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# Teratogenic Effects and The Role in The Etiology of Atopic Diseases of Erythrosine (FD&C Red No.3)

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**Abstract:** Erythrosine (FD&C Red No.3, di sodium salt of 2,4,5,7-tetra-iodofluorescein); is used extensively as a colour additive in foods, drugs and cosmetics. Previous studies have shown that some of the food additives, depending on their long-term and short-term use, have potential carcinogenic, toxic and teratogenic effects. In Turkey this colourant has temporary permission of use until studies are completed about it. In addition food colours may cause allergic reactioıns due to food intolerance.

In our study 17 pregnant rats are divided into two groups: control and erythrosine group. 7 Wistar albino rats were administered 5 ml erythrosine (2mg/ml) by gavage at 7-8-9-10-11$^{th}$ days of their pregnancy. 10 rats in positive control group are given distilled water by gavage. At 20 $^{th}$ day of pregnancy, pregnant rats in both groups sacrificed by cervical dislocation and laparotomy applied. Each live fetus was promptly weighed and examined for congenital anomalies and the crown-rump lenght was measured. Routine fetal histologic studies and light microscopic examination of the sections stained with H&E was applied. Mast cells are examined in the fetal dermal sections stained with Toluidine Blue.

In erythrosine group, there were no congenital defects, while the number of mast cells of fetal skins were increased and exocytosis was observed. This was statistically significant (U=12 $p<0.01^{**}$). With respect of the previous studies, we think that erythrosine may be one of the causes of atopic diseases.

**Key Words:** Erythrosine (FD&C Red No. 3), Food additives, Food colours, Atopy, Teratogenicity.

## Introduction

It is known that colourants have been using in foods, drugs and cosmetics as an additive since B.C. 5000. In order to make foods more delicious, protect and enhance their eye appeal, food additives are added to foods, however, they cause some reactions like urticaria, angioneurotic edema and anaphylactic shock via spesific mechanisms in sensitive individuals (1).

Erythrosine (FD&C Red No. 3), the sodium salt of 2,4,5,7-tetra iodofluorescein; is a member of xanthene dyes and used extensively as a colour additive in foods. It has been proposed that erythrosine may cause acute toxicity and it has a tumor inducer property. The WHO/FDA recommends an acceptable daily intake of 100 ppm for this additive (2).

According to certain animal experiments, erythrosine causses extremely low acute toxicity, but has no effect when administered chronically (3, 4). Erythrosine is not genotoxic and therefore does not act as a tumor initiator but perhaps it acts as a tumor promoter (4). However, in various eucariotic systems (ascites tumor cells, chiniese hamster liver cells, lymphocytes, erythrocytes, etc...) erythrosine shows an ambigious genotoxic response (3).

Increase in the number of hypersensitive individuals suggested that some of the factors influence pathogenesis of allergy may be related to use of food additives. On the other side, it is estimated that 1-5 percent of congenital malformations observed in human is related to the use of drugs and chemicals. Therefore, the aim of the present study was to investigate the effects of erythrosine on rat fetus in the means of teratogenity and atopic diseases.

## Material and Methods

**Chemical:** Erythrosine (FD&C Red No. 3) 87% pure, of certified food-grade quality was purchased from a convenient food industrial.

**Animals:** 17 female Wistar albino rats, each 13-21 week age, 130-200 g body weight, were used. Animals were housed in an environmentally controlled room (20-

Table 1. Body weight of control and erythrosine group of pregnancies (g).

| | Control Group | | | Erythrosine Group | | |
|---|---|---|---|---|---|---|
| | n | $\bar{x}$ | $S\bar{x}$ | n | $\bar{x}$ | $S\bar{x}$ |
| Before Pregnancy | 10 | 173.50 | 9.60 | 7 | 200.00 | 0.00 |
| After Pregnancy | 10 | 264.50 | 10.71 | 7 | 312.86 | 6.44 |

Table 2. Fetuse lengths of control and erythrosine groups (cm.)

