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In vivo evaluation of medicinal effects of *Myristica fragrans, Cinnamomum zeylanicum, Mucuna pruriens* on male murine fertility

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Abstract: Herbal remedies are used for managing different ailments including male sexual abnormalities. *Mucuna pruriens, Cinnamomum zeylanicum, and Myristica fragrans,* are some of the important herbs of these remedies for male sexual disorders. This study has been conducted to evaluate the effects of these drugs, individually and in combination on fertility parameters in mice. The study was carried out on male and female albino mice of BALB/c strain bearing weight of 20-25 g and age 12 to 13 weeks. Animals were divided into control and test batches (n=10). Drugs were given to the male mice test groups daily for 52 days by oral route and on 53rd day the fertility parameters were measured. Afterwards, histopathological analysis was also done. One-way analysis of variance (ANOVA) followed by post hoc was applied for statistical analysis. Important contrast was found in fertility parameters, including pregnancy outcome, serum testosterone, luteinizing hormone, follicle stimulating hormone, and histological examination of tested batches as compared to control. The fertility enhancing effect of the drugs were found in the tested doses used in this study in male albino mice of BALB/c strain. However further preclinical and clinical studies are necessary to determine the safety of these drugs

Keywords: Mice, fertility, testes, testosterone, LH, FSH, herbal drugs.

INTRODUCTION

Failure to reproduce is an important condition that needs attention in a community. A large number of couples face misery of infertility and its cause is a disorder of male reproductive system in a lot of cases. Male partners are accountable for more than fifty percent of this conjugal infertility and for reasons unidentified, of these, about forty percent male partners have defects in analysis of semen (Oud *et al*., 2022, Eisenberg *et al*., 2023). In different parts of the world, a huge number of people have fertility related abnormalities. According to a study from Yale's University published in 2022, it is estimated that up to 7% of men in USA are affected by infertility and 50% of fertility problems within a heterosexual couple are due to the man. However, according to WHO latest news report published 2023, around 17.5% of the adult population - roughly 1 in 6 worldwide - experience infertility (Harris, 2023). Nevertheless, according to a study conducted by the Pakistan Journal of Public Health, approximately 21% of couples in Pakistan face infertility issues. Furthermore, the same study found that male factors contributed to infertility in 37% of cases, female factors in 51% and both male and female factors in 12% of cases showing the urgent need to increase access to affordable, high-quality fertility treatment options (Ahmed *et al.,* 2020).

A vast number of wedded couples facing torment of infertility put up with persistent psychological ordeal in the community, more often than not, turn out to be the depressed and anxiety patients (Kiani *et al.,* 2023) making them inefficient in their daily lives. Moreover, idiopathic male infertility has no established treatment till date (Zheng *et al*, 2020). Hence it is a justified effort to find new herbal remedies for management of fertility abnormalities. In male patients the options for treatment of infertility, including *In vitro* fertilization and Intracytoplasmic sperm injection, have a lot of limitations such as monetary expenses and failure rates (Merchant *et al.,* 2011).

In addition, the technicalities/expertise required in the procedures renders these modalities out of reach for a vast majority of the sufferers. Therefore it is worth making an effort to find treatment of infertility with new herbal drugs as 80% of the world populations rely on herbal medicine owing to it being its cost-effective, reliable, safer therapeutic option. (Agarwal & Sekhon, 2011, https://www.webmd.com/infertility-and-reproduction/in-vitro-fertilization).

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Mucuna pruriens, *Cinnamomum zeylanicum* and *Myristica fragrans*, are good examples of plant derived therapeutic options. According to the Ayurveda *M. pruriens* is aphrodisiac and tonic apart from its other therapeutic uses. The M. pruriens seeds contains alkaloids, glycosides, reducing sugars, saponins, tannins, terpenoids, calcium, phosphorus and potassium, polyphenolic substances, protease inhibitor, phytic acid and L-dopa. The *Mucuna pruriens* seeds are one of the best sources of protein content. *M. pruriens* seeds possess various activities including anabolic, androgenic, analgesic, anti-inflammatory, aphrodisiac, cholesterol lowering, hypoglycemic, immune modulator and general debility (Divya *et al*, 2017).

Nutmeg is aphrodisiac apart from other effects It is used in tonics and electuaries. In Unani medicine, *M. fragrans* (nutmeg) has been mentioned to be of value in the management of male sexual disorders. Numerous studies have indicated that *M. fragrans* contains diverse phytochemicals such as Myristicin, Myristic Acid, Trimyristin, Elemicin, Safrole, Lignans, Neolignans, Maceneolignans etc. (https://www.colmed-alnahrain.edu.iq/upload/res/921.pdf).

