Results:

4.1 Myocardial ischemia/reperfusion model

4.1.1 Effect of PG and Lipoic acid and their combination on myocardial I/R injury:

The protocol involved five groups- 1) sham operated vehicle treated group (SO), 2) vehicle treated reperfusion injury control group (I/R control), 3) PG (2 mg/kg i.p.) treated reperfusion injury group (PG), 4) Lipoic acid (100 mg/kg i.p.) receiving reperfusion injury group (Lipo) and 5) co-administration of PG with Lipoic acid (PG + Lipo) reperfusion injury group were studied for the I/R injury experiment as described in section 3.2.1.1.1, 3.2.1.1.2 and 3.2.1.1.6 of materials and methods.

4.1.1.1 Effects of PG and Lipoic acid on hemodynamic and electrocardiographic parameters-

Reperfusion injury induced changes in hemodynamic and electrocardiographic parameters were observed and recorded using Biopac MP 30 system and analysed with Biopac Student Lab Pro software (Version 3.6.7).

The actual tracings of recordings showing changes in these parameters are depicted in **Fig. 1.1 a to 1.1 l.**

Fig. 1.2 a to 1.2 c represents the changes in Mean arterial blood pressure (MAP), ST elevation and ST prolongation with respect to time of induction of ischemia and reperfusion. The time period of recording shown here are Baseline, End-Ischemia and End-Reperfusion.

Induction of ischemia resulted into significant fall in systolic, diastolic and mean arterial blood pressure in I/R control and PG treated groups subjected to ischemia at End-ischemia period as compared to sham operated control (SO) ($P< 0.05$). The hemodynamic changes were not significantly different among the animals from all the groups (I/R control, PG, Lipo and PG + Lipo) subjected to ischemia (End-ischemia period) till reperfusion. After reperfusion i.e at End-reperfusion period, MAP of (Lipo and PG + Lipo) treated groups was significantly higher with Lipo ($P< 0.05$) and PG + Lipo ($P< 0.05$) as compared to I/R control group. The change in blood pressure with respect to time measured in the form of maximum (+) and minimum (–) dp/dt was significantly higher in

Lipo and PG + Lipo treated groups as compared to I/R control group. PG + Lipo group exhibited significant change in values (*P*< 0.05) through out ischemia and reperfusion for dp/dt max. The (+) dp/dt in Lipo and PG + Lipo groups was not significantly changed at End-Ischemia but and was significantly (*P*< 0.05) higher than I/R control group at End-Reperfusion period. Animals in Lipo and PG + Lipo groups also exhibited higher fall (*P*< 0.05) in minimum dp/dt as compared to vehicle treated reperfusion injury (I/R control) group at both the intervals. **(Table 1.1 and 1.2)**

Ischemia led to significant increase in ST wave amplitude (ST elevation) (*P*< 0.01) and duration of ST segment (*P*< 0.01) in all the groups subjected to ischemia as compared to sham operated control. However there was no significant difference in the groups either receiving vehicle, PG, Lipo and PG + Lipo except at the end of reperfusion where Lipo and PG + Lipo groups shown significant (*P*< 0.05) fall in ST elevation and duration of ST segment as compared to I/R control group. The incidences of VT were significantly (*P*<0.05) reduced after the treatment.(**Table 1.3 and Fig. 1.2 a to 1.2 c)**

The changes in heart rate were not significant even at the time of reperfusion in the animals subjected to ischemia-reperfusion as compared to SO group.

4.1.1.2 Effects of PG and Lipoic acid and their combination on Infarct area and Area At Risk (AAR) :

The infarct area of the reperfused myocardium was measured with the TTC staining technique. The area of infarction turns pale white due to lack of colouration of the cardiac myocytes. Absence of dehydrogenase enzyme in this area, turns infarct pale white. Viable cells which contain dehydrogenase enzyme, reacts with TTC and forms formazan crystal that give the cells a pink colouration. The area subjected to ischemic injury has pale pink colour and was marked as Area At Risk (AAR). The transverse section of heart was demarked into ischemic, non-ischemic and AAR by computer based Image J software 1.30 V (rsb.info.nih/ij.)

Sham operated animals were not exposed to I/R injury and hence the AAR and ischemic area does not develop, whereas, I/R control group has the maximum infarct area after I/R injury. The PG, Lipo and PG+Lipo groups has significantly (*P*<0.01, *P*<0.001 and *P*<0.001) reduced the area of infarction as compared to I/R control group. This was no significant difference in AAR in PG and PG+Lipo group except for Lipo group (*P*<0.05) in comparison with I/R control animals. **(Fig. 1.3)**

4.1.1.3 Effects of PG and Lipoic acid and their combination on Biomarkers of oxidative stress-

Oxidative stress induced by I/R injury caused significant ($P< 0.001$) depletion of endogenous antioxidants like reduced glutathione (GSH), superoxide dismutase (SOD) and catalase with highly significant increment in levels ($P< 0.001$) of lipid peroxidation marker, malondialdehyde (MDA) as compared to sham operated animals. PG in combination with Lipoic acid did change in these markers significantly ($P<0.05$) as compared to vehicle treated (I/R control) groups except the catalase activity. However, PG alone did not show any significant alteration in antioxidant biomarkers after induction of I/R injury as compared to vehicle treated (I/R control) group. **(Table-1.4)**

4.1.1.4 Effects of PG and Lipoic acid and their combination on inflammatory reactivity and CK activity-

Inflammatory reactivity due to neutrophil infiltration was substantially elevated after I/R injury, which was observed from the significant ($P<0.001$) increase in MPO activity of I/R control animals as compared to SO animals. Treatment with Lipoic acid, PG and their combination showed significant ($P<0.05$) decrease in MPO activity after induction of I/R injury. Whereas, Lipoic acid alone and in combination with PG significantly ($P<0.01$, $P<0.001$) reduced CK activity as compared to I/R control group. PG alone did not affect the CK activity significantly after I/R injury. **(Table 1.5)**

4.1.1.5 Effects of PG and Lipoic acid and their combination on Apoptotic cell death-

The apoptotic cell death induced by I/R injury was analyzed by observing the interneucleosomal DNA fragmentation. Vehicle treated I/R control animals showed marked evidences of DNA damage, whereas treatment with PG and Lipoic acid substantially reduced the apoptotic cell death indicated by reduced DNA fragmentation. **(Fig. 1.4)**

4.1.1.6 Effects of PG and Lipoic acid and their combination on histological features-

The photomicrographs revealed severe necrotic injury in hearts from vehicle treated groups was indicated by eosinophilia, splaying of muscle fibres. I/R injury induced severe cellular edema, heavy neutrophil infiltration and cellular necrosis in vehicle treated animals. Treatment with PG and Lipoic acid considerably restored the

Fig. 1.1 a

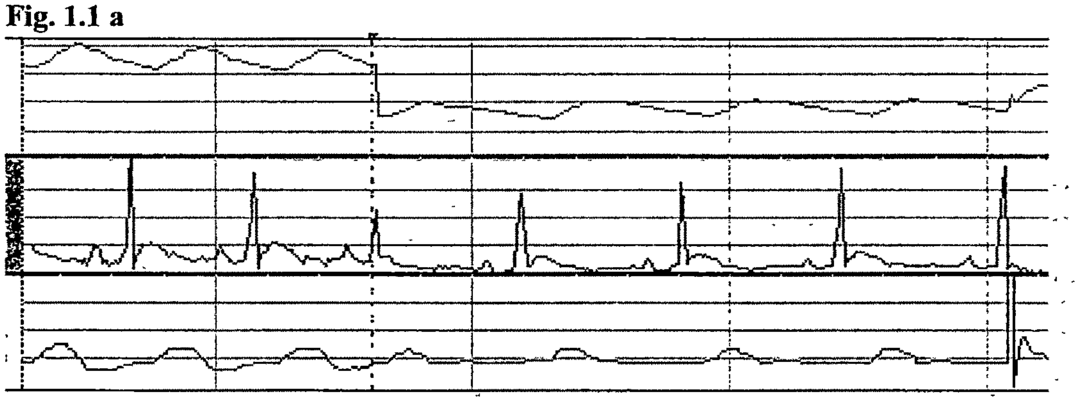


Fig. 1.1 b

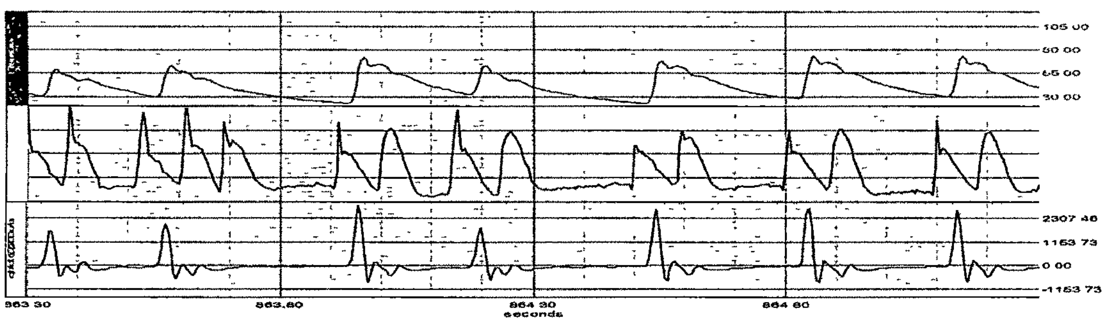


Fig. 1.1 c

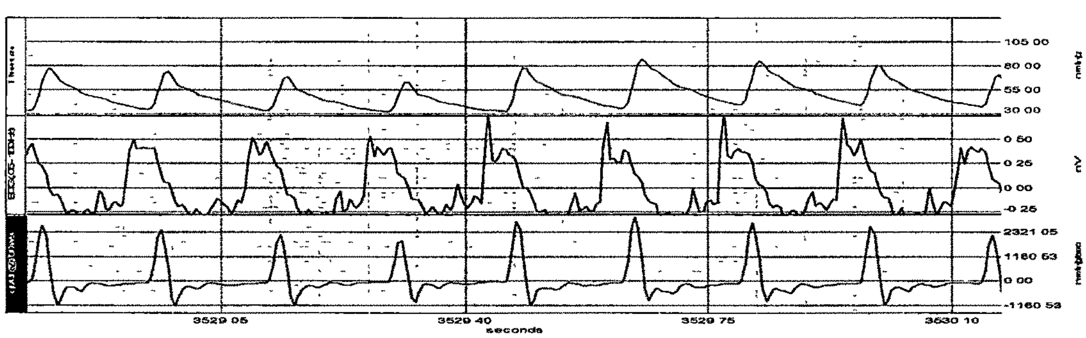
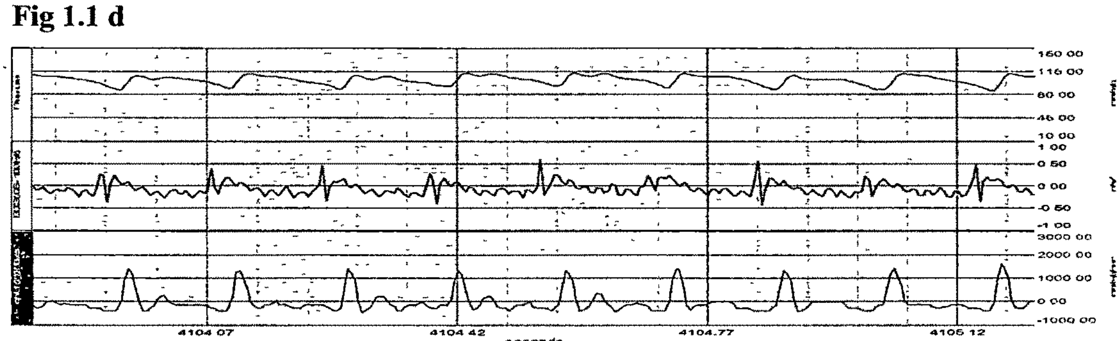


Fig 1.1 d



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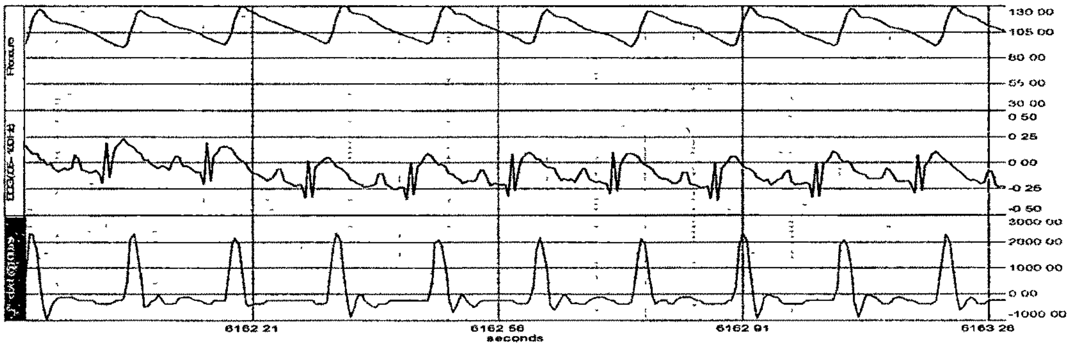

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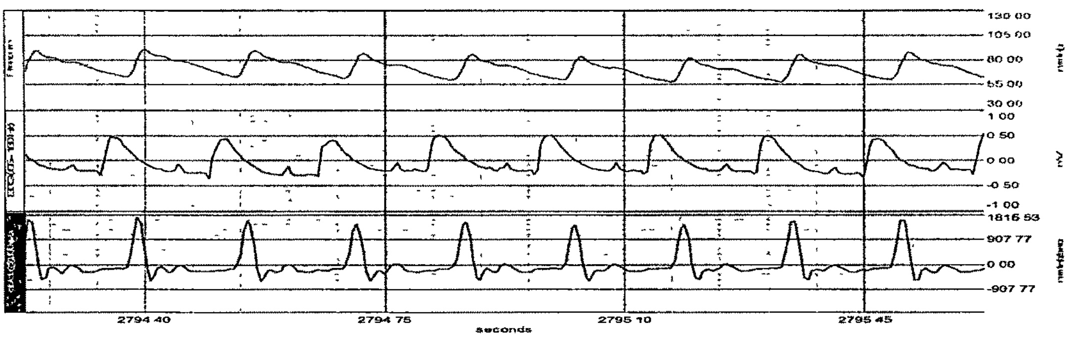


Fig 1.1 g

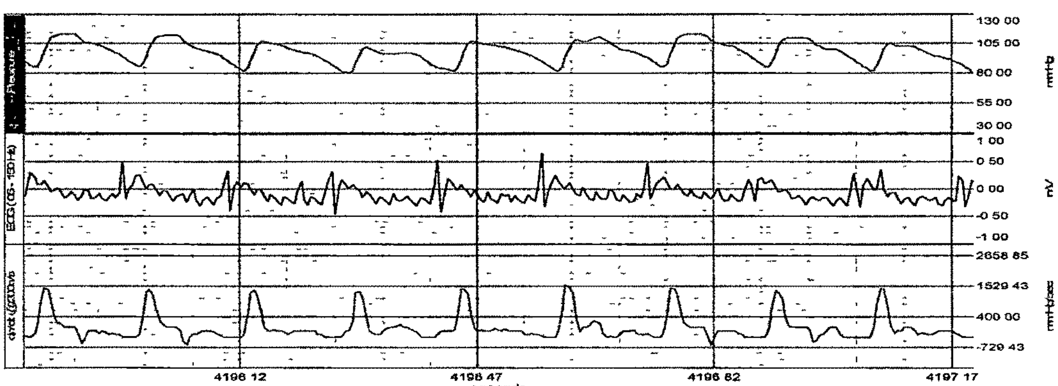


Fig 1.1 h

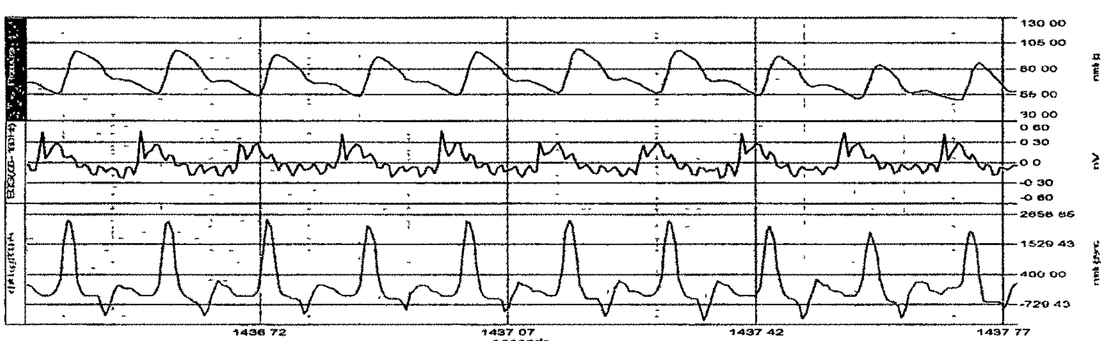


Fig 1.1 i

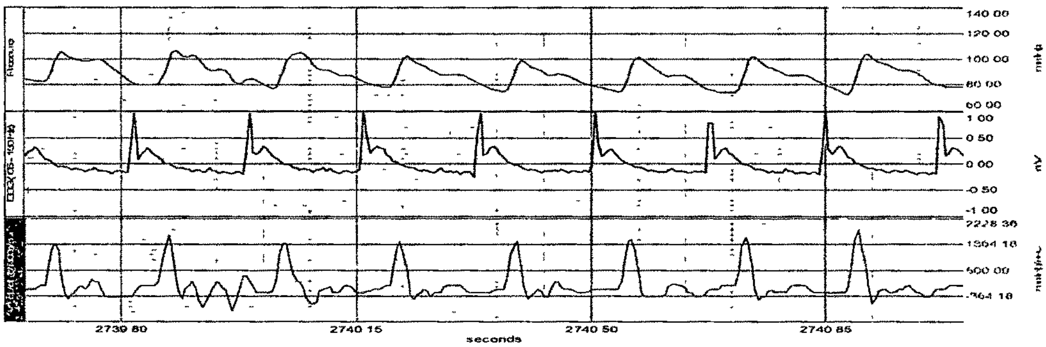


Fig 1.1 j

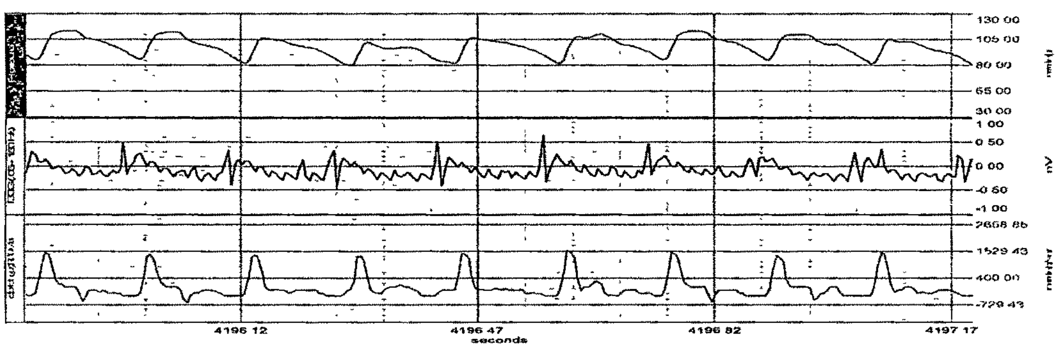


Fig 1.1 k

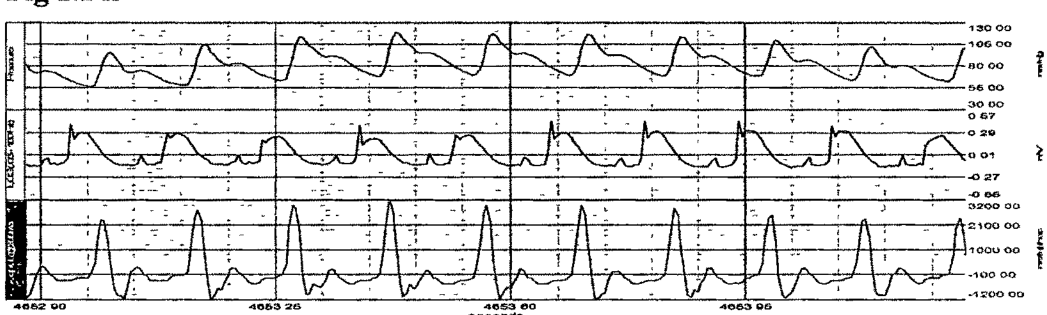


Fig 1.1 l

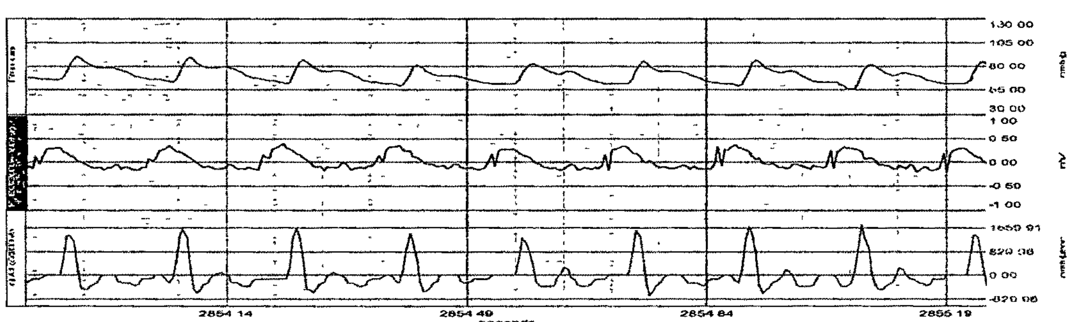


Fig. 1.2 a to 1.2 c

Changes in Mean arterial blood pressure (MAP) and ST segment with respect to time of induction of ischemia and reperfusion in the animals during study.

1) SO- Sham Operated animals (No ischemia-reperfusion) + vehicle,

2) I/R- Vehicle treated rats undergoing reperfusion injury (I/R control)

3) I/R+PG – PG treated rats subjected to reperfusion injury

4) I/R+ Lipo – Lipoic acid treated rats subjected to reperfusion injury

5) I/R+PG+Lipo – PG with Lipoic acid (Combined treatment) treated rats subjected to reperfusion injury

Arrows indicates time of 1. Baseline (Before induction of ischemia), 2. End-Ischemia (30 min after induction of ischemia) and 3. End-Reperfusion (60 min after induction of ischemia)

1.2 a. Changes in mean blood pressure (mm Hg)

1.2 b. Changes in ST elevation (mv)

1.2 c. Changes in ST prolongation (mv/s)

All values are mean ± SEM (n=6). The group treated with vehicle alone (I/R control) was compared with Sham operated group (*-$P < 0.05$, **-$P < 0.01$, *** - $P < 0.001$) while groups treated with PG, Lipoic acid and Combination treatment were compared with the I/R control group (^- $P < 0.05$, ^^-$P < 0.01$; ^^^- $P < 0.001$)

Fig.1.2 a. Changes in Mean arterial blood pressure with respect to time before and after ischemia/reperfusion

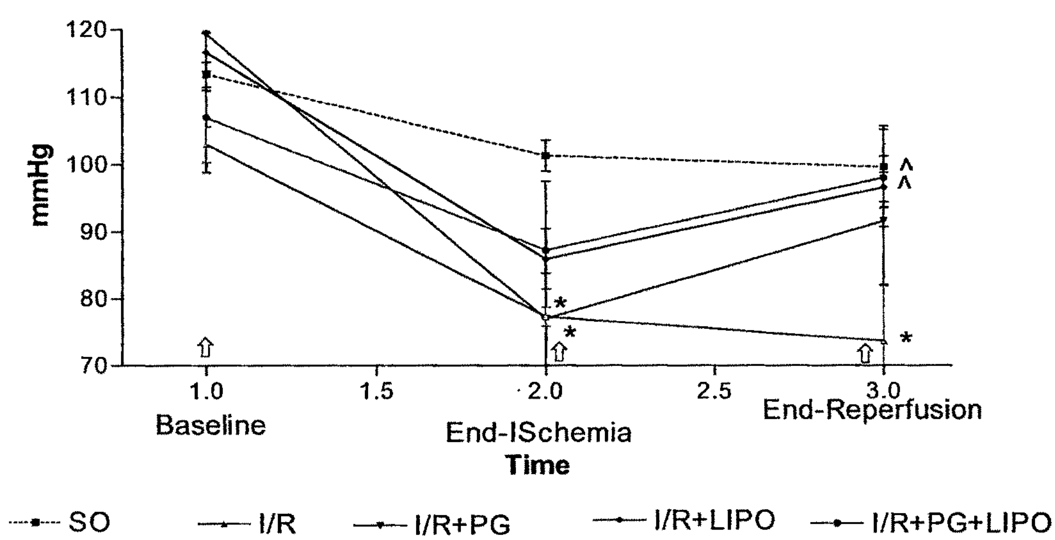


Fig. 1.2 b. Changes in ST segment elevation with respect to time, before and after ischemia/reperfusion

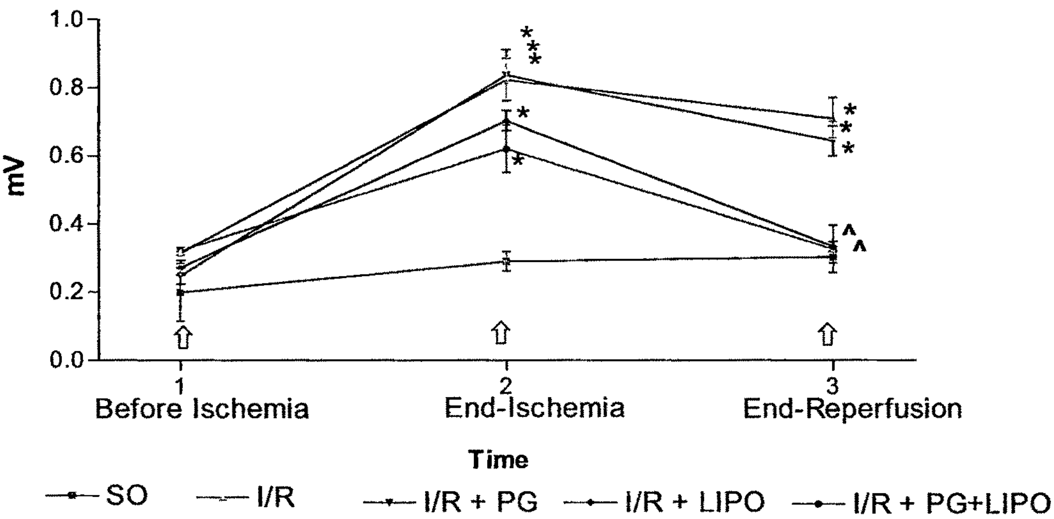


Fig. 1.2 c. Changes in ST segment prolongation with respect to time, before and after ischemia/reperfusion

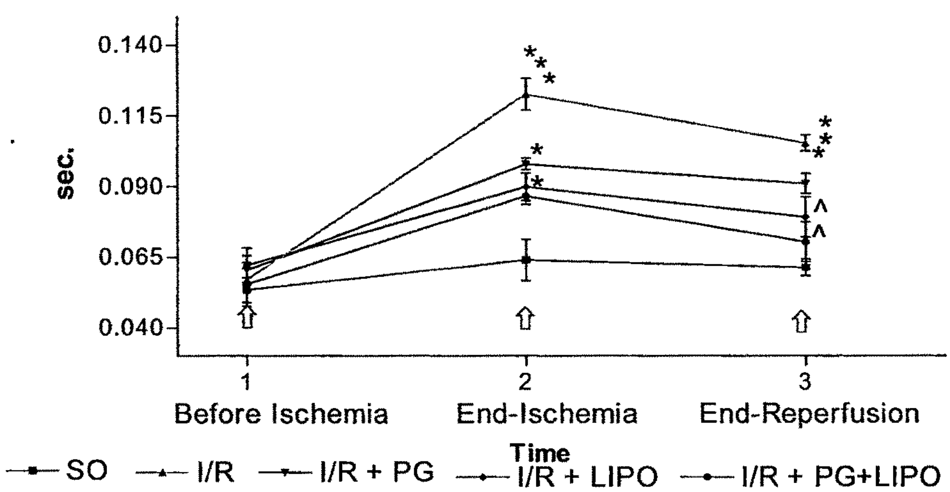


Fig. 1.3 a and 1.3 b

Effect of PG, Lipoic acid and their combination on infarct area and area at risk (AAR) after myocardial I/R injury in rats.

1.3 a. Infarct area in percentage

1.3 b. Area at risk (AAR) in percentage

All values are mean ± SEM (n=6). The group treated with PG, Lipoic acid and Combination treatment were compared with the I/R control group (^ - $P < 0.05$, ^^ -P < 0.01; ^^^- P < 0.001)

Fig.1.3 a

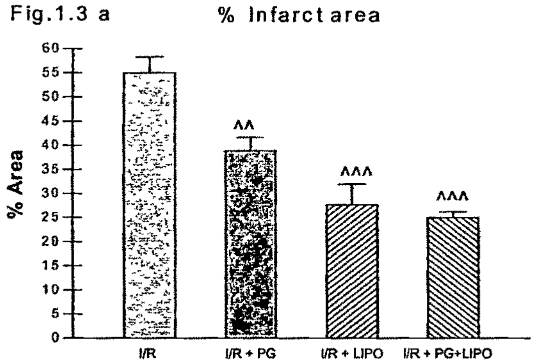


Fig. 1.3 b

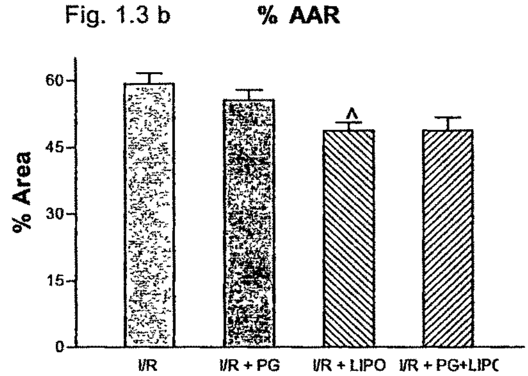
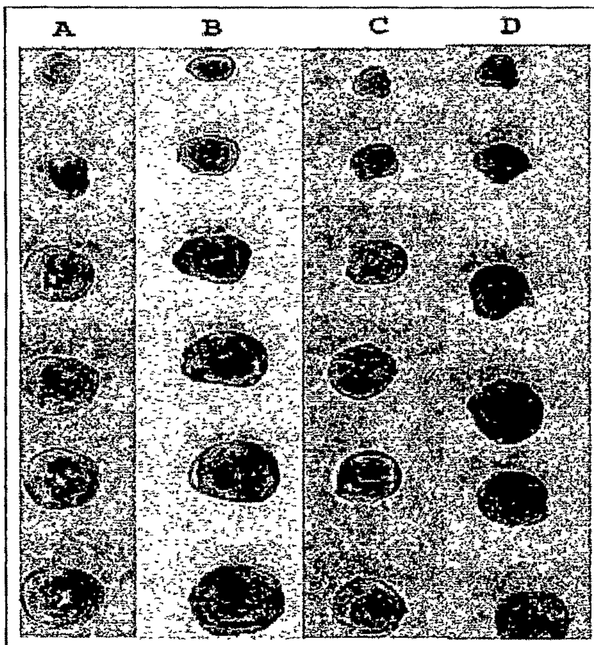
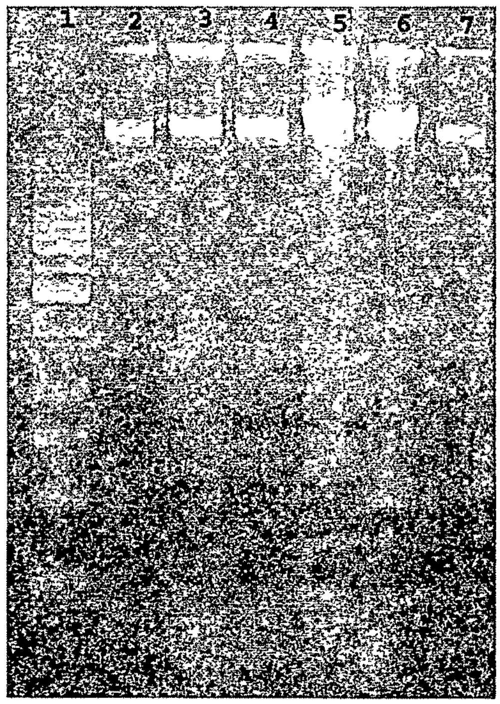


Fig 1.3 C Infarct area in TTC stained slices:



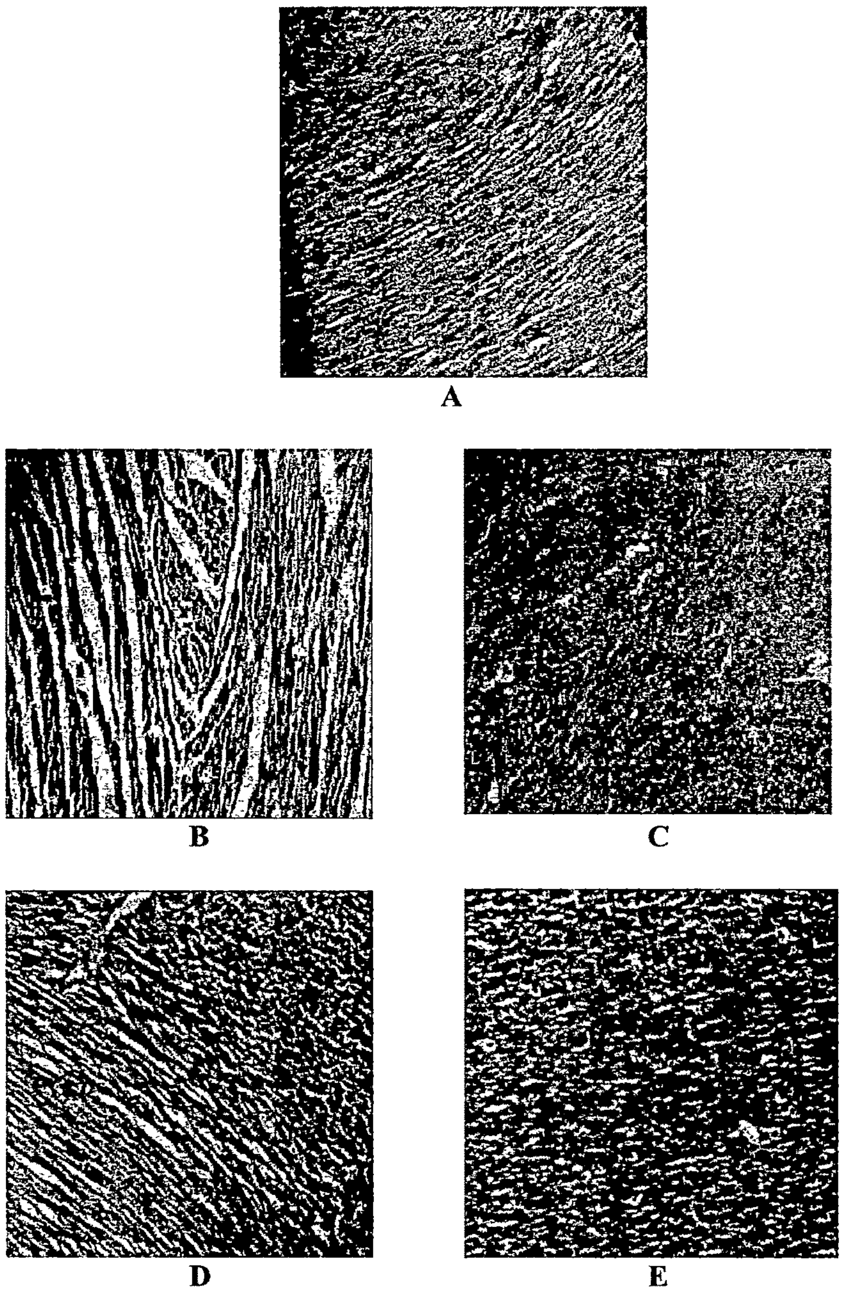
Lane A: Heart Slices of I/R control animals; **Lane B**: Heart Slices of PG treated I/R animal; **Lane C**: Heart Slices of Lipoic acid treated animals; and **Lane D**: PG and Lipoic acid combination treated I/R animal.

Fig. 1.4: Agarose gel electrophoresis of rat heart DNA after ischemia/reperfusion injury.



Lane 1, 1-kb DNA standard; **Lane 2**, displaying the pattern of PG+Lipo treatment; **Lane 3,** Lipoic acid treated I/R (I/R +Lipo) without evident DNA laddering pattern; **Lane 4,** PG treated I/R (I/R +PG) with out evident DNA laddering pattern, **Lane 5 and 6,** Ischemic control (I/R control) displaying a laddering pattern a indication of oligointernucleosomal DNA fragmentation; **Lane 7,** Sham control with out any prominent DNA fragmentation.

Fig 1.5 Photomicrographs (100X) of histological slides stained with H& E stain after ischemia/reperfusion injury.



A: Sham operated animals **B:** Vehicle treated I/R animal showing fragmented myofibrils and leukocyte infiltration; **C:** Lipoic acid treated I/R animals with improved cellular structures, continuity with adjacent myofibrils and slight cellular damage.; **D:** PG treated I/R animal with structural damage; **E:** PG+LIPO treated animal with marked improvement.

Table 1.1

The effect of PG (2 mg/kg), Lipoic acid (100 mg/kg) and their combination on ± d*p*/d*t* of rats with 1 h of ischemia and 1 h of reperfusion induced by left coronary descending artery (LCA) occlusion.

Group	+ d*p*/d*t*(mmHg/sec)			- d*p*/d*t*(mmHg/sec)		
	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)
SO	2402 ± 215	2668 ± 108	2567 ± 180	2015 ± 328	2053 ± 326	1958 ± 127
I/R control	2566 ± 166	1879 ± 148 *	1779 ± 102*	1811 ± 158	1146 ± 126*	1216 ± 93*
I/R + PG	2729 ± 72	1845 ± 130*	1810 ± 94*	1834 ± 108	1209 ±127*	1154 ± 181*
I/R + Lipo	2623 ± 132	1951 ± 193	2090 ± 132	2002 ± 156	1655 ± 101	1791 ± 86
I/R +PG+Lipo	2750 ± 172	1908 ± 197*	2355 ± 177^	1974 ± 167	1234 ± 92*	1822 ± 109^

All values are mean ± SEM (n=6). The groups treated with vehicle alone (I/R control), PG and Lipoic acid and their combination were compared with sham operated control (SO)-(* $P < 0.05$, **-$P < 0.01$, ***- $P < 0.001$) while the groups treated with PG, Lipoic acid and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P < 0.01$, ^^^- $P < 0.001$)

Table 1.2

The effect of PG (2 mg/kg), Lipoic acid (100 mg/kg) and their combination on heart rate (HR) and systolic blood pressure (SBP) of rats with 1 h of ischemia and 1 h of reperfusion induced by left coronary descending artery (LCA) occlusion.

Group	HR/min			SBP (mmHg)		
	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)
SO	419 ± 19.52	416 ± 12.05	405 ± 15.09	117.13 ± 4.46	106.66 ± 3.98	118.5 ± 2.58
I/R control	392 ± 18.21	325 ± 13.01*	308 ± 26.15*	110.52 ± 6.05	81.05 ± 3.56*	79.59 ± 5.98*
I/R + PG	397 ± 18.18	326 ± 15.16*	313 ± 16.15*	106.28 ± 6.74	82.67 ± 5.21*	73.52 ± 7.61*
I/R + Lipo	404 ± 22.21	319 ± 15.29*	307 ± 10.66*	108.44 ± 4.48	85.83 ± 6.47	92.11 ± 5.13
I/R+ PG+Lipo	390 ± 16.74	327 ± 22.56*	325 ± 18.12*	104.11 ± 5.22	87.33 ± 4.83*	96.66 ± 4.02

All values are mean ± SEM (n=6). The groups treated with vehicle alone (I/R control), PG and Lipoic acid and their combination were compared with sham operated control (SO)-(* $P < 0.05$, **-$P < 0.01$, ***- $P < 0.001$) while the groups treated with PG, Lipoic acid and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P < 0.01$, ^^^- $P < 0.001$)

Table 1.3

The effect of PG (2 mg/kg), Lipoic acid (100 mg/kg) and their combination on occurrence of ventricular tachycardia (VT) in rats with myocardial I/R injury

Group	Occurrence of VT	
	Ischemia period	Reperfusion period
I/R control	11/12	10/12
I/R + PG	7/12	8/12
I/R+ Lipo	4/12^	3/12^
I/R+ PG+Lipo	6/12	4/12^

All values are mean ± SEM (n=6). The group treated with PG, Lipoic acid and their combination were compared with the I/R control group (^ $P < 0.05$, ^^-$P < 0.01$ ^^^ - $P < 0.001$)

Table 1.4

Effect of treatment with PG (2 mg/kg), Lipoic acid (100 mg/kg) and their combination on oxidative stress markers in rats with myocardial I/R injury

Groups	SOD IU/mg of protein	CAT H_2O_2/consu /min/mg of protein	GSH μg/mg of protein	MDA nmol /mg of protein
SO	12.89 ± 1.30	410.35 ± 68.61	9.06 ± 0.84	1.05 ± 0.12
I/R control	3.02 ± 0.49***	274.01 ±62.23	3.91 ± 0.32***	3.02 ± 0.33 **
I/R+ PG	5.12 ±0.46***	170.99 ± 26.49	5.78 ± 0.43**	2.08 ± 0.35
I/R + Lipo	6.63 ± 0.58***^	234.23 ± 47.83	7.93 ± 0.64^	1.77 ± 0.13^
I/R+ PG+Lipo	7.18 ± 0.68***^	458.14 ± 51.76	7.69 ± 0.40^	1.50 ± 0.18^

All values are mean ± SEM (n=6). The groups treated with vehicle alone (I/R control), PG, Lipoic acid and their combination were compared with sham operated control (SO)-(* P< 0.05, **-P< 0.01, ***- P< 0.001) while the groups treated with PG, Lipoic acid and their combination were compared with the I/R control group (^- P < 0.05, ^^- P< 0.01, ^^^ - P< 0.001)

Table 1.5

Effect of treatment with PG (2 mg/kg), Lipoic acid (100 mg/kg) and their combination on MPO and CK activity in rats with myocardial I/R injury.

