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Development of Method for Quantitative Determination of Bromelain in Gel Formulation

Nana Gorgaslidze

Tbilisi State Medical University, Georgia

Gel of bromelain has anti-inflammatory properties and hence is used for treatment of edema and inflammations. This was developed at TSMU Iovel Kutateladze Institute of Pharmacochemistry. On previous researches we published the data of some properties of papain and bromelain containing gels. The presented work belongs to the development and validation of accurate and sensitive assay method for quantitative determination of bromelain in gel based on UV-spectrophotometric analysis. Elastasin was used as substrates for their analysis. Absorbance was recorded at 595 nm. The developed method is linear, precise and sensitive, Intra- and inter-day measurements. All strategies were approved according to ICH rules and can be received for the standard analysis of bromelain in gel formulations. Bromelain (Br), a proteolytic catalyst removed from the stem of the pineapple, is known to have mitigating action and has been appeared to decrease blood consistency, forestall the total of blood platelets, and improve ischemia-reperfusion (I/R) injury in a skeletal muscle model. We researched the limit of Br to restrain myocardial injury in a worldwide I/R model. Grown-up male Sprague-Dawley rodents were separated into two gatherings: control (PBS) and Br at 10 mg/kg in PBS controlled by means of intraperitoneal infusion (twice/day) for 15 continuous days. On day 16, the hearts were excised and subjected to 30 min of global ischemia followed by 2 h of reperfusion. Br treatment demonstrated higher left ventricular utilitarian recuperation all through reperfusion contrasted and the controls [maximum pace of ascend in intraventricular weight (dP/dtmax), 2,225 versus 1,578 mmHg/s at 2 h reperfusion]. Aortic flow was also found to be increased in Br treatment when compared with that in untreated rats (11 vs. 1 ml).Besides, Br treatment decreased both the infarct size (34% versus 43%) and the level of apoptosis (28% versus 37%) contrasted and the control creatures. Western smear examination indicated an expanded phosphorylation of both Akt and FOXO3A in the treatment bunch contrasted and the control. These results demonstrated for the first time that Br triggers an Akt-subordinate endurance pathway in the heart, uncovering a novel system of cardioprotective activity and an expected restorative objective against I/R injury. Bromelain is a general name for a group of sulfhydryl containing, proteolytic catalysts acquired from Ananas comosus, the pineapple plant. It can function in the pH range 3 to 9 but once it is combined with substrate, the activity is no longer susceptible to the effect of the pH. The compelling temperature run is 40-65 ºC with the ideal being 50 ºC. Bromelain can be activated by calcium chloride, cysteine, bisulfate salt, NaCN, H2S, Na2S and benzoate. Hg2+, Ag+, Cu2+, α-1-antitrypsin, estatin A and B, idoacetate, inhibits bromelain. First presented as a restorative compound1 in 1957, bromelain's activities include: hindrance of platelet aggregation2-4; fibrinolytic activity5 ; mitigating action6 ; antitumor action7 ; adjustment of cytokines and immunity8 ; skin debridement properties9 ; upgraded ingestion of other drugs10; mucolytic properties11; stomach related assistance12; upgraded wound healing13 and cardiovascular and circulatory improvement14,15 . Various steps that include this research work are, extraction of juice from pineapple, enzyme assay of the crude extract, purification of crude extracts by salt precipitation, dialysis, ion exchange chromatography, quantitative estimation of protein by FolinLowery's method. The Venezuelan chemist Vicente Marcano et al. 16 recorded the first isolation of bromelain in 1891 from the fruit of pineapple. MYOCARDIAL ISCHEMIA-REPERFUSION (I/R) injury occurs in a wide spectrum of disorders ranging from cardiac arrest to acute myocardial infarction and represents a major public health concern. Ischemia induces several pathological changes due to lack of oxygen supply to the myocardium, and postischemic reperfusion worsens the injury. Modulation of the adaptive response to ischemic heart disease has become a major research interest. Pharmacological preconditioning plays a prominent role in reducing such tissue damage in response to I/R injury. In this respect, bromelain (Br), which is a descriptor for a family of sulfhydryl proteolytic enzymes extracted from the stem of Ananas comosus, the common pineapple plant, has shown promise. Br is composed of several distinct cysteine proteolytic fractions ranging in size from 15 to 27 kDa and is commonly delivered as a powder in a gelatin or enteric-coated capsule. Reports suggest that oral administration of Br inhibits time-dependent thrombus formation in a laser thrombosis model and reduces human platelet aggregation both in vitro and in vivo. Br, when combined with rutin and trypsin, was also shown to have a protective effect on the skeletal muscle during I/R injury in a rabbit hindlimb model, as demonstrated by a prevention of no flow and a preservation of the muscle tissue. Previous studies have shown that Br has the capacity to reduce angina, exert antihypertensive action, and significantly reduce the incidence of coronary infarct when administered with potassium and magnesium orotate. Although earlier reports suggested the protective role of Br against I/R injury, its mechanism of action is not known. Therefore, the objective of the present study was to investigate the effect of Br pretreatment on the degree of I/R injury in an ex vivo isolated rat heart model. Moreover, the upregulation of survival kinases is known to attenuate the process of apoptosis. In particular, the serine or threonine kinase Akt is well established to play an important role in endothelial and cardiomyocyte cell biology that activates an antiapoptotic or prosurvival signaling cascade. In addition, reports suggest that the targets of phospho (p)-Akt action are localized in the nucleus. Akt regulates the activity of a variety of other targets that includes the proapoptotic protein Bad, caspase-9, and the members of the forkhead box transcription factor/protein (FOXO) family such as FOXO1, FOXO3A, and FOXO4. FOXOs inhibit Fast ligands and Bcl-2 like the protein Bim. In addition, an Akt-dependent phosphorylation leads to an inhibition of forkhead transcription factor activity and, thereby, prevents proapoptotic signaling. Hence, in the present study we investigated the effect of Br on myocardial functions, infarct size, apoptosis, and the

status of p-Akt and p-FOXO following I/R injury. As expected, Br treatment was found to exert a cardioprotective effect as demonstrated by the reduction in both infarct size and the degree of apoptosis in association with an improvement in functional changes in the myocardium such as heart rate, left ventricular developed pressure, maximum rate of rise in intraventricular pressure (dP/dt_{max}), aortic flow, and coronary flow.