RESEARCH ARTICLE

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SHORT TERM TOXICITY OF FOOD ADDITIVE AZO DYE, SUNSET YELLOW (E 110), AT LOW DOSES, IN MALE SPRAGUE-DAWLEY RATS

ABSTRACT:

Dietary toxicity of Sunset Yellow (SY) (E 110), a synthetic food coloring additive, was evaluated in male S.D. rats, to examine the possible modulating effects of SY on the liver, kidney functions and lipid profile. Twenty-four male S.D. rats were divided into four groups with six rats in each. The control (Group 1) was fed a basal diet without any treatment. Group (2, 3, and 4) received SY by intragastric gavage (i.g.) daily for 13 weeks (161.4, 80.7, and 40.35 mg/kg/day BW, respectively). The doses were chosen below the acceptable daily intake (ADI) of the WHO/FAO guidelines. After 13 weeks, a significant elevation in the biochemical parameters of total protein, albumin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), creatinine, urea and in the lipid profile was found in group (2) as compared with the normal control. Histopathological examinations showed no changes in all body organs except in the livers of groups (2) and (3) which displayed dose-dependent histopathological alterations such as necrosis, interlobular fibrosis, edema and inflammation. The liver's histological architecture of rats in group (4) was almost similar to the normal control. Long term exposure or high daily intake of synthetic colorants such as SY may be considered for risk to human health.

KEY WORDS:

Sunset Yellow, Sprague Dawley rat, Short term Toxicity.

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INTRODUCTION:

Food additives are any substance added to food, not normally consumed or a typical ingredient of the food, they may be natural or synthetic (Yilmaz *et al.*, 2009; El-Samragy, 2012). Therefore, they are classified into various categories such as antimicrobial agents, antioxidants, artificial colors, artificial flavors and flavor enhancers and chelating, thickening and stabilizing agents (Sasaki *et al.*, 2002).

Coloring agents, play a significant role in enhancing the aesthetic appeal of food (Newsome, 1986). This is an important reason why food dyes stand out as essential additive class for food industry. All color additives are alike in terms of the Food and Drug Administration's (FDA) regulatory definition, they are regulated in two classes; color additives requiring certification (synthetic), and the color additives exempt from certification (natural). There is an ever growing concern about the adverse effects of synthetic food colorants on human health (Van Bever *et al.*, 1989).

Sunset Yellow is a synthetic yellow azo dye manufactured from aromatic hydrocarbons from petroleum. It is used in fermented foods which must be heat treated. It may be found in orange sodas, margarine, snack chips, ice creams, chocolates, cake decorations, pharmaceutical pills, prescription medicines and others (Wood *et al.*, 2004). Despite its role in our food, azo dyes could be serious threat to human health. Some azo

dyes are metabolized in the intestinal wall and liver, producing free aromatic amines that are potentially carcinogenic and mutagenic (Ben Mansour *et al.*, 2009; Shimada *et al.*, 2010).

The safety of repeated exposure to permitted synthetic food additives (colorants or preservatives) has been questioned. In relation to the toxicological limit, the FAO/WHO, Joint Expert Committee on Food Additive (JECFA) established an acceptable daily intake (ADIs) of many food additives that can be used every day throughout the life time of an individual without any substantial health effects (WHO, 1987). Certain food colors such as Carmoisine (E122), Amaranth (E123), Sunset Yellow (E110), Tartrazine (E102) and Allura red (E129) have been examined in bacterial and animal studies and it has been found that their mutagenicity varies widely, depending on the dose, implying that they may also act as mutagenic and/or carcinogenic agents in humans (Tsuda *et al.*, 2001; Macioszek and Kononowicz, 2004).

Sunset Yellow may be responsible for causing an allergic reaction in people with an aspirin intolerance (Ibero *et al.*, 1982), resulting in various symptoms, including gastric upset, diarrhea, vomiting, nettle rash (urticaria), swelling of the skin (angioedema) and migraines. It may have immunomodulatory effects (Yadav *et al.*, 2013), while some reports showed that SY have been linked to hyperactivity in children (Sarhan *et al.*, 2014).

