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Review Paper

Bromelain: An Overview

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Abstract

Bromelain is a crude extract from the fruit or stem of pineapple [***Ananas comosus* (Linn.) Merr.**] plant. It consists of different closely related proteinases which are good anti-inflammatory, antithrombotic and fibrinolytic agents. The active fractions have been characterized biochemically and found to be effective after oral administration. It has earned universal acceptability as a phytotherapeutical drug because of its history of safe use and zero side effects. This communication deals with the biochemistry and applications of bromelain in therapeutic purposes.

Keywords: Bromelain, Pineapple plant, *Ananas comosus*, Proteinase, Phytotherapeutic.

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Introduction

Bromelain belongs to a group of a protein digesting enzymes obtained commercially from the fruit or stem of pineapple plant [***Ananas comosus* (Linn.) Merr.**][1-2]. It is non-toxic compound with therapeutic values in modulating. Bromelain is most notable for its effectiveness in reduction of inflammation and decreasing swelling but scope of its benefits are increasing. As a natural anti-inflammatory enzyme, bromelain has many uses. In patients of arthritis it may reduce the swelling that causes joint pain. It may also be helpful in relieving the pain, numbness, tingling and loss of motor and sensory function in fingers. This protease is beneficial in reducing the clumping of platelets, formation of plaques in arteries and the formation of blood clots. All these effects help in the treatment of cardiovascular diseases. It is widely believed that most of the orally ingested enzymes are destroyed by the digestive juices prior to being absorbed. However, there is evidence that significant amount of bromelain can be absorbed with negligible toxic effect[3].

The reason for synthesis of bromelain proteinases in pineapple plants is a big mystery in plant science. The carnivorous plants get their supply of nitrogen and phosphorus by degradation of organic matter (insects, microbes) by using proteinases and other digestive enzymes is well known. The pineapple plants grow as epiphyte in forest. Normally they grow on other plants which do not provide any nutritional support. The rosette like arrangements of pineapple leaves develops funnel-type rain water reservoirs. This so-called phytotelmata are always filled with water, as well as with nitrogen and phosphorous suppliers[4]. This hypothesis is supported by recent findings that leaves react to mechanical stimuli by

producing protein-kinases[5].

The pineapple fruit was eventually carried to India, Africa, China and other countries from Guadeloupe. According to the estimation of FAO (UN) for the year 2003, Thailand, Philippines and Brazil are the top three producers along with India ranking forth in the world. Isolation and purification of bromelain can be achieved by several methods. The commercial preparation of bromelain is done by centrifugation, ultrafiltration and lyophilization. When some proteolytic fractions of bromelain are purified, they may be physiologically inactive *in vivo* under conditions where bromelain has a beneficial effect. It was found that a great deal of the physiological activity of bromelain may not be due to its proteolytic fraction[6].

Some of the biochemical properties, absorption and bioavailability, pharmacological properties and toxicity related research reports on bromelain have been discussed in this paper.

Biochemical properties

The crude aqueous extract from stems and fruits of pineapple is known as bromelain. It is a mixture of different thiol endopeptidases and other components like phosphatases, glucosidases, peroxidases, cellulases, glycoproteins, carbohydrates and several protease inhibitors. Depending on the source bromelain is usually distinguished as stem bromelain (EC. 3.4.22.32) or fruit bromelain (EC. 3.4.22.33)[4]. From the stem of pineapple plant eight basic proteolytically active components have been detected. F4 (24,397 dalton) and F5 (24, 472 dalton) are the two main components. The proteinase considered to be the most active fraction has been identified as F9, which comprises about 2% of the total proteins. It was estimated that 50% of the proteins in F4 and F5 are glycosylated, whereas F9 was found to be unglycosylated. F9 (ananain) has a molecular weight of 23,464 dalton and has highest specific proteinase activity. The F4, F5 (212 amino acids) and F9 (216 amino acids) fractions have been completely sequenced. The optimum *p*H for the F4 and F5 fractions is 4.0 to 4.5 and that of F9 is close to a neutral *p*H(Ref. 7). The crude extract of bromelain exhibited its activity over a *p*H range of 4.5 to 9.8(Ref. 8). There are also different protein fractions which can be obtained by means of various biochemical methods [SDS-polyacrylamide gel electrophoresis (PAGE), isoelectric focusing (IEF), multicathodal-PAGE]. The substrate spectrum of the enzymes are broad, extending from synthetic low molecular mass amides and dipeptides up to high molecular substrates such as fibrin, albumin, casein, angiotensin II and bradykinin. The enzyme activity of commercial preparations of bromelain is determined with different substrates such as casein (FIP units), gelatin (gelatin digestion units) or chromatographic tripeptides[9-11]. Bromelain is not very specific in action but preferentially cleaves glycol, anayl and leucyl bonds[4].

