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Biochemical Studies On The Effect Of Ponceau 4R And/Or Vitamin E Treatment On Young Male Albino Rats

***Eman Helal; *Al-Kassas, M; **Ghada Z A Soliman; *Zahkok, S; *Abdel Wahed, H.**

*Zoology Dep. Faculty of Science, Al-Azhar University (Girls),
**National Nutrition Institute, Cairo, Egypt;

Abstract

Introduction: Due to now a day's high technology, which make foods more attractable to children.
This study aims to study the effect of Ponceau 4R (food colorant) and/or vitamin E (antioxidant) on rats.

Material And Methods: Certain parameters were measured as percentage of body weight change, body temperature, heart beat, Red & white blood cells count, hemoglobin concentration, hematocrite value, serum total lipids, serum cholesterol, serum total protein, serum albumin, globulin, A/G ratio, serum glucose, serum alanine transaminase and aspartate transaminase (ALT & AST) activity and serum total cholinesterase. The organs (brain, liver, kidney, heart and skeletal muscle) were removed, cleared then weighted. Organs were prepared for total protein, total lipid determination and other biochemical analysis.

Results: Body weight, respiration rate was significantly decreased till the 3rd month followed by a significant increase till the 6th month. WBCs count; serum total lipids, liver total lipids, serum glucose, serum cholinesterase activity were significantly reduced. Muscle total lipids, serum total cholesterol, serum total protein, serum total albumin, serum AST activity, liver AST activity were significantly increased.

Conclusion: Due to the hazardous effect of food colourants as Ponceau 4R, it is recommended that the use of Ponceau 4R as food colourant must be limited and use vitamin E as antioxidant to prevent the toxic effect of food colouring substance.

Key Words: Ponceau 4R, Rats, Vitamin E

Introduction

Food additives are substances that are not normally consumed as food by itself or considered as food ingredient. Whether it has nutritive value or not, its addition to foods during manufacture must be according to FAO & WHO (1991) regulations. Antioxidant is a molecule stable enough to donate an electron to trap the free radicl and neutralize it. Vitamin E is natural antioxidant present in foods and stored mainly in adipose tissue (Bagchi and Puri, 1998). It protects polyunsaturated fatty acids in cell membrane from peroxiation, it metabolize free radicls and reduces the risk of disease outcomes and plays a role in cancer prevention and control (Bagchi and Puri 1998; and Metin *et al.*, 2002). Ponceau 4R is food colourant that is used allover the world in great amounts. This colourant (Ponceau 4R) was evaluated for acceptable daily intake by the Joint FAO/WHO Expert Committee on Food Additives (Bär & Grienpentrog 1960; Gaunt *et al.*, 1967; and Stevenson *et al.*, 1980, 1981). Since the previous evaluation, additional data have become available. Metabolism studies indicate that Ponceau 4R is rapidly absorbed from the gastrointestinal tract and undergoes extensive metabolism with the formation of naphthionic acid and 7-hydroxy-8-amino-naphthalene-1,3-disulfonic acid. No marked accumulation of radioactivity in any tissue was found following a single dose of ^{14}C-labelled colouring to untreated rats, mice and guinea pigs or rats given repeated doses of unlabelled colouring for 28 days. Ponceau 4R consists essentially of trisodium-1- (4-sulpho-1- naphthylazo) 2-naphol--6, 8 disulfonate. It is also known

asFood Red 7, C. I. 16255, Cochineal Red A, New Coccine, Acid Red 18, SX purple, and E 124. Ponceau 4R is a synthetic red azo dye which can be used in a variety of food products. Since it is an azo dye, it may elicit intolerance in people allergic to salicylates (aspirin). Additionally, it is a histamine liberator, and may intensify symptoms of asthma. Ponceau 4R is considered carcinogenic in some countries, including the USA, Norway, and Finland, and it is currently listed as a banned substance by the U.S. Food and Drug Administration (FDA)[1], although it is still used in developing countries.

Aim Of The Work

Due to the increasing rate of consuming foods containing food colorants (as Ponceau 4R) especially by children with no control on them, this works aims to study its effect on rats lipid profile, kidney and liver function, and some enzymes and to see if presence of the natural antioxidant as vitamin E can minimize the hazards effect and protect our children.

