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Therapeutic Application of Pineapple Protease (Bromelain): A Review

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Abstract: Bromelain (EC 3.4.22.32) is a crude extract from the pineapple (*Ananas comosus*) plant that contains, among other components various closely related proteinases (stem bromelain, fruit bromelain, comosain and ananain) demonstrating both *in vitro* and *in vivo* several therapeutic properties including malignant cell growth, thrombus formation, inflammation, control of diarrhoea, dermatological and skin debridement among others. Bromelain also contains peroxidase, acid phosphatase, several protease inhibitors and organically bound calcium and remains stable over a wide range of pH 2 to 9. Available evidence indicates bromelain is well absorbed orally with its therapeutic effects being enhanced in a dose dependent manner. It has been demonstrated to be safe and an effective food supplement. However, all the mechanisms of its action remain unresolved.

Key words: Bromelain, pineapple, proteinases, inflammation

Introduction

Pineapple (*Ananas comosus*) native to Central and South America, is grown in several tropical and sub-tropical countries including Hawaii, India, China, Kenya, South Africa, Malaysia, the Philippines and Thailand.

It has been used as a medicinal plant in several native cultures and bromelain has been chemically known since 1876 (Peckoldt *et al.,* In: Taussig, 1988). Bromelain obtained from the stems of the pineapple plant contains all the soluble components of the pineapple stem in their original properties, which may involve malignant cell growth, thrombus formation, inflammation, control of diarrhoea, dermatological and skin debridement (Cohen, 1964; Taussig and Batkin, 1988; Kelly, 1996; Maurer, 2001).

Heinecke and Gortner (1957) found that bromelain concentrations is high in pineapple stems necessitating its extraction and use as a phytomedical compound because unlike the pineapple fruit which is normally used as food, the stems are a waste by-product and thus inexpensive.

The main proteolytic constituents contained in pharmacological preparations or food supplements of bromelain (stem bromelain, fruit bromelain and ananain) are also present in the pineapple fruit (Hale *et al.,* 2005). Bromelain's primary component is a Sulfhydryl proteolytic fraction. Bromelain also contains a Peroxidase, acid Phosphatase, several protease inhibitors and organically bound calcium (Kelly, 1996). Bromelain activity is stable over a wide pH range (Cohen, 1964; Taussig and Batkin, 1988; Kelly, 1996; Maurer, 2001; Heinecke and Gortner, 1957; Hale *et al.,* 2005; Mynott *et al.,* 1999; Cooreman *et al.,* 1976). Therefore it may not be necessary to enteric-protect the protease from acid conditions in the stomach. However, it may be necessary to protect the enzyme from digestion by acid proteases in the gut. It may be administered with a buffering agent, for example bicarbonate or in water or in a solution containing nutrients to assist with absorption of fluid and nutrients (Mynott *et al.,* 1999).

Several studies have been carried out and results generated indicate bromelain has useful phytomedical applications. However, these results are yet to be amalgamated and critically compared so as to chat the way forward as to whether bromelain will gain wide acceptance as a phytomedical supplement. The purpose of the present paper is to highlight some relevant contributions regarding bromelain's phytomedical applications that have been reported in recent times.

Anti - inflammatory agent: Botanicals such as *Ananas comosus* (Pineapple) and their extracts (bromelain) have been used clinically as anti-inflammatory agents in rheumatoid arthritis, soft tissue injuries, colonic inflammation, chronic pain and asthma (Taussig and Batkin, 1988; Kelly, 1996; Maurer, 2001; Cooreman *et al.,* 1976; Izaka *et al.,* 1972; Hale *et al.,* 2005; Jaber, 2002) and are currently in use as anti-inflammatory agents (Ammon, 2002; Lemay *et al.,* 2004; Darshan and Doreswamy, 2004).

