A 90-DAY ORAL TOXICITY STUDY OF TARTRAZINE, A SYNTHETIC FOOD DYE, IN WISTAR RATS

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ABSTRACT
The following study is about Tartrazine (E 102) which is known as an azo dye used in food products, drugs and cosmetics. As a part of the safety assessment of Tartrazine, a 13-week subchronic oral toxicity study was performed on Wistar rats of both sexes. The animals were divided into 5 groups of 6 animals each, 3 of each sex, and fed a diet containing 5, 7.5, or 10 mg/kg b.w, of Tartrazine and 3.75 mg/kg b.w, of Sulfanilic acid. There were no treatment-related adverse effects with regard to body weight, food and water consumption. Their blood samples were analyzed for hematological measurements, Glucose, Creatinine, Blood urea nitrogen, Cholesterol total, Triglycerid, alanine aminotransferase, aspartate aminotransferase. The Stomach, Jejunum, Liver, Kidneys tissues were also processed for histological examination. The present study shows that Tartrazine and Sulfanilic acid induced a morphological change from the discoid shape to an echinocytic form in rat RBCs. Relative weights of the liver were significantly increased in group treated with 10 mg/kg b.w, of Tartrazine and 3.75 mg/kg b.w, of Sulfanilic acid. The histopathological changes of Liver and Kidney were in accordance with the biochemical findings.

Keywords: Tartrazine, Subchronic toxicity, Hepatotoxicity, Nephrotoxicity, Wistar rats.

INTRODUCTION
Food additives are products added to the basic foodstuffs with an aim of improving its aspect, savour, taste, colour, texture, food value and conservation.

Food dyes are added with a principal aim to give a colour to a foodstuff, or to restore its natural colour. From the organoleptic point of view, the visual aspect is an important factor for the choice of the products by the consumer. Thus, the synthetic food dyes occupy an important place in the class of the essential additives for food industry in the conquest of markets. Among the food dyes which are widely used is Tartrazine. It is an orange-coloured, water soluble powder used worldwide as food additives to colour several foods, drugs and cosmetics. It has the following chemical structure illustrated in Fig. 1.

Moreover, this food colorant is used in cooking in many developing countries as a substitute for saffron 1. The Acceptable Daily Intake (ADI) for humans is 0-7.5 mg kg⁻¹ body weight 5.

Tartrazine has been implicated as the food additive which is most often responsible for allergic reactions in specific human populations 5,4,5.

The study of the carcinogenetic and mutagenetic effects of Tartrazine were established by some authors which gives variable results 6-15.

When the Tartrazine reaches the intestine, it can undergo metabolic reduction by intestinal microflora 17; and the reductive cleavage products are rapidly absorbed 18.

Following reductive cleavage of the azo linkage by intestinal bacteria, Sulfanilic acid and aminopyrazolone are produced. The pyrazolone fragment is further degraded by intestinal bacteria to yield a second molecule of Sulfanilic acid 19.

The studies described here were performed to investigate the oral subchronic toxicity of Tartrazine in Wistar rats.

MATERIALS AND METHODS

Chemicals
Tartrazine (CAS 1934-21-0, Purity 86.7%), Sulfanilic acid (CAS 122-57-3, Purity) were purchased from Alfa Aesar (Germany), Sigma-Aldrich (Japan) respectively.

Animals and housing
Male and female Wistar rats weighing between 170 and 200 g were housed in a controlled room with a 12 h light-dark cycle and temperature of 22 ± 2°C (animal house of the department of biology, faculty of sciences, Oujda, Morocco). They were kept in transparent polypropylene cages with free access to water and dry rat pellets feeds (Société SONABETAIL, Oujda, Morocco).

Experimental design
The animals were divided into 5 groups of 6 animals each, 3 of each sex. All animals were treated by daily oral gavage for 90 days with a volume of 10ml/kg. Tartrazine and Sulfanilic acid were dissolved in distilled water. Tartrazine was administered at 5, 7.5, or 10 mg/kg b.w. Sulfanilic acid was administered at 3.75 mg/kg b.w. For the control group it was administered with distilled water.

The animals were observed daily for general conditions. They were weighed once every ten days during the administration period, on the first and the last days of the period, and on the day of necropsy. Daily food consumption was measured once a week.
At the end of week 13, all rats were deprived of food, but not water, overnight and then blood samples were collected via the abdominal aorta for hematology and serum biochemistry. Animals were then killed by exsanguination from the abdominal aorta.

