Biological evaluation of some synthetic and natural food colorants

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ABSTRACT

This study was carried out to evaluate and compare the possible toxic effect among some synthetic and natural food colorants on biochemical parameters as well as liver and kidney histological of experimental rats. A total number of 45 young albino male rats (weight about 100-120 g) were used in this study. All rats were divided randomly into nine groups and fed on tested diets for four weeks (carmoisine, raspberry, sunset-yellow, and fast green as synthetic colorants; and anthocyanin, betalain, carotenoids, and chlorophyll as natural food colorants; as well as a control group). The results showed a significant increase in levels of serum alanine aminotransferase, aspartate aminotransferase, urea, creatinine, total protein, and albumin, with decreased levels of immune-globulins were observed in all groups treated by synthetic colorants groups compared to the groups treated by natural food colorants and control group. Histological examinations revealed alterations in kidneys including congestion and hemorrhage with infiltration, and deformation of the glomeruli structure. Whereas, alterations in the liver including congestion, hemorrhage, and dilatation of sinusoids and central vein with microvascular steatosis. Therefore, it is advisable to limit the uses of synthetic food colorants or replacing them with natural ones, especially for children's foods.

Keywords*:* Natural food colorants; Synthetic food colorants; Biochemical parameters; Liver histology; Kidney histology.

INTRODUCTION

Color is the most important characteristic of food. Based on the color of the food, first impressions are made. For example, the color indicates the ripeness degree of the fruit, food freshness, and toast burning. Based on these first impressions, a judgment is made whether the food is safe to eat or not and whether it can be expected to taste good or not. Since color is closely associated with expectations, the addition of color to food is a way to fulfill these expectations (Aberoumand, 2011).

Colorants are added to food to serve several purposes, including accounting for the loss during processing or storage, to enhance color already present, to minimize batch to batch variations, and color otherwise uncolored food (Lakshmi, 2014). Food colors can be divided, in respect of their origin, into natural, identical to natural and synthetic ones (Janiszewska-Turak *et al.,* 2016).

Synthetic food colorings have been associated with adverse reactions in some individuals, with claims of links to hyperactivity, asthma, and other allergic reactions (Larsen, 2008).

These claims have generated much debate as well as scientific research and have led to negative consumer perceptions of synthetic colors .The current consumer perception of synthetic food ingredients and food colorants, in particular, is that they have negative health implications and that foods containing the natural ingredient are safer, healthier, and hence a better choice (Crino *et al.,* 2013).

Regarding food color industry trends, the use of natural colorants has increased in foods and beverages as substitutes for their synthetic counterparts. This is mainly due to the growing awareness of the environmental hazards and the potential side-effects of the chemicals used in the synthesis of food colorants (Carocho *et al.,* 2014). Also, to satisfy consumers who demand natural ingredients, major food and beverage companies have committed to removing synthetic substances, including synthetic colors, from their products. This marketing strategy is in line with the so-called "clean label" trend (Cortez *et al.,* 2017).

In Egypt, there has been a sharp increase in the use of synthetic food colorants in the past few years and additionally, there is uncontrolled use of synthetic color particularly in food mostly consumed by children (Salah, 1994). More attention must be focused on the physiological and pathological effects of color additives (Helal *et al.,* 2000).

The purpose of this study is to compare and illustrate the health effects of four natural (anthocyanin, betalain, carotenoids, and chlorophyll) and four synthetic food colorants (carmoisine, raspberry, sunset yellow, and fast green) on biochemical and histological parameters of experimental rats.

MATERIAL AND METHODS

Materials

Natural colorants

Anthocyanin, betalain, carotenoids, and chlorophyll pigments were extracted from different plant sources includes: Roselle (*Hibiscus sabdariffa*), red beetroot (*Beta Vulgaris*), orange navel peels (*Citrus aurantium*) and green peas peel (*Pisum sativum*), respectively by ultrasound-assisted extraction (UAE) according to the methods of Sivakumar *et al.* (2009); Almahy *et al.* (2017) and Boukroufa *et al.* (2017). Encapsulation of pigment extracts was prepared by mixing 100 ml of the pigment extract with 20 g of matrix consist maltodextrin DE 20 and Arabic gum (at ratio 19: 1), then were mixed homogeneously. The mixture was constantly and thoroughly stirred to ensure homogeneity during freeze-drying process as described by Ravichandran *et al.* (2012).

Synthetic colorants

The synthetic colorants (carmoisine, raspberry, sunset yellow, and fast green) were purchased from Kamena Company, Kafr Tohormous, Giza, Egypt.

Methods

Experimental design

A total number of 45 young albino male rats (weighing about 100-120 g) were used in the present study. All rats were fed on a balanced diet (AIN-93) for one week according to Reeves *et al.* (1993). Such feed contained 15% casein, 5% cellulose, 3.5% mineral mixture, 1% vitamin mixture, 8% oil, 10% sugar and 57.5% starch. The rats were then divided randomly into nine groups (five rats each) and were fed on the following diets for 4 weeks:

Group 1: rats fed on the basal diet (as a control group), the standard diet was prepared according to Reeves et al. (1993). Group 2: rats fed on the basal diet and ingested orally with a dose of anthocyanin microcapsules (50 mg/kg body weight). Group 3: rats fed on basal diet and ingested orally with a dose of carmoisine (10 mg/kg body weight). Group 4: rats fed on basal diet and ingested orally with a dose of betalain microcapsules (50 mg/kg body weight). Group 5: rats fed on basal diet and ingested orally with a dose of raspberry (10 mg/kg body weight). Group 6: rats fed on basal diet and ingested orally with a dose of carotenoids microcapsules (50 mg/kg body weight). Group 7: rats fed on basal diet and ingested orally with a dose of sunset yellow (10 mg/kg body weight). Group 8: rats fed on basal diet and ingested orally with a dose of chlorophyll microcapsules (50 mg/kg body weight). Group 9: rats fed on basal diet and ingested orally with a dose of fast green (10 mg /kg body weight).

The biological investigation was carried out at the Animal House Lab, Food Science and Technology, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

At the end of the experiment, the rats were fasted overnight, and total food intake, final rat's body weights, and body weight gain were recorded.

The food efficiency ratio was calculated according to this equation:

Food efficiency ratio = body weight gain/food intake (Proll *et al.,* 1998).

Blood Samples

At the end of the experimental period (28 days), blood samples were obtained after fasting (overnight) using the orbital sinus technique of Soltan and Shehata (2012). The blood samples were collected in a dry tube and were left to clot, then centrifuged at 3000 rpm for ten min. Serum was separated and frozen at -20 °C for the subsequent analyses.

Afterward, the rats were decapitated and some internal organs such as liver and kidneys were dissected out, trimmed of excess fat, and weighted before performing histological studies. The organ weight was presented as relative organ weight and calculated according to Ping *et al.* (2006) and Elgawish and Ghanem (2014), as follows:

Relative organ weight = (Absolute organ weight/ whole body weight) × 100.

Biochemical Investigation

Measuring blood glucose

Blood glucose levels were measured according to Dafallah *et al.* (2015).

Determination of liver function enzymes activity

Serum aspartate –aminotransferase (AST) and alanine-amino transferase (ALT) were determined according to the method described by Abd El-Wahab and Moram (2012).

Determination of total protein and albumin concentration

Total protein and albumin concentration were measured by the method described by Helal *et al.* (2000).

Determination of kidney functions

Blood urea and creatinine concentration were estimated by the enzymatic method as described by Abd El-Wahab and Moram (2012).

Lipid profile

Total lipids, total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol were determined according to the method described by Ibrahim (2001).

Determination of plasma immune-system (Ig G, Ig A, and Ig M)

The kit is intended for measuring plasma Ig G, Ig A, and Ig M according to the method described by Dafallah *et al.* (2015).

Histological Examination

The liver and kidneys of rats were dissected out and fixed instantaneously in 10% formal saline for 24 h. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax (melting point of 50-56 °C). Paraffin sections were cut at 6μm thicknesses using a rotary microtome (Model MR 60, Russian), then the sections were stained with Harris' hematoxylin and eosin. The observations were performed using a light microscope (Zeiss Axiophot, Germany) and photographs were taken with an automatic photomicrographic system (Sarkar *et al*., 2005).

Statistical Analysis

The data obtained from three replicates were analyzed by (ANOVA) using the SPSS statistical package program, and the differences among the means were compared using the Duncan's Multiple Range test of SPSS (1998). A significant level of 0.05 was chosen.

Results and Discussion

Effect of oral ingestion of colorants on the behavior of rats

It was noticed that after oral administration of synthetic colorants, rats became more active, nervous, and aggressive. Also, a skin irritation was noticed after the administration of sunset yellow. These additives are a common cause of chronic urticarial, angioedema in rats, and also caused positive skin and intestinal reaction irritability (Soltan and Shehata, 2012). These results are nearly in agreement with the findings of Helal *et al.* (2000) and Soltan and Shehata (2012).