The major mechanism of action of bromelain appears to be proteolytic in nature, although evidence also suggests an immunomodulatory and hormone like activity acting via intracellular signalling pathways. *In vitro* studies have shown that bromelain can inhibit pre-incubated with medium alone (PMA) - induced T cell production of the Th_2 cytokine IL - 4 and to a lesser degree the Th_1 cytokines IL-2 and induced interferon-gamma (IFN-() via modulation of the extracellular

regulated kinase-2 (ERK-2) intracellular signalling pathway (Mynott *et al.,* 1999). Bromelain has also been shown to reduce cell surface receptors such as hyaluronan receptor CD44, which is associated with leukocyte migration and induction of proinflammatory mediators (Engwerda *et al.,* 2001; Eckert *et al.,* 1999; Hale *et al.,* 2002). Manhart *et al.* (2002) have shown bromelain to significantly reduce $CD4^+$ T lymphocytes, which are primary effectors in animal models of inflammation.

Beneficial effects of bromelain have been suggested or proven in a variety of inflammatory disease and animal models of inflammation. These include immunologically mediated arteriosclerosis in rat aortic allografts (Gaciong *et al.,* 1996), the experimental allergic encephalomyelitis (EAE) model for the human autoimmune disease multiple sclerosis (Targoni *et al.,* 1999; Hale *et al.,* 2005), IgE- mediated perennial allergic rhinitis (Thornhill and Kelly, 2000) and collagen-induced arthritis in the rat (Rovenska *et al.,* 2001). In their study on bromelain's anti-inflammatory effects in an ovalbumin - induced murine model of allergic airway disease (AAD), Secor *et al.* (2005) observed that bromelain demonstrated both anti-inflammatory and immunomodulatory effects. In this particular study, they found that bromelain treatment significantly reduced the primary outcomes of murine AAD: Total bronchoalveolar lavage (BAL) leukocytes (eosinophilis and lymphocytes), IL-13, $CD4^+$ T cells, $CD8^+$ T cells and $CD4^+CD25^+$ T cells, while also altering the $CD4^+/CD8^+$ ratio. These findings indicate that systemic bromelain treatment reduces an allergen induced localized airway inflammatory process.

Kane and Goldberg (2000) gave a description of two patients suffering from ulcerative colitis (UC) that was refractory to conventional treatment, who rapidly entered and remained in clinical and endoscopic remission after self treatment with oral bromelain obtained from a healthy food store. Studies by Hale (2004) show that daily treatment with 5mg of oral bromelain significantly decreased spontaneous colon inflammation in $IL\text{-}10^{-1-}$ mice. They further did show that anti-inflammatory activity of bromelain is dependent on its proteolytic activity.

Wen *et al.* (2006) while studying the effect of bromelain on postoperative defecation in rats, supports Hale's (2004) findings that proteolytic activity of bromelain in the colonic micro-environment is not only responsible for its anti-inflammatory activity but may be involved in the improvement of post operative ileus. That orally administered bromelain retains its proteolytic activity, was previously documented only in the small intestine of pigs (Mynott *et al.,* 1996; Chandler and Mynott, 1998). The bromelain used in this study was enterically protected. In his studies however, Hale (2004) showed that oral bromelain retains its proteolytic activity throughout the entire gastrointestinal tract of mice in the absence of encapsulation or other classic enteric protection techniques. These results could explain earlier reports that bromelain decreased intestinal inflammation in human with UC (Kane and Goldberg, 2000).

Walker *et al.* (2002), in their pilot study while investigating the effect of bromelain on acute knee pain, reported significant improvement after a month's intervention. These results were consistent with earlier reports of bromelain supplementation (Uhlig, 1981; Vogler, 1988; Lotti *et al.,* 1993) even though they could not be compared directly. Meanwhile Akhtar *et al.* (2004) in their study where they assessed the efficacy of an oral enzyme combination (ERC: Enzyme-rutin combination which contains rutin and enzymes bromelain and trypsin) versus diclofenac (a non-steroidal anti-inflammatory drug-NSAID) among patients with knee osteoarthritis (OA) in a double blind randomized version, found ERC to be as equally efficacious to diclofenac. These results are consistent with those reported earlier by (Vogler, 1988; Klein and Kullich, 2000; Tilwe *et al.,* 2001). More so, unlike diclofenac, which exhibits inherent toxicities, ERC has a well known superior safety and tolerable profile (Akhtar *et al.,* 2004).

