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Liver Cirrhosis in Albino Male Rats Induced by Sunset Yellow (E110) Dyes

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Abstract: The toxic effect of sunset yellow (E110) were studied in albino male rats. Twenty albino male rats mean weight (75± 2 gm) were used and divided into two equal groups: 1st group (Control) group contain (10) rats feed on healthy pellet, 2nd group (treated group) contain 10 rats' daily intake for 120 days by gavage (2g /1 kg b.w) Sunset yellow (E110) dissolved in distal water. Biochemical analysis of serum at day 60 & 120 of experimental by analysis catalase (Iu/mg), Malonaldehyde (MDA) (mol/L), serum Alanine transaminase (ALT) and Aspartate transaminase (AST)(Iu/l). Liver tissue were taken for histopathology changes at days (120) and stained by H&E and masson trichrome. Result showed significant decrease $P<0.05$ in catalase & significant increase in MDA, ALT &AST in treated group at 60 & 120 days. Liver cirrhosis & granuloma with necrosis is the most impudent lesion in treated group.

Keywords: Liver, Cirrhosis, Granuloma, Sunset Yellow, (E110), Food dyes.

Introduction

Food dyes are a large class of food additives added to food to increase consumer acceptability without any nutritional important (1). Food additives are any substances add to food during production to enhance or preserve flavor directly or indirectly (2).They increase the shelf life of food by maintaining product consistency , it added in regulated quantities and acceptable daily intake (3). It is chemical substances deliberately for preservation of food, flavor and texture (4). Sunset yellow molecular weight (452.36) is an azo dye orange yellow in color like sweet, jams and jellies, Soft drinks, candies Ice cream & Sauces(5). Its formula (C16H10N2 Na20 752) sunset yellow F C F (E110) belongs to azo group, in the past few years uses of some food dyes including sunset yellow was banned in United States and Japan owing to its mutagenicity which has been evidenced from several mammals bioassays (6). Sunset yellow cause several disease like asthma, anaphylaxis, urticarial, angioneurotic edema and immune suppression(7). New studies showed that seven of these dyes contributed induced carcinogenesis in lab animal including brain & testicular tumor, colon cancer and mutation(8).

*Materials & Methods

Experimental animals and management

A total of twenty albino male rats with mean of body weight (75 ± 2 gm) were used in our experiment they housed in animal house of veterinary college , Tikrit university, Iraq. After 14 days of adaptation, then kept in plastic cages (120 * 100 * 100 cm) dimensions & under 12 hours light & 12 dark at (22 ± 25 C°) for 120 day. All experimental groups were provided identical management ethics.

* Rats Intoxications

Twenty albino male rats were randomly equally divided into two groups: 1st group control group (C) contain (10) male rats feed on healthy pellet, 2nd group (treated group(T) daily and orally by gavage intake for 120 days (2gm / kg B.W) Sunset yellow (E110) which prepared from Iraqi local market in Baghdad city. The sunset yellow dissolved in distilled water when given by gavage to rats (9)

* Biochemical assay

Fasting blood obtained from rats at days (60 & 120) of experiment from heart puncher / after centrifugation at 300 rpm for 15 min, serum separated and analyzed for follow:

1) Catalase activity (U/mg) was determined according to (10).
2) Determination of malondialdehyde (MDA) according to (11).

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3) Serum Alanine aminotransferase (ALT) and serum Aspartate amino transferase (AST) (Iu/l)were assay by Commercial Kits according to biosystem Chemical Kits (Germany) and analyzed the level by spectrophotometer.

Pathological examination

At end of experiment (120) days all male rats were sacrificed by intramuscular administration of Xylazine and ketamine. Liver were taken from 1st & 2nd group and kept in 10 % neutral buffered formalin and then processed routinely tissue processing apparatus (Histokinette),the section were embedded in paraffin & Sectioned by rotary microtome & stained section with a hematoxyline and eosin (H&E) stain, also by Masson trichrome stain for fibrous tissue (12)

Statistical analysis

Biochemical assay data of 1st and 2nd groups were read by statistical analyses were carried out using SPSS software package (version 17)(10) and performed using one-way ANOVA for comparison between groups. P value of less than 0.05 were considered statistical Significance. All data were expressed as mean ± standard error (SE) (13)

Results & Discussion

* Biochemical assay:

* Catalase assay (Iu/mg) .

Catalase activity assay at table (1) indicated significant decreased (mean ± SE), in 2nd group(treated group) at day 60 & 120 and highly Significant at day 60 of experiment.