**Hematology**

Hematological measurements and calculations were performed by using Coulter® AcT 5D Hematology Analyzer (Beckman Coulter Inc., Fullerton, CA, USA). Hematological evaluations included red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell volume distribution (RDW), blood platelet count (PLT), mean platelet volume (MPV), and white blood cell count (WBC), differential leukocyte percentage and reticulocyte ratio were measured.

For morphological examination differential counts of leukocytes were made by light microscopic observation of smear specimens stained with a routine May–Glinwald–Giemsa protocol.

**Clinical biochemistry**

Clinical chemistry determinations were performed by using ILab 300 (Instrumentation Laboratory Corporate Headquarters, Barcelona, Spain). Parameters included Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), blood urea nitrogen (BUN), CREatinine (CRE), GLucose (GLU), Total CHOLEsterol (T-CHO), Triglyceride (TG), Total Protein (TP).

Activity of serum ALT and AST were determined by the method of Henry and al. (1960) 20. Total protein was determined according to the method of Gornall and al. (1949) 21. Urea was determined in serum by the method of Tiffany and al. (1972) 22. Creatinine in serum was estimated by the method of Fabiny and al. (1971) 23. Total cholesterol was estimated in serum by enzymatic colorimetric method according to Trinder (1969 a) 25a. Glucose concentration in serum was estimated by enzymatic colorimetric method according to Trinder (1969 b) 25b.

**Clinical pathology and histopathology**

At the end of the treatment, animals were exsanguinated by transecting the abdominal aorta. The body surface, the intrathoracic, intra-abdominal organs and tissues were observed macroscopically.

Organ weights were obtained for the heart, lung, liver, spleen, and stomach. The left and right kidneys were weighed separately. Relative organ weights were calculated based on body weights measured on the day of sacrifice.

A portion of each organ was fixed in 10% neutral buffered formalin for histopathology, and was further processed using standard method. The micro-sections of 5 µm thicknesses were stained with hematoxylin–eosin and the prepared slides were examined under light microscope for histopathological.

**Statistical analysis**

Data are presented in tables or figures as the mean ± SEM. The statistical significance of the differences between control and experimental groups was evaluated by Student’s t-test using GraphPad Instat 3.06 26.

**RESULTS**

**Clinical observations, Body weight, Organ weights and intake food**

Compared to the water control group, treatment with Tartrazine and Sulfanilic acid did not affect mortality, clinical signs, intake food and body weights. Body weight curves for Wistar rats during the treatment period are shown in Fig.2. Data for the intact food and Organ weight are shown in Table 1.

With regard to organ weights, statistically significant decrease of the absolute right kidney weight and increases of the relative weight of the liver were observed in group treated with 10 mg/kg b.w of Tartrazine. Moreover, absolute lung and stomach weights were significantly increased in group treated with 3.75 mg/kg b.w of Sulfanilic acid.

**Hematology results**

Hematology results after the treatment period did not revealed significant changes in group control and group treated with 10 mg/kg b.w of Tartrazine. But we revealed a significantly higher mean platelet volume, neutrophile and basophile; and a significantly lower blood platelet count in group treated with 7.5 mg/kg b.w of Tartrazine. In group treated with 3.75 mg/kg b.w of Sulfanilic acid changes included a significantly lower blood platelet count, neutrophile, lymphocyte, monocyte, basophile, and eosinophile. Hematology data are shown in Table 2.
Table 1: Intact food and organ weight for Wistar rats sacrificed on day 90 of subchronic feeding of Tartrazine and Sulfanilic acid

