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# Mate tea-mediated reduction in catecholamine synthesis improves cutaneous wound healing of chronically stressed mice

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Ketamine (PubChem CID: 3821)
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Formalin (PubChem CID: 712)
Hematoxylin (PubChem CID: 442514)
Eosin (PubChem CID: 11048)
Potassium phosphate (PubChem CID: 62657)
2,4-Dinitrophenylhydrazine (PubChem CID 3772977)
Xylenol orange (PubChem CID: 16220156)
L-Hydroxyproline (PubChem CID 5810)
Sodium dodecylsulfate (PubChem CID: 3423265)
Polyacrylamide (PubChem CID: 6579)
Penicillin (PubChem CID: 23668834)
Karamycin (PubChem CID: 441374)
Streptomycin (PubChem CID: 19649)
Amphotericin B (PubChem CID: 5280965)
Epinephrine (PubChem CID: 5816)
3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (PubChem CID: 64965)
Sirius Red (PubChem CID 9571131)

ABSTRACT

Chronic stress stimulates oxidant production and oxidative damage which compromise cutaneous wound healing. Mate tea rich is in antioxidant compounds and may be a good alternative for treatment of oxidative diseases. Therefore, the aim of this study was to investigate the effects of supplementation with mate tea on cutaneous healing in stressed mice. Mice were submitted to rotational stress (ST) and treated with mate tea (MT) daily until euthanasia. An excisional lesion was created on each mouse and 4 or 10 days later, the lesions were analyzed. In addition, murine skin fibroblasts were exposed to elevated epinephrine (E) levels plus mate tea, and fibroblast activity was evaluated. In *in vivo* assays, the supplementation with mate tea inhibited the stress-induced increase in: catecholamine synthesis (ST: 23.3 ± 2.5; ST + MT: 18.0 ± 0.9 ng/μl, $p < 0.05$), carbonyl protein content (ST: 962.0 ± 35.6; ST + MT: 35.7 ± 8.9 nmol/μg protein, $p < 0.05$), lipid peroxide levels (ST: 18.5 ± 2.3; ST + MT: 11.8 ± 1.2 nmol/mg protein, $p < 0.05$), wound contraction (ST: 44 ± 4; ST + MT: 17 ± 2%, $p < 0.05$), re-epithelialization (ST: 908 ± 35; ST + MT: 2081 ± 138 μm, $p < 0.05$), transforming growth factor-β (ST: 5.0 ± 0.02; ST + MT: 1.3 ± 0.06 u.a., $p < 0.05$), and myofibroblast density (ST: 19 ± 2; ST + MT: 9 ± 1%, $p < 0.05$). Stress-induced reduction in the amount of macrophages (ST: 133 ± 19; ST + MT: 392 ± 33 cells, $p < 0.05$) and neutrophils (ST: 1161 ± 62; ST + MT: 1313 ± 103 cells, $p < 0.05$) and hydroxyproline levels (ST: 1.3 ± 0.2; ST + MT: 4.6 ± 0.9 ng/mg tissue, $p < 0.05$), and this was reversed by mate tea. In *in vitro* assays, the treatment with mate tea reversed the reduction in collagen deposition (E: 1.7 ± 0.3; E + MT: 3.7 ± 0.01 pixels, $p < 0.05$) and the increase in cell proliferation (E: 331 ± 2; E + MT: 203 ± 3% of control, $p < 0.05$), accumulation of lipid peroxides (E: 0.66 ± 0.009; E + MT: 0.55 ± 0.01 nmol/mg protein, $p < 0.05$), and increase of tenascin-C protein expression (E: 3.8 ± 0.004; E + MT: 3.4 ± 0.004 a.u., $p < 0.05$) induced by epinephrine. In conclusion, the supplementation with mate tea improves the cutaneous wound healing of chronically stressed mice.



