Roxana Báez[1]
Miriam T. P. Lopes[1]
Carlos E. Salas[2]
Martha Hernández[3]

# *In Vivo* Antitumoral Activity of Stem Pineapple (*Ananas comosus*) Bromelain



**Abstract**

Stem bromelain (EC 3.4.22.32) is a major cysteine proteinase, isolated from pineapple (*Ananas comosus*) stem. Its main medicinal use is recognized as digestive, in vaccine formulation, antitumoral and skin debrider for the treatment of burns. To verify the identity of the principle in stem fractions responsible for the antitumoral effect, we isolated bromelain to probe its pharmacological effects. The isolated bromelain was obtained from stems of adult pineapple plants by buffered aqueous extraction and cationic chromatography. The homogeneity of bromelain was confirmed by reverse phase HPLC, SDS-PAGE and N-terminal sequencing. The *in vivo* antitumoral/antileukemic activity was evaluated using the following panel of tumor lines: P-388 leukemia, sarcoma (S-37), Ehrlich ascitic tumor (EAT), Lewis lung carcinoma (LLC), MB-F10 melanoma and ADC-755 mammary adenocarcinoma. Intraperitoneal administration of bromelain (1, 12.5, 25 mg/kg), began 24 h after tumor cell inoculation in experiments in which 5-fluorouracil (5-FU, 20 mg/kg) was used as positive control. The antitumoral activity was assessed by the survival increase (% survival index) following various treatments. With the exception of MB-F10 melanoma, all other tumor-bearing animals had a significantly increased survival index after bromelain treatment. The largest increase (~318 %) was attained in mice bearing EAT ascites and receiving 12.5 mg/kg of bromelain. This antitumoral effect was superior to that of 5-FU, whose survival index was ~263 %, relative to the untreated control. Bromelain significantly reduced the number of lung metastasis induced by LLC transplantation, as observed with 5-FU. The antitumoral activity of bromelain against S-37 and EAT, which are tumor models sensitive to immune system mediators, and the unchanged tumor progression in the metastatic model suggests that the antimetastatic action results from a mechanism independent of the primary antitumoral effect.

**Key words**
Bromeliaceae · *Ananas comosus* · cysteine proteinase · stem bromelain · antitumoral · antimetastatic





## Introduction

Bromelain is a complex mixture of cysteine proteinases present in stems and immature fruits of the pineapple plant, *Ananas comosus* (Bromeliaceae). Crude commercial bromelain preparations contain in addition to the thiol-proteinases, stem bromelain (EC 3.4.22.32), fruit bromelain (EC 3.4.22.33), ananain (EC 3.44.22.31), comosain, proteinase inhibitors, phosphatases, glucosidases, peroxidases, glycoproteins, carbohydrates, and as yet other uncharacterized substances [1].

The fraction containing bromelain offers a wide spectrum of therapeutic properties, including anti-inflammatory, antiedematous, antithrombotic, fibrinolytic, immunostimulatory, antiar-

**Affiliation**
[1] Facultad de Ciencias Médicas, Ciego de Ávila, Cuba
[2] Departamentos de Farmacología, Bioquímica e Imunologia, Instituto de Ciencias Biológicas, Universidade Federal Minas Gerais, Belo Horizonte, Brasil
[3] Laboratorio de Ingeniería Metabólica, Centro de Bioplantas, Universidad de Ciego de Avila, Cuba

**Correspondence**
Carlos E Salas, PhD · Departamento Bioquímica e Imunologia Instituto de Ciências Biológicas · UFMG · Antonio Carlos 6627 · Belo Horizonte 31270–901 · Brasil · Phone/Fax: +55-31-3499-2646 · E-mail: cesbufmg@icb.ufmg.br

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thritic and antitumoral activities [2], [3]. The anti-inflammatory effect of bromelain applies to several disorders characterized in animal models, including arthritis and urogenital inflammation [4]. However, most studies using commercial bromelain lack control assays to measure differences in sample composition and proteolytic activity of the extracts [5], [6], [7]. Some of the pharmacological actions of bromelain such as inhibition of platelet aggregation and the anti-inflammatory effects depend on the proteolytic activity of these enzymes, but other effects such as wound debridement, tumor cell growth inhibition and metastasis are independent of its proteolytic function [8], [9], [10].

