The European Research Journal
http://www.eurj.org
Original Article
e-ISSN: 2149-3189
DOI: 10.18621/eurj.335550

The effect of *Arnebia purpurea* extract on the survival of random pattern skin flaps in rats

Erkan Orhan[1], Merve Yüzbaşıoğlu[2], Yağmur Reyyan Erol[3], Erem Bilensoy[4], Nazlı Erdoğar[4], Lütfiye Ömür Demirezer[2], Ayşe Kuruüzüm-Uz[2]

[1]Department of Plastic and Reconstructive Surgery, Namık Kemal University School of Medicine, Tekirdağ, Turkey
[2]Department of Department of Pharmacognosy, Hacettepe University Faculty of Pharmacy, Ankara, Turkey
[3]Department of Plastic and Reconstructive Surgery, Eskişehir State Hospital, Eskişehir, Turkey
[4]Department of Pharmaceutical Technology, Hacettepe University Faculty of Pharmacy, Ankara, Turkey

ABSTRACT

Objectives. One of the important causes of flap losses is ischemia-reperfusion damage. *Arnebia* species are rich in naphthoquinone derivatives which known to have anti-inflammatory and anti-oxidant effects. The aim of the present study was to investigate the effects of *Arnebia purpurea* extract on viability of the skin flaps. ***Methods.*** Eighteen Wistar rats were divided in three groups. Caudal-based 9×3 cm size skin flap was applied on dorsum of the all rats and following surgery, 2 cc of *A. purpurea* extract topically was applied to the group 1 and 2 cc of 0.2% Nitrofurazon cream topically was applied to the group 2, daily. The Group 3 only received flap surgery (control group). Seven days later, all subjects were euthanatized and necrosis rate in their flaps was calculated and compared to each other. ***Results.*** The necrosis rate was calculated as 20.25% ± 1.59% in Group 1, 32.05% ± 2.23% in the Group 2 and 37.33% ± 4.12% in the Group 3. It was found that necrosis rate in the Group 1 was statistically significantly less than Groups 2 and 3 ($p < 0.001$) and that difference in necrosis rate between Group 2 and Group 3 was not statistically significant ($p > 0.05$). ***Conclusion.*** Necrosis of skin flaps was reduced through topical application of *A. purpurea* extract.

Eur Res J 2018;4(3):152-156

Keywords: Arnebia purpurea, skin flaps, necrosis, naphthoquinones, plant extracts

Introduction

The flaps have been used for repair of tissue defects and partial or complete tissue loss still remains as an important problem despite technological advanced in time and advanced techniques. One of the important causes of flap losses is ischemia-reperfusion damage. Ischemia is a condition during which part of a certain tissue receives insufficient blood supply [1]. If ischemia lasts longer than the tissue tolerates then necrosis occurs. If reperfusion is achieved before necrosis occurs, several physiopathological events occur which are first reversible and then become irreversible as a consequence of continuing condition, leading to so-called ischemia-reperfusion (I-R) damage. Main reason for I-R damage is reactive

Address for correspondence:
Abdullah Erkan Orhan, MD., Assistant Professor, Namık Kemal University School of Medicine, Department of Plastic and Reconstructive Surgery, 59030 Tekirdağ, Turkey E-mail: eorhan@yahoo.com Fax: +90 282 2509928
Received: August 21, 2017; Accepted: December 8, 2017; Published Online: December 23, 2017

oxygen species and inflammation [2].

Many pharmacological agents such as sympatholytics, vasodilators, prostaglandin inhibitors, glucocorticoids, anti-coagulant, and free radical scavenging agents have been used experimentally to ischemia and I-R damage to the skin flaps and flap viability but no clinically usable method has been developed for this purpose so far [3, 4].

Chemicals found in nature have been used for therapeutic purposes since ancient times. Despite competition from other drug discovery methods, natural products are still providing their fair share of new clinical candidates and drugs. Natural products are still a significant source of new drugs, especially for anticancer, antimicrobial, and antihypertensive therapies [5].

Alkanna in Europe and North Africa, Lithospermum in far-east and Arnebia in Anatolia has been used for centuries in traditional medicine [6]. All of them are members of *Boraginaceae* family and roots of the plants in *Boraginaceae* family are rich in naphthoquinone [7]. Naphthoquinone are used by industry as drug and food dye [8]. Naphthoquinone derivatives, Shikonin (R-configuration) and its enantiomer alkannin (S-configuration) has been shown to have wide variety of biological properties including accelerated wound healing, anti-oxidant, anti-bacterial, anti-fungal, anti-viral, anti-inflammatory , anti-tumor effects [9-13]. Previously we showed that the roots of *Arnebia purpurea* which is one of the *Boraginaceae* family member in Turkey contain high amounts of naphthoquinones [14]. Our hypothesis is the roots of *A. purpurea* which contain high amounts of naphthoquinones may reduce I-R damage on flap by anti-oxidant effects. In this study, we aimed to demonstrate the effects of *A. purpurea*'s root extract on viability of the skin flaps.

