

Microscopic Studies Of The Effect Of Some Food Additives On The Kidney Of Albino Rat

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Abstract

Recently the use of synthetic food coloring additives was increased and the levels of human exposure to such agents are very broad, thus feeding over long periods may continually possess potential hazards to the human health. Also most of the food colors tested in the conventional toxicity experiments showed toxic effects at very high level of intake i.e. 1-5 % in the diet.

The aim of this study was to evaluate the histological and histochemical effects of some of these substances (Sodium nitrate and sunset yellow) on the liver of adult albino rats. The study included three main parts:

- A. Histological studies on the liver under the effect of (Sodium nitrate and sunset yellow) substances. Using paraffin sections, which were stained with Hx & Eosin, Masson Trichrome
- B. Evaluation of histochemical activity of both alkaline phosphates enzyme and succinic dehydrogenase enzymes on the renal tissue.
- C. Statistical evaluation using image analyzer to detect glomerular area, area percentage of collagen fibers distribution and optical density of both alkaline phosphatase enzyme activity in glomerulus and succinic dehydrogenase enzyme activity in renal tubules. Seventy adult male albino rats were used. Nitrate and sunset yellow were given orally through a gastric tube in dose of 1 mg / kg / b.w. daily. The animals were classified into seven groups.

1. **Group I** (Control group)
2. **Group II:** The animals were given sodium nitrate in a dose of 1mg/kg/bwt for one month.
3. **Group III:** The animals were given sun set yellow in a dose of 1mg/kg/bwt/day for one month.
4. **Group IV:** The animals were given sodium nitrate and sunset yellow for one month in a dose of 1mg/kg/bwt/for each drug in a separate manner.
5. **Group V:** The animals were given sodium nitrate similar to the previous dose as group II for one month and left 2 weeks without oral intubations.
6. **Group VI:** The animals were given sun set yellow No 6 in a dose and route of administrations as group III for one month and kept without intubations for 2 weeks.
7. **Group VII:** The animals were given both sodium nitrate & sunset yellow by the same dose and route of administration as in group IV for one month and kept without intubations for 2 weeks.

Introduction

Food additives are common in our life and plays an important role in human being's life. Nitrate and nitrite are ubiquitous in nature and their presence are essential for the fertility of the soil, and used as

international food additive added to the original food or mixture of foods for specific purposes (*Kilgore and Li.,1980*).

The continuous use of nitrogenous fertilization in agriculture is the major source

of nitrate and nitrite which have been shown to be present in a wide variety in relatively high concentration (Walker, 1975). Nitrate itself is not toxic to mammals but it can be reduced to nitrite, most importantly by bacterial action during the storage of nitrate containing food. (Swann, 1975).

Synthetic food coloring additives are products from modern organic chemistry, which have been added to the list of additives used by the food industries. However, the available literature regarding their toxicity or metabolism revealed that little work has been published in the last decade (El-saadany, 1991).

Colors as an additives pigment or other substances synthesized, extracted, isolated or otherwise derived from vegetables or other sources (Furia, 1972; Shaker *et al.*, 1989).

Coloring additives are vital constituents of food. They are probably the first characteristics perceived by the senses and are indispensable to the modern day consumer as a means for the rapid identification and affiants acceptance of food (Furia, 1972; Taylor, 1980; Marmian, 1984; Babu & Shenalikar, 1995).

In recent years colors have attracted more attention than any other food additives, because they are the most obvious cosmetics and partially because people have made connection between some artificial dyes and adverse reactions to food (Marmian, 1984). However such levels of intake is not normally encountered. Therefore, more attention has been forced on the biological activity and effect of such colors which include chemical and physical specifications, short term and long term toxicity, carcinogenicity and metabolic studies (Salah, 1994; Babu & Shenolikar, 1995).

Material And Methods

Seventy male albino rats (*Rattus Novigisus*) were used in of average body weight about 120 gms and there average age about 2 months.

The animals were divided into 7 groups 10 rats / each. Each group of animals were placed in stain steel cage & maintained on standard pellet diet & water.

The substances used in this study were sodium nitrate and sunset yellow; both of them are available in powder form. Both of substance is given orally by gastric tube in a dose (1mg/bw\day). The dose was calculated according (Paget and Barnes - 1964)

The animals were divided into 7 main rroups (10 rats /each).

1-Group I (Control group)

2-Group II: The animals were given odium nitrate in a dose of 1mg/kg/b wt for one month.

3-Group III: The animals were given sun set yellow in a dose of 1mg/kg/bwt/day for one month.

4-Group IV: The animals were given sodium nitrate and sunset yellow for one month in a dose of 1mg/kg/bwt/for each drug in a separate manner.

5-Group V: The animals were given sodium nitrate similar to the previous dose as group II for one month and left 2 weeks without oral intubations.

6-Group VI: The animals were given sun set yellow No 6 in a dose and route of administrations as group III for one month and kept without intubations for 2 weeks.

7-Group VII: The animals were given both sodium nitrate & sunset yellow by the same dose and route of administration as in group IV for one month and kept without intubations for 2 weeks.

Paraffin section were prepared (6 um thick) stained with H&E and Masson trichrome. Frozen sections (6um) were prepared to study the activity of both alkaline phosphatase and Succinic dehydrogenase enzymes.

Quantitative study was done by using image analyzer for estimating the glomerular area, area percentage of collagen fibers distributions and optical density of both alkaline phosphatase enzyme activity in glomerulus and succinic dehydrogenase enzymes activity in renal tubules.

Results

I-Morphological changes (fig1,11)

(A)- *Hamatoxylin and Eosin stain sections*

The kidney divided into an outer cortex and inner medulla. The cortex contains numerous renal corpuscles, proximal and distal convoluted tubules. The proximal convoluted tubules are lined with tall cubical darkly acidophilic cells. The distal convoluted tubule is lighter in staining and wider lumen. The medulla contain loops of Henle and collecting tubules. Loop of Henle is lined by simple squamous epithelium in thin segment and low cuboidal epithelium in thick segment. Collecting tubules and ducts are pale in staining and lined by a simple cuboidal epithelium.

