Author's response to reviews

Title: Anti-hyperglycemic effects of three medicinal plants in diabetic pregnancy: modulation of T cell proliferation

Authors:

Akadiri Yessoufou (yeskad2001@yahoo.fr)
Joachim Gbenou (gjdjim@yahoo.fr)
Oussama Grissa (grissa_oussama@yahoo.fr)
Aziz Hichami (aziz.hichami@u-bourgogne.fr)
Anne-Marie Simonin (anne-marie.simonin@u-bourgogne.fr)
Zouhair Tabka (zouhair.tabka@rns.tn)
Mansourou Moudachirou (moudmans@yahoo.fr)
Kabirou Moutairou (kamoutairo@yahoo.fr)
Naim A Khan (naim.khan@u-bourgogne.fr)

Version: 2 Date: 31 December 2012

Author's response to reviews: see over
Dear Editor-in-Chief,

Please find enclosed herewith the duly revised version of our MS entitled as above by Akadiri Yessoufou, Joachim Gbenou, Oussama Grissa, Aziz Hichami, Anne-Marie Simonin, Zouhair Tabka, Mansourou Moudachirou, Kabirou Moutairou, Naim A. Khan, for publication in *BMC Complementary and Alternative Medicine*.

We have revised the MS as per instructions of the Referees. I am also herewith enclosing the rebuttal / answers to the referee’s comments for your kind and perusal consideration.

Our work has been neither published nor submitted for publication and it has not been sanctioned by any editorial committee. All the experiments have been conducted as per European ethical guidelines, and the protocols have been approved by the Regional Ethical Committee (Comité d’Ethique de l’Expérimentation Animale of University of Bourgogne, Dijon, France; Researcher Authorization number n°: 21 CAE 069)”. All the authors have given their consent for submission of the same for publication in *BMC Complementary and Alternative Medicine*.

We are submitting the revised version of the MS within stipulated time, i.e., no latter than December 31st 2012.

We hope that this MS will find a suitable place in your esteemed journal.

Sincerely yours,

Akadiri Yessoufou

*Dr. Akadiri YESSOUFOU, PhD*
University of Abomey-Calavi and Institute of Applied Biomedical Sciences (ISBA), 01 BP 918 Cotonou, Benin; Tél: +229.21.30.55.65 (laboratory); E-mail: yeskad2001@yahoo.fr
Answers, point by point, to Reviewers’ comments

The reviewers appreciated the paper; however, they addressed some major and minor points which we have now rectified accordingly in this revised version of the MS. The answers to their comments are as follows:

**Answers to Editorial comments:**

Dear Editor,

- Yes, we have now included the full name of the ethical committee which approved the study. Please, see Materials and Methods section “The experimental protocol was approved by the Regional Ethical Committee (Comité d’Ethique de l’Expérimentation Animale of University of Bourgogne, Dijon, France; Researcher Authorization number n°: 21 CAE 069)”.

- As per your recommendation, we have now improved the language skill by a Colleague that we thank in Acknowledgement section.
Reviewer 1: Mohammed Aqil

Minor Essential Revisions

1. As per Reviewer’s instructions, we have now, in new Table-1 (old Table-2), written name of plant in italic and removed name of the plant family.

2. We agree with the Reviewer; we have now mentioned name of test for each secondary metabolite used for phytochemical screening in new Table-1. Please see the revised MS.

3. In new Table-1 (old Table-2) [1 cm at 8/10]. It means that the decocted extract was diluted at 80% (8/10) and the foam index (FI) was at the height ≥ 1 cm. We have now clearly mentioned in the new Table-1 to make it more comprehensive: Foam index (FI) of diluted aqueous decoction (positive (+) if FI ≥ 100, meaning foam height ≥1 cm).

Other minor Revisions

As per Referee’s instructions, we have made all changes as mentioned in the Table below and included all changes accordingly in the MS.

