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Review Article

The bromelain and rutoside advantage in systemic enzyme therapy: pharmacological basis of combination with trypsin

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ABSTRACT

Inflammation involves various interlinked pathways and processes. In its uncontrolled form, inflammation results in variety of diseased sates. Current therapy for inflammatory diseases is limited to steroidal and non-steroidal anti-inflammatory drugs (NSAIDs). But these are associated with safety concerns and have a deleterious effect on wound healing. Proteolytic enzymes, also called proteases, which are naturally occurring substances derived from animal or plant sources, are believed to be effective and safer alternatives to the conventional medications. Combined with the bioflavonoid rutoside, the proteases trypsin and bromelain have been extensively investigated as alternatives to conventional therapies for pain and swelling associated with diverse conditions. Their individual mechanisms of action and the advantages of combining bromelain and rutoside with trypsin has been discussed. The combination not only covers a wider range of processes involved in inflammation, but they also complement each other's actions and provide a more well-rounded control of the inflammatory processes.

Keywords: Inflammation, Proteases, Flavonoids, Pain, Swelling

INTRODUCTION

Inflammation

Inflammation is a protective response of the body, resulting from the triggering of immune system by stimulants like pathogens and damaged tissue. The most common inflammatory signalling pathways include the nuclear factor kappa-B (NF-κB), mitogen-activated protein kinase (MAPK), and Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathways.[1] In its uncontrolled form, inflammation results in variety of diseased sates. These, primarily, involve permeability changes in the small blood vessels, activation, recruitment, and accumulation of leukocytes, and release of inflammatory mediators. Although the precise nature of inflammation is dictated by the site and stimulus, the basic mechanisms and flow involved are quite similar-recognition of specific molecular patterns by cell surface pattern receptors leading to activation of inflammatory pathways, followed by release of inflammatory mediators, which ultimately recruit and activate inflammatory cells. The first cells to be recruited are neutrophils, followed by monocytes, lymphocytes (natural killer cells [NK cells], T cells, as well as B cells), and mast cells. The chemical mediators activate cells and platelets, and the vascular system interact in a complex manner with each other and lead to changes in the tone as well as the permeability of vessels, dysregulation of coagulation pathways with the microthrombi formation, and destruction of pathogens or dead tissue by the activated cells, accompanied by collateral damage to normal tissue.[2] A summary of the basic pathways involved in the inflammation is illustrated in the Figure 1 given below.[3]

