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BIOLOGICAL EVALUATION OF MACARONI SUPPLEMENTED WITH DRIED MALLOW LEAVES AND TOMATO WASTES AS A SOURCE OF NATURAL COLORANTS

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ABSTRACT

Nowadays colors play an important role in food and drug industries. In this investigation, synthetic dyes which are used in food industry (Tartrazine/Brilliant Blue and Sunset yellow) either single or as a mixture of natural colors (dry mallow leaves and dry tomato wastes) with the synthetic dyes at a ratio (1:1) were used to reduce the harmful effect of synthetic dyes alone on the health of mankind. Natural colors, synthetic colors and their mixture (1:1) were exposed to gamma rays at different doses (0, 10 and 15 KGy) to produce a safe product. Nutrition experiments were conducted to assess the effect of natural, and synthetic colors, and their mixtures added to macaroni product on some biological and biochemical parameters in the serum of rat groups, *i.e.* total protein, albumin, globulin, A/G ratio, total lipid, total cholesterol, glucose, creatnine, alkaline phosphates activity, transaminases enzyme activity "ALT & AST", hemoglobin and hematocrate. In addition, rat organ weights (liver, kidney, spleen and gain in body weight) were detrmined. From the obtained results, it can be concluded that the natural pigments (colorants) in the diet of natural and synthetic food colorant mixture can reduce the toxic influences of the synthetic ones.

Key words: *mallow leaves, natural colorants , serum analysis , synthetic colorant , tomato waste.*

1. INTRODUCTION

Coloring substances play an important role in accepting the different foodstuffs and the assessment of their quality. They are usually added to foods to be more attractive to the purchasers or consumers as well as replacements to natural colors that might be deteriorated during processing (Rizk, 1997). In Egypt, colors in the developing food industry had available vast array of synthetic colors. This led to colors being added for decorative purposes and unfortunately produce low quality foods. There was no control over such use of color and so inevitably legislation came into force. Food colorants may come from natural sources or may be a synthetic product. Of all the food additives, perhaps the addition of color is the hardest process to justify. However, 90% of the colors used in food come from a small group of synthetic colors, the diverse group of natural color sources is used in about 10% of foods only (Ensminger *et al*., 1995). All the synthetic food colorants were liable to rise to potentially toxic degradation products, either by their metabolic transferrations or by the action of intestinal microorganisms. Synthetic food colorants were more effective than the natural ones. Moreover, the natural pigments in the ingestion of natural and synthetic food colorant mixtures reduced the toxic influences of ingested synthetic dyes (Ramadan and El-Damhogy, 1994; Abdel-Rahim *et al*., 1995; Abdou *et al*., 1997 and Salah *et al*., 1999).

The intake of foods rich in carotenoids appeared to be associated with optimal health, and a reduction in the risk of cancer, cardiovascular disease, macular degeneration and cataract formation. Specific dietary carotenoids might be responsible for these specific protective effects. Hydrocarbon carotenoids such as alpha and beta carotenes and lycopene might reduce the risk of cancer and heart diseases, whereas oxygenated carotenoids, such as lutein and zeaxanthin, might be important for the protection of the eye. Dietary carotenoids , such as lutein, cryptoxanthin, alpha and beta carotenes, and lycopene could be readily obtained from the diet. Green leafy vegetables, such as spinach and broccoli were found to contain both oxygenated and hydrocarbon carotenoids. Yellow or orange vegetables, such as carrots, have high levels of alpha and beta carotenes; and tomatoes were found to contain high amounts of lycopene. Besides, being important vitamin A sources, provitamin A carotenoids, such as alpha and beta carotenes, and cryptoxanthin appear to participate in cell defense systems that are associated with radical quenching. Non-provitamin A carotenoids, such as lutein and lycopene, major carotenoids in human plasma, have also been reported to possess strong antioxidant capability. The alteration of dietary sources of carotenoids could modify their levels in the circulation and target tissues, and thus might prevent or delay the onset of these chronic diseases (Yeum 1996).

In this respect, Abdel-Rahim *et al.* (1987) found that, when rats were administered chlorophyll or tartrazine /green–s mixture at doses of 2.35/0.1 and 4.7/0.2 mg/kg body weight dye for 4 weeks, significant increases, in liver soluble proteins, and plasma AST enzyme activity with the high dose, and liver AST and ALT enzyme activities using both doses were obtained. Ibrahim *et al.* (1988 a & b) administered chlorophyll orally to adult male albino rats in a single dose of 8 gm/kg diet for only one day, and multiple dose of 4gm/kg diet for 3 weeks. They found that AST and ALT enzyme activities in plasma, and liver homogenate, as well as, plasma glucose were significantly higher than the control; while liver glycogen was significantly lower than the control. They also reported that the total soluble proteins in plasma, and in organ tissues were stimulated by pigment administration. Abdou *et al.*, (1997) observed that the natural colorants (curcumin, chlorophyll and anthocyanine) decreased the level of total lipids and cholesterol content of blood and different tissues. In addition, increases were recorded for plasma bilirubin as well as plasma and liver soluble protein, Hb, RBC and WBC contents. GOT and GPT activity in both plasma and liver were stimulated by natural pigments. The effect of food colorants on plasma transaminases activity was less than its effect on liver. Furthermore , they reported that, pronounced increase in serum and liver transaminase activities of rats by ingestion of synthetic colorants. The load and species of food colorants ingested into animals for assimilation at any time might alter the activity of GOT and GPT, followed by changes of overall protein metabolism.

Abdel Aziz and El-Ashmawy (1993) found that, tartrazine affected hemoglobin content, hematocrate percentage and the number of red and white blood cells. In addition hemoglobin and hematocrate percentage decreased in a highly significant manner after the treatment for 21 days. The number of red blood cells was also significantly decreased after treatment for 14 and 21 days. While, the number of white blood cells was significantly increased. On the other hand, El-Sherbeny (1993) mentioned that, the body weight gain of rats fed chlorophyll and Green-S (higher dose) was significantly lower than the control group. The food consumption of rats fed both dyes was slightly lower than the control and the difference was non-significant. The activity of serum and liver homogenate AST enzyme was significantly higher than the control group. Serum urea, and creatnine in rats fed either Green-S or chlorophyll were higher than the control.

