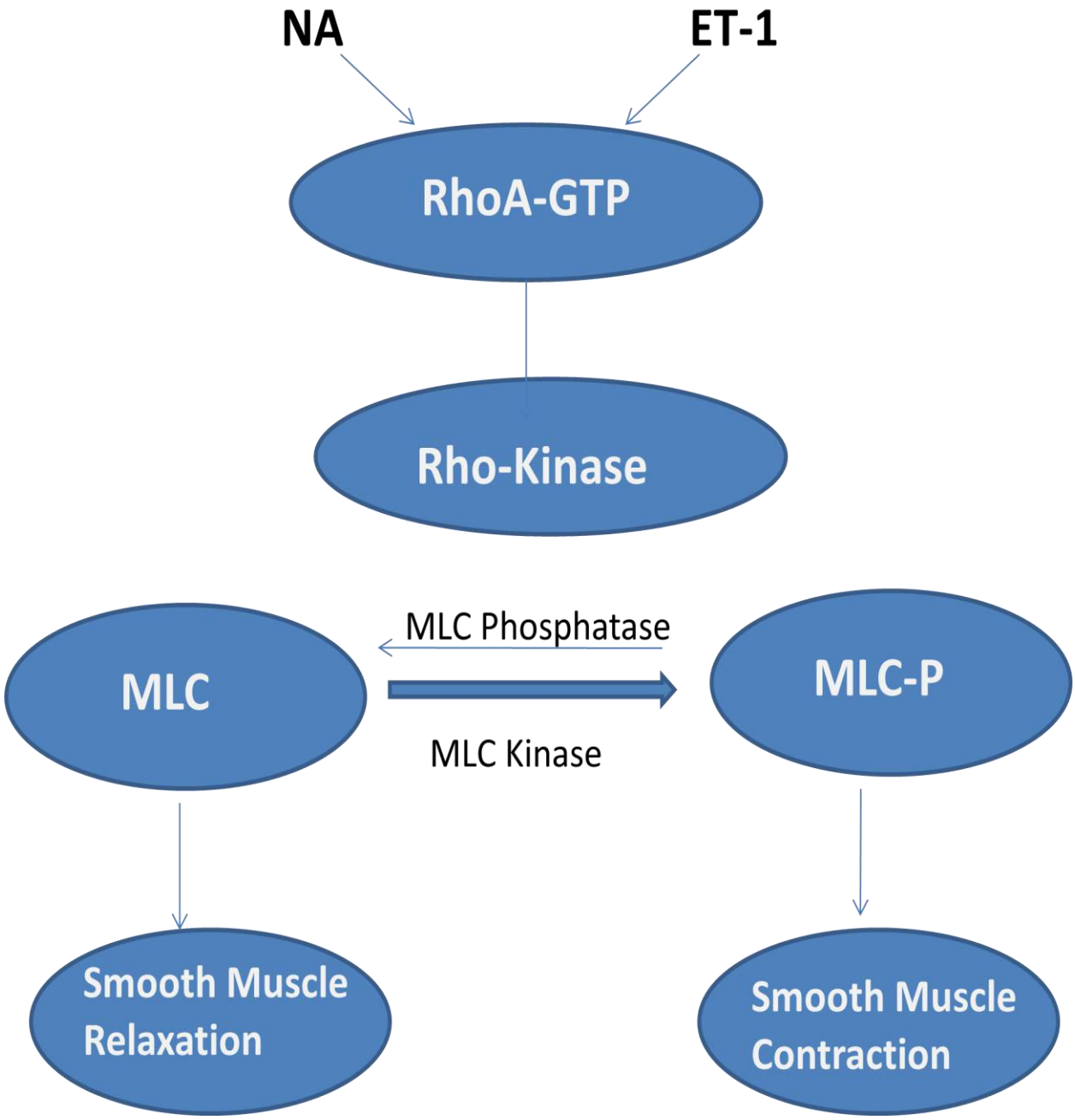


Figure 4: RhoA/Rho kinase pathway: the calcium sensitization pathway. (Dean et al., 2005)

Hormonal system: (Joseph et al, 2002)

Testosterone provides stimulus to libido and sexual drive in male. In that case 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 not enough, a patient may not develop erection, but the relationship with testosterone level is a complicated one. Approximately 1/3rd 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.

Classification (Robert and Tom, 2005)

There are 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

Classification of Male Erectile Dysfunction: Organic:

1. **Vasculogenic**
   - A. Arteriogenic
   - B. Cavernosal
   - C. Mixed
2. Neurogenic
3. Anatomic
4. Endrocrinologic

2. **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)

Oligospermia:- (Padubidri Daftary 2011)

Terms **oligospermia** and **oligozoospermia** refer to semen with a low sperm concentration and 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 referred as 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 concentration shows fluctuation and further 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

OBJECTIVE:-

The main objective of our study is to investigate the Aphrodisiac potential of Vanari gutika herbal formulation prepared using different Milk and Ghrita obtained from indigenous cow (*Bos indicus*), Buffalo and Jersey cow (*Bos Taurus*).

PLAN OF WORK:-

- Procurement of plant material and its authentication.
- Preparation of Vanari gutika formulation using different ghee and milk obtained from Jersey cow (*Bos taurus)*, indigenous cow (*Bos indicus*), buffalo.
- Standardization of Vanari gutika.
- Physiochemical evaluation.
- Qualitative phytochemical study.
- Quantitative phytochemical study.
- Chromatographic evaluations using suitable marker.
- Preclinical evaluation of different formulation of Vanari gutika by using suitable animal model for its aphrodisiac activity.
- Hormonal analysis.
- Histological activity.
- Sperm analysis.

PLANT PROFILE:-

Geographical Distributions **(Kumar et al., 2013)**

Mucuna pruriens is probably a native of tropical South or Southeast Asia, and has been widely distributed throughout the tropics. It was introduced into Florida in 1876, from where its range was extended into temperate and subtropical areas by breeding. In the south-eastern United States, it used to be the most important cover crop grown in combination with maize in an area of about 1 000 000 ha around 1920. Later, soya bean and commercial fertilizers rapidly replaced it and it disappeared from agricultural statistics in 1965. As a cover crop, it is now most important in Australia, Hawaii, the Fiji Islands, Indonesia, Malaysia and the Philippines.

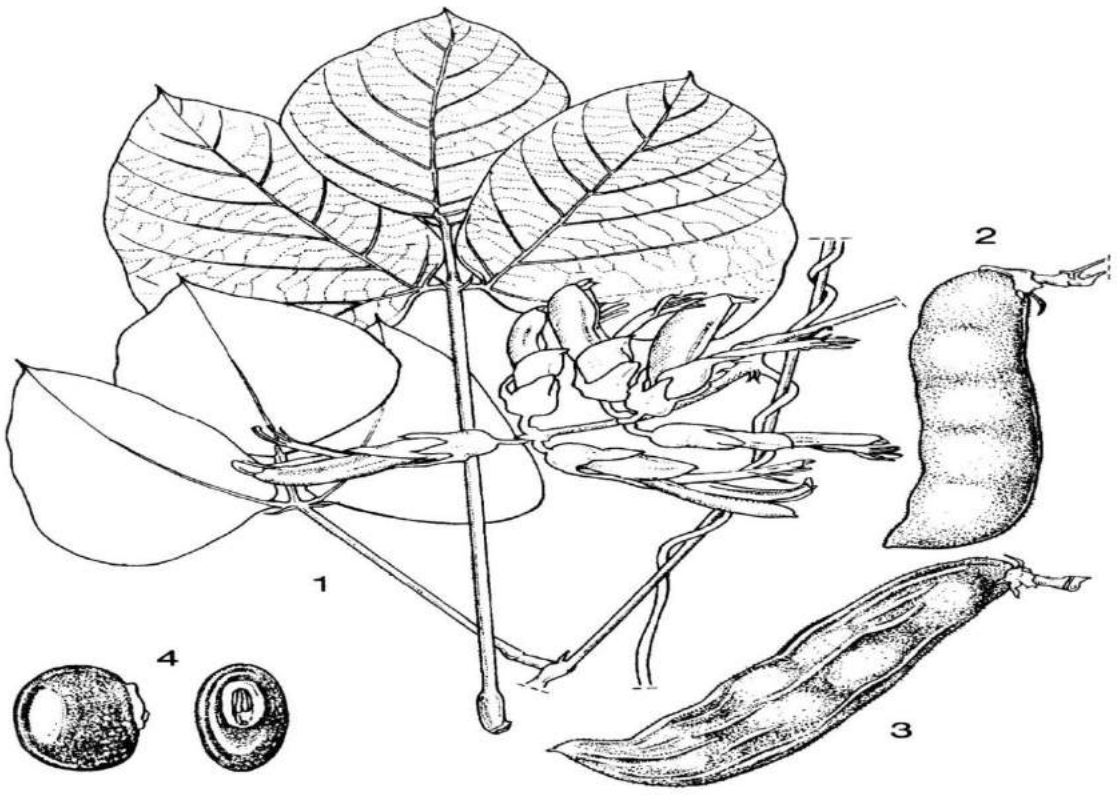
(A) (B) (C) (D)

Figure 5: Morphology of *Mucuna prurience* plant (A) whole plant (B) white seed (C) black seed (D) legumes

Description

Mucuna pruriens is a vigorous, climbing, pubescent annual herb which can grow up to 2-18 m long. The roots are numerous, 7-10 m long and with many lateral taproots. The stem is slender, cylindrical, slightly pubescent with white, straight, short and long hairs and nearly hairless.The 3-foliolate leaves are arranged alternate. The stipules are 0.5 cm long, caducous, subulate, white-hairy outside and hairless inside. The petiole is (3-)4-9(-13.5) cm long, slightly grooved above and generally slightly pubescent. The rachis is (0.5-)1-2 cm long, grooved above and slightly pubescent. The stipels are slender. The lateral leaflets are conspicuously asymmetrical, obovate, rhombic, ovate or elliptical, measuring (5-)7-15(-19) cm x (3-)5-

12(-17) cm and with symmetrical terminal leaflets. The apex is acute to acuminate-mucronate, rounded at base, and covered with appressed, grey or silvery hairs that turn black when dry.



Mucuna pruriens (L.) DC. cv. group Utilis – 1, climbing branches with inflorescence and leaves; 2, young pod; 3, mature pod; 4, seeds.

Figure 6: *Mucuna pruriens* climbing branches, leaves, young pod, mature pod, seeds.

