MALE SEXUAL BEHAVIOR IMPROVING EFFECT OF LIPID BASED EXTRACT OF *MUCUNA PRURIENS* IN RATS

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Summary

Present work emphasizes on the extraction of *Mucuna pruriens* (Linn.) DC. (MP) using hydrophilic lipids Gelucire® to achieve better extractability and to improve its therapeutic efficiency in terms of sexual behavior in male rats. The lipid extract of *Mucuna pruriens* (LEMP) was compared with conventional aqueous, ethanol:water (1:1), and methanolic extract of MP (AEMP, EWMP and MEMP respectively) for its L-DOPA content, the major constituent of MP using HPTLC. The extracts were also compared for their effects on sexual behavior in male rats. All the standardized extracts of MP were administered orally at a dose of 75mg/kg body wt. to different groups. Sildenafil (5mg/kg body wt.) was administered intraperitoneally to the standard group. Mount frequency (MF), intromission frequency (IF), mount latency (ML), intromission latency (IL), ejaculation latency (EL), post ejaculatory interval (PEI), self-genital grooming and licking were the factors evaluated during sexual behavior study. Results indicate that LEMP achieved maximum amount of L-DOPA along with other constituents as compared to that of the conventional extracts (AEMP, EWMP and MEMP). The LEMP have improved characteristics, which are required for further processing of an extract. Moreover LEMP showed better enhancement in the sexual behavior of rats as compared to the other extracts of MP. The study concludes that the proposed lipid based extraction method for MP is rapid and gives better extractability with respect to L-DOPA. The application of lipid based extraction method has also enhanced therapeutic effect of MP in improving male sexual behavior in animals.

Keywords: *Mucuna pruriens*, Gelucire®, L-3,4 dihydroxyphenylalanine (L-DOPA), Sexual behavior.

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Introduction

Mucuna pruriens (Linn.) DC. (MP) also known as Velvet bean or Cowhage is an annual herbaceous twining belonging to the family Leguminosae. Traditionally, it has been used as an aphrodisiac for male virility, as a nerve tonic for disorders of nervous system. The seeds of MP have been proved to be effective in Parkinson's disease (Hussain and Manyam, 1997). The MP is also reported to have antineoplastic, antioxidant, antidiabetic, antimicrobial, analgesic and anti-inflammatory activities (Sathiyanarayanan and Arulmozhi, 2007). The seed powder of MP helps in reducing stress, increases secretion of semen and acts as a restorative and invigorating tonic or an aphrodisiac (Shukla et al., 2007). Moreover MP regulates steroidogenesis and improves semen quality in infertile men (Shukla et al., 2008).

L-DOPA is the major constituent of MP seeds. The other chemical constituents include tetrahydroisoquinoline alkaloids, proteins, amino acids, carbohydrates, fats, minerals, lecithins, saponins, etc (Sathiyanarayanan and Arulmozhi, 2007). L-DOPA and the other constituents of the MP seeds have been proved to be effective in improving the sexual performance of male albino rats (Kumar et al., 1994). The L-DOPA has also been reported to enhance the libido and thereby improving the sexual behavior (Harvey et al., 1998).

Several attempts have been made by various researchers for the extraction of L-DOPA and other bioactives from seeds of MP using different solvents and different techniques. L-DOPA and other polar components were extracted using different solvents such as water under SO_2 protection, water-ethanol (1:1) under ascorbic acid protection, n-propanol and methanol. The maximum yield of L-DOPA and other bioactives was reported in water-ethanol (1:1) extract. Extraction with chloroform in basic medium reported to achieve maximum yield of non-polar nitrogenous substances from the seeds of MP (Misra and Wagner, 2007). L-DOPA was isolated from seeds of MP by Guggenheim's method using water containing 0.5% acetic acid and SO_2 (Damodaran and Ramaswamy, 1937). Isolation of alkaloids mucunine and mucunadine from MP seeds was reported. (Mehtha and Majumdar, 1944). Alkaloids prurienine and prurienidine were also extracted from MP seeds (Mazumdar and Zalani, 1953). L-DOPA recovery from MP seed using hot water extraction and ion exchange procedure was reported (Daxenbichler et al., 1972). Indole alkylamines were isolated from MP (Bhattacharya et al., 1971). The protein fractions were extracted from MP using ethanol and alkaline substances like sodium chloride, sodium hydroxide (Yemisi and Kayode, 2007).

The correct choice of solvents and use of heat and / or agitation to increase the solubility of the desired phytoconstituents and improve the process of mass transfer forms the basis of conventional methods involving solvent extraction for plant materials. (Mandal et al., 2007). The conventional techniques require longer extraction time, different organic solvents to achieve better extraction of relatively water-insoluble constituents. The extracts form sticky mass creating major hurdle in further processing for developing of extract in to formulations. The literature reveals that there is a need of a simplified and faster method for extraction of phytoconstituents giving better yield and avoiding or

eliminating use of organic solvents. The present work focuses on the use of well-defined lipids Gelucire® for the extraction of L-DOPA and other bioactives from the seeds of MP to achieve better extractability without using organic solvents and produce an extract with improved qualities desired for formulation development.

