Discussion

5.1 Myocardial Ischemia/Reperfusion injury:

Induction of myocardial I/R injury imparted various changes in the normal myocardium. These changes range from metabolic, homodynamic, biochemical, histological to electrocardiographic alterations. The effect of these basic physiological, metabolic or biochemical alterations may be far reaching and may affect the outcome in the applied models of ischemia and reperfusion injury. The present study showed that these changes do occur in the acute model of I/R injury (1 h of ischemia and 1 h of reperfusion) by using LCA occlusion model for induction of myocardial I/R injury in rats. The induction of injury effectively led to increase in oxidative stress, which was observed by various biomarkers (SOD, CAT, GSH and MDA) in heart. Numerous studies reported that the free radical generated in reperfused myocardium leading to injury mediated by oxidative stress and this injury can be managed by restoring the delicate balance of defensive antioxidant mechanism[368,369]. The generation of reactive oxygen species (ROS) leaves the myo75cardium extremely vulnerable to the ischemic damage. Free radicals react readily with cellular phospholipids and proteins paving the way for lipid peroxidation and oxidation of thiol groups, causing dysfunction of various cellular proteins of the myocardium[241]. The results of the present study showed a significant decrease in SOD activity but catalase activity did not show any substantial change after reperfusion injury. The GSH, with its thiol group experienced extensive oxidation and resulted in severe depletion of the endogenous GSH pool of the I/R control animals. Lipid peroxidation, which is one of the instrumental factors in exerting cellular protein dysfunction, was estimated by measuring the formed MDA levels in the heart tissue. I/R injury cause significant increase in MDA level. The area of infarction was significantly higher in the I/R control animals. However, the area at risk was not substantially different for all the groups in the study. The increase in ischemic area is directly proportional the generation ROS, inflammatory mediators and the extent of ischemia and reperfusion to which the myocardium is exposed. The proposed mechanism for occurrence of arrhythmia has been that free radicals in sufficiently high concentrations damage the sarcoplasmic reticulum[370], thereby causing calcium overload and consequently promotion of calcium-dependent arrhythmias.

The hemodynamic parameters like SBP, MBP and ± d*p*/d*t* were measured using the pressure transducer of the Biopac system. There was a significant fall in SBP and MBP at End-ischemic as well as End-reperfusion periods after the induction of I/R injury. This fall in blood pressure could be attributed to the myocardial hypoxia induced by ischemic milieu[371,372]. Likewise, there is a close inverse relationship among cytoplasmic phosphorylation potential, O_2 consumption, and coronary blood flow[373,374]. I/R injury caused severe decrease in ± d*p*/d*t* at both the observational intervals as compared to the sham operated animals. These results are in consent with the findings of Wang et al, (2004)[341]. This decrease in ± d*p*/d*t* may be initiated by the depressed contractile function caused by the production of oxidative stress during I/R injury[376] and the presence of polymorphonuclear leukocyte at the time of reperfusion[141]. MPO, a PMN derived heme protein, is a biomarker index for neutrophil infiltration and inflammatory reactivity. Role MPO activity plays major role in manifestation of myocardial reperfusion injury[243,354], and MPO activity in the present study showed steep increase in I/R animals[244].

CK activity proved to be a pivotal cardiac marker due to its rapid appearance and marked increase in serum after acute ischemia[237]. CK contains the SH groups which are easily oxidisable at the exposure of ROS[377]. In the present study, CK activity in serum of the animals was increased after the I/R injury. This increase in serum level might be the result of leakage of CK from myofibrils after the period of ischemia and reperfusion, which has resulted in the cellular necrosis. The estimation of CK release is a quantification of infarction and its correlation with histological and an ECG finding establishes the proportionate increase of CK activity to the extent of myocardial damage[238,239].

It is widely accepted that ST segment elevation is a clear index of myocardial ischemia. Epicardial ST elevation in transmural ischemic condition results, when the injury currents flow between the ischemic myocardial regions to normal myocardium[238,247]. ST segment displacement occur due to two major mechanisms at cellular level; one is a localized shorting of duration of action potential and the decreased amplitude of action potential, while the other is related to a localized decrease in resting membrane potential[238]. The present study shows that, the ST segment elevation was evident in all the ischemic animals at the onset of ischemia and was continued till reperfusion, this could be traced back to the subendocardial ischemic injury. Similarly, there was a ST segment prolongation in all vehicle treated I/R control animals. Myocardial ischemia and I/R injury will induce ventricular arrhythmia resulting in the circulation collapse and end in sudden death[378,379]. The underlying pathophysiological mechanisms that have been suggested include a burst production of reactive oxygen intermediates (superoxide radical, hydrogen peroxide, hydroxyl radical, singlet oxygen)[380], and intracellular calcium overload[381] that occurs during early moments of myocardial ischemia and I/R. The incidences of ventricular tachyarrhythmia observed in the present study could be related to the major burst of ROS, which might have generated due the presence of PMN and the other pro-inflammatory mediators like TNF-α and IL-6.

The apoptotic cell death observed in the myocardium was studied by Gel electrophoresis technique. The apoptotic activity was indicated by the ladder formation pattern, which was induced by the internucleosomal DNA fragmentation. Even though apoptosis is influenced by various factors (acidosis, ROS generation) and pathways (mitogen activated protein kinase), other mechanisms do play vital role in stimulating apoptosis. It is well established that the cytoarchitecture of the myocardium is not strictly fixed, but it reorganizes under mechanical stress by undergoing a side-to-side slippage of cardiomyocytes with respect to each other. This process causes a gradual, often eccentric, increase of ventricular volume. Although these changes typically constitute a beneficial adaptation during the acute phase of a cardiac infarction by maintaining critical pump function, such remodeling necessarily results in inefficient pump function that leads, in turn, to gradual hemodynamic deterioration. Additionally, the burden of increased work required by an abnormally high myocardial wall tension is often amplified by a further decreased perfusion of oxygen and nutrients, coupled with a compensatory increase in heart rate in an attempt to maintain adequate cardiac output. A reduced number of myocardiocytes performs all this work but the continued work-related injury further reduces the number of adequately functioning cardiomyocytes by triggering apoptosis[382]. Histologically, reperfused hearts present hypercontracted myofibrils and ruptured cellular membranes. After enduring the regional ischemia and reperfusion, myocardium shows a pattern of contracture and necrotic cell injury, which is also known as "contraction band necrosis"[116]. The histological picture is characterized by the existence of contracted sarcomeres and sarcolemmal disruption, which might be the result of strong and inhomogeneous mechanical forces. In some in situ experiments of transient coronary occlusion in heart, the area of necrosis was composed almost exclusively of contraction band necrosis[383,384]. The extent of contraction band necrosis correlates well with the magnitude of macroscopic myocardial shrinkage during the first few minutes of reperfusion, and with the magnitude of enzyme release occurring during the initial minutes of reflow[117]. However, the heavy neutrophil infiltration and the presence of edema observed in the histological slides of the reperfused hearts in the present study could be attributed to the exacerbated inflammatory reactivity in the hearts exposed to I/R injury.

All these observations conforms the severity of damage inflicted on myocardium of the I/R control animals subjected to reperfusion injury induced by LCA occlusion model in rats.

5.1.1 Effect of Progesterone on Myocardial I/R injury:

Induction of I/R injury inflicted damage the myocardium of the vehicle treated animals that was indicated by the increased infarct area, excessive MDA concentration and reduced activity of SOD. Other biomarkers like CK and MPO activity were significantly increased than sham operated animals indicating the reperfusion injury. In addition, hemodynamic and ECG parameters also provided the vital clues for the conformation of the myocardial damage. Apoptotic activity was increased and histological observations showed presence of edema, fibrosis and infiltration of neurophils.