It was estimated that the low acute oral toxicity of SY, reflected by LD_{50} values was greater than 2,000 mg/kg BW (Lu and Lavalle, 1964) and 10,000 mg/kg BW (Gaunt *et al.*, 1967) in rats. After short term studies on rats by Mathur *et al.* (2005 a&b) it was estimated that LD_{50} doses of SY for rats were around 1500 mg/kg BW/day. Due to significant effects on the animal weights without observed histological changes at a dose of 250 mg/kg BW/day it was concluded that 250 mg/kg is a Lowest Observed Adverse Effect Level (LOAEL) dose which is lower than the No Observed Adverse Effect Level (NOAEL) of 500 mg/kg BW from the rat and dog study previously used by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) to derive the Acceptable Daily Intake (ADI) by WHO (2015). For humans, both committees of JECFA in 1982 and the EU Scientific Committee for Food (SCF) in 1984, established an ADI of 0-2.5 mg/kg BW/day. However, the European Food Safety Authority (EFSA) under both EU and WHO/FAO guidelines have decided in 2009 to lower temporarily the human acceptable daily intake for SY from 2.5 to 1 mg/kg BW/day and provided a document to assist in replacing the SY with other colors. Currently, SY is forbidden in many countries such as Norway and Finland however, it is still widely used in many countries worldwide including Egypt and the Arab world.

The effect of dyes in general is not the same for every individual; it may vary according to dose, age, gender, nutritional status, genetic factors and most important on time of exposure (Sasaki *et al.*, 2002). There is some concern that SY may be contaminated with cancer-causing substances such as unsulphonated aromatic amines. Although some aromatic amines may be associated with genotoxicity or even carcinogenicity.

The present investigation was undertaken to investigate the cytotoxicity of doses of SY in rats extrapolated from its acceptable daily intake (ADI) for human, in a 13-week short term toxicity bioassay.

MATERIAL AND METHODS:

Chemicals and reagents

Pure Sunset Yellow FCF (E110) was obtained in powder form from El-Gomhouria Chemicals Co. (Cairo, Egypt), all other reagents and chemicals were purchased from Sigma-Aldrich, USA.

Animals and experimental design

Twenty-four, 6-weeks old male Sprague-Dawley (S.D.) rats weighting about 170-175 gm, were obtained from The Holding Company for Biological Products and Vaccines (Vaccera), Helwan - Egypt. Rats were maintained in plastic cages covered with metal grids with dry husk padding and allowed to acclimate in the animal facility conditions before being divided into groups for experimentation. The institutional animal care and use facility of the Zoology Department, Faculty of Science, Tanta University-Egypt, approved the experimental design. Rats were housed in a room maintained on a 12 h. light/dark cycle, at constant temperature of 23 ± 2^{o}C and humidity of 50 ± 5% with free access to water and balanced pelleted food.

Experimental groups:

Following two-weeks acclimation period in the animal facility conditions, the animals were divided according to doses and their body weights into four groups: G (1): Normal control; fed on basal diet and served as –ve control, G (2): received SY (161.4 mg/kg BW), G (3): received SY (80.7 mg/kg BW) and G (4): received SY (40.35 mg/kg BW).

Rats were administered the dedicated doses (i.g.) of SY dissolved in 0.5 ml of distilled water per day. The doses were estimated to be below the Lowest Observed Adverse Effect Level (LOAEL) dose for rats (Mathur *et al.*, 2005 a&b). The animals were carefully observed daily for clinical signs and mortality. Their body weights, food consumption and water intakes were measured precisely every week and the average was calculated during the course of

the experiment as g/rat/day for food and ml/rat/day for water.

Blood and serum samples:

At the end of the experimental period (13 weeks), all animals were fasted overnight and sacrificed under diethyl ether anesthesia. Blood was collected from the abdominal aorta of each rat for hematological and biochemical assays. Heparin was added to collected blood for hematological parameters, while the blood samples for biochemical parameters were collected without any anticoagulant to collect serum.

Complete blood picture (CBC):

A complete blood picture was estimated in heparin collected blood samples using an automated hematology analyzer; Sysmex kx-21n JAPAN CARE Co., LTD).

