

## CHAPTER 5

### RESULT

#### 5.1. Atherosclerotic Induction

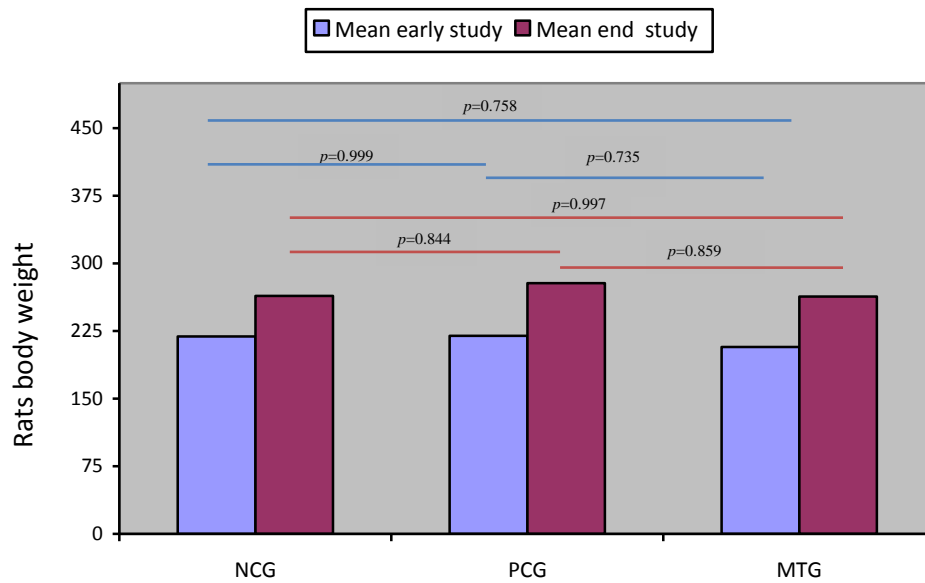
The study sample comprised 20 SD rats, of which 6 rats for negative control group, 6 rats for positive control group, and 8 rats for MSC treatment group.

Comparisons of baseline body weight are given in Table 5.1.

**Table 5.1.** Baseline characteristic of sample

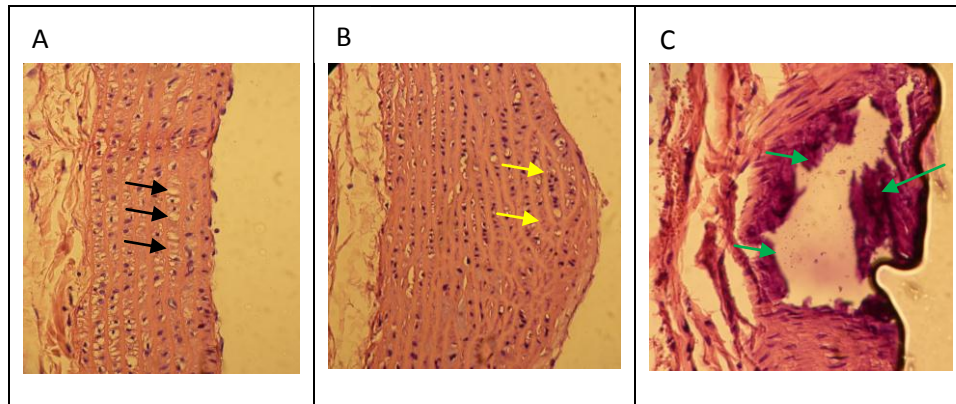
	Negative control group		Positive control group		MSC treatment group	
	Body weight early experiment (gram)	Body weight end experiment (gram)	Body weight early experiment (gram)	Body weight end experiment (gram)	Body weight early experiment (gram)	Body weight end experiment (gram)
Sample 1	172	240	225	260	235	236
Sample 2	210	270	278	277	136	290
Sample 3	240	253	209	224	228	280
Sample 4	230	280	208	252	246	280
Sample 5	223	290	200	261	204	250
Sample 6	239	250	198	272	203	245
Sample 7	-	-	-	-	196	269
Sample 8	-	-	-	-	210	255
Mean	219	263.83	219.67	278	207.25	263.125

The data distribution of sample body weight of all groups was normal. The comparisons of sample body weight at early study were statistically not significant ( $p \geq 0.05$ ), as showed in Figure 5.1. It means that each group did not have a different body weight. At the end of study, their body weight increased, but the result of their data distribution was normal and their comparison was not different too. Oneway anova test was used to compare the body weight.



**Figure 5.1.** Body weight comparison of sample at early and end of the study. NCG, negative control group; PCG, positive control group; MTG, MSC treatment group.

All groups were maintained under controlled environment (28-32<sup>0</sup>C), placed individually, enough ventilation, and water ad libitum. For negative control groups, they were fed with the standard diet all along the study. While, positive control and MSC treatment groups were induced to be atherosclerotic according to the methodology as mentioned in chapter 4. Result of atherosclerotic induction was identified by hematoxylin and eosin staining. It is shown in Figure 5.2.