Macaroni mixed with industrial 10% vegetable by-products (carrot, pea and spinach) as hypolimic and hypocholesterolemic agents reduced the hyper-effects, but values were more than that of negative control rats (normal) (Ibrahim, 1998). Tomato wastes (TW) were irradiated at doses of 0.75 and 100 K.Gy, and then incorporated into the diets. No significant differences in feed intake were observed between animals fed on the control diet and those fed on diets containing 10% raw TW, irradiated TW. (Hamza, 2001).

Hamouda (1994) studied the biological and biochemical effects of natural (chlorophyll) and synthetic (Green-S and sunset yellow) colorants as well as its mixtures feeding using rats. He found that the body weight gain of the synthetic and natural food colorants fed rats was lower than that of the control. However, synthetic colorants were more effective than natural ones. The mixtures of synthetic and natural food colorants (1:1) fed rats had more gain in body weight similar to the check. The food consumption of the tested rats during the time course of the experiments was slightly increased for rats administrated either natural or synthetic or its mixture food colorants relative to the check. Also, the food efficiency under the effect of natural food colorant was higher than that of synthetic one, but both were less than the check. The ingestion of colorant mixtures (natural & synthetic) prevented these decrease effects. Meanwhile, the liver weight/body weight ratio was reduced in rats fed either synthetic or natural food colorants. However, in the case of synthetic dyes, it was lower than that of natural colorants. This means that synthetic dyes ingestion caused emaciation in liver tissues. In contrast, the administration of synthetic and natural colorant mixtures did not change the ratio relative to control.

Abdel-Rahim *et al.* (1995) studied the hypo intensive effects of natural food colorants (curcumin, carotene and chlorophyll) on synthetic dyes (yellow 2GG, tartrazine and green-s) toxicity in their mixtures. They found that the rate of body weight gain of rats ingested either synthetic food colorant or natural pigments, as well as their mixtures was lower than the control. The liver/ body weight ratio was increased in rats ingested synthetic dyes (enlargement or tumefaction) unlike rats ingested the naturals or mixtures relative to control.

Salah *et al.*(1999) determined the total hemoglobin plasma total soluble proteins, GOT and GPT activities; and GPT and GOT activities in the liver to study the effects of carotene as a natural food colorant on the toxicity of tartrazine (synthetic food colorant) when used as food additives in albino rats. The obtained results indicated that no mortality was observed under the ingestion of tartrazine, carotene and their mixtures in rats. Synthetic and natural colorants as well as their mixture had changed all the above determinations compared to the control animals. The synthetic dye as food additive had the highest effects compared with the natural one or the mixture of synthetic and natural food colorants. On the other hand, the mixture of carotene and tartrazine showed around normal values,. In this respect, total Hb content of blood decreased total soluble protein content of plasma but still more than that of the control. In addition, GOT and GPT activities of plasma and liver were stimulated by the ingestion of synthetic or natural food colorant and their mixture. They also showed that carotene acted as an antagonistic factor in the toxicity of tartrazine in animal tissues for all experimental analysis. This indicated that natural food colorants reduced the toxicity of synthetic food colorants when the natural pigment was ingested together with the synthetic dye used as food additives.

In the present study, γ-radiation was used in an attempt to inhibit pathogenic organisms and molds in the used materials. In this respect, raw materials (dry mallow leaves , tomato wastes flour and semolina wheat flour), synthetic colors (tartrazine/brilliant blue "green" and sunset "yellow") and their mixtures (1:1) were exposed to γ-radiation at 0, 10 and 15 K.Gy. The investigated samples were applied to produce different mixtures of macaroni supplemented with natural and synthetic colors. The produced macaroni was then used in a biological experiment to investigate the effect of toxic residues of synthetic colors on the biochemical parameter of rat serum.

2. MATERIALS AND METHODS

2.1 Materials

Two species of vegetables obtained from the local markets in Cairo were used as a source of natural colors, mallow leaves (*Malta parviflora*) and Tomato wastes (*Lycopersicon esculentum L.*). While the synthetic colors, *i.e.* green (code No 404) and sunset yellow (code No 500) were obtained from Kamina Co., Egypt. Hard wheat flour (*Triticum aestivum*) was obtained from the cylinder mills of South Cairo Company.

2.2.Methods

2.2.1.Preparation of the natural and mixture colors

Washed mallow leaves and tomato wastes were dried in the oven at 65^0C under vaccum for 24 hr, then milled to fine powder by using a laboratory disc pass 20-mesh (Bahnassy and Khan 1986). All samples (natural, synthetic and their mixture (1:1) were packed under nitrogen in metalized polypropylene bags and irradiated at 10 or 15 K.Gy. using Co^{60} gamma cell (at a dose rate of 6.776K.Gy/h) located at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo.

2.3. Preparation of different blends of macaroni

Different blends of macaroni were prepared by partial replacement of semolina with powdered dry mallow or tomato waste and their mixture (1:1) as shown in Table (1). Then, the different blends of substituted macaroni were processed according to Dexter *et al.*, (1990).

Table (1): Different blends of macaroni supplemented with the colors under investigation.

