JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

STUDY THE EFFECT OF TARTRAZINE AND ITS BIODEGRADATION PRODUCTS ON THE LIVER AND KIDNEY OF FEMALE ALBINO RATS

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https://doi.org/10.55251/jmbfs.9505

ARTICLE INFO

Received 22. 9. 2022
Revised 28. 2. 2023
Accepted 9. 3. 2023
Published 1. 6. 2023

Regular article

OPEN ACCESS

ABSTRACT

Food additive azo dyes are toxic substances added to food to enhance taste or appearance. Synthetic dyes are widely used in the color of some products such as jelly, sweets, or medicines.
Tartrazine (E102) is one of the synthetic dyes used in food coloring. World health organization (WHO) recommended the acceptable daily intake of this dye is (7.5 mg/kg) body weight. In the human body, the microorganism can degrade these substances to intermediate compounds that can be more toxic in case of increasing these substances in the human body. *Pseudomonas aeruginosa* is one of the human intestinal microflorae which is able to degrade tartrazine. Decolorization assays by spectroscopy and chromatography analysis confirmed that *P. aeruginosa* can degrade tartrazine to toxic products such as Sodium 2- amino benzenesulfonate (195.15) m/z. This study discussed the effect of tartrazine and its degradation products on liver and kidney tissues. Twenty-eight female albino rats were examined by injecting them with this dye for two months. We divided the Rats into four groups each one received different treatment. Results showed a significant decrease in the body weight of rats in the control group in G2, G3, and G4. Physiological analysis of kidney and liver parameters including AST, ALT, and ALP, were markedly elevated in rats of all treated groups. Histological examination of liver tissues indicated hepatic changes in all tested groups as compared with the control group. Treatment with tartrazine and its products led to mild hepatocytes lesions. Histopathological analysis of the kidney tissues revealed an average renal capsule and other interstitial tissues in the control group, other groups received different doses of tartrazine, revealed that blood vessels appeared mildly congested with interstitial inflammatory infiltrate.

Keywords: Tartrazine, Aromatic amines, *Pseudomonas aeruginosa*, liver and kidney of albino rats

INTRODUCTION

Food additives are most common in the food industry. As colorants and preservatives, more than 2500 food additives have reportedly been employed. They are used to enhance the color and taste of the food (**Rehman *et al*., 2019**). Synthetic colorants are divided into three classes: a) azo compounds such as acidic, basic, and reactive dyes. b) triphenylmethane group, such as Malachite green, crystal violet, etc., and chinophthalon derivatives of Quinoline Yellow (**Minioti *et al*., 2007**). Azo dyes are aromatic compounds and highly water soluble usually stable. Some dyes have three (triazo), two (diazo), or more azo groups (-N=N-), however, they are all organic molecules (**Bell *et al*., 2000**). Azo dyes are widely used for coloring food, cosmetics, and pharmaceutical products (**Lorimer *et al*., 2004**). These aromatic amines have carcinogenic, mutagenic, and toxic effects (**Chung, 2000**). Due to their harmful side effects, some azo dyes have been restricted in European nations and United States for use as food additives. Azo dyes can potentially lead to numerous disorders including contact dermatitis, skin irritation, lacrimation, chemosis, permanent blindness, exophthalmos, acute tubular necrosis supervene, rhabdomyolysis, hypertension, vomiting gastritis, vertigo and, upon edema of the face, ingestion, neck, tongue, pharynx, and larynx along with respiratory irritation (**Young and Yu, 1997**). One of the most well-known artificial dyes is tartrazine (E102), commonly known as (C & FD Yellow No. 5). The World Health Organization (WHO) assigned 7.5 mg/kg/day of tartrazine as the maximum permissible daily dose (**IARC, 1982**). It is utilized as a food ingredient, to enhance characteristics and provide food products color. Numerous indicate that tartrazine might make asthma and chronic urticaria (**Lockey, 1959**). Additionally, studies have shown tartrazine dyes, which are found in various foods like ice cream, and sweets, which can make kids more sensitive to hyperactivity, allergies, irritability, learning disabilities, and aggression (**Romieu, 2005**). Histological research confirmed that tartrazine influences the organs of experimental animals, tartrazine can induce necrosis, swelling, and vacuolation of the liver cells. Also, it can cause damage to the kidneys and stomach (**Upadhyay, 1997**). In the human body, azo dyes are mostly processed via azo reductase in the liver and eliminated by the urine. They do not accumulate in the cells (**Hassan and Elnmer**, **2017**). These chemicals are dangerous not because of the dye itself but because of the breakdown byproducts of these dyes **(Varjani *et al*., 2020**). Microorganisms with the ability to use and break down azo dyes. The synthesis process makes use of some azo dyes and their intermediary metabolites. Microbial degradation byproducts have the potential to be poisonous, mutagenic, and carcinogenic (**IARC, 1982; Houk *et al*., 1999**). Numerous studies covered the subject of human intestine bacteria' capacity to break down azo dyes. The small intestine is home to these microorganisms (**Sherwood *et al*., 2013**). The elimination of the N=N linkage (azo group) results in the aromatic production chemicals, which are high poisonous and are famous to cause cancerous disorders and mutations (**Puvaneswari *et al*., 2006**). By manufacturing azo reductase within the body of human, the role of microbiota was significant part in azo dyes biodegradation (**Alshehrei, 2020**). They are broken down by liver enzymes found in the cytosolic and microsomal fractions as well as, to a lesser extent, azo reductases from gut bacteria. The initial catabolic stage in azo dyes reduction of is the reduction in the link to form aromatic amines, which is followed by visible light absorption decrease and finally dye discoloration. After azo dyes were administered to test subjects, the urine of color stuff animals and workers included aromatic amines, of them some are known carcinogens. (**Cerniglia *et al*., 1982**).

