



# Nicotine induced Erectile Dysfunction and herbal drugs for treating Erectile Dysfunction in Male albino wistar rats

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**Abstract** -The word “Aphrodisiac” is derived from “Aphrodite” the Greek goddess of love. By definition aphrodisiacs are the substance, which stimulate sexual desire (Greek-Aphrodisiakos-sexual). A variety of plants have been used as sex stimulants or sexual performance enhancer(eg; Ashwagandha, Musli, Brahmi, Shilajit, Macca etc) in traditional systems of medicine of various countries. The use of plant or plant-based products to stimulate sexual desire and to enhance performance and enjoyment is almost as old as the human race itself. A number of herbal drugs have been validated for their effect on sexual behavior and fertility and can therefore serve as basis for the identification of new chemical leads useful in sexual and erectile dysfunction. An aphrodisiac is a substance that increases libido when consumed. Orally or taken by other routes. Aphrodisiacs are distinct substances that address fertility issues or secondary sexual dysfunction such as erectile dysfunction (ED). ED has also been identified as the most common sexual dysfunction problem in male. Sex power boosting Ayurvedic herbs are called as aphrodisiacs. In this review various plants are listed which has the aphrodisiac activity but no methodical study is accomplished or lack of scientific data. Table no -1 [20].

**Keywords-** aphrodisiac, Eugeny, ED, Oligozoospermia, vajikaran

## Introduction

Male reproductive capacity was found to be deficient in nearly 50% of infertile couples according to a study carried out by the World Health Organization in 1987. The stressful lifestyle increases the sexual dysfunction in male as well as in female also. Factors behind the decrease in the in female conception are

congenital, endocrine causes, or ovulation disorder, fallopian tube damage, endometriosis and some other numerous cases. In male Oligozoospermia, sexual, and ejaculatory dysfunction are further responsible for inability to conceive [1]. So many synthetic as well as

herbal drugs are available to treat the erectile dysfunction in male. Although some of the drawbacks synthetic 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 [2].

**Vajikarna in Ayurveda-** In Sanskrit, Vaji means horse, the symbol of sexual potency and performance thus Vajikaran means producing a horse's vigor, particularly the animal's great

capacity for sexual activity in the individual. Literally the Vajikaran is not exactly aphrodisiac but the current connotational meaning is same [21]

As per Charak Samhita

एतैः प्रयोगैर्विधियद्गुणान् वीर्योपपन्नो बलवर्णयुक्तः ।  
हर्षान्वितो वाजिवदप्रवर्षो भवेत् समर्थश्च वराङ्गनास ॥ ३० ॥

After utilizing these kind of formulations, one becomes furnish with better anatomy, potency, strength, complexion and sexually excited and potent like an 8-year-old colt [22]

वाजीकरणमन्विच्छेत्सततं विषयी पुमान् ।  
तुष्टिः पुष्टिरपत्यं च गुणवत्तत्र संश्रितम् ॥ १ ॥  
अपत्यसन्तानकरं यत्सद्यः संप्रहर्षणम् ।  
वाजीवातिबलो येन यादयप्रतिहतोऽङ्गनाः ॥ २ ॥  
भयत्यतिप्रियः स्त्रीणां येन येनोपषीयते ।  
तद्वाजीकरणं तद्धि देहस्योर्जस्करं परम् ॥ ३ ॥

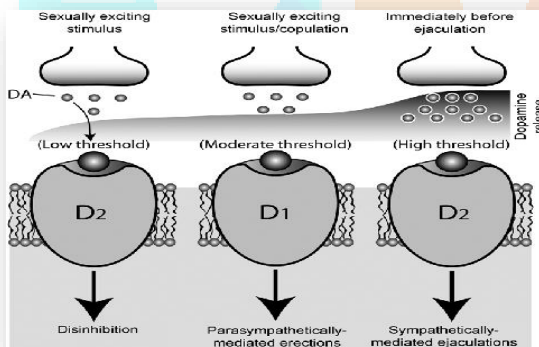


Fig.2 Brain And Neurochemical basis of sexual behaviour

“The person who is looking for sexual pleasure should turn to Vajikaran, i.e., vilification therapy regularly. The Vajikaran grants contentment, nourishment, continuity of progeny and great satisfaction. The medicines or therapy by which the man becomes capable of sexual intercourse with the woman with great strength like a colt, which endears him to women and which nourishes the body of the person is known as Vajikaran. It is best promoter of solidity and Stamina.” [23]