The most important constituents of cinnamon are cinnamaldehyde and trans-cinnamaldehyde, thus contributing to the fragrance and to the various biological activities. It contains coumarins, acids, essential oil, fiber, tannin, mucilage and volatile contents. There are many authentic scientific research articles which have scientifically proved its beneficial actions in various diseases. Cinnamon possesses various therapeutic values like digestive, carminative, neuroprotective, antiseptic, diaphoretic, hypoglycemic, aphrodisiac, Anti-carcinogenic, Anti-microbial and Anti-lipidaemic. According to my literature review cinnamon is a valuable source in medicinal field. It acts as many characters and used for unlimited diseases which can be cured. This will help Pharmaceutical manufactures to synthesis of new drugs and of physicians to practice new remedies (https://www.siddha.jfn.ac.lk/wp-content/uploads/2022/09/PostConference_eMagazine.pdf)

MATERIALS AND METHODS

Animals and environmental conditions

Permission from Board of Advanced Studies and Research was taken to conduct the present study at Department of Pharmacology, University of Karachi. The animals selected were adult male and female albino mice of BALB/c strain whose weight ranged from 20-25 g and age 12 to 13 weeks. Animal house of Pharmacology department, University of Karachi, was utilized for breeding of mice and further study protocol. For one week time period, the mice were kept for habituation and their wellbeing, physical condition, and fitness were assessed before the start of actual research protocol through general observations. Stable surrounding temperature (22±2^{o}C) and environmental condition of humidity (fifty to sixty percent) were managed to maintain for the animals during study period. In the laboratory, the typical diet was prepared in the laboratory as per National Institute of Health description (http://www.nap.edu/catalog/4758.html).

All animals were given this diet and a free access to water. Fifty healthy albino mice each male and non-pregnant females bearing close identical age and weight were engaged in the study. Mice were allocated to control and treated groups as follows in random order to minimize bias.

Animal groups

Animals were divided into following 5 batches (control and treated), each batch having ten mice (table 1).

Crude extract preparation and collection

The drugs were identified by Dr. Mohtasheemul Hasan, Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi after being purchased from local market. [Herbarium number: Myritica fragrans, Houtt., Fruit, MFF-03-10; *Cinnamomum zeylanicum* L., Bark, CZB-01-10; *Mucuna pruriens* (L.) DC., Seed, MPS-02-10]. To clean the seeds and remove dust, the *Mucuna pruriens* seeds were washed with tap water two times then washed again with distilled water. Then about 12 days were given for the seeds to dry up in shade. Then the seeds were crushed to coarse material. Similarly, *Cinnamomum zeylanicum* bark and *Myritica fragrans* seeds were also rinsed, dried up and then ground up to a coarse texture. Then methanol was used to immerse the drugs for 8 weeks. During that time, intermittently the material was shaken gently. After that the solutions were filtered and then rotary evaporator (Buchi Rotavapor R-300 rotation: 35 ppm/rpm) was used for evaporation at 40^{o}C, followed by drying of crude extract (Najmus-Saqib *et al.*, 2009, Ibrahim & Kebede, 2020). Extracts were covered and stored in cool dry place in refrigerator till further use.

Drug administration

Suspension of crude extract of drugs with 0.5% carboxymethylcellulose were administered orally to male mice for a time period of 52 days in the following doses:

Drugs	mg / Kg of body weight
Mucuna pruriens	200 (Suresh *et al*, 2010)
Cinnamomum zeylanicum	500 (Priyanga *et al*, 2013)
Myritica fragrans	500(Tajuddin *et al*, 2005)

Control group of male mice were given the 0.5% carboxymethylcellulose in the amount that was comparable to the amount of the drugs given to treated groups.

Fertility parameters
Pregnancy result, serum hormonal parameters (luteinizing hormone and follicle stimulating hormone), and histology of testes were the fertility parameters studied.

Protocol for Pregnancy result
Before mating with males, the female mice were kept separately for a time period of 30 days to make certain that they were not pregnant before study period. Male mice were given the drugs for 45 days. After that each male mouse kept randomly with a receptive female mouse for next seven days. The female mice which are not receptive were substituted with receptive ones. Drugs were given to male mice throughout this period of 7 days also (with a total period of drug administration of 52 days). Then females were kept separate from male mice. The numbers of female mice that became pregnant were documented in each batch, and after parturition, the pups born were documented in each batch (Morrissey *et al.*, 1988, Gaskill & Pritchett-Corning, 2015).

Serum hormonal parameters assessment
1 ml of blood was taken from the heart by syringe from each male animal under ketamine anesthesia to do serum hormonal assessment after the 52 days when protocol ended. Then the blood samples were centrifuged without delay to get the serum. Serum hormonal assessments were performed, using reagent kits with standard environmental and other conditions. Siemens Immulite 1000 Immunoassay system was used to evaluate the serum testosterone (LOT Number 52983063), luteinizing hormone (LOT Number 2994701) and follicle stimulating hormone (LOT Number XE2023) (Shaikh *et al.*, 2009, Bohn *et al*, 2021).

Histopathology
After getting the blood for hormonal analysis, the male mice were sacrificed after cervical dislocation to get testes for histopathological study. Autopsy was done to observe all important features. 10% buffered formalin was used immediately to immerse testes. Weight of the testes was taken after two days of fixation. Blocks of tissue were prepared, processed and the cut sections were obtained for study under microscope (Bancroft and Stevens 1990, Bancroft and Layton, 2012).

Slide formation
An automatic tissue processor (Gilford 101 system) was used to process all the tissue blocks. Tissue blocks embedded in paraffin were formed. Rotary manual microtome was used to cut 3 micron thick sections of tissue. The sections of tissue were taken to glass slides and dried overnight at 37ºC in incubator (Bancroft and Stevens 1990, Bancroft and Layton, 2012).

Staining
Two Xylene filled boxes were utilized for immersing slides for three minutes to be dewaxed. Then rising grade of alcohol were used to immerse slides for one minute. Then slides were rinsed with distilled water. After that hematoxylin and eosin were used to stain the slides. Slides were examined under microscope after staining (Bancroft and Stevens 1990, Bancroft and Layton, 2012).

