RESULTS

A-In vitro Experiments:

1- Isolated rabbit aortic strip:

Addition of different concentrations of methanol (0.01%, 0.1% and 1%) as a solvent used to dissolve carvedilol and valsartan didn't produce any change in norepinephrine and angiotensin II induced contractions of isolated rabbit aortic strip (figure 8).

Regarding the interaction of carvedilol with norepinephrine, it was observed that incubation in doses of carvedilol (2, 4, 8 and 16 ug/ml) for 15 minute produced significant decrease of the norepinephrine induced contractile response of the rabbit aortic strip. (Table 4)(Figure 9, 11). On the other hand, it was observed that incubation of gradually increasing doses of valsartan (2, 4, 8 and 16ug/ml) for 15 minutes before addition of norepinephrine in a dose of 6ug/ml produced no change in the aortic strip response to noreinephrine (Table 4) (figure 10, 11).

Moreover, it was observed that preincubation of gradually increasing doses of carvedilol (2, 4, 8 and 16 ug/ml) for 15 minutes before the addition of angiotensin II in a maximal dose (2ug/ml) produced no change in the angiotensin II induced contractile response of the rabbit aortic strip (Table 5) (figure 12, 14). While, preincubation of gradually increasing doses of valsartan (2, 4, 8 and 16 ug/ml) for 15 minutes before the addition of angiotensin II in a maximal dose (2ug/ml) produced significant ($p<0.05$) decrease of the angiotensin II induced contractile response of the rabbit aortic strip. (Table 5) (figure13, 14).

Table (4): showing the effect of carvedilol versus valsartan on mean ± SE amplitude of norepinephrine- induced contraction on isolated rabbit aortic strip.

Dose / Drug	Control	2ug/ml	4ug/ml	8ug/ml	16ug/ml
Carvedilol	3.55±0.1 {cm}	* 3.03±0.03 P<0.05	* 2.37±0.03 P<0.05	* 2.23±0.026 P<0.05	* 1.45±0.145 P<0.05
valsartan		# 3.55±0.01 P>0.05	# 3.51±0.021 P>0.05	# 3.50±0.049 P>0.05	# 3.49±0.043 P>0.05

P: compared the inhibitory effect of carvedilol and valsartan in doses of (2, 4, 8 and 16 ug/ml) on mean amplitude of norepinephrine induced contraction on isolated rabbit aortic strip compared with control value.

N.B.:

*: statistically significant inhibitory effect ($p<0.05$).

#: statistically insignificant inhibitory effect ($p>0.05$).

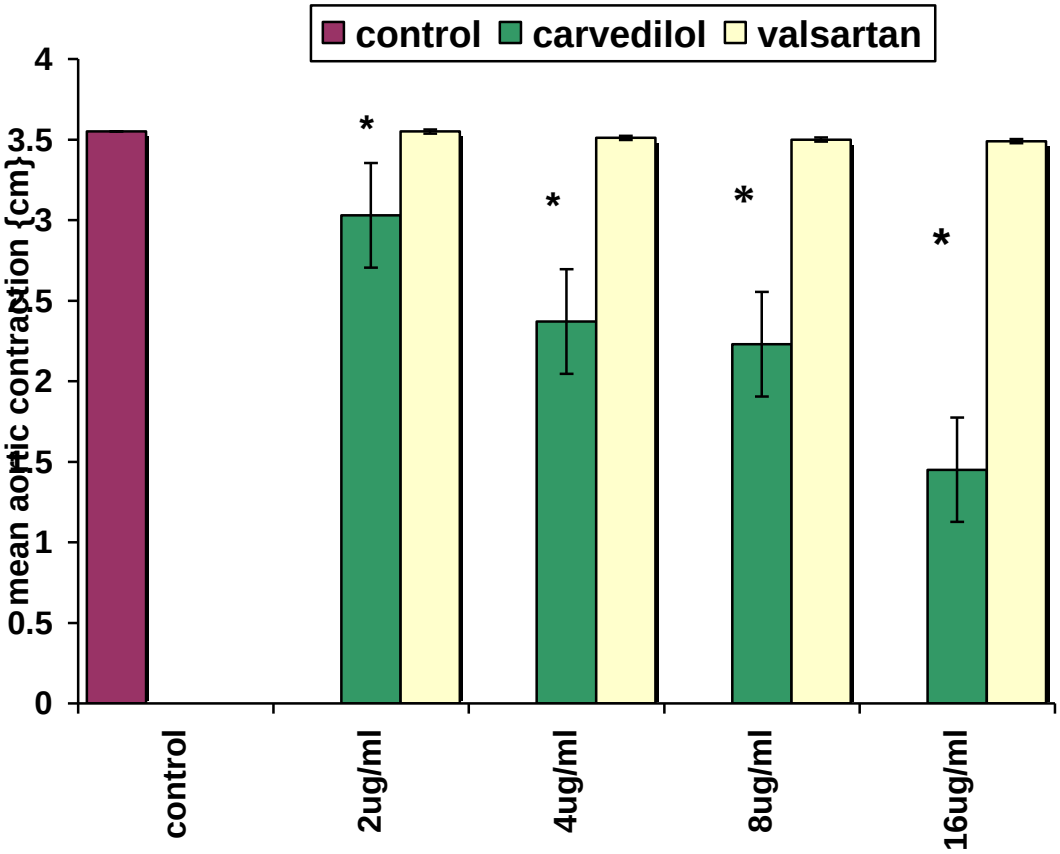


Figure (11): a chart showing the effect of carvedilol and valsartan on norepinephrine induced contraction on isolated rabbit aortic strip.

N.B.:

*****: significant inhibitory effect of carvedilol at the doses of 8 and 16ug/ml on norepinephrine induced contraction on isolated rabbit aortic strip.

Table (5): showing the effect of carvedilol versus valsartan on mean ± SE amplitude of angiotensin II- induced contraction on isolated rabbit aortic strip.

