(Jurnal Plastik Rekonstruksi, 2023; Vol 10, No 1, 23-29) WOUND HEALING/EXPERIMENTAL

Article

THE EFFECT OF ORALLY ADMINISTERED COENZYME Q10 ON THE VIABILITY OF RANDOM SKIN FLAP IN NICOTINE EXPOSED WISTAR RATS

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ABSTRACT

Introduction : Nicotine was assumed to be the cause of compromised flap. The administration of antioxidants can eliminate such effect, and one of the most promising antioxidants is Coenzyme Q10. The research aims to find the effect of orally administered Coenzyme Q10 on the viability of random skin flaps in nicotine-exposed wistar rats.
Method : Twenty-seven white rats (*Rattus norvegicus*) were assigned in three groups. The first group received no nicotine, while the other two groups were nebulized with nicotine for 4 consecutive weeks, and treated as random skin flaps. In third group, Coenzyme Q10 was administered for 7 days. Then, a standard histopathological staining and vascular endothelial growth factor (VEGF) measurement were performed on flap sample to examine the extent of neovascularization and VEGF cell expression.
Result : The average number of capillaries in group I was 5.33 ± 1.323, in group II was 5.89 ± 0.782, and in group III was 7.78 ± 2.587. There was no significant difference (p = 0.317, 95% CI) in groups I and II of VEGF expression. However, significant differences were found in the intensity of VEGF (p = 0.009, 95% CI) in groups I and group III, and in groups II and group III (p = 0.011, 95% CI). Thus, the intensity was stronger on the subject with coenzyme Q10 compared with the other two groups.
Conclusion: Coenzyme Q10 increases the viability of random flaps by increasing the number of capillaries and VEGF expression.
***Keywords**: Capillary density; Coenzyme Q10; Nicotine; Random Skin Flap; VEGF*

Latar Belakang Nikotin adalah zat yang diasumsikan sebagai penyebab flap. Pemberian antioksidan dapat menghilangkan efek tersebut, salah satunya adalah Coenzyme Q10. Penelitian ini bertujuan untuk mengetahui pengaruh pemberian Coenzyme Q10 secara oral terhadap viabilitas *random skin flap* pada tikus wistar yang terpapar nikotin.
Metodologi: Sebanyak 27 tikus putih (*Rattus norvegicus*) dibagi menjadi tiga kelompok. Kelompok pertama tidak menerima nikotin, sedangkan dua kelompok lainnya dinebulisasi dengan nikotin selama 4 minggu berturut-turut, dan diperlakukan sebagai flap kulit acak. Pada kelompok ketiga diberikan Coenzyme Q10 selama 7 hari. Selanjutnya pewarnaan histopatologi standar dan pengukuran faktor pertumbuhan endotel vaskular (VEGF) dilakukan pada sampel flap untuk memeriksa tingkat neovaskularisasi dan ekspresi sel VEGF.
Hasil: Rata-rata jumlah kapiler pada kelompok I adalah 5,33 ± 1,323, pada kelompok II adalah 5,89 ± 0,782, dan pada kelompok III adalah 7,78 ± 2,587. Tidak ada perbedaan bermakna (p = 0,317, 95% CI) pada ekspresi VEGF kelompok I dan II. Namun terdapat perbedaan yang bermakna pada intensitas VEGF (p = 0,009, 95% CI) pada kelompok I dan kelompok III, serta pada kelompok II dan kelompok III (p = 0,011, 95% CI). Hasil tersebut menunjukkan jika intensitas VEGF lebih kuat pada kelompok subjek dengan koenzim Q10 dibandingkan dengan dua kelompok lainnya.
Kesimpulan: Koenzim Q10 meningkatkan viabilitas flap acak dengan meningkatkan jumlah kapiler dan ekspresi VEGF.
***Kata Kunci:** Densitas kapiler; Koenzim Q10; Nikotin; Random Skin Flap; VEGF*

Conflicts of Interest Statement:
The author(s) listed in this manuscript declare the absence of any conflict of interest on the subject matter or materials discussed.

