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Short communication

Anti-osteoarthritic activity of *Bungarus fasciatus* venom fraction BF-F47 involving molecular markers in the rats

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ABSTRACT

A heat stable protein BF-F47 was purified from the crude venom of *Bungarus fasciatus* by CM cellulose ion exchange chromatography and HPLC. Osteoarthritis (OA) was developed in male albino Wistar rats by collagenase injection. BF-F47 treatment significantly restored urinary hydroxyproline and glucosamine in OA rats. Serum acid phosphatase, alkaline phosphatase, creatinine and serum molecular markers TNF-α, IL-1β, IL-17, cytokine induced neutrophil chemoattractant-1, matrix metalloproteinase-1, cathepsin-K, osteocalcin and PGE2 were also significantly altered. BF-F47 showed partial restoration of osteoarthritis joints. Thus, BF-F47 induced anti-osteoarthritic activity in Wistar rats acted through molecular markers of arthritis and inflammation.

Osteoarthritis (OA), the 'wear and tear' disease, is a progressive bone joint disorder, caused by different factors like injury, aging, overload, etc. (Felson et al., 2000). It affects mostly the knee joint (6%) and hip joint (3%) (Hedbom and Hauselmann, 2002), ultimately leading to bone and cartilage damage and erosion, causing pain, inflammation and restricted movements in the elderly people. OA may be idiopathic with no identifiable etiology or results from injury of the joint (Martel-Pelletier et al., 1999). Studies have indicated that inflammation of the synovium may play an important role in the pathogenesis of OA (Smith et al., 1997; Fiorito et al., 2005). Synovial inflammation is characterized by an infiltration of neutrophils, T lymphocytes and monocytes as well as vascularization and hyperplasia of the synovium (Schulte et al., 1994; De Clerck et al., 1995). From non-pharmacological treatments (exercise, weight loss therapies, therapeutic ultrasound, bracing, etc.), complementary treatments (glucosamine and chondroitin supplements) to pharmacological treatments (acetaminophen, NSAIDs, opioids, intra-articular injections of corticosteroids, hyaluronic acid, lidocaine, etc.), several other treatment options are there depending on the severity of the disease. These treatment options are either expensive or have adverse effects after prolonged use. Alternative medicines and natural products are showing promising results as anti-arthritic agents. In pathological conditions like arthritis, cancer, etc., snake venom was used from the ancient time as mentioned in Ayurveda, Unani and folk medicine (Pal et al., 2002). However, the rational therapeutic use of snake venom needs scientific validation. Anti-arthritic activity of Indian monocellate cobra (*Naja kaouthia*) venom and *Naja naja* venom purified protein NN-32 has been reported on FCA-induced arthritic rats (Gomes et al., 2010, 2014).

Bungarus fasciatus (Banded krait) an elapid snake, is prevalent in North-East India. Its venom is a cocktail of many enzymes and protein molecules. Among the several venom constituents, an antibiotic compound, Cathelicidin BF has been identified which is used to treat acne vulgaris (Wang et al., 2008, 2011), proved to be effective against salmonellosis (Xia et al., 2015) and possesses anticancer activity (Wang et al., 2013). A protein, BF-CT1 has been isolated from *Bungarus fasciatus* venom, promotes apoptosis of leukemic cancer cells and inhibits cell cycle progression at the

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check points (Bhattacharya et al., 2013). *Bungarus fasciatus* venom showed anti-arthritic and anti-inflammatory activity in experimental models (Ghosh et al., 2015). The present study was an effort to evaluate the anti-osteoarthritic activity of *Bungarus fasciatus* venom fraction BF-F47 in experimental osteoarthritic rat models.