Inagami and Murachi[12] reported catalytic activity of bromelain. They studied the bromelain-catalysed hydrolyses of N^2-benzoyl-L-arginine ethyl ester and N^2-benzoyl-L-arginine amide. These results were analysed in consideration with strong non-productive binding for the bromelain catalysed hydrolysis of N^2-benzoyl-L-arginine amide[13]. The *p*H dependent bromelain catalysed hydrolysis was also reported[13]. The *p*H dependence of the value of K_m for the bromelain-catalysed hydrolysis of N-benzoyl-L-arginine ethyl ester is anomalous. In that the profile is bell shaped having the highest values of K near neutrality. The *p*H-K_m profile is characterized also by an apparent additional ionization, required in the base form for lowering K_m, producing a 'hump' in the profile, in the region of *p*H 4-5(Ref. 14).

Absorption and bioavailability

Bromelain is absorbed through the gastrointestinal tract. It was detected (up to 40%) in blood after oral administration in rats. Bromelain concentration was found highest in the blood after one hour of administration. It is also reported that up to 40% of bromelain is absorbed from the intestine. In an experimental study it is determined the half-life (6-9h) and plasma concentration (2.5-4ng/ml) of bromelain after oral administration of 8.6g each day[15-17].

Medicinal uses

Clinical studies have shown that bromelain may help in the treatment of several disorders:

Platelet aggregation: In 1972 the conclusive evidence that bromelain prevents aggregation of blood platelets was reported by Heinicke *et al*[18]. A group of 20 volunteers with a history of heart attack or stroke, or with high platelet aggregation values were given bromelain orally as preventive dose. It decreased aggregation of blood platelets in 17 of them and normalized values in 8 of the 9 persons who previously had high aggregation values[18]. Morita *et al*[19] conducted *in vitro* studies which showed that bromelain inhibits platelet aggregation in a dose-dependent manner. They also reported the isolation and characterization of platelet aggregation inhibitory factors from bromelain. Metzig *et al*[20] studied in details the aggregation and adhesion of platelets to endothelial cells. They found that if the platelets are incubated with bromelain prior to activation with thrombin, aggregation is completely prevented.

Fibrinolysis: The effectivity of bromelain as effective fibrinolytic agent was tested in both *in vitro* and *in vivo* conditions. But its efficacy is more evident in purified

fibrinogen solutions than in plasma. It may be due to the presence of antiproteinases in plasma. A dose dependent reduction of serum fibrinogen level is seen in rats following administration of bromelain. The result showed that at the higher concentrations of bromelain, both prothrombin time (PT) and activated partial thromboplastin time (APTT) are markedly prolonged[21]. The fibrinolytic activity of bromelain has been attributed to enhanced conversion of plasminogen to plasmin, which limits the spread of coagulation processed by degrading fibrin[22].

Anti-inflammatory activity: Bromelain have actions involving other enzyme systems in exerting its anti-inflammatory effect on soft tissue injury. It can also inhibit the inflammatory pain in rats in a dose dependent manner[23]. Pre-clinical and clinical trials of systemic enzyme therapy in rheumatic disorders showed that proteolytic enzymes certainly have analgesic and anti-inflammatory effects[24]. As a result of its anti-inflammatory effect, bromelain has been found to dramatically reduce post operative swelling and pain[25]. Plasmakinins and prostaglandins have an important role in playing as mediators of pain and inflammation. Oh-Ishi *et al*[26] reported that bromelain can lower the plasmakinin level. It was also demonstrated that oral administration of bromelain can reduce the level of both PGE_2 and thrombaxane B_2 (Ref. 27).