Effect of oral ingestion of the colorants on weights of rats

The data given in Table (1) shows that the bodyweight of rats ingested oral synthetic colorants (carmoisine, raspberry, sunset yellow, and fast green) was significantly lower than the control and the other groups ingested natural colorants (anthocyanin, betalain, carotenoids, and chlorophyll).

Also, Table (1) illustrates that, except fast green group, rats fed synthetic colorants recorded a significant increase ($p < 0.05$) in feed intake values compared to the control group, but the amount of increase in feed intake differed among groups. Thus, the decrease in gaining body weight was not due to the decrease in feed consumption. For these reasons, the feed efficiency of the diet was calculated.

The results of the food efficiency ratio indicated that the rats that ingested oral natural colorants and control had higher ($p \leq 0.05$) food efficiency ratios than the other groups. The changes in food intake were not parallel to the growth rate, feed efficiency of the different colorants. These results supported the hypothesis that, the digestion of diet may be inhibited in a certain manner by the addition of synthetic colorants.

The data revealed that the synthetic food colorants had a negative effect of on growth rate (body weight gain) which may be related to the disturbance effects of colorants on different metabolic systems (Abd El-Wahab and Moram, 2012). These results are in harmony with the results of EFSA (2009), ANS (2009), and Abd El-Wahab and Moram (2012).

Relative organs weight

From Table (2), it can be found that the relative weight of tested organs showed an increase in the relative liver, kidney, and heart weights in the rats that had oral synthetic colorants compared to all the other treatments. These results are in agreement with the results of Abd El-Wahab and Moram (2012) who recorded a significant increase in the relative weight of the liver for some treatments that had diets containing synthetic colorants mixed with synthetic flavors.

Effect of oral ingestion of colorants on biochemical properties

Blood glucose levels

Results in Table (3) show that the highest increase ($p \leq 0.05$) in blood glucose level was noticed in treatments that treated with synthetic colorants, especially in the case of raspberry

treatment which amounted to (122 mg/dL), compared to the control (96 mg/dL). The elevation of glucose level can be explained by stimulation of glycogenolysis and gluconeogenesis in the liver with a temporary loss of endocrine functions of the pancreas which leading to hyperglycemia (Al-Shinnawy, 2009). These results are nearly in agreement with the reports of Amin *et al.* (2010), Himri *et al.* (2011), Dafallah *et al.* (2015), and Wopara *et al.* (2019) who indicated that the blood glucose in rats treated by synthetic colorants was significantly ($p \leq 0.05$) increased compared to all the other treatments.

Liver functional enzyme activity

Data in Table (4) revealed a marked increase ($p \leq 0.05$) in serum AST level of all groups treated by synthetic colorants as compared to the control group. On the other hand, rats treated with natural colorants (except rats fed on carotenoids) showed a significant decrease ($p \leq 0.05$) in AST level compared with rats fed on synthetic colorants.

As found in Table (4), the groups treated by carmoisine and raspberry had the highest levels ($p \leq 0.05$) of ALT (46 and 48 μ/mL, respectively) compared to the other groups (ranged between 36 – 40 μ/mL). The significant increase in the activities of serum AST and ALT for rats treated with synthetic colorants may be due to the haptic potency of these colors resulting in destructive changes in the hepatic cells. This increase is considered to be a result of related to destruction of the liver cells, while leads to liberation of these enzymes in the blood stream. Similarly, Tameda *et al.* (2005) considered the increase in ALT and AST activities as sensitive indicators of a liver cell injury. These results are approximately in agreement with the results of Helal *et al.* (2000), Amin *et al.* (2010), Abd El-Wahab and Moram, (2012), and Soltan and Shehata (2012) who indicated that rats that consumed high dose synthetic color showed a significant increase in serum ALT and AST compared to the control rats.

Total proteins, albumin, and globulin levels

Data in Table (5) revealed that the oral administration of carmoisine and raspberry induced a significant change in serum total proteins, while other groups showed insignificant differences ($p > 0.05$) between them. Also, the results showed that the levels of the plasma albumin (g/dL) were increased ($p < 0.05$) in rats fed on carmoisine, raspberry, sunset yellow, and chlorophyll compared to the other groups. The present data illustrated a significant decrease ($p \leq 0.05$) of globulin in groups treated by sunset yellow and chlorophyll. These results are in agreement with the report of Helal *et al.* (2000).

Creatinine and urea levels

Concerning the kidney functions, the present data in Table (6). The experimental rats treated by raspberry showed the highest increase ($p \leq 0.05$) in serum creatinine level (1.40 mg/dL) compared to other groups. This may be indicating protein catabolism, kidney dysfunction, or both actions (Abdel-Wahhab and Aly, 2003). Also, the experimental rats that orally ingested with raspberry showed a significant increase ($p \leq 0.05$) in serum urea level (66.20 mg/dL), compared all the other groups.

Also, the synthetic colorants, especially raspberry induced alteration in kidney functions which had a highly significant increase ($p < 0.05$) in serum urea and creatinine levels. These changes may indicate a reduction in the glomerular filtration rate as a result of acute renal dysfunction. The serum level of these two parameters depends largely on the glomerular filtration (Abd El-Wahab and Moram, 2012). These results are partially in agreement with those obtained by Abd El-Wahab and Moram (2012), Soltan and Shehata (2012), and Dafallah *et al.* (2015).

Total lipids, total cholesterol, and triglyceride levels

The data in Table (7) shows that a significant decrease ($p \leq 0.05$) in total lipids and total cholesterol for all groups that treated by natural colorants compared to synthetic colorants. The highest values were 430 and 211 mg/dL, respectively for sunset yellow, while the lowest values were 260 and 165 mg/dL, respectively in anthocyanin treatment. Also, the results showed that there was a slight significant decrease in triglyceride level in rats treated by natural colorants, except for chlorophyll compared to those groups treated by synthetic colorants. The highest value of the triglyceride level was 136 mg/dL in carmoisine, while the lowest value was 108 mg/dL in the group treated by anthocyanin.

These results are in agreement with those reported by Abou El-Zahab *et al.* (1997), Himri *et al.* (2011), Dafallah *et al.* (2015), and Wopara *et al.* (2019) who observed significant increases in serum total lipids, cholesterol, and triglycerides in rats had supplemented with some food colorants in different concentrations. The current results are contrary to the results of Sharma *et al.* (2006) who reported that two doses of tomato red (blend of carmoisine and

ponceau 4R) showed a significant decrease in serum total cholesterol and triglycerides when Swiss albino mice consumed these colorants for 21 days as short term or 42 days as long-term. Also, these results are opposite to those reported by Ashour and Abdelaziz (2009) who noticed a significant reduction in serum total cholesterol and triglycerides levels when food color azo dye (fast green) was orally consumed by male albino rats for 35 days.

Serum HDL-cholesterol, LDL-cholesterol levels

Data in Table (8) showed a significant increase ($p \leq 0.05$) in HDL and a decrease in serum LDL cholesterol for all groups treated by natural colorants compared to synthetic colorants. The highest value of LDL cholesterol was observed in the group treated by sunset yellow (134.6 mg/dL), while the lowest value was noticed in the group treated by anthocyanin (86.8 mg/dL). On the other hand, the current data showed a significant increase ($p \leq 0.05$) in HDL/LDL ratios for all groups treated by natural colorants compared to synthetic colorants. The improvement of HDL/LDL ratios were related to a significant increase in HDL levels as well as to the significant decrease in LDL levels in all groups treated by natural colorants compared to synthetic colorants. These results are approximately in agreement with the findings of Dafallah *et al.* (2015).

Immune-System

Regarding the effect of oral administration of colorants on the plasma immune-system, the data obtained in Table (9) revealed that, a significant decrease ($p \leq 0.05$) in Ig A, Ig G, and Ig M was observed in the groups treated with synthetic colorants compared to the control and natural colorants. These results are nearly in agreement with those reported by Dafallah *et al.* (2015).

Histological alterations in some functional organs of rats as affected by oral administration of colorants

Histological alterations of the liver

Data presented in Figure (1) indicated that the histological examination of the liver sections in the control group shows a normal histological photomicrograph. The liver of the control group showed average portal tract, portal veins and average hepatocytes in peri-portal area, central veins, hepatocytes arranged in single-cell cords with average intervening blood sinusoids. While, the liver in the rats treated with anthocyanin showed average portal tracts, portal veins and average hepatocytes in the peri-portal area and mildly dilated central veins with average intervening blood sinusoids and average hepatocytes in the peri-venular area (Figure 2). Anthocyanin is a functional food factor and plays an important role in the prevention of liver diseases (Bendokas *et al.,* 2020).

