

CHAPTER IV

RESULTS

This chapter of result composed of five major parts which were served to examine the effects of vitamin C supplementation on change of endothelial function in diabetic rats. These five major parts were listed in followings:

Part 1. The antioxidant effects of vitamin C supplementation on metabolic changes

- body weight (BW)
- blood glucose (BG)
- glycosylated hemoglobin (HbA_{1c})
- plasma vitamin C
- tissue lipidperoxidation

Part 2. The antioxidant effects of vitamin C supplementation on leukocyte-endothelial interaction

Part 3. The antioxidant effects of vitamin C supplementation on hemodynamic change

- mean regional iris blood-flow perfusion
- systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MAP)

Part 4. Study relationship on iris blood-flow perfusion and leukocyte-endothelial cell interaction

Part 5. Histological study for transmigration of leukocyte in iris area

Part 1. The antioxidant effects of vitamin C supplementation on metabolic and hemodynamics changes

1.1 Metabolic changes

The tail vein injection of streptozotocin 55 mg/kg/BW into 200-250g Wistar Furth rat resulted in polydipsia, polyuria, polyphagia and hyperglycemia within 48 hours and showed persistent hyperglycemia through out the experiment. In the present study, the criteria used for diabetic rats was the blood glucose level that had to be higher than 250 mg/dl.

Blood glucose, body weight and glycosylated hemoglobin of control, STZ and STZ-Vit C rats. Eight weeks after streptozotocin injection body weight were 39% lower in STZ-rats compared with non diabetic control rats ($p < 0.001$) and up to 51% in 36 weeks (Table 3. and Figure 12.). Blood glucose levels were significantly elevated in STZ rats (397.12 ± 65.48 mg/dl to 463.37 ± 106.52) compared with non diabetic control rats (83.5 ± 11.86 to 100.9 ± 11.51 ; $p < 0.001$) (Table 4. and Figure 13.). Plasma glycosylated hemoglobin level was significantly elevated in STZ rats (9.52 ± 1.66 to $10.13 \pm 1.88\%$) compared with non diabetic control rats (3.91 ± 0.15 to $4.51 \pm 0.58\%$; $p < 0.001$) in all five monitored time points (Table 5. and Figure 14.).

Interestingly, at 36week vitamin C supplemented of diabetic rats significantly improved in body weight, decrease blood glucose and decrease HbA_{1c}.

1.2 Plasma vitamin C was significantly reduced in 12, 24, and 36 weeks STZ-rats (23.01 ± 0.92 , 21.47 ± 1.87 and 15.95 ± 2.02 $\mu\text{mol/L}$ respectively compare with 12, 24 and 36 week of control rats (44.59 ± 2.12 , 43.58 ± 1.19 and 44.89 ± 2.93 ; respectively). Vitamin C supplementation

can improved the plasma vitamin C concentration in all three monitored time points of STZ-Vit C rats (43.66 ± 3.92 , 39.44 ± 2.04 and 38.65 ± 2.02 ; $p < 0.01$).

1.3 Tissue lipidperoxidation

In this present study malondialdehyde (MDA), product of lipidperoxidation was used as indicator of oxygen free radical. The MDA values were significant higher in 8, 12, 16, 24, and 36 weeks diabetic rats eyes (103.32 ± 25.28 , 107.11 ± 35.75 , 146.26 ± 25.73 , 137.59 ± 37.88 and 145.63 ± 28.80) than in 8, 12, 16, 24, and 36 weeks control rats (58.36 ± 27.76 , 72.95 ± 21.41 , 69.85 ± 23.64 , 70.49 ± 31.39 and 73.65 ± 27.91 ; respectively). Interestingly vitamin C supplemented can reduced MDA values in 16, 24, and 36 weeks of STZ-Vit C rats (84.93 ± 13.93 , 81.70 ± 21.39 and 80.88 ± 20.84 ; respectively) compare with STZ rats ($p < 0.01$) (Table 7. and Figure 16.).

Part 2. The antioxidant effects of vitamin C supplementation on leukocyte-endothelial cell interaction

The leukocyte that was counted as adherent one has to remain stationary for equal or longer than 30 second (Joussen AM et al., 2001). The leukocyte adhesion was count per field of view totally of postcapillary venule (diameter 20-50 μm) as described previously.

In the present video microscopic visualization showed clear image of leukocytes adhering to the endothelium of postcapillary venules in control non diabetes and diabetes rats all five monitored time points of per field of view. The field of view which is represent number of leukocyte adhesion in rats all five monitored time points of STZ rats (6.14 ± 1.60 , 6.05 ± 0.57 , 6.93 ± 1.79 , 12.90 ± 3.20 , and 22.28 ± 4.35 ; respectively)

were significantly high compared with rats all five monitored time points of control rats (2.20 ± 0.65 , 2.48 ± 0.79 , 2.45 ± 0.69 , 2.27 ± 0.48 and; 2.56 ± 1.03 respectively). Interestingly, vitamin C supplementation had effects to reduced the number of leukocyte adhesion to endothelial of postcapillary venule in STZ-Vit C rats. However, vitamin C supplemented STZ-rats still had significant decreased in vitamin C plasma compared with the controls (Table 6. and Figure 15).

Part 3. The antioxidant effects of vitamin C supplementation on hemodynamics changes

3.1 By using laser doppler flowmetry the regional iris blood-flow perfusion was evaluated from 8 areas of the iris around the pupil as described previously. Means regional iris blood-flow perfusion were summarized in The Table 12. and Figure 23. Means regional iris blood-flow perfusion of STZ rats were significantly reduced to 33.64 %, 50.46 %, 57.95, 57.60 % and 56.12 %, respectively compared with control rats on 8,12, 16, 24, and 36 weeks. However, the significant difference between STZ and STZ-Vit C were observed on 24 and 36 weeks.

3.2 Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean regional iris blood-flow perfusion of control, STZ and STZ-Vit C rats. The results SBP value were shown in Table 9. And the results DBP value were shown in Table 10. In the present study, diabetic state had effect on change of SBP and SBP in 16 and 24 weeks of STZ and control rats. The SBP and SBP were significantly different between STZ and STZ-Vit C at 16-36 weeks.

Part 4. Study relationship on iris blood-flow perfusion and leukocyte endothelial cell interaction

Linear regression analysis was used to examine the relationship on iris blood-flow perfusion and leukocyte-endothelial cell interaction. The result of this relationship was shown in Figure 24. Iris blood-flow perfusion values and leukocyte adhesion (cell per field of view of postcapillary venule of all five monitored time points of diabetic rats were plotted and these two parameters were correlated equation $y = -0.447x + 32.80$, $r = -0.317$, $p < 0.034$. Interestingly, the correlation was more clarified and supported by the results of STZ-Vit C, $y = -1.862x + 47.103$, $r = -0.517$, $p < 0.001$. The data demonstrates that the presences of diabetes are necessary for measurable effect on iris blood-flow perfusion can occur in diabetes, not founded relationship in control rats. From the result, it can be explained that when leukocyte adhesion was raised as the iris blood-flow perfusion was reduced in diabetic rats.

Part 5. Histological study for transmigration of leukocyte in iris area

The example of Haematoxylin-eosin stain of iris specimens of each groups were demonstrated in Figure 25. The results indicated that the abnormality of ultrastructure occurred in STZ-rats, especially on 24 weeks. The formation of cataract of 24 week STZ was observed. However, the leukocyte tranmigration in tissue area of iris has not seen in the histological area of iris.

Table 3. Mean \pm SD of body weight (g) of control rats (CON), streptozotocin rats (STZ) and streptozotocin rats supplementation with vitamin C (STZ-Vit C)

Duration (weeks)	Body weight (g)		
	CON	STZ	STZ-Vit C
8	393 \pm 31.38 (n=10)	238.8 \pm 28.75 ⁺⁺ (n=10)	236.5 \pm 35.11 ^{** , ns} (n=10)
12	443.07 \pm 22.41 (n=13)	223.25 \pm 36.80 ⁺⁺ (n=8)	229.6 \pm 47.71 ^{** , ns} (n=10)
16	471 \pm 37.10 (n=10)	261 \pm 35.71 ⁺⁺ (n=9)	245.12 \pm 38.33 ^{** , ns} (n=8)
24	507 \pm 35.55 (n=13)	246.00 \pm 19.77 ⁺⁺ (n=8)	276.88 \pm 39.25 ^{** , ns} (n=9)
36	566.45 \pm 44.13 (n=11)	278.33 \pm 55.17 ⁺⁺ (n=9)	362.33 \pm 38.91 ^{** , ##} (n=9)