The inflorescence is an axillary raceme, up to 32 cm long, 1-many-flowered and silvery pubescent. The tubercled rachis is without lateral branchlets. The bracts are 5-10 mm long, early caducous and narrowly triangular-elliptical. The pedicel is 1.5-10 mm long, and with two bracteoles measuring 10 mm x 2 mm near the base of the sepal. The sepal is bell-shaped, with tube 4-7 mm long, 5-lobed, appressed silvery pubescent outside and hairless inside. The upper pair of lobes is connate while the other 3 lobes are subequal, triangular, measure 3-9 mm long and acute. The petal is blackish-purple, pale lilac or white. It

is clawed and auricled. The upper part of it is hood-shaped, much shorter than other petals, measuring 17-22 mm x 11-15 mm, fleshy especially towards the base and rounded at the top. The wings are narrowly obovate, measuring 32-35 mm x 8-10 mm, fleshy especially towards the base, rounded at the top and finely and patently pubescent at the base. The keel measures about 35 mm x 5 mm, narrow in the middle, entirely split dorsally, ciliolate at the edges, nearly hairless towards the top, ventrally split near the base and apex, with apical part hard and ending in a horny tip. The 10 stamens are in two bundles.The fruit is an oblong, (1-)3(-7)seeded pod with oblique top, somewhat compressed laterally, slightly bulging over the seeds, measuring 4-13 cm x 1-2 cm and finely pubescent with white to light brown hairs. The valves are thick and leathery, with prominent, complete rib and with 2-3 partial and less prominent ribs.The seed is oblong-ellipsoid, somewhat laterally compressed, measuring about 15 mm x 10 mm x 5 mm and with variable colour. It is light or pinkish-brown with dark brown mosaic, mottled with grey, purple or black background, almost entirely black, grey, greyish-black or white. The hilum is oblong, lateral, eccentric, measures about 4 mm long, surrounded by a prominent, cream-coloured aril and with scale-like extension at the rim. Seedling is with hypogeal germination. (Kumar et al., 2013)

Ecology (Tropical forages)

Soil requirements

Prefers well drained, medium to high fertility soils but can be grown successfully on sandy soils and will tolerate and be productive in a very wide soil acidity range (pH <5.0–8.0).

Moisture

Prefers hot, humid climates with annual rainfall of 1,000–2,500 mm, but will grow in environments with annual rainfall as low as 400 mm. Has some tolerance to drought but is not tolerant to waterlogging .

Temperature

Is susceptible to frost but, because of its short life span, can be grown in the subtropics. Performs best at altitudes from 0–1,600 m, but can be grown up to 2,100 m asl. For grain production, altitudes of 1,200–1,500 m asl are best. Optimum temperature range is 19–27ºC.

Plant profile:-

Light

Requires high light intensity.

Reproductive development

Responds to shorter day lengths, flowering being also stimulated by higher (21ºC) night temperatures. Period between flowering and mature seed is long with pods starting to ripen 2–3 months after flowering. *Mucuna* usually dies off 45–60 days after producing seed.

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.

Plant profile:-

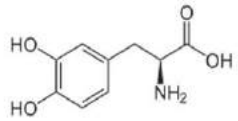
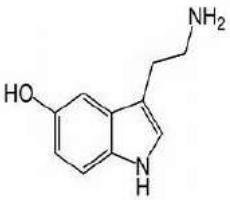
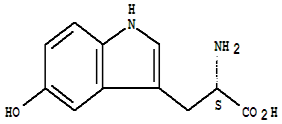
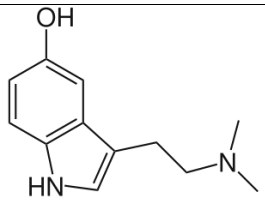
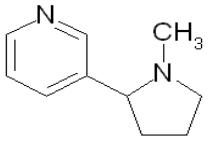
Flowers : Dark purple, lavender (6-30), pea like but larger, with distinctive curved petals and occur in drooping recemes. .

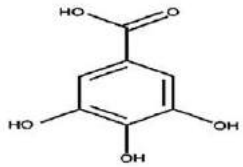
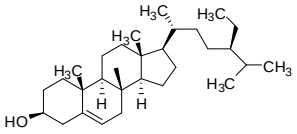
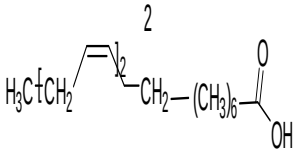
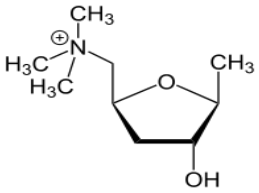
Leaflets : Membranous, terminal leaflets are smaller, lateral very unequal Sized.

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

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

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.	Bufotenine		(Dr. Duke Phytochemical database)
5.	Nicotine		(Dr. Duke Phytochemical database)

6.	Gallic acid		(Anonymous, 1996))
7.	β-Sitosterol		(Anonymous, 1996)
8.	Linoleic acid		(Anonymous, 1996)
9.	Mucunadine		(Dr. Duke Phytochemical database)

Traditional uses:

The seeds are traditionally used as nervine tonic, emmenagogue, astringent, aphrodisiac, leucorrhoea and paralysis. The hairs of the pods are vermifuge and treated as round worm infections. *Mucuna monosperma* is used as an expectorant and sedative given in cough and asthma (Khory and Katrat 1999). Bark powder well 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

able to restore and are sometimes consumed as a vegetable. Seed diet produced by 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).

MATERIAL AND METHODS:-

1. **FORMULATION:**
   a. **Procurement:** procurement of raw material like kaunch seeds, milk (Jersey cow, indigenous cow, buffalo), Ghee (Jersey cow, indigenous cow, buffalo) and honey 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).

Figure 7: Authentication of Plant

c. Preparation: In-house three different formulations are prepared by using the following formula.

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

S/n	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:

Approximately 1 kg of *Mucuna pruriens* seed was taken, dried, cleaned and boiled in about 4 liter of fresh different milks. It was concentrated until milk gets viscous, then the container was removed from flame and allowed to cool, all boiled seeds were collected, their coatings was removed and were triturated with concentrated viscous milk obtained after separating the boiled seeds until a lump mass was obtained. After that small sphere shaped gutikas were prepared from the lump mass, allowed to dry and fried in different fresh ghees until brownish red. Lastly gutika were coated with the help of sugar syrup and stored in honey.

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

Figure 8: Preparation of Vanari gutika formulations

Fig A: process of making a lump mass from boiled seed.

Fig B: manually formation of gutika.

Fig C: process of frying a Vatika

Fig D: final vanari gutika (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 desiccators 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.

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.

ESTIMATION OF PROTEIN BY LOWRY'S METHOD

Reagents: Folin –ciocalteau reagent (reagent D)-reflux gently for 10 hours a mixture consisting of 100g Sodium tungstate (Na2WoO4.2H2O), 25g Sodium molybdate

(Na2WoO4.2H2O), 700ml water, 50ml of 80% phosphoric acid, and 100ml of concentrated hydrochloric acid in a 1.5L flask. Add 150g lithium sulfate, 50ml water and a few drops of bromine water. Boil the mixture for 15 min without condenser to remove excess bromine. Cool, dilute to 1L and filter. The reagent should have no greenish 20% Sodium carbonate in 0.1N sodium hydroxide (Reagent A). 0.5% Copper Sulphate (CuSO4.5H2O) IN 1% potassium sodium tartrate (Reagent B). Alkaline copper solution.: Mix 50ml of A and 1ml of B prior to use (Reagent C). Protein Solution (Stock Standard): Weigh accurately 50mg of bovine serum albumin (fraction V) and dissolve in distilled water and make up to 50ml in a standard flask. Working Standard Solution: Dilute 10ml of the stock solution to 50ml with distilled water in a standard flask. 1.0ml of this solution contains 200µg protein.

Estimation of Protein: Pipette out 0.2, 0.4, 0.6, 0.8 and 1.0ml of the working standard into a series of test tubes. Pipette out 0.1 ml and 0.2 ml of the sample extract in two other test tubes. Make up the volume to 1.0 ml in all the test tubes. A tube with 1.0ml of water serves as the blank. Add 5.0 ml of reagent C to each tube including the blank. Mix well and allowed to standing for 10mins. Then add 0.5 ml of reagent D, Mix well and incubate at room temperature in the dark for 30min, blue color is developed. Take the reading at 660nm.Draw a standard graph and calculate the amount of protein in the sample. A

ESTIMATION OF TOTAL STEROID by Zak's method (Zak, 1954)

Procedure: 0.1ml and 0.2 ml of triple acid extract is taken and a set of standards (0.5 to 2.5 ml) were taken and made up to 5 ml with ferric chloride diluting reagent. A blank was prepared simultaneously by taking 5.0 ml diluting reagent. Then add 4.0 ml of concentrated sulphuric acid to each tube. After 30 minutes incubation, intensity of the color developed was read at 540 nm.

6. CHROMATOGRAPHIC EVALUATION:

A. Thin Layer Chromatography: (Stahl, 1969, Ketan et. al., 2008)

The formulations 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 and R_f values (Retention Factor) were calculated.

B. 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 formulation of Vanari gutika having *Mucuna pruriens* 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 Corporation 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 with Acetic acid 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/2017/37), 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 14/08/2017).

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 as 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. Further formulations were triturated and suspended in distilled water using CMC (0.5%) for oral administration. Similarly 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 (milk + ghee)	3	3
2	Standard (Sildenafil citrate tablet)	3	3
3	High dose jersey cow formulation (HJC)	3	3
4	Medium dose jersey cow formulation (MJC)	3	3
5	High dose indigenous cow formulation (HIC)	3	3
6	Medium dose indigenous cow formulation (MIC)	3	3
7	High dose buffalo formulation (HB)	3	3
8	Medium dose buffalo formulation (MB)	3	3

Females are for only mating purpose*

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 female mice 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 were recorded 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 of mounts 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 8 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 milk and ghee orally. 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. Group 3rd, 5th and 7th received High dose of specified Vanari gutika formulations in table no. 6. While, Group 4th ,6th and 8th received Medium dose of specified Vanari gutika formulations in table no. 6, once a day in the evening (3:00 PM) for 7 days. 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 7th 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 two-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 anesthesia 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 SS Pathlab Dhantoli, 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's haemocytometer 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.