## 1. Introduction

Cutaneous wound healing is a complex process which involves inflammation, dermal reconstruction, and scar remodeling. Psychological stress, which is the process in which environmental demands exceed an individual's ability to cope, resulting in affective, behavioral, and physiological changes, significantly affects cutaneous wound healing (Vileikyte, 2007). Psychological stress activates the sympathetic–adrenal medullary and hypothalamic–pituitary–adrenal axes that stimulate the release of the catecholamines from the adrenal medulla and glucocorticoids from the adrenal cortex (Dunn & Koo, 2013). High levels of stress hormones compromise inflammatory response,

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wound contraction, and dermal reconstruction impairing the cutaneous wound closure in human and rodents (Kiecolt-Glaser, Marucha, Malarkey, Mercado, & Glaser, 1995; Marucha, Kiecolt-Glaser, & Favagehi, 1998; Romana-Souza, Assis de Brito, Pereira, & Monte-Alto-Costa, 2014; Romana-Souza, Porto, & Monte-Alto-Costa, 2010; Romana-Souza et al., 2010; Rosa, Bandeira, Monte-Alto-Costa, & Romana-Souza, 2014; Sivamani et al., 2009). Recently, it was demonstrated that stress-induced high epinephrine levels increase oxidative damage on wound area contributing to the delay in wound closure (Rosa et al., 2014). In addition, dietary supplementation with olive oil rich in antioxidant compounds inhibits the effect of chronic stress on cutaneous wound healing in mice (Rosa et al., 2014). Thus, the administration of antioxidant donors could be an efficacious strategy to improve cutaneous wound healing of stressed animals.

Mate tea, produced from the roasted *Ilex paraguariensis* herb, is one of the most commonly consumed beverages in several South American countries such as Brazil, Paraguay, Uruguay, and Argentina (Bracesco, Sanchez, Contreras, Menini, & Gugliucci, 2011). Aqueous extract of mate powder is rich in phenolic (gallic acid, chlorogenic acid, coumaric acid, syringic acid, caffeic acid, ferulic acid, dicaffeoylquinic acid, and caffeoylquinic acid), methylxanthines (caffeine and theobromine), and tannins with a prominent antioxidant activity (Arcari et al., 2009; Vieira et al., 2010). Some studies demonstrate that antioxidant compounds as plant-derived polyphenols may improve cutaneous wound healing (Klass, Branford, Grobbelaar, & Rolfe, 2010; Park et al., 2008; Pastore et al., 2012; Potapovich et al., 2011; Tsuruya et al., 2014). In human keratinocyte culture, plant polyphenols as resveratrol and verbascoside may modulate inflammatory response and increase cell migration (Pastore et al., 2012; Potapovich et al., 2011). The epigallocatechin-3-gallate, the most abundant polyphenol in green tea, may decrease the contraction of human dermal fibroblast-populated collagen lattices and suppress collagen deposition and proliferation of keloid fibroblasts (Klass et al., 2010; Park et al., 2008). In addition, 3T3-L1 mouse fibroblasts treated with active polyphenolic compounds such as catechin, caffeic acid, and chlorogenic acid present an increased rate of the proliferation (Tsuruya et al., 2014). High levels of polyphenols in mate tea may have also beneficial effects on human health due to its antioxidant and anti-inflammatory activities (Korkina, De Luca, & Pastore, 2012). Chronic dietary supplementation with mate tea may protect DNA against hydrogen peroxyde-induced damage and unsaturated fatty acids in the mouse liver from oxidation (Martins et al., 2009; Miranda et al., 2008). Chronic or acute oral administration of mate tea may protect mouse lung from damage caused by cigarette smoke exposure reducing inflammatory and oxidative damages (Lanzetti et al., 2008; Lanzetti et al., 2011). In obese mice, chronic or acute treatment with mate tea restores serum levels of cholesterol, triglycerides, and glucose, reduces weight gain, insulin resistance, and adipogenesis (Arcari et al., 2009; Arcari et al., 2011; Gosmann et al., 2012). In addition, acute or chronic administration of mate tea may minimize the effects of aging increasing the life span of mice and reducing dermal fibroblast senescence (Dudonne, Coutiere, Woillez, Merillon, & Vitrac, 2011; Lanzetti et al., 2013). Nevertheless, neither study demonstrated whether the dietary supplementation with mate tea may inhibit the adverse effects of stress on cutaneous wound healing in chronically stressed mice.