This study focus will be on

I. Examine the potential of human intestinal microflora *Pseudomonas aeruginosa* to breakdown tartrazine into toxic byproducts because it can be crucial in producing harmful toxic compounds, mutagenic and carcinogenic.
II. Study the effect of tartrazine and its degradation products on the kidney and liver tissues of rats.

MATERIALS AND METHODS

Chemicals

Tartrazine was purchased from Merck (Germany), 534.36 is the molecular weight, and $C_{16}H_9N_4Na_3O_9S_2$ is the formula. The chemical composition of tetrazine is shown in Figure.1

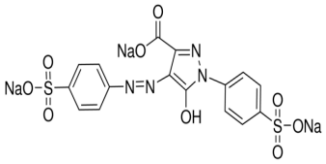


Figure 1 Chemical Structure of Tartrazine (NIH)

Bacterial strain

Pseudomonas sp. was obtained from a Umm AlQura university medical laboratory and maintained on nutrient agar. To confirm the identification of this strain, molecular identification by using 16S ribosomal RNA was done as follows:

DNA Extraction and PCR amplification

At 6000 g/min Centrifugation was done for 10 min to obtain the bacterial strain. Using the technique of genomic DNA was isolated (Cardinal *et al*., 1997). PCR amplification was done utilizing **16S8FWD** (5'AGAGTTTGATCCTGGCTCAG'3) and reverse primer **16S1510RVS** (5'GGTTACCTTGTTACGACTT'3). The first denaturation step of the PCR amplification took place for 5 min at 94 °C, then at 94 °C by 10 cycles of for 1 min, for 1 min at 60 °C, at 72 °C for 1 min, and at 94 °C 20 cycles of 1 min, at 50 °C for 1 min, at 72 °C for 1 min, and 10 minutes at 72 °C for the final extension. Utilizing agarose gel electrophoresis 1% (w/v), the amplified PCR products were seen.

Decolorization Assay

To prepare solutions to contain Tartrazine and its products for rat experiments, the bacterial strain was grown on a Nutrient broth medium containing (g/L): Peptone: 5.0, NaCl: 5.0, Yeast extract: 2.0, Beef extract: 1.0, and Tartrazine: 0.003. The medium's pH was adjusted to 8.0. It was incubated at 37°C for 72hours. After that, the medium was collected for centrifuging at 5000 rpm for 30 minutes to separate the bacterial cell mass. At 428 nm, the supernatant samples were measured. for tartrazine (E102) using UV-1800 UV/VIS Spectrophotometer (RAYLEIGH, Beijing Beifen-Ruili Analytical Instrument (Group) Co., Ltd.). Each assay was carried out in triplicate and contrasted with the control. Using the following equation, the decolorization efficiency (%) of several isolates was calculated:

$$\text{Decolorization (\%)} = \frac{\text{Initial absorbance - Final absorbance}}{\text{Initial absorbance}} \times 100$$

Assay of Tartrazine products

Spectroscopy

The degraded metabolites of Tartrazine dye were analyzed by UV-Vis Spectrophotometer. The liquid was kept and used for treating the second group of rats to measure the effect of dyes metabolites products on the tissues and functions of tested animals.

Chromatography

In order to identify the metabolites of degradation of Tartrazine and its metabolites by gas chromatography-mass spectrum (GC-MS), metabolites were collected from the supernatants using ethyl acetate with equal volume, after that it evaporated on a dry layer of Na_2SO_4. until dry. A 0.22 m membrane was used to filter the final extracts after they had been dissolved in a little amount of methyl alcohol.

GC-MS Analysis

Using a GC/MS system and an HP-5MS column, metabolite analysis was carried out (7800A-7900B; Agilent Technologies Inc., Santa Clara, CA, USA). At 1 mL/min, helium of flow rate was utilized as the gas carrier. With the following oven settings, the injector temperature was kept at 280°C: After maintaining 60 degrees for two minutes, the temperature was raised by 10 degrees each minute to 280 degrees. Mass spectra and the NIST library were used to identify the chemicals.

