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Research Paper

Effect of Blend of Metanil Yellow and Tartrazine on Different Organs of Albino Rat

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ABSTRACT

Background: Tartrazine and metanil yellow are organic azo dyes widely used in food products, drugs, and cosmetics. The present study was conducted to evaluate the toxic effect of these food colors on the liver, and kidneys of albino rats. The study also evaluates the protective effect of quercetin as an antioxidant against the toxic effect of these food colors.

Methods: Eighty adult albino rats were divided into 8 groups. Control group, 3 groups were treated with a blend of tartrazine and metanil yellow in 3 doses of 25, 50, and 75 mg/kg for 30 days by gavage, one positive control group was treated with quercetin 50 mg/kg for 30 days, and three groups were treated with the blend of tartrazine and metanil yellow plus 50 mg/kg of quercetin. At the end of the experiment, serum samples were collected to evaluate liver and kidney functions. Liver, kidney samples were fixed in 10% formalin and routinely prepared for paraffin sectioning and staining for histopathological examination.

Results: The study showed a significant elevation of liver and kidney function after treatment with the food color blend. Also, a significant improvement in liver and kidney function was observed after treatment with quercetin. Histopathological examination showed mild to moderate changes in the liver and kidney which improved after quercetin treatment.

Conclusion: The current sub-chronic study concluded that a blend of tartrazine and metanil yellow caused significant biochemical and histological changes in different organs of albino rats. Therefore, prolonged consumption of these substances leads to adverse effects on human health. Also, quercetin is vital in protecting the body against the toxic effects of food color blends.

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Introduction

For years, color additives have been used to cover the low quality of spoilt food goods and give them a better look. Many synthetic and natural food colors are used to improve the appearance of foods; however, synthetic food colors are used more often because they are more readily available, cheaper, and provide a better color.

The most common synthetic food color is tartrazine. It is the most widely used food color in candies, jellies, juices, jams, mustard, and sodas to give yellow hues. It is also widely applied for human color medications, including vitamins, capsules, and antacids [1]. Metanil yellow is commonly used as a food color in candies, meat, ice creams, and drinks in addition to many industries as a wool, paper, and leather dye in third-world nations. It is a highly water-soluble synthetic food color used as a common adulterant in food due to its easy availability and low cost [2]. Quercetin (Que) is a polyphenolic flavonoid found in many natural sources, such as tomatoes, tea, apples, broccoli, coffee, and onions. It has potential health benefits due to its anti-oxidant properties which help quercetin's cardioprotective, anti-inflammatory, and DNA-protective effects [3]. This study aims to detect the toxic effect of a blend of metanil yellow on the liver, kidney, testis, and ovary of albino rats. Also, to evaluate the protective effect of quercetin against toxicity induced by a blend of tartrazine and metanil yellow on the liver, and kidney of albino rats.

Materials and Methods

Chemicals: Tartrazine ($C_{16}H_9N_4Na_3O_9S_2$), (CAS 1934-21-0), metanil yellow ($C_{18}H_{14}N_3O_3SNa$), (CAS 587-98-4), and quercetin dihydrate ($C_{15}H_{10}O_7$), (CAS 117-39-5) were all bought from Alpha Aeser company (Germany). All of the compounds utilized were of analytical grade. Tartrazine and metanil yellow were mixed in equal ratios then dissolved in distilled water and then given to experimental animals by gavage. Also, quercetin was freshly dissolved in distilled water before treatment.

Experimental animals

In the experiment, 80 adult male and female albino rats weighing 150–200 g were used. They were purchased from the National Research Centre's breeding unit (Giza, Egypt). All rats were kept in steel mesh cages with a 12-hour light-dark cycle and a controlled temperature (21˚C–24˚C) and 50%–60% relative humidity. The animals were fed a balanced meal and had a daily water supply. The Ethics Committee for animal research authorized the complete experimental protocol, including the use of animals, and it was carried out in compliance with institutional and national rules for animal care and use.

Experimental design

At the beginning of the experiment, the rats were weighed and randomly divided into eight groups of 10 animals each, 5 of each sex. The albino rats were given a daily oral dose of different treatments by gavage needle for 30 consecutive days as follows:

Group I (sham group): Rats in this group orally administered distilled water (solvent of the tested substances) and used as a normal control.

Groups II-IV (negative control): The animals of this group were given 25, 50, and 75 mg/kg of the blend of tartrazine and metanil yellow once daily for 30 days by gavage.

The doses were chosen according to Saxena and Sharma. [4]

Group V (positive control): The animals of this group were given 50 mg/kg of quercetin once daily for 30 days by gavage [5, 6].

Group VI-VIII (treatment group): The animals of this group were given 25, 50, and 75 mg/kg of the blend+50 mg/kg of quercetin once daily for 30 days by gavage.

Body weight

The experimental rats, including the control group, were carefully monitored before starting the administration of the blend and also at the end of the experiment. The Equation 1 was used to calculate the percentage of body weight gain.

1. Body weight gain percentage=Mean final weight-Ean initial weight/Mean initial body weight×100 [7]

Sampling

At the end of the experimental period, animals were anesthetized using diethyl ether, and blood samples were collected from the orbital sinus of rats [8].

Biochemical analysis

The blood samples were centrifuged at 3000 rpm for 20 minutes to obtain serum. Supernatant sera were separated and frozen at -80°C for biochemical analysis:

Reitman and Frankel determined serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity [9]. It is a colorimetric method. Procedure: Mix the reagents. Wait 5 minutes. Measure the absorbance at 505 nm (490–520 nm) against d. Water using a 1 cm cuvette light path. The color is stable for one hour. Linearity for glutamic oxaloacetic transaminase (GOT) up to 150 units/mL and for glutamic pyruvic transaminase (GPT) up to 120 units/mL.

Total bilirubin was determined according to Walter and Gerade [10]. It is a colorimetric method. Procedures: Mix the reagents. Incubate at room temperature for 10 minutes away from light. Read the absorbances of the sample (a sample) against its blank at a wavelength of 535 nm. (530–540 nm). Using a cuvette 1 cm light path. Linearity up to 25 mg/dL (425 µmol/L). Color stability for one hour.

Albumin was determined according to Doumas et al [11]. It is a colorimetric method. Mix well, then measure after 5 minutes the absorbance of the sample and the standard (against reagent blank at 630 nm (620–640 nm). If albumin concentration exceeds 6 g/dL dilute the sample and repeat the assay. Multiply by the dilution factor.

Urea was determined according to Fawcett and Scott [12]. It is the urease-Berthelot method. Procedure: Mix the reagents, and incubate for 10 minutes at 37°C. Measure the absorbance of the sample and the standard against the blank at 550 nm, (530-570 nm). Color stability for 5 hours. Linearity up to 200 mg/dL (33.3 mmol/L) in serum or plasma and 4 g/dL (665 mmol/L) in urine.

