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ARTICLE

Effect of dipyridamole on random pattern skin flap viability in rats

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ABSTRACT

Ischemic necrosis has been most dreaded complication of flap reconstruction. Therefore, researchers have tried to improvise modalities to prevent or treat it since the onset of flap surgery. So far these researches have failed to identify a pharmacological therapy equally effective as surgical delay in augmenting skin flap viability. In the path of search for this substance, dipyridamole attracted our attention as an antiaggregant agent. Put together with pathophysiological mechanisms underlying ischemic flap necrosis, we concluded dipyridamole might have beneficial effect on survival of skin flaps. In this research random pattern dorsal rat skin flap model of McFarlane is used. Subjects are separated in a randomized fashion between two groups. Experiment group is given dipyridamole with a dose of 20 mg/kg twice daily. Control group is given same amount of saline. At seventh day viability of skin flaps is assessed and compared between groups. Also on 7th day, pathologic specimens are obtained and evaluated histopathologically in terms of neutrophil and lymphocyte infiltration, edema and fibrosis. Necrosis percentage in experiment group is found to be significantly lower than that of control group ($p < 0.01^*$). Neutrophil infiltration and edema found to be significantly lower in dipyridamole group ($p < 0.05^*$). No significant difference is observed in lymphocyte infiltration and fibrosis. Dipyridamole is shown in this research to be effective in augmenting viability of random pattern skin flaps in rats. Nevertheless, more extensive researches are needed to fully determine its precise mechanism, side effects and appropriate doses.

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Flap viability; random pattern; rat; dipyridamole; necrosis

Introduction

Despite major advances in flap surgery, ischemic necrosis continues to be the most dreaded drawback of flap reconstruction [1,2]. Surgical delay is the foremost used method to prevent distal flap necrosis [3–7]. On the other hand, surgical delay requires at least one additional surgery. Another alternative for the sme purpose is the use of various pharmacologic agents. A wide array of medications and methods are investigated clinically and experimentally with the aim of preventing distal flap necrosis [8–24]. These include agents diminishing blood viscosity, raising room temperature, calcium channel blockers, peripheral vasodilators, antithrombotic and anticoagulant agents. Anti-inflammatory, anti-oxidant agents, steroids and hyperbaric oxygen therapy are also used clinically to reduce the effects of ischemia [8,10,25].

Dipyridamole is an antiaggregant agent devised to be used in coronary artery disease due to its vasodilating effect. It also blocks platelet adhesion and aggregation. It shows its effect through inhibition of phosphodiesterase enzyme located in platelets, blocking adenosine intake in erythrocytes and increasing prostaglandin I_2 (PG I_2) production in endothelium [9,26–35]. Clinically it is used in ischemic stroke, transient ischemic attack, acute coronary syndrome and prevention of thrombosis in patients with a prosthetic valve [34–36]. Cilostazol is an antiaggregant agent -similarly to dipyridamole- acting through phosphodiesterase inhibition. Considering proved beneficial effects of cilostazol and other anti aggregant agents such as acetylsalicylic acid, hirudin and clopidogrel on flap survival, we have concluded that dipyridamole might have beneficial effect in preventing distal flap necrosis in random pattern skin flaps [11,14,16–18,21,23,30,31].

To our knowledge there is no study in literature to date, investigating the effect of dipyridamole on flap survivability in random pattern rat dorsal skin flap model. This study might be the preliminary research for the clinical use of dipyridamole with the aim of preventing ischemic flap necrosis in patients undergoing flap surgery.

Materials and methods

This study is carried out in Ankara Training and Research Hospital Animal Studies Laboratory with local ethical committee approval date and number 8/15/2017-477. Ethical principles of animal studies are followed during every step in planning and execution of the study.

Twenty Wistar-Albino male rats, bred in same facility, weighting between 200 and 300 g (mean 240 g.) are used. Test subjects are randomly assigned to control (Group 1) and experiment (Group 2) groups, each consisting of 10 subjects.

In this study, Mc Farlane's rat dorsal skin flap model is used. This model -with its adjustable necrosis ratio- is described to be used in investigating effects of different factors acting on flap survivability [5] (Figure 1).

