Original Article

In-vitro anti-inflammatory activity of leaves of *Cicer arietinum*

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ABSTRACT

Cicer arietinum also known as Bengal gram. This plant belongs to the family *Fabaceae*. It is cultivated in India in Madhya Pradesh, Uttar Pradesh, Rajasthan, and Maharashtra. This study shows that *Cicer arietinum* leaves anti-inflammatory activity. It is used to decrease inflammation. This work estimates the anti-inflammatory activity of leaves of *Cicer arietinum* by using a UV-Visible spectrophotometer at 660nm. The maximum peak of inhibition of protein denaturation by using ethanolic and hydro-alcoholic extract is observed at 500µg/ml. By comparing both the extract ethanolic extract shows maximum percentage inhibition.

Keywords: *Cicer arietinum*, Diclofenac sodium, Protein denaturation, Inflammation, Bovine serum albumin.

Natural products are compared to pure chemicals, they are less toxic. Therapeutic activity is seen in plant or plant extract, but may not be in isolated pure components. One component may decrease the toxicity and increases the usefulness of another. The herbs have a wide range of applications in the medicinal field. From ancient times herbs are used as medicines. Herbs are used in the treatment of diseases. ROS like superoxide ($O_2^{\bullet-}$) and hydroxyl ($OH^{\bullet}$), hydroperoxyl ($OOH^{\bullet}$), peroxy ($ROO^{\bullet}$) and alkoxy ($RO^{\bullet}$) radicals, and non-free radicals, e.g., hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl), which are constantly produced in the human body during cell metabolism. Others are reactive nitrogen species (RNS) consisting of nitric oxide ($NO^{\bullet}$), peroxynitrite ($ONOO^{\bullet}$), and nitrogen dioxide (NO_2). Signal transduction, gene expression, and activation of receptors can be regulated by free radicals [1].

Excessive free radicals if not eliminated immediately then they can also be toxic to living cells which can lead to oxidative damage to functional macromolecules like DNA, proteins, and lipids. As a result, it is critical to look for endogenous antioxidant substances that act as direct scavengers of free radicals or as metal ion chelating agents that catalyze the generation of radical species, which could delay the progression of many chronic diseases or reduce chronic inflammation. Antioxidant plant components can decrease the formation of free radicals and also decreases diseases by oxidative stress [2].

The phenolics and flavonoids of plant extract involve in the antioxidant activities of plants and act as anti-inflammatory agents. The formation of proinflammatory molecules like TNF-α and nitric oxide (NO) can regulate inflammation. Free radicals react with inflammatory molecules leading to cell death and tissue damage [3]. Chickpea (*Cicer arietinum L.*) is high in protein (17-23%), as well as carbs (70%), lipids (4-10%), vitamins (B group), and minerals such as potassium, phosphorus, magnesium, and calcium. Chickpea proteins have excellent bioavailability, and peptides with hypolipidemic, antioxidant, and antibacterial action, among others, have been found due to their amino acid composition after hydrolysis. However, no anti-inflammatory bioactive peptides extracted from enzymatically degraded chickpea proteins have been reported [3].

Access this article online

Received – 21st July 2023

Initial Review – 26th July 2023

Accepted – 1st August 2023

Quick Response Code

MATERIALS AND METHODS

Extraction method

Soxhlet apparatus is used for the extraction of 50gm of

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powdered drug and 200 ml of the solvent system. Wet the powder by using a solvent system and take three cycles of solvent on heating mantle at 55^0c and continuous water supply to condenser for cooling of solvent. Due to this it again comes in powdered drug and again goes in the round bottom flask and continues the cycle up to solvent becomes colorless [4].

In-vitro anti-inflammatory activity

Inflammation: The body's response (defense) to injury or infection is called inflammation. It shows redness, pain, swelling, and heat-like symptoms [5].

Treatment of inflammation

1. Acute inflammation: Rest, ice, and good wound care.

2. Chronic inflammation: Vitamin supplements (Vitamins A, C, and D), zinc [5].

Bovine serum albumin is used to search for anti-inflammatory drugs at an early stage without the use of animals. It combines with water, salt, fatty acid, vitamins, and hormones and transfers it to tissues and cells. Chickpea is the preferred protein source during inflammation [6].

Inhibition of protein denaturation

Chemicals used for the assay: Potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, HCl, bovine serum albumin (BSA), diclofenac sodium, methanol, ethanol, 70% ethanol [1].