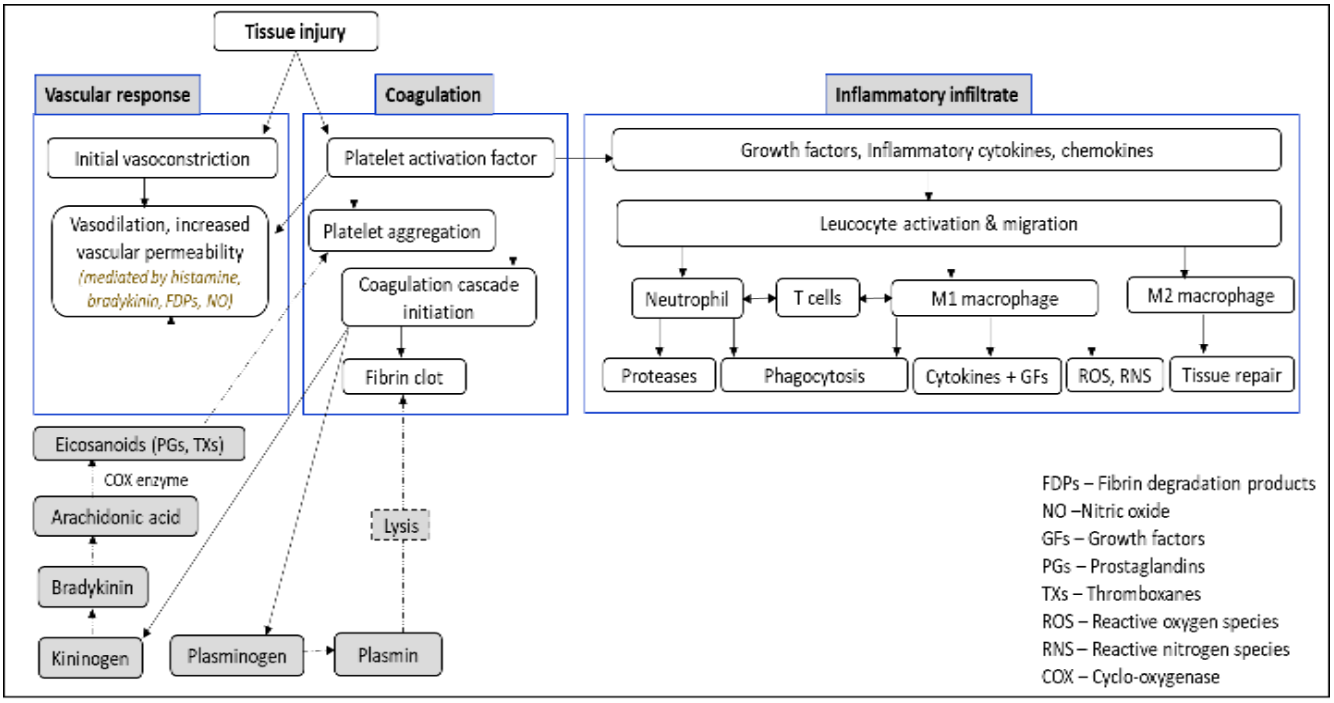

Figure 1: Basic processes in the pathophysiology of inflammation. Adapted from "Systemic therapy with bromelain- trypsin-rutoside combination in inflammation: A narrative review of the pharmacodynamics.[3]

Current conventional therapy for inflammatory conditions

Current therapy for inflammatory diseases is limited to steroidal and non-steroidal medications. Moreover, the anti-inflammatory drugs on the market and used in research usually have significant side effects, particularly when long-term use is involved. Among the medications used, non-steroidal anti-inflammatory drugs (NSAIDs) are some of the most frequently administered ones. They serve to limit inflammation and swelling. NSAIDs are, however, associated with the risk of adverse gastrointestinal events and bleeding. Both therapeutic and adverse effects of NSAIDs are due to inhibition of cyclooxygenase (COX) enzyme. More importantly, NSAIDs have been recognized to delay wound healing, especially in the long-term and reduce the tensile strength of wound.[4,5] Use of NSAIDs in wound-associated inflammation have shown to impair the wound healing process. Part of it is believed to be due to the anti-proliferative effect of NSAIDs on blood vessels and skin. It was demonstrated, in a pre-clinical study that analysed the effects of NSAIDs on incisional wound healing after surgery, that the animals treated with NSAIDs had significant reduction in fibroblasts, thereby inhibiting proliferation. The negative impact of NSAIDS on healing has been demonstrated on a wide variety of cells and tissues like mesenchymal stem cells, osteoblasts, tendon and ligament.[6] They are also believed to increase scar formation, especially if used during the proliferative phase of healing, by inhibiting PGE2 production.[7] Importantly, although NSAIDS are quite effective in providing analgesia, they have limited effectiveness in reduction of edema.[6] This has significant consequences, as presence of edema is detrimental to tissue function due to compression of small vessels, increased diffusion distance for oxygen and other nutrients, limited diffusional removal of potentially toxic by-products and compromised cellular metabolism in the swollen tissue.

Proteolytic enzymes as alternative therapy

As steroidal and NSAID medications are associated with significant side effect profiles, there is a great deal of interest in natural compounds, which have been used for centuries to reduce pain and inflammation. Systemic enzyme therapy refers to the use of proteolytic (hydrolytic) enzymes, also called proteases, which are naturally occurring substances derived from animal or plant sources. Though proteases are mostly known for role in the digestion, they are necessary for some critical processes in the body like protein recycling, immune function, cell division and blood clotting. In our body, these proteases are produced by the stomach and the pancreases; but they are also available in plant and animal sources. The role of proteases is commonly thought of as degradative, but they also contribute to effective immune responses by conveying hormone-like signals and intracellular signal transduction via the specific cell surface receptors.