Bromelain as an anti-tumour agent: Pharmacological agents with modulation of anti-inflammatory, proteolytic, platelet aggregation inhibition and prostaglandin synthesis have been considered to be beneficial in regulating tumour growth and its metastasis (Batkin *et al.,* 1985; Honn, 1983; Sato *et al.,* 1983). Bromelain, with similar regulating actions, has shown protective properties on tumour cell growth retardation and lung metastasis (Batkin *et al.,* 1985; Batkin *et al.,* 1988a; Batkin *et al.,* 1988b; Taussig and Batkin, 1988).

Batkin *et al.* (1988b) while studying the antimetastatic effect of bromelain with or without its proteolytic and anticoagulant activities in the animal model of Lewis lung carcinoma, reported significant reduction in total number of metastasis in both active and inactive bromelain as compared to control groups. This phenomenon had been reported earlier by Batkin *et al.* (1985) whose study of three cell lines was done *in vitro*. In both studies, they conclude that bromelain could be having other pharmacological entities besides its recognized proteolytic anticoagulant functions.

Recently, study results reported by researchers at the Queensland Institute of Medical Research-QIMR (QIMR, 2005), give a window of hope for this phenomenon. While studying bromelain, researchers at QIMR reported the discovery of two proteins they named CCS and CCZ and found that they could block growth of a broad range of tumour cells including breast, lung, colon, ovarian and melanoma. However, the study is on going and these results are not reliable at the moment. Batkin *et al.* (1985 and 1988a) noted that *in vitro* Lewis lung cancer cell growth retardation was a necessary correlate to antimetastatic activity. In this regard therefore,

peroxidase and proteolytic anticoagulant activities may not be relevant features of bromelain's antimetastatic potential.

Maurer *et al.* (1988) found that bromelain may induce differentiation of leukemic cells *in vitro* and proposed this phenomenon as a possible mechanism of action. In their studies, Grabowska *et al.* (1997) found that B16F10 mouse melanoma cells, pre-incubated *in vitro* with bromelain, significantly reduced lung metastatic tumour weight to about three times. However, no survival benefit was seen. Furthermore bromelain diminished the capacity of these cells to migrate through an extracellular matrix layer in an in *vitro* invasion assay and inhibited the growth of tumour cells in a concentration dependent manner, whereas the anti-proliferation effect did not correlate with the proteolytic activity. Earlier studies by Goldstein *et al.* (1975) and Taussig and Goldstein (1976) reported that bromelain feeding enhanced the resistance of mice to the harmful effect of UV (Ultra Violet) irradiation. It took twice as long for the bromelain fed group to develop pre-cancerous lesions as compared to the control group. Finally, human platelets pre-treated *in vitro* with bromelain lost their capacity to stimulate the invasiveness of several metastatic tumour cells in the *in vitro* invasion assay. Meanwhile it has been shown that metastasized cells, while migrating through the vessels, carry CD44 adhesion molecules on their surface by which they adhere to endothelial cells via the ligand hyaluron. Bromelain preferentially cleaves off CD44 molecules by virtue of its proteolytic activity, thus inhibiting the first steps of the metastatic process (Eckert *et al.,* 1999; Hale *et al.,* 2002; Hale and Haynes, 1992).

Maurer (2001) noted that metastasized tumour cells carry the receptor (uPAR) for urokinase plasminogen activator (uPA), which generates plasmin from plasminogen. Plasmin degrades the extracellular matrix (ECM), composed of collagen type IV, laminin and fibronectin. Tumour cells also secrete matrix metalloproteinases (MMPs), enabling the malignant cells to invade through the ECM. Bromelain diminishes uPAR expression and uPA activity, thus inhibiting the invasion step of metastasis. Maurer further notes that interactions between tumour cells and platelets take place on different levels i.e. intravasal distribution, adhesion on endothelial cells, invasion and extravasation. Platelets he notes, directly bind to tumour cells, a process promoted by the release of factors such as platelet factor 4, thrombospondin, thrombin and gelatinase A from platelets, which facilitate thrombus formation. Apart from this, transforming growth factor- $ (TGF-$), produced by both platelets and tumour cells, plays an important role: it induces the synthesis of ECM proteins and stimulates the activity of uPA, MMPs and angiogenesis. Thus, disturbance of the blood coagulation system may lead to the formation of thrombi by aggregating platelets and tumour cells. Bromelain is capable of inhibiting both platelet aggregation *in vitro* and *in vivo*, as well as platelet-stimulated invasiveness of tumour cells. Thus what was described and used as folk medicine by the natives of the tropics over a Century ago as over time been confirmed to have pharmaceutical applications. However, more research is required to determine the structure and characteristics of the two compounds (CCS and CCZ) that were reported by researchers at the QIMR (QIMR, 2005).