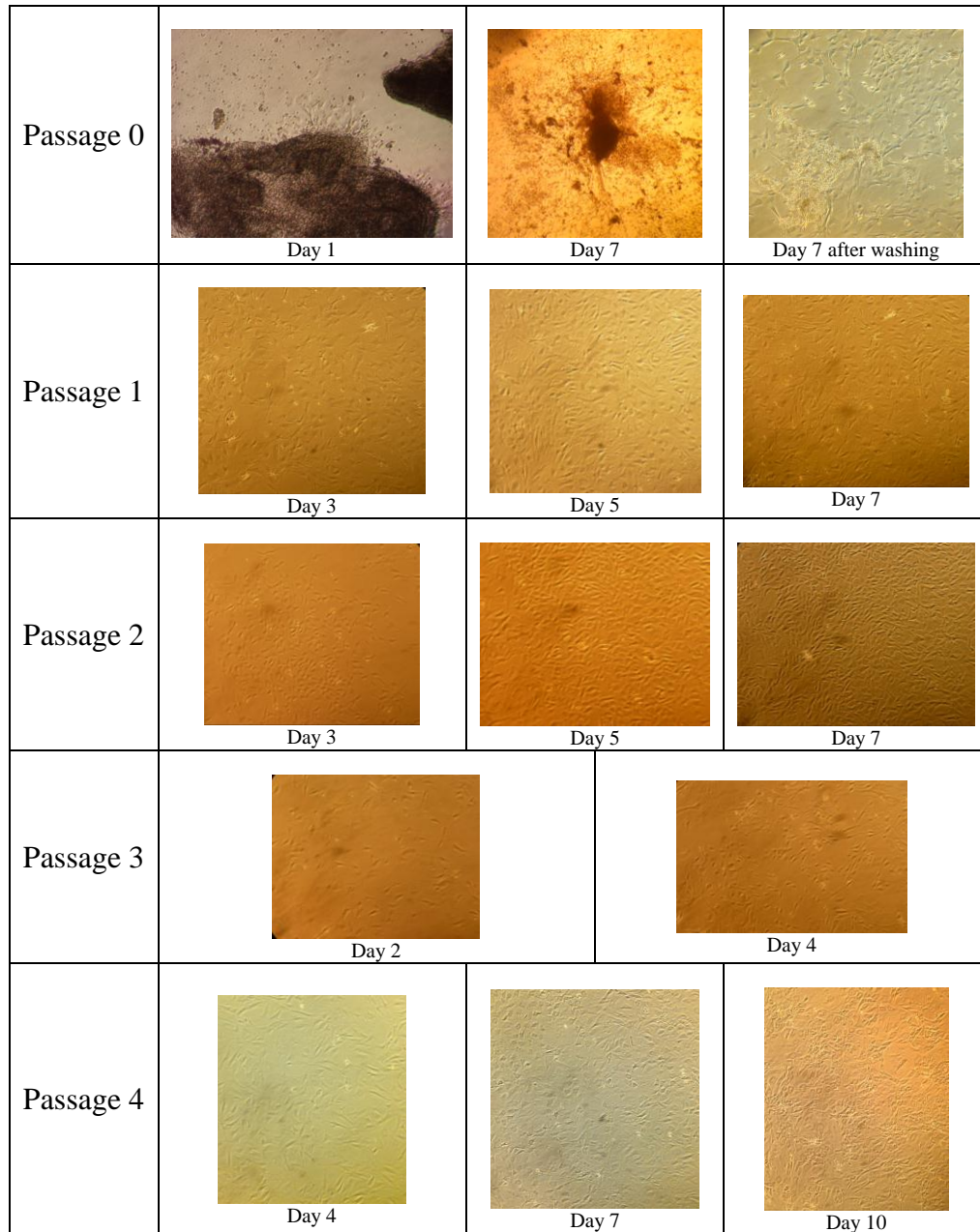


**Figure 5.2.** Hematoxylin and eosin staining result in abdominal rats after 12 (A), 14 (B), and 17 (C) weeks atherosclerotic induction. Black arrows show foam cell. Yellow arrows show hyperplasia of smooth muscle. Green Arrows show calcification plaque.

Hematoxylin and eosin staining showed foam cells in abdominal rats after 12 weeks and calcification plaques were formed in the aortas after 17 weeks of the high-fat diet. Hence, we conducted 18 weeks for atherosclerotic induction. After atherosclerotic calcification plaque occurred, negative control group was fed with the standard diet. While, MSC treatment group was injected with MSC intravenous and fed with the standard diet.

## 5.2. Mesenchymal Stem Cell Isolation and Culture

Umbilical tissue was obtained from 2 Sprague Dawley rats which 19-20 days pregnant, then it was processed according to methodology as mentioned in chapter 4. In this study, MSCs were cultured until passage 4 before they were injected to atherosclerotic SD rats. The comparison of MSC in tissue culture dish is shown in Figure 5.3.



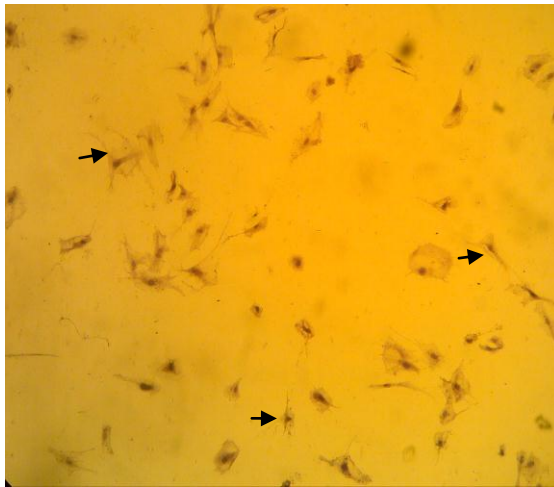
**Figure 5.3.** Passage 0 – 4 of MSC culture. Passage 0 showed that the culture was still mixed with debris. Passage 0 day 1 showed that MSCs starting growth from explant. Passage 1-4 showed that the culture were clean from debris and morphology cells were more homogen than passage 0.

### 5.3. Mesenchymal Stem Cell Identification

For MSC identification, fibroblast like stem cells were stained their Stro-1 surface marker by immunocytochemistry, and cultured in osteogenic differentiation media for 23 days, followed by alizarine red staining.