Dose / Drug	Control	2ug/ml	4ug/ml	8ug/ml	16ug/ml
Carvedilol	3.38±0.1 {cm}	# 3.38±0.03 P>0.05	# 3.38.±0.03 P>0.05	# 3.37±0.2 P>0.05	# 3.37±0.1 P>0.05
Valsartan		* 3.1±0.017 P<0.05	* 2.48±0.017 P<0.05	* 2.3±0.052 P<0.05	* 2.2±0.1 P<0.05

P: compared the inhibitory effect of carvedilol and valsartan in doses of (2, 4, 8 and 16 ug/ml) on mean amplitude of angiotensin II induced contraction on isolated rabbit aortic strip compared with control value.

N.B.:

*: statistically significant inhibitory effect ($p<0.05$).

#: statistically insignificant inhibitory effect ($p>0.05$).

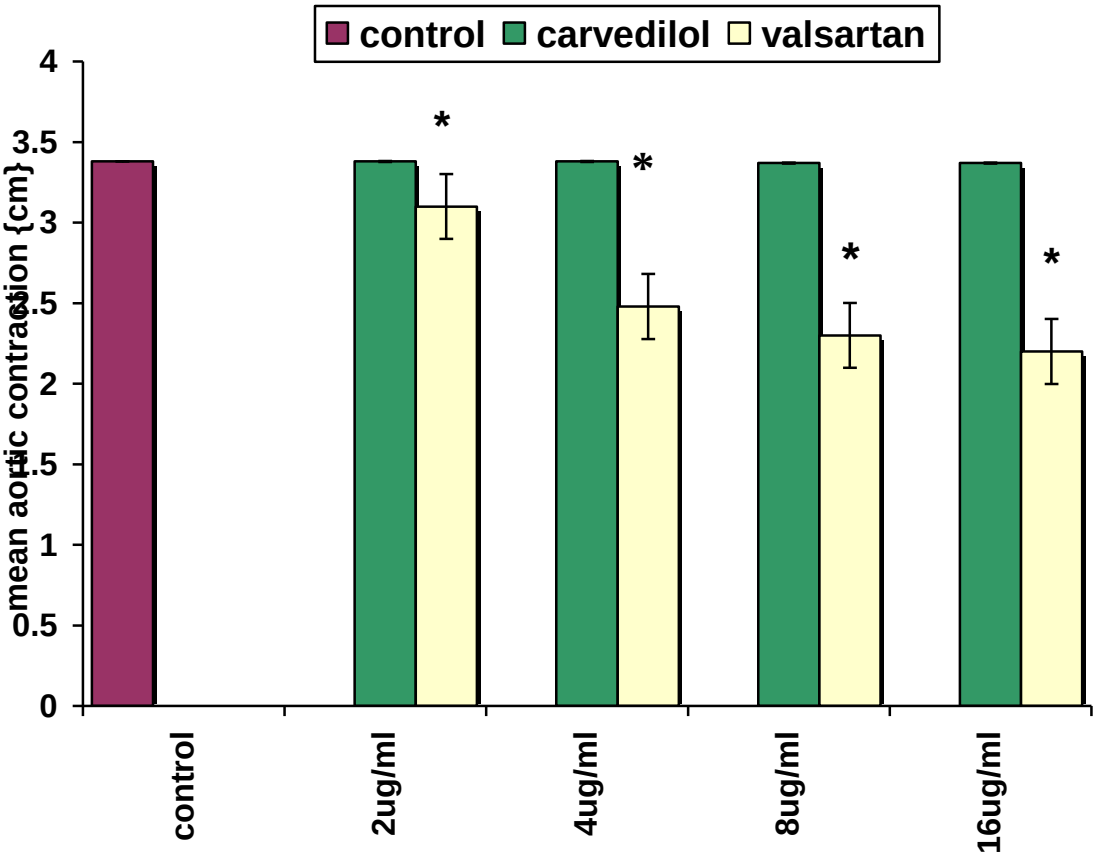


Figure (14): a chart showing the effect of carvedilol and valsartan on angiotensin II induced contraction on isolated rabbit aortic strip.

N.B.:

*****: significant inhibitory effect of valsartan at the doses of 2, 4, 8 and 16ug/ml on angiotensin II induced contraction on isolated rabbit aortic strip.

2- Isolated rabbit heart:

Addition of different concentrations of methanol (0.01%, 0.1% and 1%) the solvent used to dissolve carvedilol and valsartan produced negligible decrease in basal myocardial contractility (figure 15)

To show the effect of carvedilol and valsartan on basal cardiac contractility in isolated rabbit heart, carvedilol and valsartan was added in gradually increasing doses (2, 4, 8, 16, and 32ug/ml). It was observed that carvedilol and valsartan produced no change in the amplitude of contraction of isolated rabbit heart Table 6 (figure 16, 17, 18).

Regarding the effect of carvedilol on isoprenaline induced contraction in isolated rabbit heart, it was observed that addition of carvedilol in a dose of 16ug/ml significantly ($p<0.05$) decreased the amplitude of contractions of isolated rabbit heart in response to isoprenaline (6ug/ml) (figure 19).

On the other hand, addition of valsartan in a dose of 16ug/ml significantly ($p<0.05$) decrease amplitude of contractions of isolated rabbit heart in response to angiotensin II (2ug/ml) (figure 20).

Table (6): showing the effects of carvedilol and valsartan on mean ± SE of amplitude of contraction of isolated rabbit heart.

