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Effect of using Sage on the Biological Characterization of Experimental Rats Treated with Allura Red and Brilliant Blue Pigments

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ABSTRACT

Food additive is substance that is not regarded as food and defined as chemical substances deliberately added to foods, directly or indirectly in known quantities during production or processing to improve the different properties of food products: taste, colour, aroma, texture, duration, fitness for consumption, etc. The present study was conducted to evaluate the possible influence impacts of an azo dye (allura red) and triphenylmethane dyes (brilliant blue)on some physiological and biochemical parameters of male albino rats.All rats groups were treated orally with synthetic colorant . Data acquired revealed a marked decrease on body weight of rats, R.B.Cs , Hb and HDL-c of experimental rats treated with allura red and brilliant blue (400 mg/kg b.w) on the other hand, a noticeable increase in WBCs, activities AST, ALT, ALP, serum total protein, albumin , globulin total bilirubin, urea, creatinine, uric acid, triglycerides (TG), total cholesterol (TC) and LDL-c. Meanwhile treatment with ethanolic extract of *Salvia officinalis* leaves at dose of 200 mg/kg b.w reduced hazardous effects induced by synthetic colorants. In conclusions, *Salvia officinalis* leaves extract reduced the hazards resulting from using of synthetic colorant.

Keywords: Food additive, *Salvia officinalis,* Allura red, Brilliant blue.

INTRODUCTION

Food additives were known since the ancient civilizations to introduce special color or taste in food. Nowadays, food production companies are using a wide range of additives, more than 2500 types of additives for various purposes, including preservation, sweetening and coloring to preserve the quality of their products and to meet customers desires especially children (Montaser and Alkafafy 2013 and Sabah., 2015).

The food coloring history dates back to early Egyptians and Romans civilization, when people used saffron, various flowers, carrots, mulberries, beets, and so forth to put color to their foods suggesting use of coloring agents from prehistoric times. Later during the middle of the nineteenth century people had started using synthetic colors in place of natural colors. Since then the extensive use of synthetic food azo dyes has become very common due to increasing canned and fast food culture, despite their legislative ban (Okafor *et al* ., 2016 and Shimada *et al*.., 2010). Food coloring came in many forms consisting of liquids, powders, gels, and pastes (Shanmugam *et al*., 2013).

Allura Red belongs to synthetic mono azo dye group with strong vivid colors and widely used as food colorants to make them visually aesthetic (Rovina,2018). The molecular formula of allura Red is $C_{18}H_{14}N_2Na_2O_8S_2$ (MW: 496.42 g/mol). Allura red consisted essentially of 6-hydroxy-5-(2-methoxy-5-methyl-4-sulfophenyl) azo-2,naphtalenesulfonic acid sodium salt. It has three aromatic systems (EFSA, 2009). Allura red is extensively used in many manufactured foods including sauces, baked goods, snacks, cereal, cotton candy, soft drinks, and dairy products. Also, added to food products such as orange soda, gelatins, puddings, confections, and condiments, paper, drugs, textile industries and cosmetic substances (Zhang *et al.*, 2010).

Brilliant Blue FCF (Blue 1) is an organic compound classified as a triarylmethane dye, The molecular formula is $C_{37}H_{34}N_2Na_2O_9S_3$ (MW: 792.9 g/mol). Its IUPAC name of brilliant blue is ethyl - [4 - [4 - [ethyl -[(3 - sulfophenyl) methyl] amino] phenyl] - (2 - sulfophenyl) methylidene] - 1 - cyclohexa - 2, 5 - dienylidene] - [(3 - sulfophenyl) methyl] azanium (Okafor *et al.*, 2016). The Brilliant Blue is one of the most common dyes used in food and cosmetic preparations and medicines. It was approved in various countries to be used as a food additive in dairy products, candies, cereals, cheese, toppings, jellies, liquors, and soft drinks. This dye is also used in cosmetics such as shampoos, nail polishes, lip gloss, and lip sticks and in the textile sector. The uses of this dye are justified due to its high cost-benefits as blue is not a color currently found in secretions in the body (Watharkar, 2013).

S. officinalis L. is one of the most appreciated herbs for richness of the essential oil content and its numerous biologically active compounds. It is considered as one of the greatest forms of healing medicine. The leaves of the plant have a wide range of biological activities, such as anti-bacterial, fungistatic, virustatic, anticancer, astringent, eupeptic and antihydrotic effects (Hasanein *et al.*, 2017). These plants have been traditionally employed for their cerebrovascular and cardiac benefits, anti-inflammatory, antirheumatic, antimicrobial, tranquilizing, anticancer, antidiabetic, hepatoprotective and many other medicinal properties (Kintzios 2003). The present study aims to investigate the role of ethanolic *S. officinalis* extract to reduce hazards of synthetic coloring in albino rats.