| | n | $\bar{x}$ | $S\bar{x}$ |
|---|---|---|---|
| Control | 10 | 3.79 | 0.15 |
| Eryhrosine Group | 7 | 3.87 | 0.07 |

Table 3. Fetuse weights of control and eryhrosine groups (g).

| | n | $\bar{x}$ | $S\bar{x}$ |
|---|---|---|---|
| Control | 10 | 3.78 | 0.37 |
| Eryhrosine Group | 7 | 4.11 | 0.18 |

Table 4. Mast cell findings of control and erythrosine groups (g.)

| | Scales | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 |
| Control Groups | 1 | 220 | 93 | 2 | - | - |
| Erythrosine Group | 6 | 6 | 47 | 51 | 101 | 18 |

21˚C, 40-60% relative humidity) and 12 hour light/dark cycle and fed ad lib.

**Experimental Procedure:** On mating days two females were randomly mated with one male at approximately 4.30 p.m. The following morning, a vaginal smear was obtained from each female to determine whether copulation had taken place. Sperm positive dams were considered to be at day 0 of gestation. 10 pregnants served as controls and 7 served as erythrosine group. In eryhrosine group, each animal was treated daily, from day 7 to day 11, with the dose of 5 ml of the solution which has 2 mg/ml erythrosine dissolved in distilled water by gavage. Controls were received only 5 ml distilled water in the same time period. On 20$^{th}$ day of gestation starting at 1 p.m., the females were sacrificed by cervical dislocation. Laparotomy was performed. The uterus was opened and examined in situ. The uterine positions of all implantation sites were noted and their condition (early or late resorption, living or dead fetuses) was determined. Each live fetus was promptly weighed and examined for congenital anomalies the crown-rump length was measured.

**Histology:** Fetuses were fixed in 10% neutral formaline solution, embedded in paraffin blocks and 5µ thick slides were prepared and stained with Haematoxylin and Eosin (H&E) and to evaluate the distribution of mast cells Toluidine Blue stain was used. In the subcutaneous tissue of fetuses granulated and degranulated mast cells were classified depending on their morphologic features by Wingren and Enerbach's score.

Semiquantitative grading of staining:

0 point= no mast cells.

1 point= few, faintly metachromatic and mostly immature mast cells.

2 point= in a normal number and almost all of them degranulated mature and immature mast cells.

3 point= in a normal number, almost half of them exocytosed mature mast cells.

4 point= in a high number, strongly metachromatic, mostly granulated, few exocytosed mature mast cells.

5 point= in a high number, strongly metachromatic, almost all of them exocytosed mature mast cells, diffused granules within tissue(5).

**Statistical analysis:** Mann-Whitney-U Test and t-test.

## Results

### Macroscopic Results:

Table 1 lists the findings of the control and erythrosine groups before and after body weight of pregnancy.

According to t-test, there was a significant difference between control and erytrosine groups in the means of body weights of mothers before pregnancy (t=-2.29 SD=15 $p<0.05$*).

According to t-test, there was a significant difference between control and eryhrosine groups in the means of