Subjects in experiment group are given dipyridamole (Drisentin® 75 mg 90 tablets Sanovel, Turkey) twice daily for 7 days with a dose of 20 mg/kg dissolved in 0.3 ml saline per oral. Control group is given same amount of saline.

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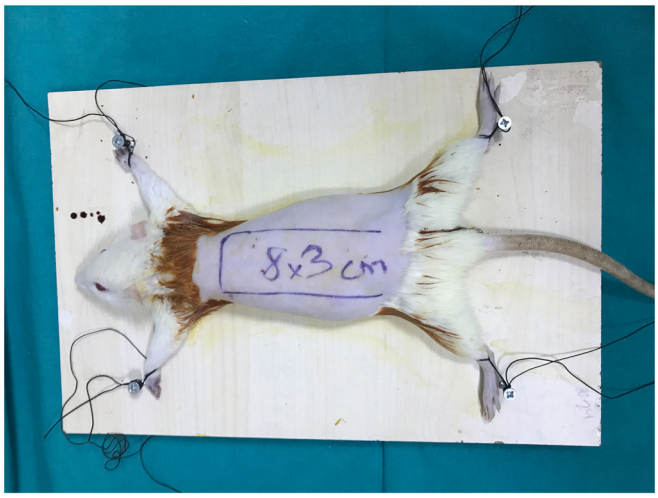

Figure 1. Markings of McFarlane's dorsal skin flap measuring 8 × 3 cm.

Figure 2. Planning of the flap.

Ketamine HCl 50 mg/kg (Ketalar® 50 mg/mL Pfizer, Turkey) and Xylazine HCL 2% 10 mg/kg (Rompun® %2 25 ml Bayer, Germany) is used for anesthesia. Following the confirmation of surgical anesthesia depth, markings are made on the back of the subject which include scapulae and posterior iliac spine. A caudally based flap measuring 3 × 8 cm is planned (Figure 2). Incisions are made bilaterally and distally through skin and panniculus carnosus and deep muscle fascia is reached (Figure 3). Using sharp dissection, flap is elevated in the anatomically natural, avascular plane, including the panniculus carnosus in the flap (Figure 4). Following total elevation of flap except for its caudal base, bleeding vessels are cauterized, flap is returned to donor site and adapted with 3/0 cutting polyglactin continuous sutures (Figure 5).

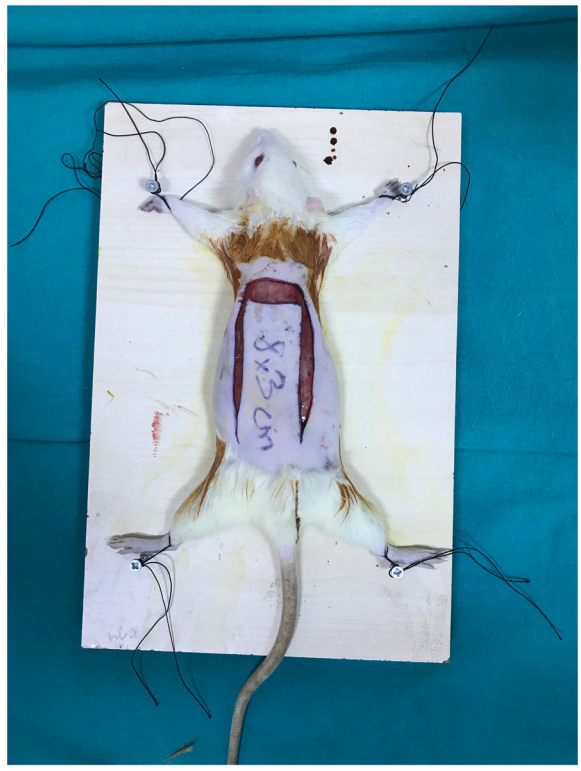

Figure 3. Making of incisions.