Doses / Drugs	control	2ug	4ug	8ug	16ug	32ug
Carvedilol	3.7±0.15 {cm}	# 3.6±0.14 P>0.05	# 3.4±0.14 P>0.05	# 3.42±0.09 P>0.05	# 3.37±0.11 P>0.05	# 2.89±0.22 P>0.05
valsartan		# 3.43±0.01 P>0.05	# 3.43±0.01 P>0.05	# 3.43±0.017 P>0.05	# 3.42±0.03 P>0.05	# 3.43±0.03 P>0.05

P: compared the effect of carvedilol and valsartan in doses of (2ug, 4ug, 8ug, 16ug and 32ug/ml) on mean amplitude of contraction on isolated rabbit heart compared with control value.

N.B.:

#: statistically insignificant inhibitory effect (p>0.05)

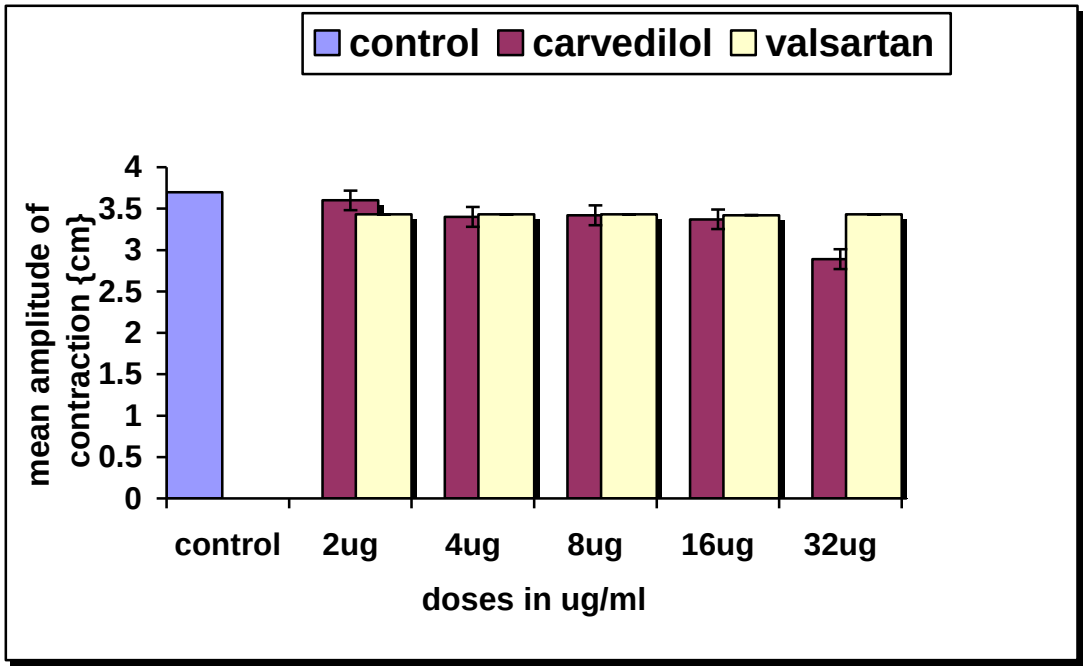


Figure (18): histogram showing the changes on mean amplitude of contraction of isolated rabbit heart after addition of carvedilol and valsartan in doses of 2ug, 4ug, 8ug, 16ug and 32ug/ml. It showed a statistically insignificant inhibitory effect of carvedilol and valsartan on mean amplitude of contraction of isolated rabbit heart ($p>0.05$) compared with control value.

In vivo experiments:

I- Effect of carvedilol on isoprepnaline induced heart failure in rats versus valsartan:

A-Effect on arterial blood pressure:

It was observed that the recorded basal systolic aortic pressure (SAP) and mean aortic pressure (MAP) in control rats were 138.6±2.66 mmHg and 118.6±3.81 mmHg respectively, (Table 7) (Figure 21, and 28A, 28B).

In the group of heart failure, the SAP was 124±1.76 mmHg while MAP was 96.0 ± 2.9 mmHg. When these values were compared to the corresponding values in normal rats, there was significant decrease ($p<0.05$) in both SAP and MAP in the group of heart failure (Table 7) (Figure 22, 28A, 28B).

In carvedilol treated group, the recorded SAP and MAP were 110.1± 2.47 mmHg and 93.6 ± 1.31 mmHg respectively. When these values were compared to the corresponding values of the heart failure group, they revealed significant decrease ($p<0.05$) of both SAP and MAP, (table 7) (Figure 23, 28A, 28B).

In valsartan treated group, the recorded SAP and MAP were 100.2± 3.12 mmHg and 80.3 ± 1.63 mmHg respectively. When these values were compared to the corresponding values of the heart failure group, they revealed significant decrease ($p<0.05$) of both SAP and MAP, (table 7) (Fig. 24, 28A, 28B,).

By comparing the effect of carvedilol with valsartan on mean SAP there was no statistically difference between effects of both drugs.

B-Effect on ECG:

ECG tracing of the group of heart failure revealed insignificant increase ($p>0.05$) in heart rate (HR) in comparison to control group, as the mean value of the heart rate in normal rats was 310 ± 15.8 b/min, while the corresponding value in control group of heart failure was 335.8 ± 26.4 b/min., (table 7) (Figure 21,22, 28C).

In carvedilol treated group of heart failure the mean value of HR was 317.7±21.7b/min. when this value was compared to corresponding value of the heart failure there were no statistical ($p>0.05$) difference (table 7) (figure 23, 28C).

In valsartan treated group of heart failure the mean value of HR was 310± 23.8 b/min. when this value was compared to corresponding value of the heart failure there were no statistical ($p>0.05$) difference (table 7) (figure 24, 28C).

By comparing the effect of carvedilol with valsartan on mean HR there was no statistically difference between effects of both drugs.

C- Effect of carvedilol and valsartan treatment on myocardic infarction size:

The mean value of infarct size percentage in control group of rats was 35.87±1.73 (Table 7) (figure 25, 28D).

In carvedilol treated group the mean value of infarct size percentage in this group of rats was 35.18%±1.94. When this value was compared to corresponding value in sham control group there was insignificant decrease ($p>0.05$) (table 7) (figure 26, 28D).

