

## Effect of *Euterpe oleracea* Mart. (acai berry) Extract on Skin Flap Survival in Mice

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Skin flap necrosis remains a major complication of reconstructive surgery. *Euterpe oleracea* Mart., popularly known as "acai berry" contains hydroxybenzoic acid, antioxidant polyphenolics and anthocyanins. These and other compounds within the acai berry confer anti-inflammatory and anti-oxidative effects. In this current study, we evaluated the protective effect of acai berry extracts on survival of random-pattern skin flaps in a murine model by histologic analysis. ICR mice were subjected to skin elevation surgery and orally administered acai berry extract (100 mg/kg) daily for 7 days. Tissues were stained with hematoxylin-eosin or Masson's trichrome to observe tissue integrity and collagen deposition. In addition, TGF- $\beta$  and VEGF was stained by immunofluorescence to determine anti-inflammatory cell infiltration and neovascularization, respectively. We found a decrease in inflammatory cell infiltration and increase in collagen deposition in the acai berry extract treated mice compared to control mice. Immunofluorescence staining reveal a higher number of TGF- $\beta$  positive cells and enhanced VEGF staining in the acai berry extract treated mice. The results from this study indicate that oral uptake of acai berry extract can promote healing and survival of surgical skin flaps in mice providing an augmentative therapeutic approach to enhancing skin flap survival.

**Key Words:** Skin flap, *Euterpe oleracea* Mart., Acai berry, Inflammation

Random-pattern skin flaps are widely used in reconstructive plastic surgery. However, development of ischemic necrosis in the distal skin flap is a major concern. Disruption of vascular distribution occurring during inappropriate reconstruction surgery plays a pivotal role in failure of skin flap attachment (Kerrigan, 1983; Atalay et al., 2003). Various studies have shown that pharmacologic drugs such as alpha adrenergic blockers, vasodilators, anti-coagulation agents, prostaglandin E1, free radical scavengers and calcium

channel blockers are effective in increasing flap survival (Jurell and Jonsson, 1976; Kjartansson et al., 1988; Ercocen et al., 1998; Davis et al., 1999; Smith and Dolan, 1999). However, prolonged administration of these drugs often lead to severe side effects precluding excessive or long-term doses. Recently, local application of exogenous growth factors such as vascular endothelial growth factor (VEGF) has emerged to augment blood flow and viability of the skin flap (Kryger et al., 2000).