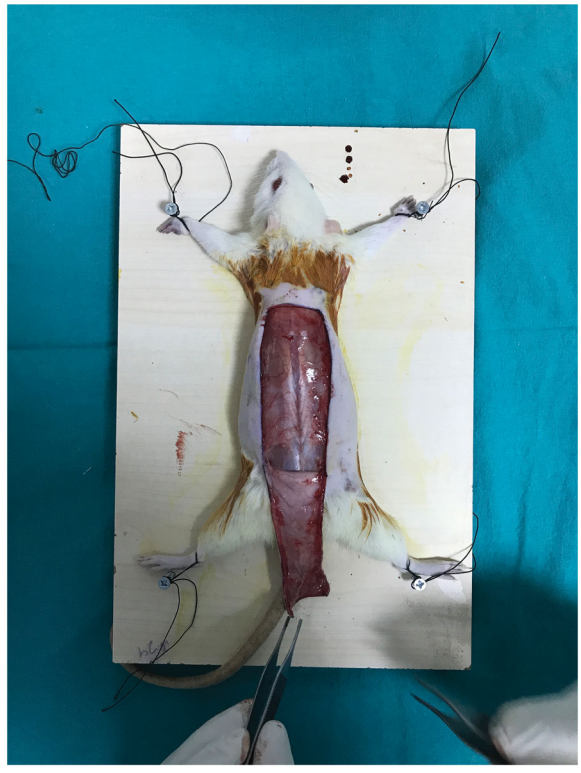

Figure 4. Elevation of the flap.

Clinical assessment

At seventh day postoperatively, photographs are taken with a Canon EOS 600 D (Canon® Tokyo, Japan) camera from a standard distance (40 cm) to assess necrosed flap area. Photographs are imported digitally to Adobe Photoshop CC (Adobe® California,

USA) digital imaging program and viable flap area, necrosed flap area and percentage of necrosed area to total flap area is measured (Figure 6). In addition, necrosed area and total flap area is measured by grid method using millimetricly checkered transparent sheet (Figure 7). During calculations, final flap area is used instead of initial one in order to avoid miscalculations due to flap contraction. Necrosed flap area is stated as percentages for each subject in both groups.

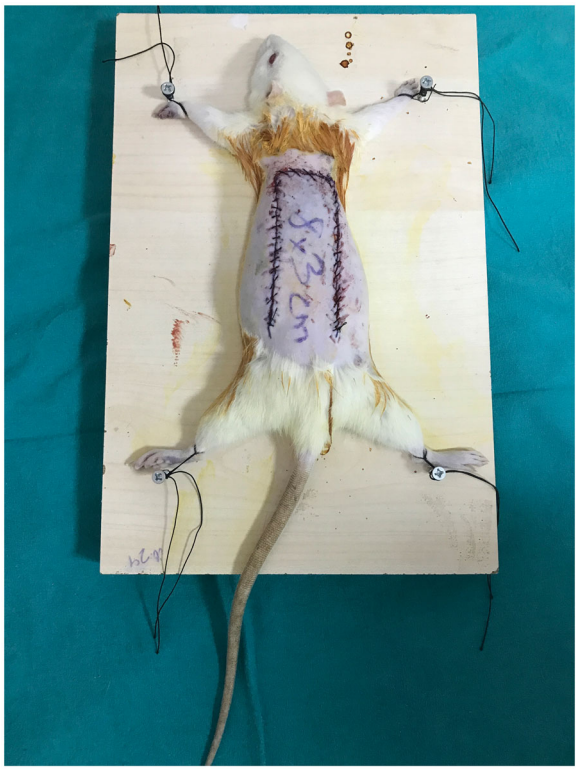

Figure 5. Readaptation of the flap using sutures to the donor area.

Histopathologic assessment

At seventh day postoperatively a tissue sample from demarcation zone between viable and necrotic tissue, measuring 1×1 cm is obtained under general anesthesia from each subject and fixed in 10% buffered formaldehyde solution (Figure 8). Following obtainment of histopathologic specimens, subjects are sacrificed by cervical dislocation. Sections are stained with Hematoxylin & Eosin (H&E) and Mallory-Azan histologic stains, examined with a Nikon E600 light microscope (Nikon® Minato, Tokyo, Japan) and photographed.

Specimens are examined in terms of polymorphonuclear leukocytes (PMNL), lymphocyte, edema and fibrosis density. A four point scoring scale is used to assess and compare the results for these four parameters (Table 1).