F. 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: Physicochemical properties of *Mucuna Pruriens* seed powder

Sr. no.	Parameter	Observation
1	LOD (%w/w)	4.6
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 8: Organoleptic Properties of Vanari gutika Formulation

Sr. no.	Properties	Jersey Cow	Indigenous Cow	Buffalo
1	Colour	Dark brownish	Dark brownish	Dark brownish
2	Odour	Pleasant	Pleasant	Characteristics
3	Taste	Sweet	Sweet	Sweet
4	Shape	Sphere	Sphere	Sphere

Table 9: Pharmaceutical parameters of Vanari gutika formulation

Sr. no.	Parameter	Jersey Cow	Indigenous Cow	Buffalo
1	Weight variation	**Pass**	**Pass**	**Pass**
2	Hardness (kg/cm)	**2.7 ± 0.05**	**3.0 ± 0.05**	**2.7 ± 0.05**
3	Diameter (mm)	**8.5**	**8.5**	**8.3**
4	pH	**5.84**	**6.21**	**6.11**

Values are mean ± SD from observations

Table 10: Physicochemical properties of Vanari gutika formulation

Sr. no.	Parameter	Jersey Cow	Indigenous Cow	Buffalo
1	Total Ash	0.08	0.08	0.10
2	Acid insoluble Ash	0.06	0.05	0.07
3	Water soluble Ash value	0.81	0.90	0.66
4	Alcohol extractive value	9.27	7.8	12
5	Aqueous soluble extractive value	30.8	29.9	32.6
6	Ether extractive value	3.2	5.9	2.6

Table 11: Phytochemical Screening

Plant constituents	Test reagents	Jersey Cow	Indigenous Cow	Buffalo
Steroids	Salkowski reaction	+	+	+
Alkaloids	Mayer's reagent	+	+	+
	Hager's reagent	+	+	+
	Wagner's Reagent	+	+	+
Tannins	Bromine water Test	-	-	-
	Potassium Dichromate	+	+	+
	10% Lead acetate test	+	+	+
Carbohydrates	Molisch's test	+	+	+
Proteins	Biuret test	+	+	+
Saponins	Foam test	-	-	-
Phenolic content	Ferric chloride test	+	+	+
Amino acid	Ninhydrin solution	+	+	+
Flavonoids	Sulphuric acid test	+	+	+
Triterpenoids	Libermann Burchard test	+	+	+

+ indicates presence – indicates absence

Table 12: Quantitative estimations of Vanari gutika formulation

Sr.no	Parameters	Jersey cow	Indigenous cow	Buffalo
1	Total alkaloids (%w/w)	0.46	0.60	0.52
2	Total Phenol content (mg/g) Eq gallic acid	0.90	1.21	1.08
3	Flavonoid content (mg/g) Eq quercetin	2.18	1.92	1.38
4	Tannin content (mg/g) Eq catechin	5.10	2.83	2.15
5	Carbohydrate content (mg/g) Eq dextrose	0.39	1.29	1.80
6	Steroids content (mg/g) Eq cholesterol	16.70	16.83	15.60
7	Protein content (mg/g)	23.82	22.83	27.44

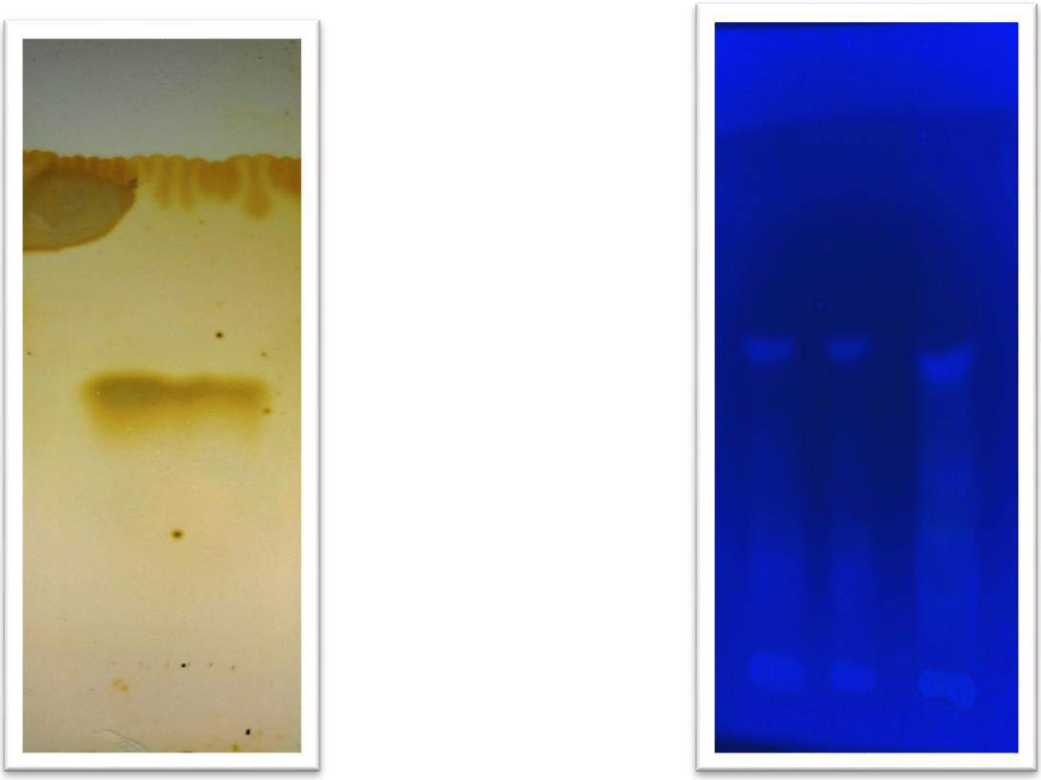
TLC Fingerprinting-

Standard prepared using Levodopa Stock Solution (0.5 mg/ml)

Sample preparation – Sample (5mg/ml) + Methanol

Mobile Phase - (n-butanol : Glacial Acetic Acid : Water)

(20 : 5 : 5)



Standard (l-dopa) **J** **I** **B**

Figure 9: TLC Fingerprinting (Jersey cow-J, Indigenous cow-I, Buffalo-B)

Table 13: TLC Fingerprinting

Sample	No. of Spots	Rf Values
Standard (L-dopa)	1	0.60
Jersey Cow (J)	1	0.58
Indigenous Cow (C)	1	0.60
Buffalo (B)	1	0.57