The second group which treated with Sodium nitrate, and The fourth group which treated with sodium nitrate and sun set show congested glomeruli and blood capillaries. Both proximal and distal convoluted tubules are dilated with some degenerated cells and significant increase in glomerular area. Changes in the seventh group it was noticed recovery changes. No significant changes in glomerular area, while in the other groups the observed changes are more or less similar to the normal renal tissues.

(B)- Masson Trichrome Stain sections

In the control group normal fibrous tissue distributions between renal tissues are observed.

The second group and the fourth groups shows marked increase in fibrous connective tissue distribution mainly in glomerulus and in between renal tubules

The seventh group shows no distinct changes in the distribution of the connective tissue in the renal tissue.

11-Histochemical results

Changes in the Alkaline phosphatase and succinic dehydrogenase enzymes activities were estimated in optical density (O.D) unit and was measured by using the image analyzing computer system. The area examined was mainly the glomerulus in Alkaline phosphatase and the renal tubules in succinic dehydrogenase.

(A) Changes in Alkaline phosphatase enzyme activity.

The first group (Control group) shows normal distribution of alkaline phosphatase enzyme activity in both glomerulus and renal tubules The second group Which represents sodium nitrate treated group and fourth group shows decrease in the alkaline phosphatase enzyme activity in glomerulus while in the seventh group no changes were observed.

(B)-Changes in succinic dehydrogenase enzyme activity:

The first group (control group) shows intense succinic dehydrogenase enzyme activity in renal tubules .The second group, The fourth group and seventh group shows decrease in the succinic dehydrogenase enzyme activity when compared the control group..

Table (1) Changes in Renal Glomerular Area Under the Effect of Sodium Nitrate and Sun Set Yellow.

Area (μm^2)	Group1	Group2	Group 3	Group 4	Group5	Group 6	Group 7
Mean	3956.474	8183.720	5442.089	7202.862	4581.335	6248.504	4006.670
Min	2318.126	4996.294	3874.700	3288.197	4623.240	3175.357	3080.411
Max.	6269.833	15575.387	7717.752	10857.721	5869.239	11328.313	4645.688
SD	1502.488	3280.079	1133.233	2452.132	1327.194	2600.264	606.450
SEM	531.210	1159.683	400.658	680.099	593.539	694.950	247.582
T-Test		Control VS Group2	Control VS Group3	Control VS Group4	Control VS Group5	Control VS Group6	Control VS Group7
		0.0051	0.0424	0.0033	0.4629	0.0343	0.9402
P value		p> 0.01	p>0.05	p>0.01	p>0.05	P < 0.05	p>0.05
Significance		Sig. Inc.	Sig. Inc.	Sig. Inc.	NS	Sig. Inc.	NS

Figure (1) Changes in Renal Glomerular Area Under The Effect of Sodium Nitrate and Sun Set Yellow.

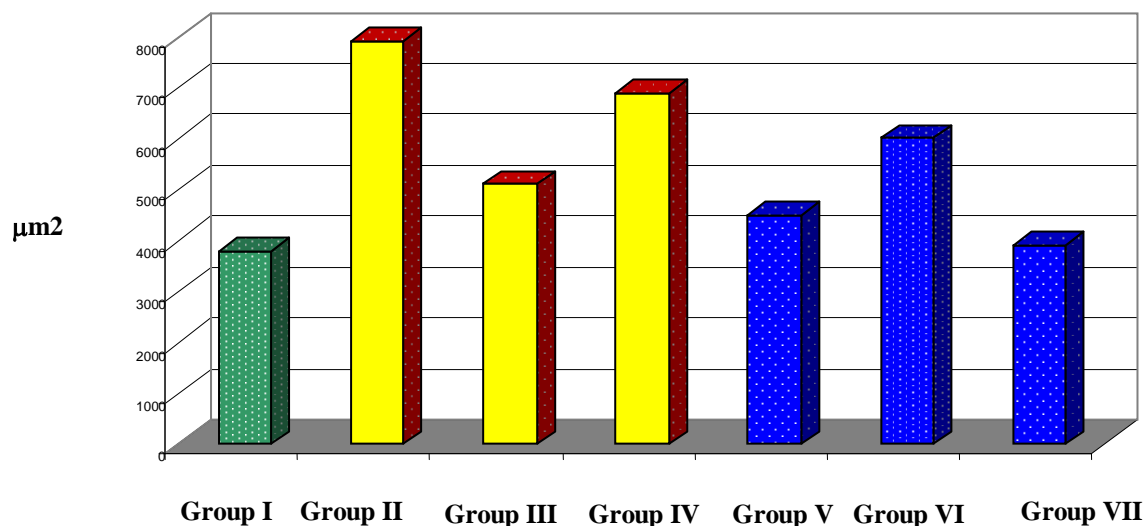


Table (2) Changes in Renal Fibrous Tissue Under the Effect of Sodium Nitrate and Sun Set Yellow.

Area (um ²)	Group1	Group2	Group 3	Group 4	Group5	Group 6	Group 7
Mean	0.154	0.263	0.154	0.166	0.094	0.237	0.172
Min	0.107	0.170	0.071	0.085	0.080	0.096	0.083
Max.	0.225	0.332	0.223	0.225	0.126	0.349	0.298
SD	0.040	0.063	0.054	0.046	0.022	0.108	0.075
SEM	0.014	0.021	0.019	0.017	0.011	0.044	0.030
T-Test		Control VS Group2	Control VS Group3	Control VS Group4	Control VS Group5	Control VS Group6	Control VS Group7
		0.0008	0.9913	0.5993	0.0210	0.0670	0.5752
P value		P < 0.01	P > 0.01	p>0.05	P< 0.05	P > 0.05	p>0.05
Significance		Sig. Inc	NS	NS	Sig. Dec	NS	NS

Figure (2) Changes in Renal Fibrous Tissue Under The Effect of Sodium Nitrate and Sun Set Yellow.