Some of the effects of bromelain are attributed to its capacity for selective proteolytic removal of cell surface molecules affecting lymphocyte activation and migration. On the other hand, bromelain-containing fractions promote secretion of cytokines, induce phagocytosis and cytotoxicity by leukocytes [11], [12], [13]. In 1972, it was demonstrated that oral administration of crude bromelain brought remarkable remissions of malignant tumors with relatively little side effects to cancer patients [14]. Subsequent studies confirmed the inhibitory effect of crude extracts and bromelain fractions on tumor cells [15], [16]. A possible mechanism for this action was the *in vitro* differentiation of leukemic cells mediated by bromelain. Batkin et al. [17] reported that bromelain administered subcutaneously to mice drastically reduced the subcutaneous uptake of Lewis lung tumor cells, compared to untreated controls. This and other subsequent reports confirm the protective antitumoral and antimetastatic effect of bromelain.

Although many studies support the oncostatic effect of bromelain, the identity of the active compound and the mechanism responsible for its action are poorly understood. Most of the data available with bromelain as an antitumoral/antimetastatic substance involve *in vitro* models, but *in vivo* confirmation of these results is lacking. To consolidate the antitumoral/antimetastatic role attributed to the protease, we analyzed the *in vivo* antitumoral effect of a purified fraction containing stem bromelain on a panel composed of six tumor cell lines transplanted into mice.

## Materials and Methods

### Protein purification

The enzyme fraction was isolated from stems following the third fruit harvest of *Ananas comosus* L. Merr cv. Red Spanish cultivar, grown in Ciego Avila, Cuba. A voucher specimen of the plant (# 10400) was deposited by Dr. Reinaldo Trujillo at the herbarium Julian Acuña of the Botanical Garden, Universidad de Camaguey. The reagents used were analytical grade. A bromelain sample from Sigma (St Louis, MO, USA) was used as a chromatographic and electrophoretic standard.

Plant stems (500 g) were rinsed with distilled water and chopped with a steel blade into small fragments before homogenization in a solution containing 0.1 – 0.5 mM $Na_2S$ buffer, pH 2, 1 : 1.5 w/v with a Waring blender [18]. Following extraction during 30 min at 4 °C, the homogenate was filtered through glass wool and centrifuged at 12,000 × g at 4 °C during 15 min (Beckman J-21; Palo Alto, CA, USA). The supernatant (100 mL) was exhaustively dialyzed against 1 L of 5 mM sodium acetate pH 5.0 and the dialysate loaded onto a CM-52 cellulose (Whatman Ltd; Balston, United Kingdom) column (2.5 × 17 cm) and stepwise eluted with 0.3 M, 0.5 M and 1 M sodium acetate buffer, pH 5.0. The proteolytic activity of eluted fractions was determined with hemoglobin substrate [19]. One unit of activity is the amount of enzyme that catalyzes the formation of 1 $\mu$mol of tyrosine per min at pH 6.8 at 37 °C. Protein concentration was estimated with the Lowry method [20].

The composition of the eluted active fractions was analyzed by HPLC chromatography (RP-18, 4 × 250 mm; LKB-Pharmacia; Uppsala, Sweden), using a 0 – 80 % acetonitrile gradient, containing 0.1 % TFA during 60 min at a flow rate of 0.5 mL/min.

### Protein electrophoresis

Protein electrophoresis was performed in SDS-denaturing gels as described previously [21]. The isolated protein was subjected to N-terminal sequencing by Edman degradation in a Beckman LF 3000 sequencer (Beckman).

### Antitumoral activity

The tumoral cell lines provided by the Cell Bank from National Institute of Oncoradiobiology (Havana City, Cuba) were leukemia P-388, sarcoma S-37, Ehrlich ascites tumor cells (EAT), Lewis lung carcinoma cells (LLC), M-B16F10 (MB-F10) melanoma and ADC-755 mammary adenocarcinoma cells. Tumor cells were routinely maintained in mice strains B6D2/F1 and NMRI provided by the National Center for Laboratory Animals (Havana City, Cuba).

P-388 leukemia cells were intraperitoneally injected in B6D2/F1 mice, S-37 and EAT tumor cells were intraperitoneally injected in NMRI mice, LLC cells were intramuscularly transplanted in the left hind leg of B6D2/F1 mice, MB-F10 and ADC-755 cells were injected subcutaneously in the left axillary region of B6D2/F1 animals. Each animal received approximately $10^6$ cells suspended in RPMI-1640 medium containing 10 % FBS (Gibco Life Technologies; Gaithersburg, MD, USA) in a volume equivalent to 1 % of animal weight, regardless of the tumor type. Food and water were supplied ad libitum during the experimental period.