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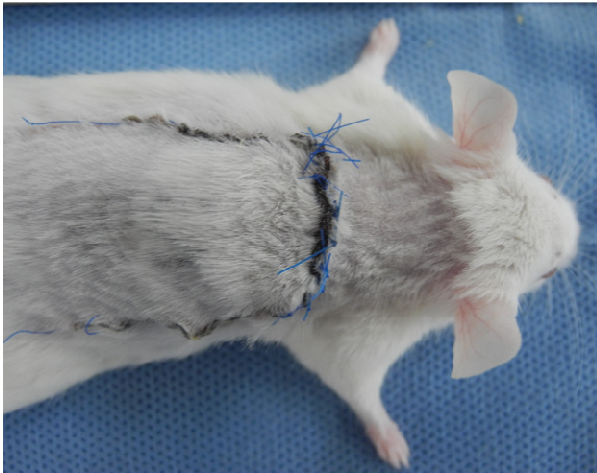
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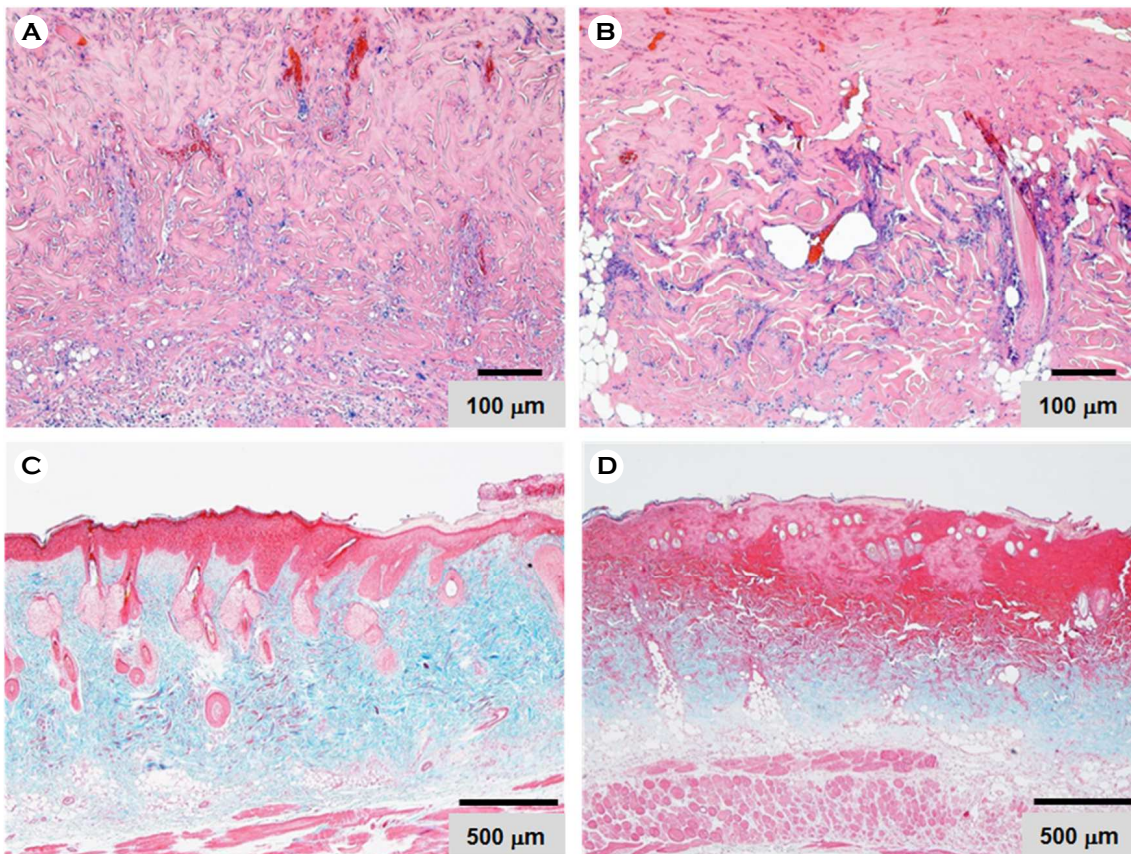
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**Fig. 1.** Post-operative photograph of random pattern skin flap surgery in ICR mice. Photograph was taken on day 7 post-surgery.

*Euterpe oleracea* Mart. (acai berry) is widely cultivated in the Amazon region of Brazil. Chemical studies have shown that acai berry contains hydroxybenzoic acids, antioxidant polyphenolics, flavan-3-ols, and anthocyanins, predominantly cyanidin 3-O-rutinoside and cyanidin 3-O-glucuronide (Mullen et al., 2002; Pacheco-Palencia et al., 2008; Cassidy et al., 2013). These complex compounds act in concert to inhibit cyclooxygenase-1 and cyclooxygenase-2, inhibit nitric oxide production and promote antioxidative effects (Schauss et al., 2006; Moura et al., 2012). Although the anti-inflammatory, antioxidant and vasodilation effects of acai berry has been shown, to the best of our knowledge, there are no studies on the effects of acai berry on random-pattern skin flap survival (Lee et al., 2016). Therefore, the aim of the study was to