STATISTICAL ANALYSIS

Serum hormonal parameters were analyzed as Mean ± SEM. Statistical scrutiny of data was performed on SPSS version 21. One way ANOVA followed by post hoc analysis was used. In comparison to control, values of $p< 0.05$ were considered as significant and $p<0.01$ was taken as highly significant.

RESULTS

Fertility parameters
Fertility enhancing ability were assessed by comparing (a) pregnancy results; (b) serum testosterone, Luteinizing Hormone and Follicle Stimulating Hormone levels and (c) histology of testes.

(a) Pregnancy Results
The pregnancy results shown in table 2, that is how many pups born after giving different drugs for fifty-two days in comparison to control group. All drugs treated groups demonstrated more number of pups born in comparison with the control group. The largest difference was in combination batch (8.1 + 0.28) in contrast to control batch (5.4 + 0.16), with p value of 0.002. Then there was difference in batch treated with *Myristica fragrans* (7.9 + 0.72), with p value of 0.005. Then in batch treated with *Mucuna pruriens* (7.7 + 0.65), with p value of 0.012. Smallest difference was in batch treated with *Cinnamomum zeylanicum* group (7.5 + 0.31), with p value of 0.026. Difference in pregnancy results was highly significant in batches treated with combination of three drugs and *Myristica fragrans*, while it was significant in batches treated with *Mucuna pruriens* and *Cinnamomum zeylanicum*.

Serum hormonal measurements
The table 3 shows the serum hormonal measurements that is serum testosterone, LH and FSH after 52 days period of drugs administration versus the control batch. There was raise in testosterone in all the batches given drugs in contrast to the control batch. Most raise was in the batch treated with combination of drugs in contrast to control batch. Then there was raise in the batch treated with *Myristica fragrans*, and then in the batch treated with *Mucuna pruriens*. The smallest raise was in the batch treated with *Cinnamomum zeylanicum*. The raise in testosterone was deemed to be significant statistically, while in the batch of combination of drugs it was highly significant versus the control batch. Similarly, there was raise in luteinizing hormone in all the batches treated with drugs in contrast to the control batch.

Table 1: Batches of Mice

Batches	Administered Drugs
A	Control; Carboxy methylcellulose (CMC) 0.5%
B	*Myritica fragrans*
C	*Cinnamomum zeylanicum*
D	*Mucuna pruriens*
E	*M. fragrans + C. zeylanicum + M. pruriens*

Table 2: pregnancy results in drug treated and control batches after 45 to 52 days drugs treatment

Animal Batches (n=10)	Number of pups born Mean values + S.E.M. (p value)
Control	5.4 ± 0.16
Mucuna pruriens	7.7 ± 0.65 (0.012)
Cinnamomum zeylanicum	7.5 ± 0.31 (0.026)
Myristica fragrans	7.9 ± 0.72 (0.005)
Combination	8.1 ± 0.28 (0.002)

Combination = *Myristica fragrans + Cinnamomum zeylanicum + Mucuna pruriens*

Table 3: serum hormonal measurements in control and treated batches after fifty two days drugs dosing

Animal Batches (n=10)	Parameters		
	Testosterone (ng/mL) Average values ± S.E.M (p value)	LH (mIu/mL) Average values ± S.E.M (p value)	FSH (mIu/mL) Average values ± S.E.M (p value)
Control	4.04 ± 0.27	3.5 ± 0.34	2.53 ± 0.37
Mucuna pruriens	6.11 ± 0.56 (0.027)	5.6 ± 0.41 (0.031)	4.49 ± 0.52 (0.035)
Cinnamomum zeylanicum	6.02 ± 0.46 (0.039)	5.57 ± 0.46 (0.034)	4.46 ± 0.38 (0.040)
Myristica fragrans	6.3 ± 0.5 (0.013)	5.73 ± 0.63 (0.019)	4.61 ± 0.62(0.023)
Combination	6.5 ± 0.52 (0.005)	6.23 ± 0.54 (0.002)	5.07 ± 0.39 (0.003)

Combination=*Mucuna pruriens + Myristica fragrans + Cinnamomum zeylanicum*

Most increment was in the batch treated with combination of drugs in contrast to control. Then there was increment in the batch treated with *Myristica fragrans* and then in the batch treated with *Mucuna pruriens*. The smallest raise was in the batch treated with *Cinnamomum zeylanicum*. Raise in luteinizing hormone was determined to be significant statistically, while in the batch of combination of drugs it was highly significant versus the control batch. Similarly, serum follicle stimulating hormone levels went up in all the batches treated versus the control batch. Most raise was in the batch treated with combination of drugs when compared to control. Then there was raise in the batch treated with *Myristica fragrans* and then in the batch treated with *Mucuna pruriens*. The smallest raise was in the batch treated with *Cinnamomum zeylanicum*. All these changes in serum follicle stimulating hormone levels were found to be significant statistically, while in the batch treated with combination of drugs it was highly significant as judged against the control batch.

Testes Microscopy

Under microscopic examination of testes of all treated batches of mice demonstrated the raise number of spermatozoa, spermatids, primary and secondary spermatocytes and spermatogonia (typical fried egg appearance) and interstitial cells in the interstitial spaces in the testes compared to control batch (figs. 1 & 2).

