Author’s response to reviews

Title: Effects of polyphenols and lipids from Pennisetum glaucum grains on T-cell activation: modulation of Ca2+ and ERK1/ERK2 signaling

Authors:
Abdelhafid Nani (nanifleches2003@hotmail.com)
Meriem Belarbi (me.belarbi@hotmail.fr)
Wided Ksouri-Megdiche (ksouriwided@yahoo.fr)
Souleymane Abdoul-Azize (bontasso@yahoo.fr)
Chahid Benammar (chabena62@yahoo.fr)
Francois Ghiringhelli (francois.ghiringhelli@yahoo.fr)
Aziz Hichami (Aziz.Hichami@u-bourgogne.fr)
Naim A Khan (Naim.Khan@u-bourgogne.fr)

Version: 4 Date: 18 October 2015

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Point-by-point answers to the reviewers comments

Reviewer: Roshanara Mishra

1. Mentioning n3:n6 ratio and whether it is in the required range will strengthen the claim that the lipid is immunomodulatory.

Answer Q1.

Nowadays, it has been understood that modern Western diet is poor in n-3 PUFAs (Marventano et al. Int J Food Sci Nutr. 2015). Indeed, increased intake of vegetable oils (sunflower, corn oil, safflower oil, and soybean oil) rich in n-6 PUFA has shifted the n-6 vs n-3 PUFA ratio from 10:1 to #50:1 (Umesha and Naidu, Food Chemist. 2012). In our study, we have estimated n-6:n-3 ratio in PGL about 14:1 which is close to the recommended ratio (Ma et al. Crit Rev Food Sci Nutr. 2013).

We have added this description in the revised MS (line 300).

2. There are few more reports available on beneficial role of pearl millet in health and disease. Those references might be added.

Answer Q2.

Although several reports have dealt with the beneficial role of other millet species such as finger millet (Shobana et al. Br J Nutr. 2010; Shukla et al. J Food Sci Technol. 2014), foxtail and proso millet (Lee et al. Nutr Res. 2010), there are a few available studies related to the beneficial effects of pearl millet on health (Shahidi et Chandrasekara J. Func Foods. 2013; Nani et al. Ann Biol Res. 2011; Nambiar et al. J App Pharmaceut Sci. 2011). We have cited our study (Nani et al.
Ann Biol Res. 2011) in the revised MS wherein we have reported that pearl millet-enriched diet improves hyperglycemia and weight in streptozotocine (STZ)-induced diabetic Wistar rats. We have also cited the study of Nambiar et al. (2011) who have proposed, in the view of its chemical composition, that pearl millet could exert several beneficial effects in health (anemia, constipation, cancer, and diabetes).

We have added in the revised MS, some references that have dealt with beneficial role of pearl millet in health and diseases; line 20.

3. High concentration of both tannin and total phenol was considered anti-nutritional (Sade, 2009) - a clarification will be appreciated in discussion with regards to its effect on T cell. Whether millet diet can be consumed normally or only as adjuvant for treatment of autoimmune diseases.

Answer Q3.

We agree with Sade FO (J Food Tehnol. 2009) who claimed that a high concentration of both tannin and total phenol should be considered anti-nutritional.

Generally, pearl consumption does not exhibit any toxic affect as millet-based foods are consumed in many African and Asian countries (Nambiar et al. J Appl Pharmaceut Sci. 2011). Furthermore, it has been reported that pearl millet contains negligible tannin content (Badau et al. Int J Food Prop. 2002). Indeed, Sade FO (J Food Technol. 2009) estimated the tannin content at 0.51 mg/ 100 g of raw pearl millet flour. The quantity of tannin present is ineffective to exhibit any side effect on health as it has been reported that in rats, proanthocyanidins, the more common tannins in our diet, may be effective beyond the doses of 1% of diet (Vallet et al. Ann Nutr Metab. 1994).


Since pearl millet seeds contain negligible tannin content, the inhibitory effect of PGPC on splenic T cells proliferation could be attributed to other phenolic compounds like ferulic acid (see our MS; line 298) rather than to tannins. Pearl millet could be, hence, consumed normally for treatment of autoimmune diseases.

4. Rowe et al, 2012 have reported that ferulic acid enhances {gamma}{delta}-T cell proliferation - kindly discuss.

Answer Q4.

Numerous studies have shown that plant polyphenols, like ferulic acid, enhance ## T cell proliferation (Rowe et al. FASEB J. 2012; Holderness et al. J Immunol. 2007; Jill et al. J Leuk Biol 2007). As ## T cells represent a small population of T
cell in spleen (2- 4%) (Lawetzky et al. J Immunol. 1990), the probable stimulatory effect of PGPC on such population might be masked because of T cells dominance (96%).

We have added in our MS (line 298) the probable effect of ferulic acid on IL-2 induced proliferation of splenic cells.

Reviewer : YungChia Chen

Figure 1 is not easy to know and its figure legend is not completely matched the figure. Authors described that they use trypan blue and WST-8 proliferation assays to determine the cell viabilities, however, there is no trypan blue exclusion data??

Answer

We removed trypan blue exclusion test from the text (line 138 in MS Nani et al.) and from the legend of Fig.1.

Reviewer: Gee Jun Tye

I do not agree with the term adjuvant in line 336 as it suggests for a vaccination programme. The use of "dietary supplements" would be far better suited as it seems slightly over claim.

Grammatical error can be seen in the paper and would be best if a native English user reviews it.

Answer

We agree with you that such results are not enough to consider pearl millet as adjuvant, so we have changed the term adjuvant to "dietary supplements" in the MS (line 349).

The revised Ms has been re-read and corrected appropriating for English language grammar.

References discussed / mentioned in the answers:


Rowe CA, Bonard SP, Stanilka JM, Percival SS. Ferulic acid enhances ##-T cell proliferation. FASEB J. 2012; 26:644.2.


Vallet J, Rouanet JM and Besancéon P. Dietary grape seed tannins: effects on nutritional balance and on some enzymic activities along the crypt-villus axis of rat small intestine. Ann Nutr Metab. 1994; 38:75-84.