Biochemical assays:

Total protein was estimated using the Biuret method (Doumas, 1975). Albumin was evaluated according to the method of (Doumas, 1997). Total bilirubin was evaluated according to (Ehrlich, 1883). The levels of AST and ALT were accomplished using the method of (Reitman and Frankel, 1975). Gamma-glutamyl transpeptidase was estimated by the method of (Meister *et al.*, 1981). Alkaline phosphatase level was measured according to the method of (Bellfield and Goldberg, 1971). Serum urea nitrogen level was determined by (Fawcett, 1960) and creatinine concentration were calculated according to (Slot, 1965). Moreover, serum cholesterol was determined as described by (Allain *et al.*, 1974). Triglycerides were determined by the method of (Rojkin *et al.*, 1974). Blood levels of electrolytes such as calcium were estimated at the Central Laboratory of Tanta University using the Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), Optima 7000 DV ICP-OES-PerkinElmer, USA.

At termination all the animals were subjected to a complete necropsy. They were examined grossly for pathological changes, and the heart, liver, spleen, kidneys, adrenal glands, testes, brain, thymus and lung were weighed. In addition to these organs, lymph nodes (cervical, mesenteric), the aorta, salivary gland, bone and bone marrow (sternum, femur), trachea, thyroid, tongue, esophagus, stomach, duodenum, small intestine, large intestine, pancreas, urinary bladder, seminal vesicle, prostate gland, epididymis, pituitary gland, sciatic nerve, skeletal muscle, spinal cord, eyes and their accessory organs were weighed. For microscopic examination, parts of right lobe of liver were fixed in 10% phosphate buffered formalin, dehydrated with 50–100% ethanol solutions, and then embedded in paraffin. Tissues were sectioned at 5 µm thickness, followed by haematoxylin-eosin staining before subjecting to photomicroscopic observation.

Statistical analysis:

The data are presented as Means ± Standard deviations (SD) and were statistically analyzed using SPSS (Statistical Package for the Social Sciences) ver. 20, USA. ANOVA test was employed for comparison of body weights, organ weights, hematology and serum biochemistry data between control and treated groups. For all comparisons, p-values less than 5% ($P < 0.05$) were considered to be statistically significant.

RESULTS:

In-life parameters:

No mortality or obvious clinical signs were evident in any of the animals throughout the experimental period. The body weights and the cumulative body weight gains were significantly increased at the end of the experiment (Fig. 1).

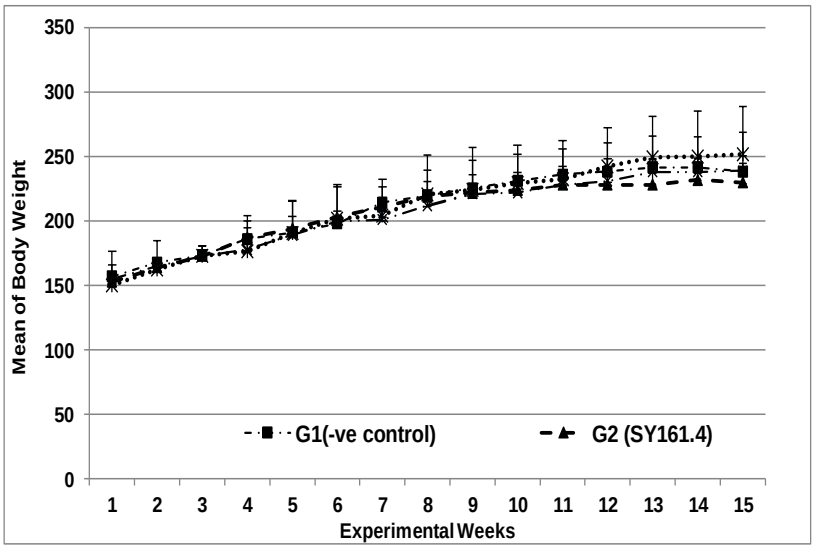

Fig. 1. Growth curve of body weights of control rats and treated with SY.

