Journal of Ethnopharmacology 155 (2014) 222–229

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Involvement of NFκB in the antirheumatic potential of *Chenopodium album* L., aerial parts extracts

Sumit K. Arora [1], Prakash R. Itankar *, Prashant R. Verma [2], Ashish P. Bharne [3], Dadasaheb M. Kokare [4]

University Department of Pharmaceutical Sciences, Department of Pharmacognosy and Phytochemistry, Rashtrasant Tukadoji Maharaj Nagpur University, Amravati Road, Nagpur 440033, Maharashtra, India

ARTICLE INFO

Article history:
Received 19 October 2013
Received in revised form
24 April 2014
Accepted 19 May 2014
Available online 24 May 2014

Keywords:
Chenopodium album
DPPH
Nitric oxide
Complete Freund's adjuvant
NFκB
Immunohistochemistry

ABSTRACT

Ethnopharmacological relevance: *Chenopodium album* L. (*C. album*) is commonly known as Bathua in Hindi (Family: Chenopodiaceae). Traditionally, the plant is used as a laxative, diuretic, sedative and the infusion of the plant is used for the treatment of rheumatism. However, no scientific validation is available on the antirheumatic potential of the plant. In the present investigation, role of NF kappa B (NFκB) in the antiarthritic potential of extracts of aerial parts of *Chenopodium album* was explored and evaluated.

Methods: The defatted aerial parts of *Chenopodium album* were successively extracted with ethylacetate, acetone, methanol and 50% methanol to study their antioxidant capacity followed by antiarthritic potential using Complete Freund's adjuvant (CFA) induced arthritis model in rats. The polyphenol, flavonoid and flavanone contents of different extracts were quantified and correlated with their antioxidant capacity, antiarthritic activity and NFκB inhibition potential.

Results: The experimental data indicated that the acetone extract of *Chenopodium album* (ACCA) has shown significant reduction in rat paw edema (80.13%) at dose level of 200 mg/kg *per oral* in 21 days of this study. On 22nd day, hematological and biochemical parameters were estimated and it was observed that the altered hematological parameters (Hb, RBC, WBC and ESR), biochemical parameters (Serum creatinine, total proteins and acute phase proteins) and loss in body weight in the arthritic rats were significantly brought back to near normal level by the ACCA extract. ACCA extract significantly decreased the NFκB expression in paraventricular nucleus of hypothalamus and this effect is comparable with standard indomethacine in CFA treated rats. The polyphenolic and flavonoid content of different extracts were in the range of 14.56 ± 0.21–42.00 ± 0.2 mg (gallic acid equivalent/g extract) and 2.20 ± 0.003–7.33 ± 0.5 mg (rutin equivalent/g extract) respectively.

Conclusion: The antiarthritic activity possessed by ACCA extract can be correlated directly to its antioxidant potential, high flavonoidal content achieved by successive extraction and its capacity to inhibit the NFκB protein, as proven by immunohistochemistry study.

1. Introduction

Rheumatoid arthritis (RA) is a chronic disease of the joint, where pro-inflammatory mediators are believed to be responsible for the cartilage destruction and bone erosion that characterizes the disease (Kingsley and Panayi, 1997). These pro-inflammatory molecules are regulated by the Nuclear factor Kappa B (NFκB) family of transcription factors (Pahl, 1999). Much evidence indicates a pivotal role for NFκB in the etiology of RA. Nuclear localization of subunits of NFκB (p50 and p65) has been shown to be significantly increased in synovial tissue of RA patients (Han et al., 1998). Similarly, it was demonstrated that fibroblast-like synoviocytes from RA synovium contain constitutively active NFκB and spontaneously produce large quantities of Interleukin-6 (IL-6) consistent with the clinical data. Increased NFκB binding activity has been demonstrated in the synovium of rodents following the development of collagen induced arthritis (CIA), adjuvant induced arthritis (AIA) and streptococcal cell wall-induced arthritis.