Methods

Approval was taken for the present study from the University Local Ethics Committee for the Experimental Animals. The study was conducted in the Production and Research Laboratories for Experimental Animals of University. Eighteen female Wistar Albino rats weighting between 250 and 350 grams produced in the Production and Research Laboratories for Experimental Animals of University. There are no differences on weight measurements of animals in the groups. The rats were housed in appropriate cages at 20°C and under 12 hours of light and dark periods. The animals were kept in separate cages in order to keep them from harming to each other prior to the surgical procedure. All rats were fed on standard rat food and no water restriction was applied.

Preparation of the extract of A. purpurea

Plant Material

The roots of *A. purpurea* were collected from Taşkent, Konya, Turkey. The plant was collected, identified and authenticated by Prof. Dr. H. D. and Dr. B. B (Department of Biology, Faculty of Sciences, Gazi University). A voucher specimen (No 3765) has been deposited in Faculty of Science, Gazi University.

Preparation of Plant Extract

The dried and powdered roots of *A. purpurea* (300 g) were extracted with n-hexane (3×500 ml, 40˚C). Each extract was collected and evoporated under reduced pressure at a temperature below 40˚C to yield dark red viscous residue (*A. purpurea*, 8.5 g).

Preparation of Cold Cream Formulation

n-hexane extract from the root of *A. purpurea*, was used as 1% concentration which was dissolved in cold cream. The cream formulation was prepared briefly as follows; 12.5 g cetyl esters wax, 12.5 g white wax, 56 g mineral oil, 0.5 g sodium borate and 19 g purified water were accurately weighed to obtain about 100 g cold cream formulation. Cetyl esters wax and white wax were reduced to small pieces and melted on a steam bath. Mineral oil was added and the mixture was heated until the temperature reached 70 °C. 1 g active extract was then added to the oily phase and mixed. Sodium borate was dissolved in purified water, warmed to 70 °C and gradually added to the melted mixture. It was stirred rapidly and continuously until congelation as described in the USP United States of Pharmacopeia. For control group, same cold cream was prepared but 1 g Nitrofurazon (Furacin®) was added instead of *A. purpurea* extract.

Surgical Procedure

All surgical procedures were performed with intramuscular administration of 50 mg/kg ketamine-HCl (Alfamine®: Ege-VET) and 10 mg/kg xylazine HCl (Rompun®: Bayer) for anesthesia by the same surgeon under sterilized conditions. The rats were fixed in prone position after their back had been

shaved. Surgical site was cleaned with 10% povidon iodine solution and covered sterilely. From their back, modified McFarlane flap included panniculus carnosus of 9×3 cm in size was removed and then flap was re-sutured with 4/0 nylon sutures [15].

The animals were divided in 3 groups so that each group contains 6 rats. Group 1 (*Arnebia purpurea* group): Following surgery 2 cc of *A. purpurea* cream was applied topically daily on the flap. Group 2 (Nitrofurazon group): Following surgery, 2 cc of 0.2% Nitrofurazon (Furacin®) cream was applied topically daily on the flap. Group 3 (control group): No substance was applied on the flap.

Evaluation of Flap Size

On the 7th day after the flap surgery all subjects were euthanized with high doses anesthesia. All flaps' photographs were taken from the same distance (60 cm) with a Sony DSC-F828 camera (Sony Corporation, Tokyo, Japan) by a different researcher who was blind about groups. The total area of necrosis was determined using the percentage of flap viability was calculated using the program Adobe Photoshop CS 5 (Adobe Systems, Inc., San Jose, CA). The necrotic area was marked on the software and the area was noted in pixels and then the percentage was calculated by a different researcher who was blind about groups [16].

Statistical Analysis

Data analysis was performed using the package program IBM SPSS Statistics 17.0 (IBM Corporation, Armonk, NY, USA). Normality of distribution of the continuous variables was tested using Shapiro-Wilk's test and homogeneity of the variances was tested using Levene's test. Definitive statistics were presented as mean ± standard deviation. Significance of the difference in ratio of necrotic area to the total area between the groups was examined with one-way analysis of variance (one-way ANOVA). In the case of significant difference being detected in one-way ANOVA, multiple comparisons of the groups were performed using with Tukey's test. A p value less than 0.05 was considered as statistically significant.