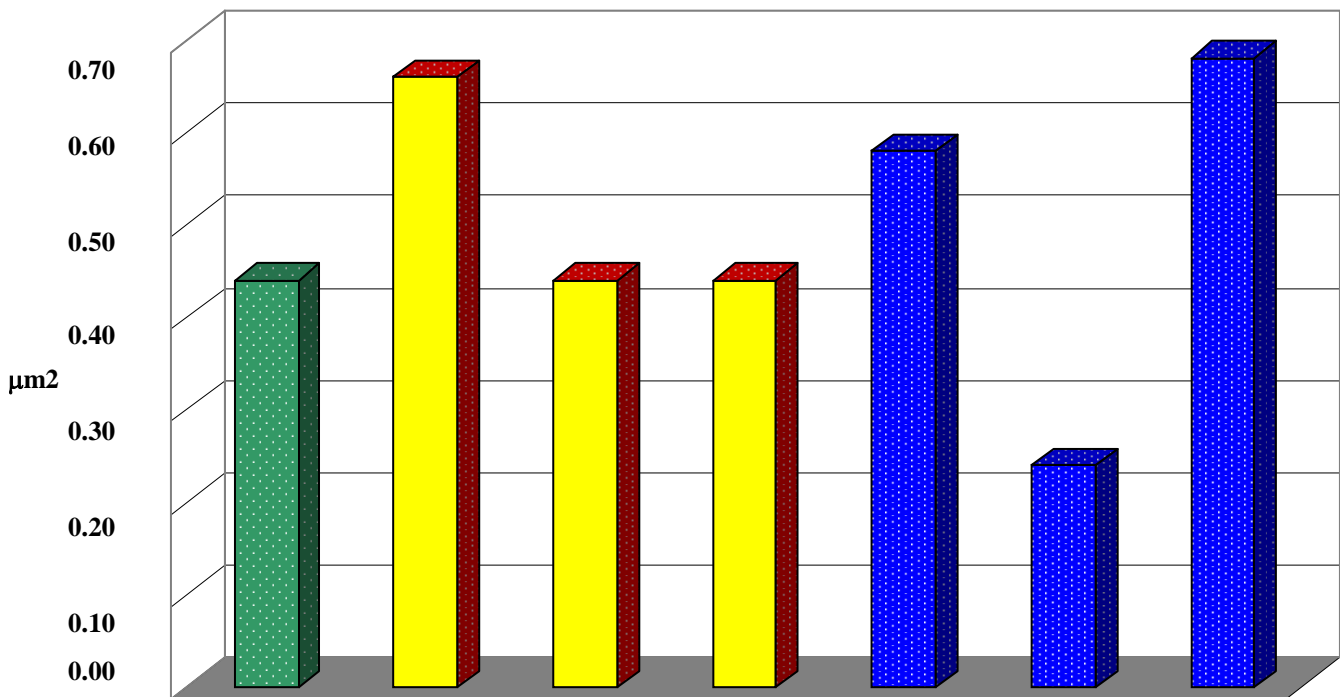


Table (3) Changes in Renal Glomerular Alkaline Phosphatase Enzyme Activity Under the Effect of Sodium Nitrate and Sun Set Yellow.

O.D.	Group1	Group2	Group 3	Group 4	Group5	Group 6	Group 7
Mean	1.365	0.894	0.775	0.271	1.051	0.936	1.381
Min	1.590	1.138	0.937	0.570	0.752	1.026	1.381
Max.	2.007	1.503	1.111	1.305	2.171	1.521	2.170
SD	0.153	0.138	0.063	0.306	0.593	0.189	0.328
SEM	0.062	0.056	0.026	0.125	0.242	0.007	0.134
T-Test		Control VS Group2	Control VS Group3	Control VS Group4	Control VS Group5	Control VS Group6	Control VS Group7
		0.0002	0.0003	0.0001	0.1257	0.0002	0.8846
P value		p> 0.001	p>0.001	p>0.001	p>0.05	P < 0.001	p>0.05
Significance		Sig. Dec.	Sig. Dec.	Sig. Dec.	NS	Sig. Dec.	NS

fig. (3) Changes in Renal Glomerular Alkaline Phosphatase Enzyme Activity Under The Effect of Sodium Nitrate and Sun Set Yellow.