Statistical assessment

SPSS 20 (IBM Corp.® Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY, USA: IBM Corp.) software is used for statistical analysis of the results. Variables are described as mean ± standard deviation and median (maximum-minimum) percentage and frequency values are used. During the data analysis, Student's *t*-test for independent samples is used for comparison of independent variables between two groups when standard distribution is confirmed and Mann Whitney-*U* test is used when not. Categorical data is analyzed using Fisher's Exact Test and Chi-square test. Monte Carlo Simulation Method is used when expected frequency is lower than 20% to allow these frequencies in analysis. For statistical significance levels of tests $p < 0.05$ values are deemed significant.

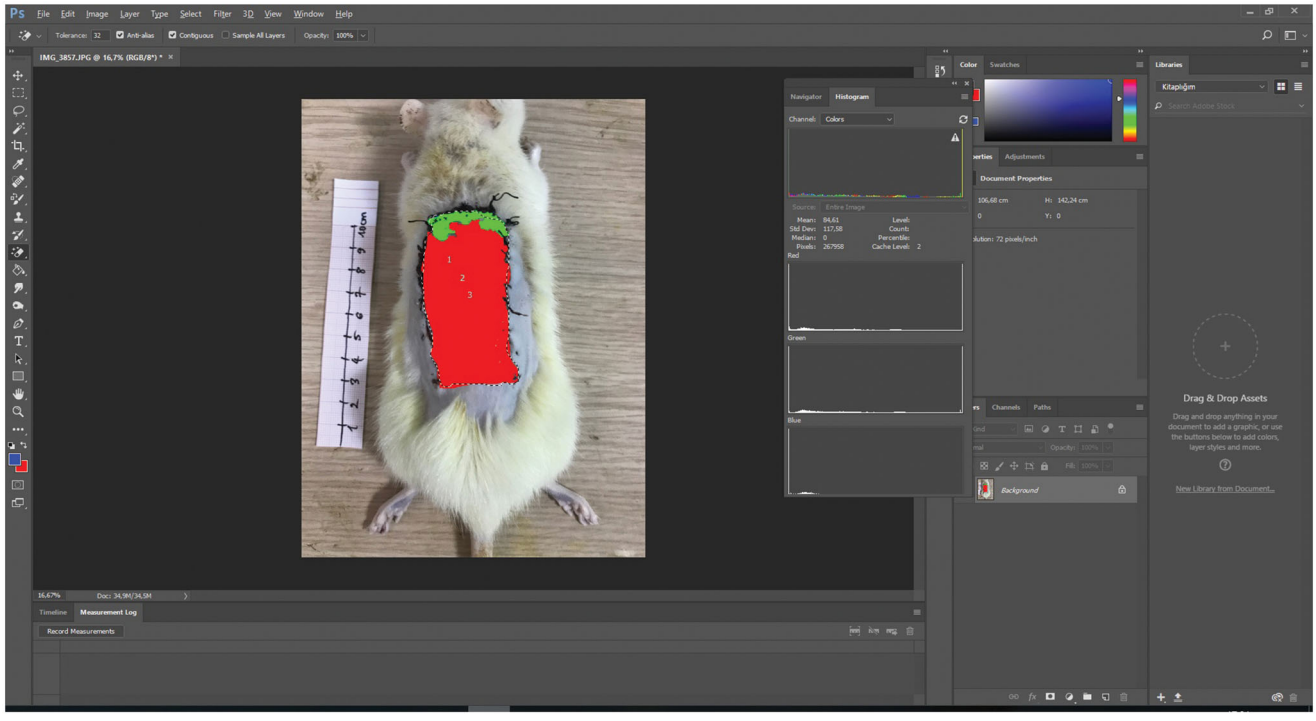

Figure 6. Calculation of the rate of necrosed flap area using Adobe Photoshop (Adobe® California, USA) software.