The antitumoral activity determination in one of the experimental models used six groups of mice containing ten animals per group. Four of the groups were given 1, 5, 12.5 and 25 mg of bromelain/kg, respectively during fifteen days (Monday through Friday), beginning the day after cell inoculation. The fifth group received 20 mg/kg of 5-fluoruracil (5-FU) from Shanghai Pharmaceutical Industry (Shangai, China) and the sixth control group received saline during the same period. The drugs were given intraperitoneally in a volume equivalent to 1 % of the animal's weight diluted in sterile water at a concentration equivalent to saline. The antileukemic effect following intraperitoneal injection of P-388 cells was assessed after nine consecutive days of treatment with the various doses of bromelain.

The antitumoral and antileukemic activities were determined by the increase in survival (% SI) rate according to the relation:

$$\% SI = \frac{St - Sc}{Sc} \times 100$$

where St represents the survival mean of treated samples and Sc the survival mean of controls, expressed as the percent value.

P-388 leukemia cells were also inoculated using a different injection site. In this case, six groups of animals (n = 10 each) were inoculated in the ocular plexus with leukemic cells followed by the drug treatment or saline during nine consecutive days. In addition, a group of non-transplanted animals treated with saline were sacrificed on day 21 to asses the spleen size. Following these treatments mice were sacrificed by cervical dislocation and spleens removed to assess growth inhibition according to the following relation:

$$\% \text{ GI} = \frac{\text{Wts} - \text{Wcs}}{\text{Wcs}} \times 100$$

where GI represents % growth inhibition, Wts is mean weight of treated spleen, and Wcs is mean weight of control spleen.

**Antimetastatic activity**

The LLC antimetastatic activity was studied using three bromelain doses; 12.5, 25 and 50 mg/kg, the positive and negative controls were 5-FU and saline solution, respectively. Bromelain or 5-FU was given daily beginning 24 h after transplantation. The animals were sacrificed on day 21 by cervical dislocation. The lungs were dissected and rinsed with Ringer's solution and fixed with Bouin's solution (75 mL picric acid, 20 mL formaldheyde, 5 mL acetic acid), during 48 h at room temperature, and the metastasis number scored with a stereotaxic microscope (Carl Zeiss, Jena, Germany). The statistical analyses for the parametric Student-Newman-Keuls test and the non-parametric Mann-Whitney, Kruskal-Wallis and Student Newman-Keuls tests were done with the Statistical Package for Social Sciences (SPSS), version 11.5 for Windows.

**Animal handling**

Housing and manipulation complied with the Cuban guidelines established by our local Institutional Animal Welfare Section, Ethics Committee in Animal Experimentation, established at the Institute of Oncoradiobiology, Havana City, Cuba Protocol # 34/2002.

## Results

Bromelain stem extracts purified by ion exchange chromatography were eluted stepwise with 0.3, 0.5 and 1 M NaCl. The peak with highest activity (eluted with 0.5 M NaCl, 19% yield) was selected for further analysis (Fig. **1**, vertical arrow). The purity of the pooled fractions was verified by SDS-PAGE and HPLC, as shown in Fig. **2**. The inset of Fig. **2** confirms the purity of a representative bromelain preparation. Moreover, the identity of the proteinase was confirmed by N-terminal sequencing.

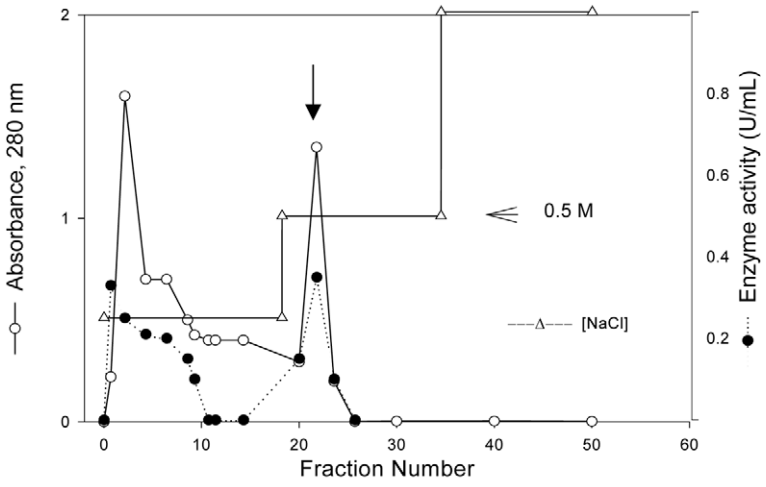


Fig. **1** CM chromatography of stem bromelain. The stem extract from bromelain (50 mg) was applied onto CM cellulose. After removing the unbound material by washing with the equilibrating buffer, the protein was eluted stepwise (0.3, 0.5 and 1 M) at a flow rate of 20 mL/h with sodium acetate pH 5.0. The vertical arrow shows the bromelain peak collected.