Groups	MPO (units/mg protein)	CK (U/L)
SO	0.23 ± 0.91	1033.67 ± 36.23
I/R control	0.82 ± 0.04**	4490.54 ± 120.99***
I/R + PG	0.47 ± 0.03^	4324.00 ± 286.27***
I/R + Lipo	0.49 ± 0.02^	3260.83 ±187.58***^^
I/R+ PG+Lipo	0.44 ± 0.02^	2235.53 ± 209.30**^^

All values are mean ± SEM (n=6). The groups treated with vehicle alone (I/R control), PG, Lipoic acid and their combination and their were compared with sham operated control (SO)- (* $P < 0.05$, **-$P < 0.01$, ***- $P < 0.001$) while the groups treated with PG, Lipoic acid and their combination werè compared with the I/R control group (^- $P < 0.05$, ^^- $P < 0.01$, ^^^ - $P < 0.001$)

4.1.2 Effect of PG, Coenzyme Q-10 (CoQ) and their combination on myocardial I/R injury:

Five groups were involved in this protocol study- 1) sham operated vehicle treated group (SO), 2) vehicle treated reperfusion injury control group (I/R control), 3) PG (2 mg/kg i.p.) treated reperfusion injury group (PG), 4) CoQ (100 mg/kg i.p.) administered to reperfusion injury group (CoQ) and 5) co-administration of PG with CoQ-10 (PG +CoQ) reperfusion injury group were studied in the I/R injury experiment as described in section 3.2.1.1.1, 3.2.1.1.3 and 3.2.1.1.7 of materials and methods.

4.1.2.1 Effect of PG, Coenzyme Q-10 (CoQ) and their combination on hemodynamic and electrocardiographic parameters-

Reperfusion injury induced changes in hemodynamic and electrocardiographic parameters were observed and recorded with Biopac MP 30 system and analysed using Biopac Student Lab Pro software (Version 3.6.7).

The actual tracings of recordings showing changes in these parameters are shown as **Fig. 2.1 a to 2.1 d**

Fig. 2.2 a to 2.2 c represents the changes in Mean arterial blood pressure (MAP), ST elevation and ST prolongation with respect to time of induction of ischemia and reperfusion. The time period of recording shown here are Baseline, End-Ischemia and End-Reperfusion.

There was a significant ($P<0.05$) decrease in systolic, diastolic and mean arterial blood pressure in I/R control , PG and CoQ treated groups subjected to ischemia at End-ischemia period in comparison to sham operated control (SO) group. At the first observational interval (End-ischemia period), all the groups showed no significant change in hemodynamic parameters as compared to I/R control animals. At End-reperfusion period (second observational interval) , MAP of treated (CoQ and PG +CoQ) groups was increased but not significantly as compared to I/R control group. The change in blood pressure with respect to time measured in the form of maximum (+) and minimum (–) dp/dt was not significantly altered in CoQ and PG + CoQ treated groups as compared to I/R control group. **(Table 2.1 and 2.2)** The incidences of VT significantly changed only in CoQ alone treated group as compared to I/R control animals. **Table 2.3**

ST wave amplitude (ST elevation) was significantly ($P<0.001$) increased and duration of ST segment ($P<0.001$) was prolonged in I/R control groups subjected to ischemia as compared to sham operated control. However, there was no significant difference in the groups receiving PG except for CoQ and PG+ CoQ ($P<0.05$) at the end of both the intervals (End-ischemia and End-reperfusion) on ST elevation and duration of ST segment as compared to I/R control group. **Fig. 2.2 a to 2.2 c**

The changes in heart rate were not significant even at the time of reperfusion in the animals subjected to ischemia-reperfusion as compared to SO group.

4.1.2.2 Effect of PG, Coenzyme Q-10 (CoQ) and their combination on Infarct size and Area At Risk (AAR) -

The slices of the hearts from various groups stained with Evans blue and TTC are shown in **Fig. 2.3 c**

Sham operated (SO) control rats were not exposed to I/R injury, the AAR was zero and so the infarct size (IS). The AAR in groups subjected to ischemia reperfusion was not significantly different among themselves. AAR for I/R control, PG, CoQ and PG+CoQ groups were 43.97 ± 2.22, 44.16 ± 2.39, 44.21 ± 1.76 and 43.51 ± 1.93 % respectively.

The CoQ treatment led to significant ($P<0.001$) decrease in infarct size as compared to I/R control group. Moreover, the change was statistically significant ($P<0.001$) in PG +CoQ treated group as compared to I/R control group. The infarct sizes in I/R control, PG, CoQ and PG+CoQ groups were 54.98 ± 3.59, 38.97 ± 3.37, 29.25 ± 3.49 and 21.26 ± 1.06 % respectively. **(Fig. 2.3 a &2.3 b)**

4.1.2.3 Effect of PG, Coenzyme Q-10 (CoQ) and their combination on Oxidative stress biomarkers in myocardial injury-

Ischemic reperfusion injury is manifested by marked increased in oxidative stress observed by significant ($P<0.001$) depletion of endogenous antioxidants like reduced glutathione (GSH), superoxide dismutase (SOD) and catalase. The lipid peroxidation induced by I/R injury was indicated by significant elevation ($P<0.001$) of malondialdehyde (MDA) as compared to sham operated animals. Treatment with CoQ alone and its combination with PG significantly ($P<0.01$) changed the activity of SOD. GSH and MDA levels were also significantly ($P<0.01$) changed with the treatment. CoQ with PG treated animals did not show any significant increase in GSH levels. However, CAT activity was not significantly different in any of the treated group as compared to I/R control animals. **Table-2.4**

4.1.2.4 Effect of PG, Coenzyme Q-10 (CoQ) and their combination on inflammatory reactivity and CK activity-

MPO was measured as a bio-index of inflammatory reactivity, which was found to increase significantly (*P*<0.001) in I/R control group as compared to SO animals. Treatment with CoQ, PG and their combination significantly (*P*<0.05) decreased MPO activity after induction of I/R injury. CK in serum was leaked out from the tissue, and this CK activity was significantly elevated in I/R control group and CoQ treatment alone and in combination with PG significantly reduced the serum levels of CK. **Table 2.5**

4.1.2.5 Effect of PG, Coenzyme Q-10 (CoQ) and their combination on Apoptosis -

I/R injury induced cell death by apoptosis was analyzed by internucleosomal DNA fragmentation. Vehicle treated I/R control animals showed marked evidences of DNA damage, whereas the band formation due to fragmentation was markedly diminished in animals treated with CoQ and PG. **Fig. 2.4**

4.1.2.6 Effect of PG, Coenzyme Q-10 (CoQ) and their combination on histological features-

Cellular injury induced by I/R was observed with photomicrographs of the hearts from vehicle treated groups. Damage was indicated by edema of cells, heavy neutrophil infiltration and cellular necrosis in vehicle treated animals. Damage was considerably reduced by CoQ and PG treatment and was indicated by restored the normal cellular structure and reduced incidences of edema and minimal neutrophil presence in the myofibrils. **Fig. 2.5**

Fig. 2.1

Recordings showing changes occurring in hemodynamic and Electrocardiographic (ECG) parameters after induction of ischemia-reperfusion in vehicle, CoQ, PG and their combination treated rats-

The Fig.s represent electrocardiograph changes in the animals at various time intervals.

2.1 a. ECG Changes occurring a during ischemia in CoQ treated group (End –Ischemia period)

2.1 b. ECG Changes occurring during reperfusion in CoQ treated group (End – Reperfusion period)

2.1 c. ECG Changes occurring a during ischemia in PG with CoQ treated group (End – Ischemia period)

2.1 d. ECG Changes occurring during reperfusion in PG with CoQ treated group (End –Reperfusion period)

Fig 2.1 a

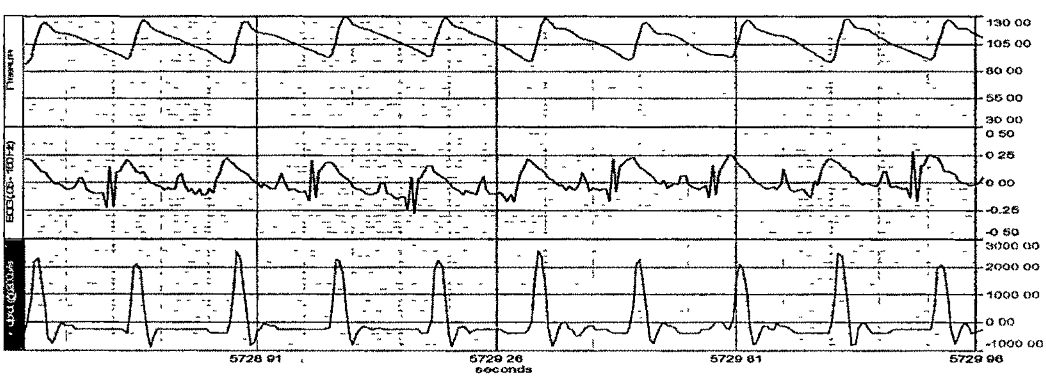


Fig 2.1 b

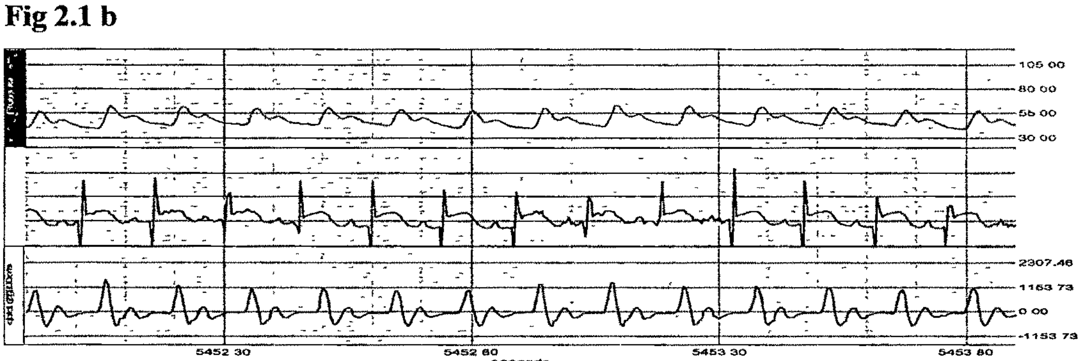


Fig 2.1 c

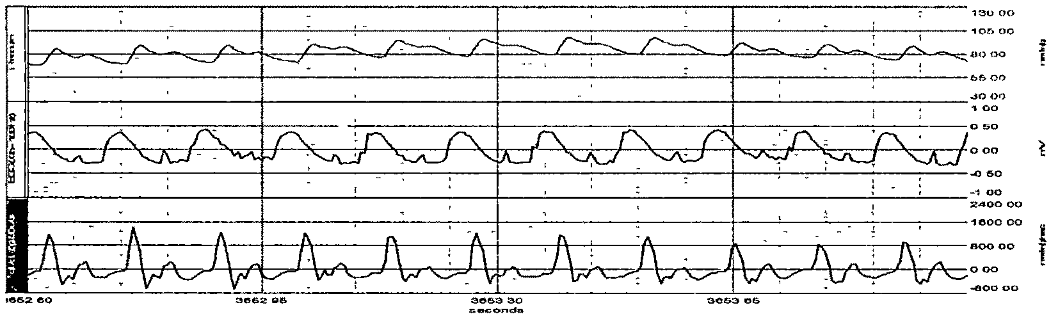


Fig 2.1 d

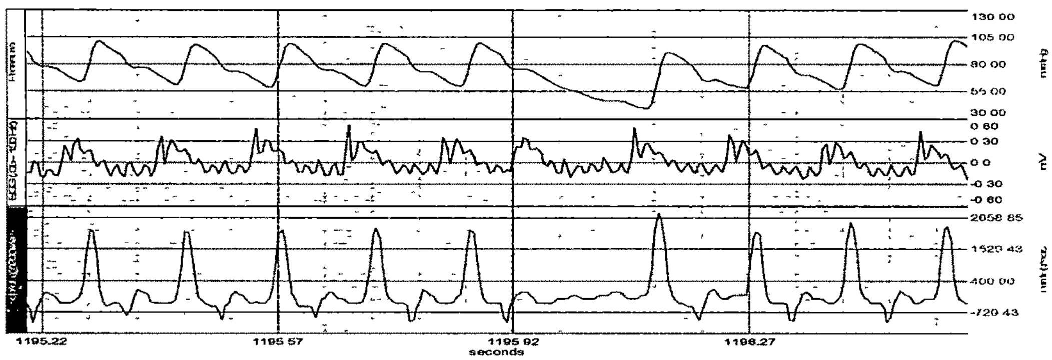


Fig. 2.2 a to c

Changes in Mean arterial blood pressure (MAP) and ST segment with respect to time of induction of ischemia and reperfusion in the animals during study.

1) **SO- Sham Operated animals (No ischemia-reperfusion) + vehicle,**
2) **I/R- Vehicle treated rats undergoing reperfusion injury(I/R control)**
3) **I/R+PG – PG treated rats subjected to reperfusion injury**
4) **I/R+ CoQ – CoQ treated rats subjected to reperfusion injury**
5) **I/R+PG+CoQ – PG with CoQ (Combined treatment) treated rats subjected to reperfusion injury**

Arrows indicates time of 1. Baseline (Before induction of ischemia), 2. End-Ischemia (30 min after induction of ischemia) and 3. End-Reperfusion (60 min after induction of ischemia)

2.2 a. Changes in mean blood pressure (mm Hg)

2.2 b. Changes in ST elevation (mv)

2.3 c. Changes in ST prolongation (mv/s)

All values are mean ± SEM (n=6). The group treated with vehicle alone (I/R control) was compared with Sham operated group (*-$P < 0.05$, **-$P < 0.01$, ***- $P < 0.001$) while groups treated with PG, CoQ and Combination treatment were compared with the I/R control group (^- $P < 0.05$, ^^-$P < 0.01$; ^^^- $P < 0.001$)

Fig.2.2 a. Changes in Mean arterial blood pressure with respect to time before and after ischemia/reperfusion

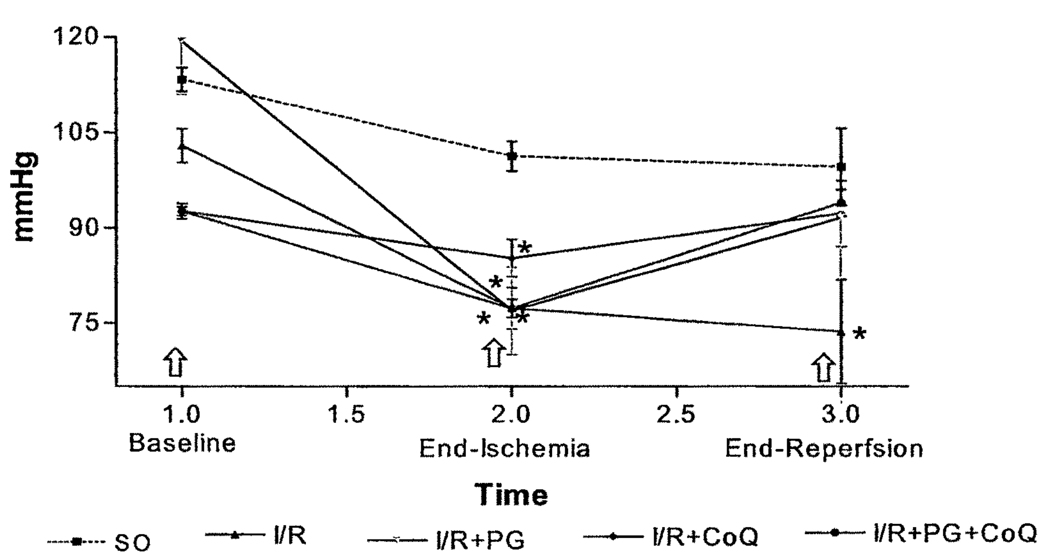


Fig. 2.2 b. Changes in ST segment elevation with respect to time, before and after ischemia/reperfusion

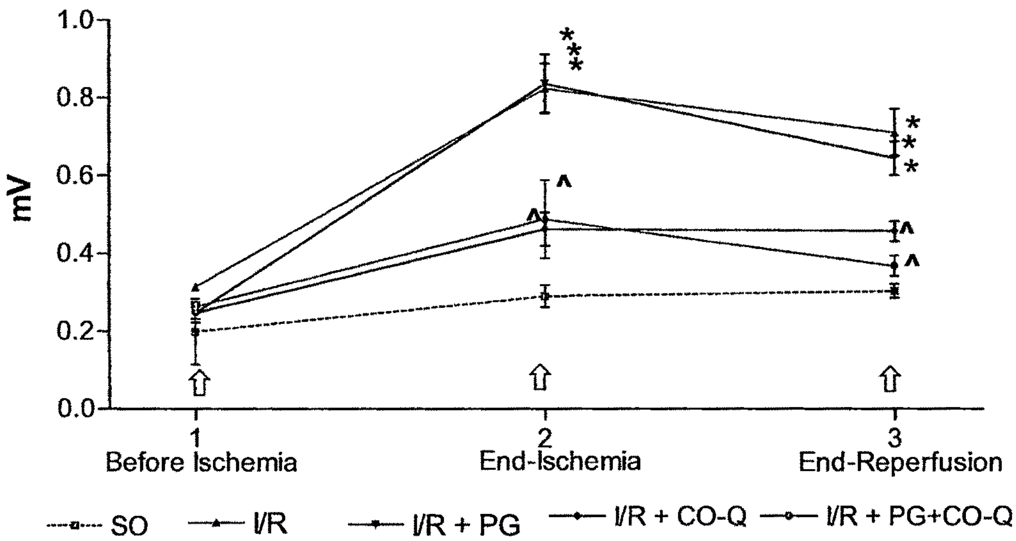


Fig. 2.2 c. Changes in ST segment prolongation with respect to time, before and after ischemia/reperfusion

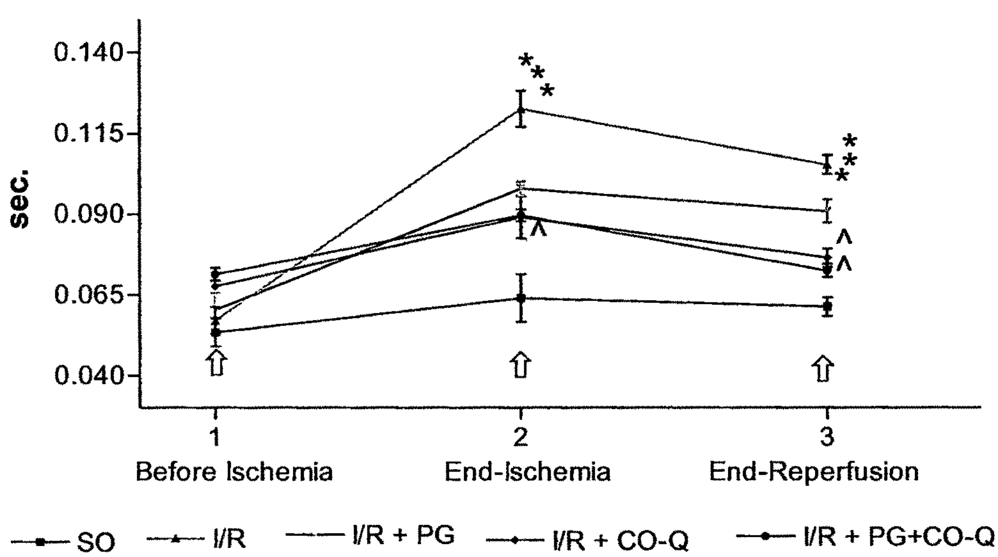


Fig. 2.3 a and 2.3 b

Effect of PG, CoQ and their combination treatment on infarct area and Area At Risk (AAR) after myocardial I/R injury in rats.

2.3 a. Infarct area in percentage

2.3 b. Area at risk (AAR) in percentage

All values are mean ± SEM (n=6). The group treated with PG, CoQ and Combination treatment were compared with the I/R control group (^ - $P < 0.05$, ^^ -$P < 0.01$; ^^^- $P < 0.001$)

Fig. 2.3 a. % Infarct area

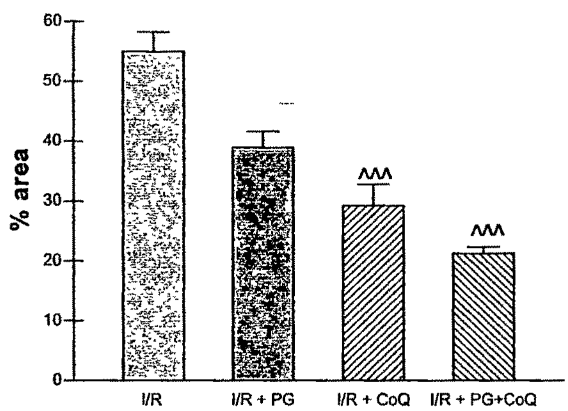
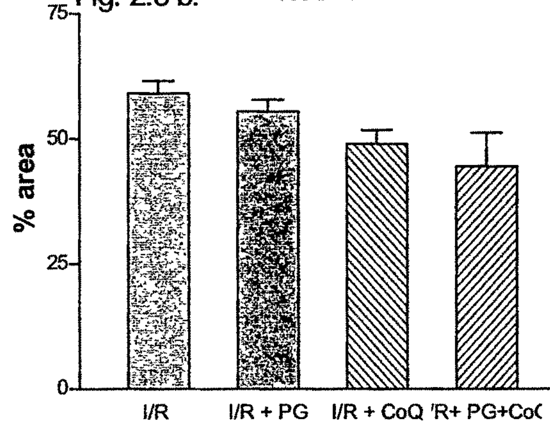


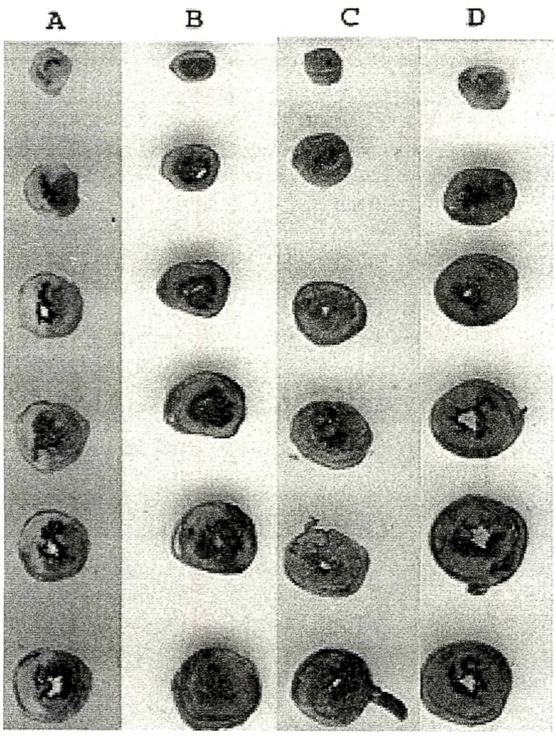
Fig. 2.3 b. %AAR



SO- Sham Operated, I/R- Vehicle treated rats undergoing reperfusion injury

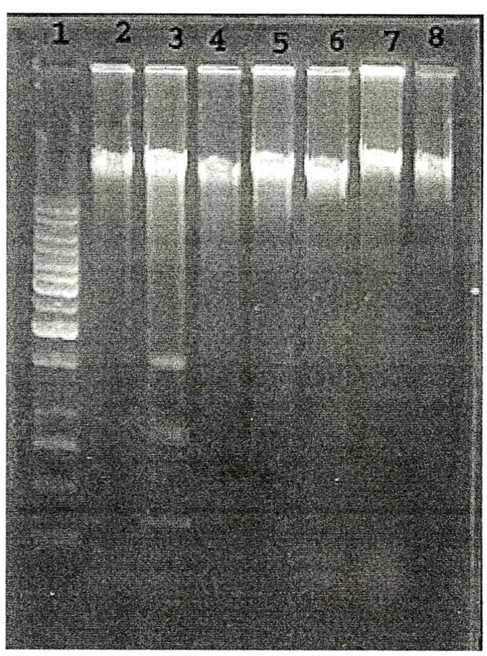
I/R+PG- Progesterone treated, I/R+CoQ – CoQ treated and I/R+PG+CoQ- combination treated reperfused animals.

Fig 2.3 c Infarct area in TTC stained slices:



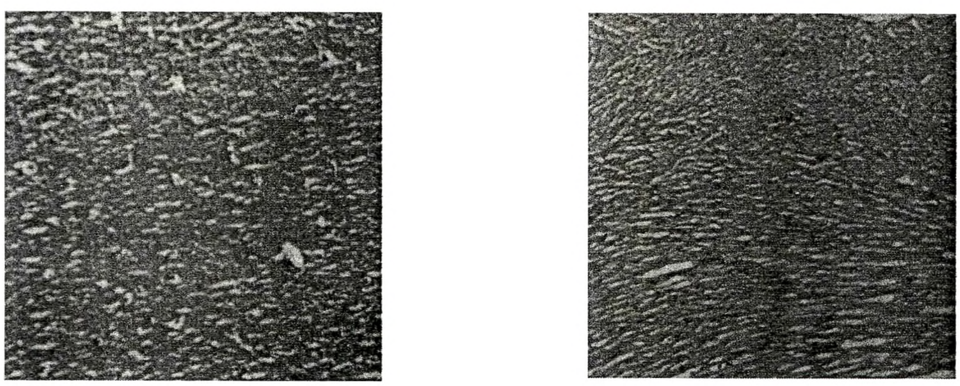
Lane A: Heart Slices of I/R control animals; **Lane B**: Heart Slices of PG treated I/R animal; **Lane C**: Heart Slices of CoQ treated I/R animals; and **Lane D**: PG and CoQ combination treated I/R animal.

Fig 2.4 Agarose gel electrophoresis of rat heart DNA after ischemia/reperfusion injury.



Lane 1, 1-kb DNA standard; **Lane 2**, Sham control with out any prominent DNA fragmentation; **Lane 3,** Ischemic control (I/R control) displaying a laddering pattern a indication of oligointernucleosomal DNA fragmentation; **Lane 4,** CoQ treated I/R (I/R + CoQ) without evident DNA laddering pattern but showed a smear pattern; **Lane 5,** PG treated I/R (I/R +PG) with out evident DNA laddering pattern: **Lane 6,** PG and CoQ combination treatd animal; **Lane 7,** Banaba treated animal showing no fragmentation; **Lane 8,** PG+BNA treated animal no DNA fragmentation.

Fig 2.5 Photomicrographs (100X) of histological slides stained with H& E stain after ischemia/reperfusion injury.



A **B**

Photomicrographs (100X) of histological slides stained with H& E stain after I/R injury.

A: CoQ treated I/R animal showing slight distorted myofibrils; **B:** PG+ CoQ treated I/R animals with improved cellular architecture, and minimum neutropil infiltration.

Table 2.1

The effect of PG, CoQ and their combination on ± d*p*/d*t* of rats with 1 h of ischemia and 1 h of reperfusion induced by left coronary descending artery (LCA) occlusion.

Group	+ d*p*/d*t*(mmHg s^{-1})			- d*p*/d*t*(mmHg s^{-1})		
	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)
SO	2402 ± 215	2668 ± 108	2567 ± 180	2015 ± 328	2053 ± 326	1958 ± 127
I/R control	2566 ± 166	1879 ± 148 *	1779 ± 102*	1811 ± 158	1146 ± 126*	1216 ± 93*
I/R + PG	2729 ± 72	1845 ± 130*	1810 ± 94*	1834 ± 108	1209 ±127*	1154 ± 181*
I/R + CoQ	2109 ± 192	1798 ± 247*	1894 ± 338*	1742 ± 56	1255 ± 191*	1493 ± 281*
I/R +PG+CoQ	2350 ± 372	1878 ± 397*	1958 ± 217*	2149 ± 157	1438 ± 199*	1732 ± 128

All values are mean ± SEM (n=6). The group treated with vehicle alone (I/R control), PG, CoQ and their combination were compared with Sham operated (SO)(*-P < 0.05, **-P < 0.01, ***- P < 0.001) while the groups treated with PG, CoQ and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P < 0.01$, ^^^ - $P < 0.001$)

Table 2.2

The effect of PG, CoQ and their combination on heart rate (HR) and systolic blood pressure (SBP) of rats with 1 h of ischemia and 1 h of reperfusion induced by left coronary descending artery (LCA) occlusion.

Group	HR (min^{-1})			SBP (mmHg)		
	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)
SO	419 ± 19.52	416 ± 12.05	405 ± 15.09	117.13 ± 4.46	106.66 ± 3.98	118.5 ± 2.58
I/R control	392 ± 18.21	325 ± 13.01*	308 ± 26.15*	110.52 ± 6.05	81.05 ± 3.56*	79.59 ± 5.98*
I/R + PG	397 ± 18.18	326 ± 15.16*	313 ± 16.15*	106.28 ± 6.74	82.67 ± 5.21*	73.52 ± 7.61*
I/R + CoQ	367 ± 52.21	348 ± 25.29*	287 ± 29.66*	111.54 ± 2.48	82.23 ± 8.47*	89.17 ± 6.13*
I/R+ PG+CoQ	450 ± 39.74	301 ± 29.59*	325 ± 18.12*	103.81 ± 9.22	79.33 ± 4.83*	90.96 ± 6.02*

All values are mean ± SEM (n=6). The group treated with vehicle alone (I/R control), PG, CoQ and their combination were compared with Sham operated (SO)(*-P < 0.05, **-P < 0.01, ***- P < 0.001) while the groups treated with PG, CoQ and their combination were compared with the I/R control group (^- *P* < 0.05, ^^- *P*< 0.01, ^^^ - *P*< 0.001)

Table 2.3

The effect of PG, CoQ and their combination on occurrence of ventricular tachycardia (VT) in rats with myocardial I/R injury

Group	Occurrence of VT	
	Ischemia period	Reperfusion period
I/R control	11/12	10/12
I/R + PG	7/12	8/12
I/R+ CoQ	5/12^	4/12^
I/R+ PG+CoQ	6/12	7/12

All values are mean ± SEM (n=6). The group treated with PG, CoQ and their combination were compared with the I/R control group (^ $P < 0.05$, ^^-$P < 0.01$ ^^^ - $P < 0.001$)

Table 2.4

Effect of treatment with PG, CoQ and their combination on oxidative stress markers in rats with myocardial I/R injury

Groups	SOD IU/mg of protein	CAT H_2O_2/consu /min/mg of protein	GSH µg/mg of protein	MDA nmol /mg of protein
SO	12.89 ± 1.30	410.35 ± 68.61	9.06 ± 0.84	1.05 ± 0.12
I/R control	3.02 ± 0.49***	274.01 ±62.23	3.91 ± 0.32***	3.02 ± 0.33 **
I/R+ PG	5.12 ±0.46***	170.99 ± 26.49	5.78 ± 0.43**	2.08 ± 0.35
I/R + CoQ	7.87 ± 0.82 **^^	344.51 ± 33.53	7.14 ± 0.61^	1.21 ± 0.47^^
I/R+ PG+CoQ	7.36 ± 0.55 ***^^	539.82 ± 110.21	6.76 ± 1.12	1.26 ± 0.37^^

All values are mean ± SEM (n=6). The group treated with vehicle alone (I/R control), PG, CoQ and their combination were compared with Sham operated (SO) (*-$P < 0.05$, **-$P < 0.01$, *** - $P < 0.001$) while the groups treated with PG, CoQ and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P < 0.01$, ^^^ - $P < 0.001$)

Table 2.5

Effect of treatment with PG, CoQ and their combination on MPO and CK activity in rats with myocardial I/R injury.

Groups	MPO units/mg of protein	CK (U/L)
SO	0.23 ± 0.91	1033.67 ± 36.23
I/R control	0.82 ± 0.04***	4490.54 ± 120.99***
I/R + PG	0.47 ± 0.03^	4324.00 ± 286.27***
I/R + CoQ	0.46 ± 0.12^	2870.66 ± 115.19***^^
I/R+ PG+CoQ	0.47 ± 0.38^	3497.54 ± 390.57***^

All values are mean ± SEM (n=6). The group treated with vehicle alone (I/R control), PG, CoQ and their combination were compared with Sham operated (SO)(*-P < 0.05, **-P < 0.01, *** - P < 0.001) while the groups treated with PG, CoQ and their combination were compared with the I/R control group (^- *P* < 0.05, ^^- *P*< 0.01, ^^^ - *P*< 0.001)

4.1.3 Effect of PG, Banaba and their combination on myocardial I/R injury:

Banaba (100mg/kg i.p.) was administered 30 min after of induction of ischemia and the group was indicated as BNA. This study protocol involved five groups - 1) sham operated vehicle treated group (SO), 2) vehicle treated reperfusion injury control group (I/R control), 3) PG (2 mg/kg i.p.) treated reperfusion injury group (PG), 4) Banaba (100 mg/kg i.p.) administered to reperfusion injury group (BNA) and 5) co-administration of PG with Banaba (PG +BNA) reperfusion injury group were studied in the I/R injury experiment as described in section 3.2.1.1.1, 3.2.1.1.4 and 3.2.1.1.8. of materials and methods.

4.1.3.1 Effects of PG, Banaba and their combination on hemodynamic and electrocardiographic parameters in myocardial I/R injury-

Biopac MP 30 system was used for investigating I/R injury induced changes in hemodynamic and electrocardiographic parameters during the study. These changes were analysed using Biopac Student Lab Pro software (Version 3.6.7).

The actual tracings displayed the alterations taken place in the hemodynamic and ECG parameters with respect to time. **(Fig. 3.1 a to 3.1 d)**

It was found that the induction of ischemia led to significant ($P<0.05$) fall in systolic, diastolic and mean arterial blood pressure in I/R control and PG treated groups in comparison to SO group, whereas the treatment with Banaba and its combination with PG caused significant ($P<0.05$) improvement in MAP after initiation of reperfusion. The changes in hemodynamic parameters observed in other treated groups were not significant as compared to I/R control group as at both End-Ischemia and End-Reperfusion intervals. The change in positive and negative fraction of blood pressure with respect to time (± dp/dt) was significantly changed only in PG+BNA treated groups as compared to I/R control group. **Table 3.1 & 3.2**

I/R injury imparted significant ($P<0.01$) ST elevation and ST prolongation in the vehicle treated (I/R control) group subjected to ischemia as compared to sham operated control. However, there was a significant ($P<0.05$) change in the groups receiving Banaba alone and PG plus Banaba (BNA and PG+BNA) at the End-ischemia period and ($P<0.001$) at End-reperfusion on ST elevation and duration of ST segment as compared to I/R control group. Incidences of VT were significantly reduced in Banaba treated group in comparison to I/R control animals. **Table 3.3 and Fig. 3.2 a to 3.2 c**

4.1.3. Effects of PG, Banaba and their combination on Infarct size and At Risk (AAR) -

The slices of the hearts from various groups stained with Evans blue and TTC are shown in **Fig. 3.3 c.**

The AAR was not significantly different in all the groups. AAR for BNA and PG+BNA groups was 44.91 ± 3.23 and 35.48 ± 2.54 respectively.

Banaba treatment caused significant ($P<0.001$) decrease in infarct size as compared to I/R control group. Banaba with PG significantly ($P<0.001$) reduced infarct size. The infarct sizes in I/R control, PG, BNA and PG+BNA groups were 54.98 ± 3.59, 38.97 ± 3.37, 25.00 ± 1.33 and 19.37 ± 0.95 respectively. **(Fig. 3.3 a and 3.3 b)**

4.1.3.3 Effects of PG, Banaba and their combination on Oxidative stress biomarkers -

Ischemic reperfusion injury significantly ($P<0.001$) depleted endogenous antioxidants like reduced glutathione (GSH), superoxide dismutase (SOD) and catalase. The lipid peroxidation due to I/R injury induction was indicated by significant elevation ($P<0.001$) of malondialdehyde (MDA) as compared to sham operated animals. Banaba treatment alone and simultaneous administration with PG significantly ($P<0.01$) altered the activity of SOD. GSH and MDA levels were also significantly ($P<0.01$) changed with the treatment. There was a significant ($P<0.01$) increase in GSH levels after BNA+PG treatment. Activity of catalase enzyme was not significantly different in any of the treated group as compared to I/R control animals. **Table-3.4**

4.1.3.4 Effects of PG, Banaba and their combination on inflammatory reactivity and CK activity-

Inflammatory activity of the animals subjected to I/R was estimated by activity of MPO, which was found to increase significantly ($P<0.001$) in I/R control group as compared to SO animals. Banaba and PG treatment significantly ($P<0.05$) reduced MPO activity after induction of I/R injury. CK levels in serum, which was supposed to be leaked out from the tissue, was significantly elevated in I/R control group and Banaba alone and in combination with PG treatment significantly reduced the serum levels of CK. **Table- 3.5**

4.1.3.5 Effects of PG, Banaba and their combination on Apoptosis -

Apoptosis induced by I/R injury observed as the interneucleosomal DNA fragmentation using Gel electrophoresis. I/R control animals showed marked evidences of DNA damage, whereas Banaba, PG and their combination treatment substantially reduced the intensity of the band formation. **Fig. 2.4**

4.1.3.6 Effects of PG, Banaba and their combination on histological features-

Injury to the cardiac myocytes induced by I/R was observed with the help of photomicrographs of the hearts from control and treated groups. Damage was indicated by edema of cells, heavy neutrophil infiltration and cellular necrosis in vehicle treated I/R control animals. Banaba showed considerable protection of the cellular structure. This treatment led to reduction in the incidences of edema formation and neutrophil infiltration. **Fig. 3.4**

Fig. 3.1

Recordings showing changes occurring in hemodynamic and Electrocardiographic (ECG) parameters after induction of ischemia-reperfusion in Banaba, PG and vehicle treated rats-

The figures represent electrocardiograph changes in the animals at various time intervals.

