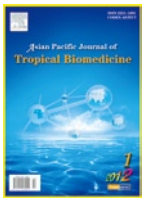
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

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



Document heading

Evaluation of *in vitro* anti-inflammatory activity of coffee against the denaturation of protein

Sangita Chandra, Priyanka Chatterjee, Protapaditya Dey, Sanjib Bhattacharya*

Pharmacognosy Division, Bengal School of Technology (A College of Pharmacy), Delhi Road, Sugandha, Hooghly 712102, West Bengal, India

ARTICLE INFO

Article history:
Received 18 January 2012
Received in revised form 21 February 2012
Accepted 15 April 2012
Available online 28 April 2012

Keywords:
Coffea arabica
Anti-inflammatory
Protein denaturation
Viscosity

ABSTRACT

Objective: To evaluate the *in vitro* anti-inflammatory effect of aqueous extract of coffee (*Coffea arabica*) against the denaturation of protein. **Methods:** The extract at different concentrations was incubated with egg albumin in controlled experimental conditions and subjected to determination of absorbance and viscosity to assess the anti-inflammatory property. Diclofenac sodium was used as the reference drug. **Results:** The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by the coffee extract. The effect of diclofenac sodium was found to be less when compared with the test extract. **Conclusions:** From the present study it can be concluded that coffee possessed marked *in vitro* anti-inflammatory effect against the denaturation of protein. The effect was plausibly due to the polyphenols contents of coffee.

1. Introduction

Inflammation is a bodily response to injury, infection or destruction characterised by heat, redness, pain, swelling and disturbed physiological functions. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells[1]. The commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs, which have several adverse effects especially gastric irritation leading to formation of gastric ulcers[2]. Natural products have contributed significantly towards the development of modern medicine. Recently traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their active therapeutic principles. The rich wealth of plant kingdom can represent a novel source of newer compounds with significant anti-inflammatory activities. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects, and low cost.

Coffee, also known as coffee bean consists of the dried ripe seeds of *Coffea arabica* Linn. (Rubiaceae). Coffee beverage is the widely consumed beverage worldwide. Coffee plant is an evergreen shrub or small tree indigenous to Arabia, grown and cultivated throughout India. Traditionally, it has been used for several important medicinal purposes in different parts of the world[3]. Previous researchers have reported some pharmacological properties on coffee[3-5]. The present study was conducted to evaluate the possible *in vitro* anti-inflammatory effect of coffee extract against the denaturation of protein.

2. Materials and methods

2.1. Plant material

Dried coffee beans (*Coffea arabica* Linn. Family: Rubiaceae) were procured in the month of July, 2011 from Sarkar & Sons., Kolkata, India. Just after procurement, the beans were ground mechanically into a coarse powder and

*Corresponding author: Sanjib Bhattacharya, Pharmacognosy Division, Bengal School of Technology (A College of Pharmacy), Delhi Road, Sugandha, Hooghly 712102, West Bengal, India.
Tel: + 91 9874331777
Fax: + 91 33 26864281.
E-mail: sakkwai@yahoo.com

kept into an air-tight container for use in the study.

2.2. Drugs and chemicals

Diclofenac sodium was procured from Organic Chemical Industries Pvt. Ltd., Kolkata 70001, West Bengal, India. All the other chemicals were of analytical grade obtained commercially. Double distilled water from all-glass still was used throughout the study.

2.3. Preparation of extract

The powder plant material (50 g) was extracted with 400 mL distilled water by boiling for 45 minutes. The extract was filtered and evaporated to dryness to yield the dry extract (AQCA, yield: 27.28%). The dry extract was kept in a vacuum desiccator until use.

2.4. Evaluation of in vitro anti-inflammatory activity

The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of AQCA so that final concentrations become 31.25, 62.5, 125, 250, 500, 1 000 μg/mL. Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37±2) ℃ in a BOD incubator (Labline Technologies) for 15 min and then heated at 70 ℃ for 5 min. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using vehicle as blank and their viscosity was determined by using Ostwald viscometer. Diclofenac sodium at the final concentration of (78.125, 156.25, 312.5, 625, 1250, 2 500 μg/mL) was used as reference drug and treated similarly for determination of absorbance and viscosity. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100 \times (V_t / V_c - 1)$$

Where, V_t = absorbance of test sample, V_c = absorbance of control.

The extract/drug concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration.

3. Results

In the present investigation, the *in vitro* anti-inflammatory effect of AQCA was evaluated against denaturation of egg albumin. The results are summarized in Table 1. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by AQCA throughout the concentration range of 31.25 to 1 000 μg/mL. Diclofenac sodium (at the concentration range of 78.125 to 2 500 μg/mL) was used as reference drug which also exhibited concentration dependent inhibition of protein denaturation (Table 2); however, the effect of diclofenac sodium was found to be less when compared with AQCA. This was further confirmed by comparing their IC_{50} values. AQCA possessed IC_{50} value 40 μg/mL whereas that of diclofenac sodium was found to be 625 μg/mL.