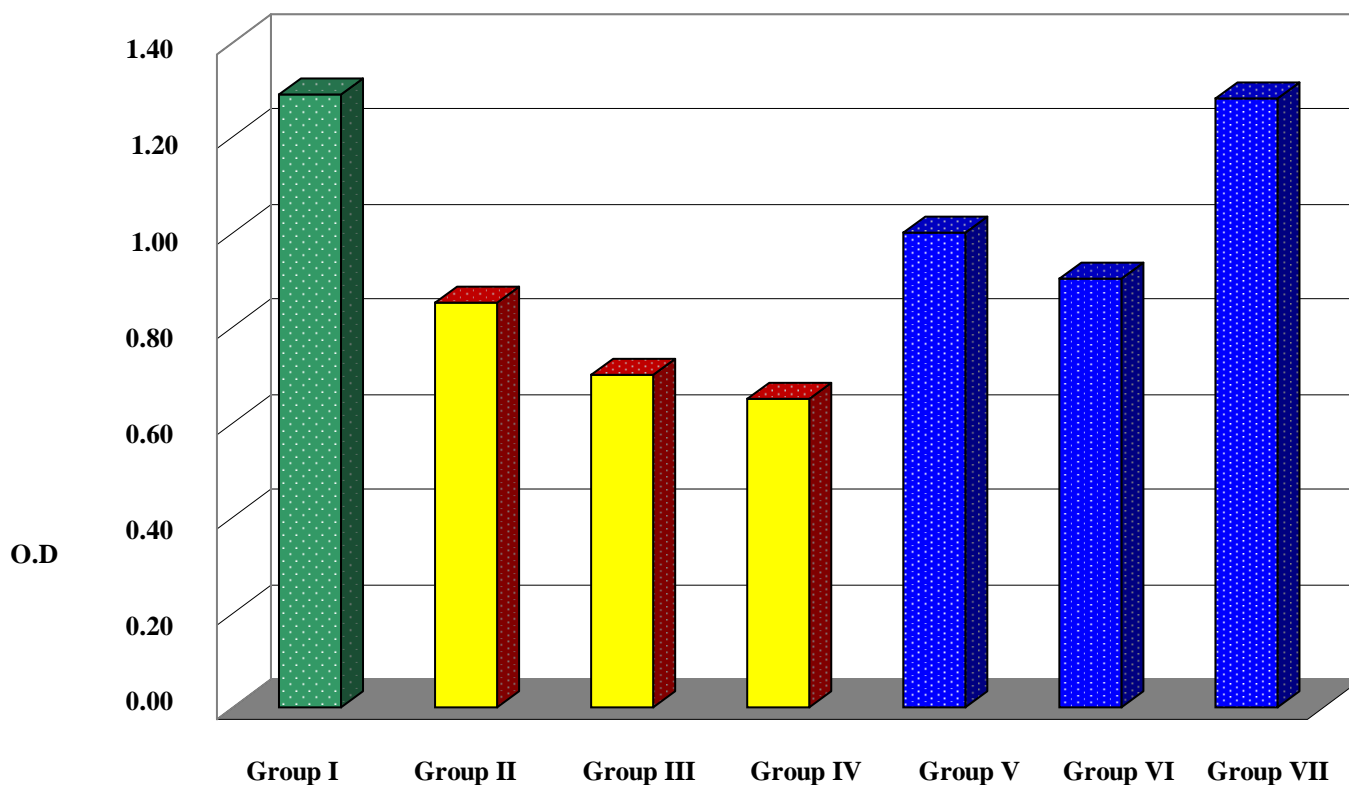
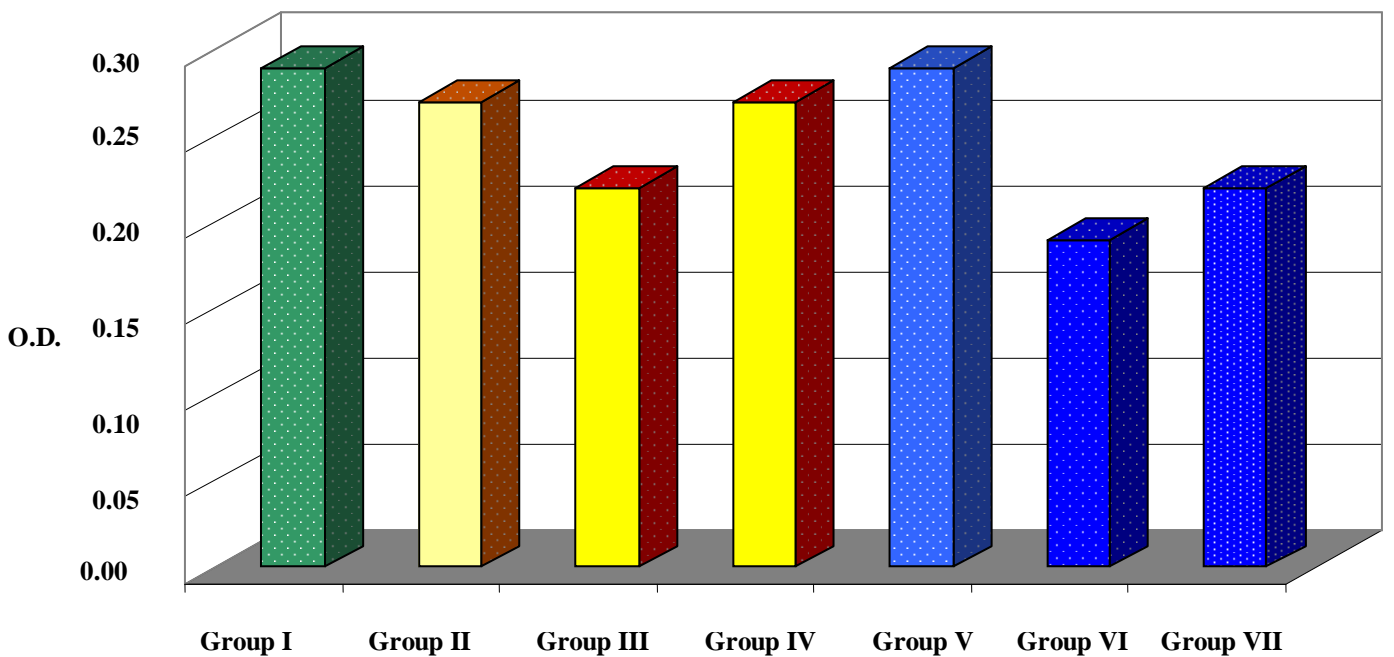


Table (4) Changes in Succinic Dehydrogenase Enzyme Activity in renal tubules Under the Effect of Sodium nitrate and sun set yellow.

O.D.	Group1	Group2	Group 3	Group 4	Group5	Group 6	Group 7
Mean	0.303	0.282	0.221	0.271	0.299	0.204	0.226
Min	0.252	0.280	0.199	0.209	0.241	0.180	0.187
Max.	0.556	0.574	0.403	0.538	0.665	0.380	0.420
SD	0.129	0.130	0.090	0.125	0.155	0.085	0.097
SEM	0.053	0.053	0.037	0.051	0.063	0.035	0.040
T-Test		Control VS Group2	Control VS Group3	Control VS Group4	Control VS Group5	Control VS Group6	Control VS Group7
		0.8105	0.1202	0.5753	0.9498	0.0640	0.1514
P value		p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
Significance		NS	NS	NS	NS	NS	NS

figure (4) : Changes in Renal Tubular Succinic Dehydrogenase Enzyme Activity Under The Effect of Sodium Nitrite and Sun Set Yellow.



