

CHAPTER IV

RESULTS

General observation

In this study, the streptozotocin treated rat was used as an experimental model of diabetes mellitus. The results of general observations in these investigations were body weights and symptoms of diabetes such as polyphagia, polydypsia, and polyuria. And, the body weights were shown in Table 1 and Figure 7.

1. All three different groups (8,12,16 weeks) of STZ-rats had significantly decreased body weights compared to age-matched control animals. Moreover, the diabetic rats also manifested other symptoms of the disease including polyphagia, polydypsia, polyuria, diarrhea, and cataracts.

2. Approximately 8,12,and 16 weeks after treatment with STZ, body weights were significantly less in the STZ-rats compared to age-matched garlic-treated STZ-rats. In addition, the STZ-rats also exhibited more polyphagia, polydypsia, and polyuria than garlic-treated STZ-rats.

Studies of cardiovascular functions

In this experiment, cardiovascular functions such as systolic pressure (SP), diastolic pressure (DP), mean arterial pressure (MAP), heart rate (HR), and aortic flow rate (AFR) were determined for all three different groups (8,12,16 weeks) of age-matched control rats, STZ-rats, and garlic-treated STZ-rats as summarized in Table 2-6 by using the experimental protocol as described previously in chapter III. Moreover, all these assessed cardiovascular parameters were also demonstrated graphically as showed in Figure 8-12.

1. In the STZ-rats of all three different groups (8,12,16 weeks), SP, DP, MAP were significantly higher than their age-matched controls. And, in these STZ-rats also had

significantly increased SP, DP, and MAP compared to garlic-treated STZ-rats in all three different groups.

2. In all three different groups (8,12,16 weeks) of STZ-rats, HR were significantly lower than the age-matched controls. And, all three different groups of STZ-rats also found that HR were significantly lower than the age-matched garlic-treated STZ-rats.

3. AFR of all three different groups (8,12,16 weeks) of STZ-rats were significantly lower than the age-matched controls. And, from comparisons between all three different groups of STZ-rats and garlic-treated STZ-rats demonstrated that AFR of STZ-rats were significantly lower than the garlic-treated STZ-rats.

Studies of does responses to vasoactive agents

In normal rats, the coronary arteriolar responses to different doses of Ach (10^{-7} , 10^{-6} , 10^{-5} , and 10^{-4} M), SNP (10^{-7} , 10^{-6} , 10^{-5} , and 10^{-4} M), and NE (10^{-7} , 10^{-6} , 10^{-5} , and 10^{-4} M) were determined. This study found that coronary arterioles did not response to 10^{-7} M of Ach, SNP, and NE. The highest dose (10^{-4} M) of Ach, SNP, NE causes maximum response more than 10^{-6} M and 10^{-5} M of these vasoactive agents. The image of coronary arteriolar response to SNP (10^{-4} M) was shown in Figure 7.

Studies of coronary arteriolar responses to vasoactive agents

1. Endothelium-dependent relaxation

The coronary arteriolar responses to topical application of Ach (10^{-4} M) were examined in controls, STZ-rats, and garlic-treated STZ-rats at three different groups (8,12, and 16 weeks). The results were shown in Table 8 and Figure 15. Significant impairment of Ach-induced relaxation was observed in coronary arterioles from the STZ-rats compared with age-matched controls. The garlic treatment in STZ-rats significantly improve relaxation to Ach compared with untreated-diabetic rats.

2. Endothelium-independent relaxation

The relaxative responses to topical administration of SNP (10^{-4} M) were examined in coronary arterioles of controls, STZ-rats, and garlic-treated STZ-rats at three different groups (8,12 and 16 weeks). The results obtained for SNP in all animals are summarized in Table 9 and Figure 16. The topical application of SNP elicited increase in coronary arteriolar diameters of controls, STZ-rats, and garlic-treated STZ-rats at all three different groups. Moreover, the relaxations produced by SNP in STZ-rats were not significantly difference compared to age-matched controls. And, SNP-induced relaxations were also not significantly difference between STZ-rats and garlic-treated STZ-rats.

3. Contractile response to norepinephrine

The response to NE (10^{-4} M) by topical application were investigated in coronary arterioles of controls, STZ-rats, and garlic-treated STZ-rats at 8,12and 16 weeks. Data obtained in these experiments are shown in Table 10 and Figure 17. In STZ-rats demonstrated that the arteriolar responses to NE was enhanced as compared with age-matched controls. Interestingly, the treatment with garlic in STZ-rats led to a significant reduction of the NE-induced contraction compared to untreated-diabetic rats.

4. Effect of cyclooxygenase inhibitor

The coronay arteriolar responses to topical application of indomethacin (10^{-4} M) were examined in controls, STZ-rats, and garlic-treated STZ-rats at 8,12,and 16 weeks The results were shown in Table 11 and Figure 18. In both controls and garlic-treated STZ-rats, the results indicated that indomethacin produced contraction at all three different groups. In contrast, indomethacin could cause the increase of coronary arteriolar diameter of STZ-rats at all three different groups.

Table 1 Body weight (g) of controls , STZ-rats , and garlic-treated STZ-rats at 8 , 12 , and 16 weeks .

groups	body weight (g)		
	8 wk	12 wk	16 wk
controls	358.00 ± 37.58	396.17 ± 51.67	447.83 ± 19.27
STZ - rats	261.00 ± 33.13 *	267.17 ± 19.61 *	273.17 ± 39.36 *
garlic - treated STZ - rats	306.33 ± 19.46 #	294.17 ± 19.80 #	351.17 ± 28.55 #

Value are means ± SE ; n = 6 rats .

* Significant difference as compared to controls (P < 0.05) .

Significant difference as compared to STZ-rats (P < 0.05) .