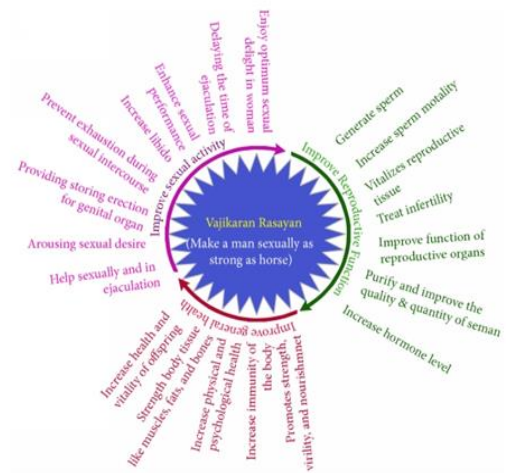


Fig. 1 Action of Vajikaran Rasayan

The main goal of Vajikaran is to produce a successful copulation for a healthy reproduction along with maximum sexual pleasure. therefore, it is considered a part hence it is taken as part of ‘eugeney.’ However, this therapy is also described under various sexual and reproductive disease i.e., Klaibya or Erectile dysfunctions, Bandhyatva or Infertility, Shukraghata Vata or azoospermia and premature ejaculation etc.

### Mechanism of Sexual Behaviour:

#### Brain and Neurochemical Basis of Sexual Behaviour

-Drugs which are used for sexuality are either affecting on the CNS(Brain) and/or on the peripheral nervous system. [fig.2] Medicines that are affecting the brain and presumably sex centres are generally attributed with an increase or decrease in sexual arousal. Drugs that affect peripheral nerves will not affect arousal directly but may affect sexual function. A bit cases, drugs action is direct and involves chemical alteration of the neurons, which governs sexual desire. Substituent, few drugs may act indirectly by altering blood flow to the sexual organs. Assumptions concerning the neurochemical basis of sexual behaviour are

derived from studies in animals, but in some cases, support has been provided by clinical studies. The transmitters include norepinephrine, dopamine, serotonin,

acetylcholine, and histamine [6]. Hypothesis suggests that sexual functions are controlled by serotonin [inhibitory] and dopamine [ excitatory]. Dopamine plays a crucial role in the central control of sexual behaviour in males [7]. Along with that it is liberal to Increase in the activity of central dopaminergic systems correlates with sexual activity and penile erection [8]. It

can also attune the activity of brain nuclei directly involved in the control of penile erection [9]. While Dopamine blockers acts as loss of libido. It has mistrusted that monoamine having pivotal role in the controlling of sexual behaviour, particularly that of dopaminergic transmission which is facilitatory to masculine activity and both dopaminergic and adrenergic receptors are involved.

### Nitric Oxide-Based Mechanism of Sexual Behaviour

Nitric oxide (NO) is an atypical regulatory molecule having the dual role as a secondary messenger/neurotransmitter. It has been implicated in diverse physiological functions [10]. [Findings so far indicate that NO may also be a major neuronal messenger [11]. In particular, it is an established physiological mediator of penile erection [12] and in the brain; NO synthase is highly in structures directly