Animals

Twenty-eight Adults female albino rats with strain weighting (100 -110) g which obtained from the Histopathology lab, Faculty of Medicine, Al-Azhar university, Cairo, Egypt. Rats were acclimatized for two weeks in polyethylene plastic cages under standard conditions. The rats were supplied water *ad libitum* and a chow diet. All animals and microbial disposable procedures in this research have been carried duo to the regulations of an institutional live cell and use committee (IACUC) at the genetic engineering and biotechnology research center, University of Sadat city, Egypt.

Animal grouping and study design

Animals were divided into four groups as shown in (Figure.2), tested groups were treated for two months as follows:

G1 (N=7): control group received distilled water + balanced diet, G**2 (N=7):** were injected orally treated dye with bacteria, **G3 (N=7):** were injected with the recommended dose of ADI of TZ 3.75 mg/kg body weight, and **G4 (N=7):** were injected with 7.5 mg/kg body weight of ADI.

*In groups (3 and 4), TZ day was dissolved in distilled water and injected via oral gavage.

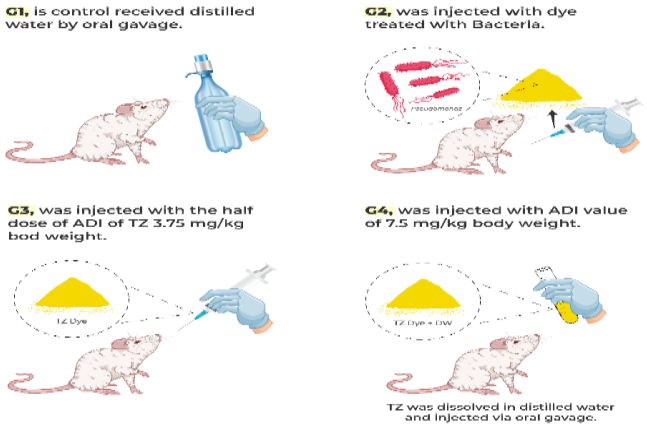


Figure 2 Tested groups of rats receiving different doses of tartrazine dyes.

Physiological studies

Measurement of body weight

Rats of control and tested groups were weighed at the beginning of the experiment and after two months to examine the feeding of tartrazine doses on their body weight.

Serum Biochemistry

Blood samples were collected from rats of each group through Orbital sinus punctures in plastic tubes then the serum was separated for analysis of liver function parameters containing including total protein, alkaline Phosphatase (ALP) (u/l), albumin concentration (ALB) (g/dl), aspartate aminotransferase (AST) (u/l), and alanine aminotransferase (ALT) (u/l), these activities were determined by the method of **(Reitman and Frankel, 1957)** Also, kidney function parameters including concentration analysis of creatinine (mg/dl), Globulin (g/dl), Urea (mg/dl), and Total protein (g/dl). Urea and creatinine were determined in serum by the method of (**Patton and Crouch, 1977**), while Total protein was determined according to the method of (**Henry, 1964**).

Histological examinations

Rats were sacrificed, liver and kidney tissue samples were taken, processed for histopathological analysis, and subjected to Hematoxylin and eosin staining protocol according to (**Robert *et al*., 2014**) for microscopical examination.

RESULTS AND DISCUSSION

Molecular identification of bacterial strain

Molecular analyses of bacterial 16S r RNA sequences contribute a powerful method for assessing fungal diversity at the genus level. The isolate used for the sequencing analysis, its code, and Gene Bank accession number are listed in Table.1. Results showed that bacterial strain similar to *Pseudomonas* aeruginosa with (99.66%) according to the report of genetic engineering and biotechnology research center, University of Sadat city, Egypt.

Table 1 Identified isolate related to the species and the identity percentage found in the gene bank.

Organism name	Acc. number	Query cover	E-value	Identity %
Pseudomonas aeruginosa	NR_113599.1	100%	0.0	99.66%

AGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCC
GGAAACGGGCGCTAATACCGCATACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCT
AGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTC
CTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAG
CACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTATCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCC
GCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGATGTGAAATCCCCGGGC
TCAACCTGGGAACTGCATCCAAAACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGA
AGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACTCTGGTAG
TCCACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGC
CGCAAGGTTAACACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGA

Figure 3 Fragment length after sequencing: ~ 881bp

Effect of incubation period on decolorization percentage

The resulted supernatant was assayed spectrophotometrically to measure absorbance at 427 nm, table 2 shows increase of decolorization percentage during seven days of incubation, while the absorbance of treated dye is decreasing as shown in figure 4.

Table 2 Decolorization percentage of Tetrazine during 7 days of incubation

Incubation time (hours)	*Decolorization (%)*
zero	00
48	61
72	66
96	72
120	79
144	83
168	83

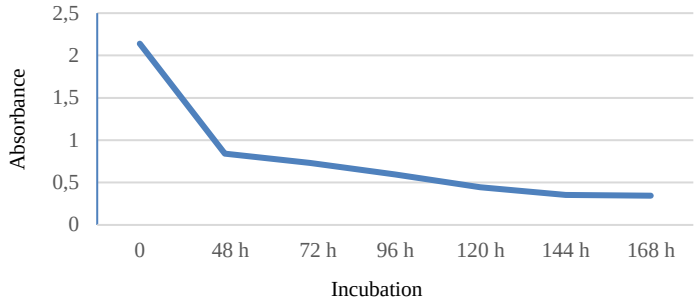


Figure 4 Decrease of absorbance of Tetrazine dye at 427 nm during 7 days of incubation.