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group control</th>
<th>5 mg/ (kg BW) of Tartrazine</th>
<th>7.5 mg/ (kg BW) of Tartrazine</th>
<th>10 mg/ (kg BW) of Tartrazine</th>
<th>3.75 mg/ (kg BW) of Sulfanilic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact food (g)</td>
<td>9.19 ± 0.39</td>
<td>10.18 ± 0.60</td>
<td>10.20 ± 0.58</td>
<td>9.81 ± 0.51</td>
<td>10.02 ± 0.33</td>
</tr>
<tr>
<td>Absolue (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>7.12 ± 0.62</td>
<td>7.07 ± 0.37</td>
<td>8.71 ± 0.71</td>
<td>8.17 ± 1.27</td>
<td>9.43 ± 1.13</td>
</tr>
<tr>
<td>Right kidney</td>
<td>0.75 ± 0.04</td>
<td>0.71 ± 0.04</td>
<td>0.77 ± 0.07</td>
<td>0.74 ± 0.08</td>
<td>0.84 ± 0.04</td>
</tr>
<tr>
<td>Left kidney</td>
<td>0.76 ± 0.05</td>
<td>0.69 ± 0.05</td>
<td>0.76 ± 0.06</td>
<td>0.77 ± 0.09</td>
<td>0.82 ± 0.04</td>
</tr>
<tr>
<td>Heart</td>
<td>0.81 ± 0.03</td>
<td>0.86 ± 0.05</td>
<td>0.85 ± 0.06</td>
<td>0.79 ± 0.06</td>
<td>0.83 ± 0.03</td>
</tr>
<tr>
<td>Lung</td>
<td>1.57 ± 0.06</td>
<td>1.66 ± 0.08</td>
<td>1.84 ± 0.1</td>
<td>1.74 ± 0.11</td>
<td>1.83 ± 0.09</td>
</tr>
<tr>
<td>Stomach</td>
<td>2.25 ± 0.12</td>
<td>2.14 ± 0.14</td>
<td>2.35 ± 0.13</td>
<td>2.17 ± 0.22</td>
<td>2.52 ± 0.12</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.55 ± 0.03</td>
<td>0.52 ± 0.12</td>
<td>0.57 ± 0.04</td>
<td>0.52 ± 0.05</td>
<td>0.57 ± 0.04</td>
</tr>
<tr>
<td>Relative (g/100g BW)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>2.86 ± 0.10</td>
<td>3.03 ± 0.07</td>
<td>3.23 ± 0.15</td>
<td>2.97 ± 0.15</td>
<td>3.23 ± 0.16</td>
</tr>
<tr>
<td>Right kidney</td>
<td>0.304 ± 0.008</td>
<td>0.308 ± 0.06</td>
<td>0.28 ± 0.02</td>
<td>0.270 ± 0.007</td>
<td>0.29 ± 0.009</td>
</tr>
<tr>
<td>Left kidney</td>
<td>0.311 ± 0.009</td>
<td>0.29 ± 0.01</td>
<td>0.28 ± 0.01</td>
<td>0.280 ± 0.008</td>
<td>0.291 ± 0.009</td>
</tr>
<tr>
<td>Heart</td>
<td>0.33 ± 0.018</td>
<td>0.37 ± 0.01</td>
<td>0.31 ± 0.01</td>
<td>0.30 ± 0.01</td>
<td>0.29 ± 0.01</td>
</tr>
<tr>
<td>Lung</td>
<td>0.65 ± 0.04</td>
<td>0.72 ± 0.05</td>
<td>0.701 ± 0.06</td>
<td>0.67 ± 0.05</td>
<td>0.64 ± 0.03</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.925 ± 0.05</td>
<td>0.92 ± 0.03</td>
<td>0.88 ± 0.04</td>
<td>0.82 ± 0.05</td>
<td>0.89 ± 0.02</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.22 ± 0.012</td>
<td>0.22 ± 0.04</td>
<td>0.21 ± 0.01</td>
<td>0.198 ± 0.007</td>
<td>0.201 ± 0.009</td>
</tr>
</tbody>
</table>

Note: values represent the mean ± SEM (n=6); * p<0.05. Significantly different from controls.

Table 2: Hematological data of Wistar rats Fed with Tartrazine and Sulfanilic acid for 90 days