Received: 22 11 2022, Revised: 18 01 2023, Accepted: 14 05 2023

 | P-ISSN 2089-6492; E-ISSN 2089-9734 | DOI: 10.14228/jprjournal.v10i1.345

INTRODUCTION

The first flap reconstruction was documented in 600 BC where Sushruta Samhita used a cheek flap to copy the nose. The development of a modified flap includes a combination of skin, bone, or fascia that carries its vascularization when transferred from the donor region to the. Over time, the flap has undergone many modifications, including skin, bone, or fascia that carries its vascularization when transferred from the donor region to the recipient.[1] In the present and future, the flap concept is essential to understand. The newly removed tissue will compromise and even die if the displaced flap does not supply enough nutrients and oxygen.[2]

Indonesia is the world's highest cigarette consumption country by 2015, about 76.2% of the population.[3] Cigarette exposure in patients is one of the causes of the compromised cause of a flap, according to research conducted by Goldminz and Bennett in 1991, which explained the significant relationship between cigarette exposure and the occurrence of necrosis in the flap.[4]

The cause of the occurrence of necrosis in random skin flaps may be extrinsic or intrinsic. Extrinsic factors may be systemic (hypotension, vascular disease, infection, etc.), local (compression, temperature, etc.), or matters related to surgical techniques. However, blood flow is the single most crucial intrinsic factor.[5] Of the more than 4,000 chemicals in cigarette smoke, nicotine is one of the most studied chemicals. Nicotine promotes tissue injury mediated Reactive Oxygen Species (ROS) and induces vasoconstriction mediated by norepinephrine, reducing skin circulation.[6,7] In addition, Nitric oxide is a free radical that many smokers absorb. Absorption of free radicals causes oxidation catalysis of low-density lipoprotein (LDL), thus promoting endothelial activation, macrophage activation, and atherosclerosis in the arterial branching area.[8]

One of the efforts to improve survival flap is to eliminate the effects of nicotine exposure on the cigarette in the patient. Coenzyme Q10 is a powerful antioxidant that plays a role in stabilizing the membrane by participating in the lipid membrane structure that surrounds the cell. Endogenous coenzyme Q10 (CoQ10) plays an essential role in preventing protein oxidation.[9] Effectiveness of CoQ10 as antioxidant stems from the fact that this compound disrupts lipid peroxidation both in initiation and propagation where its steps, contrary to the effects of vitamin E as a chain-breaking antioxidant, and only inhibits propagation.[10] This research aims to determine the effect of giving coenzyme Q10 peroral to the viability of random skin flaps in nicotine-exposed wistar rats.

METHOD

Animals' management

The subjects of this study were random skin flaps performed on healthy male rats (*Rattus novergicus*) Wistar strain aged about 3 months with bodyweight 250 – 300 g. Each rat was kept in the same cage, in the same room, and fed the exact amount and type. Rats were kept in polypropylene cage size 30 cm x 40 cm x 15 cm with ventilation and room temperature around 32^{o}C. Each cage contains 4 - 5 animals. The enclosure was covered with woven wire and husked and adapted for 1 week. The food given to the experimental animals is PAR-G pellet feed with a dose of 20 grams/day/animal try. Drinks given to the experimental animals are drinking water (Danone, AQUA) and given ad libitum by using a special bottle that will remove the water in it if the animal try sucks it up. The rats were then killed by cremation.

Ethics approval

This reseach was approved by Ethics Committee of Veterinary Faculty, Universitas Airlangga, Surabaya, Indonesia, No. 2.KE.007.01.2018.

Nicotine inhalation

Nicotine inhalation was used with Nicvape brand (Nicvape, USA). The preparation is then dissolved with sterile aquadest and administered by inhalation with a dose of 2 mg/kg twice daily for 4 weeks.

Treatment and surgical procedures

Twenty-seven animals were divided into three groups of 9 animals:

P-ISSN 2089-6492; E-ISSN 2089-9734 | DOI: 10.14228/jprjournal.v10i1.345

Group I: The skin flap on the rat's back with a 2 x 5 cm size and then checked on the day k-7 (control).

Group II: The skin flap on the rat's back with a size of 2 x 5 cm, given 2 mg/kg of inhaled nicotine 2 times a day for 4 weeks, then checked on the 7th day.

Group III: The skin flap on the rat's back with a size of 2 x 5 cm which has been given 2 g of nicotine inhalation 2 mg/kg twice a day for 4 weeks, and then given Coenzyme Q10 dissolved with normal warm saline as much as 150 mg/kg per day through Mediflo 24G (*gavage*) was then checked on the 7th day.