Table(1) :Effect of sunset Yellow on Catalase in male rats

Groups	Days 60	Days 120
1st group (C)	8.25 ± 0.20a	7.12 ± 0.12a
2nd group (T) 2 gm/kg BW sunset yellow	4.20 ± 0.12c	5.32 ± 0.23b

Values are expressed as mean± with different letters are latest significantly different P<0.05 (n:10)

2 Malondialdehyde (MDA) (mole /L)

MDA level showed Significant increased at treated group & highly significant P<0.05 at days 60 at experiment Table(2)

Table(2). Effect of sunset yellow on MDA in albino male rats.

Groups	Days 60	Days 120
1st group (C)	5.220 ± 0.23c	5.250 ± 0.31c
2nd group (T) treated with 2 gm/kg BW sunset yellow	9.87 ± 0.25a	8.21 ± 0.20b

Values are expressed as mean± with different letters are latest significantly different P<0.05 (n:10)

3) Ast (Iu/L)

Treated group at days 60 showed signal increased P<0.05 in Ast when compared with 120 day and control group , table (3)

Table (3): Effect of sunset yellow on AST In albino male rats

Groups	Days 60	Days 120
1st group (C)	25.30 ± 0.32c	23.21 ± 0.30c
2nd group (T) treated with 2 gm/kg BW sunset yellow	60.41 ± 0. 41a	52.32 ± 0.38b

Values are expressed as mean± with different letters are latest significantly different P<0.05 (n:10)

4) ALT (Iu/L)

a treated group as in table (4) indicated at days 60 & 120 significant increased P<0.05 alt when compared to control group table (4)

T table (4) Effect of Sunset Yellow on ALT (Iu/L) In albino rats

Groups	Days 60	Days 120
1st group (C)	8.13 ± 0.12	8.24± 0.20
2nd group (T) treated with 2 gm/kg BW sunset yellow	36.25 ± 0.25	35.00 ± 0.21

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Values are expressed as mean± with different letters are latest significantly different P<0.05

Liver is the main organ responsible of metabolism of toxic material and primary target organ, so damaged liver caused by chemicals like sunset Yellow cause dysfunction of cytochrome 450, mitochondrial dysfunction & oxidative stress with cell injury decrease in catalase enzymes treated group with 2gm/kg bw Sunset yellow and increased in MAD due to oxidative stress and imbalance between free radical & cause lipid peroxidation & inactivation of many enzyme (14, 15) . Significant increase in Ast & Alt due to biochemical and pathological effect of sunset yellow causing failure & disturbance of imbalance in intermediary metabolism: &leakage of enzymes from effected liver to increase in serum (15).

Pathological changes

Control group: No important pathological changes were observed in liver of 1st group (control group).

2nd group (treated with 2 gm/kg BW sunset yellow) swollen hepatocyte by fat droplet other hepatocyte cells were necrotic fig(1). All hepatocytes were necrotic with sever dilated sinusoids and sever hemorrhage with increased in number kuffer cells fig (2). Sever Sinusoid hemorrhage with multiple thrombus formation & thickening of medial layer artery & increase fibrous connective and mononuclear cells infiltration with surrounded the arteries fig(3) .

All blood vessels thrombosed with increased thickening of media layer and adventitia layer by thick fibrous connective tissue capsule (dark blues) fig (4).

liver leukosis with a heavy leukocytes , infiltration mostly mononuclear cells specially lymphocytes fig(5).

these cells arraignment as lymphocytic granuloma surrounded by thick fibrous tissue capsule (dark blue), all hepatocyte cells were necrotic with foreign body giant cell fig(6).

liver cirrhosis characterized and hepatocyte cell necrosis with thick fibrous tissue capsule and calcified center of necrosis fig(7).

Sever fibrous connective tissue capsule surrounded the cirrhotic lobules (pseudolobules) of liver with infiltration mononuclear cells fig(8) & fig.(9).

Nodular formation (pseudolobules) characterized by multiple irregular nodules without central vein and portal area, these nodule surrounded by thick fibrous connective tissue capsule (blue color) and congested blood vessels fig (10).

Sunset yellow (E 110) is food color which induced hepatic damage specially in rats and cause increase in catalase, MDA, AST and ALT enzymes. These artificial dyes associated with liver fatty change and acute cellular swelling due to drastic alteration in the antioxidant defense system (16) granuloma change cause inability of liver to detoxification & metabolized of lipid specially when hepatosteatosis occur(17).