On the other hand, the liver sections of rats treated with an oral dose of carmoisine showed mildly edematous portal tracts with mildly dilated congested portal veins and average hepatocytes in the peri-portal area, and mildly dilated central veins with mildly congested blood sinusoids and average hepatocytes in the peri-venular area (Figure 3). Montaser and Alkafafy (2013) found that with increasing the doses of carmoisine, there were degenerative changes of hepatocytes.

Also, the liver sections of rats treated with an oral dose of betalain showed mildly edematous portal tracts and average hepatocytes in peri-venular area, and mildly dilated central veins (Figure 4). Betalain was demonstrated as having a close relationship with hepatic tissue protection (da Silva *et al.,* 2019).

The liver of rats treated by an oral dose of raspberry showed mildly edematous portal tracts with mildly dilated congested portal veins and average hepatocytes in peri-portal area, mildly dilated central veins with detached lining with moderate micro-vesicular steatosis of hepatocytes in peri-venular area, and markedly dilated congested blood sinusoids (Figure 5). These results are in agreement with those obtained by Sharma *et al.* (2008) and Soltan and Shehata (2012) who reported that the synthetic color has an adverse effect on the vital organs. At a low dose of synthetic color, the liver exhibited a disruption of hepatic cells near the central vein, and hepatocellular damage.

The liver of rats treated an oral dose of carotenoids showed average portal tracts, portal veins, and hepatocytes in the peri-portal area; and mildly dilated congested central veins and average hepatocytes in the peri-venular area (Figure 6). Okechukwu *et al.* (2019) reported that the consumption of β carotene-rich foods (such as fruits, and vegetables) have a significant effects in modulating the hepatic functions and histological structures related to liver damages especially related to nonalcoholic fatty liver disease.

While, the liver of rats treated by sunset yellow showed mildly edematous portal tracts with mild portal inflammatory infiltrate, markedly dilated congested portal veins, and average hepatocytes in peri-portal area, and

mildly dilated central veins with mild micro-vesicular steatosis of hepatocytes in the peri-venular area (Figure 7). Al-Dahhan *et al.* (2014) observed fatty degeneration of rat liver in the sunset yellow group, in a histological examination.

Also, the liver of rats treated by chlorophyll showed average portal tracts, portal veins, hepatocytes in the peri-portal area, and mildly dilated central veins with average hepatocytes in the peri-venular area (Figure 8). These results are nearly in agreement with the results of Suparmi *et al.* (2016) who noted thet the structure was insignificantly changed to normal values in a histological study in the liver of rats treated with chlorophyll from *Sauropus androgynus* L. Merr. leaf.

The liver of rats treated by the fast green group showed portal tracts with mildly dilated congested portal veins and average hepatocytes in the peri-portal area, and markedly dilated congested central veins with detached lining and mild micro-vesicular steatosis of hepatocytes in the peri-venular area (Figure 9). Mekkawy *et al.* (1998) found that synthetic colorants (ponceau, carmoisine, erythrosine, sunset yellow, tartrazine, fast green, indigotin, brilliant blue, and brilliant black) had a toxic effect which appeared as hepatocellular damage indicated by vacuolation, swelling, necrosis, and pyknosis in liver cells.

Histopathological alterations of the kidney

Data presented in Figure (10) showed the kidney section of the control group. The data indicated average renal capsule, glomeruli, Bowman's spaces, proximal tubules with preserved brush borders, distal tubules, and renal medulla showed average collecting tubules with an average interstitium. Under the light microscope, the kidney sections from the control group did not show any damage, with the glomeruli and tubuli having a normal appearance.

The kidney of the rats treated by anthocyanin showed average renal capsule, average glomeruli with average Bowman's spaces, average proximal tubules with preserved brush borders, and renal medulla showed average collecting tubules with average interstitium (Figure 11). Yarijani *et al.* (2019) found that anthocyanins reduces the renal toxicity which was induced by gentamicin and can improve kidney function and a decrease tissue injuries.

On the other hand, kidney sections of rats treated with an oral dose of carmoisine showed average renal capsule, mildly congested glomeruli with average Bowman's spaces, proximal tubules with scattered apoptotic epithelial lining and partial loss of brush borders, mildly dilated interstitial blood vessels with areas of hemorrhage, marked peri-glomerular and peri-tubular inflammatory infiltrate, and the renal medulla showed collecting tubules with scattered apoptotic epithelial lining and mildly congested peri-tubular capillaries (Figure 12). Rus *et al.* (2010) observed phenomena represented by stasis and edema, congestion, hepatocyte and kidney apoptosis, with atrophy of renal corpuscles.

While the kidney of rats treated by betalain showed average renal capsule, average glomeruli with average Bowman's spaces, average proximal tubules with preserved brush borders, and the renal medulla showed collecting tubules with average epithelial lining and mildly congested peri-tubular capillaries (Figure 13). These results are nearly in agreement with the results of Almeer *et al.* (2019) who found that the kidney tissue of the control rats and rats treated with red beetroot extract had normal kidney structure with normal renal tubules and glomeruli.

From Figure (14), the kidney of rats treated with raspberry showed average renal capsule, mildly congested glomeruli with average Bowman's spaces, average proximal tubules with preserved brush borders, mildly congested interstitial blood vessels with marked areas of hemorrhage, and the renal medulla showed collecting tubules with average epithelial lining and mildly congested peri-tubular capillaries. Sharma *et al.* (2008) reported that the presence of synthetic color in food causes kidney injuries.

Also, the kidney section of rats treated with an oral dose of carotenoids showed average renal capsule, average glomeruli with average Bowman's spaces, proximal tubules, mildly dilated interstitial blood vessels, and the renal medulla showed average collecting tubules with average interstitium (Figure 15). These results are in agreement with the results of Ezejindu *et al.* (2014), who found that the consumption of carotenoids at low or high doses is not a risk factor for kidney disorders.

The kidney sections of rats treated an oral dose of sunset yellow showed average renal capsule, mildly congested glomeruli with average Bowman's spaces, average proximal tubules with preserved brush borders, mildly dilated congested interstitial blood vessels with areas of hemorrhage, and the renal medulla

showed collecting tubules with average epithelial lining and mildly congested peri-tubular capillaries (Figure 16). Al-Dahhan *et al.* (2014) observed nephritis in the kidney of rats treated by sunset yellow, in a histological study.

The kidney of the rats treated by chlorophyll group showed average renal capsule, average glomeruli with average Bowman's spaces with mildly edematous epithelial lining, mildly dilated interstitial blood vessels, and the renal medulla showed average collecting tubules with average interstitium (Figure 17). These results are nearly in agreement with the results of Suparmi *et al.* (2016) who noted that rats treated with chlorophyll from *Sauropus androgynus* (L) Merr were insignificantly changed compared to the normal values.

Also, the kidney of the rats treated by fast green showed average renal capsule, average glomeruli with average Bowman's spaces, average proximal tubules with preserved brush borders, markedly dilated congested interstitial blood vessels, and the renal medulla showed average collecting tubules with mildly congested peri-tubular capillaries (Figure 18). Ghonimi and Elbaz (2015) found that the examined kidney sections of rats treated with synthetic colorants showed degenerative changes in the renal tubules and glomeruli. The same authors observed an enlargement of renal glomeruli, with separation and vacuolation of its lining epithelial cells as well as congestion of its blood capillaries.

CONCLUSION

It could be concluded that the synthetic colorants adversely affected hepatic and renal parameters compared to natural colorants. Synthetic colorants caused increases in the levels of blood glucose, AST, ALT, creatinine, urea, total lipids, total cholesterol, and LDL. While, such synthetic colorants decreased each of body weight gain, globulin, HDL, Ig A, Ig M, and Ig A. The histological examinations revealed alterations in kidneys that included congestion and hemorrhage with infiltration and deformation of the structure of glomeruli. Also, alterations in the liver included congestion, hemorrhage, and dilatation of sinusoids and central vein with microvesicular steatosis occurred. Therefore, it is necessary to be aware of the hazardous effects of consuming such synthetic food colorants. More attention should be paid for using natural colorants. If these results have applied to humans, it is advised that the application of synthetic food colorants consumption should be drastically minimized as these colorants possess detrimental physiological effects.

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Table 1. Initial and final body weight, body weight gain (g), total food intake (g/month), and food efficiency ratio of rats fed on tested diets for 4 weeks.

