ERYTHROSINE, A COLOUR ADDITIVE, INDUCED HEPATOTOXICITY IN ALBINO RATS: HISTOLOGICAL, ULTRASTRUCTURAL, AND BIOCHEMICAL STUDIES

ABSTRACT:
The present work was designed to test the toxic impacts exerted by the colour additives erythrosine on the histology, ultrastructure and some serum liver function parameters in male albino rats. Erythrosine in a dose level equals 136 mg/kg bw was given orally to male albino rats for 4, 6 and 8 weeks. This caused time-dependent histological alterations in liver such as cytoplasmic vacuolization of hepatocytes, leucocytic infiltration and congestion in blood vessels. Ultrastructurally, erythrosine induced changes in the size of mitochondria, reduction in the rough endoplasmic reticulum, and nuclear changes in the form of degeneration of the chromatin, variation in the size of nuclear pores and formation of side projections or lateral outgrowths. In addition, erythrosine treatment induced body weight decrease and elevated liver function enzymes, mainly aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum of the treated rats. However, most of these changes subsided after one week of stopping erythrosine treatment.

INTRODUCTION:
The use of food additives is greatly increased in modern life. Additives are also used in making drugs and cosmetics. Meanwhile, avoiding usage of food additives is difficult if not possible, because some are used to improve the nutritional value of foods, to maintain food freshness, keep the consistency of food texture, or to improve taste of foods. Other types are used to give flavour, sweeteners or colour that make our food more acceptable. In addition, some additives are actual micronutrients such as ascorbic acid and carotenes (FDA, 1993). On the other hand, exposure to food additives causes discomfort, asthma, rhinitis and skin disorders. Other symptoms in different body organs were also reported. Among the symptoms appeared are migraine, irritable bowel syndrome, psychological disturbances, urinary incontinence and arthralgia. Meanwhile, the food additives cause children hyperactivity that is characterized by constant restlessness, disorganization and inattention.

Effects of food additives had gained public and scientific attention; this is partly due partly to their widespread utilization and partly to the action imposed by them on human health. It has been shown that flavouring agents such as cinnamonaldehyde and cinnamon in chewing gums and dentifrices were etiologic in the development of plasma cell gingivitis (PCG) (Marker and Kragdahl, 2002). In addition, these authors recorded that flavoring substances in chewing gums and dentifrices caused an inflammatory reaction of both free and attached gingival which is characterized by intense hyperemic and erythematous changes. In this situation, it is common for patients to suffer from mouth bleeding.

More recently, Li et al. (2005) indicated that the food additive Sudan red caused liver and urinary bladder tumours in rats and it is classified as category 3 human carcinogens. In addition, Roglans et al. (2007) indicated that fructose administered at 10 % wt/ vol. in drinking water to rats caused reduction in hepatic fatty oxidation and increased pro-inflammatory transcription factor activity and hypertriglycerideremia.

KEY WORDS:
Erythrosine, Liver Histology, Ultrastructure, ALT, AST.

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In accordance to hazards imposed by food additives, interest has been aroused driving the scientific research towards disclosing the mystery about such effects. Among the food additives widely utilized for human consumption is erythrosine. Erythrosine is a colouring agent used in making drugs, cosmetics, as dental-plaque-disclosing agent and in food manufacture (SCF, 1987).

Diemair and Haussen (1951) recorded an observable inhibition of the action of pepsin at doses 200- 400 mg/l erythrosine. Reduction in hemoglobin content, red blood cell count and cholesterol levels was recorded in rats after three months of giving erythrosine at doses of 5, 10, 15 and 50 mg/ 200- 250 g/ rat wt twice weekly (Bowie et al., 1966).

Formation of liver tumours became evident after approximately 200 days in mice aged 50- 100 days given 1 mg erythrosine / mice for 500 days (Waterman and Lignac, 1958). Willheim and Ivy (1953) recorded development of liver cirrhosis in one rat out of five treated with erythrosine at 4 % for 18 months. Hansen et al. (1973) showed formation of tumours in rats subcutaneously injected with aqueous solution of erythrosine at 12 mg/ animal once per week for two years.

