4.1. Introduction

Inflammation is body's response to disturbed homeostasis caused by infection or injury, resulting in systemic and local effects. The Roman writer Celsus in 1st century AD named the famous four cardinal signs of inflammation as Rubor (redness), Tumor (swelling/edema), Calor (heat), and Dolor (pain) (Mohan, 2010). It was Hunter who proposed that inflammation is essentially a response to injury, rather than a disease entity itself. Hunter suggested that the inflammatory response is the same for all types of injury (Sedgwick and Willoughby, 1985). Many types of cell injury can cause inflammation, including hypoxia, physical agents such as trauma and burns, chemical agents and drugs, infectious agents, immunologic reactions, genetic derangements and nutritional imbalances involving both deficiencies and excess of various nutrients (Cotran et al., 1994). The inflammatory response is a complex self-limiting process precisely regulated to prevent extensive damage to the host. When the self-limiting nature of this protective mechanism is inappropriately regulated, it is transformed to a detrimental, chronic state of inflammation. All chronic diseases are interrelated as they contain an element of increased inflammatory response, often observed long before the disease is clinically documented (Bengmark, 2004).

Inflammation is not merely considered as a component of the healing process; but also considered as a key promoter of degenerative diseases, which kills millions of people each year worldwide. Indeed, 6 of the 10 leading causes of death in Americans are coronary heart disease, cancer, stroke, diabetes mellitus, atherosclerosis, and chronic liver disease and cirrhosis (Surgeon, 1988; Famighetti et al., 1997), all of which have been previously described as inflammatory diseases. Many diseases are the manifestation of
chronic inflammation. For example, rheumatoid arthritis, characterized by chronic inflammation of the synovial membrane, is classified as a disease and not as a transitional phase in the healing process (Fries, 1992). The chronic inflammation promotes the development of Alzheimer's disease (McGeer et al., 1991). Asthma is viewed as a disease characterized by chronic airway inflammation (Tekin et al., 2000). An acute respiratory distress syndrome, sarcoidosis, glomerular nephritis, psoriasis, inflammatory bowel diseases, osteoarthritis are all inflammatory diseases (Tekin et al., 2000). Among gastrointestinal diseases, “ulcerative colitis and Crohn's diseases are characterized by chronic inflammation with superimposed acute inflammatory exacerbations” (Epstein, 1998).

Inflammation usually involves a sequence of events which can be categorized under three phases viz. acute transient phase, delayed sub acute phase and chronic proliferate phase. In the first phase, inflammatory exudates develop due to enhanced vascular permeability and leads to local edema. It is followed by the migration of leukocytes and phagocytes from blood to vascular tissue which is the second phase. In the third phase; tissue degradation is followed by fibrosis. Inflammation results in the liberation of endogenous mediators like histamine, serotonin, bradykinin, prostaglandins etc. Prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation. These mediators even in small quantities can elicit pain response (Anilkumar, 2010).
4.1.1. Role of inflammatory enzymes in diseases

Neutrophils (neutrophilic polymorphonuclear leukocytes) represent the body's primary line of defense against invading pathogens. Nevertheless, recently they have been increasingly studied as active participants in the initiation and progression of many pathological states, such as rheumatoid polyarthritis, carcinoma, allergy or ischaemia-reperfusion. All these conditions are generally accompanied by dysregulated, persistent and excessive activation of neutrophils, resulting in damage of adjacent tissues by neutrophil “destructive hardware” - by reactive oxygen species, cytotoxic proteins and proteolytic enzymes (Cascao et al., 2009; Cascao et al., 2010; Fialkow et al., 2007; Wright et al., 2010). In rheumatoid arthritis, neutrophil derived oxidants can induce cartilage degradation, depolymerise hyaluronan and decrease its lubricative properties. Further, they can reduce the protective antioxidant and antiproteinase capacity of synovial fluid and thus participate in joint erosion (Cascao et al., 2009; Edwards and Hallett, 1997). Besides, neutrophils are capable to release inflammatory mediators (eicosanoids, chemokines, cytokines), which along with their altered recruitment and delayed apoptosis, have the potential to maintain permanent inflammation (Cascao et al., 2010; Wright et al., 2010).