HPLC Analysis-

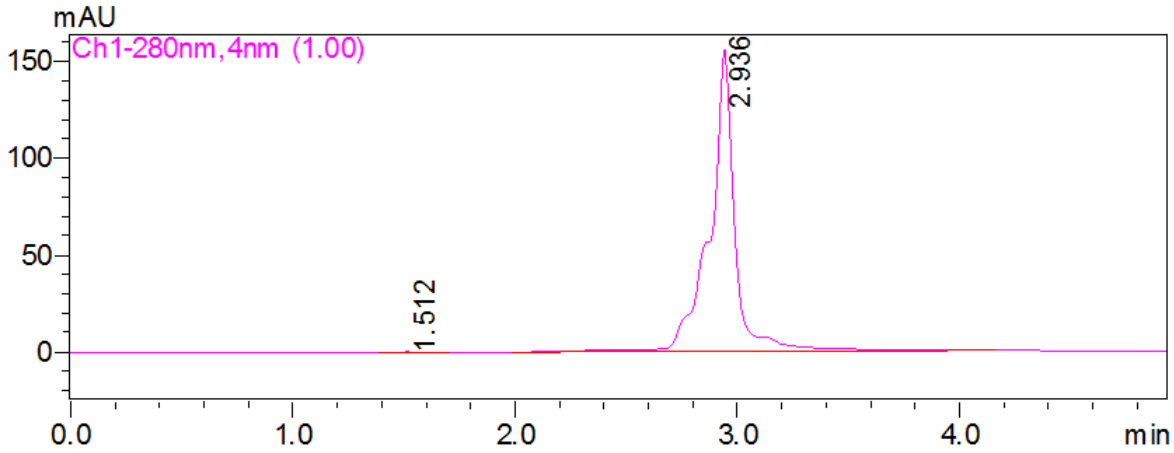


Figure 10: Chromatogram of Standard Levodopa 10 mg

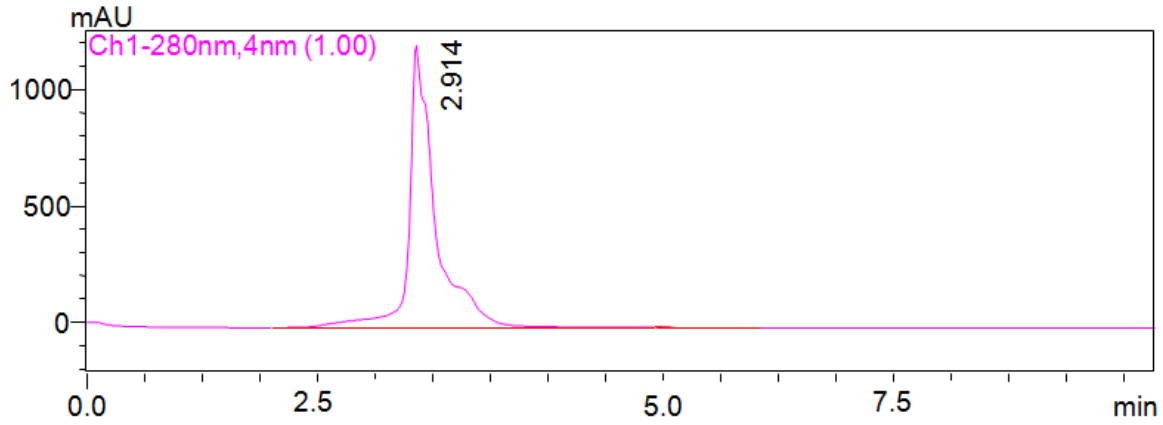


Figure 11: Chromatogram of Jersey Cow Vanari gutika formulation 100mg

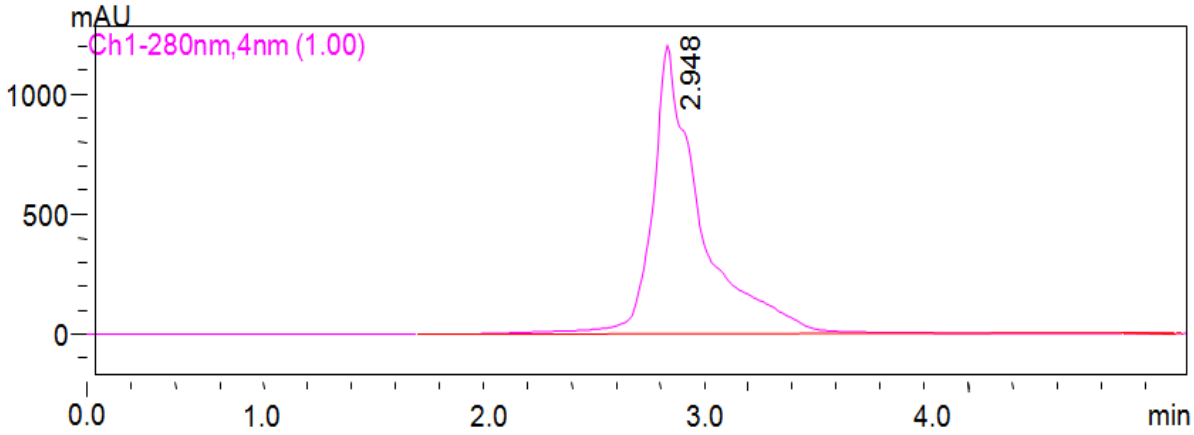


Figure 12: Chromatogram of Indigenous Cow Vanari gutika formulation 100mg

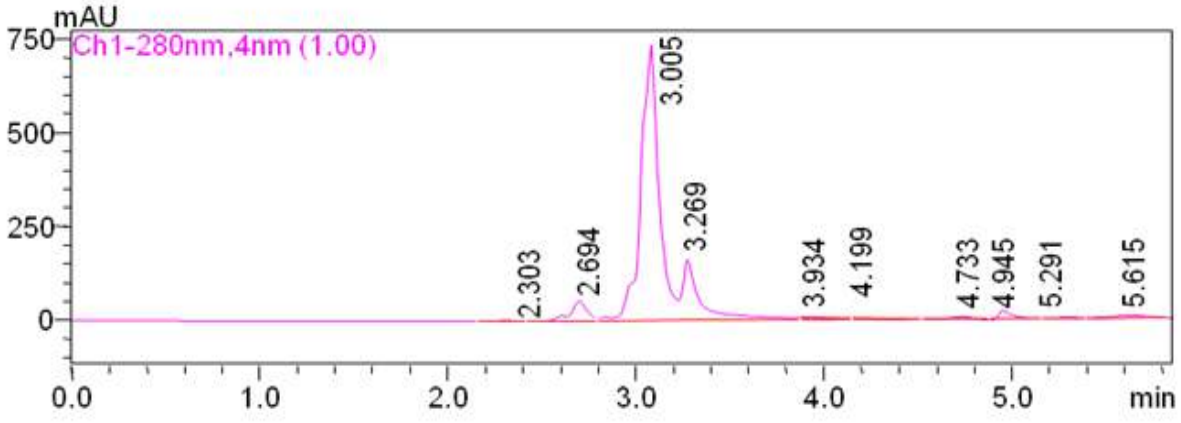


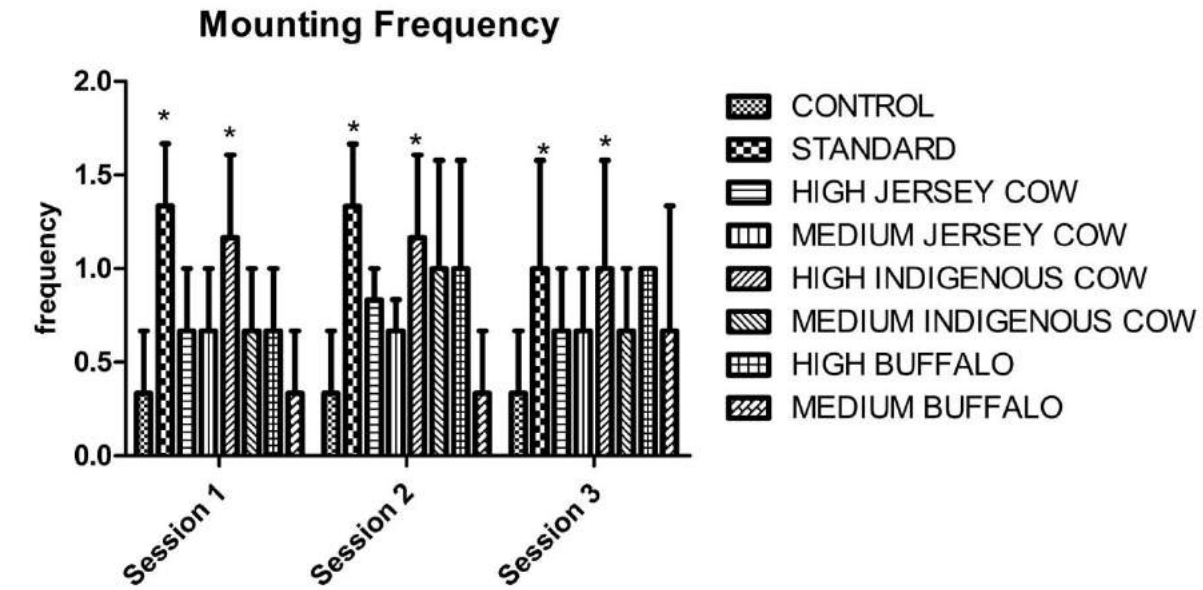
Figure 13: Chromatogram of Buffalo Vanari gutika formulation 100mg

Table 14: Results of HPLC analysis:

Sr. no	Sample	Concentration taken in µg/ml	Retention time (minutes)	Peak area	Concentration µg/ml
1	Standard	10	2.936	1238237	-
2	Jersey Cow	100	2.914	1551444	2.50
3	Indigenous Cow	100	2.948	1845453	2.98
4	Buffalo	100	3.005	1669313	2.69

Preclinical Study Results:

1. Mounting behavior

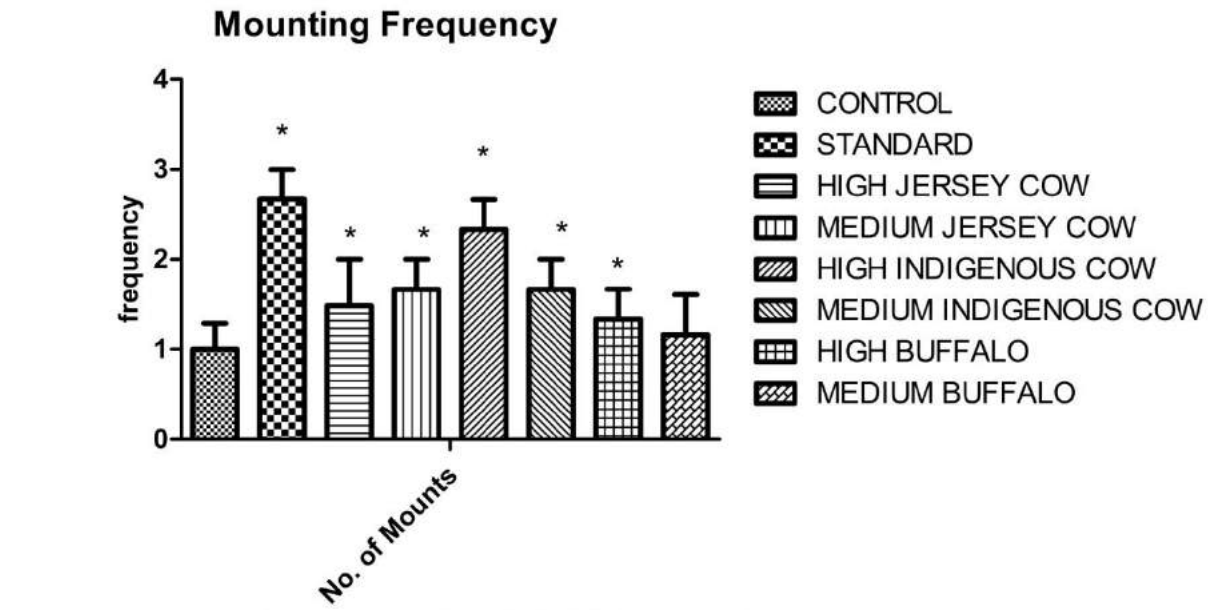


Session 1- 1st 15min of 1st hour, Session 2- 2nd 15min of 2nd hour, Session 3- 3rd 15min of 3rd hour

Figure 14: Mounting Behavior Sessions

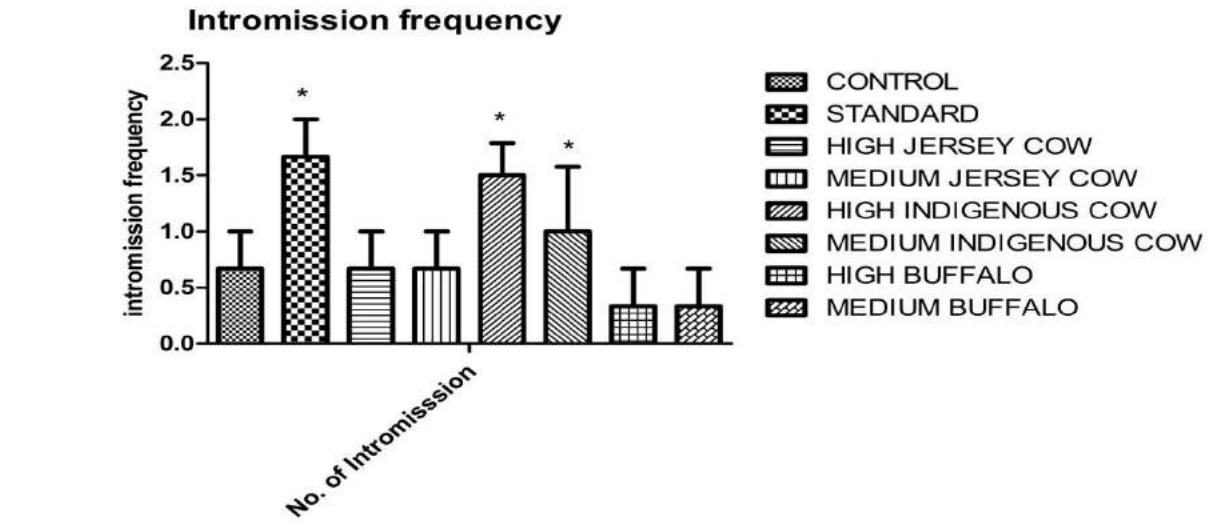
Test on libido:

2. **Mounting and Intromission frequency**



value are expressed as mean ± S.E.M. (n=3) *P≤0.05 vs. control.
Two way ANOVA followed by Bonferroni multiple comparision test.