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MATERIALS AND METHODS

Raw material

The leaves of sage or *S. officinalis* were purchased from the experimental farm Faculty of Agriculture Minia University**.**

Preparation of S. *officinalis* extract:

The fresh plant leaves were cleaned carefully, washed several times with running tap water and dried for 5 days in the shade. These leaves were ground by monlinex blinder to fine powder. leaves powder (1:10 w ⁄ v)was extracted with ethanol 80% (v ⁄v) for 4 h with constant stirring at room temperature (25°C),while, extraction suspensions were filtered through whatman No.1filter paper and the residue was re-extract with the same method El-Shemy *et al., (*2007) and khalafalla *et al.,* (2009). The supernatant combined and evaporated to dryness in rotary evaporator at 40ºC. and finally stored in freezer at 4ºC until further tests were carried out.

Experimental Animals:

Adult male albino rats of Sprague-Dawley strain, weighting 180± 10 g were obtained from farm in Cairo. The rats were housed in plastic cages in an air conditioned room at 25°C ± 2. A commercial balanced diet and tap water were provided ad libitum for two weeks before starting the experiment. All experimental procedures were conducted according to the ethical standards approved by the Institutional Animal Ethics committee guidelines for animal care and use, Minia University, Egypt.

Experimental Design:

Forty eight healthy rats were randomized assigned into 6 groups (8 rats each), after an acclimatization period two weeks ,at the following.

(1) Animals were received distilled water as control group.

(**2) *S.offcinalis* group:** Animals were received *S.offcinalis* ethanolic extract 200mg / kg (Kishore *et al.,* 2014).

(3)Allura: Animals were feed with synthetic colorant allura red with the ratio of 400mg/kg diet for 60 days. (EFSA, 2009).

(4) Allura+S: Animals were feed with synthetic colorant allura red 400mg/kg diet and *S. offcinalis* extract 200 mg / kg for 60 days

(5) Brilliant: Animals were feed with synthetic colorant brilliant blue with the ratio of 400mg/kg diet for 60 days (EFSA, 2010).

(6) Brilliant+S: Animals were feed with synthetic colorant brilliant blue 400mg/kg diet and *S. offcinalis* extract with 200 mg / kg for 60 days.

Determination of total phenolic compounds and Flavonoids:

Determination of total phenolic **by** (Maurya and Singh, 2010) and Ebrahimzadeh *et al.,* (2008).

Sampling

Blood samples:

At the end of 60 days, rats were fasted overnight and anesthetized to collect the blood samples from the retro-orbital plexus (Schermer, 1967). Suitable volumes of fresh blood were immediately taken in a heparinized tube for hematological examinations. The other parts of blood samples were allowed to coagulate at room temperature, and then centrifuged at 4ºC the clear non-haemolysed sera were separated and stored at-20ºC till used in biochemical analysis. Animals were disseted as quickly as possible and liver, kidney, brain and testies were excised, wiped with filter paper and weighted.

Hematological evaluation:

Blood was taken from the retro-orbital plexus in a heparinized tube for determined red blood cells (RBCs) and white blood cells (WBCs) counts, haemoglobin concentration (Hb), using Animal Blood Counter (Genius-KT-6400).

Biochemicalassays:

Serum enzyme activity of aspartate aminotransferase (AST, E.C.2.6.1.1.) and alanine aminotransferase (ALT; E.C.2.6.1.2.) activities were measured according to the method described by Reitman rand Fankel (1957). Alkaline phosphatase (ALP) was measured with colorimetric method (Belfield and Goldberg, 1971). Total protein, albumin, urea,creatinine and uric acid level were determined according to the methods described by Gornall *et al*., (1949), Doumas *et al.,* (1971), Fawcett and Soctt, (1960), Bartles *et al.,* (1972) Barham and Trinder (1972). Globulin was determined by difference between total protein and albumin. Total and direct bilirubin were determined calorimetrically (Walters and Gerarde, 1970). Total lipids (TL), Triglycerides (TG), Cholesterol(TC), HDL-c and LDL-c were determined according to the methods described by (Zollner and Kirsch (1962), Burstein, *et al*., (1970), Allain, *et al.,* (1974), Fassati and Prencipe (1982), and Wieland and Seidel (1983), using enzymaticcolorimetric procedures Kits from Bio- Diagnostic Co., Egypt.