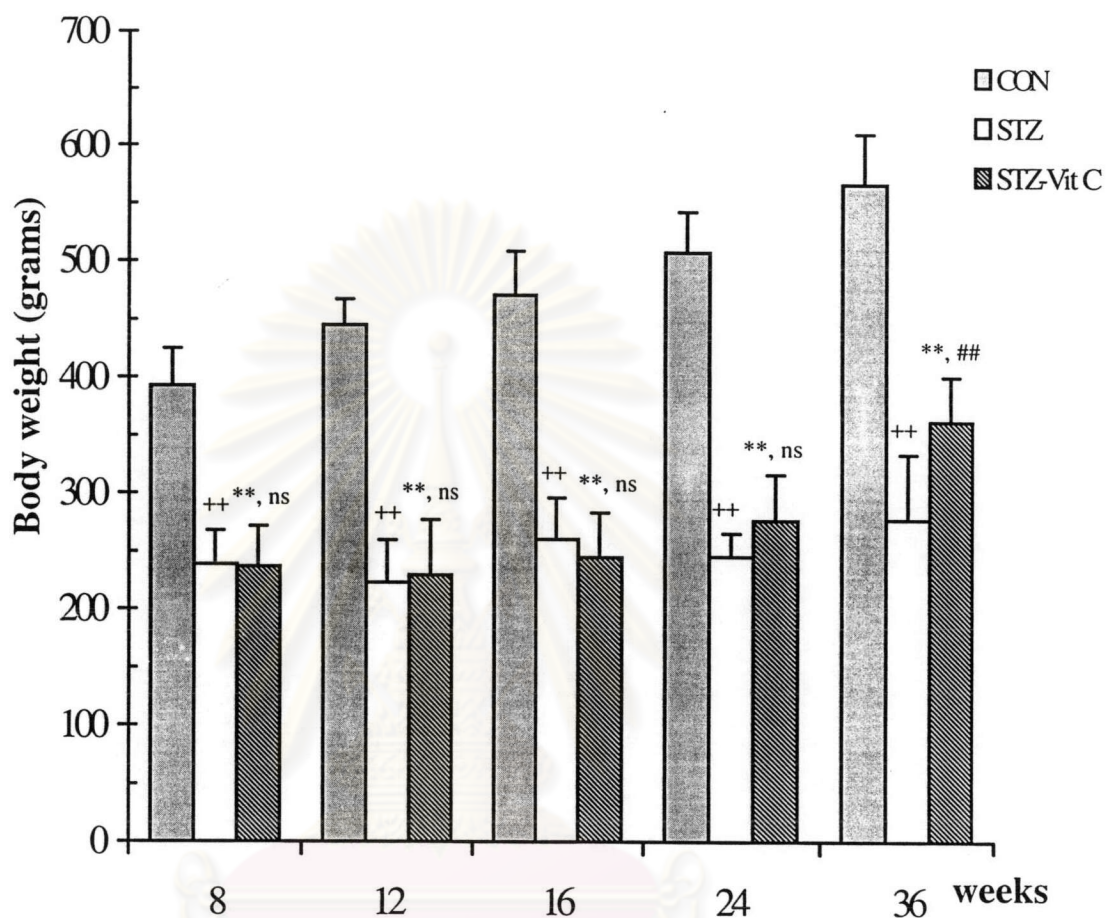
** Significantly different as compared to CON (p<0.001)

++ Significantly different as compared to CON (p<0.001)

Significantly different as compared to STZ (p<0.01)

ns no significantly different as compared to STZ

Figure 12. The antioxidant effects of vitamin C supplementation on body weight (BW)



Values: mean \pm SD

CON ; control rats

STZ ; streptozotocin rats

STZ-Vit C ; streptozotocin rats supplementation with vitamin C

- * Significantly different as compared to CON ($p < 0.05$)
- ** Significantly different as compared to CON ($p < 0.001$)
- ++ Significantly different as compared to CON ($p < 0.001$)
- # Significantly different as compared to STZ ($p < 0.05$)
- ## Significantly different as compared to STZ ($p < 0.01$)
- ns no significantly different as compared to STZ
- NS no significantly different as compared to CON

Table 4. Mean \pm SD of blood glucose (mg/dl) of control rats (CON), streptozotocin rats (STZ) and streptozotocin rats supplementation with vitamin C (STZ-Vit C)

Duration (weeks)	Blood glucose (mg/dl)		
	CON	STZ	STZ-Vit C
8	83.5 \pm 11.86 (n=10)	408.62 \pm 97.82 ⁺⁺ (n=9)	413.62 \pm 94.09 ^{**} , ns (n=8)
12	97 \pm 13.81 (n=12)	397.12 \pm 65.48 ⁺⁺ (n=8)	431.4 \pm 83.22 ^{**} , ns (n=10)
16	97.9 \pm 11.20 (n=10)	456.37 \pm 94.05 ⁺⁺ (n=8)	433.42 \pm 87.98 ^{**} , ns (n=7)
24	106.84 \pm 10.49 (n=13)	425.00 \pm 64.43 ⁺⁺ (n=7)	380.27 \pm 68.96 ^{**} , ns (n=11)
36	100.9 \pm 11.51 (n=11)	463.37 \pm 106.52 ⁺⁺ (n=8)	279.37 \pm 77.04 ^{**} , ## (n=8)

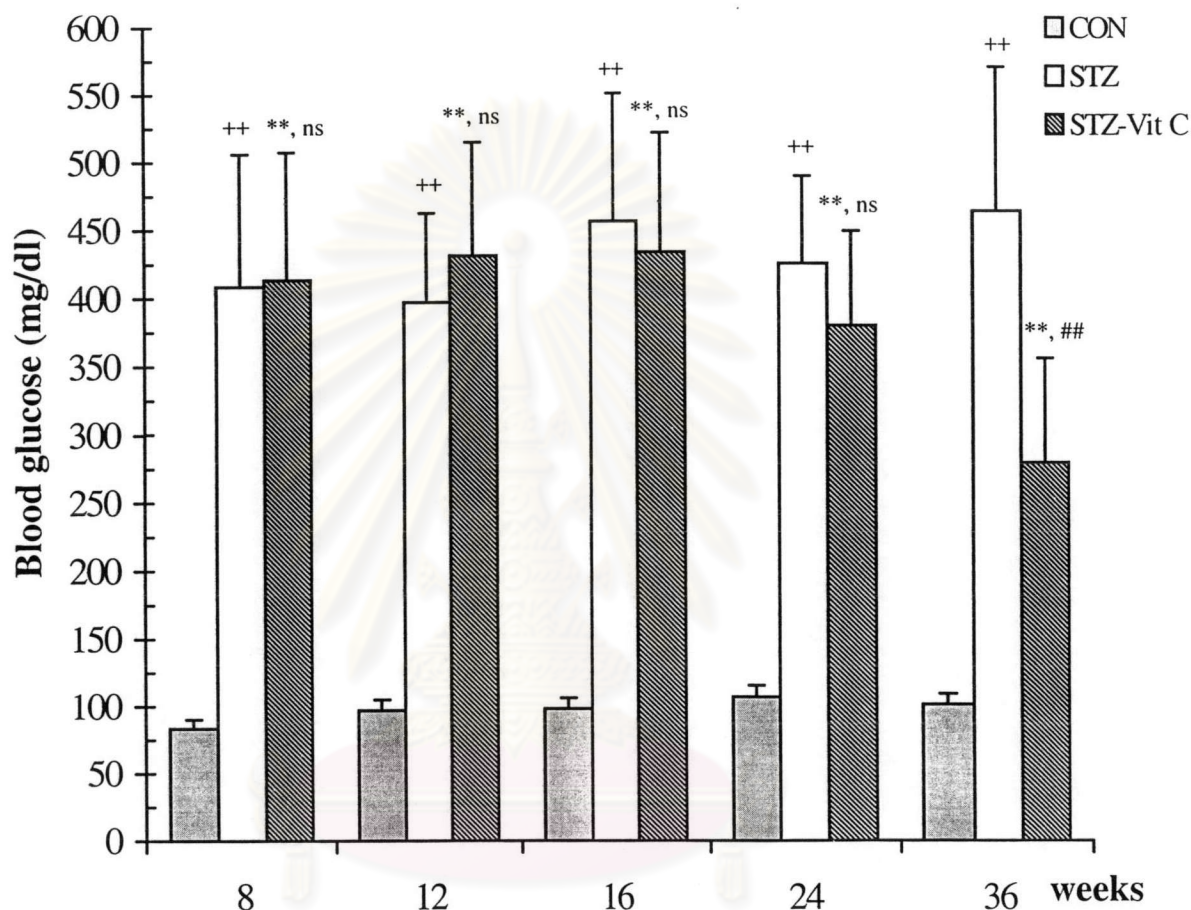
** Significantly different as compared to CON (p<0. 001)

++ Significantly different as compared to CON (p<0. 001)

Significantly different as compared to STZ (p<0. 01)

ns no significantly different as compared to STZ

Figure13. The antioxidant effects of vitamin C supplementation on blood glucose



Values: mean \pm SD

CON ; control rats

STZ ; streptozotocin rats

STZ-Vit C ; streptozotocin rats supplementation with vitamin C

** Significantly different as compared to CON ($p < 0.001$)

++ Significantly different as compared to CON ($p < 0.001$)

Significantly different as compared to STZ ($p < 0.01$)

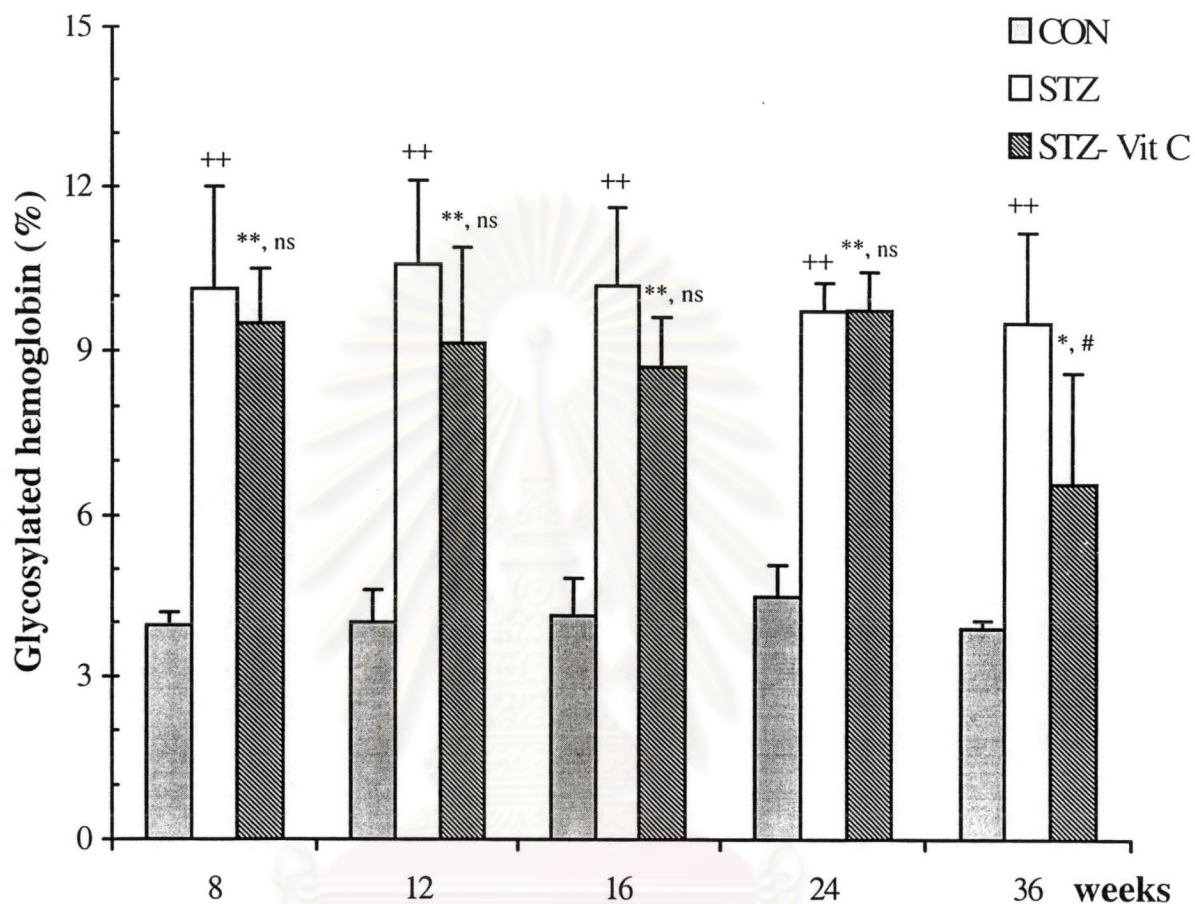
ns no significantly different as compared to STZ

