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An experimental study of Mounting frequency & Penile reflection of *Gossypium herbaceun* Linn. Seed (Panbadana) on Wistar rats.

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Introduction: *Gossypium herbaceun* Linn. occurs in Africa, Middle East countries, Central Asia and Western India, cultivated in the area as a rainy season crop, often mixed with other subsidiary crops [1,2]. This race includes nearly all types of Gossypium herbaceum grown in India belong to the malvaceae family [1, 2,3,4,5]. Cotton seed is well known in India as Binola. It is found in Asia and African countries particularly in India, North West Frontier Province, Baluchistan, Afghanistan, Persia, Mesopotamia, Syria, Egypt, Mediterranean, United State of America, North and Middle East [1,2,3,6,7,8]. **Taxonomical Classification:** Botanical classification of *Mangifera indica* Kingdom: Plantae, Subkingdom: Viridaeplantae, Class: Magnoliopsida, Order: Malveles, Family: Malvaceae, Subfamily: Malvoideae, Tribe: Gossypieae, Genus: Gossypium, Species: herbacium [3, 5, 7,9,10.]

Botanical Name: *Mangifera indica* Linn.

Vernacular name: Hindi: *Binola*, Arabic: *Habbul qutn*, Persian: *Panbadana*, Eng: Cotton seed
Latin: *Mangifera indica* Linn. [1,2,3,5,7,9,10,11.]

Unani Description of *Gossypium herbaceun* Linn.[3,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27]**:** Although this drug finds in many classical Unani literatures but most of them have not discussed its morphology in detail. It is said that it is the seed of cotton, which is very famous. Seeds are dried, small, peer shaped, brown / black in colour, its kernel as used as medicine.

Parts used (*Azja-e-Mustemil*): Kernel (*Maghz-e-Binola*).
Temperament (*Mizaj*): Hot & Moist in 2nddefree [3, 12, 13].
Action (*A'afaal*): *Moallid-e-Mani* (Spermatogogue), *Muqawwi-e-Baah* (Aphrodisiac), *Maan-e-Taaffun* (Anti-infective), *Maan-e-Sara* (Antiepileptic), *Munaffis-e-Balgham* (Expectorant), *Moallid-e-Sheer* (Lactogogue), *Jaali* (Detergent), *Musammina* (Adipogenous), *Mullayyin* (Laxative), *Muqawwi-e-Maidaa* (Gastro tonic), *Mushil* (Resolvent) [13,28,29,30,31,32].
Uses (*Istemaal*): *Qillat-e-Mani* (Deficiency of semen), *Zoaf-e-Bah* (Sexual weakness), *Ufoonat* (Infections), *Saraa* (Epilepsy), *Suaal* (Cough), *Qillat-e-Sheer* (Deficiency of milk), *Zoaf-e-Badan* (Weakness of body), *Qabz* (Constipation), *Zoaf-e-Maida* (Gastro weakness) etc. [13, 28, 29, 30, 31, 32, 33, 34.35.36].

Main action (Naf-e-Kkaas): *Muqawwi-e-Bah aur Mulayyin-e-Sinah* (Aphrodisiac & Expectorany).

Adverse effects (*Muzir Asaraat*): *Gurdon ke liye wa Garam Mizaaj ko Muzir hai* (Harmful for kidneys and Hot Temperament humans).
Antidote (*Musaleh*): *Khameera Banafsha & Sharabat-e-Banafsha* (Compound formulation of *Banafsha-Viola odorata* Linn.).
Substitute (*Badal*): Tukhm-e-Keekar aur Qurtum (seeds of *Acacia arabica* Willd. var. Indica Benth. & *Carthamus tinctorius* Linn.)
Miqdaar-e-Khuraak (Dose): 3 to 7 gm.

Compound formulations (*Murakkab*): Maajoon-e-Aardh Khurmaa.

Methods:
Plant Materials: Panbadana (seeds of Gossypium herbaceum) was procured from the market of Bangalore. The plant material was authenticated by the expert botanist Dr. Siddhamaliyya of Regional Research Institute (Ay.) Central Council for Research in Ayurveda and Siddha, Central Pharmacy, Bangalore vide authentication

voucher No. RRI/BNG/SMP/Drug Authentication /2009-10/87. The sample of the drug has been deposited in Deptt. of Ilmul Advia, National Institute of Unani Medicine, Bangalore and Regional Research Institute (Ay.) for future reference.

Preparation of the test Drug: Owing to bulky nature of test drug which makes it difficult for oral administration to rats, hydro-alcoholic extract was prepared. The seeds were powdered using an electric grinder. 100 gm course powder was extracted in 150 ml of ethanol (50%) and 150 ml of distilled water (50%) in Soxhelt apparatus for 8 hours at continuous boiling (80°C). The extract was filtered and evaporated on water bath till it dried. The yield (w/v) of the hydro-alcoholic extract of the test drug was found to be 14.84%.