In valsartan treated group the mean value of infarct size percentage in this group of rats was 35.27%±1.34. When compared to corresponding value in sham control group. There was insignificant decrease ($p>0.05$) (table 7) (figure 27, 28D).

By comparing the effect of carvedilol and valsartan treatment on infarct size there were insignificant difference between them.

Table (7): Mean ± SD of blood pressure (mmHg), heart rate (b/min.) and percentage surface area of infarct size.

Groups	Blood pressure(mmHg)		Heart rate (b/min)	Infarct size (%)
	SAP	MAP		
Control group	138.6±2.66	118.6±3.81	310.4±15.8	None
H.F. group Sham control	* 124±1.76 P1<0.05	* 96.0±2.9 P1<0.05	335.8±26.4 P1>0.05	35.87±1.73
Carvedilol group	* 110.1±2.47 P2>0.05	* 93.6±1.31 P2>0.05	317.7±21.7 P2>0.05	35.18±1.94 P2>0.05
Valsartan group	* 100.2±3.12 P3>0.05	* 80.2±1.63 P3>0.05	310±23.8 P3>0.05	35.27±1.34 P3>0.05

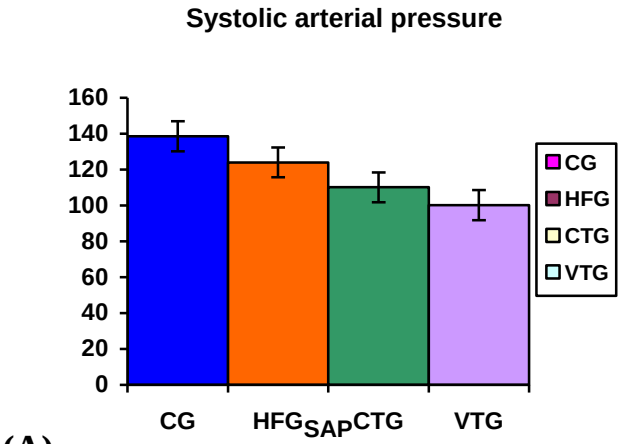
N.B:

P1: sham control (Heart failure group) compared with control group.

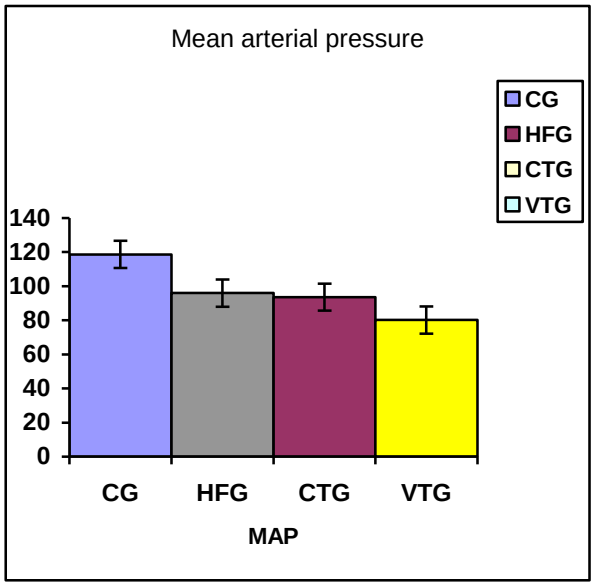
P2: carvedilol group compared with control

P3: valsartan group compared with control.

*: significant ($p<0.05$) compared to control.



(A)



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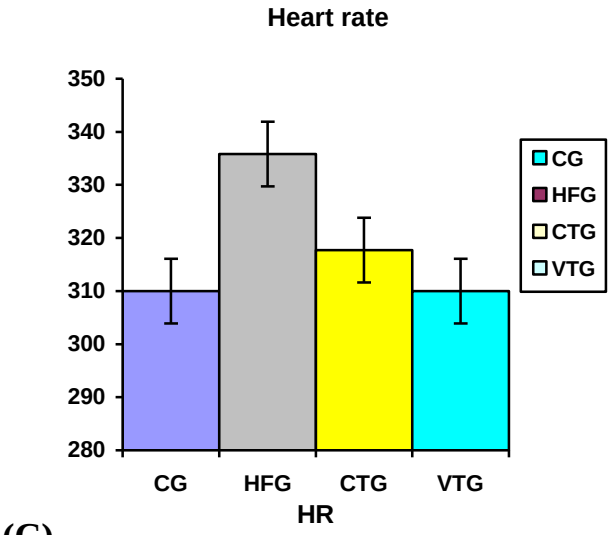
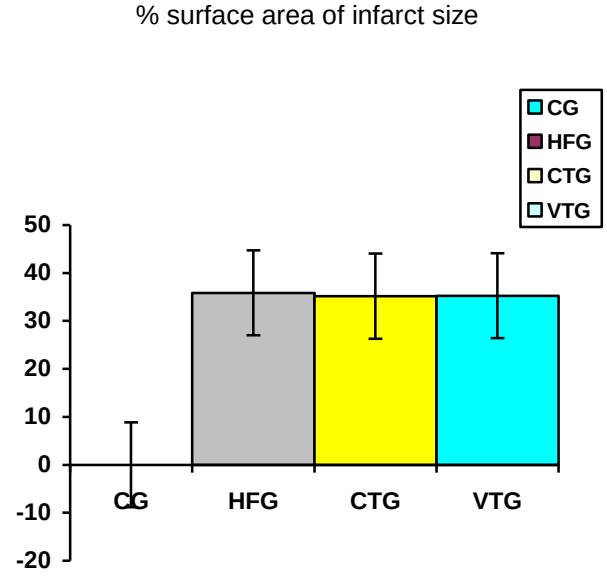


(C)



(D)

Fig. (28): A histogram showing the effect of carvedilol versus valsartan treatment on arterial blood pressure, heart rate and %surface area of infarct size.