Material And Methods

The present study was carried out on forty immature growing male albino rats of about one month age. The weight range was from 40-50 g. The animals were kept in clean cages. Food and water were added ad-libitum. The Experimental animals were divided into four groups (10 rats/group) as follows: Group 1(G 1): serve as control group; Group 2 (G 2): supplemented with vitamin E (150 mg/kg BW); Group 3 (G 3): supplemented with Ponceau 4R (0.19 mg/kg BW); Group 4 (G 4): supplemented with Ponceau 4R and vitamin E. These treatments were administered orally by stomach tube for six months. All animal groups were weighted before beginning and monthly during the experiment period. Half of each group was decapitated after 3 month, while the other half were left till the end of 6 months. At the end of the experiment, animals were decapitated and blood samples were taken in dry clean centrifuge tube. Serum was separated and kept at –20 °C until analysis. Blood samples for hematological analysis were taken by heparinized capillary tube. The organs (brain, liver, kidney, heart and skeletal muscle) were removed and cleared in isotonic saline solution then weighted. A piece of each organ were weighted and put in appropriate amount of 30 % KOH for total protein determination and in conc. H_2SO_4 for total lipid determination. Other pieces of organ were weight and put in saline solution then homogenized for biochemical analysis.

Measured Parameters

Certain parameters were measured. Percentage of body weight change was recorded monthly. Heart rates and respiration rate were recorded according to the method of Soliman e*t al.* (1973). Red & white blood cells were counted according to Mitruka *et al.* (1977). Hemoglobin level was determined according to Van Kampen and Zijlstra (1961). Hematocrite value was determined according to Rodak (1995). Serum total lipids were determined according to Knight *et al.* (1972). Serum cholesterol was determined according to Martinek *et al.* (1970). Serum total protein was determined according to Doumas (1975). Serum albumin was determined according to Doumas *et al.* (1971). Serum glucose was determined according to Trinder (1969). Serum alanine transaminase and aspartate transaminase (ALT & AST) activity were determined according to Reitman & Frankel (1957). Serum total cholinesterase was determined according to Gorun *et al.* (1978).

Statistical Analysis

Data are expressed as Mean ±SE. Data were assessed by t-test (Armitage 1974; and Lenter *et al.,* 1982). P-values < 0.05 were considered statistically significant.

Results

Table (1) shows percentage of body weigh, heart rate, rectal temperature and respiration rate changes of male albino rats treated with Ponceau 4R and vitamin E for

[1] "Artificial Food Colouring Warning" , BBC News, May 8, 2007.

three and six months. Data of table (1) shows insignificant change in body weight, heart rate, rectal temperature in rats treated with Ponceau 4R and/or vitamin E in comparison with the control group. Also data of table (1) revealed that a significant decrease ($P < 0.05$) in respiration rate was recorded in rats treated with Ponceau 4R after 3 months and the opposite was noticed after 6 months where a significant increase ($P < 0.05$) was recorded when compared with the control group. Rats treated with vitamin E alone or in combination with Ponceau 4R showed insignificant differences in respiration rate when compared with the control group during the experimental period.

Data of table (2) shows insignificant change in the percentage of organ /body weight changes of male albino rats treated with Ponceau 4R and/or vitamin E for three and six months in brain, kidney and heart. On the other hand, a highly significant decrease ($P < 0.01$) was recorded in hepatosomatic index in rats treated with Ponceau 4R after 3 & 6 months when compared with the control group.

Data of table (3) shows insignificant change in the red blood corpuscles (RBCs) count, hemoglobin (Hb) concentration and hematocrite (Hct) value of rats treated with Ponceau 4R and/or vitamin E for three and six months. Also table (3) revealed non significant difference in white blood corpuscles (WBCs) count in all treated groups after 3 & 6 months in comparison with the control group except rats treated with Ponceau 4R where they showed a significant decrease ($P < 0.05$) after 6 months.

From table (4), it was clear that treating rats with vitamin E, combination of vitamin E and Ponceau 4R revealed insignificant difference in total lipids, total cholesterol, total protein, albumin, globulin levels after 3 & 6 months of treatment. On the other hand, a significant decrease ($P < 0.01$) in total lipid, total protein, albumin and globulin level was observed in rats treated with Ponceau 4R (only) after 3 & 6 months of treatment in comparison with the control group, while rats treated with Ponceau 4 R alone after 3 & 6 months of treatment showed a significant increase ($P < 0.01$) in total cholesterol level in comparison with the control group.