3.1 a. ECG Changes occurring a during ischemia in Banaba treated group (End – Ischemia period)

3.1 b. ECG Changes occurring during reperfusion in Banaba treated group (End – Reperfusion period)

3.1 c. ECG Changes occurring a during ischemia in PG with Banaba treated group (End –Ischemia period)

3.1 d. ECG Changes occurring during reperfusion in PG with Banaba treated group (End –Reperfusion period)

Fig 3.1 a

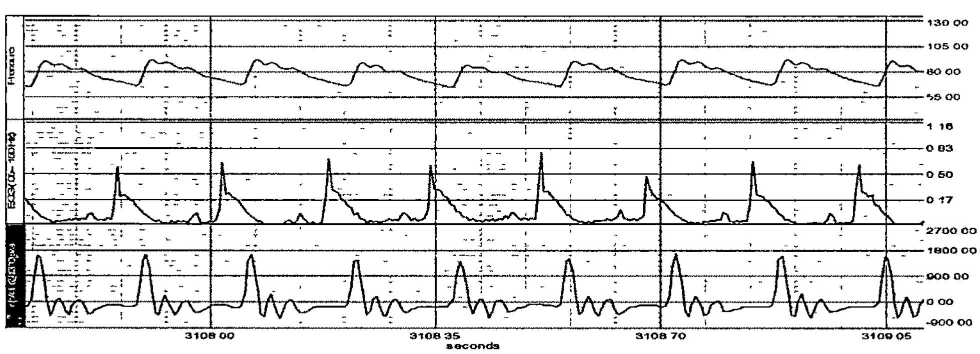


Fig 3.1 b

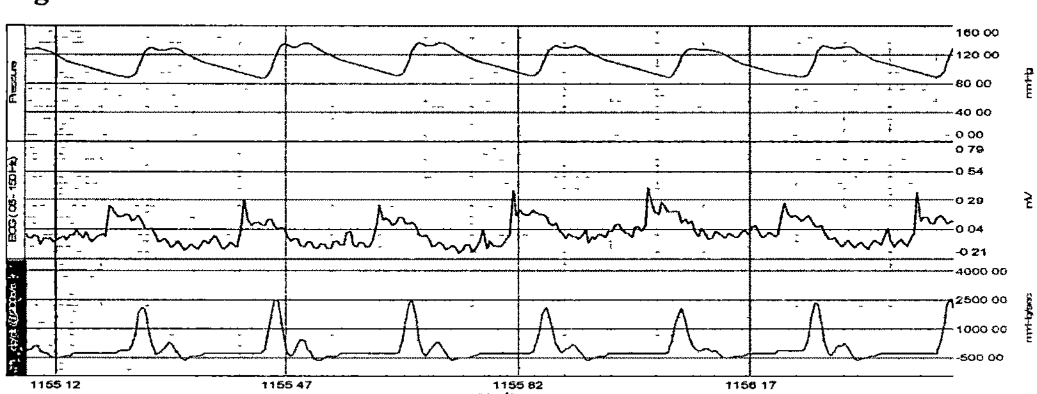


Fig 3.1 c

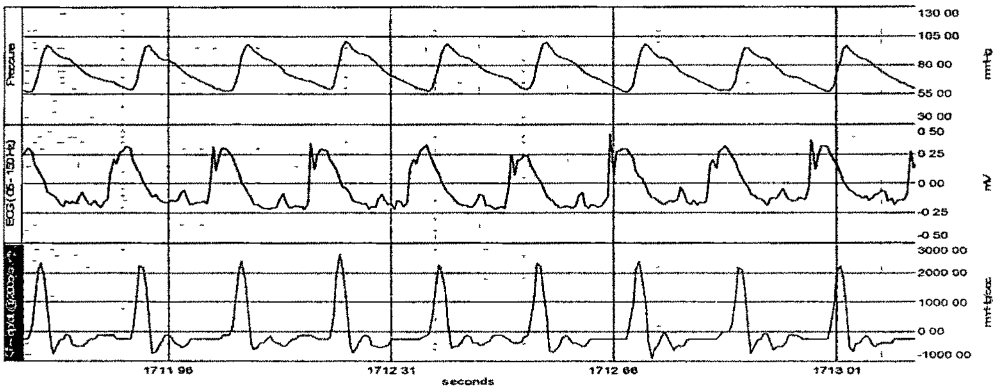


Fig 3.1 d

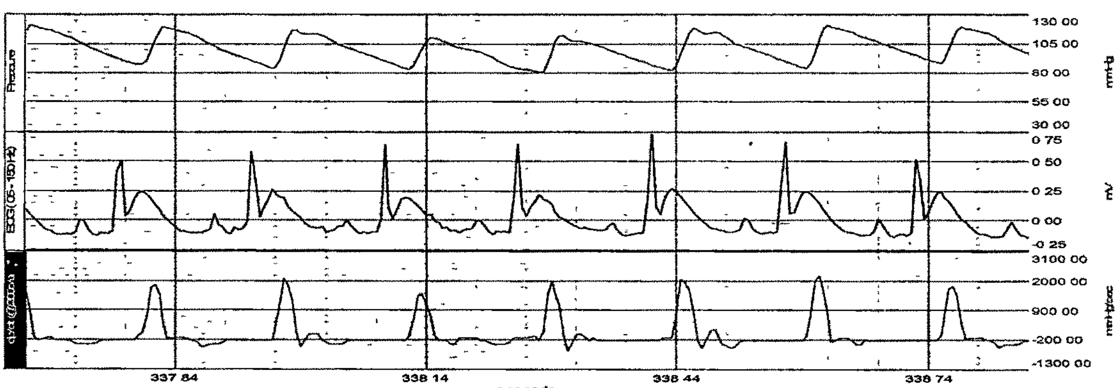


Fig. 3.2 a to 3.2 c

Changes in Mean arterial blood pressure (MAP) and ST segment with respect to time of induction of ischemia and reperfusion in the animals during study.

1) **SO- Sham Operated animals (No ischemia-reperfusion) + vehicle,**
2) **I/R- Vehicle treated rats undergoing reperfusion injury(I/R control)**
3) **I/R+PG – PG treated rats subjected to reperfusion injury**
4) **I/R+ BNA – Banaba treated rats subjected to reperfusion injury**
5) **I/R+PG+BNA – PG with Banaba (Combined treatment) treated rats subjected to reperfusion injury**

Arrows indicates time of 1. Baseline (Before induction of ischemia), 2. End-Ischemia (30 min after induction of ischemia) and 3. End-Reperfusion (60 min after induction of ischemia)

3.2 a. Changes in mean blood pressure (mm Hg)

3.2 b. Changes in ST elevation (mv)

3.2 c. Changes in ST prolongation (mv/s)

All values are mean ± SEM (n=6). The group treated with vehicle alone (I/R control) was compared with Sham operated (*-$P < 0.05$, **-$P < 0.01$, *** - $P < 0.001$) while groups treated with PG, Banaba and their Combination treatment were compared with the I/R control group (^- $P < 0.05$, ^^-$P < 0.01$; ^^^- $P < 0.001$)

Fig.3.2 a. Changes in Mean arterial blood pressure with respect to time before and after ischemia/reperfusion

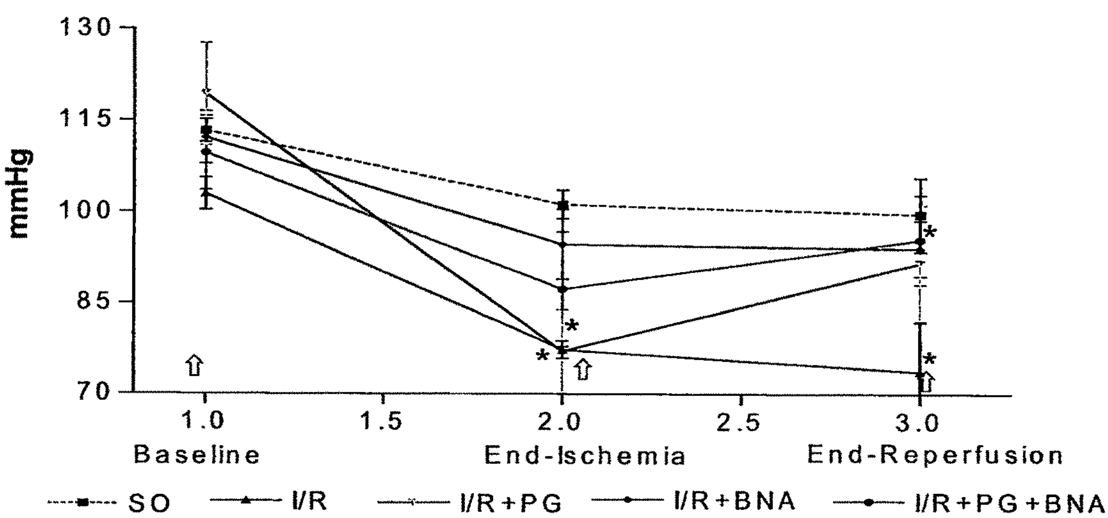


Fig. 3.2 b. Changes in ST segment elevation with respect to time, before and after ischemia/reperfusion

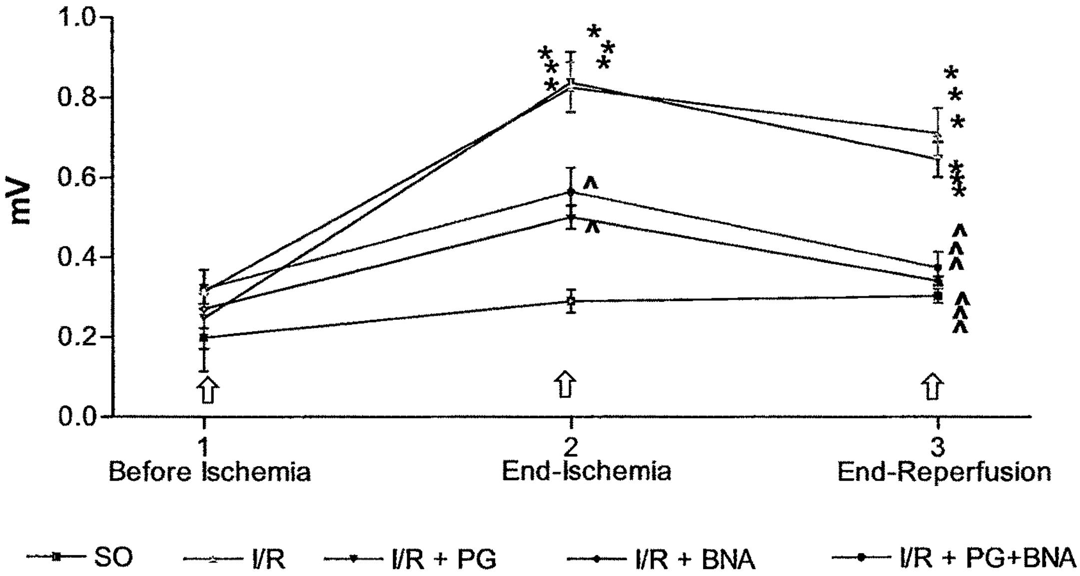


Fig. 3.2 c. Changes in ST segment prolongation with respect to time, before and after ischemia/reperfusion

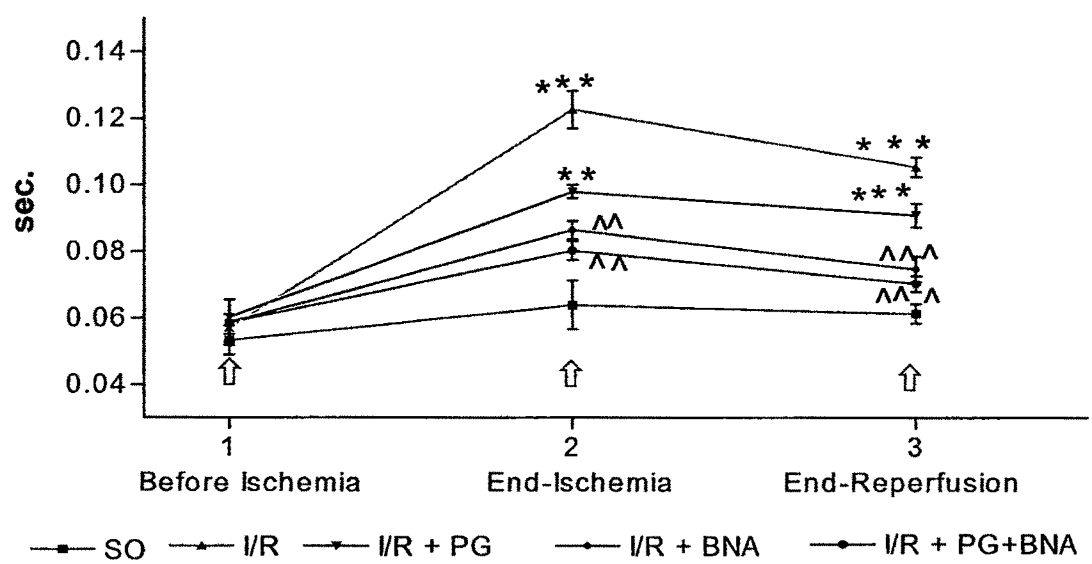


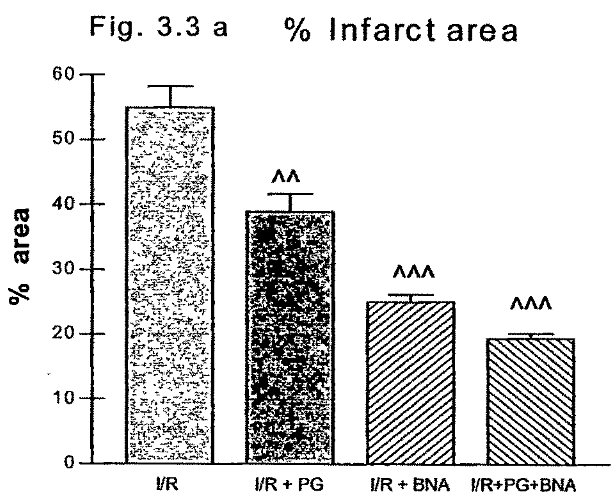
Fig. 3.3 a and 3.3 b

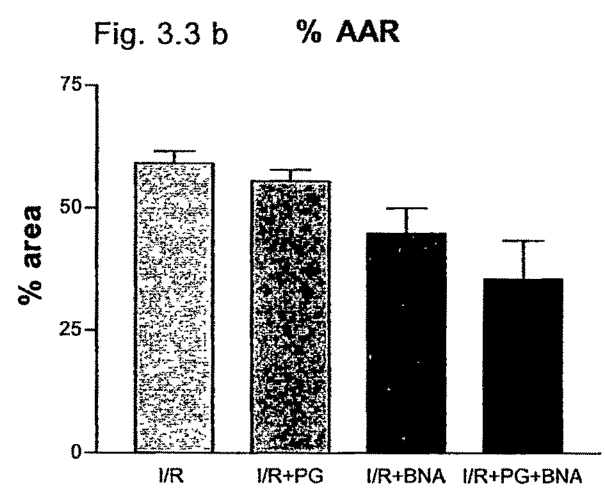
Effects of PG, Banaba and their combination on infarct area and Area At Risk (AAR) after myocardial I/R injury in rats.

3.3 a. Infarct area in percentage

3.3 b. Area at risk (AAR) in percentage

All values are mean ± SEM (n=6). The group treated with PG, Banaba and Combination treatment were compared with the I/R control group (^ - $P < 0.05$, ^^ -P < 0.01; ^^^- P < 0.001)

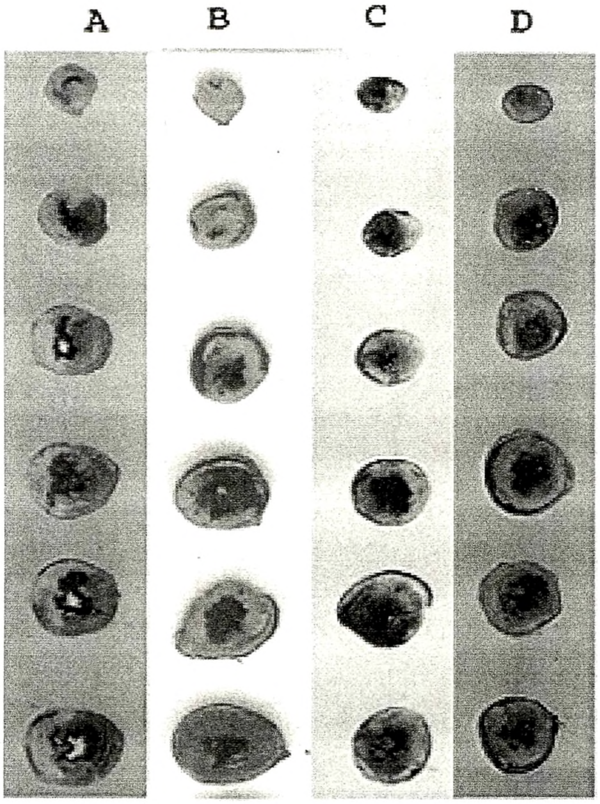




SO- Sham Operated, I/R- Vehicle treated rats undergoing reperfusion injury

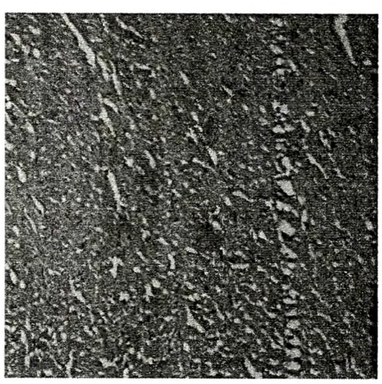
I/R+PG- Progesterone treated, I/R+BNA – Banaba treated and I/R+PG+BNA- combination treated reperfused animals.

Fig 3.3 c Area of Infarct in TTC stained Slices:



Lane A: Heart Slices of I/R control animals; **Lane B**: Heart Slices of PG treated I/R animal; **Lane C**: Heart Slices of Banaba treated I/R animals; and **Lane D**: PG and Banaba combination treated I/R animal.

Fig 3.4 Photomicrographs (100X) of histological slides stained with H& E stain after I/R.



A

B

A: Banaba treated I/R animal showing marked improvement in myofibrils; **B:** PG+ BNA treated I/R animals with nicely arranged myofibris adjacent to each other.

Table 3.1

The effect of PG, Banaba and their combination on ± d*p*/d*t* of rats with 1 h of ischemia and 1 h of reperfusion induced by left coronary descending artery (LCA) occlusion.

Group	+ d*p*/d*t*(mmHg/sec)			- d*p*/d*t*(mmHg/sec)		
	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)
SO	2402 ± 215	2668 ± 108	2567 ± 180	2015 ± 328	2053 ± 326	1958 ± 127
I/R control	2566 ± 166	1879 ± 148 *	1779 ± 102*	1811 ± 158	1146 ± 126*	1216 ± 93*
I/R + PG	2729 ± 72	1845 ± 130*	1810 ± 94*	1834 ± 108	1209 ±127*	1154 ± 181*
I/R + BNA	2329 ± 301	1981 ± 298*	1990 ± 238*	2142 ± 198	1687 ± 114*	1523 ± 168*
I/R +PG+BNA	2424 ± 242	1657± 348*	2219 ± 147	2149 ± 157	1348 ± 121*	1847 ± 354

All values are mean ± SEM (n=6). The group treated with vehicle alone (I/R control), PG, Banaba and their combination were compared with sham operated (SO)(*-P < 0.05, **-P < 0.01, *** - P < 0.001) while groups treated with PG, Banaba and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P < 0.01$, ^^^- $P < 0.001$)

Table 3.2

The effect PG, Banaba and their combination on heart rate (HR) and systolic blood pressure (SBP) of rats with 1 h of ischemia and 1 h of reperfusion induced by left coronary descending artery (LCA) occlusion.

Group	HR/min			SBP (mmHg)		
	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)
SO	419 ± 19.52	416 ± 12.05	405 ± 15.09	117.13 ± 4.46	106.66 ± 3.98	118.5 ± 2.58
I/R control	392 ± 18.21	325 ± 13.01*	308 ± 26.15*	110.52 ± 6.05	81.05 ± 3.56*	79.59 ± 5.98*
I/R + PG	397 ± 18.18	326 ± 15.16*	313 ± 16.15*	106.28 ± 6.74	82.67 ± 5.21*	73.52 ± 7.61*
I/R + BNA	435 ± 82.09	308 ±30.09*	287 ± 29.66*	112.14 ± 4.34	94.75 ± 5.75	94.06 ± 4.60
I/R+ PG+BNA	441 ± 21.74	354 ±10.59*	336 ± 23.12*	109.66 ± 6.06	87.33 ± 9.45	95.51 ± 7.43

All values are mean ± SEM (n=6). The group treated with vehicle alone (I/R control), PG, Banaba and their combination were compared with sham operated (SO)(*-$P < 0.05$, **-$P < 0.01$, ***- $P < 0.001$) while groups treated with PG, Banaba and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P < 0.01$, ^^^- $P < 0.001$)

Table 3.3

The effect of PG, Banaba and their combination on occurrence of ventricular tachycardia (VT) in rats with myocardial I/R injury

Group	Occurrence of VT	
	Ischemia period	Reperfusion period
I/R control	11/12	10/12
I/R + PG	7/12	8/12
I/R+ BNA	3/12^	6/12^
I/R+ PG+BNA	4/12^	5/12^

All values are mean ± SEM (n=6). The group treated with PG, Banaba and their combination were compared with the I/R control group (^ $P < 0.05$, ^^-$P < 0.01$ ^^^- $P < 0.001$)

Table 3.4

Effect of treatment with PG, Banaba and their combination on oxidative stress markers in rats with myocardial I/R injury

Groups	**SOD** IU/mg of protein	**CAT** H_2O_2/consu /min/mg of protein	**GSH** µg/mg of protein	**MDA** nmol /mg of protein
SO	12.89 ± 1.30	410.35 ± 68.61	9.06 ± 0.84	1.05 ± 0.12
I/R control	3.02 ± 0.49***	274.01 ±62.23	3.91 ± 0.32***	3.02 ± 0.33 **
I/R+ PG	5.12 ±0.46***	170.99 ± 26.49	5.78 ± 0.43	2.08 ± 0.35
I/R + BNA	8.58 ± 0.97**^^^	266.61 ±50.53	8.13 ± 0.45 ^	1.5 1 ± 0.17^^
I/R+ PG+BNA	9.10 ± 0.67*^^^	679.66 ± 177.21	7.95 ± 0.53^	1.34 ± 0.22^^^

All values are mean ± SEM (n=6). The group treated with vehicle alone (I/R control), PG, Banaba and their combination were compared with sham operated (SO)(*-P < 0.05, **-P < 0.01, *** - P < 0.001) while groups treated with PG, Banaba and their combination were compared with the I/R control group (^- *P* < 0.05, ^^- *P*< 0.01, ^^^ - *P*< 0.001)

Table 3.5

Effect of treatment with PG, Banaba and their combination on MPO and CK activity in rats with myocardial I/R injury.

Groups	MPO units/mg of protein	CK (U/L)
SO	0.23 ± 0.91	1033.67 ± 36.23
I/R control	0.82 ± 0.04***	4490.54 ± 120.99***
I/R + PG	0.47 ± 0.03^	4324.00 ± 286.27***
I/R + BNA	0.44 ± 0.03^	3507.16 ± 206.28***^
I/R+ PG+BNA	0.40 ± 0.02^	2059.83 ± 302.58*^^^

All values are mean ± SEM (n=6). The group treated with vehicle alone (I/R control), PG, Banaba and their combination were compared with sham operated (SO) (*-P < 0.05, **-P < 0.01, *** - P < 0.001) while groups treated with PG, Banaba and their combination were compared with the I/R control group (^- *P* < 0.05, ^^- *P*< 0.01, ^^^ - *P*< 0.001)

4.1.4 Gender specific effect of PG on myocardial reperfusion injury:

The animals aged around 12-14 weeks old, were randomized in seven groups (n =18 in each group). 1) A Sham-operated (SO) control group and 6 ischemic groups. The ischemic groups include 2) a vehicle treated I/R injury control of male (I/R-M), 3) female (I/R-F) and 4) ovariectomised (I/R-OVR) animals. Progesterone (PG 2 mg/kg i.p.) was administered to the animals 30 min after ischemia induction to 5) male, 6) female and 7) OVR animal (I/R-M+PG, I/R-F+PG, I/R-OVR+PG) groups, respectively. Pea nut oil was used as a vehicle and administered by i.p. route.

The experiment was performed in three sets of animals (n=6 in each set) for every group. The first set was used measurement of infarct area. Second set was utilized for estimation of oxidative stress parameters (SOD, GSH, CAT and LPO) and measurement of myeloperoxidase (MPO) activity. Third set was employed for DNA fragmentation analysis and histological observations. Ovariectomy was performed on the animals at the age of around 10-12 week. The animal was allowed to recover for 2 to 4 weeks after the surgical procedure for ovariectomy before performing the I/R protocols.

4.1.4.1 Effect of PG on Hemodynamic parameters in male, female and OVR animals subjected to myocardial reperfusion injury:

There was a significant ($P<0.05$) decrease in MAP, HR and ±dp/dt in all ischemic groups (I/R-M, I/R-F, I/R-OVR) as compared to sham operated (SO) animals at the end of ischemia as well as reperfusion. Similar fall in SBP and DBP was observed in all I/R groups (data not given). After the treatment with PG, only female animals restored MAP and maximal positive pressure change rate by preventing the significant ($P<0.05$) fall at the end-reperfusion interval when compared to SO group. The treatment with PG of male and OVR groups did not show any significant improvement in MAP (as well for SBP, DBP) and HR at both the intervals (End-ischemia and End-reperfusion) as compared to SO group. **(Table 4.1, 4.2)**

4.1.4.2 Effect of PG on ECG in male, female and OVR animals subjected to myocardial reperfusion injury:

ST segment prolongation and elevation in vehicle treated I/R-M, I/R-F and I/R-OVR groups was significantly ($P<0.05$) increased at the end-ischemia and end-reperfusion period as compared to SO group. The treatment with PG resulted in a significant ($P<0.05$) decrease in ST segment prolongation and elevation at both the intervals as compared to ischemic female (I/R-F) group, whereas, the PG treatment did not alter ST segment changes significantly in male and OVR female counterparts. **(Fig 4.1a to 4.1 b)**

The treatment with PG in ischemic female group significantly ($P<0.05$) reduced incidences of VT as compared to vehicle treated I/R female group. However, treatment with PG in male and OVR animals did not show significant effect on arrhythmia as compared to vehicle treated I/R male and OVR animals. **(Table 4.3)**

4.1.4.3 Effects of PG on Infarct size and Area At Risk in myocardial I/R injury -

Myocardial infarct area after I/R injury in vehicle treated I/R-M, I/R-F and I/R-OVR groups was found to be 50.26 ± 2.51 %, 49.10 ± 1.30 % and 48.69 ± 1.99 %, respectively. The treatment with PG significantly ($P<0.05$) reduced area of infarction in female rats. I/R-M and I/R-OVR rats did not show any significant reduction in the area of infarction after the PG treatment. The area at risk was not altered significantly in all the groups after I/R injury. AAR in all the treated groups did not change significantly. **(Fig. 4.2.)**

4.1.4.4 Effects of PG on Oxidative stress markers in male, female and OVR animals subjected to myocardial reperfusion injury:

SOD activity and GSH levels in I/R injury (I/R-M, I/R-F, I/R-OVR) induced animals were significantly (P<0.001) decreased as compared to SO animals. Treatment with PG showed significant (P<0.05) increase in SOD activity and GSH levels in I/R-F animals; however, the I/R-M+PG and I/R-OVR+PG rats did not show any significant increase in SOD activity and GSH levels as compared to I/R animals. The effect to on CAT activity was not significant in all the vehicle treated I/R and PG treated I/R groups as compared to SO group of animals. Lipid peroxidation, measured by formed malondialdehyde (MDA) levels was significantly (P<0.01) elevated in I/R animals as compared to SO group. MDA levels are significantly (P<0.05) reduced in PG treated female rats as compared to vehicle treated I/R-F animals. **(Table 4.4)**

4.1.4.5 Effects of PG on Myeloperoxidase activity in male, female and OVR animals subjected to myocardial reperfusion injury:

Inflammatory reactivity due to neutrophil infiltration was substantially elevated after I/R injury, which was evidenced from the significant ($P<0.01$) increase in MPO activity of I/R-M, I/R-F and I/R-OVR as compared to SO animals. The female rats treated with PG (I/R-F + PG) showed a significant ($P<0.05$) decrease in MPO activity as compared to vehicle treated I/R females (I/R-F). PG treatment in I/R-M and I/R-OVR rats did not alter the MPO activity significantly as compared to respective I/R groups. I/R injury significantly ($P<0.001$) elevated CK activity in I/R groups as compared to SO group. PG treatment significantly ($P<0.001$) reduced CK activity in I/R female animals as compared to vehicle treated I/R females. Male and OVR I/R animals did not show any significant alteration in CK activity after treatment with PG. **(Table 4.5)**

4.1.4.6 Effect of PG on Apoptosis- DNA fragmentation Analysis:

I/R injury induced prominent DNA fragmentation in all the I/R animals indicating activation of apoptotic processes. Treatment with PG of female rats showed decreased DNA fragmentation. Although visualization of DNA fragmentation with ethidium bromide is not quantitative, the reduced visualization of fragmentation in I/R F + PG group could be attributed to reduced number of apoptotic bodies observed in heart. **(Fig. 4.4)**

4.1.4.7 Histological changes in Cardiomyocytes:

The light microscopy of heart section of SO animals showed normal structures of myofibrils whereas I/R animals showed focal hemorrhage, heavy leukocyte infiltration and the presence of cellular edema with distracted striations suggestive of cellular necrosis. The section of the heart in I/R-F + PG animals showed reduced leukocyte infiltration and edema along with considerable recovery of the cellular structures as compared to I/R-F animals. **(Fig. 4.5)**

Fig. 4.2 a to 4.2 c

Changes in Mean arterial blood pressure (MAP) and ST segment with respect to time of induction of ischemia and reperfusion in the animals during study.

1) **SO- Sham Operated animals (No ischemia-reperfusion) + vehicle,**
2) **I/R-M - Vehicle treated male rats undergoing reperfusion injury**
3) **I/R-F – Vehicle treated female rats undergoing reperfusion injury**
4) **I/R-OVR– Vehicle treated ovariectomised female rats with I/R injury**
5) **I/R-M+ PG – PG treated male rats subjected to reperfusion injury**
6) **I/R-F+ PG – PG treated female rats subjected to reperfusion injury**
7) **I/R-OVR+ PG – PG treated ovariectomised female rats with I/R injury**

Arrows indicates time of 1. Baseline (Before induction of ischemia), 2. End-Ischemia (30 min after induction of ischemia) and 3. End-Reperfusion (60 min after induction of ischemia)

4.3 a. Changes in mean blood pressure (mm Hg)

4.3 b. Changes in ST elevation (mv)

4.3 c. Changes in ST prolongation (mv/s)

All values are mean ± SEM (n=6). The groups treated with vehicle (I/R-M, I/R-F, I/R-OVR) were compared with sham operated (*-P < 0.05, **-P < 0.01, *** - P < 0.001) while groups treated with PG (I/R-M+PG, I/R-F+PG, I/R-OVR+PG) were compared with the I/R group (^- P < 0.05, ^^-P < 0.01; ^^^- P < 0.001)

Fig. 4.2 a. Changes in Mean arterial blood pressure with respect to time before and after ischemia/reperfusion

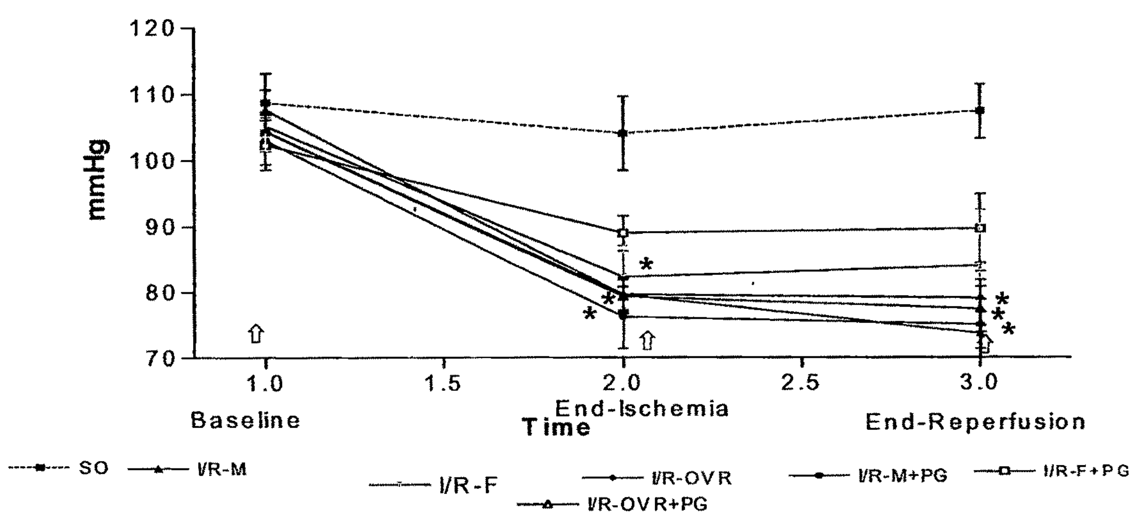


Fig. 4.2 b. Changes in ST segment elevation with respect to time, before and after ischemia/reperfusion

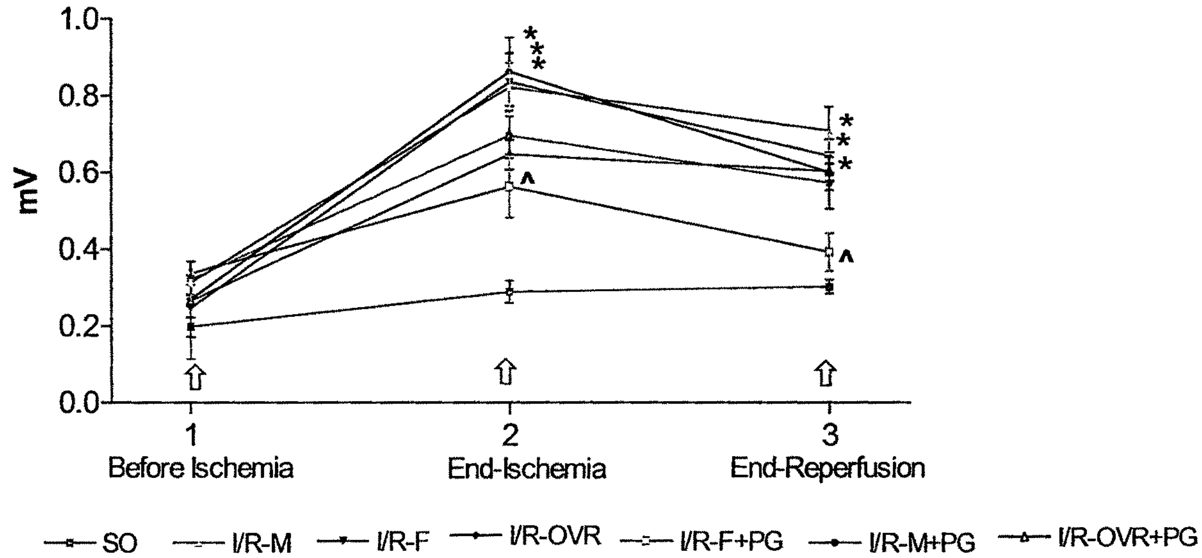


Fig. 4.2 c. Changes in ST segment prolongation with respect to time, before and after ischemia/reperfusion

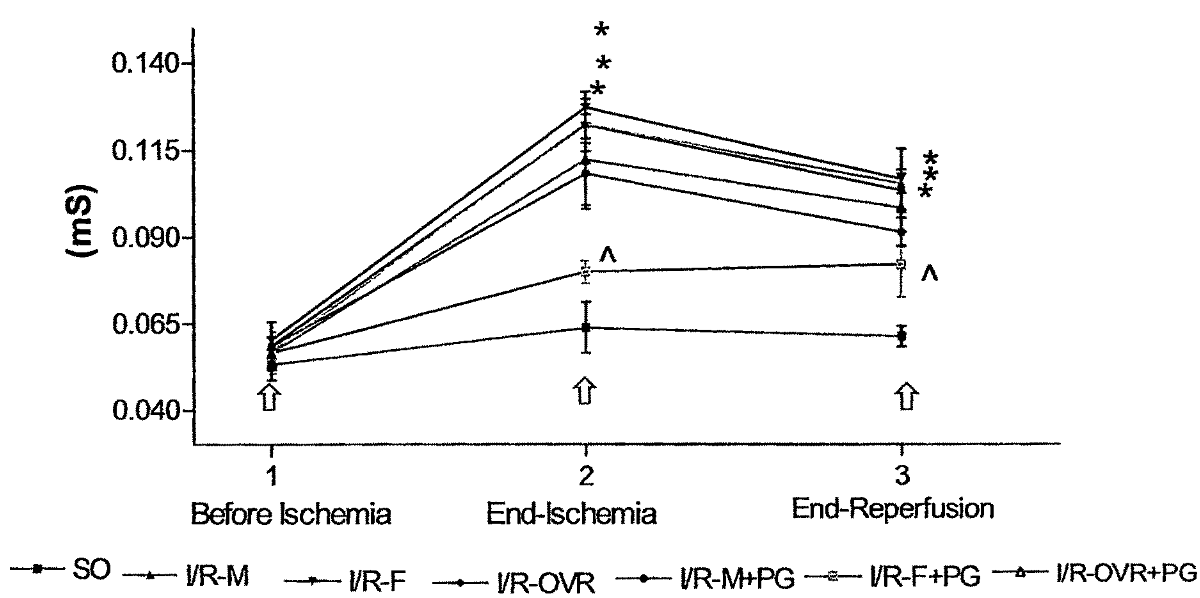


Fig. 4.3 a and 4.3 b

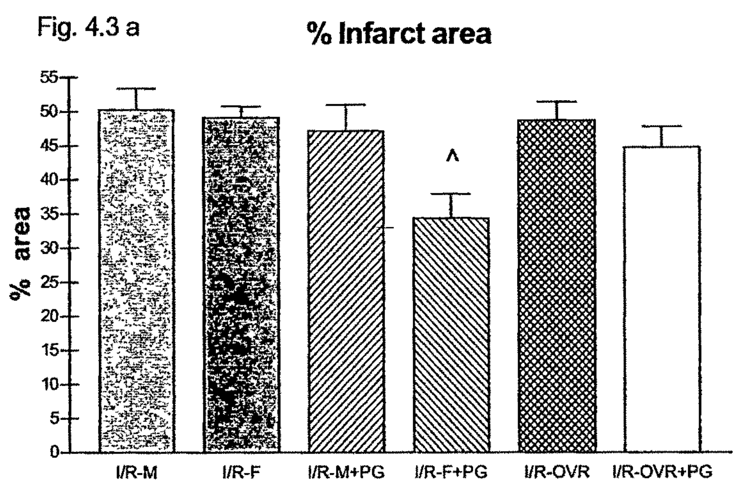
Effect of PG on infarct area after myocardial I/R injury in rats.

4.3 a. Infarct area in percentage

4.3 b. Area at risk (AAR) in percentage

All values are mean ± SEM (n=6). The groups treated with PG (I/R-M+PG, I/R-F+PG, I/R-OVR+PG) were compared with the I/R group (^- $P < 0.05$, ^^-$P < 0.01$; ^^^- $P < 0.001$)

Fig. 4.3 a

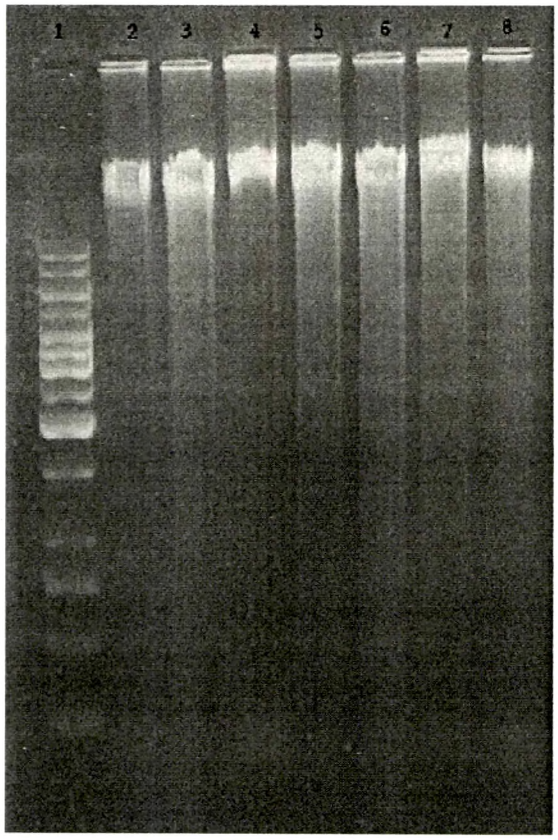


SO- Sham Operated, I/R-M, I/R-F, I/R-OVR - Vehicle treated male, female and OVR rats undergoing reperfusion injury

I/R-M+PG, I/R-F+PG, I/R-OVR+PG – PG treated male, female and OVR rats undergoing reperfusion injury.

Fig 4.4

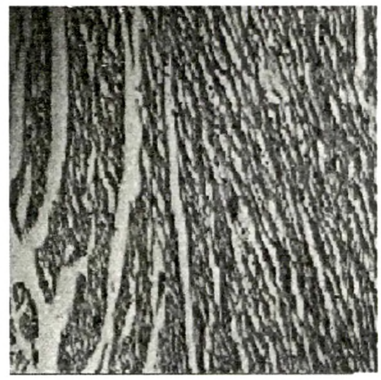
Agarose gel electrophoresis of rat heart DNA after ischemia/reperfusion injury.



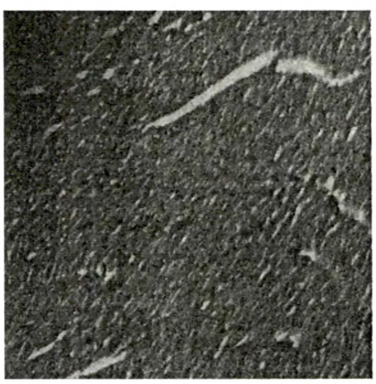
Lane 1, 1-kb DNA standard; **Lane 2,** Sham control with out any prominent DNA fragmentation; **Lane 3,** Female (I/R-F) displaying a laddering pattern a indication of oligointernucleosomal DNA fragmentation; **Lane 4,** Progesterone treated I/R female (I/R-F + PG) without evident DNA laddering pattern; **Lane 5**, Male (I/R-M) showing DNA fragmentation; **Lane 6**, Progesterone treated I/R male (I/R-M+ PG); **Lane 7**, I/R-OVR OVR females with DNA fragmentations; **Lane 8**, Progesterone treated I/R OVR female (I/R-OVR + PG) . I/R-M,: vehicle treated male ischemic group; I/R-F,: vehicle treated female ischemic group; I/R-OVR,: vehicle treated ovariectomised female ischemic group.

Fig 4.5 Photomicrographs (100X) of histological slides stained with H& E stain after ischemia/reperfusion injury.

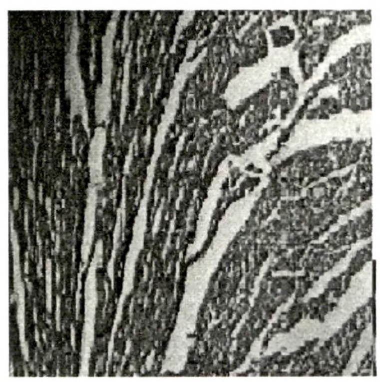
A



B



C



D

Photomicrographs (100X) of histological slides stained with H& E stain after I/R injury.