Dose: The extract was extrapolated from the human clinical dose as described by Unani physicians. The dose of crude drug was multiplied by conversion factor of 7 as described by Freirich, E.J. et al [35,36] and it was found to be 10.38 mg/kg.

Chemicals: Sildenafil citrate was obtained from Cheminnova Pharmaceuticals 82/12 A, HPSIDC, Baddi, H.P. 173205, Mumbay Central, Mumbay 400008, marked by CIPLA Ltd.

Animals: Thirty-six male and female albino rats of Wistar strain of 3 months of age and weighing 200-250 gm were obtained from the Animal House Faculty of National Institute of Unani Medicine, Bangalore. The rats were housed in individual plastic cages (size of the cages 25cm x 40cm) in room temperature 23°C ± 2 °C and 55 ± 5% humidity kept on 12 hrs light & 12 hrs dark cycles. The rats kept on laboratory chow and tap water ad libitum. The Departmental ethical committee for animal care and use approved the experimental design.

Penile Reflection Test: Test for potency: The test was carried out by the methods of Hart and Haugen [35, 36, 37] modified by us. The male rats were divided in to three groups each consisting of six rats and placed individually in separate propylene cages during the experiment. Group I represented the control group, which received 1 ml/kg of distilled water orally, animal of group II (test group) were administered the test drug in the dose of 4.71 mg/100 gm and animals of III group (standard group) were administered Sildenafil citrate in the dose of 100 µgm orally for seven days. The treatment was given at least 30 minutes before the commencement of the experiment. On the 8th day, the test for penile reflexes was carried out. Each rat was given a series of 15 minutes for penile reflex by placing the rat on its back, in a narrow glass cylinder for partial restraint. The preputial sheath was pushed behind the glans by means of thumb and index finger and held in this manner for a period of 15 minutes. Such stimulation elicits a cluster of genital reflexes. The following components were recorded: Erections (E), Quick Flips (QF) and Long Flips (LF). The frequency of these parameters observed in control, test and standard groups was statistically analysed by using the Tukey- Kramer Multiple Comparison test.

Chemical Analysis:

Determination of Serum Cholesterol, Serum Testosterone & Testicular Cholesterol: Testicular and serum cholesterol concentration were determined by the Chod-PAP method [40]. The same Wistar rats used for sexual behaviour parameters were also used for the serum cholesterol and testosterone assay. The male Wistar rats were sacrificed after dosing the drug Sildenafil and test drug. Under the anaesthesia agent Thiopentone sodium, 30-50 mg/kg.[35] after giving the anaesthesia the neck areas was quickly cleared of fur and skin to expose the jugular vein. The jugular vein was slightly displaced from the neck region (to prevent contamination of the blood with interstitial fluid) and then cut with a sharp sterile surgical blade. The blood was collected into clean and dry test tube.

After collection of blood, it was centrifuge which was left at room temperature for 10 minutes. After that the tubes were centrifuged at 22.5 x g. for 15 minutes. The sera were thereafter collected using the test tube given by Thyrocare Technologist Ltd., which were cleaned and dried for sampling and then stored frozen overnight 51 before used the testosterone assay.

The serum testosterone concentration was quantitatively determined using the serum testosterone enzyme immunoassay kit by Chemi Luminescent Immuno Assay (CLIA), which was done in the Laboratory Thyrocare Technologist Ltd. The serum cholesterol determines by the analyser [41, 42, 43] using the Aspen Laboratory Ltd., in Laboratory of National Institute of Unani Medicine.

Briefly, 0.02 cm^3 of the sample (testicular homogenate and or serum) is mixed with 2.00 cm^3 of working reagent and the absorbance of the resulting mixture read after 5 minutes at 546 nm wavelength. The blank and standard were composed in a similar way except that they were replaced with 0.02 cm^3 each of distilled water and standard solution respectively.

Results:

Effect of the extract on libido: The results obtained with the test for libido show that the extract at the dose of 4.71 mg/kg, significantly increased the Mounting Frequency (MF) ($P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively) as compared to control group. The standard drug also significantly increased the MF ($P < 0.001$) as compared to control animals. Intromission and Ejaculation were absent in control, test and standard groups (Table 1).

Table 1: Effect of hydro-alcoholic extract of *Gossypium herbaceum* (Panbadana) on mounting frequency (test for libido) in male rats:

Parameters	Mean ± SEM		
	Control (10 ml/kg)	Panbadana (10.38 mg/kg)	Sildenafil citrate (100 µgm)
Mounting Frequency (MF)	6.17 ±0.98	7.83 ± 0.47**	23.00 ± 2.17***
Intromission Frequency (IF)	Nil	Nil	Nil
Ejaculation (EJ)	Absent	Absent	Absent

Tabular values are mean ± SEM, n = 6 (number of animals in each group); significant difference from control, *P < 0.05, **P < 0.01; ***P < 0.001.