Table (5) shows tissue total lipids (mg/g tissue) levels of male albino rats in organs as brain, liver, kidney, muscle and heart treated with Ponceau 4R and vitamin E for three and six months. It can be seen that rats supplemented with vitamin E showed insignificant difference in total lipid content in brain, liver, muscle, kidney and heart tissues after 3 & 6 months of treatment in comparison with the control group. Rats treated with Ponceau 4R showed a significant decrease ($P< 0.05$) in total lipids of liver tissue, while a significant increase ($P< 0.01$) in total lipids of muscle tissue was observed. Also rats treated with Ponceau 4R combined with vitamin E revealed a significant increase ($P< 0.05$) in total lipids in muscle tissue in comparison with the control group after 3 & 6 months.

Data of table (6) shows that rats treated with Ponceau 4R and/or vitamin E revealed insignificant change in total cholesterol in brain, liver, kidney, muscle and heart tissues after three and six months in comparison with the control group.

Table (7) shows tissue total protein (mg/g tissue) of different organs of male albino rats treated with Ponceau 4R and/or vitamin E for three and six months. Data of table (7) shows that rats treated with Ponceau 4R and/or vitamin E revealed insignificant change in total protein in brain, liver, kidney, muscle and heart tissues after three and six months in comparison with the control group.

Table (8) shows serum glucose, AST, ALT and cholinesterase of male albino rats treated with Ponceau 4R and vitamin E for three and six months. Data of table (8) revealed that rats treated with vitamin E or with dual treatment Ponceau 4R and vitamin E showed insignificant change in serum glucose, AST, ALT and cholinesterase level after 3 & 6 months. Rats treated with Ponceau 4R alone showed significant decrease in glucose level after 3 & 6 months ($P< 0.05$). Serum activity of AST of rats treated with Ponceau 4R showed a significant increase ($P< 0.05$) after 3 & 6 months when compared with the control group. Rats treated with Ponceau

4R alone showed insignificant change in ALT activity after 3 & 6 months. Obtained data (in table 8) revealed insignificant difference in cholinesterase activity in all treated groups after 3 & 6 months in comparison with the control group, except rats treated with Ponceau 4R, which showed highly significant, decrease ($P< 0.01$) in cholinesterase activity after 3 & 6 months.

Table (9) shows tissue AST & ALT activity (U/g tissue) of different organs of male albino rats treated with Ponceau 4R and vitamin E for three and six months. It is clear from the table that rats treated with vitamin E alone or in combination with Ponceau 4R or treated with Ponceau 4R alone revealed insignificant change in AST, ALT activity in brain, liver, kidney, muscle, and heart tissues after 3 & 6 months when compared with the control group except AST activity of rats liver treated with Ponceau 4R alone where it showed a significant increase ($P< 0.05$). Data of the table (9) reveal that despite the insignificant change of AST activity in brain, but the activity is increased insignificantly especially with rats treated with vitamin E alone (41.37 %).

Table (1): Percentage of body weigh, heart beat rate, rectal temperature and respiration rate changes of male albino rats treated with Ponceau 4R and vitamin E for three and six months.

	3 Months				6 Months			
	Control	Vitamin E (150 mg/kg)	Ponceau 4R (0.19 mg/kg)	Ponceau 4R & Vitamin E	Control	Vitamin E (150 mg/kg)	Ponceau 4R (0.19 mg/kg)	Ponceau 4R & Vitamin E
Body Weight	23.56±2.83	25.13±1.30	27.36±2.14	25.39±0.93	22.11±1.27	19.48±1.72	16.42±2.84	20.91±1.59
Heart Beat Rate	315.0±4.01	311.0±3.48	318.0±3.27	314.0±3.73	312.0±3.74	316.0±4.00	310.0±3.16	314.0±4.31
Rectal Temperature	37.35±0.04	37.41±0.07	37.40±0.06	37.42±0.05	37.26±0.05	37.24±0.08	37.27±0.06	37.37±0.08
Respiration Rate	110.6±1.82	115.3±1.59	103.1±1.93*	113.6±1.49	109.4±2.79	113.2±1.96	120.4±3.2*	114.2±2.33

***: Significant, P < 0.05**

Table (2): Percentage of organ /body weight changes of male albino rats treated with Ponceau 4R and vitamin E for three and six months