A: Vehicle treated I/R animal showing fragmented myofibril; **B:** Progesterone treated I/R male showing leukocyte infiltration; **C:** Progesterone treated I/R female with improved cellular structures, continuity with adjacent myofibrils and minimum damage; **D**: Progesterone treated I/R ovariectomised animal with edema and damaged myofibrils.

Table 4.1

The effect of PG (2 mg/kg) on ± dp/dt of rats with 1 h of ischemia and 1 h of reperfusion induced by left coronary descending artery (LCA) occlusion.

Group	+ dp/dt(mmHg/sec)			- dp/dt(mmHg/sec)		
	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)
SO	2402 ± 215	2668 ± 108	2567 ± 180	2015 ± 328	2053 ± 326	1958 ± 127
I/R-M	2566 ± 166	1879 ± 148 *	1779 ± 102*	1811 ± 158	1146 ± 126*	1216 ± 93*
I/R-F	2623 ± 177	1895 ± 78*	1773 ± 106*	1953 ± 185	1132 ± 135*	1039 ± 50*
I/R-OVR	2580 ± 160	1800 ± 139*	1785 ± 93*	1913 ± 188	1264 ±103*	1115 ± 97*
I/R-M + PG	2729 ± 72	1845 ± 130*	1810 ± 94*	1834 ± 108	1209 ±127*	1154 ± 181*
I/R-F + PG	2623 ± 132	1951 ± 193	2090 ± 132	2002 ± 156	1655 ± 101	1791 ± 86
I/R-OVR + PG	2750 ± 172	1708 ± 197*	1755 ± 177*	1974 ± 167	1234 ± 92*	1222 ± 139*

All values are mean ± SEM (n=6). The groups treated with vehicle (I/R-M, I/R-F, I/R-OVR) were compared with sham operated (*-$P < 0.05$, **-$P < 0.01$, *** - $P < 0.001$) while groups treated with PG (I/R-M+PG, I/R-F+PG, I/R-OVR+PG) were compared with the I/R group (^- $P < 0.05$, ^^-$P < 0.01$; ^^^- $P < 0.001$)

Table 4.2

The effect of PG (2 mg/kg) on heart rate (HR) and Mean arterial pressure (MAP) of rats with 1 h of ischemia and 1 h of reperfusion induced by left coronary descending artery (LCA) occlusion.

Group	HR/min			MBP (mmHg)		
	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)
SO	419 ± 19.52	416 ± 12.05	405 ± 15.09	107.13 ± 4.46	106.66 ± 3.98	108.5 ± 2.58
I/R-M	392 ± 18.21	325 ± 13.01*	308 ± 26.15*	107.52 ± 6.05	80.05 ± 3.56*	76.59 ± 5.98*
I/R-F	398 ± 15.78	323 ± 20.15*	318 ± 18.18*	104.75 ± 6.52	79.72 ± 2.08*	77.66 ± 4.31*
I/R-OVR	404 ± 15.11	330 ± 21.14*	336 ± 20.18*	108.61 ± 6.81	81.16 ± 5.27*	77.43 ± 5.15*
I/R-M + PG	397 ± 18.18	326 ± 15.16*	313 ± 16.15*	106.28 ± 6.74	77.67 ± 5.21*	73.52 ± 7.61*
I/R-F + PG	404 ± 22.21	319 ± 15.29*	307 ± 10.66*	100.44 ± 4.48	88.83 ± 6.47	88.11 ± 5.13
I/R-OVR + PG	390 ± 16.74	327 ± 22.56*	325 ± 18.12*	104.11 ± 5.22	79.33 ± 4.83*	77.66 ± 4.02*

All values are mean ± SEM (n=6). The groups treated with vehicle (I/R-M, I/R-F, I/R-OVR) were compared with sham operated (*-P < 0.05, **-P < 0.01, ***- P < 0.001) while groups treated with PG (I/R-M+PG, I/R-F+PG, I/R-OVR+PG) were compared with the I/R group (^- P < 0.05, ^^-P < 0.01; ^^^- P < 0.001)

Table 4.3

The effect of PG (2 mg/kg) on occurrence of ventricular tachycardia (VT) in rats with myocardial I/R injury

Group	Occurrence of VT	
	Ischemia period	Reperfusion period
I/R-M	11/12	10/12
I/R-F	10/12	8/12
I/R-OVR	11/12	7/12
I/R-M + PG	7/12	8/12
I/R-F + PG	4/12^	3/12^
I/R-OVR + PG	8/12	5/12

All values are mean ± SEM (n=6). The groups treated with PG (I/R-M+PG, I/R-F+PG, I/R-OVR+PG) were compared with the I/R (I/R-M, I/R-F, I/R-OVR) group (^- $P < 0.05$, ^^-$P < 0.01$; ^^^- $P < 0.001$)

Table 4.4

Effect of treatment with PG (2 mg/kg) on oxidative stress markers in rats with myocardial I/R injury

Groups	SOD IU/mg of protein	CAT H_2O_2/consu /min/mg of protein	GSH µg/mg of protein	MDA nmol /mg of protein
SO	12.71 ± 0.89	442.35 ± 66.61	9.26 ± 0.84	1.11 ± 0.09
I/R-M	3.82 ± 0.62***	315.01 ± 43.23	3.89 ± 0.32***	3.14 ± 0.27**
I/R-F	3.75 ± 0.56***	353.91 ± 46.14	4.06 ± 0.31***	3.07 ± 0.26**
I/R-OVR	2.97 ± 0.49***	303.78 ± 44.66	4.80 ± 0.24***	3.26 ± 0.19**
I/R-M + PG	5.00 ±0.63***	289.99 ± 20.49	4.90 ± 0.49***	1.99 ± 0.26
I/R-F + PG	7.17 ± 0.56***^	357.23 ± 29.83	7.30 ± 0.45^	1.40 ± 0.07^
I/R-OVR + PG	4.61 ± 0.55***	307.14 ± 33.76	5.97 ± 0.55**	1.93 ±0.57

All values are mean ± SEM (n=6). The groups treated with vehicle (I/R-M, I/R-F, I/R-OVR) were compared with sham operated (*-P < 0.05, **-P < 0.01, *** - P < 0.001) while groups treated with PG (I/R-M+PG, I/R-F+PG, I/R-OVR+PG) were compared with the I/R group (^-P < 0.05, ^^-P < 0.01; ^^^- P < 0.001)

Table 4.5

Effect of treatment with PG (2 mg/kg) on MPO and CK activity in rats with myocardial I/R injury.

Groups	MPO (units/mg of protein)	CK (U/L)
SO	0.23 ± 0.91	1033.67 ± 36.23
I/R-M	0.82 ± 0.04**	4490.54 ± 120.99***
I/R-F	0.80 ± 0.02**	4324.52 ± 228.97***
I/R-OVR	0.81 ± 0.10**	3648.00 ± 270.15***
I/R-M + PG	0.71 ± 0.26*	3507.00 ± 136.27***
I/R-F + PG	0.37 ± 0.02^	2059.83 ± 226.58^^^
I/R-OVR + PG	0.69 ± 0.05*	3057.83 ± 291.30***

All values are mean ± SEM (n=6). The groups treated with vehicle (I/R-M, I/R-F, I/R-OVR) were compared with sham operated (*-$P < 0.05$, **-$P < 0.01$, *** - $P < 0.001$) while groups treated with PG (I/R-M+PG, I/R-F+PG, I/R-OVR+PG) were compared with the I/R group (^-$P < 0.05$, ^^-$P < 0.01$; ^^^- $P < 0.001$)

4.1.5 Effect of Estrogen, Lipoic acid and their combination on myocardial I/R injury:

The effect of estrogen with various antioxidants was observed in this study. The protocol involved five groups- 1) sham operated vehicle treated group (SO), 2) vehicle treated reperfusion injury control group (I/R control), 3) Estrogen (8 µg/kg s.c. 24 h before induction of ischemia) treated reperfusion injury group (Estro), 4) Lipoic acid (100 mg/kg i.p.) receiving reperfusion injury group (Lipo) and 5) co-administration of Lipoic acid (Estro + Lipo) after estrogen injection to the animals with I/R injury. These groups were studied for the I/R injury experiment as described in section 3.2.1.1.2, 3.2.1.1.9 and 3.2.1.1.10 of the materials and methods.

4.1.5.1 Effects of Estrogen, Lipoic acid and their combination on hemodynamic and electrocardiographic parameters-

Biopac MP 30 system was used to study these parameters and the observations were analysed with Biopac Student Lab Pro software (Version 3.6.7).

The actual tracings of recordings showing changes in ECG parameters are shown as **Fig. 5.1 a to 5.1 f**

The time period of recording shown here are Baseline, End-Ischemia and End-Reperfusion.

The significant fall in systolic, diastolic and mean arterial blood pressure in I/R control animals subjected to ischemia was observed at End-ischemia period as compared to sham operated control (SO) ($P< 0.05$). The hemodynamic changes were not significantly different among the animals from the groups (I/R control, Lipo) subjected to ischemia (End-ischemia period) till reperfusion. At End-reperfusion period, Lipo, Estro and Estro + Lipo treated groups shown to have significant increase in MAP, with Lipo, Estro ($P< 0.05$) and Estro + Lipo ($P< 0.05$) treatment as compared to I/R control group. The maximum (+) and minimum (–) dp/dt was significantly higher in Estro and Estro+ Lipo treated groups. Estro + Lipo group showed significant ($P< 0.05$) change in values through out ischemia and reperfusion for dp/dt max. The (+) dp/dt in Estro and Estro + Lipo groups was significantly changed at End-Ischemia and was significantly ($P< 0.05$) higher than I/R control group at End-Reperfusion period. Animals in Estro + Lipo group exhibited significant change ($P< 0.05$) in minimum (-) d*p*/d*t* as compared to vehicle treated reperfusion injury (I/R control) group at End-reperfusion period. **Table 5.1 and 5.2**

There was a significant (*P*< 0.01) ST elevation and ST prolongation (*P*< 0.01) in all the groups (except Estro treated groups) subjected to ischemia as compared to sham operated control. However there was no significant difference in the groups either receiving vehicle and Lipo except at the end of reperfusion where Lipo, Estro and Estro + Lipo groups shown significant (*P*< 0.05) fall in ST elevation and duration of ST segment as compared to I/R control group. The incidences of VT were significantly (*P*<0.05) reduced after Estro and Lipo treatment. **Table 5.3 and Fig. 5.2 a to 5.2 c**

The changes in heart rate were not significant even at the time of reperfusion in the animals subjected to ischemia-reperfusion as compared to SO group.

4.1.5.2 Effects of Estrogen, Lipoic acid and their combination on Infarct area and Area At Risk (AAR) :

Estrogen treated group individually and in combination with Lipoic acid significantly (*P*<0.001) reduced the infarct area after I/R injury. Lipo alone treatment also restricted the development of infarct area significantly (*P*<0.001) as compared to I/R control group. There was no significant difference in AAR in treated groups except for Lipo and Estro+ Lipo group. **Fig. 5.3 a to 5.3 b**

4.1.5.3 Effects of Estrogen, Lipoic acid and their combination on Biomarkers of oxidative stress in myocardial I/R injury-

Endogenous antioxidants like reduced glutathione (GSH) and superoxide dismutase (SOD) were significantly reduced in I/R control group, whereas Estrogen and Lipoic acid treated animals exhibited significant increase in levels of all these parameters. Catalase activity was not showing any significant change at any stage. Elevated MDA level after I/R injury was significantly (*P*<0.001) reduced as compared to sham operated animals treatment of these agents. However, Estro alone group also showed significant change in antioxidant biomarkers after induction of I/R injury as compared to vehicle treated (I/R control) group. **Table- 5.4**

4.1.5.4 Effects of Estrogen, Lipoic acid and their combination on inflammatory reactivity and CK activity in I/R injury-

A significant (*P*<0.001) increase in MPO activity after I/R injury was observed in I/R control group as compared to SO animals. Estrogen treatment in combination with Lipoic acid showed significant (*P*<0.05) decrease in MPO activity after induction of I/R injury, whereas, Lipoic acid alone and in combination with Estro significantly ($P<0.01$, $P<0.001$) reduced CK activity as compared to I/R control group. CK activity in serum was significantly reduced in treated animals as compared to control animals after I/R injury. **Table 5.5**

4.1.5.5 Effects of Estrogen, Lipoic acid and their combination on Apoptotic cell death in myocardial I/R injury-

Vehicle treated I/R control animals showed marked evidences of DNA damage, whereas treatment with estrogen and Lipoic acid substantially reduced the apoptotic cell death indicated by reduced DNA fragmentation. **Fig. 5.4**

4.1.5.6 Effects of Estrogen, Lipoic acid and their combination on histological features-

I/R injury induced severe cellular damage, heavy neutrophil infiltration and necrosis in vehicle treated animals. Estrogen treatment with Lipoic acid considerably restored the normal cellular architecture and reduced the myocardial damage indicated by decreased edema and minimal neutrophil presence in the myofibrils. **Fig. 5.5**

Fig. 5.1

Recordings showing changes occurring in hemodynamic and Electrocardiographic (ECG) parameters after induction of ischemia-reperfusion in Lipoc acid, Estrogen and vehicle treated rats-

The figures represent electrocardiograph changes in the animals at various time intervals.

5.1 a. ECG Changes occurring before induction of ischemia in Estrogen treated group (Baseline)

5.1 b. ECG Changes occurring during ischemia in Estrogen treated group (End – Ischemia period)

5.1 c. ECG Changes occurring during reperfusion in Estrogen treated group (End – Reperfusion period)

5.1 d. ECG Changes occurring before induction of ischemia in Estrogen with Lipoic acid treated group (Baseline)

5.1 e. ECG Changes occurring a during ischemia in Estrogen with Lipoic acid treated group (End –Ischemia period)

5.1 f. ECG Changes occurring during reperfusion in Estrogen with Lipoic acid treated group (End –Reperfusion period)

Fig 5.1 a

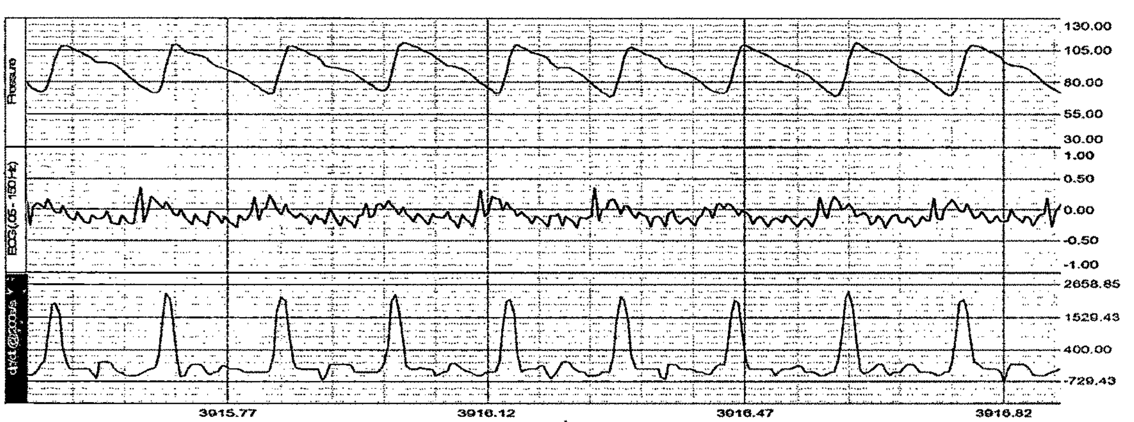


Fig 5.1 b

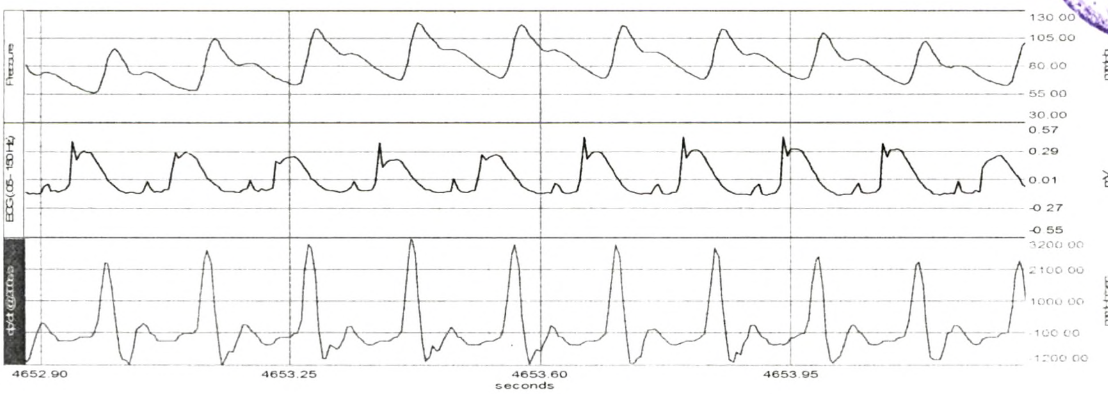


Fig 5.1 c

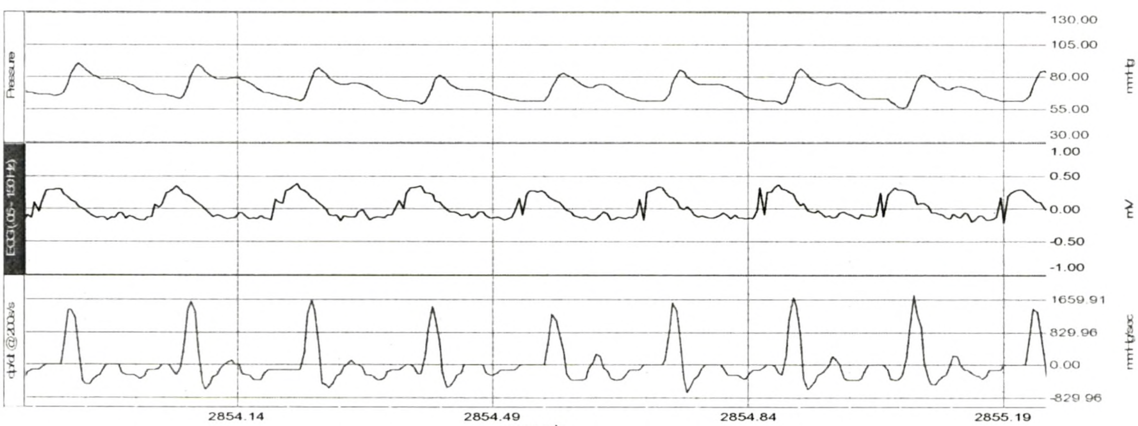


Fig 5.2 d

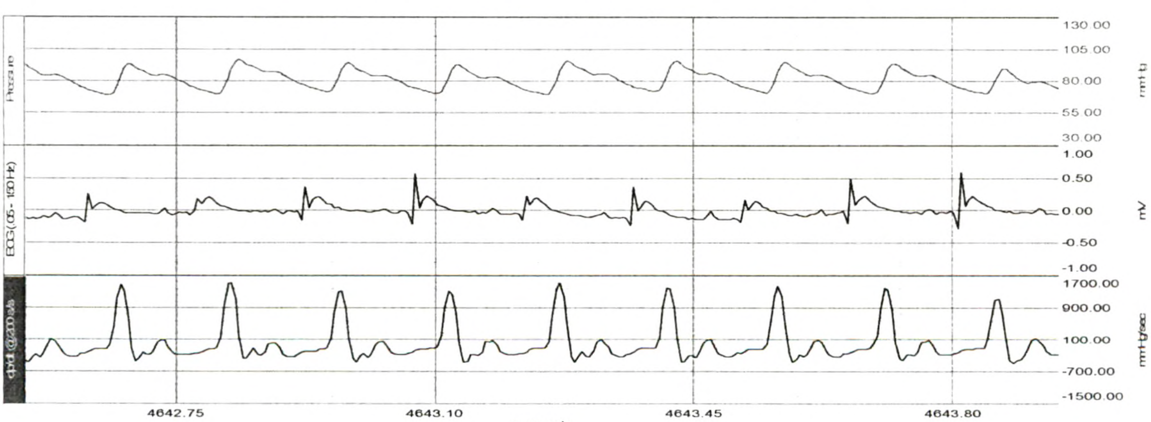


Fig 5.1 e

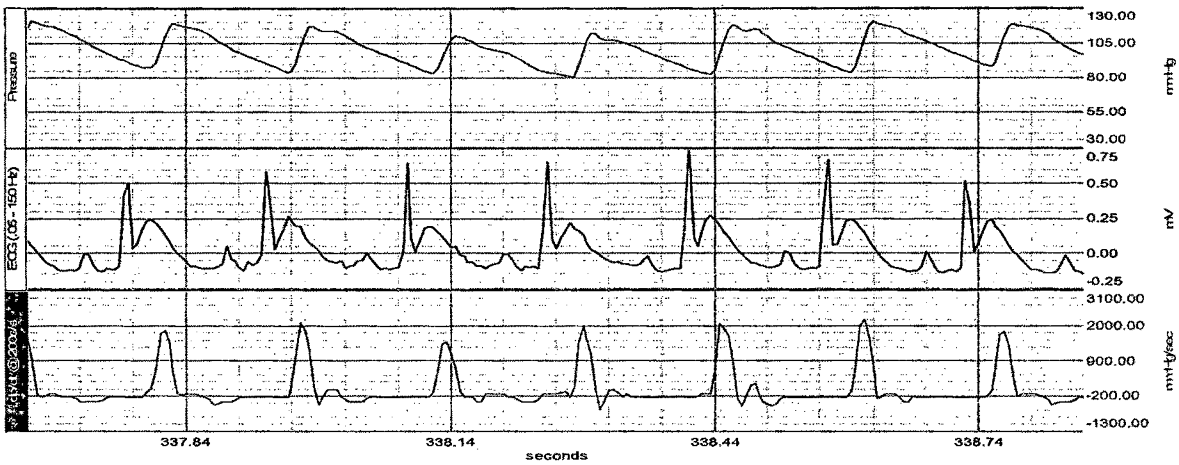


Fig 5.1 f

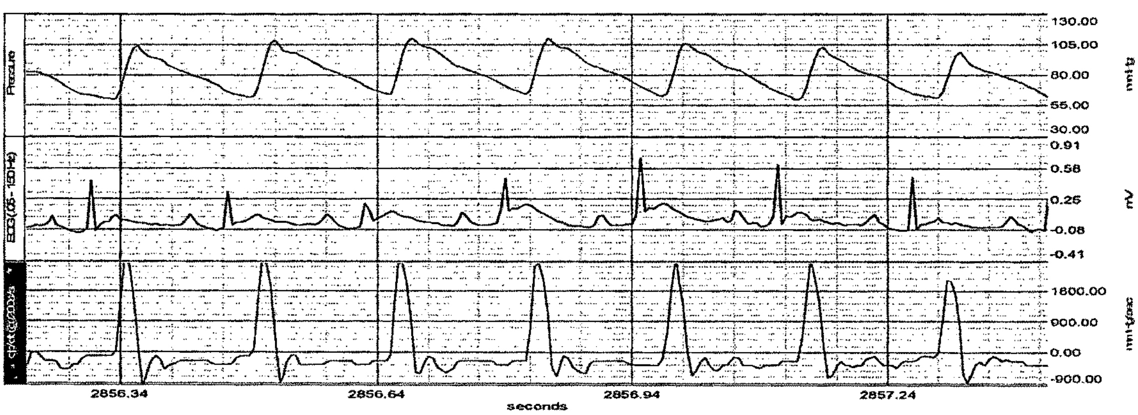


Fig. 5.2 a to c

Changes in Mean arterial blood pressure (MAP) and ST segment with respect to time of induction of ischemia and reperfusion in the animals during study.

1) **SO- Sham Operated animals (No ischemia-reperfusion) + vehicle,**
2) **I/R- Vehicle treated rats undergoing reperfusion injury(I/R control)**
3) **I/R+ESTRO – Estrogen treated rats subjected to reperfusion injury**
4) **I/R+ Lipo – Lipoic acid treated rats subjected to reperfusion injury**
5) **I/R+ESTRO+Lipo – Estrogen with Lipoic acid (Combined treatment) treated rats subjected to reperfusion injury**

Arrows indicates time of 1. Baseline (Before induction of ischemia), 2. End-Ischemia (30 min after induction of ischemia) and 3. End-Reperfusion (60 min after induction of ischemia)

5.2 a. Changes in mean blood pressure (mm Hg)

5.2 b. Changes in ST elevation (mv)

5.2 c. Changes in ST prolongation (mv/s)

All values are mean ± SEM (n=6). The groups treated with vehicle alone (I/R control), Estrogen, Lipoic acid and their combination were compared with Control (*-$P < 0.05$, **-$P < 0.01$, ***- $P < 0.001$) while groups treated with Estrogen, Lipoic acid and their Combination treatment were compared with the I/R control group (^- $P < 0.05$, ^^-$P < 0.01$; ^^^- $P < 0.001$)

Fig. 5.2 a. Changes in Mean arterial blood pressure with respect to time before and after ischemia/reperfusion

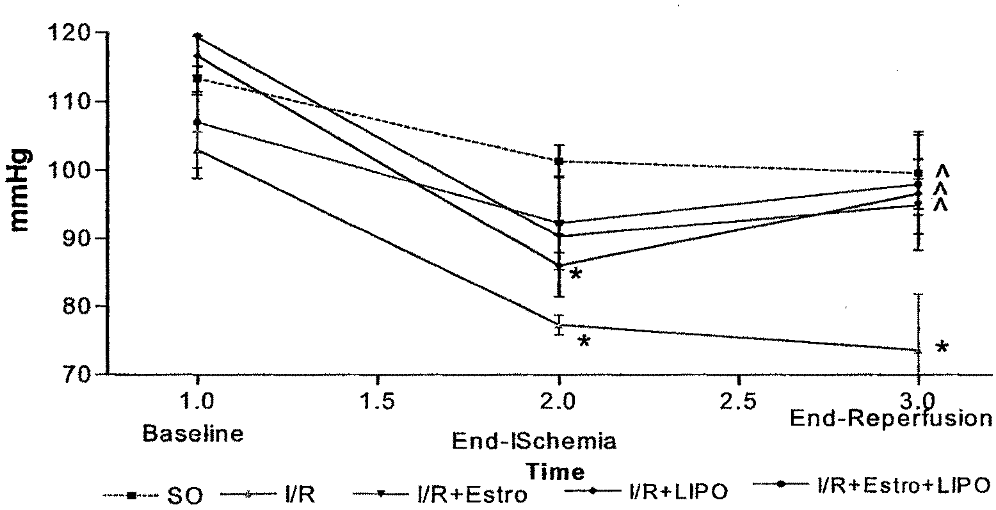


Fig. 5.2 b. Changes in ST segment elevation with respect to time, before and after ischemia/reperfusion

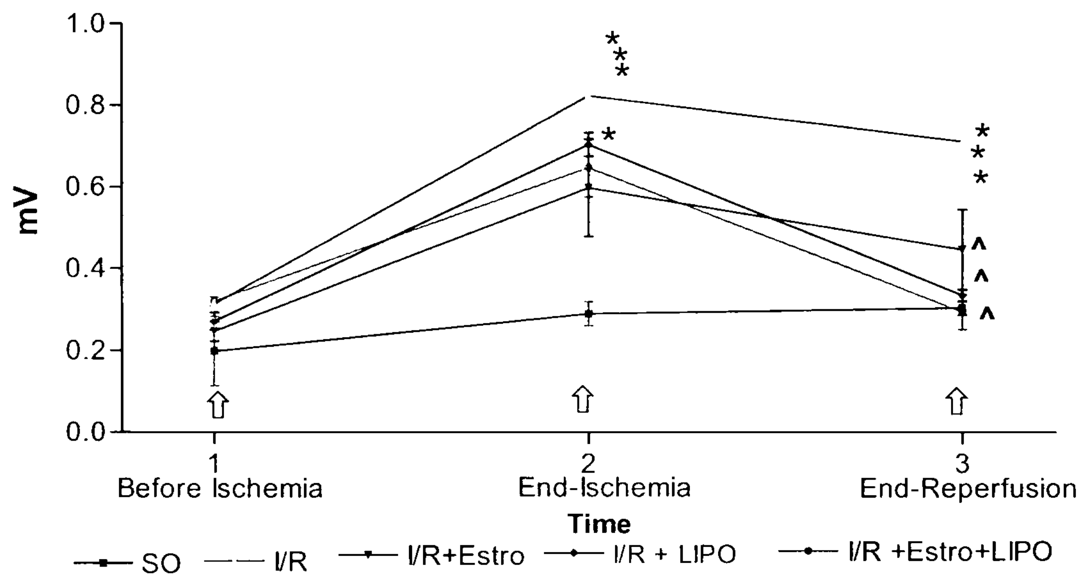


Fig. 5.2 c. Changes in ST segment prolongation with respect to time, before and after ischemia/reperfusion

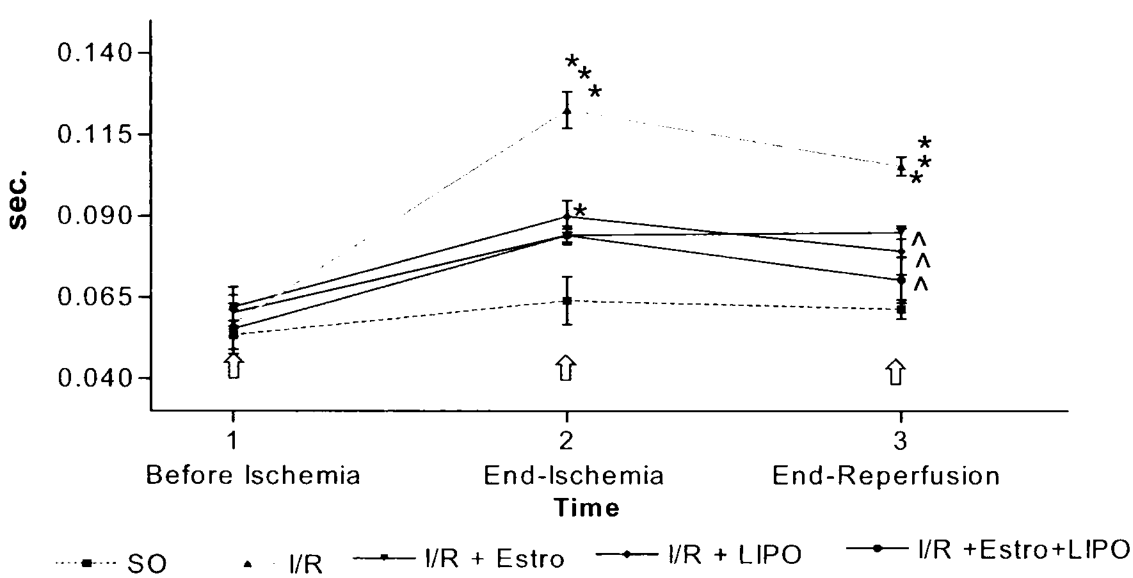


Fig. 5.3 a and 5.3 b

Estrogen, Lipoic acid and their combination on infarct area and Area At Risk (AAR) after myocardial I/R injury in rats.

5.3 a. Infarct area in percentage

5.3 b. Area at risk (AAR) in percentage

All values are mean ± SEM (n=6). The group treated with Estrogen, Lipoic acid and Combination treatment were compared with the I/R control group (^ - $P < 0.05$, ^^ -P < 0.01; ^^^- P < 0.001)

Fig. 5.3 a % Infarct area

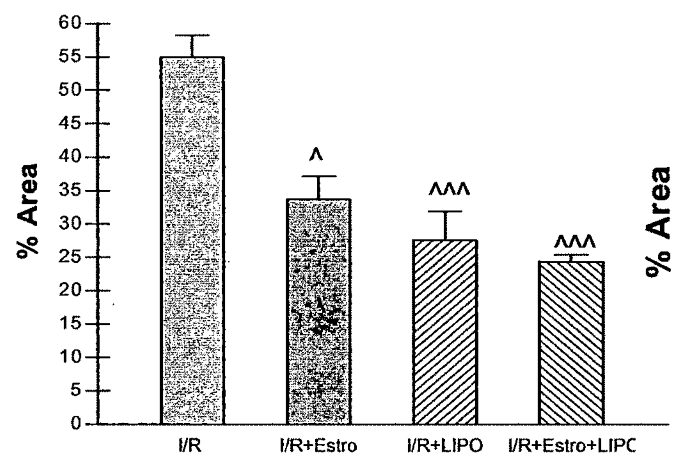
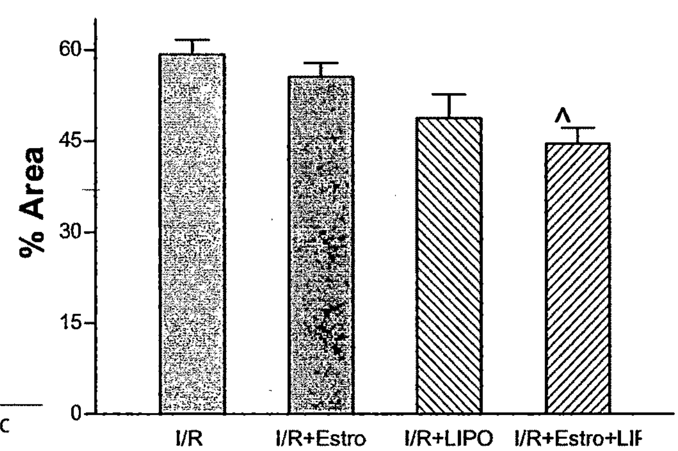


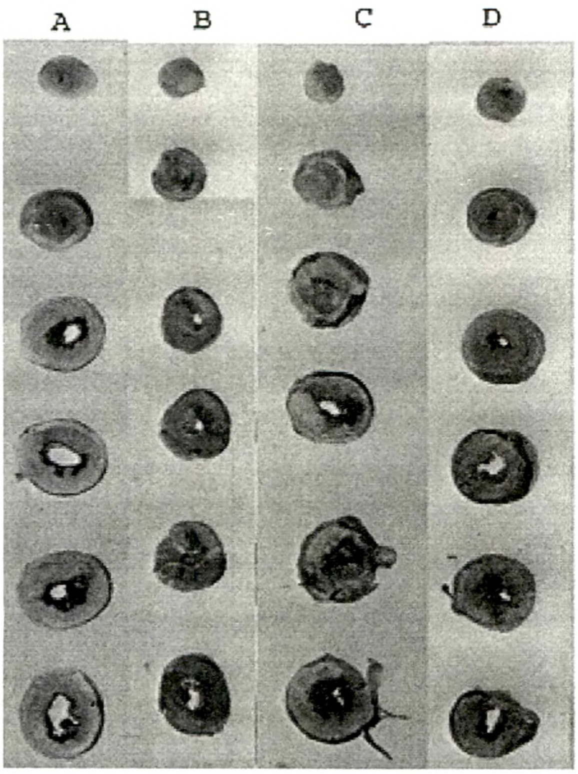
Fig. 5.3 b %AAR



SO- Sham Operated, I/R- Vehicle treated rats undergoing reperfusion injury

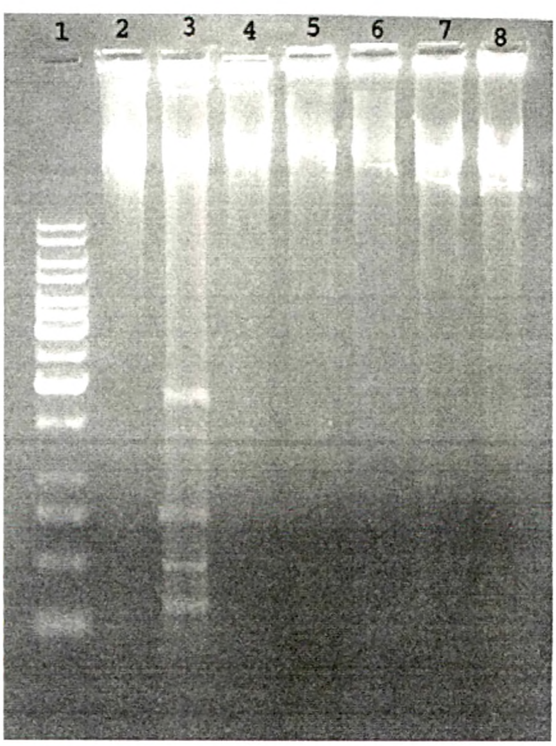
I/R+ESTRO- Estrogen treated, I/R+Lipo – Lipoic acid treated and I/R+ESTRO+Lipo-combination treated reperfused animals.

Fig. 5.3 C Infarct area in TTC stained slices:



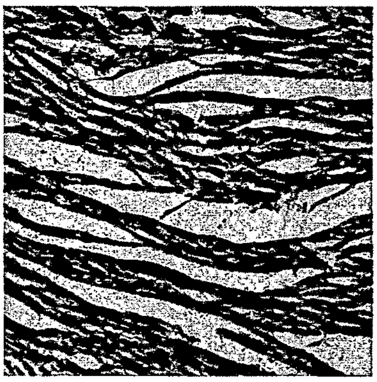
Lane A: Heart Slices of I/R control animals; **Lane B**: Heart Slices of Estrogen treated I/R animal; **Lane C**: Heart Slices of Lipoic acid treated I/R animals; and **Lane D**: Estrogen and Lipoic acid combination treated I/R animal.

Fig 5.4 Agarose gel electrophoresis of rat heart DNA after ischemia/reperfusion injury.

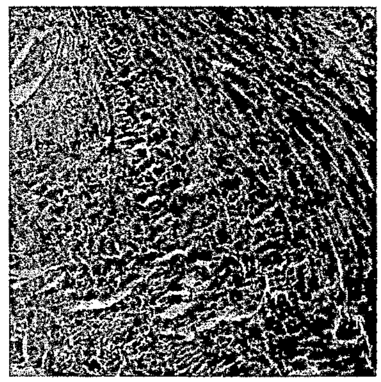


Lane 1, 1-kb DNA standard; **Lane 2**, Sham control; **Lane 3,** Ischemic control (I/R control) displaying a laddering pattern a indication of; **Lane 4,** Estrogen treated I/R (I/R + Estro) with out evident DNA laddering pattern; **Lane 5,** Lipoic acid treated I/R without evident DNA laddering pattern; **Lane 6,** Estro and Lipoic acid combination treated animal; **Lane 7,** CoQ treated animal showing no fragmentation; **Lane8,** Estro+CoQ treated animal no DNA fragmentation.

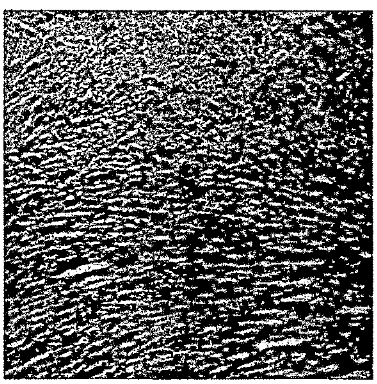
Fig 5.5 Photomicrographs (100X) of histological slides stained with H& E stain after ischemia/reperfusion injury.



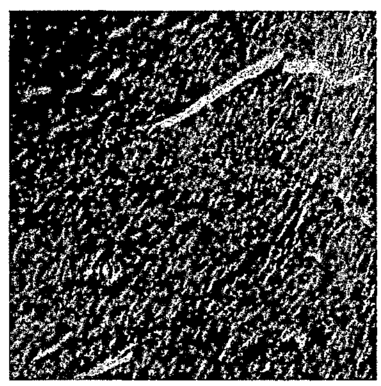
A



B



C



D

Photomicrographs (100X) of histological slides stained with H& E stain after I/R injury.

A: Vehicle treated I/R animal showing highly distorted and fragmented myofibril; **B:** Estrogen treated I/R animal showing few neurophils and relative recovery of myofibril damage; **C:** Lipoic acid treated I/R animals with improved cellular structures, continuity with adjacent myofibrils and minimum damage; **D**: Estro+LIPO treated I/R animal with reduced edema and improved cellular structure.

Table 5.1

The effect of Estrogen, Lipoic acid and their combination on ± d*p*/d*t* of rats with 1 h of ischemia and 1 h of reperfusion induced by left coronary descending artery (LCA) occlusion.

Group	+ d*p*/d*t*(mmHg/sec)			- d*p*/d*t*(mmHg/sec)		
	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)
SO	2402 ± 215	2668 ± 108	2567 ± 180	2015 ± 328	2053 ± 326	1958 ± 127
I/R control	2566 ± 166	1879 ± 148 *	1779 ± 102*	1811 ± 158	1146 ± 126*	1216 ± 93*
I/R + Estro	3357 ± 298	2475 ± 391	2891 ± 156^	1951 ± 238	1564 ± 316	1713 ± 286
I/R + Lipo	2623 ± 132	1951 ± 193	2090 ± 132	2002 ± 156	1655 ± 101	1791 ± 86
I/R+Estro+Lipo	2870 ± 172	2317 ± 247	2651 ± 254^	1358 ± 111	1136 ± 124	2054 ± 168^

All values are mean ± SEM (n=6). The groups treated with vehicle alone (I/R control), Estrogen, Lipoic acid and their combination were compared with Sham operated (SO) (*-P < 0.05, **-P < 0.01, *** - P < 0.001) while groups treated with Estrogen, Lipoic acid and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P < 0.01$, ^^^ - $P < 0.001$)

Table 5.2

The effect of Estrogen, Lipoic acid and their combination on heart rate (HR) and systolic blood pressure (SBP) of rats with 1 h of ischemia and 1 h of reperfusion induced by left coronary descending artery (LCA) occlusion.