#### 5.3.1. Stro-1 Surface Marker Identification

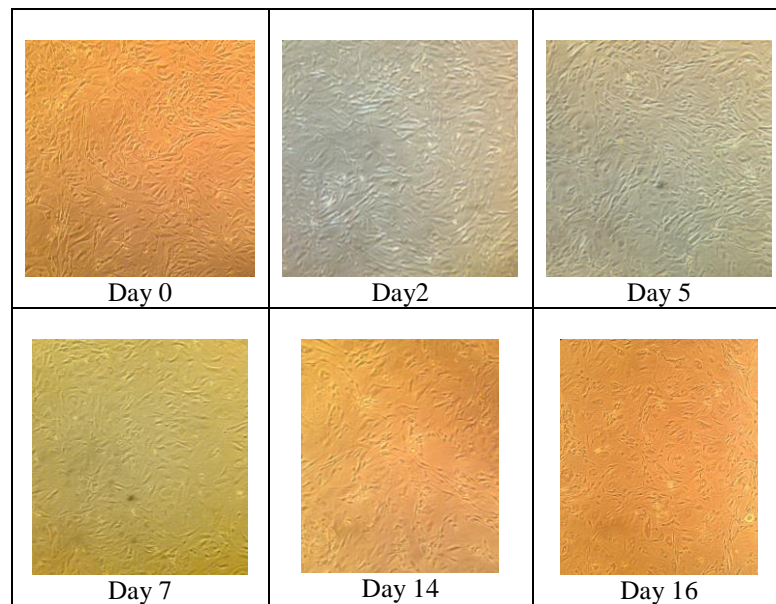
Stro-1 is one of the surface markers of MSC. By immunocytochemistry, it was expressed by fibroblas like stem cells. It means that fibroblas like stem cells found in the culture were mesenchymal stem cells.



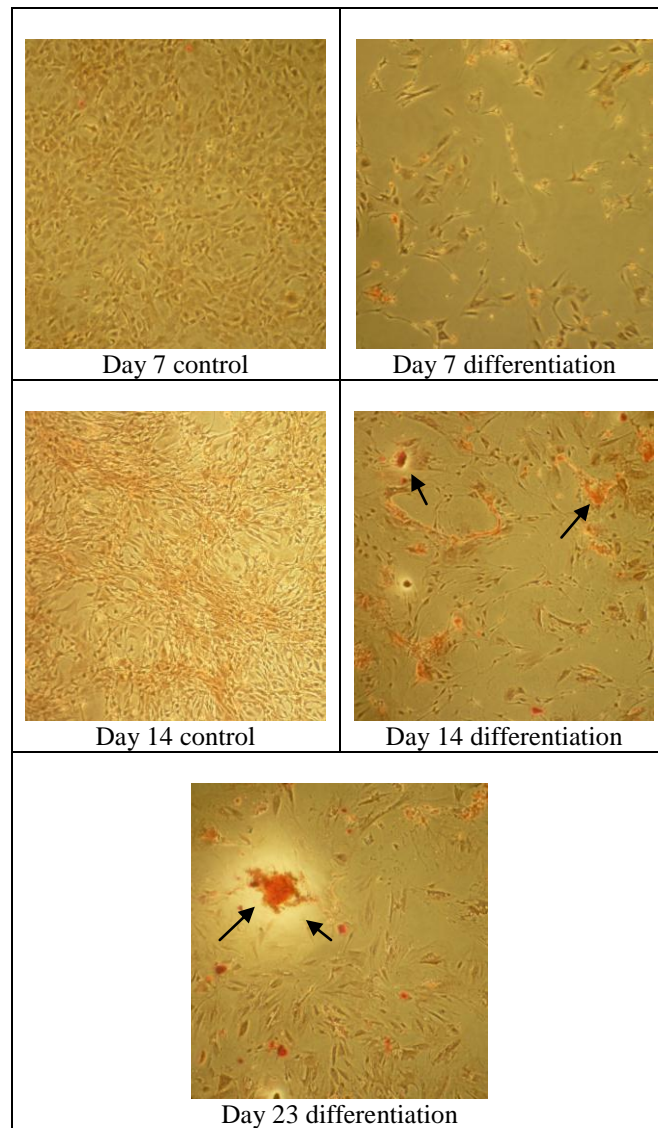
**Figure 5.4.** Immunocytochemistry MSC using Stro-1 antibody. Black arrows show Stro-1 expression

### 5.3.2. Osteoblas Differentiation of Mesenchymal Stem Cell

Fibroblast like stem cells were cultured in osteogenic differentiation media for 23 days. Morphology of cells which were cultured in osteogenic differentiation media is showed in Figure 5.5. There was no significant different in their morphology at the end of osteoblas induction. But, for the point of their proliferation capability, it seems to down slope, based on the reduction of confluent cell number when compared to control. To prove that there was calsium deposit, they were stained by alizarin red staining. Results of alizarin red staining were showed in Figure 5.6. Compared to the control, fibroblast like stem cell culture in osteogenic differentiation media showed calsium deposit. It proved that fibroblast like stem cells were MSC.



**Figure 5.5.** Process of MSC differentiation to osteoblas in tissue culture dish



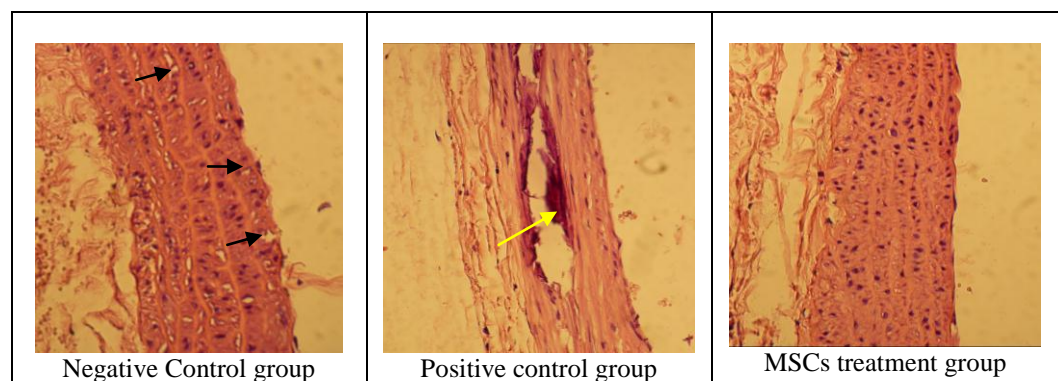
**Figure 5.6.** Alizarin red staining control and differentiation. Black arrows show calcium deposit.