N.B

* CG: control group
* HFG: Heart failure group
* CTG: Carvedilol treated group
* VTG: Valsartan treated group

D- Histopathological examination:

In the control group, no detectable changes in the cardiac smooth muscle cells were seen (figure 29).

In the group of heart failure, isoprenaline induced cardiac myocyte degenerative changes and necrosis in the form of cell swelling, loss of cytoplasmic striations and pyknotic nucleoi. Also, interstitial fibrosis was detected (fig. 30).

In carvedilol treated group, the isoprenaline induced myocardial damage was improved in comparison to the group of H.F. as myocyte degeneration and cellular necrosis as well as interstitial fibrosis was decreased (fig. 31).

Also, in valsartan treated group, the isoprenaline induced myocardial damage was improved in comparison to the group of H.F. as myocyte degeneration and cellular necrosis as well as interstitial fibrosis was decreased and there were insignificant differences between this group and carvedilol treated group (fig. 32).

II- Effect of carvedilol and valsartan on isoprenaline- induced acute myocardial infarction in rats:

II.1- On ECG:

a) T-wave voltage changes:

1- Control group:

Rats of this group received only normal saline. The mean of T-wave voltage (mV) at 0 time (immediately before isoprenaline injection) was 0.38 ± 0.001. After isoprenaline injection, the T- wave voltage tend to be elevated reaching a maximum level after 4 hours with mean of 0.76±0.004 (mV), when this value was compared with the value of T-wave voltage at zero time it releaved a significant increase ($p<0.05$) (table 8), (Figure 33, 38).

2-carvedilol pretreated group:

In this group of rats carvedilol 1mg/kg was given i.v. 15 min. prior to isoprenaline injections (S.C) the mean value of T-wave voltage at zero time was 0.31±0.001 (mV), after 4 hours the mean T-wave voltage was 0.41±0.004 (mV). When this value was compared to corresponding value in sham control group, there is a significant ($p<0.05$) decrease (table 8) (figure 34, 38).

3-carvedilol posttreated group:

In this group of rats, carvedilol 1mg/kg was given 30 min. after isoprenaline injections (S.C.) the mean value of T-wave voltage at zero time was 0.32±0.004 (mV), after 4 hours the mean T-wave voltage was 0.41±0.001 (mV). When this value was compared to corresponding value in sham control group, there is a significant ($p<0.05$) decrease (table8) (fig. 35, 38).

4-valsartan pretreated group:

In this group of rats valsartan 3mg/kg was given intraperitoneal 15 min. prior to isoprenaline injections (S.C). The mean value of T-wave voltage at zero time was 0.33 ±0.002 (mV), after 4 hours the mean T-wave voltage was 0.41±0.009 (mV). When this value was compared to corresponding value in control group, there was a significant ($p<0.05$) decrease (table8) (fig. 36, 38).

5-valsartan posttreated group:

In this group of rats, valsartan 3mg/kg was given intraperitoneal 30 min. after isoprenaline injection (S.C). The mean value of T-wave voltage at zero time was 0.34± 0.003 (mV) after 4 hours, the mean value of T-wave voltage was 0.42 ±0.009 when this value was compared to corresponding value in control group there is a significant decrease ($p<0.05$) (table 8) (fig. 37, 38).

b. Heart rate:

1- Control group:

The heart rate at the start of experiment (0 time) was 375.1± 21 while at 4 hours after isoprenaline injection, it was 400.3±28 when both values were compared to each other, there was insignificant increase ($p>0.05$) (table 9, fig. 33, 39).

2-carvedilol pretreated group:

The heart rate at the start of experiment (0 time) was 375.8±17 while at 4 hours after isoprenaline injection, it was 320.6±12 when both values were compared to each other, there is insignificant decrease ($p>0.05$) (table 9, fig. 34, 39).

3-carvedilol posttreated group:

The heart rate at the start of experiment (0 time) was 375±16 while at 4 hours after isoprenaline injection, it was 330.2±19 when both values were compared to each other, there is insignificant decrease ($p>0.05$) (table 9, fig. 35, 39).

4-valsartan pretreated group:

The heart rate at the start of experiment (0 time) was 300.8±14 while at 4 hours after isoprenaline injection, it was 250.1±12 when both values were compared to each other, there is insignificant decrease ($p>0.05$) (table 9, fig. 36, 39).

6-valsartan posttreated group:

The heart rate at the start of experiment (0 time) was 350.2±15 while at 4 hours after isoprenaline injection, it was 300.6±12 when both values were compared to each other, there is insignificant decrease ($p>0.05$) (table 9, fig. 37, 39).

Table (8): effect of carvedilol and valsartan on mean ± SE of T-wave voltage (mV) in pretreated and posttreated groups compared to control group.

Group / Time	control	Pre-treated		Post-treated	
		carvedilol	valsartan	carvedilol	valsartan
0 min.	0.38±0.001	0.31±0.001 P1>0.05	0.33±0.002 P1>0.05	0.32±0.004 P2>0.05 P3>0.05	0.34±0.002 P2>0.05 P3>0.05
30 min.	0.76±0.002	0.41±0.002 P1<0.05	0.42±0.005 P1<0.05	0.41±0.003 P2<0.05 P3>0.05	0.42±0.004 P2<0.05 P3>0.05
60 min.	0.76±0.003	0.41±0.003 P1<0.05	0.42±0.003 P1<0.05	0.41±0.002 P2<0.05 P3>0.05	0.42±0.003 P2<0.05 P3>0.05
120 min.	0.76±0.002	0.41±0.004 P1<0.05	0.42±0.001 P1<0.05	0.41±0.001 P2<0.05 P3>0.05	0.41±0.002 P2<0.05 P3>0.05
240 min.	0.76±0.004	0.41±0.004 P1<0.05	0.42±0.003 P1<0.05	0.41±0.001 P2<0.05 P3>0.05	0.41±0.009 P2<0.05 P3>0.05

N: B:
P1: compares carvedilol and valsartan pretreated groups with control.
P2: compares carvedilol and valsartan posttreated group with control
P3: compares carvedilol and valsartan pretreated group with carvedilol and valsartan post-treared group.