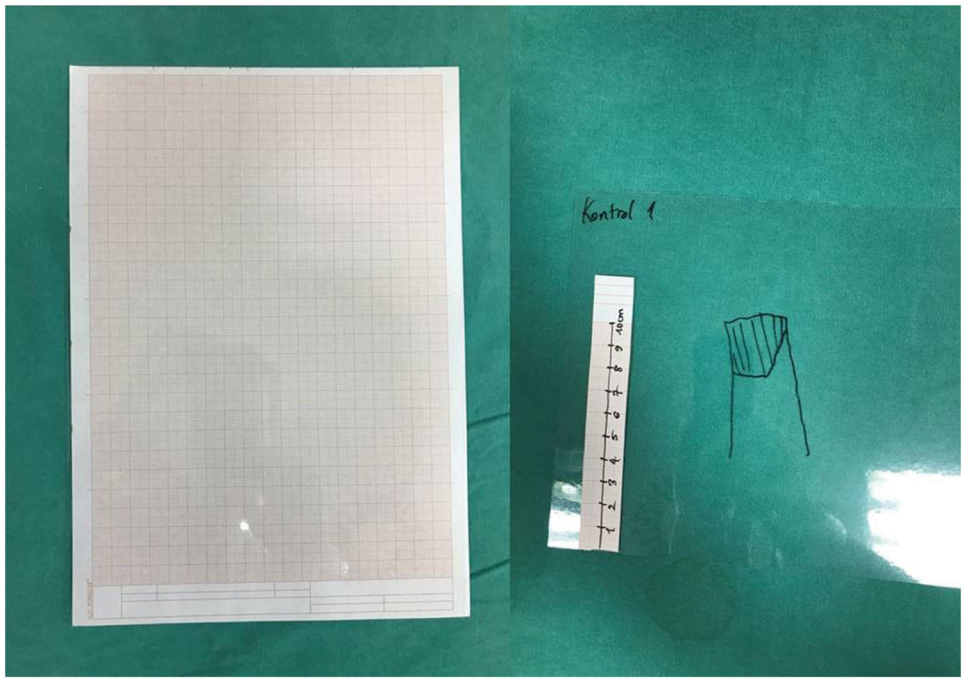

Figure 7. Transfer of the viable and necrosed flap area to transparent sheet and measurement with grid method.

Figure 8. Preparing, classification and marking of histopathologic specimens.

Table 1. Histopathologic assessment scale.

	None	Mild	Moderate	Dense	Very dense
PMNL density	0	+	++	+++	++++
Lymphocyte density	0	+	++	+++	++++
Edema in dermis	0	+	++	+++	++++
Fibrosis	0	+	++	+++	++++

Results

Clinical assessment

All flaps have shown certain amount of contraction by the seventh day postoperatively. Necrosis percentages for each subject are determined by digital and planimetric measurements and noted (Figure 9). Necrosis rate was 12.21 ± 1.64% in experiment

