Abstract

Rheumatoid arthritis (RA) is one of the most prevalent, multifactorial, chronic, inflammatory and progressive autoimmune disease which consistently affects 0.5–1.0% of the population worldwide. Currently, a number of standard treatments are used which alleviates the symptoms of RA, but their long-term use is associated with significant side effects. Thus, complementary alternative medicines from plant natural products proves effective to ameliorate the symptoms of RA with minimum side effects. The current research investigates the anti-arthritic potential of *Nigella sativa* (NS) against FCA induced RA in mice. For rheumatoid arthritis induction, Swiss albino mice was injected with FCA in sub-plantar region of left foot pad. Disease induction assessment was confirmed by biochemical (increased level of CRP, RF, Anti-CCP2, TNF-α and decreased level of IL-10 as compared to control group), histopathological (cartilage and bone erosion, synovitis development, matrix degradation and joint destruction in comparison to control group) and radiographic (soft tissue swelling, hyperostosis, edema, periarticular bone erosion as compared to control group) analysis at day 21. Rheumatoid arthritis induced groups were treated with *Nigella sativa* extract (dose 400 mg/kg orally), *Nigella sativa* conjugated silver nanoparticles (dose 400 mg/kg) and Methotrexate (0.5 mg/kg) for 38 days. At the end of the trial at day 60, anti-arthritic potential of these biomaterials was assessed by biochemical, histological and radiographic analysis. Various immunological markers (RF, anti-CCP, CRP and PGE2), pro-inflammatory (TNF-α, IL-6 and IL-1β) and antiinflammatory (IL-10) cytokines, oxidative stress (CAT, SOD, GSH and MDA), liver function test (AST, ALT and ALP) and hematological biomarkers (RBCs, WBCs, Hemoglobin and Platelets) were evaluated. *Nigella sativa* conjugated silver nanoparticles (NSNP) showed most favourable results among all treatment groups. NSNP alleviates the levels of RF (7.1±0.2 IU/l), Anti-CCP2 (16.00±0.71 U/ml), CRP (5.1±0.3 mg/l), PGE2 (42.0±1.4 ng/ml), TNF-α (33.0±1.8 ng/ml), IL-6 (16.0±0.7 pg/ml), IL-1β (299.8±3.7 pg/ml), MDA (5.6±0.2 nmol/l), AST (284.60±6.00 U/l), ALT (129.20±3.65 U/l) and ALP (212.00±4.47 U/l), WBC (6.65±0.17×103/µl) and platelets (1399.24±8.39×103/µl) as compared to FCA group (disease control); RF (13.5±0.4 IU/l), Anti-CCP2 (32.76±1.74 U/ml), CRP (8.4±0.4 mg/l),

PGE2 (73.0±2.6 ng/ml), TNF-α (53.0±2.6 ng/ml), IL-6 (31.8±2.2 pg/ml), IL-1β (451.0±12.2 pg/ml), MDA (10.4±0.2 nmol/l), AST (465.20±11.66 U/l), ALT (233.60±4.11 U/l) and ALP (374.00±5.96 U/l), WBC (11.42±0.29×103/µl) and platelets (1894.01±16.32×103/µl) ($P<0.001$) respectively. Moreover, it also showed significant increased levels of IL-10 (27.2±1.2 pg/ml), CAT (164.00±2.07 nmol/l), SOD (118.2±4.2 U/ml), GSH (3.16±0.12 umol/l), RBCs (7.68±0.23×106/µl) and Hemoglobin (11.7±0.2 g/dl) as compared to FCA (disease control) group. IL-10 (7.0±0.4 pg/ml), CAT (89.20±3.12 nmol/l), SOD (50.4±3.2 U/ml), GSH (1.62±0.10 umol/l), RBCs (5.72±0.15×106/µl) and Hemoglobin (10.5±0.3 g/dl) ($P<0.001$) respectively. Histopathological and radiographic analysis also showed alteration in tibiotarsal joint architecture in FCA treated group, which restores most significantly in NSNP treated group among all other groups. Thus it is concluded that NSNP served as a promising treatment for RA and enhanced the anti-inflammatory, antioxidant and anti-arthritic potential of *Nigella sativa*, thus significantly ameliorated rheumatoid arthritis symptoms effectively.