Gelucire® are inert semisolid waxy material, ampiphilic in nature and have defined composition. These semi synthetic glycerides have proved their ability to enhance the solubility and bioavailability of various poorly water-soluble drugs (Angust et al., 1997; Barkera et al., 2003; Gershanik and Benita, 2000). These are available in different grades like 44/14, 43/01, 50/13, 39/01, 33/01 depending upon the drop point and HLB value respectively of the lipid material (Chambin and Jannin, 2005). To utilize the property of Gelucire® for achieving better extractability an attempt was made to prepare MP extract using different grades of Gelucire® lipids. This lipid extract of MP (LEMP) was compared with the conventional AEMP, EWMP, MEMP for its L-DOPA content using HPTLC analysis. Further these extracts were compared for their effects on sexual behavior in male rats.

Materials And Methods

Plant material

Dried *Mucuna pruriens* seeds were procured from Tembe Traders; Pune, India. The plant materials were identified and authenticated by Botanical Survey of India; Pune, India. [No.BSI/WC/Tech/2008/1061].

Chemicals

The L-DOPA i.e. L-3, 4 dihydroxyphenylalanine was procured from Sigma-Aldrich Co. Sildenafil was supplied as a gift sample by The Varma Pharmacy Pvt. Ltd. Pune. Progynon® Injection (estradiol valerate) and Fetaron® Injection (hydroxy-progesterone caproate) were procured from local suppliers, Pune, India. Different grades of Gelucire® (44/14, 43/01, 50/13) were generously gifted by Gattefosse, France and supplied by Colorcon Asia Pvt Ltd; Mumbai, India. Ascorbic acid was purchased from E.Merck India Ltd, Mumbai, India. All the chemicals used were of analytical grade, from Merck Co. Ltd.

Animals

Male Wistar rats ranging 200-250 gm and female Wistar rats ranging 150-200 gm were procured from Raj Biotech Ltd; Pune, India. Animals were adapted for 7 days to laboratory conditions (controlled temperature of 25 ± 1^0C and relative humidity of 44-55% under 12/12 light/dark cycle). Food and water given ad libitum. All animal experiments were carried out as per the protocol approved by CPCSEA (Committee for the Purpose of Control and supervision of Experiment on Animals).

Extraction of MP using Gelucire® lipids

The seeds of MP were finely powdered, passed through 30 mesh screen. The seed powder was used for extraction procedure. Gelucire® 44/14, Gelucire® 43/01 and Gelucire® 50/13 grades of Gelucire® were screened for extraction. Different ratios of drug to lipid were tried in the increasing amount of lipid i.e. 0.25, 0.5, 0.75, 1, 1.25 and 1.5 parts of lipid to 1 part of drug. Final selection of the Gelucire® grade and drug to lipid ratio was made on the basis of maximum percent yield of L-DOPA in the extract. Weighed amount of Gelucire® lipid was melted in beaker at 43-50°C using water bath. The seed powder of MP (500mg) was added to the different grades of molten lipid mass separately, mixed thoroughly and allowed to cool. The above extracts get solidified at room temperature and are ready to use for further processing.

Extraction of MP using different solvents viz. Water, Ethanol:Water (1:1) and Methanol

The dried milled seed powder of MP (10 gm) was defatted with petroleum ether (100ml three times) by shaking for 24 hours at room temperature. Defatted material was dried and extracted with water (100 ml three times) for 48 hours using 0.1% ascorbic acid as a protectant. The supernatant was filtered through 0.45µm membrane filter, concentrated to dryness in a rotary evaporator to produce aqueous extract of MP (AEMP). The similar procedure was followed to produce ethanol: water (1:1) extract of MP (EWMP) and methanol extract of MP (MEMP) using each (10gm) dried milled seed powder of MP with solvents ethanol: water (1:1) and methanol respectively.

Chromatographic conditions and calibration curve for L-DOPA

Stock solution of standard L-DOPA 100µg/ml was prepared using 0.01 M hydrochloric acid in methanol. Various amounts 1,2,3,4,5,6,7,8,9 and 10 µg/ml of stock solution were spotted in triplicates as bands (width, 6mm) with a CAMAG microlitre syringe, on precoated silica gel aluminium plate 60F-254 (20cmX10cm with 250 µm thickness, E.Merck, Germany) using a CAMAG Linomat IV (Switzerland) to get the final concentration in the range of 100-1000ng/spot considering the possible variability of percentage of L-DOPA present in different extracts. A constant application rate (0.1µl/s) was employed and space between two bands was 6mm. A Model III TLC scanner with CATS 4.0 integration software was used for data generation. Slit dimension was kept at 6mmX 0.45mm, and 10 mm/sec scanning speed was employed. Calibration curve was plotted between mean peak area Vs concentration per spot. Mobile phase used was n-butanol: acetic acid: water (4:1:1 v/v/v) (Gupta, A.K., 2003). Linear ascending development was carried out in twin through glass chamber saturated with the mobile phase. Optimized chamber saturation time for mobile phase was 20 minutes at room temperature. Densitometric scanning was performed on CAMAG TLC scanner III in the absorbance mode at 282 nm.