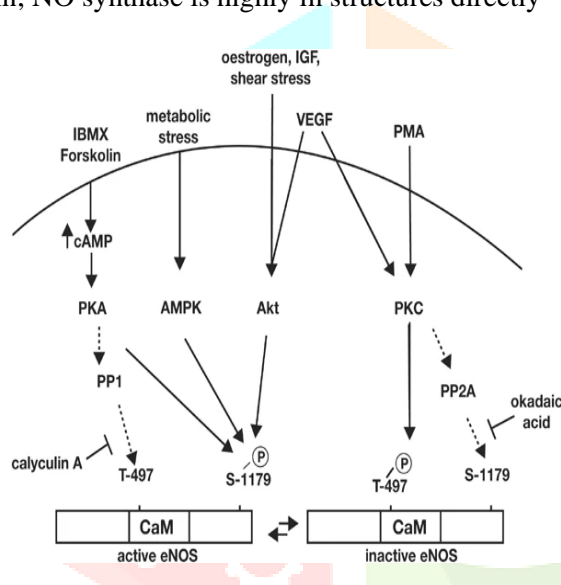


Fig.3 Signaling pathways controlling eNOS phosphorylation at Thr-497 and Ser-1179

or indirectly involved in sexual behaviour [13].

The phosphorylation of eNOS represents an important new area of investigation for the regulation of this enzyme (Figure 3) [15]. Several precise sites for phosphorylation of Enos under the influence of specific protein kinases have been identified. The phosphorylation of Enos as activated by the PI3-kinase/Akt pathway occurs at the stimulatory site human Ser-1177 (bovine Ser-1179) in the enzyme's C-terminus region, reducing the enzyme's calcium dependence, increasing the rate of electron flux from the reductase domain to the oxygenase domain, and increasing the rate of NO formation [16,17,18]. In contrast, the phosphorylation of Enos at the inhibitory site

human Thr-495 (bovine Thr-497) located in the calmodulin-binding sequence leads to deactivation of the enzyme by increasing its calcium-calmodulin dependence. [17,18,19] Importantly, since phosphorylation of Enos at Ser-1177 is accompanied by dephosphorylation at Thr-495, and vice versa, the phosphorylation and dephosphorylation reactions at the two sites are thought to be coordinated (Figure 3).

### Androgen-Based Mechanism of Sexual Behaviour-

Androgens play a very important role in the development of secondary male sexual organs such as the epididymis, vas deferens, seminal vesicle, prostate, and the penis. Along with that, androgen is important during puberty, male fertility, and male sexual function [29]. Primary androgen is testosterone which is secreted by testes and synthesised by Leydig cells. Synthesis of testosterone is stimulated by luteinizing hormone (LH). Testosterone helps in stimulation of spermatogenesis in seminiferous tubules. The testosterone- or dihydrotestosterone-receptor complex next crosses the nuclear membrane, binds to DNA, and stimulates new mRNA synthesis and, thereby, new protein synthesis. The effect of testosterone on libido may require conversion of testosterone to oestradiol in the hypothalamus. The mechanisms whereby testosterone affects muscle, bone, and the erythron do not appear to require prior molecular conversion [30].

Drugs used to treat various sexual problems are found to modify the action of neurotransmitters which could be facilitatory, inhibitory, or both. Androgens are known to influence NO production in the brain as well as in the periphery [31, 32]. NO is synthesized by the enzyme nitric oxide synthase (NOS) which plays an important role in many brain functions. NO function as a neurotransmitter and NOS is present in the regions of the brain that regulate sexual functions [33]. Interestingly, administration of testosterone to castrated male rats increases the number of NO synthase-labelled neurons in the mPOA, indicating an increase in NO synthesis [34]. NO is capable of stimulating dopamine (DA) release in the mPOA, which in turn stimulates penile erection. This mechanism may constitute one way in which androgens stimulate sexual arousal [35].