It was noticed that most scientific researches on erythrosine dealt with its endocrine influences especially on the pituitary and thyroid glands. In this concern, Paul et al. (1988) found that at a dose of 1500 mg/ day/ human for 14 days, slight increase in serum TSH and decrease in T₃ and T₄ were noticed. According to studies extended up to 2 years on rats, Capen (1994) recorded that erythrosine inhibited T₃ secretion; this is due to increase in TSH secretion, hyper trophy of follicular cells, hyperplasia and increased follicular cell tumours of the thyroid.

Exposure to erythrosine affected estrogen secretion that may be involved in the development of breast cancer (Dess et al., 1997) implying that erythrosine increased binding of the estrogen receptor from MCF-7 cells to estrogen responsive element and has estrogen-like growth stimulating properties and it may be genotoxic.

Abdel-Aziz et al. (1997) indicated that mice exposed daily to erythrosine for 21 days at doses 68 and 136 mg/ kg induced decrease in testicular lactic dehydrogenase isoenzyme activity and a pachytenic spermatogenesis marker of testicular toxicity. Still, erythrosine disrupted the normal morphology in sperm head. The authors also found that erythrosine given at doses 860 and 1360 mg/ kg caused sperm head abnormality by about 50 and 65 %, respectively, which in turn enhanced the spermatogenic dysfunction and germ cell mutagenesis.

When erythrosine was mixed with rat's diet at dose 2464 mg/ kg bw/day for 30 months, a decrease of weight gain in females was induced. In both sexes increased thyroid weight and higher follicular hypertrophy, hyperplasia and thyroid adenoma were also recorded (Borzelleca et al., 1987). This work was planned to test the histological and ultrastructural changes induced in the liver of rats and changes in AST and ALT enzymes in the serum under erythrosine treatment.

The present work was conducted to study the effect of erythrosine on histology, electron examination of liver and liver function enzymes.

**MATERIAL AND METHODS:**

**A- Experimental Animals:**

Healthy adult male albino rats (Rattus norvegicus) of inbred strain approximately two months old, each weighing 120 ± 5 g, were used in this study. Rats were kept in the laboratory for at least one week before experimentation under almost constant temperature (30± 2 °C) with 12: 12 hour dark-light cycle and relative humidity equal 45±5%. Rats were fed on standard commercial rodent chow diet and water was supplied ad libitum.

**B- The Chemical used:**

1- Erythrosine: 2, 4, 5, 7-tetraiodofluorescien (C₂H₁₂I₄Na₂O₈) as a powder manufactured by Oxford Laboratory, Mumbai, was utilized.

**C- The Experimental protocols:**

Rats were divided randomly into the two following groups:

1- Control group: Animals (20 rats) fed on normal rodent diet throughout the experimental period and administered orally with mammalian saline.

2- Erythrosine-administered group: Rats of this group (30 rats) were orally administered daily with 136 mg/ kg bw/day of aqueous erythrosine according to Abdel-Aziz et al. (1997). Animals were exposed to erythrosine for a total period of 8 weeks. Five rats were taken from each treatment and specimens were inspected after 4, 6 and 8 weeks and two weeks post-cessation of the treatment. Rats were anesthetized by halothane (Pharco, Egypt), dissected and small pieces of the liver were fixed either in 10 % neutral formalin for histological preparation or in glutaraldehyde for electron microscope examination. Sera were collected in centrifuge tubes, kept at -20°C for biochemical determination of ALT, AST.

**Histological and ultrastructural preparation**

Small pieces of liver from both control and erythrosine-administered rats were fixed in 10 % neutral formalin, washed dehydrated, cleared and embedded in parablast. Sections of 5 μm thickness were cut using rotary microtome (Leica, model Rm 2125, Germany),
mounted on clean slides and stained with 
Ehrlich's hematoxylin and counterstained with 
eosin (Lillie and Fulmer, 1976).