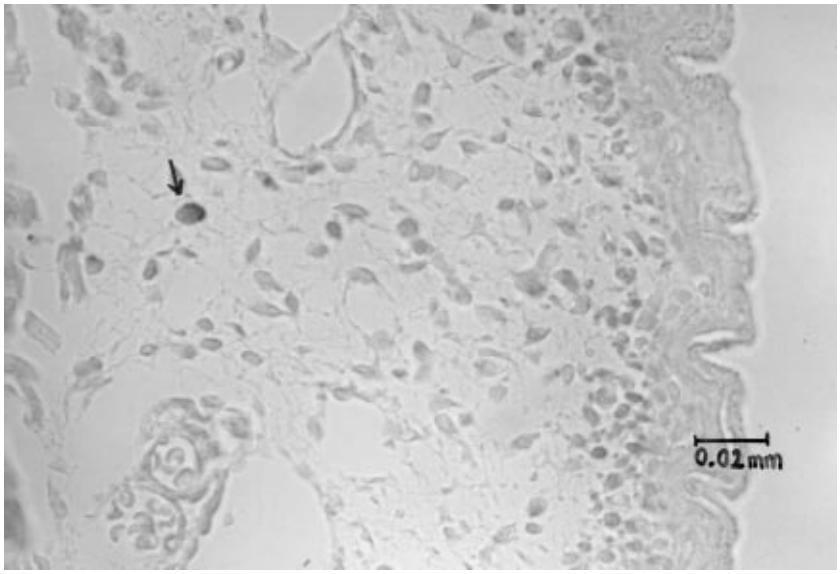


Figure 1. A number of mast cell (→) in dermis of control group Toluidine Blue.

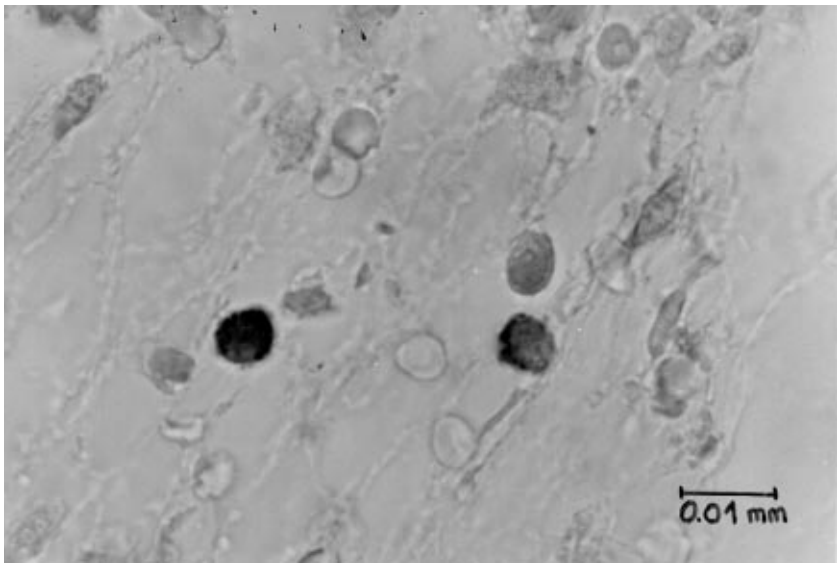


Figure 2. Mast cells including few numbers of granules in dermis of control group. Toluidine Blue.

body weights of mothers after pregnancy (t=-3.46 SD=15 p<0.01**).

There was no significant difference between control and erythrosine groups fetuses in the means of crown-rump lenght (t=-0.43 SD=15 p>0.05) (Table 2).

According to t-test, there was no significant difference between control and erythrosine group fetuses in the means of fetuses weights (t=-0.72 SD=15 p>0.05) (Table 3).

Microscopic Results:

The development of nervous system, face, skull, skeleton, endocrine, cardiovascular, respiratory, digestive, urogenital systems and sensory organs was evaluated on series of sections stained with H&E. Our results showed that there was no difference between control and erythrosine groups.

In order to determine the number of the mast cells in fetal subcutaneous tissues, the distribution of mature and immature mast cells, the presence of granulation and degranulation of these cells and the diffusion of these granules within tissues, the slides stained with Toluidine Blue were evaluated. According to our results in the fetuses subcutaneous tissues of control group, there were a few number of immature mast cells (Fig. 1, 2). In the fetuses of eryhrosine group subcutaneous sections mast cell numbers and degranulation intensity increased were determined (Fig. 3, 4). This differences between control

