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Influence of hydrogen peroxide induced oxidative stress on survival rate of early chick embryo development

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Abstract: Reactive oxygen species (ROS) induced oxidative stress influences embryonic growth and development. Hydrogen peroxide (H_2O_2) is a major source of ROS generation that induces oxidative stress by altering redox status. In present study, direct effect of H_2O_2 generated oxidative stress on survival rate and associated toxicity in chick embryo was studied during early development. Chick embryos at 96 hrs of incubation were treated with single doses of 2.5µg, 5.0µg, 10µg, 50µg, 100µg, 300µg and 500µg H_2O_2 concentrations per embryo. The toxic effects recorded after 144 hrs of development. The results showed that treatments of 2.5 and 5.0µg H_2O_2 doses did not affect survival rate and embryo development. 10µg and 50µg H_2O_2 doses treatment exhibited slightly reduced survival rate without affecting normal morphology. Administration of 100 and 300µg H_2O_2 doses caused predominant decrease in the survival rate in comparison with the normal and PBS treated control embryos with deformities such as growth retardation, defected brain, limbs and vascular development with hemorrhage. Treatment of 500µg H_2O_2 exhibited no survival of embryos. These results indicated that post-omphalomesentric stages of early chick embryos are more susceptible to elevated level of H_2O_2 induced oxidative stress leading to significant reduction in survival rate with associated deformities. These results are discussed with probable reasons.

Key words: Hydrogen Peroxide; Oxidative Stress; Reactive Oxygen Species; deformities Survival Rate.

Introduction

Oxidative stress is associated with various physiological and pathological conditions. It is induced by reactive oxygen species (ROS) that can alter many cellular activities [1]. It can affect embryonic development by influencing cell signaling pathways involved in proliferation, differentiation and apoptosis [2,3]. Hydrogen peroxide (H_2O_2) is a strong oxidizing agent used in a number of medical, industrial and food applications [4]. It is a major source of ROS generation at high concentrations it changes the redox status of surrounding cells that induce oxidative stress [5,6]. The embryo is more susceptible to oxidative stress and teratogens which modify redox status that disrupt fetal development.

The studies on effects of H_2O_2 (0.5mM, 1mM and 1.5mM) and free radical scavenging by vitamin C indicated H_2O_2 influenced mortality with associated toxicity in embryo [7,8]. These doses of H_2O_2 have shown to affect cardiomyocytes of embryonic ventricular regions [9]. Earlier studied concentrations of H_2O_2 viz; 2.5, 5.0, 10, 50, 100, 300 and 500µg also influence vasculogenesis [10]; where early doses have shown non-significant pro-vasculogenic effects while 50µg onwards showed dose dependent vasculogenesis inhibition in survived embryos. In present work similar doses were used to study influence of H_2O_2 induced oxidative stress on survival rate and associated toxicity if any; in early chicken development.

Materials and Methods

Preparation of H_2O_2 solution: The H_2O_2 powder was dissolved in phosphate buffer saline (PBS) to prepare test solutions of suitable concentrations (2.5, 5.0, 10, 50, 100, 300 and 500µg/ml) that can be used for applications during embryonic studies.

Experimental protocol: Fertilized eggs of *Gallus gallus* chick embryo were obtained from local area of Karad (MH, India) and were disinfected with 70% alcohol and incubated at 37^{0}C with 55-60% relative humidity in sterile condition. 20 eggs/concentration of H_2O_2 were used to study the effects. The treatment of H_2O_2 doses to induce oxidative stress was initiated at 96 hrs of incubation, as a single dose of graded concentrations i.e. 2.5, 5.0, 10, 50, 100, 300 and 500µg H_2O_2/ embryo by window method [11]. Control embryos were treated with PBS only. The eggs were incubated further up to 144 hrs of development.

On completion of experiment, the embryos were removed from the incubator and heartbeat of embryos were observed to assess survival rate using dissection microscope. The number of beats per minute from embryos of different groups were recorded. During readings desiccation of embryo was prevented.

The development of embryos was photographed using digital camera (Powershot A2200). Embryos were dissected and washed with PBS. Observations were made for any noticeable morphological abnormalities of treated and control embryos.