Detection of metabolic products

UV spectrophotometer

To confirm decolorization of Tetrazine to metabolic by-products, Figure.5 Shows the absorbance of Tetrazine at 427nm before treatment with *P. aeruginosa*. The metabolite products appear at a different area of the ultraviolet zone after seven days of incubation with bacterial strain. The colorless peaks indicate the formation of derivates of Tetrazine, figure 6.

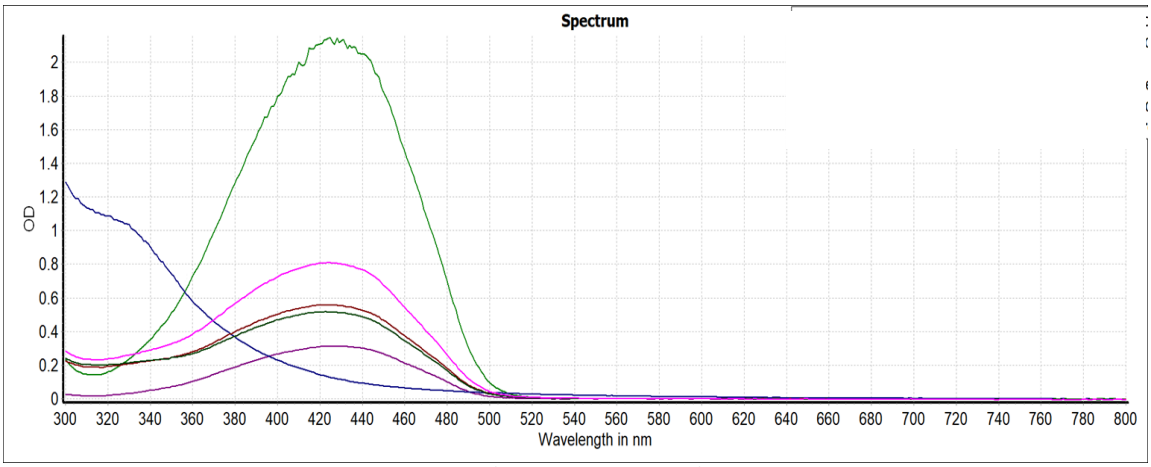


Figure 5 Spectroscopic absorbance of Tetrazine at 427nm before treatment with *P.* aeruginosa

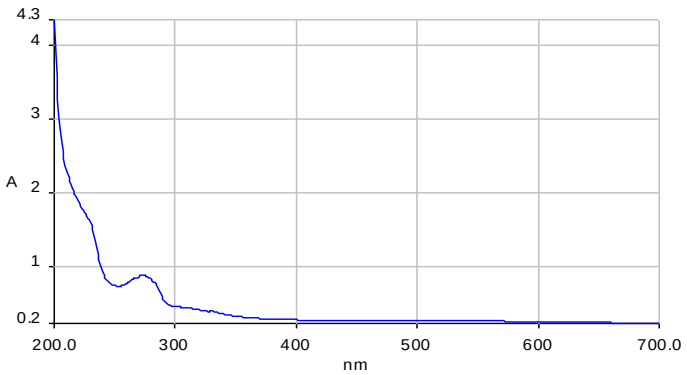
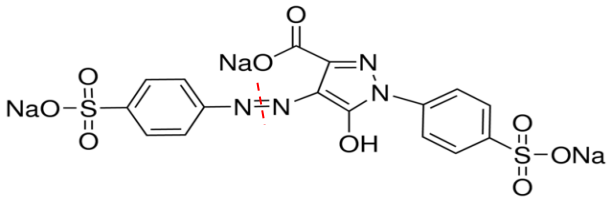


Figure 6 Spectroscopic absorbance of metabolites products after treatment with *P.* aeruginosa

GC-MS

The metabolites product of Tetrazine biodegradation by *P. aeruginosa* was detected by using GC-MS analysis. Tetrazine has a high molecular weight of 534.36 and after treatment by bacterial strain, the azo bond (N = N) breaks down into small fragments as shown in the proposed mechanism in Figure 7., The result demonstrates that the appearance of peak related to one of the proposed products which show in Figure 8., this compound corresponding to sodium 2- amino benzenesulfonate and the molecular weight is (195.15) m/z. This agrees with a study by (**Mout-inho *et al.*, 2007**) who mention that, once the gastrointestinal bacteria have digested tartrazine, it becomes aromatic amine sulfanilic acid.