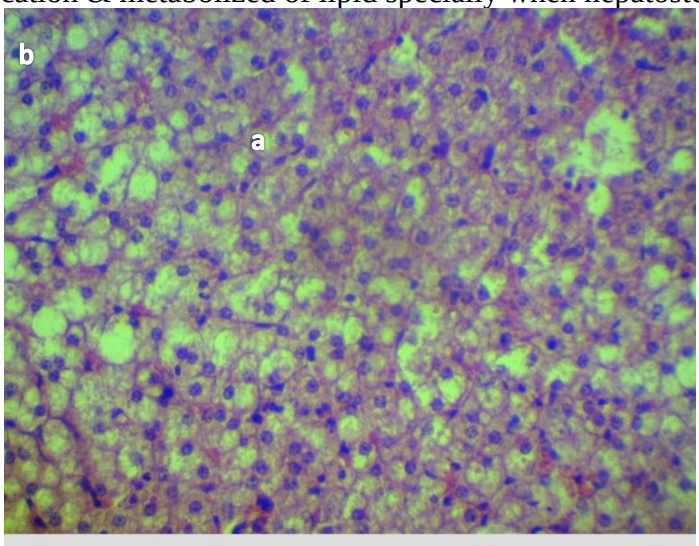

Figure 1 Histopathological section in liver of albino male rat 2nd group (treated with 2 gm/kg BW sunset yellow) showed a) fat vacuoles b) necrotic hepatocytes H & E stain X20

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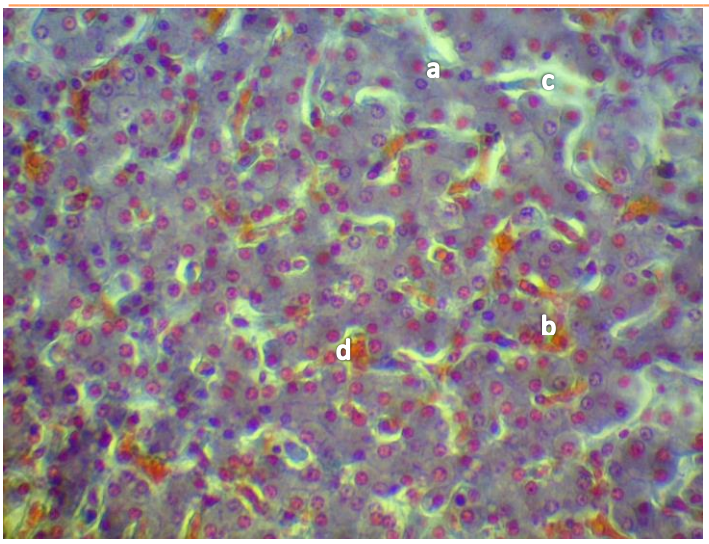

Figure 2 Histopathological section in liver of albino male rat 2nd group (treated with 2 gm/kg BW sunset yellow) showed a) necrotic hepatocytes b) increased in number kuffer cells c) dilated sinusoid d) Sinusoid hemorrhage H & E stain X40

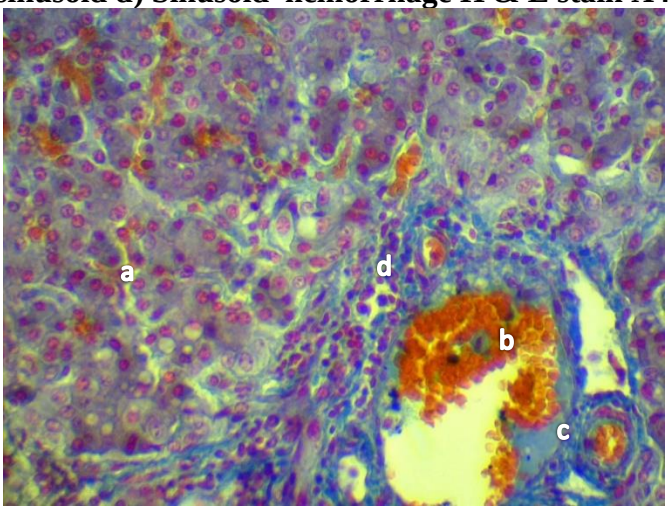

Figure 3 Histopathological section in liver of albino male rat 2nd group (treated with 2 gm/kg BW sunset yellow) showed a) Sinusoid hemorrhage b) thrombus formation c) thickening of medial layer artery d) mononuclear cells infiltration Masson trichrome stain X20

