MUTAGENIC EFFECTS OF THE FOOD COLOUR ERYTHROSINE IN RATS

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ABSTRACT: Male rats were treated with a diet supplemented daily by the synthetic food colour erythrosine (0.08 and 0.4 g/kg diet) for 30 days. Changes in mutagenic activities, as an index for evaluating of possible toxic effects, were monitored by measuring chromosomal aberrations of rat bone marrow, nucleic acids and total protein concentrations of rat liver and brain. The present study found that erythrosine induced chromosomal aberrations. Chromosomal aberrations of bone marrow cells were centromeric attenuation, centric fusion, deletions, ring shape, stickiness, end-to-end association and polyploidy. The mitotic index was statistically increased with a small dose of erythrosine but was inhibited with the higher dose. Biochemical assays revealed that the nucleic acids and the total protein increased markedly during the various periods of treatment. Results indicated that the two doses of erythrosine were found to be mutagenic agents, the high dose of erythrosine having more effect than the lower one.

KEY WORDS: Erythrosine; Mutagenics; Chromosomal aberration.

INTRODUCTION

Erythrosine (CI Food Red FD and C Red number 3) (Iodoeosin; 2,4,5,7-tetraiod fluorescenic disodium salt; C₂₀H₆I₄Na₂O₅ mol. wt. 879.87. Erythrosine has been listed as a permitted food colouring agent by the FAO/WHO Expert Group [19]. However, the Group did recommend that this substance be properly evaluated for its mutagenic potential [37]. Although data on acute toxicity of this compound is not available, short-term studies conducted on rats fed for 28 days on a diet containing 65 mg erythrosine reveal adverse effects compared with controls [8, 21]. Similarly long-term studies carried out on rats and does fed for 2 years on a diet containing erythrosine reveal reduced growth and spleen weight [24, 25].

The aim of this work is to evaluate the effect of erythrosine at two doses on the chromosomes of bone marrow cells of the laboratory rats. Biochemical assays were
done to determine the effect of erythrosine on the amount of DNA, RNA and total proteins in brain and liver homogenates of rats.

MATERIALS AND METHODS

Adult male rats (*Rattus norvegicus*) weighing 100–120 g each were used. They were randomly selected from an experimental breeding station in Helwan, Egypt. They were offered a balanced standard maintenance diet with free access of water [10, 26].

The experiments were carried out on 60 male rats which were divided into three groups including control and treated groups. The first subgroup served as a control and the second subgroup was daily given a high oral dose (0.4 g/kg diet) of erythrosine [23], and the third subgroup was daily given small oral dose (0.08 g/kg diet) – 1/10 dose of Ibrahim et al. [27].

Ten of the control and treated animals were used in each of the biochemical and chromosomal studies and were sacrificed after 6 hours at the end of the experimental period. The method used for chromosomal preparation from the bone marrow cells was based on the techniques devised by Yosida and Amano [38].

The liver and brain were removed immediately and homogenized in 0.9% NaCl solution for the determination of nucleic acid and total protein. Nucleic acids were extracted from liver and brain homogenates [35], deoxyribonucleic acid [14], ribonucleic acid [33], and total protein [12] were determined.

The results obtained were statistically analysed by Kurtz’s method [30].

RESULTS

Chromosome analysis

In the present work, the effect of both doses 0.4 and 0.08 g/kg diet supplement of erythrosine on the chromosomes of bone marrow cells of male rats (*Rattus norvegicus*) after 30 days was studied.

In 30 days of treatment, the mitotic index (number of divided cells per 1000 of the rat bone marrow cells) in the group treated with the small dose of erythrosine increased statistically but in the group was inhibited with the higher dose (Table I). Chromosomal aberrations were centromeric attenuation, centric fusion, deletions, ring shaped, stickiness, end-to-end association, polyploidy, gaps and break (Table II).
TABLE I. EFFECT OF ERYTHROSINE ON THE MITOTIC INDEX IN RATS (*RATTUS NORVEGICUS*)

<table>
<thead>
<tr>
<th>Doses [g/kg diet]</th>
<th>Treatment [days]</th>
<th>No. of dividing cells/1000 cells/rat</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean ± S.E.</td>
</tr>
<tr>
<td>Low dose (0.08 g/kg diet)</td>
<td>Control</td>
<td>40.0 ± 1.23</td>
<td>Changes of control [%]</td>
</tr>
<tr>
<td></td>
<td>erythrosine (30 days)</td>
<td>48.0 ± 1.02**</td>
<td>20.0</td>
</tr>
<tr>
<td>High dose (0.40 g/kg diet)</td>
<td>Control</td>
<td>41.0 ± 1.77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Erythrosine (30 days)</td>
<td>25.2 ± 2.30**</td>
<td>38.5</td>
</tr>
</tbody>
</table>

Results are expressed of mean ± S.E. of five rats.  
S.E.= Standard error.  
** High significant.