For preparation of sections to be 
examined by TEM, the method of Karnovsky 
(1965) was followed. Ultrathin sections were 
stained, examined and photographed under 
JEOL JEM-100S electron microscope, Unit of 
Electron Microscope, National Cancers 
Institute, Egypt.

Biochemical analysis:

a- Liver Function Enzymes:

Alanine-aminotransferase (ALT) and 
aspartate aminotransferase (AST) were 
measured according to the method adopted by 
Gella et al. (1985) using Spinreact Kit (S. A. 
Ctra. Santa Coloma, Spain). Results were 
analyzed statistically using Student's "t" test 
at P<0.01 level of significance.

RESULTS:

Changes in Body Weight

Treatment with erythrosine induced 
statistically significant reduction in the body 
weight in all time intervals reaching its 
maximum level at the end of the 8th week 
while an increase in body weight was induced 
two weeks after stopping such treatment 
(Table 1).

<table>
<thead>
<tr>
<th>Treatment period</th>
<th>Control group</th>
<th>Erythrosine group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td></td>
</tr>
<tr>
<td>4 weeks</td>
<td>143 ± 1.21</td>
<td>133.2 ± 1.46*</td>
</tr>
<tr>
<td>6 weeks</td>
<td>144 ± 1.41</td>
<td>127.2 ± 1.07*</td>
</tr>
<tr>
<td>8 weeks</td>
<td>143.8 ± 1.77</td>
<td>126.2 ± 1.39*</td>
</tr>
<tr>
<td>2 weeks post-treatment</td>
<td>144.2 ± 1.46</td>
<td>128.6 ± 1.03*</td>
</tr>
</tbody>
</table>

n=5 animals for each group. 
* Statistically significant different from control (P<0.01).

Histological Examination

Liver sections of control animals 
appeared without any histological 
abnormalities (Fig. 1). Animals treated with 
erythrosine for 4 weeks exhibited abnormal 
arrangement of hepatic strands, congested 
blood vessels and leucocytic infiltration (Fig. 
2). After 6 weeks, sections exhibited loss of 
normal hepatic architecture, marked 
leucocytic infiltration, and cytoplasmic 
vacuolization of hepatocytes. Enlargement of 
bile ductules was also evident (Fig. 3). Still, 
after 8 weeks had lapsed, the condition 
became worse where liver sections inspection 
revealed congestion of blood vessels, 
cytoplasmic vacuolization of hepatocytes, 
presence of macro- and micronuclei and loss 
of the characteristic hepatic strand pattern 
(Fig. 4). However, two weeks following 
stopping such treatment, the condition of the 
liver became much better where sections 
inspection revealed that the hepatic cells 
began to be arranged in normal strands with 
no cytoplasmic vacuolization. Neither 

hemorrhage nor infiltration was found, Kupffer 
cells became smaller and the hepatic blood 
sinusoids became narrow (Fig. 5).

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Fig. 5. Section in the liver of a rat 2 weeks post-cessation of erythrosine administration showing almost normal liver histology, where the hepatocytes are arranged in characteristic strands pattern radiating from the central vein (CV) and sinusoids (S) became nearly normal. Kupffer cells: arrowhead. X400