<table>
<thead>
<tr>
<th>Page number</th>
<th>Revised manuscript</th>
<th>First version</th>
</tr>
</thead>
<tbody>
<tr>
<td>2- abstract</td>
<td><em>Nauclea latifolia</em> (root and stem)</td>
<td><em>Nauclea latifolia</em> (root stem)</td>
</tr>
<tr>
<td>3 - background</td>
<td><em>Oxytenanthera abyssinica</em> (Gramineae)</td>
<td><em>Oxytenanthera abyssinica</em> (Gramineae family):</td>
</tr>
<tr>
<td>5- Preparation of different plant extracts:</td>
<td>root and stem of <em>Nauclea latifolia</em> (Please see 2nd Paragraph)</td>
<td>root stem of <em>Nauclea latifolia</em></td>
</tr>
<tr>
<td>5- Preparation of different plant extracts:</td>
<td>The ethanolic extract was obtained and subjected to liquid-liquid extraction (Please see 2nd Paragraph)</td>
<td>Then, we obtained the Ethanol-extract (a) that will be used:</td>
</tr>
<tr>
<td>5- Preparation of different plant extracts:</td>
<td>all the extracts were used for study (Please see 2nd Paragraph, last sentence)</td>
<td>All of the extracts were then used for experiments below</td>
</tr>
<tr>
<td>New Table-1 (old Table-2)</td>
<td><em>Nauclea latifolia</em> (root and stem)</td>
<td><em>Nauclea latifolia</em>: (root stem)</td>
</tr>
<tr>
<td>New Figure-4 and Figure-3 (old Figure 1 and Figure 2)</td>
<td><em>Picralima nitida</em></td>
<td><em>Picralima nitida</em></td>
</tr>
<tr>
<td>New Figure-4 and Figure-3 (old Figure 1 and Figure 2)</td>
<td><em>Oxytenanthera abyssinica</em></td>
<td><em>Oxytenanthera abyssinica</em></td>
</tr>
<tr>
<td>New Figure-4 and Figure-3 (old Figure 1 and Figure 2)</td>
<td><em>Nauclea latifolia</em></td>
<td><em>Nauclea latifolia</em></td>
</tr>
</tbody>
</table>
Reviewer 2: Gustavo Volpato

Major Compulsory Revisions

The real objective of this work is to investigate the efficacy of three different anti-diabetic plants on hyperglycemia during diabetic pregnancy in rats. As per Reviewer’s instructions, we have now largely reviewed and rearranged this manuscript in order to make this objective clearly understandable. The title is also modified to make it more close to the results in this study. The new title proposed is as follows: “Anti-hyperglycemic effects of three medicinal plants in diabetic pregnancy: modulation of T cell proliferation”.

Figures and Tables are now in properly order to understand this objective. Introduction, Materials and Methods are now reorganized. Discussion is modified. Please see the revised manuscript.

Briefly, after plants’ collection and extracts’ preparation, we present, in this revised manuscript, results of the effect of extracts on glycemia of pregnant diabetic rats. To explain these effects, we analyze phytochemical composition of these plants (fatty acids, chemical compounds, vitamin C) and their antioxidant capacity. We also examine the effects on the plant extracts on T cell proliferation, since antioxidants have been reported to modulate immune system. T cell proliferation was made in human Jurkat T cells (non-diabetic, non-pregnant person). We believe that these results are the preliminary one and further investigations are required to examine plants effects on lipids, body weight (mother, foetus and offspring), insulin and other diabetes- and macrosomia-related parameters in diabetic pregnant rats and their offspring.

Essential Minor Revisions

1. The Reviewer raises the question of diabetes model which leads to macrosomia. In fact, in the present work, we have referred into many of our works [17-22] in which experimental diabetes, induced by five low doses of STZ, leads to macrosomia in offspring. However, another model of diabetes could, in contrast, lead to microsomia (low birth weight). Indeed, the group of Van Assche has exhaustively investigated the consequences of experimental maternal diabetes induced by streptozotocin on fetus and adult progeny [9 Exp Diab Res]. In fact, experimental diabetes during pregnancy, induced by a high single dose of streptozotocin, occurs by direct toxic effects on pancreatic β-islet cells [9 Exp diab res]. The fetal growth is retarded, leading to fetal microsomia (low birth weight) [9 Exp diab res]. Postnatal development is also retarded, and these offspring remain small at adulthood; however, they develop insulin resistance [Van Hassche 9]. However, streptozotocin, administered at low doses during 5 consecutive days, induces mild type 1 diabetes, following a T-lymphocyte-dependent process, an autoimmune destruction of pancreatic β cells [22 Herold exp diab res]. This model of diabetes during pregnancy leads to macrosomia in offspring. Macrosomic (large-sized) offspring of diabetic dams maintained an accelerated weight gain until adulthood [17-22]. Since, in this paper, we only focus on maternal diabetes, but not on macrosomia, we just evoked, in the Introduction, macrosomia which appears as main consequence of maternal diabetes in offspring. However, we have now added this precision to the Introduction (fifth paragraph) and have discussed the model of maternal diabetes that we induced in this study. Please, see the Discussion section.