Inflammation is a complex pathophysiological process mediated by a variety of signaling molecules and enzymes produced by leukocytes involved in both acute and chronic inflammatory disorders (Heras et al., 2001). It is well known that cyclooxgenase (COX), 5-lipoxygenase (5-LOX), matrix metalloproteinases (MMPs) and hyaluronidases are the common mediators of inflammation. These enzymes are increased by neutrophil...
stimulation in a variety of inflammations and hypersensitivity based human diseases (Mayatepet and Hoffmann, 1995; Surh et al., 2001; Bernstein et al., 1994).

Lipoxygenases (LOXs) are involved in the biosynthesis of various bioregulators, which are closely related to the pathogenesis of allergies, atherosclerosis and some cancers (Spector et al., 1988). 5-LOX (EC.1.13.11.34) belongs to the class of iron containing lipoxygenases that catalyze the incorporation of dioxygen into unsaturated fatty acid, preferentially arachidonic acid (AA), at C-5, giving rise to the production of biologically active compounds hydroperoxyeicosatetraenoic acids (HpETEs) and 5-hydroxyeicosatetraenoic acid (5-HETE) (Yamamoto, 1992). It is also the key enzyme in leukotriene (LT) biosynthesis, catalyzing initial steps in conversion of AA to biologically active leukotrienes through lipoxygenase pathway such as leukotriene C4, leukotriene D4 and leukotriene E4 which are powerful spasmogens implicated in inflammatory and allergic responses (Shibata1, 2003). The biological effects of 5-HETE and LTs can be antagonized or prevented by targeting the production of 5-HETE and LT through inhibition of 5-LO pathway and thus may have a therapeutic potential in a variety of inflammatory and allergic diseases (Prasad et al., 2004).

Hyaluronidase [EC 3.2.1.35] (hyaluronoglucosaminidase) is a hydrolytic enzyme which catalyzes the degradation of hyaluronic acid (hyaluronan) and chondroitin sulfate (Watson, 1993; Girish and Kemparaju, 2007). Hyaluronic acid and chondroitin sulphate are the constituents of amorphous substances of connective tissue. Hyaluronic acid is a natural and sugar-like biopolymer consisting of D-glucuronic acid and N-acetyl-D-glucosamine units. These days, hyaluronidase has received much attention due to its ability to abruptly alter hyaluronic acid homeostasis. Degradation products of hyaluronic
acid accumulated during tissue injury can stimulate the expression of inflammatory genes by a variety of immune cells at the site of injury (Jiang et al., 2007). The interaction of hyaluronidase with hyaluronic acid disrupts the basement membrane integrity and produces an angiogenic response. For example, the enzyme mediates inflammation via histamine released from mast cells. Therefore inhibition of hyaluronidase (inhibition of hyaluronic acid degradation) may be crucial in reducing disease progression of allergies and inflammation (Girish and Kemparaju, 2007; Furusawa et al., 2011). Hyaluronidase has also been reported to be implicated in tumor invasiveness and metastasis (Madan et al., 1999). Hyaluronidase inhibitors are potent regulators that maintain hyaluronidase homeostasis and might serve as anti-inflammatory agents (Girish et al., 2009). Some typical anti-allergic drugs such as disodium cromoglycate (DSCG), transilist, liquiritigenin, and baicalein exhibited strong hyaluronidase inhibitory activity (Yingprasertchai et al., 2003; Kakegawa et al., 1992). Many natural compounds, such as phenolics, flavonoids and tannins that are derived from plants have been intensively studied as hyaluronidase inhibitors (Hertel et al., 2006; Mio and Stern, 2002).