The food consumption and water intake values was not constant during the experimental weeks (Table 1).

Table 1 . Food and water consumption and SY intake of both control and SY treated groups of male rats.

Groups	G1 (-Ve control)	G2 (SY 161.4)	G3 (SY 80.7)	G4 (SY 40.35)	Unit
Average total intake of SY during 13 weeks	0	12.09	6.09	1.58	mg
Average intake of SY daily	0	0.93	0.47	0.122	mg/kg/day
Average daily intake of food	12.8	16.95	16.46	11.85	grams
Average daily intake of water	12.04	16.08	15.46	14.59	ml

Organ weights:

Body weights, weight gains, absolute and relative organ weights are represented in **table 2**. The body weights and the cumulative body weight gains were significantly increased at the end of the experiment and was related to the age of rats. Absolute prostate weights were significantly increased in group (2) ($P<0.05$) versus –ve control. Testes weights were generally lower in the three treatment groups (2-4) than the control data (group 1) albeit statistical significance was obtained only when the data of group (3) was compared to that of the controls.

Table 2. Body weights, absolute and relative organ weights of male rats in control and SY treated groups.

Groups	1	2	3	4
Sunset Yellow (mg/kg/day)	0	161.4	80.7	40.35
No. of rats examined	6	5	5	6
Initial Body Weight (g)	173.5 ± 7.8	173 ± 7.6	173 ± 5.7	173.3 ± 7.5
Final Body Weight (g)	238.3 ± 33.8	230 ± 14.6	239 ± 29.9	251.7 ± 37.4
Weight gain	64.8 ± 26	57 ± 7	66 ± 24.2	78.4 ± 29.9
Organ Weight (g)				
Liver Weight (g)	5.96 ± 0.55 (2.5)[a]	6.06 ± 0.21 (2.6)[b]	5.93 ± 0.50 (2.48)	6.25 ± 0.87 (2.48)
Left Kidney Weight (g)	0.59 ± 0.07 (0.25)	0.66 ± 0.08 (0.29)	0.64 ± 0.07 (0.27)	0.69 ± 0.15 (0.28)
Right Kidney Weight (g)	0.61 ± 0.08 (0.26)	0.70 ± 0.07 (0.13)	0.59 ± 0.06 (0.25)	0.72 ± 0.16 (0.28)
Spleen Weight (g)	0.85 ± 0.16 (0.36)	0.78 ± 0.07 (0.34)	0.66 ± 0.09 (0.27)	0.89 ± 0.23 (0.35)
Adrenal gland Weight(g)	0.11 ± 0.01 (0.05)	0.35 ± 0.39 (0.15)	0.19 ± 0.29 (0.08)	0.09 ± 0.05 (0.03)
Testes Weights (g)	2.77 ± 0.51 (1.16)	2.83 ± 0.22 (1.22)	2.33 ± 0.47 (0.97)	2.79 ± 0.35 (1.11)
Lung Weight (g)	1.45 ± 0.23 (0.61)	1.92 ± 1.28 (0.83)	1.49 ± 0.15 (0.22)	1.64 ± 0.29 (0.65)
Prostate Weight (g)	0.49 ± 0.07 (0.2)	0.73 ± 0.19* (0.32)	0.54 ± 0.15 (0.23)	0.64 ± 0.17 (0.25)
Heart Weight (g)	0.74 ± 0.04 (0.31)	0.76 ± 0.10 (0.33)	0.76 ± 0.06 (0.32)	0.89 ± 0.22 (0.35)

a: Mean ± SD. absolute wts.; b: Numbers between brackets is relative organ wt. (%) = ratio of organ wt/BW (%)., *: Significant vs. G1 at $P < 0.05$.

Hematology and serum biochemistry:

Several alterations were observed in the hematological parameters (Table 3) including: WBCs and platelets counts were significantly increased in groups 2 and 3 with dose-dependence. RBCs counts, HB values and lymphocytes numbers were significantly increased in group 2, while the monocytes numbers were significantly increased in groups 3 and 4. Interestingly, the MCV levels were significantly increased in both groups 3 and 4 while it was found significantly decreased in group 2 as compared with the control levels of group 1. Furthermore, the numbers of granylocytes was found to be significantly increased in groups 2 and 4 but decreased in group 3.

