Original Article

DOI: 10.7860/JCDR/2022/58609.17301

Sinapis arvensis-Wild Mustard as an Anti-inflammatory Agent: An In-vitro Study

Pharmacology Section

T ASHWINI[1], ARUL AMUTHA ELIZABETH[2], SNEHA AISHWARYA[3], I GLORY JOSEPHINE[4], S BRIGIDA[5], R SRINIVASAN[6]

ABSTRACT

Introduction: Inflammation is body's immune response to harmful stimulus. Commonly used conventional anti-inflammatory agents are Non-Steroidal Anti-inflammatory Drugs (NSAIDs). But on prolonged long-term use, it causes serious adverse events. So, the search towards natural agents which have anti-inflammatory property are increasing nowadays. *Sinapis arvensis* is an annual flowering plant which has proven multipurpose medicinal phytoconstituents.

Aim: To evaluate in-vitro anti-inflammatory effects of flower extracts of *Sinapis arvensis* with diclofenac as standard.

Materials and Methods: This in-vitro study assessed the laboratory based anti-inflammatory activity, performed using Bovine Serum Albumin (BSA) assay in December 2021 at Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India. BSA at pH of 6.8 generated denatured proteins. The anti-inflammatory activity of the sample (flower extracts of *Sinapis arvensis*) and standard (Diclofenac) was assessed by adding to BSA and percentage of inhibition of denaturation were calculated using the formula based on the absorbance measured. Descriptive statistics was used for analysis of collected data.

Results: The concentration-dependent inhibition of protein denaturation was observed for both *Sinapis arvensis* and Diclofenac. At 100 µg concentration, percentage of inhibition reached up to 81.8% and 100% for *Sinapis arvensis* and Diclofenac, respectively.

Conclusion: The present study showed that flower extracts of *Sinapis arvensis* exhibited concentration dependent anti-inflammatory property invitro which proves to be nearly equivalent with that of the standard Diclofenac.

Keywords: Bovine serum assay, Diclofenac, Flower extracts, Protein denaturation

INTRODUCTION

Inflammation is the human body's immune response to harmful intruders. Inflammatory response is basically the cellular and vascular response to trauma and the main purpose of it is to initiate the healing of the injured tissues [1]. Inflammation can be classified into acute or chronic inflammation [2]. Acute inflammatory response is initial response which is usually beneficial lasting for few days. It is characterized by rubor (redness), tumour (swelling), calor (heat), dolor (pain) and functio laesa (loss of function) [3]. An uncontrolled acute inflammation may end upto chronic inflammation, which may contribute to chronic inflammatory diseases [1]. Chronic inflammation may result from various reasons like failure of acute inflammatory response, autoimmune diseases (rheumatoid arthritis, rheumatic fever, lupus, asthma, psoriasis, etc.) [2].

Owing to its maladaptive and non-resolving inflammatory nature, the use of conventional anti-inflammatory therapy with drugs like NSAIDs, corticosteroids are crucial [4]. The most commonly used NSAIDs are paracetamol, indomethacin, aspirin, diclofenac which inhibits cyclooxygenase enzyme thereby preventing the synthesis of inflammatory mediators like prostaglandin leading to analgesic, anti-inflammatory and anti-pyretic effects [5]. Diseases like autoimmune disorders need long-term management but drugs like NSAIDs on prolonged use can cause serious adverse events such as cardiovascular, renal, gastrointestinal complications [6]. So, the switch towards re-evaluating the medicinal plants which has anti-inflammatory effect is increasing nowadays because of their less reported adverse effects [7].

Sinapis arvensis (family Brassicaceae) is an annual plant, commonly called as 'wild mustard' [8]. It has been traditionally used against rheumatic pain [9]. The family (Brassicaceae) species, *Sinapis alba* are also known to have anti-inflammatory effect [10]. Diclofenac, a non-steroidal anti-inflammatory drug NSAID, which also has analgesic antipyretic activity, displays its action by inhibiting the activity of cyclo-oxygenase 1 (COX1), cyclo-oxygenase (COX2) enzymes, thereby inhibiting synthesis of inflammatory mediators like thromboxanes, prostacyclins, prostaglandin E2. In addition, it also has activity in decreasing the already raised substance P in synovial fluid of rheumatoid arthritis patients [11]. Diclofenac has also proved to possess superior intrinsic anti-inflammatory activity than the other NSAIDs [12]. So, in this study diclofenac was used as the standard. The aim of the present study was to evaluate the anti-inflammatory effect of *Sinapis arvensis* by using Bovine Serum Albumin (BSA) assay, with diclofenac as standard.

