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ANTI-INFLAMMATORY ACTIVITY OF ALLYLISOTHIOCYANATE - AN *IN VITRO* STUDIES

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ABSTRACT

Majority of chronic diseases are stem from unresolved inflammation process. Some diet and phytochemicals act as inhibitors of inflammation and prevent the occurrence of several inflammation related pathogenesis. The present study was aimed to investigate the anti-inflammatory property of allylisothiocyanate (AITC), a naturally occurring phytochemical by using in vitro studies such as albumin denaturation assay, heat induced haemolysis and hypotonicity effect on human erythrocytes (membrane stabilization). Three different concentrations of AITC (1, 2, 4µg/ ml) was used to study the in-vitro anti-inflammatory activities and Diclofenac sodium (2µg/ ml) was used as reference drug. The experiment was conducted in triplicate. The percentage of inhibition of protein denaturation and hemolysis occur in dose dependent manner and significant effects are observed (47, 37, and 25) in 4µg AITC treated group which are close to the action of standard drug (49, 42, and 30). But no significant difference between 2µg (45, 36, and 22) and 4µg AITC treated groups. The results reveal the better anti-inflammatory effect of AITC at minimum dose of (2µg/ml) and further in vitro studies are needed to understand the mechanism of action.

KEYWORDS

Inflammation Protein denaturation, Haemolysis, Allylisothiocyanate and Diclofenac sodium.

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INTRODUCTION

Inflammation is a natural, healthy response to cellular damage and immune response to a perceived risk. All diseases or ailments stem from inflammation. Chronic inflammation can eventually cause several diseases and conditions, including some cancers, rheumatoid arthritis, atherosclerosis, periodontitis, and hay fever.

Membrane stabilization is an important for the maintaining of structural integrity of biological membranes such as erythrocyte and lysosomal membranes. These are disturbed on osmotic pressure and heat (Sadique *et al.,* 1989)[1]. Biological membranes are formed by varying composition of proteins, lipids and carbohydrates. They are asymmetric structures, fluidity of the membrane are thermodynamically stable and metabolically active. The erythrocyte membrane is similar to lysosomal membrane and the result obtained in erythrocyte membrane can be extrapolated to the stabilization of lysosomal membrane (Omale *et al.,* 2008)[2]. The energy of cells depends on the integrity of their membranes, exposure of erythrocyte in extreme condition such as hypotonic medium which results in lysis of membrane followed by haemolysis and oxidation of haemoglobin. An injury to RBC membrane will make cells susceptible to secondary damage through free radical induced lipid peroxidation (Ferrali *et al.,* 1992)[3].

Anti-inflammatory phytochemical foods are most colourful fruits and vegetables, oily fish (which contain higher levels of omega-3 fatty acids), nuts, seeds, and certain spices, such as ginger, garlic and cayenne. Traditional system of medicine continued to be widely used as various diseases. Global estimate indicates that 80% of about 5 billion population offered the uses of traditional medicines which are mainly derived from plant materials (Karthishwaran *et al.,* 2010)[4]. Phytochemicals are present in a variety of plants, and are utilized as important components of both human and animal diets. These include fruits, seeds, herbs and vegetables (Okwu *et al.,* 2005)[5]. Phytochemicals are occurrence of all the secondary metabolites and they posses significant protection of the cell membrane against injurious substances (Maxwell *et al.,* 1995)[6].

AITC is an aliphatic isothiocyanate and it is a natural compound present in all plants of the Cruciferae family such as brussels sprouts, cauliflower, cabbage, kale, horseradish and wasabi and a hydrolysis product between the enzyme myrosinase and a glucosinolate known as sinigrin. It consists of anti-oxidative, anti-bacterial and anti-fungal anti-inflammatory activity (Zhang *et al.,* 2010)[7]. AITC is also used as a food additive and flavouring agent. Allyl isothiocyanate can also be liberated by dry distillation of the seeds. Synthetic allyl isothiocyanate is used as an insecticide, bacteriocide, and nematocide, and is used in certain cases for crop protection (Romanowski *et al.,* 2005)[8].

