Author's response to reviews

Title: Mondia whitei (Periplocaceae) prevents and Guibourtia tessmannii (Caesalpiniaceae) facilitates fictive ejaculation in spinal male rats

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Author's response to reviews:

some changes have been made on Figures 2, 3 and 4 regarding the dose and unit for dopamine

Dear Editor

I am very pleased to submit the revised MS following the reviewer requirements. Corrections are indicated in the MS with green color. The title of the paper had been changed since the Review 1. It reads now as: `Mondia whitei (Periplocaceae) prevents and Guibourtia tessmannii (Caesalpiniaceae) facilitates fictive ejaculation in spinal male rats`.

Reviewer's report

Title: Mondia whitei (Periplocaceae) prevents and Guibourtia tessmannii (Caesalpiniaceae) facilitates fictive ejaculation in spinal male rats

Version: 4 Date: 24 August 2012

Reviewer: Gabriela Rodriguez-Manzo

Reviewer's report:

The manuscript has been importantly improved. However there are still some issues that have to be solved.

Major compulsory revisions

1) Background: The modification made to the first sentence of the manuscript transformed a correct statement into an incorrect concept that is central to the understanding of the functioning of the spinal generator for ejaculation. The spinal generator for ejaculation is not a relay system for sensorial and descending information. It is the neural integrative center for ejaculation, the neural circuit that controls ejaculation. Please eliminate this false concept. The
statement appearing in the first version was adequate.

Answer 1: Corrections adequately done

2) Hypothesis (page 2): the affirmation that the plant extracts’ aphrodisiac effect upon sexual potency can be exerted by interfering with the expression of ejaculation is a contradiction.

I think there is confusion as to what the term aphrodisiac means. An inhibitory action of a treatment on sexual responses cannot be considered as an aphrodisiac effect. Another issue is that the inhibitory action of a drug has the potential to be used to treat a given disorder, for instance premature ejaculation.

This confusion appears also in the discussion section. Thus, on page 9 appears a sentence, difficult to understand (in italics) Mondia whitei extracts exert an inhibitory effect ..... on the ejaculatory response. Together, these results support the hypothesis that natural compounds contained in Mondia whitei extracts, which exerts an aphrodisiac effect in copulating rat, might be useful in preventing the ejaculatory response in a rat model for the study of ejaculation. Moreover, on page 10, it is stated that the main aphrodisiac effect of Guibourtia tessmannii extracts is due to the enhancement of the inhibitory intraspinal tone that determines the ejaculation occurrence.

Authors have to separate these notions. Actually they provide a definition of aphrodisiacs in the introduction that clearly does not include inhibitory actions of these substances on sexual responses.

The whole discussion should be reviewed in this sense, limiting the use of the term aphrodisiac where pertinent and separating it from the potential use of some inhibitory actions of the extracts for the treatment of specific sexual disorders.

Answer 2: The hypothesis of the study has been reformulated; in order to be constant in the use of the notion of `aphrodisiac`, the use of this word has been revisited especially in the discussion section. This part of the work has been rewritten accordingly.

3) Haloperidol is a nonspecific antagonist for all dopamine receptors, not only for D2-D3 subtypes. Please correct the information throughout the manuscript.

Answer 3: change done

4) Methods: the dose of dopamine used is huge (60 mg/kg, i.v.). The statement that the dose was chosen on the bases of unpublished previous studies does not support its use. Based on the reported weight of the rats employed, a single animal could have received up to 20 mg, i.v.! With this dose level you cannot conclude that the effects are due to the specific stimulation of DA receptors. For instance, it has been reported that i.v. injection of a dose as low as 100 µg/kg to rats elevates plasma LH levels (Vijayan & McCann, 1978. Neuroendocrinology 25:221-35)

What happens with lower doses of DA? Administration of 1, 3 or 10 mg/kg dopamine i.v. would still be a very high dose, but within the range of i.p. administered doses. A dose-response curve would give a better idea of the
effects of the neurotransmitter on fictive ejaculation and allow you to select the best dose for your purposes. Compare the DA dose used with the one selected for haloperidol (0.01 mg/kg), it is three orders of magnitude lower.

Answer 4: In this study, we used the same dose of dopamine (0.1uM/kg) as reported in our previous paper (Reference Watcho P, Carro-Juarez, M: Evaluation of the excopula ejaculatory potentials of Bersama engleriana in spinal male rats. Asian J Androl 2009, 11:533-539). This dose of dopamine (0.1uM/kg) was selected during my research visit at The Universidad Autonoma de Tlaxcala, Mexico, from May 26, 2007 to August 24, 2007 and is being now used in our Laboratory in Cameroon. We extremely apologize for this inconvenience. The doses of the standard drugs (dopamine and haloperidol) used in the present study are now expressed in molar/kg rather than mg/kg.

