Anti-inflammatory and analgesic effect of bromelain in mice and rats

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ABSTRACT

Bromelain is a crude aquaeous extract obtained from both the stem and fruit of the pineapple plant, which contains a number of proteolytic enzymes. The analgesic and anti-inflammatory activities of bromelain were investigated. Bromelain was evaluated for anti-inflammatory effect by carrageen-induced rat paw edema. The analgesic activity was tested by acetic acid-induced writhing response and hot plate method in albino mice. Twenty adult male Balb/c mice (20-25 g) were used to evaluate the analgesic effect. And 25 male Sprague-Dawley rats (200-250 g) were used to study the anti-inflammatory activy. This study showed that bromelain in doses of 10; 20 and 40 mg/kg showed 11.3; 45.1 and 56.3% inhibition of paw edema respectively at the end of three hours, and the percentage of protection from writhing was 11.1; 23.4 and 40.8% respectively. In the hot plate model, the bromelain at 20 and 40 mg/kg increased the pain threshold significantly after 30 min, 1, 2 and 4 h of administration. Bromelain showed dose-dependent action in all the experimental models. These study suggest that bromelain has anti-inflammatory and analgesic properties.

Keywords : Bromelain, analgesic, anti-inflammatory, mice, rats

Efek analgesik dan anti-inflamasi dari bromelain pada mencit dan tikus

ABSTRAK

Bromelain merupakan ekstrak cairan yang diperoleh dari batang dan buah tumbuhan nanas yang mengandung beberapa enzim proteolitik. Telah diteliti aktivitas analgesik dan anti-inflamasi dari bromelain. Efek anti-inflamasi bromelain dievaluasi pada udem telapak kaki tikus yang disebabkan oleh karagen. Aktivitas analgesik diuji pada geliatan yang disebabkan oleh asam asetat, dan metode lempeng panas pada mencit. Duapuluh ekor mencit jantan balb/c (20-25 g) digunakan untuk menilai efek analgesik. Dan 20 ekor tikus jantan Sprague-Dawley (200-250 g) digunakan untuk menguji aktifitas anti-inflamasi. Hasil penelitian menunjukkan bromelain dosis 10, 20 and 40 mg/kg menunjukkan hambatan pada udem telapak kaki tikus sebesar 11,3; 45,1 and 56,3% berturut-turut pada 3 jam terakhir, dan persentase proteksi pada geliatan tikus adalah 11,1; 23,4 dan 40,8% berturut-turut. Pada lempengan panas, bromelain 20 and 40 mg/kg meningkatkan ambang nyeri secara bermakna setelah 30 menit, 1, 2 dan 4 jam dari pemberian. Pada semua model penelitian menunjukkan bahwa efek dari bromelain tergantung pada dosis. Study ini membuktikan bahwa bromelain mempunyai efek anti-inflamasi dan analgesik.

Kata kunci : Bromelain, analgesik, anti-inflamasi, mencit, tikus

INTRODUCTION

In recent years, a number of clinical studies have appeared to substantiate one of the traditional therapeutic uses of extracts of bromelain namely in thet treatment of inflammatory disorders of the musculoskletel system.[1] Bromelain is a general name for a family of sulfhydryl proteolytic enzymes obtained from both the stem and fruit of the Ananas comosus, the pineapple plant.[2] In vitro studies have demonstrated that bromelain inhibits platelet aggregation stimulated by ADP or epinephrine, as well as by prostaglandin precursors, in a dose-dependent manner.[3] Research has indicated that bromelain prevents aggregation of human blood platelets in vivo and in vitro, prevents or minimizes the severity of angina pectoris and transcient 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. If administered for prolonged time periods, bromelain also exerts an anti-hypertensive effect in experimental animals.[1,3,4]

Bromelain may have digestant activity and has putative anti-inflammatory, immunomodulatory, anti-diarrheal, anti-carcinogenic and wound healing actions and arthritis.[5-8] Although this bromelain has many useful claims, the mechanism of its medicinal effects are not understood. The objectives of this study were to evaluate the analgesic and anti-inflammatory activities of bromelain in mice and rats.

MATERIAL AND METHODS

Chemicals

Acetic acid, bromelain, carrageenin and other standard laboratory chemicals were obtained from Sigma Chemi-cals, Dorset, England.