DISCUSSION

So far there is no reported research that used pregnancy outcome as a measure of success for the tested herbal drugs. We discovered that increased rate of pregnancy was highly significant in mice treated with combination of drugs and with *Myristica fragrans*, while in batches treated with *Mucuna pruriens* and *Cinnamomum zeylanicum* it was significant as compared to the control batch.

In this study, the rise in serum levels of testosterone, luteinizing hormone and follicle stimulating hormone in the batch treated with combination of drugs was highly significant, while in the batches treated with *Myristica fragrans*, *Mucuna pruriens* and *Cinnamomum zeylanicum* the rise in serum levels of these hormones was found to be significant statistically as compared to the batch of control mice. These results are also endorsed by microscopic examination of testes which exhibited seminiferous tubules with regular architecture and basement membrane intact, more spermatozoa, spermatids, primary and secondary spermatocytes, more spermatogonia (typical fried egg appearance) in all the batches of animals treated by drugs.

Similarly *Mucuna pruriens* is reported to have enhancing effect on fertility in several studies. In rats, raised numbers of spermatozoa are caused by bark extract of

Mucuna pruriens. Similarly, *Mucuna pruriens* exhibited sustained androgenic and sexual activities in adult male normal rats (Suresh *et al.,* 2009; Suresh *et al.,* 2010). Likewise there was enhanced number and motility of spermatozoa with raised testosterone levels after treatment with oral suspension of ethanol extract of seeds of *Mucuna pruriens* in adult albino male rats of Wistar strain. The sexual function activity was significantly enhanced including sexual behavior before coitus, behavior during mating, potency and libido at dosage of 200 mg of *Mucuna pruriens*. Sexual stimulating effect of *Mucuna pruriens* involves a sound coordination of vascular, endocrine and neuronal systems (Suresh *et al.,* 2009).

Action at hormonal level may be related with the increased spermatogenic effect with raised sperm count and motility. Raised testosterone levels by *Mucuna pruriens* could be responsible for total sexual behavior improvement (McGinnis *et al.,* 1989, Choowong-In *et al*, 2022). Aphrodisiac effect through dopaminergic pathway may be related with the increased L-Dopa level in *Mucuna pruriens* (Nagashayana *et al.,* 2000). Increased spermatogenesis due to positive effect on Hypothalmic-Pituatory-Testicular axis is correlated with the increased activity of dopaminergic system by L-DOPA of *Mucuna pruriens* (Herberg and Rose 1990, Sriraman *et al.,* 2003, Suresh *et al.,* 2010). Hormones of adrenal glands and the autonomic nervous system control the steroid hormones synthesis and sperm production. Any abnormality of autonomic nervous system and secretion of adrenal hormones may affect the serum levels of testosterone, gonadotropins (follicle stimulating hormone and luteinizing hormone), and luteinizing hormone releasing hormone (Qiu *et al*, 2023). In the brain, dopaminergic neurons activity is known to affect the number of sperms, sperm motility, and fertility. Thus fertility augmenting effect of *Mucuna pruriens* may be attributed to its content of L-Dopa. In brain, increased level of dopamine not only increases the levels of plasma testosterone but also increases the sexual drive, enhanced sexual performance, and activation of sexual behavior (Melis *et al*, 2022).

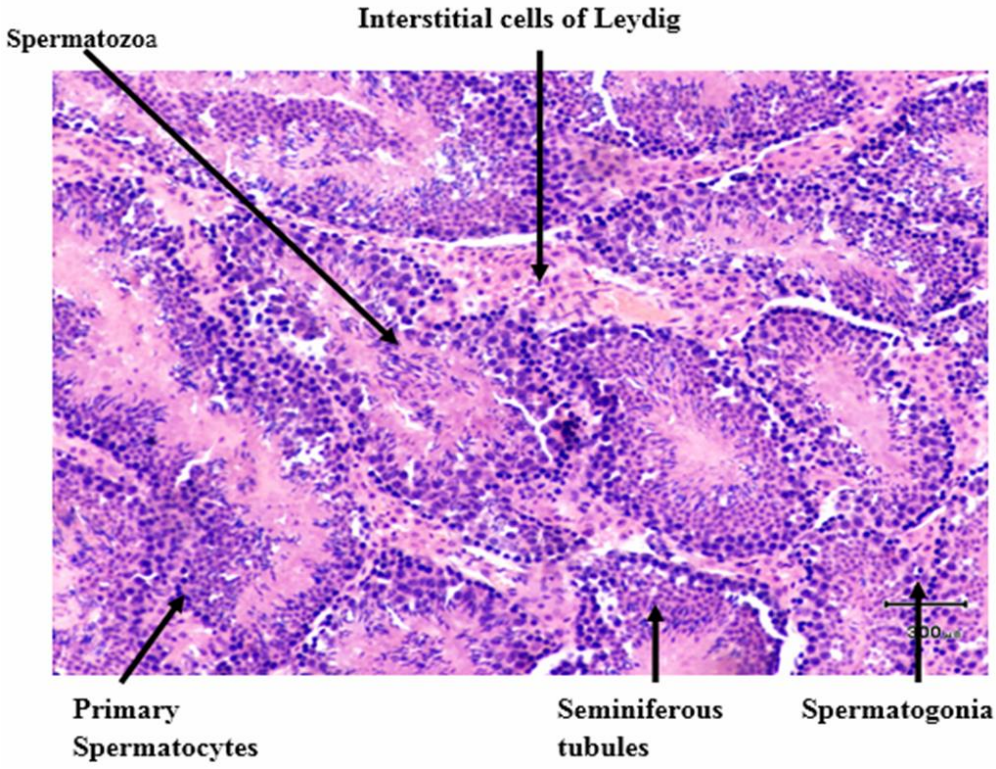
In another study, increase motility and concentration of sperm was seen in all the male patients with infertility in contrast to control after 3 months oral treatment with 5 g of seeds powder of *Mucuna pruriens* every day with milk. Some central effect may be implicated in the aphrodisiac action of *Mucuna pruriens* with restoration and rejuvenation of sexual power (Suresh & Prakash, 2012). Antioxidant and neuro-stimulatory effects may also be related to it. *Mucuna pruriens* contains many bioactive constituents like flavonoids, alkylamines, coumarines, and alkaloids which may be responsible for its antioxidant effect. Methanol extract of *Mucuna pruriens* seeds is reported to have strong antioxidant activity. In men with infertility, there is involvement of lipid peroxidation, a free radical mediated process. In men with infertility, the raised lipid peroxide in seminal plasma is decreased by *Mucuna pruriens*. Copper, iron, magnesium and zinc contained in *Mucuna pruriens* have a significant positive impact on fertility and spermatogenesis (Ahmed *et al.,* 2008).