Table 5. Mean \pm SD of glycosylated hemoglobin A_{1c} (%) of Control rats (CON), streptozotocin rats (STZ) and streptozotocin rats supplementation with vitamin C (STZ-Vit C)

Duration (weeks)	Glycosylated hemoglobin A _{1c} (HbA _{1c} ;%)		
	CON	STZ	STZ-Vit C
8	3.96 \pm 0.23 (n=6)	10.13 \pm 1.88 ⁺⁺ (n=9)	9.5 \pm 1.00 ^{** , ns} (n=8)
12	4 \pm 0.61 (n=6)	10.57 \pm 1.55 ⁺⁺ (n=7)	9.13 \pm 1.76 ^{** , ns} (n=6)
16	4.13 \pm 0.71 (n=8)	10.18 \pm 1.43 ⁺⁺ (n=6)	8.71 \pm 0.90 ^{** , ns} (n=7)
24	4.51 \pm 0.58 (n=6)	9.72 \pm 0.52 ⁺⁺ (n=5)	9.75 \pm 0.70 ^{** , ns} (n=6)
36	3.91 \pm 0.15 (n=7)	9.52 \pm 1.66 ⁺⁺ (n=4)	6.60 \pm 2.03 ^{* , #} (n=5)

- * Significantly different as compared to CON (p<0.05)
 ** Significantly different as compared to CON (p<0.001)
 ++ Significantly different as compared to CON (p<0.001)
 # Significantly different as compared to STZ (p<0.05)
 ## Significantly different as compared to STZ (p<0.01)
 ns no significantly different as compared to STZ

Figure 14. The antioxidant effects of vitamin C supplementation on glycosylated hemoglobin



Values: mean \pm SD

CON ; control rats

STZ ; streptozotocin rats

STZ-Vit C ; streptozotocin rats supplementation with vitamin C

* Significantly different as compared to CON ($p < 0.05$)

** Significantly different as compared to CON ($p < 0.001$)

++ Significantly different as compared to CON ($p < 0.001$)

Significantly different as compared to STZ ($p < 0.05$)

Significantly different as compared to STZ ($p < 0.01$)

ns no significantly different as compared to STZ

Table 6. Mean \pm SD of plasma vitamin C of control rats (CON), streptozotocin rats (STZ) and streptozotocin rats supplementation with vitamin C (STZ-Vit C)

Duration (weeks)	Plasma vitamin C ($\mu\text{mol} / \text{L}$)		
	CON	STZ	STZ-Vit C
12	44.59 \pm 2.12	23.01 \pm 0.92 ⁺⁺	43.66 \pm 3.92 ^{NS,##}
24	43.58 \pm 1.19	21.47 \pm 1.87 ⁺⁺	39.44 \pm 2.04 ^{NS,##}
36	44.89 \pm 2.93	15.95 \pm 2.02 ⁺⁺	38.65 \pm 2.02 ^{NS,##}

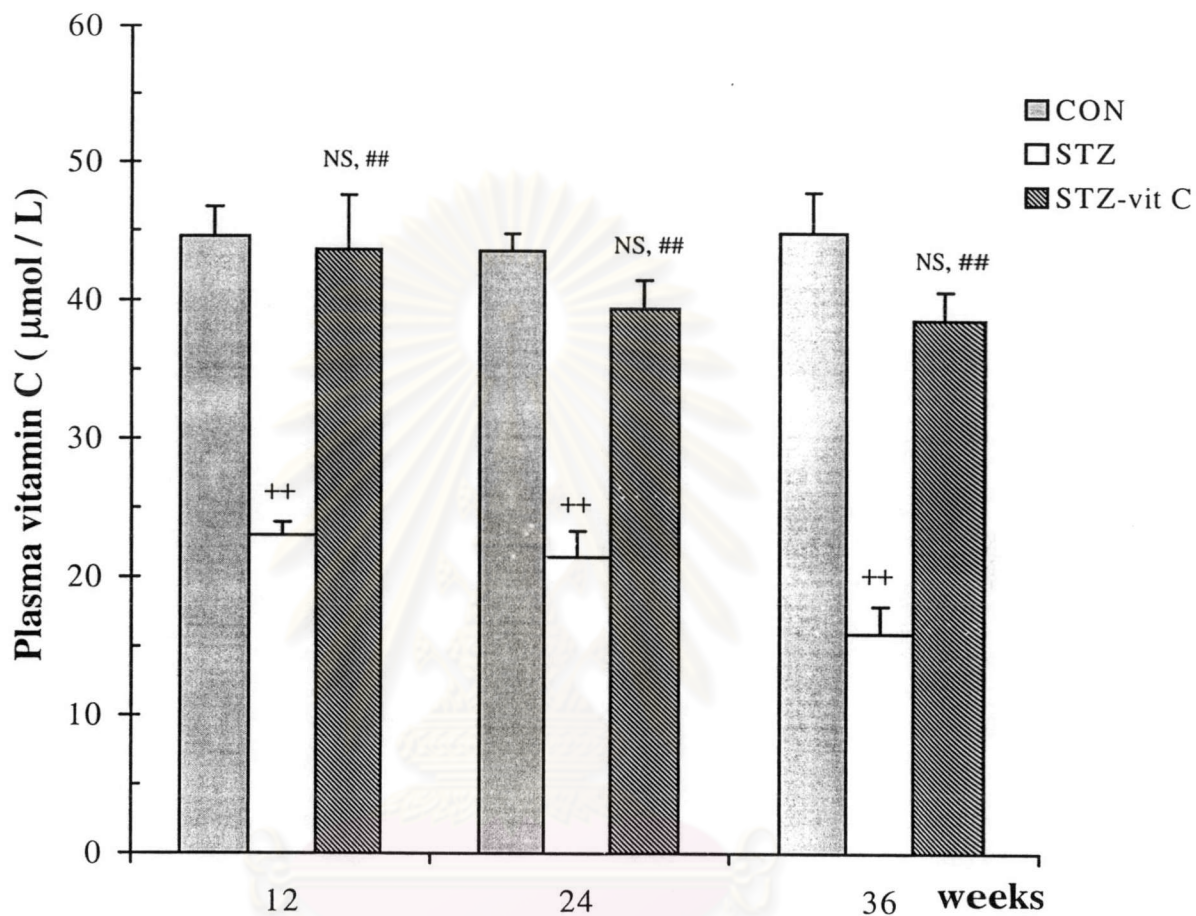
++ Significantly different as compared to CON (p<0.01)

Significantly different as compared to STZ (p<0.01)

NS no significantly different as compared to CON

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Figure 15. The antioxidant effects of vitamin C supplementation on plasma vitamin C



Values: mean \pm SD

CON ; control rats

STZ ; streptozotocin rats

STZ-Vit C ; streptozotocin rats supplementation with vitamin C

++ Significantly different as compared to CON ($p < 0.001$)

Significantly different as compared to STZ ($p < 0.01$)