Groups	Initial body weight	Final body weight	Bodyweight gain	Total food intake	Food efficiency ratio
Control	113^{ab}	195^{bc}	82.0^{d}	1861^{c}	0.044^{b}
Anthocyanin	115^{a}	200.7^{b}	85.7^{bc}	1892^{b}	0.045^{ab}
Carmoisine	110^{b}	181.6^{d}	71.6^{g}	1879^{b}	0.038^{d}
Betalain	112^{ab}	199.2^{b}	87.2^{b}	1925^{a}	0.045^{ab}
Raspberry	114^{ab}	184^{d}	70.0^{g}	1880^{b}	0.037^{d}
Carotenoids	113^{ab}	204.4^{a}	91.4^{a}	1894^{b}	0.048^{a}
Sunset yellow	116^{a}	191.3^{c}	75.3^{f}	1879^{b}	0.040^{c}
Chlorophyll	115^{a}	198.5^{b}	83.5^{cd}	1885^{b}	0.044^{ab}
Fast green	110^{b}	188.6^{c}	78.6^{e}	1854^{c}	0.042^{bc}

Means within the column having the same superscript are not significantly different ($p \leq 0.05$).

Table 2. Relative organs weight (%) of rats fed on tested diets for 4 weeks.

Groups	Relative liver weight	Relative kidney weight	Relative heart weight	Relative spleen weight
Control	3.40^{c}	0.70^{b}	0.31^{c}	0.29^{bc}
Anthocyanin	3.68^{b}	0.82^{ab}	0.38^{bc}	0.27^{c}
Carmoisine	3.84^{ab}	0.89^{a}	0.44^{a}	0.26^{c}
Betalain	3.38^{c}	0.77^{ab}	0.29^{c}	0.31^{bc}
Raspberry	3.92^{a}	0.85^{ab}	0.37^{bc}	0.29^{bc}
Carotenoids	3.54^{bc}	0.80^{ab}	0.33^{bc}	0.34^{ab}
Sunset yellow	3.90^{a}	0.85^{ab}	0.41^{ab}	0.37^{a}
Chlorophyll	3.21^{d}	0.77^{ab}	0.30^{c}	0.26^{c}
Fast green	3.47^{c}	0.78^{ab}	0.34^{bc}	0.31^{bc}

Means within the column having the same superscript are not significantly different ($p \leq 0.05$).

Table 3. Blood glucose levels (mg/dL) of rats fed on tested diets for 4 weeks.

Groups	Initial Glucose	Final Glucose
Control	92^{b}	96^{cd}
Anthocyanin	93^{b}	94^{d}
Carmoisine	98^{a}	116^{b}
Betalain	95^{ab}	101^{c}
Raspberry	95^{ab}	122^{a}
Carotenoids	89^{c}	93^{d}
Sunset yellow	97^{a}	111^{b}
Chlorophyll	90^{bc}	93^{d}
Fast green	92^{b}	99^{c}

Means within the column having the same superscript are not significantly different ($p \leq 0.05$).

Table 4. Plasma aspartate–aminotransferase (AST) and alanine-amino transferase (ALT) enzymes activities (μ/ mL) of rats fed on tested diets for 4 weeks.

Groups	AST	ALT
Control	32^{c}	38^{b}
Anthocyanin	32^{c}	40^{b}
Carmoisine	36^{b}	46^{a}
Betalain	32^{c}	40^{b}
Raspberry	40^{a}	48^{a}
Carotenoids	36^{b}	36^{b}
Sunset yellow	36^{b}	40^{b}
Chlorophyll	32^{c}	40^{b}
Fast green	36^{b}	38^{b}

Means within the column having the same superscript are not significantly different ($p \leq 0.05$).

Table 5. Plasma total proteins, albumin, and globulin levels (g/dL) of rats fed on tested diets for 4 weeks.

Groups	Total proteins	Albumin	Globulin
Control	6.6^{bc}	3.6^{c}	3.0^{b}
Anthocyanin	7.0^{b}	3.2^{d}	3.8^{a}
Carmoisine	7.8^{a}	4.1^{b}	3.7^{a}
Betalain	7.0^{b}	3.7^{c}	3.3^{b}
Raspberry	7.5^{a}	4.0^{b}	3.5^{ab}
Carotenoids	6.7^{b}	3.6^{c}	3.1^{b}
Sunset yellow	7.2^{ab}	4.8^{a}	2.4^{c}
Chlorophyll	6.4^{c}	4.0^{b}	2.4^{c}
Fast green	7.0^{b}	3.5^{c}	3.5^{ab}

Means within the column having the same superscript are not significantly different ($p \leq 0.05$).

Table 6. Plasma creatinine, urea levels (mg/dL) of rats fed on tested diets for 4 weeks:

Groups	Creatinine	Urea
Control	0.80^{c}	52.60^{e}
Anthocyanin	0.80^{c}	57.40^{cd}
Carmoisine	1.20^{ab}	63.40^{ab}
Betalain	1.00^{bc}	56.20^{cd}
Raspberry	1.40^{a}	66.20^{a}
Carotenoids	1.00^{bc}	51.60^{e}
Sunset yellow	0.80^{c}	61.20^{bc}
Chlorophyll	0.80^{c}	57.80^{cd}
Fast green	1.00^{bc}	59.80^{bcd}

Means within the column having the same superscript are not significantly different ($p \leq 0.05$).

Table 7. Plasma total lipids, total cholesterol, and triglyceride (mg/dL) of rats fed on tested diets for 4 weeks.

Groups	Total lipids	Total cholesterol	Triglyceride
Control	310^{e}	181^{cd}	120^{cd}
Anthocyanin	260^{f}	165^{f}	108^{e}
Carmoisine	400^{b}	195^{b}	136^{a}
Betalain	320^{d}	174^{e}	118^{d}
Raspberry	350^{c}	186^{c}	130^{b}
Carotenoids	320^{d}	169^{ef}	122^{c}
Sunset yellow	430^{a}	211^{a}	132^{b}
Chlorophyll	342^{c}	176de	129^{b}
Fast green	422^{a}	201^{b}	130^{b}

Means within the column having the same superscript are not significantly different ($p \leq 0.05$).

Table 8. Plasma HDL-cholesterol, LDL-cholesterol levels (mg/dL), and HDL/LDL ratios of rats fed on tested diets for 4 weeks.

Groups	HDL-Cholesterol	LDL-Cholesterol	HDL/LDL ratio
Control	50^{bc}	91.6^{ef}	0.545^{bc}
Anthocyanin	56^{a}	86.8^{f}	0.645^{a}
Carmoisine	45^{ef}	117^{b}	0.384^{de}
Betalain	52^{b}	90.4^{ef}	0.575^{ab}
Raspberry	47de	111.2^{c}	0.422^{d}
Carotenoids	52^{b}	93^{e}	0.559^{ab}
Sunset yellow	43^{f}	134.6^{a}	0.319^{e}
Chlorophyll	48^{cd}	103^{d}	0.466^{cd}
Fast green	46de	115.4^{bc}	0.398^{de}

Means within the column having the same superscript are not significantly different ($p \leq 0.05$).

Table 9. Effect of natural and synthetic colorants on immune-system in plasma of male albino rats for 4 weeks.

Groups	Ig A	IgG	Ig M
Control	302^{ab}	2270^{b}	188^{b}
Anthocyanin	311^{a}	2480^{a}	182^{c}
Carmoisine	214^{f}	1648^{h}	113^{f}
Betalain	308^{a}	2200^{c}	178^{c}
Raspberry	268^{d}	1840^{g}	136^{e}
Carotenoids	300^{ab}	2220^{c}	208^{a}
Sunset yellow	256^{e}	1920^{f}	138^{e}
Chlorophyll	284^{c}	2160^{d}	206^{a}
Fast green	264^{d}	1960^{e}	150^{d}

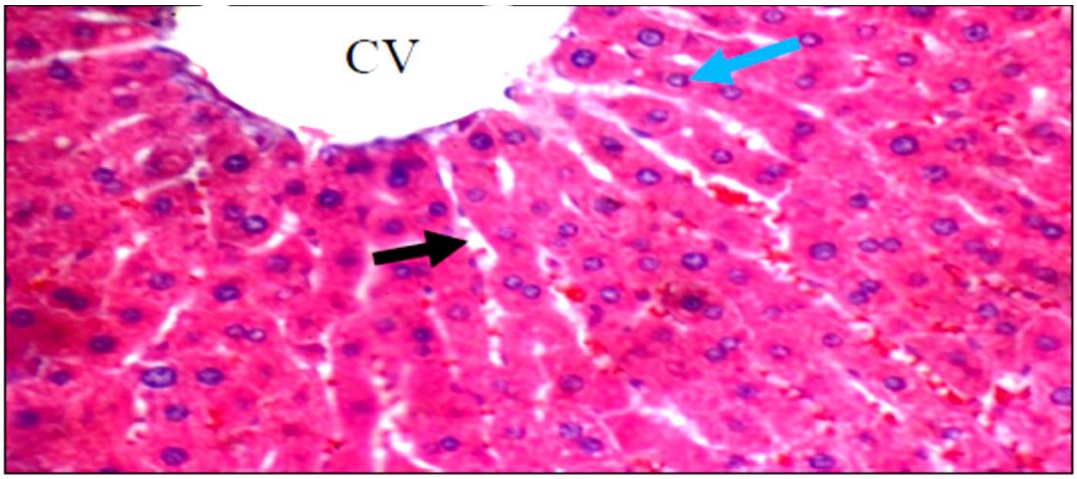

Figure 1. Liver of control group showing average central vein (CV) and average hepatocytes arranged in single-cell cords (black arrow) with average intervening blood sinusoids (blue arrow) (H&E X 400).
Means within the column having the same superscript are not significantly different ($p \leq 0.05$).