Treatment with PG showed better cardioprotection in treated rats as compared to vehicle treated rats after I/R injury but this protective effect of PG was not significant. PG treated animals showed significant reduction in inflammatory reactivity, whereas, free radical scavenging ability and the improvement in contractile (increased $\pm dp/dt$) function of the myocardium did not change significantly as compared to I/R control animals. The present study demonstrates minimum effect of PG on blood pressure and heart rate. PG did not alter MAP and heart rate significantly after I/R injury. Blood vessel walls contain smooth muscle cells and an endothelial cell lining. These structures bind estrogen and progesterone with high affinity, and ERs (estrogen receptors) have been identified in both types of vascular cells in women and men[385]. The presence of estrogen and progesterone binding sites in endothelial cells and in the vessel walls have been documented in animal and human arteries. In diseased vessels with dysfunctional endothelium, the release of NO is reduced even after the PG treatment. Thus, these stimuli cause the contraction of smooth muscle-cell (and paradoxical) vasoconstriction[386]. This lead to non-significant change in blood pressure of the treated animals after I/R injury. The (±) dp/dt was decreased during ischemia and continued to decrease after the reperfusion of the myocardium in I/R animals.

This decrease in cardiac contractility leading to fall in ±dp/dt can be linked to the presence of polymorphonuclear neutrophils (PMNs) during I/R[141]. The treatment with PG during ischemia reduced the ST segment disturbances in rats but not substantially, whereas, vehicle treated I/R control rats continued to show the shortened action potential and diminished resting membrane potential visible from the elevated ST segment. The natural progesterone and its derivatives exert a partial androgenic effect[387,388] explaining some of the negative effects observed on cardiovascular risk factors or surrogate markers of risk. However, the moderate cardioprotection provided by the PG treatment could be attributed to coronary relaxation by inhibiting Ca^{2+} mobilization into coronary smooth muscle. PG prevents the multiplication of and migration of smooth muscles, which are involved in formation of plaque that blocks the arteries in the heart[319]. In the present study, the animals of either sex were exposed to the I/R injury and the possible gender specific effect of progesterone was not considered. It is well established that the increased accumulation of the neutrophil in reperfused myocardium increases apoptosis[389]. The agarose gel electrophoresis showed the evident DNA fragments of the reperfused rat myocardium supported by Fliss et al., (1996)[390]. Treatment with PG reduced the intensity of internucleosomal DNA fragmentation in treated animals and similarly the neutrophil content determined by MPO activity was also reduced as compared to the I/R control animals. Thus the effect of progesterone on myocardial I/R injury was found to be mixed one, where the PG treated animals showed cardioprotection which was not substantially better than vehicle treated I/R control animals.

5.1.2 Lipoic acid on Myocardial I/R injury:

Lipoic acid treatment in the present study significantly reduced the myocardial damage caused by the reperfusion injury. Lipoic acid showed significant antioxidant activity, which was evidenced from the increased SOD activity and GSH level as compared to I/R control animals. The MDA that is marker for lipid peroxidation was also significantly reduced. Lipoic acid has been shown to quench hydroxyl radicals, hypochlorous acid, singlet oxygen, nitric oxide radical, peroxynitrite and hydrogen peroxide. It also scavenges superoxide and peroxyl radicals and preventing the initiation of lipid peroxidation[391]. Besides quenching of free radicals, Lipoic acid has few more other antioxidant properties, including the chelation of transition metals and the regeneration of other antioxidants, such as glutathione, α-tocopherol and ascorbate[391]. Mitochondrial parameters are impaired in a more complex way with reduced function in hypoxia as well as in the reoxygenation period[392]. The course of ischemia:hypoxia and reperfusion:re-oxygenation is characterized by a) loss of mitochondrial -SH groups due to oxidative stress, b) an activation of mitochondrial ATP hydrolysis and c) a decrease of mitochondrial ATP synthesis due to impaired coupling of oxidative phosphorylation. Lipoic acid reported to act on each of these factors and possess excellent protective activity. Freisleben et al, (2000)[52] stated that Lipoic acid showed an increase in ATP rat heart mitoplasts and concomitantly reduces the hydrolysis of ATP, thus stabilizing the delicate energy balance during the I/R injury. Since Lipoic acid quenches hypochlorous acid, it ultimately reduces the activity of MPO enzyme. This enzyme regulates the production of hypochlorous acid and works as a potent biomarker for the inflammatory reactivity. The increased level of CK in serum is due the leakage of enzyme into the blood as a result of cellular necrosis[393]. This CK increase was observed in I/R animals and the end of reperfusion period. Lipoic acid is sufficiently hydrophobic to permeate the biological membrane at high rates and the lipophilicity may furthermore be the part of its pharmacological activity in terms of its kinetics and site of interaction. Sensitive thiols of membrane proteins in hydrophobic environment may especially be protected by Lipoic acid via -SH:-S-S- interchange. Hence, an influence of Lipoic acid on the reactivity of mitochondrial thiols, on ATPase activity and ATP synthesis in rat heart mitochondria, and on creatine kinase can be expected. During the early reoxygenation phase, where CK fluxes abruptly decline in the controls indicating either a decrease in flux of phosphate PCr (cellular phosphocreatine) to g-ATP or a loss of creatine kinase. Lipoic acid either maintains the flux of phosphate or impedes the loss of enzyme in heart. However, in Lipoic acid treated hearts also, a transient decline between 40 and 60 min of reoxygenation may appear which is immediately repaired[394]. The significantly decrease in infarct size could be related to all these intriguing factors which in turn restricts the myocardial damage. To addition of its protective antioxidant effects, Haramaki et al (1993)[328], proposed that Lipoic acid improves the cardiac recovery after ischemia.

Apoptosis was influenced by varying levels of the neutrophils and oxidative stress in myocardial I/R injury[395-397]. Lipoic acid treatment in this study reduced the intensity of internucleosomal DNA fragmentation in treated animals and similarly the neutrophil content determined by MPO activity was also reduced which was observed in the histological studies of the heart tissue. Histological observations showed reduced edema formation and marked reduction in neutrophil infilatration in the treated animals. Considering all these results and observation, it can be stated that Lipoic acid, a lipophilic antioxidant agent exhibit a potent cardioprotective activity.

5.1.3 CoQ-10 on Myocardial I/R injury:

CoQ treatment in myocardial reperfusion injury was conducted in the present study. The results of this study indicate the protective action of CoQ on LCA induced myocardial I/R injury in rats.

Crestanello et at (1996)[337], documented that exogenously administered CoQ appears to increase the concentrations of high-energy phosphates, as observed by ATP and phosphocreatine levels during equilibration. This increase in high-energy phosphate levels was not translated into better mechanical function before ischemia. However, during reperfusion CoQ hearts showed higher phosphocreatine, ATP levels but also improved recovery of mechanical function. The improvement in mechanical function during reperfusion could be explained by this increase in aerobic energy production in treated hearts, interestingly it was reported that before ischemia the elevation in high-energy phosphate levels did not improve mechanical function. Here in, our study showed minimal improvement in the hemodynamic parameters, which were not significant as compared to the control animals. This discrepancy could have developed due to acute dose administration of CoQ to the animals exposed to the I/R injury. However, the antioxidant activity of CoQ was clearly evidenced from the favorable changes in all the antioxidant biomarkers estimated during the study. CoQ administration led to increase myocardial CK activity and higher ATP levels during reperfusion[337]. We found that the CK activity was increased in serum due to leakage of the damaged myocardial cells in I/R control animals. The theory of this cellular leakage was contested by Crestanello et at (1996)[337], who earlier provided the observations supporting this very belief of increased serum CK. In our study, treatment with CoQ significantly reduced the serum CK activity as compared to I/R control animals. This decrease in serum CK activity, could be attributed to the better cardioprotection and the reduced oxidation of CK in myocardium by CoQ. In addition to this, CoQ exhibited significant anti-inflammatory effect observed by the reduced inflammatory reactivity.