	3 months				6 months			
	Control	Vitamin E (150 mg/kg)	Ponceau 4R (0.19 mg/kg)	Ponceau 4R & Vitamin E	Control	Vitamin E (150 mg/kg)	Ponceau 4R (0.19 mg/kg)	Ponceau 4R & Vitamin E
Brain	0.95±0.06	1.03±0.12	0.75±0.08	0.78±0.08	0.59±0.01	0.63±0.04	0.58±0.03	0.63±0.03
Liver	3.43±0.10	3.36±0.09	2.90±0.04**	2.99±0.18	3.01±0.08	2.85±0.09	2.61±0.051**	2.92±0.07
Kidney	0.62±0.02	0.64±0.04	0.53±0.04	0.57±0.01	0.65±0.04	0.63±0.04	0.62±0.02	0.56±0.02
Heart	0.34±0.03	0.51±0.18	0.31±0.01	0.29±0.01	0.29±0.01	0.31±0.02	0.33±0.02	0.34±0.03

****: Highly Significant, P < 0.01**

Table (3): Mean values of red blood corpuscles (RBCs) count, white blood corpuscles (WBCs) count, hemoglobin (Hb) concentration and hematocrite (Hct) value male albino rats treated with Ponceau 4R and vitamin E for three and four months.

	3 months				6 months			
	Control	Vitamin E (150 mg/kg)	Ponceau 4R (0.19 mg/kg)	Ponceau 4R & Vitamin E	Control	Vitamin E (150 mg/kg)	Ponceau 4R (0.19 mg/kg)	Ponceau 4R & Vitamin E
RBCs	7.88±0.12	8.06±0.25	7.40±0.18	7.90±0.29	7.50±0.41	6.82±0.31	6.52±0.14	6.74±0.37
WBCs	11.56±0.88	9.90±0.34	8.12±0.78	10.20±0.34	12.32±0.40	11.38±0.24	10.34±0.49*	12.14±0.25
Hb g/dl	15.18±0.59	15.80±0.25	13.25±0.80	16.18±0.22	14.38±0.38	14.40±0.27	12.34±0.86	14.72±0.64
Hct %	47.06±1.93	50.24±1.10	47.72±0.93	50.56±1.67	45.76±1.37	44.62±2.39	42.58±2.17	49.42±1.36

****: Highly Significant, P < 0.01**

Table (4): Serum total lipids, total cholesterol, total protein, albumin, globulin and A/G ratio of male albino rats treated with Ponceau 4R and vitamin E for three and six months.

	3 Months				6 Months			
	Control	Vitamin E (150 mg/kg)	Ponceau 4R (0.19 mg/kg)	Ponceau 4R & Vitamin E	Control	Vitamin E (150 mg/kg)	Ponceau 4R (0.19 mg/kg)	Ponceau 4R Vitamin E
Total Lipids (mg/dl)	352.32±8.21	352.54±12.3	262.7±5.12**	334.36±14.53	357.52±8.05	358.94±8.52	311.78±5.04**	338.78±7.13
TotalCholesterol (mg/dl)	123.24±2.01	122.32±1.54	145.8±5.37**	127.06±3.32	114.34±4.91	118.76±5.81	133.0±2.45**	115.92±2.91
Total Protein (g/dl)	6.90±0.07	6.98±0.12	5.62±0.12**	6.76±0.24	6.76±0.10	6.56±0.13	5.76±0.10**	6.56±0.14
Albumin (g/dl)	4.50±0.08	4.16±0.19	3.60±0.07**	4.25±0.15	4.43±0.07	4.64±0.13	3.64±0.09**	4.48±0.07
Globulin (g/dl)	2.40±0.14	2.28±0.10	2.02±0.07*	2.51±0.14	2.33±0.04	1.92±0.21	2.12±0.16	2.08±0.17
A/G ratio	1.88±0.14	1.48±0.18	1.78±0.05	1.69±0.16	1.90±0.02	2.42±0.34	1.72±0.17	2.15±0.21

*: Significant, P < 0.05, **: Highly Significant, P < 0.01

Table (5): Tissue total lipids (mg/g tissue) of male albino rats treated with Ponceau 4R and vitamin E for three and six months.