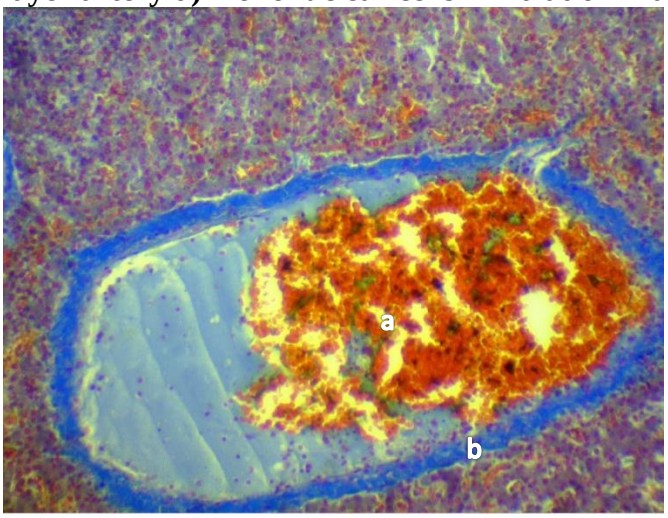

Figure 4 Histopathological section in liver of albino male rat 2nd group (treated with 2 gm/kg BW sunset yellow) showed a) thrombus formation b) thick fibrous connective tissue capsule (dark blues). Masson trichrome stain X40

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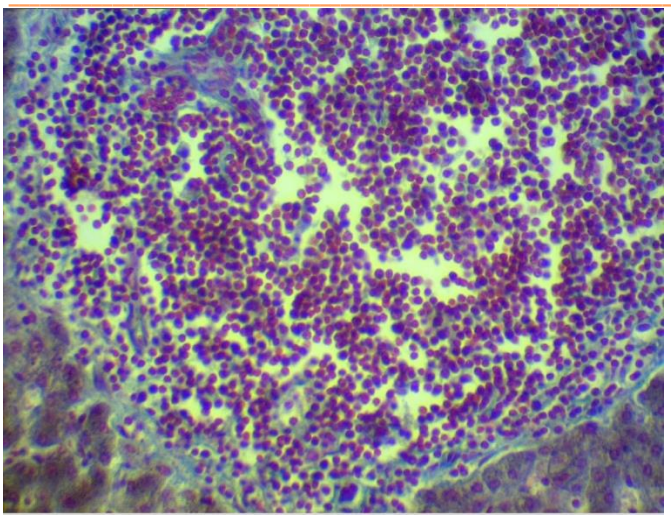

Figure 5 Histopathological section in liver of albino male rat 2nd group (treated with 2 gm/kg BW sunset yellow) showed liver leucosis with infiltration mononuclear cells . Masson trichrome stain X40

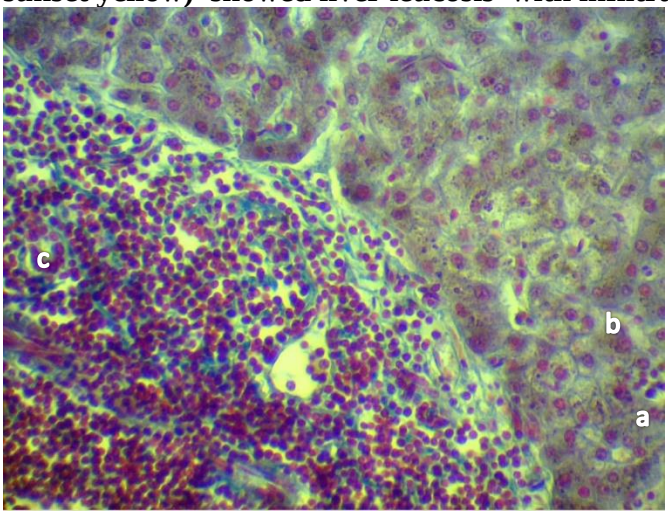

Figure 6 Histopathological section in liver of albino male rat 2nd group (treated with 2 gm/kg BW sunset yellow) showed a) lymphocytic granuloma b) necrotic hepatocyte cells c) foreign body giant cell .Masson trichrome stain X40