All the chemicals and reagents used in this study were of analytical grade unless otherwise mentioned. Male albino Wistar rats (120 ± 10 g) were purchased from approved animal breeders of University of Calcutta. Animal ethical clearance was availed before conducting experiments [Approval no. IAEC-III/Proposal/AG-02/2012 dated 07.06.2012]. Lyophilized *Bungarus fasciatus* venom (BFV) was collected commercially from Calcutta Snake Park, Kolkata, India. BFV (60 mg) was dissolved in distilled water, heat treated at 70 °C for 30 min, then centrifuged at 1500 rpm for 15 min. Supernatant was collected, applied on Ion exchange chromatography using CM-cellulose column (100 × 20 mm), equilibrated with 0.02 M phosphate buffer, pH 7.2. With increasing concentrations of NaCl (0.02–1.0 M), protein fractions were eluted, desalted in sephadex G-10 column and lyophilized. The fraction (0.5 M Nacl gradient) was further purified by RP-HPLC using protein pak 60 column (Shimadzu HPLC system, Japan, Model LC 20AD). Purified fractions from CM cellulose column/HPLC were subjected to protein estimation (Lowry et al., 1951), molecular weight determination by SDS-PAGE (Laemmli, 1970) and anti-osteoarthritic activity studies.

Experimental osteoarthritis was induced by intra-articular injection of 20 μl bacterial collagenase (5CDU) in the right knee joint of male albino Wistar rats (120 ± 10 gm) (Van der Kraan et al., 1989). Rats (120 ± 10 g) were divided into four groups (n = 6). Gr.1: Sham control, Gr.2: OA control, Gr.3: Standard drug, Indomethacin treated (0.25 mg kg^{-1}, p.o.×5 days, alternatively), Gr.4: BF-F47 treated (0.3 mg kg^{-1}, i.p. × 14 days). Urinary hydroxyproline (Neuman and Logan, 1950) and glucosamine (Elson and Morgan, 1933) levels were measured spectrophotometrically (Analab UV-180). After 14 days of treatment, rats were anaesthetised, sacrificed and blood was collected from hepatic portal vein in siliconized vials. Serum was separated by centrifugation at 3000 rpm for 30 min. Serum acid phosphatase, alkaline phosphatase and creatinine levels were measured using biochemical kits (Merck, India). Serum pro-inflammatory molecular markers (TNF-α, IL-1β, CINC-1, IL-17, MMP-1, Cathepsin-K, PGE2 and Osteocalcin) levels were measured using ELISA kits (R & D, USA) and ELISA reader (Biotek, USA). Ankle joints were collected from the rats and fixed in 10% buffered formalin for 1 day. Joints were kept in Osteomol solution for 4–5 days for decalcification, followed by dehydration in 50%, 70%, 90% and 100% alcohol, clearing in xylol, embedding in liquid paraffin (56–58 °C) and preparation of paraffin blocks. Sections (5 μm) were cut in rotary microtome (Weswox Optic, India), stained with hematoxylin-eosin, observed in bright field microscope (Motic, Germany) and photographed using motic software (Motic Images Plus 2.0 software). Data expressed in terms of mean ± SEM (n = 6). One way ANOVA followed by Tukey's test was done using OriginPro8 software for determination of significant differences between groups. $p < 0.05$ was considered to be statistically significant.

BFV resolved into four protein peaks (P1, P2, P3, P4) through ion exchange chromatography (Fig. 1A). The fourth peak (P4) was eluted by 0.5 M NaCl, showed anti-osteoarthritic activity. P4 was termed as BF-F47 for convenience. SDS-PAGE showed that BF-F47 contains four distinct bands. RP-HPLC using Protein pak 60 column exhibited four peaks with retention time of 10.15, 14.46, 16.13 and 23.56 min respectively. The HPLC purified fraction (peak no. 3) with retention time 16.13 min, showed molecular weight of 14 KDa by SDS-PAGE (Fig. 1B) and found to be anti-osteoarthritic. For the sake of convenience, the active compound having anti-osteoarthritic activity was provisionally designated as BF-F47. BF-F47 (0.3 mg kg^{-1}) treatment in group 4 rats significantly reduced urinary hydroxyproline and glucosamine levels by 28.94% and 61.21% respectively; whereas indomethacin treated group 3 rats showed reduction in urinary hydroxyproline and glucosamine levels by 39.18% and 73.13% respectively in comparison to OA control group 2 rats (Table 1). Serum ACP, ALP, CRE and Osteocalcin levels were significantly increased in OA control group 2 rats as compared with sham control group 1 rats. BF-F47 (0.3 mg kg^{-1}) treatment in group 4 rats significantly reduced serum ACP, ALP, CRE and Osteocalcin level by 49.33%, 51.01%, 41.90% and 51.44%; Whereas Indomethacin treated group 3 rats showed significant reduction of the above parameters by 43.74%, 32.09%, 36.19% and 49.92% as compared with OA control group 2 rats (Table 1). Serum TNF-α, IL-1β, CINC-1, IL-17, MMP-1, PGE_2 and Cathepsin-K levels

