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# Effect of *Platelet Rich Plasma* to Increasing of *Endothelial Nitric Oxide Sinthetase* (eNOS), Angiogenesis, and Decreasing of Necrotic Tissue in Acute Ischemic because of Vascular Lesion in *Oryctolagus cuniculus* Lower Extremity

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**ABSTRACT**

Periferal Arterial Occlusion Disease is obstructive artery disease in lower extremity because of decreasing arterial flow when activity, and in advanced case, it happened also in resting. Because the occlusion, there was dereasing blood flow to the distal with the effect of hypoxia. This situation will stimulate *Vascular Endothelial Growth Fator* (VEGF). VEGF will stimulate angiogenesis. *Endothelial Nitric Oxide* (eNOS) also have important impact in angiogenesis process. Platelet also have impact, when the platelet is activated and aggregated by hypoxia, can stimulate VEGF to start angiogenesis cascade. Analyze PRP effect in eNOS expression, angiogenesis, and necrotic area in ischemic condition because vascular lesion. This study is an experimental research. The study samples are *orcytolagus cuniculus* with weight 700-1000 grams, male, age 8-10 months with acute ischemic in lower extremity because vascular lesion. The samples will divide in 2 groups, one groups have PRP injection and the other one not. Every group will divide again to 4 groups with different ligation time, 2,4,6, and 8 hours. There was significant difference in eNOS expression, with PRP group has better outcome in 2,4,6, and 8 hours ligation and the correlation is strong and directly proportional with ligation duration. There was significant difference in angiogenesis, with PRP group has better outcome in 2,4,6, and 8 hours ligation and the correlation is strong and directly proportional with ligation duration. There was significant difference in necrotic area, with PRP group has better outcome in 2,4, and 6 hours, but didn't significant in 8 hours ligation and the correlation is strong and inversely proportional with ligation duration. PRP significantly increasing eNOS expression and angiogenesis, and decreasing necrotic area in inchemic lesion because vascular lesion.

## 1. INTRODUCTION

Peripheral Oclusion Arterial Disease is the disease with decreasing of arterial flow in extremity inferior. This disease start with asymptomatic, and and the abnormality can be seen in non invasive technique. When this disease become symptomatic, this classic syptoms is *claudicatio intermitten*. This disease can make hypoxia problem in distal tissue. This condition will increase signalling to release of *Vascular Endothelial Growth Factor* (VEGF). VEGF will make angiogenesis in ischemic tissue (Rutherford, 2010). Nitric oxide (NO) is a main factor in vasomotor tonus, angiogenesis, and vascular permeability. NO is made from NO sintase (NOS), and made from 3 isoform: neuronal NOS (nNOS), inducible NOS dan endotelial NOS (eNOS) (Ha, et al., 2016). eNOS effect in angiogenesis is proven with Luciano et al, who do experimental with mice in ischemic condition and did not have enough Akt1. Mice with low level of Akt1 show low level of eNOS. This effect make disturbance in blood flow after ischemia. Permeability, who affect by VEGF and mobilization of precursor will disturb by low level of eNOS. Platelet Rich Plasma (PRP) is from plasma fraction in blood autolog who have platelet. Platelet will agregrated and activated because of trauma and hipoxia who will release growth factor, with one of them is VEGF to stimulate angiogenesis cascade. This research will give exogenous PRP in rabbit with ischemic in inferior extremity because vascular lesion for calculate the effect of Platelet Rich Plasma (PRP) to endothelial NOS (eNOS) expression, so can increasing angiogenesis in ischemic acute condition in lower extremity because vascular lesion.

## 2. Methods

### *2.1 Experimental Study and Data Sources*

This research will use rabbit Orictolagus cuniculus. We will divide into 4 group with different duration of duration of ligation, 2,4,6, and 8 hours. Every group consist of 6 rabbit. After that, each group devide into 2 group. First group will have PRP injection after ligation, and second group will have NaCl 0,9% injection after ligation. We take the data 1 week after injection. We have 3 dependent variable: Angiogenesis, eNOS expression, and necrotic area. We see angiogenesis from the neovascularization in microscopic examination. From eNOS expression we see the results from percentage, and we calculate the necrotic area with vernier caliper. From the results, we analyze and do correlation and comparation study between variable.

## 3. Results

### *3.1 eNOS Expression*

We have 8 groups with data of eNOS expression. We did correlation test and we found the strong and linear correlation between PRP injection and duration of ligation with p score > 0,01. We also do comparation study in every group with 2,4,6, and 8 hours ligation. We found there was significant difference with PRP group have higher level of eNOS expression with p score <0,005.