One of the different aspects in the pathogenesis of male infertility is involvement of free radicals (Rajeshwar *et al.,* 2005). Herbal drugs like *Mucuna pruriens* have invigorating and aphrodisiac effects which can restore the sexual power. *Mucuna pruriens* exhibits antioxidant effects (Shukla *et al.,* 2010). In male rats, negative changes due to older age were reported to be reverted by *Mucuna pruriens* (Suresh *et al.,* 2010). Oxidative stress is directly or indirectly involved in reproductive dysfunction and infertility in advance age in male animals and humans (Levy *et al.,* 1999, Feldman *et al.,* 2000) and this can be reverted by the augmented antioxidant systems due to *Mucuna pruriens* (Rajeshwar *et al.,* 2005, Ahmed *et al.,* 2008, Shukla *et al.,* 2010)

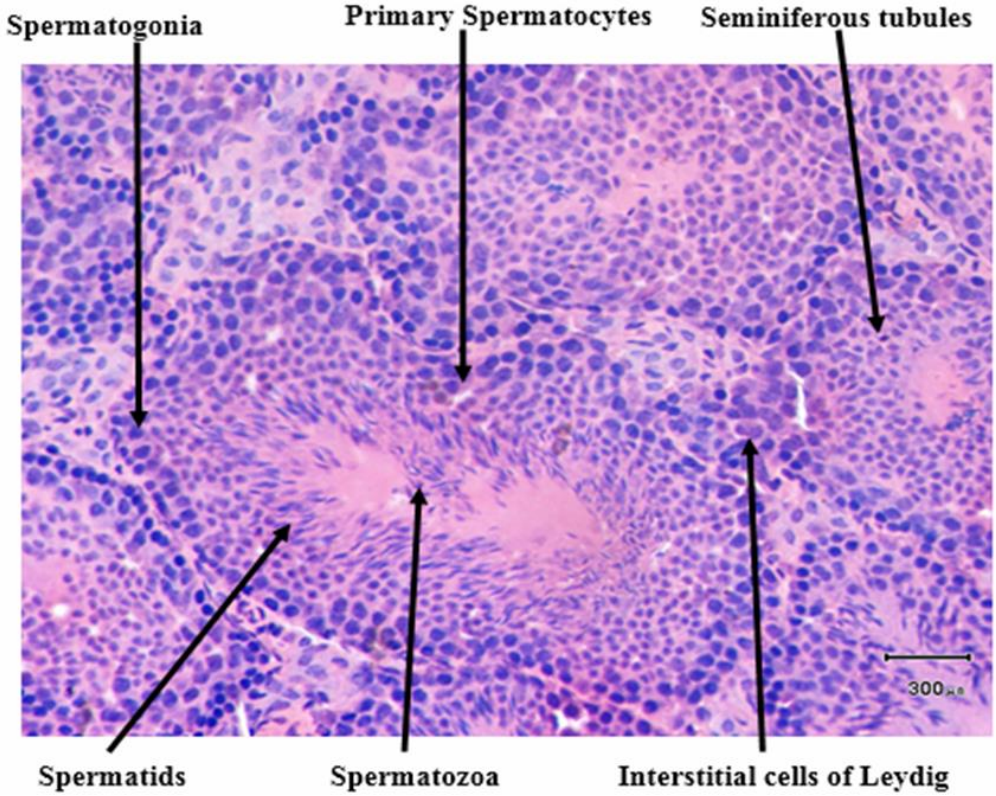
Free radical mediated damage is a well recognized factor in many diseases and among these is infertility in males because of increasing age (Vaidya *et al.,* 1978). In these diseases, *Mucuna pruriens* has clinical effectiveness due to its action on lipid peroxidation. This effectiveness depends on duration of treatment and its dose. Elimination of the free radicals produced due to interaction of iron and catecholamine, or action on the nervous system are two possible reasons for the protective effect of *Mucuna pruriens* against infertility (Chukwudi *et al.,* 2011).