* Corresponding author. Tel.: +91 712 2550324; mobile: +91 9766191818; fax: +91 712 2500355.
E-mail addresses: sumitkishanarora@gmail.com (S.K. Arora), pri.200672@gmail.com (P.R. Itankar), prashantrkverma@rediffmail.com (P.R. Verma), ashishbharne2@gmail.com (A.P. Bharne), kokaredada@yahoo.com (D.M. Kokare).
[1] Mobile: +918793484246.
[2] Mobile: +919423100313.
[3] Mobile: +919970309402.
[4] Mobile: +919850318502.

http://dx.doi.org/10.1016/j.jep.2014.05.026
0378-8741/

AIA causes activation of hypothalamus pituitary adrenal (HPA) axis which ultimately leads to glucocorticoid secretion, a factor responsible for regulation of cytokine (Chesnokova and Melmed, 2000; Eskandari et al., 2003). It is already reported that the cytokines causes the activation of NFκB. In addition, novel therapies for RA are based on addressing and correcting the dysregulation of these neural and neuroendocrine pathways. The number of antirheumatic agents including glucocorticoids, sulfasalazine, gold salts, leflunomide and aspirin are inhibitors of NFκB which explain the part of their anti-inflammatory effects (Tak and Firestein, 2001). Above evidences suggest that NFκB signaling pathway are potential targets for the development of novel RA therapeutics.

Chenopodium album L., (*C. album*) (family: Chenopodiaceae) is an annual shrub widely grown in Asia, Africa, Europe and North America. It is commonly known as Bathua (in Hindi), pigweed, fat hen or lamb-quarters. The plant is used in folk medicine in different parts of the world as diuretic, laxative, sedative, hepatoprotective and antiparasitic. The leaves possesses anthelmentic, antiphlogistic, antirheumatic, mildly laxative and odontalgic properties, applied as wash or poultice to bug bites, sunstroke, rheumatic joints and swollen feets (Kokanova-Nedialkova et al., 2009). Additionally, decoction of its aerial parts mixed with alcohol was used in the rheumatism (Gupta et al., 2008).

However, no scientific validation is available on the antirheumatic potential of the plant. Phytochemical analysis revealed the presence of phenolic glycoside cinnamic acid (Nahar and Sarker, 2005), sinapic acid, ferulic acid and their derivatives, quercetin and kaempferol glycosides (Kowalewski, 1997), xyloside, cinnamic acid amide alkaloid, chenoalbicin (Cutillo et al., 2003).

In view of the above folklore claim, it was thought worthwhile to evaluate the effect of extracts of aerial parts of *Chenopodium album* in Complete Freund's adjuvant (CFA) induced RA in rats. Furthermore, to explore the mechanism of action, modulation of NFκB in hypothalamic brain nucleus is also studied employing immunohistochemistry using antibody against p65 subunit of NFκB.

2. Material and methods

2.1. Chemicals and standard drugs

NFκB p65 Antibody (A) (cat. No. Sc-109 Santa Cruz Biotechnology, USA) was obtained from NCCS, Pune as a generous gift. Indomethacin and CFA reagent were obtained from Sigma Aldrich chemicals, Powai, Mumbai respectively.

2.2. Plant material and preparation of extract

The aerial parts were collected from Ramtek region in the month of August, authenticated by Dr. (Mrs.) Alka Chaturvedi, Department of Botany, R.T.M. Nagpur University, Nagpur. A voucher specimen has been deposited in the Herbarium of Department of Botany, with collection number RA 9576.

The aerial parts of *Chenopodium album* were dried under shade and pulverized to a coarse powder. The powdered crude material (1 kg) was defatted with petroleum ether and then extracted successively with ethyl acetate, acetone and methanol using Soxhlet extractor followed by cold maceration (7 days) with 50% methanol. The extracts were concentrated using rotary vacuum evaporator to yield ethyl acetate extract (EACA, yield: 3.9% w/w), acetone extract (ACCA, yield: 4.79% w/w), methanolic extract (MECA, yield: 13.58% w/w) and 50% methanolic extract (HACA, yield: 12.76% w/w). These extracts were subsequently subjected to phytochemical and pharmacological screening.