Reagent preparation

1) Phosphate buffer

For 200ml buffer, weigh 0.136gm potassium dihydrogen phosphate, 0.176gm disodium hydrogen phosphate, and 0.7gm sodium chloride. Mix all the chemicals in a 250ml beaker and add 100ml distilled water. Maintain the pH at 6.3 with the help of HCl. Make up the volume up to 200ml [1,9].

2) Bovine Serum Albumin (BSA)

Weigh 5gm BSA and add in a 100 ml buffer [1].

Standard preparation: Diclofenac sodium is used as a standard. For the preparation of the stock solution, dissolve 100mg diclofenac sodium in 100ml methanol to produce 1000µg/ml. From this stock solution, 3 different concentrations of 100, 200, and 500µg/ml were prepared by pipetting 1, 2, and 5ml of stock solution and diluting it up to 10ml [1].

Test solution preparation: Weigh 100mg semisolid drug extract and dissolve it in 100ml solvents (Ethanol and 70% Hydroethanol) to produce 1000µg/ml. From this stock solution, 3 different concentrations of 100, 200, and 500µg/ml were prepared by pipetting 1, 2, and 5ml of stock solution and diluting it up to 10ml [1,7].

Procedure: In different 7 test tubes, add 0.9ml BSA. In the first 3 test tubes add 0.1ml standard i.e. diclofenac sodium of different concentrations (100µg/ml, 200µg/ml, and 500µg/ml), in the next 3 add 0.1ml test solution of *Cicer arietinum* leaves extract of different concentration (100µg/ml, 200µg/ml and 500µg/ml), in remaining 1 test tube add 0.1ml distilled water (Figure 1). Then the test tubes were incubated at room temperature for 5 minutes and then add 2.5ml phosphate buffer in each test tube. Measure the absorbance at 660nm by using a UV Visible spectrophotometer [1,9].

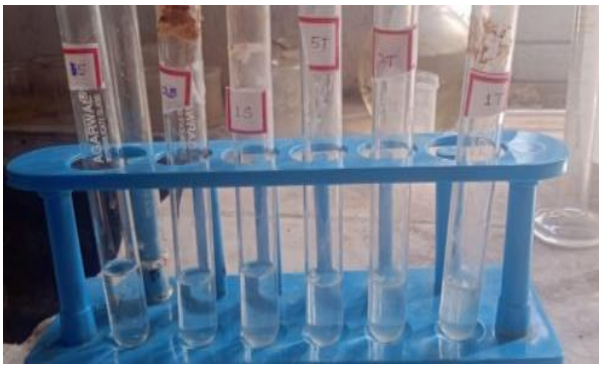

Figure 1 - Dilution for Anti-inflammatory Activity

Calculation: Percentage inhibition was calculated by using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Abs.of control} - \text{Abs.of test}}{\text{Abs.of control}} \times 10$$

Results and Discussion: Inflammation is a physiological response to bacterial infection or damaged vascularized living tissue. Although this tissue's response is normally to protect itself, when it becomes uncontrollable, recurring, or chronic, it can be linked to disorders such as asthma, obesity, and rheumatoid arthritis, among others. The most common treatments for inflammation are steroidal and non-steroidal medicines; however, their usage is limited due to their numerous adverse effects; thus, interest is growing in the development of alternative therapies derived from natural sources that do not pose health hazards [10]. *Cicer arietinum* plant shows anti-

inflammatory activity due to the presence of alkaloids, glycosides, steroids, carbohydrates, amino acids, and inorganic elements.

Due to injury to the body, the body's defense system gets activated and releases some mediators which can lead to an increase in blood flow which causes an increase in the size of the blood vessel and then swelling of that body part i.e. inflammation. To overcome the inflammation, anti-inflammatory agents are used. Inhibition of protein denaturation activity is first performed to study the anti-inflammatory activity of the leaves of *Cicer arietinum*.

The determination of inhibition of protein denaturation activity by ethanolic and hydro-alcoholic extract was shown by this study. This study gives the percentage inhibition. The ethanolic extract shows high percentage inhibition at 500µg/ml i.e. 66.67%. The percentage inhibition increases as concentration increases, which is shown in the table and graph. The hydro-alcoholic extract shows high percentage inhibition at 500µg/ml i.e. 36.11%. The percentage inhibition increases as the concentration increases, which is shown in the table and graph. These values are compared with the standard (Table 1, Figure 2) [8].