**Effect of Nicotine in male rats-** Cigarette smoke (CS) is a risk factor for developing ED on its own [24]. At up to 10 years of follow-up in the Massachusetts Male Aging Study [29], CS nearly increased the risks of developing mild or complete ED in males aged 40–70 years at baseline [25]. While the negative effects of CS

on ED are well known, the widely current animal model for ED, there have been relatively few studies using rats for the study of CS related ED [27-29]. Xie et al. demonstrated pathophysiological processes of ED in cigarette smokers are yet unknown. Passive smoking for 1 hour per day, 5 days per week for 8 weeks in rats resulted in hypertension and decreased penile neuronal nitric oxide synthase (nNOS) content compared to controls; interestingly, cavernous erectile response to electrostimulation was not significantly reduced in tobacco exposed animals compared to controls in this study.

### Nicotine preparation-

The investigation used nicotine hydrogen tartrate (95 percent Nicotine) from BDH Chemicals Ltd. in Poole, England. Each group of animals received nicotine doses of 0.5 mg/kg and 1.0 mg/kg per body weight, freshly produced in normal saline. The working solutions were kept at 4°C for no more than ten days in a foil-wrapped glass bottle.

### Animals And treatment -

Experiments were carried out on forty male and twenty-five female Sprague-Dawley rats, aged 2 to 2.5 months and weighing between 150 and 180 grammes, procured from the Animal House, University of Ibadan, Oyo State, Nigeria. The rats were placed into five equal groups and given unlimited rat chow and water. Animals were kept in a well-ventilated room at room temperature with a 12/12-hour light/dark cycle. The five groups of male rats being treated for 30 days, with the control group receiving 0.2 ml/kg normal saline 0.5 mg/kg nicotine-treated group, 1.0 mg/kg nicotine-treated group, 0.5 mg/kg nicotine-treated group but left untreated for another 30 days and 1.0 mg/kg nicotine-treated group but left untreated for another 30 days

**Test for libido-** Non-estrous untreated female rats were coupled on the 30th day at 6.00 pm to observe libido-oriented mounting behaviour. The Overtaking the copulatory position are male rats. the female rats, but without success in achieving intromission was thought to be a mount rat males from each group was carefully selected and appropriately labelled. The rodents were kept in a clear tank. Allow 15 minutes for acclimatisation. After a non-estrous female rat was brought to the wards. in to the ring There were a lot of mounts. 15 minutes of sound was captured. This procedure was likewise somewhat lengthy. done for the rehabilitation group.<sup>[35]</sup>

**Fertility studies:** For the fertility test, a total of 25 untreated fertile, prestrous female rats were employed. On the 31st day of treatment, five untreated female rats were cohabited with a male rat

from one of the five male groups, with the exception of the recovery groups, who cohabited on the 31st day of the recovery period. According to previous studies<sup>[31]</sup>, For five days, all of the animals were kept together. A vaginal plug was recognised as a sign of a successful mating and was documented on the first day of pregnancy<sup>[32]</sup>. The following formula was used to calculate a fertility test: The number of litters delivered as well as their weights were calculated.

**Collection of sperm:** The left testis was removed, along with its sperm. Epididymis is a type of epididymis. As previously described, the caudal epididymis was detached from the testis and lacerated to collect the semen on a microscope slide for study of semen characteristics<sup>[20]</sup> Analysis of sperm characteristics: The ability to move in a progressive manner was immediately tested. Two drops of warm 2.9 percent sodium citrate were applied to the squeezed sperm on a pre-warmed slide. After that, it was covered with a cover slip and examined and scored under a microscope with an x40 objective and low light<sup>[33]</sup>. Using the eosin/nigrosine stain, a viability study (% of living spermatozoa) was performed. Two drops of the stain were put to the compressed sperm on a microscope slide. The non-motile (dead) sperms absorbed the stain while the motile (living) sperms were left unstained. Using x40 microscope objectives, the stained and unstained sperm cells were counted, and an average value was recorded from which percentage viability was computed. After air drying the sperm smears on microscope slides, two drops of Walls and Ewas dye were used to assess sperm morphology. Those slides were viewed with an oil immersion microscope with an x100 objective. The aberrant sperm cells were counted and the percentage was estimated using Wyrobek and Bruce's approach<sup>[34]</sup>. The epididymis was immersed in 5 mL normal saline in a measuring cylinder, and the volume displaced was recorded as the epididymis volume. The enhanced Neubauer hemocytometer was used to count sperm under a microscope. Five Thoma chambers were used to count<sup>[35]</sup>. Statistical analysis: For each group, the findings are shown as mean SEM. The differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range Post hoc test for pairwise comparisons. For sperm abnormalities, the data was analyzed using  $\chi^2$  test. All statistical comparisons and tests were performed using ANOVA test.