Figure 15: Mounting Frequency



value are expressed as mean ± S.E.M. (n=3) *P≤0.05 vs. control.
Two way ANOVA followed by Bonferroni multiple comparision test.

Figure 16: Intromission Frequency

3. Mounting and intromission latency

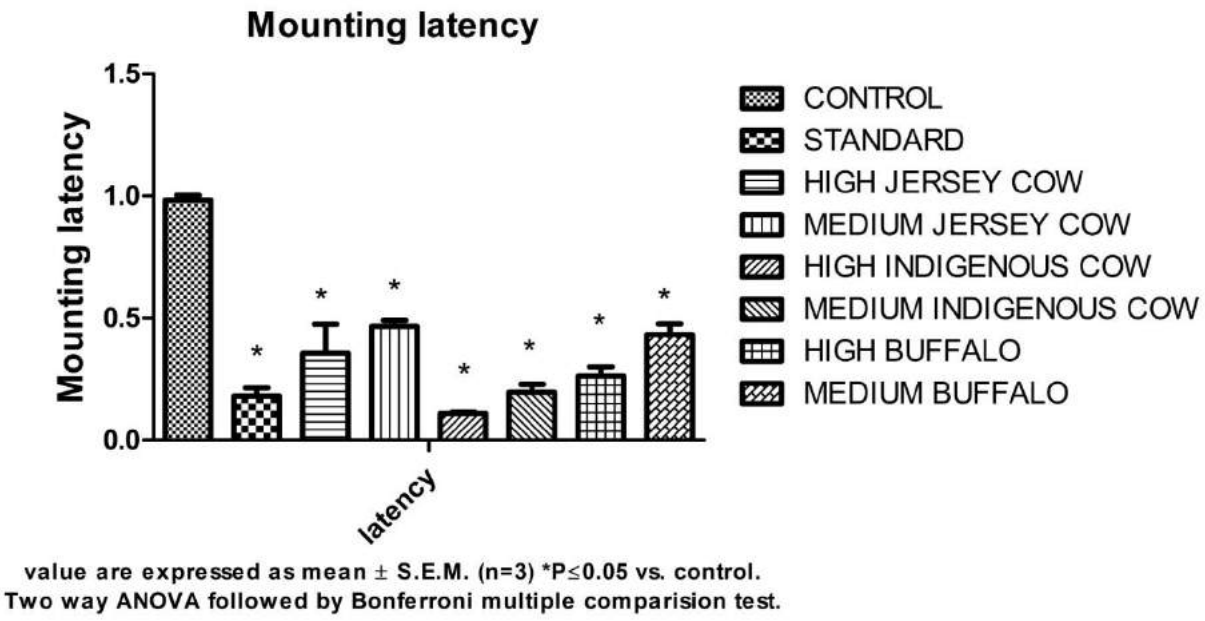


Figure 17: Mounting Latency

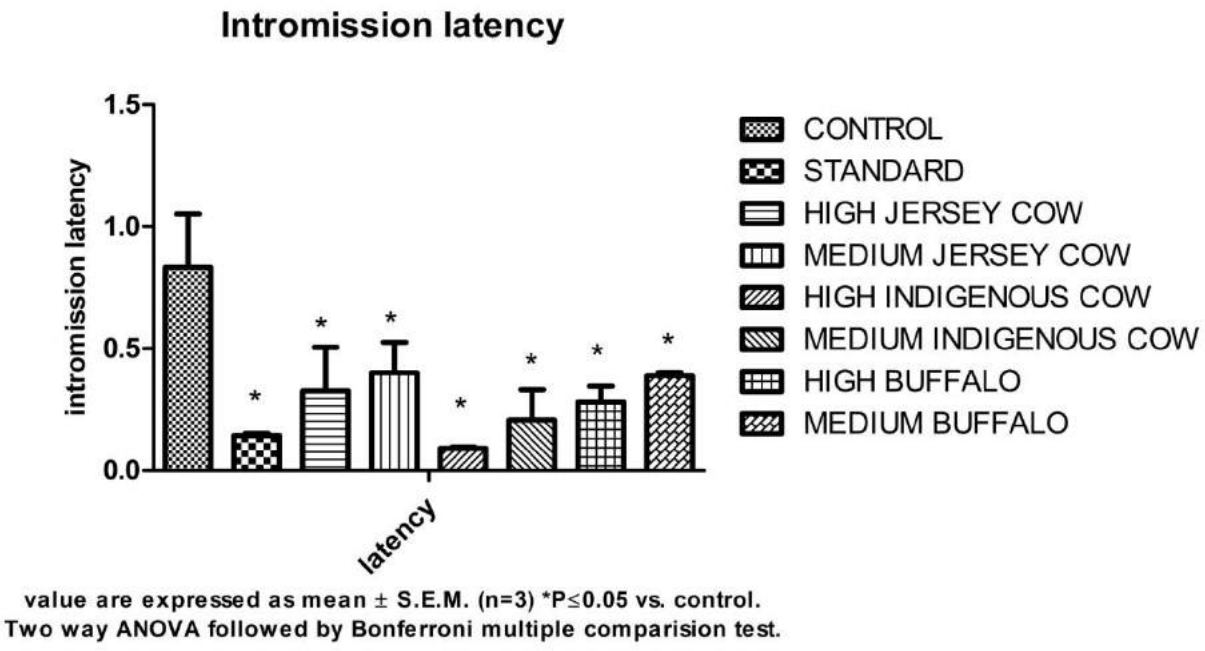


Figure 18: Intromission Latency

4. Ejaculation frequency

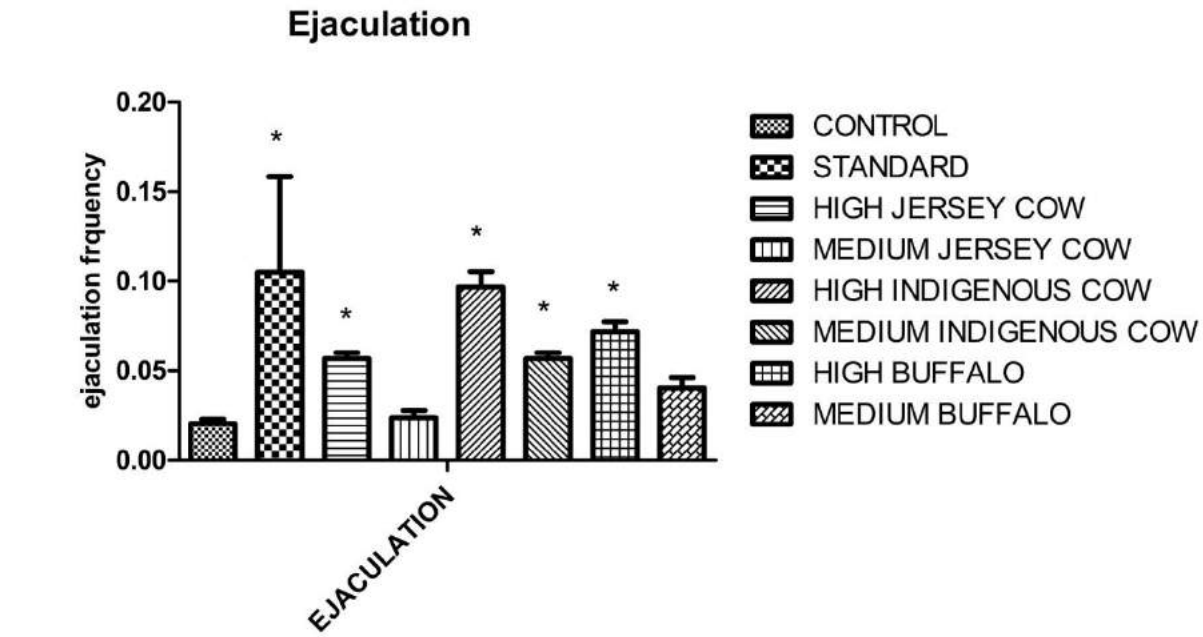


Figure 19: Ejaculation Frequency

5. Epidydimal Sperm Count

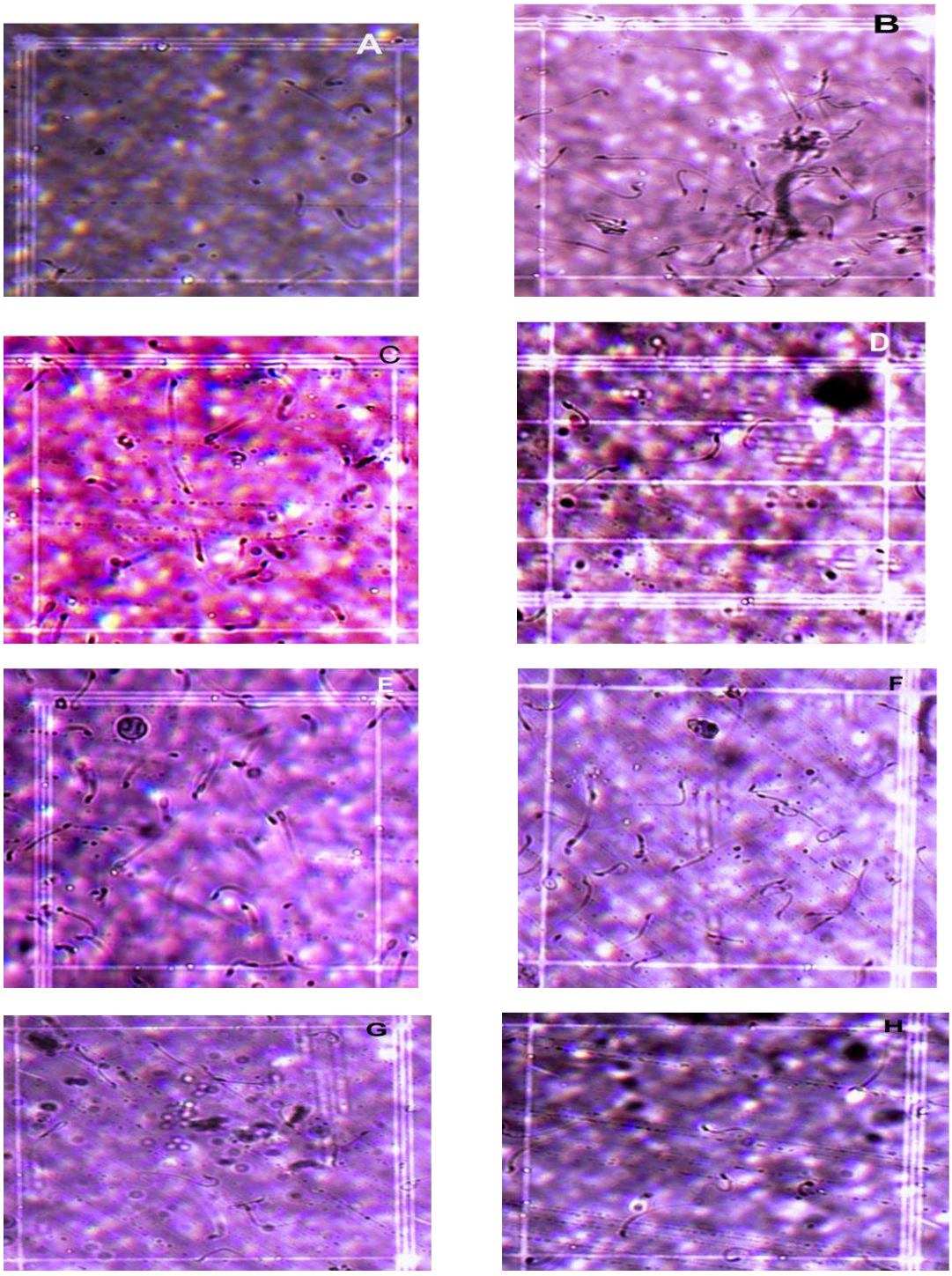


Figure 20: Epididymal sperm count at 40x. (A): Control group, (B): Standard group, (C): High Jersey Cow group, (D): Medium Jersey Cow group, (E): High Indigenous group, (F): Medium Indigenous group, (G): High Buffalo group, (H): Medium Buffalo group.

