PREFACE

Free radicals which are naturally formed ($O^{\cdot}$ and $-OH^{\cdot}$) and if increased due to the stress during development aught to have impact on growth and function of brains. Thus in present work stress of H_2O_2 was generated by window method at different stages of brain development to analysis its effect and stress related metabolisms. Using free radical scavenger vitamin C, which also occurs naturally in animals was used to protect the H_2O_2 generated stress using proper controls.

H_2O_2 dose, vitamin C doses were selected using survival, hatching abnormality data while treatment initiations and durations of treatment were based on brain differentiation , development and growth hrs.

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

The results were interpreted to reveal their importance in embryonic brain development.

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

For the reviews presented in the different parts of introduction, the parameters assayed from total embryo in early stages and total brain on its distinct appearance. Thus free radical generated malondialdehyde product of lipid peroxidation was studied in brain as Thiobarbaturic Acid Reactive Species (TBARS).

Similarly stressed metabolism indicator formaldehyde was assayed in brain.

Additionally to evaluate antioxidant vitamin C supplementation influence on in vivo free radicals scavengers glutathione was also estimated along with total proteins.

Thus under this project H_2O_2 (0.5 mM per developing embryo) was given at 24 hrs, 34 hrs, 40 hrs, 48 hrs, 72 hrs, 96 hrs and 120 hrs and were observed for its impact on brain development study mortality and abnormalities on hatching. To study the role of antioxidant vitamin C (3 mg/ developing embryo) was simultaneously given with H_2O_2 treatment to reveal improvement in mortality and abnormalities at the different stages to brain development viz. neural tube closure (24 hrs), formation of three primary brain vesicles (34 hrs), formation of five neuromeres in hind brain (40 hrs), Flexion and torsion (48 hrs), differentiation of different brain regions (72 hrs), enlargement of brain regions (96 hrs) and further enlargement of brain regions (120 hrs). The controls of HBSS (medium of treatment) and of vitamin C (antioxidant/vitamin independent effect) were conducted along with the corresponding normal animals.

The reasons for dose selection and experimental protocol have been justified.

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

Thus this thesis opens with preface and acknowledgement and is arranged further in four chapters.

Chapter I: Introduction: Which deals with reasons to take the problem, selection of the animals , their developmental status, organ, H_2O_2 as free radical inducer, vitamin C as antioxidant, the doses of both free radical free radical scavenger (mortality based). Treatment initiation hrs and hrs of treatment intervals and parameters studied (lipid peroxidation, glutathione, formaldehyde and proteins). Besides it includes relevant review literature on embryonic development, free radical impacts on developing brain, neurons,

role of glutathione its role in free radical scavenger, formaldehyde production under H_2O_2 generated stress condition.

Chapter II: Material and Methods: This chapter includes details about animals used in experimental work, selected hrs of incubation. Integrated experimental protocol with details of treatments given, bioassay methods of parameters (proteins, lipid peroxidation, glutathione and formaldehyde).

Chapter III: Observations and Discussion: Chapter is divided into following sections.

Section I: Mortality and Abnormalities

Section II : Proteins

Section III : Lipid peroxidation

Section IV : Glutathione

Section V : Formaldehyde

Chapter IV: General discussion

Integrated discussion of above parameters is presented.

Dissertation ends with the bibliography used in present project.