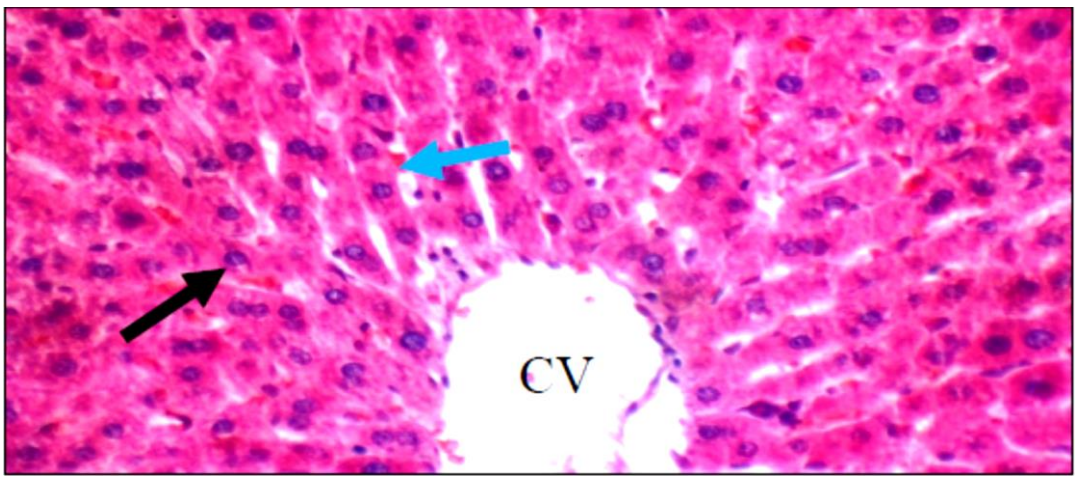

Figure 2. Liver of rats group treated by anthocyanin showing mildly dilated central vein (CV), average intervening blood sinusoids (black arrow), and average hepatocytes in the peri-venular area (blue arrow) (H&E X 400).

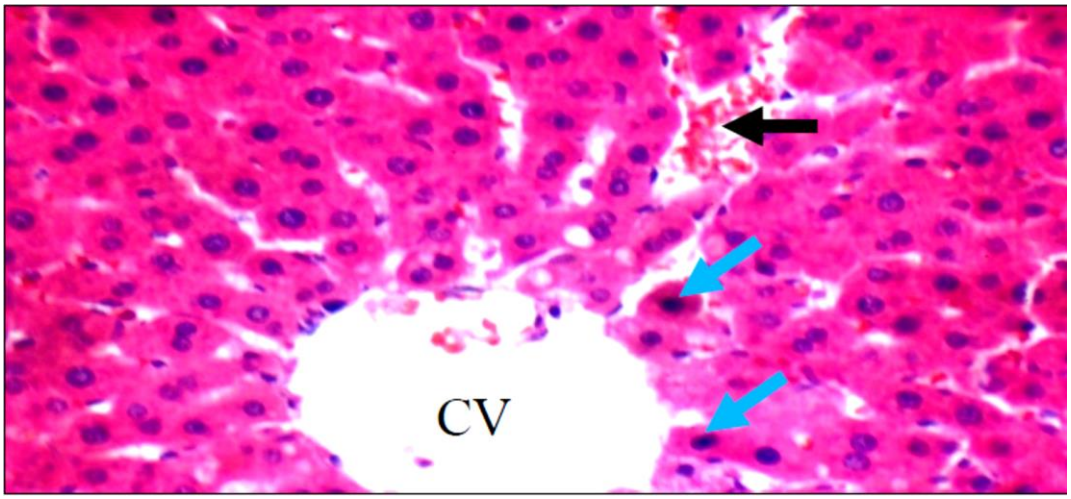

Figure 3. Liver of rats group treated by carmoisine showing mildly dilated central vein (CV) with mildly dilated blood sinusoids (black arrow), and average hepatocytes in the peri-venular area (blue arrow) (H&E X 400).

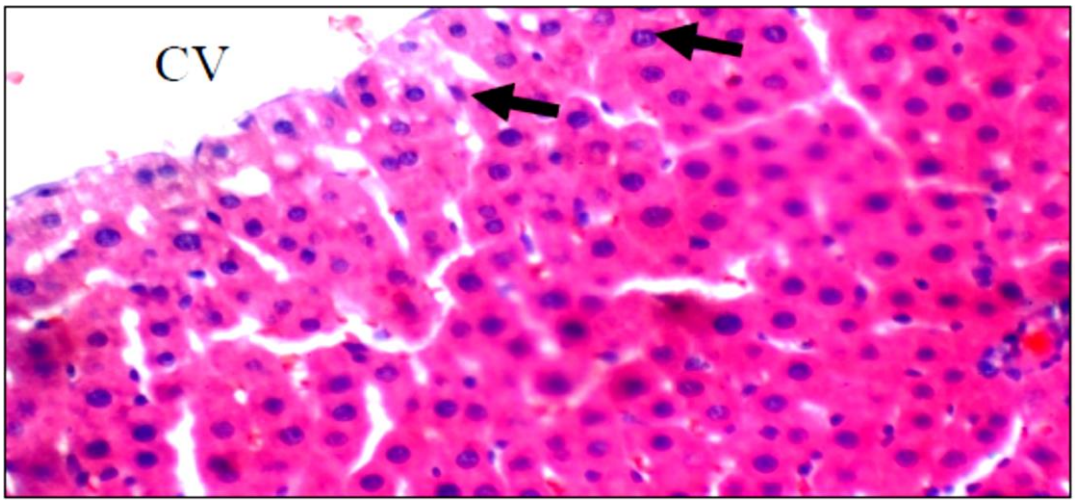

Figure 4. Liver of rats group treated by betalain showing mildly dilated central vein (CV), average hepatocytes in the peri-venular area (black arrow) (H&E X 400).

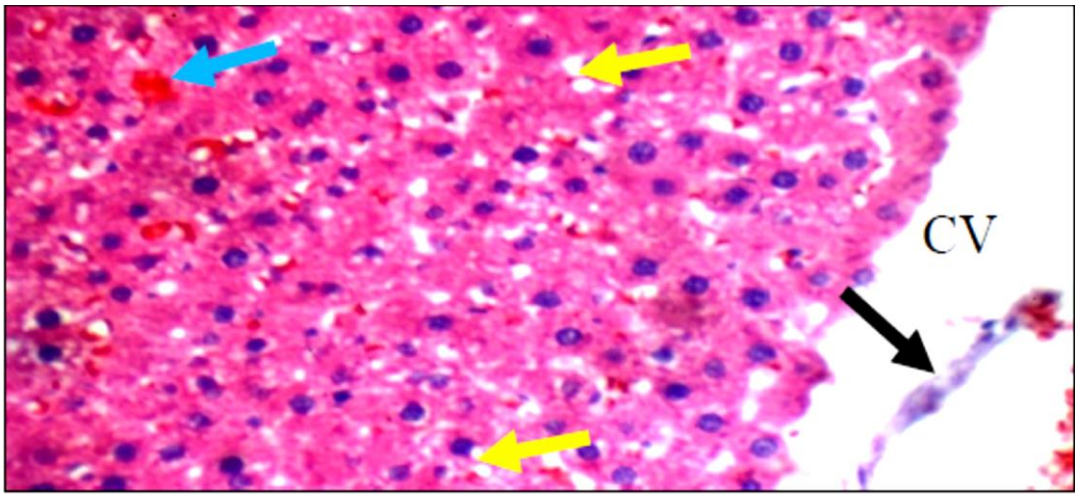

Figure 5. Liver of rats group treated by raspberry showing mildly dilated central vein (CV) with detached lining (black arrow), mildly congested blood sinusoids (blue arrow), and moderate micro-vesicular steatosis of hepatocytes in the peri-venular area (yellow arrow) (H&E X 400).