High levels of lipid peroxide cause peroxidation of polyunsaturated fatty acids of sperm cell membrane that affect the anatomy and physiology of sperms. Many bioactive constituents of *Mucuna pruriens* such as coumarins, flavonoids, alkylamines, alkaloids, and others are related to its increase antioxidant activity. Stimulation of hypothalamus and forebrain by *Mucuna pruriens* content of Levo dopa and its metabolite dopamine is responsible for gonadotropin releasing hormone secretion (Vermes *et al.,* 1979). This results in anterior pituitary gland stimulation that causes increase luteinizing hormone and follicle stimulating hormone secretion. This in turn stimulates interstitial cells of Leydig in testes that results in stimulation of synthesis and secretion of testosterone. Sperms formation and production is controlled by combined working of hypothalamus and anterior pituitary gland. *Mucuna pruriens* also plays a role in facilitation to male genital system to function properly, contraction of seminal vesicles, and transport of sperms (Shukla *et al.,* 2010).

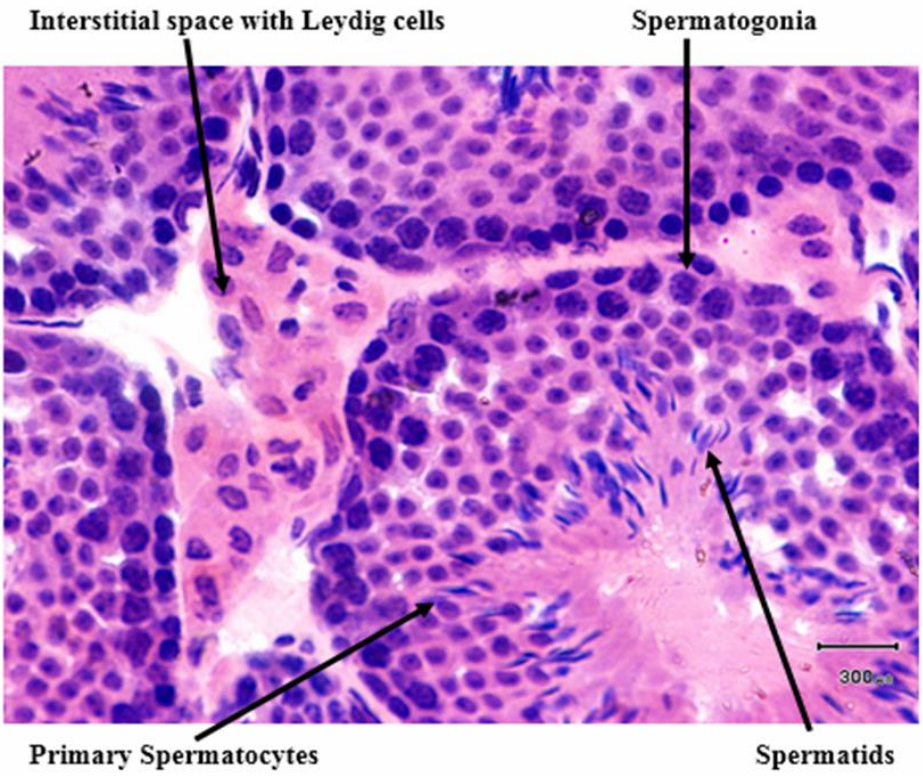
Cinnamomum zeylanicum is reported to have many roles traditionally including as an aphrodisiac (Shah *et al.,* 1998) and in the treatment of impotence (Barceloux, 2009).



(1a) (photograph 100X): Seminiferous tubules illustrate regular arrangement and basement membrane is intact. Lumen shows Spermatozoa. Architecture appears normal. Tubules seem normal with active spermatogenesis. Interstitium also appears with Interstitial cells of Leydig scattered around.

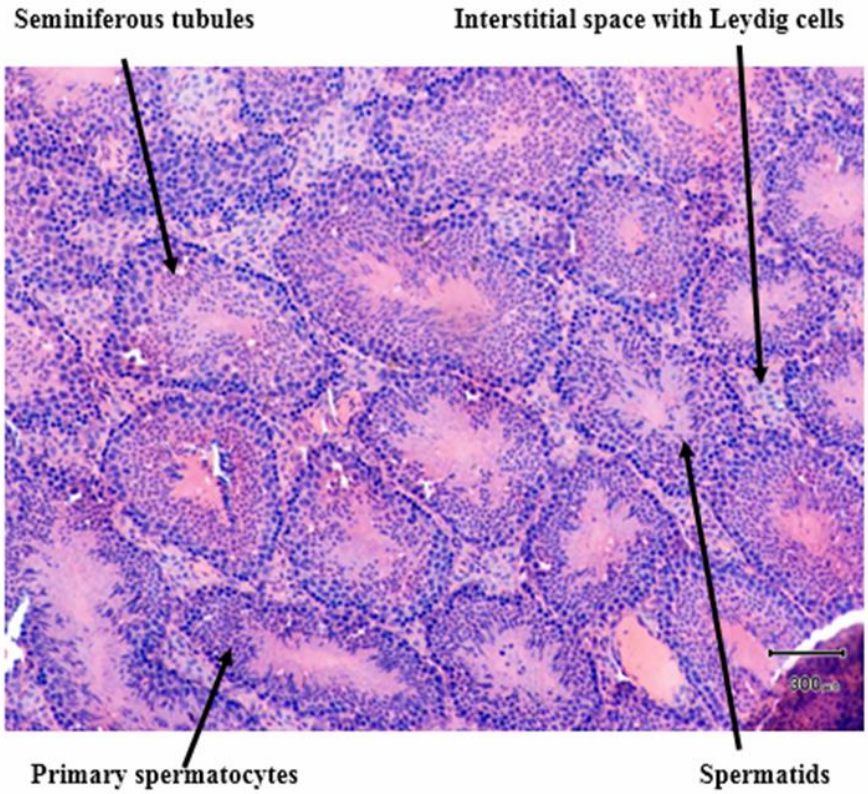


(1b) (photograph 200 X): Seminiferous tubules illustrate regular arrangement and basement membrane is intact. Spermatozoa, spermatids, primary spermatocytes and Spermatogonia with typical fried egg appearance, are seen.