Parameters                  | Group control | 5 mg/ (kg BW) of Tartrazine | 7.5 mg/ (kg BW) of Tartrazine | 10 mg/ (kg BW) of Tartrazine | 3.75 mg/ (kg BW) of Sulfanilic acid |
<table>
<thead>
<tr>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (10^3/µl)</td>
<td>4.53 ± 1.18</td>
<td>4.58 ± 0.43</td>
<td>6.26 ± 1.54</td>
<td>6.80 ± 1.25</td>
<td>9.21 ± 1.25</td>
</tr>
<tr>
<td>RBC (10^6/µl)</td>
<td>7.39 ± 0.26</td>
<td>6.99 ± 0.24</td>
<td>7.73 ± 0.22</td>
<td>7.56 ± 0.19</td>
<td>7.75 ± 0.23</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>13.13 ± 0.33</td>
<td>12.46 ± 0.51</td>
<td>13.71 ± 0.38</td>
<td>13.51 ± 0.24</td>
<td>13.65 ± 0.18</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>38.93 ± 1.19</td>
<td>36.23 ± 1.59</td>
<td>40.40 ± 1.18</td>
<td>40.36 ± 0.88</td>
<td>40.71 ± 0.90</td>
</tr>
<tr>
<td>MCV (FL)</td>
<td>52.50 ± 0.42</td>
<td>52.33 ± 0.71</td>
<td>52.16 ± 0.16</td>
<td>53.33 ± 0.42</td>
<td>52.50 ± 0.56</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>17.91 ± 0.26</td>
<td>17.41 ± 0.37</td>
<td>17.75 ± 0.14</td>
<td>17.91 ± 0.22</td>
<td>17.66 ± 0.32</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>33.76 ± 0.26</td>
<td>33.41 ± 0.28</td>
<td>33.98 ± 0.22</td>
<td>33.50 ± 0.21</td>
<td>33.56 ± 0.30</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>12.00 ± 0.38</td>
<td>12.10 ± 0.79</td>
<td>12.15 ± 0.23</td>
<td>11.13 ± 0.35</td>
<td>11.98 ± 0.37</td>
</tr>
<tr>
<td>PLT (10^3/µl)</td>
<td>72500 ± 1156</td>
<td>62750 ± 43.90</td>
<td>60983 ± 45.58*</td>
<td>72716 ± 1675</td>
<td>65416 ± 233.3*</td>
</tr>
<tr>
<td>MPV (FL)</td>
<td>64.55 ± 0.08</td>
<td>68.66 ± 0.23</td>
<td>68.00 ± 0.03</td>
<td>63.88 ± 0.01</td>
<td>6.63 ± 0.07</td>
</tr>
<tr>
<td>NE (%)</td>
<td>19.43 ± 1.86</td>
<td>32.6 ± 4.55</td>
<td>25.35 ± 2.17*</td>
<td>22.40 ± 2.19</td>
<td>18.50 ± 0.74*</td>
</tr>
<tr>
<td>LY (%)</td>
<td>72.75 ± 1.27</td>
<td>59.70 ± 3.97</td>
<td>65.78 ± 3.09</td>
<td>68.98 ± 1.97</td>
<td>70.05 ± 0.43*</td>
</tr>
<tr>
<td>MO (%)</td>
<td>3.48 ± 0.63</td>
<td>3.98 ± 1.66</td>
<td>6.08 ± 2.10</td>
<td>4.05 ± 0.69</td>
<td>5.58 ± 1.07*</td>
</tr>
<tr>
<td>EO (%)</td>
<td>3.86 ± 1.18</td>
<td>3.28 ± 1.21</td>
<td>1.88 ± 0.55</td>
<td>4.20 ± 1.41</td>
<td>5.36 ± 0.76*</td>
</tr>
<tr>
<td>BA (%)</td>
<td>0.46 ± 0.03</td>
<td>0.31 ± 0.03</td>
<td>0.90 ± 0.16*</td>
<td>0.36 ± 0.03</td>
<td>0.50 ± 0.03*</td>
</tr>
</tbody>
</table>

Note: values represent the mean ± SEM (n=6); * p<0.05. Significantly different from controls.** p<0.01. very significantly different from controls.

The morphological changes of rat RBCs were made by light microscopical observation. All the treated groups showed morphological changes in the form of echinocytes. The percentage of echinocytes significantly increased among the treated groups in a dose-response manner (*** p<0.001) (Fig 3, Fig 4).

Fig. 3: The percentage of echinocytes significantly increased among the treated groups in a dose-response manner (P<0.001)

Note: values represent the mean ± SEM (n=12); *** p<0.001. extremely significantly different from controls
Biochemical examination

The levels of GLU, CREA, were significantly increased in all groups compared to group control (Fig.5, Fig.6). The levels of CHOL, TG were significantly increased in group treated with 7.5, 10 mg/kg b.w of Tartrazine and group treated with 3.75 mg/kg b.w of Sulfanilic acid (Fig.7, Fig.8). There was no significant difference in the level of BUN and ALT among the different groups. The level of AST was significantly increased in group treated with 10 mg/kg b.w of Tartrazine and group treated with 3.75 mg/kg b.w of Sulfanilic acid. The total Protein was significantly increased in group treated with 7.5, 10 mg/kg b.w of Tartrazine.

Note: values represent the mean ± SEM (n=6); *p<0.05. significantly different from controls; **p<0.01. extremely significantly different from controls

---

Fig. 4: Effects of Tartrazine on the morphology of rat RBC. (A): untreated RBC and RBC treated with (B): 5 mg/ (kg BW), (C): 7.5 mg/ (kg BW), (D): 10 mg/ (kg BW) and (E): treated with 3.75 mg/ (kg BW) of Sulfanilic acid. The echinocytes can be observed in (B), (C) and (D)

Biochemical examination

The levels of GLU, CREA, were significantly increased in all groups compared to group control (Fig.5, Fig.6). The levels of CHOL, TG were significantly increased in group treated with 7.5, 10 mg/kg b.w of Tartrazine and group treated with 3.75 mg/kg b.w of Sulfanilic acid (Fig.7, Fig.8). There was no significant difference in the level of BUN and ALT among the different groups. The level of AST was significantly increased in group treated with 10 mg/kg b.w of Tartrazine and group treated with 3.75 mg/kg b.w of Sulfanilic acid. The total Protein was significantly increased in group treated with 7.5, 10 mg/kg b.w of Tartrazine.