The aim of this study was to investigate the effect of dietary supplementation with mate tea on cutaneous wound healing in chronically stressed mice. In addition, this study evaluated the effects of high epinephrine concentrations, as observed in stress, and mate tea on murine dermal fibroblast cultures.

## 2. Material and methods

### 2.1. Chronic stress model

In this study, male Swiss mice two months old were housed in groups of five animals per cage in a room with controlled humidity and temperature (22 °C) on a 12-h light/dark cycle and air exhaustion cycle (15 min/h). To induce rotational stress, a psychological stress model that compromises the animals' housing through spatial disorientation leading to anxiety, animals were kept in plastic cages (30 × 20 × 13 cm) (Insight; Ribeirão Preto, Brazil) with a stainless steel grid cover. Animal's cages were daily spun at 115 rpm for 15 min every hour, 24 h/day, until euthanasia (Riley, 1981; Romana-Souza et al., 2010). Another group of animals was also kept in the same plastic cages, but not submitted to stress. All procedures were carried out in accordance with Brazilian legislation for experimentation with animals (no. 11.794, October 8th, 2008) and were approved by the Ethical Committee for Animal Use of the State University of Rio de Janeiro (no. CEUA/022/2012).

### 2.2. Supplementation with mate tea and total polyphenol concentration

The roasted mate tea beverage was prepared by dissolving instant mate tea powder (Leao Jr, Curitiba, Brazil) in filtrated water (0.3 mg/ml) using a homogenizer. The solution was prepared fresh each day (Arcari et al., 2009; Miranda et al., 2008). Mice of nonstressed or stressed groups were treated with mate tea or water by gavage every day between 9 and 10 a.m. The supplementation with mate tea (1 mg/kg body weight) or water (using the same volume as that of the mate tea) began 2 days after beginning of the stress model and was daily maintained until euthanasia (Fig. 1) (Arcari et al., 2009). The duration of mate tea supplementation was a total of 14 days. Thus, each mate tea-treated mice received 0.03 mg per day of mate tea (100 μl of a solution of 0.3 mg/ml). The total concentration of phenolic compounds in the mate tea solution used in this study was measured using the Folin–Ciocalteu method as described (Ainsworth & Gillespie, 2007; Lakenbrink, Lapczynski, Maiwald, & Engelhardt, 2000). The mate tea solution (0.3 mg/ml) presented 47 μg/ml of the total polyphenols and each animal received 4.7 μg of polyphenols per day.

### 2.3. Experimental groups

Mice were divided into four groups as follows (20 animals per group): *nonstressed group*: animals not submitted to stress that received only water; *nonstressed + mate tea group*: animals not submitted to stress that received mate tea; *stressed group*: animals submitted to stress that received only water; *stressed + mate tea group*: animals submitted to stress that received mate tea.

### 2.4. Wounding model and macroscopic analysis

Three days after beginning of the stress protocol, mice were intraperitoneally anesthetized with ketamine (150 mg/kg) and xylazine (15 mg/kg). A full-thickness excisional wound (1 $cm^2$) was created in the dorsum's animals and was not sutured or covered with a dressing and healed by secondary intention. To evaluate wound contraction, the lesion was measured soon after wounding and 4, 7, and 10 days later without scab removal as described (Romana-Souza et al., 2010). The data were expressed as percentage of the original wound area.

### 2.5. Tissue processing and microscopic analysis

Mice were intraperitoneally anesthetized with ketamine (150 mg/kg) and xylazine (15 mg/kg) and killed by decapitation 4 or 10 days after wounding. After four days of wounding, the peripheral blood was collected and plasma was frozen at −70 °C to measure normetanephrine levels. In this day of wounding, 5 lesions per group were also collected and frozen at −70 °C to measure carbonyl protein and lipid peroxide levels (Fig. 1). Ten days after wounding, 5 lesions and adjacent normal skin were collected per group, and these samples were formalin-fixed (pH 7.2) and paraffin-embedded (Fig. 1). In addition, 10 lesions per group were collected and frozen at −70 °C (Fig. 1). The frozen lesions