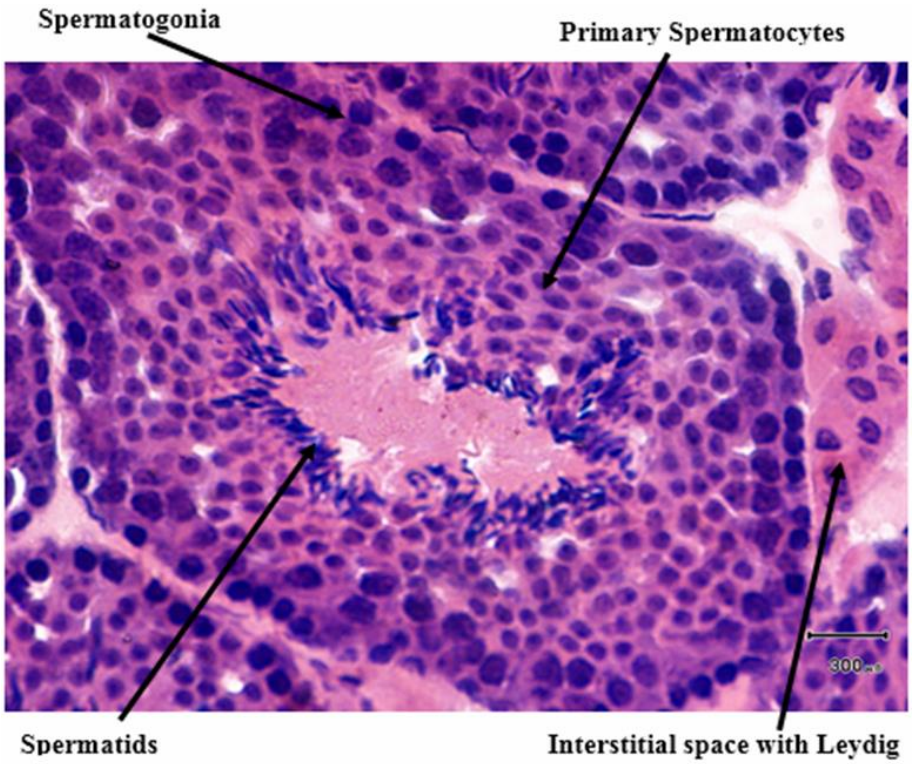


(1c) (photograph 400 X): Interstitial cells with nuclei of normal diameter in interstitial space.

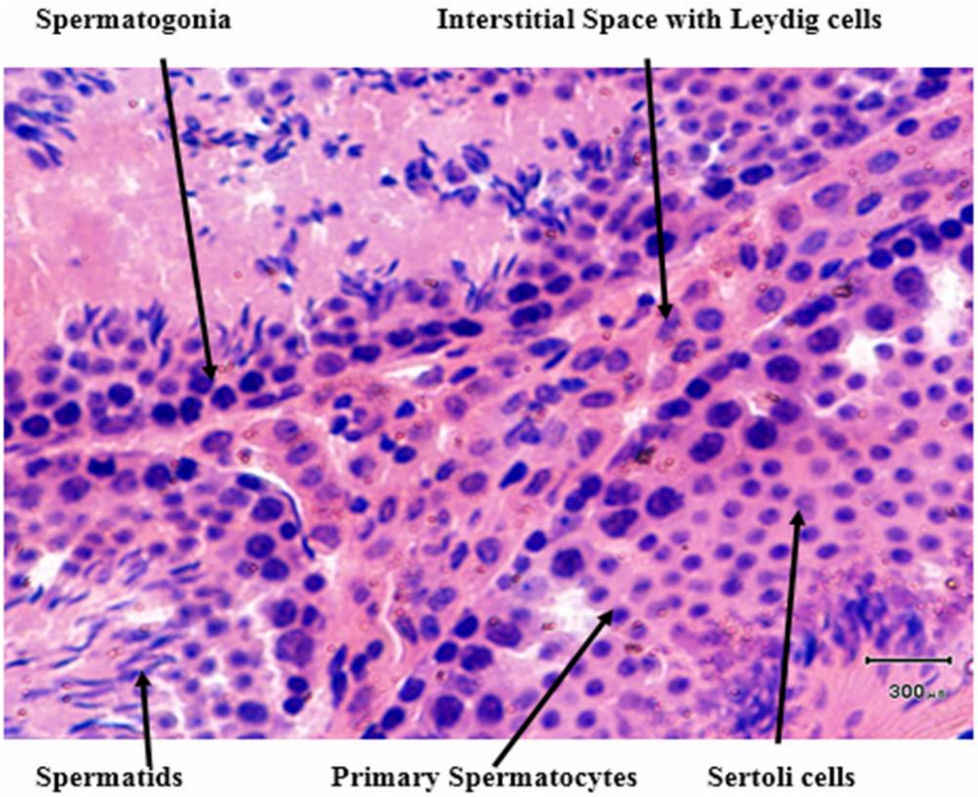
Fig. 1: Histological analysis of testis of mouse from control batch



(2a) (photograph 100X): Seminiferous tubules illustrate regular arrangement and basement membrane is intact. Lumen shows more Spermatozoa in contrast to mouse of control batch.