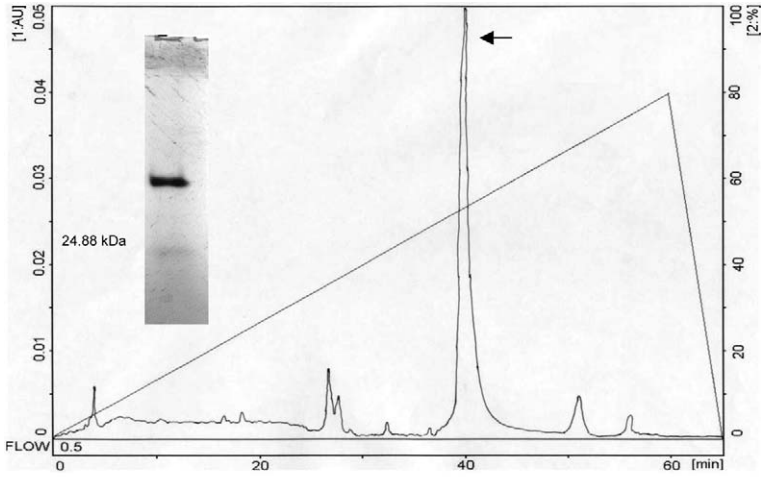


Fig. **2** HPLC and electrophoresis of stem bromelain. The HPLC chromatography of stem bromelain on RP-18 (4×250 mm, LKB-Pharmacia) is shown. The protein is eluted with a linear gradient (0–80% B) of 0.1% trifluoroacetic acid-acetonitrile at 0.5 mL/min. The data shows the 280 nm elution profile as a function of time. The inset shows the SDS-PAGE electrophoretic profile of 15 μg stem bromelain obtained from CM cellulose. The horizontal arrow shows the bromelain peak.

Stem bromelain purified as described above was used in antitumoral assays. Its efficacy was first studied in mice injected with P-388 leukemia cells, followed by daily intraperitoneal treatment with bromelain. Mice injected with P-388 leukemia cells developed ascites in the peritoneal cavity. In these experiments, transplanted animals had a reduced survival of 11 ± 2.4 days (100%), (Table **1**) while mice treated with bromelain had increased life expectancies of between 140% and 169% depending on the bromelain dose. The 5-FU treated positive controls significantly increased their survival in a similar manner to the 5 mg/kg bromelain dose. It has been established that the spleen mass of P-388 leukemia-infected animals increases following the alternative ocular plexus infection model, therefore we measured the effect of two bromelain doses on spleen size. The untreated transplanted control confirms this increase (Table **1**) and shows that bromelain or the 5-FU positive control reduced the spleen size. The optimal bromelain dose was 5 mg/kg regardless of the injection site (intraperitoneal vs. plexus).

Based on these results we extended the screening by including five tumorigenic non-hematopoietic cell lines by using similar transplantation protocols. Table **2** summarizes the *in vivo* results obtained in mice transplanted with LLC, ADC-755, EAT, S-37 and MB-F10 cell lines treated with the optimal bromelain dose. The survival rate expressed as the number of days and the % survival index are shown in the untreated transplanted control, 5-FU and bromelain conditions. The untreated control showed a mean survival of 11 days with ascites and 28 days with LLC tumors while the corresponding groups treated with bromelain at the optimal dose lived between 21 and 35 days, respectively. With the exception of MB-F10 melanoma, all other tumor-bearing animals significantly increased their survival index after bromelain treatment. The longest effect on survival (~318%, over untreated control) was attained in mice bearing EAT ascites and treated with12.5 mg/kg of bromelain. This antitumoral effect was superior to that of 5 FU, whose survival attained ~263% relative to the untreated control.