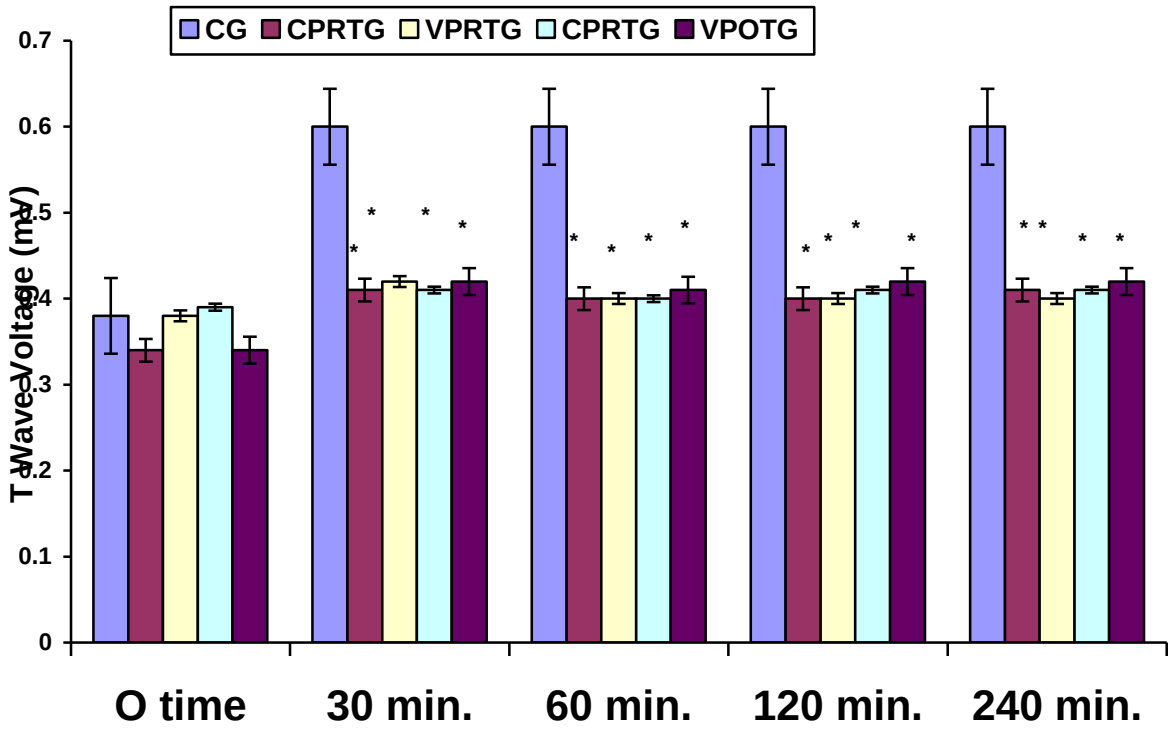


Figure (38): effect of carvedilol and valsartan treatment on mean ± SE of T.wave voltage (mV) in pretreated and posttreated groups compared to control group

0 time time of isoprenaline injection

* Significant ($p<0.05$) compared to control group

Table (9): effect of carvedilol and valsartan treatment on mean ± SEof heart rate {b/min.} in pretreated and posttreated groups compared to control group.

Group / Time	control	Pre-treated		Post-treated	
		carvedilol	valsartan	carvedilol	valsartan
0 min.	375.1±21	375.81±17	300.8±14	375±16	350.2±15
30 min.	400.2±22	375.81±16 P1>0.05	300.6±14 P1>0.05	375±17 P2>0.05 P3>0.05	350.3±14 P2>0.05 P3>0.05
60 min.	400.6±23	350.1±16 P1>0.05	275.3±13 P1>0.05	350.1±18 P2>0.05 P3>0.03	300.1±13 P2>0.05 P3>0.05
120 min.	400.4±24	320.4±15 P1>0.05	250.2±12 p1>0.05	330.3±17 P2>0.05 P3>0.05	300.8±12 P2>0.05 P3>0.05
240 min.	400.3±28	320.6±15 P1>0.05	250.1±12 p1>0.05	330.2±19 P2>0.05 P3>0.05	300.6±12 p2<0.05 P3>0.05

N: B:

P1: compares carvedilol and valsartan pretreated group with control.

P2: compares carvedilol and valsartan posttreated group carvedilol control.

P3: compares carvedilol and valsartan post-treared group with pretreated group.