6. Epididymal sperm count

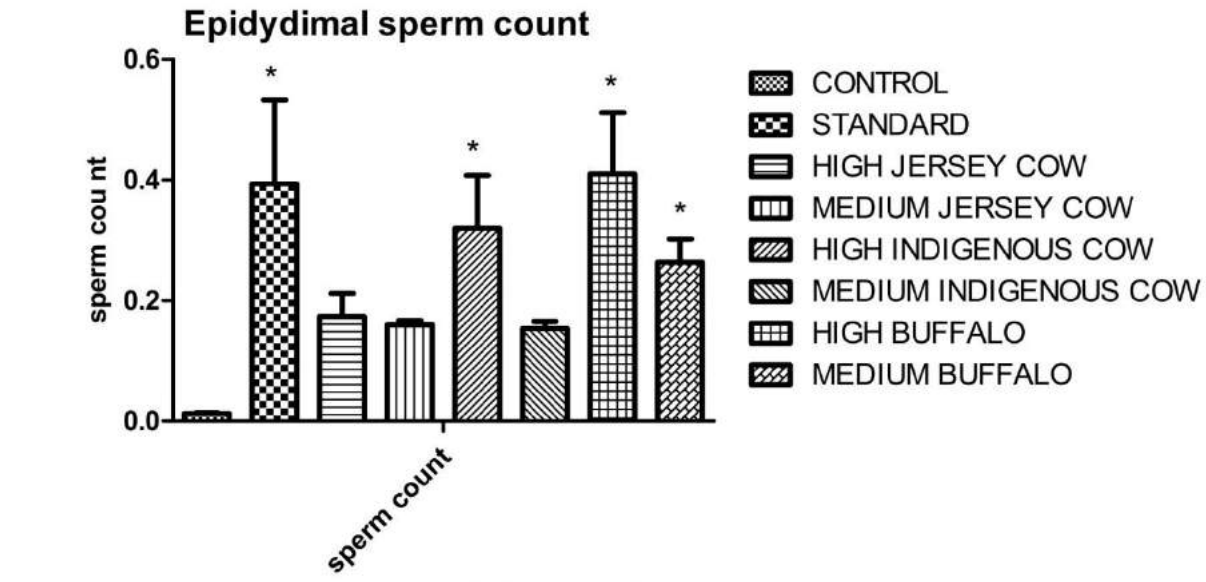


Figure 21: Epididymal Sperm Count

7. Biochemical analysis

1. Testosterone Level

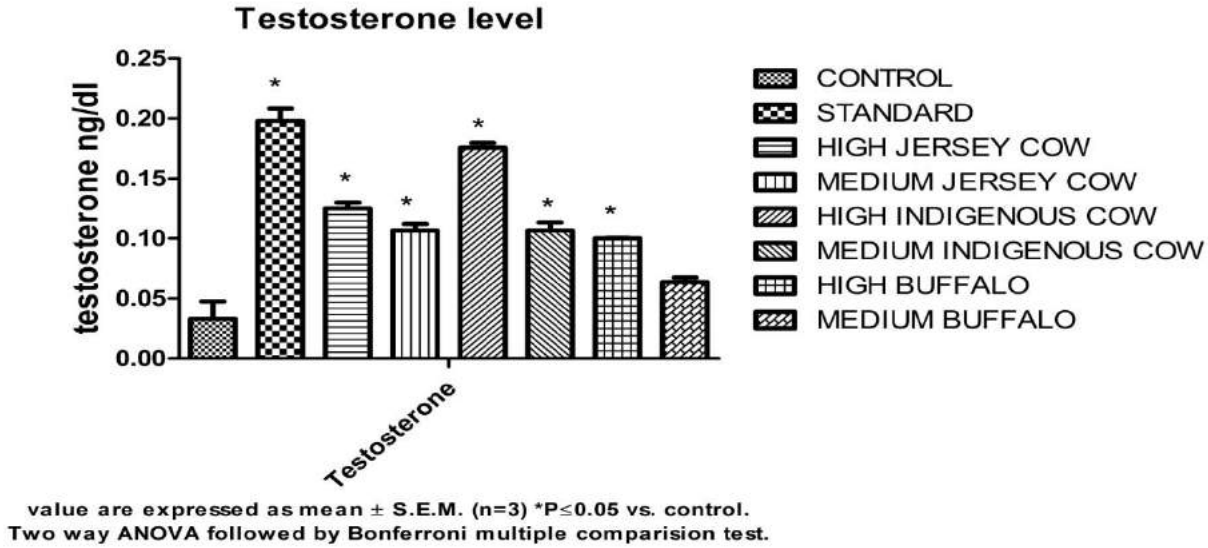


Figure 22: Testosterone level

2. **Luteinizing Hormone Level**

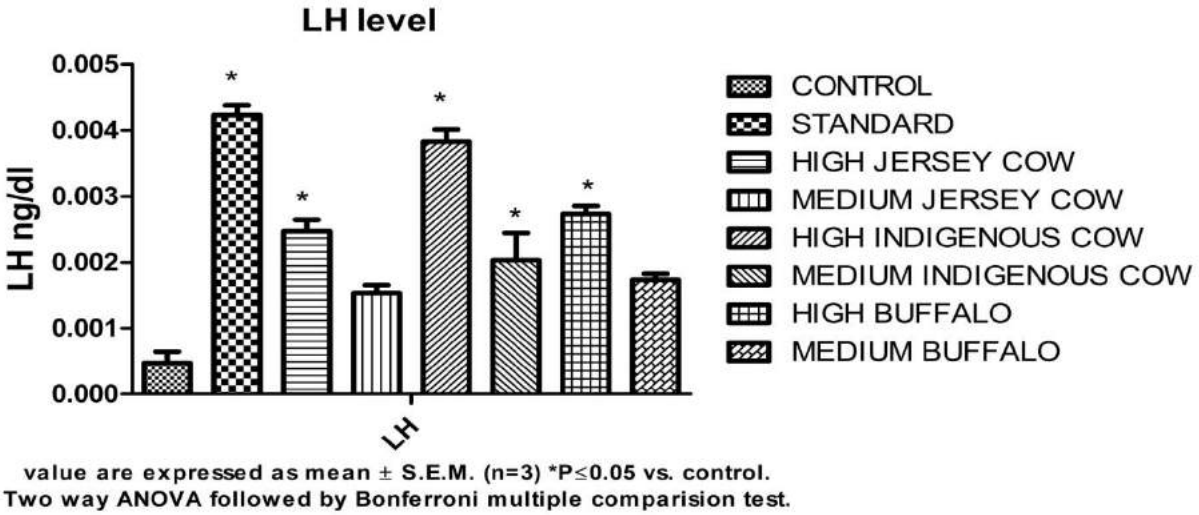


Figure 23: Luteinizing Hormone Level

3. **Follicle Stimulating Hormone**

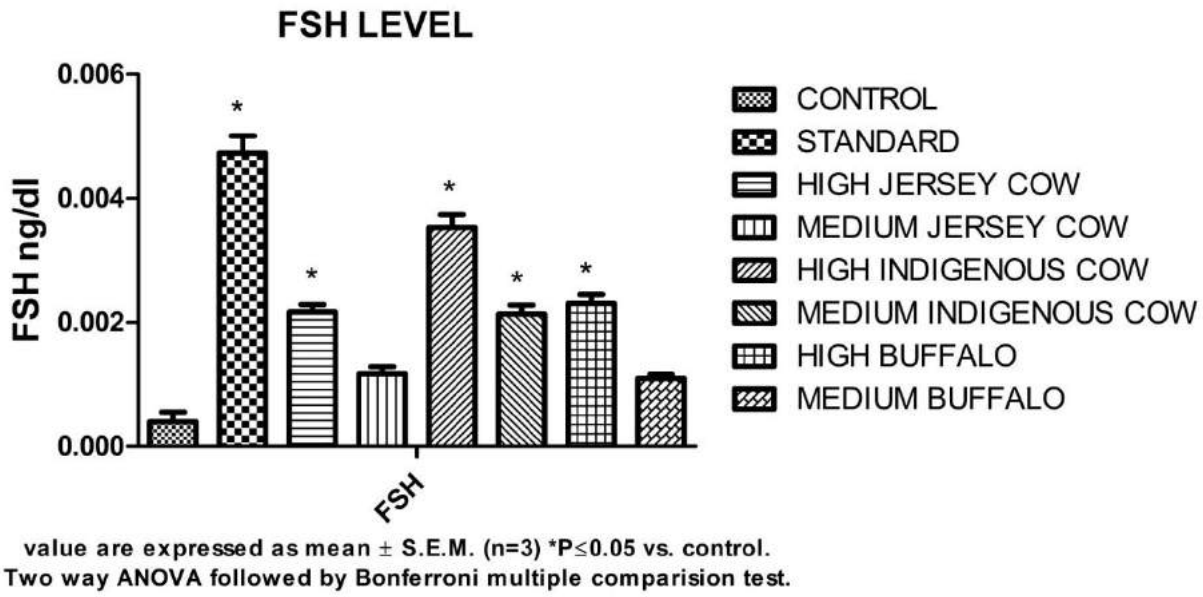


Figure 24: Follicle Stimulating Hormone

Histopathology Study

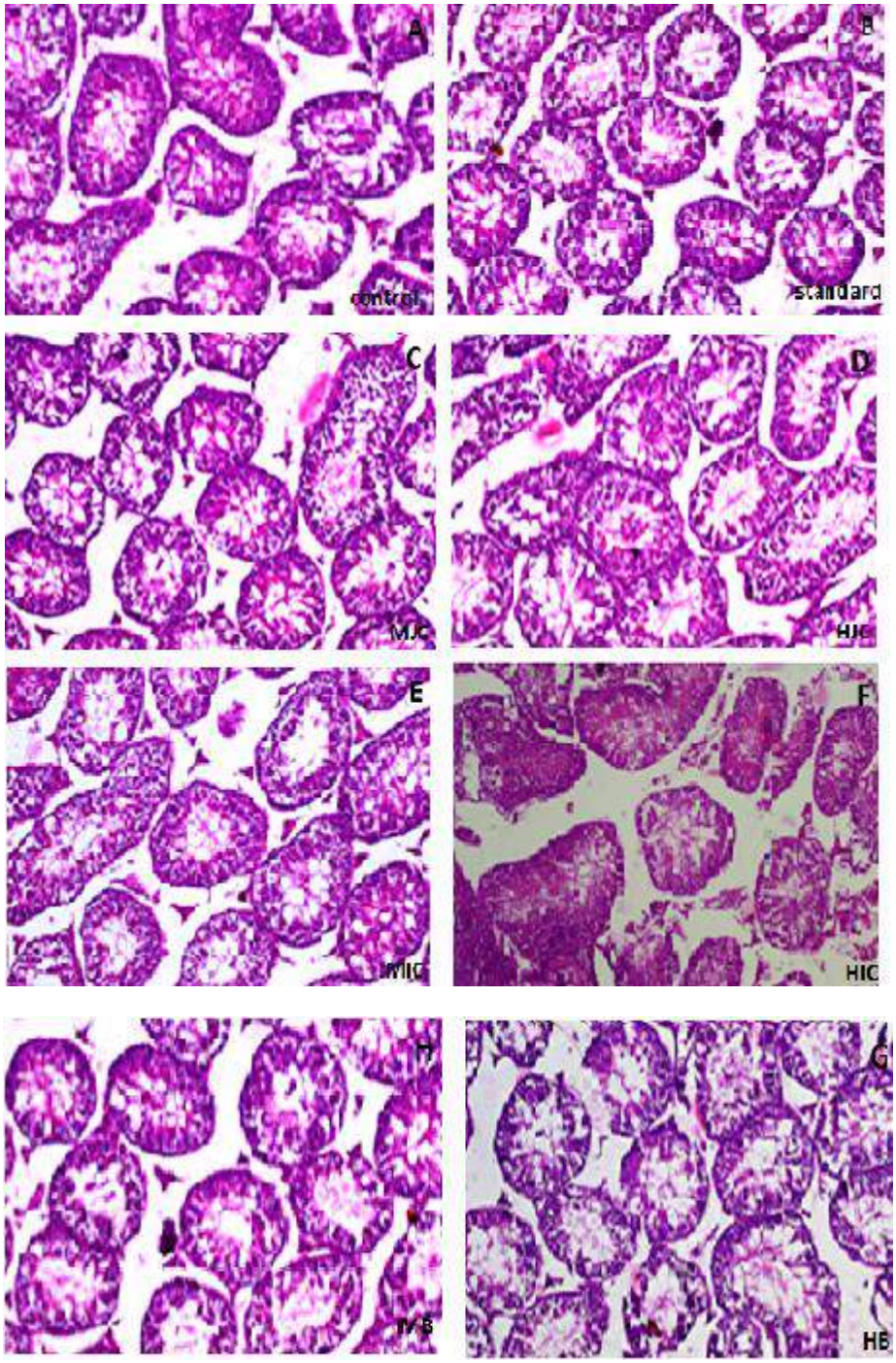


Figure 25: Histopathology of Testis (A): Control group, (B): Standard group, (C): High Jersey Cow group, (D): Medium Jersey Cow group, (E): High Indigenous cow group, (F): Medium Indigenous cow group, (G): High Buffalo group, (H): Medium Buffalo group.

SUMMARY AND DISCUSSION:-

Ten years of research that has provided data regarding the prevalence of sexual dysfunctions has been reviewed. A thorough review of the literature identified 52 studies that have been published in the 10 years since an earlier review by Spector and Carey (1990). Community samples indicate a current prevalence of 0 - 3% for male orgasmic disorder, 0 - 5% for erectile disorder, and 0 - 3% for male hypoactive sexual desire disorder. Pooling current and 1-year figures provides community prevalence estimates of 7 - 10% for female orgasmic disorder and 4 - 5% for premature ejaculation. Stable community estimates of the current prevalence for the other sexual dysfunctions remain unavailable. Prevalence estimates obtained from primary care and sexuality clinic samples are characteristically higher. Although a relatively large number of studies have been conducted since Spector and Carey's (1990) review, the lack of methodological rigor of many studies limits the confidence that can be placed in these findings (Jeffrey Simons and Michael P. Carey).

There are a lot of synthetic drug available in market to treat male infertility, erectile dysfunction and oligospermia. The synthetic drugs available in market, like siledenafil, taladafil, varedelafil are expensive. Instead of opting for expensive allopathic medicines, many people rely on economic herbal medicines for health care. So there must be interest and remedies towards herbal formulations functional in the area of treating infertility diseases.

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, milk (Jersey cow, indigenous cow, buffalo), Ghee (Jersey cow, indigenous cow, buffalo), honey and sugar. Clinically, it has been found to be useful in cases of male infertility.

In present research work Vanari gutika formulation is made with the help of different Ghee and Milk obtained from Jersey cow (*Bos taurus*), Indigenous cow and Buffalo. The aim of the study was to study which formulation provided more potent activity amongst the above said varieties of cow milk, along with its quality control profiling and evaluation of 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 components of the Vanari gutika and having a potent aphrodisiac potential (Sekar et. el., 2009). But, Comparative study of Vanari gutika formulation prepared with Ghee and Milk of Jersey cow (*Bos taurus*), indigenous cow and buffalo formulations 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 of different Vanari gutika formulations 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 5 hr in case of Jersey cow formulation, 6 hr in case of Indigenous cow formulation and 8 hr in case of buffalo formulation. The result obtained in the present study is a contrasting one as the maximum disintegration time for sugar coated tablet in our study is reported to be 60 min. Thus, in our case the disintegration is very high which may be attributed to the presence of milk and ghee which is highly rich in carbohydrate that may act as natural binder which kept the gutika intact for a longer period of time. Further the preparation procedure of gutika 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. It is found that buffalo formulation takes more time for disintegration than Jersey cow formulation and indigenous cow formulation due to high fats present in buffalo milk and ghee. 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 loss on drying, 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 exists. 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 found to be absent.