Creatinine was determined according to Schirmeister, et al [13]. It is a colorimetric method. Procedures: Mix equal volumes of reagents before the assay. Incubate for 5 minutes at 37°C. Measure the absorbance of the sample and standard against the blank at 520 nm. (500–550 nm). Linearity up to 10 mg/dL in serum or plasma and 300 mg/dL in diluted urine.

Histological examination

At the end of the experiment, the animals were slaughtered by decapitation, and a laparotomy was performed to remove the tissues (liver, kidneys) that had been kept in a formalin solution. Sections of about 5 µm thick were prepared and stained using hematoxylin and eosin (H and E).

Statistical analysis

SPSS software, version 16 (Inc, Chicago, IL, USA) was used; categorical variables were presented as frequency and percentage. Quantitative variables were presented by Mean±SD. Qualitative variables were compared using the chi-square test. Quantitative variables were compared using the analysis of variance (ANOVA) test. $P \leq 0.05$ was considered significant. Post hoc test was performed as the next step after analysis of variance (ANOVA) for inter-group comparison to test the difference between both groups.

Results

The study was conducted on Swiss albino rats. By feeding on the blend of food colors at different doses, the following results were found:

Body weight

Table 1 presents a significant reduction of weight gain with $P<0.001$ in groups treated with 25, 50 and 75 mg/kg of the blend compared to the control group. In group II, the animal gained more body weight i.e. 16.19% compared to animals of groups III (13.66%) and IV (11.59%).

Hepatic biomarkers

In the current study, a statistically significant increase is observed in ALT, AST, and bilirubin levels and a significant decrease of albumin in a dose-dependent manner in groups II, III, IV treated with 25, 50, and 75 mg/kg/BW of the blend compared to the control group as illustrated in Tables 2, 3 and 4 which indicates a negative effect of the blend on the liver. Our study also showed a statistically significant decrease of ALT and AST, and bilirubin levels with ($P<0.001$) in groups VI, VII, and VIII compared to II, III, and IV after quercetin treatment as shown in Tables 5 and 6.

Renal biomarkers

The current study illustrates a statistically significant increase in urea and creatinine levels in a dose-dependent manner in groups II, III, and IV treated with 25, 50 and 75 mg/kg BW of the blend compared to the control group as illustrated in Table 2 and 7. This indicates a negative effect of the blend in the kidneys. Also, a statistically significant de-

Table 1. Weight changes comparison among different study groups

Groups	Mean±SD		
	Before Experiment	After Experiment	Gain in Body Weights (%)
G I	171±14.8	202.3±18.8	18.29±5.13
G II	176±18.3	204.5±22.3	16.19±4.47
G III	165±17.1	186.6±17.6	13.66±2.58
G IV	170.5±11.8	190.2±12.1	11.59±2.52
G V	175.5 ±18.1	208.8±20.02	19.04±2.6
G VI	173.5±11.5	203.6±19.3	17.18±4.6
G VII	171±17.2	195.5±19.5	14.3±1.88
G VIII	167.1±8.5	188.2±7.8	12.67±3.08
P of ANOVA test	0.721	0.039*	<0.001**

*Significant, **Highly significant; Value represents the Mean±SD.

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Group I (sham group) and groups II-IV (negative control): Rats treated with 25, 50, and 75 mg of a blend of tartrazine and metanil yellow/kg body weight; Groups V (positive control): Rats treated with 50 mg/kg quercetin; Groups VI-VIII (treatment group): Treated with 25, 50, and 75 mg metanil yellow/kg body weight plus 50 mg/kg quercetin daily by gavage for 30 days.

Table 2. Biochemical parameters of liver and kidney function tests of rats of different groups

Groups	ALT	AST	Bilirubin	Albumin	Urea	Creatinine
G I	25.01±2.4	29.8±2.1	0.72±0.17	4.38±0.61	28.9±3.1	0.72±0.14
G II	74.7±3.4	77.9±7.7	1.28±0.21	3.07±0.19	75.5±3.3	0.89±0.11
G III	96.09±2.6	99.5±4.2	2.05±0.23	2.44±0.14	83.1±3.1	1.2±0.11
G IV	109.6±9.7	118.6±12.2	3.04±0.32	1.75±0.19	93.6±3.7	1.6±0.11
G V	22.7±2.5	25.3±2.7	0.52±0.11	4.40±0.57	25.2±1.8	0.61±0.08
G VI	53.3±7.41	56.9±7.9	0.82±0.12	3.66±0.29	63.3±6.2	0.66±0.08
G VII	77.7±3.4	81.8±2.5	1.71±0.23	2.99±0.22	75.04±3.9	1±0.07
G VIII	92.9±13.3	99.5±10.9	2.57±0.31	2.38±0.34	81.2±4.4	1.33±0.17
P	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**

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ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

The value represents the Mean±SD; *P<0.05: Significantly different from control; **P<0.001: Highly significantly different from control. Group I (sham group) and groups II-IV (negative control): Rats treated with 25, 50, and 75 mg of a blend of tartrazine and metanil yellow/kg body weight; Groups V (positive control): Rats treated with 50 mg/kg quercetin; Groups VI-VIII (treatment group): Treated with 25, 50, and 75 mg metanil yellow/kg body weight plus 50 mg/kg quercetin daily by gavage for 30 days.