Ingredients	Flour g	Water ml	Natural color g	Synthetic color g
Dry mallow leaves	1000	300	107.0	-
Synthetic green	1000	300	-	0.1
Mixture green	1000	300	107.7	0.1
Dry tomato wastes	1000	300	292.5	-
Synthetic yellow	1000	300	-	0.1
Mixture yellow	1000	300	293.2	0.1
Semolina	1000	300	-	-

2.4. Experimental Animals

A number of 192 male albino rats obtained from Helwan Station, Cairo, with an initial body weights of approximately 50-60 g was used for the biological experiment. Rats were kept on standard diet (according to Lane-Peter and Pearson (1971), for two weeks (adaptation period), and then transferred to the experimental diets for 90 days (experimental period). The rats were divided according to the feeding treatment into 20 groups as follows: Group (1) control animals fed on normal diet, group (2) negative control animals fed on diets of macaroni without colors; groups (3 to 11) animals fed on diets containing macaroni with green color and groups (12 to 20) animals fed on diets containing macaroni with yellow color (Table 2). At the end of the experimental period, body weight gain was calculated. The serum samples were collected and kept at $-10^{\circ}C$ for biochemical analysis.

Rats were killed by decapitation and the liver, kidney and spleen were separated from each rat and weighted.

Table (2): Different animal groups according to the tested diets.

Group	Content	Casein	Cor	Cellulos	Salt	Vit.	Starch	macaroni
1	Control	20	10	5	4	1	60	-
2	N. Control	20	10	5	4	1	-	60
3	N.M.	20	10	5	4	1	-	60
4	M.M.	20	10	5	4	1	-	60
5	Sy.G.	20	10	5	4	1	-	60
6	N.M. 10	20	10	5	4	1	-	60
7	M.M. 10	20	10	5	4	1	-	60
8	Sy.G.	20	10	5	4	1	-	60
9	N.M. 15	20	10	5	4	1	-	60
10	M.M. 15	20	10	5	4	1	-	60
11	Sy.Y. 15	20	10	5	4	1	-	60
12	N.T.	20	10	5	4	1	-	60
13	M.T.	20	10	5	4	1	-	60
14	Sy.Y.	20	10	5	4	1	-	60
15	N.T. 10	20	10	5	4	1	-	60
16	M.T. 10	20	10	5	4	1	-	60
17	Sy.Y.	20	10	5	4	1	-	60
18	N.T. 15	20	10	5	4	1	-	60
19	M.T.15	20	10	5	4	1	-	60
20	Sy.Y. 15	20	10	5	4	1	-	60

N. control = Negative control (macaroni without color); N.M.=Natural mallow leaves; M.M.=Mixture (dried mallow leaves and synthetic green color (1:1); Sy.G= synthetic green color; N.T.= Natural dry tomato waste; M.T.= Mixture (dried tomato waste and the synthetic sunset yellow color (1:1); Sy.y.= synthetic yellow color.

2.5. Biochemical analysis

Serum total protein was determined calorimetrically using Biuret method according to Henry (1964). Serum albumin was determined according to the method of Doumas *et al.*, (1971). Globulin was calculated by the difference between total soluble protein and albumin. Serum lipids including both free fatty acids and cholesterol were determined as mentioned by (Schmit, 1964). Total cholesterol was determined by the enzymatic method described by Richmond (1973). Serum glucose was measured according to the enzymatic method of Trinder (1969). Aspartate and alanine transaminases (AST and ALT) activities were estimated colorimetrically according to the method of Reitman and Frankel (1957). Serum alkaline phosphatase activity was determined according to the method of Roy (1970). Creatnine was estimated using the kinetic method of Henry (1974). Hemoglobin concentration (HB) and Hematocrate were estimated according to the method of Dacie and Lewis, (1991).

The statistical analyses were performed according to Daniel(1991).

3. RESULTS AND DISCUSSIONS

A few investigators observed the healthy effects of natural colorants, which have hypo intensive influences on the toxic effects of synthetic dyes used as food additives. However, most experiments were carried out to evaluate the relationship between chlorophyll and carotenoids as natural food additives and tartrazine / brilliant blue and sunset yellow as synthetic food colorants used for foodstuff.

3.1.Effects of natural and synthetic food colorants (green color)

Different sources of unirradiated or irradiated color *i.e.* natural (dry mallow leaves), synthetic (tartrazine / brilliant blue) and their mixtures were biologically evaluated.

The results in Table (3) show that the highest values in serum total protein, serum total lipid, A/G ratio, hemoglobin and hematocrate were found in rats fed on diet contained natural mallow leaves (N.M.) being 7.33, 4.78, 1.87, 15.57 and 43.10 g/dL, respectively,. Rats fed on natural mallow leaves at 15 K.Gy (N.M 15 k.Gy) showed the highest levels of aspartate aminotransferase (AST) activity, alkaline phosphates (ALP) activity, creatnine and glucose being 139.94 U/ml, 157.37 U/ml, 0.96 mg/dL and 111.41 mg/dL, relative to control. While the lowest values were recorded in the case of globulin, total lipid (T.L.), triglyceride (T.G.) and cholesterol for rats fed on diet contained N.M.15K.Gy being 2.51 g / dL ,3.08 g / dL , 87.58 mg / dl and 162.26 mg / dL, relative to control . On the other hand , no change was observed in alanine aminotransferase (ALT) value in all feeding groups, relative to control.

Diets supplemented with natural food colorants only had a slight stimulation in serum analysis. The results showed that the lowest reductions in ALP activity, T.L, T.G and cholesterol were in the serum of rats fed on diets with natural food colorants (N.M.15k.Gy.).

Data in Table (3) reveal that rats fed on the diets containing mixture color (dry mallow leaves with tartrazine / brilliant blue (M.M) either unirradiated or irradiated show some changes in rat serum constituents. Results show that the values of total protein ranged between (6.60 – 6.80 g/dL), albumin (4.00 – 4.20 g/dL) globulin (2.50 – 2.90 g/dL), A/G ratio(1.40 – 1.70). While the activities of AST, and ALP enzymes ranged between 129–150, 25–29 and 135–165 U/ml respectively. On the other hand, the amounts of creatnine, T.L, T.G, cholesterol, glucose, hemoglobin and hematocrate were in the range of 0.90–1.00 mg/dL, 3.10–3.30 g/L, 105–116 mg/dL, 170–203 mg/dL, 81–100 mg/dL, 13–16 g/dL and 37–48 g/dL, respectively. It was observed that natural food colorants in their mixture with synthetic ones decreased the stimulation influences of the present dyes which were used as food additives .