## Results Effect of nicotine on semen characteristics

**Motility:** The mean percentage of live sperms in rats treated with both 0.5 mg/kg and 1.0 mg/kg body weight treated percent **Fertility Success = Pregnancy Females X 100 Mated females** was significantly decreased (P0.05) in rats treated with both 0.5 mg/kg and 1.0 mg/kg body weight treated percent **Fertility Success = Pregnancy Females X 100 Mated females**.

Table 1. Semen parameters of experimental rats treated with nicotine

| Dose                    | Motility (%)                  | Live/Dead ratio (%)           | Volume ( $\mu$ l)            | Count ( $\times 10^6/ml$ )    |
|-------------------------|-------------------------------|-------------------------------|------------------------------|-------------------------------|
| Control                 | 91.00 $\pm$ 2.00 <sup>a</sup> | 96.80 $\pm$ 1.47 <sup>a</sup> | 5.20 $\pm$ 0.00 <sup>a</sup> | 103.60 $\pm$ 3.8 <sup>a</sup> |
| 0.5 mg/kg B.W.          | 60.00 $\pm$ 6.33 <sup>b</sup> | 92.60 $\pm$ 3.33 <sup>a</sup> | 5.18 $\pm$ 0.04 <sup>a</sup> | 71.80 $\pm$ 5.20 <sup>b</sup> |
| 1.0 mg/kg B.W.          | 44.00 $\pm$ 4.90 <sup>b</sup> | 91.20 $\pm$ 6.65 <sup>a</sup> | 5.16 $\pm$ 0.05 <sup>a</sup> | 51.40 $\pm$ 5.20 <sup>b</sup> |
| 0.5 mg/kg B.W. recovery | 82.00 $\pm$ 4.00 <sup>a</sup> | 94.60 $\pm$ 2.58 <sup>a</sup> | 5.18 $\pm$ 0.04 <sup>a</sup> | 93.40 $\pm$ 6.50 <sup>a</sup> |
| 1.0 mg/kg B.W. recovery | 68.00 $\pm$ 7.48 <sup>b</sup> | 91.00 $\pm$ 3.74 <sup>a</sup> | 5.18 $\pm$ 0.04 <sup>a</sup> | 70.80 $\pm$ 3.60 <sup>b</sup> |

Values are expressed as Means  $\pm$  SEM of 8 rats per group. Means in columns with different superscript letters are significantly different  $p < 0.05$ .

There are a number of plants that boast aphrodisiac properties but lack scientific evidence, as listed below, Table -1<sup>[20]</sup>

