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The injection of rice bran oil to avian egg: focus on carotenoids content of liver and brain in embryonic period

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ABSTRACT

Introduction: The brain and liver are highly vulnerable to oxidative stress in embryo developmental periods. The levels of antioxidant in these tissues are correlated with the mother's nutrition during pregnancy. The present study was conducted to assess the level of carotenoids in liver and brain following the injection of Rice Bran Oil (RBO) to the chicken embryo.

Methods: The eggs were divided into three groups (n=10, for each group). 0.1 cc of RBO was injected into the chorioallantoic membrane and into the egg yolk on the day 4 of incubation. The experiment was terminated on the day 20 of incubation, then, the liver and brain sample tissues were collected. The carotenoids level was measured and compared in the groups.

Results: The levels of carotenoids of the eggs yolks in which RBO were injected in them were 0.31±0.08 and 1.2±0.08 (µg/g tissue) in brain and liver, respectively. These changes were significant as compared with control group (P<0.05).

Conclusion: RBO exposed embryo significantly increased carotenoids level of liver and brain. Therefore, the result of this study confirms health benefit of RBO consumption during embryonic development.

Implication for health policy/practice/research/medical education:
The results of this study demonstrated that rice bran oil could enhance the level of carotenoids of brain and liver. Therefore, it might protect these organs from oxidative damages.

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Introduction

Oxidative stress results from excessive levels of reactive oxygen species (ROS). ROS are generated in two pathways: Endogenous sources and exogenous sources. Major sites of Endogenous generation of ROS in cells are mitochondria, microsomes, endoplasmic reticulum, and nuclei. Exogenous sources are xenobiotics, ionizing radiation, and ultraviolet light. The embryos are exposed to a much higher arterial oxygen level, which results in an increased ROS (1). The birth defects can be result from oxidative stress during embryonic development. The reduced antioxidants protect biological membranes and DNA during the early stages of embryonic development (1). It has been suggested that antioxidant rich food are essential in pregnancy periods.

Carotenoids have also been documented for health promoting function. These components are known to provide a range of biological effects (provitamin, antioxidant, coloring etc.). The role of carotenoids as a source of pigment and in immune defense system has been established. Exogenous antioxidant increases the chance of embryos, even those of fair-quality, to develop to blastocyst (2).

Avian embryos undergo extremely rapid development over a relatively short period of time, and so are likely to suffer high levels of oxidative damage**.** Avian embryos model is useful for studying embryonic development time (3). Moreover, the liver and brain are the most susceptible organs to the stress oxidative. For this purpose, we studied the ability of RBO to increase carotenoids level of liver and brain in chicken embryos. The protocol used for this study is a sensitive, inexpensive and rapid toxicity test,

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providing information on embryonic toxicity, lethality, teratogenicity, growth retardation and metabolism. Studies have shown that a positive correlation exists between the magnitude of effective concentration in chick embryotoxicity test and those in other systems including mammal tests.

Material and Methods

Experimental design

Fertile leghorn eggs were collected from a breeding farm two days after lay. Infertile and damaged eggs were discarded. Thirty eggs were divided into three groups (n= 10, for each group). The eggs were candled on the day 4 and injected with edible grade RBO. 0.1 ml of RBO was injected into the chorioallantoic membrane (group a) and this amount was also injected into the egg yolk (group b). Chorioallantoic has a number of useful qualities, particularly with respect to toxicity testing (4). The injection sites were closed with paraffin and the eggs were incubated at 37.5±0.1°C and 50%–60% relative humidity. The eggs were candled the day after injection and thereafter every 48 hours for checking dead embryos. The experiment was terminated on the day 20 of incubation. The tissues were washed with isotonic saline.

Determination of total carotenoids of organs

The homogenization of tissues was conducted by the Teflon homogenizer. Total carotenoids were determined by β carotene standard curve and by spectrophotometric method at 470 nm. The total carotenoids content of samples was calculated on the basis of the standard curve of β carotene (5).

Statistical analysis

The data were tested by analysis of T-Test in SPSS software. The evaluation was made by comparing group results. The difference more than 95% (P≤0.05) was considered significant. The data values were expressed as mean±SD.

Results

The level of carotenoids is shown in Table 1. There was significant different between control group compared to (a) group (P=0.04) and control compared to (b) group (P=0.000) in the brain samples.

The carotenoids were not significantly different between controls compared to (a) group (P>0.05) in liver. But, there was significant difference between controls compared to (b) group (P<0.05) in liver.

Discussion

The changes in the concentrations of carotenoids of brain and liver (Table 1) confirmed that carotenoids were transferred to these organs following the injection of RBO into embryonated eggs.

In the last years, there has been growing interest in finding enrichment food by antioxidant. Take of this kind of food, leads to increased level of antioxidants in fetal organs during pregnancy and effective antioxidants will improve fetal outcomes (6).

Table 1. The carotenoids level of liver and brain of chicken embryo (µg/g tissue)

Groups	Liver	Brain
Control	0.96±0.12	0.12±0.04
a (RBO was injected into the chorioallantoic membrane)	0.99±0.12	0.18±0.06
b (RBO was injected into the yolk)	1. 2±0.08	0.31±0.08

Pregnancy is a period of increased metabolic demands with changes in a physiology of mother and requirements of a growing fetus (7). Especially, nutrition of the mother during pregnancy is very important for development of the fetal brain. The eating of low quality oils has been shown to induce oxidative stress especially in embryonic time. Furthermore, the exposures to environmental pollutants are highly correlated with an elevated risk of congenital anomalies (8).

In this study, carotenoids levels were significantly increased in (a) and (b) groups (P<0.05; Table 1) in brain. The brain is highly enriched with polyunsaturated fatty acids which makes it most vulnerable to lipid peroxidation by oxygen-free radicals (9).

The level of carotenoids was also significantly different between (b) group compared to control group in the liver. Table 1 shows that the content of carotenoids in liver is more than brain in all groups. The concentration of carotenoids in the liver far exceeds that any other tissue (10). Liver is susceptible organ because of its metabolizing and detoxifying functions. Therefore, this organ needs a strong antioxidant defense system. Carotenoids are efficient scavengers of free radicals particularly at low oxygen tensions that prevail in embryonic tissues. Previous studies showed that tissues of chickens hatching from carotenoid-enriched eggs were found to resist to lipid peroxidation (10). Carotenoids are soluble in the polar solvents including edible fats and oils. They are usually entered into the oil. They play an important role in the prevention of oxidative stress, several chronic diseases and maintaining good health (11). Therefore, protective effect of this oil on brain and liver might be from its oxidative stress.

In conclusion, RBO has the potential to be a good source of natural carotenoids. The active components that are considered to be powerful antioxidants such as tocotrienols and γ-oryzanol are present in RBO. These components are associated with the prevention of cardiovascular diseases and cancer (12). In another study, rice bran extract potentially was natural antioxidant (13). Therefore, it could be considered by food industries as health promotion agent. Our findings offer an opportunity for using RBO for medical and food purposes.

Authors' contributions

All authors contributed to this research.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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