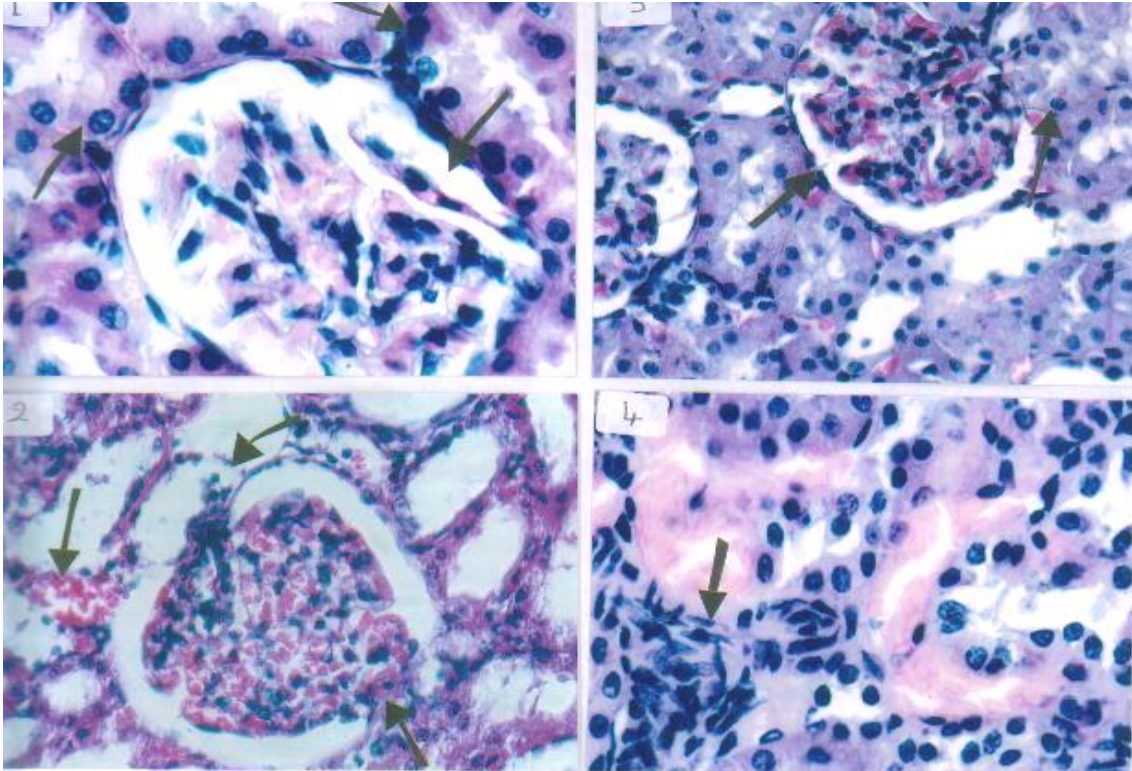


Fig 1: Collective photomicrograph of sections in the rat kidney

- 1- Group 1"control group " shows the normal glomeruli, proximal and distal convoluted tubules.
- 2- Group 2"treated with sodium nitrate" shows congested blood capillaries and dilated proximal and distal convoluted tubules and degenerated cells.
- 3- Group 4"treated with sodium nitrate and sun set" shows congested glomerulus & dilated proximal and distal convoluted tubules vacuolations and degeneration of some cells.
- 4-Group 7"sodium nitrate and sun set recovery" shows some degenerated cells surrounded by fibroblasts. (Hx&E X500).

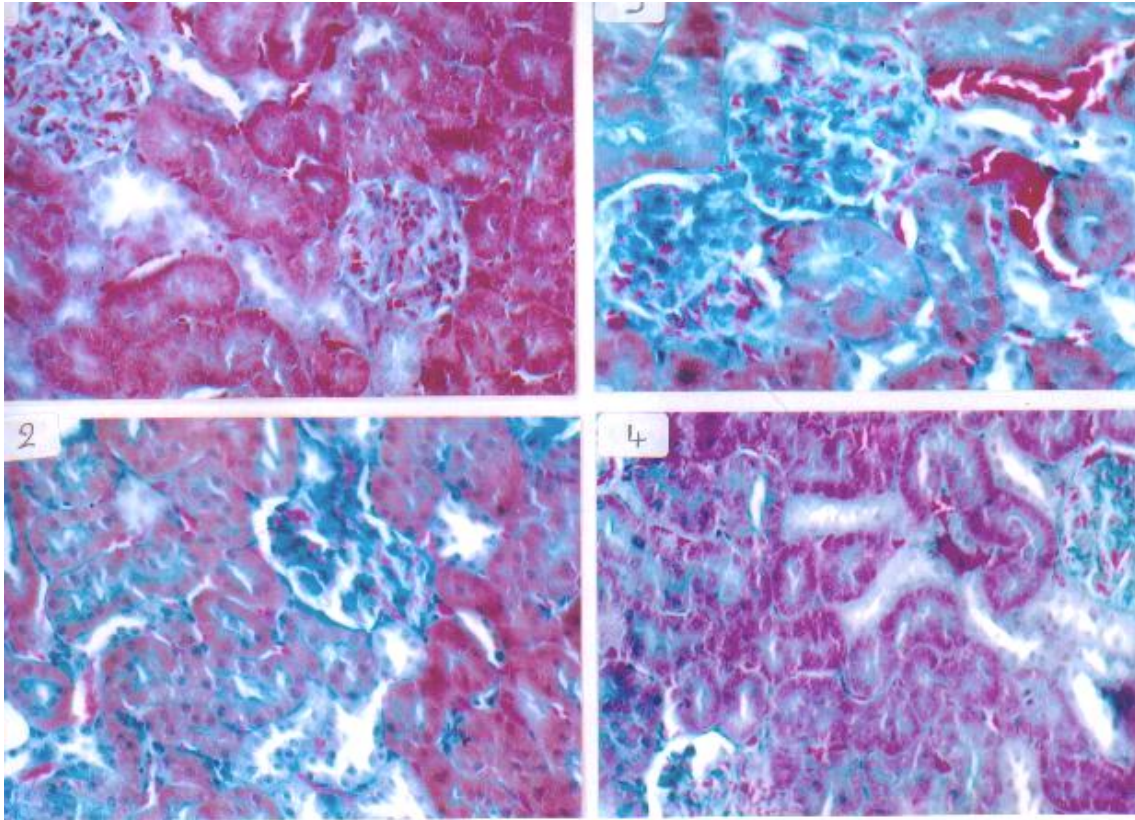


Fig 2: Collective photomicrograph of sections in the rat kidney

- 1- Group 1 "control group" shows normal C.T. distributions.
- 2- Group 2 "treated with sodium nitrate" shows marked increase in fibrous C.T. in glomerulus and in-between renal tubules.
- 3- Group 4 "treated with sodium nitrate and sun set" shows C.T. distributions similar to control group.
- 4- Group 7 "sodium nitrate and sun set" shows C.T. distributions similar to control group. (Masson trichrome stain x 500).

