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Some Biochemical Parameters Of Young Male Albino Rats Treated With Ponceau 4 R And Vitamin E.

Eman G. E. Helal*, Mahmoud A. El-Kassas*, Samer A. M. Zaahkouk*, Amany F. Mahmoud** and Hesham G. Abdel-Wahed*

Departments of Zoology –Faculty of Science - Al-Azhar University(Girls) * and Faculty of Home Economics- jeddah, K.S.A. **

Abstract

The current study aimed to clarify hazardous effect of ponceau 4R as food colour on rats and the effect of vitamin E as an antioxidant to prevent its toxic effect. Fourty premature growing male albino rats were divided into four groups, the first served as control group, the second supplemented with vitamin E (150 mg/kg),the third treated with ponceau 4R(0.19 mg/kg) and fourth treated with ponceau 4R(0.19 mg/kg) and vitamin E (150 mg/kg). Half of each group was treated for 3 m, the other half was treated for 6 months.

Rats treated with ponceau 4R showed highly significant decrease ($P<0.01$) in serum total lipids, proteins, albumin levels and cholinesterase activity throughout the experimental period, while recorded a significant decrease ($P<0.05$) in glucose level till the end of the experiment and in globulin level and A/G ratio after 3 months of treatment only. On the other hand, significant increase was detected in total cholesterol level ($P < .01$) and AST activity ($P < .05$) after both periods of treatment, while ALT activity showed insignificant change. It seams from the presents study that vitamin E has protective effect against the side effect of ponceau 4R on rats.

Key words: Ponceau 4R , vitamin E, food colour and antioxidant.

Introduction

Food additives are substances that not normally consumed as a food by itself or as a typical ingredient of the food. The importance of chemical food additives has been increasingly emphasized in recent years for a technological purpose in the manufacture, processing preparation, packing and transport *(FAO & WHO,1991)* and also to enhance the quality of food products *(Hannaa and Azzah, 1999).*

But also, food additives are conceded to be one of the difficult problems which cause some toxicological effects on children, specially in the age of nursery, because they used food containing colorants and additives in great amounts which attracts their attention.

Food colourants such as ponceau 4R are (is use all over the world on great amounts) one of synthetic colourants which are derived from chemical substances *(Hannaa and Azzah, 1999).*

Antioxidant is a molecule stable enough to donate an electron to rampaging free radical and neutralize it. Vitamin E is one of the lighter antioxidants found in the diet (mainly stored in adipose tissues, liver and muscle) *(Abdel- Khalek et al., 2003).*

Vitamin E is a principle antioxidant in the body and providing a protective role to prevent oxidantion of unsaturated fats. It plays a role in cancer preventation *(Metin et al.,2003 and Guyton&Hall,2004).*

Many toxicological aspects have been noticed in children in the age of nursery. So, this attracts our attention to study the changes of biochemical parameters of premature rats which produced after long term administration of one resample food colour (ponceau 4R) and to exam one of

natural antioxidant vitamin E to see if it could minimize the additive hazards, if present.

Animal and Methods

***Animals*:**

Forty immature growing male albino rats of local strain with body weight (b. wt.) ranging between (40-50 gm) were used in the current work . Rats were divided into four equal groups:

Group I : Served as control.

Group II : Supplemented with vitamin E (150 mg/kg).

Group III : Treated with ponceau 4R (0.19 mg /kg).

Group IV : Treated with ponceau 4R (0.19 mg /kg) and vitamin E (150 mg/kg).

After three months of treatment, half of the rats of each group was decapitated, while the other half was continuing treatment for three months more, then decapitated and blood sera were collected for determination of total lipids level *(Knight et al., 1972)*, cholesterol *(Martinek, 1970)*, transaminases (aspartate (AST) and alanine (ALT)) *(Reitman and Frankel, 1975)*, total protein *(Doumans, 1975)*,albumin *(Doumas et al., 1971)*, glucose *(Trinder, 1969)* and cholinesterase activity *(Gorun et al.,1978)*.

The statistical analysis of the obtained data was done according to *Armitage (1974) and Lenter et al. (1982*). Significant differences between the means of control and treated groups were considered at $P< 0.05$.

Results

The present study recorded insignif-icant difference in serum total lipid, choles-terol, protein, albumin, globulin, A/G ratio, glucose, AST& ALT levels and choline-sterase activity in rats which were treated with vitamin E and rats which were treated with vitamin E combined with ponceau 4R after three and six months in comparison with the control rats (Tables 1&2).

Otherwise, rats treated with ponceau 4R showed significant decrease ($P<0.01$) in total lipids, total proteins, albumins levels and cholinesterase activity throughout the experimental periods and recorded a significant decrease ($P<0.05$) in globulin level and A/G after 3 months of treatment only. Otherwise, they recorded significant increase in serum cholesterol level ($P<0.01$), and AST activities ($P<0.05$) when compared with control group after three and six months while no significant change was recorded in ALT activity till the end of the experiment (Tables 1&2).