MECHANISMS OF ACTION OF ENZYME COMBINATION

Some of the popular enzymes and their combinations are trypsin-chymotrypsin, bromelain and papain. Combined with the bioflavonoid rutin (Rutoside), the proteases

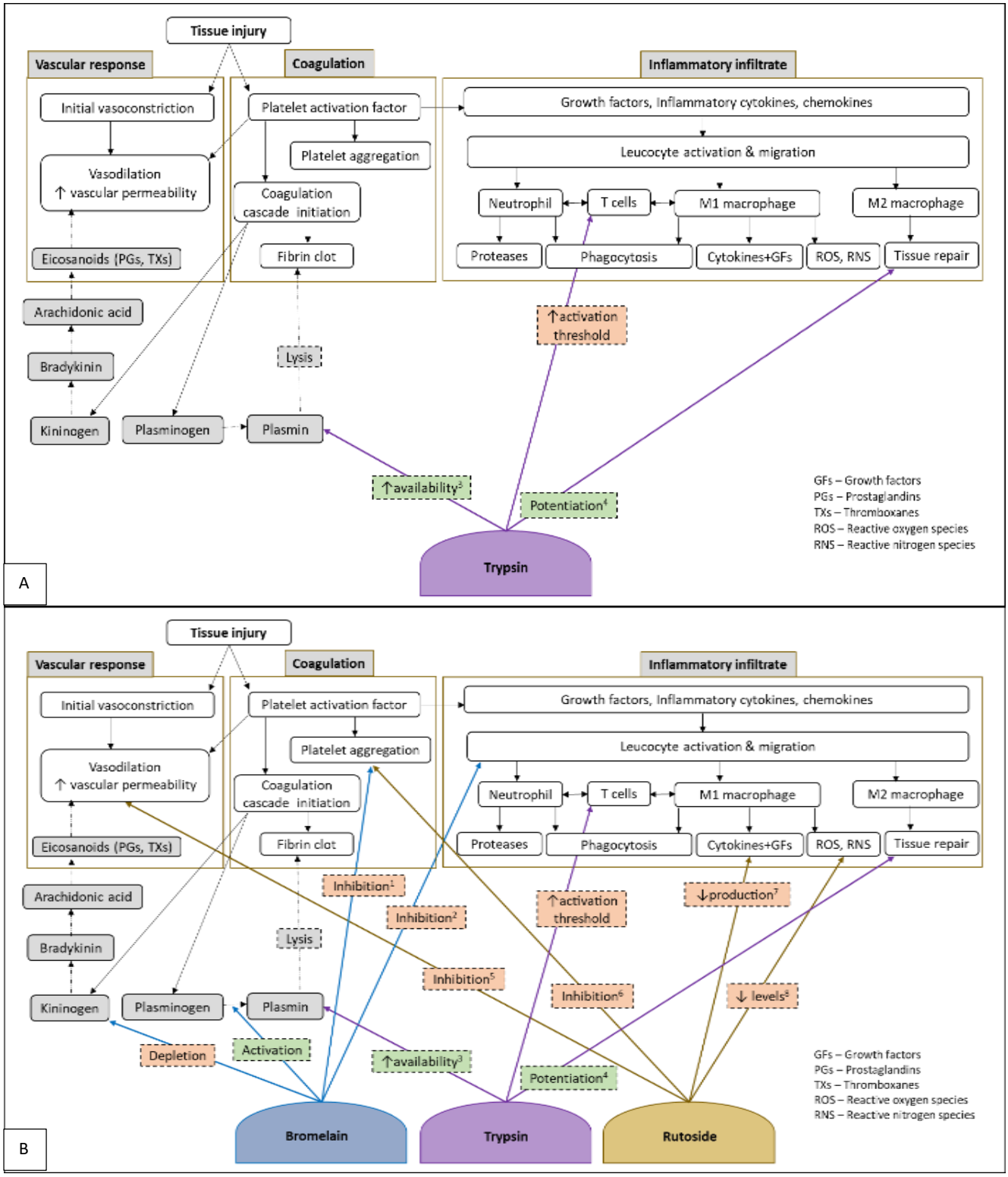

Figure 2 (A and B): Major mechanisms of action of trypsin/chymotrypsin in inflammation. Combined mechanisms of trypsin, bromelain and rutoside against different processes involved in inflammation.[3]

1-by alteration of adhesion molecules on the platelet surface; 2-by alteration of cell surface molecules involved in leucocyte activation and adhesion; 3-by displacing "bound" plasmin from plasma proteins; 4-by the activation of Protease activated receptor (PAR)1 and PAR2 receptors on macrophages; 5-by decreased production of nitric oxide (NO) by inducible nitric oxide synthase (iNOS); 6-by inhibiting intracellular Ca^{2+} mobilization in platelets; 7-by inhibiting the transcription of genes encoding for pro-inflammatory cytokines and 8-by scavenging action & iNOS inhibition.

Table 1: Mechanism of action of trypsin/ chymotrypsin.