Note: values represent the mean ± SEM (n=6); *p<0.05. significantly different from controls; **p<0.01. extremely significantly different from controls

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Fig. 5: Effects of Tartrazine on plasma Glucose. (Group control) untreated and treated with (Group 1) 5 mg/ (kg BW), (Group 2) 7.5 mg/ (kg BW), (Group 3) 10 mg/ (kg BW), and group treated with Sulfanilic acid

Note: values represent the mean ± SEM (n=6); *p<0.05. significantly different from controls; **p<0.01. extremely significantly different from controls

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Fig. 6: Effects of Tartrazine on plasma Creatinine. (Group control) untreated and treated with (Group 1) 5 mg/ (kg BW), (Group 2) 7.5 mg/ (kg BW), (Group 3) 10 mg/ (kg BW), and group treated with Sulfanilic acid

Note: values represent the mean ± SEM (n=6); *p<0.05. significantly different from controls; **p<0.01. extremely significantly different from controls; ***p<0.001. extremely significantly different from controls
Fig. 7: Effects of Tartrazine on plasma Cholesterol. (Group control) untreated and treated with (Group 1) 5 mg/ (kg BW), (Group 2) 7.5 mg/ (kg BW), (Group 3) 10 mg/ (kg BW), and group treated with Sulfanilic acid. Note: values represent the mean ± SEM (n=6); * p<0.05. significantly different from controls.

Fig. 8: Effects of Tartrazine on plasma Triglycerid. (Group control) untreated and treated with (Group 1) 5 mg/ (kg BW), (Group 2) 7.5 mg/ (kg BW), (Group 3) 10 mg/ (kg BW), and group treated with Sulfanilic acid. Note: values represent the mean ± SEM (n=6); * p<0.05. significantly different from controls; ** p<0.01. very significantly different from controls.

Table 3: Effects of Tartrazine on plasma Urea, Total Protein, AST, ALT. (Group control) untreated and treated with (Group 1) 5 mg/ (kg BW), (Group 2) 7.5 mg/ (kg BW), (Group 3) 10 mg/ (kg BW)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group control</th>
<th>5 mg/ (kg BW) of Tartrazine</th>
<th>7.5 mg/ (kg BW) of Tartrazine</th>
<th>10 mg/ (kg BW) of Tartrazine</th>
<th>3.75 mg/ (kg BW) of Sulfanilic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (g/l)</td>
<td>0.386 ± 0.024</td>
<td>0.395 ± 0.018</td>
<td>0.36 ± 0.02</td>
<td>0.40 ± 0.039</td>
<td>0.39 ± 0.03</td>
</tr>
<tr>
<td>Total Protein</td>
<td>63.5 ± 2.56</td>
<td>58.83 ± 0.47</td>
<td>71.5 ± 2.41 *</td>
<td>69.83 ± 0.70 *</td>
<td>57.66 ± 0.91</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>120 ± 5.64</td>
<td>131.33 ± 7.50</td>
<td>119 ± 7.68</td>
<td>165.16 ± 17.82 *</td>
<td>159.16 ± 12.755 *</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>54.16 ± 5.33</td>
<td>67.83 ± 4.27</td>
<td>58 ± 3.66</td>
<td>62 ± 2.84</td>
<td>60.66 ± 1.97</td>
</tr>
</tbody>
</table>

Note: values represent the mean ± SEM (n=6); p<0.05. Significantly different from controls.

Histopathological studies

The results of histological examination are shown in Table 4. In control group no structural changes were identified by histopathology in the liver, kidneys, stomach, or jejunum, suggesting that these animals were healthy and the conditions under which the experiment was conducted were proper. All the tissue sections obtained from the stomach of experimental Wistar rats fed with 5, 7.5, 10 mg/ (kg BW) of Tartrazine, and with 3.75 mg/kg b.w of Sulfanilic acid were not different from the control animals tissues. All the sections were essentially normal without any inflammatory lesion (Fig.9).