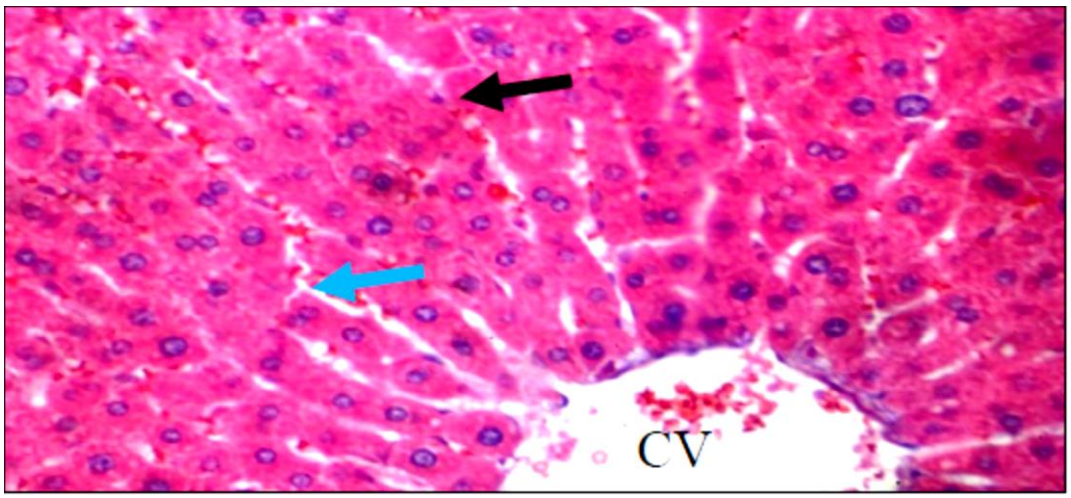

Figure 6. Liver of rats group treated by carotenoids showing mildly dilated congested central vein (CV), mildly congested blood sinusoids (blue arrow), and average hepatocytes in the peri-venular area (black arrow) (H&E X 400).

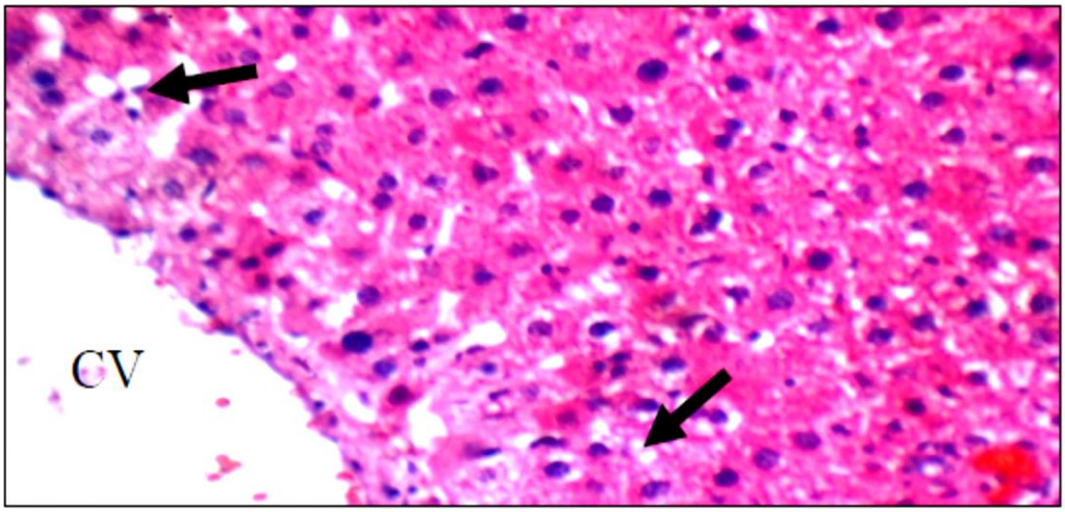

Figure 7. Liver of rats group treated by sunset yellow showing mildly dilated central vein (CV) and mild micro-vesicular steatosis of hepatocytes in the peri-venular area (black arrow) (H&E X 400).

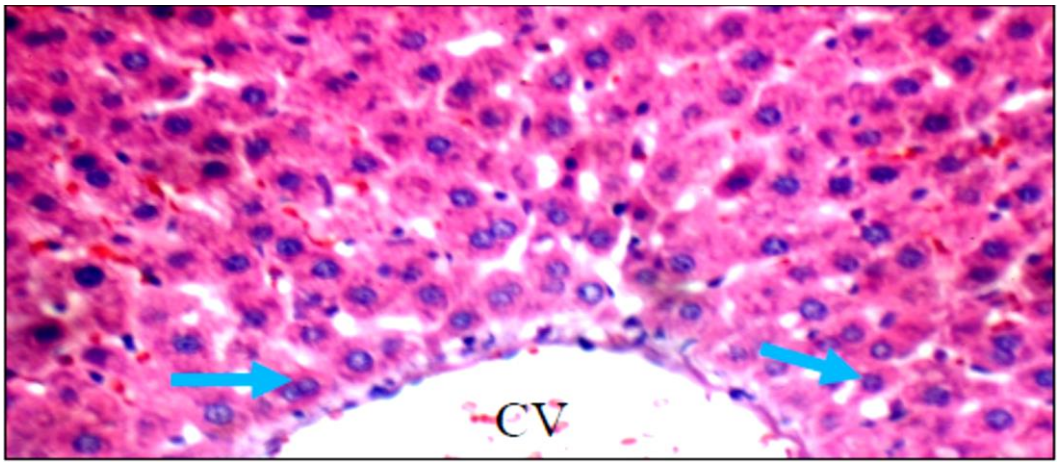

Figure 8. Liver of rats group treated by chlorophyll showing mildly dilated central vein (CV) with average hepatocytes in the peri-venular area (blue arrow) (H&E X 400).

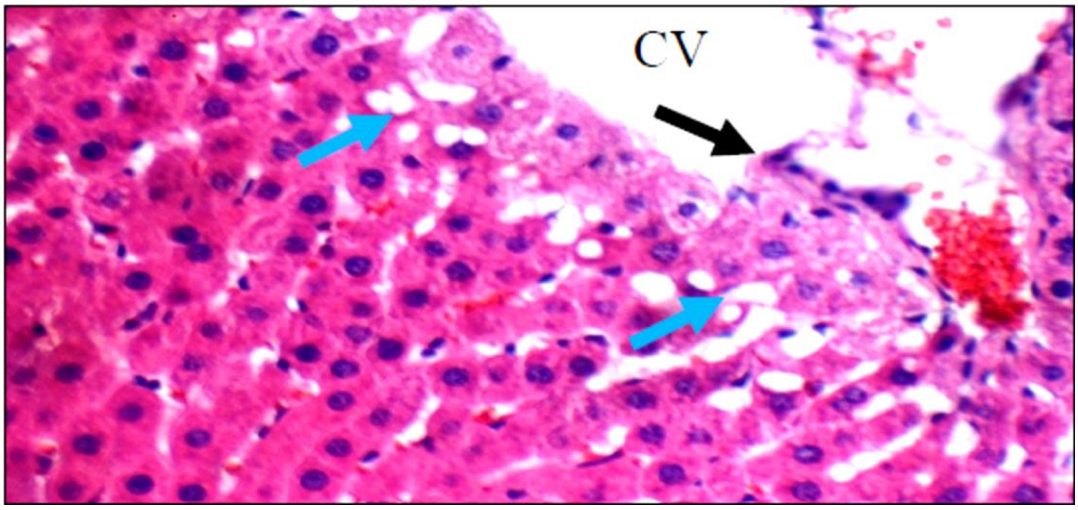

Figure 9. Liver of rats group treated by fast green showing markedly dilated congested central vein (CV) with detached lining (black arrow), and mild micro-vesicular steatosis of hepatocytes in the peri-venular area (blue arrow) (H&E X 400).

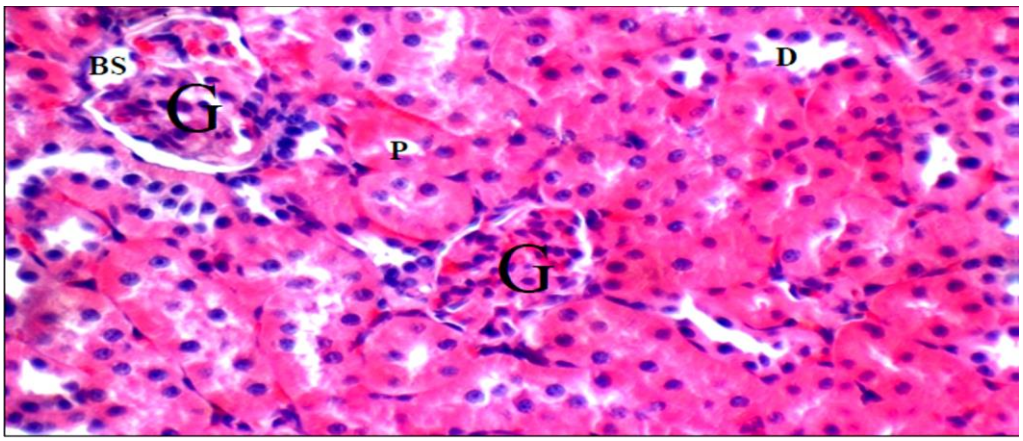

Figure 10. High power view of control group kidney showing average glomeruli (G) with average Bowman's space (BS), average proximal tubules (P) with preserved brush borders (black arrow), and average distal tubules (D) (H&E X 400).

