PREFACE

Free radicals are constantly formed in the body their presence in the body in low amount is may play important role in the many biological processes including oxidative brust reaction essential for phagocytes. They also act as second messenger in the in the cellular signaling path way. But production of the Reactive oxygen species or the free radicals in the more concentration causes the damage to the cell and may alter the physiological processes. Free radical pathology may occur when the body antioxidant mechanism can not keep pace with rate at which free radicals and other oxidants are being formed. Thus in present project oxidative stress was generated by H_2O_2.By window method at different stages of Chorioallontoic membrane development for analysis of its stress and effect on the angiogenesis of the Chick CAM. Using free radical scavenger Vitamin C, which also occurs naturally in animals was use to protect the H_2O_2 generated stress using proper control.

H_2O_2 dose, Vitamin C doses were selected using survival, hatching abnormality data while treatment initiation and duration were based on the development of the CAM.

The alterations were evaluated using parameters of lipid peroxidation,glutathione content, formaldehyde content and protein contents.

The results are important for the stress related alterations on embryonic growth especially development of the CAM.

Thus free radical generated Malonaldehyde product of Lipid peroxidation of was studied in CAM of Chick as Thiobarbituric Acid Reactive oxygen species (TBARS).

Similarly stressed metabolism indicator formaldehyde was assayed in CAM. Additionally to evaluate antioxidant Vitamin C supplementation influence on in vivo free radicals scavenger glutathione was also estimated along with total proteins.

Thus under the project H_2O_2(0.5 mM/embryos) was given at 48 hrs.55 hrs,66hrs, 72hrs,88hrs, and 96 hrs and were observed for its impact on CAM angiogenesis of Chick and mortality study. To study the role of antioxidant Vitamin C

(3 mg) / embryo was given with H_2O_2 treatment to improve the mortality and angiogenesis at different stages of the vitelline veins development of the CAM.
The reasons for the dose selection and experimental protocol have been justified.

The alterations in above parameters under the above experimental conditions presented under different section in the following chapters.

Chapter I: Introduction : Which deals with the reasons to take the problem, types of Angiogenesis, The Angiogenesis processes: How do new blood vessels grow?, Growth factors and angiogenesis, Reasons to take chick embryo as an animal model, Reasons to take Chorioallontoic membrane, Reasons to select the developmental stages, Development of Chorioallontoic membrane in birds, Free radicals, Biological significance of the free radicals, Oxidative stress, Free radicals and antioxidants, Free radicals antioxidants ,and angiogenesis, Reasons to use H_2O_2 as a free radical inducing agent, reasons to select doses of H_2O_2 (Mortality based), Generation of H_2O_2 in the body, Vitamin C as a free radical scavenger, Reasons to select doses of Vitamin C, The other parameters also studied are Protein, Lipid peroxidation , Glutathione and Formaldehyde.

Chapter II : Material and Methods: This chapter includes details about animals used in the experimental work, Selected hrs of incubation, experimental protocol, with details of treatment given , bioassay methods of parameters (Protein, Lipid peroxidation, Glutathione , and Formaldehyde).Also the method used for the quantification of the Angiogenesis of the CAM is also given.

Chapter III: Observations and Discussion: Chapter is divided in to following sections.

Section I: Mortality

Section II: CAM- Angiogenesis

Section III: Protein

Section IV: Lipid peroxidation

Section V: Glutathione

Section VI: Formaldehyde

Integrated discussion of the above parameters is presented.

Dissertation ends with the bibliography used in present work.