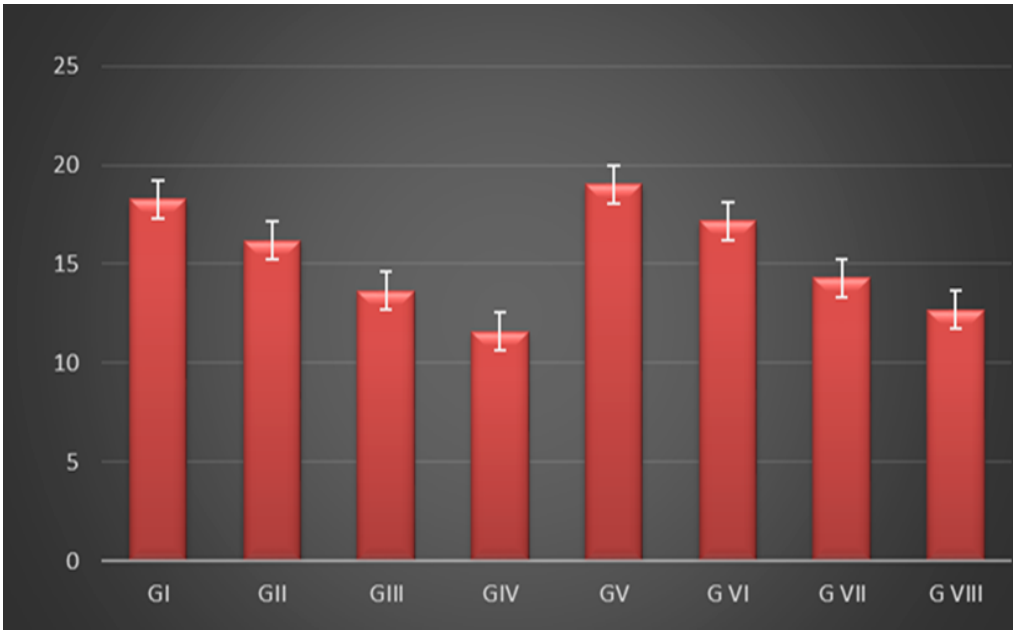

Figure 1. Percentage of body weight gain between different study groups

Table 3. Mean ALT and AST levels in different study groups

Groups	Mean±SD	
	ALT (U/mL)	AST (U/mL)
G I	25.01±2.4	29.8±2.1
G II	74.7±3.4	77.9±7.7
G III	96.09±2.6	99.5±4.2
G IV	109.6±9.7	118.6±12.2
P of ANOVA	<0.001**	<0.001**
P Between Groups#		
G I and G II	<0.001**	<0.001**
G I and III	<0.001**	<0.001**
G I and IV	<0.001**	<0.001**
G II and G III	<0.001**	<0.001**
G II and G IV	<0.001**	<0.001**
G III and G IV	<0.001**	<0.001**

**Highly significant, #Post Hoc test (Tukey HSD).

Abbreviations: ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ANOVA: Analysis of variance; HSD: Honestly significant difference. Group I (sham group) and groups II-IV (negative control): Rats treated with 25, 50, and 75 mg of a blend of tartrazine and metanil yellow/kg body weight; Groups V (positive control): Rats treated with 50 mg/kg quercetin; Groups VI-VIII (treatment group): Treated with 25, 50, and 75 mg metanil yellow/kg body weight plus 50 mg/kg quercetin daily by gavage for 30 days.

Table 5. Mean ALT and AST levels before and after treatment with quercetin

Group	Mean±SD	
	ALT	AST
GII	74.7±3.4	77.9±7.7
G VI	53.3±7.4	56.9±7.9
P	<0.001**	<0.001**
III	96.09±2.6	99.5±4.2
VII	77.7±3.4	81.8±2.5
P	<0.001**	<0.001**
IV	109.6±7.79	118.6±12.2
VIII	92.9±13.3	99.5±10.9
P	<0.001**	<0.001**

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**Highly significant. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

Group I (sham group) and groups II-IV (negative control): Rats treated with 25, 50, and 75 mg of a blend of tartrazine and metanil yellow/kg body weight; Groups V (positive control): Rats treated with 50 mg/kg quercetin; Groups VI-VIII (treatment group) treated with 25, 50, and 75 mg metanil yellow/kg body weight plus 50 mg/kg quercetin daily by gavage for 30 days.

Table 4. Mean bilirubin and albumin levels in different study groups

Group	Mean±SD	
	Bilirubin (mg/dL)	Albumin (gm/dL)
G I	0.72±0.17	4.38±0.61
G II	1.28±0.21	3.07±0.19
G III	2.05±0.23	2.44±0.14
G IV	3.04±0.32	1.75±0.19
P of ANNOVA	<0.001**	<0.001**
P Between Groups#		
G I and G II	<0.001**	<0.001**
G I and III	<0.001**	<0.001**
G I and IV	<0.001**	<0.001**
G II and G III	<0.001**	0.005*
G II and G IV	<0.001**	<0.001**
G III and G IV	<0.001**	<0.001**

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Abbreviations: ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; HSD: Honestly significant difference.

*Significant, **Highly significant, #Post Hoc test (Tukey HSD).

Group I (sham group)and groups II-IV (negative control): Rats treated with 25, 50, and 75 mg of a blend of tartrazine and metanil yellow/kg body weight; Groups V (positive control): Rats treated with 50 mg/kg quercetin; Groups VI-VIII (treatment group) treated with 25, 50, and 75 mg metanil yellow/kg body weight plus 50 mg/kg quercetin daily by gavage for 30 days.

Table 6. Mean bilirubin, albumin levels before and after treatment with quercetin

Group	Mean±SD	
	Bilirubin	Albumin
II	1.28±0.21	3.07±0.19
VI	0.82±0.12	3.66±0.29
P	<0.001**	<0.001**
III	2.05±0.23	2.44±0.14
VII	1.71±0.23	2.99±0.22
P	<0.001**	0.025*
IV	3.04±0.32	1.75±0.19
VIII	2.75±0.31	2.38±0.34
P	<0.001**	0.006*

*Significant, **Highly significant.

Group I (sham group) and groups II-IV (negative control): Rats treated with 25, 50, and 75 mg of a blend of tartrazine and metanil yellow/kg body weight; Groups V (positive control): Rats treated with 50 mg/kg quercetin; Groups VI-VIII (treatment group) treated with 25, 50, and 75 mg metanil yellow/kg body weight plus 50 mg/kg quercetin daily by gavage for 30 days.

Table 7. Mean creatinine and urea levels in different study groups

Group	Mean±SD	
	Creatinine (mg/dL)	Urea (mg/dL)
G I	0.72±0.14	28.9±3.1
G II	0.89±0.11	75.5±3.3
G III	1.2± 0.11	83.1±3.1
G IV	1.6±0.11	93.6±3.7
P of ANOVA	<0.001**	<0.001**
P Between Group#		
G I and G II	0.028*	<0.001**
G I and III	<0.001**	<0.001**
G I and IV	<0.001**	<0.001**
G II and G III	<0.001**	0.001*
G II and G IV	<0.001**	<0.001**
G III and G IV	<0.001**	<0.001**

*Significant, **Highly significant, #Post Hoc test (Tukey HSD).

Abbreviations: HSD: Honestly significant difference; ANOVA: Analysis of variance.

Group I (sham group) and groups II-IV (negative control): Rats treated with 25, 50, and 75 mg of a blend of tartrazine and metanil yellow/kg body weight; Groups V (positive control): Rats treated with 50 mg/kg quercetin; Groups VI-VIII (treatment group): Treated with 25, 50, and 75 mg metanil yellow/kg body weight plus 50 mg/kg quercetin daily by gavage for 30 days.

Table 8. Mean blood creatinine, urea levels before and after treatment with quercetin

Group	Mean±SD	
	Creatinine	Urea
II	0.89±0.11	75.5±3.3
VI	0.66±0.088	63.3±6.2
P	0.001*	<0.001**
III	1.24±0.11	83.1±3.1
VII	1±0.07	75±3.9
P	0.001*	<0.001**
IV	1.63±0.11	93.6±3.7
VIII	1.33±0.17	81.2±4.4
P	0.001*	<0.001**

*Significant, **Highly significant.