Histopathological examination:

Liver and kidney specimens were fixed in 10% formalin solution. After 24 h of fixation followed by embedding in a paraffin block, it was cut into sections of 5µm thickness and stained with hematoxylin -eosin (H&E) for routine histopathological examination according to Bancroft *et al.* (1996).

Statistic alanalysis:

The results obtained in the present study were evaluated by One Way ANOVA test. The results were expressed as mean ± standard error and values of $P<0.05$ were considered statistically significant (Snedecor and Cochran, 1986).

RESULTS AND DISCUSSION

Total phenolic and flavonoids of *S. officinalis*

Results in Table (1) showed that total phenolic and flavonoids contents of *S. officinalis* extract 95 and 13.5 respectively**.**

Solvent	Total phenolic compound (mg/g)[a]	Flavonoids (mg/g)[b]
	S. officinalis	*S. officinalis*
Ethanol 80%	95 ±0.66	13.50±1.2

a*: mg GAE/g of dry leaves extracts b: mg QE/g of dry leaves extracts

Sage leaves are a considerable source of phenolic compounds, the highest content oftotal polyphenols, flavonoids(Gird *et al.,*2014)

Effect on body weight and feed efficiency of rats:

Results in Table (2) showed that there were no significant differences in rat group treated with *S. officinalis* extract comparing with normal control in body

weight gain and Feed Efficiency Ratio for 60 days. The body weight gain was a significantly ($P < 0.05$) decreased in groups treated with allura and brilliant blue about 39.9% and 29.7% respectively comparing with normal control.

Table 2. Effect of *S. offcinalis* on body weight gain, daily feed intake and feed efficiency on rats treated with allura red and brilliant blue.

parameters / Groups	Final Weight (g)	Body weight gain (g)	Daily feed intake (g)	Feed Efficiency Ratio(%)
Control	309.5±1.5	115.3±1.4	19.65	9.6
S.*offcinalis*	321±1.5	133.7±2.5	18.73	11.7
Allura	246^{a}±2.3	69.2^{a}±4.7	18.52	6.4
Allura +S	270.8^{ad}±1.3	90.8^{ad}±1.8	18.95	8
Briliant blue	255.4^{a}±5.3	81^{a}±14.90	17.66	7.3
Briliant +S	277.6^{a}±5.09	101.4^{e}±5.3	19.62	8.3

Data represent the mean ±S.E of observations from eight rats .a significantly different from control group at p <0.05 ,d significantly different from allura and e significantly different from brilliant blue .

Our results agreement with the obtained data of many authors who found that synthetic colorants caused a significant reduction in body weight gain in rats (Amin *et al*., 2010 Al-shinawy 2009).Meanwhile groups treated with *S. offcinals* (200mg) extracts plus allura red improved the reduction of body weight gain about 31.2% comparing with allura alone whereas groups treated with *S. offcinals* (200mg) plus brilliant blue alleviated the reduction of body weight gain about 25.1% comparing with brilliant blue alone. Allura red and brilliant blue reduced the feed efficiency ratio about 33.3% and 23.9% comparing control. The concomitant with *S. offcinals* (200mg) increased these reduction about 25% in group treated with allura red and about 13.6% in group treated with brilliant blue.

Changes in relative weight of some organs:

The relative weight of liver, kidney, testes and brain are shown in Table (2). There were no significant differences in group treated with *S. officinalis* extract compared to normal control in all relative weight organs expect the liver in animal group treated with brilliant blue which decreased significantly ($P < 0.05$)about 35.2% and in brain about 31.8% comparing with control.

Table 3. Effect of S.*offcinalis* extract on relative organs of rats in rats treated with allura red and brilliant blue.

parameters / Groups	liver	Kidney	Testes	Brain
Control	3.4±0.2	0.77±0.04	1±0.07	0.66±0.01
S. offcinalis	3.07±0.18	0.76±0.04	1.2±0.1	0. 6±0.05
Allura	3.42±0.44	0.7±0.005	1.18±0.12	0.68±0.04
Allura+S	3.3±0.05	0.73±0.05	1.2±0.07	0.6±0.07
Briliant blue	2.2^{a}±0.1	0.62±0.04	0.97±0.02	0.45^{a}±0.02
Briliant+S	2.8±0.08	0.72±0.03	1.02±0.05	0.52±0.02

Data represent the mean ±S.E of observations from eight rats .a significantly different from control group at p <0.05,d significantly different from brilliant blue.

These results are in agreement with Abd El-Wahab and Moram(2012) who showed no significant differences ($p > 0.05$)in relative weight of organs such as the liver, kidney and heart in groups treated with synthetic colorants comparing with control expect the liver in group treated with brilliant blue.

