Author's response to reviews

Title: Termite usage associated with antibiotic therapy: Enhancement of aminoglycoside antibiotic activity by natural products of Nasutitermes corniger (Motschulsky 1855)

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Version: 2 Date: 1 June 2009

Author's response to reviews: see over
Reviewer's report
Title: Zootherapy associated with antibiotic therapy: Enhancement of aminoglycosides antibiotic activity by natural products of Nasutitermes corniger (Motschulsky 1855)

Version: 1 Date: 18 April 2009
Reviewer: Tsutomu Hatano
Reviewer's report:

This manuscript deals with the effects on extracts from an insect species on the antibacterial activity of aminoglycoside antibiotics. The results shown by the authors seem to be somewhat fragmentary, but are interesting, and I think the publication of this will be valuable for the readers of this journal, although the following problems should be fixed by the authors.

Minor Essential Revisions
1) Title of the paper: The word “Zootherapy” is not adequate in this case, since that may be regarded as some animal-assisted therapy. The authors should indicate the usage of insect in the title clearly.
THE TITLE WAS MODIFIED

2) The sentences on Lines 6-12 in page 6 are practically the same as the latter half of the previous paragraph. These sentences should be removed.
THE SENTENCE WAS REMOVED

3) Line 11 in Page 5: 1 g/ml must be 1 microgram/ml.
THE UNIT WAS CORRECTED

4) Lines 17-18 in Page 5: “Synergy was defined as an FIC > 0.5,” should be “Synergy was defined as an FIC < 0.5.”
THE SIGNAL WAS CORRECTED

5) Line 12 in Page 6: The word “neomycin” should be removed, since Table 1 indicates the synergism between CPZ and neomycin.
THE NAME WAS REMOVED

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests.

Reviewer's report
Title: Zootherapy associated with antibiotic therapy: Enhancement of aminoglycosides antibiotic activity by natural products of Nasutitermes corniger
This is an interesting study but the authors should be ashamed of submitting an MS with so many typos and English errors. There are now plenty of companies who will proof read for small amounts of money and in future I would urge the authors to send their MS out for English evaluation. Another reviewer is unlikely to be as tolerant!

**THE ARTICLE WAS REVISED BY AN NATURAL ENGLISH-SPEAKER CITIZEN**

Major compulsory revisions

Chlorpromazine has very recently been shown to alter expression of efflux pumps rather than work directly as an EPI and the authors should mention this in their MS. See the work of Piddock LJV.

**THIS INFORMATION WAS ADDED IN THE METHODOLOGY**

The revision MUST be proof read by a native English speaker.

**THE ARTICLE WAS REVISED BY AN NATURAL ENGLISH-SPEAKER CITIZEN**

Minor essential revisions.

Title - aminoglycoside singular.

**CORRECTED**

Page 2 line 2 insert the before Northeaster. Line 3 were not was. Line 4 up please rewrite the sentence 'being a new artifact form against....' as this makes no sense at all.

**ALL POINTS WERE REVISED**

Page 3 line 1 delete more inseret well. Line 5 delete of before Brazil. Line 6 insert the before West, and insert an before spreads. Line 11 evidence singular, delete 'had become' and insert 'have revised'. Liune 12 insert 'as a' before synonym.

**ALL POINTS WERE REVISED**

Line 13 insert are before also and delete can all be.. Line 14 represent singular. Line 5 up bacteria and insert such before as.

**CORRECTED**

Page 4 line 3 delete the. Line 9 delete second 'clinical'. Line 4 up nests plural. Line 2 up - do you mean reserved or removed?

**ALL POINTS CORRECTED. MEANS RESERVED**

Page 5 line 1 insert by before Prof and Systematics plural.