Standardization of the extracts AEMP, EWMP, MEMP and LEMP by HPTLC

Weighed 10 mg of each extract AEMP, EWMP, MEMP and LEMP was reconstituted, made upto 10ml with water, ethanol:water (1:1), methanol and water respectively. The mixtures were subjected to sonication for 5 minutes and filtered through 0.45µm membrane filter. Aliquots of 5µl of these solutions were applied to TLC plate in triplicate to get concentrations of 5000ng/spot. Densitometric scanning was performed on CAMAG TLC scanner III in the absorbance mode at 282 nm. The mean peak areas were considered for further calculations.

Acute oral toxicity tests for the extracts of MP

Acute oral toxicity tests were carried for the prepared extracts of MP in various groups of female Wistar rats as per OECD guidelines and data was analyzed using AOT 425 software.

Effect of MP extracts on sexual behavior in male rats

Selection of male rats: (Kumar et al., 1994)
Normal male rats were trained for sexual experience. To provide sexual experience, each male rat was allowed 30-minute exposure to a female rat in behavioral estrous, l0 days before testing for copulatory performance. The animals were tested three times over a 10 days period for copulatory behavior by exposing them to receptive females. Sexually responding animals were selected for the study. The animals, which did not show any sexual interest during training period were considered as inactive and were replaced with active ones.

Treatment of animals:
Male Wistar rats capable of mounting over female rats were selected for the study. These male rats were divided into 6 groups comprising 6 rats in each group. Group I received only distilled water served as control group. Group II received Sildenafil 5 mg/kg body wt. orally 1 hour before the commencement of the experiment on observation day only. Groups III, IV, V and VI received AEMP, EWMP, MEMP and LEMP respectively at a dose of 75 mg/kg. (Kumar et al., 1994). The overectomized female rats were brought in to estrous by sequential administration of estradiol valerate (12µg/kg body weight) and hydroxy progesterone (1.5 mg/kg body weight), through subcutaneous injections, 48 hours and 4 hours before the copulatory studies respectively. Sexual behavior studies were carried out in a separate room under dim red illumination. The male rat was placed in a rectangular plexiglass chamber, 10 minutes before the introduction of primed female, for it to get acclimatized to the chamber conditions. The primed female was introduced in to the chamber. The drug extracts were administered for 14 days and various sexual behavior parameters were observed on 1^{st}, 7^{th}, and 14^{th} day of drug administration. To access the copulatory sexual behavior of rats following parameters were recorded for 30 min period. (I) mount frequency (MF)**:** number of mounts without intromission from the time of introduction of the female until ejaculation; (II) intromission frequency (IF)**:** number of intromissions from the time of introduction of the female until ejaculation;

(III) mount latency (ML)**:** time interval between the introduction of the female and the first mount by the male; (IV) intromission latency (IL)**:** interval from the time of introduction of the female to the first intromission by the male. It is characterized by pelvic thrusting and springing dismount. (V) ejaculation latency (EL): time interval between the first intromission and ejaculation. It is characterized by deeper and longer pelvic thrusting and slow dismount followed by a period of inactivity; (VI) post-ejaculatory interval (PEI): time interval between ejaculation and the first intromission of the following series. Also the parameters indicating orientational activities were studied viz. (I) licking: male rats towards the female and (II) self-genital grooming (SGG): male rats towards self (Tajuddin et al., 2004; Tyagi et al., 2008).

Statistical Analysis

The data obtained from the experiments are expressed as mean± SEM. Comparison of different groups was done by one-way ANOVA followed by Dunnett Test.

Results

Extraction of MP using Gelucire® lipids

Selection and optimization of lipid grade and drug to lipid ratio was carried out using different grades of Gelucire® lipids in different ratios of drug to lipid as shown in Table 1. The yield of L-DOPA was found to be maximum in MP extract produced using Gelucire® 44/14 than with Gelucire® 43/01 and Gelucire® 50/13. The yield of L-DOPA in the extract with Gelucire® 44/14 was found to be maximum at a drug to lipid ratio of 1:1.25 as shown in table (1). Further increase in lipid proportion did not result in increase in L-DOPA content. The extract produced using Gelucire® 44/14 was dull brown in color and when the extract was treated with water it readily got dispersed and required less time and shear to disperse.