The Vanari gutika formulation were subjected to quantitative estimations such as to polyphenol, flavonoid content, tannin content, steroid content, protein content and alkaloidal content and it was found that total alkaloids content, total phenol content and steroid content higher in indigenous cow formulation, while protein content and carbohydrate content was found higher in buffalo formulation. As buffalo milk and ghrita rich in fat.

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 color, preservatives and sweetening agents (Kasture et al., 2004). In the present study, TLC was done for the determination of different components present in the different formulations of Vanari gutika. The TLC of formulations of Vanari gutika (Jersey cow, indigenous cow, buffalo) sample confirm the presence of L-dopa, having Rf value 0.60 as that of standard L-dopa. Rf values of Jersey cow sample, Indigenous cow sample, Buffalo sample were found to be 0.58, 0.60, 0.57 respectively.

Aphrodisiac potential of Vanari gutika:-

The present study is designed in two different parts, first one is short term effect session (on 1st day) and another one is long term effect session (for seven days). The aphrodisiac potential can be evaluated on the basis of mating behavior parameters 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 high/medium dose of vanari gutika formulations. All the parameter of mating behavior was observed on 1st day and after 7 day.

In 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 of indigenous cow Vanari gutika formulation shows increasing in mounting frequency as compare to another formulation groups. While the medium dose of jersey cow and buffalo formulation groups didn't 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 days at 3 pm. On the 8th 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 high dose of indigenous cow formulation group and standard group significantly increase MF as compare to another formulation groups. Further, the intromission frequency was significantly increased in Standard, high and medium dose of indigenous cow formulation group.

Also the mounting and intromission latency was found significantly decreased in all groups as compared to the control group. This showed at high dose of indigenous cow formulation and standard groups have intensified the sexual activity.

The high dose of indigenous cow formulation group was found to have promising sexual behavior improving effect than medium dose of indigenous formulation and other medium groups.

Further the ejaculation frequency shows the positive effect in standard and high dose of indigenous cow formulation group as compared to control, medium and high dose of other groups.

The epididymal sperm count was significantly increased in high dose of buffalo formulation, high dose of indigenous cow formulation and standard group as compared to control group. But, the medium dose of buffalo formulation group also showed the increase in sperm count as compared to control group. The improvement in sperm count confides that the formulation was also useful in oligospermia condition.

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

The histopathology of testis of treated mice with high dose formulation groups (Jersey cow, indigenous cow, and buffalo) revealed the ruptured seminiferous tubules. While in control, standard and medium dose formulation groups (Jersey cow, indigenous cow, and buffalo), it showed continue seminiferous tubules intact in interstitial connective tissue and the Leydig cells have been observed.