Fig. **3A** shows the number of lung metastasis following LLC transplantation along with data for bromelain and 5-FU. A significant reduction in metastatic foci per animal was stereotaxically observed, between 85% and 74% for bromelain doses of 50 and 12.5 mg/kg, respectively, compared to the untreated control; however, 5-FU afforded the largest reduction in metastasis, equivalent to 93%.

Since the tumor size could not be determined in EAT, we scored the number of tumor cells in the ascitic fluid, following 40-day survival. Fig. **3B** summarizes the results obtained by counting the ascites cells in treated and control animals. Both 25 mg/kg bromelain and 5-FU drastically reduced the number of ascites compared to the untreated transplanted control.

Table **1** The antitumoral activity of bromelain on P-388 leukemia

| *Condition* | *Dose (mg/kg)* | *Survival (days)* | *Survival index (%)* | *Spleen mass (g)* |
|---|---|---|---|---|
| Non-transplanted | | ND | ND | 141.13[d] |
| Transplanted w/o treatment | | 11 ± 2[b] | 100 | 1190.75[a] |
| 5-FU treated | 20 | 19 ± 3[a] | 174[a] | 473.75[c] |
| Bromelain treated | 1 | 16 ± 3[a] | 141[c] | 564.57[b] |
| | 5 | 19 ± 2[a] | 169[a] | 602.65[b] |
| | 12.5 | 18 ± 1[a] | 157[b] | ND |
| | 25 | 18 ± 2[a] | 160[b] | ND |

The mean survival (days), the survival index (%) and the mean mass of spleen is shown as function of the bromelain dose. Each group (n = 10) of inoculated animals received leukemia cells in the ocular plexus. Data with identical superscript letters within each column represent values which are non-significantly different by Kruskal-Wallis, Student-Newman-Keuls tests ($p < 0.05$). In addition, a group of non-transplanted animals treated with saline were sacrificed by day 21 to asses the spleen size. ND = not determined.

## Discussion

Bromelain has been used as a traditional medicine for many years before the basis for its therapeutic actions were investigated [14], [15], [17], [22]. More recently, many studies shed light on the pharmacological actions of this natural substance; however the mechanisms underlying the beneficial properties are far from being elucidated. The complex composition of commercial bromelain preparations is one of the challenges faced in pharmacological studies with bromelain, casting doubts on the identity of the active principle responsible for its therapeutic effect.

Table **2** The antitumoral activity of bromelain using several tumor models

| *Tumor type* | *Bromelain (mg/kg)* | *Survival (days)* | | | *(%) Survival Index* | |
|---|---|---|---|---|---|---|
| | | *Untreated Tumor* | *5-FU* | *Bromelain* | *5-FU* | *Bromelain* |
| LLC | 12.5 | 28 ± 4[b] | 36 ± 7[a] | 36 ± 4[a] | 128.6[a] | 128.6[a] |
| ADC-755 | 25.0 | 16 ± 5[b] | 23 ± 4[a] | 24 ± 4[a] | 143.7[b] | 150.0[a] |
| EAT | 12.5 | 11 ± 6[c] | 29 ± 4[b] | 35 ± 6[a] | 263.6[b] | 318.2[a] |
| S-37 | 12.5 | 14 ± 5[c] | 35 ± 3[a] | 31 ± 4[b] | 250[a] | 221.4[b] |
| MB-F10 | 12.5 | 20 ± 4[b] | 23 ± 3[a] | 21 ± 6[b] | 116[a] | 105.0[b] |

The antitumoral activity was assayed by using six groups of mice containing ten animals per group that received tumor cells and the drug, including one control, as described in the Methods. The mean survival (days) and the survival index (%) are shown compared to 5-FU as the positive control. Data shown with identical superscript letters when comparing survival (days) of untreated, 5-FU and bromelain columns in each tumor cell line (row) are not significantly different by Kruskal-Wallis, Student-Newman-Keuls tests ($p < 0.05$). Data shown with identical superscript letters comparing the % survival in 5-FU and bromelain columns, for each tumor line (rows) represent values non-significantly different by Mann-Whitney test ($p < 0.05$).