Results

Mean necrosis rate in Group 1 (*A. purpurea* group) was 20.25% ± 1.59%, in Group 2 (Nitrofurazon group) was 32.05% ± 2.23% and in group 3 (control group) was 37.33% ± 4.12% (Figures 1 and 2). Necrosis rate in the Group 1 (*A. purpurea* group) was statistically significantly less than Groups 2 (Nitrofurazon group) and Group 3 (control group) (p<0.001) and that difference in necrosis rate between Group 2 and Group 3 was not statistically significant (p=0.150) (Table 1).

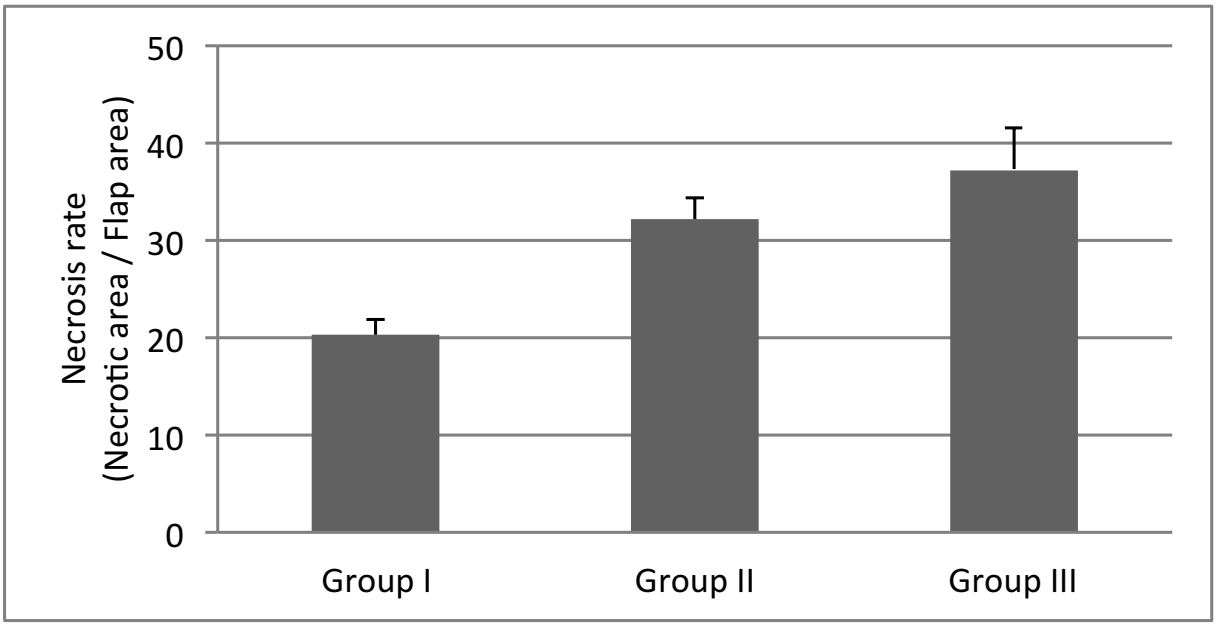
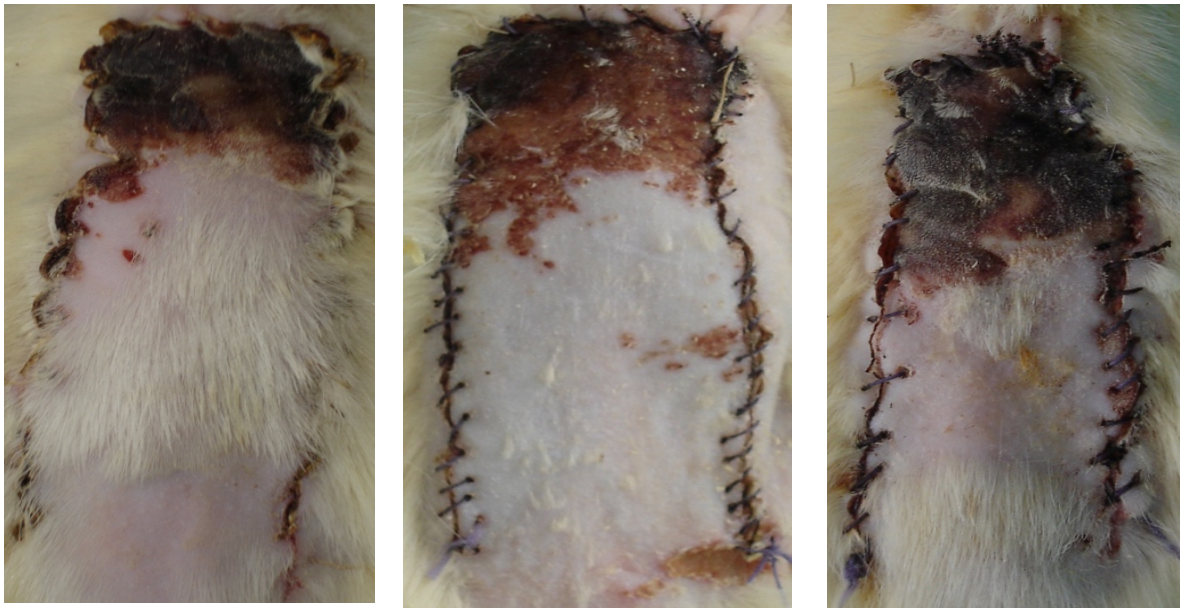