Group	HR/min			SBP (mmHg)		
	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)
SO	419 ± 19.52	416 ± 12.05	405 ± 15.09	117.13 ± 4.46	106.66 ± 3.98	118.5 ± 2.58
I/R control	392 ± 18.21	325 ± 13.01*	308 ± 26.15*	110.52 ± 6.05	81.05 ± 3.56*	79.59 ± 5.98*
I/R + Estro	458 ± 45.24	333 ± 25.16*	303 ± 21.54*	107.24 ± 11.84	92.87 ± 13.11	98.12 ± 4.71
I/R + Lipo	404 ± 22.21	319 ± 15.29*	307 ± 10.66*	108.44 ± 4.48	85.83 ± 6.47	92.11 ± 5.13
I/R+Estro+Lipo	413 ± 31.14	387 ± 42.36	441 ± 36.65	100.21 ± 8.22	93.53 ± 4.83	94.36 ± 6.12

All values are mean ± SEM (n=6). The groups treated with vehicle alone (I/R control), Estrogen, Lipoic acid and their combination were compared with Sham operated (SO) (*-P < 0.05, **-P < 0.01, *** - P < 0.001) while groups treated with Estrogen, Lipoic acid and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P < 0.01$, ^^^ - $P < 0.001$)

Table 5.3

The effect of Estrogen, Lipoic acid and their combination on occurrence of ventricular tachycardia (VT) in rats with myocardial I/R injury

Group	Occurrence of VT	
	Ischemia period	Reperfusion period
I/R control	1/12	10/12
I/R + Estro	3/12^	3/12^
I/R+ Lipo	4/12^	3/12^
I/R+Estro+Lipo	4/12^	2/12^

All values are mean ± SEM (n=6). The groups treated with Estrogen, Lipoic acid and their combination were compared with the I/R group (^ $P < 0.05$, ^^-$P < 0.01$ ^^^- $P < 0.001$)

Table 5.4

Effect of treatment with Estrogen, Lipoic acid and their combination on oxidative stress markers in rats with myocardial I/R injury

Groups	SOD (IU/mg protein)	CAT H_2O_2 consu/min/mg of protein	GSH µg/mg of protein	MDA nmol /mg of protein
SO	12.89 ± 1.30	410.35 ± 68.61	9.06 ± 0.84	1.05 ± 0.12
I/R control	3.02 ± 0.49***	274.01 ±62.23	3.91 ± 0.32***	3.02 ± 0.33**
I/R+Estro	6.41 ±0.38***^	315.09 ± 69.49	7.12 ± 0.63^	1.66 ± 0.19^
I/R + Lipo	6.14 ± 0.41 ***^	234.23 ± 47.83	7.93 ± 0.64^	1.77 ± 0.13^
I/R+Estro +Lipo	7.24 ± 0.78***^	368.40 ± 71.76	7.52 ± 0.69^	1.37 ± 0.11^^

All values are mean ± SEM (n=6). The groups treated with vehicle alone (I/R control), Estrogen, Lipoic acid and their combination were compared with Sham operated (SO) (*- $P < 0.05$, **-$P < 0.01$, ***- $P < 0.001$) while groups treated with Estrogen, Lipoic acid and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P < 0.01$, ^^^- $P < 0.001$)

Table 5.5

Effect of treatment with Estrogen, Lipoic acid and their combination on MPO and CK activity in rats with myocardial I/R injury.

Groups	MPO units/mg of protein	CK (U/L)
SO	0.23 ± 0.91	1033.67 ± 36.23
I/R control	0.82 ± 0.04**	4490.54 ± 120.99***
I/R +Estro	0.45 ± 0.05^	2140.00 ± 326.62*^^^
I/R + Lipo	0.48 ± 0.02^	3260.83 ±187.58***^^
I/R+ Estro+Lipo	0.39 ± 0.03^	2091.93 ±308.30^^^

All values are mean ± SEM (n=6). The groups treated with vehicle alone (I/R control), Estrogen, Lipoic acid and their combination were compared with Sham operated (SO) (*- $P < 0.05$, **-$P < 0.01$, ***- $P < 0.001$) while groups treated with Estrogen, Lipoic acid and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P < 0.01$, ^^^- $P < 0.001$)

4.1.6 Effect of Estrogen, Coenzyme Q-10 (CoQ) and their combination on myocardial reperfusion injury:

1) Sham operated vehicle treated group (SO), 2) vehicle treated reperfusion injury control group (I/R control), 3) Estrogen (8 µg/kg s.c. 24 h before induction of ischemia) treated reperfusion injury group (Estro), 4) CoQ (100 mg/kg i.p.) administered to reperfusion injury group (CoQ) and 5) treatment of CoQ-10 after pre-administration of estrogen (Estro+CoQ) were studied in this myocardial I/R injury experiment. The details of the groups are described in section 3.2.1.1.3, 3.2.1.1.9 and 3.2.1.1.11 of materials and methods.

4.1.6.1 Effects of Estrogen, CoQ and their combination on hemodynamic and electrocardiographic parameters in I/R injury-

The changes in ECG parameters with respect to time of induction of ischemia and reperfusion are represented in **Fig. 6.1 a to 6.1 c.**

There was a significant (P<0.05) decrease in systolic, diastolic and mean arterial blood pressure in I/R control and CoQ treated groups subjected to ischemia at End-ischemia period in comparison to sham operated control (SO) group. At the first observational interval (End-ischemia period), CoQ treated group showed no significant change in hemodynamic parameters as compared to I/R control animals. At End-reperfusion period (second observational interval), MAP of treated (CoQ and Estro+CoQ) groups was increased but not significantly as compared to I/R control group. Only Estro alone treated group exhibited significant (P<0.05) increase in MAP at End-reperfusion interval. The change (+) and (–) dp/dt was not significantly altered in Estro, CoQ and Estro + CoQ treated groups as compared to I/R control group. **Table 6.1 & 6.2**

ST segment was significantly (P<0.001) elevated and prolonged in I/R control group subjected to ischemia as compared to sham operated control. Estro alone and Estro + CoQ group showed the changes in ST segment but these were not significant as compared to SO group. At the end of both the intervals (End-ischemia and End-reperfusion), there was no significant difference between the groups receiving vehicle and CoQ for ST elevation and duration of ST segment as compared to I/R control group. However, Estro alone group showed significant (P<0.05) alterations in ST segment as compared to I/R control group.(**Fig. 6.2 a to 6.2 c**)There was a significant difference in VT incidences in the treated group as compare to the I/R control group. **Table 6.3**

The changes in heart rate were not significant even at the time of reperfusion in the animals subjected to ischemia-reperfusion as compared to SO group.

4.1.6.2 Effects of Estrogen, CoQ and their combination on Infarct size and Area At Risk (AAR) in myocardial I/R injury -

Infarct sizes of the treated animals were measured using TTC staining Technique. AAR for Estro, CoQ and Estro+CoQ groups were 44.49 ± 4.09, 41.19± 2.20 and 44.39 ± 5.43 % respectively.

The CoQ treatment led to significant ($P<0.001$) decrease in infarct size as compared to I/R control group, moreover, the change was statistically significant ($P<0.001$) PG +CoQ treated group. The infarct sizes in I/R, Estro, CoQ and Estro+CoQ groups were 54.98 ± 3.28, 32.08 ± 3.10, 29.02 ± 3.60 and 29.34 ± 4.28 % respectively. **(Fig. 6.3 a to 6.3 b)**

4.1.6.3 Effects of Estrogen, CoQ and their combination on Oxidative stress biomarkers in myocardial I/R injury -

There was a depletion of GSH and SOD caused by I/R injury in the I/R control group. MDA levels were significantly elevated ($P<0.001$) as compared to sham-operated animals. Estrogen, CoQ and their combination significantly ($P<0.01$) altered the activity of SOD. GSH levels were significantly ($P<0.01$) elevated in treated groups and this treatment led to significant ($P<0.01$) reduction in MDA level as compared to I/R control group. CAT activity was not significantly different in any of the treated group as compared to I/R control animals. **Table-6.4**

4.1.6.4 Effects of Estrogen, CoQ and their combination on inflammatory reactivity and CK activity in myocardial I/R injury -

MPO activity was significantly ($P<0.05$) decreased by estrogen and CoQ treatment. CK levels in serum significantly ($P<0.001$) depleted in treated groups as compared to I/R control group. **Table 6.5**

4.1.6.5 Effects of Estrogen, CoQ and their combination on Apoptosis in myocardial I/R injury -

Apoptotic activity was observed by internucleosomal DNA fragmentation. Vehicle treated I/R control animals showed marked evidences of DNA damage, whereas

Estrogen and CoQ treatment considerably reduced the intensity of the band formation. **Fig. 5.4**

4.1.6.6 Effects of Estrogen, CoQ and their combination on histological features-

Damage caused by I/R injury was indicated by neutrophil infiltration and disturbed architecture of the myocytes in the vehicle treated I/R control animals. This damage was markedly reduced by Estrogen and CoQ treatment and overall improvement of the cellular structure was observed. **Fig. 6.4**

Fig. 6.1

Recordings showing changes occurring in hemodynamic and Electrocardiographic (ECG) parameters after induction of ischemia-reperfusion in CoQ, Estrogen and vehicle treated rats-

The figures represent electrocardiograph changes in the animals at various time intervals.

6.1 a. ECG Changes occurring before induction of ischemia in Estrogen with CoQ treated group (Baseline)

6.1 b. ECG Changes occurring a during ischemia in Estrogen with CoQ treated group (End –Ischemia period)

6.1 c. ECG Changes occurring during reperfusion in Estrogen with CoQ treated group (End –Reperfusion period)

Fig 6.1 a

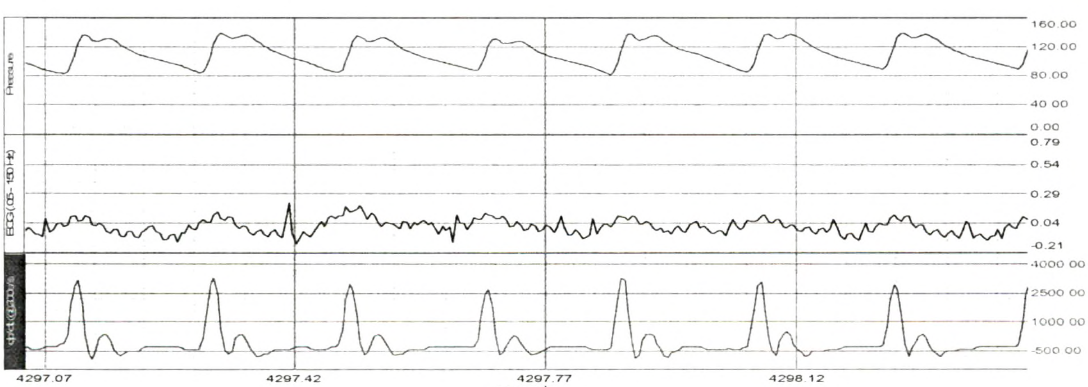


Fig 6.1 b

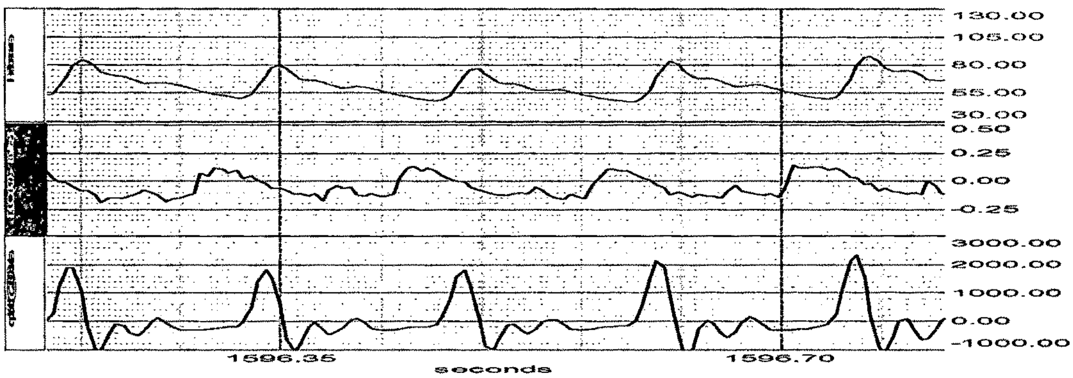


Fig 6.1 c

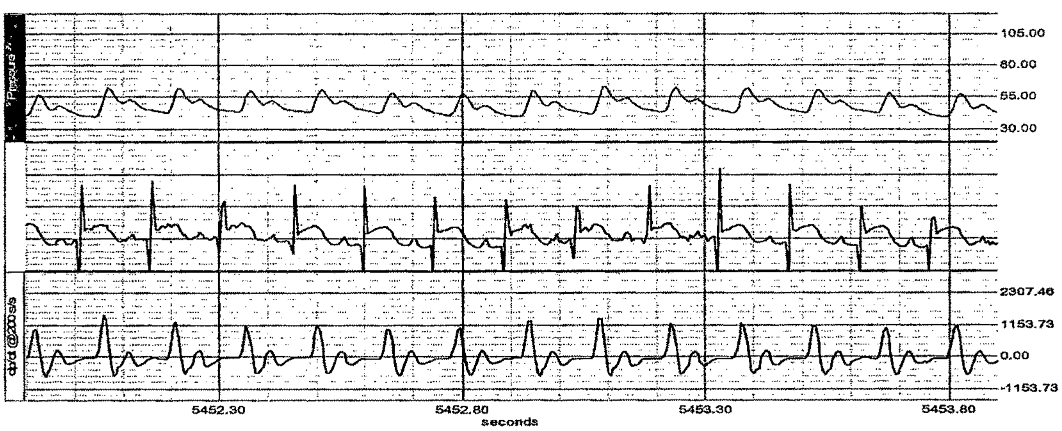


Fig. 6.2 a to c

Changes in Mean arterial blood pressure (MAP) and ST segment with respect to time of induction of ischemia and reperfusion in the animals during study.

1) **SO- Sham Operated animals (No ischemia-reperfusion) + vehicle,**
2) **I/R- Vehicle treated rats undergoing reperfusion injury(I/R control)**
3) **I/R+Estro – Estrogen treated rats subjected to reperfusion injury**
4) **I/R+ CoQ – CoQ treated rats subjected to reperfusion injury**
5) **I/R+Estro+CoQ – Estrogen with CoQ (Combined treatment) treated rats subjected to reperfusion injury**

Arrows indicates time of 1. Baseline (Before induction of ischemia), 2. End-Ischemia (30 min after induction of ischemia) and 3. End-Reperfusion (60 min after induction of ischemia)

6.2 a. Changes in mean blood pressure (mm Hg)

6.2 b. Changes in ST elevation (mv)

6.2 c. Changes in ST prolongation (mv/s)

All values are mean ± SEM (n=6). The group treated with vehicle alone (I/R control), Estrogen, Lipoic acid and their combination were compared with Sham operated group (*-$P < 0.05$, **-$P < 0.01$, *** - $P < 0.001$) while groups treated with Estrogen, CoQ and their Combination treatment were compared with the I/R group (^- $P < 0.05$, ^^-$P < 0.01$; ^^^- $P < 0.001$)

Fig. 6.2 a. Changes in Mean arterial blood pressure with respect to time before and after ischemia/reperfusion

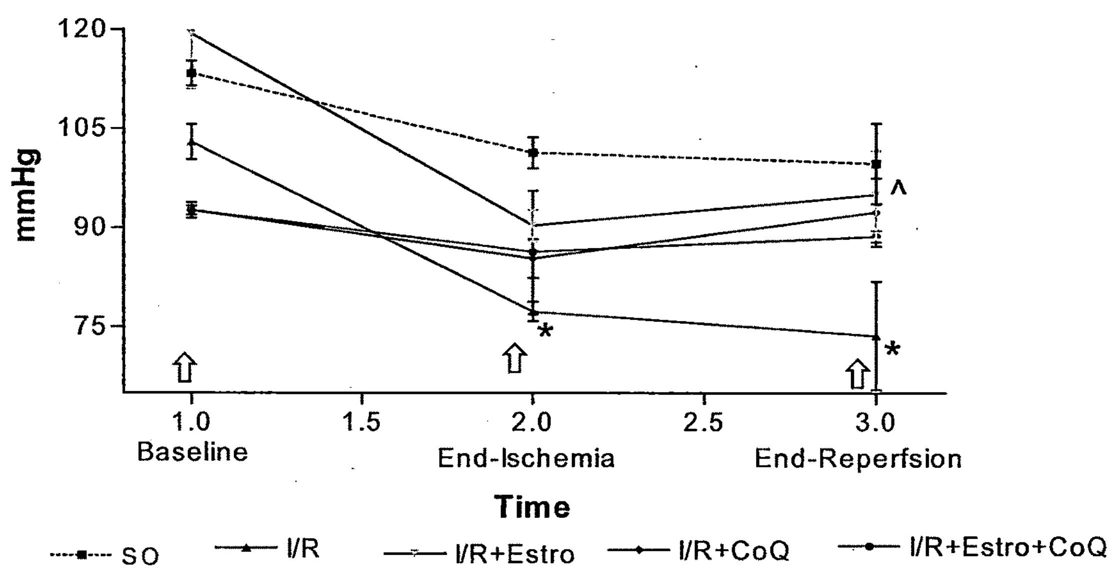


Fig. 6.2 b. Changes in ST segment elevation with respect to time, before and after ischemia/reperfusion

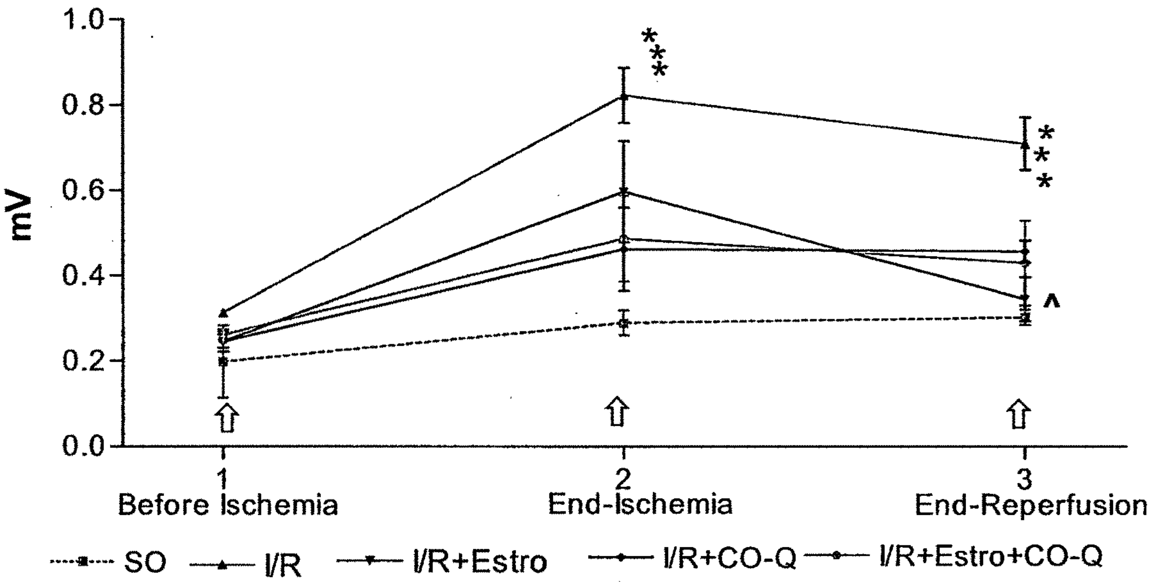


Fig. 6.2 c. Changes in ST segment prolongation with respect to time, before and after ischemia/reperfusion

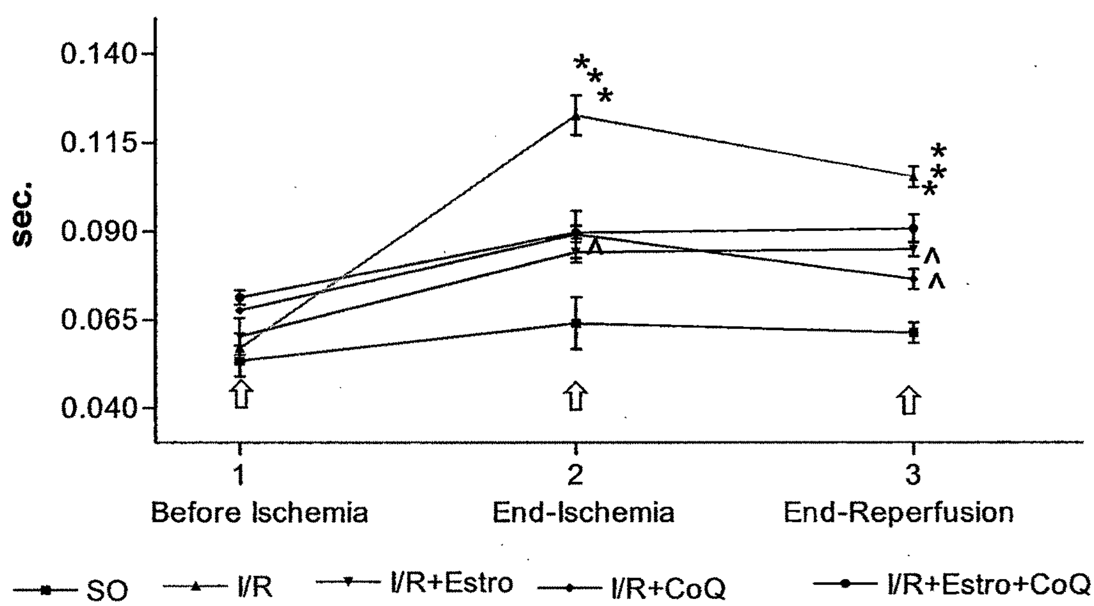


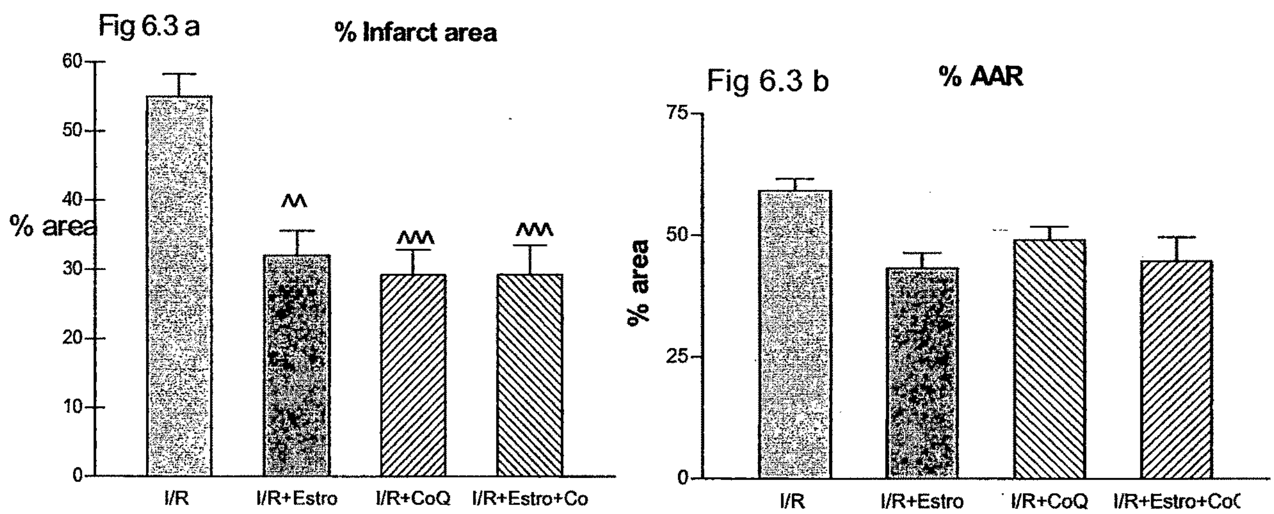
Fig. 6.3 a and 6.3 b

Effect of Estrogen, Lipoic acid and their combination on infarct area and area at risk (AAR) after myocardial I/R injury in rats.

6.3 a. Infarct area in percentage

6.3 b. Area at risk (AAR) in percentage

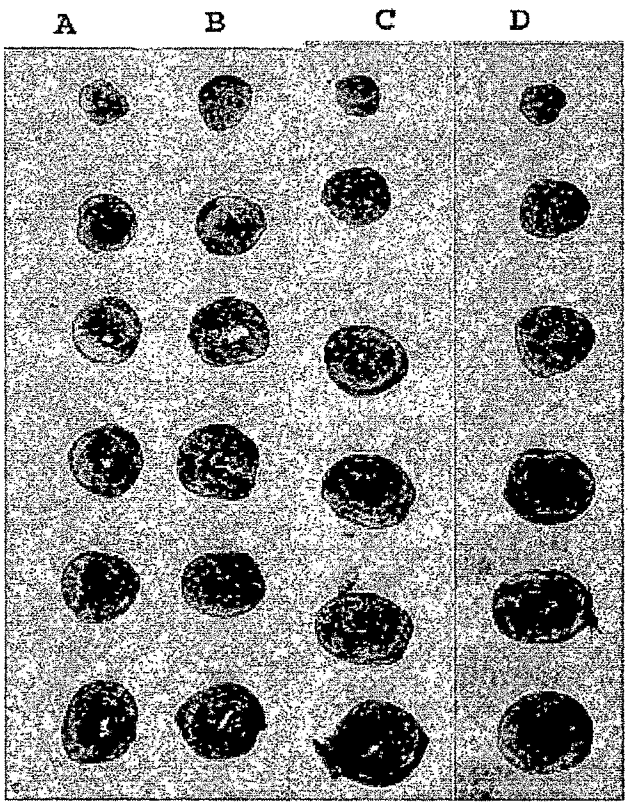
All values are mean ± SEM (n=6). The groups treated with Estrogen, Lipoic acid and their combination were compared with the I/R control group (^ - $P < 0.05$, ^^ -P < 0.01; ^^^- P < 0.001)



SO- Sham Operated, I/R- Vehicle treated rats undergoing reperfusion injury

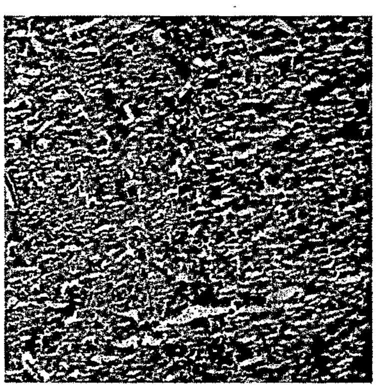
I/R+Estro - Estrogen treated, I/R+CoQ – CoQ treated and I/R+Estro +CoQ- combination treated reperfused animals.

Fig 6.3 c : Infarct area in TTC stained slices.

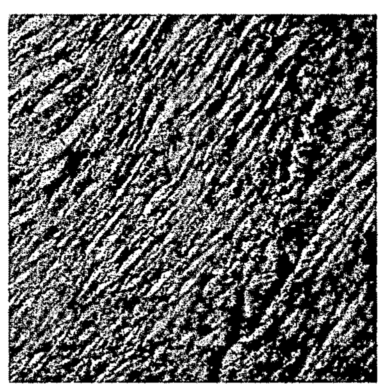


Lane A: Heart Slices of I/R control animals; **Lane B**: Heart Slices of Estrogen treated I/R animal; **Lane C**: Heart Slices of CoQ treated I/R animals; and **Lane D**: Estrogen and CoQ combination treated I/R animal.

Fig 6.4 Photomicrographs (100X) of histological slides stained with H& E stain after ischemia/reperfusion injury.



A



B

A: CoQ treated I/R animal showing neutrophils but improvement in overall structure; **B:** Estro+ CoQ treated I/R animals with few edema cells and striated myofibrils, however the distortion of myofibrils is reduced.

Table 6.1

The effect of Estrogen, CoQ and their combination on ± d*p*/d*t* of rats with 1 h of ischemia and 1 h of reperfusion induced by left coronary descending artery (LCA) occlusion.

Group	+ d*p*/d*t*(mmHg/sec)			- d*p*/d*t*(mmHg/sec)		
	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)
SO	2402 ± 215	2668 ± 108	2567 ± 180	2015 ± 328	2053 ± 326	1958 ± 127
I/R control	2566 ± 166	1879 ± 148 *	1779 ± 102*	1811 ± 158	1146 ± 126*	1216 ± 93*
I/R + Estro	3357 ± 298	2475 ± 391	2891 ± 156^	1951 ± 238	1564 ± 316	1713 ± 286
I/R + CoQ	2109 ± 192	1798 ± 247*	1894 ± 338*	1742 ± 56	1255 ± 191*	1493 ± 281*
I/R+Estro+CoQ	3179 ± 114	2221 ± 113	2427 ± 364	1578 ± 451	1391 ± 135	1454 ± 126

All values are mean ± SEM (n=6). The group treated with vehicle alone (I/R control), Estrogen, CoQ and their combination were compared with Sham operated group (SO) (*-P < 0.05, **-P < 0.01, *** - P < 0.001) while groups treated with Estrogen, CoQ and their combination were compared with the I/R group (^- *P* < 0.05, ^^- *P*< 0.01, ^^^ - *P*< 0.001)

Table 6.2

The effect of Estrogen, CoQ and their combination on heart rate (HR) and systolic blood pressure (SBP) of rats with 1 h of ischemia and 1 h of reperfusion induced by left coronary descending artery (LCA) occlusion.

Group	HR (min^{-1})			SBP (mmHg)		
	Baseline	End-ischemia	End-reperfusion	Baseline	End-ischemia	End-reperfusion
SO	419 ± 19.52	416 ± 12.05	405 ± 15.09	117.13 ± 4.46	106.66 ± 3.98	118.5 ± 2.58
I/R control	392 ± 18.21	325 ± 13.01*	308 ± 26.15*	110.52 ± 6.05	81.05 ± 3.56*	79.59 ± 5.98*
I/R + Estro	458 ± 45.24	333 ± 25.16*	303 ± 21.54*	107.24 ± 11.84	92.87 ± 13.11	98.12 ± 4.71
I/R + CoQ	367 ± 52.21	348 ± 25.29*	287 ± 29.66*	111.54 ± 2.48	82.23 ± 8.47*	89.17 ± 6.13*
I/R+Estro+CoQ	447 ± 43.22	371 ± 32.96	401 ± 59.65	109.51 ± 11.82	89.83 ± 9.71	91.66 ± 12.12

All values are mean ± SEM (n=6). The group treated with vehicle alone (I/R control), Estrogen, CoQ and their combination were compared with Sham operated group (SO) (*-P < 0.05, **-P < 0.01, *** - P < 0.001) while groups treated with Estrogen, CoQ and their combination were compared with the I/R group (^- P < 0.05, ^^- P< 0.01, ^^^ - P< 0.001)

Table 6.3

The effect of Estrogen, CoQ and their combination on occurrence of ventricular tachycardia (VT) in rats with myocardial I/R injury

Group	Occurrence of VT	
	Ischemia period	Reperfusion period
I/R control	11/12	10/12
I/R + Estro	3/12^	3/12^
I/R+ CoQ	5/12^	4/12^
I/R+Estro+CoQ	5/12^	3/12^

All values are mean ± SEM (n=6). The group treated with Estrogen, CoQ and their combination and CoQ were compared with the I/R group (^ P < 0.05, ^^-P < 0.01 ^^^ - P < 0.001)

Table 6.4

Effect of treatment with Estrogen, CoQ and their combination on oxidative stress markers in rats with myocardial I/R injury

Groups	SOD (IU/mg of protein)	CAT H_2O_2 consu/min/mg of protein	GSH µg/mg of protein	MDA nmol /mg of protein
SO	12.89 ± 1.30	410.35 ± 68.61	9.06 ± 0.84	1.05 ± 0.12
I/R control	3.02 ± 0.49***	274.01 ±62.23	3.91 ± 0.32***	3.02 ± 0.33 **
I/R+Estro	6.41 ±0.38***^	315.09 ± 69.49	7.12 ± 0.63^	1.66 ± 0.19^
I/R + CoQ	7.87 ± 0.82**^^	344.51 ± 33.53	7.14 ± 0.61^	1.21 ± 0.47^^
I/R+Estro+CoQ	6.99 ± 0.89***^	395.70 ± 128.16	6.81 ± 0.21^	1.46 ± 0.31^^

All values are mean ± SEM (n=6). The group treated with vehicle alone (I/R control), Estrogen, CoQ and their combination were compared with Sham operated group (SO) (*-P < 0.05, **-P < 0.01, *** - P < 0.001) while groups treated with Estrogen, CoQ and their combination were compared with the I/R group (^- *P* < 0.05, ^^- *P*< 0.01, ^^^ - *P*< 0.001)

Table 6.5

Effect of treatment with Estrogen, Lipoic acid and their combination on MPO and CK activity in rats with myocardial I/R injury.

Groups	MPO units/mg of protein	CK (U/L)
SO	0.23 ± 0.91	1033.67 ± 36.23
I/R control	0.82 ± 0.04**	4490.54 ± 120.99***
I/R +Estro	0.45 ± 0.05^	2140.00 ± 326.62*^^^
I/R + CoQ	0.46 ± 0.12^	2870.66 ± 115.19***^^^
I/R+ Estro+CoQ	0.46 ± 0.03^	2694.93 ± 436.21^^^

All values are mean ± SEM (n=6). The groups treated with vehicle alone (I/R control), Estrogen, Lipoic acid and their combination were compared with Sham operated (SO) (*-P < 0.05, **-P < 0.01, *** - P < 0.001) while groups treated with Estrogen, Lipoic acid and their combination were compared with the I/R control group (^- *P* < 0.05, ^^- *P*< 0.01, ^^^ - *P*< 0.001)

4.1.7 Effect of Estrogen, Banaba and their combination on myocardial reperfusion injury:

Banaba (100mg/kg i.p.) was administered 30 min after of induction of ischemia. Other groups involved in this study were, 1) vehicle treated Sham operated (SO) group of animals, 2) vehicle treated ischemic control (I/R control), 3) Estrogen (8 µg/kg s.c. 24 h before induction of ischemia) treated reperfusion injury group (Estro), 4) Banaba treated animals and 5) the treatment with the combination of Estrogen and Banaba (Estro+BNA) were studied in this protocol of the myocardial reperfusion injury. The details of he groups are given in section 3.2.1.1.9, 3.2.1.1.4 and 3.2.1.1.12 of materials and methods.

4.1.7.1 Effects of Estrogen, Banaba and their combination on hemodynamic and electrocardiographic parameters in myocardial reperfusion injury -

Three observational intervals were followed for ECG observationsviz., Baseline, End-Ischemia and End-Reperfusion. **(Fig.7.1 a to 7.1c)**

The treatment with Banaba and Estrogen in combination caused significant ($P<0.05$) improvement in MAP after initiation of reperfusion. The changes in hemodynamic parameters observed in other treated groups were not significant as compared to I/R control group as at both End-Ischemia and End-Reperfusion intervals. The changes in (±) dp/dt were significantly altered only in Estro+BNA and Estro treated groups as compared to I/R control group. **Table 7.1, 7.2 and Fig. 7.2 a to 7.2 c.**

I/R induced ST elevation and ST prolongation were significantly ($P<0.05$) change in the groups receiving Banaba, Estro and Estro with Banaba (Estro+BNA) at the End-ischemia period and ($P<0.001$) at End-reperfusion as compared to I/R control group. Incidences of VT were significantly reduced in Banaba and Estro treated group in comparison to I/R control animals. Incidences of VT in treated group were significantly ($P<0.05$) reduced. Changes in heart rate were not significant. **Table 7.3**

4.1.7.2 Effects of Estrogen, Banaba and their combination on Infarct size and Area At Risk in myocardial I/R injury -

The AAR was not significantly different among the groups except Estro+BNA which showed significant ($P<0.01$) reduction in AAR as compared to I/R group. AAR for BNA, Estro and Estro+BNA groups was 44.91 ± 3.23, 44.49 ± 4.09 and 36.11 ±6.18 % respectively.

Banaba and Estrogen treatment caused significant (P<0.001) decrease in infarct size as compared to I/R control group. The infarct sizes of I/R, Estro, BNA and Estro+BNA groups were 54.98 ± 3.59, 32.08 ± 3.10, 25.00 ± 1.33 and 22.29 ± 1.46 % respectively. **(Fig.7.3 a to 7.3 b)**

4.1.7.3 Effects of Estrogen, Banaba and their combination on Oxidative stress biomarkers in myocardial reperfusion injury -

Estrogen and Banaba treatment significantly (P<0.05, P<0.001) increased the activity of SOD in heart. GSH level was significantly (P<0.05) restored after treatment in comparison to I/R control group. There was a significant (P<0.05, P<0.01) increase in GSH level after BNA and Estro treatment. Activity of catalase enzyme was not significantly different in any of the treated group as compared to I/R animals. **Table-7.4**

4.1.7.4 Effects of Estrogen, Banaba and their combination on MPO and CK activity in myocardial reperfusion injury -

MPO activity was significantly (P<0.01) reduced by Estrogen and Banaba treatment as compared to the elevated levels recorded in I/R group. Similarly, CK serum activity significantly (P<0.01) reduced by the treatment of I/R animals with Estrogen and Banaba. **Table 7.5**

4.1.7.5 Effects of Estrogen, Banaba and their combination on Apoptosis in myocardial reperfusion injury -

The interneucleosomal band formation was reduced by Banaba, estrogen and co-administration of both (Estro+BNA) as observed form the photographs. **Fig. 7.4**

4.1.7.6 Effects of Estrogen, Banaba and their combination on histological features-

The histologial observations of the treated animals showed marked reduction in the characteristic damage after I/R injury. The neutophil presence and development of edema was restricted by the treatment of Banaba and Estrogen. **Fig. 7.5**

Fig. 7.1

Recordings showing changes occurring in hemodynamic and Electrocardiographic (ECG) parameters after induction of ischemia-reperfusion in Banaba, Estrogen and vehicle treated rats-

The figures represent electrocardiograph changes in the animals at various time intervals.

7.1 a. ECG Changes occurring before induction of ischemia in Estrogen with Banaba treated group (Baseline)

7.1 b. ECG Changes occurring a during ischemia in Estrogen with Banaba treated group (End –Ischemia period)

7.1 c. ECG Changes occurring during reperfusion in Estrogen with Banaba treated group (End –Reperfusion period)

Fig 7.1 a

Fig 7.1 b

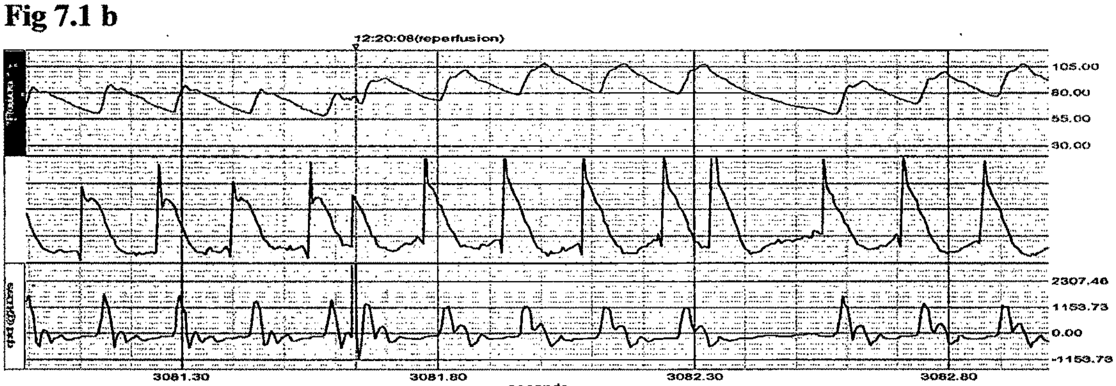


Fig 7.3 c

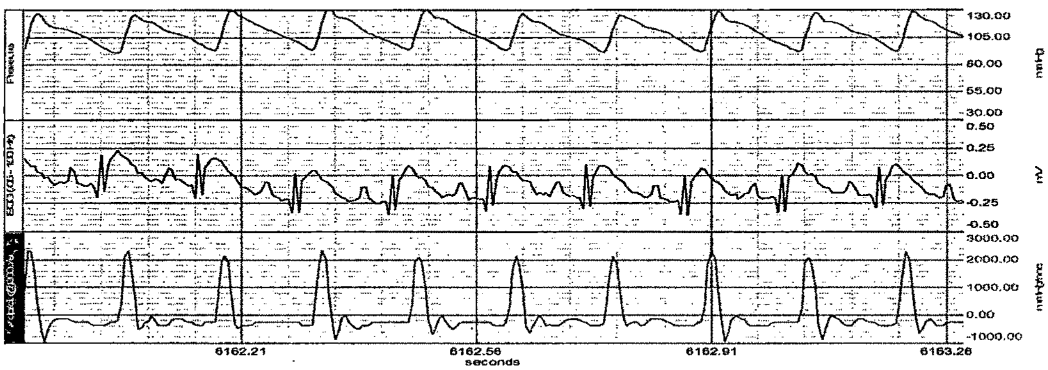

130.00
105.00
80.00
55.00
30.00
0.50
0.25
0.00
-0.25
-0.50
3000.00
2000.00
1000.00
0.00
-1000.00
6162.21
6162.56
seconds
6162.91
6163.26

Fig. 7.2 a to 7.2 c

Changes in Mean arterial blood pressure (MAP) and ST segment with respect to time of induction of ischemia and reperfusion in the animals during study.