MATERIALS AND METHODS

This laboratory-based in-vitro study was conducted at Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India, in December 2021.

Procedure

Preparation of *Sinapis arvensis* flower extraction: The *Sinapis arvensis* flower (100 g) was taken, washed, dried, and grinded into powder. An amount of 250 mL of ethanol was added to the flower powder. The extraction was carried out using Soxhlet apparatus for 8 hours at 70° Celsius (C),and then the extract was kept in rotatory evaporator following which the solvent was evaporated, and the extract was taken and kept at 4°C for further use.

BSA, at pH of 6.8, generates denatured proteins (inflammatory markers). Various concentrations of samples (flower extracts of *Sinapis arvensis* and Diclofenac) such as 20 µg, 40 µg, 60 µg, 80 µg, 100 µg were taken in separate test tubes and serial dilution were made upto 1 ml using methanol in respective test tubes

Control test tubes were also prepared with 50 µL methanol without the sample. An amount of 5 mL of 0.2% BSA was prepared in tris buffered saline at pH=6.8 and were added to all the test tubes (samples and controls). Samples and control test tubes were

incubated at 37°C for 20 min and then at 72°C for 5 min. After incubation, samples and control were allowed to cool for 10 min and the absorbance was measured at 660 nm. Control test tubes did not show inhibition of denaturation. Both the samples (*Sinapis arvensis* and Diclofenac) denaturation inhibition were calculated and compared [13]. Inhibition of denaturation (%) was calculated using the following formula: [14]

$$\text{Inhibition of denaturation (\%)} = \frac{\text{absorbance of control-absorbance of sample}}{\text{absorbance of control}} \times 100$$

STATISTICAL ANALYSIS

Descriptive analyses were done used to analyse the collected data.

RESULTS

[Table/Fig-1] shows percentage of inhibition were best at 100 µg sample concentration (Diclofenac -100% at 100 µg, sample *Sinapis arvensis* -81.8% at 100 µg). Based on the BSA, percentage of inhibition was calculated for both diclofenac and flower extracts of *Sinapis arvensis*. The absorbance was noted at varying sample concentration ranging from 20 to 100 µg.

Diclofenac showed the concentration-dependent inhibition of protein denaturation which reached up to 100 percent at 100 µg concentration. Flower extracts of *Sinapis arvensis* also showed concentration-dependent inhibition reaching upto 81.8 percent at 100 µg concentration [Table/Fig-1].

Sample concentration (µg)	20 (µg)	40 (µg)	60 (µg)	80 (µg)	100 (µg)
Diclofenac (standard) (Absorbance unit)	0.04	0.03	0.02	0.01	0
% Inhibition	63.6	72.7	81.8	90.9	100.0
Sinapis arvensis (sample) (Absorbance unit)	0.07	0.05	0.04	0.03	0.02
% Inhibition	36.3	54.4	63.6	72.7	81.8

[Table/Fig-1]: Bovine Serum Albumin (BSA) Assay: percentage inhibition of Diclofenac and *Sinapis arvensis* in increasing concentration.

DISCUSSION

Protein denaturation is a process by which protein loses its secondary and tertiary structure and its biological function may also be lost. Denaturation of protein is acclaimed as a marker for inflammation [15].

Denatured proteins end up forming the antigens which ultimately leads to the inflammation and inflammatory diseases like rheumatoid arthritis. BSA assay is the widely accepted and used in-vitro protein denaturation assay to evaluate the anti-inflammatory property of the products. In this assay, denatured proteins are generated by using BSA at pH of 6.8, anti-inflammatory property of the substance can be identified by adding the substance to the inflammation (through the generation of denatured proteins) induced BSA. Protein denaturation inhibition by substances indicate anti-inflammatory property. Higher the degree of its inhibition, greater would be the anti-inflammatory potential [11].

