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Original Research Article

The effects of bromelain on angiogenesis, nitric oxide, and matrix metalloproteinase-3 and -9 in rats exposed to electrical burn injury

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ABSTRACT

This study was aimed to investigate the effects of bromelain on angiogenesis, nitric oxide, and matrix metalloproteinase-3 and -9 in rats exposed to 1200 mV electrical burn injury. Thirty-five, male Wistar albino rats was divided into five groups ($n = 7$ each), including control (untreated) group; electrical burn injury (EI) group; electrical burn injury + 21.25 mg/kg BW bromelain group (EIB_1); electrical burn injury + 42.50 mg/kg BW bromelain group (EIB_2); and electrical burn injury + 85.00 mg/kg BW bromelain group (EIB_3). Rat models of electrical burns done by providing electricity in rats that had been anesthetized, a voltage of 1200 mV and strong currents 15 mA for 10 s. The VEFG, NO, and MMP-9 levels were significantly greater in the EI group compared to the untreated group. Out of the 21.25 mg/kg BW, 42.50 mg/kg BW, and 85 mg/kg BW doses of Bromelain extract, only the lowest doses prevented EI-induced increase in NO and MMP-9 level ($P < 0.05$). Bromelain at the lowest dose (21.25 mg/kg BW) act as anti-inflammatory and modulate the matrix metalloproteinase-9 in rats exposed to 1200 mV electrical burn injury.

1. Introduction

Electrical burn trauma happen due to a contact with live electric wires or lightning. The degree of electrical injury is dependent on the current, voltage, duration of contact, tissue resistance, and the path of the current flow through the body [1–3]. Initial medical care after electrical injury focuses on the most common sequellae of electrocution: infection of the burn wounds, myonecrosis leading to acute renal failure, cardiac arrest or arrhythmia, pneumonia, nausea, and vomiting [4,5].

Bromelain (pineapple enzyme, *Ananas comasus*) is an aqueous extract obtained from the stem and fruit of the pineapple plant that contains high levels of proteolytic enzymes and which composition varies depending on the source and purification method [6,7]. It is an aqueous extract of pineapple contains a complex mixture of proteases and non-protease components. Bromelain is used in food processing for meat tenderization [8]. Bromelain directly influences pain mediators such as bradykinin [9], although its analgesic properties are closely linked to its anti-inflammatory properties [10,11]. It has been shown that this fibrinolytic agent promotes reabsorption of edema in the blood circulation [12]; it reduces swelling, bruising, pain, and healing time after trauma and surgical procedures [11]. Evidence has shown that bromelain digests fibrin, thus allowing the elimination of edema [13]. Indirectly, bromelain increases the time required for the conversion of prothrombin into thrombin, and thus activating plasminogen into plasmin; by these means, it prevents the formation of fibrin [13]. All these cause a reduction in vascular permeability. In addition, bromelain inhibits the synthesis of pro-inflammatory prostaglandins, particularly PGE2 [14].

Wound healing involving a number of processes is an orderly, but complex phenomenon. An essential feature of wound healing is re-epithelialization which depends on two basic functions of keratinocytes, proliferation and migration [15]. Re-epithelialization process is influenced by a combination of growth factors and cytokines, including VEGF and TGF-β [16]. VEGF is one of the most potent angiogenesis stimulating growth factors and functions as an inducer of vascular permeability and an endothelial cell mitogen [17–19]. Several reports showed that VEGF increase re-epitheliali-zation in wounded sites [20–22].

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As far as we know, there is no study to applied bromelain in wound healing processes of electrical burn injury. Therefore, this study aimed to investigate the effects of bromelain on angiogenesis, nitric oxide, and matrix metalloproteinase-3 and -9 in rats exposed to 1200 mV electrical injury.

2. Material and methods

2.1. Animal

Thirty-five, male Wistar albino rats, 16 weeks aged, weighing 160–200 g were used for the present investigation. They were housed in a clean wire cage and maintained under standard laboratory conditions (temperature 25 ± 2 °C with dark/light cycle 12/12 h). They were fed a standard pellet diet and received water *ad libitum*. The animals were acclimatized to laboratory conditions for one week prior to the experiment. The rats were divided into five groups ($n = 7$ each), including control (untreated) group; electrical burn injury (EI) group; electrical burn injury + 21.25 mg/kg BW bromelain group (EIB_1); electrical burn injury + 42.50 mg/kg BW bromelain group (EIB_2); and electrical burn injury + 85.00 mg/kg BW bromelain group (EIB_3) (Fig. 1).