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Group I (sham group) and groups II-IV (negative control): Rats treated with 25, 50, and 75 mg of a blend of tartrazine and metanil yellow/kg body weight; Groups V (positive control): Rats treated with 50 mg/kg quercetin; Groups VI-VIII (treatment group): Treated with 25, 50, and 75 mg metanil yellow/kg body weight plus 50 mg/kg quercetin daily by gavage for 30 days.

crease in urea and creatinine levels (P<0.05) was observed in groups VI, VII, and VIII compared to II, III, and IV after quercetin treatment as shown in Table 8.

Histopathological examination

Liver

The liver tissue of G I (control group) showed normal hepatic tissues with hepatic strands of cells around the central vein. Liver tissue in G II showed congestion of the central vein (CV) and hydropic degeneration of hepatocytes (5 rats). G III showed congestion of CV and hydropic degeneration of hepatocytes (5 rats), and necrosis (5 rats). G IV showed congestion of CV and sinusoids, and hydropic degeneration of hepatocytes (4 rats). Focal necrosis, peritonitis, subcapsular congestion, formation of new bile ductules, and interface hepatitis with mononuclear cellular infiltration (6 rats). Treatment with quercetin showed improvement in hepatic histology.

Kidneys

Histological examination of kidney tissues in G I showed normal renal tissues with normal glomeruli and tubules. Renal tissue in G II showed congestion of glomerular tuft (5 rats), G III showed congestion of glomerular Tuft (9 rats), and mononuclear cellular infiltrate (1 rat). G IV showed congestion of glomerular tuft (9 rats), mononuclear cellular infiltration, degenerated glomeruli, degeneration in proximal convoluted tubules, and tubular casts (3 rats). Treatment with quercetin showed improvement in renal histology.

Discussion

Food dyes are chemical or natural coloring products used to give colors to food. Body weight monitoring is considered a good marker of animal or human growth. Some researchers use body weight loss as a reliable sensitive indicator for toxicity [7]. The study revealed a statistically significant decrease in body weight gain in rats receiving the food color blend. The study referred to this finding to decrease caloric intake due to the Tartrazine and metanil yellow component of the diet but was not related to the treatment used. The current study is consistent with Al seeni et al.'s study where a decrease was observed in body weight and body weight gain due to tartrazine treatment in a dose of 10 mg/kg BW for 8 weeks [14]. Similarly, our study is consistent with Al malki, et al.'s study, who showed significant retardation in the body weight gain of metanil-yellow-treated groups which received 50 mg/kg BW for 8 weeks of metanil yellow [15].

The study is inconsistent with Sharma G's study, which showed a significant increase in body weight gain in rats treated with low and high doses (1

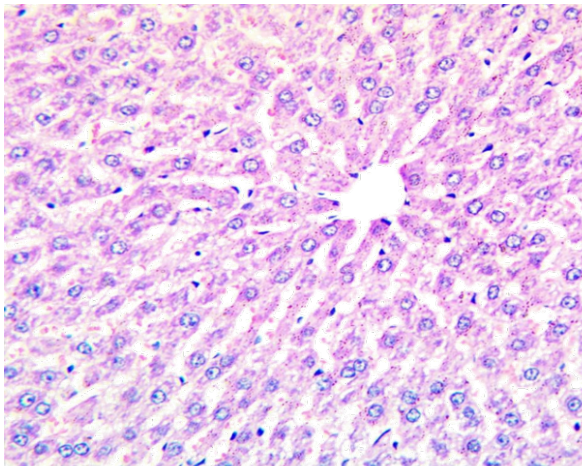

Figure 2. Liver of rat from group I showing the normal histological structure of hepatic paranchyma (H & R X400)

gm, 2 gm/kg) of kerasi powder (blend of tartrazine and sunset yellow) for 35 days [16]. This occurs when the sunset yellow raised the body weights and caused obesity in the experimental animals. Also, our study is inconsistent with Himri et al's study, which reported no effect on body weight gain after administration of 5, 7.5 mg/kg BW of tartrazine for 13 weeks [17].

The current study showed normal levels of ALT, AST, albumin, and bilirubin in the control group as well as in the positive control group, while a significant increase is observed in ALT, AST, and bilirubin and a significant decrease of albumin are observed in a dose-dependent manner (P<0.001) in groups II, III, and IV receiving

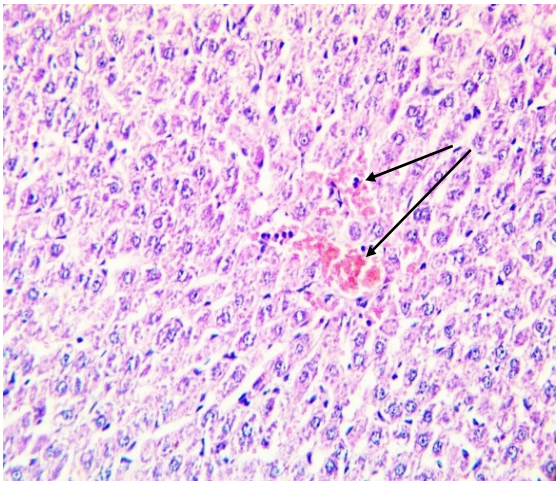

Figure 3. Liver of rat from group II showing congestion of the central vein (arrow) and slight hydropic degeneration of hepatocytes (H & E X400)

25, 50, and 75 mg/kg BW of the blend compared to the control group. These results were attributed to the liver damage caused by the toxic effect of these synthetic dyes. This study is consistent with the previous study of El Rabey et al., which had a similar effect on liver enzymes, bilirubin, and albumin due to treatment with 10 mg/kg tartrazine for 8 weeks [18]. The present study is also consistent c with Tawfik et al's study which reported a highly significant increase in levels of ALT, AST, and bilirubin, and albumin after treatment with 20 mg/kg tartrazine for 60 days [19]. Our study is inconsistent with Himri et al.'s study, which revealed that rats

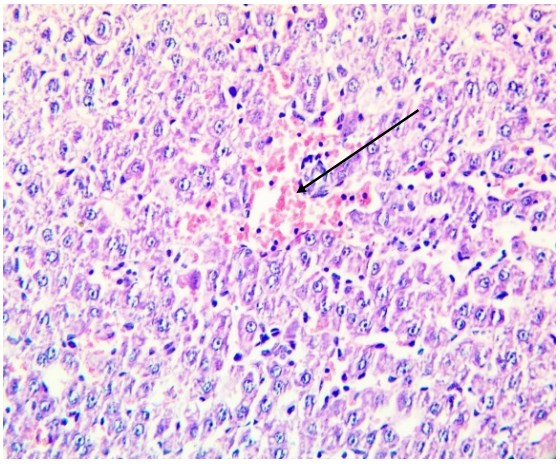

Figure 4. Liver of rat from group III showing congestion of the central vein (arrow) and slight hydropic degeneration of hepatocycts (H & E X400)