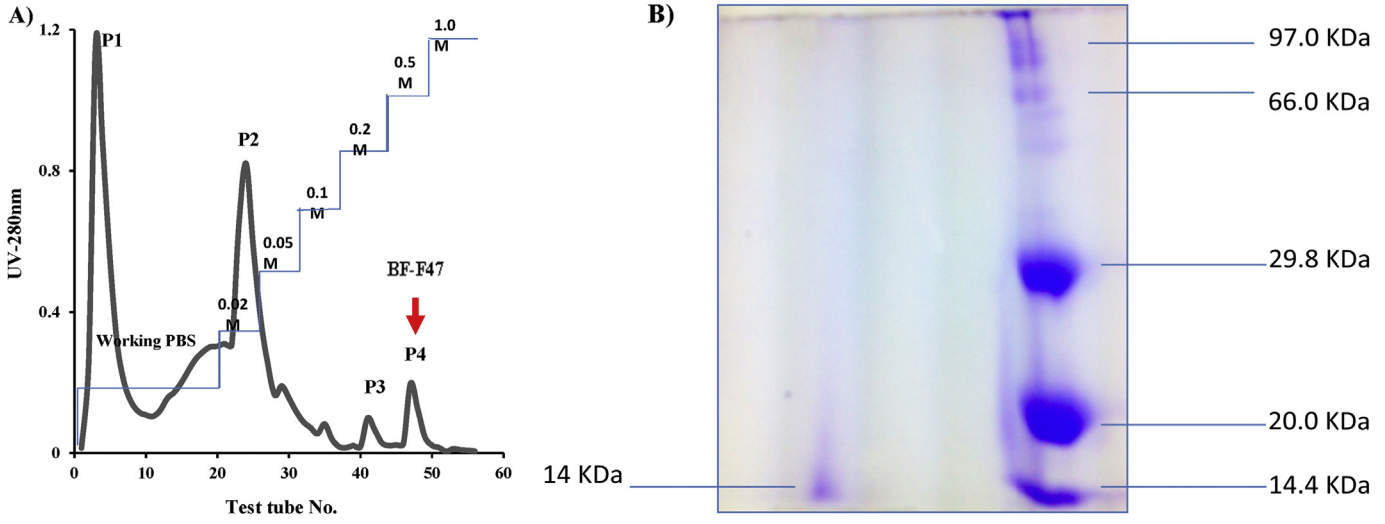

Fig. 1. Purification of BF-F47 from *Bungarus fasciatus* venom through CM-cellulose ion exchange chromatography. A) CM-cellulose ion exchange chromatography of *Bungarus fasciatus* venom. Lyophilized BFV (60 mg) was dissolved, heat-treated at 70 °C and applied to CM-cellulose column equilibrated with 0.02 M phosphate buffer, pH 7.2. Proteins were eluted with increasing concentrations of NaCl (0.02–1.0 M). Among the eluted protein peaks, peak 4 (P4) was eluted by 0.5 M NaCl. B) Molecular weight determination of HPLC purified fraction by SDS-PAGE.