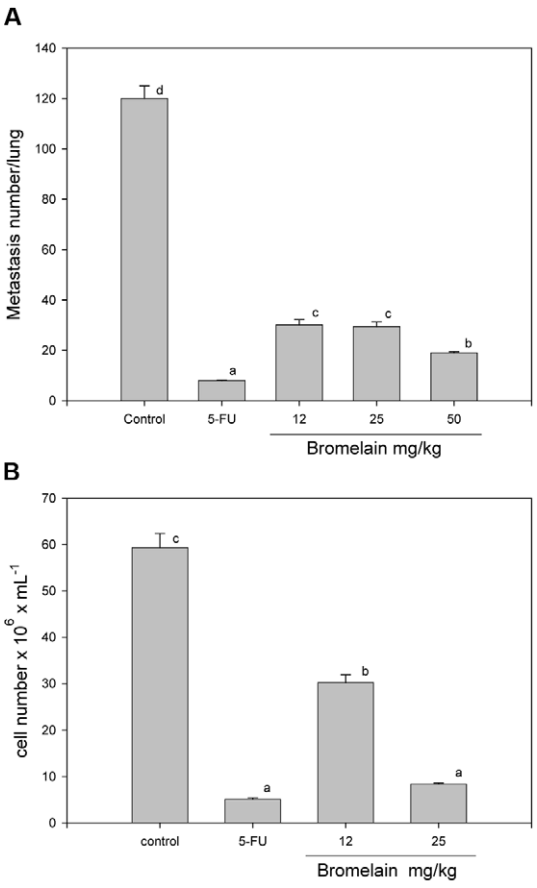


Fig. **3** The bromelain effect on Lewis lung metastasis and Ehrlich ascites. **A** The mean values for Lewis pulmonary metastasis are shown using different doses of bromelain according to the procedure described in the Methods section. A positive control containing 5-FU (20 mg/kg) and a negative saline control were included. Means bearing different letters are statistically different (Kruskal-Wallis, Student-Newman-Keuls tests, $p \leq 0.05$). **B** The effect of bromelain on the number of ascite cells is shown according to the procedure described in the Methods section. The mean ascites values are compared with the positive control 5-FU (20 mg/kg) and the saline control. Means bearing different letters are statistically different (Kruskal-Wallis, Student-Newman-Keuls tests, $p \leq 0.05$).

In this study, we evaluated the antitumoral effect of stem bromelain purified by cationic chromatography. The pooled fractions contained the bulk of the proteolytic activity, (0.54 U/mg), although a second heterogeneous protein peak displaying proteolytic activity eluted at low salt concentration. The results illustrated in Fig. **1** and **2** show that the fractions recovered after ion-exchange chromatography were essentially free of major contaminants, assuring that subsequent pharmacological assays involved genuine stem bromelain. The identity of stem bromelain was confirmed by N-terminal amino acid sequencing: (VPQSIDWRDYGAVTSVKNQNPCGAC)

By using this fraction we show that bromelain displays antitumoral activity in mice inoculated with LP-388 leukemic cells regardless of the injection site, in agreement with prior data showing a similar *in vitro* effect in three types of leukemic cells [23]. The authors suggested that the antitumoral effect was associated with the induction of leukocyte differentiation, or, by a direct proteolytic effect of bromelain on tumoral Ag/Ab complexes triggering the action of T cytotoxic cells.

Bromelain exhibits antitumoral activity on each of the murine tumor models except for MB-F10 melanoma (Table **2**). The non-significant survival increase in the latter case is less pronounced than that observed with 5-FU. Similarly, previous data using MB-F10 cells pretreated with bromelain showed a three-fold size reduction of metastasized tumors, but without increase in life survival [16], although the doses and sites of injection are different in our experimental model. The unaltered survival observed in both studies minimizes the role played by dose differences and route of administration.

The antimetastatic effect of bromelain was also demonstrated in the LLC model by using three bromelain doses between 12.5 – 50 mg/kg. This is the only case in which we used a relatively high (50 mg/kg) dose without visible adverse effects. In this case, bromelain did not change the lag time for tumor growth of transplanted cells, or alter the progression of the primary tumor. We interpret this observation as if the antimetastatic effect results from a mechanism independent of the primary antitumoral effect. Similar studies using bromelain in combination with LLC cells applied subcutaneously yielded similar results [17], in spite of different doses and sites for drug application. Our results also confirm earlier data obtained *in vitro* attributing this action to the inhibitory effect of bromelain on platelet aggregation by endothelial cells and down-regulation of tpA receptor [10]. On the other hand, the selective inhibitory migration of glioma cells reported for bromelain correlates with its inhibitory action on PGE2, thus blocking tumor growth, progression, immunosuppression, and angiogenesis [23], [24], [25]. In addition, the fibrinogenolytic and the platelet anti-aggregative effect of bromelain enhanced brain blood circulation and protection from thrombus formation.