Table 1. Selection and optimization of lipid grade and drug: lipid ratio on the basis of L-DOPA content

Lipid (Gelucire®) grade	Mucuna: Lipid proportion	Peak area for L-DOPA[a]
50/13	1: 0.25	1674.66±0.1
50/13	1: 0.5	1819.33±0.62
50/13	1: 0.75	1886.00± 1.57
50/13	1: 1	1737.00±1.06
50/13	1: 1.25	1817.66±1.06
50/13	1: 1.5	2000.66±1.12
44/14	1: 0.25	1737.33±0.83
44/14	1: 0.5	1795±0.55
44/14	1: 0.75	2040.33±1.45

44/14	1	2190±0.85
44/14[b]	1.25	2593±0.28
44/14	1.5	2292.67±0.82
43/01	0.25	862.67±0.74
43/01	0.5	934.66±0.59
43/01	0.75	984±1.13
43/01	1	980±0.63
43/01	1.25	1079.67±1.086
43/01	1.5	1117±1.02

[a]Values are expressed as Mean ± SEM (n=3); [b] The optimized drug lipid ratio.

Calibration curve for L-DOPA

Calibration curve was found to be linear over the concentration range of 100 to 1000 ng/spot with the linear regression equation, y= 7.6197x + 24.5 and correlation coefficient r^2=0.9996. The Rf Value of L-DOPA was found to be 0.44.

Standardization of the extracts AEMP, EWMP, MEMP and LEMP by HPTLC

HPTLC analysis of all the extracts showed that L-DOPA content is maximum with LEMP i.e. Gelucire® extract of MP than with other conventional extracts as mentioned in Table 2. The HPTLC overlay chromatogram and spectra of the extracts and standard L-DOPA are given in Fig 1. and Fig 2. respectively.

Table 2. Quantitative estimation of L-DOPA content in prepared extracts.

Extracts of *Mucuna pruriens* (MP)	**L-DOPA content (%) w/w[a]**
Aqueous extract (AEMP)	6.433± 0.20
Ethanol:water(1:1)extract (EWMP)	6.490±0.08
Methanolic extract (MEMP)	5.943±0.04
Lipid extract (LEMP)	7.063±0.06

[a]Values are expressed as Mean ± SEM (n=3)

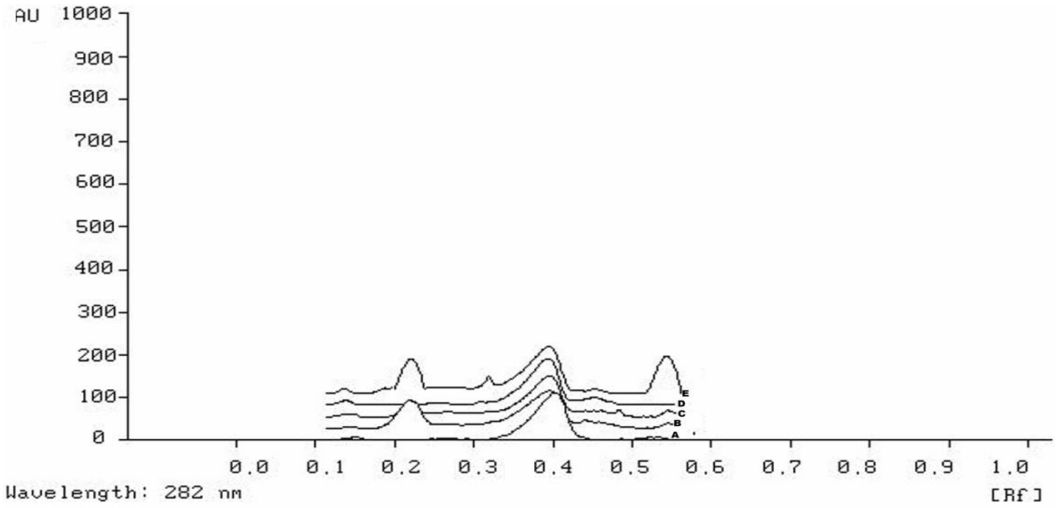

Fig 1. Overlay of chromatograms of all the extracts of *Mucuna pruriens*, A. Standard L-DOPA in methanol, B. Ethanol: water (1:1) extract, C. Aqueous extract, D. Methanolic extract and E. Lipid extract.