Conclusion

- In the present study, attempts were made for the quality control profiling and preclinical evaluation of herbal formulation Vanari gutika prepared using different milk and ghrita obtained from indigenous cow (*Bos indicus)*, Buffalo and Jersey cow *(Bos taurus)* for its aphrodisiac potential.
- The physicochemical and pharmaceutical parameters of three different formulations were evaluated and were found to be within the prescribed limits as per standard values mentioned in Active Pharmaceutical Ingredients.
- 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 powder 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, amino acids, phenol, proteins, triterpenoids and carbohydrates which were found to be in considerable amount in three different Vanari gutika formulation, whereas saponins were found to be absent.
- Quantification studies of three different formulations showed presence of total alkaloid content, total phenol content, flavonoid content, tannin content, carbohydrate content. Whereas Steroid content and Protein content showed greater values due to presence of milk and ghrita content in formulation.
- The TLC analysis of standard L-dopa, Jersey cow formulation, Indigenous cow formulation and buffalo showed the spot at Rf value 0.60, 0.58, 0.60, 0.57 respectively.
- In High Performance Liquid Chromatography study three different Vanari gutika formulations were compared with standard Levodopa, and Retention time was found nearby to standard.
- In the pharmacological evaluation we have successfully evaluate the aphrodisiac property of three different formulation of Vanari gutika using short term as well as long term animal models.
- From the overall observation it was found that the high dose of indigenous cow Vanari gutika formulation shows maximum aphrodisiac potential as compare to other group. However the high dose of buffalo formulation shows maximum number of sperm count as compare to other group.
- Thus, from the overall study it may be concluded that Vanari gutika formulation has significant aphrodisiac activity, which is mainly due to presence of *Mucuna pruriens*.
- Although further work is needed to be done. Investigations regarding the detailed mechanisms underlying the pharmacological activities, which would show the exact mode of action through which high indigenous cow formulation can found to be more effective than others.

Conclusion

- During Disintegration studies, the disintegration time of Vanari gutika formulation was found to be greater than the limits given by IP.
- Thus, it can be proposed that, administration of Vanari gutika along with hot milk may decrease the total disintegration time.

REFERENCES-

- Anantha Kumar, K. V., Srinivasan, K. K., Shanbhag, T., & Gurumadhava Rao, S. (1994). Aphrodisiac activity of the seeds of *mucuna pruriens*. *Indian Drugs*, *31*(7), 321-327.
- Anonymous, the Wealth of India, Publication and Information Directorate, CSIR, New Delhi, 947-949. 1996.
- 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.
- Anonymous. The Wealth of India Raw Materials, NISCAIR Publishing New Delhi, L-M, Vol VI: 439-444. 2005.
- Bhaskar A, Vidhya VG, Ramya M "Hypoglycemic effect of *Mucunaprurien*sseed 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.
- Chang 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.
- Chaudhari, B. Ankita; Nehal, K. Gohil; Komal, A. Kale; Lambole, Vijay; Gajera, Vipul, a review: phytochemistry, therapeutic use and pharmacological activity of *Mucuna pruriens* linn., EBSCO's Information Services.
- *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.

References

- Desai 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.
- Elizabeth West, Micheal Krychman, Natural Aphrodisiacs—A Review of Selected Sexual Enhancers, Willey online library, October 2015, Vol. 3, Pg no. 279-288.
- 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.
- Gitanjali Deokar, Pratiksha Anil Deore, Sanjay Kshirsagar., Phytochemistry and Pharmacological activity of *Mucuna pruriens*: a review, vol 3 (2016).
- 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.
- Hagerman, 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.
- Harshada Kakulte., Phytochemistry and pharmacological activity of *Mucuna pruriens*: a review. Pharmaceutical and Biological Evaluations, Vol 3, No 1(2016).
- Himanshu Bhusan Sahoo et al., Aphrodisiac activity of polyherbal formulation in experimental models on male rats Pharmacognosy research, 2014 april-june, PMCID: PMC3996747.
- 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.

References

- J. R. Kovac, C.Labbate, R.Ramasamy, D.Tang, L.Lipshultz., Effect of Cigarette Smoking on erectile dysfunction, NCBI, 2016, PMCID- PMC4485976.
- James Daramola et al., Effect of (*Mucuna pruriens*) on spermiograms Of West African dwarf bucks. ResearchGate, Tropical and Subtropical Agroecosystems, 18 (2015): 145 – 150.
- Jeffrey Simons and Michael P. Carey., National Institute of health public access, Arch Sex Behav. 2001 April ; 30(2): 177–219.
- 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.
- Jyoti Pandey, Rajshree Pandey., Phytochemical study of seeds of *Mucuna pruriens,* International Journal for Research in Applied Science & Engineering Technology,Volume 4 Issue I, January 2016.
- K. Muthu and P. Krishnamoorthy,Evaluation of androgenic activity of *Mucuna pruriens* in male rats. African Journal of Biotechnology Vol. 10(66), pp. 15017-15019, 26 October, 2011.
- Kasture, 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 "*Mucunapruriens*in Parkinson's disease: a double blind clinical and pharmacological study" J. Neurol. Neurosurg. Psychiatry 75:1672-1677, 2004.
- Kavita C. and Thangamani ., Amazing bean "*Mucuna pruriens*": A comprehensive review, Journal of Medicinal Plants Research, January, 2014.
- Kazim Sahin et al., Comparative evaluation of the sexual functions and NF-κB and Nrf2 pathways of some aphrodisiac herbal extracts in male rats.BMC Complementary & Alternative Medicine, PMCID: PMC5000417.
- Ketan Pravinbhai Modi, NatvarlalManilal Patel and Ramesh Kishorilal Goyal, "Estimation of L-Dopa from *Mucuna pruriens* linn and formulation containing *M. pruriens* by HPTLC Method" Chem. Pharm. Bull. 56(3) 357-359 (2008).
- Khandelwal, K.R., Practical Pharmacognosy Techniques and Experiments,149-146, 2007.
- Kokate 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.

References

- Mayank Thakur,Nagendra S. Chauhan,Shilpi Bhargava,Vinod K. Dixit., A Comparative Study on Aphrodisiac Activity of Some Ayurvedic Herbs in Male Albino Rats. Archives of Sexual Behaviour, December 2009, Volume 38, Issue 6, pp 1009–1015.
- 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.
- 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.
- Mukesh Kumar Yadav et al., Phytochemist and Pharmacological review of *Mucuna pruriens*: a review, International Journal of Green Chemistry, Aug 16, 2017.
- Mukesh Kumar Yadav et al., Phytochemistry and pharmacological activity of *Mucuna pruriens*: A review. International Journal of Green Pharmacy, Apr-Jun 2017, 11 (2).
- Nagendra Singh Chauhan, Vikas Sharma, V. K. Dixit and Mayank Thakur., A Review on Plants Used for Improvement of Sexual Performance and Virility, BioMed Research International, Volume 2014 (2014), Article ID 868062, 19 pages.
- Ovallath S, Deepa P "The history of parkinsonism: descriptions in ancient Indian medical literature" MovDisord. 28(5):566-568, 2013.
- 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. 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.
- Paul R. Brezina, Fahd N. Yunus, Yulian Zhao, "Effects of Pharmaceutical Medication on Male Fertility" journal of reprod infertile, 3-11, 2012.

References

- Pradeep Kumar , Sudipta Saha., An updated review on Taxonomy, Phytochemistry, Pharmacology and Toxicology of *Mucuna pruriens*, Journal of Pharmacognosy and Phytochemistry, Volume 2 Issue 1,2013.
- Pujari S. A. and Gandhi M. B., Studies On Effects Of Seed And Leaf Extracts Of *Mucuna Pruriens* On Some Common Bacterial Pathogens. Journal of Environmental Research And Development, Vol. 8 No. 1, July-September 2013.
- 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.
- Rawat Swati and Motwani Sapna, International Journal of Research and Development in Pharmacy and Life Sciences, August - September, 2012, Vol. 1, No.3, pp 122-125.
- Robert C. Dean, Tom F. Lue, Physiology of Penile Erection and Pathophysiology of Erectile Dysfunction. *UrolClin North Am.* 2005 November, 32(4): pp-379.
- Robert C. Dean, and Tom F. Lue., Physiology of Penile Erection and Pathophysiology of Erectile Dysfunction, NCBI, 2006, PMCID- PMC1351051.
- Roberto Guerranti et al., The Magic Velvet Bean of *Mucuna pruriens*. Journal of Traditional and Complementary Medicine, PMCID: PMC3942911
- 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.
- Sachchida Nand Rai, Hareram Birla, Walia Zahra, Saumitra Sen Singh, Surya Pratap Singh., Immunomodulation of Parkinson's disease using *Mucuna pruriens* (Mp), Journal of Chemical Neuroanatomy, June 2017.
- Sanjay Kasture, Mahalaxmi Mohan, Veena Kasture *Mucuna pruriens* seeds in treatment of Parkinson's disease: pharmacological review, oriental pharmacy and experimental medicine, September 2013, Volume 13, Issue 3, pp 165–174.
- 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) 497-501.230-231.

References

- Sekhar Suresh et al., Dose- and time-dependent effects of ethanolic extract of *Mucuna pruriens* Linn. seed on sexual behaviour of normal male rats. Journal of Ethnopharmacology, Volume 122, Issue 3, 21 April 2009, Pages 497-501.
- Seppan Prakash et al., Effect of *Mucuna pruriens* (Linn.) on Sexual Behavior and Sperm Parameters in Streptozotocin-Induced Diabetic Male Rat. The Journal of Sexual Medicine, Volume 9, Issue 12 December 2012 Pages 3066–3078.
- Shanbhag, 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.
- Taymour mostafa, "Cigarette smoking and male infertility" Cairo university, Journal of Advanced Research, 179-186, 2010.
- Wagner H and Bladt S, Plant Drug Analysis, 2Nd edition, Springer, Berlin, (1996)
- Wallter B. Shelley et al., Studies on Cowhage (*Mucuna Pruriens*) and Its Pruritogenic Proteinase, Mucunain. JAMA Dermatology, November 1955.
- 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
- Yerra Rajeshwar, Malaya Gupta And Upal Kanti Mazumder., *In Vitro* Lipid Peroxidation and Antimicrobial Activity of *Mucuna pruriens* Seeds, Iranian Journal Of Pharmacology & Therapeutics June, 2005.