Table 3. Blood picture (CBC) of control and SY treated groups of male rats.

Group	1	2	3	4	Unit
Sunset Yellow	0	161.4	80.7	40.35	Mg/kg/day
Item					
WBC	7.9 ± 1.8[a]	10.4 ± 1.13	8.15 ± 0.35	7.95 ± 0.07	$X10^3$ /µL
RBC	7.8 ± 0.028	9.7 ± 1.13	7.82 ± 0.45	7.75 ± 0.98	$X10^6$ /µL
HGB	13.8 ± 0.28	15.25 ± 1.06	13.85 ± 0.21	13.75 ± 1.63	g/dl
HCT	40.5 ± 1.41	48 ± 3.54	42.5 ± 2.83	41.25 ± 2.47	%
MCV	51.92 ± 0.21	49.48 ± 0.7	55.12 ± 0.56	53.5 ± 3.5	fL
MCH	17.65 ± 0.35	15.77 ± 1.6	17.69 ± 0.21	17.7 ± 0.07	Pg
MCHC	34.07 ± 0.07	31.66 ± 0.98*	32.47 ± 0.71	33.2 ± 0.6	g/dl
PLT	175 ± 35.36	375 ± 35.36*	250 ± 70.7	185 ± 49.49	$X10^3$ /µL
LYM.	5.25 ± 0.35	8.75 ± 1.06*	6.05 ± 0.64	5.1 ± 0.85	$X10^3$ /µL
MON.	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	$X10^3$ /µL
GRA.	0.35 ± 0.07	0.55 ± 0.35	0.2 ± 0	0.4 ± 0.42	$X10^3$ /µL

a: Mean ± SD; *: Significant vs. G1 at $P < 0.05$.

Serum biochemistry data shown in table 4, indicates that the total protein levels were significantly increased only in group 2 as compared with the normal control levels. The albumin levels, AST, ALT, and ALP values were significantly increased in groups 2 and 3, while the levels of GGT and total bilirubin were significantly increased only in group 2 as compared to control values. In addition, the creatinine and Urea serum levels were significantly increased in group 2. Importantly, the lipid profile including the cholesterol and triglycerides levels were significantly increased in all SY-treated groups in a dose dependent manner. The electrolytes levels of Ca ion levels were increased in all SY treated groups.

Table 4. Biochemical levels of some parameters in control and SY treated groups in male rats.

Group	1	2	3	4	Unit
Sunset Yellow	0	161.4	80.7	40.35	Mg/kg/day
		Liver Function			
Total protein	6.3 ± 0.28[a]	7.85 ± 0.49	6.45 ± 0.49	6.35 ± 0.49	g/dl
Albumin	3.8 ± 0.57	5.2 ± 0.98	5.2 ± 0.14	3.87 ± 0.67	g/dl
AST	52.5 ± 10.61	90 ± 14.1	60 ± 14.14	53.5 ± 2.12	IU/l
ALT	22.5 ± 10.61	45 ± 7.07	32.5 ± 3.54	23.5 ± 2.12	IU/l
GGT	5.03 ± 4.77	5.75 ± 0.64	5.15 ± 0.07	5 ± 0.71	IU/l
ALP	112 ± 11.31	132.5 ± 10.61	116 ± 1.41	112.5 ± 17.68	IU/l
Total bilirubin	0.25 ± 0.07	0.75 ± 0.07*	0.33 ± 0.04	0.28 ± 0.04	Mg/dl
		Kidney Function			
Creatinine	0.28 ± 0.08	0.85 ± 0.07*	0.33 ± 0.03	0.27 ± 0.07	Mg/dl
BUN	18.5 ± 3.54	22.5 ± 3.54	19.5 ± 3.53	20.5 ± 0.71	Mg/dl
		Lipid Profile			
Cholestrol	41.5 ± 2.12	69.5 ± 0.71*	66.35 ± 3.75*	56.5 ± 10.61	Mg/dl
Triglyceride	109.5 ± 2.12	119 ± 1.41	116.5 ± 6.36	112.5 ± 3.54	Mg/dl
		Electrolytes			
Ca I	1.19 ± 0.14	1.51 ± 0.24	1.26 ± 0.007	1.33 ± 0.04	mmol/L
Ca T	2.33 ± 0.28	3.14 ± 0.37	2.65 ± 0.007	2.6 ± 0.08	mmol/L

a : Mean ± SD; Ca I : ionized Calcium, Ca T: Total Calcium; *: Significant vs. G1 at $P<0.05$.