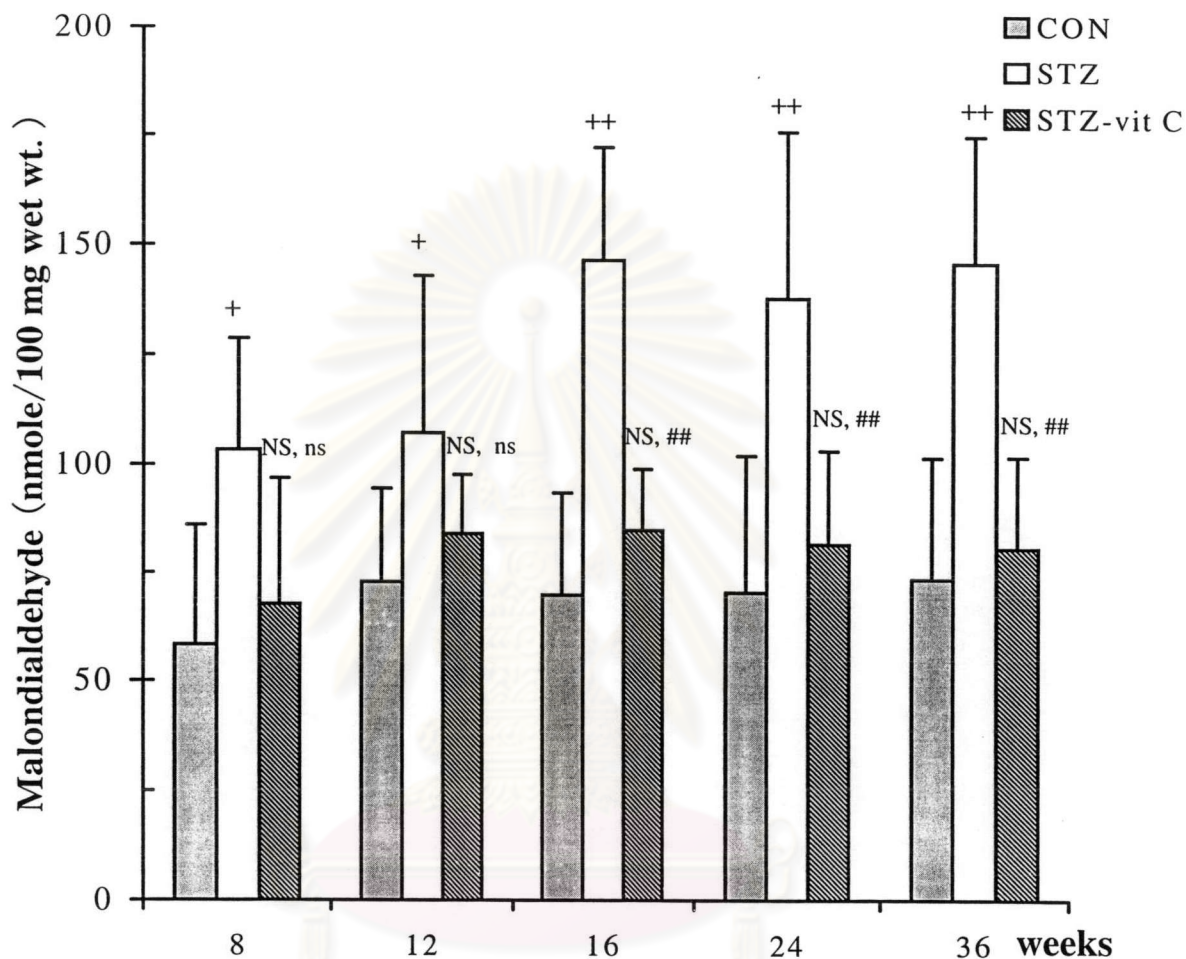
NS no significantly different as compared to CON

Table 7. Mean \pm SD Malondialdehyde (MDA) level in eye of control rats (CON), streptozotocin rats (STZ) and streptozotocin rats supplementation with vitamin C (STZ-Vit C)

Duration (weeks)	Malondialdehyde (nmole/100 mg wet wt.)		
	CON	STZ	STZ-Vit C
8	58.36 \pm 27.79 (n=7)	103.32 \pm 25.28 ⁺ (n=9)	67.71 \pm 29.08 ^{NS, ns} (n=6)
12	72.95 \pm 21.41 (n=10)	107.11 \pm 35.75 ⁺ (n=6)	84.15 \pm 13.44 ^{NS, ns} (n=6)
16	69.85 \pm 23.64 (n=9)	146.26 \pm 25.73 ⁺⁺ (n=8)	84.93 \pm 13.93 ^{NS, ##} (n=7)
24	70.49 \pm 31.39 (n=11)	137.59 \pm 37.88 ⁺⁺ (n=6)	81.70 \pm 21.39 ^{NS, ##} (n=10)
36	73.65 \pm 27.91 (n=10)	145.63 \pm 28.80 ⁺⁺ (n=11)	80.88 \pm 20.84 ^{NS, ##} (n=8)

- + Significantly different as compared to CON (p<0.05)
 ++ Significantly different as compared to CON (p<0.001)
 ## Significantly different as compared to STZ (p<0.01)
 ns no significantly different as compared to STZ
 NS no significantly different as compared to CON

Figure 16. The antioxidant effects of vitamin C supplementation on malondialdehyde (MDA) level in eye



Values: mean \pm SD

CON ; control rats

STZ ; streptozotocin rats

STZ-Vit C ; streptozotocin rats supplementation with vitamin C

+ Significantly different as compared to CON ($p < 0.05$)

++ Significantly different as compared to CON ($p < 0.001$)

Significantly different as compared to STZ ($p < 0.01$)

ns no significantly different as compared to STZ

NS no significantly different as compared to CON

Table 8. Mean \pm SD of leukocyte adhesion of control rats (CON), streptozotocin rats (STZ) and streptozotocin rats supplementation with vitamin C (STZ-Vit C)

Duration (weeks)	Leukocyte adhesion (per field of view)		
	CON	STZ	STZ-Vit C
8	2.20 \pm 0.65 (n=7)	6.14 \pm 1.60 ⁺⁺ (n=9)	5.14 \pm 1.12 ^{** , ns} (n=8)
12	2.48 \pm 0.79 (n=9)	6.05 \pm 0.57 ⁺⁺ (n=6)	5.29 \pm 0.84 ^{** , ns} (n=6)
16	2.45 \pm 0.69 (n=8)	6.93 \pm 1.79 ⁺⁺ (n=8)	5.47 \pm 1.52 ^{** , ns} (n=6)
24	2.27 \pm 0.48 (n=9)	12.90 \pm 3.20 ⁺⁺ (n=7)	9.33 \pm 2.68 ^{** , ##} (n=10)
36	2.56 \pm 1.03 (n=9)	22.28 \pm 4.35 ⁺⁺ (n=7)	11.89 \pm 2.77 ^{** , ##} (n=8)

++ Significantly different as compared to CON (p<0.001)

** Significantly different as compared to CON (p<0.001)

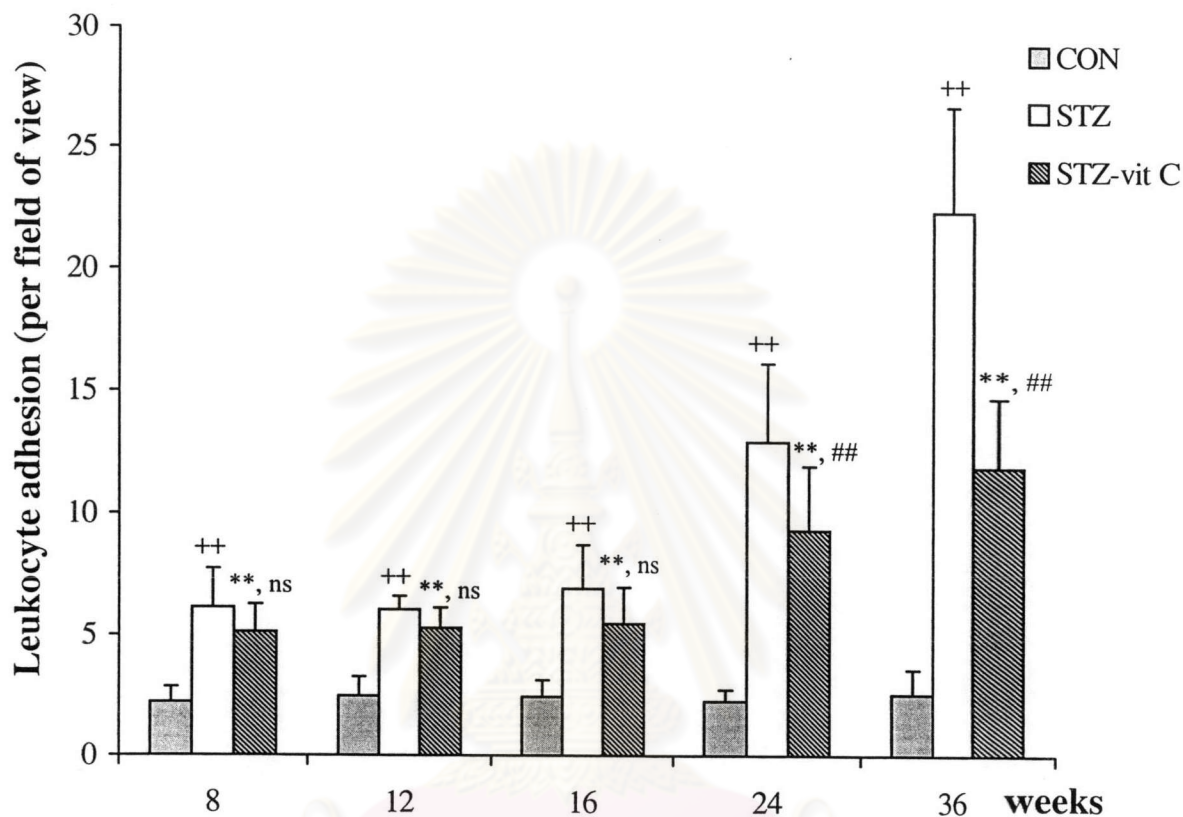
Significantly different as compared to STZ (p<0.05)

Significantly different as compared to STZ (p<0.01)

ns no significantly different as compared to STZ

NS no significantly different as compared to CON

Figure 17. The antioxidant effects of vitamin C supplementation on leukocyte adhesion



Values: mean \pm SD

CON ; control rats

STZ ; streptozotocin rats

STZ-Vit C ; streptozotocin rats supplementation with vitamin C

⁺⁺ Significantly different as compared to CON ($p < 0.001$)

^{**} Significantly different as compared to CON ($p < 0.001$)