Eosinophils most likely play important roles in the pathophysiology of bronchial asthma and other allergic diseases (Butterfield et al., 1995). In such diseases, mediators released by T cells and other inflammatory cells induce migration of eosinophils from blood into the affected tissues (Kita et al., 1998). Subsequently, appropriate stimuli trigger eosinophil activation, resulting in the local release of a series of inflammatory mediators, such as lipid metabolites (Lee et al., 1984), superoxide anion (Sedgwick et al., 1988), and toxic cationic granule proteins (Abu-Ghazaleh et al., 1989). During allergic inflammation, eosinophils can be exposed to a milieu of inflammatory mediators.
potentially able to activate and to induce mediator release from eosinophils. Out of many mediators released by inflammatory cells (e.g., mast cells), proteases are found to be potential proinflammatory agents (Schwartz, 1994; Schwartz et al., 1981) and are abundant at the sites of allergic inflammation (Broide et al., 1991).

Trypsin, a serine protease and a stimulus for protease-activated receptor-2 (PAR2) induced superoxide anion production and degranulation by eosinophils. A serine protease inhibitor, 4- (2-aminoethyl) benzene sulfonyl fluoride hydrochloride (AEBSF), inhibited trypsin-induced eosinophil activation. These findings indicate that, human eosinophils are activated when exposed to certain serine proteases, and release inflammatory mediators. These observations may have important implications in our understanding of the regulation of eosinophil activation at inflammation sites in patients with asthma and allergic diseases. Furthermore, during an allergic response, mast cells release their granular contents that include serine proteases, such as trypsin (Schwartz et al., 1981; Broide et al., 1991; Smith et al., 1984) and chymotrypsin (Sayama et al., 1987). Therefore, once eosinophils are recruited into an allergic inflammation site, these allergens or granule enzymes may directly activate eosinophils through the interaction of their serine protease activities and eosinophil PARs and may induce inflammatory mediator release from eosinophils (Miike, 2001). Therefore, once allergic responses are initiated, eosinophils interacting with serine proteases may exacerbate the inflammation and disease process by producing more inflammatory mediators. Conversely, removal of protease activity from the sites of inflammation or blockade of eosinophil PARs may benefit patients with allergic diseases by dampening this vicious cycle of allergic inflammation.
4.1.2. Role of diet and dietary polyphenols in the treatment of inflammatory diseases

Worldwide morbidity and mortality from infectious diseases is being replaced by chronic diseases, such as cancer, obesity and type II diabetes, cardiovascular diseases, neurodegenerative diseases and aging (Bengmark, 2004; Kennedy, 2006). Today many of the Americans are plagued by proinflammatory degenerative diseases because of their lifestyle is somehow proinflammatory in nature. During the past several decades, the incidence of obesity has significantly raised worldwide (Park et al., 2007). Obesity is associated with a state of chronic, low-grade inflammation, particularly in white adipose tissue (Wellen and Hotamisligil, 2005) demonstrating a close link between metabolism and immunity. The integration of metabolism and immunity, under normal condition can be viewed as a central homeostatic mechanism, but whose dysfunction can lead to a cluster of chronic metabolic disorders, particularly obesity, type 2 diabetes and cardiovascular diseases (Hotamisligil, 2006; Semenkovich, 2006). It is clear that chronic excess of nutrients engages common or overlapping pathways regulating both metabolic and immune functions through common key regulatory molecules and signalling systems. A variety of dietary factors play a significant role in creating a “diet-induced proinflammatory state” and the degenerative inflammatory diseases which are promoted by dietary imbalances (Surgeon, 1988). The concept that a diet rich in vegetables, fruits, and the proper fats can prevent a host of proinflammatory degenerative diseases is now well accepted and are evidently proved that they are associated with a reduced risk of various forms of cancer, cardiovascular disease, arthritis, diabetes and even aging (Gerster, 1997).
Although many of antiinflammatory drugs are in use, their continuous administration leads to adverse side effects (Harris, 1990; Watts and Isaac, 1992). Therefore, there is a need to explore alternative strategies to lower the formation of inflammatory mediators with the help natural dietary products. Polyphenols constitute one of the most numerous and ubiquitously distributed group of plant secondary metabolites, present in all plants that are commonly consumed in the diet including grains, legumes, fruits, vegetables, spices, olive oil, red wine and tea (Bravo, 1998). Epidemiological studies show that populations consuming predominantly a Mediterranean diet exhibit lower incidence of coronary heart disease than those eating a Northern, European or North American diet. This diet is rich in olive oil, which contains phenolic compounds, leading to the suggestion that the high consumption of this fat, at least in part, contribute to the health benefits (Wahle et al., 2004; Trichopoulou et al., 2003; Rietjens et al., 2007; Singh et al., 2008; Fito et al., 2007). Polyphenols have been described to have a wide range of biological activities and many reports, published during recent years, have highlighted the beneficial effects of phenolic compounds illustrating their promising role as therapeutic tools in several acute and chronic disorders (Visioli and Galli, 1998; Middleton et al., 2000; Urquiaga and Leighton, 2000; Visioli and Galli, 2001; Simonyi et al., 2005; Scalbert et al., 2005; Tian, 2006). Particularly, epidemiological and experimental studies have been focused on the anti-inflammatory activity of dietary polyphenols (Bengmark, 2004; Yoon and Baek, 2005).