MATERIAL AND METHODS

Analysis of *in vitro* anti-inflammatory activity

Inhibition of albumin denaturation

The anti-inflammatory activity of allyl isothiocyanate was studied by using inhibition of albumin denaturation technique which was studied according to Mizushima *et al.* (1968)[9] and Sakat *et al.* (2010)[10] followed with minor modifications. The 1ml of AITC and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N Hcl. The sample were incubated at 37ºC for 20 min and then heated to 51ºC for 20 min, after cooling the samples the turbidity was measured at 660 nm using colorimeter. The experiment was performed in triplicate.

The percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition = (OD of Control –OD of Sample) X 100/ OD of control.

Membrane stabilization

Preparation of Red Blood Cells (RBCs) suspension

The blood was collected from healthy human volunteer who has not taken any NSAIDs (Non Steroidal Anti-Inflammatory Drugs) for 2 weeks prior to the experiment and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 revolution per 10 minutes and washed three times with equal volume of normal saline. The volume of blood was measured and re constituted as 10% v/v suspension with normal saline **(**Sakat *et al.,* 2010[10], Sadique *et al.,* 1989)[1].

Heat induced haemolysis

The reaction mixture consisted of 1 ml test sample of different concentrations of AITC (1, 2 and 4µg/ml) and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Diclofenac sodium was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56ºC for 30 min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was read at 560 nm. The experiment was performed in triplicates for all the test samples (Sakat *et al.,* 2010[10], Shinde *et al.,* 1999)[11].

The percentage inhibition of haemolysis was calculated as follows:

Percentage inhibition = (OD of control –OD of sample) X 100/ OD of control

Hypotonicity-induced haemolysis

Different concentration of AITC (1,2 and 4µg/ml) and control was separately mixed with 1ml of phosphate buffer, 2ml of hyposaline and 0.5ml of RBC suspension. Diclofenac sodium (2µg/ml) was used as a standard drug. All the assay mixtures were incubated at 37ºC for 30 min and centrifuged at 3000 rpm. The supernatant was decanted and the haemoglobin content was estimated by a spectrophotometer at 560 nm **(**Azeem *et al.,* 2010)[12].

The percentage of hemolysis was estimated by assuming the haemolysis produced in the control as 100%.

Percentage protection = 100- (OD sample/OD control) x 100

RESULTS

Inhibition of albumin denaturation

Protein denaturation is a process. In which proteins lose their structures and properties by external stress or compounds, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most proteins lose their biological functions when denatured. Denaturation of protein causes inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of AITC to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. Maximum inhibition of 47% was observed at 4µg/ml. Diclofenac sodium a standard anti-inflammation drug showed the maximum inhibition 49% at the concentration of 2µg/ml (Table No.1).

Heat induced haemolysis

The AITC was effective in inhibiting the heat induced haemolysis with increasing the concentrations. Maximum 42% of inhibition of erythrocyte membrane lysis induced by heat was observed. Diclofenac sodium 2µg/ml offered a significant 42% of inhibition of erythrocyte lysis.

Hypotonicity induced haemolysis

The results showed that AITC at concentration range of 1, 2 and 4µg/ml protect the erythrocyte membrane against lysis induced by hypotonic solution. Diclofenac sodium (2µg/ml) offered a significant protection against the damaging effect of hypotonic solution. At the concentration of 2µg/ml, AITC showed 25% protection, whereas, Diclofenac sodium (2µg/ml) showed 30 % inhibition of RBC haemolysis.

DISCUSSION

The prevention of erythrocyte lysis is known to be a very good index of anti-inflammatory activity of AITC. RBC membrane is similar to lysosomal membrane components. Lysosomal enzymes are being released during inflammation which leads to the tissue damage and inflammation. The inflammatory responses get limited by preventing the release of lysosomal constituents of activated neutrophils thereby the damage of the tissue is reduced. AITC inhibits the release of the lysosomal content of neutrophils at the site of inflammation. Prevention of protein denaturation may also help in prevent inflammatory conditions (Sakat *et al.,* 2010)[10] and prove that AITC indicates its anti-inflammatory properties. The results are coincides with the *in vitro* study on *Trigonella foenum graecum* extracts demonstrate the depression of inflammation due to the presence of active principle compounds flavonoids and related polyphenols

(Thinagaran Rajan *et al.,* 2014)[13]. Hence, *Trigonella foenum graecum* leaves can be used as a potent anti-inflammatory agent.