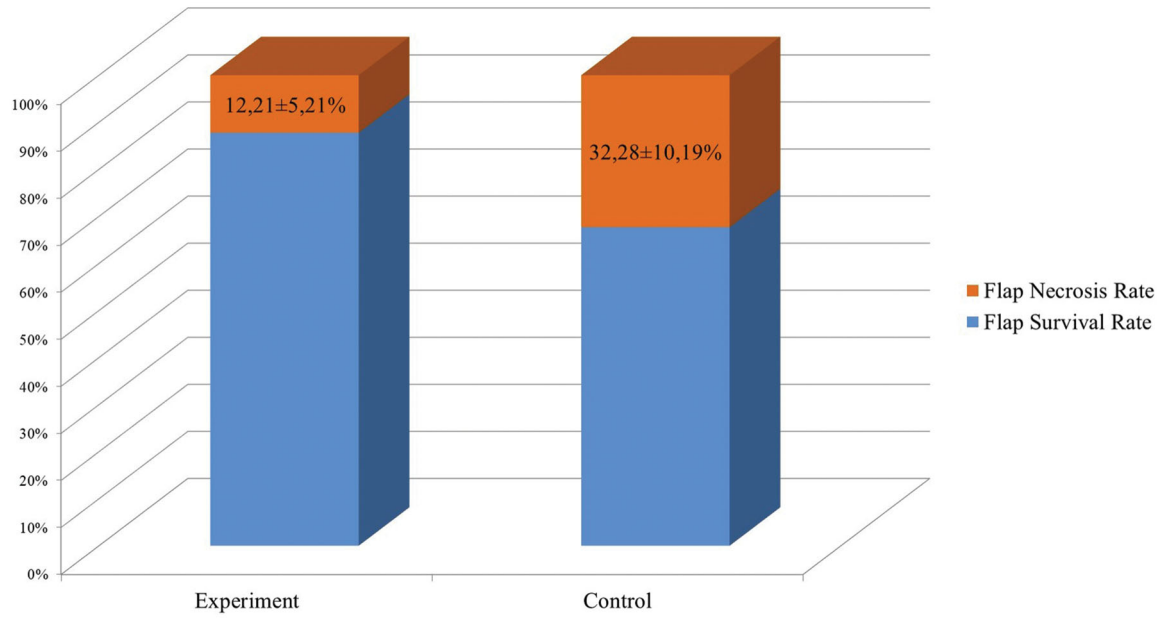

Figure 9. Flap necrosis rate measurements (mean ± standard deviation).

Table 2. Descriptive statistical values of groups in regard of necrosis rate.

Group statistics

	Group	*N*	Mean	Std. deviation	Std. error mean
Necrosis_Rate	Experiment	10	12.2130	5.20658	1.64646
	Control	10	32.2870	10.19134	3.22278

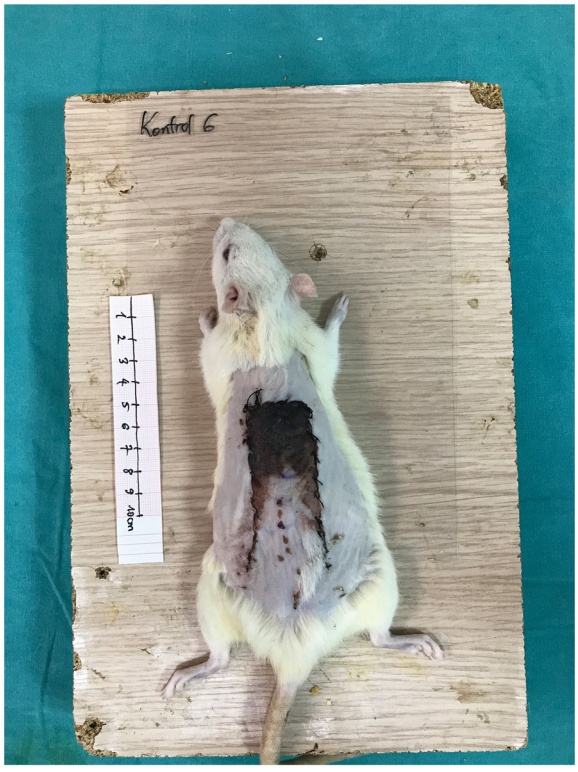

Figure 10. Necrosed flap area of subject number 6 in control group at 7th post operative day.

group and 32.28 ± 3.22% in control group (Table 2). Following the confirmation of standard distribution of variables, data is compared statistically between groups using Student *t*-test for independent variables.

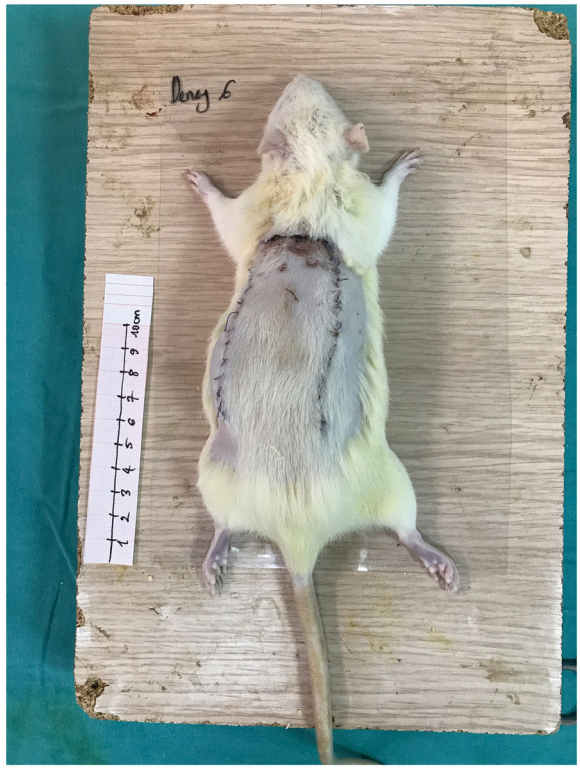

Figure 11. Necrosed flap area of subject number 6 in experiment group at 7th post operative day.