It has been shown that phenolic compounds can exert modulatory action in cells by interacting with a wide spectrum of molecular targets central to the cell signalling machinery. The molecular mechanisms involved in the anti-inflammatory activities of
polyphenols have also been suggested to include the inhibition of pro-inflammatory enzymes. Dietary polyphenols have been found to inhibit cellular enzymes such as 5-lipoxygenase, hyaluronidase and trypsin, thus exerting an important anti-inflammatory action (Huang et al., 2006; Prasad et al., 2004; Laughton et al., 1991).

The work presented in this chapter deals with the modulatory effect of caraway phenolic extract on inflammatory enzymes such as 5-lipoxygenase, hyaluronidase and trypsin.

4.2. Materials and Methods

4.2.1. Chemicals

Arachidonic acid, phenidone and NDGA, hyaluronidase, sodium hyaluronate, glycyrrhizin, trypsin, N-benzoyl-D,L-arginine-\(p\)-nitroanilide (BAPNA) and ovomucoid were obtained from Sigma Chemical Co., St. Louis, MO, USA. All the solvents and other chemicals used in this study were of analytical grade and obtained from Hi Media, Mumbai, India.

4.2.2. Caraway phenolic extract

Caraway phenolic extract was prepared as explained in chapter 2.

4.2.3. Effect of caraway phenolic extract on human 5-lipoxygenase activity

4.2.3.1. Isolation of 5-lipoxygenase crude enzyme from PMNLs of human blood

Human peripheral venous blood from healthy individuals who have not received any medication was collected with anticoagulant EDTA and PMNLs were separated from blood by Ficoll-histopaque density gradient method and hypotonic lysis of erythrocytes (Boyum, 1976). All the procedures were performed at 4°C, PMNLs were suspended in
phosphate buffer saline and sonicated for 20-30s at 20kHz to release the cytosolic 5-LOX enzyme into solution. This solution was centrifuged at 10,000rpm for 30min at 4°C and the supernatant was used as the source of enzyme. The concentration of protein was estimated by Lowry’s method using BSA as standard (Lowry et al., 1951).

4.2.3.2. Inhibition of 5-lipoxygenase by caraway phenolic extract

Assay of 5-LOX was performed according to the method of Aharony and Stein (1986). The standard reaction mixture for the 5-LOX assay contained 100µM phosphate buffer, pH 7.4, 75µM of dithiothreitol, 200µM of ATP, 300µM of CaCl₂, 60µM of AA and 5µg protein. The enzymatic reaction was carried out at room temperature and the 5-LOX activity was measured at 234nm indicating the formation of 5-HETE. The molar extinction coefficient of 25mM/cm was used to calculate the specific activity of the enzyme. The enzyme activity was expressed as µmoles of 5-HETE formed/min/mg protein.