Similarly, Karthik *et al.* (2013)[14] evaluated the *in vitro* anti-inflammatory activity of *Canthium parviflorum* by protein denaturation method. Denaturation of proteins is a well-documented cause of inflammation. The data of our studies suggests that allylisothiocyanate having significant anti-inflammatory activity. Therefore, AITC can be used for treating inflammation related diseases.

Table No.1: Effect of AITC on heat induced protein denaturation

S.No	Treatment(s)	Concentration (µg/ml)	Absorbance at 660nm	% inhibition of protein denaturation
1	Control	-	0.157 ± 0.03	-
2	AITC	1	0.094 ± 0.01	40
3	AITC	2	0.086 ± 0.04	45
4	AITC	4	0.082 ± 0.02	47
5	Diclofenacsodium	2	0.080 ± 0.05	49

Each value represents the mean ± SD. N=3.

Table No.2: Effect of AITC on heat induced haemolysis of erythrocyte

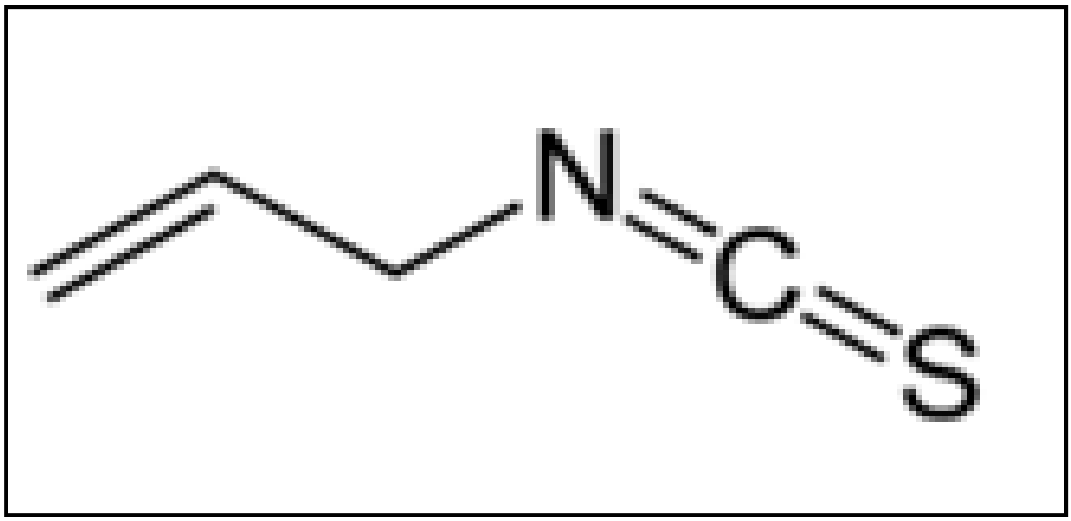
S.No	Treatment(s)	Concentration (µg/ml)	Absorbance at 560 nm	% inhibition of haemolysis
1	Control	-	3.82 ± 0.02	-
2	AITC	1	2.60 ± 0.05	31
3	AITC	2	2.43 ± 0.03	36
4	AITC	4	2.37 ± 0.04	37
5	Diclofenac sodium	2	2.21 ± 0.01	42

Each value represents the mean ± SD. N=3.

Table No.3: Effect of AITC on hypotonicity induced haemolysis of erythrocyte

S.No	Treatment(s)	Concentration (µg/ml)	Absorbance at 560 nm	% inhibition of haemolysis
1	Control	-	3.21 ± 0.03	-
2	AITC	1	2.73 ± 0.02	15
3	AITC	2	2.51 ± 0.04	22
4	AITC	4	2.41 ± 0.06	25
5	Diclofenac sodium	2	2.21 ± 0.05	30

Each value represents the mean ± SD. N=3.



Structure of Allylisothiocyanate (AITC)

CONCLUSION

In vitro studies prove that AITC possess anti-inflammatory activity. The cruciferous vegetables consist of AITC and they could be considered as a natural source of membrane stabilizers and is capable of providing an alternative remedy for the treatment of inflammatory disorders.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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