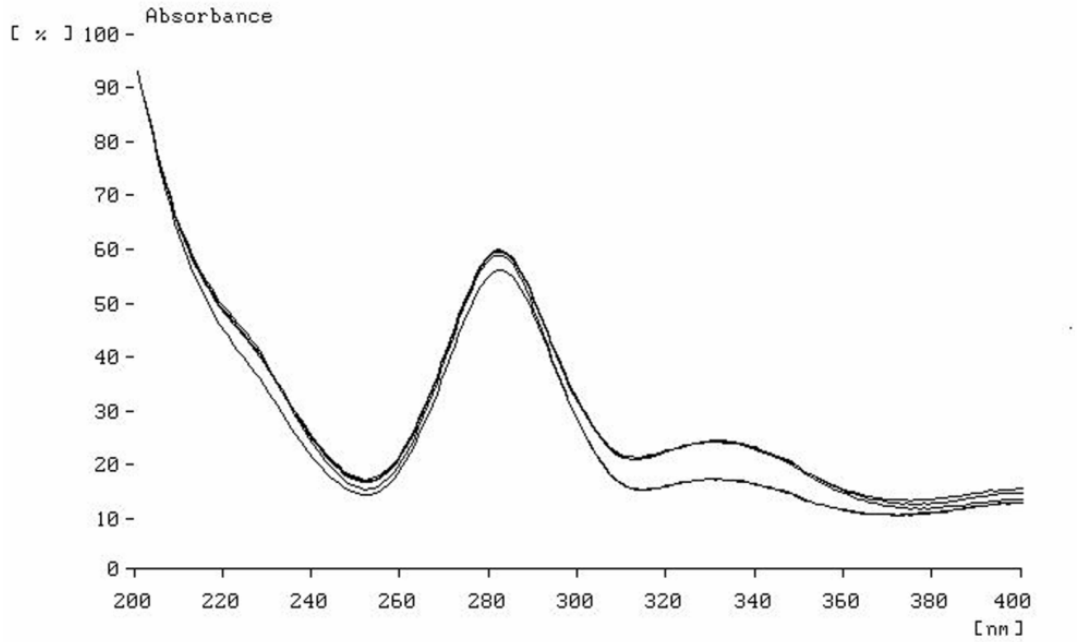

Fig 2. Overlay spectra of extracts and standard L-DOPA

Acute Oral Toxicity

No mortality or changes in behavior were observed in Wistar rats groups with any extract.

Effect of MP extracts on sexual behavior in male rats

The results indicate that LEMP showed significant increase in the mounting frequency ($p<0.01$), intromission frequency ($p<0.01$) and ejaculation latency ($p<0.01$) on 1st, 7th and 14th days of the treatment as compared to the control group. The LEMP also showed significant decrease in mount latency ($p<0.01$), intromission latency ($p<0.01$) and post ejaculatory interval ($p<0.01$) on 1st, 7th and 14th days of the treatment as compared to the control group.

The MEMP showed significant increase in the mounting frequency ($p<0.01$), intromission frequency ($p<0.01$) and in ejaculation latency ($p<0.05$) on 1st, 7th and 14th days of the treatment and showed significant decrease in mount latency ($p <0.01$), intromission latency ($p<0.01$) and post ejaculatory interval ($p<0.01$) on 1st, 7th and 14th days of the treatment as compared to the control group.

The EWMP showed significant increase in mount frequency ($p<0.05$) and ejaculation latency ($p<0.01$) on 1st, 7th and 14th days of the treatment as compared to the control group. The same extract also showed significant decrease in intromission latency ($p<0.05$) as compared to the control group.

The AEMP showed significant increase ($p<0.01$) in ejaculation latency. Also it showed significant decrease in intromission latency ($p<0.01$) and post ejaculatory interval ($p<0.01$).

The effect of Sildenafil was found to be predominant than all the extracts tested for mount frequency and ejaculation latency. Both Sildenafil group and the LEMP group showed significant increase ($p<0.001$) in self-genital grooming as compared to control group. The LEMP also showed significant increase ($p<0.01$) in licking behavior as compared to control group. The results of copulatory sexual behavior study in male rats treated with different extracts of MP were shown in Table 3.

Table 3. Copulatory sexual behavior study in male rats treated with different extracts of MP (*Mucuna pruriens*)