For determining the inhibitory activity of the phenolic extract, varying concentrations (2.5, 5, 10, 15, 20, 25, 30, 35, 40 and 45µg/mL) of phenolic extract was incubated with the enzyme for 2min and the reaction was initiated by the addition of AA and the 5-LOX activity was measured as 5-HETE formed at 234nm spectrophotometrically as described above. The experiment was carried out in triplicates.

4.2.4. Inhibitory effect of caraway phenolic extract on hyaluronidase activity

The inhibitory effect of caraway phenolic extract on activated hyaluronidase was determined by the modified method described by Asada et al., (1997). Hyaluronidase was activated by incubating 100µL hyaluronidase (4.15mg/mL in 0.1M acetate buffer,
pH 3.8) with 50μL sodium chloride (26.3mg/mL in 0.1M acetate buffer, pH 3.8) for 20 minutes at 37°C. Following activation, the enzyme mixture was pre-incubated with 200μL of test samples/reference standard at various concentrations for 20min at 37°C. After pre-incubation, 150μL of sodium hyaluronate (6mg/mL in 0.1M acetate buffer, pH 3.8) was added and the reaction mixture was incubated at 37°C for 40min. The reaction was arrested by the addition of 0.1mL (0.4N) of sodium hydroxide and 100μL (0.8M) potassium tetraborate. The mixture was kept in boiling water bath for 3 minutes, cooled to room temperature and 3mL of 67mM DMAB (p-dimethyl amino benzaldehyde) was added and incubated at 37°C for 20min. The absorbance was measured at 585nm. Controls were run which are devoid of test samples. The percentage inhibition of hyaluronidase was calculated as follows:

\[
\text{% inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100
\]

4.2.5. Inhibitory effect of caraway phenolic extract on trypsin activity

Trypsin inhibition was determined by a modified method of Cannell et al., (1988). Trypsin was dissolved in 50mM Tris-HCl, pH 7.6, to a concentration of 150units/mL. BAPNA (4.6mg) was dissolved in 100μL DMSO and used as substrate. 400μL of 0.4M Tris-HCl, pH 7.5, 400μL of enzyme solution, 800μL of test solution / reference standard of different concentrations were pre-incubated at 37°C for 30min. After pre incubation, 800μL of substrate solution was added, incubated at 37°C for one hour and the absorbance was read at 410nm using UV-visible spectrophotometer. A control reaction
was carried out without the test sample. The percentage inhibition of trypsin was calculated as follows:

\[
\text{\% inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100
\]

4.2.6. Statistical analysis

Statistical analysis was done using the software SPSS. The differences in mean values were tested using one-way analysis of variance (ANOVA) and Duncan’s multiple range test (DMRT) was used to determine the significant differences amongst the test materials. Differences were considered to be significant at P≤0.05.

4.3. Results

4.3.1. Inhibitory effect of caraway phenolic extract on human 5-lipoxygenase

The inhibitory activity of caraway phenolic extract on 5-LOX is shown in Figure 16. Caraway phenolic extract at 2.5-45µg concentration significantly inhibited 5-LOX activity compared to synthetic inhibitors.

The inhibitory effect of phenolic extract of caraway on human PMNLs 5-LOX activity was evaluated by measuring the decreased concentration of 5-HETE, by spectrophotometric method. The caraway extract inhibited human PMNLs 5-LOX activity in a concentration dependent manner (Figure 16). The IC\textsubscript{50} value for the caraway extract was found to be 21.4µg/mL. The effect of known synthetic 5-LOX inhibitors viz., phenidone and NDGA were tested and compared with that of caraway extract. The IC\textsubscript{50} values of phenidone and NDGA were found to be 24.6µg/mL and 29.1µg/mL, respectively. The data indicates that the caraway phenolic extract effectively inhibited 5-LOX as compared to synthetic inhibitors, phenidone and NDGA.
4.3.2. Inhibitory effect of caraway phenolic extract on hyaluronidase

The inhibitory activity of caraway phenolic extract on hyaluronidase is shown in Figure 17. Caraway phenolic extract at 25-400µg concentration significantly inhibited hyaluronidase activity as compared to the synthetic inhibitor glycyrrhizin.