NSAIDs also has a property of preventing protein denaturation [16]. Even though synthetic chemical drugs like diclofenac have potent anti-inflammatory property, on prolonged use it can cause complications. Because of complications induced by prolonged conventional synthetic drug treatment, the search towards natural agents which has anti-inflammatory properties are increasing nowadays. Studies have shown that substances derived from plants are found to be effective, safe and alternative as an anti-inflammatory agent [17].

Sinapis arvensis had been used as rheumatic pain treatment traditionally [9]. The anti-inflammatory activity of the flower extracts has been assessed by invitro BSA assay, by taking Diclofenac as standard. Results of this study revealed that Diclofenac showed 100% percentage of inhibition at 100 µg of sample concentration whereas flower extracts of *Sinapis arvensis* showed 81.8% percentage of inhibition at 100 µg sample concentration in the concentration dependent manner.

This study proves that *Sinapis arvensis* may exhibit similar anti-inflammatory effect in incremental manner probably with reduced complications when compared to that of conventional standard drug Diclofenac. There are not much researches proving the anti-inflammatory potential of *Sinapis arvensis.*

Plants have secondary metabolites as major constituents through secondary metabolism. These secondary metabolites have various medicinal properties. Secondary metabolites like phenolic compounds such as flavonoids, tannins, and alkaloids, saponins, terpenoids can be used as anti-inflammatory agents [18]. Phytoconstituents of *Sinapis arvensis* were studied and found that they have more than 40 phytoconstituents including phenolic compounds, flavonoids, tannins, terpenes, alkaloids, which were known to possess anti-inflammatory property [19]. Flavonoids has been extracted and isolated from flowers of *Sinapis arvensis* and the mixture of flavonoids were administrated to the inflammation-induced rat paw. The results showed that flavonoid mixture from the flower have a potent and prominent anti-inflammatory property [20].

Limitation(s)

Being an in-vitro and preliminary study, the results cannot be extrapolated to human beings Further in-vivo studies and clinical trials are required to prove the anti-inflammatory activity of *Sinapis arvensis*.

CONCLUSION(S)

This study proves that at a maximum tested concentration of 100 µg Sample flower extracts of *Sinapis arvensis* showed 81.8% inhibition which is almost equivalent to that of standard Diclofenac. It is concluded that sample flower extracts of *Sinapis arvensis* have a potential anti-inflammatory effect in concentration dependent manner in-vitro.