1) **SO- Sham Operated animals (No ischemia-reperfusion) + vehicle,**
2) **I/R- Vehicle treated rats undergoing reperfusion injury(I/R control)**
3) **I/R+ESTRO – Estrogen treated rats subjected to reperfusion injury**
4) **I/R+ BNA – Banaba treated rats subjected to reperfusion injury**
5) **I/R+ESTRO+BNA – Estrogen with Banaba (Combined treatment) treated rats subjected to reperfusion injury**

Arrows indicates time of 1. Baseline (Before induction of ischemia), 2. End-Ischemia (30 min after induction of ischemia) and 3. End-Reperfusion (60 min after induction of ischemia)

7.2 a. Changes in mean blood pressure (mm Hg)

7.2 b. Changes in ST elevation (mv)

7.2 c. Changes in ST prolongation (mv/s)

All values are mean ± SEM (n=6). The group treated with vehicle alone (I/R control), Estrogen, Banaba and their combination were compared with sham operated (*-$P < 0.05$, **-$P < 0.01$, *** - $P < 0.001$) while groups treated Estrogen, Banaba and their combination treatment were compared with the I/R control group (^- $P < 0.05$, ^^-$P < 0.01$; ^^^- $P < 0.001$)

Fig.7.2 a. Changes in Mean arterial blood pressure with respect to time before and after ischemia/reperfusion

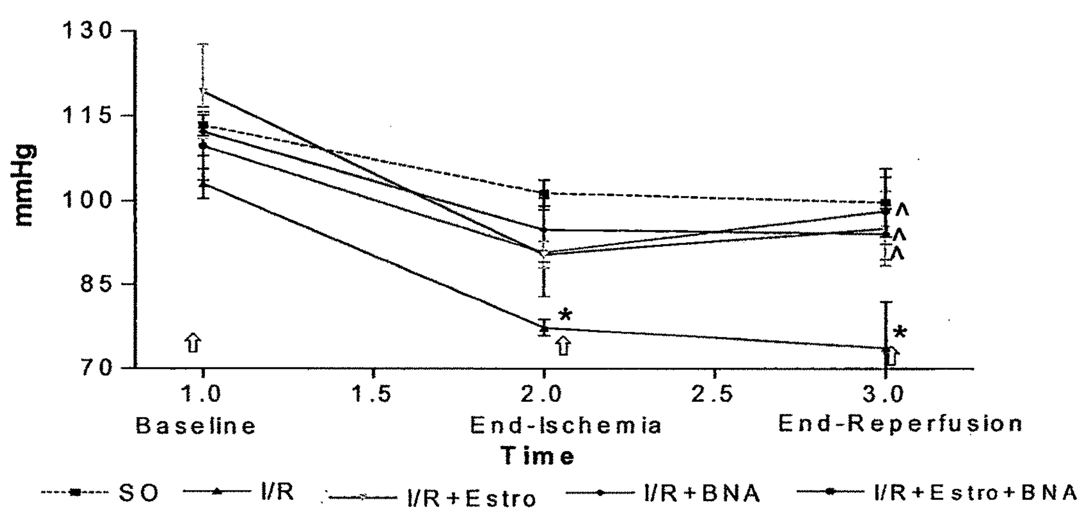


Fig. 7.2 b. Changes in ST segment elevation with respect to time, before and after ischemia/reperfusion

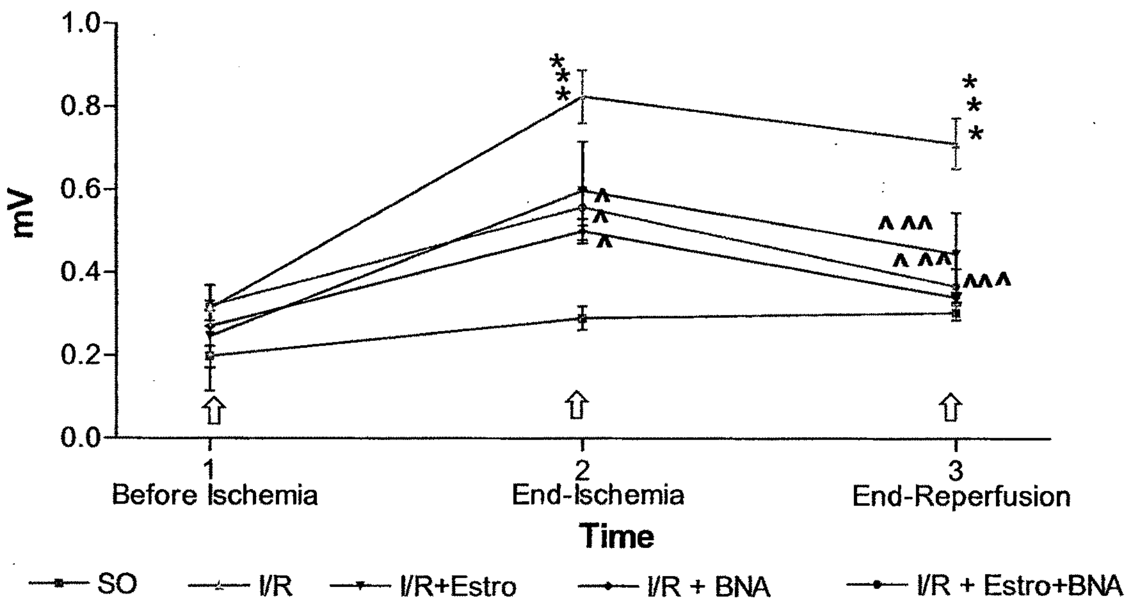


Fig. 7.2 c. Changes in ST segment prolongation with respect to time, before and after ischemia/reperfusion

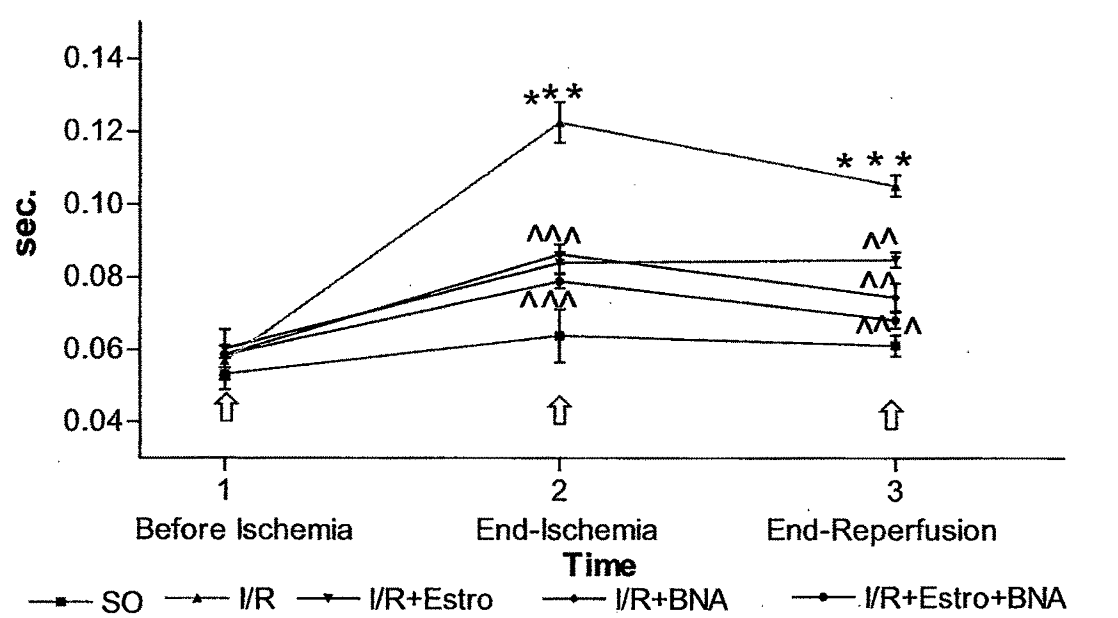


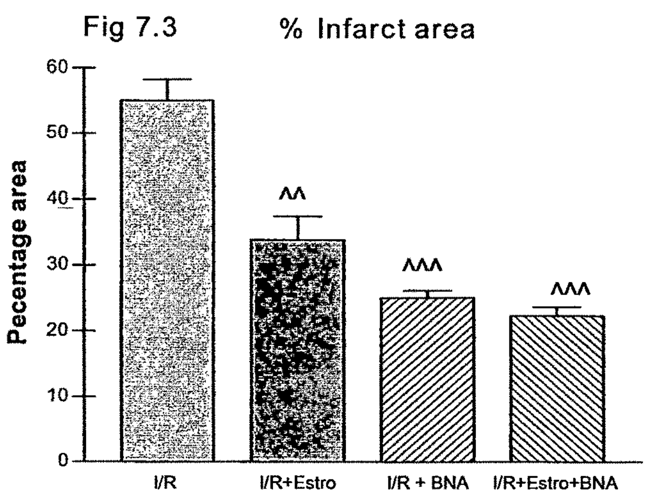
Fig. 7.3 a and 7.3 b

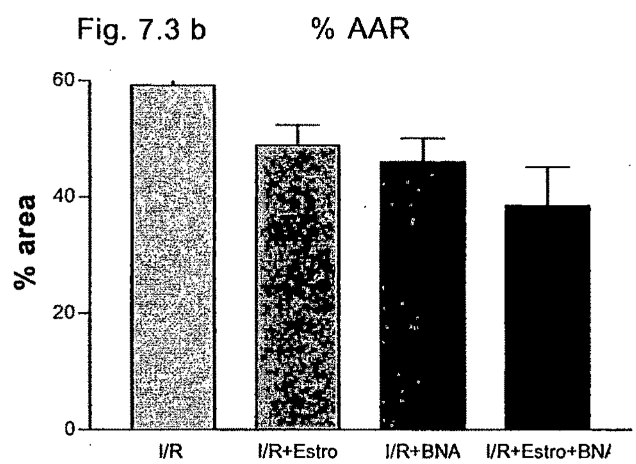
Effect of Estrogen, Banaba and their combination treatment on infarct area and Area At Risk (AAR) after myocardial I/R injury in rats.

7.3 a. Infarct area in percentage

7.3 b. Area at risk (AAR) in percentage

All values are mean ± SEM (n=6). The group treated with Estrogen, Banaba and Combination treatment were compared with the I/R control group (^ - *P* < 0.05, ^^ -P < 0.01; ^^^- P < 0.001)

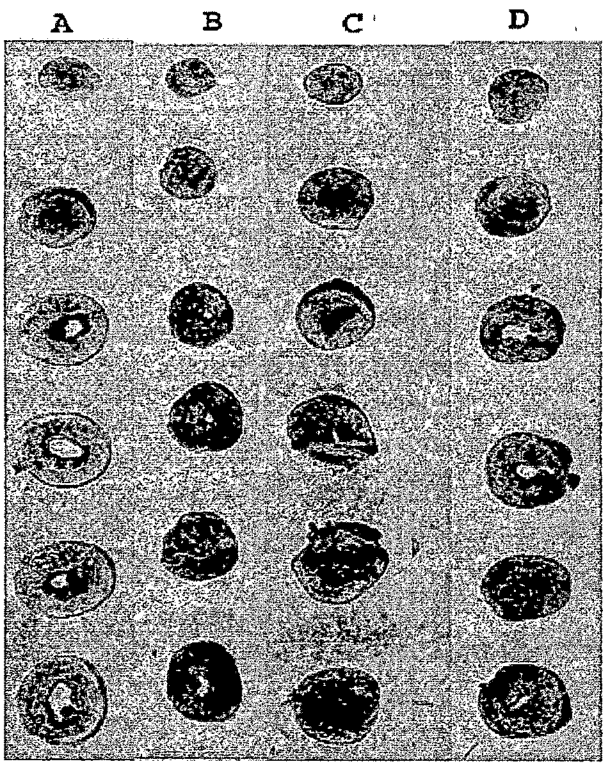




SO- Sham Operated, I/R- Vehicle treated rats undergoing reperfusion injury

I/R+ESTRO- Estrogen treated, I/R+BNA – Banaba treated and I/R+ESTRO+BNA- combination treated reperfused animals.

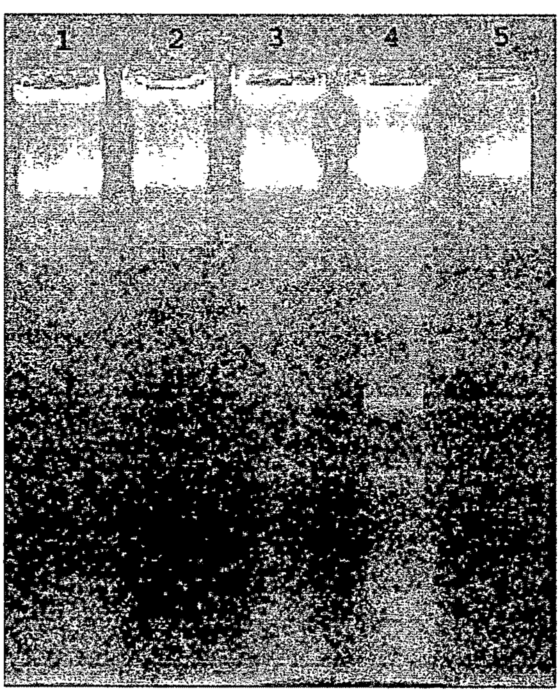
Fig. 7.3 Infarct area in TTC stained slices.



Lane A: Heart Slices of I/R control animals; **Lane B**: Heart Slices of Estrogen treated I/R animal; **Lane C**: Heart Slices of Banaba treated I/R animals; and **Lane D**: Estrogen and Banaba combination treated I/R animal.

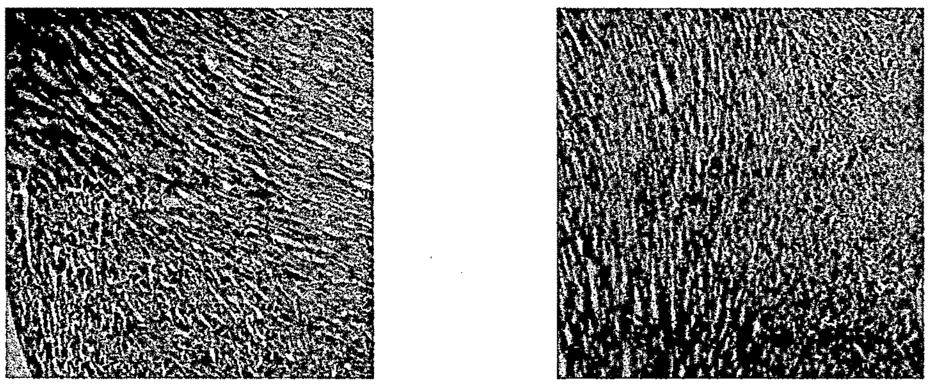
Fig 7.4

Agarose gel electrophoresis of rat heart DNA after ischemia/reperfusion injury.



Lane 1 , Estro and Banaba combination treated animal; **Lane 2,** Banaba treated I/R without evident DNA laddering pattern; **Lane 3,** Estrogen treated I/R (I/R + Estro) with out evident DNA laddering pattern; **Lane 4,** Ischemic control (I/R control) displaying a laddering pattern a indication of; **Lane 5**, Sham control.

Fig 7.5 Photomicrographs (100X) of histological slides stained with H& E stain after ischemia/reperfusion injury.



A **B**

A: Banaba treated I/R animal; **B:** Estro+ BNA treated I/R animal.

Table 7.1

The effect of Estrogen, Banaba and their combination on ± d*p*/d*t* of rats with 1 h of ischemia and 1 h of reperfusion induced by left coronary descending artery (LCA) occlusion.

Group	+ d*p*/d*t*(mmHg/sec)			- d*p*/d*t*(mmHg/sec)		
	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)	Baseline (0 min)	End-ischemia (60 min)	End-reperfusion (120 min)
SO	2402 ± 215	2668 ± 108	2567 ± 180	2015 ± 328	2053 ± 326	1958 ± 127
I/R control	2566 ± 166	1879 ± 148 *	1779 ± 102*	1811 ± 158	1146 ± 126*	1216 ± 93*
I/R + Estro	3357 ± 298	2475 ± 391	2891 ± 156^	1951 ± 238	1564 ± 316	1713 ± 286
I/R + BNA	2329 ± 301	1981 ± 298*	1990 ± 238*	2142 ± 198	1687 ± 114*	1523 ± 168*
I/R+Estro+BNA	3079 ± 119	2321 ± 133	2628 ± 314	1978 ± 451	1490 ± 245	1554 ± 226

All values are mean ± SEM (n=6). The groups treated with vehicle alone (I/R control), Estrogen, Banaba and their combination were compared with Sham operated (SO)(*-P < 0.05, **-P < 0.01, *** - P < 0.001) while groups treated with Estrogen, Banaba and their combination were compared with the I/R group (^- $P < 0.05$, ^^- $P < 0.01$, ^^^- $P < 0.001$)

Table 7.2

The effect of Estrogen, Banaba and their combination on heart rate (HR) and systolic blood pressure (SBP) of rats with 1 h of ischemia and 1 h of reperfusion induced by left coronary descending artery (LCA) occlusion.

Group	HR/min			SBP (mmHg)		
	Baseline	End-ischemia	End-reperfusion	Baseline	End-ischemia	End-reperfusion
SO	419 ± 19.52	416 ± 12.05	405 ± 15.09	117.13 ± 4.46	106.66 ± 3.98	118.5 ± 2.58
I/R control	392 ± 18.21	325 ± 13.01*	308 ± 26.15*	110.52 ± 6.05	81.05 ± 3.56*	79.59 ± 5.98*
I/R + Estro	458 ± 45.24	333 ± 25.16*	303 ± 21.54*	107.24 ± 11.84	92.87 ± 13.11	98.12 ± 4.71
I/R + BNA	435 ± 82.09	308 ±30.09*	287 ± 29.66*	112.14 ± 4.34	94.75 ± 5.75	94.06 ± 4.60
I/R+Estro+BNA	417 ± 40.22	381 ± 22.96	431 ± 49.65	110.51 ± 11.82	88.83 ± 8.71	99.66 ± 10.12

All values are mean ± SEM (n=6). The groups treated with vehicle alone (I/R control), Estrogen, Banaba and their combination were compared with Sham operated (SO)(*-P < 0.05, **-P < 0.01, *** - P < 0.001) while groups treated with Estrogen, Banaba and their combination were compared with the I/R group (^- P < 0.05, ^^- P< 0.01, ^^^ - P< 0.001)

Table 7.3

The effect of Estrogen, Banaba and their combination on occurrence of ventricular tachycardia (VT) in rats with myocardial I/R injury

Group	Occurrence of VT	
	Ischemia period	Reperfusion period
I/R control	11/12	10/12
R + Estro	3/12^	3/12^
I/R+ BNA	3/12^	6/12^
I/R+Estro+BNA	5/12^	4/12^

All values are mean ± SEM (n=6). The group treated with Estrogen, Banaba and their combination were compared with the I/R group (^ $P < 0.05$, ^^-$P < 0.01$ ^^^ - $P < 0.001$)

Table 7.4

Effect of treatment with Estrogen, Banaba and their combination on oxidative stress markers in rats with myocardial I/R injury

Groups	SOD IU (mg/protein)	CAT H_2O_2 consu/min/mg of protein	GSH µg/mg of protein	MDA nmol /mg of protein
SO	12.89 ± 1.30	410.35 ± 68.61	9.06 ± 0.84	1.05 ± 0.12
I/R control	3.02 ± 0.49***	274.01 ±62.23	3.91 ± 0.32***	3.02 ± 0.33**
I/R+Estro	6.41 ±0.38***^	315.09 ± 69.49	7.12 ± 0.63^	1.66 ± 0.19^
I/R + BNA	8.58 ± 0.97*^^^	266.61 ± 50.53	8.13 ± 0.45 ^	1.5 1 ± 0.17^^
I/R+Estro+BNA	9.10 ± 0.89*^^^	445.36 ± 54.54	7.95 ± 1.00^	1.36 ± 0.23^^

All values are mean ± SEM (n=6). The groups treated with vehicle alone (I/R control), Estrogen, Banaba and their combination were compared with Sham operated (SO)(*-P < 0.05, **-P < 0.01, *** - P < 0.001) while groups treated with Estrogen, Banaba and their combination were compared with the I/R group (^- *P* < 0.05, ^^- *P*< 0.01, ^^^ - *P*< 0.001)

Table 7.5

Effect of treatment with Estrogen, Banaba and their combination on MPO and CK activity in rats with myocardial I/R injury.

Groups	MPO (units/mg of protein)	CK (U/L)
SO	0.23 ± 0.91	1033.67 ± 36.23
I/R control	0.82 ± 0.04***	4490.54 ± 120.99***
I/R +Estro	0.45 ± 0.05^	2140.00 ± 326.62*^^^
I/R + BNA	0.44 ± 0.03^	3507.16 ± 206.28**^
I/R+ Estro+BNA	0.38 ± 0.02^^	2694.93 ± 436.21^^^

All values are mean ± SEM (n=6). The groups treated with vehicle alone (I/R control), Estrogen, Banaba and their combination were compared with Sham operated (SO)(*-P < 0.05, **-P < 0.01, *** - P < 0.001) while groups treated with Estrogen, Banaba and their combination were compared with the I/R group (^- $P < 0.05$, ^^- $P< 0.01$, ^^^ - $P< 0.001$)

4.2 Cerebral ischemia/reperfusion model

4.2.1 PG and Lipoic acid and their combination treatment on cerebral reperfusion injury:

The effect of treatment of various drugs on cerebral I/R injury was studied using MCAO model. This study protocol involving contains five groups- 1) sham operated vehicle treated group (SO), 2) vehicle treated cerebral reperfusion injury control group (I/R control), 3) PG (2 mg/kg i.p. 1 h after ischemia induction) treated reperfusion injury group (PG), 4) Lipoic acid (100 mg/kg i.p. 1 h after ischemia induction) receiving reperfusion injury group (Lipo) and 5) co-administration of PG with Lipoic acid (PG + Lipo) reperfusion injury group. These groups were studied for the cerebral I/R injury experiment and the groups are described in section 3.2.2.1.1, 3.2.2.1.2 and 3.2.2.1.6 of materials and methods.

4.2.1.1 Effects of PG and Lipoic acid and their combination on Neurological deficit-

Using the scale described in protocol 1 of section 3.2.2, the quantification of the neurological deficit induced by cerebral I/R was performed. There was a severe neurological deficit in vehicle treated (I/R control) animals after 2 h of ischemia and 22 h of reperfusion. Even though Lipoic acid and progesterone treatment of the animals caused mild reduction in neurological deficit, the changes were not significant as compared to the I/R control group of animals. But it was found that the combined treatment of PG+Lipo group showed significant (P< 0.05) reduction in neurological deficit after the induction of I/R injury. **Table 8.1**

4.2.1.2 Effects of PG and Lipoic acid and their combination on Cerebral infarct area and Brain swelling-

The cerebral infarct and the brain swelling of the ipsilateral and contralateral hemispheres of treated and untreated animals were measure using the TTC staining technique which is explained in protocol 1 of section **3.2.2.**

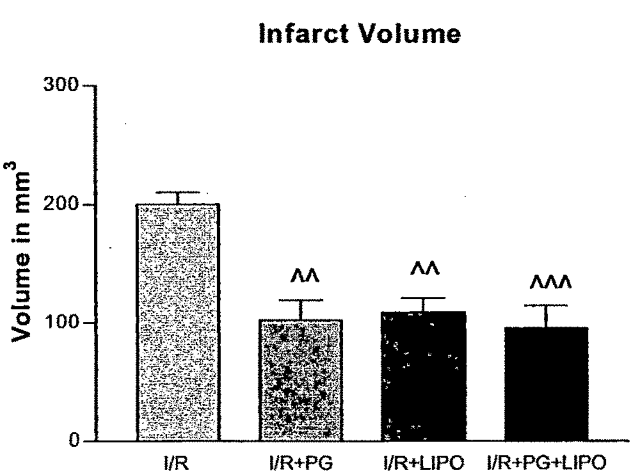


Fig. 8.1 Volume of infarct in ipsilateral hemisphere.
^ Compared to I/R group; ^P < 0.05

Induction of cerebral I/R injury resulted in increased volume of infarction and percentage of brain swelling in ipsilateral hemisphere (200.29 ± 18.95 mm^3 and 24.19 ± 1.78 %) of I/R control animals. Lipoic acid treatment of the animals significantly (P< 0.01) reduced the volume of infarction (108.83 ± 12.07 mm^3) and brain swelling (13.20 ± 1.89 %) in ipsilateral hemispheres of the animals after the I/R injury. Similarly PG had an ameliorating effect on the treated animals, where the volume of infarct and the brain swelling of the ipsilateral hemisphere were found to be 102.23 ± 17.55 mm^3 and 11.64 ± 1.48 % respectively. This difference in the volume of infarction and brain swelling was significant (P< 0.01). The combined treatment of PG and Lipoic acid (PG+Lipo) showed synergistic effect on infarct volume, where the animals showed significant (P< 0.001) protection against the I/R injury. The volume of infarct and the brain swelling were 95.39 ± 16.99 mm^3 and 12.69 ± 2.08 % respectively. **Fig. 8.1 and 8.2.**

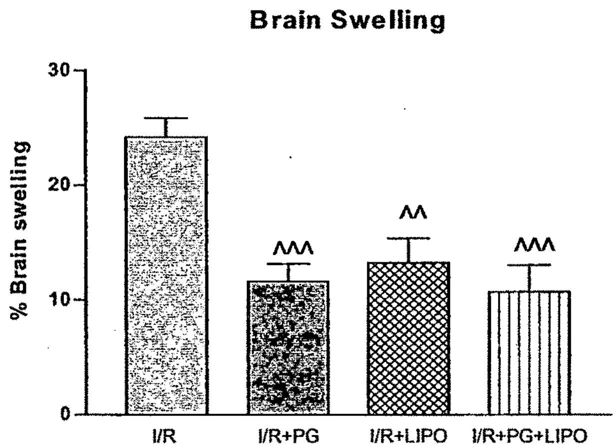


Fig. 8.2 % Brain swelling of ipsilateral hemisphere, ^ Compared to I/R group; ^P < 0.05

4.2.1.3 Effects of PG and Lipoic acid and their combination on antioxidant biomarkers in brain-

The antioxidant biomarker like some endogenous enzymes (SOD & Catalase), GSH and LPO were substantially changed after MCAO induced I/R injury in brain. Lipoic acid treatment significantly restored the depleted SOD activity as compared to I/R control group in ipsilateral hemisphere. CAT activity had some changes but these were not significant. The GSH levels were found to increase significantly. LPO measured by estimating MDA levels was decreased due to Lipoic acid treatment. PG in combination with Lipoic acid found to have enhanced the antioxidant effect of Lipoic acid alone treatment. SOD and GSH were significantly increased as compared to I/R control group only in ipsilateral hemisphere of the animal's brain, whereas, MDA levels were significantly decreased in the ipsilateral hemispheres of the treated animals. However, the change in the activity of the CAT was inconclusive. **Table 8.2**

4.2.1.4 Effects of PG and Lipoic acid and their combination on inflammatory reactivity in cerebral I/R injury-

Inflammatory response due to neutrophil infiltration was substantially elevated after I/R injury, which was observed from the significant ($P<0.001$) increase in MPO activity of I/R control animals as compared to SO animals. Treatment with Lipoic acid and PG showed significant ($P<0.05$) decrease in MPO activity of animals with I/R injury. **Table 8.3**

4.2.1.5 Effects of PG and Lipoic acid and their combination on Blood brain barrier (BBB)-

Evan's blue (EB) extravasation in brain was considered as a marker for damage induced by cerebral I/R to BBB. I/R injury caused severe damage to BBB, which was observed from heavy EB content (0.97±0.19 μg/ml) in ipsilateral hemisphere of brain. Animals treated with Lipoic acid significantly reduced the permeability of BBB and the EB content was restricted at 1.48 ± 0.20 μg/ml. PG with Lipoic acid showed excellent protective activity and significantly ($P<0.01$) reduced the EB content as compared to I/R control animals. **Table 8.3**

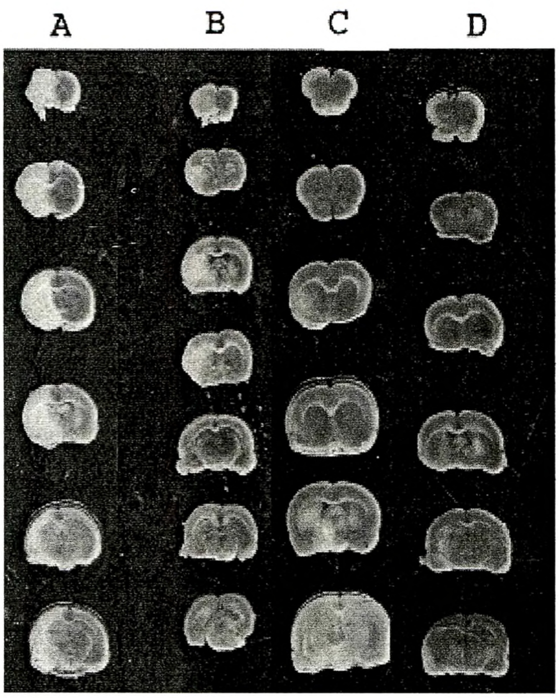
4.2.1.6 Effects of PG and Lipoic acid and their combination on Apoptotic cell death-

The apoptotic cell death induced by cerebral I/R injury was studied by observing the interneucleosomal DNA fragmentation. Vehicle treated I/R control animals showed clear band formation pattern as the marked evidences of DNA damage, whereas treatment with PG and Lipoic acid substantially reduced the apoptotic cell death indicated by reduced DNA fragmentation. **Fig. 8.3**

4.1.1.7 Effects of PG and Lipoic acid and their combination on histological features-

The photomicrographs indicated cellular injury to brain in vehicle treated groups, was observed by discontinued neuronal cells. I/R injury induced severe cellular edema and caused heavy neutrophil vehicle treated animals. Treatment with PG and Lipoic acid considerably restored the normal cellular architecture and reduced the damage indicated by decreased edema and minimal neutrophil presence in the cells. **Fig. 8.4**

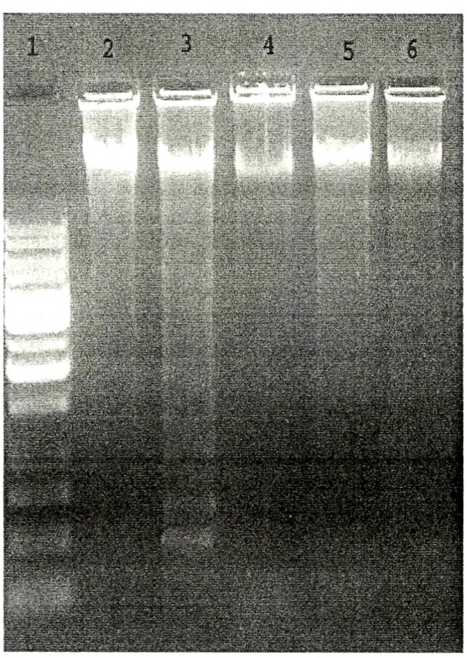
Fig 8.2 b. Infarct Volume in TTC stained slices



Lane A: Brain Slices of I/R control animal; **Lane B**: Brain Slices of PG treated I/R animal; **Lane C**: Brain Slices of Lipoic acid treated I/R animal; and **Lane D**: PG and Lipoic acid combination treated I/R animal.

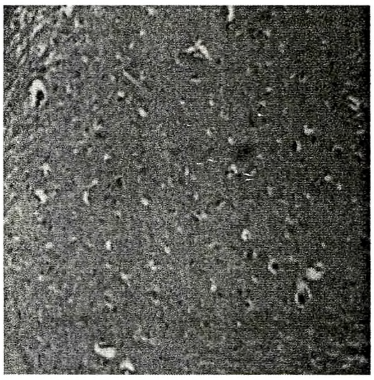
Fig 8.3

Agarose gel electrophoresis of rat brain (Ipsilateral) DNA after ischemia/reperfusion injury.

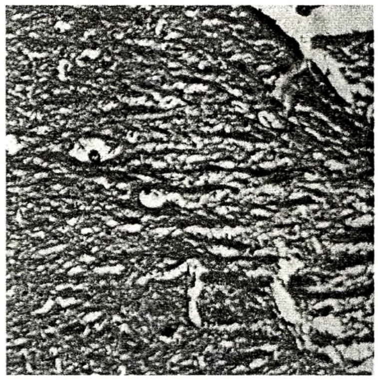


Lane 1, 1-kb DNA standard; **Lane 2,** Sham control; **Lane 3,** Ischemic control (I/R control, ispsilateral hemisphere) displaying a laddering pattern a indication of; **Lane 4,** PG treated I/R (I/R +PG) with out evident DNA laddering pattern; **Lane 5,** Lipoic acid treated I/R without evident DNA laddering pattern; **Lane 6,** PG and Lipoic acid combination treated animal.

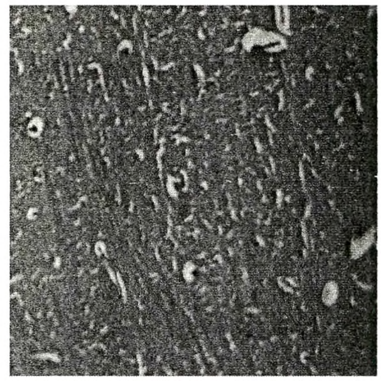
Fig 8.4 Photomicrographs (100X) of histological slides (Brain) stained with H& E stain after ischemia/reperfusion injury.



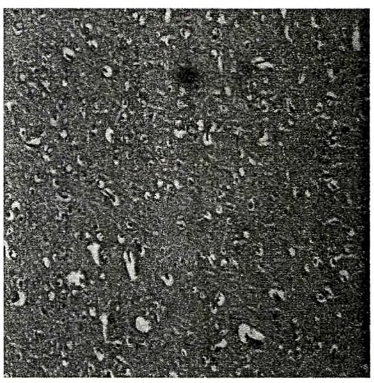
A



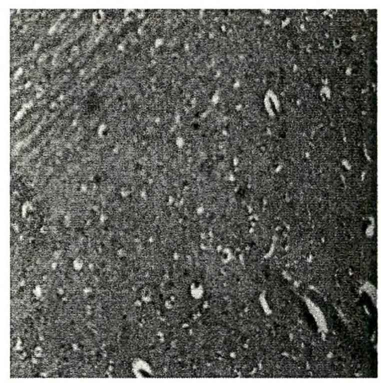
B



C



C



D

Photomicrographs (100X) of histological slides (Brain) stained with H& E stain after I/R injury.

A: Sham operated animals **B:** Vehicle treated I/R animal showing distorted neuronal cells , edematous cells and leukocyte infiltration; **C:** PG treated I/R animals with improvedneuronal architecture, few edematous cells; **D:** Lipoic acid treated I/R animal with few neurophils and edema. Slides showed overall recovery; **E:** PG+LIPO treated animal with marked improvement in cellular structure with few neutropils.

Table 8.1

Neurological deficit measurement in reperfused, PG (2mg/kg), Lipoic acid (100 mg/kg) and their combination treated groups

Treatment	N	Neurological score
I/R control	6	3.5
I/R+PG	6	2.5
I/R+Lipo	6	2.0
I/R+PG+Lipo	6	1.5^

Neurological deficit was assessed after 22 hours of reperfusion. The values are represented as median of the individual scores.

^ *P*< 0.05 as compared to I/R control group.

Table 8.2

Effect of PG (2mg/kg), Lipoic acid (100 mg/kg) and their combination treatment on Oxidative stress markers

Groups	SOD IU/mg of protein	CAT H_2O_2/consu /min/mg of protein	GSH µg/mg of protein	MDA nmol /mg of protein
SO				
Ipsilateral	9.08 ± 0.57	232.35 ± 5.16	21.39 ± 1.19	5.56 ± 0.29
Contralateral	8.72 ± 0.26	244.30 ± 11.77	21.67 ± 0.98	5.69 ± 0.35
I/R control				
Ipsilateral	4.06 ± 0.53**	185.39 ± 22.92	5.20 ± 0.22***	13.79 ± 1.74**
Contralateral	6.10 ± 0.58	218.75 ± 21.49	15.69 ± 0.26	6.83 ± 1.04
I/R+PG				
Ipsilateral	8.24 ± 0.83^	149.41 ± 12.69	9.97 ± 0.82**^	6.88± 1.16^^
Contralateral	8.33 ± 0.70	190.8 ± 6.92	16.8 ± 0.51	6.6 ± 0.43
I/R+Lipo				
Ipsilateral	8.37± 0.94^	230.42 ± 41.10	12.92 ±1.32*^^	6.95 ± 0.63^^
Contralateral	8.79 ± 1.25	202.14 ± 40.64	19.34 ± 4.31	6.12 ± 0.69
I/R+Lipo+PG				
Ipsilateral	8.71 ± 0.69^	363.42 ± 38.02	13.11 ± 1.21*^^	4.80 ± 0.45^^^
Contralateral	8.12 ± 0.97	312.42 ± 42.32	16.34 ± 2.63	6.12 ± 0.69

All values are mean ± SEM (n=6). I/R control, PG, Lipoic acid and their combination were compared with sham operated control (SO)-(* $P< 0.05$, **-$P< 0.01$, ***- $P< 0.001$) while the groups treated with PG, Lipoic acid and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P< 0.01$, ^^^ - $P< 0.001$)

Table 8.3

Effect of PG (2mg/kg), Lipoic acid (100 mg/kg) and their combination treatment MPO and Blood Brain Barrier permeability estimation

Groups	N	MPO units/mg of protein	Evans blue μg/ml
SO			
Ipsilateral	6	0.12 ± 0.02	0.97 ± 0.19
Contralateral		0.09 ± 0.02	0.87 ± 0.14
I/R control			
Ipsilateral	6	0.52 ± 0.07**	3.86 ± 0.53***
Contralateral		0.20 ± 0.03	1.79 ± 0.92
I/R+PG			
Ipsilateral	6	0.27 ± 0.05^	1.53 ± 0.17*^
Contralateral		0.19 ± 0.03	1.19 ± .013
I/R+LIPO			
Ipsilateral	6	0.24 ± 0.05^	1.48 ± 0.20*^
Contralateral		0.15 ± 0.01	1.07 ± .019
I/R+PG+LIPO			
Ipsilateral	6	0.19 ± 0.04^^	1.31 ± 0.10*^^
Contralateral		0.12 ± 0.03	1.09 ± 0.27

All values are mean ± SEM (n=6). I/R control, PG, Lipoic acid and their combination were compared with sham operated control (SO)-(* $P < 0.05$, **-$P < 0.01$, ***- $P < 0.001$) while the groups treated with PG, Lipoic acid and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P < 0.01$, ^^^ - $P < 0.001$)

4.2.2 Effect of PG, CoQ alone and their combination on cerebral I/R

This study protocol involves five groups- 1) Group 1 was sham operated vehicle treated group (SO), 2) Group 2 was vehicle treated cerebral reperfusion injury control group (I/R control), 3) Group 3 was PG (2 mg/kg i.p. 1 h after ischemia induction) treated reperfusion injury group (PG), 4) CoQ (100 mg/kg i.p. 1 h after ischemia induction) was administered to reperfusion injury animals and this was considered as Group 4 (CoQ). 5) Group 5 received simultaneous administration of PG with CoQ (PG +CoQ) 1 h after induction of ischemia. All these groups are described in section 3.2.2.1.1, 3.2.2.1.3 and 3.2.2.1.7 of material and methods.

4.2.2.1 Effects of PG, CoQ and their combination on Neurological deficit-

I/R control animals showed substantial neurological deficit at the end of reperfusion injury protocol. Treatment with CoQ and PG did not reduce the neurological deficit after 22 h of reperfusion. The simultaneous administration of PG and CoQ did not take the deficit any further; this treatment did not show any significant improvement in animal on neurological scale. **Table 9.1**

4.2.2.2 Effects of PG, CoQ and their combination on Cerebral infarct are and Brain swelling in cerebral reperfusion injury-

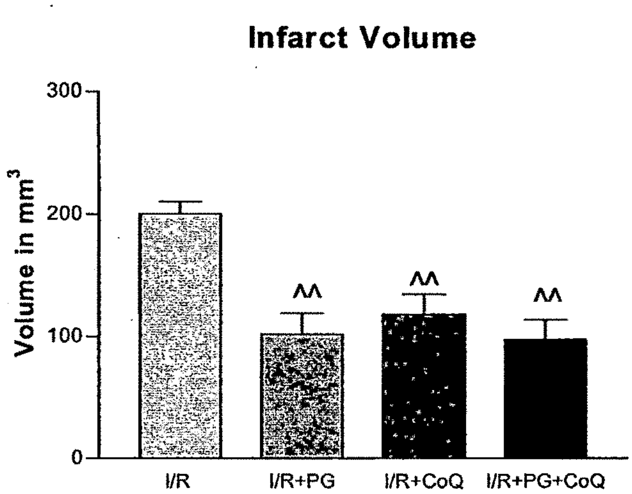


Fig. 9.1 Volume of infarct in ipsilateral hemisphere; ^ Compared to I/R group; ^$P < 0.05$

Cerebral I/R injury induced increased volume of infarction and brain swelling in ipsilateral hemisphere of I/R control animals. Treatment with CoQ significantly ($P < 0.01$) reduced the volume of infarction (118.51 ± 16.60 mm^3) and brain swelling (16.54 ± 1.86 %) in ipsilateral hemispheres of the animals after the I/R injury. The simultaneous treatment with PG and CoQ (PG+CoQ) showed protective effect on infarct volume. However, the animals (PG+CoQ) showed similar significance ($P < 0.01$) in protection of brain against the I/R injury. The volume of infarct and the brain swelling for (PG+CoQ) group was 97.92 ± 15.88 mm^3 and 9.11 ± 0.96 % respectively.

