Effect of food preservative and food coloring agent on some physiological and hematological parameters in albino rats and the protective role of garlic

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Abstract:

Most children, in the age of nursery consume food that contains both colorants and preservatives with great amount. This observation led us to study the interaction between sodium nitrite as a food preservative and sun set yellow as a coloring agent. The mixture of the two agents at the limited dose of each was found to be a lethal dose. So, 1/10th of this dose was used daily for 30 days. Animals were divided into three groups. The first group served as a control, while the second group was orally administered a mixture of 10 mg sod.nitrite (NaNO3)/kg mixed with 0.5 mg/kg/day sun set yellow (S.S.Y). The third group received garlic (5 mcg/kg) in addition to the above mentioned mixture. After 30 days of treatment, half of the animals from each group were decapitated. The other half of the animals was left for another 15 days without any additional treatment as a recovery period.

Ingestion of the mixture of (NaNO3 and S.S.Y) significantly decreased rat body weight, RBCs and WBCs counts, Hb%, Hct%. No changes were recorded for organ/ body weight, respiratory rate, heart rate, rectal temperature.

A complete recovery from the abnormalities of most physiological and hematological parameters was observed after the recovery period or when garlic was administered.

Key Words: Garlic, antioxidant, preservatives, colorants.

Introduction:

Food additives are substances internationally added to food. They may be natural or synthetic (Harris, 1986). The principal classes of food additives are coloring agents, preservatives, flavors, emulsifiers and stabilizers (Lindsay, 1985). Common anti-microbial preservatives include sodium nitrate, sodium nitrite, sulphur dioxide, sodium bisulphate, potassium bisulphate, ect and disodium EDTA.

One of the principal preservatives are the nitrates which are used in the form of salts or free acids (HMSO, 1987). The use of sodium nitrate as a preservative is common in cooked meat, sausages and the milk used for some types of cheese. Because of the use of more than one type of such food, the percentage of nitrite content of the daily food ratio may be higher than the admissible level (Bilczuk et al., 1991). Apparently very little nitrates are formed by endogenous synthesis and most, if not all, are of dietary origin (Bartholomew and Hill, 1984).

Food colorants may often be considered simply cosmetic in nature, but its role is very significant. Both food quality and flavor are closely associated with color. Consumers are conditioned to expect food of certain colors and to reject any deviation from their expectations (Amerine et al.,1965).

Every food designer knows that consumers judge a product not only on its
flavor, but on its appearance as well. One important class of ingredients exists solely to enhance the appearance of what we eat: food colors. In recent years, product designers have been asked to formulate using so-called natural colors with increasing frequency. This presents a set of challenges that is totally different to those presented when using certified colors. (Kuntz, 1994).

Garlic has played an important dietary and medical role through the history of mankind. In some western countries, the sale of garlic preparations ranks with those of leading prescription drugs. The therapeutic efficacy of garlic encompasses a wide variety of ailments, including cardiovascular, cancer, hepatic microbial infections. Various preparations of garlic, mainly aged garlic extract (AGE), have been shown to have promising antioxidant potential. However, the presence of more than one compounds in garlic, with apparently opposite biological effects, has added to the complexity of the subject. Raw garlic homogenate has been reported to exert antioxidant potential but higher doses have been shown to be toxic to the heart, liver and kidney (Banerjee et al., 2003).

It has been noticed that children often eat food containing preservatives and at the same time drink some drinks containing colorants. The question arises here whether food preservatives and colorants would interact with each other. The aim of this work is therefore to test this possibility by mixing a limited dose of sodium nitrite (one of the most used preservatives) with a limited dose of sunset yellow (one of the most attractive colorants and test their recombinant effect on rats). The interaction of both limited doses resulted in a recombinant lethal dose that led to the death of all rats used. So, this study was planned to achieve two goals: The first is; to follow up the biological effect of this mixture on young male albino rats; and the second; is to study the effect of garlic (one of the most potent antioxidant) in order to illustrate the possibility of ameliorating the expected hazards.

Material and method:
Thirty young male albino rats (weighing about 70-80 gm) were used in this study. Animals were housed in stainless steel cages, fed on rat chew and offered water ad libitum. The animals were divided into three equal groups (10 rats each) as follows:

The first group: (control group)
Orally administered 10 mg Na No3/Kg and 0.5 mg sun set yellow (S.S.Y.) daily for a month.

The second group: Received the same dose of Na No3 and S.S.Y in addition to 50 mcg/ Kg/ day of garlic administered by oral intubation to each rat, for one month.

Body weights, respiratory rate, heart rate and rectal temperature were recorded once a week throughout the experimental period. After 30 days of treatment, 5 animals of each group were weighed and then decapitated. While the other half was kept for two weeks without any additional treatment for recovery.