Effect of *S.offcinalis* extract on biochemical assay in experimental rats treated with allura red and brilliant blue.

Effect of hematological parameters:

Results in Table (4) showed that there were no significant differences in animal group treated with *S. officinalis* extract in Hb, WBCs and RBCs levels compared to the control. Hemoglobin level in groups treated with allura red and brilliant blue were decreased about 23% and 27.7% respectively comparing to normal control whereas, animals treated with *S.officinalis* extract plus synthetic colorants restored hemoglobin level to nearly normal control.

Table 4. Effect of *S. offcinalis* extract on hematological parameters in blood rats treated with allura and brilliant blue.

parameters / Groups	WBC (10^3/mm3)	RBC (10^6/mm^3)	HB (mg/dl)
	8±0.8	**5.7±0.02**	**10.13±0.7**
S.officinalis	7.4±0.4	5.9±0.07	10.15±0.25
Allura	10.63^{a}±0.14	4.63^{a}±0.13	7.8^{a}±0.5
Allura+s	8.1^{d}±0.7	5.5± 0.09	9.5^{d}±0.2
Briliant blue	12.65^{a}±1.7	4.3^{a}±0.13	7.32^{a} ±0.5
Briliant+s	9.22^{e}±2.2	5.4±0.4	10.03^{e}±0.4

Data represent the mean ±S.E of observations from eight rats .a significantly different from control group at p < 0.05 ,d significantly different from allura and e significantly different from brilliant blue .

In addition, treatment with allura and brilliant blue caused a reduction in red blood cell count about 18.7% and 24.4% respectively comparing with normal control. Whereas, treatment with *S.officinalis* extracts plus synthetic colorants restored RBCs level to nearly normal control meanwhile, white blood cell count was significantly ($P < 0.05$) increased in groups treated with allura and brilliant blue about 1.3 and 1.6 fold compared to normal control whereas groups treated with, *S.officinalis* extract with allura decreased WBCs about 23.8% comparing with treatment with allura only and about 27.1% in concomitant treatment of *S.officinalis* extract with brilliant blue comparing with brilliant blue only. These results are in accordance with many authors who found that a significant decrease ($P<0.01$) in R.B.Cs and Hb content in animal group treated with synthetic colorant .These changes induced may be due to the prevention of red blood cell synthesis via inhibition of erythropoisis in the bone marrow.(AL-shinnawy 2009and Al Reza *et al*.,2018).

Effect on liver function .

Data in Table (5) showed that there were no significant differences in group treated with *S. officinalis* extract comparing with normal control in AST, ALT and ALP activities Also, total bilirubin, direct bilirubin and in direct bilirubin levels for 60 days. Synthetic coloring, allure red and brilliant blue caused changes in liver function in rats.

All animal group treated with allura caused highly significant ($P < 0.05$) increased in serum AST, ALT, ALP, TB and DB about 3.7, 8.0, 2.8, 2.9 and 4.8 fold respectively comparing with normal control. Meanwhile co-administration of *S. officinalis* with allura decreased AST, ALT and ALP activities and TB and DB about 26.6%, 52.7%, 61.06%, 41.2 % and 56% respectively comparing with group treated with allura alone. Additionally, treatment

with brilliant blue caused a significant (P < 0.05) increase in serum activity AST, ALT, ALP, TB and DB about 3.9, 5.6, 2.2, 2.1 and 2.6 fold respectively comparing with normal control. These results came in accordance with Mahmoud (2006) who reported that increasing in ALT, AST, ALP activates, and SBIL-T concentration in rats treated with brilliant blue that changes in serum ALT and AST activities are due to cellular degradation by brilliant blue dye, perhaps on the liver or heart muscle.

While co-administration of *S. officinalis* plus brilliant blue decreased AST, ALT, and ALP and TB activity about 48.2%, 38.8%, 55% and 36.2% respectively comparing with group treated with brilliant blue while restored DB to normal control.

S.officinalis, one of the richest sources of antioxidants its medicinal value is directly related to this property (Medjahed *et al*., 2016).

