Title: Effect of 50% ethanolic extract of Syzygium aromaticum (L.) Merr. & Perry. (clove) on Sexual behaviour of normal male rats.

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Author’s response to reviews:

Title of manuscript: Effect of 50% ethanolic extract of Syzygium aromaticum (L.) Merr. & Perry. (clove) on sexual behaviour of normal male rats

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The changes have been made in the manuscript in accordance with the suggestions made by Mr. Mohammad sayyah in his review report. Point-by-point response to the comments is provided herewith.

Major compulsory Revision

1. The route of administration of clove in the traditional unani system of medicine is essentially oral.
2. The voucher number of drug (S721) has now been mentioned in the manuscript.
3. The test drug viz clove (s.aromaticum) is widely used as an ingredient of human diet. It is quite non toxic. Therefore, it is likely to have, very high practically not determinable LD 50. However, it was tested for a possible side effect namely gastric irritation by the test for ulcerogenicity.
4. We have examined previously the aphrodisiac effect of the extracts in normal male mice. But for that study, methods and parameters were quite different from the present study. In previous study we examined only mounting frequency and mating performance of animals as the marker for sexual function. Further, mounting frequency was observed in I & III hour after drug administration and for mating performance of the animals the drug was administered at 6 pm. And in the next day morning the presence of sperm in vaginal smear was examined. Hence, the comparison between the previous and present work is not viable.
5. During the course of study all drug-treated rats at high dose level (500mg/kg) were observed at least once daily for any overt signs of toxicity (salivation, ptosis, Wrighting, conversion, exophthalmia) and changes in behaviour (such as spontaneous movement in the cage, climbing, grooming, cleaning of face etc). There was found no abnormal sign of toxicity or behaviour of rats, therefore, it was not expressed. In our previous study too any change in behaviour of animals was not found. This corroborates our findings.
6. As commented that components like eugenol etc. are found in essential oil of clove and not in the extract. Taking into account the valuable comment we have again prepared the ethanolic (50%) extract of clove by the same method first and later in the extract the oil was distilled using Cleavenger’s apparatus (B.P.method) and it found to be 4.70%. Simultaneously by the same method with reference to crude drug it was found as 8.30%. Hence, when the essential oil is present in the extract (even if in half quantity) it means the component like eugenol may be present therein.

Further, we have only discussed the possible mechanism action of the drug in context of essential oil/eugenol etc. but not commented for the exact mechanism of action. In clove apart from essential oil some other components like gallotannic acid, oleoanolic acid, vanillin, chrome and eugenin are also found. They may also be attributed to its property. It may kindly be noted that in the discussion section we have already mentioned in the last that further research should be aimed to isolating the active principle(s) responsible for its aphrodisiac activity and mechanism.

Minor Essential Revision

1. Typographical errors as indicated have been corrected.
   i) page 1, line 11: gasstric.....corrected ad gastric
   ii) Reference 16, line 2: antioxident .... Corrected as antioxidant.

Title of manuscript: Effect of 50% ethanolic extract of Syzygium aromaticum (L.) Merr. & Perry. (clove) on
The changes have been made in the manuscript in accordance with the suggestions made by Mr. Srinivas NAMMI in his review report. Point-by-point response to the comments is provided herewith.

**Major compulsory Revisions**
1) The route of administration for animals in group 1 was oral and it has now been mentioned in the manuscript.
2) The rationale behind the administration of ethinyl oestradiol and progesterone was to bring the female rats into oestrus [1] as the female rat allows mating only during the estrus cycle.
3) The dose of the two hormones were administered in the light of the method of szechtman et al (1981) in which dose of hormones were mentioned not in terms of body weight but dose per animal [2]
4) The animals of all the groups were tested for mating behaviour at 8pm in dim artificial light (1 w fluorescent tube in a lab of 14’x14’) and now this is also mentioned in the manuscript on page 3.
5) All the parameters studies are now discussed more clearly in the results section namely, Mounting Frequency (MF), Intromission Frequency (IF), Mounting Latency (ML), Intromission Latency (IL), Ejaculatory Latency in first series (EL1) Ejaculatory Latency in second series (EL2), Post Ejaculatory Interval (PEI) in mating behaviour test and MF, IF, EJ (Ejaculation), in context of test for libido. In context of test for potency Erections (E), Quick Flips (QF), Long Flips (LF), Total Penile Reflexes (TPR) have been described. As per your suggestion, the results have also been co-related with our study [3] in discussion section. This was the very first study on sexual function improving effect of the test drug.
6) In Unani classical literature it is generally mentioned that spices affect the gastric as well as intestinal mucosa. However, we cite one experimental study carried out for observing the effect of spices on gastric acid secretion. The study conducted on rats concluded that spices produced an increase in gastric acid secretion by a cholinergic mechanism [4]

**Minor Essential revisions**
1) Page 1, line 29: The sentence, the clove is reported.... have been changed as "clove is also reported..."
2) Page 1, line 29: .... in low doses [7], useful .... has been changed as .......in low doses [7] and useful ......as recommended.
3) Page 1, line 31: Italicized the name Herps simplex and used low case for species as suggested.
4) Page 2, line 3: The sentence, Results... has been modified.
5) All the headings (first and second level) have now been written uninformly in sentence case and colon mark at the end of headings has also been removed.
6) All the drugs names appeared in middle of sentences have been changed into lower case.
7) Page 3, lines 1 and 2: The sentence, these preparations... has been modified as, all the drug solution were prepared just before administration.
8) Page 3, line 17: The sentence, they were administered suspension of ...... has been modified as, they were administered with suspension of .......
9) The dose of ethinyl oestradiol used is 100mg/animal and has been mentioned in the manuscript.
10) We have used one way analysis of variance (ANOVA) with post-hoc ‘t’ test for statistical analysis.
11) The manuscript is checked again for possible typographical and grammatical errors and omissions and some corrections are made wherever required.
12-13) Tables are corrected as suggested.
14) As suggested all the reference are checked for formatting (including journal abbreviations) and corrected in accordance with journals’ instructions wherever required.

**References**