From the results in Table (3) it could be noticed that all diets with synthetic green color varied in their effects on the different serum constituent's values. A significant increase was observed in rats fed on diets either unirradiated or irradiated (Sy.G) in AST, ALT and ALP activities ; and glucose values . In addition to the

Table (3): Effect of natural food colorants (dry mallow leaves), colorants mixture (mallow leaves +tartrazine/brilliant blue) and synthetic food colorants (tartrazine/ brilliant blue) on some serum constituents.

Group	Control	N.M.			M.M.			Sy.G.		
Analysis		**Unirradiated**	**10K.Gy**	**15K.Gy**	**Unirradiated**	**10K.Gy**	**15K.Gy**	**Unirradiated**	**10K.Gy**	**15K.Gy**
Total protein g/dl	6.84 ± 0.31	7.33 ± 0.51	7.20 ± 0.62	6.96 ± 0.93	6.73 ± 1.03	6.72 ± 0.98	6.64 ± 0.26	7.64 ± 0.51	7.53 ± 0.9	7.52 ± 0.64
Albumin g/dl	3.99 ± 0.41	4.78 ± 0.39	4.64 ± 0.46	4.45 ± 0.64	4.20 ± 0.49	4.05 ± 0.48	4.02 ± 0.71	3.97 ± 0.33	3.84 ± 0.71	3.73 ± 0.5
Globulin g/dl	2.86 ± 0.40	2.56 ± 0.51	2.55 ± 0.96	2.51 ± 0.79	2.53 ± 0.93	2.67 ± 0.77	2.62 ± 0.87	3.67 ±0.58	3.69 ± 1.02	3.80 ± 0.72
A/G ratio	1.40	1.87	1.82	1.77	1.66	1.52	1.53	1.08	1.04	0.98
AST u/ml	129.38 ± 3.08	131.44 ± 3.59	132.25 ± 3.6	139.94 ± 2.46 *	131.94 ± 2.61	132.25 ± 2.36	152.56 ± 1.59 *	164.00 ± 5.18 *	169.56 ± 0.86 *	177.25 ± 4.73 *
ALT u/ml	25.98 ± 1.05	24.63 ± 1.95	25.33 ± 0.91	25.60 ± 1.82	25.08 ± 0.69	25.55 ± 0.87	28.80 ± 0.91	30.20 ± 1.07 *	30.83 ± 0.64 *	31.30 ± 0.49 *
ALP u/ml	135.23 ± 2.35	138.10 ±30.89	155.00 ±12.84	157.37 ±16.01	161.36 ± 6.44	163.01 ± 8.66	164.89 ± 4.17	227.49 ± 5.92 *	241.88 ± 21.78 *	244.28 ± 4.84 *
Creatnine mg/dl	0.92 ± 5.39E^{-02}	0.89 ± 6.6 E^{-02}	0.94 ± 7.75 E^{-02}	0.96 ± 0.11	0.97 ± 0.11	1.01 ± 0.15	1.02 ± 7.3 E^{-02}	1.06 ± 4.05 E^{-02}	1.16 ± 9.91 E^{-02}	1.18 ± 6.33 E^{-02} *
Total lipid g/l	3.22 ± 0.26	3.29 ± 0.22	3.19 ± 0.4	3.08 ± 0.19	3.33 ± 0.24	3.22 ± 0.24	3.12 ± 0.25	2.74 ± 0.3	2.59 ± 0.22	2.42 ± 0.12 *
Triglyceride mg/dl	115.96 ± 4.92	105.77 ±11.02	92.81 ±11.83	87.58 ± 7.81 *	110.13 ± 7.93	109.17 ± 4.73	105.12 ±14.32	93.46 ± 7.92	91.39 ± 4.18	83.88 ± 8.55 *
Cholesterol mg/dl	203.30 ± 4.78	171.23 ±25.98	167.92 ±18.5	162.26 ±33.58	182.55 ±19.67	176.41 ±21.46	169.34 ±24.65	158.49 ±27.63	154.72 ±27.53	137.17 ±22.5 *
Glucose mg/dl	81.39 ± 3.32	100.90 ± 2.61 *	102.24 ± 2.47 *	111.41 ± 4.43 *	96.19 ± 2.56	99.94 ± 5.7 *	100.37 ± 3.79 *	120.99 ± 7.13 *	127.52 ± 4.72 *	131.04 ± 16.4 *
Hemoglobin g/dl	12.85 ± 0.47	15.57 ± 1.21 *	14.67 ± 0.59	12.94 ± 0.41	16.02 ± 0.81 *	15.66 ± 0.37 *	14.68 ± 1.25	12.32 ± 0.69	11.56 ± 1.44	10.55 ± 0.3
Hematocrate g/dl	37.06	43.10	41.41	39.02	47.87	46.13	45.29	38.16	36.44	35.76

* Significant at P $\leq$ 0.05, N.M. = Natural mallow leaves, M.M. = Mixture between mallow leaves and tartrazine / brilliant blue (1:1), , Sy. G.= Tartrazine/brilliant blue

creatnine value in the case of irradiated diet (Sy.G.15 K.Gy). Meanwhile, the values of serum T.L, T.G and cholesterol were decreased in rats fed on irradiated diets (Sy.G.15 K.Gy) relative to their control values. While, serum T.P, albumin, globulin, A/G ratio, hemoglobin and hematocrate had slight changes relative to their control.