CoQ prevents myocardial damage during ischemia by preserving cell structure and function, which was observed in histological and apoptotic studies of this experiment. The CoQ may protect reperfused myocardium through its antioxidant and membrane stabilizing properties, as well as through its ability to increase ATP levels to support energy-consuming processes of the myocardium.

5.1.4 Effect of Banaba treatment on Myocardial I/R injury:

The results from the present study demonstrate that the treatment of Banaba (100 mg/kg) protects myocardial I/R injury in rats. Treatment with Banaba showed significant reduction in inflammatory reactivity, better free radical scavenging ability and stabilized ECG (decreased ST elevation and VT incidences) after 1 h of ischemia followed by 1 h of reperfusion. Treatment with Banaba restored MAP but did not show any significant difference to I/R control group. The positive and negative pressure change ($\pm dp/dt$) values were increased but not significantly as compared to I/R control group. The fall in HR in the Banaba treated group was less pronounced as compared to I/R control group but this treatment did not restore HR significantly. A significant ST segment prolongation and elevation was observed in I/R control group as compared to SO group. Epicardial ST elevation in transmural ischemic condition results when the injury currents flow between the ischemic myocardial regions to normal myocardium[238,247].

The free radical generated in reperfused myocardium leading to injury mediated by oxidative stress. This injury can be managed by restoring the delicate balance of defensive antioxidant mechanism[368,369]. The present study demonstrated that the Banaba treated ischemic rats significantly restored SOD activity and GSH levels as compared to I/R control animals. The lipid peroxidation was also reduced in treated animals. The infarct area of the ischemic heart is directly related to the free radical scavenging activity[342,398]. Banaba treatment significantly reduced the infarct area. Inflammatory reactivity due various mediators induced by I/R, precipitates into myocardial functional suppression[241,306]. MPO, which is a biomarker index for neutrophil infiltration and inflammatory reactivity, plays a pivotal role in myocardial reperfusion injury[243,354]. Elevated MPO activity in ischemic rats was significantly decreased by Banaba treatment. CK is an important cellular enzyme mediating energy transduction in muscle cells. The increased level of CK in serum is due the leakage of enzyme into the blood[393]. The proportionate increase of CK activity is linked to the extent of myocardial damage[239,393]. Banaba treatment significantly reduced serum CK levels. Presence of neutrophils and oxidative stress inflences apoptotic activity in myocardial I/R injury[395-397]. Increased accumulation of the neutrophil in reperfused myocardium increases apoptosis[389]. Treatment with Banaba reduced the intensity of internucleosomal DNA fragmentation in ischemic rats.

The cardioprotective effect of Banaba could be attributed to its ability to improve the antioxidant mechanism, reduction in the inflammatory reactivity and apoptotic activity in ischemic animals. One of the major phytoconstituent of Banaba is corosolic acid, which is reported to exhibit potent antioxidant, anti-inflammatory and glucose reuptake activity[284,296,353]. Corosolic acid component of Banaba extract might be responsible for the observed cardioprotective activity in myocardial I/R injury.

5.1.5 Estrogen on Myocardial reperfusion injury:

Treatment of estrogen prior to induction of I/R injury in rats showed potential cardioprotective action. This action of estrogen on the myocardium could be mediated by wide array of mechanisms and its effect on various intriguing factors. Estrogen treatment showed significant change in the contractile mechanism of the heart, as the hemodynamic parameters like MAP, $(\pm)dp/dt$ altered substantially in comparison with the control I/R animals. ST segment changes were considerably stabilized after the pre-treatment of estrogen before 24 of induction of ischemia to the animal. The effect on hemodynamic parameters could be mediated by the estrogen induced vasorelaxation. Estrogen can induce vasorelaxation by endothelium independent and endothelium dependent mechanism[321]. The endothelium independent mechanism occurs by hyperpolarization of vascular smooth muscles mediated by an increase in outward potassium current[399] and competitive inhibition of Ca^{2+} entry through L-type calcium ion channels[400]. Santos et al, (2004)[321], reported that, estrogen stimulates the release of three distinct endothelium-derived relaxing compounds in coronary arteries: NO, PG I_2 and EDHF. The contribution of each of these factors to endothelium-dependent vasodilation varies across vascular beds and also according to which agent is used to stimulate the endothelium. The endothelium, in general, predominantly releases NO in large arteries[401] such as carotid arteries[402], while the contribution of EDHF is more important in smaller resistance arteries[403] contribute to the vasodilating response. Involvement of prostanoids in maintaining vascular tone in the study also can be looked upon. Estrogen treated animals significantly reduced the infarct area after I/R injury. In the present study, estrogen exhibited excellent antioxidant activity; it substantially increased SOD activity and GSH levels, whereas the lipid peroxidation was significantly reduced. CK contains the SH groups which are easily oxidisable at the exposure of ROS[236]. Results showed that the CK activity in serum of the animals was increased after the I/R injury. Estrogen reduced this serum CK activity. Estradiol has been reported to inhibit low-density lipoprotein oxidation and lipid peroxidation of microsomes. Estrogen acts as an antioxidant by chelating iron[404], thus reducing oxygen free radical production via the Fenton reaction. Secondly, it may protect against oxidative stress by regenerating endogenous oxidant defenses. Acute estrogen administration reduces myocardial necrosis, and suggests that, this cardioprotection is, in part, due to the ability of estrogen to attenuate PMN-mediated reperfusion injury. The estrogen found to have potent inhibitory effect on MPO activity. MPO, a PMN derived heme protein, is a biomarker index for neutrophil infiltration and inflammatory reactivity.

Apoptosis is influenced by various factors (acidosis, ROS generation) and pathways (mitogen activated protein kinase). Inflammatory mediators like TNF-α and interleukins play major role in stimulating apoptosis. Similarly, the heavy neutrophil infiltration and the presence of edema observed in the histological slides of the reperfused hearts in the present study could be attributed to the exacerbated inflammatory reactivity in the hearts exposed to I/R. Since estrogen effectively counters oxidative stress as well as inflammation the reduced DNA fragmentation observed in estrogen treated animals could be mediated by the cardioprotective effect of estrogen on myocardial reperfusion injury.

5.1.7 Effect of combined treatment of PG and Lipoic acid on Myocardial reperfusion injury:

The effect of this sex hormone (PG) in combination with a lipid soluble antioxidant (Lipoic acid) was evaluated on myocardial I/R injury. PG and Lipoic acid were simultaneously administered to the animal 30 min after induction of ischemia. The effect was observed for the total protocol duration. After the completion of this I/R injury protocol, the animals were subjected to estimation of various parameters to analyze the effect of this combined treatment. Oxidative stress parameter measurement indicated potent antioxidant effect and reduced MPO activity confirmed the anti-inflammatory activity of combined treatment of PG and Lipoic acid. The hemodynamic parameters showed significant improvement in PG+Lipo group of animals this indicates the improved contractility of combination treatment after induction of I/R injury.