The histopathological examination showed lymphoid infiltrates in the Jejunum of experimental Wistar rats fed with 7.5, 10 mg/ (kg BW) of Tartrazine (Fig.10).

The histopathological studies showed brown pigment deposition in the Kupffer cells and fatty degeneration of the liver in groups treated with 7.5, 10 mg/ (kg BW) of Tartrazine (Fig.11).

They also revealed tubular dilatation with thickened basement membrane in group treated with 5 mg/ (kg BW) of Tartrazine, tubular degeneration, and dilatation of the glomerular capillaries in group treated with 7.5 mg/ (kg BW) of Tartrazine, and intercapillary sclerosis, atrophy of glomerulus in group treated with 10 mg/ (kg BW) of Tartrazine (Fig.12).
### Table 4: Histological data for the main organs of Wistar rats treated orally with different concentration of Tartrazine and Sulfanilic acid for 90 days

<table>
<thead>
<tr>
<th>Dose</th>
<th>Tartrazine (mg/kg b.w)</th>
<th>Sulfanilic acid (mg/kg b.w)</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>No. of animals examined</td>
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<tr>
<td>Kidneys</td>
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<td>Eosinophilic body</td>
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<tr>
<td>Liver</td>
<td>Centrilobular necrosis</td>
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Note: values represent the mean ± SEM (n=6); * Significantly different from controls at p<0.05 by Fisher exact test; ** Very significantly different from controls p<0.01 by Fisher exact test

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**Fig. 9:** Photomicrographies of paraffin embedded rat stomach. All sections were stained with hematoxylin and eosin

(A): Section of control rats showing normal histological appearance of the stomach. (B): Section of rat stomach treated with 5 mg/ (kg BW) of Tartrazine showing normal architecture. (C): Section of rat stomach treated with 7.5 mg/ (kg BW) of Tartrazine showing normal architecture. (D): Section of rat stomach treated with 10 mg/ (kg BW) of Tartrazine showing normal architecture. (E): Section of rat stomach treated with 3.75 mg/ (kg BW) of Sulfanilic acid showing normal architecture. (Scale bar = 40μm).
Fig. 10: Photomicrographies of paraffin embedded rat jejunum. All sections were stained with hematoxylin and eosin.

(A): Jejunum section of control rats showing normal histological appearance of the jejunum. (B): Section of rat jejunum treated with 5 mg/ (kg BW) of Tartrazine showing lymphoid infiltration. (C): Section of rat jejunum treated with 7.5 mg/ (kg BW) of Tartrazine showing lymphoid infiltration. (D): Section of rat jejunum treated with 10 mg/ (kg BW) of Tartrazine showing lymphoid infiltration. (E): Section of rat jejunum treated with 3.75 mg/ (kg BW) of Sulfanilic acid showing lymphoid infiltration. (Scale bar = 40μm).

Fig. 11: Photomicrographies of paraffin embedded rat liver. All sections were stained with hematoxylin and eosin

(A): Liver section of control rats showing normal histological appearance of the liver. (B): Section of rat liver treated with 5 mg/ (kg BW) of Tartrazine revealed fatty degeneration (arrow). (C): Section of rat liver treated with 7.5 mg/ (kg BW) of Tartrazine revealed fatty degeneration (black arrow) and brown pigment deposition in Kupffer cells (white arrow). (D): Section of rat liver treated with 10 mg/ (kg BW) of Tartrazine revealed fatty degeneration (black arrow) and brown pigment deposition in Kupffer cells (white arrow). (E): Section of rat liver treated with 3.75 mg/ (kg BW) of Sulfanilic acid showing near normal architecture. (Scale bar = 40μm).
when Tomato Red was consumed by Swiss albino mice. The present study shows that Tartrazine and Sulfanilic acid induced a morphological change from the discoid shape to an echinocytic manner. This is in agreement with Helal and al. (2000) who found a significant increase in serum total protein concentration when compared to control rats.

The differences in mean body weight, organ weights and intake food between control and treated groups (5, 7.5, 10 (mg/kg bw) of Tartrazine and 3.75 (mg/kg bw) of Sulfanilic acid) were non-significant. These results are in accordance with the data from Borzelleca and Hallagan (1988b) who suggested that the decrease of body weight was due to decreased caloric intake due to the Tartrazine component of the diet, and that there were a few differences between control and treated groups in the haematological, clinical chemistry and urine analysis parameters but none of the differences appeared to be treatment-related.