Figure 1. Mean necrosis rate of groups. Necrosis rate in Group I (*Arnebia purpurea* group) was statistically significantly lower than the Group II (Nitrofurazon group) and Group III (control group) (p <0.001) (post hoc Tukey's test)



a b c

Figure 2. View of necrosis of the flaps. a = Group 1 (*Arnebia purpurea* group), b = Group 2 (Nitrofurazon group) and c = Group 3 (control group).

Discussion

Previously we showed that the roots of *A. purpurea* involve naphthoquinones highly [14] and in the current study we think that because of anti-inflammatory and anti-oxidant activity, naphthoquinone derivatives in the *A. purpurea* extract reduced I-R damage occurring in the flap so amount of necrosis was found to be statistically significantly lower in the groups receiving *A. purpurea.*

In the experimental model of caudal-based randomly patterned skin flap on back of the rats we used in the present study, no tissue loss occurred on the area close to the pedicle when the flap was removed because it had sufficient perfusion but irreversible tissue damage and necrosis occurred due to ischemia at the tip of flap away from the pedicle. In so-called transitional zone between these areas, ischemia occurred in early stage due to hyperadrenergic condition because of noradrenaline released from the sympathetic nerve endings intersected during flap removal but perfusion was regained in 12 to 24 hours after the hyperadrenergic condition ended. Reperfusion occurring after ischemic period causes ischemic-reperfusion damage [3].

Basic role in physiopathology of I-R damage is played by reactive oxygen species (ROS) During ischemia period, high amount of xanthine oxidase-mediated ROS from endothelial cells and during

Table 1. Mean necrosis rate of groups

	Necrosis Rate (%)
Group 1	20.25 ± 1.59
Group 2	32.05 ± 2.23
Group 3	37.33 ± 4.12
p^a	< 0.001
Group 1 - Group 2	$p < 0.001^b$
Group 1 - Group 3	$p < 0.001^b$
Group 2 - Group 3	$p = 0.150^b$

Data are shown as mean ± standard deviation. Group I = *Arnebia purpurea* group), Group II = Nitrofurazon group, Group III = control group, [a] one-way analysis of variance (One-way ANOVA), [b] post hoc Tukey's test

reperfusion period high amount of nicotine amide adenine dinucleotide phosphate (NADPH) oxidase-mediated ROS from neutrophils generated in skin flap. ROS damage to DNA by oxidizing the nucleic acids, impair protein structure by oxidizing the amino acids, and cause lipid peroxidation by reacting with fatty acids [4, 6].

Shikonin/alkannin which are naphthoquinone derivatives, gives electron to the reactive oxygen radicals and make them more stable, reduces lipid peroxidation, increase amount of such anti-oxidant enzymes as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), prevents attraction of the neutrophils by reducing capillary permeability, and decreases neutrophil derived ROS release by inhibiting the NADPH oxidase enzyme in the neutrophils [6, 17] and through anti-oxidant activity occurring this way, shikonin/alkannin reduces I-R damage [17].

Rohrich *et al.* [18] defined an ideal pharmacological agent to be used to improve viability of the flap as being able to exert effects in the post-operative period, being easy to apply, reliable, cheap, having fully clarified mechanism of action, and protective effects against flap necrosis. *Arnebia* extracts have been used in traditional medicine for centuries and thus is reliable. Herbal products can be prepare as easy and cheap. As in traditional medicine, it was used in the present study topically and its topical use was shown to be effective.

Although we think that *A. purpurea* increases flap viability with antioxidant effect but as a limitation of our study, we didn't have any analysis about it so our study should be accepted as preliminary work and further studies on the mechanism of that flap protective effect are needed.