The fall in blood pressure in I/R could be attributed to the myocardial hypoxia induced by ischemic conditions[371,372]. Blood vessel walls contain smooth muscle cells and an endothelial cell lining. These structures bind estrogen and progesterone with high affinity[385]. PG alone treatment shown to have improved vascular tone and this could be attributed to coronary relaxation by inhibiting Ca^{2+} mobilization into coronary smooth muscle. PG prevents the multiplication of and migration of smooth muscles, which are involved in formation of plaque that blocks the arteries in the heart[319]. The decrease in $\pm$ d*p*/d*t* may be initiated by the depressed contractile function caused by the production of oxidative stress during I/R injury[376] and the presence of polymorphonuclear leukocyte at the time of reperfusion[141]. Lipoic acid quenches hypochlorous acid; it ultimately reduces the activity of MPO enzyme, which indicates the decreased inflammation after Lipoic acid treatment. As Lipoic acid is acting as a very potential antioxidant, which has significant effect on inflammation, its combination with PG, which takes care of smooth muscles, could defiantly improve the vascular reactivity of the animal when subjected to I/R injury. All the other related parameters like ST segment changes, CK activity were also influenced by the combined effect of PG and Lipoic acid. Combination of these two agents, lead to significant antioxidant activity in the present study, which is evidenced form increased endogenous antioxidant enzymes and the reduction in lipid peroxidation. Inflammation and the serum CK activity is reduced as compared to I/R control group. These effects of combined treatment lead to reduced apoptotic cell death observed from the reduced intensity of the band formation. The apoptosis is partly governed by ROS generation and these free radicals were scavenged by Lipoic acid and hence the additive effect of PG and Lipoic acid treatment could provide excellent myocardial protection to the animals exposed to I/R injury.

5.1.8 Effect of combined treatment of PG and COQ on Myocardial reperfusion injury:

The combined treatment of PG and CoQ produced non-significant increase in hemodynamic parameters of the treated animals. The ST segment changes were significantly stabilized and the antioxidant activity was prominent as compared to the control I/R animals. This combination treatment exerted favorable effects on various biomarkers like MPO and CK activity. MPO activity was significantly reduced, thereby indicating decreased inflammatory reactivity. The histological observations showed decreased presence of neutrophils and edema cells in the myocytes after the I/R injury. The Gel electrophoteric studies showed the fragmentation of DNA is considerably prevented, which signifies the decreased apoptotic activity in heart.

PG exerted its protective effects through the actions on endothelium may be by direct or indirect mechanism. However, in diseased vessels with dysfunctional endothelium, the release of NO is reduced even after the PG treatment. Thus, these stimuli cause the contraction of smooth muscle-cell (and paradoxical) vasoconstriction[386]. Even if, CoQ has a good effect on energy metabolism which may lead to mechanical function recovery, it may not be able to reverse the fall in MAP and (±) d*p*/d*t* after the induction of reperfusion injury as the dosage schedule for this combined treatment of PG and CoQ was acute. The infarct size of the treated animal was significantly reduced. The effect of the combination treatment on oxidative stress markers and inflammatory bio-index was also significant but these effects never seem to be additive in action to the individual activity of these drugs. The apoptotic activity and histoloical observations showed overall improvement in the cellular structure of the cells after the induction of myocardial reperfusion injury.

5.1.9 Combination of PG with Banaba and its effect on myocardial reperfusion injury:

The results from the group of animals treated with simultaneous administration of PG and Banaba demonstrate that this combined treatment protects myocardial I/R injury in rats. Co-administration showed significant reduction in inflammatory reactivity, potent free radical scavenging ability and stabilized ECG parameters (decreased ST elevation and VT incidences) after 1 h of ischemia followed by 1 h of reperfusion. Administration of PG and Banaba simultaneous after 30 min of ischemia induction restored MAP but did not show any significant difference in treated group after I/R injury. The (±) d*p*/d*t* values were increased but not significantly as compared to I/R group. The fall in HR in the treated group was decreased as compared to I/R control group but this treatment did not restore HR significantly. There was a significant improvement in ST segment changes at both the observational intervals in treated animals as compared to I/R control animals. The combination (PG+BNA) treatment showed better antioxidant activity and reduced inflammation in the treated animals. The effect of Banana on glucose reuptake and oxidative stress[284,296,353], would have assisted in the improvement of hemodynamic parameters by stabilizing the energy balance and reducing the damage to endothelium from oxidants. Moreover, PG could cause coronary relaxation by inhibiting Ca^{2+} mobilization into coronary smooth muscle[319]. Progesterone was also documented to have anti-platelet aggregating effect by releasing NO from the endothelium. From all these studies, it is observed that the combination of PG and Banaba could provide a potent cardioprotective activity to the hearts subjected to I/R injury.

5.1.10 Gender specific effect of PG on myocardial reperfusion injury:

The results from the present study demonstrate that the effect of treatment with PG on myocardial I/R injury was greatly influenced by gender of the animals. Treatment with PG provided better cardioprotection in female rats as compared to male and OVR rats after I/R injury. Females showed significant reduction in inflammatory reactivity, better free radical scavenging ability and the improvement in contractile (increased ±d*p*/d*t*) function of the myocardium and stabilized ECG (decreased ST elevation and VT incidences) after the low dose administration of PG during I/R injury.

The present study demonstrates minimum influence of PG on heart rate in male and ovariectomised rats. However, PG did alter MAP significantly after I/R injury in female rats. The maximal positive and negative first derivative of pressure (±d*p*/d*t*) was decreased during ischemia and continued to decrease after the reperfusion of the myocardium in I/R animals. The treatment with PG markedly improved the contractile function in female rats, whereas males and OVR rats had unaltered contractility after the treatment. The gender based difference on contractile functions is in consent with the reports of Wang et al., (2005)[405] and Jain et al. (2002)[406]. The treatment with PG during ischemia considerably reduced the ST segment disturbances in female rats, whereas male and OVR rats continued to show the shortened action potential and diminished resting membrane potential visible from the elevated ST segment.

Results of this study demonstrated that the PG treated ischemic female rats significantly restored SOD activity and GSH levels as compared to the vehicle treated I/R animals. The lipid peroxidation was also reduced in females treated with PG. However, male and OVR animals exhibited higher MDA levels and low SOD activity along with marked reduction in endogenous GSH pool even after PG treatment. This difference could be attributed to the synergistic effect of low dose of PG and the endogenous circulating estrogen content in the female ratS[36,304,306]. These findings were consistent with the significantly higher myocardial infarct area in male and OVR female animals after PG treatment. Female animals treated with PG have protected hearts against I/R injury, may be due to a diminished inflammatory response[405]. The results showed that the treatment with PG substantially reduced the MPO activity in female rats after I/R. Ischemic male and OVR rats after PG treatment may have lacked the natural beneficial edge over females as far as inflammation is considered, which is in harmony with the accepted views[405,407]. PG treatment significantly reduced serum CK in female animals after I/R injury than that of vehicle treated females. This observation is consistent with stabilized ST segment and elevated GSH levels in females treated with PG. CK activity in males and OVR counterparts treated with PG remains to be on the higher side and it is in consent with the depleted levels of GSH and elevated ST segment.

In present study, agarose gel electrophoresis showed the evident DNA fragments of the reperfused rat myocardium which was also supported by reports of Fliss et al., (1996)[390]. However, PG treated animals showed varying degree of the laddering pattern of DNA. Treatment with PG reduced the intensity of internucleosomal DNA fragmentation in female animals and similarly the neutrophil content determined by MPO activity was also reduced. These findings can state that the effect of PG is different on males and females. Male gender and ischemia could interact crucially and promote apoptotic changes and contribute to gender influenced cardiovascular complications and other diseases[408].

The effect of PG treatment on acute myocardial I/R injury in rats is greatly influenced by gender of the animals. Generally males, females and reproductive females do differ in number of ways, but special aspect of this difference is the circulating levels of estrogen and progesterone. Thus the most discreet justification of the observed differences of the treatment with PG on I/R injury induced myocardial damage is that, PG reduces the myocardial infarct size in intact females but not in males and OVR females. The present study demonstrates the gender specific effect of progesterone on experimentally induced myocardial I/R injury in rats.