4.2.2.3 Effects of PG, CoQ and their combination on Antioxidant biomarkers in brain-

Cerebral I/R injury induced significant decrease in SOD, activity and GSH level in control animals. The lipid peroxidation was found to have marked increase in vehicle treated control animals. The treatment with the CoQ significantly increased the SOD and GSH levels in treated animals, whereas the MDA content was significantly reduced as compared to I/R control animals. Simultaneous administration of CoQ and PG showed better antioxidant activity. This PG+CoQ group showed significant elevation in SOD activity (9.20 ± 0.68), GSH levels (11.81 ± 1.63) and marked depletion of MDA content (5.49 ± 0.84) in the treated animals.

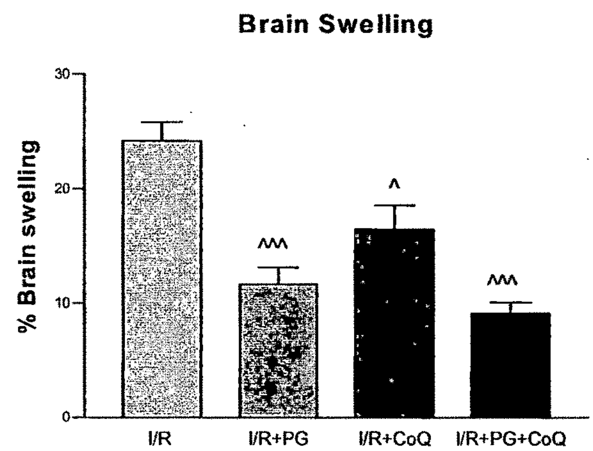


Fig. 9.2 % Brain swelling of ipsilateral hemisphere; ^ Compared to I/R group; ^$P < 0.05$

Catalase levels did not change significantly in any of the groups of this study. **Table 9.2**

4.2.2.4 Effects of PG, CoQ and their combination on MPO activity in cerebral I/R injury -

MPO activity, which was significantly (P<0.01) increased in I/R control animals as compared to SO animals was reduced by CoQ administration to the I/R control animals. However, there was no significant change observed between CoQ alone and CoQ+PG group of animals. **Table 9.3**

4.2.2.5 Effects of PG, CoQ and their combination on Blood brain barrier (BBB)–

Cerebral I/R injury caused severe damage to BBB, which was quantified by EB content (0.97±0.19 µg/ml) in ipsilateral hemisphere of brain of control animal. Treatment with CoQ significantly reduced the permeability of BBB (.48 ± 0.20 µg/ml), whereas simultaneous treatment with PG and CoQ further reduced the extravasation of EB in brain. **Table 9.3**

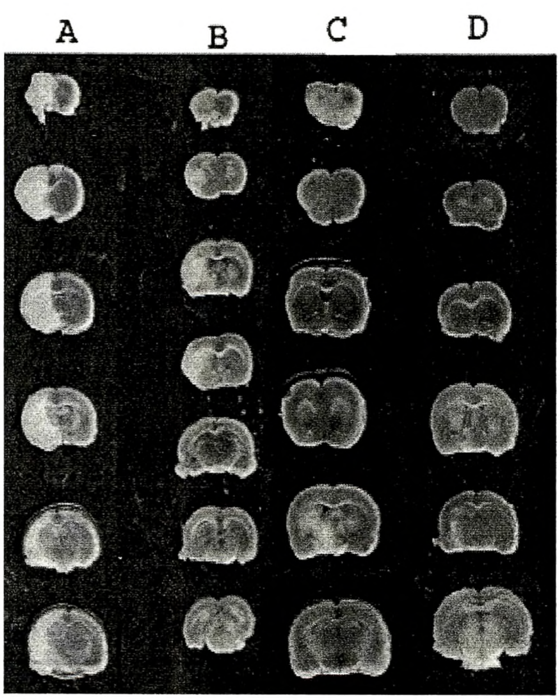
4.2.2. Effects of PG, CoQ and their combination on Apoptotic cell death-

The band formation pattern is the indication of internucleosomal DNA fragmentation, which was observed in control I/R animals. Treatment with COQ and PG slightly reduced the intensity of this fragmentation of DNA in the ipsilateral hemispheres of the treated animals. **Fig. 9.3**

4.2.2.7 Effects of PG, CoQ and their combination on histological features-

The characteristic cellular damage induced by I/R injury was reduced by CoQ treatment and the reduced neutrophil infiltration is one of the indicators for the decreased cellular destruction in the treated animals. The co-administration PG and CoQ, markedly reduced the oedematous cells and the overall cellular architecture was improved. **Fig. 9.4**

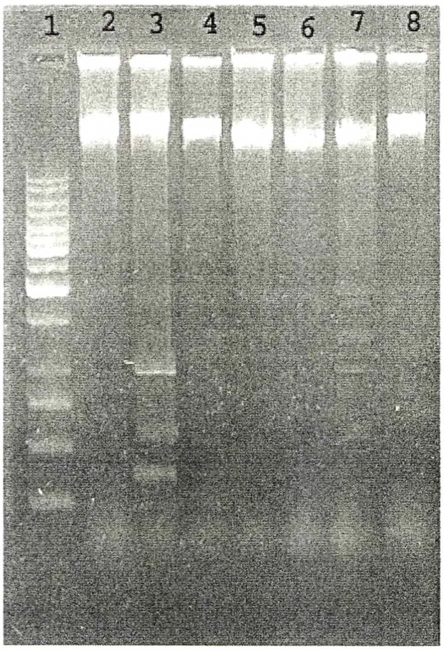
Fig 9.2 b. Infarct Volume in TTC stained slices



Lane A: Brain Slices of I/R control animal; **Lane B**: Brain Slices of PG treated I/R animal; **Lane C**: Brain Slices of CoQ treated I/R animal; and **Lane D**: PG and CoQ combination treated I/R animal.

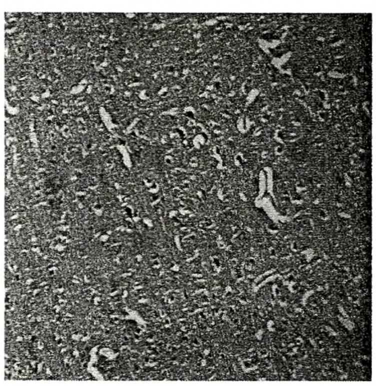
Fig 9.3

Agarose gel electrophoresis of rat brain (Ipsilateral) DNA after ischemia/reperfusion injury.

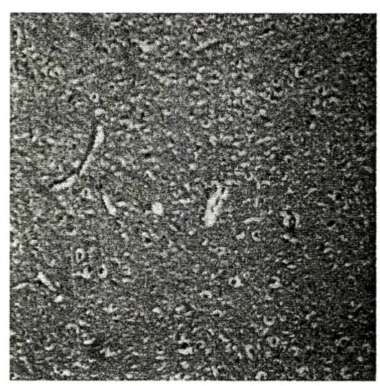


Lane 1, 1-kb DNA standard; **Lane 2**, Sham control; **Lane 3,** Ischemic control (I/R control, ispsilateral hemisphere) displaying a laddering pattern; **Lane 4,** PG treated I/R (I/R +PG) with out evident DNA laddering pattern; **Lane 5,** Banaba treated I/R without evident DNA laddering pattern; **Lane 6,** PG and Banaba combination treated animal; **Lane 7**, CoQ treated animals showing a slite fragmentation od DNA: **Lane 8,** PG+ CoQ treated animal showing no fragmentation of DNA

Fig 9.4 Photomicrographs (100X) of histological slides (Brain) stained with H& E stain after ischemia/reperfusion injury.



A



B

A: CoQ treated I/R animal with neurophils infiltrations and edematous cells; **B:** PG+CoQ treated animal with reduced cellular damage with few neutropils edematous cells.

Table 9.1

Neurological deficit measurement in reperfused, PG (2mg/kg), CoQ (100 mg/kg) and their combination treated groups

Treatment	N	Neurological score
I/R control	6	3.5
I/R+PG	6	2.5
I/R+CoQ	6	2.5
I/R+PG+CoQ	6	2.0

Neurological deficit was assessed after 22 hours of reperfusion. The values are represented as median of the individual scores. ^ $P< 0.05$ as compared to I/R control group.

Table 9.2

Effect of PG (2mg/kg), CoQ (100 mg/kg) and their combination treatment on Oxidative stress markers

Groups	SOD IU/mg of protein	CAT H2O2 consu /min/mg of protein	GSH µg/mg of protein	MDA nmol/mg of protein
SO				
Ipsilateral	9.08 ± 0.57	232.35 ± 5.16	21.39 ± 1.19	5.56 ± 0.29
Contralateral	8.72 ± 0.26	244.30 ± 11.77	21.67 ± 0.98	5.69 ± 0.35
I/R control				
Ipsilateral	4.06 ± 0.53**	185.39 ± 22.92	5.20 ± 0.22***	13.79 ± 1.74**
Contralateral	6.10 ± 0.58	218.75 ± 21.49	15.69 ± 0.26	6.83 ± 1.04
I/R+PG				
Ipsilateral	8.24 ± 0.83^	149.41 ± 12.69	9.97 ± 0.82***^	9.18± 1.16*
Contralateral	8.33 ± 0.70	190.8 ± 6.92	16.8 ± 0.51	6.6 ± 0.43
I/R+CoQ				
Ipsilateral	7.87± 0.39 ^	314.79 ± 70.42	11.45 ± 0.87**^^	5.88 ± 0.77^^
Contralateral	7.96 ± 0.49	300.65 ± 46.64	17.11 ± 1.13	5.55 ± 0.95
I/R+PG+CoQ				
Ipsilateral	9.20 ± 0.68^^	331.36 ± 40.69	11.81 ± 1.63**^^	5.49 ± 0.84^^
Contralateral	7.23 ± 0.43	312.42 ± 42.32	15.67 ± 1.79	5.32 ± 0.89

All values are mean ± SEM (n=6). I/R control, PG, COQ and their combination were compared with sham operated control (SO)-(* $P< 0.05$, **-$P< 0.01$, ***- $P< 0.001$) while the groups treated with PG, CoQ and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P< 0.01$, ^^^- $P< 0.001$)

Table 9.3

Effect of PG (2mg/kg), CoQ (100 mg/kg) and their combination treatment MPO and Blood Brain Barrier permeability estimation

Groups	N	MPO units/mg of protein	Evans blue μg/ml
SO			
Ipsilateral	6	0.12 ± 0.02	0.97 ± 0.19
Contralateral		0.09 ± 0.02	0.87 ± 0.14
I/R control			
Ipsilateral	6	0.52 ± 0.07***	3.86 ± 0.53***
Contralateral		0.20 ± 0.03	1.79 ± 0.92
I/R+PG			
Ipsilateral	6	0.27 ± 0.05^	1.53 ± 0.17*^
Contralateral		0.19 ± 0.03	1.19 ± .013
I/R+CoQ			
Ipsilateral	6	0.19 ± 0.03^^	1.30 ± 0.10*^
Contralateral		0.12 ± 0.05	1.09 ± 0.02
I/R+PG+CoQ			
Ipsilateral	6	0.16 ± 0.04^^	1.23 ± 0.22*^^
Contralateral		0.11 ± 0.02	1.04 ± 0.35

All values are mean ± SEM (n=6). I/R control, PG, CoQ and their combination were compared with sham operated control (SO)-(* $P< 0.05$, **-$P< 0.01$, ***- $P< 0.001$) while the groups treated with PG, CoQ and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P< 0.01$, ^^^ - $P< 0.001$)

4.2.3 Effect of treatment with PG, Banaba and their combination on cerebral I/R injury:

Treatment with PG and Banaba comprises of five groups- 1) Group 1 was sham operated vehicle treated group (SO), 2) Group 2 was vehicle treated cerebral reperfusion injury control group (I/R control), 3) Group 3 was PG (2 mg/kg i.p. 1 h after ischemia induction) treated reperfusion injury group (PG), 4) Group 4 was the Banaba treated (100 mg/kg i.p. 1 h after ischemia induction) animals with reperfusion injury (BNA). 5) Group 5 received simultaneous administration of PG with Banaba (PG + BNA) 1 h after induction of ischemia. All these groups are described in section 3.2.2.1.1, 3.2.2.1.4 and 3.2.2.1.8 of materials and methods.

4.2.3.1 Effects of PG, Banaba and their combination on Neurological deficit-

Neurological deficit induced by the cerebral I/R injury was scored according to the various locomotor activities of the animals after 22 h of reperfusion. The I/R control group had maximum deficit due to the extent of damage inflicted by the I/R injury. Treatment with Banaba did change the deficit score of the animals but it was not significant. Similar results were observed for PG alone treated animals and the animals treated with simultaneous administration of Banaba and PG. **Table 10.1**

4.2.3.2 Effects of PG, Banaba and their combination on Cerebral infarct are and Brain swelling-

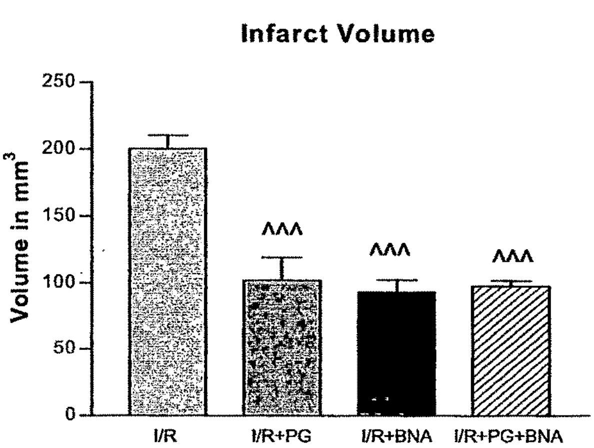
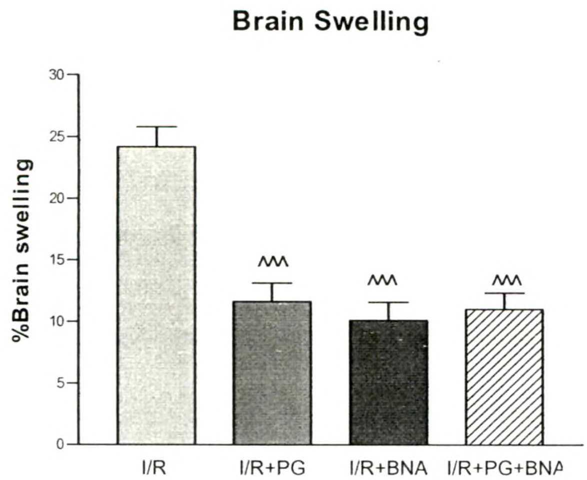


Fig. 10.1. Volume of infarct in ipsilateral hemisphere; ^ Compared to I/R group; ^$P < 0.05$

Banaba treatment significantly (P<0.001) reduced the volume of infarction (93.30 ± 9.15 mm^3) and brain swelling (10.11 ± 1.32 %) in ipsilateral hemispheres of the animals after the I/R injury. The co-administration of Banaba with PG showed ameliorating effect on infarct volume and brain swelling which was significantly (P<0.001) reduced as compared to I/R control group rendering protection to brain against the I/R injury.



The volume of infarct and the brain swelling for (PG+BNA) group was found to be 97.53 ± 7.90 mm^3 and 10.02 ± 1.19 % respectively.

Fig. 10.2. % Brain swelling of ipsilateral hemisphere; ^ Compared to I/R group; $^P < 0.05$

4.2.3.3 Effects of PG, Banaba and their combination on Antioxidant biomarkers in brain-

SOD activity and GSH level in ipsilateral hemispheres of the control animals were significantly depleted by severe cerebral I/R. The MDA levels indicating the extent of lipid peroxidation were also found to have substantial increase as compared to sham operated animals. Banaba administration effectively reduced the lipid peroxidation and shown significant increase in GSH levels. This treatment led to significant restoration of SOD activity in the ipsilateral hemispheres of brain in the treated animals. The Banaba administration with PG, found to have good antioxidant property but there was no significant difference observed in Banaba alone treatment group and combined treatment group of animals receiving Banaba and PG simultaneously. Catalase change was not significant except for PG+BNA group, which showed significant ($P<0.001$) increase as compared to I/R control group. **Table: 10.2**

4.2.3.4 Effects of PG, Banaba and their combination Inflammation in cerebral I/R injury-

Inflammation was quantified by MPO activity measurement, which was significantly ($P<0.001$) higher in vehicle treated I/R control animals as compared to SO group. MPO activity in Banaba treatment group was significantly reduced; similarly for PG treated animals too, whereas the MPO activity found to have highly significant ($P<0.01$) decrease in PG+BNA treated animals. **Table 10.3**

4.2.3.6 Effects of PG, Banaba and their combination Blood brain barrier (BBB)–

BBB permeability was increased due to the damage to the endothelial cell junctions, which was indicated by EB extravasations in ipsilateral hemisphere of the animal. After 22 h of reperfusion, EB content in ipsilateral hemisphere of brain of control animal estimated was 0.97±0.19 μg/ml. Banaba treated animals showed decreased EB extravasation (1.36 ± 0.07), which was significant (P<0.01) as compared to I/R control animals. Co-administration of PG and Banaba to animals also significantly restricted the EB content in brain and its content in ipsilateral hemisphere of the treated animal was found to be 1.20 ± 0.32 μg/ml. **Table 10.3**

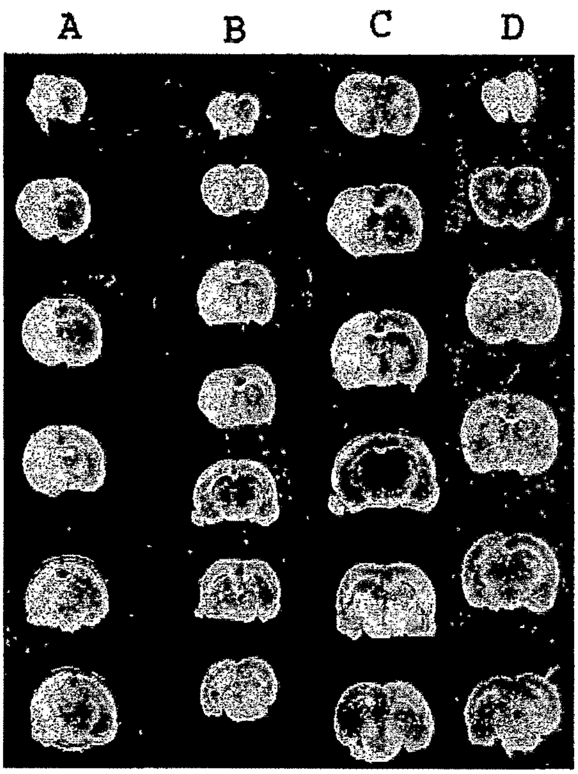
4.2.3.6 Effects of PG, Banaba and their combination on Apoptotic cell death-

The intensity of band formation due to internucleosomal DNA fragmentation observed in control I/R animals was very high. Banaba and PG treatment reduced this fragmentation of DNA in the ipsilateral hemispheres of the treated animals. Similarly PG+Banaba i.e. the co-administration of PG and Banaba prevented the band formation in the neuronal cells. **Fig. 9.3**

4.2.3.7 Effects of PG, Banaba and their combination on histological features-

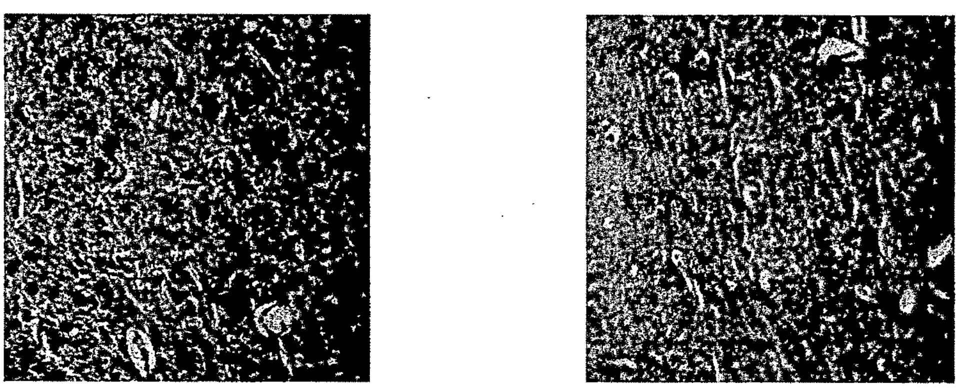
The edema, neutrophils observed in the histological slides of the brain in I/R animals indicated the severity of the impact exerted by cerebral I/R injury on the animals. Banaba protected the animals from the cellular damage, which was observed by reduced number of infiltrated neurophils and the almost intact cellular architecture after 22 h of reperfusion. **Fig. 10.3**

Fig 10.2 b Infarct volume in the TTC stained brain slices.



Lane A: Brain Slices of I/R control animal; **Lane B**: Brain Slices of PG treated I/R animal; **Lane C**: Brain Slices of Banaba treated I/R animal; and **Lane D**: PG and Banaba combination treated I/R animal.

Fig 10.4 Photomicrographs (100X) of histological slides (Brain) stained with H& E stain after ischemia/reperfusion injury.



A B

A: Banaba treated I/R animal with cellular recovery but with few edematous cells; **B:** PG+BNA treated animal withminimum neutrophil infiltration and reduced damage.

Table 10.1

Neurological deficit measurement in reperfused, PG (2mg/kg), Banaba (100 mg/kg) and their combination treated groups

Treatment	N	Neurological score
I/R control	6	3.5
I/R+PG	6	2.5
I/R+BNA	6	3.0
I/R+PG+BNA	6	2.5

Neurological deficit was assessed after 22 hours of reperfusion. Results are displayed as the median of the score. ^ $P< 0.05$ as compared to I/R control group.

Table 10.2

Effect of PG (2mg/kg), Banaba (100 mg/kg) and their combination treatment on Oxidative stress markers

Groups	SOD IU/mg of protein	CAT H_2O_2 consu /min/mg of protein	GSH µg/mg of protein	MDA nmol/mg of protein
SO				
Ipsilateral	9.08 ± 0.57	232.35 ± 5.16	21.39 ± 1.19	5.56 ± 0.29
Contralateral	8.72 ± 0.26	244.30 ± 11.77	21.67 ± 0.98	5.69 ± 0.35
I/R control				
Ipsilateral	4.06 ± 0.53**	185.39 ± 22.92	5.20 ± 0.22***	13.79 ± 1.74**
Contralateral	6.10 ± 0.58	218.75 ± 21.49	15.69 ± 0.26	6.83 ± 1.04
I/R+PG				
Ipsilateral	8.24 ± 0.83^	149.41 ± 12.69	9.97 ± 0.82***^	9.18± 1.16*
Contralateral	8.33 ± 0.70	190.8 ± 6.92	16.8 ± 0.51	6.6 ± 0.43
I/R+BNA				
Ipsilateral	8.47 ± 0.90^	232.94 ± 18.18	11.45 ± 0.87**^^	5.88 ± 0.77^^
Contralateral	8.49 ± 0.63	161.83 ± 64.52	17.11 ± 1.13	5.55 ± 0.95
I/R+PG+BNA				
Ipsilateral	8.98 ± 0.85^	342.65±24.72^^	12.81 ± 1.63**^^	5.49 ± 0.84^^
Contralateral	7.68 ± 1.02	340.79 ± 27.43	15.67 ± 1.79	5.32 ± 0.89

All values are mean ± SEM (n=6). I/R control, PG, Banaba and their combination were compared with sham operated control (SO)-(* $P< 0.05$, **-$P< 0.01$, ***- $P< 0.001$) while the groups treated with PG, Banaba and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P< 0.01$, ^^^ - $P< 0.001$)

Table 10.3

Effect of PG (2mg/kg), Banaba (100 mg/kg) and their combination treatment MPO and Blood Brain Barrier permeability estimation

Groups	N	MPO units/mg of protein	Evans blue µg/ml
SO			
Ipsilateral	6	0.12 ± 0.02	0.97 ± 0.19
Contralateral		0.09 ± 0.02	0.87 ± 0.14
I/R control			
Ipsilateral	6	0.52 ± 0.07***	3.86 ± 0.53***
Contralateral		0.20 ± 0.03	1.79 ± 0.92
I/R+PG			
Ipsilateral	6	0.27 ± 0.05^	1.53 ± 0.17*^
Contralateral		0.19 ± 0.03	1.19 ± .013
I/R+BNA			
Ipsilateral	6	0.18 ± 0.04 ^^	1.36 ± 0.07*^
Contralateral		0.19 ± 0.02	1.01 ± 0.12
I/R+PG+BNA			
Ipsilateral	6	0.17 ± 0.06^^	1.20 ± 0.32*^^
Contralateral		0.11 ± 0.03	1.10 ± 0.27

All values are mean ± SEM (n=6). I/R control, PG, Banaba and their combination were compared with sham operated control (SO)-(* $P< 0.05$, **-$P< 0.01$, ***- $P< 0.001$) while the groups treated with PG, Banaba and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P< 0.01$, ^^^ - $P< 0.001$)

4.2.4 Effect of Trimetazidine (TMZ), PG and their combination on MCAO induced cerebral I/R injury

Animals were randomly divided in five groups containing 30 animals per group. 1) Group 1: Sham operated control group (the filament was only introduced in ICA and removed immediately, animals received saline 2 ml/kg i.p as a vehicle). 2) Group 2: Reperfused I/R Control group (MCAO rats received vehicle). 3) & 4) Group 3 and 4: Trimetazidine (5 mg/kg and 25 mg/kg i.p. respectively) administered 1 h after MCAO followed by 22 h of reperfusion, while 5) Group 5 was treated with the combination of PG (2 mg/kg) and TMZ (25 mg/kg) 1 h after ischemia induction. All these groups are described in section 3.2.2.1.1 and 3.2.2.1.5 of materials and methods.

4.2.4.1. Effect of TMZ, PG and their combination on Neurological Deficit

There was a severe neurological deficit in RI group of animals after 2 h of ischemia and 22 h of reperfusion. Even though TMZ treatment caused mild reduction in neurological deficit, the changes were not significant (P = 0.0587 by Kruskal-Wallis One Way Analysis of Variance on Ranks). Similarly the combination treatment did not reduce the deficit significantly. The median of total deficit score for I/R control group was 3.5. **Table 11.1**

4.2.4.2. Effect of TMZ, PG and their combination on cerebral infarction and brain swelling

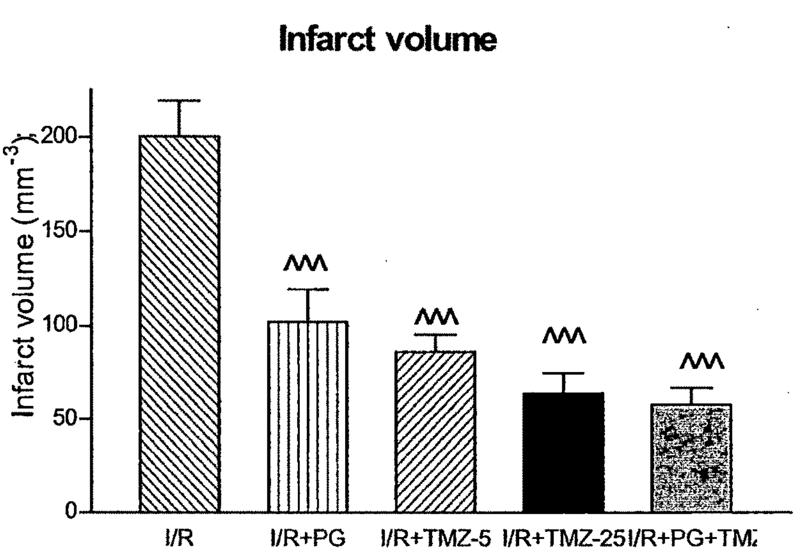


Fig. 11.1 Volume of infarct in ipsilateral hemisphere; ^ Compared to I/R control group; ^^^P < 0.001

Cerebral I/R injury resulted in increased volume of infarction in ipsilateral hemisphere (200.29 ± 18.95) of I/R control animals. Treatment with TMZ (5 mg/kg i.p. and 25 mg/kg i.p.) significantly (P<0.001) reduced the volume of infarct as compared to I/R control animals at the end of 22 h of reperfusion. Similarly, the % brain swelling in treated groups (5 mg/kg i.p. and 25 mg/kg i.p.) was also found to have a significant ($P<0.01$, $P<0.001$ respectively) decrease as compared to I/R control animals. The combination of PG and TMZ showed significant ($P<0.001$) changes in both volume of infarction and brain swelling after the reperfusion injury. **Fig. 11.1 and 11.2.**

4.2.4.3. Effect of TMZ, PG and their combination on Cerebral Oxidative stress biomarker levels:

Treatment with TMZ (5 mg/kg i.p. and 25 mg/kg i.p.) significantly ($P<0.05$) elevated SOD activity as compared to I/R control animals. TMZ (5 mg/kg i.p. and 25 mg/kg i.p.) significantly ($P<0.05$, $P<0.01$ respectively) decreased MDA levels in ipsilateral hemispheres of reperfused animals as compared to I/R animals. Similarly, the GSH levels in the ipsilateral hemispheres of TMZ treated animals were found to have significant ($P<0.05$) increase as compared to I/R control animals. It is worthwhile to note that the CAT activity has not significantly altered after TMZ treatment at the end of 22 h of reperfusion. The treatment of PG and TMZ (25 mg/kg) combination significantly restored the SOD and GSH levels in the ipsilateral hemispheres of the brain, whereas the MDA levels were significantly reduced after the treatment as compared to the I/R control animals. **Table 11.2**

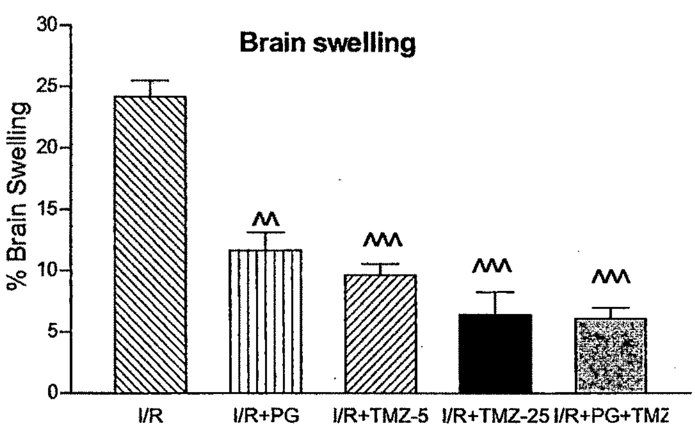


Fig. 11.2 % Brain swelling of ipsilateral hemisphere; ^ Compared to I/R control group; ^^$P < 0.01$, ^^^$P < 0.001$

4.2.4.4. Effect of TMZ, PG and their combination on MPO activity in brain

MPO enzyme activity is a vital biomarker of infiltrated neutrophils in rats. Treatment with TMZ (5 mg/kg i.p. and 25 mg/kg i.p.) and its combination with PG significantly ($P<0.05$ $P<0.01$ and $P<0.001$) reduced MPO activity in ipsilateral hemisphere of treated animals as compared to I/R control animal respectively. **Table 11.3**

4.2.4.5. Effect of TMZ, PG and their combination on BBB permeability assessment

EB content measurement in ipsilateral and contralateral hemispheres assessed BBB permeability. The EB content of ipsilateral hemisphere of TMZ and combination treated animals was significantly ($P<0.05$) reduced as compared to I/R control animals. **Table 11.3**

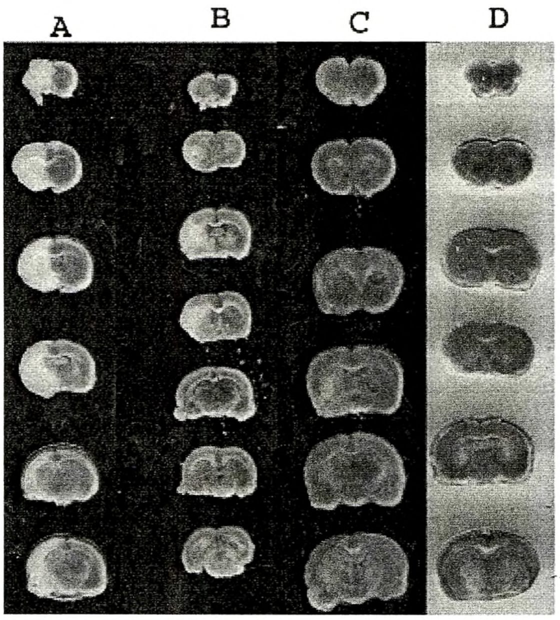
4.2.4.6. Effect of TMZ, PG and their combination on DNA fragmentation

In agarose gel electrophoresis, I/R animals showed strong evidences of DNA fragmentation in the form of DNA laddering pattern which is a characteristic of oligoneucleosomal DNA fragmentation. Treatment with TMZ and its combination with PG showed decreased DNA fragmentation. Although visualization of DNA fragmentation with ethidium bromide is not quantitative, the reduced visualization of fragmentation in treatment group could be attributed to reduced number of apoptotic bodies observed in brain. **Fig. 11.3**

4.2.4.7. Effect of TMZ, PG and their combination on Histological changes

The light microscopy of brain section of Sham operated animals showed continuous intact pattern of the cells, whereas I/R control animals showed disturbed cellular structures, heavy leukocyte infiltration and the presence of cellular edema. The section of the brain in TMZ treated animals showed reduced leukocyte infiltration and edema along with considerable recovery of the cellular structures as compared to I/R control group. **Fig. 11.4**

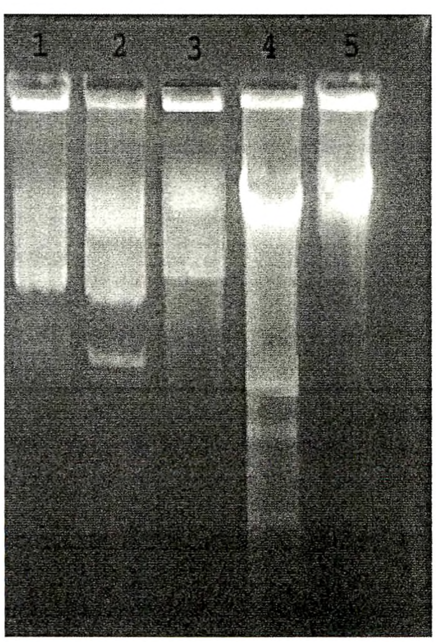
Fig. 11.2 b Infarct volume in the TTC stained brain slices.



Lane A: Brain Slices of I/R control animal; **Lane B**: Brain Slices of PG treated I/R animal; **Lane C**: Brain Slices of TMZ-25 treated I/R animal; and **Lane D**: PG and TMZ-25 combination treated I/R animal.

Fig. 11.3

Agarose gel electrophoresis of rat brain (Ipsilateral) DNA after ischemia/reperfusion injury.

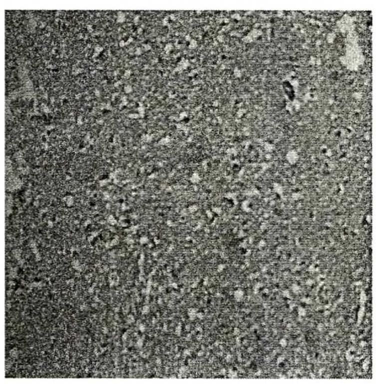


Lane 1, 1 PG and TMZ-25 combination treated animal; **Lane 2**, TMZ-25 treated animal with few band formations; **Lane 3,** PG treated I/R (I/R +PG); **Lane 4,** Ischemic control (I/R control, ispsilateral hemisphere) displaying a laddering pattern; **Lane 5,** Sham control.

Fig 11.4 Photomicrographs (100X) of histological slides (Brain) stained with H& E stain after ischemia/reperfusion injury.

A

B



C

A: TMZ-5 treated group exhibits leukocyte infiltration but restored the cellular structure;

B: TMZ-25 treated animal with few edematous cells and substantial recovery;

C: PG+TMZ-25 treated animals showed neutrophils but overall recovery of the neuronal damage.

Table 11.1

Neurological deficit measurement in TMZ (5 & 25 mg/kg) and its combination with PG (2mg/kg + TMZ 25 mg/kg) treated groupss

Treatment	N	Neurological score
I/R control	6	3.5
I/R+TMZ-5	6	2.5
I/R+TMZ-25	6	2.0
I/R+PG+TMZ-25	6	2.0

Neurological deficit was assessed after 22 hours of reperfusion. The values are represented as median of the individual scores. All values shown are mean ± SEM and n=6. ^ Compared to I/R control group; ^$P < 0.05$

Table 11.2 Effect of TMZ (5 & 25 mg/kg) and its combination with PG (2mg/kg + TMZ 25 mg/kg) treatment on Oxidative stress markers

Groups	SOD IU/mg of protein	CAT H2O2 consu/ min/mg of protein	GSH µg/mg of protein	MDA nmol/mg of protein
SO				
Ipsilateral	9.08 ± 0.57	232.35 ± 5.16	21.39 ± 1.19	5.56 ± 0.29
Contralateral	8.72 ± 0.26	244.30 ± 11.77	21.67 ± 0.98	5.69 ± 0.35
I/R control				
Ipsilateral	4.06 ± 0.53**	185.39 ± 22.92	5.20 ± 0.22***	13.79 ± 1.74**
Contralateral	6.10 ± 0.58	218.75 ±21.49	15.69 ± 0.26	6.83 ± 1.04
I/R+PG				
Ipsilateral	8.24 ± 0.83^	149.41 ± 12.69	9.97 ± 0.82***^	9.18± 1.16*
Contralateral	8.33 ± 0.70	190.8 ± 6.92	16.8 ± 0.51	6.6 ± 0.43
I/R+TMZ-5				
Ipsilateral	7.84 ± 0.26^	213.2 ± 5.10	9.44 ± 0.31***^	7.6 ± 0.34^
Contralateral	9.53 ± 0.73	190.8 ± 6.92	16.8 ± 0.51	6.6 ± 0.43
I/R+TMZ-25				
Ipsilateral	8.64 ± 0.78^	206.2 ± 12.10	10.11 ± 0.31**^^	6.63 ± 0.52^^
Contralateral	9.12 ± 0.56	167.6 ± 9.64	11.34 ± 1.31	6.12 ± 0.69
I/R+PG+TMZ-25				
Ipsilateral	8.95 ± 1.46^^	279.56 ± 20.36	14.84 ± 0.87*^^	6.09 ± 0.67^^
Contralateral	9.56 ± 0.94	209.16 ± 15.79	15.03 ± 1.57	5.93 ± 1.65

All values are mean ± SEM (n=6). I/R control, TMZ, PG and their combination were compared with sham operated control (SO)-(* $P< 0.05$, **-$P< 0.01$, ***- $P< 0.001$) while the groups treated with TMZ, PG and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P< 0.01$, ^^^- $P< 0.001$)

Table 11.3

Effect of TMZ (5 & 25 mg/kg) and its combination with PG (2mg/kg + TMZ 25 mg/kg) treatment on MPO and Blood Brain Barrier permeability estimation

Groups	N	MPO units/mg of protein	Evans blue µg/ml
SO			
Ipsilateral	6	0.12 ± 0.02	0.97 ± 0.19
Contralateral		0.09 ± 0.02	0.87 ± 0.14
I/R control			
Ipsilateral	6	0.52 ± 0.07***	3.86 ± 0.53 ***
Contralateral		0.20 ± 0.03	2.79 ± 0.92
I/R+PG			
Ipsilateral	6	0.27 ± 0.05^	1.53 ± 0.17*^
Contralateral		0.19 ± 0.03	1.19 ± .013
I/R+TMZ-5			
Ipsilateral	6	0.26 ± 0.04^	1.76 ± 0.11 *^
Contralateral		0.19 ± 0.03	1.58 ± .014
I/R+TMZ-25			
Ipsilateral	6	0.18 ± 0.03^^	1.54 ± 0.13 *^
Contralateral		0.15 ± 0.01	1.50 ± 0.1
I/R+PG+TMZ-25			
Ipsilateral	6	0.15 ± 0.04^^^	1.35 ± 0.18 *^
Contralateral		0.16 ± 0.07	1.31 ± 0.15

All values are mean ± SEM (n=6). I/R control, TMZ, PG and their combination were compared with sham operated control (SO)-(* $P< 0.05$, **-$P< 0.01$, ***- $P< 0.001$) while the groups treated with TMZ, PG and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P< 0.01$, ^^^ - $P< 0.001$)

4.2.5 Estrogen and its combination with Lipoic acid on cerebral I/R injury

Estrogen was studied here for its effect in combination with various antioxidants on cerebral I/R injury. This study protocol involved following groups- 1) sham operated vehicle treated group (SO), 2) vehicle treated cerebral reperfusion injury control group (I/R control), 3) Estrogen (8 µg/kg s.c. 24 h before induction of ischemia) treated cerebral reperfusion injury group (Estro), 4) Lipoic acid (100 mg/kg i.p.) administered 1 h after ischemia induction to reperfusion injury animals in this group (Lipo) and 5) co-administration of Lipoic acid (Estro + Lipo) after estrogen injection to the animals with cerebral I/R injury, as described in section 3.2.1.1.2, 3.2.2.1.9 and 3.2.2.1.10 of materials and methods.