There is evidence suggesting a link between the growth of solid tumor and angiogenesis [26]. The imbalance between proangiogenic and antiangiogenic factors and their endothelial cell receptors may determine the outcome of tumor growth and invasiveness. The role of TGF-$\beta$ produced by platelets and tumoral cells, known for its tumorigenic action, may upset this balance thereby promoting angiogenesis. By contrast, bromelain is known to act by reducing TGF-$\beta$ levels and platelet aggregation can counteract the effects of the proangiogenic factor [10]. Hale et al. [27] proposed that the effect of bromelain is mediated by its action on surface cell molecules of lymphocytes, monocytes and granulocytes, as more than 59 surface targets are removed or modified upon *in vitro* incubation with bromelain. CD44, one of these surface molecules, is reduced in leukemic and melanoma cells upon incubation with bromelain, both, *in vitro* and *in vivo*, thus hindering leukocyte activation and migration, consequently facilitating the action of T killer cells.

In mammary tumor 755, the protease increased the survival index by 150%, slightly above the index attained by 5-FU (143.7%) (Table **2**). This survival increase is associated with retardation in tumor growth. This is a slowly growing tumor and, like LLC, is resistant to most antineoplasics. The monocytes from mammary tumor patients treated with bromelain show a reversible increase in cytotoxic activity compared to monocytes from similarly treated healthy donors [28]. *In vitro* experiments with cultured mammary adenocarcinoma cells incubated with brome-

lain showed an $IC_{50} = 5 \times 10^{-7}$ M, while the toxicity against B16F10 melanoma and Ehrlich cells was less pronounce ($IC_{50} > 10^{-6}$ M) (not shown).

The survival of mice treated with EAT and S-37 tumor cells was siginificantly increased by bromelain treatement (Table **2**). The increase in EAT mice was more pronounced than for the positive control 5-FU while 5-FU was more effective in S-37 mice. It appears that bromelain is more efficient in these models, which are ascitic variants of spontaneous tumors. Similarly, intraperitoneal (*i.p.*) or subcutaneous (*s.c.*) administration of bromelain significantly reduced local tumor weight, following inoculation of sarcoma L-1 cells, however, lung colonization was non-significantly reduced [29]**.** The increase in efficiency might be related to the immunomodulatory property assigned to bromelain. In line with this notion, it has been established that bromelain stimulates monocyte secretion of IL-1$\beta$, TNF-$\alpha$, phagocytosis and cytotoxicity by lymphocytes and granulocytes. These results highlight the relevance of the immunomodulatory role of bromelain but fail to identify a particular mechanism underlying its protective role. The immunomodulatory property of bromelain is unique since Mynott et al. [5], reported that the protease blocks Erk1, Erk2 from leukocyte cells, while other plant proteases and animal serine proteinases stimulate the Erk1/Erk2 signaling pathway [30], [31]. The antitumoral effect seen here seems to be more efficient when tumor cells and bromelain are applied within the same area (i.e., EAT, S-37), suggesting that a direct interaction between the drug and the tumor cell enhances the antitumoral effect. Similarly, when glioma cells were preincubated with bromelain before implantation, there was an improved reduction of glioma cell adhesion, migration and invasiveness without affecting cell viability [24].

The involvement of endogenous cathepsins during tumor invasiveness is being shown in many cancer models; these cysteine proteinases belong to the same family as the plant cysteine proteinases, the subject of this study. The protective effect of plant bromelain may be explained by induction of antibodies that can also inhibit the activity of cathepsins.

The promising effect of proteinases has prompted the therapeutic application of proteinases as adjuvant factors during treatment of neoplastic disease. The aim of this therapy is to decrease the side effects resulting from chemo- and/or radiotherapy by enhancing the immunological response and in some cases decreasing the frequency of metastasis.

The results from this study were obtained with a purified bromelain preparation; thus, we propose that the antitumoral effect depends on the bromelain molecule, albeit it is uncertain if the intactness of proteolytic function is essential for the antitumoral effect. Unpublished results obtained by our group using a similar cysteine proteinase from Caricaceae suggest that enzyme inhibition does not impair some of its pharmacological effects. The *in vivo* results presented support the use of bromelain as antitumoral and antimetastatic substance, even though the mechanism underlying its action remains elusive.


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