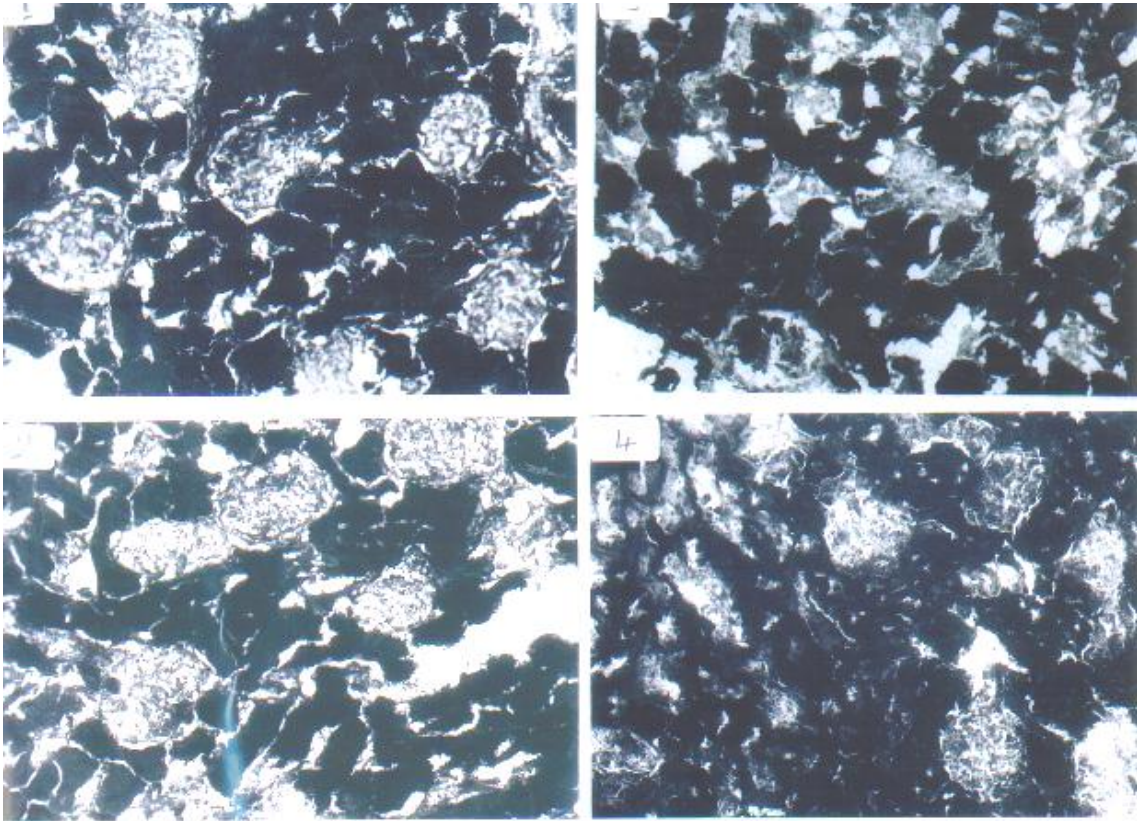


Fig 3: Collective photomicrograph of a sections in the rat kidney

- 1-Group 1 "control group" shows alkaline phosphatase enzyme activity distribution in the tubules and glomerulus.
- 2-Group 2"treated with sodium nitrate" shows decrease alkaline phosphatase enzyme activity in glomeruli.
- 3-Group4"treated with sodium nitrate and sun set" shows decrease alkaline phosphatase enzyme activity.
- 4-Group 7)"sodium nitrate and sun set recovery" shows alkaline phosphatase enzyme activity similar to control group. (Gomori stain x 500)