2.2. Bromealin treatment

Bromelain was obtained from Sigma, USA (catalog number: Sigma B-4882). Bromelain was dissolved in saline was given based on lethal dose. Mode of administration was intraperitoneally at single dose.

2.3. Electrical burns

Rat models of electrical burns done by providing electricity in rats that had been anesthetized. Rats were injected intraperitoneally with ketalar 15 mg (100 mg/kg) that had been diluted with saline. Anesthetized rat was put to sleep on her back on the table applicator. Left front leg that has been coated with a wet cloth clamped AC power flow as inflow. The right hind leg that has been coated with a wet cloth clamped AC power as an outflow. Furthermore, given a voltage of 1200 mV, strong currents 15 mA for 10 s [23].

2.4. Analysis of VEGF levels

The amount of VEGF protein in the serum was determined using the Mouse VEGF (VEGF-A) Quantikine ELISA kit Cat MMV000 (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Optical density was read by using a microplate reader at 450 nm. The amount of VEGF (pg/mL) was calculated from a standard curve. The sensitivity of this kit was 5 pg/mL.

2.5. Analysis of NO levels

The amount of NO in the serum was determined using the Total Nitric Oxide and Nitrate/Nitrite Parameter Assay kit Catalog KGE001 (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Optical density was read by using a microplate reader at 540 nm. The amount of NO (pg/mL) was calculated from a standard curve.

2.6. Analysis of MMP-3 and MMP-9 levels

The amount of MMP-3 and MMP-9 in the serum was determined using the Mouse Total MMP-3 (Catalog: MMP300) and MMP-9 (Catalog MMPT90) Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Optical density was read by using a microplate reader at 450 nm. The amount of MMP-3 and MMP-9 (pg/mL) was calculated from a standard curve. The sensitivity of this kit was 5 pg/mL.

2.7. Ethics

This research has been approved by the research ethics committee, Faculty of Medicine, University of Brawijaya, Malang, Indonesia.

2.8. Statistical analysis

Data are presented as mean ± SD and differences between groups were analyzed using 1-way ANOVA with SPSS 15.0 statistical package. The post Hoc test was used if the ANOVA was significant. $P < 0.05$ was considered statistically significant.

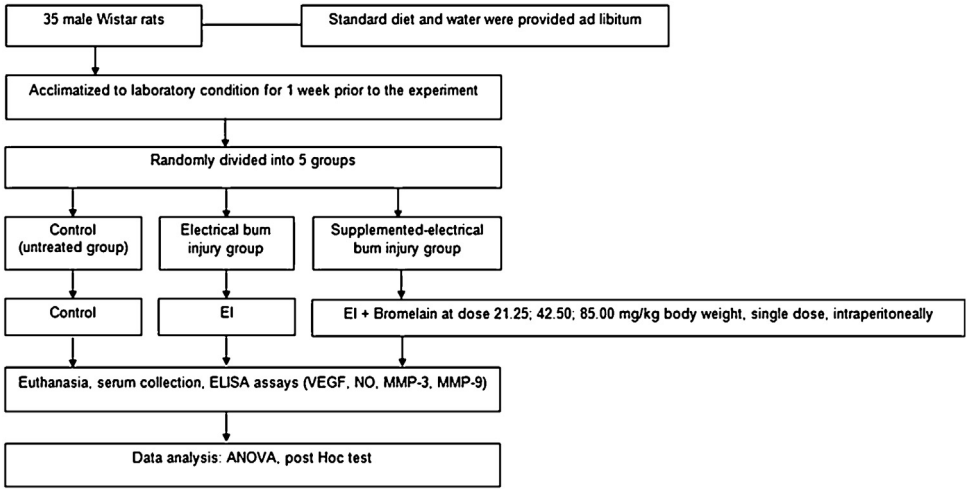

Fig. 1. The schematic design of this study. Thirty-five male Wistar rats were randomly divided into seven groups. One group is a non-exposure group (control). One group is an electrical injury group (EI). Three groups were exposed to electrical injury and intraperitoneal administration of bromelain at dose of 21.25 mg/kg BW (EIB_1); 42.50 mg/kg BW (EIB_2); and 85.00 mg/kg BW (EIB_3).