Rats were given anesthesia with 30 mg/kg of intramuscular ketamine hydrochloride and 10 mg/kg of xylazine hydrochloride. After anesthesia administration, a flap elevation of 2 cm x 5 cm (ratio 1: 2.5) was performed with dissected areolar tissue above *panniculus carnosus* and *fascia profundus* of the *dorsal musculature* using the dorsal musculature the "flap McFarlane" method. The formed flap was laid back over the donor wound that has been covered with a transparent dressing to avoid vascular encounters between the flap and the donor surface. The end of the flap was then sutured using 4-0 nylon (Figure 1). For infection prophylaxis, 150 mg/kg of single-dose Ampicillin was administered subcutaneously to all groups. Specimens for groups I, II, and III were taken at day 7.1 x 1 cm at the distal boundary of the flap pedicle.

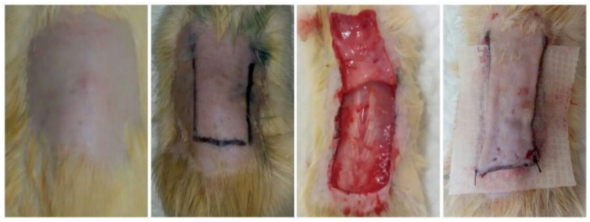

Figure 1. Flap elevation process. Shaved ridges, 5x2 cm incision design, incision, and flap elevation, then applied tulle between flap and bed wound and fixed on the distal flap with one stitch.

Coenzyme Q10 oral

This study used Ubidecarenone coenzyme Q10 150 mg (Blackmores, Australia). The preparation was then dissolved with sterile aquadest and administered orally with a *gavage* system at a dose of 150 mg/kg/day (Figure 2). Determining the doses was obtained from studied before by Lee et al.[11], that showed the treatment of coenzyme Q10 with dosage 150 mg/day had anti-inflammatory effect on interleukin-6 (IL-6), and antioxidization effect.

Figure 2. Giving oral coenzyme Q10

Capillary density

The number of capillaries in this study is the number of capillaries in each field of view on histological examination using hematoxylin-eosin staining. Capillary density was assessed by measuring the number of capillaries in 10 planes of view on each preparation and expressed as the average number of capillaries per square millimeter. Three independent reviewers analyzed under 400x magnification blinded.

Vascular endothelial growth factor (VEGF) expression

Vascular endothelial growth factor (VEGF) is the most important growth factor in adult tissue that undergoes physiological and angiogenic pathogenesis in chronic inflammation, wound healing, tumor, and diabetic retinopathy.[12] For VEGF expression examination, immunohistochemical staining technique was used. This study used VEGFA antibody reagents in rat hosts (VEGF/1063 Novusbio®). Readings by reviewers using light microscope (Nikon H600L) blindly using a 400x magnification microscope at 10 sites and counted all the positive cells for VEGF antibodies, whether the paint intensity was weak, medium, or strong. Cells that express VEGF will be brownish in the vicinity of the lumen and endothelial cell cytoplasm. The cells can be spindle-like fibroblasts up to cobblestone (round-oval). Then calculated the mean percentage of VEGF expression from 5 fields of view. Subsequently, multiplication between percentage score of immunoreactive cell/area with color intensity score was count.

P-ISSN 2089-6492; E-ISSN 2089-9734 | DOI: 10.14228/jprjournal.v10i1.345

Statistical analysis

For comparison of histopathological data SPSS 15.0 statistical package software was used. The distributions of the continuous variables were tested using the Kolmogorov-Smirnov test. For the capillary's density, comparisons of continuous variables between 2 groups were performed by independent T-test. For the expression of VEGF, the Kruskal Wallis test also found at least one difference between the three groups (P = 0.003; 95% CI) in terms of VEGF intensity. Therefore, a Mann-Whitney test was conducted to test each pair of groups. P values of less than 0.05 were considered statistically significant at 95% confidence interval.

RESULTS

The number of capillaries was examined by hematoxylin-eosin staining of the biopsy section and was assessed by an independent reviewer at 10 sites under 400x enlargement. Group I (control) histological images, group II (inhaled and flap nicotine), and group III (inhaled nicotine, flap, and coenzyme Q10) were shown in Figure 3.