| Sr.no | Common Name             | Scientific Name                    |
|-------|-------------------------|------------------------------------|
| 1.    | Acacia                  | <i>Acacia nilotica</i>             |
| 2.    | Ajowan                  | <i>Trichyspermum ammi</i>          |
| 3.    | American Solomon's seal | <i>Polygonatum biflorum</i>        |
| 4.    | Anise                   | <i>Pimpinella anisum</i>           |
| 5.    | Apricot                 | <i>Prunus armenical</i>            |
| 6.    | Artichoke               | <i>Cynara cardunculus</i>          |
| 7.    | Autum crocus, colchicum | <i>Medow saffron</i>               |
| 8.    | Beet                    | <i>Beta vulgaris</i>               |
| 9.    | Betal pepper            | <i>Piper bettle</i>                |
| 10.   | Black dot               | <i>Cullen coryfolium</i>           |
| 11.   | Bloodroot               | <i>Sanguinaria canadensis</i>      |
| 12.   | Benzoin                 | <i>Styrax benzoin</i>              |
| 13.   | Woodruff                | <i>Gallium odoratum</i>            |
| 14.   | Winged bean             | <i>Psophocarpus tetragonolobus</i> |
| 15.   | Unicorn root            | <i>Aletris farinosa</i>            |
| 16.   | Tuliptree               | <i>Liriodendron tuliptree</i>      |
| 17.   | Tonka bean              | <i>Diptereryx odorata</i>          |
| 18.   | Tea weed                | <i>Stachytarphata jamaicensis</i>  |
| 19.   | Syrian rue              | <i>Peganum harmala</i>             |
| 20.   | Snake plant and root    | <i>Dracaena trifasciata</i>        |
| 21.   | Scarlet poppy           | <i>Papaver rhoeas</i>              |

## Plants used to treat the Erectile dysfunction-

### 1.Acacia (*Acacia nilotica*)



### 2.Apricot (*Prunus armenical*)

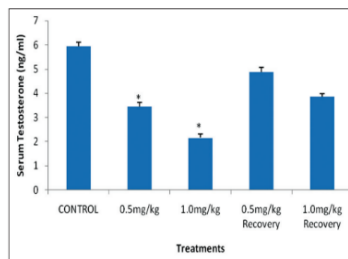


### 3.Bloodroot (*Sanguinaria canadensis*)

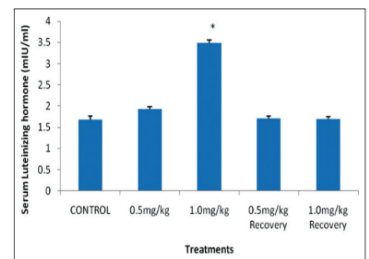


### 4.Ajowan (*Trichyspermum ammi*)

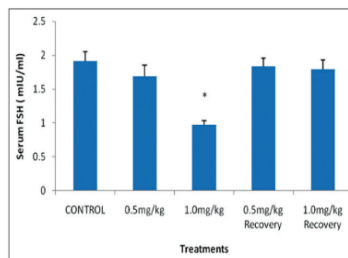


**5. Beetroot** (*Sanguinaria canadensis*)**9. Tonka Bean** (*Diptereryx odorata*)**6. Teaweed** (*Stachytarphata jamaicensis*)**Following graphs shows the nicotine effect on testosterone level** [35]

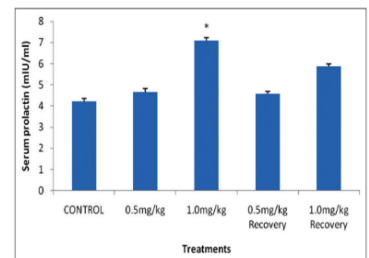
**Figure 1:** Effect of nicotine on serum testosterone level in male rats. Values are expressed as means  $\pm$  S.E.M of eight rats per group. Bar carrying the asterisk sign on each parameter are significantly different at  $P < 0.05$



**Figure 2:** Effect of nicotine on serum luteinizing hormone level in male rats. Values are expressed as means  $\pm$  S.E.M of eight rats per group. Bar carrying the asterisk sign on each parameter are significantly different at  $P < 0.05$

**7. Unicorn root** (*Aletris farinose*)

**Figure 3:** Effect of nicotine on serum follicle stimulating hormone level in male rats. Values are expressed as means  $\pm$  S.E.M of eight rats per group. Bar carrying the asterisk sign on each parameter are significantly different at  $P < 0.05$



**Figure 4:** Effect of nicotine on serum prolactin level in male rats. Values are expressed as means  $\pm$  S.E.M of eight rats per group. Bar carrying the asterisk sign on each parameter are significantly different at  $P < 0.05$

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