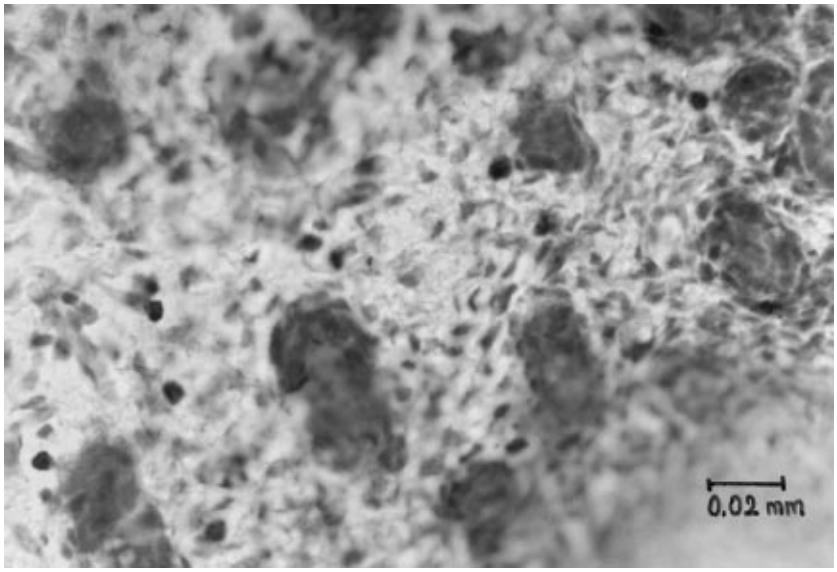


Figure 3. A lot of mast cells in dermis of erythrosine group. Toluidine Blue.

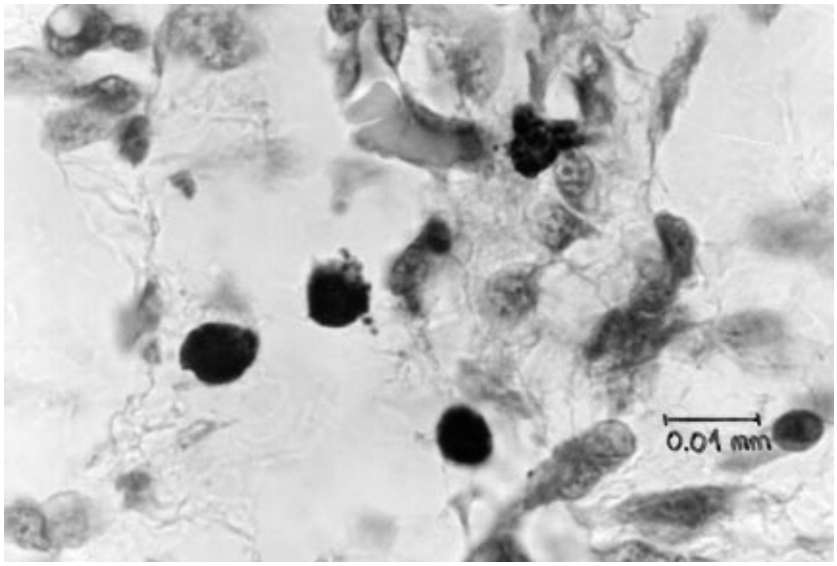


Figure 4. A lot of mast cells exhibiting exocytosis in dermis of erythrosine group. Toluidine Blue.

and erythrosine groups were evaluated according to semiquantitative grading as described before (5, 6, 7).

According to Mann-Whitney U Test results done for non-parametric values, there was a significant difference between control and erythrosine group fetuses in the means of subcutaneous mast cell scales (U=12, $p<0.01^{**}$) (Table 4).

## Discussion

Colourants must be non-toxic, since they have been commonly used in foods (8, 9, 10).

Teratogenity is depended on the genetic disposition of mother and fetus the expose with teratogen(s) during development and the dose and time-period of teratogen. However, the causes of birth defects are largely unknown, 20-25% of these defects have been derived from genetic sources (11).

"Critical period" of pregnant rats 7, 8, 9. days of pregnancy. 0-17 days in human, however, named as "rezistant period". In "embryonic period", 7-57, day of pregnancy, organ differentiation starts to develop, therefore this is a very sensitive period in which the adverse effects of teratogens are frequently seen (12).