3.2. Effects of natural and synthetic food colorants (dry tomato waste and sunset yellow color)

Different sources of unirradiated or irradiated color *i.e.* natural (dry tomato waste), synthetic (Sunset yellow) and their mixtures were biologically evaluated. Data in Table (4) show that the highest values in serum total protein, albumin, A/G ratio, total lipid, hemoglobin and hematocrate were found in the group fed on diet containing natural tomato waste (N.T.) being 7.16 g/dL, 3.87 g/dL, 1.18, 3.55 g/L, 15.09 g/dL and 42.49 g/dL respectively, relative to their control. While, the lowest values were recorded for ALT, T.G. and cholesterol in the group fed on diet containing N.T.15 k.Gy being 24.55 U/ml, 83.94 mg/dL and 124.06 mg/dL respectively, relative to control.

Data in Table (4) show the serum constituents of rats fed on unirradiated or irradiated colorants mixture (dry tomato waste and sunset yellow). It could be noticed that total protein, albumin, globulin, A/G ratio, AST, ALT, ALP, creatnine, serum total lipid, serum triglyceride, cholesterol, serum glucose, hemoglobin and hematocrate of tested rats were varied. A significant increase was noticed in the group fed on M.T.15k.GY (151.63 U/ml) for AST activity, relative to the control value (129.38 U/ml). Also, hemoglobin had a significant increase in both groups that were fed on M.T. and M.T.10 k.Gy being 15.29 and 14.47 g/dL respectively, relative to the control values (12.85 g/dL).

From the results in Table (4) it was found that the diet of irradiated synthetic color (15 k.Gy) caused the lowest decrease in A/G ratio, serum total lipid and hematocrate which were 0.61, 2.66 g/L, 31.42 g/dl respectively, relative to control. While, unirradiated and irradiated diet with synthetic color (Sy.Y) showed a significant increase in serum total protein, globulin, AST, ALT, ALP, and glucose. Also, the diet Sy.Y.15 k.Gy revealed a significant decrease in serum albumin, triglyceride, cholesterol and hemoglobin being 3.02 g/dl, 60.35 mg/dl, 103.68 mg/dl and 11.25 g/dl, respectively relative to the control.

It is well known that the serum protein consists mainly of albumin and globulin. It is obvious that the experimental food colorant diets might stimulate albumin biosynthesis relative to that of the control animals. In the same time the present treatments may be inhibited or unchanged the biosynthesis of globulin. Natural pigments in their mixture improved the ratio of A/G. It means that natural food colorants may stimulate the process of protein biosynthesis to reduce the kind of protein that decreased the toxicity of synthetic dyes used as food colorants. These results are in agreement with those of Hamouda (1994), Abdel- Rahim *et al* .(1995); Salah *et al.* (1999) and Mohamed (1999). They found that food colorants stimulated the transaminase activity but synthetic colorants were more effective than natural ones. In the meantime, natural colorants in mixtures with synthetic dyes inhibited the toxic influences of synthetic dyes in the animal. Salah (1994) found that synthetic dyes significantly stimulated ALP activity in experimental rat plasma. The present study indicated that the load and kind of synthetic and natural food colorants inducted into animals for assimilation at any time might alter the activity of enzymes such as AST, ALT and ALP followed by changes in overall protein metabolism. Accordingly, some of the amino acids were dominating and converted to glucose. This was confirmed by the data of blood glucose in the present study. In connection, the amino nitrogen was excreted as urea and creatnine. These also were confirmed by the results of kidney function partly reported in the present study. On these bases, a relation might exit between protein metabolism and feeding on food colorants. Also animals in protein biosynthesis *via* feeding on food colorants needed a high amount of rich phosphate stimulation of ALP for that stimulation of ALP was important to supply inorganic phosphate required for ATP synthesis.

The present work agreed with Salah (1994) and Mohamed (1999) who found that kidney function of rats fed on synthetic dyes used as food additives has changed in which the plasma levels of

Table (4):Effect of natural food colorants (tomato waste), colorants mixture (tomato waste+sunset yellow) and synthetic food colorants (sunset yellow) on some serum constituents.