Table 5. Effect of S.*offcinalis* extract on liver function in rats treatment with allura and brilliant blue.

parameters Groups	AST (u/ml)	ALT (u/ml)	ALP (u/ml)	T.Bilirubin (mg/dl)	D. Bilirubin (mg/dl)	Ind. Bilirubin (mg/dl)
Control	35.12±1.07	4.1±0.4	84.03 ±1.8	0.54±0.003	0.22±0.01	0.32±0.02
S.*offcinalis*	33.56±2.01	3.9±0.3	81.19±2.3	0.54±0.005	0.25±0.001	0.29±0.04
Allura	133.3^{a}±2.5	33.07 ^{a} ±1.7	240.4 ^{a}±14.07	1.6^{a}±0.17	1.07^{a}±0.09	0.7^{a}±0.33
Allura +S	97.8^{ad}±2.6	15.61^{ad}±1.01	93.6^{ad}±1.6	0.94^{ad}±0.01	0.47^{ad}±0.02	0.32^{d}±0.08
Briliant blue	139.7^{a}±8.2	23.3^{a}±2.3	182.1^{a}±6.4	1.16^{a}±0.11	0.58^{a}±0.01	0.55^{a}±0.10
Briliant +S	72.3^{ae}±2.17	14.24^{ae}±0.17	81.94^{e}±4.8	0.74^{a}±0.03	0.29^{e}±0.03	0.38^{e}±0.05

Data represent the mean ±S.E of observations from eight rats .a significantly different from control group at p < 0.05,dsignificantly different from allura and e significantly different from brilliant blue .

Effect on protein and albumin parameters.

Data in Table (6) showed that there were no significant differences in group treated with *S. officinalis*, extract comparing with normal control in total protein, albumin and globulins.

Table 6. Effect of *S.ofcinalis* extract on protein and albumin in rats treatment with allura and brilliant blue.

parameters Groups	Protein (mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)
control	7.03±0.2	2.7±0.2	4.3±0.11
S.*ofcinalis*	6.8±0.2	2.7±0.17	4.1±0.4
Allura	13.29^{a}±0.4	3.7^{a}±0.1	10.19^{a}±0.4
Allura +S	11.3^{ad}±0.3	2.9^{d}±0.11	8.4^{ad}±0.7
Briliant blue	14.84^{a}±0.8	3.8^{a}±0.19	11.04^{a}±0.4
Briliant+S	10.29^{ae} ±0.5	2.9^{e}±0.14	8.02^{ae}±0.5

Data represent the mean ±S.E of observations from eight rats .a significantly different from control group at p < 0.05,d significantly different from allura and e significantly different from brilliant blue .

Meanwhile synthetic allura caused a significant (P < 0.05) increase in serum total protein, albumin and globulin about 1.9, 1.3 and 2.3fold respectively comparing with normal control. These results are in accordance with detected by Alsolami (2017) who showed a significant increase (p<0.05) in total serum proteins and serum globulin in groups treated with allura red whereas treatment with *S. officinalis* extract plus allura decreased this elevation in serum total protein and globulin about 14.9% and 17.5% respectively comparing with allura alone and restored the albumin to nearly normal control.

Additionally, treatment with brilliant blue caused a significant (P < 0.05) increasing in serum total protein, albumin and globulin about 2.11,1.4 and 2.5fold respectively comparing with normal control. Whereas treatment with *S. offcinalis* with brilliant blue decreased serum total protein and globulin about 26.41% and 27.3% comparing with brilliant blue and restored albumin to normal control. *S. officinalis* leave's extract contain a high polyphenol content which exhibit a strong antioxidant activities (Abdelkader *et al.*, 2014).

Effect on kidney function .

Data in Table (7) showed that there were no significant differences in animal group treated with *S. officinalis* extract comparing with normal control in serum urea, creatinine and uric acid levels for 60 days. Synthetic coloring, allura red and brilliant blue caused changes in kidney function in experimental rats.

Table 7. Effect of *S.offcinalis* extract on kidney function in rats treatment with allura and brilliant blue.

parameters Groups	Urea (mg/dl)	Creatien (mg/dl)	Uric acid (mg/dl)
Control	0.53±0.02	0.4±0.11	0.414±0.02
S.*offcinalis*	0.53±0.01	0.4±0.04	0.4±0.05
Allura	4.7^{a}±0.3	1.11^{a}±0.14	1.4 ^{a}±0.1
Allura +S	2.11^{ad}±0.2	0.4^{d}±0.12	0.8^{ad}±0.07
Brilliant blue	4.4^{a}±0.15	1.04^{a}±0.2	1.37^{a}±0.07
Briliant +S	2.3^{ae}±0.16	0.4^{e}±0.12	0.9^{ae} ±0.1

Data represent the mean ±S.E of observations from eight rats .a significantly different from control group at p < 0.05, d significantly different from allura and e significantly different from brilliant blue

Data showed a significant (P < 0.05) increase in serum urea, creatinine and uric acid 8.8, 2.7 and 3.3 fold respectively in groups treated with allura comparing with normal control. While concomitant treatment with *S. officinalis* with allure decreased serum urea and uric acid levels about 55% and 42.8% respectively comparing with allura and restored creatinine to normal control. The data showed increasing significant (P < 0.05) in serum urea, creatinine and uric acid 8.3, 2.6 and 3.3 fold respectively in groups treated with brilliant blue comparing with normal control. Meanwhile co-administration of *S. officinalis* plus brilliant blue decreased in serum urea and uric acid about 47.7% and 34.3% comparing with brilliant blue alone and restored creatinine to normal control .