Larsson et al. have reported that there was no significant finding of development retardation in their

study which investigated the effects of amaranth and ponceau-4-R. (13). Similarly, Collins et al. have not observed any difference of average of fetal weights between control and allura red induced experimental groups (14). Our findings indicated that organogenesis period did not exhibit developmental retardation in erythrosine group. Therefore, it is suggested that erythrosine did not effective in organogenesis period and also did not cause any developmental retardation. Our findings are consistent with the previous studies (13, 14, 15).

Larsson et al. (1975) observed 8% fetal death in control group in their study which searched the effects of amaranth and ponceau-4R. These authors the same parameter was 3.9% in days 0-7, and 12.7% in day 6-18 in experimental groups (13). In our study, we have not observed any resorption or fetal death. This findings are corralated with the previous studies (16, 17).

Larsson et al. observed exencephalic, open eyes and tail deformities, skeletal anomalies, hydrocephalic, subcutaneous haemorrhagie and pelvis renalis dilatation with a very low frequency in their experiment. Collins et al. also found subcutaneous haemorrhagie in their allura red study (13, 14). In our study there was no sternal variation and there was no significant difference between control and erythrosine groups in the means of skeletal malformations.

Collins et al. (1992) have found that tartrazine, with the dose of 100 mg/body weight/day, did not mediated maternal, teratogenic or fetotoxic effects when administered by drinking water, suggesting that gavage methods or drinking water administration might not change the results (15). In our study resorption, abortus, neural tube defects, skeletal malformation or sternebral variation were not observed in fetuses. For this reason, we conclude that erythrosine administration in the dose of we used during the organogenesis period of pregnancy is not teratogenic. It supports other studies (14, 16, 18).

Most atopic diseases, like urticaria, asthma, eczema, migraine, show food intolerance. It is known that, cooking or digesting the foods do not cause the entire loss of antigenic properties. Intake of antigenic food materials which in protein structure has been known to cause the reactions like asthma, headache, urticaria, angioneurotic edema. The food additives, which aimed to be colourant, preventer of oxidation and enhancer of taste, causes some allergic reactions. Food intolerance plays a role in the diseases known as Restaurant Asthma and Chiniese Restaurant Asthma. Nowadays the increase in the fast and available food consumption would lead the increase in the incidence of asthma (17, 18, 19, 20).

In recent years, it has been observed that there is an increase in the number of the people who suffer from allergy. A lot of factors acts on the initiation of allergic reactions but commonly in all of them histamine and other mediators triggers acute reactions. The number of mast cells is higher in atopic individuals than that of normal ones. It has been demonstrated that there is positive correlation between histamine concentration and the distribution of mast cells. The trigger mechanism is known to mediated by mast cells, although histamine and other mediators are synthesized out of mast cells. The effectiveness of other cells coincides with later periods and the sensitivity initiated by mast cells are required (6, 21, 22, 23).

It had been reported that tartrazine, which was used to give yellow colour to foods and drinks, caused urticaria, angioneurotic edema, migraine and gastrointestinal complaints. In the same study, i.v. administered tartrazine had been shown to mediate a dose-dependent bornchoconstruction and make mast cells to degranulated. The relationship between food additives and nervous system is not only present in tartrazine, but it also observed in xanthene dyes, for instance erythrosine, which have been demonstrated that to effect cholinergic and dopaminergic neuromodulator releases in neuro-muscular junctions (10).

In our observation, erythrosine increased the number of mast cells and stimulated the degranulation of these cells, suggesting that eryhrosine may play an inductive role in atopic diseases. Therefore, we thought that the increase in the number of atopic diseases might be dependent on the gradually increasing consumption of the foods include additives.

In conclusion, we believe that people must be informed about the effects of food additives in the direction of formal regulation. Since food additives make different effects on different animal species. We believe that it is necessary to pursue further researchs which investigates different species with the parameters of haematology, biochemistry and genotoxicity. We hope our study would contribute some benefits for consumer health and especially the prevention of mother's and baby's healths.

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