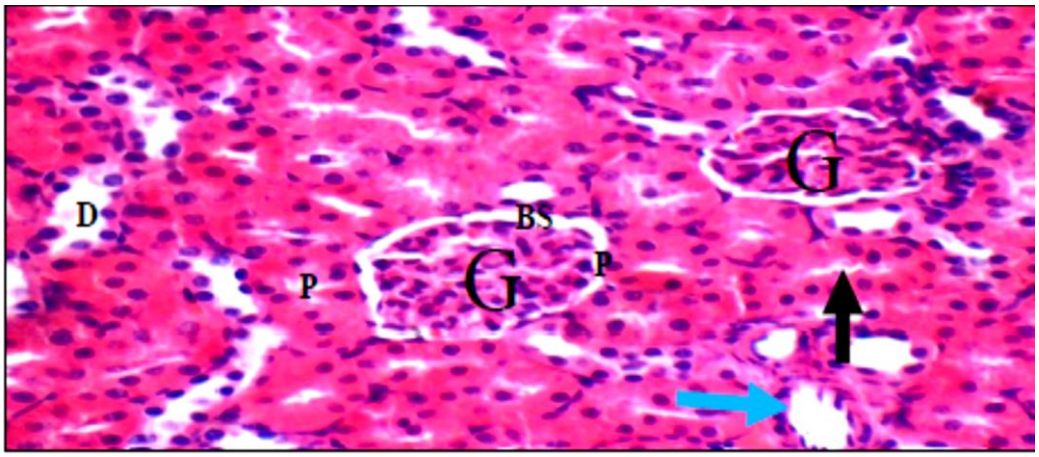

Figure 11. High power view of rats' kidney treated by anthocyanin showing average glomeruli (G) with average Bowman's spaces (BS), average proximal tubules (P) with preserved brush borders (black arrow), and average interstitial blood vessels (blue arrow) (H&E X 400).

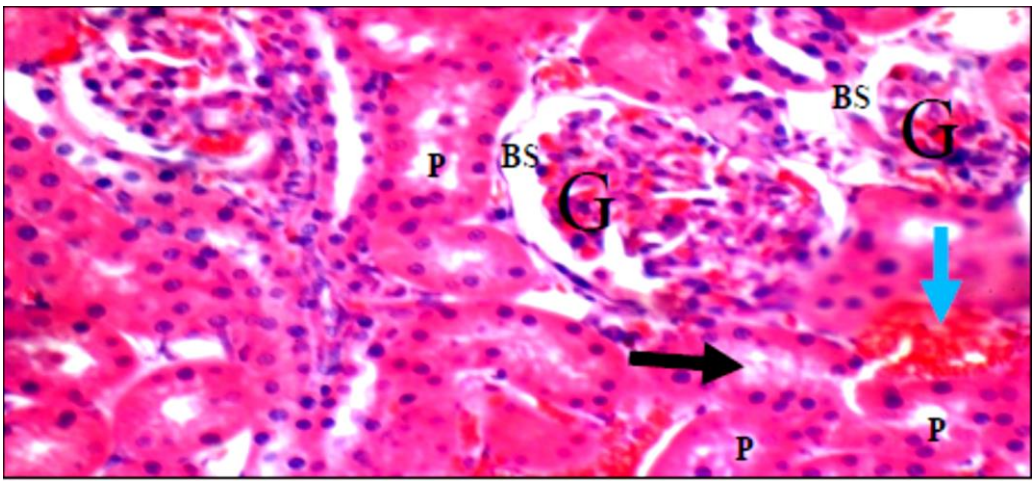

Figure 12. High power view of rats' kidney treated by carmoisine showing mildly congested glomeruli (G) with average Bowman's spaces (BS), proximal tubules (P) with partial loss of brush borders (black arrow) and areas of interstitial hemorrhage (blue arrow) (H&E X 400).

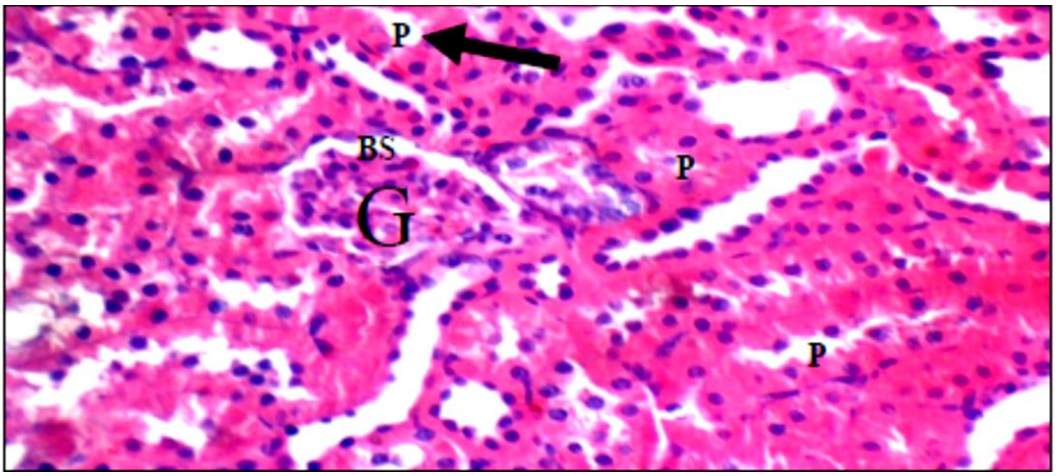

Figure 13. High power view of rats' kidney treated by betalain showing average glomerulus (G) with average Bowman's space (BS), and average proximal tubules (P) with preserved brush borders (black arrow) (H&E X 400).

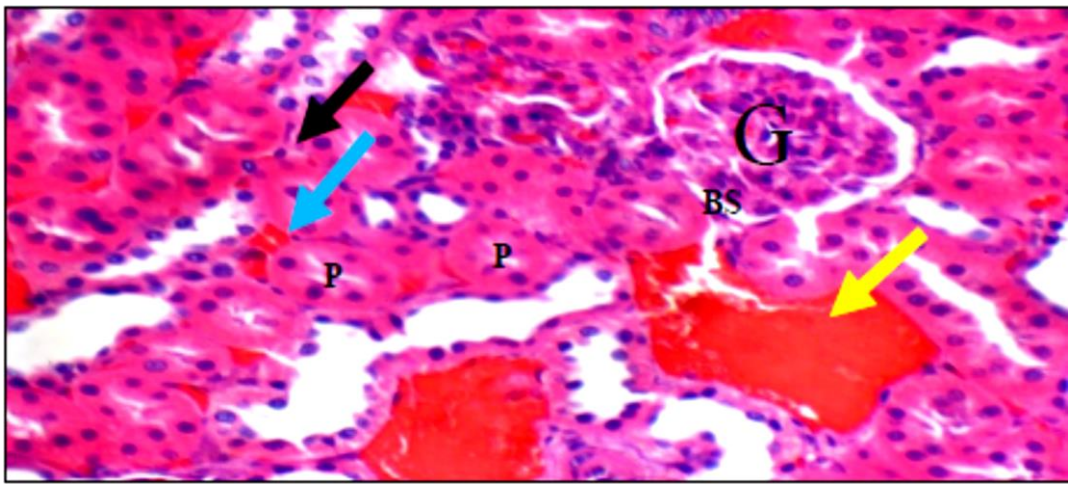

Figure 14. High power view of rats' kidney treated by raspberry showing mildly congested glomerulus (G) with average Bowman's space (BS), proximal tubules (P) with preserved brush borders (black arrow), and mildly congested interstitial blood vessels (blue arrow) with marked areas of hemorrhage (yellow arrow) (H&E X 400).

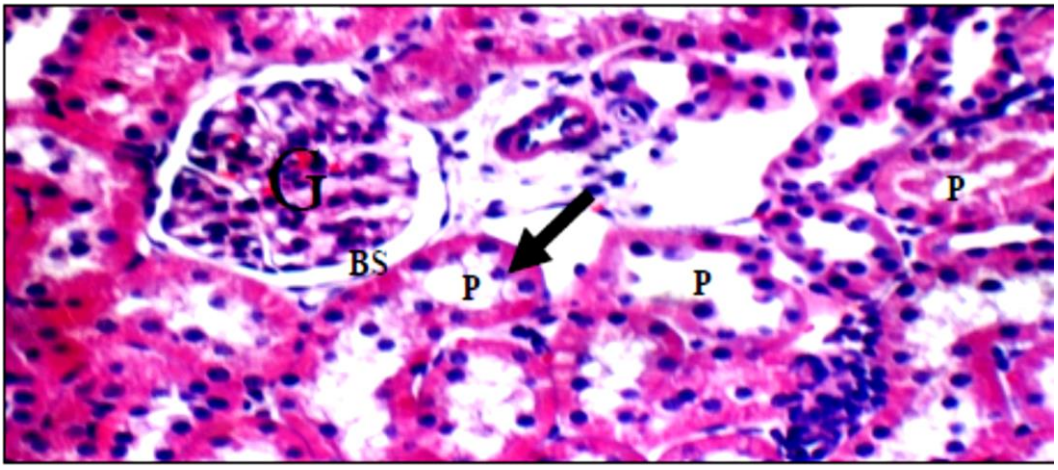

Figure 15. High power view of rats' kidney treated by carotenoids showing average glomerulus (G) with average Bowman's space (BS), proximal tubules (P) with mildly edematous epithelial lining (black arrow) and partial loss of brush borders (blue arrow) (H&E X 400).