## 5.4. Abdominal Aorta Staining

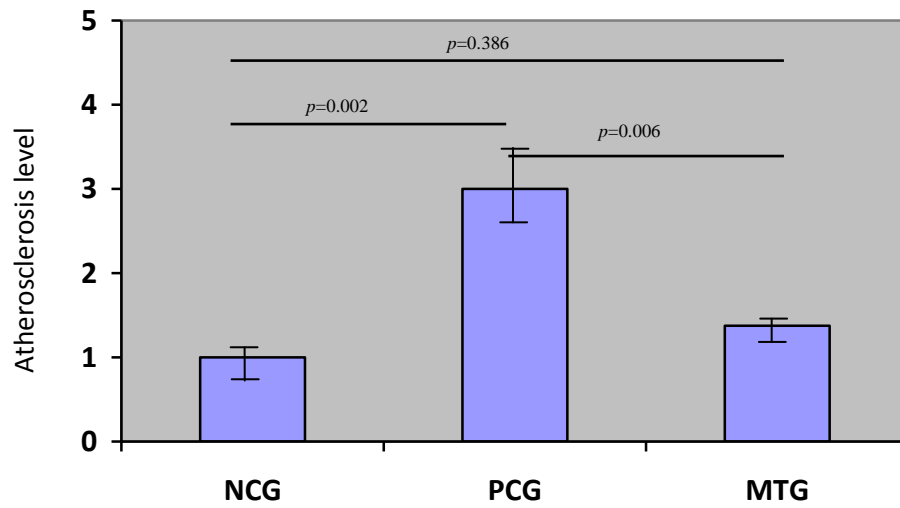
### 5.4.1. HE staining of abdominal aorta

At the end of study, abdominal aorta was stained by hematoxylin and eosin and identified by two pathology anatomy experts. Atherosclerosis plaque was obtained from this staining procedure. The result of comparison of each group is showed in Figure 5.7 and 5.8. Atherosclerosis plaque on MSC treatment group was lower than that of positive control group, and statistically significant ( $p=0.006$ ). That means intravenous injection of MSC ameliorated atherosclerosis plaque.



**Figure 5.7.** HE staining. Black arrows show foam cells. Yellow arrow shows calcification plaque.

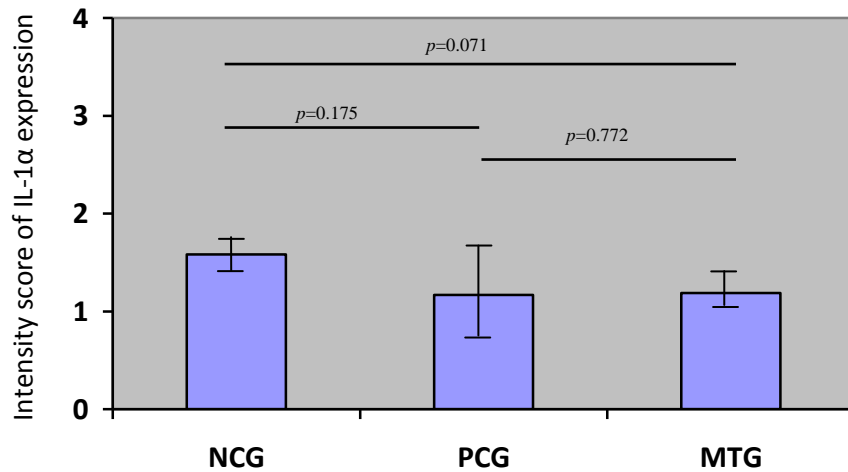




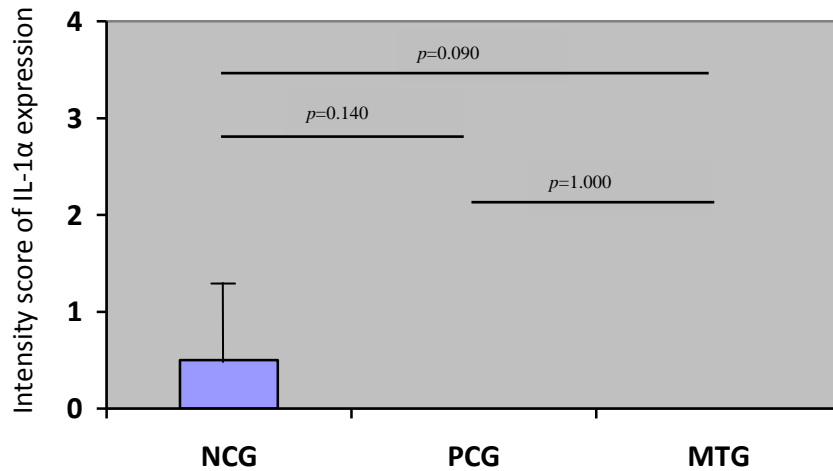
**Figure 5.8.** The comparison of the result of HE reading. NCG: Negative control group; PCG: Positive control group; MTG: MSC treatment group.