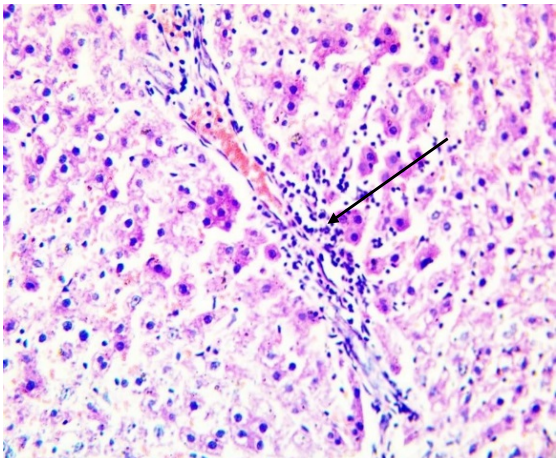

Figure 5. Liver of rat from group iv showing interface of hepatitis with monouclear cellular infiltration (arrow) (H & E X400)

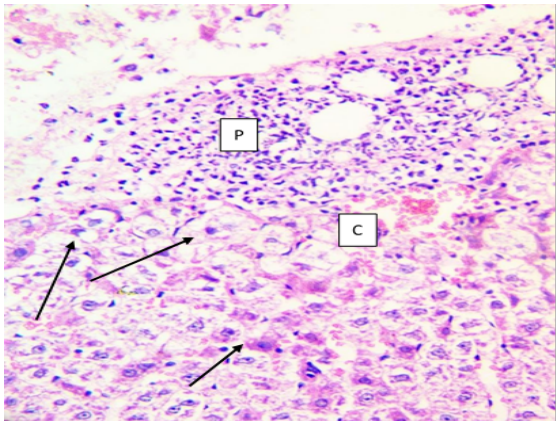

Figure 6. Liver of rat from group iv showing peritonitis (P), subcapsular congestion (C), hydropic degeneration of hepatocytes and focal necrosis (arrows) (H & E X400)

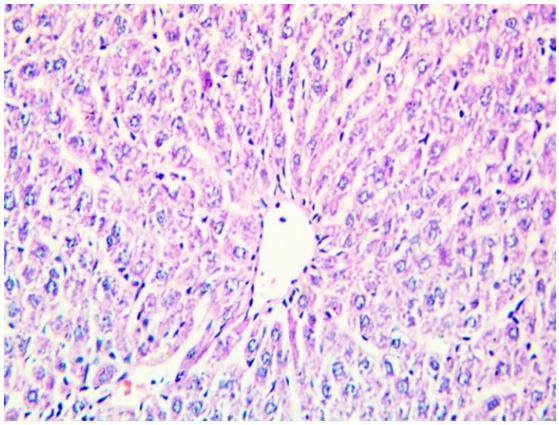

Figure 7. Liver of rat from group v showing the normal histological structure of hepatic parechyma (H & E X400)

consuming a high dose of Tartrazine (10 mg/kg BW) showed a significant increase in serum AST compared to control rats while the same dose showed no significant increase in serum III Aminotransferases elevation in the blood is an indication of tissue damage and degenerative changes caused by tartrazine and metanil yellow [20]. Hepatic cells contain high levels of liver enzymes in the cytoplasm, ALT and AST particularly exist in the mitochondria. Due to the injury caused to hepatic cells, the leakage of cytosol increases the levels of these hepatospecific enzymes in the serum. The elevated serum enzyme levels, such as AST and ALT is markers of cellular leakage, impaired functional integrity, and increase the permeability of cell membrane in the liver. So measurement of serum AST and ALT levels is vital for the indirect assessment of the liver status [15].

Our study shows a significant decrease in ALT, AST, and bilirubin and a significant increase in albumin levels toward normal values after treatment with 50 mg/kg of quercetin for 30 days. This is consistent with the results of El-Nekeety et al.'s study, which reported that treatment with quercetin at two tested doses 50, and 100 mg/kg BW overcome the biochemical and histological changes in a dose-dependent manner in rats fed aflatoxin-contaminated diet [5]. This is also inconsistent with Owumi et al.'s study, which stated that treatment with 40 mg/kg quercetin in dichloromethane-treated rats caused significantly decreased near-normal values of the serum ALT, AST [21].

The present study showed normal levels of urea and creatinine in control and positive control groups while a significant elevation of urea, and creatinine in a dose-

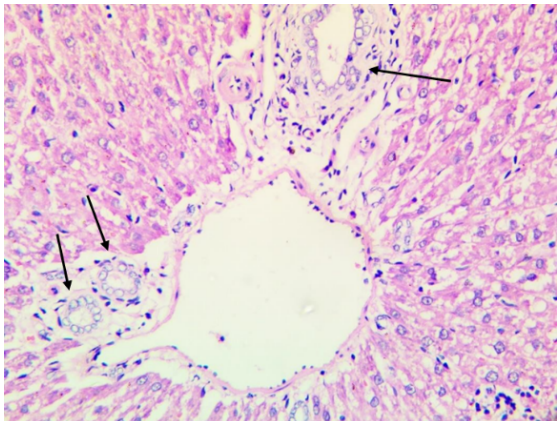

Figure 8. Liver of rat from group iv showing hydropic degeneration of hepatocytes and formation of new bileductulus (arrows) (H & E X400)

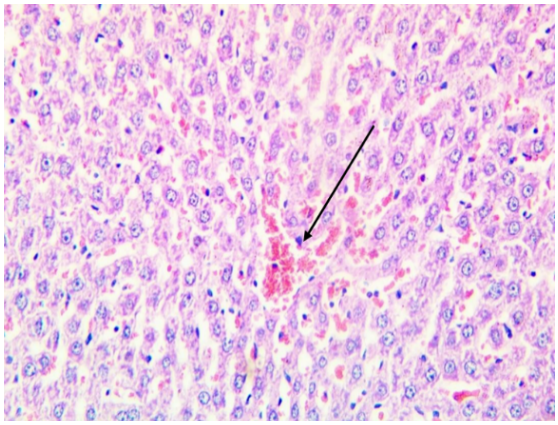

Figure 9. Liver of rat from group vi showing congestion of the central vein (arrow) and slight hydropic degeneration of hepatocytes (H & E X400)

dependent manner is observed in groups, II, III, and IV receiving 25, 50, and 75 mg/kg BW of the blend. These changes were attributed to the reduction of glomerular filtration and renal injury caused by the toxic effect of these dyes.

Our study is consistent with Tawfek et al.'s study, which showed a significant elevation in serum creatinine and urea in rats after treatment with 20 mg/kg tartrazine for 60 days [19]. Also, it is consistent with the results of Khayyat et al.'s study, which reported a significant increase in blood urea, creatinine and uric acids in rats received 7.5 mg/kg tartrazine for 30 days [22].

Our results are inconsistent with the results of Himri et al., and Abo EL-Sooud et al.'s study, where a significant increase was observed in serum creatinine but serum urea concentration showed no significant increase after treatment with 3, 7.5 mg/kg tartrazine. These results referred to a low dose of the used tartrazine [17-23].