Liver of control rats has normal-structured organelles (Figs 6 & 7). Liver sections examined under the electron microscope showed that the liver consists of hepatocytes containing spherical nuclei each having one or two nucleoli. The nucleus is surrounded by nuclear envelope that is provided with nuclear pores providing pathway between the nucleus and cytoplasm. The envelope consists of two parallel membranes separated by a narrow space. The chromatin is distinguished into dense clumps of heterochromatin and lightly-stained euchromatin. The cytoplasm exhibited granular appearance and contains spherical and elongated mitochondria and profuse amount of rough endoplasmic reticulum especially around the nuclear envelope and between the mitochondria. The hepatic sinusoids have extremely thin walls on discontinuous layer of lining cells consisted of endothelial and Kupffer cells. In addition, bile canaliculi with short microvilli are present between the hepatic cells. Treatment with erythrosine for 4 weeks caused degeneration of cristae of some mitochondria, change in the spreading of the rough endoplasmic reticulum, decrease in chromatin and appearance of few vacuoles in the cytoplasm (Fig. 8). After 6 weeks had passed, the changes became more prominent. At the end of the 8th week, the alterations became more severe. The cytoplasm contained round different-sized vacuoles (Fig. 9). Megamitochondria (some with degenerated cristae) and decreased spreading of the rough endoplasmic reticulum were evident (Fig. 10). The chromatin was decreased and the nuclear matrix became more electrons dense. The nuclei underwent morphological alterations with damaged nuclear envelopes (Fig. 11). During the recovery period, a degree of improvement in the ultrastructure was attained, where nearly normal shape and size of most mitochondria, and most nuclei appeared normal (Figs 12 & 13).
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Fig. 9. Electron micrograph of a rat’s hepatocyte treated with erythrosine daily for 8 weeks showing the presence of number of mitochondria (M) with damaged cristae and sparse endoplasmic reticulum (RER). The nucleus (N) has large nucleolus (Nu) and deteriorated heterochromatin (Hc). Widened nuclear pores (NP) were also evident. X2000

Fig. 10. Ultrathin section of a hepatocyte of a rat orally administered with erythrosine for 8 weeks showing the presence of some vacuoles (V) in the cytoplasm and highly distorted nucleus (N) suffering projections (arrowhead) and rupture of nuclear envelope (NE). Mitochondria: M. X3000

Fig. 11. Ultrathin section of a hepatocyte obtained from liver of a rat two weeks post-treatment with erythrosine showing highly distorted nucleus (N) that gives up projections (arrowhead) and density of euchromatin (Ec). Heterochromatin (Hc) depleted at certain places. Some degenerated mitochondria (M) are also observed. Nucleolus: Nu. X4000

Fig. 12. Electron micrograph of a rat’s hepatocyte two weeks post-treatment with erythrosine showing megamitochondria (M) whose cristae are distorted and other normal-sized mitochondria with degenerated cristae, in addition to the presence of little amount of rough endoplasmic reticulum (RER). The nucleus (N) has irregular boundary with loss of some heterochromatin (Hc). Nuclear pores (NP) are wider than normal. X3000

**Biochemical parameters**

1- **Serum aspartate aminotransferase (AST):**

Oral administration of erythrosine caused a significant increase in serum AST except after 4 weeks where insignificant increase was noted. Such increase reached its maximum level after 8 weeks of treatment. However, an advanced degree of normal activity was achieved during the recovery period (Table 2).

Table 2. Effects of erythrosine on serum aspartate aminotransferase (AST)(U/l).

<table>
<thead>
<tr>
<th>Periods of treatment</th>
<th>Control group</th>
<th>Erythrosine group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>4 weeks</td>
<td>84.6 ± 1.3</td>
<td>85 ± 2.9</td>
</tr>
<tr>
<td>6 weeks</td>
<td>87.2 ± 0.9</td>
<td>119.6 ± 0.7</td>
</tr>
<tr>
<td>8 weeks</td>
<td>87 ± 1.1</td>
<td>122.6 ± 2.9</td>
</tr>
<tr>
<td>2 weeks post-treatment</td>
<td>89.8 ± 0.4</td>
<td>108.8 ± 2.4</td>
</tr>
</tbody>
</table>

n= 5 animals for each group.

* Statistically significantly different from the control (P<0.01).

2- **Serum alanine aminotransferase (ALT):**

Pursuing the conditions previously described for AST enzyme, induction of statistically significant increase in ALT activity was recorded. Such increase became less pronounced during the recovery period (Table 3).

Table 3. Changes in serum alanine aminotransferase (ALT)(U/l) caused by erythrosine.