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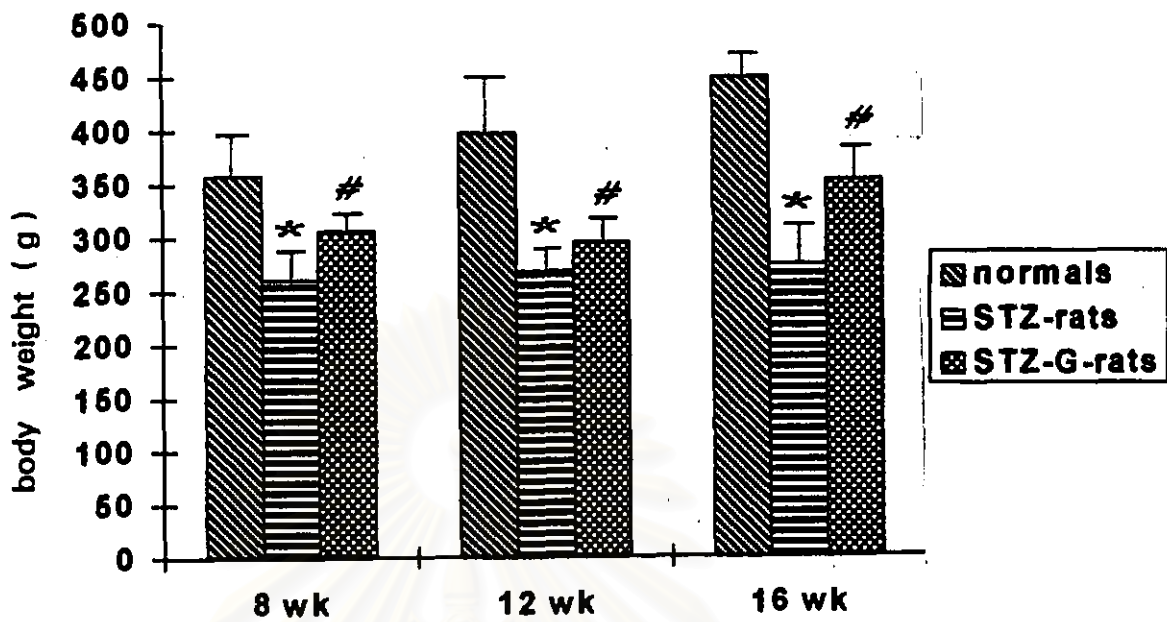


Fig. 7 Body weight (g) of controls , STZ-rats , and garlic-treated STZ-rats (STZ-G-rats) at 8 , 12 , and 16 weeks .

Values are means \pm SE ; n = 6 rats .

* Significant difference as compared to controls (P < 0.05) .

Significant difference as compared to STZ-rats (P < 0.05) .

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Table 2 Systolic pressure (mmHg) of controls , STZ-rats , and garlic-treated STZ-rats at 8 , 12 , and 16 weeks .

groups	systolic pressure (mmHg)		
	8 wk	12 wk	16 wk
controls	92.50 ± 7.58	95.00 ± 8.94	100.00 ± 3.16
STZ - rats	109.17 ± 3.76 *	135.00 ± 10.00 *	136.67 ± 13.29 *
garlic - treated STZ - rats	88.33 ± 8.16 #	90.00 ± 7.07 #	90.00 ± 7.07 #

Values are means ± SE ; n = 6 rats .

* Significant difference as compared to controls (P < 0.05) .

Significant difference as compared to STZ-rats (P < 0.05) .

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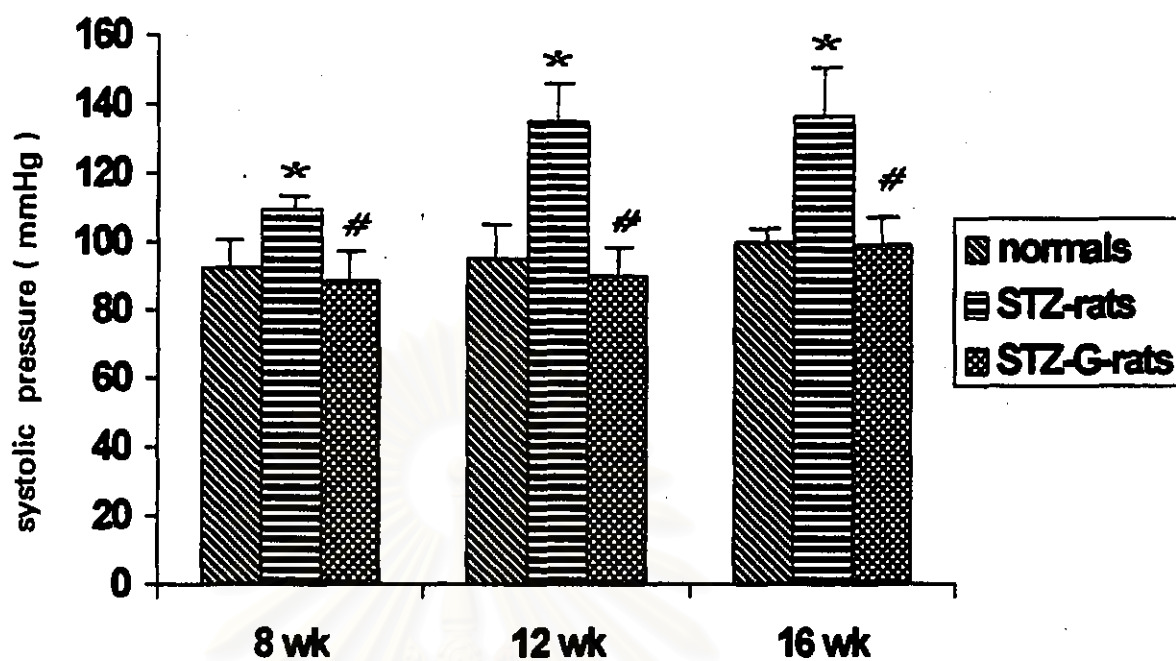


Fig. 8 Systolic pressure (mmHg) of controls , STZ-rats , and garlic-treated STZ-rats (STZ-G-rats) at 8 , 12 , and 16 weeks .