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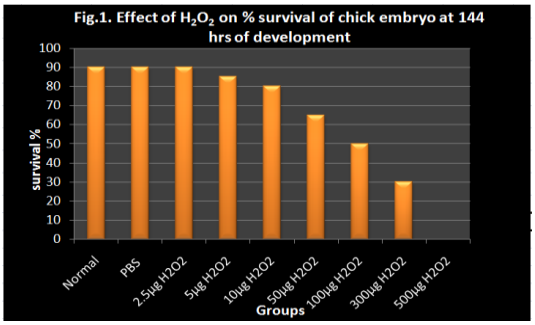
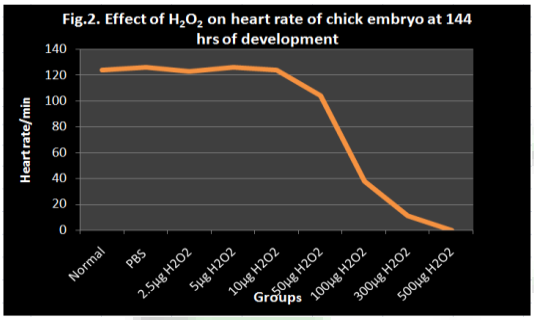
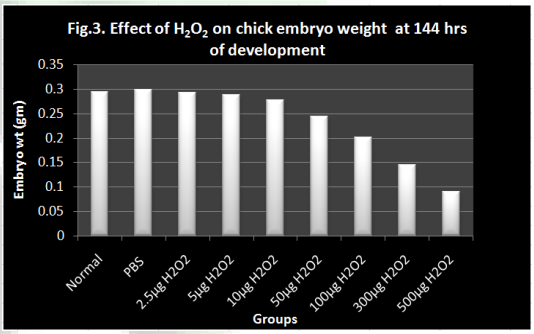
Results

Treatments with various doses of H_2O_2 affected the survival rate of embryos in a dose dependent manner which was prominently decreased with increasing H_2O_2 concentrations as compared with normal and control groups. Fig. 1 shows graphical representation of the survival rate of chick embryos and Fig. 2 showed alterations in embryo weight; while alterations in embryo weight were presented in Fig. 3.





The normal and medium control embryos showed 90% survival and 10% mortality with embryo weight 0.294 gm and 0.299 gm respectively There were no abnormalities observed at 144 hrs of development (Plate I; Fig. B & C). Treatments of 2.5µg and 5.0µg doses of H_2O_2 did not show any toxicity/ deformity and also no change in survival rate as compared to normal and PBS control embryos. Chick embryos when treated with 10µg and 50µg doses of H_2O_2, it showed 15.78% and 31.57% decrease in survival rate as compared to normal and PBS control. Abnormalities such as reduction in size and abnormal brain development (bulky brain) were also evidenced at 50µg H_2O_2 dose. However, treatments with 100µg H_2O_2 showed 44.5% decreased survival rate in comparison with the normal and PBS treated control embryos (Fig.1). All survived embryos showed morphological deformities such as reduced growth, narrow neck, malformed limbs and bulged eye (Plate I. Fig. H). Treatment of 300µg H_2O_2 showed significantly reduced survival rate (66.7%) as compare to normal and control groups. Survived embryos showed adversely influenced growth and development of embryo with increasing deformities such as reduction in the size and weight (by 51.66%) of embryos indicating growth retardation, poor limb development, twisted neck and defected vascular development with hemorrhage as observed on CAM (Plate I, Fig. I). Treatments of 500µg H_2O_2 dose showed 100% morality (weight loss by 69.70%) with severe morphological lesions/damage in dead embryos with vascular damage on CAM (Plate I, Fig. J).



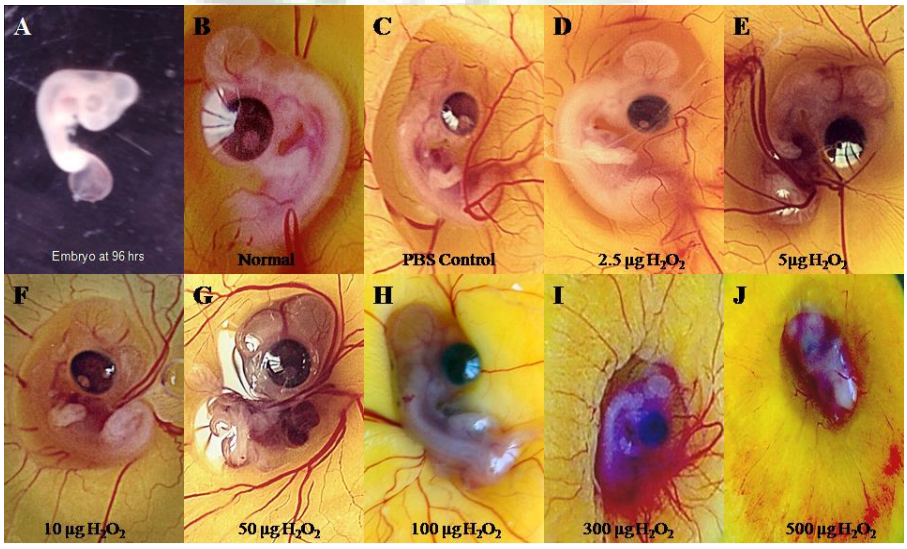


Plate I: Morphological alterations in chick embryo by the influence of H_2O_2 induced oxidative stress at 144hrs of development.

The results of survival rate were in agreement with the variable heart rates of chick embryo at treatment of different doses (Fig. 2). At 144 hrs of development, average heart rate in normal and control embryos was 124±11 beat per minute. A dose dependent decrease in heart rate was also observed with increasing concentrations of H_2O_2 compared with normal and PBS treated control groups. Administration of 2.5, 5.0 and 10µg H_2O_2 doses did not show any alterations in heart rate. While significant depletion in heart rate was noted at 50, 100 and 300µg H_2O_2 doses induced oxidative stress, where heart rate was dropped drastically and arrhythmias were noted at 300µg dose. When these results were compared with normal and control embryos it was observed that the heart rate was reduced significantly by 16.12%, 69.35% and 91.12% respectively. These results indicated that elevated level of H_2O_2 induced oxidative stress are associated with increased mortality and associated embryotoxicity.