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The present study shows that Tartrazine and Sulfanilic acid induced a morphological change from the discoid shape to an echinocytic form in rat RBCs. The percentage of RBC echinocytes forms treated by Tartrazine and Sulfanilic acid were shown in a dose-response manner. Some studies had shown that RBCs respond to various treatments by various agents by altering their morphological features (Przybylka and al., 1998; Taib and al., 2009).

Our work revealed that rats which consumed 7.5, 10 mg/kg bw of Tartrazine showed a significant increase in serum total protein concentration when compared to control rats.

These results are in accordance with Mekkawy and al. (1998) who found a significant increase in serum total protein, also these are in agreement with Aboel-Zahab and al. (1997) who found the same effect on serum total protein with rats whose diets were supplemented with chocolate colours A and B. In addition, Sharma and al. (2006) found that total protein was significantly elevated when Tomato Red was consumed by Swiss albino mice.

DISCUSSION
During the administration period, no deaths occurred in animals of any group. There were no abnormal signs in all groups.

The differences in mean body weight, organ weights and intake food between control and treated groups (5, 7.5, 10 (mg/kg bw) of Tartrazine and 3.75 (mg/kg bw) of Sulfanilic acid) were non-significant. These results are in accordance with the data from Borzelleca and Hallagan (1988b) who suggested that the decrease of body weight was due to decreased caloric intake due to the Tartrazine component of the diet, and that there were a few differences between control and treated groups in the haematological, clinical chemistry and urine analysis parameters but none of the differences appeared to be treatment-related.

Regarding the hematological finding in group treated with 3.75 mg/kg bw of Sulfanilic acid, the observed increase of WBC suggests a physiological inflammatory response of its absorption.

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Sharma and al. (2005) observed a significant increase in serum total protein and globulin in rats whose diets were supplemented with chocolate colours A and B.

K.A. Amin and al. (2010) demonstrated that high dose of Tartrazine caused a significant increase in serum total protein and serum albumin concentration when rats consumed high dose of Tartrazine (500 mg/kg bw) or low dose of Tartrazine (15 mg/kg bw).

Our study demonstrated that the daily intake for 90 day of Tartrazine exhibited a significant increase in serum Creatinine concentration when compared with control rats in a dose-response manner. This is in agreement with data reported by K.A. Amin and al. (2010) who found a significant elevation in serum Creatinine and urea level of rats dosed with organic azo dye (fast green) orally for 35 days. Our study is also in accordance with data reported by K.A. Amin and al. (2010) who observed a significant elevation in serum Creatinine and urea level when rats consumed high dose of Tartrazine (500 mg/kg bw) or low dose of Tartrazine (15 mg/kg bw).

Despite the fact that our results are in agreement with the preceding studies, they are also in a contrary because the serum Urea concentration showed no significant increase. The mechanism for these changes are unclear from the present results, but these changes may be due to the low concentration of Tartrazine given to Wistar rat. Also, these results are at the same time correlated with those reported by Aboel-Zahab and al. (1997) who observed no significant increases in serum Urea concentration, and are also in a contrary because the serum Creatinine concentration showed no significant increase in rats whose diets were supplemented with chocolate colours A and B that Tartrazine and Carmoisine were

Fig. 12: Photomicrographies of paraffin embedded rat kidney. All sections were stained with hematoxylin and eosin

(A): kidney section of control rats showing normal histological appearance of the kidney. (B): Section of rat kidney treated with 5 mg/ (kg BW) of Tartrazine revealed tubular dilatation with thickened basement membrane (white arrow). (C): Section of rat kidney treated with 7.5 mg/ (kg BW) of Tartrazine showed revealed tubular degeneration, (white arrow) and dilatation of the glomerular capillaries (black arrow). (D): Section of rat kidney treated with 10 mg/ (kg BW) of Tartrazine revealed intercapillary sclerosis (white arrow) and atrophy of glomerulus (black arrow). (E): Section of rat kidney treated with 3.75 mg/ (kg BW) of Sulfanilic acid showing near normal architecture. (Scale bar = 40μm).
among of them (sunset yellow, Tartrazine, Carmoisine and brilliant blue) in varying concentrations.

The increase in serum Cholesterol and Triglycerid levels obtained in this study are in accordance with results recorded by Aboel-Zaahab and al. (1997) 31 who observed significant increases in serum total lipids, cholesterol and triglycerides in rats whose diets were supplemented with chocolate colours A and B that Tartrazine and Carmoisine were among of them (sunset yellow, Tartrazine, Carmoisine and brilliant blue) in varying concentrations. Our results are in contrary with V. Rus and al. (2009) 39 who described changes in the liver when guinea pigs received Tartrazine in drinking water in concentrations of 1, 2 and 3% for 3 weeks. For the concentration of 1% there was a slight congestion, in both intralobular and extralobular vessels and discrete perivascular edema. In the external third of lobules they observed some apoptotic hepatocytes. For the concentration of 2%, the vascular phenomena are more pronounced, capillary congestion is present in many lobules, determining a slight compression atrophy of hepatocyte cords. Hepatocytes in various stages of apoptosis were observed in small numbers within the liver lobe, the number of hepatocytes in apoptosis being greater at the lobe periphery. For 3% concentration, liver lesions are more advanced than in other concentrations.