5.1.11 Combination of Estrogen and Lipoic acid and its effect on myocardial I/R injury:

The estrogen and Lipoic acid were administered at different time intervals. Estrogen (8 μg/kg s.c.) was injected 24 h before induction of ischemia and Lipoic acid (100 mg/kg i.p) was administered 30 min after the induction of ischemia in the animal. The animal was exposed to the I/R protocol of 2 h and all the investigational parameters were studied. The effect of estrogen and Lipoic acid combination was found to be cardioprotective. The activity of endogenous antioxidant enzyme like SOD was significantly increased, GSH levels was also elevated as compared I/R control animals. The lipid peroxidation, inflammatory reactivity and CK activity in serum showed highly significant decrease as compared to control I/R animals. Hemodynamic and ECG parameters were significantly improved at both the observational intervals. Apoptosis and the cellular structure (observed by histological studies) showed considerable improvement after this combination treatment.

Estrogen can cause vasorelaxation by endothelium independent and endothelium dependent mechanism[321], and thus exerts a major effect on hemodynamic parameters. Estrogen exhibited free radical scavenging activity which could be mediated by chelating iron[404] and regenerating endogenous oxidant defenses[409]. Estradiol inhibits low-density lipoprotein oxidation and lipid peroxidation of microsomes. Lipoic acid, when administered alone also shown cardioprotective effect but this effect is mediated by different mechanisms than that of estrogen. Freisleben et al , (2000)[394] stated that Lipoic acid showed an increase in ATP of rat heart mitoplasts and concomitantly reduced the hydrolysis of ATP, thus stabilizing the delicate energy balance during the I/R injury. Since Lipoic acid quenches hypochlorous acid, it ultimately reduces the activity MPO enzyme. The hydrophobicity and lipoplilicity of Lipoic acid might assist in its ability to permeat biological membrane and improve its kinetics at the site of interaction. Lipoic acid acts chiefly on mitochondria and helps to regenerate and maintain the energy balance of the cell. Lipoic acid either maintains the flux of phosphate or impedes the loss of enzyme in heart, thereby preventing the heart from the injury. Hence estrogen and Lipoic acid had antioxidant effect but this effect was mediated by difference mechanisms for both the drugs. The inflammatory activity also plays a very important role in apoptosis and necrosis. Thus from these observations, it can be said that the cardioprotective activity shown by this combined treatment of estrogen and Lipoic acid could be attributed to the additive effect of different mechanisms of protection in reperfusion injury.

5.1.12 Effect of combined treatment of Estrogen and CoQ on Myocardial reperfusion injury:

The administration of CoQ (100 mg/kg i.p) to the estrogen (8 μg/kg s.c.) pretreated animal provided a moderate cardioprotection. The effect of the combination is not better than individual activity of estrogen or COQ in almost all the investigational observations. The effect on hemodynamic parameters was not significantly different from the other treated or control group. However, the incidences of VT were significantly reduced as compared to I/R control animals. The combination treatment did not affect the ST segment changes signifcantly. The effect on oxidative stress was significant as compared to I/R control group but these values were not better than the individual drug treatment. This effect of the combination (Estro+CoQ) treatment cannot be explained from this particular study. However, Taggart et al, (1996)[401] reported the lack of protection by acute CoQ treatment during in cardiac operations. On the other hand, the role of female hormones is closely linked both to protection and susceptibility to vascular diseases[20]. Estrogen may also influence vasoconstriction depending upon the vascular bed and the site of action by increasing $\alpha 1$ adrenargic activity and/or catecholamine release. Current study found that estrogen has potent antioxidant effect. But the cellular mechanism underlying these major hormonal effects remains to be determined[20]. These few mixed observations may justify the moderate effect of this combination of cardioprotection of heart exposed to reperfusion injury.

5.1.13 Combination of Estrogen and Banaba and its effect on myocardial I/R injury:

Estrogen and Banaba in combination provided the protective effect on myocardial injury. There was significant improvement in ECG parameters during the study. The effects on hemodynamic parameters were substantially different from the control I/R animals. The fall in MAP after ischemia induction was blocked by the combined treatment and the SBP was non-significantly restored. Antioxidant activity was significantly elevated than that of I/R control animals. DNA fragmentation intensity was reduced and the histological studies showed better cellular structure of the cardiomyocytes in treated animals. Free radical scavenging and inflammatory activity of Banaba (due to the presence of corosolic acid) might play a vital role in extending protection to I/R hearts[284,296]. The glucose uptake effect of Banaba may contribute towards maintaining the delicate energy balance during I/R injury. The vasorelaxing activity of Estrogen[321] could be of enormous importance in maintaining and restoring the hemodynamics of the treated animal. Moreover its anitioxidant effects especially on lipid peroxidation, could reduce the oxidant induced damage to the endothelium as well as to cardiac myocytes too. Hence, both estrogen and Banaba found to be cardioprotective with potent antioxidant activity. This protective effect on myocardial reperfusion injury could be attributed to the combined effect estrogen and Banaba in treated animals.

5.2 Cerebral Reperfusion injury:

Cerebral reperfusion injury was induced by occlusion of middle cerebral artery (MACO) model. The reperfusion injury activates the cascade of events leading to energy deficits, inflammation and ultimately resulting in irreversible cerebral injury[1]. Oxidative stress induced by excessive release of glutamate, an excitatory amino acid and neutrophil infiltration following reperfusion, plays crucial role in post-ischemic cerebral insult. The amplification of this severe imbalance in neuron and astroglial cells of ischemic core and penumbra results in progression of brain infarct[133]. The destabilization of the delicate neuronal energy balance causes the depletion of ATP stores in the affected cells. ROS plays a leading role in regulating energy production, apoptosis signaling, inflammatory system, necrosis, redox homeostasis and maintaining vascular tone.

Severe depletion of SOD activity in brain by I/R injury, especially during reperfusion causes severe neuronal damage[277]. Endogenous antioxidant enzymes restrict ROS generation and reduce the cellular damage[49]. The induction of I/R injury caused severe exhaustion of SOD activity and depletion of intracellular levels of GSH in ipsilateral hemisphere of the brain reflecting oxidative stress. Redox regulation of membrane receptor-dependent signals was found to be strongly dependent on the intracellular glutathione level. Major consequence of GSH loss during oxidative stress in brain is the formation of protein glutathione mixed disulfides (PrSSG) and loss of protein thiols[219]. The loss of GSH and formation of PrSSG in the brain results in dysfunction of various membrane functions in cells, such as inhibition of Na+K+-ATPase activity and mitochondrial enzyme activities[327]. Brain has the liability of more easily peroxidizable fatty acids, which consumes an inordinately higher fraction of total oxygen consumption. Moreover, brain lacks an enriched defensive antioxidant system[226]. In the present study, I/R animals showed elevated level of MDA in ipsilateral hemisphere of brain at the end of 22 h of reperfusion as compared to SO animals. There was a severe neurological deficit in I/R animals after 2 h of ischemia and 22 h of reperfusion. Cerebral I/R injury resulted in increased volume of infarction and % brain swelling in ipsilateral hemisphere of brain.