Table 1 - *In-vitro* anti-inflammatory activity of *Cicer arietinum*

Conc. (µg/ml)	Percentage Inhibition (Diclofenac sodium) (%)	Percentage Inhibition (Ethanolic) (%)	Percentage Inhibition (Hydro-alc.) (%)
100	38.89	25	22.22
200	63.89	41.67	30.56
500	77.78	66.67	36.11

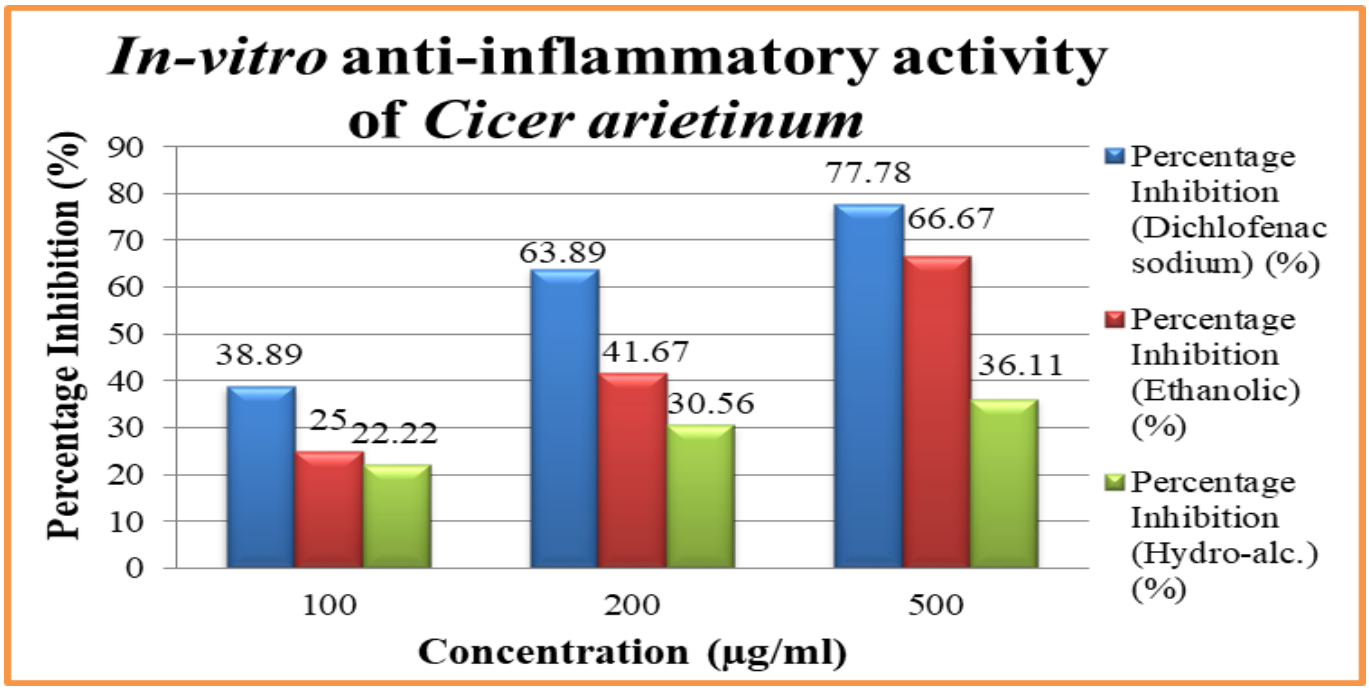

Figure 2 - *In-vitro* anti-inflammatory activity of *Cicer arietinum* for ethanolic and hydro-alcoholic extract.

CONCLUSION

Based on this study, it is concluded that the ethanolic and hydro-alcoholic extract of leaves of *Cicer arietinum* shows anti-inflammatory activity by inhibition of protein denaturation activity further studies were suggested to isolate the active principles responsible for the activity. The ethanolic extract shows a high percentage of inhibition as compared with the hydro-alcoholic extract. Ethanolic extract at 500µg/ml shows high percentage inhibition i.e. 66.67%. The percentage inhibition increases as the concentration increases from 100µg/ml to 500µg/ml and it is compared with the standard.

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How to cite this article: Supriya Mhamane, Priyanka Adat. *In-vitro* anti-inflammatory activity of leaves of *Cicer arietinum*. Indian J Pharm Drug Studies. 2023; 2(4):179-182.

Funding: None *Conflict of Interest: None Stated*