Sodium 2- aminobenzenesulfonate (195.15) m/z

Figure 7 Proposed mechanism of Tetrazine biodegradation by *P. aeruginosa*

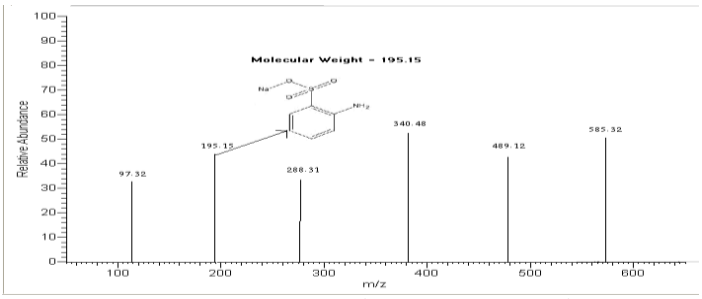


Figure 8 The intermediate metabolites of TZ degradation identified by GC–MS analysis

PHYSIOLOGICAL ANALYSIS

Effect of tartrazine dye treatment on albino female rats' body weight

Data in the table (2) describe the effect of color foods on average body weight, and results showed significant changes in body weight. In the first group (control), which received a normal diet and distil water for two months, their body weight was significantly increased ($P \leq 0.05$). While, groups 2, 3, and 4 that received treated dye with bacterial strain or half of the recommended tartrazine dose or recommended dose of tartrazine, respectively showed a noticeable decrease ($P \leq 0.05$) in their body weight.

Results indicated that no significant changes were detected in the body weight of all treated rat groups (tartrazine or its products), while a significant increase ($P \leq 0.05$) in body weight of control rats was recorded. These findings agreed well with that of (**Arefin *et al.*, 2017**) who demonstrated that rats treated with tartrazine had a significant decrease in average body weight. Also, **(Amin *et al.*, 2018)** and **(Aboel-Zahab *et al.*, 1997)** reported that the administration of high doses of tartrazine decreased significantly the gain of body weight in albino rats.

Table 2 Body weight (g) of female albino rats before and after treatment by tartrazine dye its products.

Group No. N=7	Body Weight (g)	
	Before treatments	After treatments
G1	100 ±4 [a]	127±4 [a]
G2	110 ±4 [a]	85±4 [c]
G3	110 ±4 [a]	100 ±4 [ab]
G4	110 ±4 [a]	95 ±4

Values are means ± SE, means having the same letter are significantly different at $P \leq 0.05$.

Effect of tartrazine dye on liver and kidney function parameters

In the present study, table (3) shows that the activities of serum enzymes including ALT, ALP, and AST were significantly elevated in rats of all treated groups, the elevation was markedly in rats of group 4 that received the highest doses of tartrazine as compared with ($P \leq 0.05$) the control values. Such changes detected in liver enzymes could reflect the hepatic damage induced by exposure to the toxic dye. These hepatic lesions are attributed to hepatic cell auto-oxidation brought on the increase in ROS or free radical production (**Suzuki *et al.*, 1998**) suggesting damage to both mitochondrial membrane and hepatic cells in tartrazine-administrated rats (**Amin *et al.*, 2010**). The obtained data revealed that the serum concentration of globulin, albumin, and total protein was markedly increased in rats of group 4 compared with its value in control animals, the finding that suggests the activation of the liver microsomal enzyme system by the toxic dye stimulating rate of hepatic synthesis of protein including albumin and total protein. Research has proven that environmental toxins, Drugs, carcinogens, natural dietary toxins, and even endogenous substrates like steroids are just a few of the substances that are bio-transformed by the cytochrome P-450- hepatic microsomal mixed-function oxidase (MFO) system is reliant. (**Bidlack *et al.*, 1986**).

In addition, our investigation showed that tartrazine's high dose led to a considerable rise in globulin serum, which was consistent with the findings of **(Aboel-Zahab *et al.*, 1997),** and (**Mekkawy *et al.*, 1998**).

Also, table (3) describes those rats who received Tartrazine daily intake and its products for two months presented a substantial rise in urea concentration and serum creatinine in contrast to rats' control. In addition, a dose-dependent increase in their levels was detected ($P \leq 0.05$). The obtained results agree well with those of (**Ashour and Abdelaziz, 2009**) who found that rats had higher serum levels of urea and creatinine were raised in rats who consumed a synthetic colorant.

Table (3) Effect of Tartrazine dye on liver and kidney functions in female albino rats.

Groups	ALP (u/l)	AST (u/l)	ALT (u/l)	ALB (g/dl)	Total protein (g/dl)	Globulin (g/dl)	Creatinine (mg/dl)	Urea (mg/dl)
G1	87.56 ± 2.79[a]	47.85 ± 2.70[a]	8.91 ± 0.27[a]	3.27 ± 0.84[a]	5.59 ± 0.12[a]	1.89 ± 0.08[a]	1.32 ± 0.06[a]	17.54 ± 0.68[a]
G2	114.16 ±3.85[b]	48.96 ± 0.96	10.78 ±0.65	3.81 ± 0.14	5.71 ± 0.26	1.77 ± 0.05	1.65 ± 0.05[b]	34.41 ± 1.59[b]
G3	117.16 ± 2.3[b]	52.91 ± 2.54[b]	14.2 ± 1.19[b]	4.57 ± 0.08[b]	6.66 ± 0.18[b]	3.79±2.00[b]	1.55 ± 0.05[b]	37.46 ± 0.94[b]
G4	119 ± 2.93[b]	55.38 ± 1.0[b]	15.4 ± 0.80[b]	4.76 ± 0.13[b]	6.70 ± 0.18[b]	2.13 ± 0.04[b]	1.80 ± 0.05[c]	42.15 ± 1.03[c]