	3 Months				6 Months			
	Control	Vitamin E (150 mg/kg)	Ponceau 4R (0.19 mg/kg)	Ponceau 4R & Vitamin E	Control	Vitamin E (150 mg/kg)	Ponceau 4R (0.19 mg/kg)	Ponceau 4R Vitamin E
Brain	79.48±5.63	89.58±3.88	74.86±3.20	76.12±2.89	59.44±4.28	69.84±2.97	62.72±2.27	53.94±3.19
Liver	91.76±3.51	96.10±5.93	74.74±6.41*	77.62±7.46	86.08±4.29	75.50±3.69	65.68±5.47*	73.62±4.44
Kidney	55.92±1.90	60.08±1.00	52.34±1.74	58.58±4.16	62.82±3.61	56.90±2.30	59.84±2.84	59.52±3.10
Muscle	51.08±6.74	65.60±4.30	80.10±2.41**	73.90±3.07**	40.98±2.30	47.24±4.62	58.40±1.98**	51.40±3.00*
Heart	58.60±2.61	63.62±2.88	62.90±5.69	64.36±2.08	53.58±6.50	51.66±3.30	55.90±2.71	45.56±3.83

*: Significant, P < 0.05, **: Highly Significant, P < 0.01

Table (6): Tissue total cholesterol (mg/g tissue) of different organs of male albino rats treated with Ponceau 4Rand vitamin E for three and six months.

	3 Months				6 Months			
	Control	Vitamin E (150 mg/kg)	Ponceau 4R (0.19 mg/kg)	Ponceau 4R & Vitamin E	Control	Vitamin E (150 mg/kg)	Ponceau 4R (0.19 mg/kg)	Ponceau 4R & Vitamin E
Brain	66.42±3.24	79.58±5.64	67.17±6.20	60.95±4.27	80.54±3.89	87.67±3.73	74.62±1.90	72.55 ±3.72
Liver	55.47±2.77	58.84±3.46	59.84±2.49	58.48±3.32	59.39±3.69	60.94±4.73	64.65±5.23	61.25±2.85
Kidney	61.33±2.72	62.43±3.83	62.79±3.91	58.11±3.65	61.56±2.46	63.54±2.22	66.08±2.76	59.94±2.04
Muscle	55.50± 3.79	59.60± 2.82	62.56±2.61	49.92± 2.93	55.97± 4.83	57.16± 2.06	59.74±3.31	53.11±2.18
Heart	64.20± 3.11	63.79± 2.66	63.16± 2.48	58.17±2.27	68.08± 2.49	66.58± 2.09	66.26±2.39	65.10±2.31

Table (7): Tissue total protein (mg/g tissue) of different organs of male albino rats treated with Ponceau 4R and vitamin E for three and six months.

	3 Months				6 Months			
	Control	Vitamin E (150 mg/kg)	Ponceau 4R (0.19 mg/kg)	Ponceau 4R & Vitamin E	Control	Vitamin E (150 mg/kg)	Ponceau 4R (0.19 mg/kg)	Ponceau 4R & Vitamin E
Brain	43.36±1.80	41.28±2.02	38.54±1.50	39.14±1.86	44.06±2.75	39.70±1.80	39.40±3.47	40.44±3.06
Liver	40.06±3.26	33.76±2.89	35.56±2.97	50.28±3.89	49.74±2.81	41.96±2.71	41.88±2.16	44.94±2.79
Kidney	26.04±3.77	29.20±3.32	22.18±2.69	22.20±1.99	44.22±4.44	42.78±3.94	39.34±2.15	42.18±2.38
Muscle	32.62±3.14	36.04±4.36	29.76±1.96	42.16±3.16	42.76±4.30	42.52±3.59	47.32±2.65	39.86±3.83
Heart	33.40±4.44	27.44±1.83	25.84±2.05	27.74±2.59	38.60±2.21	42.62±2.50	43.10±2.43	46,10±2.49

Table (8): Serum glucose, AST, ALT and cholinesterase of male albino rats treated with Ponceau 4R and vitamin E for three and six months.

	3 Months				6 Months			
	Control	Vitamin E I50 mg/kg	Ponccan 4R (0.19 mg/kg)	Ponccau 4R + vitamin E	Control	Vitamin E I50 mg/kg	Ponccan 4R 0.19 mg/kg	Ponccau 4R + vitamin E
Glucose (mg/dl)	99.38±6.34	100.10±8.46	76.4±3.27**	114.30±3.20	96.34±4.16	90.60±3.64	79.60±3.81*	97.22±4.47
AST (U/l)	27.94 ±1.55	29.04 ±0.96	40.6±4.12**	30.20 ±2.00	30.98 ±0.40	28.38 ±1.23	35.40±1.47*	32.64 ±1.21
ALT (U/L)	24.50 ±1.50	26.30 ±1.01	23.30 ±2.78	25.98 ±1.14	24.64 ±1.19	25.50 ±1.32	28.34 ±1.24	25.23 ±0.84
Cholinesterase	11.70 ±0.89	9.22 ±1.19	7.96±0.44**	12.18 ±0.44	10.50 ±1.05	9.62 ±0.74	6.12±0.58**	10.46 ±0.36

*: Significant, P < 0.05, **: Highly Significant, P < 0.01

Table (9): Tissue AST & ALT (u/g tissue) of different organs of male albino rats treated with Ponceau 4R and vitamin E for three and six months.