Table(1): Serum total lipids, total cholesterol, total proteins, albumin, globulin and A/G ratio of rats treated with food colour (ponceau 4R) and/or antioxidant(vitamin E) for three and six months.

Periods		Three month period				Six month period			
Paramrters	Treatments	Control	Vitamin E (150mg/kg)	Ponceau 4R (0.19mg/kg)	Ponceau 4R & vitamin E	Control	Vitamin E (150mg/kg)	Ponceau 4R (0.19mg/kg)	Ponceau 4R & vitamin E
Total lipids mg/dl	Mean	352.32	352.54	262.72	334.36	357.52	358.94	311.78	338.78
	± S.E	8.21	12.30	5.12	14.53	8.05	8.52	5.04	7.13
	prob.	------	N.S	0.01	N.S	-----	N.S	0.01	N.S
Total cholesterol mg/dl	Mean	123.24	122.32	145.84	127.06	114.34	118.76	133.04	115.92
	± S.E	2.01	1.54	5.37	3.32	4.91	5.81	2.45	2.91
	prob	------	N.S	0.01	N.S	-----	N.S	0.01	N.S
Total protein mg/dl	Mean	6.90	6.98	5.62	6.76	6.76	6.56	5.76	6.56
	± S.E	0.07	0.12	0.12	0.24	0.10	0.13	0.10	0.14
	prob	-----	N.S	0.01	N.S	-----	N.S	0.01	N.S
Albumin g/dl	Mean	4.50	4.16	3.60	4.25	4.43	4.64	3.64	4.48
	± S.E	0.08	0.19	0.07	0.15	0.07	0.13	0.09	0.07
	prob	-----	N.S	0.01	N.S	----	N.S	0.01	N.S
Globulin g/dl	Mean	2.40	2.82	2.02	2.51	2.33	1.92	2.12	2.08
	± S.E	0.14	0.10	0.07	0.14	0.04	0.21	0.16	0.17
	prob	-----	N.S	0.05	N.S	-----	N.S	N.S	N.S
A/G ratio	Mean	1.88	1.48	1.78	1.69	1.90	2.42	1.72	2.15
	± S.E	0.14	0.18	0.05	0.16	0.02	0.34	0.17	0.21
	prob	-----	N.S	0.05	N.S	-----	N.S	N.S	N.S

Prob. = Probability 0.05 = Significant at P≤ 0.05
N.S = Non significant 0.01 = Highly Significant at P≤ 0.01

Table(2): Serum glucose, AST, ALT and cholinesterase of male albino rats treated with food colour (ponceau 4R) and/or antioxidant(vitamin E) for three and six months.

Periods		Three month period				Six month period			
Paramrters	Treatments	Control	Vitamin E (150mg/kg)	Ponceau 4R (0.19mg/kg)	Ponceau 4R & vitamin E	Control	Vitamin E (150mg/kg)	Ponceau 4R (0.19mg/kg)	Ponceau 4R & vitamin E
Glucose mg/dl	Mean	99.38	100.10	76.40	114.30	96.34	90.60	79.60	97.22
	± S.E	6.34	8.46	3.27	3.20	4.16	3.64	3.81	4.47
	prob.	-----	N.S	0.05	N.S	-------	N.S	0.05	N.S
AST µ/L	Mean	27.94	29.04	40.66	80.20	30.98	28.38	35.40	32.64
	± S.E	1.55	0.96	4.12	2.00	0.40	1.23	1.47	1.21
	prob	-----	N.S	0.05	N.S	------	N.S	0.05	N.S
ALT µ/L	Mean	24.50	26.30	23.30	25.98	24.64	25.50	28.34	25.23
	± S.E	1.50	1.01	2.78	1.14	1.19	1.32	1.24	0.84
	prob	-----	N.S	N.S	N.S	------	N.S	N.S	N.S
Cholin-esterase µ/ml	Mean	11.70	9.22	7.96	12.18	10.50	9.62	6.12	10.46
	± S.E	0.89	1.19	0.44	0.44	1.05	0.74	0.58	0.36
	prob	-----	N.S	0.01	N.S	-----	N.S	0.01	N.S

Prob. = Probability 0.05 = Significant at P≤ 0.05
N.S = Non significant 0.01 = Highly Significant at P≤ 0.01
AST = Aspartat aminotransfere ALT= Alanine aminotransfere.