Increased urea and creatinine levels indicate impairment in the glomerular filtration rate of the kidney as well as diminished re-absorption at the renal epithelium [21]. These changes indicate renal dysfunction. It was previously found that the concentrations of urea and creatinine increase due to degenerative changes in kidneys caused by toxic substances. The higher levels of urea and creatinine in exposed rats may also be due to oxidative damage to the kidneys [24].

The present study showed a significant decrease in serum urea and creatinine toward normal levels after treatment with 50 mg/kg quercetin for 30 days. Our results are consistent with Alidadi et al.'s study, which found that administration of 75 mg/kg quercetin protected against kidney damage induced by titanium dioxide. Quercetin decreases the levels of the enzymes, which indicates that this flavonoid has a protective effect on the proximal cells [25].

Quercetin effectively decreases hepatorenal injury biomarkers, possibly via the preservation of membrane integrity and enhancing endogenous antioxidant capacity [21].

Quercetin protects organs from damage by stabilizing in the redox state and maintaining the antioxidant capacity offered. It can be also attributed to calcium channel-blocking activity exerted by quercetin. Calcium contents in liver cells increases during the process of experimental liver damage, and calcium channel-blocking drugs inhibit the development of hepatic damage induced by different hepatotoxins [26]. In the present study, histologic changes of the liver due to the administration of 25, 50, and 75 mg/kg food color blend for 30 days cause mild and moderate changes in the form of congestion of CV and sinusoid, hydropic degeneration, necrosis of hepatocytes, peritonitis, interface hepatitis with mononuclear cellular infiltration and formation of new bile ductules. Our results are consistent with the results of Saxena and Sharma's study, which reported that the administration of tartrazine and metanil yellow for 30 days causes degeneration, necrosis, and vacuolation of hepatocytes [4]. Our study correlates with Sharma et al.'s study, the current study also is consistent with the study of Sharma et al., (2019), in which the liver sections of the rats treated with 100 mg/kg BW metanil yellow for 28 days showed disarrangement of normal hepatic cells with centri-lobular necrosis, vascular and cellular degeneration, and inflammation [20]. Histopathological changes in the liver can be explained by reactive oxygen species released during the metabolism of food colors that play an essential role in pathological changes in the liver [27]. In the current study, histologic changes of the kidney show congestion of the glomerular tuft, degenerated glomeruli, degeneration of the proximal convoluted tubules mononuclear cellular infiltrate, and urinary casts after treatment with 25, 50, and 75 mg/kg of the blend for 30 days. These changes are confirmed by increasing the level of urea and creatinine in the blood. Our results are consistent with Sharma et al.'s study, which found that kidney slices of my yellow-treated rats showed hydropic degeneration of the glomerular and tubular cells compared to normal rat kidneys. The histopathological changes in the kidney and liver are also attributed to the hazardous effect of metanil yellow which causes lipid peroxidation in the liver through free radical generation. In addition, metanil yellow also causes depletion of glutathione (GSH), superoxide dismutase (SOD), and catalase coupled with elevation in MDA level in liver and kidney tissues which in turn causes organ damage [20].

The present study showed improvement in hepatic and renal histology after treatment with 50 mg/kg quercetin. Our study agrees with El-Nekeety et al.'s study, which found that treatment with Aflatoxin resulted in vacuolation and mononuclear cellular infiltration in between the hepatocytes and a decrease in protein reaction in hepatocytes with abnormal vacuolated nucleus and loss of the normal chromatin pattern [5]. Treatment with quercetin at the two tested doses 50, and 100 mg/kg BW overcome these histological changes in a dose-dependent manner. Also, the current study is consistent with Sifan et al.'s study, which reported that histopathological changes in kidney were more pronounced after the administration

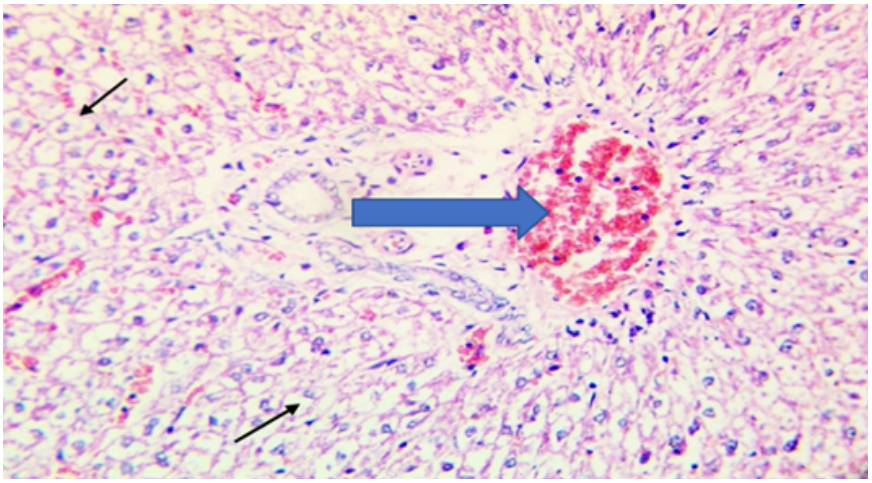

Figure 10. Liver of rat from group vii showing congestion (blue arrow) and hydropic degeneration of hepatocytes (black arrows) (H and E X 400)

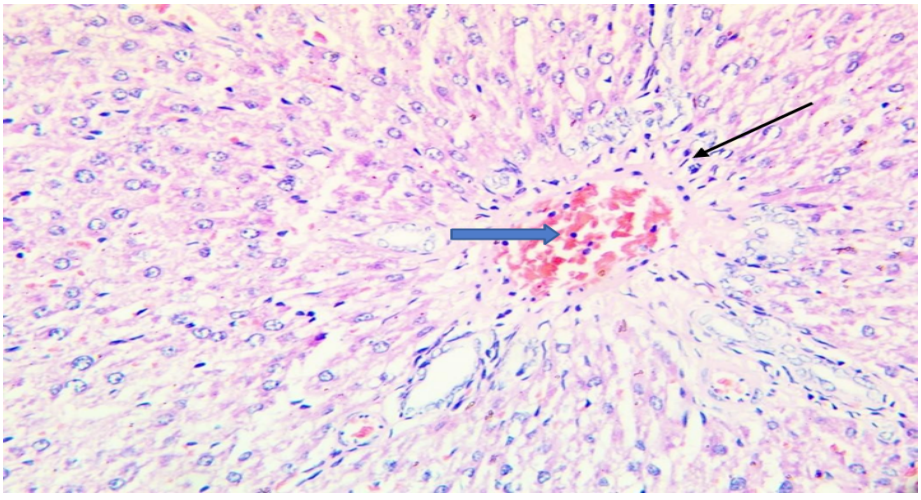

Figure 11. Liver of rat from group (viii) showing congestion (blue arrow) with mononuclear cellular infiltration (black arrow) (H and E X400)