		3 Months				6 Months			
		Control	Vitamin E I50 mg/kg	Ponccan 4R 0.19 mg/kg	Ponccau 4R + vitamin E	Control	Vitamin E I50 mg/kg	Ponccan 4R 0.19 mg/kg	Ponccau 4R + vitamin E
AST	Brain	17.84±1.92	25.22±2.32	21.40±2.90	22.30±1.91	22.30±1.88	23.02±2.14	24.66±1.10	23.12±2.21
	Liver	20.22±2.49	23.82±2.91	29.82±2.46*	20.60±2.24	21.72±2.61	22.02±1.86	30.62±2.31	19.78±1.67
	Kidney	20.60±2.27	22.90±3.52	23.12 ±2.13	19.24±2.41	21.12±1.38	18.24±1.74	19.56±1.80	18.68±1.61
	Muscle	18.62±1.64	20.26±2.10	20.70 ±2.23	20.82±2.64	18.54±1.68	17.76±1.26	17.32±1.35	18.16±2.25
	Heart	16.52±1.82	16.10±1.33	19.30 ±3.22	18.24±2.49	22.88±2.23	19.40±2.42	22.30±1.88	19.00±2.50
ALT	Brain	16.14±1.36	19.18±1.83	16.04±1.47	16.80±2.00	15.94±0.90	15.48±1.08	15.32±0.95	13.88±0.38
	Liver	14.40±1.48	20.78±2.03	15.28±1.35	13.56±1.60	15.44±1.12	16.24±1.32	16.08±1.23	16.18±1.36
	Kidney	15.22±1.77	21.54±3.44	15.32±1.80	18.92±2.97	16.66±1.13	16.52±1.17	15.78±1.40	16.84±1.37
	Muscle	13.70±1.02	15.34±1.80	15.48±1.36	15.94±2.25	19.98±2.55	16.18±1.33	18.08±1.16	16.24±1.32
	Heart	16.20±1.33	22.36±2.15	21.94±2.23	18.42±2.24	17.84±1.52	18.92±2.69	14.60±0.45	15.52±0.86

*: Significant, P < 0.05

Discussion

The present study is concerned with the effects of synthetic food colorant (Ponceau 4R) as preservative added to foodstuffs. Most of those that are used in developing countries are not permissible (AL-Sharkawi *et al.,* 1996) although now the regulation is towards using antioxidant.

Data of the present study showed that rats treated with vitamin E showed no significant change in body weight, which is in agreement with Meyer *et al.* (1989); and Eder and Kirchgessner (1998) who observed that the level of vitamin E in the diet had no effect on body weight gain. Data of the present study showed that rats treated with Ponceau 4R showed no significant change in body weight, which is in agreement with Brantom *et al.* (1987 a, b) who found no change in body weight of rats fed Ponceau 4R at dietary level (0, 50, 500, 1250 mg/kg BW), but they also found that high dose (toxic level) of Ponceau 4R lower body weight gain without any reduction in food intake. The reduction in body weight may be due to a reduction in food utilization (Grant and Butler 1989) or vitamin C deficiency according to Uchida *et al.* (1990).

The present study revealed insignificant change in rats' heart rate in any treated groups till the end of the experiment. The stability of body temperature may be due to the high ability of rats to adjust their body temperature (Helal *et al.,* 1997). Data of the present study showed insignificant change in rats respiration rate in groups treated with vitamin E alone or with Ponceau 4R. On the other hand, groups treated with Ponceau 4R showed a significant decrease in respiration till the third month then a significant increase was noticed from the fourth month till the end of the experiment. This increment may be due to more oxygen requirement in the in the internal organs and it also attributed to the decrease in the red blood cell count and Hb content (Helal *et al.,* 1997). These are in disagreement with Mcdoniel *et al.* (1993) and Isliker *et al.* (1997) who suggested that supplementation with vitamin E stabilizes the cardiomyocyte as well as the mitochondria

membranes probably by preventing the detachment of their outer membrane. The inner mitochondria membrane is not sensitive to vitamin E depletion since the respiration rate was not affected Isliker *et al.* (1997).