Values are means \pm SE ; n = 6 rats .

* Significant difference as compared to controls ($P < 0.05$) .

Significant difference as compared to STZ-rats ($P < 0.05$) .

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Table 3 Diastolic pressure (mmHg) of controls , STZ-rats , and garlic-treated STZ-rats at 8 , 12 , and 16 weeks .

groups	diastolic pressure (mmHg)		
	8 wk	12 wk	16 wk
controls	63.33 ± 7.52	65.83 ± 10.12	65.00 ± 8.94
STZ - rats	89.17 ± 3.76 *	108.33 ± 13.66 *	115.83 ± 12.81 *
garlic - treated STZ - rats	68.33 ± 6.05 #	65.00 ± 6.32 #	67.50 ± 4.18 #

Value are means ± SE ; n = 6 rats .

* Significant difference as compared to controls (P < 0.05) .

Significant difference as compared to STZ-rats (P < 0.05) .

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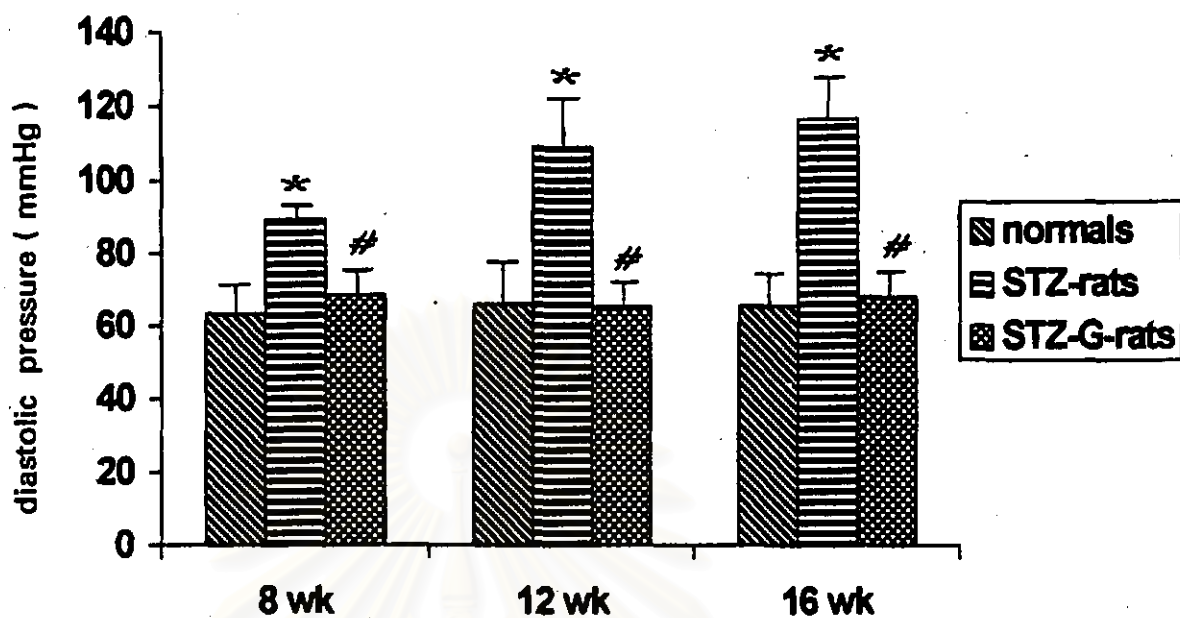


Fig. 9 Diastolic pressure (mmHg) of controls , STZ-rats , and garlic-treated STZ-rats (STZ-G-rats) at 8 , 12 , and 16 weeks . Values are means \pm SE ; n = 6 rats .

* Significant difference as compared to controls (P < 0.05) .

Significant difference as compared to STZ-rats (P < 0.05) .

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Table 4 Mean arterial pressure^a (mmHg) of controls, STZ-rats, and garlic-treated STZ-rats at 8, 12, and 16 weeks.

groups	mean arterial pressure (mmHg)		
	8 wk	12 wk	16 wk
controls	73.06 ± 7.48	74.72 ± 8.19	75.83 ± 5.34
STZ - rats	95.56 ± 3.28 [*]	117.22 ± 12.32 [*]	121.11 ± 14.67 [*]
garlic - treated STZ - rats	74.99 ± 6.32 [#]	73.33 ± 6.05 [#]	75.00 ± 4.71 [#]

Value are means ± SE ; n = 6 rats .

* Significant difference as compared to controls (P < 0.05) .

Significant difference as compared to STZ-rats (P < 0.05) .

a. Mean arterial pressure was calculated by the summation of diastolic pressure and one - third pulse pressure . And , pulse pressure was also calculated by systolic pressure minus diastolic pressure .