Action	Mechanism	Evidence
Pro-fibrinolytic	Displaces protein-bound plasmin allowing more plasmin to be available for fibrinolysis	-In rabbits and dogs, trypsin led to diminution of in situ thrombus.[8] -In an *in vitro* study, trypsin exposure led to reduced clotting of fibrinogen by thrombin[9]
Promotes tissue repair	Promotes differentiation of macrophage secretion profile towards an M2a phenotype (with a more healing/repairing profile)	-In peripheral blood mononuclear cells, trypsin led to increased differentiation of monocytes to fibrocytes.[10] -In a macrophage culture, trypsin altered the macrophages toward an M2a phenotype, which is involved in wound healing[11]
Reduces cellular inflammation	Raises the T-cell activation threshold	-In an *in vitro* study, trypsin exposure increased the threshold of T-cell activation by antigen presenting cells (APCs).[12] -In mice, oral trypsin altered 3 surface molecules on T cells, that are involved in regulating T-cell activation threshold[13]

Table 2: Mechanism of action of bromelain.

Action	Mechanism	Evidence
Pro-fibrinolytic	Stimulates conversion of plasminogen to plasmin, resulting in increased fibrinolysis.	-In rabbits, bromelain increased prothrombin time by 80-250%, increased antithrombin time and increased serum level plasmin.[14] -In rats, bromelain increased serum fibrinolytic activity[14]
Anti-platelet	Inhibits thrombin/ADP-induced platelet aggregation.	In an *in vitro* study, bromelain decreased human platelet aggregation, decreased adhesion to bovine endothelial cells and reduced thrombus formation[15]
Anti-kinin	Lowers kininogen and bradykinin levels	-In rats, bromelain reduced the levels of high molecular weight (HMW) kininogen in serum.[16] -In rat paw edema model, bromelain reduced level of plasma kininogen, accompanied by reduction in edema[17]
Reduction of prostaglandins	Decreases synthesis of inflammatory prostaglandins	In rat-inflammation models, bromelain reduced the levels of prostaglandin E2 and thromboxane B2[14]
Reduction of leucocyte migration	Alters leucocyte cell surface molecules which are responsible for leukocyte homing, cellular adhesion, and activation[18]	-In an *in vitro* chemotaxis assay, bromelain decreased the migration of human neutrophils by 40%.[19] -In 3 murine models, bromelain led to 50-85% reduction in neutrophil migration into the inflamed peritoneal cavity[19]

Table 3: Mechanism of action of rutoside.

Action	Mechanism	Evidence
Antioxidant	Chelation of ions suppression of free radical generation	-In multiple murine models, rutoside reduced the production of reactive species and inflammatory mediators.[20-22] -In rat liver microsomes, rutoside decreased iron ion-dependent lipid peroxidation[23] -In human macrophages, rutoside decreased inducible NO synthase (iNOS) -mediated production of NO.[20]
Anti-inflammatory	Inhibits transcription of >20 genes encoding critical proinflammatory factors like TNF-α, IL-1, IL-8, chemotactic factors	-In activated human macrophages, rutoside reduced the expression of inflammation-related genes and decreased the release of NO.[20] -In a rat model of arthritis, rutoside reduced the levels of inflammatory cytokines in rat sera and showed improvement in clinical signs of chronic arthritis[20]
Vaso-protection	Attenuates vascular permeability due to histamine, bradykinin and fibrin degradation products	-In rat skin, rutoside reduced the leakage of human serum albumin.[24]

Continued.

Action	Mechanism	Evidence
		-In frog mesentery venules and capillaries, rutoside decreased the fluid and macromolecules permeability in inflamed vessels.[25]
Anti-platelet	Inhibits platelet aggregation	-In rabbit platelets stimulated by platelet activating factor (PAF), rutoside reduced aggregation, reduced serotonin release and reduced the levels of intraplatelet free calcium.[26] -In human platelets stimulated by collagen, rutoside reduced aggregation, intracellular calcium mobilization and thromboxane A2 formation.[27]

Trypsin and Bromelain have been extensively investigated as alternatives to conventional therapies for pain and swelling associated with diverse conditions. In some countries, these are used as dietary supplements, while in some they are available as over-the-counter medications. In India, these are available as different pharmaceutical formulations - dispersible tablets (e.g., Disperzyme) and enteric-coated tablets (e.g., Phlogam). The mechanisms of action of the individual components of enzyme-flavonoid combination are summarized in the Tables 1-3.[8-27]

ADVANTAGES OF COMBINING BROMELAIN AND RUTOSIDE WITH TRYPSIN

The actions of each of the components has been illustrated in Figure 2.[3] From the mechanisms it is apparent that trypsin (and chymotrypsin) actions are primarily related to enhancing fibrinolysis by making more plasmin available for action, inhibition of T-cell mediated immune response and assisting wound repair mediated by M2 macrophages.

