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SHORT COMMUNICATION

In vivo wound healing and antiulcer properties of white sweet potato (*Ipomoea batatas*)

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KEYWORDS

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Abstract The potential of tuber flour of *Ipomoea batatas* (L.) Lam. cv. Brazlândia Branca (white sweet potato) as wound healing and antiulcerogenic agent was investigated *in vivo* in animal model. Excision on the back of Wistar rats was performed to induce wounds that were topically treated with Beeler's base containing tuber flour of white sweet potato at 2.5%. Number of cells undergoing metaphase and the degree of tissue re-epithelialization were investigated 4, 7 and 10 days post-treatment. The protective effect of aqueous suspension of tuber flour (75 and 100 mg/kg animal weight) on gastric mucosa of Wistar rats was also studied by using the ethanol-induced ulceration model. Ointment based on white sweet potato at 2.5% effectively triggered the healing of cutaneous wound as attested by the increased number of cells undergoing metaphase and tissue re-epithelialization regardless the time of wound treatment. Tuber flour potentially prevented ethanol-induced gastric ulceration by suppressing edema formation and partly protecting gastric mucosa wrinkles. Crude extracts also exhibited potential as free radical scavengers. The results from animal model experiments indicate the potential of tuber flour of white sweet potato to heal wounds.

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Introduction

Wound healing is a process in which an injured tissue tries to repair the damage. It comprises four phases that occur to promote an efficient healing: blood coagulation, inflammation, cell proliferation, lesion contraction and remodeling [1]. The success of this effort depends on complex physiological machinery that involves interactions between a variety of cells, biochemical mediators, and extra-cellular matrix molecules [2]. In skin, cells neighboring a wound (epithelium, fibroblast, and

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keratinocyte) migrate to start fibroblast production, followed by extracellular matrix deposition, angiogenesis, healing, and finally epithelium reconstitution [2]. The search for wound healing agents is one of the oldest challenges in medicine and remains a challenge because the tissue repair mechanism is still not fully understood.

Ulcer is an inflammatory process characterized by the loss of epithelial cells from the skin or mucosa. The cure for ulcer involves interactions between various tissue and cell types [3,4]. An ideal antiulcer agent should decrease the inflammation and enhance healing processes [5].

Humans have traditionally used a number of plant preparations to accelerate wound healing even when scientific evidence for their action and/or efficacy is not documented [6–9]. *Ipomoea batatas* (L.) Lam (sweet potato) is one of the most important food crops in Brazil [10]. Its tubers are largely used for human and animal consumption and also as raw materials for textile, paper, cosmetics and automotive fuels industries [11]. The role of *I. batatas* tubers on human nutrition, as antioxidant, antidiabetic, cancer-preventive agent [12–14] is well described. The effect of commercially available fibers of Japanese sweet potato and kumara tubers (New Zealand sweet potato) on rat wounds and human health has also been addressed [15,16]. However, no report on the wound healing and antiulcer properties of white sweet potato is documented up to date. Herein, this work brings evidence of the ability of crude flour produced from tubers of a Brazilian white sweet potato variety to heal wounds and protect gastric mucosa from developing ulcer in animal models.

Material and methods

Plant material and preparation of ointment and plant aqueous suspension

I. batatas (L.) Lam. cv. Brazlândia Branca (white sweet potato) was provided by Embrapa Hortaliças (Brasília, Brazil). The species was identified by Dr. João R. Stehman and the voucher specimen deposited in the herbarium of the Department of Botany at the Federal University of Minas Gerais under the number BHCB 157 083. Tubers were grounded to a fine powder that was further dried in the absence of light at room temperature to furnish crude flour. The ointment consisted of Beeler's base and tuber flour at 2.5%, prepared essentially as described by Lopes et al. [17]. Aqueous suspensions of tuber flour were prepared accordingly to provide doses of 75 mg/kg or 100 mg/kg of animal weight that were used in the experiments of ulceration induction.

In vivo experiments

The Animal Ethics Committee of the State University of West Parana (CEEAAP/UNIOESTE) approved all the *in vivo* experimental procedures under the protocol number 20/09.