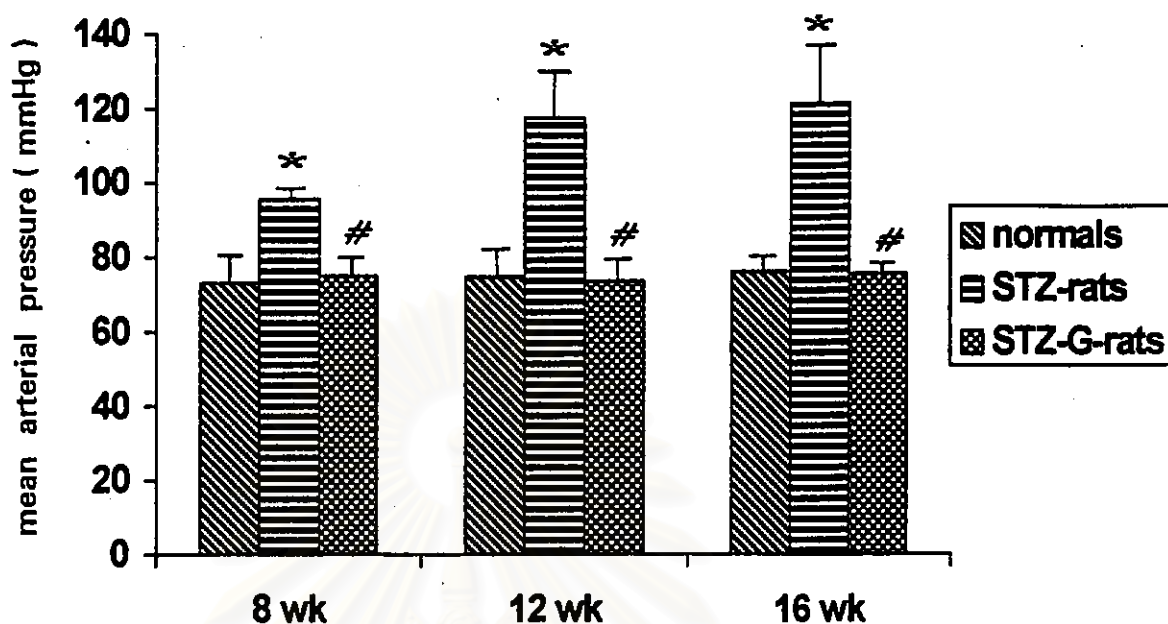


Fig. 10 Mean arterial pressure (mmHg) of controls , STZ-rats , and garlic-treated STZ-rats (STZ-G-rats) at 8 , 12 , and 16 weeks . Values are means \pm SE ; n = 6 rats .

* Significant difference as compared to controls ($P < 0.05$) .

Significant difference as compared to STZ-rats ($P < 0.05$) .

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Table 5 Heart rate (beats / minute) of controls , STZ-rats , and garlic-treated STZ-rats at 8 , 12 , and 16 weeks .

groups	heart rate (beats / min)		
	8 wk	12 wk	16 wk
controls	402.50 ± 11.29	402.50 ± 17.53	395.00 ± 22.58
STZ - rats	297.50 ± 19.94 [*]	302.50 ± 37.25 [*]	290.00 ± 24.49 [*]
garlic - treated STZ - rats	331.67 ± 30.72 [#]	349.17 ± 20.59 [#]	345.83 ± 16.25 [#]

Value are means ± SE ; n = 6 rats .

* Significant difference as compared to controls (P < 0.05) .

Significant difference as compared to STZ-rats (P < 0.05) .

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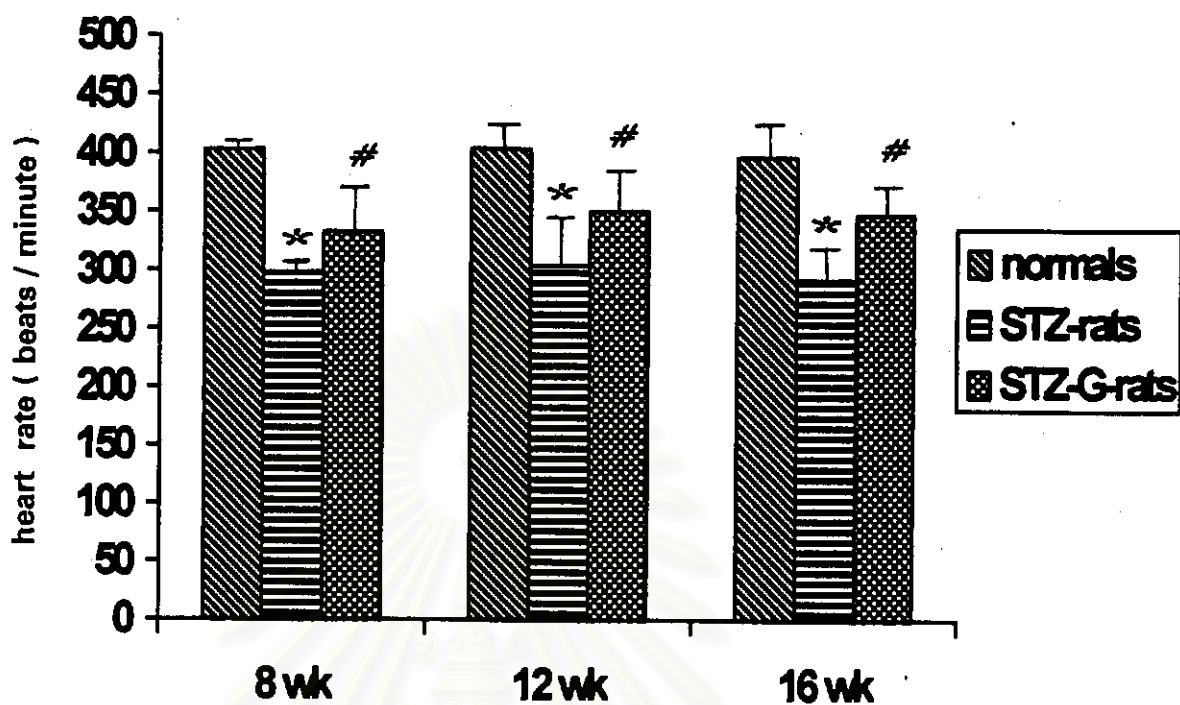


Fig. 11 Heart rate (beats / minute) of controls , STZ-rats , and garlic-treated STZ-rats (STZ-G-rats) at 8 , 12 , and 16 weeks .

Value are means \pm SE ; n = 6 rats .

* Significant difference as compared to controls (P < 0.05) .

Significant difference as compared to STZ-rats (P < 0.05) .