Macroscopic and microscopic findings:

Macroscopic examination of liver at necropsy revealed no gross lesions. Microscopic examination of liver revealed some dose-dependent histopathological alterations; these included necrosis, focal edema, inflammation and interlobular fibrosis. These toxic effects to the liver was in all rats of group 2, in about 50% of the rats in group 3 while none in group 4 in which the histological architecture of the liver parenchyma was almost similar to normal (Fig. 2).

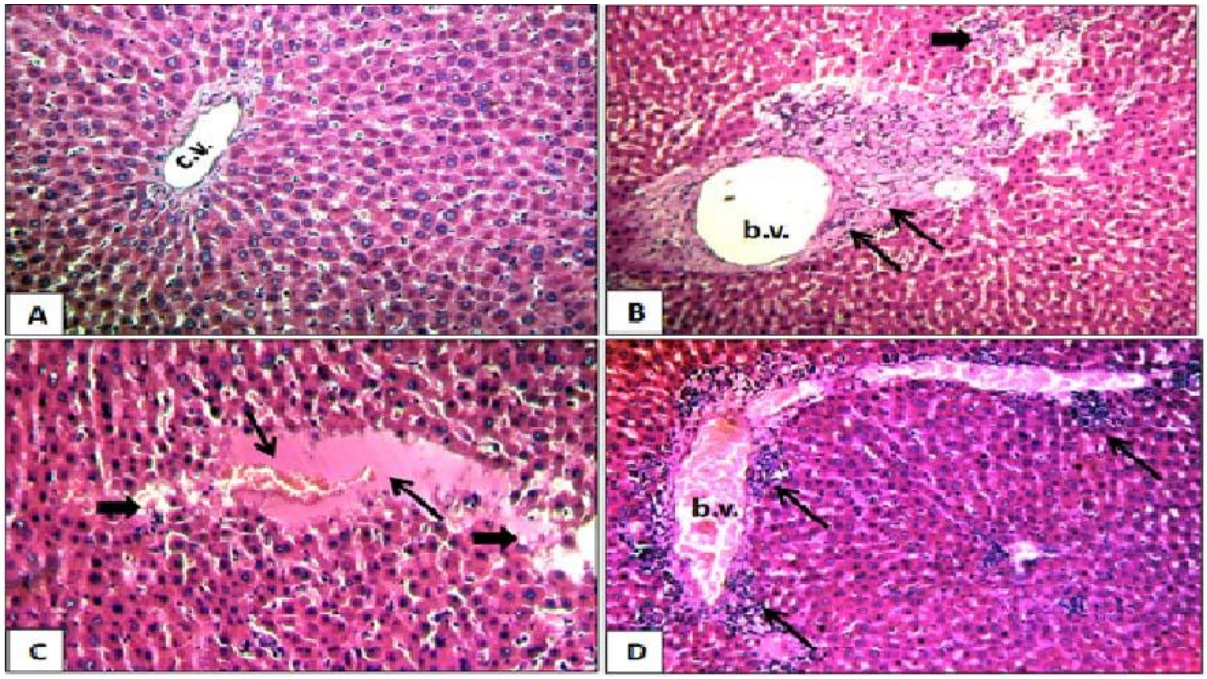

Fig. 2. A-D: Photomicrographs showing liver architecture of normal and SY-treated groups (2 &3). A): Liver section of a normal rat from G1 showing normal histological architecture and central vein (c.v.). X 400, HE; B): Liver section from G2 rats treated with 161.4 mg/kg/BW for 13 weeks showing interlobular space with a dilated blood vessel (b.v.), fibrosis (arrows) and focal necrotic areas (arrow heads). X 200, HE; C): Liver section from G2 rats treated with 161.4 mg/kg/BW for 13 weeks showing edema (arrows) and focal necrotic areas (arrow heads). X 400, HE; D): Liver section from G3 rats treated with 80.7 mg/kg/BW for 13 weeks showing dilated blood vessel (b.v.) with inflammation areas (arrows). X: 200, HE.