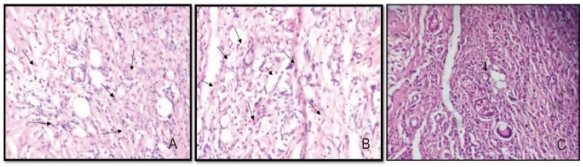

Figure 3. Histological features of specimens with hematoxylin-eosin staining, 400x enlargement. (a) Capillaries in group I (control); (b) Capillaries in group II (inhaled and flap nicotine); and (c) Capillaries in Group III (inhaled nicotine, flap, and coenzyme Q10)

Table 1 shows capillary number data on random skin flap in group I (control), group II (inhaled and flap nicotine), and group III (inhaled nicotine, flap, and coenzyme Q10).

Table 1. The number of capillaries in the skin random flap

Group I	Group II	Group III	P-value
5.33 ± 1.323	5.89 ± 0.782	7.78 ± 2.587	0.007

The mean number of capillaries in group I (control) random skin flap was 5.33 with standard deviation ± 1.323, in group II (inhaled and flap nicotine) was 5.89 with standard deviation ± 0.782, and in group III (inhaled nicotine, flap, and coenzyme Q10) was 7.78 with standard deviation ± 2.587.

In Kolmogorov-Smirnov test obtained P > 0.05, which means no significant difference between the data to be tested with normal raw data. It could be expressed the data of capillary amount on random skin flap in group I (control), group II (nicotine inhalation and flap), and group II (inhaled nicotine, flap, and coenzyme Q10) were normally distributed.

ANOVA test results obtained P-value of 0.007 (P < 0.05) showed a significant difference in the number of capillaries in the random skin flap between group I (control), group II (inhalation and flap nicotine), and group II (inhaled nicotine, flap, and coenzyme Q10) (Table 1).

Comparison of the mean number of capillaries in random skin flap group I (control), group II (inhaled and flap nicotine), and group II (inhaled nicotine, flap, and coenzyme Q10) were described in Figure 4.

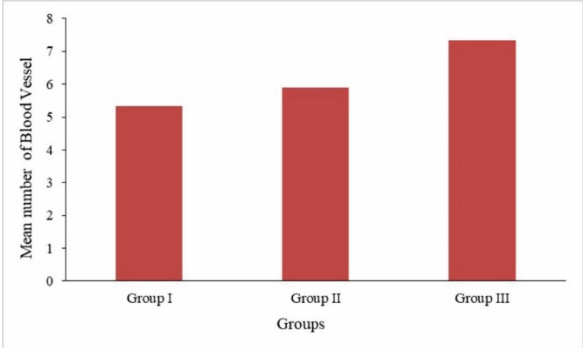

Figure 4. Average number of capillaries in random skin flap in group I (control), group II (inhaled and flap nicotine), and group II (inhaled nicotine, flap, and coenzyme Q10)

T independent tests were performed for each pair of tested groups. There was no significant difference (P = 0.733, 95% CI) between the mean number of blood vessels in group I (5.33 ± 1.323) and the mean number of blood vessels in group II (5.89 ± 0.782). However, significant differences (P = 0.008; 95% CI) were obtained between the mean number of blood vessels in group I (5.33 ± 1.323) and the mean number of blood vessels in group III (7.78 ± 2.587). In addition, significant differences (P = 0.024; 95% CI) were obtained between the average number of blood vessels in group II (5.89 ± 0.782) and the mean number of blood vessels in group III (7.78 ± 2.587). Thus, the average blood vessels in group III were significantly greater than in groups I and II.

P-ISSN 2089-6492; E-ISSN 2089-9734 | DOI: 10.14228/jprjournal.v10i1.345

Histological images of group I (control) VEGF expression, group II (inhaled and flap nicotine), and group II (inhaled nicotine, flap, and coenzyme Q10) were shown in Figure 5.

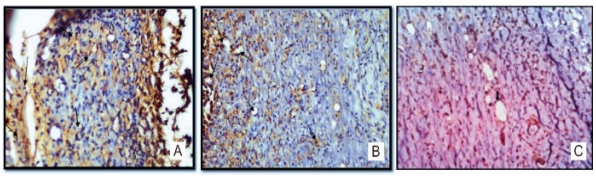

Figure 5. Histological features of specimens with immunohistochemical staining and light microscopy. (a) VEGF expression in group I (control); (b) VEGF expression in group II (nicotine and flap); and (c) VEGF expression in group III (nicotine, flap, and coenzyme Q10**)**

Table 2 showed data on the amount of VEGF expression in random skin flap in group I (control), group II (inhaled and flap nicotine), and group II (inhaled nicotine, flap, and coenzyme Q10).