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Table 6 Aortic flow rate (ml / min) of controls , STZ-rats , and garlic-treated STZ-rats at 8 , 12 , and 16 weeks .

groups	aortic flow rate (ml / min)		
	8 wk	12 wk	16 wk
controls	67.67 ± 1.50	65.83 ± 3.54	65.33 ± 1.75
STZ - rats	56.33 ± 2.25*	49.33 ± 2.66*	48.50 ± 2.66*
garlic - treated STZ - rats	62.67 ± 1.97#	62.17 ± 2.64#	63.17 ± 2.04#

Value are means ± SE ; n = 6 rats .

* Significant difference as compared to controls (P < 0.05) .

Significant difference as compared to STZ-rats (P < 0.05) .

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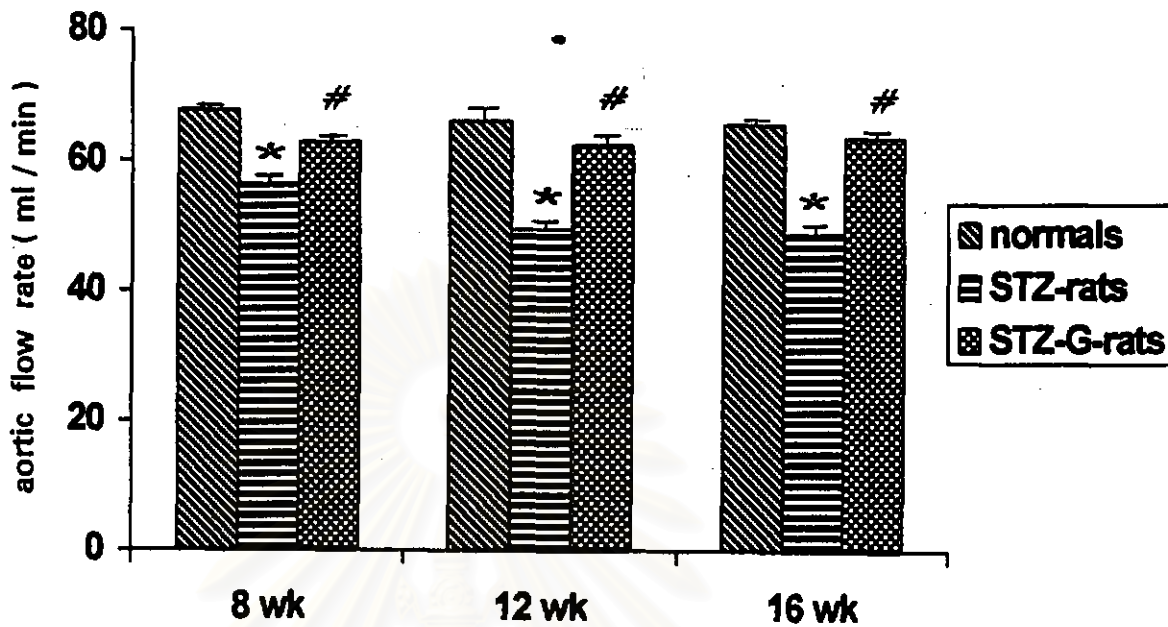


Fig. 12 Aortic flow rate (ml / min) of controls , STZ-rats , and garlic-treated STZ-rats (STZ-G-rats) at 8 , 12 , and 16 weeks . Values are means \pm SE ; n = 6 rats .

* Significant difference as compared to controls ($P < 0.05$) .

Significant difference as compared to STZ-rats ($P < 0.05$) .

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Table 7 Ratio of heart weight per 100 gram body weight (%) of controls , STZ-rats , and garlic-treated at 8 , 12 , and 16 weeks .

groups	ratio of heart weight per 100 gram body weight (%)		
	8 wk	12 wk	16 wk
controls	0.32 ± 0.05	0.33 ± 0.01	0.33 ± 0.01
STZ - rats	$0.43 \pm 0.02^*$	$0.43 \pm 0.03^*$	$0.43 \pm 0.04^*$
garlic - treated STZ - rats	$0.39 \pm 0.01^\#$	$0.34 \pm 0.04^\#$	$0.37 \pm 0.04^\#$

Value are means \pm SE ; n = 6 rats .

* Significant difference as compared to controls (P < 0.05) .

Significant difference as compared to STZ-rats (P < 0.05) .

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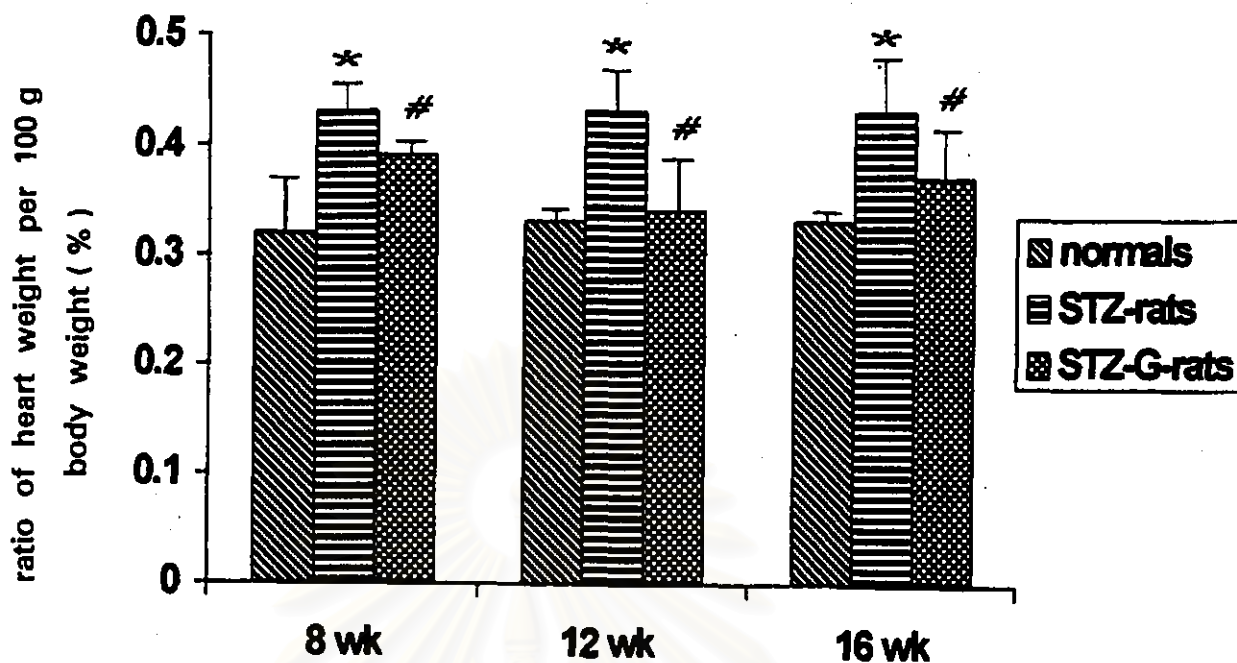