Values are mean ± SE, means within the same column having different letters are significantly different at ($P \leq 0.05$), ALT: Alanine aminotransferase, ALP: Alkaline Phosphatase, ALB: Albumin. AST: Aspartate aminotransferase

Histopathological Analysis

Liver

Histological examinations of control rats revealed regular portal tracts with average portal veins and in peri-portal area average hepatocytes, average central veins, regular hepatocytes organized in single-cell cords, and average blood sinusoids between the veins (fig.9) hepatic tissue changes of tested groups comparing with control group revealed that in group 2 , that received 7.5 mg/kg of tartrazine dye daily / two months, liver tissue showed markedly dilated central veins with disconnected lining and Hepatocyte apoptotic fragments in the peri-venular region, and mildly congested blood sinusoids (fig.10) while, in rats of group 3, that received a half dose of tartrazine dye, liver histology demonstrated portal tracts with mild edema and peri-portal inflammation infiltrate, hepatocytes and mild portal in the peri-portal area showed apoptosis was scattered, and there was mild micro-vesicular steatosis. (fig.11). Moreover, histopathological analysis of liver tissues in rats of group 4, that received a biodegradation product of tartrazine dye, indicated an average portal tract, mild intra-lobular inflammatory infiltrate with scattered apoptotic hepatocytes (fig.12). The different changes detected in tested groups appeared to be due to the different doses of tartrazine dye and the biodegradation products used. Other studies found that receiving high or low doses of synthetic dyes such as Ponceau, sunset yellow, tartrazine, and Fast green have a side effect on liver histology appearing as hepatocellular damage indicated by necrosis, swelling, and pyknosis (**Mekkawy *et al.*, 1998**; **Upadhyay, 1997**). In this study, the treatment of rats with tartrazine and its product lids to mild hepatocytes. This result coincided with a study by (**Upadhyay, 1997)** Who reported that treatment of tartrazine caused a mild hydropic degeneration of hepatocytes in the liver of rats. Other studies revealed that receiving high doses of tartrazine increased permeability, damage, and injuries of hepatocytes. (**Senthil *et al.*, 2003).**

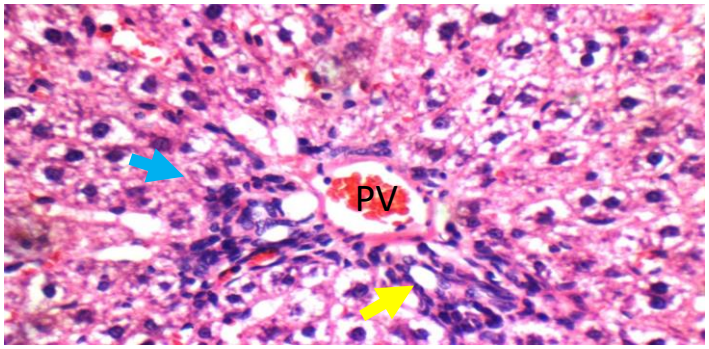


Figure 9 Control group High power picture demonstrating a typical portal tract with a typical portal vein (PV), a typical bile duct (yellow arrow), and a typical hepatocyte in the peri-portal zone (blue arrow) (H&E X 400)

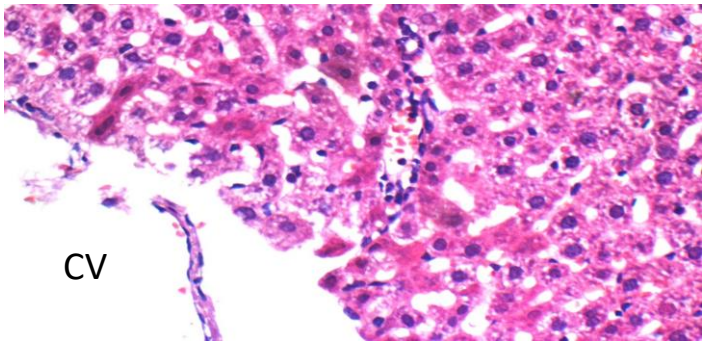


Figure 10 Group 2 liver showed markedly central veins dilated with disconnecting lining and in the peri-venular zone scattered apoptotic hepatocytes, and mildly congested blood sinusoids.