<table>
<thead>
<tr>
<th>Period of treatment</th>
<th>Control group</th>
<th>Erythrosine group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>4 weeks</td>
<td>39.8 ± 2.0</td>
<td>49.2 ± 0.3</td>
</tr>
<tr>
<td>6 weeks</td>
<td>38.8 ± 0.4</td>
<td>88.8 ± 1.2</td>
</tr>
<tr>
<td>8 weeks</td>
<td>38.2 ± 0.7</td>
<td>75.4 ± 0.5</td>
</tr>
<tr>
<td>2 weeks post-treatment</td>
<td>36.4 ± 0.6</td>
<td>50 ± 2.9</td>
</tr>
</tbody>
</table>

n= 5 animals for each group.

Statistically significantly different from the control (P<0.01).
DISCUSSION:
The importance of colour additives emerges from the fact that they are incorporated in the manufacture of some foods and in the composition of some drugs and cosmetics (FDA, 1993). Erythrosine (FD and C Red No. 3) gained wide utilization in many foods such as snacks, cakes, canned food and canned fruits. The deleterious effects of erythrosine on body organs should be dealt with considerable attention. Promotion of thyroid gland tumours (Jennings et al., 1990), abnormalities in the thyroid gland secretion that resulted in inhibition of the T3 hormone secretion (Capen, 1994), abnormalities in sperms (Abd-El Aziz et al., 1997) were among the deleterious impact of this material on some body organs. In addition, Reyes et al. (1996) recorded inhibition of mitochondrial respiration while effects on brain as a result of inhibition of Na, K-ATPase in rat brain were announced by Sibergeld et al. (1982). Earlier, Larsson (1975) recorded skeletal abnormalities, hydrocephalic, subcutaneous hemorrhage and pelvic renalis dilation upon exposure to amaranth and ponceau-4R. Added to that, Dess et al. (1997) observed occurrence of breast cancer upon exposure to erythrosine and Ashida et al. (2000) recorded reduction in the activities of gluconeogenesis and ureogenesis, reduction in DNA and consequently decrease in protein synthesis.

In the present work, highly significant depression in body weight after administration of erythrosine was recorded. Such effect was time-dependent. This finding was in agreement with the results of other researchers. Hansen et al. (1973a) recorded slight growth depression in weanling Osborne-Mendel rats fed on 5.0 % erythrosine for two years. In addition, Hansen et al. (1973b) indicated reduction in body weight in rats upon feeding on 2 and 4 % erythrosine for 86 weeks. Moreover, Brozelleca et al. (1987) confirmed the reducing effect of erythrosine on body weight when incorporated into in rats' diet in a dose 2464 mg/ kg for 3 months. The results obtained in this work run in line with those obtained by Brozelleca and Hallagan (1990) where 4 % erythrosine caused decrease in body weight of rats.

Alterations induced in the liver of rats administrated with erythrosine appeared in the form of disruption of normal hepatic architecture and congestion of the central veins. In addition, condensed hepatic cells, leucocytic infiltrations, enlarged branches of the portal veins and bile ductules, cytoplasmic vacuolization & changes in cell size. However, little studies were conducted exploring erythrosine effects on liver histological picture. With regards the lack of literature on erythrosine effect on liver, one should consider similar actions caused by other materials. In this respect, Huang et al. (2007) found that when either Topiramate (TPM) or TPM (40 or 80 mg/ kg. d, TPM) with valproate sodium (VPA, 300 mg/kg.d) (used in the treatment of childhood epilepsy) given intragastrically to male Wister rats for 3 months, induced granular degeneration of some hepatocytes near the central veins in low dose, while the high dose caused punctuate necrosis in some hepatocytes. However, the combined action of low dose with valporate sodium caused deleterious action on the hepatocytes as granular degeneration and fatty degeneration accompanied with punctuate necrosis. Coincides with the congestion of blood vessels found in this work, Aboel-Zahab et al. (1997) recorded that different synthetic chocolate agents (browen HI, indigocarmine) administered to adult albino rats for 30 and 60 days caused congestion of blood vessels, hemorrhage and pigment deposition in portal tract. Many investigators found that administration of the drug endosulfan at dose level 10 mg kg bw/day for 2 and 4 weeks to rats caused dilation of sinusoids, appearance of pyknotic nuclei, cytoplasmic degeneration, as well as various nuclear aberrations (Choudhary et al., 2003). In coincidence to leuukocytic infiltration, necrotic nuclei, and vacuolar degeneration observed in this work; that was also recorded by Gokalp et al. (2003) by utilizing the organophosphate methidathion intramuscularly at 150 and 200 mg/kg bw in rats. They found mononuclear cells infiltration, dilation of sinusoids, necrotic areas, granular degeneration, and pykoenic nuclei of the liver cells.