Modulation of cell adhesion: Bromelain has been found to remove T-cell CD44 molecules and to affect T-cell activation. The highly purified bromelain protease F9 was tested on the adhesion of peripheral blood lymphocytes (PBL) to human umbilical vein endothelial cells (HUVEC). Both bromelain and protease F9 reduced expression of CD44, but F9 was about ten times more active than bromelain, having about 97% inhibition of CD44 expression. The results showed F9 selectively decreases expression. It also indicates F9 selectively decreases the CD44 mediated binding of PBL to HUVEC[28].

Cytokine induction: The successful initiation of an immune response depends on several factors such as T cell and macrophages, along with the polypeptides factors. These factors produce cytokines which play a key role in communication during normal immunological response as well as infections, inflammatory and neoplastic disease states. Bromelain has been reported to induce cytokine production in human peripheral blood mononuclear cells. Treatment leads to the production of tumour necrosis factor alpha (TNF-alpha), interleukin-1-beta (IL-1-beta) and interleukin-6 (IL-6) in a time and dose dependent manner. The ability to induce cytokine production may explain the antitumour effects observed after oral administration of polyenzyme preparations[29, 30].

Potentiation of antibiotics: Potentiation of antibiotic molecule is one of the main uses of bromelain for several years. Bromelain can modify the permeability of organs and tissues to different drugs. It prolongs sleeping time in mice when administered with pentobarbital[31] and increases levels of penicillin and gentamycin in rats. In humans, bromelain has been well documented to increase blood and urine levels of antibiotics and results in higher blood and tissue levels of tetracycline and amoxicillin when they are administered concurrently with bromelain[32].

Digestive aid: Bromelain has been successfully used as a digestive enzyme following pancreatectomy, in case of exocrine pancreas insufficiency and in other intestinal disorders[33]. Because of its wide *p*H range, bromelain has activity in the stomach as well as the small intestine. The enzyme has also shown to be an adequate replacement of pepsin and trypsin in case of deficiency. Bromelain has been reported to heal gastric ulcers in experimental animals[34].

Cardiovascular and Circulatory applications: Bromelain can prevent aggregation of human blood platelets *in vivo* and *in vitro*. It also prevents or minimizes the severity of angina pectoris and transient ischemic attack (TIA), which is useful in the prevention and treatment of thrombophlebitis may break down cholesterol plaques and exerts a potent fibrinolytic activity. If administered for prolonged time periods, bromelain also exerts an anti-hypertensive effect in experimental animals[35, 36]. Nieper[37] found that administration of bromelain (400-1000 mg/day) to angina pectoris patients resulted in the prevention of symptoms within 4 to 90 days.

Debridement: Bromelain used topically as a cream (35% bromelain in a lipid base) for the beneficial effect of the elimination of burn debris and in acceleration of healing. A non-proteolytic component of bromelain is responsible for this effect. This component is known

as esterase which has no hydrolytic enzyme activity against normal protein substances or various glycosamino glycan substrates. Its activity varies greatly from preparation to preparation[38].

Toxicity

Moss *et al*[31] determined the oral dose of bromelain and no acute toxicity was found up to 10g/kg body weight of mice, rats and rabbits. The lethal dose (LD_{50}) also determined in *i.p.* and *i.v.* route. The LD_{50} in case of *i.p.* in mice is 37mg/kg and in rat it is 85 mg/kg. For *i.v.* administration in mice it is 30mg/kg and in rabbit, 20mg/kg. There was no toxic reaction in both the cases. No significant change in blood coagulation parameters after giving bromelain (3000 FIP units/ day) to human for 10 days was observed[39].

Conclusion

Bromelain has been used for a wide range of therapeutic applications for last four decades. But the mode of its action has not been yet completely well understood. It has been shown to be well absorbed after oral applications and it have no negative impact on health after prolonged use. All these evidences suggest that bromelain can be used as an effective supplement for alround health.

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