Author's response to reviews

Title: Modulation of T cell proliferation and cytokine response by Plumbagin extracted from Plumbago zeylanica in collagen induced arthritis

Authors:

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Author's response to reviews: see over
Abstract: Background: Plumbagin (5-hydroxy-2-methyl-1, 4-naphthoquinone) purified from Plumbago zeylanica was studied on immunomodulatory functions in Collagen induced arthritis. Methods: Effect of Plumbagin on different immunoglobulin G subclasses and Con A induced T-cell proliferation in collagen induced arthritic DBA/1 mice was observed. In addition the cytokines like Interleukin-2 and Interferon-χ levels were estimated from the T cell culture supernatants of arthritic mice primed with different concentrations of plumbagin (0.1, 0.2, 0.4 mg/mouse). Results: Plumbagin significantly suppressed the anti-collagen antibodies when compared to the indomethacin. Immunoglobulin G2a levels were also decreased in Plumbagin treated mice. We also observed that Plumbagin enhanced the decreased Con A induced T cell proliferation and Interleukin-2 production in arthritic mice. Moreover elevated levels of IFN-χ were found to be decreased in Plumbagin treated spleen cell culture supernatants. Conclusion: The results obtained in the current study indicate that Plumbagin very effectively modulates both T cell as well as B cell responses.

Dear Sir

I am sending revised manuscript with incorporation as per the suggestions made by both reviewers.

Abstract restructured into Background, Methods, Results, Conclusions.

We followed guidelines as per the animal ethical committee. I am sending our Institutional ethical clearance certificate

Mentioned about no competing interests

No financial competing interests

Financial competing interests

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Are there any non-financial competing interests (political, personal, religious, academic, ideological, intellectual, commercial or any other) to declare in relation to this manuscript? If so, please specify.

Not applicable.

Thanking you

Yours sincerely

Aparanji Poosarla
Addressing Reviewers comments

Referee-2

1. **Selection of Plumbagin and its dose**: Root of Plumbago zeylanica was one of the constituent of herbal formulation used by tribals for effective treatment of Joint pains. We carried out bioassay guided fractionation to see the effect of Individual constituents against anticollagen antibodies in collagen induced arthritic mice. Ethyl acetate fraction was potent in decreasing inflammation as well as B cell response. We performed all expts with PZE-6 of Plumbago zeylanica. After that we purified and tested with animal model subjected for structural studies as it contains Plumbagin. Dose effect was studied in male DBA/1 mice (30gm). It may vary in different haplotypes.

2. **Hypothesis**: As per your suggestion I have corrected the sentence for Ref1. Introduction was also shortened.

3. **Materials and Methods**: Treatment was given weekly thricce six weeks i.e day 0 to day 42. It was corrected. Anti collagen antibody estimated with sera samples collected at week intervals. (3,5,7). Complete ELISA protocol was written.

4. **Results**: The details of Results were stated as per figures accordingly.

5. Suggestions incorporated

6. **Discussion**: It was restructured as per your valuable advises.

7. **We carried out experiments with available cytokines in the lab. Further we would like to determine IL-1, IL-17 and TNF-A.**

8. **T cell have three subpopulations Th1, Th2 and Th17. We have observed the decrease of T cell proliferation in CIA mice.**
9. Plumbagin may stimulate the TH2 population. T cell proliferation requires IL-2.

10. Legends for all figures were corrected.

11. Other comment: All suggestions were incorporated. IFN-γ was estimated as per R&D ELISA kit protocol. May its secretion was low in 48hr culture for estimation.

Referee-1

1. Statistical analysis, two way ANOVA of results was done using SPSS software.

2. Significance levels were mentioned as p value less than 0.05.

3. Materials and methods
   
   Emulsion A and B were specified
   
   The dosage of indomethacin 3 mg/kg
   
   Regarding animal experimentations suggestions incorporated and enclosed Institutional ethical committee document
   
   Corrections incorporated
   
   Voucher number of the sample (4232) mentioned

Discussion section modified.