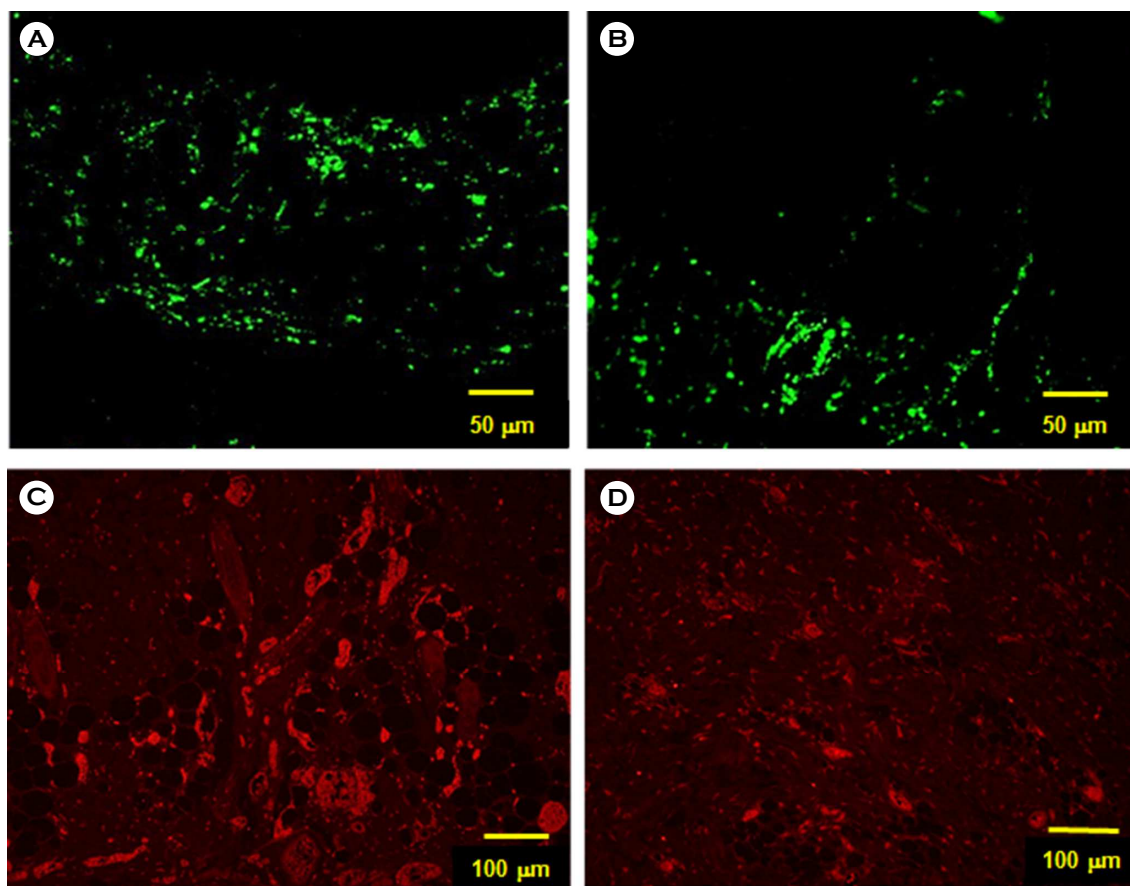


**Fig. 2. Hematoxylin-eosin and Masson's trichrome staining.** Representative histologic images of the skin flap day 7 post-surgery. FFPE tissues (5 µm) were stained with hematoxylin-eosin or Masson's trichrome stain. (A) Hematoxylin-eosin slides from acai berry treated mice. (B) Hematoxylin-eosin slides of control mice. (C) Masson's trichrome slides from acai berry treated mice. (D) Masson's trichrome slides from control mice. Bars indicate either 100 µm or 500 µm. Light green staining in the Masson's trichrome slides indicates collagen. Images were taken using a Nikon Eclipse 80i microscope and rendered on Adobe Photoshop. Total of 10 mice per group from two independent experiments.

determine whether *Euterpe oleracea* Mart. could improve the survival of random-pattern skin flaps in mouse.

Freeze-dried acai berry powder was purchased from iHerb (organic acai powder, Navitas Organics, Novato, CA, USA). 200 g of acai berry powder was mixed in 400 mL of distilled water and boiled for 10 min. Thereafter, 400 mL of 20% ethanol was added, shaken for 4 hr at room temperature and the extract filtered through Whatman filter paper. The extract was lyophilized, frozen at  $-20^{\circ}\text{C}$  until usage. Eight-week-old, male ICR mice (25~30 gram) were purchased from Daehan Biolink (Eumseong, Chungcheongbukdo, Korea) and were provided with standard chow and water *ad libitum*. Animal experiments were conducted strictly within

the guidelines of Institutional Animal Care and Use Committee of Kosin University College of Medicine (IACUC #14-05). Random pattern skin flap elevation surgery was performed under anesthesia by intraperitoneal injection of 50 mg/kg of Zoletil (Virbac, Carros, France) and 5 mg/kg of xylazine (Rompun; Bayer Korea Inc., Korea). Hair from the dorsal area was removed using depilatory cream. A  $3\times 5$  cm sized skin flap was vertically elevated from the panniculus carnosus layer, returned to the original location and the flap was sutured with a 4-0 nylon silk (Fig. 1). Following skin flap elevation, mice ( $n=5$ ) were given daily either acai berry extract per oral (100 mg/kg) or a PBS control for 7 days. Control mice ( $n=5$ ) were given PBS per oral. Mouse experi-



**Fig. 3. Immunofluorescence staining of TGF- $\beta$  and VEGF.** Representative immunofluorescence images of the skin flap day 7 post-surgery. FFPE tissues ( $5\ \mu\text{m}$ ) were deparaffinized, treated with heat induced antigen retrieval reagents and stained with rabbit anti-TGF- $\beta$  or anti-VEGF polyclonal antibodies. Goat anti-rabbit IgG polyclonal antibodies conjugated with Alexa 488 or Alexa 594 were used as secondary antibodies. (A) TGF- $\beta$  positive cells (green) of acai berry treated mice. (B) TGF- $\beta$  positive cells of control mice. (C) VEGF positive signal (red) in slides of acai berry treated mice. (D) VEGF positive signal (red) in slides of control mice. Bars indicate either  $50\ \mu\text{m}$  or  $100\ \mu\text{m}$ . Total of 10 mice per group from two independent experiments.

ments were repeated again for a total of ten mice per group.