^{##} Significantly different as compared to STZ ($p < 0.01$)

ns no significantly different as compared to STZ

NS no significantly different as compared to CON

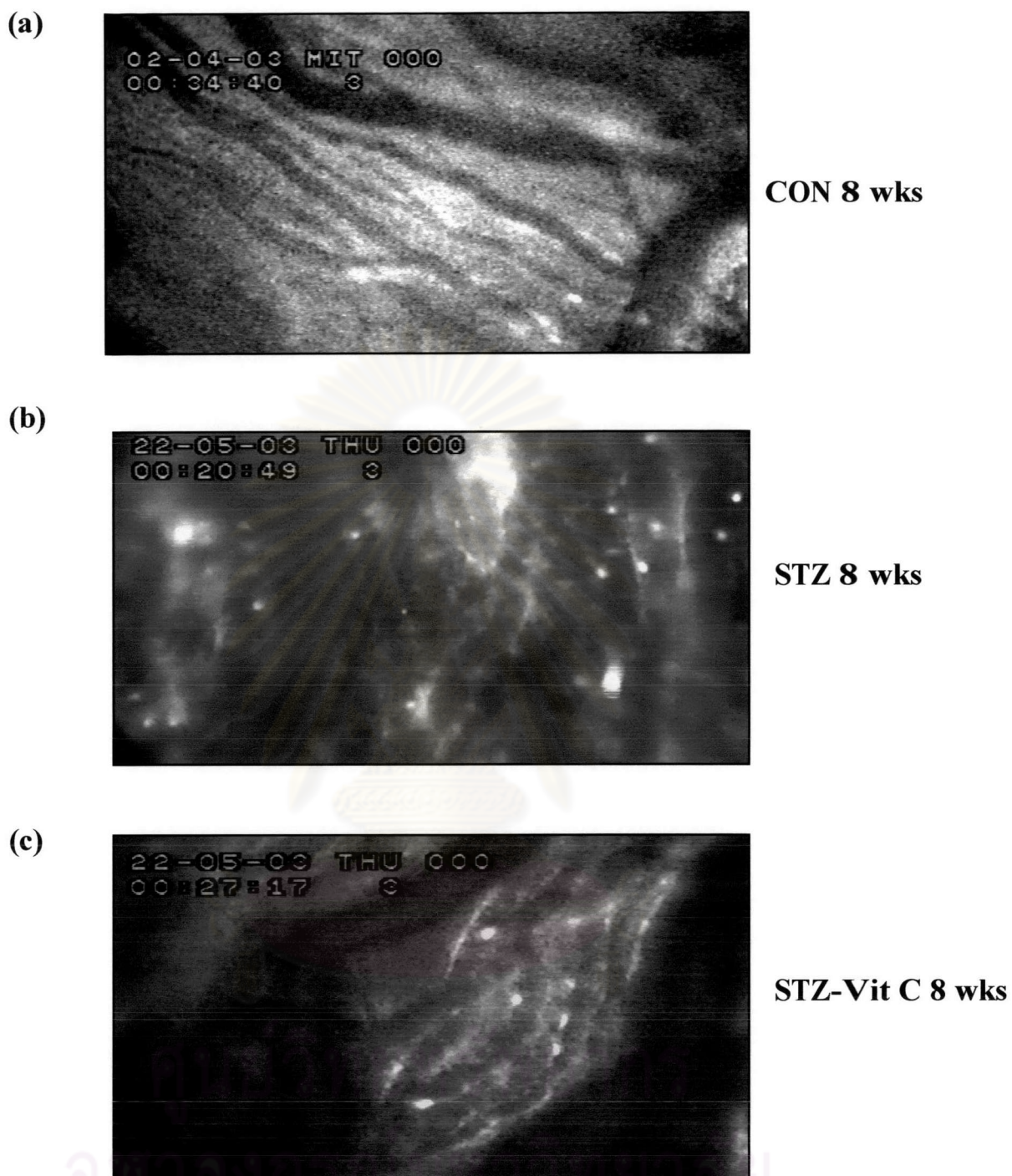


Figure 18. Intravital microscopic demonstration of leukocyte adhesion in the postcapillary venule from 8 week of a) Control, b) STZ-rat, c) STZ with vitamin C supplementation. White dots represent leukocytes stained by intravenous injection of fluorescein marker, rhodamine 6G.

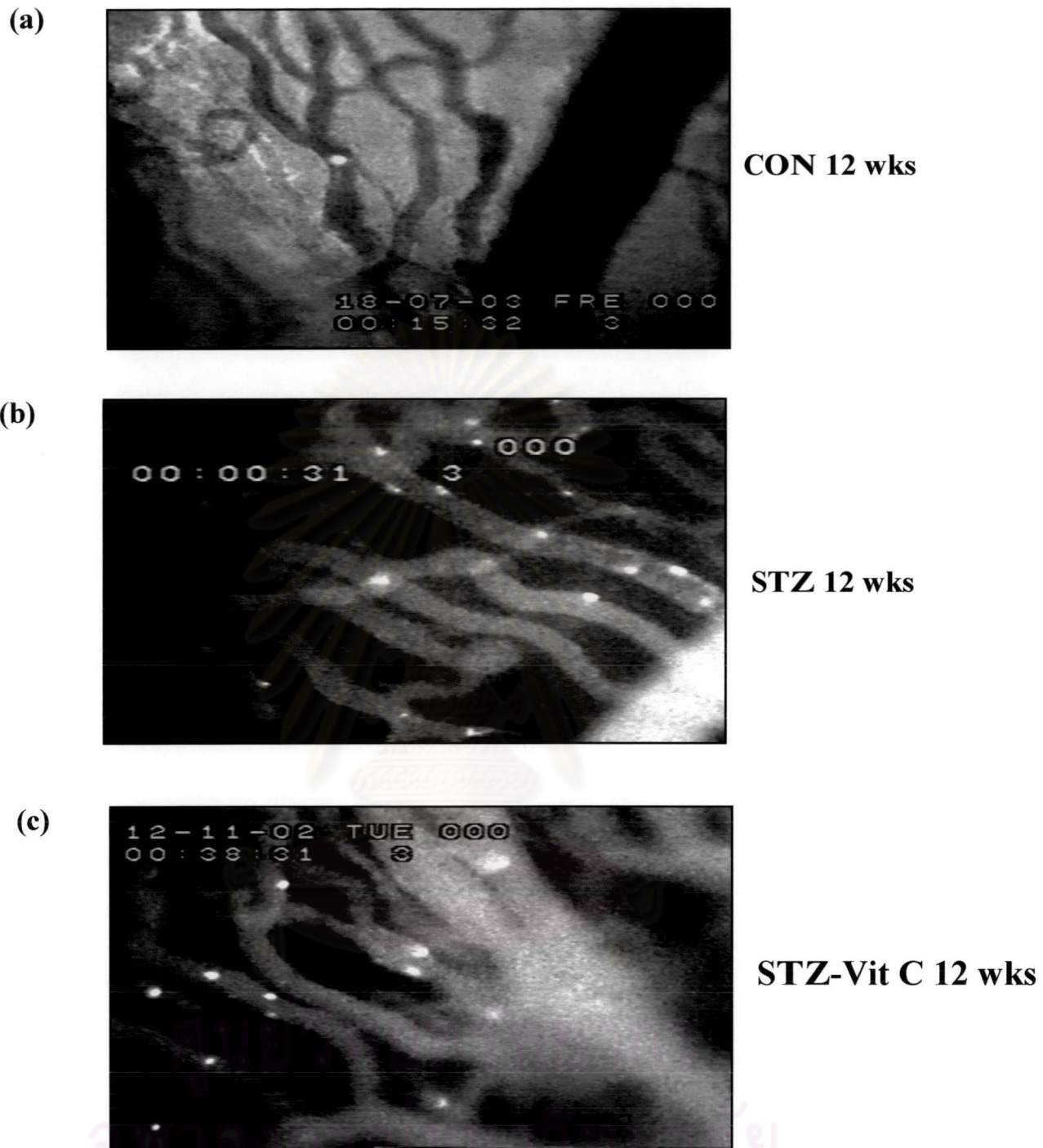


Figure 19. Intravital microscopic demonstration of leukocyte adhesion in the postcapillary venule from 12 week of a) Control, b) STZ-rat, c) STZ with vitamin C supplementation. White dots represent leukocytes stained by intravenous injection of fluorescein marker, rhodamine 6G.

