# CARDIOPROTECTIVE EFFECTS OF ERYTHROPOIETIN HORMONE: A STUDY ON A POSSIBLE ROLE IN HEART FAILURE AND MYOCARDIAL INFARCTION: ELUCIDATION OF UNDERLYING MECHANISMS.

*Thesis*
*Submitted in partial fulfillment for master degree in*
*Basic medical science*
*Physiology*

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Erythropoietin hormone Is a Glycoprotein hormone , mainly produced in the kidneys, is an essential viability and growth factor for the erythrocytic progenitors. Beside its primary role in hemopoiesis , it was found recently that it has some other non-hemopoietic actions on : embryonic development , GIT , endothelial cells , Kidney, malignant cells , CNS , and the heart. In the context of ischemia a cytoprotective effect of Epo hormone has been recently verified. Experiementally erythropoietin can have a dramatic neuroprotective effect in animal models of cerebral ischemia. This protective effect is attributed to an antiapoptic effect on neuronal cells . This raised the possibility that erythropoietin can have an antiapoptotic effect on cardimyocyte as well. Indeed some recent studies have demonstrated that in-vitro isolated perfused hearts exposed to Epo are protected against subsequent hypoxia. These protective actions have also been directly verified On isolated cultured H٩c٢ cardiac myoblasts as well. The other mechanism of EPO cardioprotection is its ability to recruit endothelial cells for proliferation and synthesis of additional blood vessels by the process known as angiogenesis. In this study we demonstrated the protective role of EPO against experimentally induced heart failure and myocardial infarction.

Material and methods :

Forty normal adult age matched male Wistar rats were used in this study, their weight ranged from ٢٠٠-٢٥٠ grams. Animals were kept in the animal facility of Cairo University.

Animals were randomly divided into four groups each one contains ١٠ rats.

- ***GROUP ١:*** CONTROL GROUP

Rats were Injected by vehicle of normal saline(٠.٢ ml) three times per week intraperitoneally for four weeks to standardize all conditions for all animals.

- ***GROUP ٢:*** EPO GROUP

Rats were Injected by Erythropoietin hormone (SEDICO pharmaceutical Co.) subcutaneously (١٠٠ IU/KG) three times /week for four weeks.

- ***GROUP ٣:*** EPO-AD group

Rats were Injected by Erythropoietin hormone subcutaneously in addition to Adriamycin intraperitoneally.

- ***GROUP ٤:*** AD group

Rats were injected by Adriamycin (PHARMACIA Italia S.p.A.) intraperitoneally (٢.٥mg/Kg body weight) in six equal doses over the period of two weeks

Mean ABP using rat tail cuff method and HCT measurements were performed at the beginning and at the end of the experimental period .

The in vivo cardiac functions were studied by the two-dimensional short-axis echocardiography of the left ventricle and M-mode tracings were

recorded to measure LV end-diastolic dimension (LVEDD) and LV endsystolic dimension (LVSD).

$$FS = \frac{\text{End-diastolic dimension} - \text{End-systolic dimension}}{\text{End-diastolic dimension}} \times ١٠٠\%$$

The In vitro cardiac functions were evaluated by the isolated rat heart preparation by measuring HR , LVSP, LVDP, left ventricular developed pressure (LVDP) , rate pressure product RPP the product heart rate×left ventricular developed pressure (HR X LVDP). And maximum rate of pressure rise $\Delta P/\Delta t$ max.

The percentage recovery was calculated by comparing each one of these parameters before and after the induction of myocardial infarction .

Immunohistochemical studies of sections from the four studied groups were performed using Caspase-٣ and CD ٣١ antibodies .

Results :

Body weight significantly increased in control, EPO, and EPO-AD groups , while no significant weight increase was detected in AD group

Recording of ABP among the four groups showed no change from baseline values for control and EPO-AD groups throughout the study. While in EPO group there was an increase in ABP at the end of the study. AD group on the

other hand showed a significant decrease in mean ABP compared to values at the start of the study

Hematocrite was also normal in control and EPO-AD groups both at the beginning and at the end of the study. A significant increase in the EPO group at the end of the study period was observed and on the contrary there was a significant reduction in heart failure AD group

Echocardiography showed that Epo conferred a protective effect on the failing heart reflected on significant improvement of the FS after ٢ weeks compared to AD group after ٤ weeks of therapy this protective role was more obvious in improving the FS of these rats

By comparing the percentage recovery for LVDP, dT/dPmax and RPP among the four groups at ١٢٠ min: EPO treated group showed a functional recovery for the three cardiac performance parameters that was significantly higher than Control, EPO-AD, AD groups

While EPO-AD functional recovery in all three parameters was significantly higher than Control and AD groups.

finally AD group showed a functional recovery in all three parameters which was significantly less than Control, EPO, EPO-AD groups.

Histopathological examination in the present study showed sever features of apoptosis in the AD group , but these features were moderate in Control and EPO-AD groups and mild with EPO group. These findings correlate with the Caspase-٣ activity among the four groups , it was strong in AD group , moderate in Control, EPO-AD , and weak with EPO treated rats which demonstrate the antiapoptotic effect of EPO.

The angiogenic effect was demonstrated in this study by the increase in CD٣١ density in the EPO and EPO-AD groups, compared to weak density in Control, AD groups.

# CONCLUSION

Erythropoietin hormone can be effectively used as prophylaxis therapy in ameliorating deteriorations in myocardial cell functions in heart failure. Its beneficial effects are demonstrated with early usage of the hormone where the hormone can induce immediate as well as long term effects. The hormone however carries the potential upon therapeutic use to induce unwanted expansion of red cell mass. However, the emergence of new generations of therapeutically available forms of the hormone that are devoid of its hemopoeitic effects can potentially extend the use both regarding beneficial doses and efficient durations of therapy. Moreover, Erythropoietin effects in preventing undesired apoptotic cell death are clearly demonstrated in neural cells. Cardiac cells as well are also protected by Erythropoietin against adverse apoptosis occurring in the setting of myocardial infarction. The present work also extended this to demonstrate such limitation and reduction in apoptosis in ischemic complications that can occur on top of heart failure. Erythropoietin hormone has been demonstrated in this study to protect not only normal hearts from infarction but also cases with doxorubicin induced failure. The protective effects of Erythropoietin in heart failure not only was demonstrated to involve antiapoptotic effects which again are clearly demonstrated here but also Erythropoietin was able recruit endothelial cells for proliferation and synthesis of additional vasculature that can cope with the demands of the failing heart in the well known process of angiogenesis. This also adds to the ability of the failing myocardial cells treated with the hormone to withstand ischemic challenges.

# ABSTRACT

Epo hormone has recently shown a protective effects against ischemic damage in different organs such as cerebral and renal ischemia . In this study we demonstrated the protective effect of EPO against the experimentally induced heart failure by Adriamycin treatment and against the experimentally induced myocardial infarction . We evaluated the role of Epo by measuring the improvement of cardiac functions in the rats treated with EPO compared to others Both *in vivo* by short axis echocardiography and *in vitro* by isolated heart perfusion study. The possible mechanisms were also evaluated by immunohistochemical staining and revealed the inhibition of Caspase-٣ activity and the increase in the angiogenic marker CD٣١ in EPO treated animals compared to others.


# RECOMMENDATIONS

١- The use of Erythropoietin Hormone in the prevention of Doxorubicin (Adriamycin ) induced cardiac toxicity.

٢- The use of Erythropoietin Hormone in high risk patients of myocardial infarction.

٣- The use of Erythropoietin hormone in the combined cases of heart failure and myocardial ischemia.