Data in the present study indicate insignificant difference in percentage of heart, brain and kidney organs weight/body weight in all treated groups (vitamin E and/or Ponceau 4R), while hepatosomatic index showed a significant decrease in rats treated with Ponceau 4R. Farag *et al.* (1991) reported no change in the weight of liver, heart, kidney, spleen and brain organs in animals receiving natural antioxidant. They attributed this to the reason that stimulation has not taken place for enzymes necessary to breakdown these antioxidants.

The present work showed insignificant change in red blood cell count, Hb concentration and hematocrite value in groups treated with vitamin E. Moriguchi (1990) found that red blood corpuscles count increased with increasing content of vitamin E. The feeding of Ponceau 4R led to a significant decrease in erythrocyte count and hemoglobin level due to iron deficiency. Also the decrease may be due to the prevention of RBC synthesis via inhibition of erythropoiesis in bone marrow or may be due to iron deficiency anemia, hemolytic anemia, microcytic anemia. These results are in agreement with Mason *et al.* (1974); and Abu El-Zahab *et al.* (1997). The feeding of Ponceau 4R (200 mg/kg BW & 200 mg/L drinking water) led to a significant decrease in erythrocyte counts by 14.2 % (Gautam *et al.* 1986). The feeding of Ponceau 4R led to a significant decrease in WBC counts till the end of the experiment, while rats treated with vitamin E showed insignificant difference. Our results are in agreement with Tan *et al.* (1992) and Soheir *et al.* (1996). The reduction in WBCs count lower the defense mechanism (immune system) which play an important role in attacking and interacting with foreign antigens and initiating a primary immune response.

Data in the present study indicate insignificant change in serum total lipids of rats treated with vitamin E alone or in combination with Ponceau 4R, while treatment with Ponceau 4R alone revealed a highly significant decrease in total lipids in serum, liver. These decreases in total lipids may be due to lipolysis, via stimulation of hormone sensitive lipase (Abd El-Dayem, 2002).

Data in the present study revealed a highly significant increase in serum total cholesterol levels of rats treated with Ponceau 4R till the end of the experiment. A highly significant decrease was observed in heart total cholesterol. The elevation in serum total cholesterol level may be attributed to the blockage of liver bile ducts, causing reduction or cessation of its secretion to the duodenum. Consequently, it appeared in the serum causing cholestasis (Hassan *et al.*, 1995 and Helal *et al.*, 1997). Also the elevation may be due to the mobilization of the free fatty acids from the adipose tissue to the blood stream and increase level of acetyl Co-A, leading to increase in the synthesis of cholesterol. The results are in agreement with the finding of Gomaa (1995); Hassan *et al.* (1995) and Helal *et al.* (1997).

The present study indicate a highly significant decrease in serum total proteins and albumin levels through out the experimental period in rats treated with Ponceau 4R. This reduction may be due to substantially of protein synthesis by the liver, this depression may be due to an alteration in the intracellular protein synthesis mechanism and that the oxidative enzyme changes were probably secondary in altering proteins. These results are in agreement with Hurkat (1977); Shakoori *et al.* (1988). Also Mekkawy *et al.* (1988) and Amr *et al.* (1994) who reported that the decreases of protein might be due to reduction of serum globulin level supports with the disturbances on the immunoglobulin production, these was accompanied by a decrease of body weight gain and this may be a result of toxicity especially on the muscle. The decrease in serum albumin level may be due to loss of protein from the elementary tract, or due to decrease protein formation in the liver (impaired ability of the liver to form albumin). This result were in harmony with that obtained by Said *et al.* (1992), who concluded that the decrease in serum albumin level was due to trap of protein from the elementary tract or due to hepatic

necrosis. Rats treated with Ponceau 4R showed significant decrease in globulin level after 3 months of treatment, which may be due to the disturbance on immunoglobulin production.

Glucose is the key molecule in carbohydrate metabolism. The present data showed significant decrease in serum glucose level of rats treated with Ponceau 4R. This may be due to the effect of Ponceau 4R on cells of pancreas which lead to increase secretion of insulin hormone which reduce glucose in serum or the decrease may be due to liver disease, or due to adrenocortical insufficiency, anterior pituitary insufficiency and hypothyroidism as indicated by Katzung (1995).