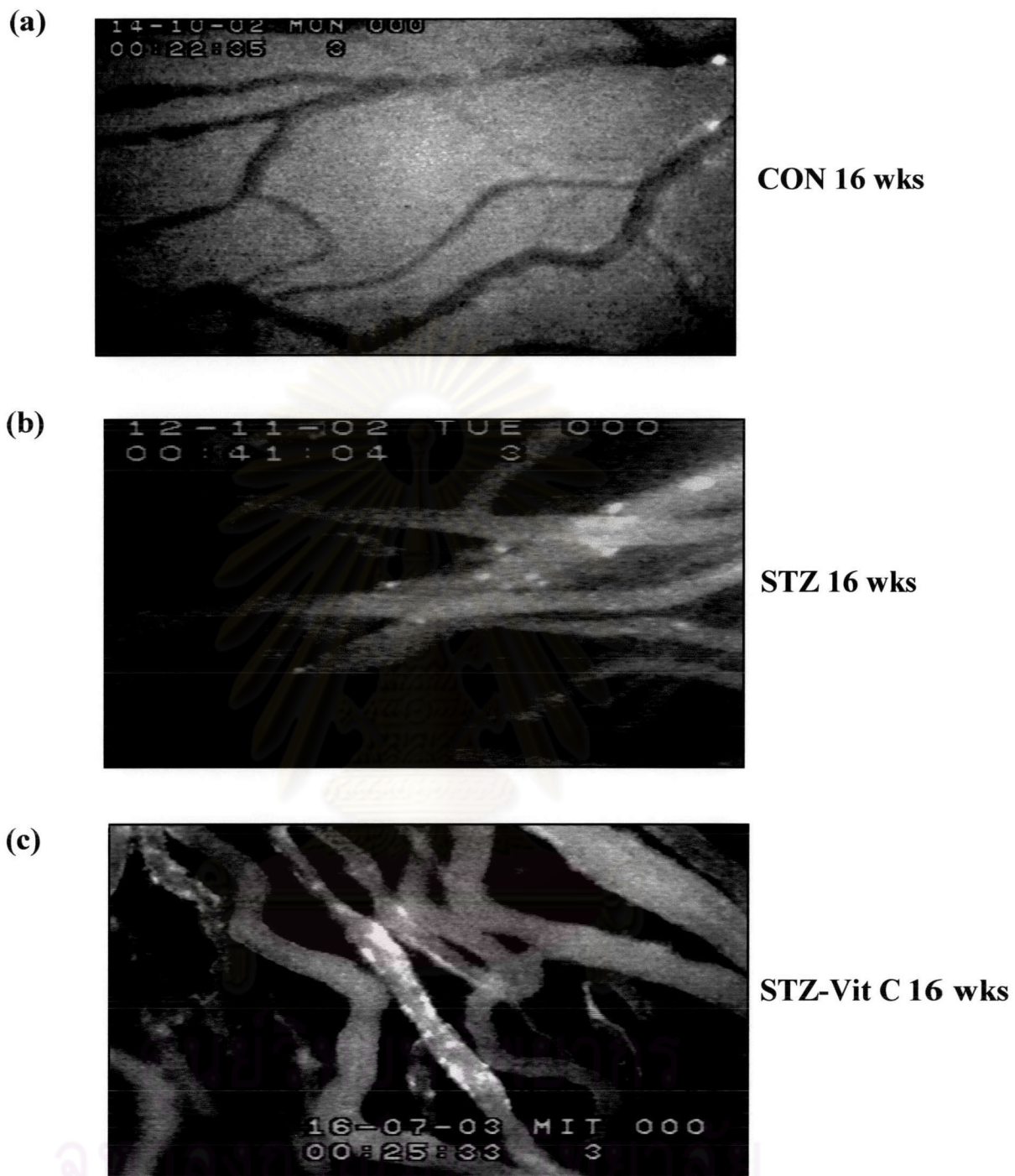


Figure 20. Intravital microscopic demonstration of leukocyte adhesion in the postcapillary venule from 36 week of a) Control, b) STZ-rat, c) STZ with vitamin C supplementation. White dots represent leukocytes stained by intravenous injection of fluorescein marker, rhodamine 6G.

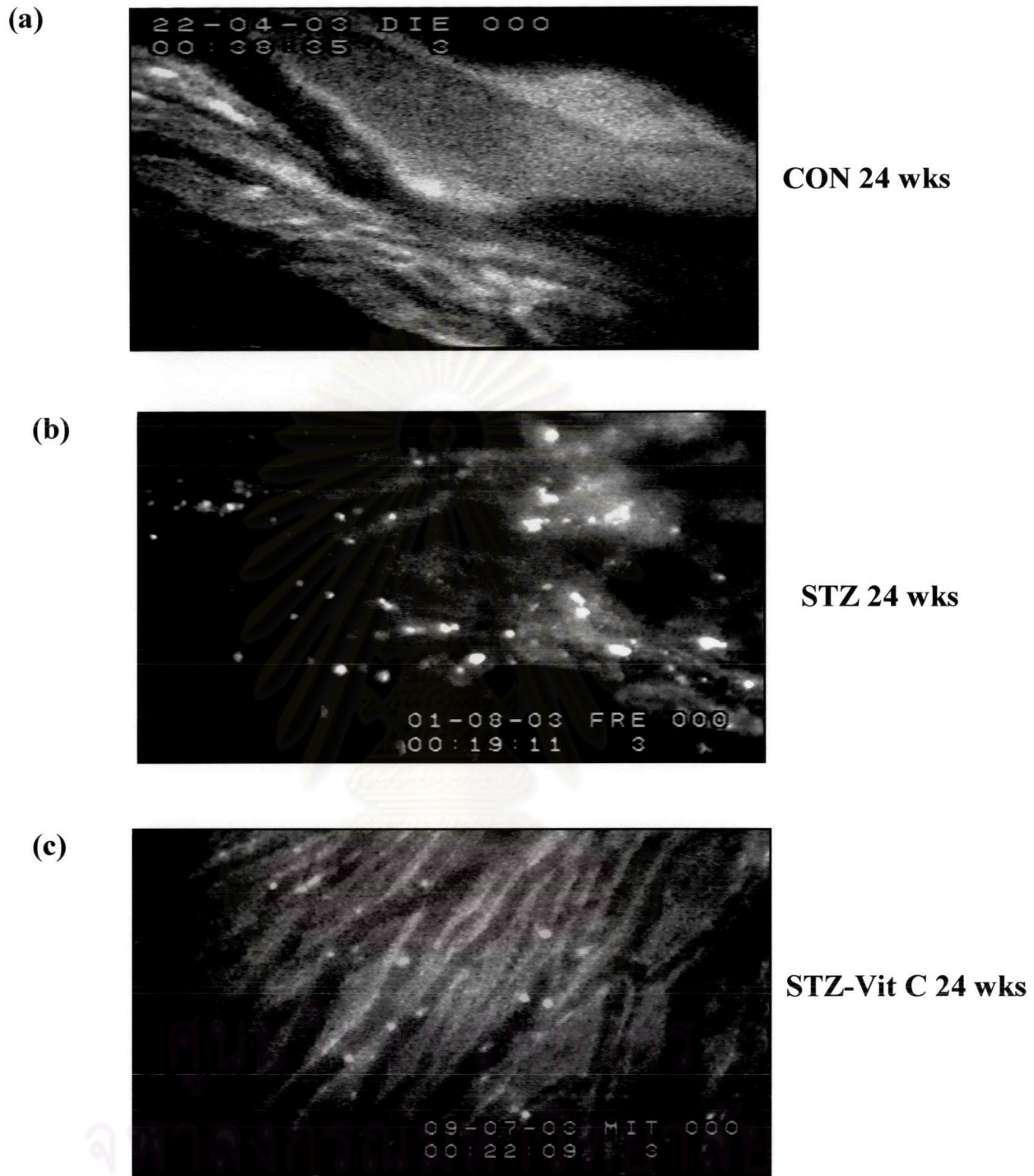


Figure 21. Intravital microscopic demonstration of leukocyte adhesion in the postcapillary venule from 24 week of a) Control, b) STZ-rat, c) STZ with vitamin C supplementation. White dots represent leukocytes stained by intravenous injection of fluorescein marker, rhodamine 6G.

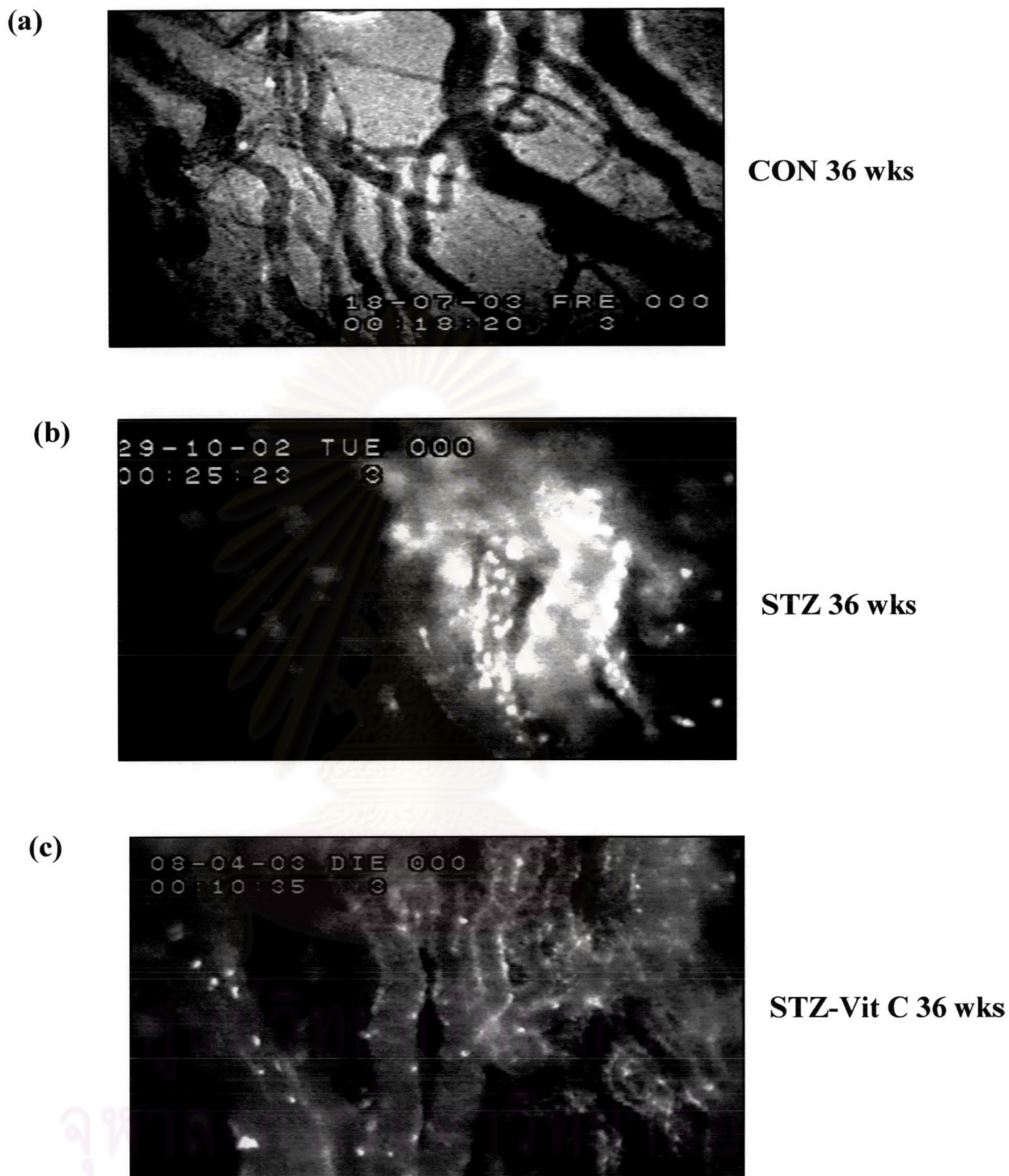


Figure 22. Intravital microscopic demonstration of leukocyte adhesion in the postcapillary venule from 36 weeks of a) Control, b) STZ-rat, c) STZ with vitamin C supplementation. White dots represent leukocytes stained by intravenous injection of fluorescein marker, rhodamine 6G.