#### 5.4.2. Abdominal Aorta IL-1 $\alpha$ Expression

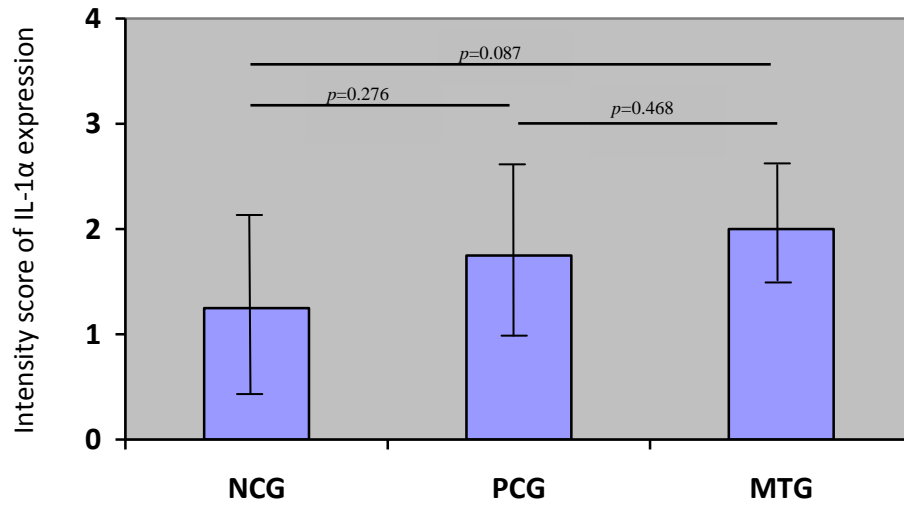
Abdominal aorta IL-1 $\alpha$  expression was measured by immunohistochemistry and quantified according to modified intensity score. The comparison of IL-1 $\alpha$  expression is showed in Figure 5.9, Figure 5.10, Figure 5.11, and Figure 5.12.



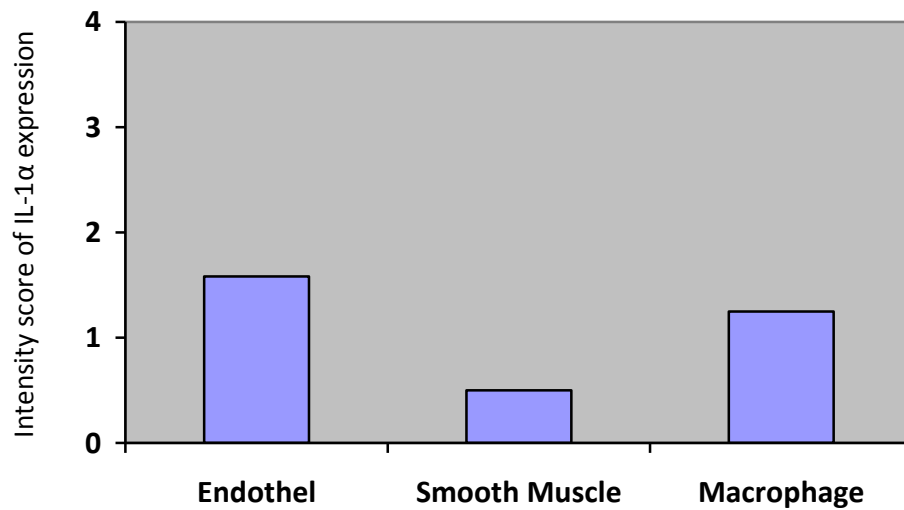
**Figure 5.9.** IL-1 $\alpha$  expression in endothel. NCG: Negative control group; PCG: Positive control group; MTG: MSC treatment group.



**Figure 5.10.** IL-1 $\alpha$  expression in smooth muscle. NCG: Negative control group; PCG: Positive control group; MTG: MSC treatment group.

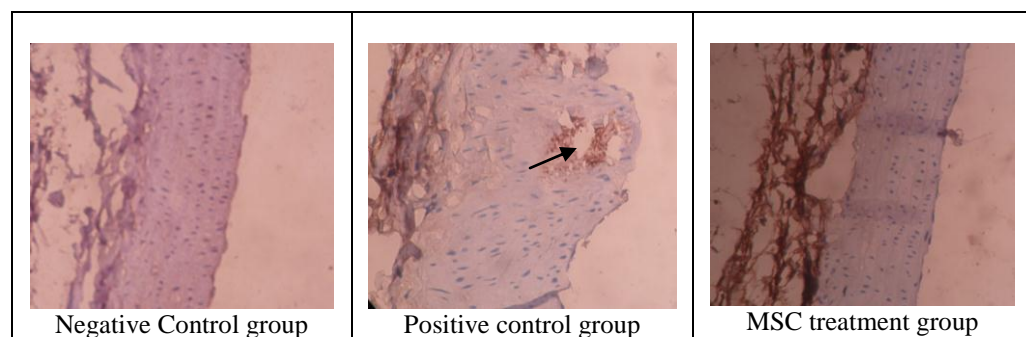


**Figure 5.11.** IL-1 $\alpha$  expression in macrophage. NCG: Negative control group; PCG: Positive control group; MTG: MSC treatment group.