(2b) (photograph 200X): Seminiferous tubules illustrate regular arrangement and basement membrane is intact. More Spermatozoa, spermatids, primary and secondary spermatocytes and Spermatogonia (typical fried egg appearance), are visible in contrast to mouse of control batch.



(2c) (photograph 400X): In interstitial space, there are more interstitial cells in contrast to mouse of control batch.

Fig. 2: Histological analysis of testis of mouse from treated batch

Many herbal drugs used as an aphrodisiac and as semenogogues contain *Cinnamomum zeylanicum* as an important component. It is reported that *Cinnamomum zeylanicum* produced increased weight of the testes, caudae epididymis, and seminal vesicles, possibly due to enhanced serum hormone level in male mice as compare to control after administration orally for ninety days in a dose of 100mg /kg /day. There was also increased number of sperms and increased sperm motility (Shah *et al.*, 1998).

Similarly, follicle stimulating hormone and luteinizing hormone levels, and count of sperms and primary spermatocytes are reported to be increased by *Cinnamomum zeylanicum* 200 and 400mg/Kg body weight. 50 and 100mg /Kg body weight. *Cinnamomum zeylanicum* increases serum testosterone level. 20 days treatment with *Cinnamomum zeylanicum* in male mice demonstrated positive effect due to hormonal changes in reproductive system and pituitary-gonadal axis as compared to the control (Modaresi *et al.,* 2009).

In conformity with our research, few works reported the fertility augmenting effect of *Myristica fragrans* (Tajuddin *et al.,* 2005). Significantly improved mating performance and the mounting behavior was noted in normal adult male Swiss mice after administration of 500 mg / Kg body weight of 50 % ethanol extract of *Myristica fragrans.* Augmented sexual behavior was produced by increased serum testosterone level. Effect of drugs on neurotransmitter levels or their actions at the cellular level also affects the sexual behavior. Sexual enhancing effect of *Myristica fragrans* may be contributed by its stimulant effect on nervous system. Improved sexual behavior by *Myristica fragrans* may be contributed by its effect of increased blood flow in the body (Tajuddin *et al.,* 2003, Tajuddin *et al.,* 2005).

Likewise, after oral administration of 500 mg / Kg the extract of *Myristica fragrans,* the 3 months old male albino Wistar strain rats exhibit significant increase of sexual activity. It noticeably increased the mounting frequency, intromission frequency, intromission latency, and caused striking lessening in the mounting latency and post ejaculatory interval. It also greatly enhanced the erections and other aphrodisiac parameters. It implies that *Myristica fragrans* has sexual excitement effect, escalating the libido and potency, which might be attributed to its nervous stimulating effect. Phytochemical analysis of extract demonstrated the presence of amino acids, alkaloids, sterols, and phenols. Consequently, the increased sexual function effect of the *Myristica fragrans* might be linked to the presence of these chemicals (Tajuddin *et al.,* 2005).

Myristica fragrans has antioxidant effects. *Myristica fragrans* showed strongest protection in the deoxyribose assay. *Myristica fragrans* improved the stability of oils (corn, olive, sunflower and olive) and fats (butter and margarine) against oxidation (110°C). In TEAC assay, *Myristica fragrans* had more antioxidant effect than BHT (butylated hydroxytoluene). The phenylpropanoid compound extracts derived from *Myristica fragrans* exhibited antioxidant activity (Murcia *et al.,* 2004). The aqueous extract of fresh nutmeg mace contain lignans which demonstrated radioprotective, antioxidant, and immunomodulatory effects in the mammalian cells (Checker *et al.,* 2008).

Nutmeg seed contain monoterpenoid rich extracts like 4-allyl-2,6-dimethoxyphenol, alpha-terpineol and terpinene-4-ol, which have immense antioxidant effects. The aril part of *Myristica fragrans* has ability to hinder superoxide radical scavenging activity and lipid peroxidation in rats due to its antioxidant effect (Yadav and Bhatnagar, 2007). In mice, prior treatment with *Myristica fragrans* effectively protected against biochemical hazards of radiation, like decreased lipid peroxidation and acid phosphatase level, and parallel increase in liver alkaline phosphatase and glutathione activity (Sharma and Kumar, 2007).

CONCLUSION

With this study, we present scientific justification for the traditional use of *Myristica fragrans, Cinnamomum zeylanicum, and Mucuna pruriens* in the treatment of male fertility abnormalities. There is scope for further research on the role of these drugs in enhancing the sexual activity, the detection of their active ingredients, and the explanation of mechanism of their action. Thorough exploration is required to establish whether these desired effects are due to the crude form of drug or because of their fractional ingredients described in phytochemical research. Whether these herbs are efficacious and safe in various preparations needs further preclinical research and research on human volunteers and patients with sexual disorders.

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