Table 9. Mean \pm SD of Systolic blood pressure (mmHg) of control rats (CON), streptozotocin rats (STZ) and streptozotocin rats supplementation with vitamin C (STZ-Vit C)

Duration (weeks)	Systolic blood pressure (mmHg)		
	CON	STZ	STZ-Vit C
8	88.57 \pm 22.49 (n=7)	109.28 \pm 9.75 ^{NS} (n=7)	95.00 \pm 22.20 ^{NS, ns} (n=8)
12	103.00 \pm 20.18 (n=5)	115.00 \pm 18.70 ^{NS} (n=5)	109.28 \pm 12.39 ^{NS, ns} (n=7)
16	93.33 \pm 23.16 (n=6)	125.83 \pm 13.57 ⁺ (n=6)	112.50 \pm 8.80 ^{NS, ns} (n=6)
24	105.55 \pm 14.45 (n=9)	116.00 \pm 12.94 ^{NS} (n=5)	103.00 \pm 5.70 ^{NS, ns} (n=5)
36	104.00 \pm 8.94 (n=5)	132.00 \pm 7.52 ⁺ (n=10)	111.66 \pm 7.52 ^{NS,##} (n=6)

+ Significantly different as compared to CON (p<0.05)

Significantly different as compared to STZ (p<0.01)

ns no significantly different as compared to STZ

NS no significantly different as compared to CON

Table 10. Mean \pm SD of Diastolic blood pressure (mmHg) of control rats (CON), streptozotocin rats (STZ) and streptozotocin rats supplementation with vitamin C (STZ-Vit C)

Duration (weeks)	Diastolic blood pressure (mmHg)		
	CON	STZ	STZ-Vit C
8	79.28 \pm 21.68 (n=7)	97.85 \pm 8.09 ^{NS} (n=7)	85.62 \pm 20.07 ^{NS, ns} (n=8)
12	90.00 \pm 19.68 (n=5)	106.00 \pm 21.62 ^{NS} (n=5)	92.85 \pm 14.09 ^{NS, ns} (n=7)
16	79.16 \pm 20.83 (n=6)	109.16 \pm 12.41 ⁺ (n=6)	105.00 \pm 7.07 ^{NS, ns} (n=6)
24	90.00 \pm 13.91 (n=9)	90.00 \pm 11.72 ^{NS} (n=5)	95.00 \pm 8.66 ^{NS, ns} (n=5)
36	88.00 \pm 10.36 (n=5)	118.50 \pm 8.83 ⁺ (n=10)	103.33 \pm 6.05 ^{*,##} (n=6)

- * Significantly different as compared to CON (p<0.05)
 ## Significantly different as compared to STZ (p<0.01)
 ns no significantly different as compared to STZ
 NS no significantly different as compared to CON

Table 11. Mean \pm SD of mean arterial blood pressure (mmHg) of control rats (CON), streptozotocin rats (STZ) and streptozotocin rats supplementation with vitamin C (STZ-Vit C)

Duration (weeks)	Mean arterial blood pressure (mmHg)		
	CON	STZ	STZ-Vit C
8	95.12 \pm 27.23 (n=10)	96.14 \pm 11.94 ^{NS} (n=9)	92.86 \pm 24.95 ^{NS, ns} (n=8)
12	107.21 \pm 24.89 (n=8)	105.86 \pm 19.85 ^{NS} (n=6)	95.76 \pm 13.93 ^{NS, ns} (n=6)
16	99.59 \pm 26.59 (n=10)	107.08 \pm 14.98 ^{NS} (n=9)	106.14 \pm 7.74 ^{NS, ns} (n=7)
24	97.26 \pm 19.40 (n=13)	95.41 \pm 16.38 ^{NS} (n=7)	105.77 \pm 14.96 ^{NS, ns} (n=8)
36	106.83 \pm 15.73 (n=8)	119.97 \pm 23.84 ^{NS} (n=14)	122.81 \pm 33.92 ^{NS, ns} (n=7)

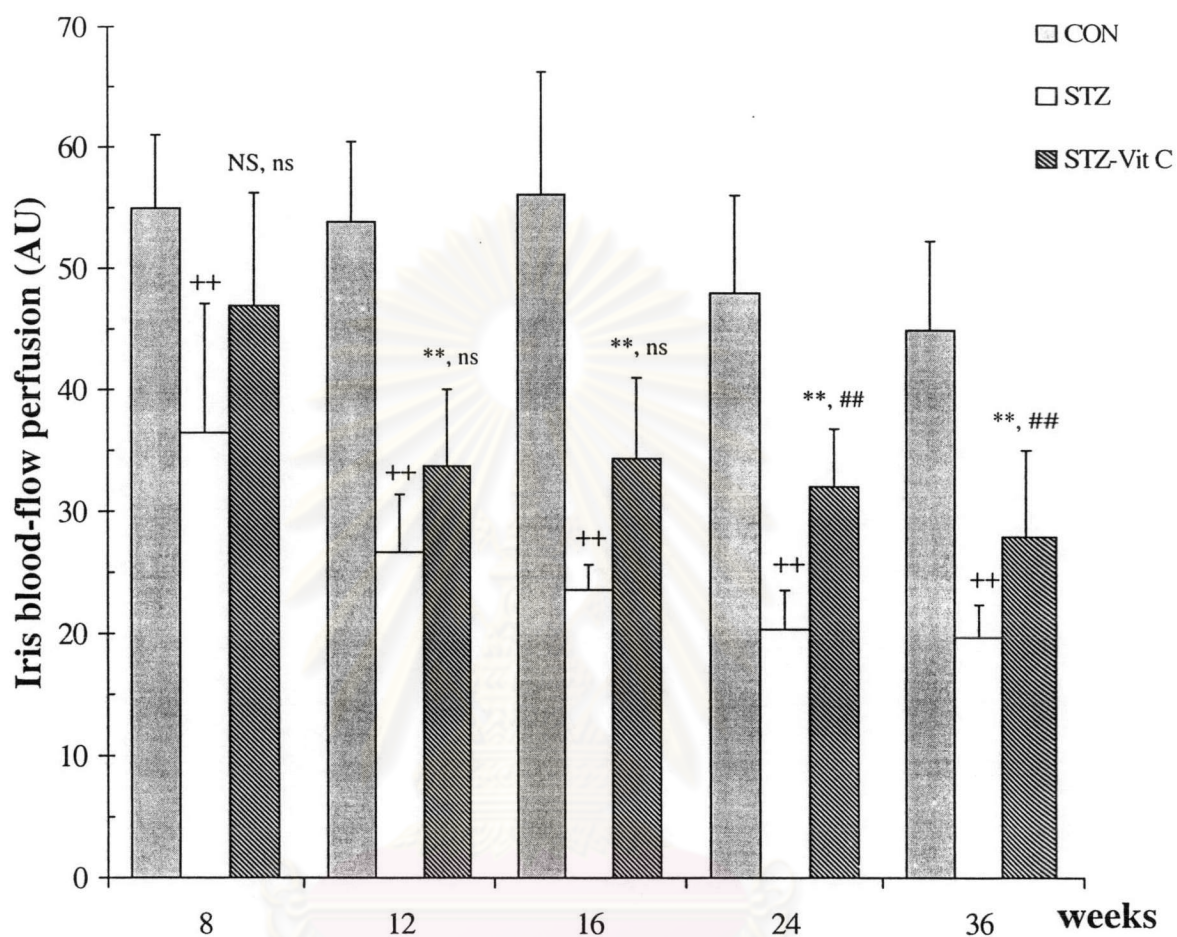
ns no significantly different as compared to STZ
 NS no significantly different as compared to CON

Table 12. Mean \pm SD of iris blood-flow perfusion of control rats (CON), streptozotocin rats (STZ) and streptozotocin rats supplementation with vitamin C (STZ-Vit C)

Duration (weeks)	Iris blood-flow perfusion (AU)		
	CON	STZ	STZ-Vit C
8	54.95 \pm 6.05 (n=10)	43.05 \pm 8.18 ^{NS} (n=7)	46.97 \pm 9.23 ^{NS, ns} (n=9)
12	53.82 \pm 6.59 (n=13)	26.66 \pm 4.76 ⁺⁺ (n=7)	33.74 \pm 6.30 ^{** , ns} (n=8)
16	56.06 \pm 10.11 (n=10)	23.57 \pm 2.08 ⁺⁺ (n=6)	34.36 \pm 6.62 ^{** , ns} (n=5)
24	48 \pm 7.97 (n=13)	20.35 \pm 3.24 ⁺⁺ (n=6)	32.06 \pm 4.72 ^{** , ##} (n=7)
36	44.94 \pm 7.30 (n=10)	19.72 \pm 2.67 ⁺⁺ (n=12)	27.99 \pm 7.07 ^{** , ##} (n=8)

- ++ Significantly different as compared to CON (p<0. 001)
 ** Significantly different as compared to CON (p<0.001)
 ## Significantly different as compared to STZ (p<0.01)
 ns no significantly different as compared to STZ
 NS no significantly different as compared to CON

Figure 23. The antioxidant effects of vitamin C supplementation on iris blood-flow perfusion