**Figure 5.12.** IL-1 $\alpha$  expression in negative control group.

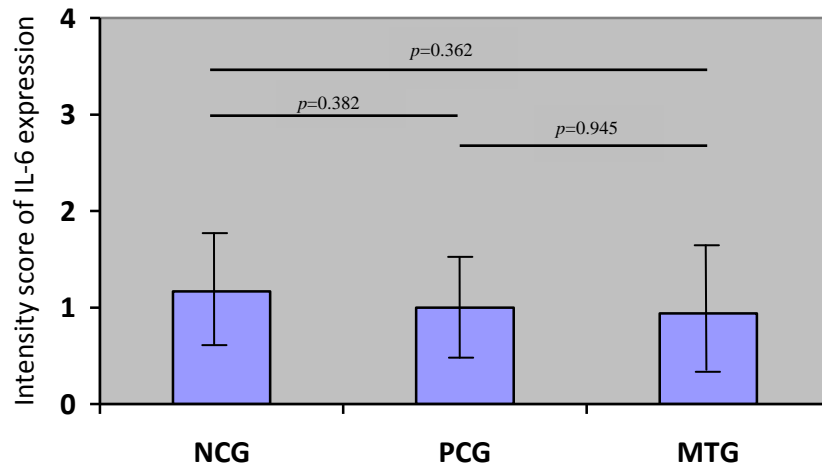
IL-1 $\alpha$  expression both in endothel and smooth muscle almost had the same pattern, where its expression in negative control group was higher than that of in positive control group and followed by its expression in MSC treatment group, but statistically not significant ( $p \geq 0.05$ ). Those pattern were different from its expression in macrophage, where it was higher in MSC treatment group than that of in positive control group and followed by its expression in negative control group, statistically not significant too ( $p \geq 0.05$ ). Both pattern are interesting, considering endothel and smooth muscle are tissue which are not mobile, different with macrophage which mobile across the tissue. Furthermore, mean of intensity score of smooth muscle cell was the lowest among all location. As we know that endothel is barrier of vessel wall and it always contact with material in lumen, whereas macrophage are mobile, those add an interesting result.



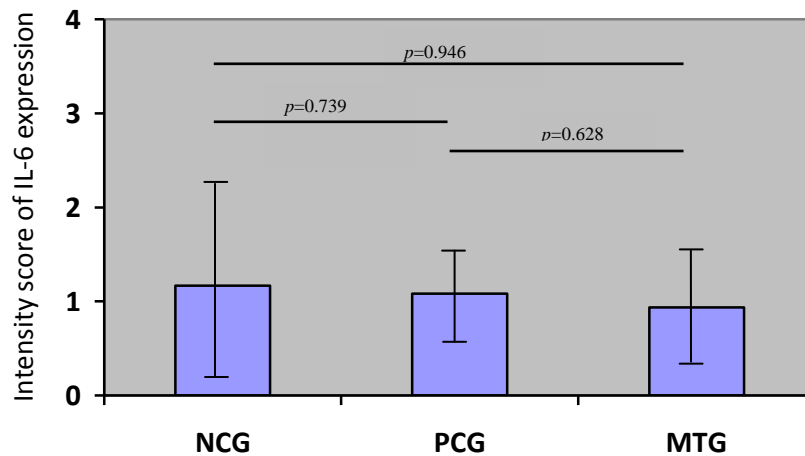
**Figure 5.13.** IL-1 $\alpha$  immunohistochemistry staining of abdominal aorta. Black arrow shows IL-1 $\alpha$  expression in calcification plaque.

### 5.4.3. Abdominal Aorta IL-6 Expression

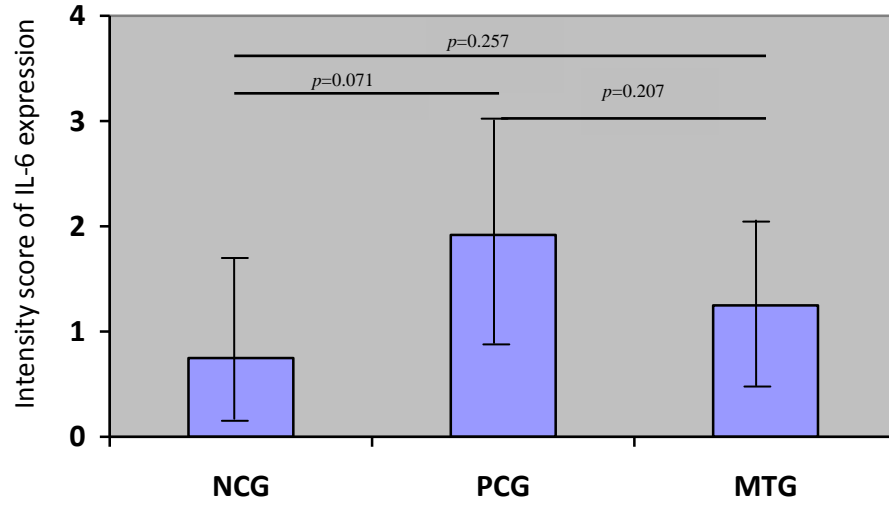
Abdominal aorta IL-6 expression was measured by immunohistochemistry and quantified according to modified intensity score. The comparison of IL-6 expression is showed in Figure 5.14, Figure 5.15, Figure 5.16, and Figure 5.17.



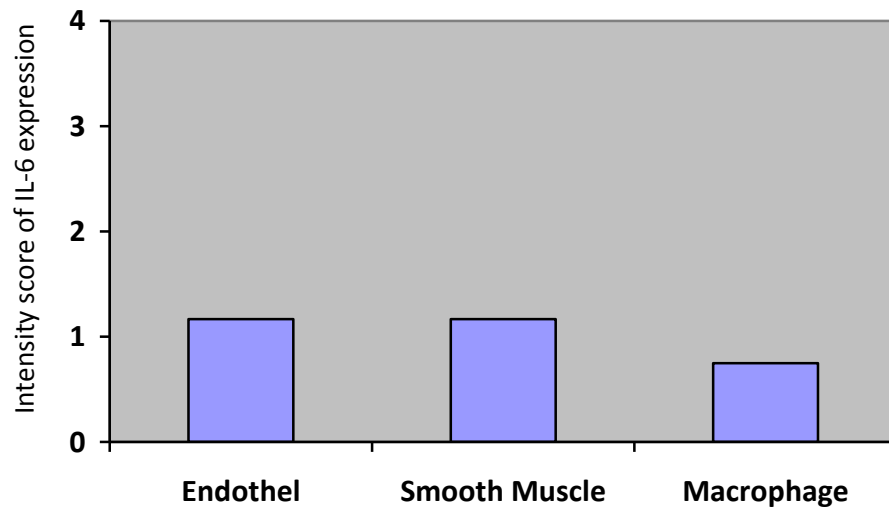
**Figure 5.14.** IL-6 expression in endothel. NCG: Negative control group; PCG: Positive control group; MTG: MSC treatment group.