The present data showed highly significant increase in serum AST activity of rats treated with Ponceau 4R through out the experiment. Elevation of transferase activity in blood has been considered as an indicator of tissue damage. However, other factors are considered for this process such as alteration in permeability of cell membrane, increasing the synthesis of the enzyme or decreasing the rate of degradation of the enzyme (Dinman *et al.* 1963; Luckens and Phelps 1969). Ignatov (1976) recorded that the elevation in serum AST is due to the degradation and necrosis of liver cells, which is accompanied by a damage of cell wall, cytolysis and so pouring a considerable amount of these mitochondrial enzymes into the blood stream. The present results are in agreement with those obtained by Yani *et al.* (1991); Abd El-Dayem (1992); and Ahmed & Mona (2000).

Generally the activities of ALT & AST are considerably increased following the administration of various hepatotoxic compounds, which lead to acute hepatocellular damage or extrahepatic obstructions by being considered as highly sensitive liver markers (Abu El-Zahab *et al.* 1997). Saleh (1986) related the elevation of ALT & AST activities to the damage of the liver cell, while Abu El-Zahab *et al.* (1997) report that coloring agents induce liver tissue damage and cause a significant increase of ALT, AST & alkaline phosphatase activities in rats serum.

Acetyl cholinesterase inhibition is one of the most important negative effects and the interaction with the enzyme results in acute cholinergic poisoning (Wills 1972; and Howard & Janice 1989). Data of the present work showed that rats treated with Ponceau 4R showed a significant decrease in serum cholinesterase activity after 3 & 6 months of treatment. These decreases may be a sign of metabolic alterations of brain cholinergic synapses, which form a part of an inhibitory mechanism controlling the activity of cholinergic neurons. The inhibitory effect of Ponceau 4R administration on cholinesterase activity could be due to a direct action on the enzyme activity (Abd El-Rahiem *et al.* 1999) or due to hepatic parenchyma disease.

Conclusion

It is clear that the administration of food colouring as Ponceau 4R to rats caused many disturbance in different parameters of rats. It is recommended that the use of Ponceau 4R as food colouring must be limited due to its hazardous effect to the children and human health. It is recommended to use vitamin E as antioxidant to prevent the toxic effect of food colouring substance.

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دراسات كيميائية حيوية على تأثير معاملة بونسيية 4R و/ أو فيتامين اي (E) على ذكور الفئران

إيمان هلال*،محمود القصاص*، غادة زع سليمان**، سمير زيتون*، هشام عبد الواحد**

* قسم علم الحيوان–كلية العلوم–جامعة الأزهر (البنات)–** المعهد القومي للتغذية

المقدمة:

نظرا للتطور الهائل في هذه الايام فان استخدام المواد الملونة بونسيية 4R في الصناعات الغذائية جعل شكل الغذاء اكثر جاذبية للاطفال ، لذا تهدف هذه الدراسة لبيان تأثير بونسيية 4R (مواد ملونة) وبيان التأثير المتعادل لفيتامين اي (E) (مضاد للأكسدة طبيعي) على الفئران.

المواد والطرق:

تم قياس بعض المتغيرات مثل نسبة التغير فى وزن الجسم، درجة حرارة الجسم، معدل ضربات القلب/دقيقة، عدد كرات الدم الحمراء والبيضاء، الهيموجلوبين، الهيماتوكريت، الدهون الكلية، الكوليسترول، البروتين الكلى، الأليبومين، الجلوكوز، آلانين ترانس اميينيز، اسبرتات تراس اميينيز، الكولين استيريز.

النتيجة:

وجد نقص معنوى فى معظم المتغيرات مثل وزن الجسم، معدل التنفس حتى الشهر الثالث ثم اخذ في الزيادة المعنوية حتى الشهر السادس. وجد نقص معنوى في عدد كرات الدم البيضاء والكوليسترول واللبيدات الكلية فى الدم و الكبد ، الجلوكوز، الكولين استيريز. بينما لوحظ ارتفاع معنوى فى كوليسترول الدم، اللبيدات الكلية فى العضلات، البروتين الكلى، الأليبومين، آلانين ترانس امينيز، اسبرتات ترانس امينيز.

الاستنتاج:

نتيجة للتأثيرات العشوائية لمضافات الأغذية مثل بونسيية 4R ، فإنه يوصى بتحديد استخدام بونسيية 4R مع استخدام فيتامين اي (E) كمادة مضادة للأكسدة لمنع التأثير الضار.