Values: mean \pm SD

CON ; control rats

STZ ; streptozotocin rats

STZ-Vit C ; streptozotocin rats supplementation with vitamin C

++ Significantly different as compared to CON ($p < 0.001$)

** Significantly different as compared to CON ($p < 0.001$)

Significantly different as compared to STZ ($p < 0.01$)

ns no significantly different as compared to STZ

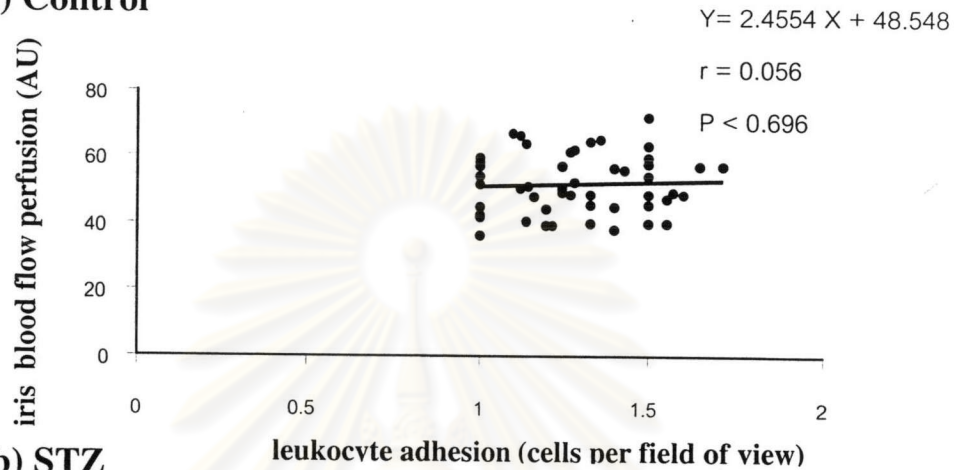
NS no significantly different as compared to CON

Table 13. Correlation factors r and p-values between iris blood-flow perfusion and leukocyte adhesion of control rats (CON), streptozotocin rats (STZ) and streptozotocin rats supplementation with vitamin C (STZ-Vit C) were summarized.

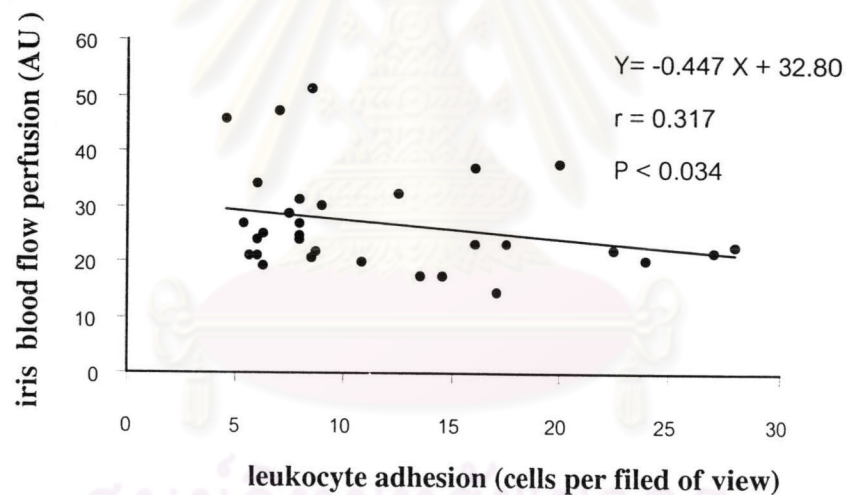
group	weeks	r	p-values
CON	8 (n=8)	0.116	0.784
	12 (n=10)	-0.465	0.175
	16 (n=10)	-0.317	0.372
	24 (n= 13)	-0.143	0.642
	36 (n=10)	0.576	0.082
	8-36 (n=41)	0.056	0.694
STZ	8 (n=9)	-0.197	0.64
	12 (n=6)	0.397	0.378
	16 (n=8)	0.431	0.393
	24 (n=7)	0.304	0.557
	36 (n=7)	-0.01	0.983
	8-36 (n= 37)	-0.317	0.068
STZ-Vit C	8 (n= 8)	0.408	0.316
	12 (n=6)	-0.338	0.512
	16 (n= 6)	-0.203	0.699
	24 (n=10)	-0.461	0.18
	36 (n=8)	-0.75	0.032
	8-36 (n=38)	-0.517	0.001

Figure 24. Correlation of leukocyte adhesion and regional blood flow in the postcapillary venule from 8-36 week of a) Control, b) STZ-rat, c) STZ with vitamin C supplementation.

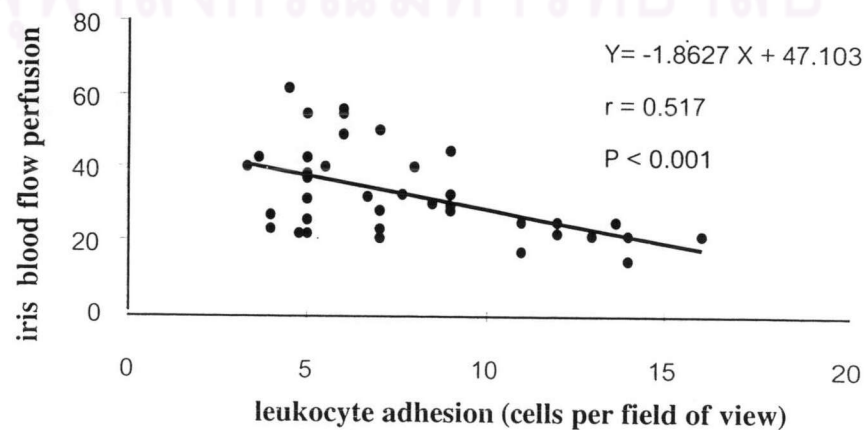
(a) Control



(b) STZ



(c) STZ- vit C



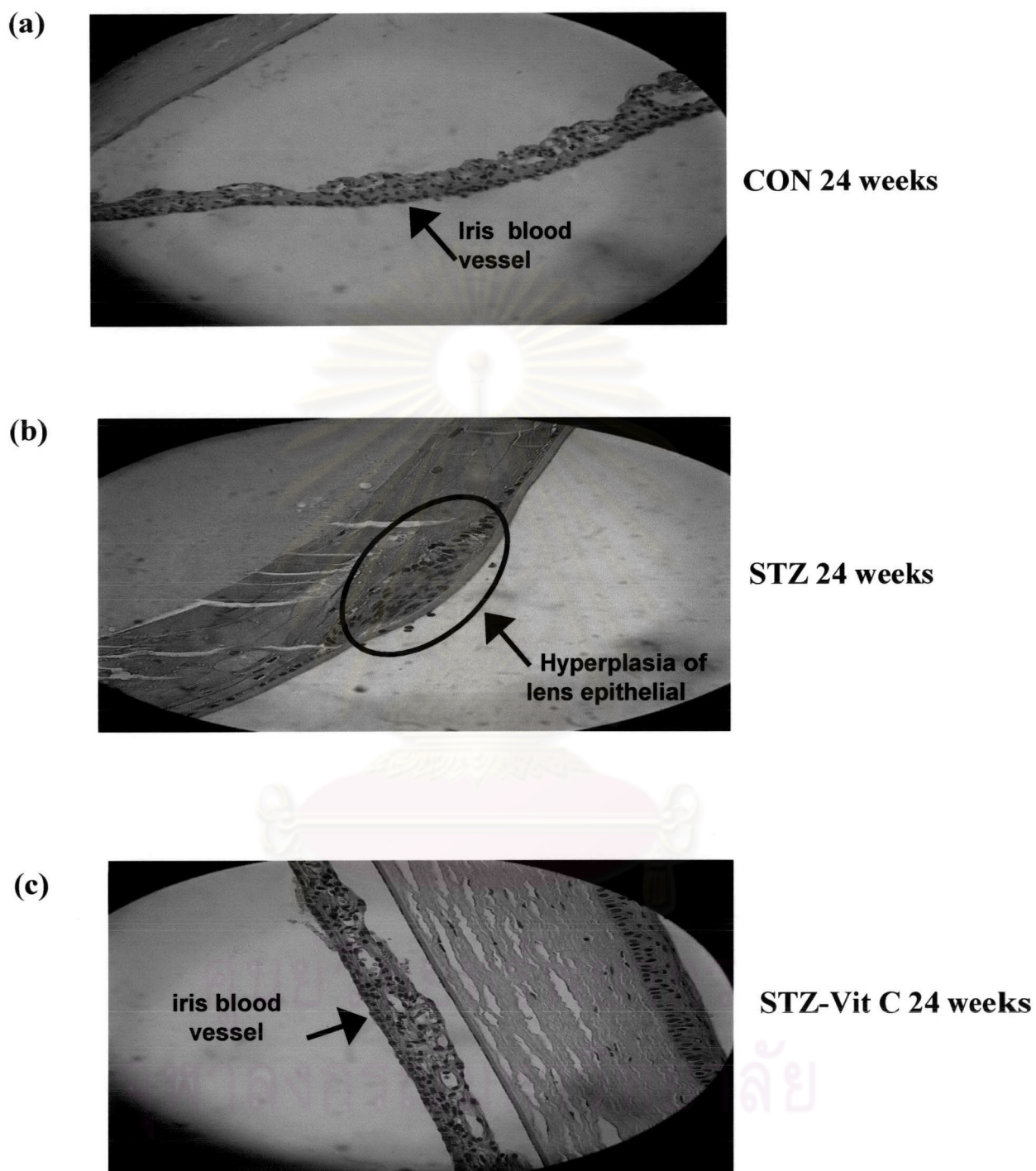


Figure 25. Histological changes of iris specimens at 24 weeks of a) Control, b) STZ-rat, c) STZ with vitamin C supplementation were demonstrated by using Haematoxylin-eosin staining. The results indicated amount increase in hyperplasia of lens epithelial formed in 24-STZ-rats.