The addition of bromelain not only complements the fibrinolytic action of trypsin, but also has broad ranging effects of limiting vascular permeability (which can help counter edema), inhibiting platelet aggregation (which can help counter dysregulated coagulation and thrombosis) and limiting inflammatory cell infiltration. From a pharmacokinetic and dosing perspective too, combining the enzymes trypsin and bromelain is suitable. This is based on results from human volunteer studies, which reported that the elimination half-life of trypsin after oral administration, was estimated to be around 9-12 hours, while for oral bromelain, it ranged from 8-14.5 hours.[28]

Additionally, it has been demonstrated that the bromelain component in these combinations lead to increased absorption of antibiotics up to 25-40%. This action has been reported with multiple antibiotics and with different routes of application including subcutaneous and intramuscular routes, including penicillin, cephalosporin, and tetracycline classes.[29-32]

Rutoside, on the other hand, has far-reaching effects in controlling the oxidative stress, vascular permeability and countering the activation of NF-κB and further release of inflammatory cytokines and chemokines. Put together the combination acts at various levels to control inflammation-vascular permeability, dysregulated coagulation, cell activation, cell migration, cell differentiation, free radical generation, prostaglandin production and even transcription of genes responsible for inflammatory cytokine production.

CONCLUSION

From the pharmacological data discussed above, it is apparent that, when combined, these ingredients not only cover a wider range of processes involved in inflammation, but they also complement each other's actions and provide a more well-rounded control of the inflammatory process, unlike the trypsin-chymotrypsin combination which has a limited range of action or conventional drugs (like NSAIDs) which focus only on a specific enzyme or pathway.

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REFERENCES

1. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J et al. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2017;9(6):7204-18.
2. Stone WL, Basit H, Burns B. Pathology, inflammation. InStatPearls. StatPearls Publishing. 2021.
3. Daftary G, Shah A, Divekar G, John J, Patil N, Khemnar B. Systemic therapy with bromelain-trypsin-rutoside combination in inflammation: A narrative review of the pharmacodynamics. Natl J Physiol Pharm Pharmacol. 2022;12(11):1-8.
4. Beitz JM. Pharmacologic Impact (aka" Breaking Bad") of Medications on Wound Healing and Wound Development: A Literature-based Overview. Ostomy Wound Manag. 2017;63(3):18-35.
5. Enoch S, Grey JE, Harding KG. Non-surgical and drug treatments. BMJ. 2006;332(7546):900-3.
6. Hess CT. Checklist for factors affecting wound healing. Adv Skin Wound Care. 2011;24(4):192.
7. Su WH, Cheng MH, Lee WL, Tsou TS, Chang WH, Chen CS et al. Nonsteroidal anti-inflammatory drugs for wounds: pain relief or excessive scar formation? Mediators inflam. 2010;2010.
8. Innerfield I, Schwarz A, Angrist A. Intravenous trypsin: Its anticoagulant, fibrinolytic and thrombolytic effects. J Clin Invest. 1952;31:1049-55.