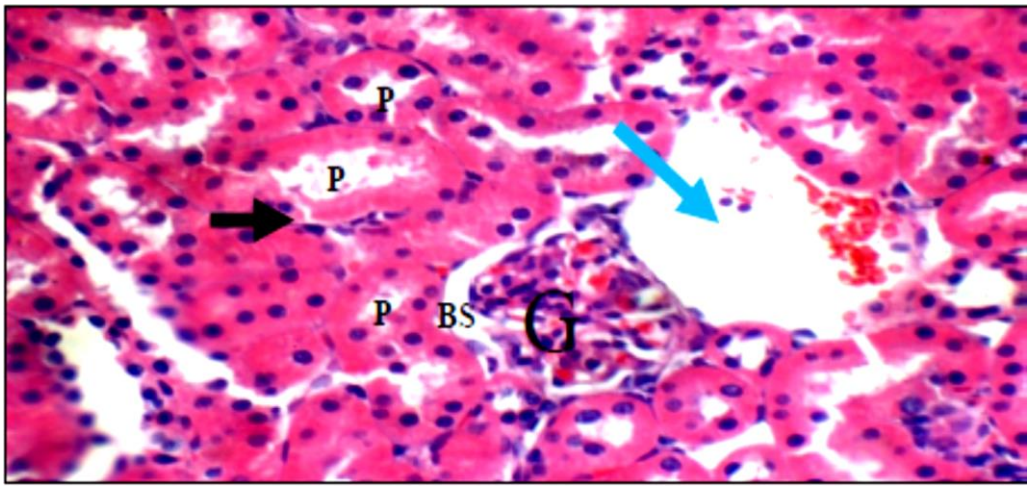

Figure 16. High power view of rats' kidney treated by sunset yellow showing mildly congested glomerulus (G) with average Bowman's space (BS), proximal tubules (P) with preserved brush borders (black arrow), and mildly dilated congested interstitial blood vessels (blue arrow) (H&E X 400).

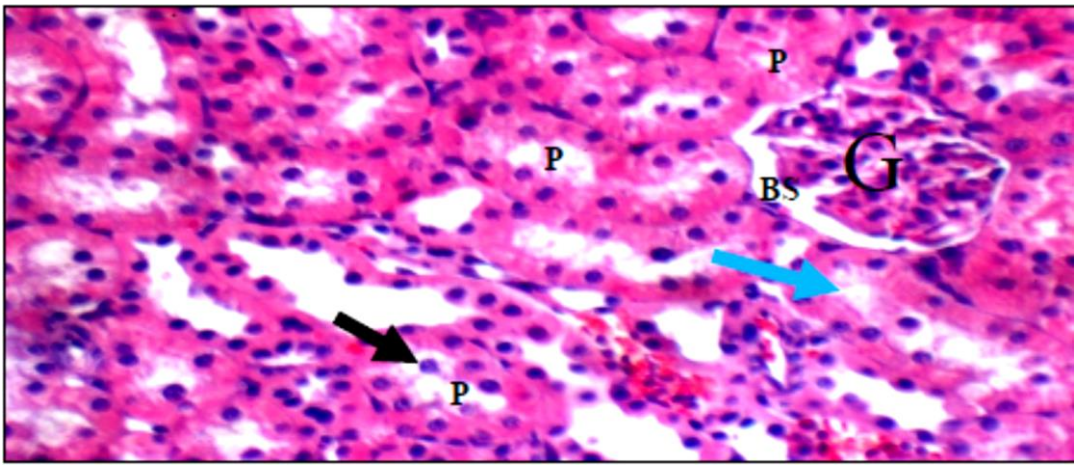

Figure 17. High power view of rats' kidney treated by chlorophyll showing average glomerulus (G) with average Bowman's space (BS), proximal tubules (P) with mildly edematous epithelial lining (black arrow) and partial loss of brush borders (black arrow) (H&E X 400).

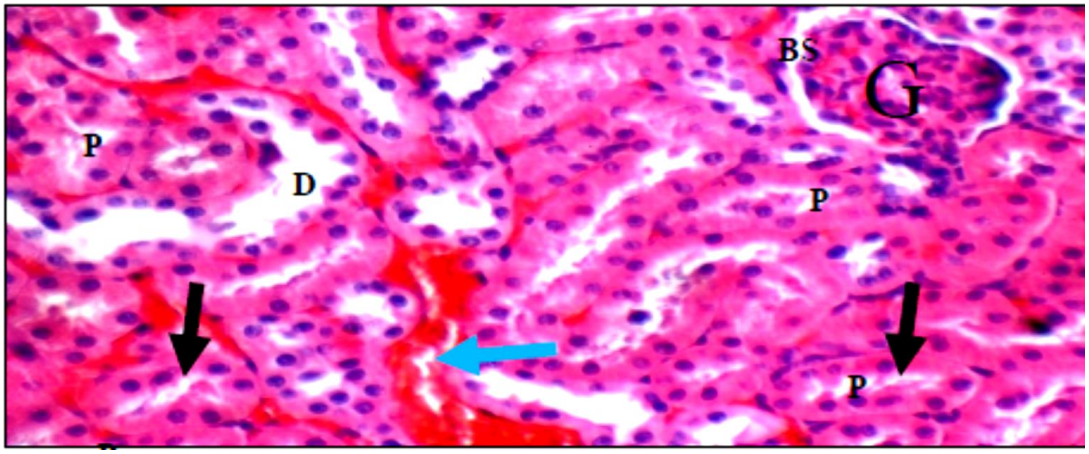

Figure 18. High power view of rats' kidney treated by fast green showing average glomerulus (G) with average Bowman's space (BS), average proximal tubules (P) with preserved brush borders (black arrow) and markedly dilated congested interstitial blood vessels (blue arrow) (H&E X 400).

التقييم البيولوجى لبعض الملونات الغذائية الصناعية والطبيعية

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الملخص العربي

أجريت هذه الدراسة لتقييم ومقارنة التأثير الضار المحتمل لبعض الملونات الغذائية الصناعية (الكارموزين، الرازبرى، أصفرغروب الشمس والأخضر السريع) ومقارنتها ببعض الملونات الغذائية الطبيعية المستخلصة من مصادر نباتية (الأنثوسيانين والبيتالين والكاروتينويدات والكلوروفيل) على العوامل البيوكيميائية لفئران التجارب وكذلك الفحوصات النسيجية للكبد و الكلى لتلك الفئران. وقد أوضحت النتائج وجود زيادة معنوية في مستويات ألانين أمينو ترانسفيريز ، الأسبارتات أمينو ترانسفيريز، اليوريا، الكرياتينين، البروتين الكلي والألبيومين، مع انخفاض مستويات الجلوبيولين المناعي في مجموعات الفئران التى تم تغذيتها على الملونات الصناعية المختبرة بالمقارنة مع مجموعات الفئران التى تم تغذيتها على ملونات الغذاء الطبيعية ومجموعة التحكم (الكنترول) . كما أظهرت الفحوصات النسيجية عن حدوث تغيرات في الكلى تشمل: احتقان ونزيف مع ارتشاح وتشوه في بنية الكبيبات الكلوية. بينما كانت التغيرات التى حدثت في الكبد: الاحتقان والنزيف وتورم أشباه الجيوب الكبدية والوريد المركزي مع حدوث تراكم لجزيئات دهنية دقيقة الحجم في مجموعات الفئران التى تم تغذيتها على الملونات الصناعية المختبرة بالمقارنة مع مجموعات الفئران التى تم تغذيتها على الملونات الطبيعية ومجموعة التحكم (الكنترول). لذلك، يُنصح بالحد من استخدام تلك الملونات الصناعية سابقة الذكر أو استبدالها بالملونات الغذائية الطبيعية خاصة فى أغذية الأطفال.

الكلمات الإسترشادية: الملونات الغذائية الطبيعية؛ الملونات الغذائية الصناعية؛ المعايير البيوكيميائية؛ الفحص النسيجى للكبد؛ الفحص النسيجى للكلية.