These results are in agreement with many authors who found that highly significantly elevated in values of serum urea and creatinine concentration upon daily oral administration of tartrazine ,sunset yellow ,fast green, and brilliant blue.(Tawfek *et al*., 2015 and Al Reza *et al.,* 2018). Alsolami (2017) found a highly significant (P<0.01) rise of serum urea and creatinine in group treated with allura red.

Effect on lipid profile :

Data in Table (8) showed that there were no significant differences in group treated with *S. officinalis* extract comparing with normal control on lipid profile for 60 days. Allure red and brilliant blue changes in lipid profile in rats.

Table 8. Effect of *S.offcinalis* extract on lipid profile in rats treatment with allura red and brilliant blue.

parameters Groups	TG (mg/dl)	TC (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Control	119.2±4	43.6±3.1	34.25±0.6	22.74±0.5
S.*offcinalis*	116.7±4.8	43.5±4.8	33.06±1.3	24.7±2.2
Allura	209.7^{a}±3	98.76^{a}±2.9	168.5^{a}±8.6	11.5^{a}±1.5
Allura +S	178.9^{ad}±9.9	62.08 ^{ad} ±3.3	60.1^{ad}±4.4	21.6^{d}±0.7
Briliant blue	207.5^{a}±7.7	101^{a}±4.2	157.2^{a}±10.7	19.4^{a}±0.8
Briliant+S	141.1^{ae} ±6.4	56.98^{ae} ±3.9	91.2^{ae}±2.7	24.06±1.7

Data represent the mean ±S.E of observations from eight rats .a significantly different from control group at p < 0.05 d significantly different from allura and e significantly different from brilliant blue

Treatment with allura caused highly significant (P < 0.05) increase in TG, TC, and LDL-c about 1.7, 2.2 and 4.9 fold respectively compared with normal control. Whereas treatment with *S. officinalis* with allura decreased serum TG, TC, and LDL-c about 14.6%, 37.14% and 64.3% respectively comparing with group treated with allure. Additionally, treated with brilliant blue caused a significant (P < 0.05) increase in TG, TC, and LDL-c about1.7, 2.3and 4.5 fold respectively comparing with normal of control group. Whereas treated with *S. officinalis* with brilliant blue decreased TG, TC and LDL-c about 32%, 43.5% and 41.9% respectively comparing with group treated with brilliant blue. The date showed decreased in HDL-c levels in rats group treated with synthetic allura red and brilliant blue about 49.4% and 14.6% respectively comparing with normal control. Meanwhile concomitant treated with *S. officinalis* plus allura increased HDL-c levels 1.8 fold comparing with group treated with allura . While treated with *S. officinalis* with brilliant blue increased HDL-c levels about 1.2 fold respectively comparing with group treated with brilliant blue .

Our results agreement with these obtained by AL-Shinnawy (2009) who found a significant elevation (P<0.05) in these parameters in groups treated with amaranth only. These results are in accordance with that obtained by Tawfek *et al*., (2015) who found a highly significant increase in LDL concentration in group treated with synthetic colorant . *S. officinalis* consumption is accountable for the improvement of the lipid profile inducing a decrease on the highly atherogenic LDL-c particles and an increase the HDL-c, these effect may be due to the ability of *S. officinalis* to suppress cholesterol biosynthesi (Elida *et al* ., 2010).

Histological examination

Histopathological examination of Liver:

Sections from group treated with allura showed cytoplasmic vacuolization of hepatocytes, focal necrosis of hepatocytes associated with inflammatory cells infiltration, Kupffer cells activation and portal infiltration with inflammatory cells. Meanwhile, liver of rats from group treated with S.*offcinalis* with allura showed Kupffer cells activation a mild fibroplasia and inflammatory cells infiltration in the portal triad (Table 9).