9. Alexander B, Pechet L, Kliman A. Proteolysis, fibrinolysis, and coagulation: Significance in thrombolytic therapy. Circulation. 1962;26:596-611.
10. White MJ, Glenn M, Gomer RH. Trypsin potentiates human fibrocyte differentiation. PLoS One. 2013;8:e70795.
11. White MJ, Gomer RH. Trypsin, tryptase, and thrombin polarize macrophages towards a pro-fibrotic M2a phenotype. PLoS One. 2015;10:e0138748.
12. Targoni O, Lehmann PV. Modulation of the activation threshold for autoreactive T cells via systemic enzyme therapy with phlogenzym®. J Neuroimmunol. 1995;56:66.
13. Lehmann PV. Immunomodulation by proteolytic enzymes. Nephrol Dial Transplant. 1996;11:952-5.
14. Lotz-Winter H. On the Pharmacology of Bromelain: An Update with Special Regard to Animal Studies on Dose-Dependent Effects. Planta Med. 1990;56(03):249-53.
15. Metzig C, Grabowska E, Eckert K, Rehse K, Maurer H. Bromelain proteases reduce human platelet aggregation *in vitro*, adhesion to bovine endothelial cells and thrombus formation in rat vessels *in vivo*. *In Vivo* (Brooklyn). 1999;13(1):7-12.
16. Oh-Ishi S, Uchida Y, Ueno A, Katori M. Bromelain, a thilprotease from pineapple stem, depletes high molecular weight kininogen by activation of Hageman factor (factor XII). Thromb Res. 1979;14:665-72.
17. Suda H, Yamauchi H, Iso T. Potentiative effect of angiotensin converting enzyme inhibitor on carrageenan edema in rats and the role of tissue kininogen. J Pharmacobiodyn. 1984;7:372-7.
18. Hale LP, Greer PK, Sempowski GD. Bromelain treatment alters leukocyte expression of cell surface molecules involved in cellular adhesion and activation. Clin Immunol. 2002;104:183-90.
19. Fitzhugh DJ, Shan S, Dewhirst MW, Hale LP. Bromelain treatment decreases neutrophil migration to sites of inflammation. Clin Immunol. 2008;128:66-74.
20. Kauss T, Moynet D, Rambert J, Al-Kharrat A, Brajot S, Thiolat D et al. Rutoside decreases human macrophage-derived inflammatory mediators and improves clinical signs in adjuvant-induced arthritis. Arthritis Res Ther. 2008;10(1):R19.
21. Adefegha SA, Leal DB, de Oliveira JS, Manzoni AG, Bremm JM. Modulation of reactive oxygen species production, apoptosis and cell cycle in pleural exudate cells of carrageenan-induced acute inflammation in rats by rutin. Food Funct. 2017;8(12):4459-68.
22. Khajevand-Khazaei MR, Mohseni-Moghaddam P, Hosseini M, Gholami L, Baluchnejadmojarad T, Roghani M. Rutin, a quercetin glycoside, alleviates acute endotoxemic kidney injury in C57BL/6 mice via suppression of inflammation and up-regulation of antioxidants and SIRT1. Eur J Pharmacol. 2018;833:307-13.
23. Afanas'ev I, Dcrozhko A, Brodskii A, Kostyuk V, Potapovitch A. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in li, pid peroxidation. Biochem Pharmacol. 1989;38(11):1763-69.
24. Gerdin B, Svensjö E. Inhibitory effect of the flavonoid O-(beta-hydroxyethyl)-rutoside on increased microvascular permeability induced by various agents in rat skin. Int J Microcirc Clin Exp. 1983;2(1):39-46.
25. Blumberg S, Clough G, Michel C. Effects of hydroxyethyl rutosides upon the permeability of single capillaries in the frog mesentery. Br J Pharmacol. 1989;96(4):913-19.
26. Chen W, Jin M, Wu W. Experimental study on inhibitory effect of rutin against platelet activation induced by platelet activating factor in rabbits. Zhongguo Zhong Xi Yi Jie He Za Zhi. 2002;22(4):283-85.
27. Sheu J, Hsiao G, Chou P, Shen M, Chou D. Mechanisms Involved in the Antiplatelet Activity of Rutin, a Glycoside of the Flavonol Quercetin, in Human Platelets. J Agric Food Chem. 2004;52(14):4414-18.
28. Lorkowski G. Gastrointestinal absorption and biological activities of serine and cysteine proteases of animal and plant origin: review on absorption of serine and cysteine proteases. Int J Physiol Pathophysiol Pharmacol. 2012;4(1):10-27.
29. Barsom S, Sasse-Rollenhagen K, Betrmann A. Erfolgreiche Prostatitisbehandlung mit hydrolytischen Enzymen. (Effects of simultaneously administered Hydrolytic enzymes on Antibiotic serum levels). Erfahrungsheilkunde. 1982;31:2.
30. Renzini G, Varengo M. Die Resorption von Tetrazyklin in Gegenwart von Bromelain bei oraler Applikation. Arzneimittel-Forsch (Drug Res). 1972;2:410-2.
31. Tinozzi S, Venegoni A. Effect of bromelain on serum and tissue levels of amoxycillin. Drug Exp Clin Res. 1978;1:39-44.
32. Friesen A, Schilling A, Hofstetter A, Adam D. Tetracyclin-Konzentration im Prostata-Sekret. Z. antimikrob. antineoplast. Chirurgie. 1987;2:61-5.

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