Group	Day	MF	ML	IF	IL	EL	PEI	SGG	Licking
Control	1	11.66±0.80	95.49±5.25	4.83±0.60	109.06±6.69	230.03±3.79	340.03±8.45	14.33±0.95	15.16±1.25
	7	9.83±0.87	98.47±4.50	5.16±0.95	106.44±6.57	234.50±3.55	341.00±6.82	13.83±1.07	13.66±1.11
	14	10.33±0.71	97.73±4.92	4.00+0.52	103.96±3.72	233.32±4.42	336.50±8.89	13.50±0.96	14.16±1.13
Sildenafil	1	43.16±1.30	12.13±1.38*	24.16±1.62**	15.70±1.17**	310.89±8.22**	99.86±8.68**	30.83±3.34**	24.50±2.06
	7	42.00±1.77	12.57±1.35*	23.66±1.12**	13.87±0.97**	317.56±6.67**	100.38±9.35*	30.50±3.31**	27.00±2.11
	14	42.50±1.91	12.67±1.42*	24.33±1.54**	14.17±0.53**	320.68±6.71**	104.19±7.40*	30.16±5.17**	25.33±1.54
AEMP	1	11.16±0.94	113.56±7.82	4.66±0.49	124.57±6.44*	201.49±7.17**	326.10±9.40*	14.00±1.09	10.67+0.88
	7	11.50±1.11	113.93±8.93	5.00±0.36	122.06±8.16*	201.48±7.09**	301.89±3.67*	17.00±1.59	21.17+1.30
	14	12.83±1.16	111.92±6.88	8.16±0.65	118.07±8.40*	202.46±6.22**	314.79±9.07*	17.66±0.88	21.83+1.64
EWMP	1	13.00±1.46	105.78±6.54	6.33±0.49	115.50±9.00*	212.97±5.42**	343.45±2.81	14.83±1.49	12.33±1.64
	7	14.17±1.07	97.56±8.25	7.16±0.60	109.89±7.55*	207.75±4.65**	322.98±8.59	17.00±1.29	25.16±2.82
	14	19.00±3.18	98.77±9.79	7.50±0.43	114.59±7.54*	216.90±7.01**	326.37±9.29	17.00±1.24	29.83±1.35
MEMP	1	21.00±1.06	38.48±2.58*	8.50±0.43**	43.24±1.70**	228.47±6.78	208.82±5.37*	16.50±2.04	12.50±1.45
	7	22.50±1.25	28.48±1.76*	10.66±1.52**	39.05±3.00**	221.58±2.84	203.69±4.30*	22.16±1.56	13.16±1.70
	14	22.83±1.99	30.79±2.64*	11.16±1.83**	37.07±2.74**	229.81±5.92	207.29±5.62*	19.33±2.42	14.50±1.60
LEMP	1	35.83±3.62	25.34±1.88*	23.33±2.33**	32.40±1.87**	328.66±3.35**	130.39±7.01*	21.83±3.33**	18.83±0.94*
	7	37.00±2.92	28.40±2.74*	27.33±3.34**	37.11±2.44**	326.69±9.58**	134.76±3.21*	30.83±1.92**	30.83±2.30*
	14	40.00±2.78	30.44±2.78*	30.16±1.78**	34.39±1.86**	328.16±5.60**	126.01±8.34*	33.00±2.73**	34.17±2.12*

Values are expressed as Mean ± SEM., (n=6), significant as compared with control group (* $p<0.05$, ** $p<0.01$).
AEMP: Aqueous extract of MP; EWMP: Ethanol water extract of MP; MEMP: Methanol extract of MP; LEMP: Lipid extract of MP; MF**:** mount frequency; IF**:** intromission frequency; ML: mount latency; IL**:** intromission latency; EL: ejaculation latency; PEI: post-ejaculatory interval and SGG: self-genital grooming.

Discussion

In the present study attempts have been made for development of an extraction method using hydrophilic lipids Gelucire® for the medicinal plant *Mucuna pruriens* (MP). Chemically Gelucire® are polyethylene glycol glycerides composed of mono-, di- and triglycerides and mono- and diesters of polyethylene glycol (PEG). These disperse or solubilize in aqueous media forming micelles, microscopic globules or vesicles and thereby improve the solublization of poorly water-soluble constituents. (Chambin and Jannin, 2005). Results of HPTLC analysis of all the extracts of MP showed that maximum extraction of L-DOPA (7.18%w/w) was achieved with the Gelucire® 44/14 at a drug to lipid ratio of 1:1.25; as compared to the conventional AEMP, EWMP and MEMP. Moreover the densitogram of this LEMP showed presence of other peaks along with the L-DOPA. This clearly indicates that the LEMP has given better extractability as compared to AEMP, EWMP and MEMP. The conventional extracts AEMP, EWMP and MEMP gave lower yield of L-DOPA as compared to LEMP; moreover, these extracts were sticky in nature and the time required for extraction process was longer (more than 48 hours). Also the process involved use of high amounts of organic solvents. However the LEMP was easily dispersed in water with minimum amount of shear and time. The total time required for extraction process was not more than 15 minutes. The method did not involve use of any organic solvent.

The results of sexual behavior study indicate that all the extracts of MP possess sexual activity and amongst these extracts, the LEMP showed better improvement in sexual behavior in male rats. The mount frequency (MF) and intromission frequency (IF) are considered as indices of libido and potency. (Tajuddin et al., 2004). The maximum increase in (MF) and (IF) by LEMP proves potential sexual function improving effect of the extract. The AEMP, EWMP and MEMP also showed increase in mounting frequency, intromission frequency as compared to the control group but to lesser extent than the LEMP. Premature ejaculation is one of the kind of sexual dysfunction (Yakubu et al., 2007). Therefore assessment of ejaculation latency (EL) was studied. Sildenafil and LEMP showed maximum increase in EL. The post ejaculatory interval (PEI) reflects the rate of recovery from exhaustion after first series of mating. (Tajuddin et al., 2004). All the extracts showed decrease in PEI but LEMP showed maximum decrease in PEI, which was comparable with Sildenafil. Genital sniffing plays a major role in the readiness of male rat for reproduction (Hernandez-Gonazalez, 2000). LEMP showed maximum increase in orientational activities viz. ano-genital sniffing (self) and licking. This showed increased sexual stimulation of male rats towards female rats. Thus, all the extracts of MP showed significant change in copulatory behavior and increased sexual urge in rats, however the effect was predominant in the groups treated with Sildenafil and LEMP respectively. The extracts of MP showed comparative pattern of the sexual activity of the order of: Sildenafil > LEMP > MEMP > EWMP > AEMP as compared to the control group.