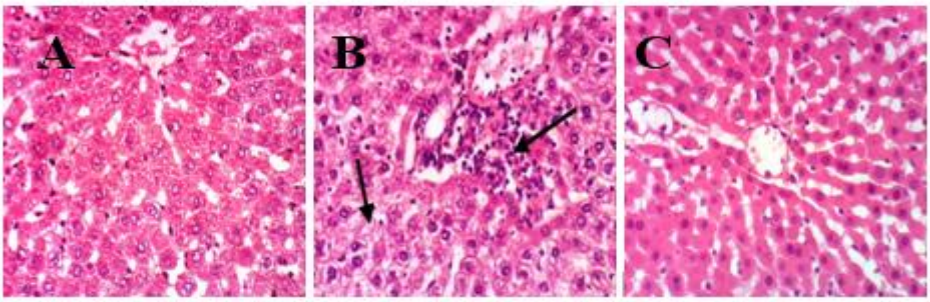


Figure 1. photomicrograph of the cross section in the liver cortex of(A) control, allura (B), , allura and S.*offcinalis* (C)(H and Ex400).

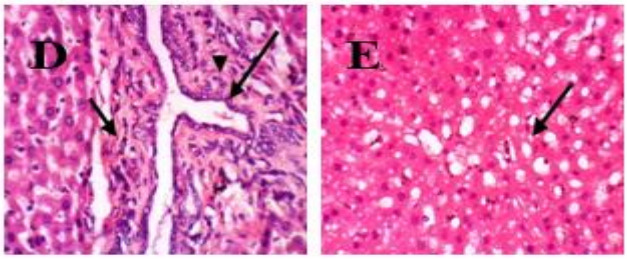


Figure 2. photomicrograph of the cross section in the liver cortex of brilliant (D), brilliant and *S.offcinalis* (E) (H and Ex400).

In contrary, liver from animal group treated with brilliant blue showed a serve cytoplasmic vacuolization of hepatocytes fibroplasia in the portal triad, hyperplasia of biliary epithelium with newly formed bile ductuoles and portal infiltration with inflammatory cells.

Examined sections from group treated with brilliant blue plus *S.offcinalis* showed no changes except cytoplasmic vacuolization of hepatocytes .

Our results agreement with that of Khayyat *et al* ., (2018) who reported that synthetic colorant, sunset yellow and allura red caused alterations in the liver include congestion, fibrosis, leucocytes infiltration, an increased number of kupffer cells, as well as necrotic and hydropic degeneration in hepatic cells. These indicate the occurrence of intracellular oedema, as a result of toxicity or immune aggressions. Also, Mahmoud (2006) revealed that treatment with Brilliant blue caused alterations in liver includes: focal necrosis of hepatocytes, infiltration and vacuolation.

Many authors found that synthetic colorant (Quinoline yellow, Ponceau4R and sunsat yellow)

caused a congestion and dilatation of portal vein with inflammatory cells infiltration in the portal area as well as the hepatic parenchyma (Mahmoud *et al* 2010 and AL-Dahhan *et al.,* 2014)

Table 9. Histopathological notes on liver tissue of rats treatment with synthetic allura and brilliant blue ,*S. offcinalis* extract plus allura red and brilliant blue.

Groups	Histopathological change			
	cytoplasmic vacuolization of hepatocytes	focal necrosis of hepatocytes associated with inflammatory cells infiltration	Kupffer cells activation	portal infiltration with inflammatory cells
Control	—	—	—	—
Allura	++	+	++	++
Allura +S	—	—	+	+
Brilliant blue	+++	++	++	++
Brilient +S	++	—	—	—

(-) no change (+) mild change (++) moderate change (+++) severe change

Histopathological examination of kidneys:

Moreover, kidneys from group treated with allura revealed vacuolation of epithelial lining renal tubules,vacuolation of endothelial lining glomerular tuft and proteinaceous material in the lumen of renal tubules (Table 9).

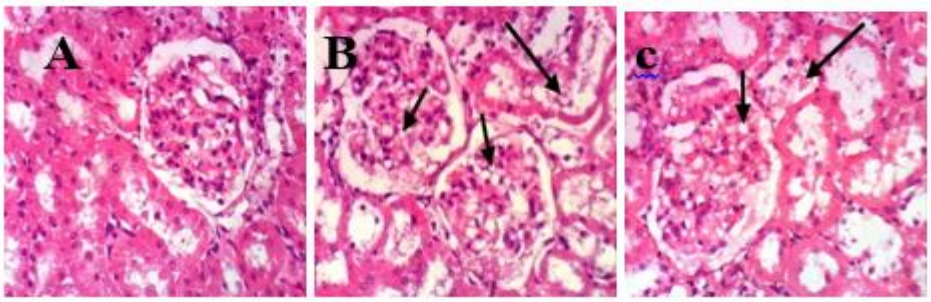
Sections from group treated with *S. officinals* with allura revealed vacuolation of endothelial lining glomerular tuft and proteinaceous material in the lumen of renal tubules