From each of the decapitated rats, liver, kidney, hearts and testes were dissected out, cleaned from adherent tissues and weighed at once.

Blood samples were collected and EDTA, an anticoagulant, was added to the collected blood for hematological parameters. Hemoglobin concentration was determined according to Van-Kampen and Zulstra (1961). Red and white blood cells were counted, and haematocrit values (Hct) were estimated using the technique of Rodak (1995).

Student t-test was used for comparison of data obtained for different parameters from different experimental animal groups. Significant differences between the means of control and treated groups were considered only at ( p < 0.05) (Sokal and Rohif, 1981).

Results:
Control young rats showed a net gain (19.6% and 18.9%) in body weight after the treatment and recovery periods respectively; while the gain of body weight in rats treated
Effect of food preservative and food coloring……

by the mixture of sodium nitrite, sunset yellow (NaNO3 and SSY) and garlic signify-cantly was higher (22.5% and 18.9%) as compared to control (p<0.01). On the other hand, there was significant (p<.01) weight loss in rats treated with a mixture (NaNO3 and SSY). After recovery period (15 days), there was a gain in the body weight (10.6%) table (1). The organs/body weight of male albino rats treated and control rats after both treated and recovery periods are presented in table (1). No significant detectable changes in the relative weight of the kidney, brain, heart, liver and testes were recorded in all treated groups.

All of the animals showed nonsignificant changes in respiratory rates, heart rate and rectal temperature, after both treated and recovery periods (Table 2).

Investigation of the effect of oral administration of the mixture of sod.nitrite and sunset yellow on red blood cells, hemoglobin, haematocreite, revealed highly signi-fycant decrease (P<.01) in all parameters. Supplementation with garlic, however resulted in values for hematological parameters which was Nansignificantly recorded from control rats. After the recovery period, no significant values were recorded in all treated groups as shown in figures (1-4). Treatment with the mixture caused significant reduction in white blood cell count as shown in figure (1).

Table (1): The change in body weight gain and in the ratio of organ weight/body, weight of rats control, rats (control, treated with NaNO3+SSY and treated with NaNO3+SSY+garlic) after experimental and recovery periods.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treated period</th>
<th>Recovery period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>NaNO+SSY</td>
</tr>
<tr>
<td>%of body weight</td>
<td>X</td>
<td>19.6</td>
</tr>
<tr>
<td></td>
<td>S.E</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Kidney/ b.wt</td>
<td>X</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>S.E</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Brain b.wt</td>
<td>X</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>S.E</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>N.S</td>
</tr>
<tr>
<td>Cardio-somatic index</td>
<td>X</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>S.E</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Hepato-somatic index</td>
<td>X</td>
<td>3.2</td>
</tr>
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<td></td>
<td>S.E</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;.01</td>
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<tr>
<td>Ganado-somatic index</td>
<td>X</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td>S.E</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

S.E=avera rage ±

118
**Table (2):** The effect of sodium nitrite and SSY and garlic on respiratory rate, heart beats and rectal temperature after experimental and recovery periods.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treated period</th>
<th></th>
<th>Recovery period</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>NaNO+SSY</td>
<td>NaNO+SSY+Garlic</td>
<td>Control</td>
</tr>
<tr>
<td>Respiratory rate (breath/min)</td>
<td>X</td>
<td>49</td>
<td>51</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>S.E.</td>
<td>1.8</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>N.S</td>
<td>&lt;.05</td>
<td>N.S</td>
</tr>
<tr>
<td>Heart beat/min.</td>
<td>X</td>
<td>136</td>
<td>139.6</td>
<td>134</td>
</tr>
<tr>
<td></td>
<td>S.E.</td>
<td>1.8</td>
<td>0.7</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
<td>X</td>
<td>34.72</td>
<td>34.52</td>
<td>34.86</td>
</tr>
<tr>
<td></td>
<td>S.E.</td>
<td>0.09</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
</tr>
</tbody>
</table>

**Fig. (1):** The effect of sodium nitrite, S.S.Y. and garlic on W.B.Cs.

**Fig. (2):** The effect of sodium nitrite, S.S.Y. and garlic on R.B.Cs.
Effect of food preservative and food coloring……

Fig.(3): The effect of sodium nitrite, S.S.Y. and garlic on hemoglobin concentration (HB%).

![Graph showing the effect of sodium nitrite, S.S.Y. and garlic on hemoglobin concentration (HB%).]

Fig.(4): The effect of sodium nitrite, S.S.Y. and garlic on hematocrit value (Hct%).

![Graph showing the effect of sodium nitrite, S.S.Y. and garlic on hematocrit value (Hct%).]