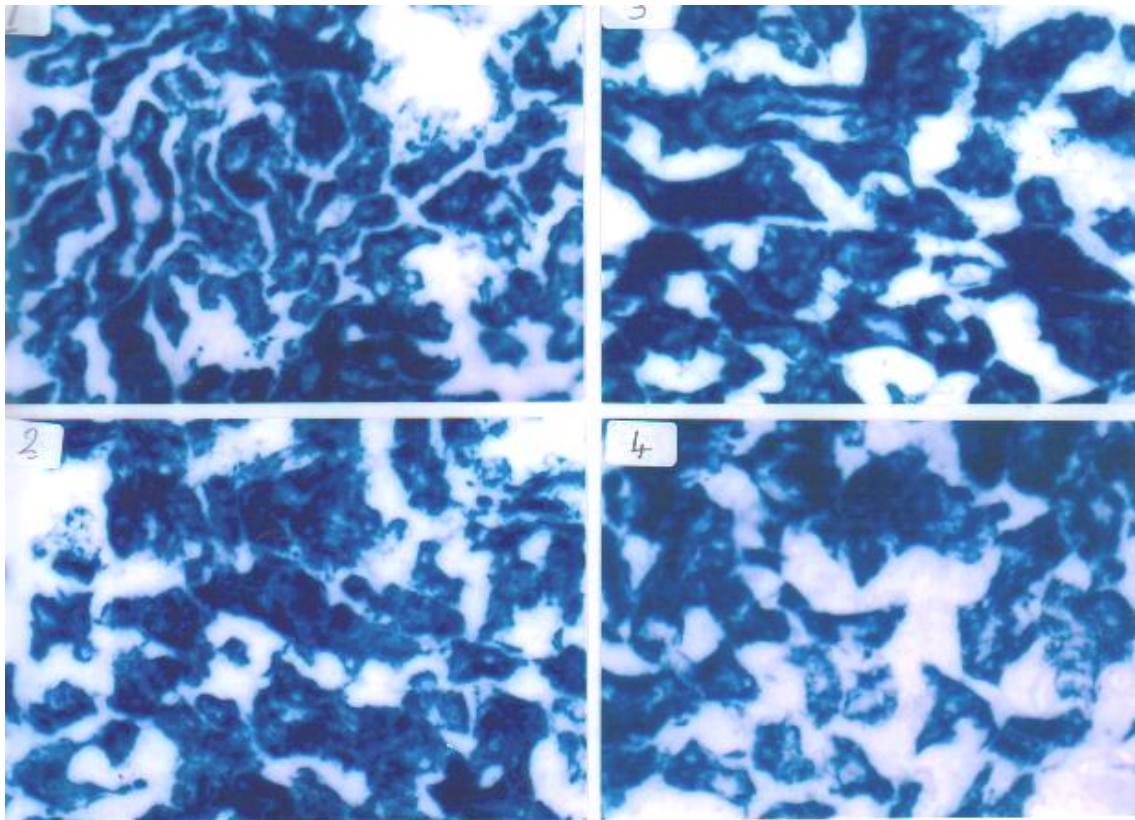


Fig 4: Collective photomicrograph of sections in the rat kidney

- 1-Group1 "control group" shows intense succinic dehydrogenase enzyme activity in renal tubules.
- 2-Group 2"treated with sodium nitrate" shows decrease succinic dehydrogenase enzyme activity.
- 3-Group 4"treated with sodium nitrate and sun set" shows decrease in succinic dehydrogenase enzyme activity in renal tubules.
- 4-Group7"treated with sodium nitrate and sun set" shows no changes in succinic dehydrogenase enzyme activity in renal tubules.
(Nachlas technique X500).

Discussion

It has been noticed that people especially children at the age of nursery usually used food containing colorant and additives with great amounts which attracts their attention.

Appetite is a concept largely influenced by vision, when we look at a certain food; we first appreciate its image. If we don't like its color, we don't feel like purchasing it. Before we buy a food, first the food has to capture our attention, seize our appetite. Colors play a large role in attracting us to foods. So, food industries, realizing this basic human tendency, play with the color concept cunningly, for the sake of having us consume their products in greater quantities with more appetite. Food additives are substances that not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value.

Nowadays, food additives are considered to be one of the difficult problems in the food industry. All food additives, whether actually in use or being proposed for use, should be subjected to appropriate toxicological testing and evaluation? The natural additives are safer and appreciated the addition of food preservatives and coloring.

Synthetic compounds should be either omitted completely or highly restricted to lower levels due to its side effects. The present study was carried out on adult albino rats to evaluate the side effects of these food additives on its kidney.

It is known that nitrate and nitrite and dimethyl amine may react in the gastrointestinal tract synthesizing, the harmful dimethylnitrosoamine substances which had adverse effects on animal and human organs (*Swan, 1975*), nitrate and nitrite also cause pathological changes in rat liver (*Gracia et al., 1987*) and kidney (*Gojer & Sawant, 1992 and Anthony et al., 1994*), pleasant chicks liver and kidney (*Storand and Persin, 1983*).

Also nitrite reacts with amines to produce nitrosamines and with amides to produce nitrosamides. Nitrosamines and nitrosomides constitute the N-nitroso

compounds (NNC) and the reaction with nitrite is called nitrosation, nitrosamines readily induced tumours of the liver, oesophagus, kidney, nasal cavity and pancreas and nitrosamine chiefly induce tumours of glandular stomach, small intestine and nervous as well as lymphoid systems the tissue affections dependences on the species (NNC) and the treatment system (*Sidney, 1986*).

Nitrates and nitrites are environmental pollutants present in food & water and it is suggested that they may be contribute to the etiology of kidney diseases and problems related to immunity in domestic fowls. Kidney has a vital physiological role in case of toxicity, in this study we try to evaluate the effects of nitrites & sunset yellow on the renal morphology.

Studies of the histochemical activities of succinic dehydrogenase & alkaline phosphatase enzymes facilitate understanding the effect of food additives on renal tissue.

We selected two agents of food additives substances to study their effects on the kidney of albino rat, the first one was the sodium nitrate as a food preservative and the second was the sunset yellow as a one of food colorants singly or in combination.

We give sodium nitrate & sunset yellow by a gastric tube for one month then stop the intubations for another 2 weeks to show the possibility of recovery.

In the present study, it was found that sodium nitrate causes dilatation and congestion of glomerular and peritubular capillaries. The lumen of renal tubules (proximal and distal convoluted tubules) becomes dilated with degenerations of its cells. This action occur when sodium nitrate given alone or in combination with sunset yellow.

Also, sodium nitrate induces fibrosis around glomerulus and renal tubules, which may be due to nitric oxide (NO) formations, which causes vascular smooth muscle relaxation. This leads to dilatations of their lumens and increases their blood flow.

The permanent vasodilatation and congestion causes cellular hypoxia and

death, which may be followed by fibrosis (Took J.E., 1996).

In sunset yellow given groups there is many areas of cellular infiltrations which may be due to allergies (Wood, 1980) and hypersensitivity to sunset antigens that leads to formations of antigen – antibody complex which deposited in glomerular subendothelial, mesangium and tubular cells ,once immune complex are deposited, injury induced by both cellular and soluble mediators including neutrophils which releases proteases and monocytes macro-phage lymphocyte and natural killer which release cytokines, cytotoxic cell mediators growth factors also mesangium cells produces inflammatory response by releasing cytokines this leads to glomerulo-nephritis and interstitial nephritis.

Chronic glomerulonephritis leads to glomerular fibrosis and chronic interstitial nephritis leads to peritubular fibrosis (Stanley et al., 1999).