Table 2. Intensity of VEGF expression on *random skin flap*

Intensity of VEGF expression		
Group I	**Group II**	**Group III**
+2	+2	+3
+2	+2	+2
+2	+2	+2
+2	+2	+3
+1	+2	+3
+2	+2	+2
+2	+2	+2
+2	+2	+3
+2	+2	+3

The Kruskal Wallis test also found at least one difference between the three groups (P = 0.003; 95% CI) in terms of VEGF intensity. Therefore, a Mann-Whitney test was conducted to test each pair of groups.

In the Mann-Whitney test, there were no significant differences (P = 0.317, 95% CI) between VEGF intensities in groups I and II. However, significant differences were found in the intensity of VEGF (P = 0.009, 95% CI) in groups I and group III. In addition, significant differences were also observed in VEGF intensity (p = 0.011, 95% CI) in groups II and III. Thus, the intensity was stronger on the subject with coenzyme Q10 compared with the other two groups.

DISCUSSION

Data and analysis in this study showed a significant difference in the number of arteries per field and VEGF intensity between group II and group III. Together, both groups received the same dose of nicotine inhalation and made random flaps in the same manner and technique. However, group III received coenzyme Q10 orally, while group II did not get the preparation.

This result can be explained by looking at the role of VEGF in the wound. In wounds, VEGF has a significant role. Inflammation and local conditions in wounds induce VEGF to relieve tissue hypoxia and metabolic deficiency by promoting angiogenesis and endothelial cell function. The Coenzyme Q10 and VEGF linkages were found by Can et al.[13] who examined coenzyme Q10 on flap viability. It was explained that elevated angiogenesis was characterized by high intensity of VEGF. This study was consistent with the findings in the above study as there was a significantly greater number of blood vessels in the group receiving coenzyme Q10 doses than those who did not receive a coenzyme dose of Q10.

According to Forrest et al.[14] administering 2 mg/kg BW/day of nicotine injection for four weeks can significantly decrease capillary blood flow, distal perfusion of random pattern skin flap in mice. Other studies mention similar things and that there is no difference between inhalation of nicotine by cigarette smoke and inhalation by intravenous administration.[15]

This study did not show any significant difference in blood vessel count and VEGF expression between the group receiving the nicotine dose and the group not receiving the nicotine dose prior to the random flap. According to the reference, this may result from a shorter nicotine delivery than nicotine in previous studies. In addition to the copolymerization obtained in conjunction with a study conducted by Cooke and Bitterman[16] explained the angiogenesis regulatory mechanisms via the cholinergic pathway associated with ischemic tissue conditions acetylcholine receptors would occur. After exposure to acute nicotine, endothelial cells secrete nicotinic cholinergic receptor receptors (nAChR). Nicotine entering the bloodstream will bind to these receptors and stimulate endothelial cells to proliferate, migrate and form new capillaries.

P-ISSN 2089-6492; E-ISSN 2089-9734 | DOI: 10.14228/jprjournal.v10i1.345

As far as our study, this research is the first study in Indonesia about testing coenzyme Q10 as a protective agent against tissue ischemia. Network ischemia is tested in vivo in a random flap created after exposure to nicotine. The advantages of this study are the direct comparison of the effects of coenzyme Q10 in vivo in mice with the same precondition, given inhalation of nicotine and random flap. This study also confirmed a previous study that mentions the positive effects of coenzyme Q10 on angiogenesis in ischemic tissue.

The lack of this study is twofold. Firstly, this study did not observe specifically the viability of random flap clinically, so that the variables used as the basis for conclusion are histopathologic observations alone. Second, coenzyme Q10 works by inhibiting lipid peroxidase and through NO production lines giving protective angiogenesis effects tissue, therefore coenzyme Q10 after exposure to ischemic induction by nicotine may not have a more optimal effect than when given nicotine exposure.

Histopathological evidence of coenzyme Q10 increases the viability of random flaps by improving the amount of capillary density and VEGF expression. However, the clinical outcomes have to studied further to develop coenzyme Q10 function for applying on significant flaps.