Fig. 13 Ratio of heart weight per 100 gram body weight (%) of controls , STZ-rats , and garlic-treated STZ-rats (STZ-G-rats) at 8 , 12 , and 16 weeks . Value are means \pm SE ; n = 6 rats .

* Significant difference as compared to controls ($P < 0.05$) .

Significant difference as compared to STZ-rats ($P < 0.05$) .

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A.



B.

Fig. 14 Coronary arteriolar responses to topical application of sodium nitroprusside (10^{-4} M) in a isolated arrested heart of control rat .

A. Before topical application of sodium nitroprusside .

B. Coronary arterioles dilate after topical application of sodium nitroprusside .

Table 8 Changes in arteriolar diameter in responses to acetylcholine (10^{-4} M) of controls, STZ-rats, and garlic-treated STZ-rats at 8, 12, and 16 weeks.

groups	changes in arteriolar diameter (%)		
	8 wk	12 wk	16 wk
controls	$+12.75 \pm 0.76$	$+12.67 \pm 1.68$	$+12.75 \pm 0.88$
STZ - rats	$+6.88 \pm 0.58^*$	$+6.27 \pm 0.83^*$	$+6.55 \pm 1.41^*$
garlic - treated STZ - rats	$+11.60 \pm 1.96^\#$	$+10.88 \pm 2.17^\#$	$+9.54 \pm 2.52^\#$

Values are means \pm SE ; n = 6 rats

A positive value indicates arteriolar vasodilation .

* Significant difference as compared to controls (P < 0.05) .

Significant difference as compared to STZ-rats (P < 0.05) .

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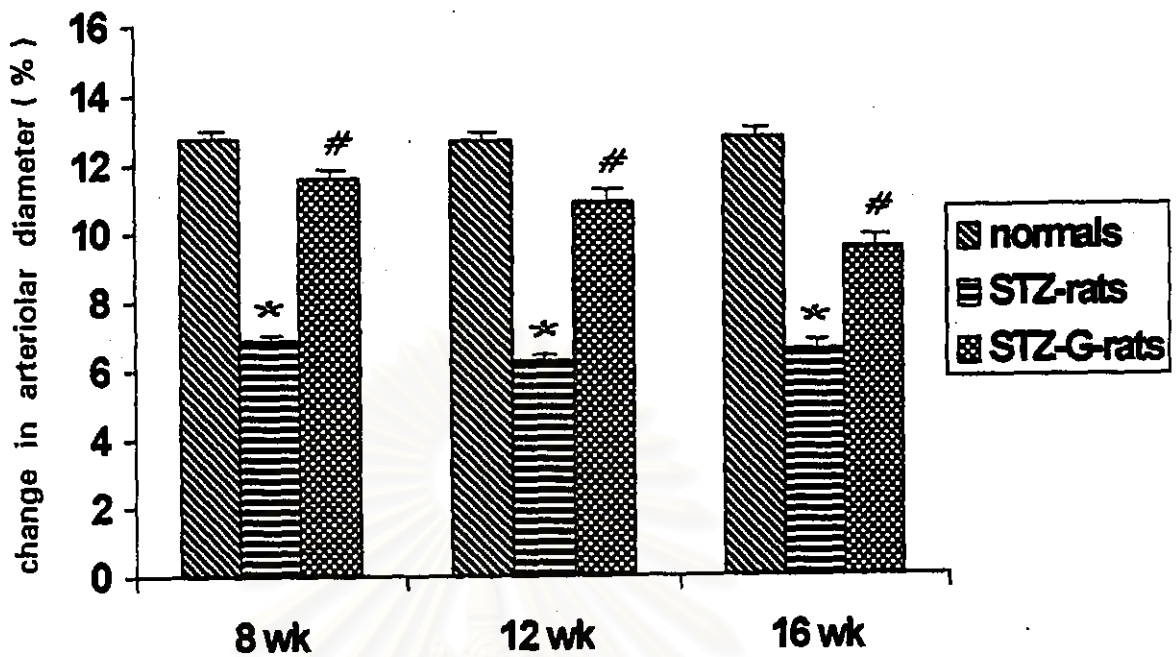


Fig. 15 Changes in arteriolar diameter in responses to acetylcholine (10^{-4} M) of controls, STZ-rats, and garlic-treated STZ-rats (STZ-G-rats) at 8, 12, and 16 weeks. Values are means \pm SE; $n = 6$ rats

* Significant difference as compared to controls ($P < 0.05$).

Significant difference as compared to STZ-rats ($P < 0.05$).