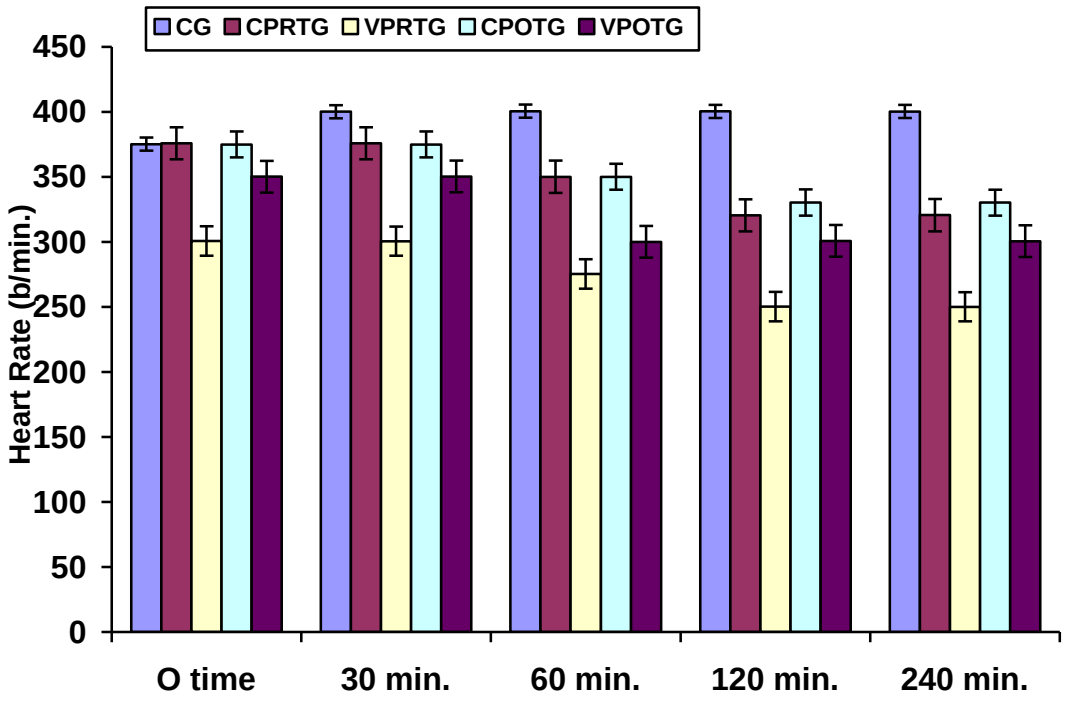


Figure (39): effect of carvedilol and valsartan treatment on mean ± S.E. of heart rate in pretreated and posttreated groups compared to control group

0 time time of isoprenaline injection

II-2 on serum level of CPK:

The mean value of serum CPK in normal rats (n= 6) was 252.5±22 U/L

1-control group:

In this group of rats, the mean serum CPK level 4 hours after isoprenaline injection was 1810.4 ± 123 U/L.

This level was highly significantly (P<0.05) increased when compared to mean value of CPK level of normal rats (Table 10, Fig.40).

2-carvedilol pretreated group:

In this group of rats, the mean CPK level was 897.3 ±52 U/L at 4 hours after isoprenaline injection. This level was significantly elevated when compared to CPK level in normal rats (P<0.05) while when this value was compared to corresponding value in control group, there was a significant decrease (P<0.05) (Fig.40 and Table 10).

3-carvedilol posttreated group:

In this group, the mean CPK level was 1112.3±102 U/L 4 hours after isoprenaline injection. This level is significantly elevated (P<0.05) in comparison to CPK level in normal rats. While when it was compared to the corresponding level of control group, there was a significant reduction of mean CPK level in carvedilol post-treated (Table 10 and Fig. 40).

4-valsartan pretreated group:

In this group of rats, the mean CPK level was 891.3 ± 48 U/L at 4 hours after isoprenaline injection. This level was significantly elevated when compared to CPK level in normal rats (P<0.05) while when this

value was compared to corresponding value in control group, there was a significant decrease ($P<0.05$) (Fig. 40 and table 10).

5-valsartan posttreated group:

In this group, the mean CPK level was 1114.6 ± 98 U/L 4 hours after isoprenaline injection. This level is significantly elevated ($P<0.05$) in comparison to CPK level in normal rats. While when it was compared to the corresponding level of control group, there was a significant reduction of mean CPK level in valsartan posttreated (Table 10 and Fig.40).

Table (10): effect of carvedilol and valsartan treatment on mean ± S.E of CPK values [U/L] in pretreated and post-treated groups compared to normal and control groups.

	Normal	control	Pretreated		Posttreated	
			carvedilol	valsartan	carvedilol	Valsartan
CPK [U/L]	252.5±22	1810.4±123 P1<0.05	897.3±58 P2<0.05	891.3±58 P2<0.05	1112.3±122 P3<0.05 P4>0.05	1114.6±133 P3<0.05 P4>0.05

P1 :compares CPK values in control group with normal values.
P2 :compares CPK values in carvedilol and valsartan pretreated treated group with control.
P3: compares CPK of carvedilol and valsartan posttreated group with control.
P4: compares CPK of carvedilol and valsartan pretreated group with carvedilol and valsartan posttreated group.

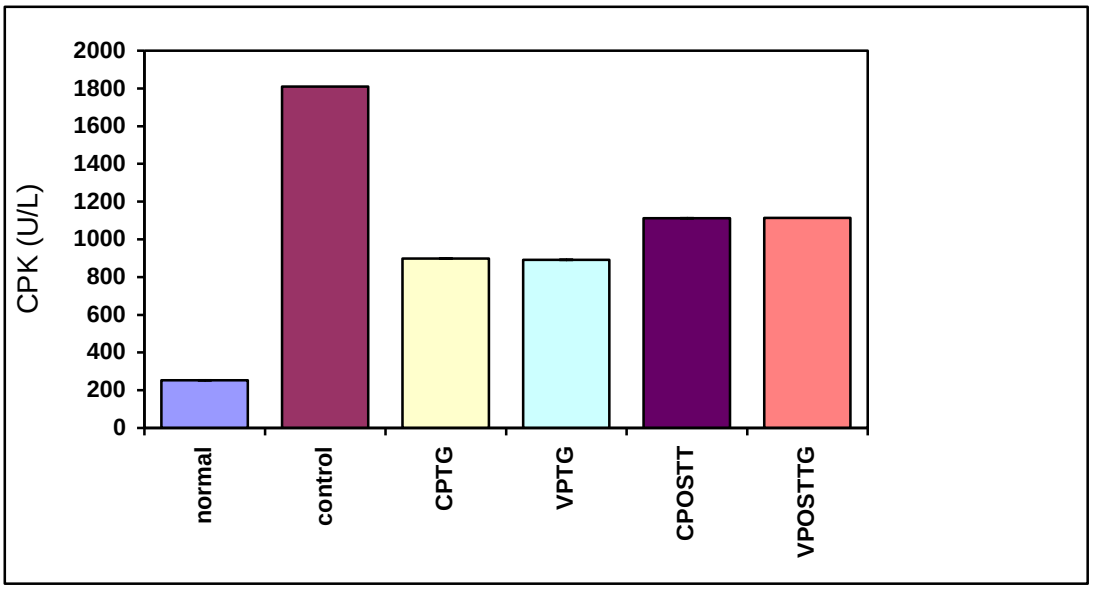


Figure (40): effect of carvedilol and valsartan on serum CPK level (U/L) in pre-treated and post-treated groups compared to normal and control group.