5) Comparison of the parameters of the motor trains obtained in the groups treated with the distinct drugs or extracts with those obtained after saline injection does not make sense, because in all cases saline solution did not induce a response. Thus, there are no parameters to be compared.

The authors could compare the features of the motor responses obtained with the different drugs or extracts with those of the motor responses obtained by genital stimulation (urethral or penile). Such a comparison would give information on the direction of the extract-induced changes in the parameters of the expressed motor trains, i.e. diminution or increase.

The comparison of the effects of the extract of Mondia whitei to those of dopamine lacks also of sense, again there are no parameters to be compared since there was no response to the extract. In this case, the only result to be reported is that the extract did not induce fictive ejaculation.

I would suggest authors to eliminate from table 2 those treatments that did not induce a motor response and elaborate a third table showing presence or absence (+ or -) after the different treatments. In such a table, it could be appreciated that the response induced by Guibourtia tessmannii is blocked by haloperidol, for instance.

Answer 5: Changes properly done. A third table has been added as requested.

6) The results described under the heading “Effects of the aqueous and hexane extracts of Mondia whitei on the expression of DA induced fictive ejaculation” are not clear. Authors refer to a Fig. 1E which is not included in the manuscript.

Thereafter, they state that the pro-ejaculatory effect of DA was not prevented in all spinal rats pretreated with Mondia whitei. Does this statement mean that in some spinal animals the effects of DA were prevented? If so, it would be important to provide the proportion of animals in which the response was inhibited, as well as the proportion of those exhibiting fictive ejaculation. This information should be provided for all treatments in which the authors observed that some animals responded and some others did not.

Answer 6: Many changes have been done. In fact, the dose 20mg/kg completely inhibited the pro-ejaculatory effect of dopamine.
7) In the last paragraph of the discussion the authors provide 2 references to support the notion that fictive ejaculation can be produced by systemic injection of DA acting at the spinal generator for ejaculation. None of the mentioned references (39 and 40) include that information.

Also, the notion in the last sentence of the discussion that “results support the existence of subtypes of aphrodisiac plants” is not sustained. Which classification of aphrodisiac plants divides them into subtypes?

Answer 7: New references have been introduced and the proposed classification of aphrodisiacs cancelled in the discussion section.

8) Conclusion: The data of this work do not show that the effects of Mondia whitei are not mediated by DA, as stated. They only show that the fictive ejaculation response induced by a very high dose of DA, presumably activating other mechanisms in addition to DA receptors, is not prevented by the Mondia whitei extract. Also, authors do not have evidence that Guibourtia tessmannii extracts enhance a putative inhibitory intraspinal tone. Their data show that these extracts induce the expression of fictive ejaculation with a delay (increased latency as compared to the one recorded after DA or genital stimulation), but give no information as to the mechanism mediating that effect.

Answer 8: Taking these remarks into consideration, the discussion has been reformulated and those speculative ideas eliminated.

9) There are still many language errors that make some paragraphs very difficult to understand. Seek for someone fluent in English to check the manuscript.

Answer 9: the English level has been improved.

Minor essential Revisions

Background:

1) A partial definition of ejaculation is provided. The ejaculatory response includes rhythmic increases in seminal vesicle pressure and contractions of perineal muscles as stated, but not only. Ejaculation is a more complex phenomenon. Please provide a more complete definition.

Answer 1: change done

2) Approximately at the middle of page 2, it is stated that “dopamine is a naturally occurring hormone directly responsible for ejaculation”. A more accurate statement would be “dopamine is a neurotransmitter playing a central role in the control of ejaculation”.

Answer: change done

3) In the following sentence the term “spinal” should be eliminated, since the second part of the sentence includes the statement that it was tested in spinal rats.
4) Please verify in the sentence that follows that the mentioned references (30 & 31) tested the effects of haloperidol or other dopaminergic antagonists in spinal rats, I don’t think so.

Answer: Reference 30 has been change (now Reference 35). however, reference 31 (now reference 36) is maintained since it supports our idea.

Methods:
1) Drugs heading, third line: what do you mean by the expression “extemporally dissolved” in saline solution?
This statement means freshly prepared; change done.
2) Activation and recording….. heading, last sentence. Introduce the following change to this sentence: The frequency of contractions …..was calculated by dividing the number of contractions by the duration of the motor train. The same applies to legend of table 2.

Answer: change done

Discussion:
Page 11, authors mention that the haloperidol-induced blockade of the fictive ejaculation response produced by the extracts of Guibourtia tessmannii support the hypothesis that the pro-ejaculatory actions of these extracts require the participation of spinal DA pathways. Since that hypothesis was not posed in the manuscript, authors should change the statement to indicate that the data obtained show the participation of spinal dopaminergic pathways in those actions.

Answer: change done

Conclusion:
What do the authors mean by “an inverse aphrodisiac”? Does this term exist?
Provide a reference or a definition.
Answer: that term has been cancelled from the paper.

Thank you for your understanding
Best regards
Pierre WATCHO
Corresponding author.