Bromelain promotes debridement of burns: Burns are characterized by formation of an eschar, which is made up of burned and traumatized tissue. The eschar not only hinders accurate diagnosis of the burn's depth but also serves as a medium for bacterial growth and therefore a source of infection, contamination and sepsis of the injury and to the neighbouring originally undamaged tissues (Rosenberg *et al.,* 2004). Rapid debridement considerably reduces morbidity and mortality of severely burned patients. It permits early skin grafting and lessens the problems of infection, contamination and sepsis thus abbreviating the convalescence period (Maurer, 2001; Sheridan *et al.,* 1994; Sheridan *et al.,* 1998; Prasanna *et al.,* 1994; Monafo, 1974; Nada *et al.,* 1998).

While surgical debridement is non-selective, chemical debridement removes only the burned denatured skin (Maurer, 2001; Sheridan *et al.,* 1994; Janzekovic, 1970; Salisbury, 1990; Miller *et al.,* 1992). Furthermore, surgical excision is painful and exposes patients to the risks of repeated anaesthesia and significant bleeding. Enzymatic debridement has been suggested with experimental runs giving positive results. Topical bromelain (35% in a lipid base) was reported to achieve complete debridement on experimental burns in rats in about 2 days, as compared with collagenase, which required about 10 days, with no side effects or damage to adjacent burned tissue (Klaue *et al.,* 1979). When topical bromelain was used for frostbite eschar removal, no debridement other than of superficial eschar layers was noted; after two topical applications of bromelain, frostbite injuries remained unaffected (Ahle and Hamlet, 1987).

Rosenberg *et al.* (2004), reported complete debridement of the eschar after only one to two brief applications with minimal side effects and no blood loss. In the same study, no specific debridase (a bromelain derived debriding agent) related morbidity or mortality was noted. However, due to incomplete data in a large number of subjects, inaccuracies may have been possible thus calling for more controlled studies to assess the safety and efficacy of a proteolytic enzyme for enzymatic skin debridement.

Effects of bromelain on diarrhoea: Diarrhoea is a major cause of illness and death in children and young animals (Cravioto *et al.,* 1988; Smith and Lingood, 1982;

Roselli *et al.,* 2007). Vibro cholerae and enterotoxigenic Escherichia coli (ETEC) are two important microorganisms that cause diarrhoea Levine *et al.* (1983). ETEC produces one or both of a heat-labile (LT) and/or heat stable enterotoxin (either STa or STb) and V.cholerae liberate cholera toxin (CT) (Mynott *et al.,* 1997). To contain this problem, drugs such as chlorpromazine, nicotinic acid, loperamide and berberine sulfate have been used in animal models to inhibit secretion by CT and LT (Guandalini *et al.,* 1984; Holmgren *et al.,* 1978; Turjman *et al.,* 1978). Berberine, chlorpromazine and indomethacin also reduce secretion induced by STa (Greenberg *et al.,* 1980; Abbey and Knoop, 1979; Guandalini *et al.,* 1987). Despite the efficacy of these antisecretory compounds in animals, none are routinely available for use in children and adults because of adverse side effects or the large doses required for efficacy (Chandler and Mynott, 1998). Over time, oral rehydration therapy which has a significant impact on morbidity and mortality of patients with acute infectious diarrhoea has been used. However, oral rehydration therapy does not interfere with the secretory process nor diminish diarrhoea (Field, 1981). Bromelain has been demonstrated to have anti-diarrhoea activity (Chandler and Mynott, 1998; Thomson *et al.,* 2001).

Studies by Mynott *et al.* (1997) have reported stem bromelain to show antisecretory properties. Using a rabbit ileum mounted in using chambers, they showed that bromelain could prevent net changes in short-circuit current (Isc) and therefore, fluid secretion mediated by secretagogues that act through cAMP (cyclic-3, 5-adenosine monophosphate), cGMP (cyclic-3, 5-guanosine monophosphate) and calcium-dependent signalling pathways. Because most toxins that cause diarrhoea activate one of these pathways, bromelain would be expected to be an effective anti diarrhoea nutraceutical drug.