**THIS IS THE NAME OF HERBARIUM**

Line 9 Minimum - M
capital. Line 11 - what is the symbol before g/ml - micro?.
CORRECTED

Line 11 move 'the FIC'
after and and insert was before calculated.
CORRECTED

Line 8 up what is the full stop under >.
Line 6 up CPZ is not an EPI - it affects expression.
CORRECTED

Line 4 and 3 up did not in full.
Line 3 up showing. Line 2 up decoct and termites lower case.
CORRECTED

Page 6 line 1 delete an and inser or additive after synergistic. The only way to be sure of synergism is by a checker board assay.
CORRECTED

Line 5 re-write Despite decocts
not showing a ....decocct lower case. Next line move growth to before medium.
CORRECTED

Line 8 delete an. Line 9 insert The before decoct and termite lower case.
Next line insert again before demonstrating. Line 13 up insert the after for. Another not other. Insert that after be. Next line lower cases for cecocct and termite.
CORRECTED

Line 9 up
this sentence is nonsense - please re-write.
THE SENTENCE WAS REWRITED

Line 7 up insert the before molecular. Line 6 delete about insert of.
Line 4 up re-write as N. corniger in terms of synergism with amino glycosides or any other....
Line 2 up insert such before as.
ALL POINTS WERE CORRECTED

Page 7 line 1 delete as insert including. Line 2 no underscore. 1980s and delete decade. Delete remaining insert belonging.
Line 3 all words in lower case.
ALL POINTS WERE CORRECTED

Line 4 delete in the insert as. Delete as insert to.
Line 6 delete to the insert for.
Line 3 up decocts lower case. Line 1 up all lower case and delete as the chlorpromazine.
ALL POINTS WERE CORRECTED

Page 8 line 3 insert the before termite.
CORRECTED
Table 1 legend - insert 'of insects' after decoct. Also throughout the whole MS - mL
Table 2 legend insert 'of insects' after decoct. Also throughout the whole MS - mL
CORRECTED

Level of interest: An article of importance in its field
Quality of written English: Needs some language corrections before being published
THE ARTICLE WAS REVISED BY AN NATURAL ENGLISH-SPEAKER CITIZEN
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests

Reviewer’s report
Title: Zootherapy associated with antibiotic therapy: Enhancement of aminoglycosides antibiotic activity by natural products of Nasutitermes corniger (Motschulsky 1855)
Version: 1 Date: 4 May 2009
Reviewer: Maria Leticia Miranda Fernandes Estevinho
Reviewer’s report:
Major Compulsory revisions
Reviewer's report
Title: Zootherapy associated with antibiotic therapy: Enhancement of aminoglycosides antibiotic activity by natural products of Nasutitermes corniger (Motschulsky 1855)
Version: 1 Date: 11 May 2009

Reviewer: Zakaria Mbwambo
Reviewer's report:

Abstract. If the topic is on the effect of a natural product on antibiotics, how does chlorpromazine get in, Does the authors know that CPZ is a neuroleptic (Antipsychotic)? They should give an explanation on why involving chlorpromazine in their study. If their interest is to provide information on the effect of decocts of a termite, then
they should concentrate on termites.

CHLORPROMAZINE WAS USED IN THIS STUDY AS A POSITIVE CONTROL BY MODULATE THE ACTIVITY OF EFFLUX PUMP, ONE OF THE MOST IMPORTANT MECHANISM OF RESISTANCE TO AMINOGLYCOSIDES. THE RESULTS OF CPZ COULD INDICATE IF THE DECOCTS CAN AFFECT THE SAME OR DIFFERENT MECHANISMS AFFECTED BY CPZ.

As such there is no connection between methodology and conclusions in the abstract.

THE TEXT WAS REWRITED AND THIS CONNECTION WAS ESTABLISHED.

Background: The synonyms of N. corniger should just be indicated in a sentence and therefore ref. 9 may be deleted.

THE SENTENCE WAS REWRITED, BUT THE REFERNCE WAS NOT DELETED BY THE NECESSITY OF THE READER KNOW WHAT THE SOURCE OF THIS INFORMATION.

Page 4. The study should be limited to evaluation of decocts of N. corniger alone and not with chlorpromazine

THE TERM CHLORPROMAZINE WAS RETIRED OF THE OBJECTIVE

Methods: Two strains of E. coli were used. What is the reason for that?