I/R injury lead to an inflammatory response mediated by various cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta and interleukin-6 (IL-1β and IL - 6)[246]. Due to initial interaction between endothelium and circulating leukocytes brain experiences stroke induced inflammation. Increased brain MPO activity after transient focal ischemia virtually reflects the neutrophil infiltration and this neutrophil infiltration into the ischemic brain is implicated in post ischemic brain injury (which was evident from the histological observations[246]. In the present study, MPO activity in the ipsilateral hemispheres of the I/R control animals was significantly increased, indicating the increased inflammatory reactivity. BBB dysfunction may be influenced by hyperthermia-induced increase in inflammatory reactions following ischemic stroke injury leading to infiltration of leukocytes. BBB is highly sensitive to temperature variation and this temperature sensitive nature of BBB is a probable cause of brain damage brought about by inflammatory reactivity. The increased permeability of BBB is responsible for the transfer of leukocyte recruitment mediators across barrier after hyperthermia during cerebral ischemic condition in rats[203]. The results showed that the BBB permeability was increased substantially. The enhanced EB extravasation observed in the brain after the reperfusion injury is a sign of damage to the tight endothelial junctions of the BBB.

Apoptosis takes place in same time period as that of inflammation[169]. Apoptosis is an energy dependent process and hence the pericore regions of affected hemispheres may provide the ideal platform for the programmed cell death. Elevated levels of TNF-α, which has a major role in inflammation, works as an extrinsic pathway for apoptosis. In ischemia, the increased levels of ROS, calcium, glutamate and DNA damage plays a role of intrinsic activators of apoptosis[169]. Apoptosis was observed by the gel electrophoresis study after the isolation DNA from the ipsilateral hemisphere of brain[359]. In agarose gel electrophoresis, I/R control animals showed strong evidences of DNA fragmentation in the form of DNA laddering pattern, which is a characteristic of oligoneucleosomal DNA fragmentation. The light microscopy of brain section of I/R control animals showed disturbed cellular structures, heavy leukocyte infiltration and the presence of cellular edema.

5.2.1 Effect of PG on Cerebral reperfusion injury:

Induction of I/R induced the metabolic, biochemical and neuronal changes in the animals. There was a severe neurological deficit in vehicle treated (I/R control) animals after reperfusion injury. Even though PG 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. The antioxidant biomarker like some endogenous enzymes (SOD & Catalase), GSH and LPO were substantially changed after MCAO induced I/R injury in brain. PG treated animals showed elevated SOD activity and increased GSH level in ipsilateral hemisphere of brain. Lipid peroxidation was significantly reduced but CAT activity was changed after the treatment of PG in the reperfused animals. Similarly PG had an ameliorating effect on the infarct volume of the treated animals, the volume of infarct and the brain swelling of the ipsilateral hemisphere was significantly reduced as compared to I/R control animals. The exogenous PG has been shown to reduce secondary neuronal loss and to attenuate brain edema via mechanism related to the reduction in free radical-induced lipid peroxidation[36]. PG protects brain from the excitotoxicity[319].

MPO activity in ipsilateral hemisphere was markedly increased, indicating the exacerbation of inflammatory response. PG treatment significantly reduces the inflammation in the treated animals. Exogenous PG supplements are reported to suppress IL-1β in cerebral ischemia; this exogenous progesterone may synergies the IL-1β suppression due to presence of endogenous circulating PG[304], thereby exerting potential anti-inflammatory effect. PG mediated enhancement of nitric oxide reduces platelet aggregation and provides neuroprotection in I/R injury[322]. I/R injury caused severe damage to BBB which was observed from heavy EB content in ipsilateral hemisphere of brain. Animals treated with PG significantly reduced the permeability of BBB and the EB content was restricted.

The improvement in BBB function after injury by PG treatment, may be modulated either by inhibiting active ion transport via $Na^{+}K^{+}$-ATPase[411], by influencing the astroglial-inducer or the endothelial-effector cell components of the BBB[412]. It also inhibits the vessel growth associated with leaky BBB function after brain injury. PG reduced the apoptotic activity in I/R injury, this effect may be influenced by the signal transduction pathways in brain. PG elicits the phosphorylation of Akt, a downstream effectors of the phosphoinositide-3(PI-3) kinase pathway and of extracellular-signal regulated kinase (ERK), a component of the mitogen-activated protein kinase pathway. These pathways offer mechanisms for neuroprotection[319]. Hence from all these observations and results, it can be said that PG exerted a neuroprotective effect by its activity to suppress inflammation, reduce lipid peroxidation and activate various neuroprotective pathways in the animals exposed to cerebral reperfusion injury.

5.2.2 Effect of Lipoic acid on Cerebral reperfusion injury:

Lipoic acid was administered to the animals to investigate its effect on cerebral reperfusion injury induced by MCAO model in rats. Lipoic acid, a proven lipid soluble antioxidant showed neuroprotection in this study. The cascade of events activated by reperfusion injury leads to energy deficits, inflammation and ultimately resulting in irreversible cerebral injury[1]. The loss of GSH during oxidative stress in the brain causes the formation of protein glutathione mixed disulfides (PrSSG) and loss of protein thiols[219]. GSH loss and formation of PrSSG in the brain results in dysfunction of various membrane functions in cells, such as inhibition of Na^+K^+-ATPase activity and decreased mitochondrial enzyme activities[327]. Reperfusion injury induced a severe neurological deficit in I/R control animals. Lipoic acid treatment of the animals caused mild reduction in neurological deficit compared to the I/R control animals. Lipoic acid treatment produced potent antioxidant effect may by chelating the metal ions and regenerating the GSH[391]. Lipoic acid scavenges the hypochlorous acid and there by reduces the MPO activity indicating decreased inflammatory response mediated by various cytokines generated during I/R injury[246].

The increased permeability of BBB is responsible for the transfer of leukocyte recruitment mediators across barrier during cerebral ischemic condition in rats[203]. Lipoic acid reduced the BBB permeability, which is evidenced from the decreased EB extravasation in brain. Lipoic acid may have protected the BBB by causing substantial reduction in inflammation and free radical generation leading reduced permeability of the BBB. Elevation in the levels of cytokines, which has a major role in inflammation, works as an extrinsic pathway for apoptosis. In ischemia, the increased levels of ROS, calcium, glutamate and DNA damage plays a role of intrinsic activators of apoptosis[169]. Gel Electrophoresis observations showed the reduction in the DNA fragmentation of brain in Lipoic acid treated animals. The histological observations indicate the reduced accumulation of leukocytes in the brain of the animal. Lipoic acid appeared to prevent the ischemia/reperfusion induced loss of GSH from cortex, striatum and hippocampus of brain[37].

There are evidences from animal and cell culture studies that Lipoic acid can counter excitotoxic damage; Lipoic acid caused a marked reduction in the volume of NMDA-induced lesions in the striatum and protected hippocampal neurons[413]. Hence the neuroprotection exhibited by the Lipoic acid in reperfusion injury could be attributed to its explicit ability to scavenge the free radicals, reduce inflammation and to counter the excitoxicity induced by ischemic insult.

5.3 Effect of CoQ on cerebral reperfusion injury:

CoQ was studied for its effects on reperfusion injury in brain. Our results showed that reperfusion injury induced severe imbalance in the endogenous antioxidant defence mechanism in brain. CoQ treatment significantly increased SOD activity and the endogenous GSH pools after the I/R injury. Endogenous SOD activity in the brain is reported to reduce the ischemic infarct area[277]. CoQ treatment improves brain metabolism by reducing acidosis in brain and accelerating recovery of the ATP level. Zhao et al, (1996)[414] reported that CoQ acts as a potent free radical scavenger during oxidative stress in cerebral ischemia in gerbils. CoQ treatment prevented the development of both neurological deficits and detectable histological damage in brain tissue of rabbits[415]. Both enzymatic and nonenzymatic antioxidants appeared to be markedly restored following CoQ treatment in our experiment. Ameliorated expression or preservation of endogenous antioxidants in the brain may result in improvement of histologically detected features of neuronal damage in reperfusion injury and decreased infarcted area in global ischemia.