The efficacy of bromelain in this study was 62% in preventing LT induced secretion, 51% effective against CT and 35% effective against STa. Bromelain also prevented secretory changes caused by prostaglandin E_2, theophylline, calcium-ionophore A23187, 8-Br-cAMP (8-bromocyclic-3, 5-adenosine monophosphate) and 8-Br-cGMP (8- bromocyclic-3, 5-guanosine mono-phosphate), well known intracellular mediators of ion secretion. The efficacy of bromelain was reported not caused by reduced tissue viability resulting from its proteolytic effects on enterocytes, indicated by experiments measuring uptake of nutrients into intestinal cells and experiments measuring short circuit responses to glucose. Meanwhile studies by Roselli *et al.* (2007) on the effect of different plant extracts and natural substances (PENS) against membrane damage induced by ETEC in pig intestinal cells showed bromelain to be among those with protective effect.

Bromelain improves decrease in defecation in ileus condition: Postoperative gastrointestinal dysmotility (ileus) is a common consequence of abdominal surgery causing significant patient discomfort (nausea, vomiting, abdominal distension and inability to eat or defecate), and often leads to more serious problems (acute gastric dilatation, aspiration, respiratory compromise, cardiac arrhythmia and perforation). Due to limited therapy specific for this procedure, ileus remains an important clinical problem (Wen *et al.,* 2006).

In the US, bromelain is sold in health food stores as a nutritional supplement to promote digestive health and as an anti-inflammatory medication for horses (Hale, 2004). It has been used successfully as a digestive enzyme following pancreatectomy in cases of exocrine pancreas insufficiency and in other intestinal disorders (Knill-Jones *et al.,* 1970). Recently, it was reported that stool discharge improved in some Japanese patients concomitantly suffering from haemorrhoids and constipation after using bromelain {private communication in (Wen *et al.,* 2006). This in essence suggests that bromelain may improve intestinal propulsive motility.

In another study, the combination of ox bile, pancreatin and bromelain was shown to be effective in lowering stool fat excretion in patients with pancreatic steatorrhoea. In addition, this combination resulted in a gain in weight in most cases as well as enhanced subjective feeling of well being. Symptomatic improvement was also noted in relation to pain, flatulence and stool frequency (Balakrishnan *et al.,* 1981). In a recent study, Wen *et al*. (2006) did show that treatment with 500 mg/kg bromelain significantly increased wet weight and water content of faecal pellets to near normal levels in postoperative rats. The results suggest that bromelain may play an important role in treatment of ileus. In the same study, bromelain treatment was shown to significantly suppress overexpression of colonic iNOS mRNA, accompanied by improvement of decrease in defecation in postoperative rats. It is therefore suggested that modulation of iNOS gene expression is involved in the improvement by bromelain of the decreased defecation in postoperative rats, at least in part, by inhibiting colonic iNOS gene expression probably through NF-$_k$B pathway.

Bromelain inhibits thrombus formation: Studies have indicated that bromelain prevents aggregation of human blood platelets *in vivo* and *in vitro*, prevents or minimizes the severity of angina pectoris and transient ischemic attacks (TIA), is useful in the prevention and treatment of thrombosis and thrombophlebitis, may break down cholesterol plaques and exerts a potent fibrinolytic activity (Taussig and Nieper, 1979; Kelly, 1996). Furthermore, it has been suggested that bromelain increases vessels wall permeability to oxygen and nutrients while increasingly thinning blood both of which aid in these conditions (Kelly, 1996).

Heinicke *et al.* (1972) were the first to report that bromelain prevents aggregation of blood platelets. In their study carried among human volunteers with a history of heart attack or stroke or with people having high aggregation values, as well as with health subjects, oral administration of bromelain (160-1000 mg per day) decreased aggregation of blood platelets in all the subjects. Later studies by Nieper (1978), who administered 400-1000 mg per day of bromelain to 14 patients with angina pectoris resulted in the disappearance of symptoms in all patients within 4 to 90 days but reappeared after bromelain administration was discontinued.