Group	Control	N.T.			M.T.			Sy.Y.		
Analysis		Unirradiated	10K.Gy	15K.Gy	Unirradiated	10K.Gy	15K.Gy	Unirradiated	10K.Gy	15K.Gy
Total protein g/dl	6.84 ± 0.31	7.16 ± 0.6	7.11 ± 0.24	7.09 ± 0.49	6.65 ± 0.18	6.54 ± 0.28	6.49 ± 0.93	8.79 ± 0.57 *	8.76 ± 0.71 *	7.99 ± 0.57
Albumin g/dl	3.99 ± 0.41	3.87 ± 0.21	3.71 ± 0.4	3.66 ± 0.27 *	3.39 ± 0.33	3.36 ± 0.34	3.32 ± 0.27	3.27 ± 0.32	3.19 ± 9.75E^{-02}	3.02 ± 0.31 *
Globulin g/dl	2.86 ± 0.40	3.29 ± 0.64	3.40 ±0.33	3.43 ±0.48	3.26 ±0.51	3.18 ±0.32	3.17 ±1.19	5.52 ±0.7 *	5.57 ±0.74 *	4.97 ±0.64 *
A/G ratio	1.40	1.18	1.09	1.07	1.04	1.06	1.05	0.59	0.57	0.61
AST u/ml	129.38 ± 3.08	131.63 ± 2.57	133.44 ± 5.24	136.69 ± 4.48	132.13 ± 3.44	133.63 ± 3.45	151.63 ± 0.78 *	156.25 ± 3.69 *	158.81 ± 0.68 *	162.00 ± 1.45 *
ALT u/ml	25.98 ± 1.05	22.73 ± 0.9	23.90 ± 2.12	24.55 ± 0.53	22.90 ± 2.08	24.38 ± 1.1	25.48 ± 1.12	27.13 ± 0.56	30.80 ± 0.4 *	31.28 ± 1.81 *
ALP u/ml	135.23 ± 2.35	134.76 ± 7.22	136.09 ± 2.45	150.76 ± 7.06	152.12 ± 5.67	154.87 ± 8.82	155.21 ± 6.48	160.76 ± 14.69 *	168.53 ± 7.75 *	172.39 ± 8.82 *
Creatnine mg/dl	0.92 ± 5.39 E^{-02}	0.91 ± 6.98 E^{-02}	0.98 ± 0.13	1.03 ± 0.19	0.92 ± 9.47 E^{-02}	1.02 ± 0.23	1.03 ± 0.15	1.07 ± 0.19	1.08 ± 0.14	1.17 ± 7.0 E^{-02}
Total lipid g/l	3.22 ± 0.26	3.55 ± 6.23 E^{-02}	3.32 ± 0.18	3.16 ± 0.32	3.24 ± 0.12	3.00 ± 0.24	2.93 ± 0.27	2.86 ± 0.23	2.80 ± 0.26	2.66 ± 0.32
Triglyceride mg/dl	115.96 ± 4.92	98.15 ± 16.83	96.78 ± 7.37	83.94 ± 13.58 *	103.36 ± 3.67	100.12 ± 3.74	99.02 ± 3.15	76.36 ± 5.79 *	76.14 ± 6.21 *	60.35 ± 1.65 *
Cholesterol mg/dl	203.30 ± 4.78	150.47 ± 27.94	127.83 ±13.48 *	124.06 ± 15.72 *	197.64 ± 26.4	185.71 ± 15.85	183.87 ± 29.01	120.28 ± 23.51 *	115.09 ± 4.43 *	103.68 ± 10.27 *
Glucose mg/dl	81.39 ± 3.32	104.90 ± 6.6	106.71 ± 16.45	108.93 ± 11.51	96.29 ± 15.75	101.18 ± 2.96	103.66 ± 22.73	131.68 ± 16.99 *	141.15 ± 16.94 *	147.02 ± 9.13 *
Hemoglobin g/dl	12.85 ± 0.47	15.09 ± 0.19 *	14.31 ± 0.46 *	13.47 ± 0.87	15.29 ± 0.62 *	14.47 ± 0.53 *	14.14 ± 0.4	11.57 ± 0.37	11.25 ± 0.35 *	11.25 ± 0.11 *
Hematocrate g/dl	37.06	42.49	40.18	35.40	47.86	42.13	39.66	35.27	32.64	31.42

* Significant at P $\leq$ 0.05, N.T. = Natural color (tomato waste), M. T. = Mixture between dry tomato waste and sunset yellow (1:1), Sy. y = Sunset yellow.

creatnine was significantly increased relative to those of the control animals. The present results are also in line with those of Hamouda (1994) and Mohamed (1999), who found that some decreases were observed in total lipids in the serum of rats fed on food colorants which may be attributed to the nature of their chemical structure and to fatty acid biosynthesis precursor (Acetyl CoA) which was transferred toward energy metabolism. The rate of lipids biosynthesis was reduced and the rate of their catabolism was stimulated by the additive induction. However the food colorants had a powerful hypolipimic effect.

Natural food colorants acted as antagonistic agents against synthetic one. These findings are in agreement with those of Hamouda, (1994), Abdel-Rahim *et al.* ,(1995), and Mohamed (1999) who found that natural pigments reduced the high level of blood glucose which was produced by synthetic dyes (food additives). Also blood glucose levels were increased in rats fed on diets with either natural or synthetic food colorants. However, liver glycogen was decreased by the same treatments. The increase of blood glucose in the present work may be due to the glucose production after food colorants induction by glycogenolysis and glucogensis processes. This result is in agreement with Salah *et al.* (1999) who reported that the toxic effects of synthetic dye diets may reduce or disappear by natural pigment application alone or with synthetic dyes. In other words, natural food colorants had antagonistic effects on the synthetic dyes used as food additive influences.

3.3. Effects of food colorants on body weight gain

The body weight gain of rats fed on diets containing natural and synthetic food colorants and their mixture was estimated during the experimental periods (Tables 5-6). The data show that the gain in body weight of rats fed normal diets (control animals) reached the value of 210.04 g.

The diets containing (M.M.) showed the maximum gain in body weight followed by rats fed on diets containing M.M. (10 k.Gy), N.M, M.M (15k.Gy),N.M.(10k.Gy),N.M.(15k.Gy),Sy.G.,Sy. G.(10k.Gy),and then Sy.G.(15k.Gy) diets, respectively. In contrary, the synthetic green color in the diet caused a decrease in body gain weight compared with the control. This means that natural colorants supplemented with synthetic ones in the animal diet had reduced the toxic effects of synthetic dyes (Table 5).

The results in (Table 6) show that the synthetic yellow color in diets decreased the gain in body weight compared with the control. The maximum decrease in value was observed in the case of Sy.Y.15 K.Gy (195.83 g.) and the highest increase value was in the treatment of M.T (10K.Gy) 254.70 g., relative to the control (210.44 g.). This means that natural colorants supplemented with synthetic ones in the animal diet reduced the toxic effects of synthetic dyes used as foodstuff. The present results are in agreement with those of Abdel-Rahim *et al*. (1995). They found that rats fed on synthetic green dyes as food colorants were characterized by low growth rates compared to the control but the mixture of natural and synthetic green color and B-carotene reduced the toxic effects of synthetic food colorants. This means that food colorants have significant antagonistic effect of synthetic ones. Also, the results are in harmony with Hamouda (1994) who reported that the decrease of the body weight gain may be due to the complex between the food colorants and lipids of basal diet and the enzymatic effective for growth (Abdel-Rahim *et al*., 1989). In addition, the natural yellow color was carotene that produced vitamin "A" needed for growth. (Suharno and Muhilal (1996), West (1996), and Salah *et al*. (1999)) .