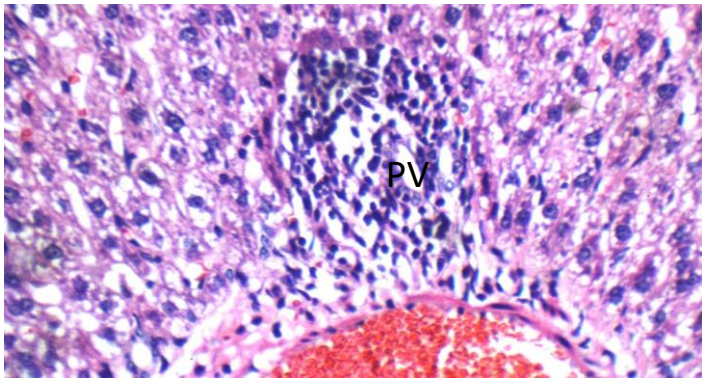


Figure 11 Group.3 view of higher power displaying moderately portal tract edematous with the little portal (black arrow) and inflammation in the peri-portal region, hepatocytes in the peri-portal area showing dispersed apoptosis (yellow arrow), and mild micro-vesicular steatosis (red arrow) (H&E X 400)

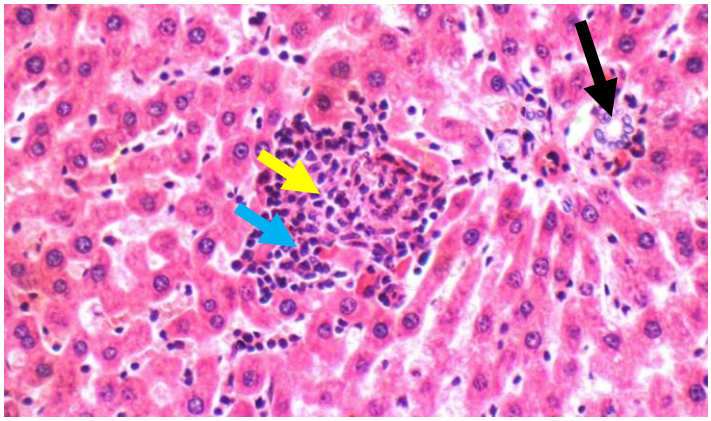


Figure 12 Group.4 high magnification view of the average portal tract (black arrow), a little intralobular inflammatory infiltration (blue arrow), and dispersed apoptotic hepatocytes (yellow arrow) (H&E X 400)

Kidney

Histopathological analysis of renal tissue of control rats revealed an average glomerulus with typical Bowman's spaces, average renal capsule, average with average epithelial lining and preserved brush borders, average collecting tubules and average interstation were visible in the renal medulla, and average distal tubules (fig.13). Histological examination of renal tissue of rats treated with a heigh dose of tartrazine dye (7.5 mg/kg) indicated an average Bowman's spaces with average glomeruli, average renal capsule, proximal tubules with apoptotic epithelial lining and partial necrosis, also we apparently with areas of hemorrhage congested interstitial blood vessels (fig.14).In rat group 3 ,that received a half dose of the dye, renal tissue analysis displayed mild renal capsule, markedly hypercellular Mild glomeruli Bowman's spaces and proximal tubules with a thin epithelial lining are present. Mild peri-vascular edema with mildly obstructed interstitial blood vessels (fig.15).However , histological examination of the kidney of rats received the tartrazine degradation product (group 4) showed proximal tubules with scattered epithelial lining apoptotic and marked interstitial inflammatory infiltrate (fig.16).

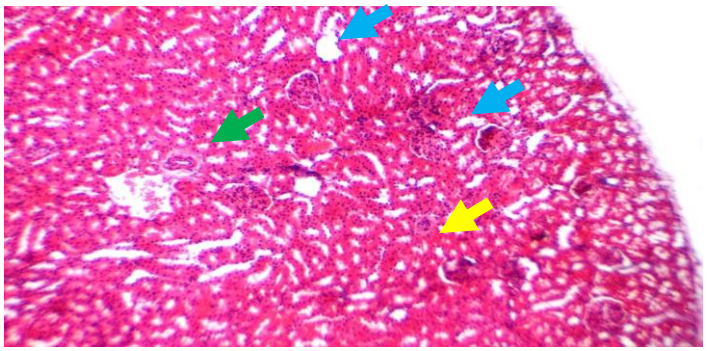


Figure 13 Control group kidney showing average interstitial blood vessels (green arrow), mild renal capsule (black arrow), mild tubules (yellow arrow), and average glomeruli (blue arrow) (H&E X 200)

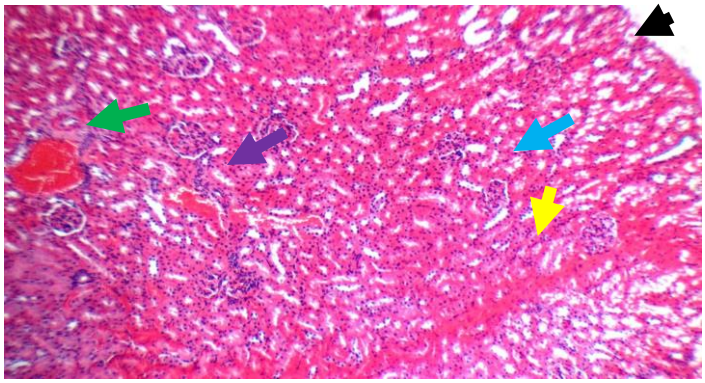


Figure 14 Group 2 kidney showing average glomeruli (blue arrow), average renal capsule (black arrow), areas of necrosis (yellow arrow), areas of hemorrhage (violet arrow), and significant obstructed interstitial blood vessels (green arrow) (H&E X 200)