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Table 9 Changes in arteriolar diameter in responses to sodium nitroprusside (10^{-4} M) of controls, STZ-rats, and garlic-treated STZ-rats at 8, 12, and 16 weeks.

groups	changes in arteriolar diameter (%)		
	8. wk	12 wk	16 wk
controls	+15.54 ± 0.97	+15.62 ± 0.53	+15.24 ± 1.12
STZ - rats	+13.89 ± 2.06 ^a NS	+15.37 ± 1.38 ^a NS	+14.90 ± 1.30 ^a NS
garlic - treated STZ - rats	+14.35 ± 1.60 ^b NS	+13.99 ± 2.20 ^b NS	+14.54 ± 1.59 ^b NS

Values are means ± SE ; n = 6 rats

A positive value indicates arteriolar vasodilation .

^a
NS Nonsignificant difference as compared to controls (P < 0.05) .

^b
NS Nonsignificant difference as compared to STZ-rats (P < 0.05) .

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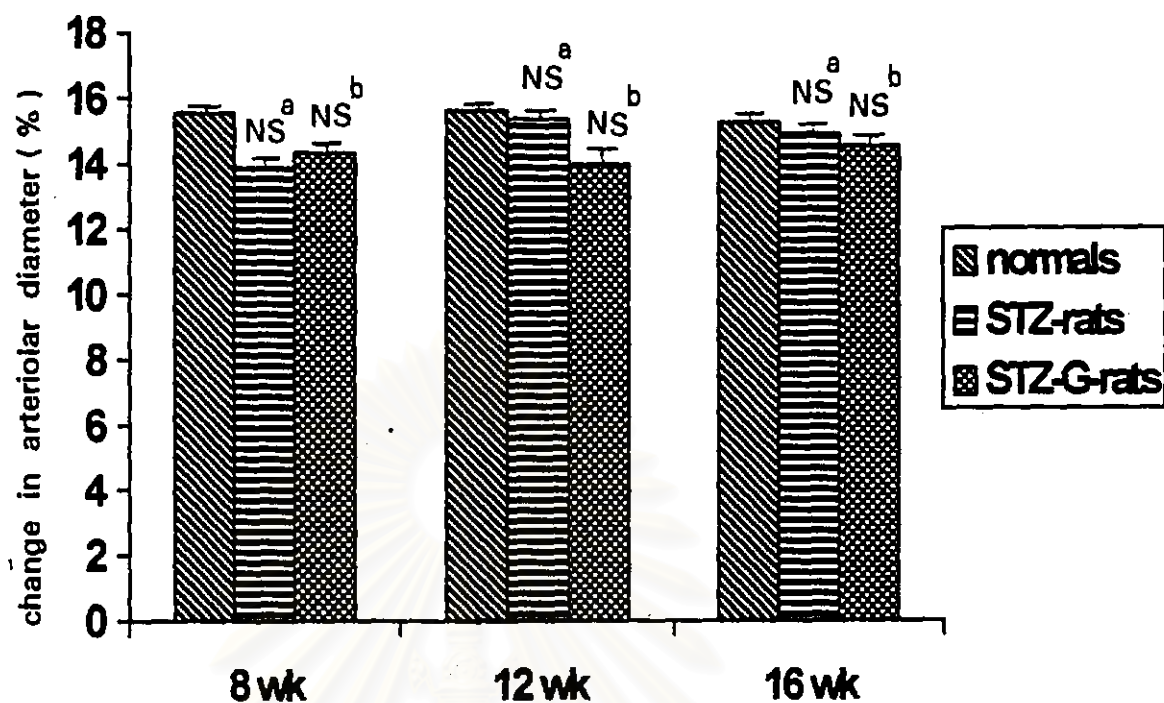


Fig. 16 Changes in arteriolar diameter in responses to sodium nitroprusside (10^{-4} M) of controls, STZ-rats, and garlic-treated (STZ-G-rats) at 8, 12, and 16 weeks. Values are means \pm SE; $n = 6$ rats

NS^a Nonsignificant difference as compared to controls ($P < 0.05$).

NS^b Nonsignificant difference as compared to STZ-rats ($P < 0.05$).

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Table 10 Changes in arteriolar diameter in responses to norepinephrine (10^{-4} M) of controls, STZ-rats, and garlic-treated STZ-rats at 8, 12, and 16 weeks.

groups	changes in arteriolar diameter (%)		
	8 wk	12 wk	16 wk
controls	-13.19 ± 1.77	-12.19 ± 1.78	-12.44 ± 1.83
STZ - rats	$-18.17 \pm 0.83^*$	$-18.25 \pm 1.38^*$	$-17.89 \pm 0.86^*$
garlic - treated STZ - rats	$-14.60 \pm 0.41^\#$	$-14.80 \pm 2.88^\#$	$-14.95 \pm 0.86^\#$

Values are means \pm SE ; n = 6 rats

A negative value indicates arteriolar vasoconstriction.

* Significant difference as compared to controls (P < 0.05).

Significant difference as compared to STZ-rats (P < 0.05).