3.4. Effects of food colorants on rat organ weight

The liver is the organ concerned with lipid metabolism like other organs, which has an effect on lipid content of the liver of most animals (averages about 5%) but it depends on the diet and animal health under the influence of various pathological and physiological disturbances. Its lipid content under the influence of different diets might be due to the accumulation or decline of fats (Hamouda, 1994).

The results in Tables (5-6) show that organ weights were changed by increasing or decreasing relative to that of control rats. Data of the liver, kidney and spleen weight of the experimental rats

Table (5): Effect of natural food colorants (dry mallow leaves), colorants mixture (mallow leaves +tartrazine/brilliant blue) and synthetic food colorants (tartrazine/ brilliant blue) on body and organ weight of rats.

Group	Control	N.M.			M.M.			Sy.G.		
Weight g		Unirradiated	10K.Gy	15K.Gy	Unirradiated	10K.Gy	15K.Gy	Unirradiated	10K.Gy	15K.Gy
Initial body weight	50.81 ±0.35	55.27 ±1.53	53.54 ±0.78	53.84 ±1.83	55.59 ±1.27	55.14 ±0.77	54.01 ±1.89	52.85 ±0.83	51.60 ±1.24	55.47 ±1.43
Final body weight	260.85 ±7.21	252.14 ±4.07	239.67 ±7.38	237.63 ±2.57	283.26 ±9.77	270.04 ±7.50	247.17 ±8.97	234.93 ±12.20	224.41 ±13.46	219.24 ±0.88
Body weight Gain	210.04 ±6.92	196.37 ±5.20	186.13 ±6.67	183.79 ±3.39*	227.67 ±9.16	214.91 ±8.00	193.16 ±7.24	182.09 ±12.09*	172.80 ±12.33*	163.77 ±1.51*
Liver weight	9.51 ±0.52	11.04 ±1.03	10.96 ±0.92	10.14 ±0.54	12.82 ±0.50*	12.14 ±0.90*	11.38 ±1.05	8.92 ±0.60	8.68 ±0.50	7.74 ±0.39
Kidney weight	1.96 ±4.99 E^{-02}	2.02 ±0.13	1.94 ±8.38 E^{-02}	1.86 ±0.11	1.89 ±5.19 E^{-02}	1.87 ±0.11	1.85 ±0.11	1.81 ±0.16	1.78 ±0.15	1.69 ±0.19
Spleen weight	0.71 ±2.77 E^{-02}	0.59 ±3.49 E^{-02}	0.65 ±3.07 E^{-02}	0.67 ±5.02 E^{-02}	0.74 ±0.10	0.74 ±5.08 E^{-02}	0.75 ±2.53 E^{-02}	0.79 ±2.32 E^{-02}	0.84 ±8.24 E^{-02}	0.86 ±0.14

N.M. = Natural mallow leaves, M.M. = Mixture between mallow leaves and tartrazine / brilliant blue (1:1), , Sy. G.= Tartrazine/brilliant blue

Table (6): Effect of natural food colorants (tomato waste), colorants mixture (tomato waste+sunset yellow) and synthetic food colorants (sunset yellow) on body and organ weight of rats.

Group	Control	N.T.			M.T.			Sy.Y.		
Weight g		**Unirradiated**	**10K.Gy**	**15K.Gy**	**Unirradiated**	**10K.Gy**	**15K.Gy**	**Unirradiated**	**10K.Gy**	**15K.Gy**
Initial body weight	50.81 ±0.35	50.90 ±0.85	50.69 ±0.89	51.20 ±0.95	51.16 ±1.0	50.73 ± 0.30	50.42 ±0.84	50.40 ±0.52	50.45 ±0.77	50.58 ±1.0
Final body weight	260.85 ±7.21	298.45 ±9.78	294.64 ±8.30	290.60 ±5.39	305.85 ±8.10	301.16 ±7.10	298.64 ±3.46	264.98 ±5.06	254.99 ±7.55	246.41 ±4.85
Body weight Gain	210.04 ±6.92	247.56 ±9.23*	243.79 ±7.41*	239.41 ±4.50*	254.70 ±7.11*	250.43 ±7.26*	248.22 ±2.69*	214.59 ±5.27	204.55 ±6.92	195.83 ±4.28
Liver weight	9.51 ±0.52	13.20 ±1.05*	12.14 ±0.9*	10.61 ±1.05	11.63 ±0.76	10.76 ±0.74	10.48 ±0.80	8.51 ±0.67	8.30 ±0.45	7.99 ±0.63
Kidney weight	1.96 ±4.99 E^{-02}	2.17 ±0.11	2.15 ±0.11	2.12 ±3.87 E^{-02}	2.14 ±0.18	2.05 ±0.19	2.05 ±0.29	2.04 ±0.14	1.92 ±5.89 E^{-02}	1.90 ±5.66 E^{-02}
Spleen weight	0.71 ±2.77 E^{-02}	.0.54 ±6.38 E^{-02}	0.62 ±5.91 E^{-02}	0.70 ± ±4.94 E^{-03}	0.65 ±8.71 E^{-02}	0.68 ±8.08 E^{-02}	0.74 ±0.11	0.71 ±8.00 E^{-02}	0.75 ±8.34 E^{-02}	0.83 ±0.12

N.T. = Natural color (tomato waste), M. T. = Mixture between dry tomato waste and sunset yellow (1:1), Sy. y = Sunset yellow.

fed on normal and upgraded different supplements are present in Table (5). From the results, it could be noticed that liver weight like other organs was increased with the increasing in body weight. The weight values of liver were 11.04, 12.82, 8.92, 10.96, 12.14, 8.68, 10.14, 11.38 and 7.74 g. relative to the control for N.M., M.M., Sy.G., N.M. 10k.Gy, M.M. 10k.Gy, Sy.G. 10k.Gy, N.M. 15k.Gy, M.M. 15k.Gy and Sy.G. 15K.Gy, respectively. Also diets of synthetic dyes were more effective than natural pigment diets. Moreover natural pigments in mixtures of natural and synthetic colorants diets reduced the influences of synthetic dyes in liver weight. The average values of liver weights were lower (Sy.G.15k.Gy) in some tested rats relative to the control. The increases produced by feeding on M.M. were more than that observed for all other treatments for tested rats.