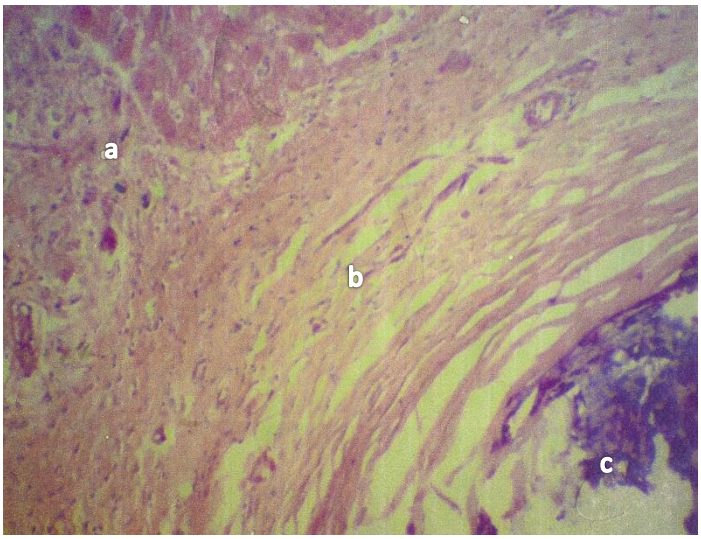

Figure 7 Histopathological section in liver of albino male rat 2nd group (treated with 2 gm/kg BW sunset yellow) showed a) necrotic hepatocyte cell b) thick fibrous tissue capsule c) calcification. H&E stain X40

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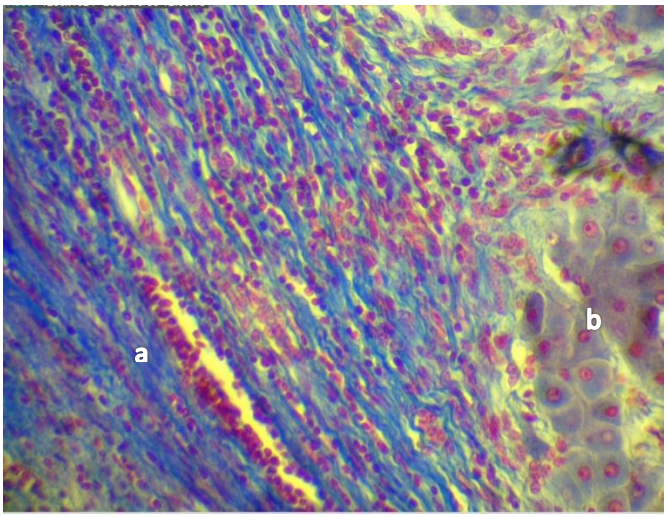

Figure 8 Histopathological section in liver of albino male rat 2nd group (treated with 2 gm/kg BW sunset yellow) showed a)connective tissue b) cirrhotic lobules (pseudolobules). Masson trichrome stain X40

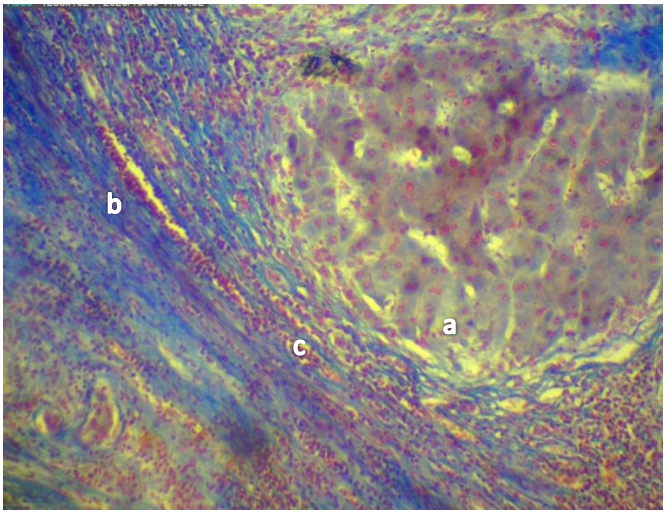

Figure 8 Histopathological section in liver of albino male rat 2nd group (treated with 2 gm/kg BW sunset yellow) showed a) pseudo hepatic lobules b) thick fibrous tissue capsule c) infiltration mononuclear cells .Masson trichrome stain X40