Chick embryo at 96 hrs of development at which H_2O_2 doses were initiated (Fig. A). Normal embryo at 144 hrs of development and PBS treated control embryos with normal morphology (Fig. B & C). Treatment of 2.5, 5 and 10 µg H_2O_2 doses showed nearly similar morphology with normal embryo (Fig. D, E & F). Treatment of 50µg H_2O_2 displayed bulky brain (Fig. G). Treatment of 100µg H_2O_2 showed reduced growth with narrow neck, bulged eye (Fig. H). Treatment of 300µg H_2O_2 showed pronounced morphological deformities such as reduced size, twisted neck, folding of legs, marked inhibition of vasculature (Fig. I). Treatment 500µg H_2O_2induced oxidative stress cause embryo death with significant vascular damage/embryo arrest (Fig. J)

Discussion

Oxidative stress highly affects embryo development as well as intracellular redox status. In present study effect of H_2O_2 induced oxidative stress by various concentrations viz., 2.5, 5, 10, 50, 100, 300 and 500µg/ embryo on survival rate of chick embryo is evaluated to determine the toxicity leading to mortality and morphological abnormalities. The doses of H_2O_2 to induce oxidative stress were initiated at 96 hrs and embryos were observed for survival rate after 48 hrs of incubation at 144 hrs.

The results show that H_2O_2 induced oxidative stress affects the survival rate of early stage of chick embryo in dose dependent manner. It is decreased with increasing oxidative stress induced by graded H_2O_2 concentrations which is predominantly noted at higher doses with increasing deformities. Treatments of 2.5 and 5µg H_2O_2/ embryo showed no mortality. The survival rate of chick embryo for H_2O_2 treated group at increasing doses i.e. at 10 (80%), 50 (65%), 100 (50%) and 300µg (30%); which is were lower than normal and control groups with morphological deformities including reduced size, weight (developmental arrest), abnormal head and neck region, reduced limbs and marked inhibition of CAM vasculature with hemorrhage on CAM noted at 100 and 500µg H_2O_2 doses. Heart rate is dropped drastically with noted arrhythmias. While administration of 500µg H_2O_2 dose exhibited 100% mortality. These results indicate that the survival rate is correlated with the concentrations of H_2O_2 doses. It might be due to overproduction of ROS resulting from impaired intracellular milieu and disturbed metabolism which are detrimental for the embryo [12].

Oxidative stress occurs as a result of an imbalance between the pro-oxidants and the ability of the antioxidants to scavenge the excess ROS production. At 2.5 and 5.0µg H_2O_2 doses, embryo seems to manage ROS production by intracellular antioxidant system. But at increasing doses of H_2O_2 pronounced embryotoxicity may be associated with impairments in other metabolisms, the mitochondrial alterations, developmental arrest, physical damage, apoptosis induction and lipid peroxidation at early development of chick embryo [13]. Decreased heart rate seems to be the consequence of impaired cardiac function through cellular dysfunction or death through apoptosis and necrosis, which is attributed to the cardiomyocytes injury especially in ventricle with the higher concentrations of H_2O_2 [9]. It is reported that cardiac outflow tract is highly sensitive to H_2O_2 induced oxidant stress [14]. Significantly reduced size and weight of embryos are due to the defects in extra-embryonic vessel formation at 100µg and 500µg H_2O_2; inhibiting angiogenesis and vasculogenesis with severely reduced heart weight; which is studied earlier with similar doses [10].

Low doses of H_2O_2 may be generating low levels of ROS; where cells may survive themselves via the induction of the inherent detoxifying systems or activating intracellular antioxidant system with associated less deformities, since high oxidative stress is known to associate with cytotoxicity, DNA fragmentation and apoptosis [15,16]. The cytotoxicity is also observed in the primary chick embryo fibroblast cells; evidenced by increase in number of apoptotic cells in H_2O_2 treated groups [17]. The normal growth and development of chick embryo is controlled by regulating pH, osmolarity and O_2 content of its own environment along with continuous synthesis of growth factors, hormones and other proteins [18-21]. These growth factors, activities of proliferating and growing cells may have altered by the induction of oxidative stress at high dose concentrations of H_2O_2 leading to significant decreased survival rate of embryo at post-omphalomesentric stages of development; while in survived embryos it seems that the normal growth pattern was altered to create deformities. This deviation can compromise future development

and/or often resulting in early embryonic death or fetal abnormalities.

In conclusion, early chick embryo at low doses of H_2O_2 survive themselves against oxidative stress but it is more susceptible to injury induced by oxidative stress at high H_2O_2 concentrations leading to significant decreased survival rate of the developing embryos with deformities that may have been the consequences of cytotoxicity, apoptosis and defected vasculature.

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