Discussion:

The present observations of body weight loss after the treatment with both food preservative and food colorant may be due to the reduction of food consumption (Grant and Butler, 1989). On the other hand, the reduction of mean body weight may be due to the increase in the level of both nitrite and sunset yellow leading to increased catabolic processes in the body. Greenblatt and Mirvish (1972), Maekawa et al. (1982) and Til et al. (1998) recorded a reduction of body weight gain due to nitrite treatments. Many investigators recorded a reduction in body weight as a result of colorants supplementation (Brozelleca et al. 1989; Osman et al., 1995 and Abu El-Zahab et al., 1997). In the present measurements, it was found that garlic ameliorates the catabolic effect of both NaNO3 and S.S.Y.

The present results showed nonsignificant changes of the relative weight of the tested organs of the male rats treated with NaNO3 + SSY or with NaNO3 + SSY + garlic. Hirose et al., (1993) and Yoshida et al., (1994) renatice d an increase of absolute and relative liver and kidney weights of rats treated with sodium nitrite. These results are in contrary with those of the present work. This could be due to the lesions and other disturbances (Dini et al., 1992, and Hirose et al., 1993) leading to loss of nutrient and fluids or to inhibition of gastrointestinal mucosa Na+ / K+, ATPase and alkaline phosphatase (Bruning-Fann and Kaneene, 1993).

The present data revealed that respiration rate, heart rate and body temperature are almost the same in all groups under the treatment conditions.

Administration of both sod.nitrite and S.S.Y. for one month to rats induced a decease of W.B.Cs,R.B.Cs,Hb% and Hct%. It is known that
Garlic has been shown to have promising antioxidant potential that defends against free radicals damage, thereby preserving the body’s healthy functioning (Hey, 2002). So, it’s well recommended to use a moderate dose of garlic daily in children and adult’s food since it protects them against pro-oxidant and other tragic effects resulting from food additives and other food toxins. Aged Garlic Extract (AGE) can protect vascular endothelial cells from oxidant injury. Also garlic contains about 0.5% of a volatile oil that is composed of sulphur-containing compo-unds. Garlic’s sulphur compounds, in addition to selenium and Vitamins A and C containing compounds, make it a potent antioxidant, protecting cell membranes and DNA from damage and disease. So, it is important to keep an array of antioxidant compounds to develop good herbal preparation, like AGE (Yamasaki & Lau, 1997).

References
Effect of food preservative and food coloring……

تأثير خلط المواد الحافظة و الملونة للأطعمة على بعض المعايير الفسيولوجية ومعايير الدم في الجرذان البيضاء والتأثير الوقائي للثوم

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معظم الأطفال يأكلون و يشربون مكواولات تحتوي على مواد حافظة و مواد ملونة في نفس الوقت. وقد لفت هذا السلوك الانتباه للدراسة التفاعل بين أحد المواد الحافظة الشائعة وهي نتريت الصوديوم مع أحد المواد الملونة وهي "صن ست الأصفر" ولقد وجد أن خلط النسبة المسموحة لكل من هانيين المادتين قد أدى إلى تأثير سام و لذلك تم استخدام (1/10) من هذه الجرعة على الجرذان لمدة 30 يوما حيث قسمت الحيوانات إلى ثلاث مجموعات. المجموعة الأولى استخدمت كمجموعة ضابطه بينما جربت المجموعة الثانية خليط من 10 مجم نترات الصوديوم و 5 مجم/ كجم/ يوم صن ست الأصفر أما المجموعة الثالثة فقد تناولت 5مجم/ كجم من الثوم بالإضافة إلى الخليط السابق واستمرت المعاملة لمدة 30 يوما ثم نجحت نصف المجاميع و ترك النصف الآخر دون أي معاملة للاستشفاء فترة 15 يوم أخرى. لقد أدى تجريع الخليط المكون مع نترات الصوديوم و صن ست الأصفر إلى نقص ملحوظ في وزن الجسم و عدد الكرات الدم الحمراء والبيضاء و نسبة معدل الهيموجلوبين والهيماتوكريبت. بينما لم يتأثر نسبة وزن الأعضاء و وزن الجسم و معدل التنفس و ضربات القلب و درجة حرارة الجسم.

وقد لوحظ أنه قد تم الشفاء من أغلب هذه التغيرات الفسيولوجية ومعايير الدم بعد فترة 15 يوم من الاستشفاء أو مع تناول عنصر الثوم ولهذه البحث هو دراسة التفاعل الذي يحدث بين المواد الحافظة والملونة على الجرذان وهذا يحدث من خلط جرعة محددة من نترات الصوديوم مع جرعة محددة من "صن ست الأصفر". و تهدف الدراسة إلى عاملين و الأول هو متابعة التأثيرات البيولوجية لهذه المخلوط على الجرذان و الثانية لدراسة تأثير عنصر الثوم وهو من العناصر القوية ضد التكسد و الذي يستخدم لتفاين الآثار الضارة المتوقعة لحمى المادتين.