Meanwhile, the results in Table (6) reveal that liver weight ranged from 7.99 g to 13.20 g for rats fed on diets of Sy.Y. 15k.Gy and N.T., respectively. Results in Table (5) reveal the effects of natural and synthetic food colorants on kidney weight of the experimental male adult' albino rats. Some treatments decreased the weight, such as rats fed on Sy.G15k.Gy., the value was 1.69 g relative to the control, and also other treatments changed the kidney weight. Data in Table (6) show that kidney weight ranged between 1.9 g and 2.2 g, also the values were 2.17, 2.14, 2.04, 2.15, 2.05, 1.92, 2.12, 2.05 and 1.90 g. for N.T., M.T., Sy.Y., N.T.10 k.Gy, M.T.10 k.Gy, Sy.Y., 10 k.Gy, N.T. 15 k.Gy, M.T.15 k.Gy. and Sy.Y.15 k.Gy, respectively.

The same trend was observed in the case of spleen weight relative to that of the control animals. The data in Table (5) show that the spleen weight values were 0.59, 0.74, 0.79, 0.65, 0.74, 0.84, 0.67, 0.75 and 0.86 g for the animals fed on the diets N.M., M.M., S.G., N.M.10 k.Gy, M.M. 10 k.Gy, S.G.10 k.Gy, N.M.15 k.Gy, M.M.15 k.Gy and S.G.15 k.Gy, respectively.

Results in Table (6) show the spleen weight values relative to the control. It appears that the highest level was 0.83 g. in the case of the diet Sy.Y.15 k.Gy, while N.T. has the lowest value of 0.54 g. relative to the control.

The induction of natural and synthetic colorants and their mixture, changes the body functions but natural colorant in the mixture was able to overcome impaired functions caused by synthetic ones; it caused also improvement in the appetite to food and increased the daily food intake which followed by readjustment in the gain in body weight. The present results are in tendency with those obtained by Abdel-Rahim *et al.* (1995) and Mohamed (1999). They found that natural food colorants reduced the toxic effects of synthetic ones in organs tissues. Also, Abdou *et al.* (1997) and Salah *et al.* (1999) found similar results in which natural pigments reduced the toxic effects of synthetic ones. The increase or decrease of liver weight relative to the control under the influence of different diets might be due to the accumulation or reduction of fats. The abnormal effects were noticed on relative organ weights of the liver and kidneys when rats were fed natural or synthetic food colorants. It can be concluded that the increase in the average liver weight by colorant ingestion may be attributed to that colorants causing a tumeric effects. These effects were reduced in the case of natural and synthetic colorant mixtures as antagonistic effect. Also,it might be attributed to that colorants causing a hypolipimic and hypocholesterolemic effects.

Many published information are available on the use of irradiation to extend the shelf-life of perishable food time and ensure their hygienic quality. However, little is known about the effect of radiation on natural or synthetic colorants. It should be emphasized that no/or very little data are available in the literature about this influence of interaction between gamma radiation and natural or synthetic colors as well as storage on different conditions. These items need more investigations and more research.

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التقييم الحيوي للمكرونة المدعمة بأوراق الخبيزة ومخلفات الطماطم الجافة كمصدر للألوان الغذائية الطبيعية

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ملخص

تلعب الألوان دورا هاما فى كثير من الصناعات الغذائية، وصناعة الأدوية. وقد استهدفت هذه الدراسة التقييم الحيوى لاستخدام الألوان الصناعية (الترترازين مع الأزرق اللامع وأصفر غروب الشمس) سواء بمفردها أو فى خليط مع الألوان الطبيعية (أوراق الخبيزة الجافة، ومخلف الطماطم الجاف) بنسبة (١:١) وذلك لتقليل التأثير الضار الذى قد ينشأ أو يصيب الإنسان من جراء الاستخدام المتكرر للون الصناعى. تم تشعيع كل من المواد الخام (الألوان) الطبيعية والصناعية والخليط بينها على جرعات مختلفة (١٠، ١٥ كيلوجراى) للحصول على منتج آمن صحيا. تم عمل تقييم حيوى لدراسة تأثير كل من الألوان الطبيعية والصناعية سواء بمفردها أو فى مخلوط بينها عن طريق إدخالهما فى منتج غذائى هو المكرونة التى استخدمت فى تجربة تغذية للفئران الألبينومع وجود مجموعة كنترول خالية من الألوان. تم بعد انتهاء مدة التجربة التى استمرت ٩٠ يوما حساب الزيادة فى وزن الفئران ووزن كل من أعضاء الفئران (الكبد والكلى والطحال) مقارنة بالكنترول. كذلك تم عمل التقديرات الحيوية المختلفة فى سيرم مجاميع الفئران المختلفة تحت الدراسة، حيث تم تقدير كل من البروتينات الكلية وبروتينات الألبيومين والجلوبيولين ونسبة الألبيومين الى الجلوبيولين والدهون الكلية والكوليستيرول والجلسريدات الكلية وسكر الدم كذلك تم تقدير نشاط الانزيمات الناقلة للأمين بالإضافة لنشاط انزيم الفوسفاتيز القاعدى أيضا تم تقدير كل من نسبة الهيموجلوبين والهيماتوكريت بالدم. وقد أوضحت النتائج أن وجود الألوان الصناعية بمفردها ادى الى حيود معظم القيم التى تم تقديرها عن الكنترول بينما أدى وجود الصبغات الطبيعية عند خلطها مع الصبغات الصناعية الى تقليل ذلك الحيود واقتراب النتائج من الكنترول، مما يدل على أن الألوان الطبيعية قد أدت الى تقليل التأثير السام الناتج من الألوان الصناعية بمفردها.