In response to oxidative stress, neuronal cells may undergo necrosis or apoptosis. Excessive glutamate exposure is a common feature of both the processes. The withdrawal of survival factors like absence of depolarising level of extracellular potassium or via direct activation of the death cascade in neurons may activate the apoptotic process[416]. In apoptotic process mitochondria play a crucial role. Both pro- and anti-apoptotic proteins such as Bcl-2 family and Bax respectively are localised in the outer mitochondrial membrane[417]. Other factors responsible for apoptosis are leakage of cytochrome-c, increased membrane permeability and its depolarization. Cytochrome-c released from mitochondria activates caspase-3, the protease as a main factor in apoptotic process. This caspase-3 enzyme whose activation requires Apaf-1 protein, initiates an apoptotic cascade[418]. The highest levels of

ROS are found in necrotic process. However, the increased ROS production may also be involved in apoptotic events. Scavengers of free radicals improve cellular respiration in neurons undergoing apoptosis[419]. Therefore it is most likely that, CoQ may prevent brain not only from necrotic but also from apoptotic insults. CoQ can reduce brain neuronal damage, as it was observed in our histological studies. CoQ, known as a free radicals scavenger improves the redox potential in the brain. It seems to be one of mechanisms of CoQ which modulates the severity of oxidative stress and speeds up the recovery of brain metabolism after ischemic insult. Thus the activity of CoQ is predominantly governed by its potent antioxidant potential in cerebral injury.

5.2.4 Effect of Banaba on reperfusion injury in brain:

Banaba was investigated for its effect on cerebral reperfusion injury in MCAO induced ischemia in forebrain of rat. Banaba reported have excellent glucose uptake activity[296]. The treatment caused significant reduction in oxidative stress and inflammatory reactivity in ipsilateral hemisphere of brain. SOD and GSH levels were improved significantly, whereas the lipid peroxidation was restricted by Banaba treatment in treated animals as compared to I/R control animals. Role of oxidative stress and lipid peroxidation in inducing damage during cerebral reperfusion injury is well reported by various researchers[219].

Generation of ROS during reperfusion injury plays a leading role in regulating energy production, apoptosis signaling and inflammatory reactivity. Toran-Allerand (1999)[420] reported that some of the hormones upregulate expression of insulin-like growth factor (IGF-I) in glia, which could also modulate the astrocytic response to trauma and protect the neuronal cells from injury. Banaba reported to have the insulin like effects and it is also reported to activate the glucose uptake in the cells2[77,296]. This activity of Banaba might have some beneficial effects on the energy metabolism of the cells under stress condition and can provide the much needed energy supply to withstand the hypoxic conditions in reperfusion injury.

Cerebral injury instigates various inflammatory pathways. The cytokines generated due to these pathways unleash the excessive inflammatory reactivity in the brain[246]. These cytokines along with the neutrophils leads to formation of hypochlorous acid, which was a component in the production of MPO enzyme. MPO is widely accepted as a reliable biomarker of inflammation[246]. Banaba significantly reduced the MPO activity in ipsilateral hemisphere of brain of the treated animal as compared to I/R control animal. Banaba reduced the BBB permeability as well as the apoptosis in the treated animals. This protection of the brain by Banaba could be mediated by reduced oxidative stress and decreased inflammatory reactivity. ROS and inflammation plays a pivotal role not only in BBB damage but also in apoptosis[169,204]. Thus Banaba has a potent neuroprotective effect in cerebral reperfusion injury, which could be governed by antioxidant and anti-inflammatory activity.

5.2.5 Effect of Trimetazidine and its combination with PG on cerebral reperfusion injury:

Trimetazidine reported to control the oxidative stress, prevents I/R induced myocardial damage to mitochondrial respiration and exerts an anti-ischemic effect in hearts subjected to ischemia[100,344]. TMZ is reported to preserve the cellular homeostasis by controlling intracellular acidosis and calcium overload in the cells there by conserving the valuable ATP stores to meet the energy requirements in heart[100]. The pathogenesis of cerebral ischemia and myocardial ischemia leading to array of critical cellular changes has few similarities. TMZ is lipophilic and crosses blood brain barrier[349] and hence, TMZ may work as a neuroprotective agent in cerebral I/R injury.

Our findings showed that, TMZ significantly elevated SOD activity as compared to I/R control animals. Depletion of SOD activity in I/R injury, especially during reperfusion causes severe neuronal damage[277]. The marked reduction in SOD activity in ipsilateral hemisphere was restored due to upregulation of SOD enzymatic activity in ischemic brain by TMZ treatment. Similarly the combination of PG and TMZ was also able to significantly elevate the SOD activity. Significant deficit in intracellular level of GSH, a thiol antioxidant reflects oxidative stress. The treatment with TMZ significantly restored the GSH content in ipsilateral hemisphere. Brain is highly susceptible to lipid peroxidation. TMZ treated animals showed reduced level of MDA in ipsilateral hemisphere of brain at the end of 22 h of reperfusion as compared to I/R control animals which is in consent with the reports of Gumuslu et al.(1997)[421] and Serteser et al.(2002)[422]. TMZ is reported to inhibit the activation of lipid peroxidation in brain[346]. The combination treatment of PG and TMZ exhibited better antioxidant property as both, PG and TMZ reported to reduce the damage induced by attenuating the free radical induced lipid peroxidation in brain[36].

Neutrophil infiltration in brain leads to inflammation during stroke. In the present study, TMZ and combination treated animals exhibited a significant decrease in MPO activity as compared I/R control animals. Enhanced BBB permeability is a probable cause of brain damage brought about by inflammatory reactivity. The increased permeability of BBB is responsible for the transfer of leukocyte recruitment mediators across the barrier. In the current study, TMZ reduced BBB permeability and MPO activity in treated animals, indicating the protection of BBB. However, PG exhibited the anti-inflammatory activity by suppressing IL-1β[306]. PG may improve the BBB functioning via active ion transport inhibition or by influencing the endothelial-effector cell components of BBB[412]. Thus the combination treatment prevented BBB damage by individual approaches, which were mediated by the different mechanisms exhibited by TMZ and PG in cerebral reperfusion injury.

Elevated levels of TNF-α, which has a major role to play in inflammation, works as an extrinsic pathway for apoptosis. Oligonucleosomal DNA fragmentation, which is a characteristic feature of apoptosis, was reduced in ipsilateral hemisphere of TMZ and combination treated animals. TMZ may provide the neuroprotective effect by the prevention or at least by restricting apoptotic cell death to some extent in cerebral I/R injury. However, the effect of PG on apoptosis is mediated by influencing the various signal transduction pathways in brain[319].

The neuroprotective effects of TMZ might be governed by multiple mechanisms. TMZ produced potent ROS scavenging activity and further attenuated infarct size. Reduced leukocyte infiltration restricted the damage induced by inflammatory reactions in post ischemic injury. TMZ increases glucose utilization by upregulating [^{3}H] glucose uptake in the brain and exert central beneficial effects[423]. Moreover, the combined treatment of TMZ and PG provided better protective effect, if not highly significant, because of the individual and unique approach of PG and TMZ to neuroprotection in cerebral injury. Hence TMZ can also be used as a safe and potent neuroprotective tool in transient cerebral I/R injury induced neuronal damage, whereas the combination treatment of TMZ and PG should be studied more elaborately to truly justify the real neuroprotective potential of this treatment in cerebral injury.

5.2.6 Effect of estrogen on cerebral reperfusion injury

Estrogen was administered to the animals 24 h before the induction ischemia. Estrogen was studies for its effect on neurobehavioral activities, impact on oxidative stress and inflammation as well as for its influences on apoptosis and BBB damage.