**Figure 5.15.** IL-6 expression in smooth muscle. NCG: Negative control group; PCG: Positive control group; MTG: MSC treatment group.

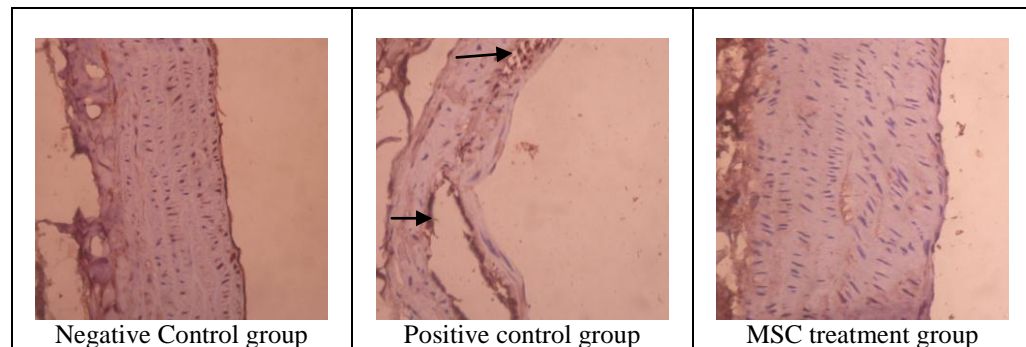


**Figure 5.16.** IL-6 expression in macrophage. NCG: Negative control group; PCG: Positive control group; MTG: MSC treatment group.



**Figure 5.17.** IL-6 expression in negative control group.

IL-6 expression in both endothel and smooth muscle had the same pattern, where its expression in negative control group was higher than that of in positive control group and followed by its expression in MSC treatment group, but statistically not significant ( $p \geq 0.05$ ). Those pattern were different from its expression in macrophage, where it was higher in positive control group than in MSC treatment group and followed by its expression in negative control group, statistically not significant too ( $p \geq 0.05$ ). Both pattern are also interesting, considering endothel and smooth muscle are tissue which are not mobile, different with macrophage which mobile across the tissue. Furthermore, another interesting result was mean of intensity score of endothel and smooth muscle relatively same, additionally the different mean among them was slight.

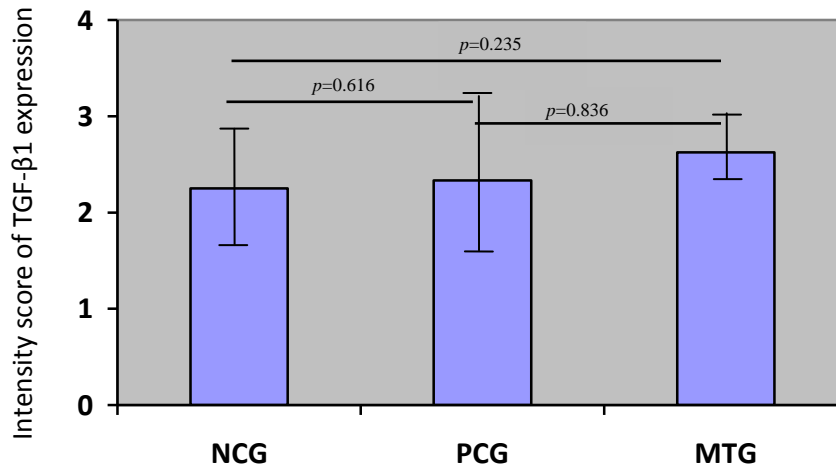


**Figure 5.18.** IL-6 immunohistochemistry staining of abdominal aortae. Black arrow shows IL-6 expression on calcification plaque.