MP treatment induces activation of sexual behavior by increasing dopamine level in the brain. It has been proposed that increased dopamine level in brain optimize the release of hormones like testosterone, which leads to increased sexual drive and better performance.

(Shukla et al., 2007). The efficacy of MP is not completely attributed to it's L-DOPA content. (Kumar et al., 1994). Other constituents of MP also contribute in improvement of sexual behavior. The better performance of LEMP in terms of improved sexual behavior suggests, Gelucire lipids have achieved better availability of L-DOPA along with other constituents of MP for absorption in body. The improved sexual behavior by administration of LEMP may be attributed possibly to increased level of dopamine in brain. The lipophilic nature of Gelucire might have contributed in improving the penetration of L-DOPA and its metabolites across blood brain barrier. P-glycoprotein of blood brain barrier can actively transport array of lipophilic drugs out of the brain capillary endothelial cells that form the blood brain barrier (Sathiyanarayanan et al., 2010). Co-administration of actives with carriers that inhibit P-gpmediated efflux or the incorporation of actives into specific lipid excipients alters the pharmacokinetics of the administered actives. (Chen et al., 2005; Ahuja et al., 2007). Gelucire 44/14 posses P-gp inhibiting property (Kristina et al., 2007) which strengthens the possibility of Gelucire lipid contributing in improving penetration of L-DOPA and its metabolites across blood brain barrier.

In summary the present study provides evidence that the lipid based extraction method proposed is rapid and gives better extractability of phytoconstituents. Also the extract produced by this method has better qualities required for further development in to formulation and the method overcomes the majority of drawbacks associated with the conventional extraction methods. The application of lipid extraction method has also enhanced the therapeutic efficiency of MP in improving male sexual behavior in rats proving the advantages of Gelucire® lipid extraction.

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References

1. Amin, K.M.Y., Khan, M.N., Zillur, R.S., Khan, N.A. Sexual function improving effect of *Mucuna pruriens* in sexually normal rats. Fitoterapia 1996; 67, 53-58.
2. Ahuja, N., Katare, O.P., Singh, B. Studies on dissolution enhancement and mathematical modeling of drug release of a poorly water-soluble drug using water-soluble carriers. European Journal of Pharmaceutics and Biopharmaceutics. 2007; 65; 26-38.
3. Aungst, B.J., Nhung, H., Nguyen, Nancy, J., R., Susan, M.R., Munir, A., Susan, J.W., Linyee, S. Ampiphilic vehicles improve the oral bioavailability of a poorly soluble HIV protease inhibitor at high doses. Int. J. Pharm. 1997; 156, 79-88.
4. Aversa, A. and Fabbri, A. New oral agents for erectile dysfunction: what is changing in your practice. Asian. J. Andrology. 2001; 3, 175-179.