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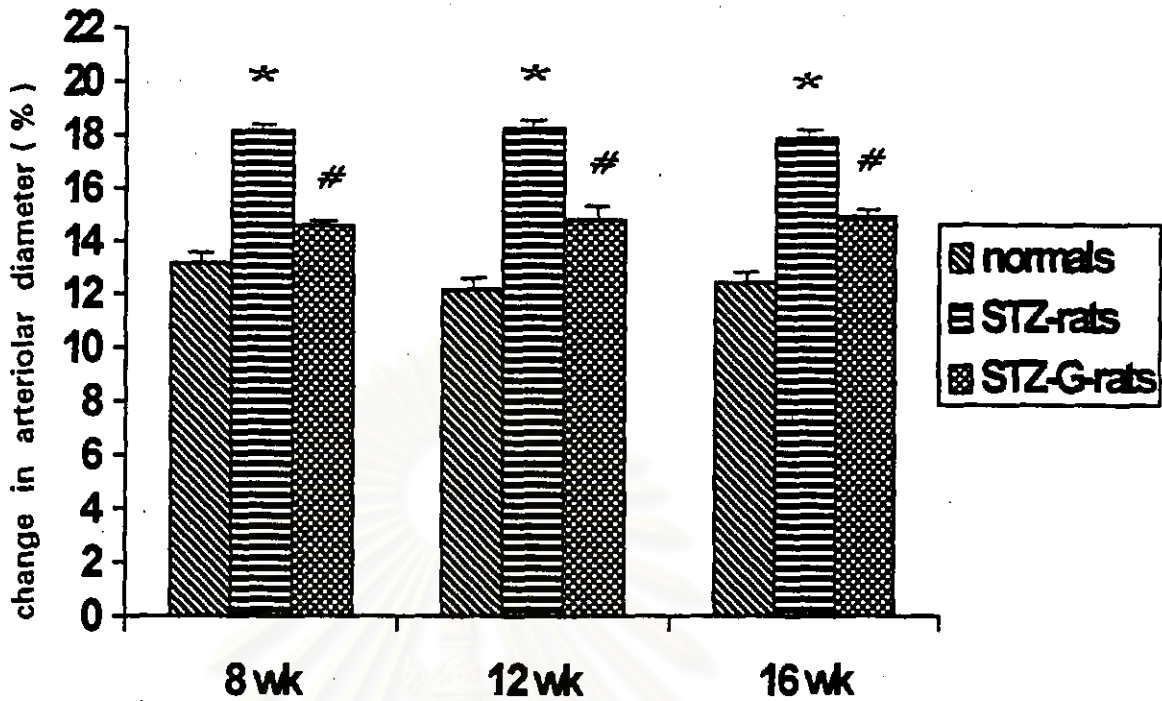


Fig. 17 Changes in arteriolar diameter in responses to norepinephrine (10^{-4} M) of controls, STZ-rats, and garlic-treated STZ-rats (STZ-G-rats) at 8, 12, and 16 weeks. Values are means \pm SE; $n = 6$ rats

* Significant difference as compared to controls ($P < 0.05$).

Significant difference as compared to STZ-rats ($P < 0.05$).

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Table 11 Changes in arteriolar diameter in responses to indomethacin⁴ (10 M) of controls, STZ-rats, and garlic-treated STZ-rats at 8, 12, and 16 weeks.

groups	changes in arteriolar diameter (%)		
	8 wk	12 wk	16 wk
controls	-9.32 ± 1.60	-8.43 ± 1.67	-9.51 ± 1.21
STZ - rats	+6.99 ± 0.97	+6.99 ± 1.47	+7.06 ± 1.29
garlic - treated STZ - rats	-8.68 ± 1.16	-7.61 ± 1.24	-7.84 ± 0.77

Values are means ± SE ; n = 6 rats

A positive value indicates arteriolar vasodilation .

A negative value. indicates arteriolar vasoconstriction .

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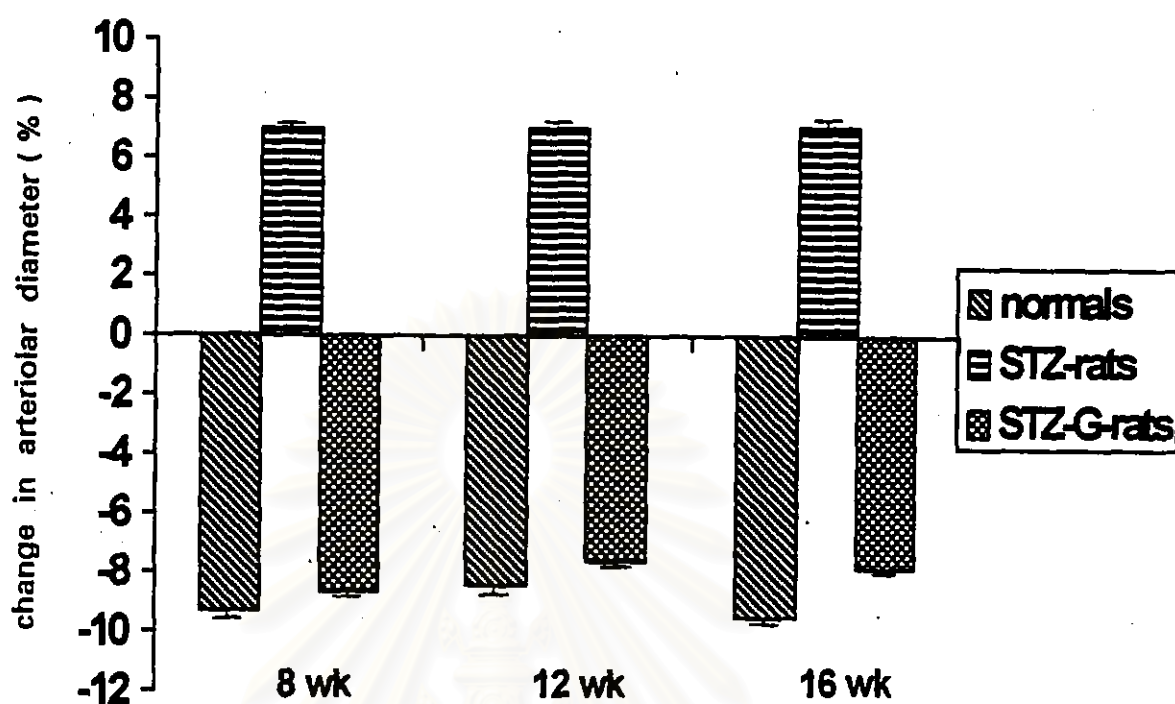


Fig. 18 Changes in arteriolar diameter in responses to indomethacin (10^{-4} M) of controls, STZ-rats, and garlic-treated STZ-rats (STZ-G-rats) at 8, 12, and 16 weeks. Values are means \pm SE; $n = 6$ rats. A positive value indicates arteriolar vasodilation. A negative value indicates arteriolar vasoconstriction.

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