REFERENCES

[1] Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et. al. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2018;9 (6):7204-18.
[2] Pahwa R, Goyal A, Jialal I. Chronic inflammation. StatPearls 2021 Sep 28.
[3] Hannoodee S, Nasuruddin DN. Acute Inflammatory Response. [Updated 2021 Nov 21]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK556083/..
[4] Odegaard JI, Chawla A. Pleiotropic actions of insulin resistance and inflammation in metabolic homeostasis. Science. 2013;339 (6116):172-77.
[5] Gunaydin C, Bilge SS. Effects of nonsteroidal anti-inflammatory drugs at the molecular level. Eurasian J Med. 2018;50 (2):116-21.
[6] Wongrakpanich S, Wongrakpanich A, Melhado K, Rangaswami J. A comprehensive review of non-steroidal anti-inflammatory drug use in the elderly. Aging Dis. 2018;9 (1):143-50.
[7] Oguntibeju OO. Medicinal plants with anti-inflammatory activities from selected countries and regions of Africa. J Inflamm Res. 2018;11:307-17. Doi: 10.2147/JIR.S167789.
[8] Zargar M, Kavhiza NJ, Bayat M, Pakina E. Wild Mustard (Sinapis arvensis) Competition and Control in Rain-Fed Spring Wheat (Triticum aestivum L.). Agronomy. 2021;11 (11):2306.
[9] Erbay MŞ, Anıl S, Melikoğlu G. Plants used as painkiller in folk medicine in Turkey–III: Rheumatic pain. Marmara Pharm J. 2018;22 (2):133-41.
[10] Xian YF, Hu Z, Ip SP, Chen JN, Su ZR, Lai XP et al. Comparison of the anti-inflammatory effects of Sinapis alba and Brassica juncea in mouse models of inflammation. Phytomedicine. 2018 Nov 15;50:196-04. Doi: 10.1016/j.phymed.2018.05.010. Epub 2018 May 19.
[11] Heendeniya SN, Ratnasooriya WD, Pathirana RN. In vitro investigation of anti-inflammatory activity and evaluation of phytochemical profile of Syzygium caryophyllatum. J Pharmacogn Phytochem. 2018;7 (1):1759-63.
[12] Pradal J. Comparison of Skin permeation and putative anti-inflammatory activity of commercially available topical products containing ibuprofen and diclofenac. J Pain Res. 2020;13:2805-14. Doi: 10.2147/JPR.S262390.
[13] Drăgan MA, Stan CD, Panzariu A, Profire LE. Evaluation of anti-inflammatory potential of some new ferullic acid derivatives. Farmacia. 2016;64 (2):194-97.
[14] Marius M, Amadou D,Donatien AA,Gilbert A,William YN, Rauf K et al., In Vitro Antioxidant, Anti-inflammatory, and In Vivo Anticolitis Effects of Combretin A and Combretin B on Dextran Sodium Sulfate-Induced Ulcerative Colitis in Mice. Gastroenterol Res Pract. 2020;2020:4253174.

[15] Ruiz-Ruiz JC, Matus-Basto AJ, Acereto-Escoffié P, Segura-Campos MR. Antioxidant and anti-inflammatory activities of phenolic compounds isolated from Melipona beecheii honey. Food and Agricultural Immunology. 2017;28 (6):1424-37.
[16] Ashok D, Madhuri EV, Sarasija M, Kanth SS, Vijjulatha M, Alaparthi MD,et al. Synthesis, biological evaluation and molecular docking of spirofurochromanone derivatives as anti-inflammatory and antioxidant agents. RSC advances. 2017;7 (41):25710-24.
[17] Nunes CDR, Barreto Arantes M, Menezes de Faria Pereira S, Leandro da Cruz L,de Souza Passos M, Pereira de Moraes L, et al. Plants as sources of anti-inflammatory agents. Molecules. 2020;25 (16):3726.
[18] Mohammed MS, Osman WJ, Garelnabi EA, Osman Z, Osman B, Khalid HS, et al. Secondary metabolites as anti-inflammatory agents. J Phytopharmacol. 2014;3 (4):275-85.
[19] Al-Rubaye AF, Kadhim MJ, Hameed IH. Determination of bioactive chemical composition of methanolic leaves extract of Sinapis arvensis using GC-MS technique. Int J Toxicol Pharmacol Res. 2017;9 (2):163-78.
[20] Ali AA, Hassanean HA, Mohammed M, Kamel MS, El-Khayat ES. Chemical and biological studies of Sinapis arvensis growing in Egypt. Bulletin of Pharmaceutical Sciences. Assiut. 2000;23(2):99-109.

PARTICULARS OF CONTRIBUTORS:
1. Postgraduate, Department of Pharmacology, Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India.
2. Professor and Head, Department of Pharmacology, Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India.
3. Assistant Professor, Department of Pharmacology, Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India.
4. Professor, Department of Pharmacology, Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India.
5. Assistant Professor, Department of Pharmacology, Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India.
6. Assistant professor, Department of General Medicine, SRM Medical College and Hospital, Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Sneha Aishwarya,
Plot. No. 78, Bharathi Street, Annai Indira Nagar, Velachery, Chennai, Tamil Nadu, India.
E-mail: snehaaishwarya24@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jun 30, 2022
- Manual Googling: Oct 10, 2022
- iThenticate Software: Oct 20, 2022 (6%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? NA
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jun 22, 2022**
Date of Peer Review: **Aug 12, 2022**
Date of Acceptance: **Oct 21, 2022**
Date of Publishing: **Dec 01, 2022**