5. Barkera, S.A., Yapb, S.P., Yuenb, K.H., McCoya, C.P., Murphya, J.R., Craiga, D.Q.M. An investigation into the structure and bioavailability of a-tocopherol dispersions in Gelucire 44/14. J. Control. Release. 2003; 91, 477-488.
6. Bhattacharya, S.K., Sanyal, A.K., Ghoshal, S. Investigation of the hallucinogenic activity of indole alkylamines isolated from *Mucuna pruriens* DC. Indian. J. Physiol 1971; 25, 53-55.
7. Chambin, O. and Jannin, V. Interest of multifunctional lipid excipients. Case of Gelucire 44/14. Drug. Dev. Ind. Pharm. 2005; 31, 527-534.
8. Chen, M.C., Wang, J.L., Tzen, J.T. Elevating bioavailability of cyclosporine A via encapsulation in artificial oil bodies stabilized by caleosin. Biotechnoloy progress. 2005; 21(4), 1297-301.
9. Damodaran, M. and Ramaswamy, R. Isolation of L-3,4-dihydroxy-phenylalnine from the seeds of *Mucuna pruriens*. Biochemistry. 1937; 136, 2149-2152.
10. Daxenbichler, M.E., VanEtten, C.H., Earle, F.R., Tallent, W.H. L-DOPA recovery from *Mucuna* seed. J. Agr. Food. Chem. 1972; 20, 1046-1048.
11. Gershanik, T. and Benita, S. Self-dispersing lipid formulations for improving oral absorption of lipophilic drugs. Eur. J. Pharm. Biopharm. 2000; 50, 179-188.
12. Gupta, A.K. *Mucuna pruriens.* Quality standards of Indian medicinal plants. 2003. Indian Council of Medical Research, New Delhi. Vol. I, 137-141.
13. Harvey, N.S. Serial cognitive profiles in levodopa-induced hypersexuality. Brit. J. Psychiat. 1998; 153, 833-836.
14. Hernandez-Gonzalez M. Prepubertal genital grooming and penile erections in relation to sexual behavior of rats. Physiol. Behaviour 2000; 71: 51-56.
15. Horita, H., Sato, Y., Adachi, H., Suzuki, N. and Kato, R. Effects of Levodopa on nocturnal penile tumescence: a preliminary study. J. Androl. 1998; 19, 619-624.
16. Hussain, G.and Manyam, B.V. *Mucuna pruriens* proves more effective than L-DOPA in Parkinson's disease in animal model. Phytother. Res. 1997; 11, 419-423.
17. Kristina, S.B., Andrea, T., Stephen, D.L., Wasan, K.M. Lipid excipients Peceol and Gelucire 44/14 decrease P-glycoprotein mediated efflux of Rhodamine 123 partially due to modifying P-glycoprotein protein expression within Caco-2 cells. J Pharm Pharm Sci . 2007; 10; 319–331.
18. Kumar A.K.V., Srinivasan, K.K., Shanbhag, T., Rao, S.G. Aphrodisiac activity of the seeds of *Mucuna pruriens*. Indian Drugs 1994; 31, 321-327.
19. Majumdar, D.N. and Santra, D.K. The *Mucuna pruriens* DC. Part II. Isolation of water insoluble alkaloids. Indian. J. Pharm. 1953; 15, 60-61.
20. Mandal, V., Mohan, Y., Hemalatha, S. Microwave assisted extraction- An innovative and promising extraction tool for medicinal plant research. Pharmacog. Rev. 2007; 1, 7-18.
21. Mishra., L.C. Scientific basis for ayurvedic therapies. 2004. CRC Press LLC. New York, Washington DC. Edition 1, 459-478.
22. Misra, L. and Wagner, H. Extraction of bioactive principles from *Mucuna pruriens* seeds. Indian. J. Biochem. Bio. 2007; 44, 55-60.
23. Misra, L. and Wagner, H. Alkaloidal constituents of *Mucuna pruriens* seeds. Phytochemistry. 2004; 65, 2565-2567.
24. Misra, L. and Wagner, H. Lipid derivatives from *Mucuna pruriens* seeds. Indian. J. Chem. Section B. 2006; 45, 801-804.

25. Saksena, S. and Dixit, V.K. Role of total alkaloids of *Mucuna pruriens* Baker in spermatogenesis in albino rats. Indian. J. Nat. Products. 1987; 3, 3-7.
26. Sathiyanarayanan, L. and Arulmozhi, A. *Mucuna pruriens* Linn-A comprehensive review. Pharmacog. Rev. 2007; 1, 157-162.
27. Sathiyanarayanan, L., Paradkar, A.R., Mahadik, K.R. In vivo and in vitro antioxidant activity of lipid based extract of *Bacopa monniera* Linn. compared to conventional extract and traditional preparation. European Journal of Integrative Medicine. 2010; 2(2); 93-101.
28. Shukla, K.K., Mahdi, A.A, Ahmad, M.K, Jaiswar, S.P, Shankwar, S.N., Tiwari, S.C. *Mucuna pruriens* reduces stress and improves the quality of semen in infertile men. 2007; Original Article by eCAM Advance Access. doi:10.1093/ecam/nem 171; 1-8.
29. Shukla, K.K., Mahdi, A.A., Ahmad, MK., Shankhwar, S.N., Rajender, S., Jaiswar, S.P. *Mucuna pruriens* improves male fertility by its action on the hypothalamus-pituitary-gonadal axis. Fertil. Steril. 2008; 6, 1934-1940.
30. Tajuddin, Shamshad, A., Abdul, L., Iqbal, A. Q. Effect of 50% ethanolic extract of *Syzygium aromaticum* (L.) Merr. & Perry. (clove) on sexual behavior of normal male rats. BMC. Complement. Alt. Med. 2004; 4, 17.
31. Tyagi, R.M., Aswar, U.M., Mohan, V.B., Bodhankar, S.L., Zambre, G.N. Thakurdesai, P.A. Study of furostenol glycoside fraction of *Tribulus terrestris* on male sexual function in rats. Pharm. Biol. 2008; 46, 191-198.
32. Yakubu, M.T., Akanji, M.A., Oladiji, A.T. Male sexual dysfunction and methods used in assessing medicinal plants with aphrodisiac potentials. Pharmacog. Rev. 2007; 1, 49-56.
33. Yemisi, A. A. and Kayode, O. A. Evaluation of the gelation characteristics of Mucuna bean flour and protein isolate. Electron. J. Environ. Agr. Food. Chem. 2007; 6, 2243-2262.
34. Zamble, A., Sahpaz, Y., Brunet, C., Bailleul, F. Effects of *Microdesmis keayana* roots on sexual on behavior of male rats. Phytomedicine. 2008; 15, 625-629.