Viable flap area is found to be higher and necrosed area smaller in experiment group at seventh postoperative day (Figures 10–11). This observation is confirmed in statistical analysis, when data is compared between groups ($p < 0.01^*$) (Table 3).

Histopathologic assessment

Neutrophil infiltration, lymphocyte infiltration, edema and fibrosis density is evaluated and scored separately for each subject

Table 3. Comparison of necrosis rate between groups. Significant values are noted italic bold.

	Independent samples test							
	Levene's test for equality of variances		*t*-Test for equality of means					
	F	Sig.	t	df	Sig. (2-tailed)	Mean difference	Std. error difference	95% Confidence interval of the difference Lower
Necrosis_rate								
Equal variances assumed	1.499	***.237****	−5.547	18	***.000****	−20.07400	3.61900	−27.67724
Equal variances not assumed			−5.547	13.398	.000	−20.07400	3.61900	−27.86881

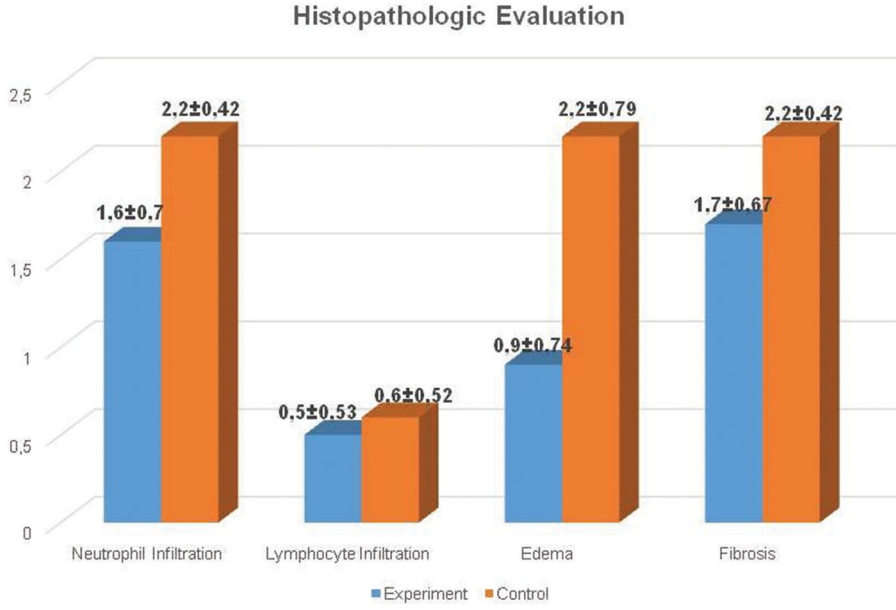

Figure 12. Histopathologic assessment results on a scale of 0–4 (mean ± standard deviation).

Table 4. Statistical analysis of histopathologic assessment between groups. Significant values are noted italic bold.

Independent samples test					
	t	Sig. (2-tailed)	Mean difference	Std. error difference	Lower
PMNL_infiltration					
Equal variances assumed	−2.324	***.032****	−.60000	.25820	−1.14246
Equal variances not assumed	−2.324	.035	−.60000	.25820	−1.15105
Lymphocyte infiltration					
Equal variances assumed	−.429	.673	−.10000	.23333	−.59022
Equal variances not assumed	−.429	.673	−.10000	.23333	−.59023
edema					
Equal variances assumed	−3.806	***.001****	−1.30000	.34157	−2.01760
Equal variances not assumed	−3.806	.001	−1.30000	.34157	−2.01783
fibrosis					
Equal variances assumed	−1.987	.062	−.50000	.25166	−1.02872
Equal variances not assumed	−1.987	.065	−.50000	.25166	−1.03611

using the previously stated scale and statistically compared between experiment and control groups (Figure 12). Neutrophil infiltration and edema is found to be statistically lower in experiment group ($p < 0.05^*$) (Table 4). Figure 13 shows reduced density of neutrophil infiltration in experiment group comparing to control group under light microscopy with hematoxylin-eosin (H&E) staining. Although mean value for lymphocyte infiltration (0.5 compared to 0.6) and fibrosis (1.7 compared to 2.2) were lower in experiment group, statistical analysis failed to reveal a significance for these parameters ($p = 0.673$ and $p = 0.062$ respectively).