CONCLUSION

Coenzyme Q10 increases the viability of random flap by increasing the number of capillaries and VEGF expression.

ACKNOWLEDGEMENT

The authors give thanks to Airlangga University for the contribution of this research.

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REFERENCES

1. Budd ME, Hovsepian R, Basseri B, Evans GRD. Flaps. In: Guyuron B, Eriksson E, Persing JA, editors. Plastic Surgery: Indications and Practice, Philadelphia: Saunders Elsevier, 2009, 67–78.
2. Prasetyono TOH. Flap: penuntun dasar dalam ilmu bedah plastik [Flap: a basic guide to plastic surgery]. Jakarta: Sagung Seto, 2011.
3. World Health Organization (WHO). WHO global report on trends in prevalence of tobacco smoking 2015. Switzerland: WHO, 2015. URL:// https://apps.who.int/iris/bitstream/10665/156262/1/9789241564922_eng.pdf. (4 February 2018).
4. Gill JF, Yu SS, Neuhaus IM. Tobacco smoking and dermatologic surgery. J Am Acad Dermatol. 2013;68(1):167–172.
5. Campos JHO, Gomes HC, dos-Santos WLC, Cardeal M, Ferreira LM. Effect of nicotine treatment and withdrawal on random-pattern skin flaps in rats. Exp Toxicol Pathol. 2008;60(6):449 – 452.
6. Black CE, Huang N, Neligan PC, Levine RH, Lipa JE, Lintlop JE, et al. Effect of nicotine on vasoconstrictor and vasodilator responses in human skin vasculature. Am J Physiol Regul Integr Comp Physiol. 2001;281:R1097–R1104.
7. Leite MT, Gomes HC, Percario S, Russo CRA, Ferreira LM. Dimethyl sulfoxide as a block to the deleterious effect of nicotine in a random skin flap in the rat. Plast Reconstr Surg. 2007;120:1819–1822.
8. Heitzer T, Yla-Herttuala S, Luoma J, Kurz S, Munzel T, Just H, et al. Cigarette smoking potentiates endothelial dysfunction of forearm resistance vessels in patients with hypercholesterolemia: role of oxidized LDL. Circulation. 1996;9:1346–53.
9. Forsmark-Andree P, Persson B, Radi R, Daller G, Ernster L. Oxidative modification of nicotinamide nucleotide transhydrogenase in submitochondrial particles: effect of endogenous ubiquinol. Arch Biochem Biophys. 1996;336(1):113–120.
10. Mitchell P. Protonmotive redox mechanism of the cytochrome B–c1 complex in the respiratory chain:

P-ISSN 2089-6492; E-ISSN 2089-9734 | DOI: 10.14228/jprjournal.v10i1.345

protonmotive ubiquinone cycle. FEBS Lett. 1975;56(1):1 – 6.
11. Lee, BD, Huang, YC, Chen SJ, Lin PT. Effects of coenzyme Q10 supplementation on inflammatory markers (high-sensitivity C-reactive protein, interleukin-6, and homocysteine) in patients with coronary artery disease. Nutrition. 2012;28(7-8):767-772.
12. Kumar V, Abbas AK, Fausto N. Tissue renewal, repair, regeneration. In: Kumar V, Abbas A, Fausto N, et al., editors. Robbins and Cotran Pathologic Basis of Disease, 8th ed. Philadelphia: Saunders Elsevier, 2010, 87-118.
13. Can A, Temel M, Dokuyucu R, Mutaf M. The effect of coenzyme Q10 (Ubiquinone) on random pattern skin flap survival in rat model. Ann Plast Surg. 2015;77(2):e9 – e14.
14. Forrest CR, Pang CY, Lindsay WK. Dose and time effects of nicotine treatment on the capillary blood flow and viability of random pattern skin flaps in the rat. Br J Plast Surg. 1987;40(3):295 – 299.
15. Rotenberg KS, Miller RP, Adir J. Pharmacokinetics of nicotine in rats after single-cigarette smoke inhalation. J Pharm Sci. 1980;69(9):1087-90.
16. Cooke JP, Bitterman H. Nicotine and angiogenesis: a new paradign for tobacco-related disease. Ann Med. 2004;26:33-40.

P-ISSN 2089-6492; E-ISSN 2089-9734 | DOI: 10.14228/jprjournal.v10i1.345