Effect of the extract on potency: The test for potency revealed that the extract at the dose of 4.71 mg/kg, significantly increased the frequency of Erections (E) ($P < 0.001$), Quick Flips (QF) ($P < 0.001$) and Long Flips (LF) ($P < 0.001$) as well as the aggregate of these penile reflexes (TPR) ($P < 0.001$) in comparison with the control group. The test drug at the dose of 250 mg/kg, significantly increased the E ($P < 0.05$), LF ($P < 0.01$) and TPR ($P < 0.05$) but did not significantly affect. The QF. Whereas, the extract at the dose of 4.71 mg/kg, did not alter the E, QF, LF and TPR. The standard drug also significantly increased the E ($P < 0.001$), QF ($P < 0.001$), LF ($P < 0.001$) and TPR ($P < 0.001$) with respect to the control animals (Table 2).

Table 2: Effect of hydro-alcoholic extract of *Gossypium herbaceum* (Panbadana) on penile reflexes (test for potency) in male rats:

Parameters	Mean ± SEM		
	Control (1 ml/kg)	Panbadana (100 mg/kg)	Sildenafil citrate (100 µgm)
Erections (E)	7.67 ± 1.63	7.50 ± 0.42 NS	19.00 ± 2.64***
Quick Flips (QF)	5.17 ± 0.75	5.50 ± 0.49 NS	17.30 ± 4.13***
Long Flips (LF)	2.17 ± 1.17	3.33 ± 0.30 NS	12.00 ± 2.26***
Total Penile Reflexes (TPR)	15.01 ± 3.55	16.33 ± 1.21 NS	48.30 ± 9.03***

Tabular values are mean ± SEM, n = 6 (number of animals in each group); significant difference from control, NS: Not significant. *P < 0.05, **P <0.01; ***P < 0.001.

Adverse effects: No treatment-related overt signs of toxicity, stress and changes in behaviour were observed. The food and water intake of all the treated animals remained similar to those of the control group.

Discussion: The present study was aimed to investigate the aphrodisiac effect of nutmeg extract (50% ethanolic) along with its acute toxicity using various animal models. The study exhibits a marked change in sexual behaviour of male rats. The results of the present investigations show the test drug also caused a significant reduction in the Mounting Latency (ML) and Intromission Latency (IL) as compared to control animals, while a highly significant decrease was observed in the ML of animals treated with the referent drug. This also provides an evidence for aphrodisiac effect of the test drug. These findings show that the test drug produces a striking enhancement of over-all sexual performance of normal animals. MF after penile anesthetization of rats is a reliable index of 'pure' libido and the penile reflexes of the rats are a good model of 'pure' potency [44]. Therefore, in the present study the extract was also studied for effect on these components of sexual behaviour.

The effect of the test drug on libido was studied by assessing the MF after genital anaesthetization which does away with the reinforcing effect of genital sensation thus affording the study of pure libido or intrinsic sexual desire. During the experiment the test drug produced a significant increase in the MF of sexually normal male rats. Whereas, the MF was much reduced in control, test and standard animals in comparison with the MF of corresponding groups in mating behaviour test where the penis had not been anaesthetized. However, the test for libido revealed that Intromission and Ejaculation were absent in all groups of animals, as the genital sensations which are absent due to penile anaesthetization are necessary for the development of these two events. Thus, it may be inferred that the test drug produced a striking increase in 'pure' libido. The test for potency exhibited that the extract significantly increased the frequency of all the components of penile reflexes: Erections (E), Quick Flips (QF) and Long Flips (LF) as compared to control group, but comparatively less than the standard drug. The aggregate of these penile reflexes (TPR) was also significantly increased in both test and standard animals.

This indicates that the test drug increases 'pure 'potency also. Although the effect of the extract on 'pure' libido and 'pure' potency was evaluated by using two different methods, a rough comparison of the results indicates that the test drug augmented both libido and to an equal extent potency. The positive inferences from the specific tests for libido and potency substantiate the indications of the mating behaviour test to show in a rather conclusive manner that the test drug enhances both the libido and potency in normal male animals. These conclusions are further supported by an earlier study reporting libido and potency increasing effect of nutmeg in mice [45]. In addition, nutmeg, a well know spice and a herbal drug is widely used in Unani medicine without any known or recorded toxicity in the management of male sexual disorders. Such herbal drugs may be directly used, without any toxicity testing. However, when an extract or active fraction of such drug is used it is better to evaluate possible toxicity. Although it is the normal practice to determine the LD50 value, now it is acceptable to limit the study to an acute toxicity test using multiple doses including reasonably high doses of the drug [46].

Conclusion: The resultant significant and sustained increase in the sexual activity of male rats, without any conspicuous adverse effects and toxicity, suggests that nutmeg possesses clinically applicable aphrodisiac activity, and also lends support to the claims for its traditional usage as sexual function enhancing medicine. Further, the study also indicates that the aphrodisiac effects of the test drug may be due to its nervous stimulating property. Thus, it may prove to be an effective and safe alternative remedy in sexual disorders.

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