Induction of wounds

Eighteen adult male albino Wistar rats weighing from 200 to 400 g were individually maintained in cages with *ad lib* water and chow at 22 °C and 12 h photoperiod. After depilation and asepsis, two excisions (1 cm^2 area each; 2–3 cm apart from each other) were done in opposite sides of the back of each ethyl ether-anesthetized animal. One wounded area was treated with the ointment formulated with tuber flour (2.5%) and the other with ointment base solely, according to the suggested elsewhere [17]. Animals were divided into three groups that were maintained for 4, 7 or 10 days under treatment, and then killed. 2 h prior to the killing, each animal was injected with vincristine sulfate (1 mg/kg body weight) to arrest metaphase of mitosis. Wounded skins were removed from ethyl ether-anesthetized animals, fixed in Buoin's solution for 12 h and embedded in paraffin. Sections of 10 μm were stained with haematoxylin and eosin and evaluated for epithelial cell growth in the basal and suprabasal layers of re-epithelialization region. Results were expressed in terms of number of metaphase/10 mm. The epithelium length in surface was measured in both re-epithelialization borders of 10 fragments of injured tissues collected from each animal by using an optical microscope (magnification of 10× or 100×). Results were expressed in mm as the length average.

Induction of ulceration

Sixteen adult male albino Wistar rats weighing from 120 to 220 g were divided into four groups. Deionized water and aqueous suspension of tuber flour (75 mg/kg or 100 mg/kg) were administrated through gavage in animals from groups I, II and III, respectively. These doses were chosen to meet the concentration of cimetidine suggested elsewhere [18]. Animals from group IV received cimetidine (100 mg/kg) intraperitoneally. One hour post-treatment, animals received 1 mL of ethanol 96% (v/v) through gavage to induce ulceration [19,20]. After another hour, animals were killed in a CO_2 chamber and the stomach removed for further analysis. Ulceration index was calculated as described elsewhere [21] and the severity of ulceration was classified according to Suffredini et al. [22].

Scavenging of free radicals

The ability of crude extracts of *I. batatas* tubers to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was determined according to da Silva et al. [23]. Ethanolic and aqueous crude extracts were prepared by grinding 30 mg of tuber flour in liquid nitrogen and extracting cellular content with 1 mL of ethanol or deionized water. Homogenates were centrifuged at 10,000g and a volume of each supernatant was incubated with equal volume of 100 μM DPPH. The systems were maintained under stirring and absence of light for 30 min and the absorbance recorded at 517 nm. Experiments were performed in triplicate.

Statistical analyses

Data obtained from wound healing and ulcer prevention experiments were analyzed by Student's *t*-test ($P < 0.05$) and non-parametric test one-way-ANOVA ($P < 0.05$), respectively.

Results

Wounds treated with *I. batatas*-based ointment exhibited a dry, dark-brown crust thicker than those observed in control ones, regardless the time of exposure to tuber flour. No exudate or contamination was observed along with the days of wound exposure to the different treatments. The number of cells undergoing metaphase was significantly higher (4.5 fold, $P < 0.001$) in tuber flour-treated wounds 4 days after exposure when compared to non-treated wounds, see Fig. 1. Higher number of cells (*ca*. 2.5 fold) in metaphase, in comparison with non-treated wounds, was still observed after exposure to *I. batatas* ointment for up to 10 days. Re-epithelialization process was 43% and 75% more intense in wounds treated with *I. batatas* ointment for 4 and 10 days, respectively, when compared to wounds treated solely with Beeler's base, see Fig. 1. No significant difference between treatments was observed after 7 days of exposure probably due to the great variability of responses among the tested animals.

The potential of tuber flour of white sweet potato to induce wound healing prompted us to check the ability of this plant material to prevent gastric ulceration. As expected, gastric mucosas treated with deionized water prior to ulceration induction showed widespread edema and absence of wrinkles. The reference drug cimetidine prevented edema formation by 50% while 75% of gastric mucosa surface presented wrinkles. Treatment with aqueous suspensions of tuber flour at 75 and 100 mg/kg prevented edema formation by 75% and 100%, respectively. While tuber flour at 75 mg/kg failed to protect mucosa wrinkles (they were completely absent), 50% of them remained intact when a higher dose of tuber flour (100 mg/kg) was used.