On day 7 after surgery, mice were euthanized and formalin-fixed paraffin embedded (FFPE) tissues of the border were stained with hematoxylin-eosin and Masson's trichrome using standard protocols (Lee et al., 2018). Histological evaluation showed infiltration of inflammatory cells less organized tissue structure in the control group compared to the acai berry treated group (Fig. 2A and 2B). The images were examined using the image J software and statistical analysis were performed using SPSS (PASW Statistics 18.0, Chicago, IL, USA) statistical software. Data was expressed as the mean standard deviation. Differences between groups were analyzed by one-way ANOVA.

The collagen deposition was calculated to be  $156764.9 \pm 20.6$  pixels/high-power field in the acai berry treated group versus  $87981.0 \pm 21.1$  pixels/high-power field in the control group (Fig. 2C and 2D) ( $P < 0.05$ ). These results indicate that daily uptake of acai berry extract ameliorated infiltration of inflammatory cells and promoted collagen synthesis in the skin flap. To detect the presence of the anti-inflammatory cytokine TGF- $\beta$ , FFPE sections were treated with heat induced antigen retrieval reagent and stained with polyclonal rabbit anti-TGF- $\beta$  Ab (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Immunofluorescence results show more TGF- $\beta$  positive cells in the acai berry treated group compared to the control group (acai berry group,  $83 \pm 9$  cells/high-power field; control group,  $67 \pm 6$  cells/high-power field;  $P < 0.05$ ) (Fig. 3A and 3B). To detect the presence of VEGF (an angiogenic factor), FFPE tissues were stained with polyclonal rabbit anti-VEGF Ab (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Immunofluorescence results show more VEGF signal in the acai berry treated group compared to the control group (acai berry group,  $18101.3 \pm 10.5$  pixels/high-power field; control group,  $10539.0 \pm 6.9$  pixels/high-power field;  $P < 0.05$ ) (Fig. 3C and 3D). Collectively, these results suggest that daily oral administration of acai berry extract in mice promotes healing of the skin flap, in part, due to induction of the anti-inflammatory cytokine TGF- $\beta$  and the angiogenesis factor VEGF.

The main cause of flap necrosis are inflammatory cell infiltration, generation of reactive oxygen species (ROS), capillary thrombus formation and decreased blood flow in

the skin flap (Ohashi et al., 2007; Staiculescu et al., 2014). Therapeutic drugs such as anti-coagulants, phenothiazines, prostaglandin E1, deferoxamine, superoxide dismutase, dimethyl sulfoxide, allopurinol, N-acetylcysteine and nitric oxide synthase inhibitors have all been used to promote wound healing (Cuzzocrea et al., 2000; Hsieh et al., 2014; Swartz et al., 2015). When tissue is subjected to hypoxia or endothelial damage, expression of the VEGF and transforming growth factor- $\beta$  (TGF- $\beta$ ) are up-regulated (Erning et al., 2007). Many studies have confirmed that VEGF and TGF- $\beta$  expression results in neovascularization, increased blood flow and subsequent improvements in tissue viability (Zhang et al., 2003; Chung et al., 2013). A recent report by de Mota et. al showed that extracts from acai berry seeds given orally enhanced microcirculation in skin flaps in hamsters (Coelho da Mota et al., 2018). Our data are consistent with these findings although the acai extract we used was from the whole fruit. We did not find any adverse effects on mice given the acai berry extracts (data not shown). In addition, in vitro cytotoxicity experiments using acai berry extracts and RAW264.7 cell lines exhibited no cytotoxic effects when treated at up to 1,000  $\mu\text{g/mL}$  in a MTT assay (data not shown). This study confirms the positive effects of treatment with acai berry extract on the survival of random pattern skin flaps in mice. These results may represent a new therapeutic approach to enhancing flap viability and achieving faster wound repair. Further studies are needed to fully understand the benefit and limit of treatment with acai berry extract and wound healing.

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None

#### CONFLICT OF INTEREST

The authors have no conflicts of interest with regards to this study.

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