2.3. Phytochemical screening (Harbone, 1976; Stahl, 1969)

The extracts were screened for the presence of different phytochemicals by employing thin layer chromatographic (TLC) techniques. Thin layer plates precoated with silica gel G (Merck, 0.25 mm thickness) were used. Development was carried out with different solvent systems such as ethyl acetate:methanol:water (100:13.5:10, v/v/v), ethyl acetate:formic acid:acetic acid:water (100:11:11:26, v/v/v/v), chloroform:methanol:water (70:30:4, v/v/v), toluene:ethyl acetate:diethylamine (70:20:10, v/v/v) and ethyl acetate:methanol: water:acetic acid (65:15:15:10, v/v/v/v). After development of chromatogram in the solvents, the plates were dried and sprayed with Dragendorff's, $AlCl_3$, hydroxylamine-ferric chloride, ninhydrin and antimony trichloride reagents for the detection of alkaloids, flavonoids, lactones/esters, protein/amino acids, unsaturated sterols and triterpenes respectively. Detection of anthraquinones, saponins, tannins, carbohydrate and/or glycosides was carried out using KOH, anisaldehyde-sulphuric acid, ferric chloride and naphthoresorcinol reagent respectively. Visualization was carried out under visible and UV light (λ: 366 nm). The important secondary metabolites such as total polyphenol, flavonoid and flavonone were quantified in EACA, ACCA, MECA and HACA extracts respectively.

2.4. Determination of total polyphenol compounds (TP), flavonoids (TFA), total flavanones (TFO) and degree of polymerization

2.4.1. Determination of total polyphenol compounds (TP)

Total polyphenol content was measured using the Folin–Ciocalteu colorimetric method (Singleton et al., 1999). Briefly, 0.1 ml of test sample was mixed with 1 ml of diluted Folin–Ciocalteu's phenol reagent (1:10 with distilled water; 0.2 N), to which 1 ml of saturated sodium carbonate (75 g/l) solution was added after 3 min and adjusted to 10 ml with distilled water. The reaction mixture was kept in the dark for 90 min and the absorbance was read at 725 nm (Shimadzu UV–VIS spectrophotometer 1600) against standard blanks prepared in the same way without the Folin–Ciocalteu's phenol reagent. Gallic acid was used as a reference for constructing the standard curve (10–100 mg/ml). The results were expressed as mg of gallic acid equivalents (GAE)/g of extract. All determinations were performed in triplicate.

2.4.2. Determination of flavonoids (TFA)

Flavonoid content was determined by the aluminum chloride method (Stanojevic et al., 2009). Briefly, to 1 ml of test solution (1 mg/ml), 1.5 ml of 95% alcohol, 0.1 ml of 10% aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$), 0.1 ml of 1 M sodium acetate (CH_3COONa) and 2.3 ml of distilled water was added. After incubation at room temperature for 40 min, absorbance of the reaction mixture was measured at 435 nm against corresponding blank, prepared in the same manner without adding $AlCl_3$. Rutin was used as a reference standard and results were expressed as mg of rutin equivalents (RE)/g of extract. All determinations were performed in triplicate.

2.4.3. Determination of total flavanones (TFO)

The modified 2,4-dinitrophenylhydrazine (DNPH) method was used for determination of flavanones (Nagy and Grancai, 1996). Naringin was used as the reference standard. Twenty milligrams of naringin was dissolved in methanol and then diluted to 500, 1000 and 2000 μg/ml. One milliliter of each of the diluted standard solutions was reacted separately with 2 ml of 1% DNPH reagent and 2 ml of methanol at 50 °C for 50 min. After cooling at room temperature, the reaction mixture was mixed with 5 ml of 1% KOH in 70% methanol and incubated at room temperature for 2 min. Then 1 ml of the mixture was taken, mixed with 5 ml of methanol and centrifuged at 1000 rpm for 10 min to remove the precipitate.