Our findings are agreement with (Rowe et al., 1994) which decided that sunset has the following side effects articularia (hives), Rhinitis (runny nose) nasal congestion. Eosinophilactic response, purpura (bruising).

In the present study the alkaline phosphatase enzyme activity was significantly decreased as ($p < 0.05$) in both groups given sodium nitrate and sunset yellow singly or in combination in comparison to control group, our findings are in agreement with (Moriyama & Nelson, 1987 and Kurimoto et al., 1984), Which postulated that nitrate in a partly diffusible anion that can cross the plasma membrane and significantly affect membrane enzymes e.g. (adenosine triphosphate) activity (Moriyama and Nelson, 1987) inhibit the energy metabolism of the cells and damage cell membrane integrity (Kurimoto et al., 1984).

Our findings are also in agreement with (Staewson et al., 1973 and Atef et al., 1991) hence food preservative as sodium nitrate and food coloring agents as sunset has many adverse effects and harmful to human health so we must avoid food stuffs containing these substances as much as possible.

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دراسة مجهرية لتأثير بعض المواد المضافة للأغذية على الكلى فى الجرذان البيضاء

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تهدف هذه الدراسة إلى معرفة تأثير بعض المواد التى تضاف للأطعمة مثل نترات الصوديوم كمادة حافظة وصبغ ست الأصفر كمادة ملونة حيث شاع استخدام هذه المواد فى الآونة الأخيرة بعد إضافتها إلى كثير من الأطعمة المعلبة مثل اللحوم والأسماك والمشروبات المختلفة بين كثير من الناس الذين يقبلون على استخدامها وهم ليسوا على دراية بالآثار الضارة لها وكان الغرض من هذه الدراسة هو تقييم مدى تأثير هذه المواد باستخدام مادة نترات الصوديوم (مادة حافظة) و مادة صبغ ست الأصفر (مادة ملونة) على الكلى الجردان البيضاء من الناحية الهستولوجية والهستوكيميائية.

أشتمل هذا البحث على ثلاثة أجزاء:

أولاً: دراسة مجهرية على نسيج الكبد تحت تأثير مادتي (نترات الصوديوم) و(صبغ ست

ست

الأصفر) كلا على حده وسويا وذلك باستعمال قطاعات شمعية مصبوغة بصبغه الهيماتوكسلين والأيوسين، الماسون ثلاثي الألوان.
ثانياً: تقييم النشاط الهستوكيميائى لأنزيم الفوسفاتيز القلوى وأنزيم السكسينك ديهيدروجينيز فى نسيج الكلى

ثالثاً: تحليل إحصائى وكمى للنتائج التى تم الحصول عليها من هذه الدراسة.
وقد استخدم فى هذه الدراسة 70 من الجرذان فى متوسط شهرين من العمر ومتوسط وزنها 120 جرام وقدمت لها وجبات مكونة من 20% بروتينات و 5% ألياف، 35% دهون وفيتامينات ومعادن و مياه من الصنبور وقد قسمت الجرذان إلى 7 مجموعات تتكون كل منها من 10 جرذان و خصصت 6 مجموعات منها لدراسة مادة نترات صوديوم و الصبغ ست الأصفر على الكلى فى جرعة (1 مجم/كجم/يوم) كل على حدا فى حين إعتبرت المجموعة الباقية مجموعته ضابطه.
- المجموعة الأولى: مجموعة ضابطة للمقارنة.
- المجموعة الثانية: تم إعطائها نترات الصوديوم لمدة 30 يوم و أخذت العينات بعد انتهاء المدة

مباشرة.

- المجموعة الثالثة: تم إعطائها الصبغ ست الأصفر لمدة 30 يوم و أخذت العينات بعد انتهاء

المدة مباشرة.

- المجموعة الرابعة: تم إعطائها كل من نترات الصوديوم و الصبغ ست الأصفر لمدة 30 يوم

و أخذت العينات بعد انتهاء المدة مباشرة.
- المجموعة الخامسة: تم إعطائها نترات الصوديوم لمدة 30 يوم ثم أوقف التجرع و تركت

الفئران لمدة 30 يوم
- المجموعة السادسة: تم إعطائها الصن ست الأصفر لمدة 30 يوم ثم أوقف التجرع و تركت
الفئران لمدة 30 يوم

- المجموعة السابعة: تم إعطائها كل من نترات الصوديوم و الصن ست الأصفر لمدة 30 يوم ثم أوقف التجرع و تركت الفئران لمدة 30 يوم أخرى ثم أخذت العينات.

وقد دلت النتائج على ما يلي:

1- التغيرات المجهرية

لوحظ وجود زيادة في حجم الشعيرات الدموية الكلوية المكببه واحتقانها بالدم وزيادة في حجم الأنسجة الليفية المحيطة بها والمحيطه بالأنابيب الكلويه في كل من المجموعات التي أعطيت نترات الصوديوم منفردا أو مع الصن ست الأصفر كما لوحظ أن هذه التغيرات لم تتغير عند توقف إعطاء هذه المواد كذلك عدم وجود تغير يذكر في كل المجموعات التي أعطيت الصن ست الأصفر.

2- التغيرات الهستوكيميائية

-نقص ذو دلالة إحصائية في نشاط نشاط أنزيم الفوسفاتيز القلوبو السكسينك ديهدروجينيز في كل من المجموعات التي أعطيت نترات الصوديوم منفردا أو مع الصن ست الأصفر ولوحظ أن هذه التغيرات لم تتغير عند توقف إعطاء هذه المواد.
-عدم وجود تغير يذكر في نشاط الأنزيم في كل من المجموعات التي أعطيت الصن ست الأصفر

وبعد استعراض نتائج هذه الدراسة في محاورها المختلفة يمكن أن نستنتج أن هناك تأثير ضار لمادة نترات الصوديوم على أنسجة الكلى تزيد حدتها بإضافة مادة الصن ست الأصفر كما لوحظ أن تأثير مادة الصن ست الأصفر بمفردها ضعيف إلى حد كبير.
وتوصى هذه الدراسة بعدم استخدام هذه المواد في الأغذية وكذلك عدم التعرض لها لفترات طويلة