However, kidneys from group treated with brilliant blue revealed vacuolation of epithelial lining renal tubules and endothelial lining glomerular tuft, proteinaceous material in the lumen of renal tubules and congestion of renal blood vessels . Moreover, kidneys from group treated with *S. officinals* with brilliant blue showed a mild change in vacuolation of endothelial lining glomerular tuft and proteinaceous material in the lumen of renal tubules

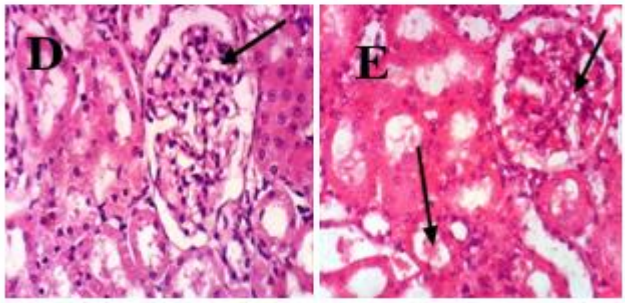
Table 10. Histopathological notes on kidenytissue of rats treatment with synthetic allura and brilliant blue ,*S.offcinalis* extract plus allura red and brilliant blue.

Groups	Histopathological change				
	vacuolation of renal tubular epithelium	vacuolation of glomerular tuft	congestion of renal blood vessel	Proteinaceous material in renal tubules	Necrosis of renal tubules
Allura	—	—	—	—	—
Allura +S	++	++	—	+	—
Brilliant blue	—	++	—	+	—
Brilient +S	+	++	—	++	—

(-) no change (+) mild change (++) moderate change (+++) severe change



Figur 3. photomicrograph of the cross section in the kidney cortex of,(A) control, allura (B), allura and *S.offcinalis* (C) (H&Ex400).



Figur 4. photomicrograph of the cross section in the kidney cortex of brilliant (D), brilliant and *S.offcinalis* (E) (H&Ex400).

Our results agreement with that of Khayyat *et al* ., (2018) who reported that synthetic colorant, sunset yellow and allura red caused inflammation, necrosis and vacuolisation of the tubular epithelium occurred, in addition to the destruction of glomeruli, with thickening of Bowman's capsule. These indicate the occurrence of intracellular oedema, as a result of toxicity or immune aggressions.

CONCLUSION

The present study concluded that it was clear that the administration of allurared and brilliant blue to rats caused many disturbances in the physiological and biochemical parameters, and groups treated with *S. officinalis* plus synthetic colorant reduced this disturbances.

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تأثير استخدام المر يميه علي الصفات البيولوجية للفئران المعامله بصبغات الالورا الاحمر والازرق اللامع .

إسراء محمد مجاهد ، محمدي عبدالحميد عيسي ،عاطف عبدالمحسن عبدالرحمن و ماجدة عويس محمود

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تعرف المضافات الغذائية بأنها مواد كيميائية تضاف عمدا إلى الأطعمة بشكل مباشر أو غير مباشر بكميات معروفة أثناء الإنتاج أو المعالجة لتحسين الخصائص المختلفة للمنتجات الغذائية: الذوق واللون والرائحة والملمس والمدة واللياقة للاستهلاك،إلخ. وهي مواد لا تُعتبر كغذاء . أجريت هذه الدراسة لتقييم تأثيرات التأثيرات المحتملة صبغة الآزو (أحمرا لألورا) وأصباغ ثلاثي فينيل ميثان (الأزرق اللامع) على بعض الدراسات الفسيولوجية والكيميائية الحيوية للجرذان ألبينو الذكور. تم اعطاء جميع مجموعات الفئران عن طريق الفم. أوضحت النتائج انخفاض ملحوظ في وزن الجسم من الفئران،كرات الدم الحمراء والهيموجلوبين والبروتين الدهني مرتفع الكثافه للفئران التي عوملت باللورا الحمراء 400 والأزرق اللامع (400 مجم / كجمB.W). من ناحية أخرى ،هنا ك زيادة ملحوظة في WBCs، وأنشطة الاسبرتات تراس امينيز والالانين تراس امينيز والالكلين فوسفاتيز،وبروتين المصل الكلي، والألبيومين والجلوبيولين البليروبين الكلى و اليوربا و الكرياتينين وحامض اليوريك الجلسيريدات الثلاثيه والكلسترول الكلي والبروتين الدهني ذو الكثافه المنخفضه التي اعطيت مستخلص الإيثانول من نبات المريمية بجرعة (200 مجم / كغم من وزن الجسم).والخلاصه ان مستخلص نبات المريمية يقلل من إخطار الملونات الصناعية.