WE USED ONE CLINICAL ISOLATE OF E. COLI PRESENTING MULTIRESISTANCE TO DEMONSTRATE THAT THE IMPORTANCE OF THE ANTIBACTERIAL ACTIVITY ALONE OF ASSOCIATED WITH OTHER SUBSTANCES COULD PREVENT THE MULTIRESISTANCE DEVELOPMENT. TO DEONSTRATE THIS, WAS NECESSARY THE USE OF AN ATCC STRAIN AS AN NEGATIVE CONTROL.

Identification of plant species in which termites grows is mixed with preparation of Decocts??? This is serious! The authors should separate identification issues from the section on Preparation of decocts!

THESE PARTS WERE SEPARATED

Identification of the organisms, namely, the termite and the two plant species (NOT just two plants) from which the termites were collected could be fused together in a separate paragraph.

DONE

Results: Pg 5. Decocts didn’t show antibacterial activity at 1024 µl/ml ......but when incorporated with the growth medium at 256 µl/ml, a reduction of the MIC for gentamycin and neomycin was observed in the strain E. coli 27 (but not with the ATCC8539), demonstrating an synergistic effect of this natural product with these aminoglycosides (Table 1). Authors could not reveal what is contained in the growth medium?. Something in the medium triggered the decocts to effect gentamycin and neomycin otherwise reduction of the MIC for these antibiotics (gentamycin and neomycin) wouldn’t have happened.

THE MEDIUM USED WAS BHI BROTH 10%. THE MEDIUM COMPOSITION IS SPECIFIED BY THE MANUFACTURER. BUT IN THE TEXT, WE MIX TO THE MEDIUM THE DECOCTS TO VERIFY IF THIS DECOCTS COULD AFFECT THE MIC OF ANTIBIOTICS WHEN TESTED BY MICRODILLUTION.

In the same paragraph, it is written that “Decocts of Termites from A. occidentale
L. showed synergism only against neomycin (Table 2), demonstrating the influence of the plant substrate in the pharmacological properties of this natural products of termites”. This is a confusing statement. Are we experimenting on decocts from termites of extracts from a plant species?

As explained in the Material and Methods, the decocts were produced using termites nests retired from 2 different species of trees. The results shown that the source of the nest could affect the modifying antibiotic activity of the decocts, maybe by the different composition of secondary metabolites of each tree. But the reviewer is correct to indicates this confusion. By this fact, the objective was modified to inform this point.

As pointed earlier, the authors should stick to one scientific hypothesis and try to defend it through well planed experiments and not just putting or mixing events and hence loosing scientific merit of the study.

This point was answered above.

After identification of a plant species with its authority, then in the succeeding para one does not have to again and again indicate authority e.g., A. occidentale L. (so this L. here should be deleted)

DONE

Para 3 page 6 on Synergism with CPZ is also superfluous and there is no need to suggest resistance mechanism for CPZ which is not a natural product.

The citation of CPZ in this point indicate the existence of an mechanism of resistance to gentamicin, affected by DCL but not by CPZ, indicating an mechanism different of efflux pump or one none affected by CPZ.

Discussion: Here I expected to see correlation of the study findings with the available literature. Again the authors provide literature search on peptides with antimicrobial activity and many other stories. This part requires revision.

Unfortunately, no report about the antibacterial or modifying antibiotic activity of natural products from termites were obtained. The only ones were reports using peptides and proteins. By this fact, we inform in the end of the first paragraph of discussion that is the first report of antibacterial activity of natural products of N. Corniger alone or associated with antibiotic.

Literature:
It has been difficult for me to do a complete search for all the quoted literature cited due to limitations for our web site down here. However, for consistence purposes, references should be inserted at the end of sentences.

DONE

Language is also poor, so authors should give an opportunity for someone who is eloquent in this language to read the manuscript several times in order to ensure no mistakes are allowed.

The article was revised by an natural English-speaker citizen.
Level of interest: An article of limited interest
Quality of written English: Needs some language corrections before being published
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
'I declare that I have no competing interests'