The inhibitory activity of caraway phenolic extract was tested in the concentration range of 25-400µg/mL on hyaluronidase. Glycyrrhizin, a synthetic inhibitor, was used as a standard. The phenolic extract as well as the synthetic inhibitor exhibited dose dependent inhibitory response. Caraway phenolic extract inhibited the enzyme at microgram level with an IC$_{50}$ value 336µg/mL, whereas the IC$_{50}$ value of glycyrrhizin was found to be 271µg/mL. These values indicate that the inhibitory activity of caraway phenolic extract was less as compared to that of glycyrrhizin.
Figure 17. Inhibition of hyaluronidase activity by caraway phenolic extract and glycyrrhizin. The values are mean ± SEM of three experiments.

4.3.3. Inhibitory effect of caraway phenolic extract on trypsin

The inhibitory activity of caraway phenolic extract on trypsin is shown in Figure 18. The caraway phenolic extract, in the concentration range of 5-170µg at which significantly inhibited the trypsin activity, as compared to the synthetic inhibitor ovomucoid.

The phenolic extract as well as the synthetic inhibitor exhibited dose dependent inhibitory response against trypsin activity. Caraway phenolic extract inhibited the enzyme with an IC$_{50}$ value 46µg/mL, where as the IC$_{50}$ value of ovomucoid was found to be 21µg/mL. The inhibitory activity of caraway phenolic extract was less as compared to that of the ovomucoid.
Figure 18. Inhibition of trypsin activity by caraway phenolic extract and ovomucoid. The values are mean ± SEM of three experiments.

4.4. Discussion

Inflammation is a normal protective response to tissue injury and it involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair (Vane et al., 1995). It is a complex process, which is frequently associated with pain and involves occurrences such as the increase in vascular permeability, increase of protein denaturation and membrane alterations (Umapathy et al., 2010). A great number of inflammatory mediators including platelet-activating factor, prostaglandins, leukotrienes, cytokines, and adhesion molecules have been found to act on specific targets. These factors lead to the release of other mediators from leukocytes which further attract leukocytes, such as neutrophils to the site of inflammation (Kim, 2009). Natural products have long been contributed to the
development of modern therapeutic drugs (Cragg, 1997). Evidence exists that drugs derived from natural products can modulate various inflammatory mediators (arachidonic acid metabolites, peptides, cytokines, etc.). These modulations serve as controlling points where the amplification of the inflammatory processes can be disconnected and thereby reduce subsequent disease risk. Polyphenols are major non-nutrient constituents of many common culinary herbs and spices. Multiple studies, both epidemiological and experimental, suggest that polyphenols possess anti-inflammatory and antioxidant activity that may contribute, via the diet, to the prevention of chronic diseases such as cancer, cardiovascular disease, inflammatory bowel disease, and Alzheimer’s (Viuda-Martos, 2011; Kris-Etherton, 2002; Singh, 2008; Kaefer, 2008; Pan, 2010).

Lipoxygenase enzyme was reported to convert the arachidonic, linoleic and other polyunsaturated fatty acids into biologically active metabolites involved in the inflammatory and immune responses (Catalano and Procopio, 2005). According to Pontiki and Hadjipavlou-Litina (2008), enzymes are correlated with inflammatory and allergic reactions because of the formation of the LTs. The LTs are potent class of biologically active lipid derivatives that play an important role in the allergic and inflammatory response (Harris, 1990). High levels of LTs could be observed in case of asthma, psoriasis, allergic rhinitis, rheumatoid arthritis and colitis ulcerosa (Schneider and Bucar, 2005). LTC₄ is one of the important LTs involved in immediate hypersensitivity, broncho-constriction, smooth muscle contraction and increased vascular permeability of epithelial mucus secretion and there has been considerable interest in the development of human 5-LOX inhibitors for therapeutic use (Cashman, 1985; Lewis, 1990). In view of the central role in mediating the inflammatory responses, significant
efforts have been directed towards inhibition of 5-LOX by phenolic acids and flavonoid compounds present in spice to modulate biosynthesis of LTs (Laughton, 1991).

