



## ABSTRACT

Inflammation-related diseases are recognized as the major cause of morbidity around the globe. In this study, the anti-inflammatory potential of sericin, curcumin and their mixture was investigated *in vivo* and *in vitro*. Edema was induced via 1% carrageenan and then sericin (0.03, 0.06, 0.09 mg/ml), curcumin (1%, 2%, 3%) doses were applied topically. Based on the preliminary results a mixture of best concentration of sericin and curcumin was successfully achieved and was used in further studies. The paw circumference and thickness was measured after 1, 2, 3, 4, 5, 6-hour post carrageenan injection. Furthermore, the blood was collected by heart puncture technique for hematological analysis to observe the mean count of erythrocytes, hemoglobin, platelets, white blood cells (neutrophils, monocytes, eosinophils and basophils) in control and experimental groups. The levels of IL-4, IL-10 were measured from the serum. In mice fibroblast cells, sericin (20, 40, 60  $\mu\text{g/mL}$ ), curcumin (5, 10, 20  $\mu\text{M}$ ) and mixture concentrations were applied and then stimulated with Lipopolysaccharide to check series of inflammatory mediators. Afterward, the cells were used for the analysis of gene expression and supernatant was collected for protein expression of IL-1 $\beta$ , IL-4, IL-10 and ICAM-1. Scratch assay was also performed to check the influence of sericin, curcumin and their mixture on the migration index of the McCoy cell lines towards the gap. The effect of sericin, curcumin and their mixture on the translocation of p65 was analysed by western blot technique. Our results demonstrated that sericin and curcumin caused a dose dependent reduction in edema whereas, the mixture-treated group reduced the paw thickness and circumference most significantly ( $p=0.0001$ ). In the carrageenan alone group the number of erythrocytes, hemoglobin and platelets were decreased, whereas count of white blood cells was increased significantly. The effect of sericin and curcumin was dose dependent. The highest concentrations of sericin (0.09 mg/ml) and curcumin (3%) reversed the blood parameters most to the normal. The hematological studies for this mixture formulation of sericin 0.09 mg/ml sericin and 3% curcumin were reported for the first time and they significantly ( $p < 0.05$ ) balanced all blood cells. Furthermore, the mixture-treatment of carrageenan-inflicted group increased the levels of anti-inflammatory cytokines IL-4 (650.87pg/ml) and IL-10 (183.14 pg/ml) in comparison to carrageenan control. The *in vitro* data revealed that among all the treatment doses



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mixture-treated group effectively reduced the gene and protein expression of IL-1 $\beta$  and ICAM-1 in comparison to McCoy cells stimulated with LPS. Moreover, mixture-treatment elevated the expression of IL-4, IL-10 at genes (4.3 folds; 3.7 folds, respectively) and protein levels (169.33 pg/ml; 141.83 pg/ml, respectively). Mixture of sericin and curcumin has markedly increased the movement of McCoy cells towards the gap in the scratch assay and also inhibited the NF-kB pathway by attenuating the translocation of p65 nuclear factor. Current study reports the enhanced anti-inflammatory effects of mixture of curcumin and sericin through modulating expressions of interleukins *in vitro* and *in vivo*. Thus, natural products (curcumin and sericin) based formulations have greater potential for clinical investigations.