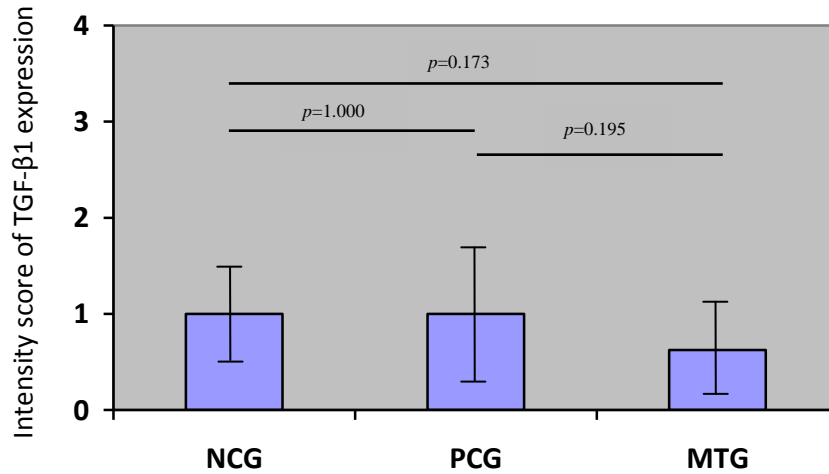


#### 5.4.4. Abdominal Aorta TGF- $\beta$ 1 Expression

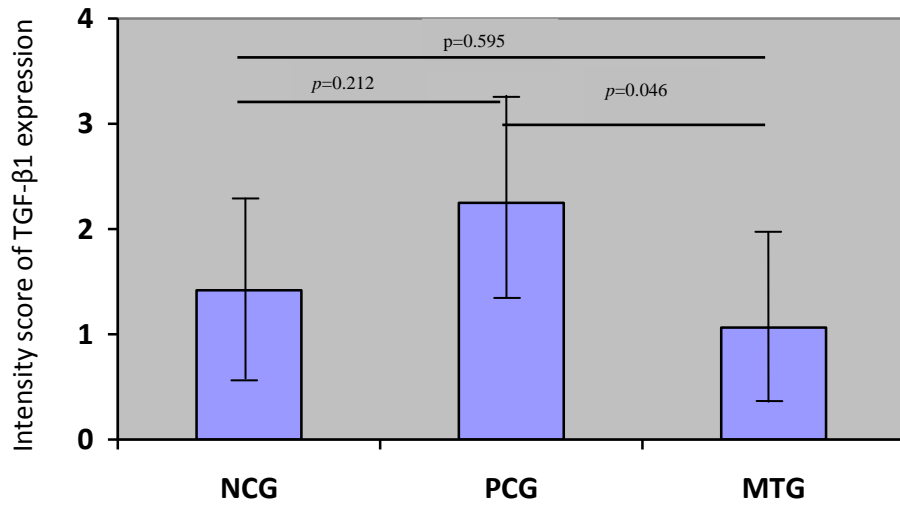
Abdominal aorta TGF- $\beta$ 1 expression was measured by immunohistochemistry and quantified according to modified intensity score. The quantification of TGF- $\beta$ 1 expression is showed in Figure 5.19, Figure 5.20, and Figure 5.21, and Figure 5.22.



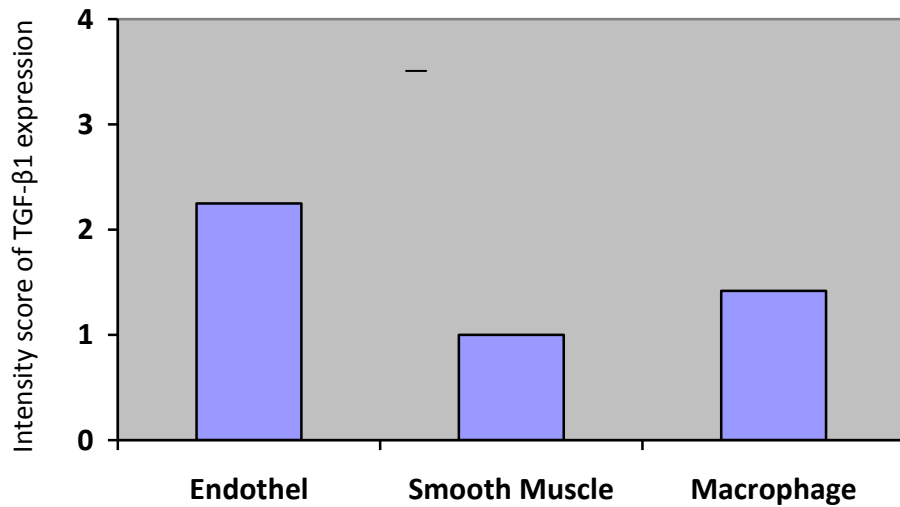
**Figure 5.19.** TGF- $\beta$ 1 expression in endothel. NCG: Negative control group; PCG: Positive control group; MTG: MSC treatment group.



**Figure 5.20.** TGF- $\beta$ 1 expression in smooth muscle. NCG: Negative control group; PCG: Positive control group; MTG: MSC treatment group.

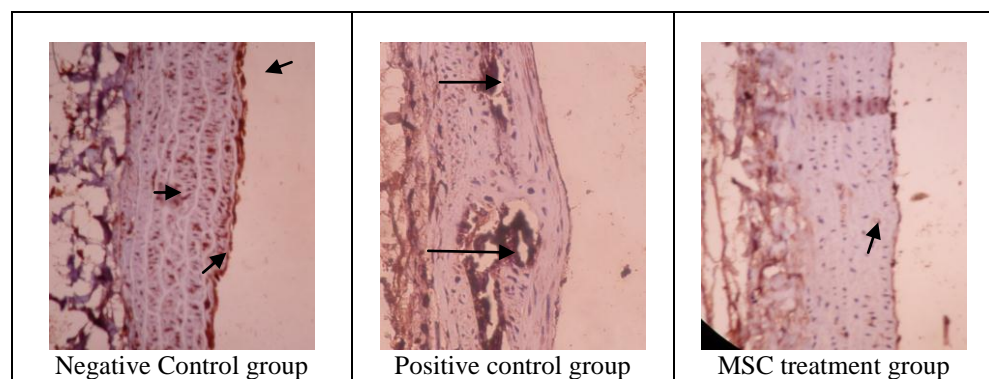


**Figure 5.21.** TGF-β1 expression in macrophage. NCG: Negative control group; PCG: Positive control group; MTG: MSC treatment group.



**Figure 5.22.** TGF-β1 expression in negative control group

TGF- $\beta$ 1 expression in almost all location in this study showed that its expression in positive control group was higher than that of in negative control group, but statistically not significant ( $p \geq 0.05$ ). Especially for TGF- $\beta$ 1 expression in MSC treatment group, it showed fluctuating pattern. Compare to the other groups, it was higher in endothel, but lower in smooth muscle and macrophage. Only its expression in macrophage which showed statistically significant lower in MSC treatment group compared to TGF- $\beta$ 1 expression in positive control group ( $p = 0.046$ ). All pattern are interesting, considering endothel and smooth muscle are tissue which are not mobile, different with macrophage which mobile across the tissue. Furthermore, when we observed mean of intensity score from all of groups, it showed interesting pattern as well. Smooth muscle have the lowest score than all of group.



**Figure 5.23.** TGF- $\beta$ 1 immunohistochemistry staining of abdominal aorta. Black arrows show TGF- $\beta$ 1 expression.