Conclusions

In conclusion, in this study we showed that with high naphthoquinones content, *A. purpurea* cream decreased randomly patterned flap necrosis.

Authorship declaration

All authors listed meet the authorship criteria according to the latest guidelines of the International Committee of Medical Journal Editors, and all authors are in agreement with the manuscript.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgements

We wish to thank Prof. Dr. Hayri Duman (Department of Biology, Faculty of Sciences, Gazi University, Ankara, Turkey) and Assist. Prof. Dr. Bilgehan Bilgili (in memory of the deceased) for collecting and authenticating the plant material.

References

[1] Orhan E, Erol YR, Deren O, Altun S, Unal S, Erdoğan B. Efficacy of liposuction as a delay method for improving flap survival. Aesthetic Plast Surg 2016;40:931-7.
[2] Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Cell biology of ischemia/reperfusion injury. Int Rev Cell Mol Biol 2012;298:229-317.
[3] de Lima Silva JJ, Guimaraes SB, da Silveira ER, de Vasconcelos PR, Lima GG, Torres SM, et al. Effects of Copaifera langsdorffii Desf. on ischemia-reperfusion of randomized skin flaps in rats. Aesthetic Plast Surg 2009;33:104-9.
[4] van den Heuvel MG, Buurman WA, Bast A, van der Hulst RR. Review: Ischaemia-reperfusion injury in flap surgery. J Plast Reconstr Aesthet Surg 2009;62:721-6.
[5] Butler MS. The role of natural product chemistry in drug discovery. J Nat Prod 2004;67:2141-53.
[6] Kourounakis AP, Assimopoulou AN, Papageorgiou VP, Gavalas A, Kourounakis PN. Alkannin and shikonin: effect on free radical processes and on inflammation-a preliminary pharmacochemical investigation. Arch Pharm (Weinheim) 2002;335:262-6.
[7] Kırımer N, Bozan B, Baser KHC. A new naphthaquinone from the roots of Arnebia densiflora. Fitoterapia 1995;66:499-500.
[8] Hu Y, Jiang Z, Leung KSY, Zhao Z. Simultaneous determination of naphtaquinone derivatives in Boraginaceous herbs by high-performance liquid chromatography. Anal Chim Acta 2006;577:26-31.
[9] Akkol EK, Koca U, Peşin I, Yilmazer D, Toker G, Yeşilada E. Exploring the wound healing activity of A. densiflora (Nordm.) Ledeb. by in vivo models. J Ethnopharmacol 2009;124:137-41.
[10] Parray JA, Hamid R, Kamili AN, Shameem N, Jan S, Ganai BA. Biological efficacy and radical scavenging potential of shikonin in Arnebia benthamii (Wall ex. G Don) Johnston. Ind Crops Prod 2015;74:434-39.
[11] Singh LK, Maheshwari DK, Shukla S. Antibacterial effect of butyryl alkannin from Arnebia euchroma against vancomycin-resistant pathogens of Enterococcus faecalis causing urinary tract infections. Nat Prod Res 2015; 29:2299-301.
[12] Zhang ZL, Fan HY, Yang MY, Zhang ZK, Liu K. Therapeutic effect of a hydroxynaphthoquinone fraction on dextran sulfate sodium-induced ulcerative colitis. World J Gastroenterol 2014;20:15310-8.
[13] Jang SY, Jang EH, Jeong SY, Kim J-H. Shikonin inhibits the growth of human prostate cancer cells via modulation of the androgen receptor. Int J Oncol 2014;44:1455-60.
[14] Yuzbasioglu M, Kuruuzum-Uz A, Guvenalp Z, Simon A, Toth G, Harput US, et al. Cytotoxic compounds from endemic Arnebia purpurea. Nat Prod Commun 2015;10:595-6.
[15] Acartürk TO, Dinçyürek H, Dağlıoğlu K. Delay by staged elevation of flaps and importance of inclusion of the perforator artery. J Hand Microsurg 2015;7:1-5.
[16] Uysal AC, Mizuno H, Tobita M, Ogawa R, Hyakusoku H. The effect of adipose-derived stem cells on ischemia-reperfusion injury: immunohistochemical and ultrastructural evaluation. Plast Reconstr Surg 2009;124:804-15.
[17] Wang ZH, Liu T, Gan L, Wang T, Yuan X, Zhang B, et al. Shikonin protects mouse brain against cerebral ischemia/reperfusion injury through its antioxidant activity. Eur J Pharmacol 2010;643:211-7.
[18] Rohrich RJ, Cherry GW, Spira M. Enhancement of skin-flap survival using nitroglycerin ointment. Plast Reconstr Surg 1984;73:943-8.