Ulceration index was calculated by considering the severity of gastric mucosal lesions as follows: (**I**) ulcer area < 1 mm^2; (**II**) 1 mm^2 $<$ ulcer area $>$ 3 mm^2; and (**III**) ulcer area $>$ 3 mm^2 [21]. Then, the ulceration index was determined according to the formula: $1 \times$ (number of lesions of grade (**I**) $+ 2 \times$ (number of lesions of grade **II**) $+ 3 \times$ (number of lesions of grade **III**). Ulceration index in gastric mucosas was 2.2- and 3.6-fold lower in animals treated with 75 and 100 mg/kg tuber flour aqueous suspension, respectively, than in those treated with deionized water prior ulceration induction, see Fig. 2. Tuber flour at 75 mg/kg was as effective as the reference drug cimetidine at 100 mg/kg, see Fig. 2.

Considering that the production of free radicals is increased after tissue wounding, crude extracts of *I. batatas* tuber were investigated for the ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Ethanolic extracts were able to scavenge 20 ± 0.9% of DPPH radicals present in the reaction medium while aqueous extracts at the same concentration captured only 13 ± 0.4% of free radicals ($P < 0.05$; Student's *t*-test).

Discussion

Sweet potato is one of the most consumed vegetables in Brazil. Its tuberous root is also used as a raw material in textile, paper, cosmetic and adhesive industries [11]. Besides its broad use, little is known about the medicinal properties of white sweet potato.