DISCUSSION:

Dietary treatment of sunset yellow (SY) for 13 weeks in the present study caused slight increase in body weights and at body weight gains at the low dose of SY. A similar finding was also reported by Gautam *et al.* (2010), he studied the toxic impact of Tartrazine, another food coloring azo dye, on Swiss albino mice and also found an increase in body weight gain in both experimental groups for low dose (200 mg/kg BW) and high dose (400 mg/kg BW) groups. Hasan (2010) also showed weight gain in experimental animals treated with 7.5 mg/kg BW Tartrazine and 0.15 and 0.3 mg/kg chocolate brown. (Chatterjee and Shinde, 2002) also reported an increase in the body weight over 20% above the mean body weight. Similar results have also been reported previously by big deal of investigators such as (Osman *et al.*,1995) in mice fed with synthetic food colorants (Sharma *et al.*, 2005a) in mice fed with orange red and in mice fed with apple green (Sharma *et al.*, 2006) and in mice fed with lead chromate (Chakravarty *et al.*, 2007).

On the contrary, Sharma *et al.* (2005b) studied the hemotoxic effect of chocolate brown on Swiss albino mice and observed no significant change in body weight of experimental mice when compared with control. Also, Sharma *et al.* (2009) showed a highly significant decrease in the body weight of experimental animals when fed with Tartrazine with higher doses, while, Abdel-Aziz *et al.* (1997) found similar results with their studies on erythrosine. Helal *et al.* (2000) found lower body weights in very young rats fed with many different other synthetic food colorant including SY indicating differential effects on body weight gains between young and older experimental animals.

In the present study, an increase in the total protein and albumin levels were shown after administration of SY at different doses particularly higher ones. Sharma *et al.* (2010) identified a significant increase in the serum protein levels at the lower dose, increase was non-significant at the higher dose in Swiss albino mice fed with Tartrazine. Also, they reported an increase in the serum protein levels in Swiss albino mice fed with chocolate brown. Himri *et al.* (2011) studied oral toxicity of Tartrazine after the 90 day and evaluated that the total protein significantly increased after treatment with 7.5 and 10 mg/kg BW, but decreases in a group treated with 5 mg/kg BW. On the contrary, in Swiss albino mice fed with kesari powder (Sharma *et al.*, 2010), a significant decrease in the total serum proteins was found at both dose levels of the powder. The present results are also in accordance with (Chakravarty *et al.*, 2005; Ashour and Abdel-aziz, 2009; Latha and Jeevaratanm, 2012) who observed a slight increase in the total protein and albumin

levels in treated groups in comparison to the control group in a 13-week oral toxicity study of carotenoid pigment in rat.

The present study on the experimental animals revealed increased in level of alkaline phosphatase (ALP) after administration of high dose of SY. Sharma *et al.* (2010) also showed an increase in ALP in rats fed with metanil yellow and maintained on low protein diet. Helal (2001) studied the effect of oral administration of a mixture of sodium nitrate and SY given daily for 30 days to rats, and observed a significant increase in the serum ALP level. Sharma *et al.* (2010) observed the effect of feeding different dose of blend (Chocolate brown) on mice serum and revealed an increase in the ALP levels, also observed an increase in ALP in female Swiss albino mice fed with kesari powder (a blend of Tatrazine and SY). Mahmoud (2006) observed an increase in ALP in rat fed with the synthetic dye brilliant blue. These data indicate pronounced effect of SY on liver functions.