Table 1.
Effect of AQCA on protein denaturation.

Concentration (μg/mL)	% Inhibition	Viscosity (cp)
Control	-	1.45
31.25	20	0.71
62.50	120	0.75
125.00	400	0.79
250.00	1 320	0.84
500.00	2 800	0.88
1 000.00	3 700	1.00

Table 2.
Effect of diclofenac sodium on protein denaturation.

Concentration (μg/mL)	% Inhibition	Viscosity (cp)
Control	-	1.45
78.125	12.5	0.80
156.25	12.5	0.86
312.5	25.0	1.00
625	50.0	1.13
1 250	212.5	1.15
2 500	812.5	1.26

4. Discussion

There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for *in vitro* assessment of anti-inflammatory property of aqueous coffee extract (AQCA). Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins *in vivo*[6,7]. Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development.

The increments in absorbances of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein (albumin) denaturation by AQCA and reference drug diclofenac sodium[8]. From the IC_{50} values it becomes evident that AQCA was more active than diclofenac sodium, being effective in lower concentrations. This anti-denaturation effect was further supported by the change in viscosities. It has been reported that the viscosities of protein solutions increase on denaturation[9]. In the present study, the relatively high viscosity of control dispersion substantiated this fact. Presence of AQCA prevented this,

implying inhibition of protein denaturation. Here, the viscosities decreased when compared with control where no test extract/drug was added. However, the viscosities were found to decrease with concomitant decrease in concentration of test extract and reference drug as well. Although, the viscosities of the test samples (extract/drug), of all concentrations were always less than that of control. This decrease in viscosities may be due to decrease in concentration of test extract/drug in reaction mixture, which resulted in decreased viscosity; and/or other uncertain physico-chemical factors. Nevertheless, the viscosity data indicated inhibition of protein (albumin) denaturation. The effect of concentration of test agent on viscosity behaviour of denatured protein dispersion requires further studies.

The major constituents of coffee bean are an alkaloid caffeine, polyphenolic compounds like tannins and a phenolic acid namely chlorogenic acid[10]. Polyphenols are well known natural products known to possess several notable biological properties[11]. In the present study, the *in vitro* anti-inflammatory activity of coffee can be attributed to its polyphenols content. The effect may be due to the synergistic effect rather than single constituent.

It has been reported that one of the features of several non-steroidal anti-inflammatory drugs is their ability to stabilize (prevent denaturation) heat treated albumin at the physiological pH (pH: 6.2-6.5)[12]. Therefore, form the results of the present preliminary study it can be concluded that coffee possessed marked *in vitro* anti-inflammatory effect against the denaturation of protein. Further definitive studies are necessary to ascertain the mechanisms and constituents behind its anti-inflammatory actions.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are thankful to the authority of the Bengal School of Technology (A College of Pharmacy), Sugandha, Hooghly, 712102, West Bengal, India, for providing necessary facilities for the present study.

References

[1] Anonymous. *New medical dictionary*. New Delhi: Oxford and IBH Publishing Co. Pvt. Ltd.; 2007, p. 376.

[2] Tripathi KD. *Essentials of medical pharmacology*. New Delhi: Jaypee Brothers Medical Publishers Ltd.; 2008, p. 189.

[3] Bisht S, Sosidia SS. *Coffea arabica*: A wonder gift to medical science. *J Nat Pharm* 2010; **1**: 58-65.

[4] Murthy PS, Manonmani HK. Physico-chemical, antioxidant and antimicrobial properties of Indian monsooned coffee. *Eur Food Res Technol* 2009; **229**: 645-650.

[5] Nayeem N, Denny G, Mehta SK. Comparative phytochemical analysis, antimicrobial and antioxidant activity of the methanolic extracts of the leaves of *Coffea arabica* and *Coffea robusta*. *Der Pharmacia Lettre* 2011; **3**: 292-297.

[6] Opie EL. On the relation of necrosis and inflammation to denaturation of proteins. *J Exp Med* 1962; **115**: 597-608.

[7] Umapathy E, Ndebia EJ, Meeme A, Adam B, Menziwa P, Nkeh-Chungag BN, et al. An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. *J Med Plants Res* 2010; **4**: 789-795

[8] Jagtap VA, Agasimundim YS, Jayachandran E, Sathe BS. *In vitro* anti-inflammatory activity of 2-amino-3-(substituted benzylidinecarbohydrazide)-4,5,6,7- tetrahydrobenzothiophenes. *J Pharm Res* 2011; **4**: 378-379.

[9] Anson ML, Mirsky AE. The effect of denaturation on the viscosity of protein systems. *Gen Physiol* 1932; **15**: 341-350.

[10] Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy*. Pune (India): Nirali Prakashan; 2010; p. 3.70-3.71.

[11] Bhattacharya S. Are we in the polyphenols era? P*harmacognosy Res* 2011, **3**: 147.

[12] Williams LAD, O'Connar A, Latore L, Dennis O, Ringer S, Whittaker JA, et al. The *in vitro* anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. *West Indian Med J* 2008; **57**: 327-331.