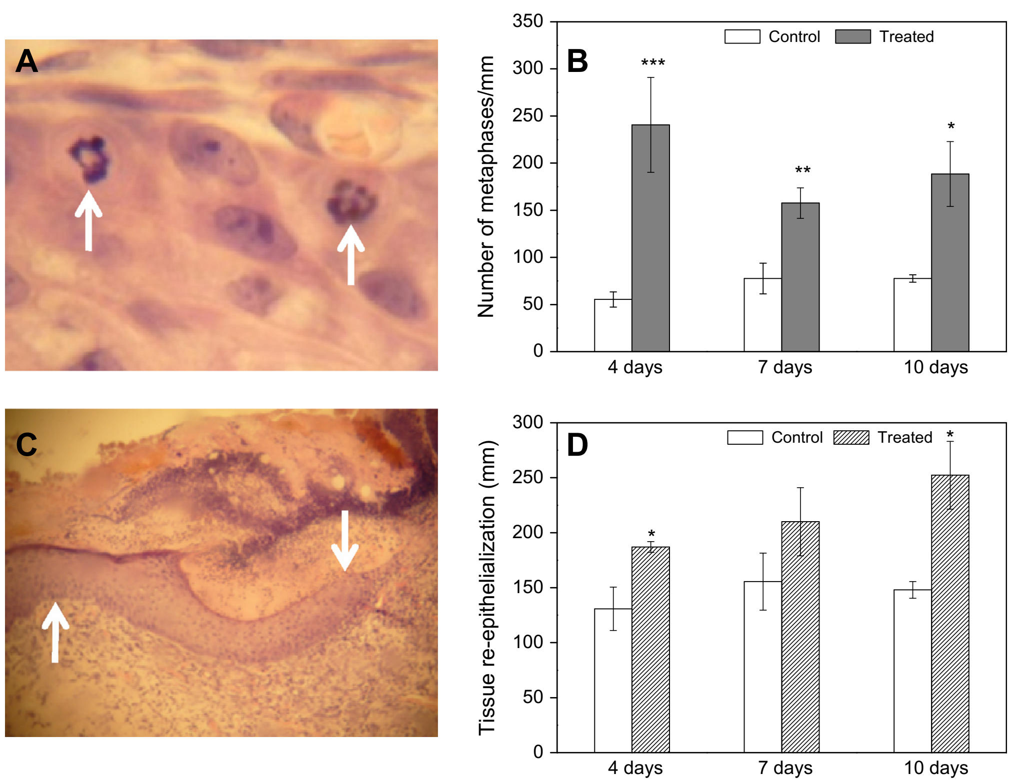

Fig. 1 Effect of tuber flour of *Ipomoea batatas* on cutaneous wounds. (**A**) Epidermis cells treated with tuber flour-based ointment (2.5%) for 10 days (magnification: 100×). Arrows indicate cells undergoing metaphase. (**B**) Quantitative analysis of cells in metaphase in wounded epidermis counted in 40 microscope fields (250 μm each). (**C**) Epidermis cells under re-epithelialization (arrows) 10 days post-treatment (magnification: 10×). (**D**) Quantification of re-epithelialization. Data are means ± SD ($n = 5$), $^*P < 0.05$ compared to control (Student's *t*-test).

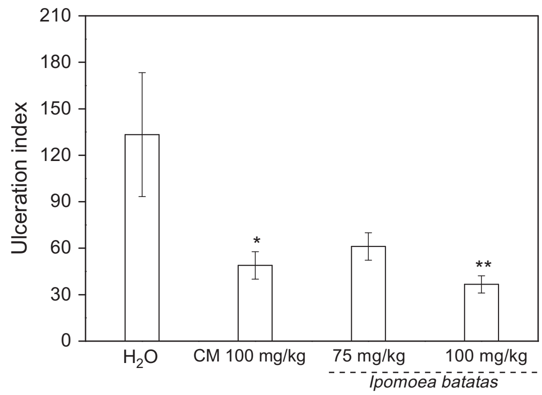

Fig. 2 Prevention of ethanol-induced ulceration on rat gastric mucosa by tuber flour of *Ipomoea batatas*. CM, reference drug cimetidine. Data are means ± SD ($n = 4$). $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to control.

Ointment based on tuber crude flour of white sweet potato at 2.5% effectively triggered cutaneous wound healing in Wistar rats as attested by the induction of metaphase in cells and tissue re-epithelialization already in the first four hours of treatment, see Fig. 1. The wound healing properties of white sweet potato may be associated to the occurrence of carotenoids and (poly)phenols known to occur in tubers of this particular plant variety [24,25]. Thus, such metabolites could account for the control of free radicals that are overproduced during the inflammation of wounded tissue [1]. Then, the decrease of free radicals in the wounded tissue would somehow favor its healing. Indeed, *in vitro* experiments performed with DPPH free radicals revealed that crude extracts of white sweet potato tubers (aqueous or ethanolic) were able to scavenge up to 20% of radicals.

Tuber flour of white sweet potato potentially prevented ethanol-induced gastric ulceration, see Fig. 2. Gastric mucosa pre-treated with an aqueous suspension of tuber flour at 75 mg/kg was twice as potent as the reference drug cimetidine (100 mg/kg) in preventing edema formation due to ethanol-induced ulceration. Edemas were completely absent in gastric mucosas pre-treated with tuber flour at the same concentration of that used for cimetidine. Although tuber flour at 75 mg/kg did not protect gastric mucosa wrinkles, a higher concentration (100 mg/kg) partly maintained wrinkles intact. Tuber flour at 75 mg/kg was shown to be as effective as the reference drug cimetidine at 100 mg/kg in reducing ulceration index in ethanol-induced rats, see Fig. 2. Although tuber flour and cimetidine were administered in rats throughout different routes, tuber flour of white sweet potato at 100 mg/kg was more effective than cimetidine at a similar dose, see Fig. 2. Ethanol causes increased vascular permeability that leads to hemorrhagic erosions or edema [26]. Our results suggest that aqueous suspension of tuber flour prevents edema formation.

Conclusions

Ointment based on white sweet potato promoted the healing of cutaneous wounds in experiments performed with animal models. Suspensions of tuber flour of white sweet potato also prevented ethanol-induced gastric ulceration while crude extracts were able to scavenge free radicals in *in vitro* experiments. To the best of our knowledge, this is the first report on the potential of tuber crude flour of *I. batatas* (L.) Lam. cv. Brazlândia Branca as wound healing and antiulcerogenic agent in animal models.

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