Summary and Conclusion

Reperfusion injury of the myocardium is a complex phenomenon consisting of several independent etiologies. During the earliest phase (ie, minutes) of reperfusion, development of cardiomyocyte contracture seems to be the primary cause for necrotic cardiomyocyte injury. Thereafter (ie, minutes to hours), various additional causes can lead to a further increase of cell death either by necrosis or apoptosis, and vascular failure may further aggravate the damage due to a burst of free radicals[3]. Myocardial reperfusion injury in the present study was signified by ECG and hemodynamic abnormalities in the animals subjected to the protocol. The infarct size of the vehicle treated (I/R) control animals found to have increased significantly. Various biomarkers measure for estimation of oxidative stress, inflammation and abnormalities of released enzymes were substantially increased as compared to the Sham control animals.

Apotosis in the myocardial tissue was increased and was observed by Gel electrophoresis. All these parameters indicated the reperfusion injury to the animal.

- Progesterone (PG) was administered alone and in combination with Lipoic acid to the I/R animals. PG in combination with Lipoic acid provided the cardioprotection to the treated I/R animals. The Oxidative stress was decreased as well as there was reduction in the inflammatory reactivity in PG alone treated animals. This led to minimum disturbances in the ECG and hemodynamic parameters during ischemia and perfusion. Ultimately infarct size and apoptosis was restricted by PG treatment. In combination with Liopic acid, the proven antioxidant activity of Lipoic acid would have augmented the effect of PG on reperfusion injury.

  Briefly, it can be said that combination of PG and Lipoic acid accorded cardioprotective effect due to the additive effect on antioxidant activity of both the agents. Improvement in vasorelaxion by PG[319] and restoration of energy balance by Lipoic acid[331] could have attribute to protective activity. However, the cardioprotective activity of combination was not substantially different from the activity of individual agents. Prolongation of the treatment may give better cardioprotection to hearts subject to reperfusion-injury.
- CoQ alone and in combination with PG was evaluated for its activity on myocardial reperfusion injury. CoQ alone exhibited cardioprotective activity owing to its status of potent antioxidant. 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. The effects of combination of PG with CoQ are encouraging. The combination imparted the protective activity due to the additive effect of PG and CoQ on reperfused heart.

- Banaba is a herb which was evaluated for its effect on reperfusion injury for the first time in this study. 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[351,352]. PG was also documented to have anti-platelet aggregating effect by releasing NO from the endothelium. It is observed that the combination of PG and Banaba could provide a potent cardioprotective activity to the hearts subjected to I/R injury.

- Gender specific effects of PG on myocardial reperfusion injury provided a interesting insight to the action of sex hormones on animals in reperfused hearts. The effect of PG treatment on acute myocardial I/R injury in rats is greatly influenced by gender of the animals. PG reduces the myocardial infarct size in intact females but not in males and OVR females. This effect could be attributed to the interaction of PG and endogenous estrogen in the female rats which would have played a decisive role in bestowing cardioprotection to the female reperfused hearts.

- Treatment of estrogen prior to induction of I/R injury in rats showed potential cardioprotective action. 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. 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. The cardioprotective activity of estrogen and Lipoic acid combination could be

attributed to the additive effect of different mechanisms of protection in 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 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, the antioxidant effect of CoQ alone and estrogen alone can be utilized in the chronic treatment or else the change in the time of administration of dose can improve the outcome of the injury.

- 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[351,352]. Moreover, Banaba may maintain the delicate energy balance during I/R injury. Coupled with vasorelaxing and antioxidant effect of Estrogen[321], the combination of Estrogen with Banaba, proved to be a potent cardio-protective tool in reperfusion injury.

Brain damage in stroke is the result of a plethora of highly complex mechanisms leading to infarct maturation. As the brain has a very high demand for oxygen and glucose, a disruption of the circulation in the areas affected by vascular occlusion leads to a depletion of substrates within minutes. 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.

- 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. 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 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.

The effect of combination of PG with Lipoic acid on cerebral injury was significantly neuroprotective as compared to the vehicle treated I/R animals. This effect could be mediated by the reduced excitotoxicity, which was reduced by PG[319] as well as Lipoic acid in reperfusion injury. Moreover the antioxidant activity of Lipoic acid would have provided excellent neuroprotection in the focal cerebral ischemia.

- 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.

Even though CoQ and PG provided good neuroprotective activity individually, the combination treatment did not show significant change in almost all the investigational parameters as compared to PG and CoQ alone treatment. Hence, it can be said that, in the current protocol PG and CoQ did not exhibit any additive or synergistic effect after the simultaneous administration to I/R animals.

- The treatment of Banaba caused significant reduction in oxidative stress and inflammatory reactivity in ipsilateral hemisphere of brain. Banaba reduced the BBB permeability as well as the apoptosis in the treated animals and thus exerted protective activity on brain in focal cerebral ischemia.

Banaba might affect the neuroprotective activity of this combination (PG+BNA) by its effect on glucose metabolism and maintaining energy balance in the cells undergoing the stress induced by reperfusion injury[352]. 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.

- 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 reperfusion induced by

attenuating the free radical induced lipid peroxidation in brain[306]. 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]. TMZ increases glucose utilization by upregulating [^{3}H] glucose uptake in the brain and exert central beneficial effects[423].

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.

- 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. Estrogen improved the neurological recovery of the animal but the neurological deficit decrease in treated animals was not significant as compared to I/R control animals. BBB damage and apoptosis was also reduced in the estrogen treated animals. Thus estrogen protected cerebral tissue from the reperfusion induced damage.

The combination of Estrogen and Lipoic acid had significant reduction in exitotoxicity in addition to their ROS scavenging effect. This combination could provide a platform to develop a potent therapeutic approach towards managing stroke injury.

- Both, antioxidant activity and preservation of mitochondrial function of CoQ may contribute to the observed neuroprotection. Estrogen is also reported to reduced inflammation and excitotoxicity in brain. And these effects of both the agents imparted the neuroprotection in the animals treated with Estro+CoQ treatment.
- Similarly Banaba and Estrogen combination showed reduced damage to the reperfused brain. Banaba reduced the MPO activity signifying the decreased inflammation. Estrogen along with its antioxidant activity and reduced excitotoxicity mediated damage, would have contributed to the neuroprotection of the animals in the treated group.

From all these observations of the study involving two different models of reperfusion injury with distinct mechanism of cellular damage, it can be said that the reperfusion injury can damage the reperfused tissue metabolically as well as structurally. The effect of hormones alone and in combination with various antioxidants on reperfusion injury is encouraging. However, the results can be of clinical importance if the time of administration of dose and the duration of the treatment is change. As we have observed the effect of these combinations in acute dosage schedule, the chronic administration may result in synergistic effects of the individual agents in the combination.

Hormonal treatment with antioxidants provides a unique opportunity to develop a therapy to reduce and restrict the damage induced by reperfusion injury. In future, modifying and combining the presently available therapeutic tools can achieve the proper management of the patients suffering from myocardial and cerebral reperfusion injury.