TABLE II. MEANS OF CHROMOSOMAL ABERRATIONS INDUCED BY ERYTHROSINE PER 50 METAPHASE NUCLEI OF RATS AFTER 30 DAYS

<table>
<thead>
<tr>
<th>Type of aberration</th>
<th>No. of cells with aberration/50 metaphase</th>
<th>Erythrosine [g/kg diet]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Low dose (0.08 g/kg diet)</td>
</tr>
<tr>
<td>Diploidy</td>
<td>0.21</td>
<td>4.5**</td>
</tr>
<tr>
<td>Centric fusion</td>
<td>0.00</td>
<td>3.6**</td>
</tr>
<tr>
<td>Break</td>
<td>0.00</td>
<td>1.1**</td>
</tr>
<tr>
<td>Gap</td>
<td>0.00</td>
<td>3.1**</td>
</tr>
<tr>
<td>Centrometric attenuation</td>
<td>0.01</td>
<td>4.2**</td>
</tr>
<tr>
<td>Deletion</td>
<td>0.00</td>
<td>3.8**</td>
</tr>
<tr>
<td>Ring shaped</td>
<td>0.00</td>
<td>1.4**</td>
</tr>
<tr>
<td>Stickness</td>
<td>0.01</td>
<td>2.2**</td>
</tr>
<tr>
<td>End-to-end</td>
<td>0.00</td>
<td>1.2**</td>
</tr>
</tbody>
</table>

Results are expressed of mean ± S.E. of five rats.  
S.E.= Standard error.  
** High significant.

Biochemical analysis

The results gained presented in table III indicate that administration of both doses of 0–08 and 4 g/kg diet of erythrosine to rats over 30 days caused a significant increase of nucleic acids (DNA and RNA) and total protein in brain and liver of rats (Table III).
TABLE III. EFFECT OF ERYTHROSINE [G/KG DIET] ON NUCLEIC ACIDS [MG/G TISSUE] OF LIVER AND BRAIN OF MALE RATS AFTER 30 DAYS

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Parameter</th>
<th>Low dose (0.08 g/kg diet)</th>
<th>High dose (0.40 g/kg diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>T</td>
</tr>
<tr>
<td>Liver</td>
<td>DNA</td>
<td>3.71 ± 0.21</td>
<td>4.67 ± 0.06**</td>
</tr>
<tr>
<td></td>
<td>RNA</td>
<td>6.81 ± 0.37</td>
<td>8.63 ± 0.12**</td>
</tr>
<tr>
<td></td>
<td>Total protein</td>
<td>276.52 ± 4.15</td>
<td>361.41 ± 3.21**</td>
</tr>
<tr>
<td>Brain</td>
<td>DNA</td>
<td>1.91 ± 0.05</td>
<td>2.22 ± 0.06**</td>
</tr>
<tr>
<td></td>
<td>RNA</td>
<td>1.82 ± 0.04</td>
<td>2.31 ± 0.05**</td>
</tr>
<tr>
<td></td>
<td>Total protein</td>
<td>280.1 ± 5.05</td>
<td>300.2 ± 4.02**</td>
</tr>
</tbody>
</table>

Results are expressed of mean ± S.E. of five rats.
S.E. = Standard error.
** High significant.

DISCUSSION

There has been increasing concern in recent years about the mutagenic potential of a variety of food additives and food colours and it is believed, that such substances may present a possible hazard to man by causing gene mutations and/or chromosomal aberrations [37].

The effect of erythrosine administration on the mitotic index (number of dividing cells/1000) was recorded.

The low dose (0.08 g/kg diet) of erythrosine provoked a significant increase at 30 days but the higher dose (0.4 g/kg diet of erythrosine) caused a significant decrease after 30 days. Our results are consistent with the results of Maugh and DeHondt et al. [13, 32] using cannabis; and Baset et al. [13] using cypermethrin). It has long been known that the inhibition of nuclear DNA synthesis by certain chemicals invariably blocks mitosis [4, 11].

Chromosomal aberrations were observed following administration erythrosine at both doses, consisting mainly of: diploidy (Figure 1a) and centric fusion (Figure 1b). These are presented in Table II.

Damage to chromosomes after the GI stage of the cell cycle causes chromatid lesions and results in chromatid breaks [36]. This finding was observed in the present study and is consistent with that of Zhurkov [39]. Therefore, it may be possible to conclude that erythrosine exerts its mutagenic effect after the GI stage of the cell cycle.
Mutagenic substances are known of causing hereditary malformation by their effect on germ cells or non hereditary malformation by their effect on somatic cells [2, 29].

From the present results, it is evident that erythrosine, at both doses of 0.4 and 0.08 g/kg diet has the effect of significantly increasing liver and brain DNA and RNA content after 30 days may have resulted from induction of RNA synthesis [20].

Brachet [6] has demonstrated that cellular RNA synthesis is a DNA-dependent process, thus the increasing RNA content after treatment with erythrosine was probably caused by the increase in DNA which is in complete agreement with the results obtained by Maugh [32]. It is clear that there an increase in RNA or DNA base. In fact Allen and Abrahamson [3] reported similar results in rats fed mixture of PCB. In the same studies a number of hepatic cells exhibited an increase in the size of nuclei an observation which

Fig. 1a. Metaphase spread from bone marrow of treated rat with erythrosine showing deploidy and endomitosis (Giemsa stain X 1250).

Fig. 1b. Metaphase spread from bone marrow of treated rat with erythrosine showing centric fusion (Giemsa stain X 1250).
coincides with those reported by Banhawy [5], El-Beih [18], Sanad [34] and El-Banhawy et al. [16].

Administration of erythrosine at 0.08 g/kg diet causes a significant increase in the total protein concentration in the rat brain and liver. Increasing the dose to 0.4 g/kg diet induced a significant increase in protein content of liver and brain. Similar increases in total proteins were found with mercuric in fishes [28] and with insecticide in rats [31] and with radiation sensitivity in human cells [15], with chloramphenicol in rats [9] and with chloramphenicol in rats [17].

The general picture of results illustrates that erythrosine influences the level of protein and nucleic acids, which probably control the genetic pattern of potential enzymes and metabolic pathways in the cell at the nuclear level. Also, erythrosine decreased mitosis, as well as causing chromosomal structural aberrations, which may be suggestive of its mutagenicity.

References:
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