In the present work, erythrosine exerted strong ultrastructure damaging action on hepatocytes such as degeneration of some mitochondrial cristae, while other mitochondria became larger than normal, the spread of rough endoplasmic reticulum decreased; and cytoplasmic vacuoles were observed. The nucleus exhibited changes in its shape. These changes will certainly leave behind highly damaged cells that without any doubt their functions would be compromised. These changes became less evident during the recovery period. Other food additives produced comparatively similar action. The butylated hydroxyltoluene caused aggregation of chromatin around periphery of nuclear envelope, proliferation of smooth endoplasmic reticulum, clumping of rough endoplasmic reticulum with broken cisternae, withering and autolysis of mitochondria, augmentation of lipid droplets and glycogen depletion (Safer and Al-Nughamish, 1999). When cinnamyl anthranilate injected to male CD1 mice and Fisher 344 rats, changes pointing to peroxisomal proliferation were recorded (Viswatigam and Caldwell, 1997). Sprague-Dawely rats fed on sucrose from 20 to 50%
(w/w), and sacrificed after 3 weeks showed increased in spread of the smooth endoplasmic reticulum (Bacon et al., 1984).

According to data obtained in this work, erythrosine was found to exert effects upon some biochemical parameters in the serum of the exposed rats. The transaminases (AST and ALT) exhibited elevated activities where rats treated with erythrosine exhibited highly significant increase in serum ALT and AST from the beginning to the end of treatment. Similar results were obtained by several investigations. Mekkawy et al. (1998) proved that when rats fed on diet containing 0.08 and 0.49 g/kg erythrosine for 30 days, there was an increase in serum ALT and AST. In addition, Aboel-Zahab et al. (1997) showed similar results of elevated activities of ALT and AST when rats administered for 30 and 60 days with synthetic colouring agents. In addition, Li et al. (2005) recorded an increase in serum ALT when wild-type mice fed on diet containing large amount of sucrose (48%) for 4-12 weeks. Also, rats administrated with 0.6 mg/g bw monosodium glutamate for 10 days exhibited increase in serum ALT and AST (Onyema et al., 2006). Mowafy et al. (2001) found that sodium nitrite induced elevation in serum AST and ALT in mothers of Sprague Dawley rats while sodium benzoate caused high serum AST only.

In conclusion, erythrosine caused alteration in some histological and biochemical parameters of male albino rats. The importance of studying effects of food additives came from the fact that they are used in canned food and making drugs and little work was conducted despite of their significance. So, the unreasonable use of different food additives should be monitored and carefully adjusted. Accordingly, scientific research should be carried out exploring their hazards in order to limit and control their wide use.

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Abdel-Samie et al., Erythrosine, a Colour Additive, Induced Hepatotoxicity in Albino Rats.

The oxidative stress induced by Erythrosine was evaluated in albino rats. Erythrosine is a common food additive used to impart color to foods. In this study, the liver and kidney tissues were evaluated for their oxidative stress levels. The results indicated that Erythrosine induced oxidative stress in rats, as evidenced by increased levels of reactive oxygen species (ROS) and decreased levels of antioxidants. These findings highlight the importance of regulating the use of food additives to prevent potential health risks associated with their consumption.