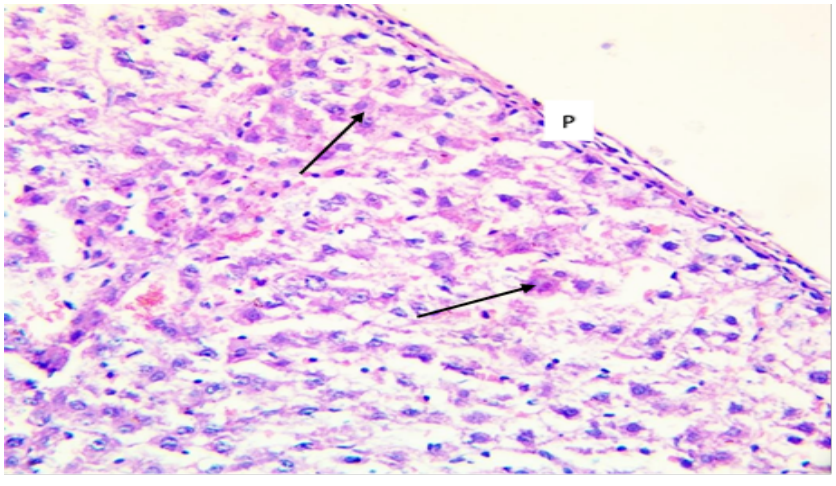

Figure 12. Liver of rat from group viii showing peritonitis (P), hydropic degeneration of hepatocytes and focal necrosis (arrows) (H and E X400)

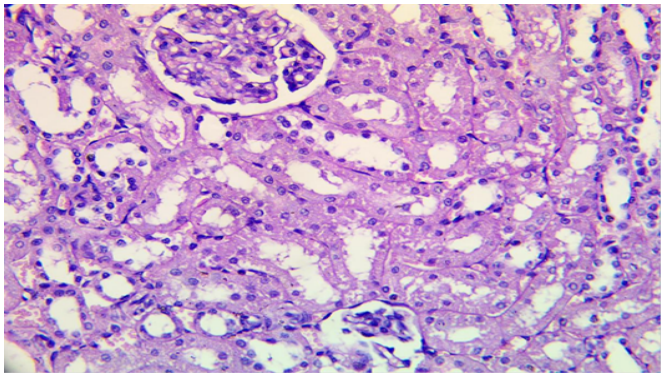

Figure 13. Kidney of rat from group i showing no histopathological changes (H and E X400)

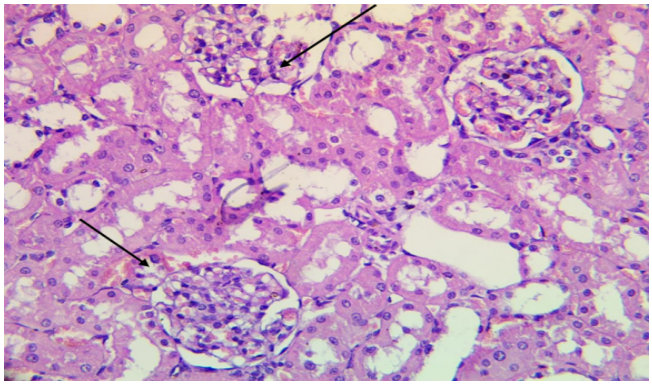

Figure 14. Kidney of rat from group ii showing slight congestion of glomerular tuft (arrows) (H and E X400)

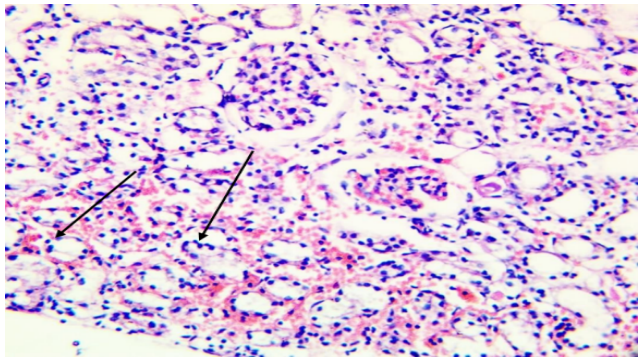

Figure 15. Kidney of rat from group iii showing congestion and mononuclear cellular infiltrate (arrows) (H and E X400)

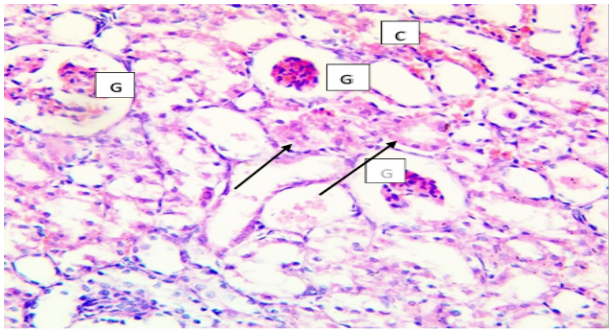

Figure 16. Kidney of rat from group iv showing congestion (C) and degenerated glomeruli (G), and degeneration of the proximal convoluted tubules (arrows) (H and E X400)

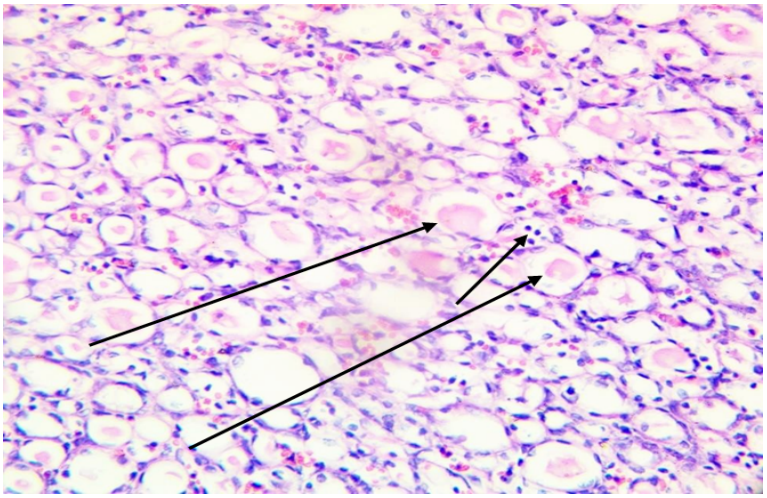

Figure 17. Kidney of rat from group iv showing mononuclear cellular infiltrate (short arrow) and urinary casts (long arrows) (H and E X 400)