The obtained results revealed, an increase in the levels of ALT, AST, and total bilirubin levels at higher doses of SY. This effect on the liver function parameters by the high doses of colorants is in accordance with the previous work of (Gaunt *et al.* 1972; Abdel-Rahim *et al.*, 1987; Ibrahim *et al.*, 1988; Aboel-Zahab *et al.*, 1997), who recorded a pronounced increase of serum and liver transaminases level in rats ingested synthetic colorants. AST is considered to be also specific for liver function tests (Ganong, 1991), its levels are known to be increased in plasma of all groups after the treatment and recovery period with annatto and SY. The liver function disturbance after use of SY was accompanied with histopathological changes in the liver parenchyma. To evaluate the potential hepatotoxicity by analyzing liver function tests in serum, hepatocellular damage in liver tissue after chronic or subchronic exposure to adult male rats is known to be confirmatory for liver toxicity of any substance in human or in experimental animal (Meshkibaf *et al.*, 2006).

Two other unfavourable side effects of SY were noted in the present study. The Ca ion levels were increased as well as the lipid profile including the cholesterol and triglycerides levels in all SY-treated groups in a dose dependent manner. Similar results were obtained when beet and curcumin extracts were studied as food colorants to rats authores. EFSA (2014) and El-Malky *et al.* (2014) mentioned that rats administrated overdose of studied synthetic colors showed significant increase in the lipid profile of blood including total cholesterol, low density lipoproteins (LDL) and high density lipoproteins (HDL). The lipid profile and calcium ion levels were increased here are in agree well with the previous author.

The microscopic examination in the liver of the rats treated with SY by intragastric gavage (i.g.) showed changes in liver architecture than normal control of rats after 13 weeks. Although several previous studies have mentioned the safe use of SY, the obtained results indicated the necessity of additional long-term studies with different doses that will be provided to rats. Moreover, the use of acceptable daily intake (ADI) of SY as recommended synthetic yellow color, and even the commercial colorants led to obtain non-acceptable histopathological and biochemical data which could be extrapolated to human health where the treatment dose of group 3 (80.7 mg/kg BW/day) which is lower than ADI of SY induce obvious histopathological alteration in hepatic cells.

In conclusion, these results support the trend of using the natural coloring agents. Also, we suppose that SY has a retard damage effect on liver function, lipid profile, hypercalcemia and histopathology. Therefore, the present study sheds light on the nutritional hazards in the liver and blood biochemistry due to the uncontrolled use of synthetic colorants.

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دراسة السمية لمادة أصفر غروب الشمس (E110) في الجرعات المنخفضة كمضافات غذائية على المدى القصير في ذكور الجرذان

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تم تقييم السمية الغذائية من مادة أصفر غروب الشمس حيث انها من المضافات الغذائية الصناعية الهامة. تم حقن 24 من ذكور الجرذان بجرعات 161.4 و 80.4 و 40.2 مل / كجم / يوم على التوالي يوميا لمدة 13 أسبوعا. وتم تقسيمها إلى أربع مجموعات متساوية. المجموعة الأولي تعمل كمجموعة ضابطة سلبية. المجموعة الثانية تم حقنها بمادة أصفر غروب الشمس بجرعة 161.4 مل / كجم / يوم، المجموعة الثالثة تم حقنها بجرعة 80.7 مل / كجم / يوم والمجموعة الرابعة حقنت بجرعة 40.35 مل / كجم / يوم. تم اختيار جرعات أقل من المعطي اليومي المقبول تبعا لمنظمة الصحة العالمية FAO. بعد 13 أسبوعا، وجد تغيير في القياسات القياسات البيوكيميائية لوظائف الكبد والكلى وفي مستوى الدهون في المجموعة (2) التي حصلت على 161.4 مل / كجم / يوم بالمقارنة مع الضابطة، وظهرت في كبد المجموعات (2) و (3) والتي تعتمد على جرعة التغيرات التشريحية المرضية مثل النخر، والتليف بين الفصوص، والاوديما والالتهابات. وكانت التركيبات النسيجية لكبد الفئران في المجموعة (4) مماثلة تقريبا إلى الضابطة. ولذلك، ينبغي توخى الحظرمن التعرض لفترات طويلة من الملونات الاصطناعية مثل مادة أصفر الغروب للخطر على صحة الإنسان.