General Conclusions
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The present study revealed several unique features with regard to induction and confirmation of CIA in rats and the effect of a novel tetrapeptide derivative PEP1261 on proliferation, apoptosis and MMP-2 expression of cultured synovial fibroblasts.

In order to find out the effects of PEP1261, collagen induced arthritis model was first standardized. Biochemical, radiological, immunological and histological observations together with the presence of gelatinolytic activity in serum showed that CIA could be induced optimally in rats using a combination of 450µg of *Mycobacterium tuberculosis* and 150µg of Collagen type II.

Addition of PEP1261 to synovial fibroblasts showed dose and time dependent inhibition of proliferation, which could be confirmed by viability of the cells and DNA synthesis by trypan blue exclusion method/MTT assay and $[^3]H$-thymidine incorporation. PEP1261 was effective in the inhibition of cultured synovial fibroblasts from both arthritic and control rats.

Synovial fibroblasts treated with peptides revealed induction of apoptosis, which resulted in cleavage of caspase-3 and its death substrate poly- (ADP-ribose) polymerase (PARP). Pre-treatment of cells with caspase-3 inhibitor prevented inhibition of $[^3]H$ thymidine incorporation, DNA fragmentation, cleavage of caspase-3 and PARP as confirmed by western blotting as well as annexin-V/PI-staining using flow cytometry and also demonstrated that apoptosis was inhibited. However, caspase-1 and caspase-2 inhibitors did not prevent the peptides from
inducing apoptosis. Hence, caspase-3 might have a critical role in apoptosis induced by peptides.

Treatment of synovial fibroblasts with NO (Nitric oxide) donor, SNAP showed significant elevation of nitric oxide levels and resulted in absence of apoptosis by preventing the inhibition of [\(^3\text{H}\)] thymidine incorporation. This was evidenced by DNA fragmentation, annexin V/ PI staining, caspase-3 and PARP cleavage as well as intra cellular caspase-3 activity. In contrast, treatment with PEP1261 and parental peptides followed by SNAP treatment, induced apoptosis as well as lowering the levels of nitric oxide. These results suggested that PEP1261 suppressed the proliferation and induced apoptosis in synovial fibroblasts from CIA.

This study also demonstrated that nitric oxide donor enhanced MMP-2 expression in synovial fibroblasts and as such, it might be an important contributor to the development of the characteristic features of RA. In addition, it was shown that nitric oxide upregulated MMP-2 mRNA expression in the fibroblasts, confirming the role of the nitric oxide in RA. Hence, studies were taken up to examine whether PEP1261 was able to inhibit nitrite levels and experiments performed on synovial fibroblasts showed inhibition of nitrite level as well as MMP-2 mRNA expression.

Apart from in vitro studies, tetrapeptide derivative (PEP1261) was tested for its antiarthritic activity in vivo in collagen induced arthritis in rats. CIA rats showed an increase in paw volume and an increase in the levels of serum hepatic
enzymes. Histological studies on ankle joint showed marked inflammatory reaction with infiltration of numerous mononuclear cells in the subepithelial stromal tissues. PEP1261, at the Median effective dose (ED$_{50}$) of 15-mg/kg-body wt., exhibited a significant antiarthritic activity as evidenced by lowering of paw volume and histopathological studies on ankle joints confirmed the above results by showing a decrease in mononuclear cell infiltration, hypertrophy, hyperplasia and pannus formation. PEP1261 was also noticed to inhibit the levels of SGOT, SGPT and alkaline phosphatase and normal histology was observed in liver tissue after PEP1261 treatment. This clearly indicated that PEP1261 did not exhibit any toxicity or systemic side effects. In addition, TUNEL positive cells were observed in ankle joints after treatment with PEP1261 indicated that apoptosis was induced.

Both in vitro and in vivo studies clearly demonstrated that the tetrapeptide derivative (PEP1261) induced apoptosis and it was very effective as an antiarthritic agent in collagen induced arthritis in rats.

In conclusion, the results of the present investigation have fulfilled the objective and scope of this study. It is earnestly hoped that the results obtained have opened up newer horizons of research for further work in the area of rheumatoid arthritis and its management by peptide drugs. Because of their comparatively less side effects, peptide drugs and peptidomimetics will have a significant role in future drug development and administration. Further intensified efforts are necessary to test the tetrapeptide derivative in in vivo models to
understand its mechanism of action. This model of Collagen induced arthritis in rats could be safely used for the pharmacological assessment of antiarithmetic drugs.