**Table 1:** eNOS Expression

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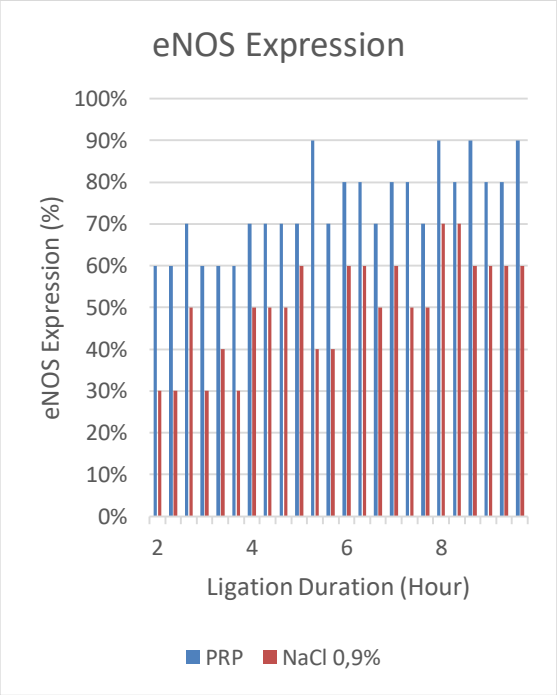
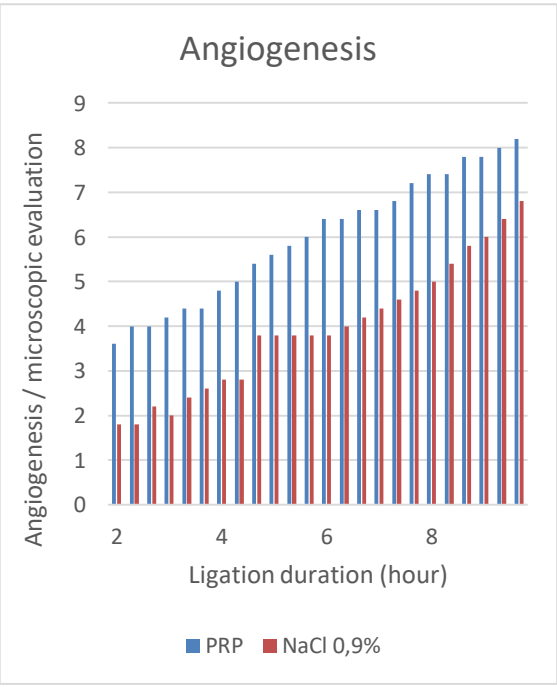



### *3.2 Angiogenesis*

We have 8 groups with data of angiogenesis. We did correlation test and we found the strong and linear correlation between PRP injection and duration of ligation with p score > 0,01. We also do comparation study in every group with 2,4,6, and 8 hours ligation. We found there was significant difference with PRP group have higher level of angiogenesis with p score <0,005.

**Table 2:** Angiogenesis

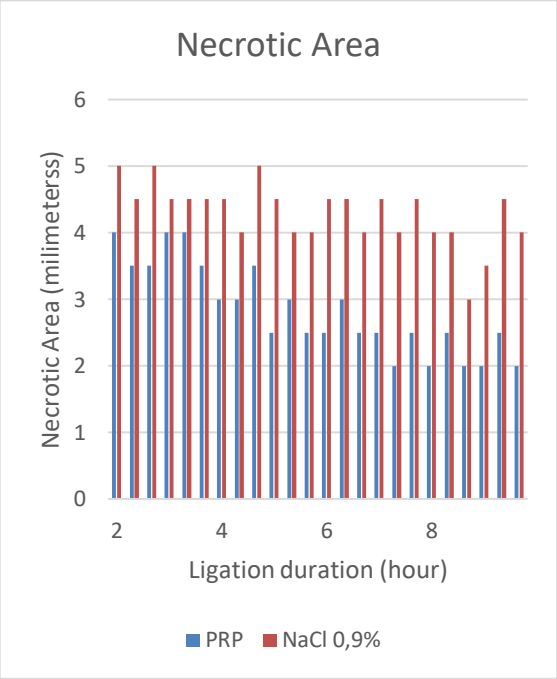



### *3.3 Necrotic Area*

We have 8 groups with data of necrotic area. We did correlation test and we found the strong and opposite

correlation between PRP injection and duration of ligation with p score > 0,01. We also do comparation study in every group with 2,4,6, and 8 hours ligation. We found there was significant difference with PRP group have lower level of necrotic area with p score <0,005. But in 8 hours ligation group, there was no significant difference with PRP group and NaCl group.

**Table 3:** Necrotic Area




**4. Discussion**

From this experimental study we found PRP can give significant results in increasing eNOS expression, angiogenesis, and decreasing necrotic area. This results show the PRP effect. PRP have a lot of growth factor, and one of them is *vascular endothelial growth factor* (VEGF). VEGF is a key factor of oxygen hungry-cells to promote angiogenesis. When ischemic happens, there will be metabolism change and give signal for VEGF production.

PRP also has antimicrobial effect, help wound healing, and repair the necrotic tissue. But from this experience, after 6 hours, there is no significant effect in necrotic area because muscle can't regeneration well after 6 hours.

**5. Conclusion**

1. There is significant difference of eNOS expression between PRP group and NaCl group in 2,4,6, and 8 hours ligation group.
2. There is significant difference of angiogenesis between PRP group and NaCl group in 2,4,6, and 8 hours ligation group.
3. There is significant difference of necrotic area between PRP group and NaCl group in 2,4,6, and 8 hours ligation group.
4. There is strong correlation and directly proportional in eNOS expression and duration of ligation in PRP group
5. There is strong correlation and directly proportional in angiogenesis and duration of ligation in PRP group

6. There is strong correlation and opposite proportional in necrotic area and duration of ligation in PRP group

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