4.2.5.1 Effects of Estrogen, Lipoic acid and their combination on Neurological deficit-

It was found that after 22 of reperfusion, I/R injury led to a severe neurological deficit in vehicle treated (I/R control) animals. Estrogen improved the neurological well being of the animals but not significantly as compared to I/R control animals. Lipoic acid administered to the animals pretreated with estrogen was unable to reduce the neurological deficit significantly. **Table 12.1**

4.2.5.2 Effects of Estrogen, Lipoic acid and their combination on infarction and brain swelling

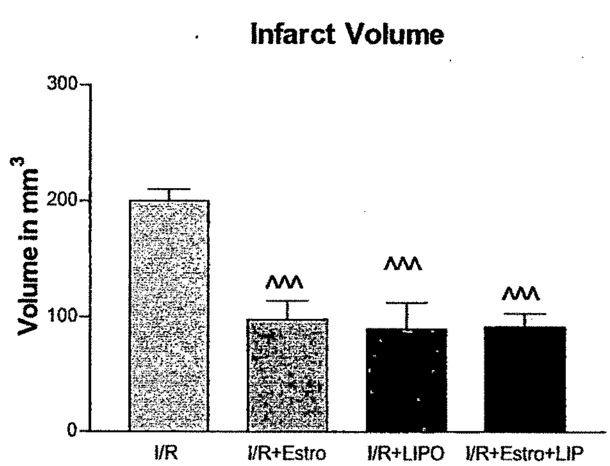


Fig. 12.1. Volume of infarct in ipsilateral hemisphere; ^ Compared to I/R control group; $^P < 0.05$

The volume of infarction and percentage of brain swelling in ipsilateral hemisphere was found to be 200.29 ± 18.95 and 24.19 ± 1.78 % in I/R control animals. Lipoic acid treatment significantly (P<0.01) reduced the volume of infarction (108.83 ± 12.07) and brain swelling (13.20 ± 1.89 %) in ipsilateral hemispheres of the animals. Estrogen treatment of the animals provided marked protection from the injury which was reflected in volume of infarct and the percentage brain swelling of the treated animals. This significant (P<0.001) reduction in infarct volume and brain swelling was further observed in Estro+Lipo group of animals, where the volume of infarct was 91.74 ± 10.32 mm^3 and brain swelling was found to be 9.63 ± 1.39 %. **Fig. 12.1 & 12.2**

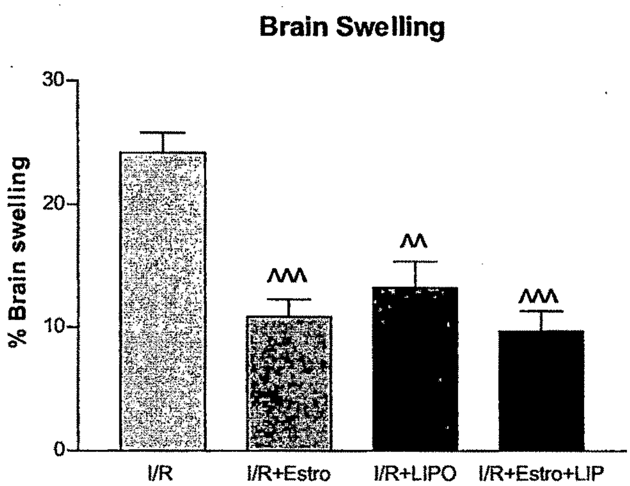


Fig. 12.2. % Brain swelling of ipsilateral hemisphere; ^ Compared to I/R control group; ^^P < 0.01; ^^^P < 0.001

4.2.5.3. Effects of Estrogen, Lipoic acid and their combination on antioxidant biomarkers in brain-

Estrogen treatment had marked effect on oxidative biomarkers after I/R injury. Estrogen significantly (P<0.05) restored the SOD activity in the ipsilateral hemisphere of the animal. GSH levels showed marked increase as compared to I/R control animals after Estrogen treatment. MDA levels were significantly (P<0.01) changed in estrogen treated animals. Lipoic acid treatment to estrogen pre-treated animals showed highly significant (P<0.001) decrease in MDA level and the GSH level was increased. Endogenous antioxidant enzymes like SOD shown significant (P<0.05) elevation in its activity, whereas the change in catalase activity was inconsistent as compared to I/R control group of animals. **Table 12.2**

4.2.5.4 Effects of Estrogen, Lipoic acid and their combination on inflammatory reactivity -

MPO activity was significantly (P<0.001) increased in of I/R control animals as compared to SO animals due to exacerbation of inflammation. Estrogen treatment reduced the MPO activity in ipsilateral hemisphere; similarly there was a highly significant difference in the MPO activity of animals with Lipoic acid administration after estrogen pre-treatment as compared to the MPO activity of vehicle treated animals with I/R injury. **Table 12.3**

4.2.5.5 Effects of Estrogen, Lipoic acid and their combination on Blood brain barrier (BBB) –

Estrogen prevented the EB extravasation and the EB content in ipsilateral hemisphere was found to be 1.78 ± 0.24 μg/ml. Animals treated with Lipoic acid significantly reduced the permeability of BBB and the EB content was restricted at 1.48 ± 0.20 μg/ml. Estrogen with Lipoic acid showed significantly (P<0.01) reduction in the EB content of ipsilateral hemisphere of the treated animal as compared to I/R control animals. **Table 12.3**

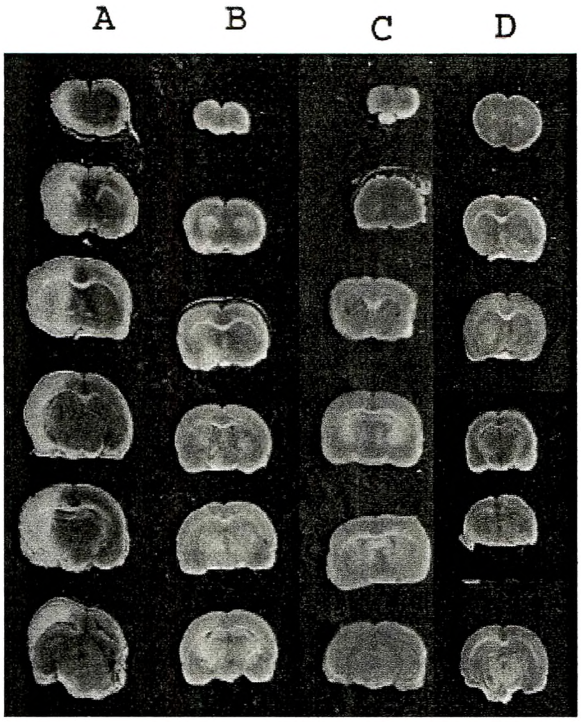
4.2.5.6 Effects of Estrogen, Lipoic acid and their combination on apoptotic cell death-

Vehicle treated I/R animals showed a specific but prominent pattern of band formation, which was due to the fragmentation of DNA, whereas treatment with estrogen and Lipoic acid substantially reduced the fragmentation ladders, formed due to the apoptotic cell induced in the brain by cerebral I/R injury. **Fig. 12.3**

4.2.5.7 Effects of Estrogen, Lipoic acid and their combination on histological features-

The photomicrographs provided the extent of cellular injury exhibited by cerebral hemispheres in vehicle treated groups after induction of I/R injury. This damage was indicated by discontinued neuronal cell architecture, cellular edema and heavy neutrophil infiltration. Treatment with Lipoic acid to estrogen-pretreated animals substantially restored the damage to neuronal cells and decreased edema and neutrophil presence in the cells of the brain. **Fig. 12.4**

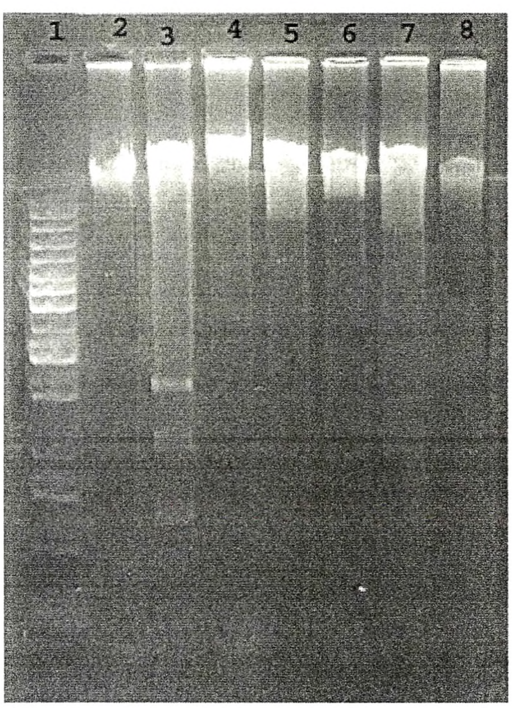
Fig.12.2 b Infarct in TTC stained brain slices



Lane A: Brain Slices of I/R control animal; **Lane B**: Brain Slices of Estrogen treated I/R animal; **Lane C**: Brain Slices of Lipoic acid treated I/R animal; and **Lane D**: Estro and Lipoic acid combination treated I/R animal.

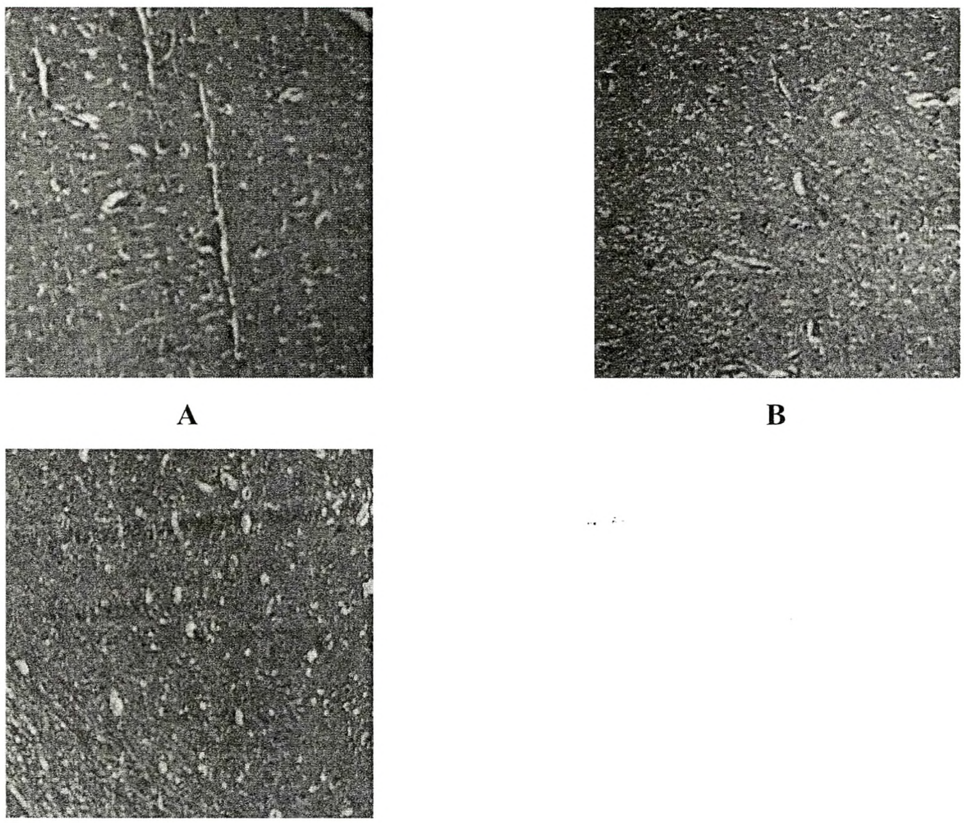
Fig. 12.3

Agarose gel electrophoresis of rat brain (Ipsilateral) DNA after ischemia/reperfusion injury.



Lane 1, 1-kb DNA standard; **Lane 2,** Sham control; **Lane 3,** Ischemic control (I/R control) displaying a laddering pattern a indication of oligointernucleosomal DNA fragmentation; **Lane 4,** Estrogen treated I/R without evident DNA laddering pattern; **Lane 5,** Lipoic acid treated I/R animals: **Lane 6,** Estrogen and Lipoic acid combination treated animal; **Lane 7,** CoQ treated animal showing slight smear; **Lane 8,** PG+CoQ treated animal no DNA fragmentation.

Fig 12.4 Photomicrographs (100X) of histological slides (Brain) stained with H& E stain after ischemia/reperfusion injury.



A B

C

A: Estrogen treated animal with few distorted cells and edema; **B:** Lipoic acid treated animal with neutrophil infiltration and recovered cellular structure; **C:** Estro+LIPO treated animals showed neutrophils and edema in the section of brain. However the cells are recoved of the injury induced damage.

4.2.6 Effect of Estrogen, CoQ and their combination on cerebral I/R-

This study protocol involves five groups- 1) Group 1 was sham operated vehicle treated group (SO), 2) Group 2 was vehicle treated cerebral reperfusion injury control group (I/R control), 3) Group 3 was estrogen (8 μg/kg s.c. 24 h before ischemia induction) treated reperfusion injury group (Estro), 4) CoQ (100 mg/kg i.p. 1 h after ischemia induction) was administered to reperfusion injury animals and this was designated as Group 4 (CoQ).5) In Group 5, CoQ was administered (1 h after induction of ischemia) to the estrogen pre-treated animals. All these groups are described in section 3.2.2.1.9, 3.2.2.1.3 and 3.2.2.1.11 of materials and methods.

4.2.6.1 Effects of Estrogen, CoQ and their combination on Neurological deficit-

Estrogen with CoQ treatment caused reduction in the neurological deficit of the treated animals after 22 h of reperfusion. This decrease in the neurological deficit was found to be significant (P<0.05) as compared to the neurological score of the I/R control animals. **Table 13.1**

4.2.6.2 Effects of Estrogen, CoQ and their combination on Cerebral infarction and brain swelling-

Vehicle treated I/R animals showed greater volume of infarction (200 ± 10.16 mm^3) and brain swelling (24.19 ± 1.78 %). Treatment with CoQ significantly (P< 0.01) reduced the volume of infarction (118.51 ± 16.60 mm^3) and brain swelling (16.54 ± 1.86 %) in ipsilateral hemispheres of the animals after the I/R injury. The effect of CoQ estrogen pre-treated animals was encouraging which showed highly significant (P< 0.01) reduction in infarction volume as well as brain swelling of the animals

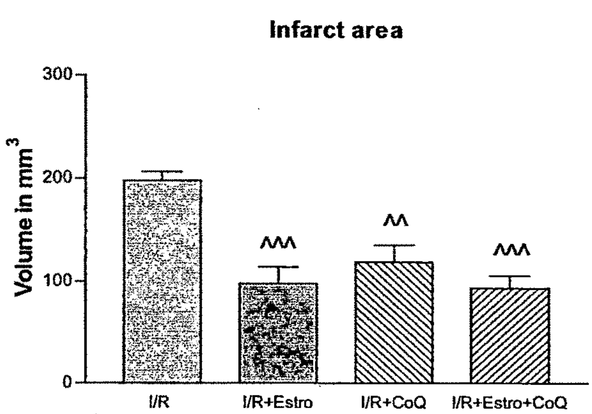


Fig. 13.1 Volume of infarct in ipsilateral hemisphere; ^ Compared to I/R control group; ^P < 0.05

as compared to vehicle treated I/R control animals. **Fig. 13.1 and 13.2**

4.2.6.3 Effects of Estrogen, CoQ and their combination on antioxidant biomarkers in brain-

SOD activity and GSH level were significantly (*P*< 0.01 and *P*< 0.001 respectively) reduced in control animals. The lipid peroxidation was found to have marked increase in vehicle treated control animals. Estrogen with CoQ treatment significantly (*P*< 0.001 and *P*< 0.01 respectively) increased the SOD and GSH levels in treated animals, whereas the MDA content was significantly(*P*< 0.01) reduced as compared to I/R control animals. Catalase levels did not change significantly in any of the groups of this study. **Table 13.2**

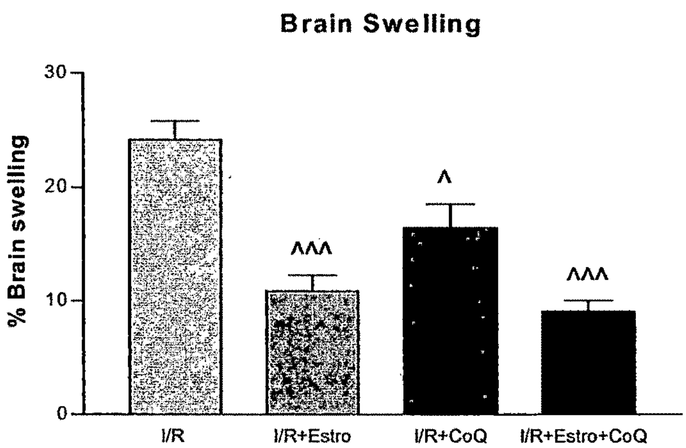


Fig. 13.2. % Brain swelling of ipsilateral hemisphere;
^ Compared to I/R control group; ^*P* < 0.05

4.2.6.4 Effects of Estrogen, CoQ and their combination on MPO activity -

Elevated MPO activity in I/R control animals as compared to SO animals was significantly (*P*< 0.01) reduced by CoQ administration to the animals pretreated with estrogen. Estrogen alone treatment also reduced the MPO activity significantly (*P*< 0.05) as compared to vehicle treated I/R animals. **Table 13.3**

4.2.6.5 Effect of Estrogen, CoQ and on their combination Blood brain barrier (BBB)-

Cerebral I/R injury caused severe EB extravastion and its content was found to be 0.97±0.19 µg/ml in ipsilateral hemisphere of brain of control animal. Treatment with CoQ significantly reduced the permeability of BBB (0.48 ± 0.20 µg/ml). Estrogen with CoQ significantly (*P*< 0.01) decreased the extravasated content of EB (1.20 ± 0.42 µg/ml) in brain of the treated animal. **Table 13.3**

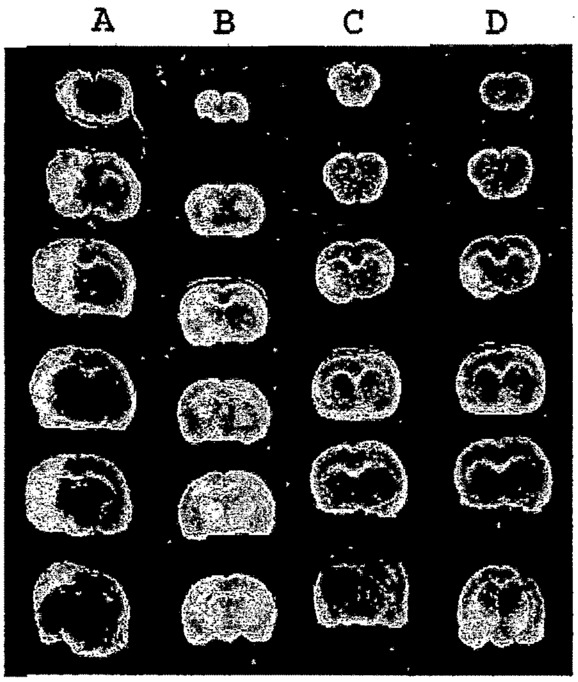
4.2.6.6 Effects of Estrogen, CoQ and their combination on apoptotic cell death-

The apoptotic cell damage observed by laddering pattern in the brains of I/R control animals was restricted due to the combined effect of estrogen and CoQ after the administration of CoQ to estrogen pretreated animals. **Fig. 12.3**

4.2.6.7 Effects of Estrogen CoQ and their combination on histological features-

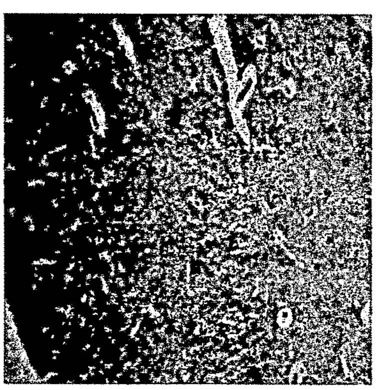
The histological damage observed with photomicrographs was reduced in the estrogen treated animals. This damage represented by the edema formation, neutrophil accumulation and cellular distortion. Estrogen with CoQ administration found to have considerable improvement in the cellular architecture of the observed histological slides of treated animals after I/R injury. **Fig. 13.3**

Fig.13.2 b Infarct in TTC stained brain slices

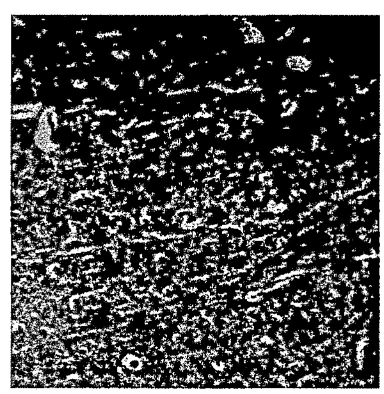


Lane A: Brain Slices of I/R control animal; **Lane B**: Brain Slices of Estrogen treated I/R animal; **Lane C**: Brain Slices of CoQ treated I/R animal; and **Lane D**: Estro and CoQ combination treated I/R animal.

Fig 13.3 Photomicrographs (100X) of histological slides (Brain) stained with H& E stain after ischemia/reperfusion injury.



A



B

A: CoQ treatment reduced the neutrophil infiltration. Edematous cells are present in few numbers; **B:** Estrogen in combination with COQ (Estro+CoQ) markedly recovered the damaged cells. Very few neutrophils and edema cells are visible.

Table 12.1

Neurological deficit measurement in Estrogen (8 µg/kg), Lipoic acid (100 mg/kg) and their combination groups

Treatment	N	Neurological score
I/R control	6	3.5
I/R+Estro	6	2.0
I/R+Lipo	6	2.0
I/R+Estro+Lipo	6	2.0

Neurological deficit was assessed after 22 hours of reperfusion. The values are represented as median of the individual scores.

^ $P< 0.05$ as compared to I/R control group.

Table 12.2

Effect of Estrogen (8 µg/kg.), Lipoic acid (100 mg/kg) and combination treatment on Oxidative stress markers

Groups	SOD IU/mg of protein	CAT H2O2 consu/min/mg of protein	GSH µg/mg of protein	MDA nmol/mg of protein
SO				
Ipsilateral	9.08 ± 0.57	232.35 ± 5.16	21.39 ± 1.19	5.56 ± 0.29
Contralateral	8.72 ± 0.26	244.30 ± 11.77	21.67 ± 0.98	5.69 ± 0.35
I/R control				
Ipsilateral	4.06 ± 0.53**	185.39 ± 22.92	5.20 ± 0.22***	13.79 ± 1.74**
Contralateral	6.10 ± 0.58	218.75 ± 21.49	15.69 ± 0.26	6.83 ± 1.04
I/R+Estro				
Ipsilateral	8.01 ± 0.32^	257.26 ± 63.69	13.47 ± 1.40*^^	8.71 ± 1.18^
Contralateral	7.85 ± 0.80	200.8 ± 17.92	15.21 ± 0.95	7.65 ± 0.51
I/R+Lipo				
Ipsilateral	8.37± 0.94^	230.42 ± 41.10	12.92 ±1.32*^	6.95 ± 0.63^^
Contralateral	8.79 ± 1.25	202.14 ± 40.64	19.34 ± 4.31	6.12 ± 0.69
I/R+Estro+Lipo				
Ipsilateral	8.87 ± 0.71^^	345.62 ± 23.58	14.09 ± 1.54*^^	5.24 ± 0.45^^^
Contralateral	8.01 ± 0.84	333.54 ± 12.65	16.01 ± 1.85	5.68 ± 0.76

All values are mean ± SEM (n=6). I/R control, Estro, Lipoic acid and their combination were compared with sham operated control (SO)-(* $P< 0.05$, **-$P< 0.01$, ***- $P< 0.001$) while the groups treated with Estro, Lipoic acid and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P< 0.01$, ^^^- $P< 0.001$)

Table 12.3

Effect of Estrogen (8 µg/kg.), Lipoic acid (100 mg/kg) and their combination on MPO and Blood Brain Barrier permeability estimation

Groups	N	MPO units/mg of protein	Evans blue µg/ml
SO			
Ipsilateral	6	0.12 ± 0.02	0.97 ± 0.19
Contralateral		0.09 ± 0.02	0.87 ± 0.14
I/R control			
Ipsilateral	6	0.52 ± 0.07**	3.86 ± 0.53***
Contralateral		0.20 ± 0.03	1.79 ± 0.92
I/R+Estro			
Ipsilateral	6	0.21 ± 0.06^	1.78 ± 0.24*^
Contralateral		0.16 ± 0.04	1.15 ± 0.13
I/R+LIPO			
Ipsilateral	6	0.24 ± 0.05^	1.48 ± 0.20*^
Contralateral		0.15 ± 0.01	1.07 ± 0.19
I/R+Estro+LIPO			
Ipsilateral	6	0.18 ± 0.07^^	1.26 ± 0.19*^^
Contralateral		0.14 ± 0.02	1.12 ± 0.31

All values are mean ± SEM (n=6). I/R control, Estro, Lipoic acid and their combination were compared with sham operated control (SO)-(* $P< 0.05$, **-$P< 0.01$, ***- $P< 0.001$) while the groups treated with Estro, Lipoic acid and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P< 0.01$, ^^^- $P< 0.001$)

4.2.7 Effect of estrogen, Banaba alone and their combination on cerebral I/R injury:

Treatment with estrogen and Banaba comprises of five groups- 1) Group 1 was sham operated vehicle treated group (SO), 2) Group 2 was vehicle treated cerebral reperfusion injury control group (I/R control), 3) Group 3 consists of animals treated with estrogen (8 µg/kg s.c. 24 h before ischemia induction) (Estro), 4) Group 4 was the Banaba treated (100 mg/kg i.p. 1 h after ischemia induction) animals with reperfusion injury (BNA). 5) Group 5 Banaba was administered (1 h after ischemia induction) to the estrogen pre-treated animals (Estro + BNA) subjected to cerebral reperfusion injury. All these groups are described in section 3.2.2.1.4, 3.2.2.1.9 and 3.2.2.1.12 of materials and methods.

4.2.7.1 Effects of Estrogen, Banaba and their combination on Neurological deficit-

The I/R control group had severe neurological deficit due to the extent of damage inflicted by the I/R injury. Treatment with estrogen and Banaba caused the change in deficit score of the animals but it was not significant. Similarly estrogen alone and Banaba alone did not cause any significant change in the neurological deficit in treated animals as compared to I/R control animals. **Table 14.1**

4.2.7.2 Effects of Estrogen, Banaba and their combination on Cerebral infarct are and Brain swelling-

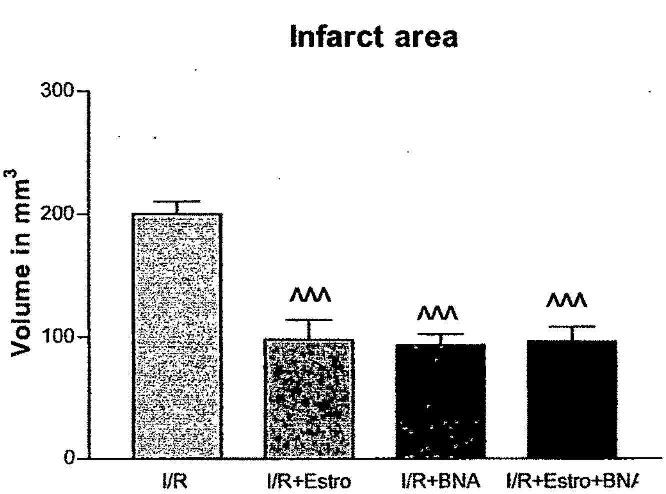
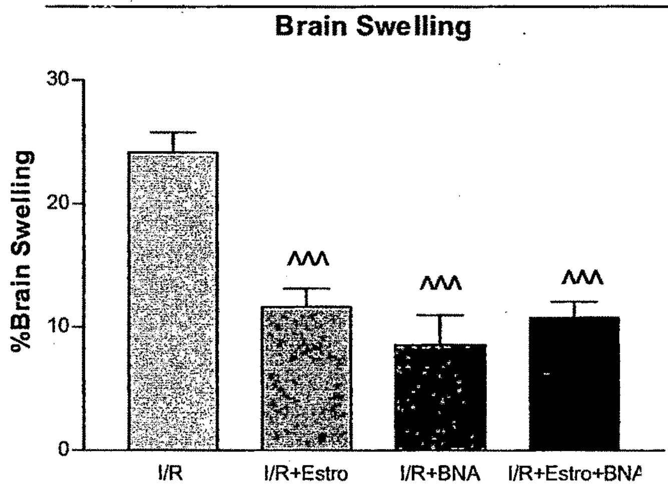


Fig. 14.1 Volume of infarct in ipsilateral hemisphere; ^ Compared to I/R control group; ^$P < 0.05$

Infarct volume was significantly (P<0.001) reduced by estrogen treatment as compared to I/R group. The volume of infarction was found to be (98.17 ± 14.52) and brain swelling (11.64 ± 1.36) in ipsilateral hemispheres of the animals after the I/R injury. The administration of Banaba to estrogen pre-treated animals showed highly significant (P<0.001) decrease in infarct



volume (96.29 ± 9.86) and brain swelling(10.75 ± 1.31%) as compared to I/R control group rendering protection to brain against the I/R injury.

Fig. 14.2 % Brain swelling of ipsilateral hemisphere; ^ Compared to I/R control group; ^$P < 0.05$

4.2.7.3 Effects of Estrogen, Banaba and their combination on antioxidant biomarkers in brain-

Banaba administration effectively reduced the lipid peroxidation (5.88 ± 0.77) and shown significant ($P<0.01$) increase in GSH (11.45 ± 0.87) levels. This Banaba treatment led to significant ($P<0.05$) restoration of SOD activity (8.47 ± 0.90) in the ipsilateral hemispheres of brain in the treated animals. The Banaba administration to the estrogen pre-treated animals showed good antioxidant property with SOD activity of 8.64 ± 0.88 IU/mg of proteins. Catalase change was not significant while MDA level (5.68 ± 0.91) was significantly ($P<0.01$) reduced as compared to I/R control group. **Table: 14.2**

4.2.7.4 Effects of Estrogen, Banaba and their combination on Inflammation in cerebral reperfusion injury-

Inflammation was quantified by MPO activity measurement, which was significantly ($P<0.001$) higher in vehicle treated (0.52 ± 0.07) I/R control animals as compared to SO group (0.12 ± 0.02). MPO activity in Banaba treatment group (0.18 ± 0.04) was significantly ($P<0.01$) reduced; similarly combined treatment of Estrogen and Banaba showed significant ($P<0.01$) decreased the MPO activity (0.16 ± 0.08) in treated animals. **Table 14.3**

4.2.7.5 Effects of Estrogen, Banaba and their combination on Blood brain barrier (BBB)-

After 22 h of reperfusion, EB content in ipsilateral hemisphere of brain of control animal estimated was 0.97± 0.19 μg/ml. Estrogen reduced the EB content in brain to 1.78 ± 0.24 μg/ml. Banaba treated animals showed decreased EB extravasation (1.36 ± 0.07 μg/ml) which was significant ($P<0.05$) as compared to I/R control animals. Combined treatment of Estrogen and Banaba to animals also restricted the EB content in brain significantly ($P<0.01$)

and its content in ipsilateral hemisphere of the treated animal was found to be 1.31 ± 0.42 μg/ml. **Table 14.3**

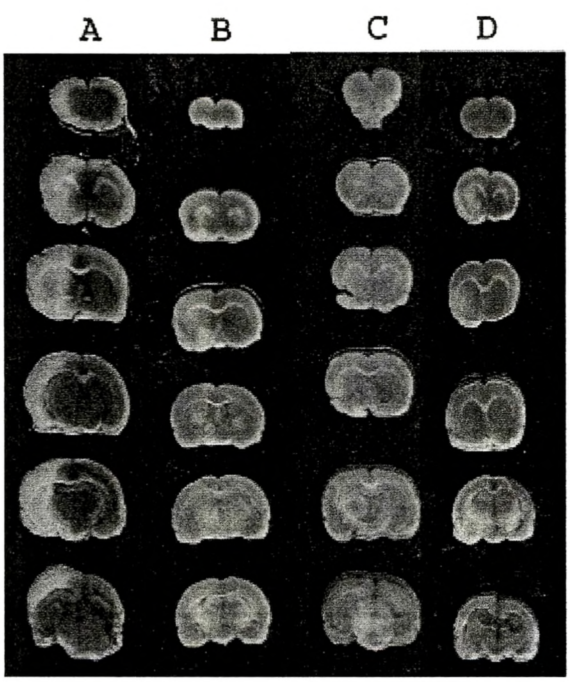
4.2.7.6 Effects of Estrogen, Banaba and their combination on apoptotic cell death-

Banaba and Estrogen combined treatment reduced the fragmentation of DNA in the ipsilateral hemispheres of the treated animals as compared to vehicle treated I/R animals. The induction of cerebral I/R injury resulted in the ladder formation in the neuronal cells due to DNA fragmentation, which was an indication of apoptotic cell death. **Fig. 14.3**

4.1.3.7 Effects of Estrogen, Banaba and their combination on histological features-

Estrogen and Banaba protected the animals from the cellular damage, which was observed by reduced number of infiltrated neurophils and the almost intact cellular architecture after 22 h of reperfusion. **Fig. 14.4**

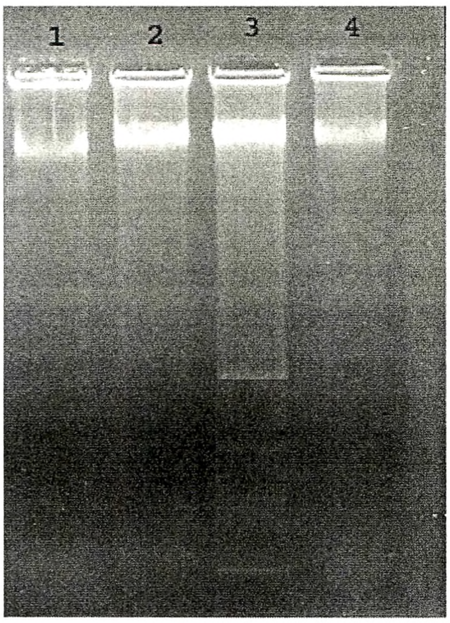
Fig.14.2 b Infarct in TTC stained brain slices



Lane A: Brain Slices of I/R control animal; **Lane B**: Brain Slices of Estrogen treated I/R animal; **Lane C**: Brain Slices of Banaba treated I/R animal; and **Lane D**: Estro and Banaba combination treated I/R animal.

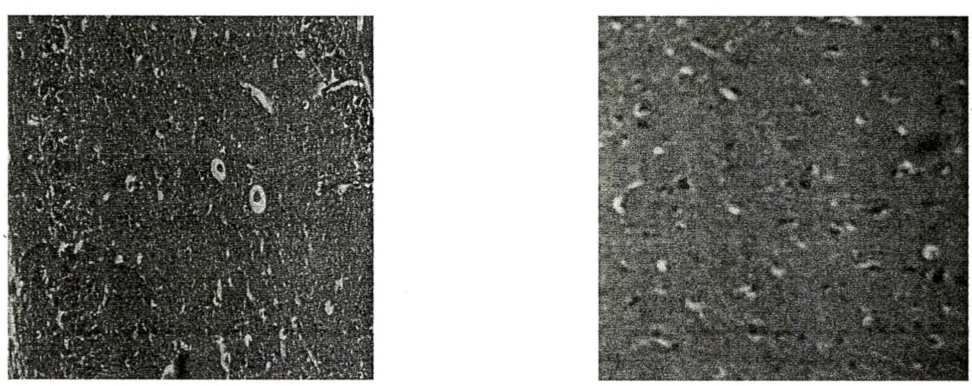
Fig. 14.3

Agarose gel electrophoresis of rat brain (Ipsilateral) DNA after ischemia/reperfusion injury.



Lane 1, Estrogen and Banaba treated animals showing no fragmentation; **Lane 2**, Banaba treated animal; **Lane 3,** Ischemic control (I/R control) displaying a laddering pattern a indication of oligointernucleosomal DNA fragmentation; **Lane 4,** Sham operated animal.

Fig 14.4 Photomicrographs (100X) of histological slides (Brain) stained with H& E stain after ischemia/reperfusion injury.



A **B**

A: Banaba treatment showed few edematous cells and improved cellular structure; B: Estrogen in combination with Banaba (Estro+BNA) showed few neutrophils but the overall cellular architecture is restored.

Table 14.1

Neurological deficit measurement in reperfused and Estrogen (8 μg/kg.), Banaba (100 mg/kg) and their combination treated groups

Treatment	N	Neurological score
I/R control	6	3.5
I/R+Estro	6	2.0
I/R+BNA	6	3.0
I/R+Estro+BNA	6	2.0

Neurological deficit was assessed after 22 hours of reperfusion. The values are represented as median of the individual scores.

^ $P< 0.05$ as compared to I/R control group.

Table 14.2

Effect of Estrogen (8 µg/kg s.c.), Banaba (100 mg/kg i.p) and their combination treatment on Oxidative stress markers

Groups	SOD IU/mg of protein	CAT H_2O_2 consu /min/mg of protein	GSH µg/mg of protein	MDA nmol/mg of protein
SO				
Ipsilateral	9.08 ± 0.57	232.35 ± 5.16	21.39 ± 1.19	5.56 ± 0.29
Contralateral	8.72 ± 0.26	244.30 ± 11.77	21.67 ± 0.98	5.69 ± 0.35
I/R control				
Ipsilateral	4.06 ± 0.53**	185.39 ± 22.92	5.20 ± 0.22***	13.79 ± 1.74**
Contralateral	6.10 ± 0.58	218.75 ± 21.49	15.69 ± 0.26	6.83 ± 1.04
I/R+Estro				
Ipsilateral	8.01 ± 0.32^	257.26 ± 63.69	13.47 ± 1.40*^^	8.71 ± 1.18^
Contralateral	7.85 ± 0.80	200.8 ± 17.92	15.21 ± 0.95	7.65 ± 0.51
I/R+BNA				
Ipsilateral	8.47 ± 0.90^	232.94 ± 18.18	11.45 ± 0.87**^^	5.88 ± 0.77^^
Contralateral	8.49 ± 0.63	161.83 ± 64.52	17.11 ± 1.13	5.55 ± 0.95
I/R+Estro+BNA				
Ipsilateral	8.64 ± 0.88^^	324.24 ± 33.87	12.61 ± 0.98**^^	5.68 ± 0.91^^
Contralateral	7.91 ± 1.23	264.28 ± 19.32	16.79 ± 1.85	5.94 ± 0.24

All values are mean ± SEM (n=6). I/R control, Estro, Banaba and their combination were compared with sham operated control (SO)-(* $P< 0.05$, **-$P< 0.01$, ***- $P< 0.001$) while the groups treated with Estro, Banaba and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P< 0.01$, ^^^ - $P< 0.001$)

Table 14.3

Effect of Estrogen (8 μg/kg s.c.), Banaba (100 mg/kg i.p) and their combination MPO and Blood Brain Barrier permeability estimation

Groups	N	MPO units/mg of protein	Evans blue μg/ml
SO			
Ipsilateral	6	0.12 ± 0.02	0.97 ± 0.19
Contralateral		0.09 ± 0.02	0.87 ± 0.14
I/R control			
Ipsilateral	6	0.52 ± 0.07***	3.86 ± 0.53***
Contralateral		0.20 ± 0.03	1.79 ± 0.92
I/R+Estro			
Ipsilateral	6	0.21 ± 0.06^	1.78 ± 0.24*^
Contralateral		0.16 ± 0.04	1.15 ± 0.13
I/R+BNA			
Ipsilateral	6	0.18 ± 0.04 ^^	1.36 ± 0.07*^
Contralateral		0.19 ± 0.02	1.01 ± 0.12
I/R+Estro+BNA			
Ipsilateral	6	0.16 ± 0.08^^	1.31 ± 0.42*^^
Contralateral		0.14 ± 0.05	1.05 ± 0.19

All values are mean ± SEM (n=6). I/R control, Estro, Banaba and their combination were compared with sham operated control (SO)-(* $P< 0.05$, **-$P< 0.01$, ***- $P< 0.001$) while the groups treated with Estro, Banaba and their combination were compared with the I/R control group (^- $P < 0.05$, ^^- $P< 0.01$, ^^^- $P< 0.001$)