Animals

Adult male Balb/c mice (20-25 g) were used for all the analgesic experiments. Adult male Sprague-Dawley rats (200-250 g) were used to study the anti-inflammatory activity. The animals (five per cage) were maintained under standard laboratory conditions (light period of 12 h/day and temperature 27° C ± 2° C), with access to food and water ad libitum. The experimental procedures were carried out in strict compliance with the Institutional Animal Ethics Committee regulations. All experiments were performed in the morning according to the guidelines for the care of laboratory animals.[9]

Acetic acid-induced writhing in mice

The analgesic activity of bromelain was assessed using writhing test (abdominal constriction test).[10,11] Acetic acid solution (10 ml/kg, 0.6%) was injected intraperitoneally, and the contraction of abdominal muscles together with stretching of the hind limbs was cumulatively counted over a period of 0.5 h beginning 5 min after acetic acid injection. The bromelain was administered (0, 10, 20 and 40 mg/kg, per orally) 0.5 h before the acetic acid injection.

Analgesic activity was expressed as the percentage inhibition of abdominal constrictions between control animals and mice pretreated with the bromelain using the ratio : (control mean – treated mean) x 100 / control mean.

Hot plate test in mice

The hot-plate test was performed to measure response latencies according to the method previously described.[11,12] The hot-plate (Model 7280, Ugo Basile, Italy) was

maintained at 55.0 ± 0.2° C and the animals were placed into the Perspex cylinder on the heated surface and the time (sec) to discomfort reaction (licking paws or jumping) was recorded as response latency, prior to and 30, 60, 120 and 150 min after administration of the bromelain (0, 10, 20 and 40 mg/kg, perorally). A latency period of 20 sec was defined as complete analgesia and the measurement was terminated if it exceeded the latency period in order to avoid injury.

Carrageenin induced paw inflammation in rats

The carrageenan induced paw inflammation model has been described previously.[13] Sprague Dawley rats were divided into the control groups were given saline perorally, bromelain groups received 10, 20 and 40 mg/kg bromelain (per oral) respectively. This was followed by the administration of 1% caragenin through the intraplantar route 30 min after administration bromelain or saline. The paw volume was measured by using plethysmometrically (Ugo Basile, Italy) at 0 and 3 hours after the carrageenan injection.[10,11,13] The difference between the two readings was taken as the volume of edema and the percentage anti-inflammatory activity was calculated as the percentage inhibition of rat paw edema between control animals and mice pretreated with the bromelain using the ratio: (control mean – treated mean) x 100 / control mean.

Statistical analysis

Numerical results are expressed as mean ± SD, unless otherwise stated. One-way analysis of variance (ANOVA) was used for statistical comparison; $P<0.05$ being the criterion for statistical significance. The significant treatment means were further subjected to Duncan multiple comparison post test.

RESULTS

Effect of bromelain on the acetic acid-induced writhing

As shown in Table 1, bromelain (10, 20 and 40 mg/kg per oral) showed a significant dose dependent reduction in the number of writhing with approximately 11.3%, 23.4% and 40.8% of inhibition respectively. Maximum inhibition (40.8%) was observed at the dose of 40 mg/kg. Bromelain at 10 mg/kg had mild analgesic effect was not significantly different from the control. Whereas, bromelain at 20 (a-b significantly) and 40 mg/kg (a-c significantly different incomparing to control), the analgesic activity was significantly different from the control ($P<0.05$).

Table 1. Effect of bromelain on acetic acid-induced writhing in mice

Group	No. of writhing movements (mean ± SEM)	Percentage inhibition
Control	45.3 ± 6.1^{a}	-
Bromelain 10 mg/kg BW	40.3 ± 5.3^{ab}	11.1
Bromelain 20 mg/kg BW	34.7 ± 3.5^{b}	23.4
Bromelain 40 mg/kg BW	26.8 ± 3.1^{c}	40.8