Discussion

Dipyridamole was first marketed as a coronary vasodilating agent to be used in coronary heart disease and acute coronary syndrome. Later on, its anti platelet and anti antithrombotic effects are observed [35]. It shows its effect through inhibition of phosphodiesterase enzyme, leading to elevated cAMP levels and vasodilatation [21]. Furthermore it potentializes the effect of PG I_2. Blass et al. has shown that dipyridamole effectively potentializes the effects of PG I_2 in rats and in in-vitro isolated rabbit myocard tissue model [29]. In another study, dipyridamole is shown to

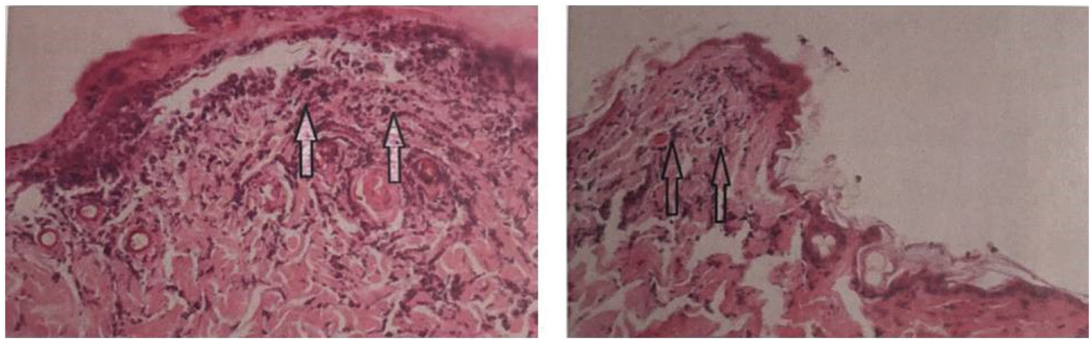

Figure 13. Polymorphonuclear leukocyte infiltration in control and experiment groups (respectively) under light microscopy with H&E staining and ×200 magnification.

decrease tissue damage in frosting injuries in rats [30]. Furthermore, it is shown to cause neurological improvement and a decrease in infarct area in focal cerebral ischemia model [37,38]. Saino et al. reported dipyridamole reduces Ca^{2+} influx and causes vessel smooth muscle relaxation in *in-vitro* human arterioles [27].

These data from literature combined with the background of flap pathophysiology and dipyridamole's mechanism of effect led to the conclusion that this substance may have favorable effect on flap viability, forming the basis for this study. Coherent with the hypothesis, viability of flaps on post-operative seventh day in experiment group was found to be significantly higher ($p < 0.01^*$) than control group. The underlying mechanism is thought to be the fact that dipyridamole potentializes the effect of PG I_2, increases PG I_2 synthesis and inhibits platelet aggregation caused by thrombogenic substances such as TX A_2, ET-1, NE, 5-HT arising following surgical trauma. However, further studies are needed to enlighten exact mechanism.

In histopathologic examination, neutrophil infiltration and edema is found to be significantly lower in experiment group ($p < 0.05^*$). These findings are coherent with the results of studies by Weyrich et al. and Iliano stating dipyridamole has anti-inflammatory effects [28,33]. In regard of lymphocyte infiltration and fibrosis, no significant difference is observed. This might be due to low rate of lymphocyte infiltration in both groups hindering accurate statistical analysis and the fact that seven days is too short a time period in which to observe significant amount of fibrosis.

This study is a preliminary research to investigate effects of dipyridamole on random skin flap survivability. This study might be guiding for the clinical use of dipyridamole with the aim of augmenting random pattern skin flap viability and prevention of flap necrosis. In the light of the results from this study, dipyridamole is shown to have protective effects against ischemic flap necrosis. On the other hand, further studies are needed to elicit underlying mechanism of effect, determine dose-response curve and possible side effects.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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