II-3-Histopathological examination:

In the control group, no detectable changes in the cardiac smooth muscle cells were seen (figure 41).

In the group of acute myocardial infarction, isoprenaline induced cardiac myocyte degenerative changes and necrosis in the form of cell swelling, loss of cytoplasmic striations and pyknotic nucleoi. Also, interstitial fibrosis was detected (fig. 42).

In carvedilol pretreated and posttreated groups, the isoprenaline induced myocardial damage was improved in comparison to the group of acute M.I as myocyte degeneration and cellular necrosis as well as interstitial fibrosis was decreased (fig. 43, 44).

Also, in valsartan pretreated and posttreated groups, the isoprenaline induced myocardial damage was improved in comparison to the group of acute M.I. as myocyte degeneration and cellular necrosis as well as interstitial fibrosis was decreased and there were insignificant differences between this group and carvedilol treated group (fig. 45,46).

II-4- Effect of carvedilol versus valsartan treatment on infarct size:

1-control group:

The mean value of infarct size percentage in this group of rats was 37.9% ± 0.09 (Table 11 and Fig. 47, 52).

2-carvedilol pretreated group:

The mean value of infarct size percentage in this group of rats was 37.8%±0.06 %. When this value was compared to corresponding value in control group there was insignificant decrease (P>0.05) (Table 11) (Fig. 48, 52).

2- carvedilol post treated group:

The mean value of infarct size in this group of rats was 37.7%±0.06%. When this value was compared to corresponding value in control group there was insignificant decrease (P>0.05). But when this value was compared to the corresponding value in carvedilol pre-treated group there was no significant change (Table 11) (Fig. 49, 52).

4-Valsartan pretreated group:

The mean value of infarct size percentage in this group of rats was 36.8%±0.09 %. When this value was compared to corresponding value in control group there was insignificant decrease (P>0.05) (Table 11) (Fig. 50, 52).

5-valsartan posttreated group:

The mean value of infarct size in this group of rats was 37.2%±0.08%. When this value was compared to corresponding value in control group there was insignificant decrease (P>0.05). But when

this value was compared to the corresponding value in valsartan pre-treated group there was no significant change (Table 11) (Fig. 51, 52).

By comparing the effect of carvedilol and valsartan treatment on myocardiac infarct size there were insignificant difference between them.

Table (11): effect of carvedilol and valsartan treatment on mean ±SE on percentage surface area of infarct size in pretreated and post-treated groups compared to control.

Group / M.I.size	control	Pre-treated		Post-treated	
		carvedilol	valsartan	carvedilol	valsartan
% M.I. size	38.9%±0.09	37.8±0.06 P1>0.05	37.8±0.09 P2>0.05	37.7%±0.06 P3>0.05	37.2±0.08 P5>0.05

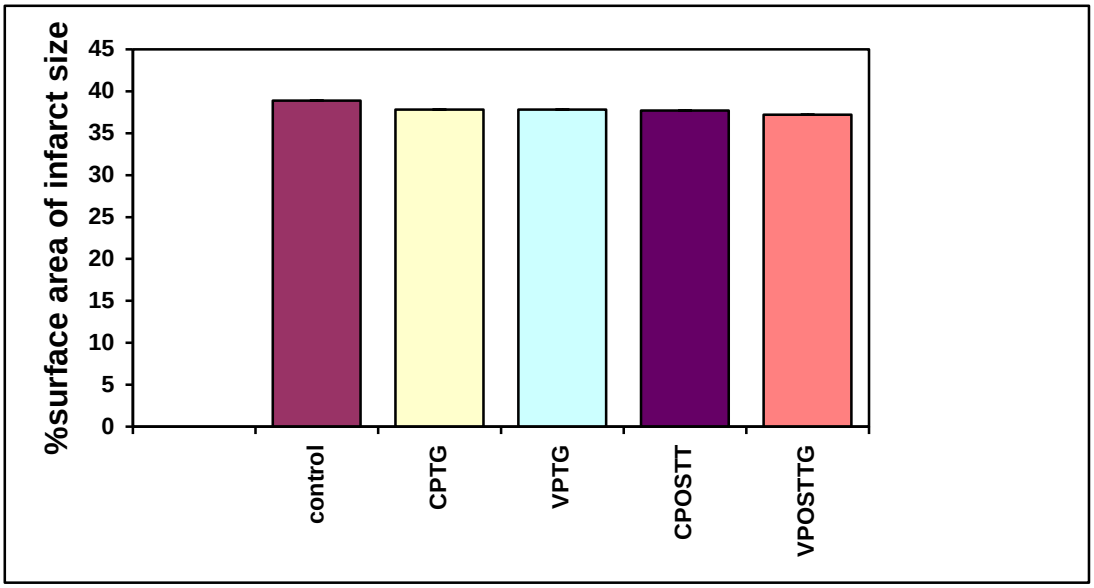


Figure (52): effect of carvedilol treatment and valsartan treatment on infarct size surface area represented as % of total surface area of the left ventricular segments in pre-treated and post-treated groups compared to control group.

N:B

CPTG: carvedilol pretreated group.

VPTG: valsartan pretreated group.

CPOSTT: carvedilol posttreated group.

VPOSTT: valsartan posttreated group