One-way ANOVA, n = 5 each group

$^{a-c}$ significantly different at $P<0.05$, compared to control

Table 2. Effect of bromelain on hot plate reaction time in mice

Group	Hot plate reaction time (min)				
	Pre	30	60	120	150
Control	7.2 ± 0.8[a]	6.8 ± 0.5[a]	7.7 ± 0.4[a]	7.2 ± 0.8[a]	8.1 ± 0.6[a]
Bromelain 10 mg/kg BW	7.4 ± 0.6[a]	6.5 ± 0.8[a]	8.2 ± 0.6[a]	8.6 ± 0.7[a]	8.4 ± 0.7[a]
Bromelain 20 mg/kg BW	6.8 ± 0.9[a]	7.4 ± 0.7[ab]	9.8 ± 0.5[b]	10.3 ± 0.5[b]	8.9 ± 0.6[ab]
Bromelain 40 mg/kg BW	7.5 ± 0.4[a]	8.9 ± 0.3[b]	10.5 ± 0.6[c*]	11.8 ± 0.4[c*]	10.6 ± 0.8[b]

One-way ANOVA, n = 5 each group
[a-c*] significantly different at P < 0.05, compared to control

Effect of bromelain on hot-plate test

Table 2 shows the time course of the analgesic produced by of bromelain (10, 20 and 40 mg/kg BW). The predrug reaction time were not significantly different between the four groups. Administration of bromelain peroral resulted in significant and dose-dependent prolongation of the response latency in the hot-plate test. The effect reached a peak at approximately 60 (10,5 ± 0.6) or 120 min (11.8 ± 0.4) min after administration and then gradually decreased.

Effect of bromelain on carrageenin induced inflammation

The results of anti-inflammatory activity are shown in Table 3. In the acute inflammation model, the bromelain in doses of 10, 20 and 40 mg/kg per oral. produced dose-dependent inhibition of rat paw edema. At 10 mg/kg BW concentration, bromelain had mild anti-inflammatory property was no significantly different from the control. Whereas, bromelain at 20 and 40 mg/kg BW, the anti-inflammatory activity was significantly different from the control (P<0.05).

DISCUSSION

The hot-plate and acetic acid-induced writhing tests are one of the most common tests for evaluating the analgesic efficacy of drugs in rodents. Administration of bromelain showed analgesic activity in the hot-plate and acetic acid-induced writhing tests (Table 1 and 2).

Table 3. Effect of bromelain on carrageenin induced inflammation

Group	Increase in paw volume (mean ± SEM) in ml	Percentage of inhibition of paw edema
Control	0.71 ± 0.13[a]	-
Bromelain 10 mg/kg BW	0.63 ± 0.15[a]	11.3
Bromelain 20 mg/kg BW	0.39 ± 0.08[b*]	45.1
Bromelain 40 mg/kg BW	0.31 ± 0.03[c*]	56.3

One-way ANOVA, n = 5 each group
[a-c*] significantly different at P<0.05, compared to control

The bromelain (10, 20 and 40 mg/kg BW per oral) suppressed the acetic acid-induced writhing response significantly in a dose-dependent manner and resulted significantly dose-dependent prolongation of the response latency in the hot-plate test. These results indicate that bromelain possesses centrally and peripherally mediated analgesic properties. However, the analgesic activity of bromelain was found to be more significant on the acetic acid-induced model than the hot plate model and thus it appears that bromelain inhibits predominantly the peripheral pain mechanism.

The carrageenin-induced edema in the rat hind paw most widely used for the screening of new anti-inflammatory agents.[10,11,13] Carrageenin is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility. Carrageenin-induced edema is mediated through the release prostaglandin and slow reacting substances which peak at 3 h.[10,11,13] The increase in the paw volume following carrageenan administration in the control. In the carrageenin-induced paw edema, the bromelain induced dose-dependent reduction of paw edema in rat. The bromelain in doses of 20 and 40 mg/kg per oral produced significant inhibition of paw edema as compared to the control. These results indicate that bromelain possesses inhibition of prostaglandin release mediated analgesic and anti-inflammatory properties.

Data have also indicated that bromelain has analgesic properties, for example in inflammatory pain in human, human urogenital inflammation. Its analgesic properties are thought to be a result of its direct influence effects through its anti-inflammatory actions.[14] Bromelain has been used as treatment for a number of disease conditions such as osteoarthritis of the knee and shoulder. Safety and tolerability at the lower dose appears to be good. But, there are also a number of other potential safety issus need to be addressed. These include investigating the possibility of renal effects, potentiating effect action of anticoagulations (e.g.warfarin) and enhanced absorption of antibiotics.[1,15]

In conclusion, this study demonstrated that the analgesic and anti-inflammatory effect of bromelain may be attributed to inhibition of prostaglandin release.

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