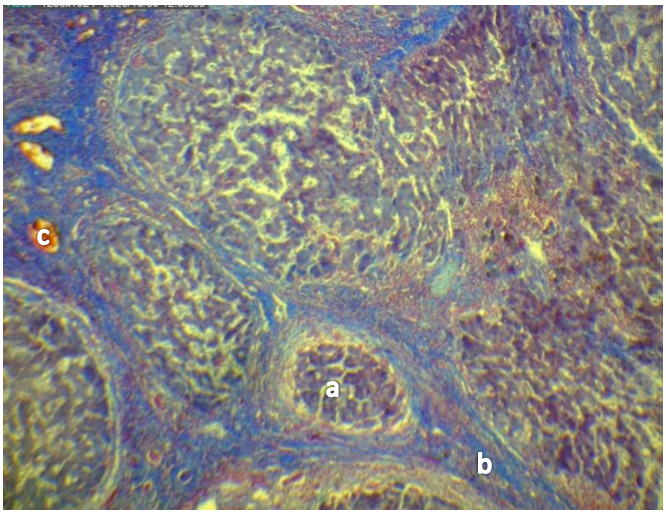

Figure 10 Histopathological section in liver of albino male rat 2nd group (treated with 2 gm/kg BW sunset yellow) showed a) multiple pseudolobules(cirrhotic lobules) (a) thick fibrous connective tissue capsule (blue color) (b) congested blood vessels (c) . Masson trichrome stain X40

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References

1. Al-Kaisei, B. I., Humadi, A., & Humadai, T. J. (2019). TOXICOPATHOLOGICAL AND MUTAGENIC OF FOOD DYES SUNSET YELLOW (E-110) ON WISTER MALE RATS. Biochemical and Cellular Archives, 19(2), 3421-3426.
2. Antakli, S. A. A. D., Nejem, L. E. O. N., & Katran, S. H. E. R. E. N. (2015). Simultaneous determination of Tartrazine and Brilliant Blue in foodstuffs by spectrophotometric method. International Journal of Pharmacy and Pharmaceutical Sciences, 7, 214-218.
3. Kunkel, E. M., & Barbara, H. D. (2004). Gale Nutrition and Well-being A to Z. The Gale Group Inc., Macmilln Refernce USA, New York.
4. Daniel M. (2007) Reactions to food Additives and preservatives Drug adminstration 131.
5. Weiss, B. (2010). Neurobehavioral Effects of Artificial Food Dyes, with Bernard Weiss. Podcasts: The Researcher's Perspective, 2010(1.(
6. Feng, J., Cerniglia, C. E., & Chen, H. (2012). Toxicological significance of azo dye metabolism by human intestinal microbiota. Frontiers in bioscience (Elite edition), 4, 568.
7. 7)Yadav, A., Kumar, A., Dwivedi, P. D., Tripathi, A., & Das, M. (2012). In vitro studies on immunotoxic potential of Orange II in splenocytes. Toxicology letters, 208(3), 239-245.
8. Curran, L. (2010). Food Dyes Linked to Cancer, ADHD, Allergies. Food safety news.
9. Choi, H. (2012). Risk assessment of daily intakes of artificial colour additives in food commonly consumed in Korea. Journal of Food and Nutrition Research, 51(1), 13-22.
10. Hadwan, M. H., & kadhum Ali, S. (2018). New spectrophotometric assay for assessments of catalase activity in biological samples. Analytical biochemistry, 542, 29-33.
11. Buege, J. A., & Aust, S. D. (1978). [30] Microsomal lipid peroxidation. In Methods in enzymology (Vol. 52, pp. 302-310). Academic press.
12. Suvarna, K. S., Layton, C., & Bancroft, J. D. (Eds.). (2018). Bancroft's theory and practice of histological techniques E-Book. Elsevier health sciences.
13. Morgan, G. A., Barrett, K. C., Leech, N. L., & Gloeckner, G. W. (2019). IBM SPSS for Introductory Statistics: Use and Interpretation: Use and Interpretation. Routledge.
14. Khayyat, L. I., Essawy, A. E., Sorour, J. M., & Soffar, A. (2018). Sunset Yellow and Allura Red modulate Bcl2 and COX2 expression levels and confer oxidative stress-mediated renal and hepatic toxicity in male rats. PeerJ, 6, e5689., 190 (2-3), 103-13
15. Amin, K. A., Hameid II, H. A., & Abd Elsttar, A. H. (2010). Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food and Chemical Toxicology, 48(10), 2994-2999.
16. Saxena, B., & Sharma, S. (2015). Food color induced hepatotoxicity in Swiss albino rats, Rattus norvegicus. Toxicology international, 22(1), 152.