As per Reviewer’s instructions, we have now added this precision the MS. Please see the fifth paragraph of Introduction.

2. We agree with the Reviewer who suggests changing the last sentence of the conclusion. We have now changed the same and replaced by the following: “However, further studies are required to elucidate the effects of different extracts of plants in the
modulation of different parameters related to diabetic pregnancy and autoimmune diseases”.

**Discretionary Revisions**

1. As per Reviewer’s suggestion, we have now removed the software item used in the study (last heading “Statistical analysis” of Materials and Methods section).
Reviewer 3: Ching-Hua Yeh

Major Compulsory Revisions

1. As per Reviewer’s instructions, we have now clearly specified in the revised MS that plant extracts diluted in sterile physiological saline solution (NaCl 0.9%) were sterilized and filtered [filter 0.20 µm (Nalge Nunc International Corp., USA)] before injecting to pregnant rats or adding into cell culture, to prevent eventual infection and bacterial contamination. Please see the Materials and Methods section (Diabetes induction and animal treatment by plant extracts, 3rd paragraph)

2. In new Table-1 (old Table-2), we have presented data of phytochemical screening of total brut extracts of plants, not those of each fraction. However, we have discussed appropriately the correlation between the polyphenol contents and the antioxidant activities of the extracts. Moreover, we have now mentioned name of test for each secondary metabolite used for phytochemical screening in new Table-1. Please see the revised MS.

3. We agree with the Reviewer who suggests that the activated T cell distribution and IL-2 concentration in pregnant-STZ rats treated with and without plant extracts will be more comprehensive. The proposed experiments could have been performed on fresh cell samples. We feel so sorry for not presenting these results. We propose to perform these experiments for further investigations on these plants.

I hope that the referee will appreciate our concern.

Essential Minor Revisions

1. We have now, in methods section, replaced RPMI-1940 by RPMI-1640.

2. As per Reviewer’s suggestions, we have now changed the glycemia unit from g/L to mg/dl, in new Figure 1 (old Figure 3). Please, see the Figure 1 legends

3. In new Table 1 (old Table 2), the levels of compounds are now indicated as follows: (++++) too high, (+++) high and (+) low, indicating the presence of compounds; (-) indicates the absence of compound in plant extracts. Please, see the Table-1 legends.

4. We have now rectified the legends of new Figure 3 (old Figure 2) accordingly. The unit is expressed as “Time of 50% of haemolysis (min)”; (not Equal to Tolox).

5. We highly do appreciate the comments of the Reviewer about the use of plant extracts in diabetic pregnant women in infectious/inflammatory conditions as the extracts inhibit T cell proliferation under stimulation. As you and we are aware, most of medicines exhibit undesirable effects and this case could be one of plant applications in diabetic pregnant patients in infectious/inflammatory conditions.

6. In this paper, we report results about effects of extracts on glycemia in pregnant diabetic rats. To explain these effects, we analyze phytochemical composition of these plants (fatty acids, chemical compounds, vitamin C) and their antioxidant capacity. We also examine the effects on plant extracts on T cell proliferation, since antioxidants have been reported to modulate immune system. We believe that further investigations are required to examine plants effects on lipids, body weight (mother, foetus and offspring), insulin and other parameters related diabetes and macrosomia in diabetic pregnant rats and their offspring.

We feel so sorry for not investigating, in this paper, all these parameters which will be the subject of our future investigations.

I hope that the referee will appreciate our concerns.