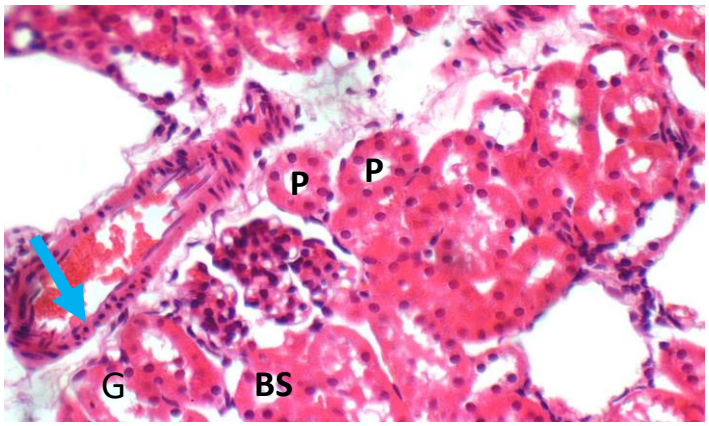


Figure 15 Group.3 another view showing mild Bowman's spaces (BS) with hypercellular glomerulus (G), average epithelial lining with proximal tubules (black arrow), and mildly obstructed interstitial blood vessels (blue arrow) with moderate peri-vascular edema (yellow arrow) (H&E X 400).

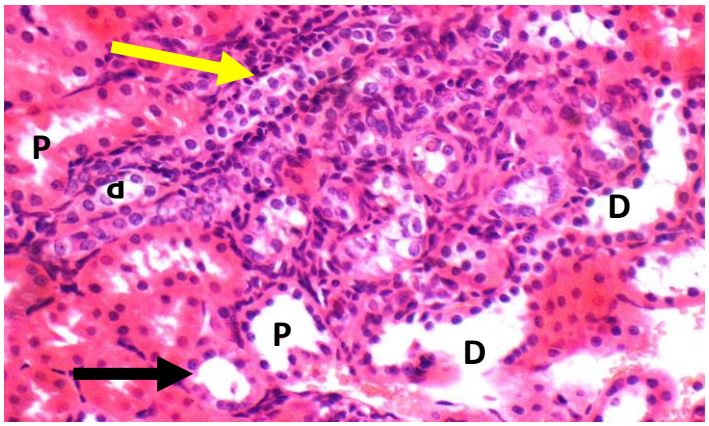


Figure 16 Group 4 another view showing apoptotic epithelial lining with proximal tubules (black arrow), and marked interstitial inflammation (Yellow arrow) (H&E X 400)

The present histopathological findings agree well with the result obtained by the work of (**Himari *et al*., 2011**) that revealed in the tartrazine (7.5 mg/kg BW) treated rat kidney, there was tubular degradation and glomerular capillary dilatation. Interpapillary sclerosis and glomerulus atrophy were visible in rats given tartrazine 10 mg / (kg BW). These alterations are consistent with the findings of (**Rus *et al*., 2009**), who reported alterations in the kidney following guinea pigs' ingestion of tartrazine in water at conc. of 1, 2, and 3% for three weeks. The kidney has varying degrees of congestion and perivascular edema, which is similar to the liver's changes in certain ways. Additionally, irregular glomerular filtration and tubular or glomerular stasis were noted. Vascular congestion is only somewhat present in animals given a low dose of tartrazine (1%). Furthermore, an area of nephrite apoptosis appeared, and the most pronounced changes were recorded with the higher dose. Authors have suggested that the glomerulus is the primary site of action for a variety of substances, and it is susceptible to damage from all toxic, metabolic, and immune mechanisms (**Jones *et al*.1985**). The kidney tissues degenerate as a result of the toxic irritants carried to the kidney by circulating blood.

CONCLUSION

Food azo dyes like tartrazine can cause acute symptoms in organs such as changes in liver and kidney tissues, biochemical parameters, and also loss in weight. Human intestinal microflora *Pseudomonas aeruginosa* can play a vital role in breakdown tartrazine into byproducts. Regrading to this research, biodegradation of this dye producing harmful toxic compounds, mutagenic and carcinogenic. Azo reduction can be accomplished by human intestinal microflora because they can produce azo reductase (**Chung, 2016**). Long-term of using high doses of toxic dyes can affect digestive system and also affect human health, causing allergies and other human diseases.

Acknowledgment: The author would like to thank Professor Kamal Attia for helping in the explanation of the physiological data in this study. The author Also would like to thank Professor Sayed Abdel Raheem for helping in explain changes in animal sections.

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