Goodman et al.(1996)[426] showed that pre-treatment with estrogen could prevent excitotoxic and oxidative stress injury and enhance cell survival in cultures of embryonic hippocampal neurons. Estrogen possesses an antioxidant activity. The present study reports, that endogenous antioxidant levels were increased in brain, whereas lipid peroxidation was reduced due to the estrogen treatment of the rats. In culture preparations it induces the growth and proliferation of reactive astrocytes[425] to scavenge free radicals and other toxic by-products of injury in addition to providing trophic factor support to damaged nerve cells. Estrogen can also upregulate expression of insulin-like growth factor (IGF-I) in glia, which could also modulate the astrocytic response to trauma (Toran-Allerand, 1999). After injury to the adult brain, estrogen administration enhances the outgrowth and regeneration of dendritic spines and axonal synapses[420]. The presence of higher levels of estrogen at the time of injury might protect nerve cells from secondary, excitotoxic damage caused by ischemia and thus lead to reduction of cognitive deficits[357,426]. We observed that estrogen improved the neurological recovery of the animal but the neurological deficit decrease in treated animals was not significant as compared to the I/R control animals.

The damage to BBB was significantly reduced by the estrogen administration. Apoptotic activity observed by the oligoneucleosomal DNA fragmentation was considerably reduced in the treated animals as compared to I/R control animals.

Estrogen has the explicit ability to affect protein synthesis that is involved in inhibiting and promoting injury-induced apoptosis. This latter effect (apoptosis) can be attenuated by the ability of estrogen to reduce oxidative stress and by down-regulating the expression of apoptosis-triggering genes.

These results are consistent with other work[424], showing that estrogen treatment might enhance both morphological and cognitive recovery in brain-damaged rats after induction of cerebral reperfusion injury

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5.2.7 Effect of PG in combination with Lipoic acid on reperfusion:

The combination of Lipoic acid with PG was found to have significant protective effect on the neuronal cell damage induced by reperfusion. Infarct area of the treated group was significantly reduced as these both the agents have moderate to higher antioxidant activity. However, this activity could be mediated by different mechanisms. Treated group showed increased SOD activity while catalase activity was unaffected by the combined treatment of this hormone with an antioxidant. Lipid peroxidation was also reduced in the animals as compared to I/R control animals. Inflammation, apoptosis was significantly decreased after the combined effect of PG and Lipoic acid. The damage induced to BBB was caused by inflammation and oxidative stress[169]; this damage was reduced as both the drugs have good free radical scavenging and anti-inflammatory activity[36,37].

Hence, the neuroprotection provided by the combined treatment could be mediated by the enhanced antioxidant effect and the reduced excitotoxicity. Lipoic acid extends its antioxidant action to brain by restoring endogenous GSH pool and increasing the high phosphate content of the neuron in treated animal[37,391], whereas, PG attributes its antioxidant action to reduced lipid peroxidation in brain. Moreover, Lipoic acid may synergies the activity of PG on excitotoxicity, as Lipoic acid can reduce NMDA induced lesions. PG is reported to decrease the excitotoxicity of the reperfused brain[319]. Thus, these two agents can provide the potent neuroprotective activity if given in combination after I/R injury.

5.2.8 Effect of combination of PG with CoQ on brain in reperfusion injury

Effect of PG with CoQ combination was significantly different from that of I/R control animals. The free radical scavenging activity of this combined treatment was significantly increased as SOD and GSH levels were elevated and lipid peroxidation was reduced after the treatment, while, CAT activity was not showing any marked change as compared to the I/R control animals. CoQ is reported to improve the brain metabolism by improving the high energy ATP content of neurons and reducing the acidosis[410]. PG may play its part in reducing the oxidative damage by decreasing the lipid peroxidation in brain. CoQ and PG combined treatment treatment significantly reduced the inflammation and BBB permeability. CoQ is documented to have protective effect on apoptotic cell death and inflammation reduction could be a crucial component of this activity on brain[419]. Thus the effect of CoQ on oxidative stress mediated apoptosis and PG's effect on excitotoxicity could improve the individual protective activity of these agents when administered simultaneously. Hence, the combination of CoQ and PG would have given better neuroprotective effect than the PG and CoQ alone treatment of the rats in reperfusion injury. However, the combination treatment did not show significant change in almost all the investigational parameters as compared to PG and CoQ alone treatment.

5.2.9 Banaba with PG on cerebral reperfusion injury:

Effect of Banaba with PG on cerebral injury was found to be protective but this activity was not significantly different from Banaba alone treatment. The combined treatment had significant antioxidant effect, and this effect could be driving factor in the neuroprotective activity in the reperfused animals. Banaba in combination with PG restricted the inflammatory reactivity and reduced the damage to BBB, similarly the histological and apoptotic study observations also confirmed the alleviating activity of this combination in the treated animals. Banaba might affect the neuroprotective activity of this combination by its effect on glucose metabolism and maintaining energy balance in the cells undergoing the stress induced by reperfusion injury[296,353]. PG is reported to reduce excitotoxicity by mediating GABA signaling pathway in specific areas of brain and protects brain[319]. The anti-inflammatory effect of this combination treatment could be influenced by the activity of exogenous progesterone to synergies the IL-1β suppression due to presence of endogenous circulating PG[306]. Hence, the potent protective activity on reperfused brain exhibited by PG with Banaba treatment could be attributed reduced ROS generation and cytokine production during cerebral reperfusion injury.

5.2.10 Effect of Estrogen in combination with Lipoic acid on cerebral reperfusion injury:

The effect of this combination treatment is protective to the brain injury. Especially the oxidative stress and BBB damage was significantly reduced in the ipsilateral hemispheres of the treated animals as compared to the control animals. However, the neurological deficit reduction of the treated animal was statistically not significant. Estrogen provided the neuroprotection by its antioxidant activity and the special ability of the neuronal regeneration after the I/R injury. The activity of Lipoic acid is also based on the free radical scavenging property by thereby increasing the endogenous GSH pool in the brain after injury. This restoration of GSH helps in maintaining the delicate energy balance of the brain metabolism in the due course of injury and the recovery of the neurons from the injury. The role of estrogen and Lipoic acid on excitotoxicity would have had the greater implication on reduced apoptotic activity. Thus the combined treatment of Estrogen and Lipoic acid holds the potential for the development of the maintenance therapy for the reperfusion injury.

5.2.11 Effect of combination of Estrogen with CoQ on cerebral reperfusion injury:

In the present study, CoQ with Estrogen were shown to have protective effect on cerebral injury. Estrogen, as discussed earlier, has multi-dimentional effect on oxidative stress, inflammation and apoptosis in the cerebral reperfusion injury. However, the animals were pretreated with estrogen before the injection of CoQ after 1 h of induction of ischemia to the animals. Thus the already initiated protective effect of estrogen would have augmented the antioxidant and mitochondrial effect of CoQ in the brain. Both antioxidant effects and preservation of mitochondrial function of CoQ may contribute to the observed neuroprotection.

5.2.12 Effect of Estrogen in combination with Banaba on cerebral reperfusion injury:

This combination of Estrogen and Banaba exerted their neuroprotecive effect probably by inhibiting the exacerbation of inflammatory reactivity during reperfusion injury. This inflammation is a major contributing factor towards the BBB damage and apoptosis. Estrogen inhibited the apoptosis by regulating the protein synthesis that is involved in initiating or inhibiting the apoptotic activity[322,424]. Thus the combination acted towards improving the oxidative status of brain and reducing the inflammation induced damage after the reperfusion injury.