The present study has shown that the caraway phenolic extract possesses significant inhibitory effect on human PMNLs 5-LOX with an IC$_{50}$ value 21.4µg/mL. This is comparable with the efficacies of synthetic inhibitors, phenidone and NDGA, with an IC$_{50}$ value of 24.6µg and 29.3µg/mL, respectively. Being a natural substance, caraway phenolic compounds may be preferred to prevent lipid peroxidation over synthetic drugs, phenidone and NDGA. The spice active principles, curcumin, eugenol, piperine, capsaicin, cinnamaldehyde, quercetin, and allyl sulfide were significantly inhibited the human PMNL 5-LOX activity and the formation of LTs in a concentration dependent manner (Prasad, 2004). These findings clearly suggest that phenolic compounds in spices might have physiological role in modulating 5-LOX pathway.

The caraway phenolic extract exhibited dose dependent inhibitory response on hyaluronidase and the activity was at microgram level with an IC$_{50}$ value of 336µg/mL. However, it was less potent as compared to that of synthetic inhibitor, glycyrrhizin. The literature indicates that polyphenolic compounds such as curcumin and tannic acid are good inhibitors of hyaluronidase with an IC$_{50}$ value of 57µM and 86µM, respectively (Girish and Kemparaju, 2005). From the data, it is clearly evident that the phenolic extract of caraway is a highly potent inhibitor of hyaluronidase activity at relatively very less concentration as compared to curcumin and tannic acid.

The proteolytic enzymes and proteins play an essential role in inflammation and other functions of the immune system. Proteolytic enzymes such as bromelain, papain,
trypsin and chymotrypsin are essential regulators and modulators of the inflammatory response (Cottrell, 2003). Trypsin has been shown to induce in vivo epidermal proliferation, vasodilation and inflammatory infiltration in the upper epidermis (Stefano and Eleonora, 2003). Denaturation of proteins is a well documented cause of inflammation. The inflammatory drugs (salicylic acid, phenylbutazone etc.) have shown dose dependent ability to protect thermally induced protein denaturation (Mizushima and Kobayashi, 1968). Similar results were observed in many reports from plant extract (Sakat et al., 2010). The extracts may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutrophils lysosomal constituents include bactericidal enzymes and proteases, which upon extracellular release cause further tissue inflammation and damage (Chou, 1997). Leukocyte proteases play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by protease inhibitors (Das and Chatterjee, 1995). An earlier report indicates that phenolic compounds and flavonoids to be a competitive inhibitor of serine protease such as trypsin (Parellada and Guinea, 1995). In the present study, the inhibitory activity of caraway phenolic extract was tested on trypsin and compared with ovomucoid, a synthetic inhibitor. The caraway phenolic extract exhibited dose dependent response and inhibited the activity of trypsin at microgram level with an IC$_{50}$ value 46µg/mL. The inhibitory activity of caraway phenolic extract thus showed less potency as compared to that of ovomucoid which is a synthetic inhibitor. Although the inhibitory activity of caraway phenolic extract is less, it could be preferred over ovomucoid as being a natural product.
Table 9. IC$_{50}$ values of caraway phenolic extract on inflammatory enzymes

<table>
<thead>
<tr>
<th>Enzyme assay</th>
<th>IC$_{50}$ values of Caraway phenolic extract (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-LOX</td>
<td>21±5</td>
</tr>
<tr>
<td>Hyaluronidase</td>
<td>336±13</td>
</tr>
<tr>
<td>Trypsin</td>
<td>46±7</td>
</tr>
</tbody>
</table>

4.5. Conclusion

The data presented here indicates that caraway seeds are rich source of polyphenolic compounds such as phenols and flavonoids. The anti-inflammatory activities of the phenolic extract of the caraway seeds could be due to the presence of these compounds. These findings provide scientific evidence for using the natural products as promising source for the treatment of several inflammatory diseases. Further, in vivo detailed studies using the caraway phenolic extract should be interesting and could lead to the development of potential sources of novel anti-inflammatory drugs.