The groups treated with 10 mg/ (kg BW) of Tartrazine revealed a marked increase in the average liver weight in the experimental groups and severe histological changes in the liver; But we did not reveal congested blood vessels and areas of haemorrhage in the liver.

In the early phase of fatty degeneration, vacuoles appear in the cytoplasm around the nucleus, because their lipid content is dissolved in the course of embedding. The vacuoles appear empty. As the damage to the cells progresses, the hepatocytes become swollen and a single large vacuole will occupy their entire cytoplasm, pushing aside the nucleus and making the hepatocyte signet-ring shaped. The degenerated hepatocytes form wide trabeculae which compress and narrow the lumen of the sinusoids 40.

The present histopathological findings revealed tubular dilatation, tubular degeneration, dilatation of the glomerular capillaries, intercapillary sclerosis, and atrophy of glomerulus. These changes are in consistency with the reporting of V. Rus and al. (2009) 39 who described changes in the kidney when guinea pigs received Tartrazine in drinking water in concentrations of 1, 2 and 3% for 3 weeks. The changes in the kidney are somewhat comparable to those of liver, meaning that there is congestion of different intensities and perivascular edema. In addition to these, abnormal glomerular filtration and glomerular or tubular stasis were observed. In animals treated with low dose (1%), vascular congestion is relatively moderate and for the group treated with Tartrazine present also altered glomerular filtration processes, with accumulation of protein material in the capsular space. In the groups treated with 2% concentration, the changes are comparable to a certain point, only to have higher intensity. Glomerular filtration disorders are present in animals treated with carmoisine, but are more pronounced at this dose for 2% Tartrazine, where stasis occurs with atrophy of renal corpuscles. Furthermore, area nephrocyte apoptosis appears. Concentration of 3% led to the kidney level the most pronounced changes. Renal corporules atrophy is present in both groups, for the group treated with Tartrazine having in addition several corporules and tubular necrosis.

The glomerulus is the primary site of action of several chemicals and it may be injured by any toxic, metabolic and immunologic mechanism 41-42. The toxic irritant substances brought to the kidney by circulatory blood cause degenerative changes in the kidney tissues 43. According to Varely, H. (1987) 43, the blood urea can be increased in all forms of kidney diseases. Also plasma Creatinine increases in renal diseases gave prognostic significance than those of other nitrogenous substances.

Tartrazine is transformed into aromatic amine sulfanilic acid after being metabolized by the gastrointestinal microflora 37. The formed aromatic amines can generate reactive oxygen species as part of their metabolism by interaction of these amino groups with nitrite.
or nitrate containing foods or in the stomach. The reactive oxygen species could be produced in the metabolism of nitrosamines and increase oxidative stress 45.

Reactive oxygen species play an essential role in pathological changes in the liver 46. Increased generation of Reactive oxygen species or free radicals is able to cause auto-oxidation of the hepatic cells, resulting in marked hepatic lesions 47.

CONCLUSION

In conclusion, the present 13-week subchronic study indicates that Tartrazine not only causes changes in hepatic and renal parameters but also their effect become more risky at higher doses because it can induce oxidative stress by formation of free radicals.

Therefore, it is necessary that people should be aware about the hazardous effects of consuming Tartrazine.

ABBREVIATIONS

Acceptable Daily Intake (ADI); Alanine aminotransferase (ALT); Aspartate aminotransferase (AST); Basophil (BA); Blood platelet count (PLT); Blood urea nitrogen (BUN); Body weight (bw); Creatinine (CRE); Esophinephile (EO); Glucose (GLU); Hematocrit (HCT); Hemoglobin concentration (HGB); Lymphocyte (LY); Mean corpuscular haemoglobin (MCH); Mean corpuscular haemoglobin concentration (MCHC); Mean corpuscular volume (MCV); Monocyte (MO); Neutrophile (NE); Red blood cell count (RBC); Red cell volume distribution (RDW); Total cholesterol (T-CHO); Total protein (T-P); Triglyceride (TG); White blood cell count (WBC).

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