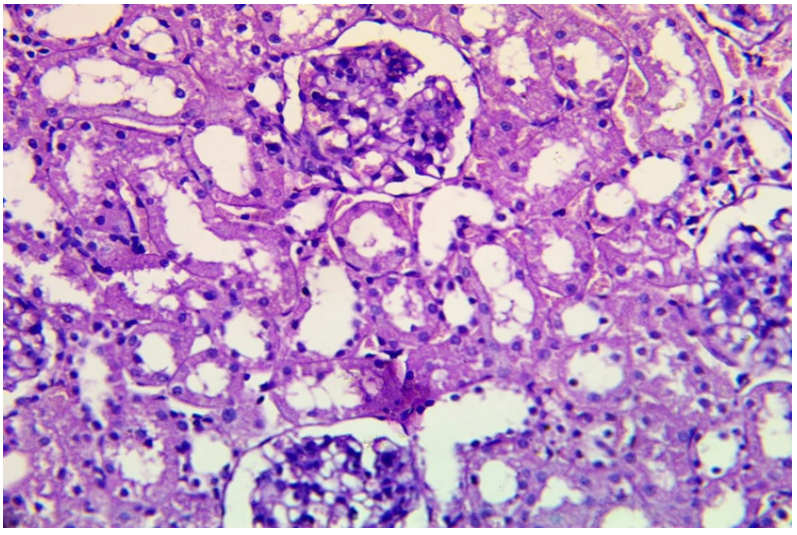

Figure 18. Kidney of rat from group v showing no histopathological changes (H and E X 400)

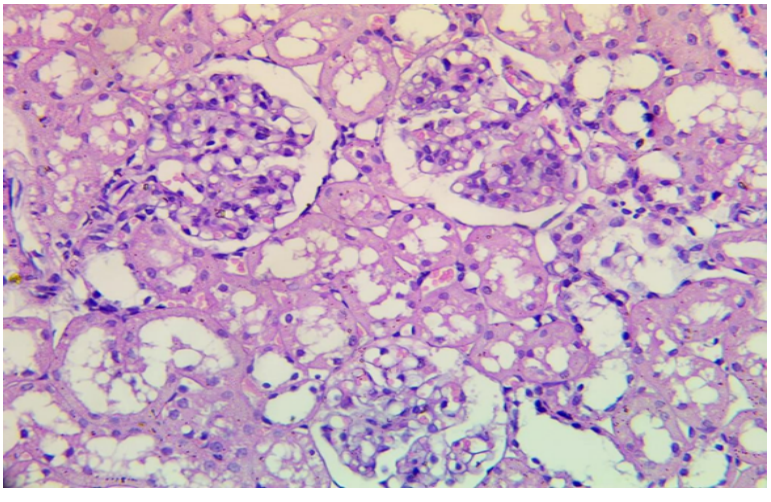

Figure 19. Kidney of rat from group vi showing no histopathological changes (H and E X 400)

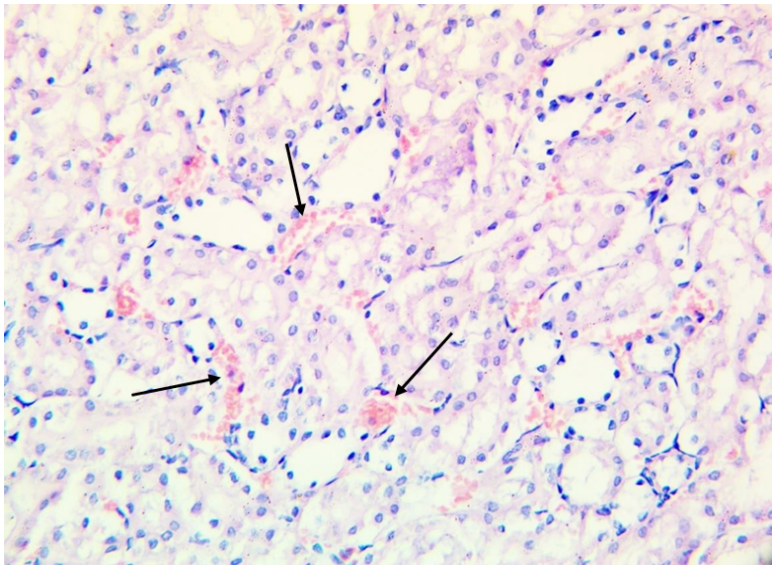

Figure 20. Kidney of rat from group vii showing congestion of the renal tubules (arrows) (H and E X 400)

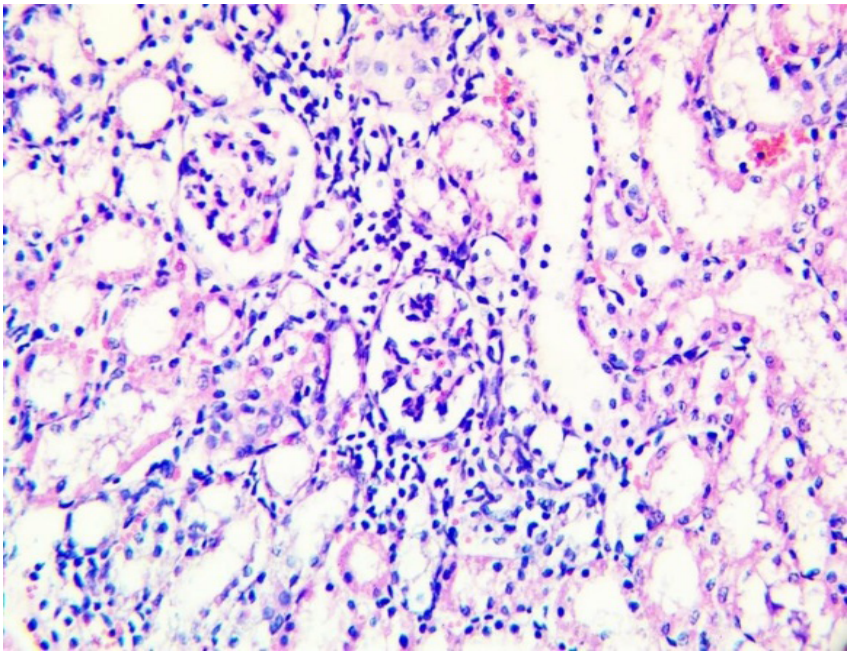

Figure 21. Kidney of rat from group viii showing congestion (C) and mononuclear cellular infiltrate (arrows) (H and E X 400)

of organophosphate pesticides. The main characteristic findings were the appearance of renal tubular epithelial cell swelling, vacuolar degeneration, and granular degeneration. Quercetin was given in 2 doses 10, 50 mg/kg BW. Histopathological changes became less pronounced in the kidneys after quercetin administration, especially at high doses than in the kidneys of the pesticide-treated group [28].

Conclusion

The current sub-chronic study concluded that a blend of tartrazine and metanil yellow caused significant biochemical and histological changes in different organs of albino rats. Therefore, prolonged consumption of these substances leads to adverse effects on human health. Also, quercetin is vital in protecting the body against the toxic effects of food color blends.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of the Faculty of Medicine, Fayoum University (Code: (D 188) on 13/1/2019.

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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