Metzig *et al.* (1999) showed that pre-incubation of human platelets with 10ug/ml bromelain completely prevents thrombin induced platelet aggregation *in vitro* and also reduced the adhesion of thrombin stimulated, fluorescently labelled platelets to bovine aorta endothelial cells. Similarly, they reported that oral (60 mg/kg) and intravenous (30 mg/kg) bromelain inhibited *in vivo* thrombus formation in a model of laser-induced thrombosis in rats. The ability of bromelain to influence these conditions could be due to its ability to breakdown fibrinous plaques. Bromelain has been shown to dissolve arteriosclerotic plaque in rabbit aorta *in vivo* and *in vitro* (Taussig and Nieper, 1979). Later, Hale *et al.* (2002) showed that *in vitro* bromelain treatment of leukocytes in whole blood proteolytically altered 14 of 59 leukocyte makers studied. It is important to note that bromelain induced loss of CD41 and CD42a via proteolysis would be expected to decrease platelet function and thus inhibit thrombus formation.

Bromelain gives strong immunogenicity: Bromelain has been shown to remove T-cell CD44 molecules from lymphocytes among other bromelain sensitive molecules (Hale *et al.,* 2002; Eckert *et al.,* 1999; Hale and Haynes, 1992; Roep *et al.,* 2002; Desser *et al.,* 1993). Munzig *et al.* (1994) did show that highly purified bromelain protease F9 reduced the expression of CD44 to about 10 times more than the crude bromelain, achieving about 97% inhibition of CD44 expression. Roep *et al.* (Roep *et al.,* 2002) reported that protease treatment reduced expression of cell surface receptors on T-cells and antigen-presenting cells. Previously, reduction of CD44 expression on lymphocytes of patients with multiple sclerosis during protease therapy had been reported (Munzig *et al.,* 1994; Stauder *et al.,* 1997; Hale and Haynes, 1992).

Roep *et al.* (2002) suggested that the generation of soluble forms of adhesion molecules by proteolytic cleavage could act as an additional benefit for immonomudulatory function of protease treatment. However, they noted that the quality of immune activation plays an important role during chronic autoimmunity. Earlier, animal models for rheumatoid arthritis and Type 1 diabetes protease treatment prevented or delayed the onset of these diseases (Wiest-Ladenburger *et al.,* 1997; Emancipator *et al.,* 1997; White *et al.,* 1991). Later, Hale (2004) unexpectedly found bromelain to exhibit strong immunogenicity following oral dosing. In further studies following this phenomenon, Hale *et al.* (2006) reported that repeated exposure was necessary for development of anti-bromelain antibodies, with exposure period ranging from 3 to 6 weeks on a dose dependent manner.

Bromelain application in dermatological disorders: Bromelain among other fruit extracts from apricots, apples, peaches, pears, papayas, pomegranates, cherries, kiwis, tangerines and oranges have been described to play an important role in treating dermatological disorders (Murad, 2003). Ozlen (1995) has disclosed a cosmetic composition containing at least one alpha-hydroxy acid, salicylic acid and at least one digestive enzyme derived from fruit. Preferably the digestive enzyme is a mixture of bromelain and papain. Bromelain is disclosed as being typically obtained from pineapple and papain is disclosed as being typically obtained from dry papaya latex. The compositions are allegedly useful for treating various cosmetic conditions or dermatological disorders, such as lack of adequate skin firmness, wrinkles and dry skin.

Conclusion: Bromelain being a plant extract, contains various components such as proteinases, peroxidises, phosphatises, protease inhibitors and organically bound calcium whose ratio to each other might vary according to soil composition, climate conditions during plant growth, geographical location where the pineapple was grown, pineapple variety and the process of extraction. These factors might contribute to the variations of bromelain's pharmacological activities.

Proteolytic activity of bromelain has been shown to play only a part in its pharmacological activity while other factors such as immunomodulatory, hormone like properties, fibrinolytic activity and uncharacterised components such as CCS and CCZ complement towards its pharmacological activity. However, there is need for further investigation on the uncharacterised components.

Bromelain's activity remains stable over a wide pH range which explains why its activity has been found to be effective over the entire gastrointestinal tract. Since it is safe and non toxic, there is need to investigate how it can be incorporated in foods. On our view, if successfully incorporated in foods, it could become more acceptable as a nutraceutical product than it now is.

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