Discussion

The present study is concerned with toxicological effects of one of synthetic food colouring (ponceau 4R) of food stuffs. Most of food additives used in growing countries are not permissible *(Al-Sharkawi et al., 1996)*. The effects of synthetic colouring (ponceau 4R) to food stuffs on some biochemical parameters of rats were investigated. Antioxidants is any substance that, when present at low concentration compared with those of an oxidisable substrate, significanttly delays or prevent oxidation of that substrate.Antioxidant protective mechanisms can be classified according to their mode (preventative, chain breaking), site (intracellular, extracellular), mechanism (enzymatic and nonenzymatic) of action *(Lavrik et al.,1993 and Grisolia &Tarahashi, 1994)*. The role of an antioxidant vitamin E were also studied throughout the experiment.

Insignificant change in serum total lipids and total cholesterol of rats treated with an antioxidant (vitamin E) and rats treated with vitamin E combined with ponceau 4R may be due to the protective role of vitamin E to prevent oxidation of the hormone-sensitive lipase which regulate lipid and cholesterol metabolism *(Gyton & Hall, 2004 and Ganong, 2005)*.

While, treatment with ponceau 4R revealed a highly significant decrease in serum total lipids and highly significant incr-ease in serum total cholesterol. The decrease in total lipids may be due to lipoysis, via stimulation of hormone-sensitive lipase *(Abdel-Dayem, 2002)*. Otherwise, the elevation in serum total cholesterol level could be attributed to the peroxidation of cell membrane lipids *(Standberg, 1977)* or to the blockage of liver bile ducts, causing reduction or cessation of its secretion to duodenum. Consequently, it appearred in the serum causing cholestasis and also may be due to the mobilization of free fatty acids from the adipose tissue to blood stream and increase level of acetyl Co.A,leading to increase in thesynthesis of cholesterol *(Ganong, 2005)*.

Concerning, serum total proteins and albumin the present data indicated highly significant decrease in rats treated with ponceau 4R till the end of the experiment. This decrease in total protein may be due to substantially of protein synthesis in the liver, this depression may be due to an alternation in the intracellular protein synthesis mechanism and the oxidative enzyme change were probably secondary in altering proteins *(Ganong, 2005)*. This result in agreement with *Amer et al.(1994)* who reported that the decrease of protein may be attributed to reduction of serum globulin level supports with the disturbance on the immunoglobulin production, these was accompanied by a decrease of body weight gain. It may be result of toxicity especially on the muscle. These data were in disagreement with *Al-Shinnawy (1994) and Gomaa (1995)*. The results of the present study showed that treatment with poncea 4R after three and six months period respectivily caused a highly significant decrease of serum albumin level, these decrease may be due to loss of protein fromation from the alimentary tract, or to decrease formation of protein in the liver (impaired ability of the liver to form albumin) *(Ganong, 2005)*.

Rats treated with ponceau 4R revealed significant decrease in serum globulin and A/G ratio after three months periods, these decrease may be due to the disturbance on immunoglobulin production or may be due to block protein synthesis while fast breakdown occurs *(Eremin and Yanni et al., 1981)*. This leads to an increase of free amino acids and to decrease of protein turnover *(Yanni et al., 1991)*.

Glucose is a key molecule in carbohydrate metabolism. It is formed as a result of the digestion of complex carbohydrates or as a reults of synthesis within the body (glucoeogenesis) *(Gyton & Hall, 2004)*.

The present data showed a significant decrease in rats treated with ponceau 4R which showed after three and six months

periods. 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 may be due to liver disease or due to adrenocorticol insufficiency, anterior pituitary insufficiency and hypothyrodisum *(Ganong, 2005)*.

The present results showed that rats treated with ponceau 4R revealed a significant elevation in serum AST activity throughout the experiment which was considered as an indicator of tissue damage and necrosis of liver cells as a result to tissue toxicity which disturbs the integrity of cell membrane resulting an increase of the enzyme level in blood specialy transaminases (AST& ALT) which present within the cytoplasm of living cells *(Luckens and Phelps, 1969, Ignatov, 1976 and Begum & Vijayaraghvan, 1995)*. Or may be due to alteration in permeability of cell membrane, increasing the synthesis of the enzyme or decreasing rate of degradation of enzyme *(Ganong, 2005)*.

Rats treated with vitamin E or vitamin E combined with ponceau 4R recorded insignificant differences in serum AST & ALT activities till the end of the experiment may be related to the protective role of vitamin E in modulating the toxic effect of ponceau 4R on liver function (AST & ALT) and indicated that vitamin E improved liver function *(Ahmed and Mannaa, 2000)*.

Acetyl cholinesterase inhibition is one of the most important negative effects and the interaction with the enzyme results in acute cholinergic poisoning *(Howared & Janice, 1980)*. The results in the present work showed a significant decrease in cholinesterase activity in group treated with ponceau 4R after three and six months. This decrease may be due to a sing of metabolic alternations 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 *(Abdel-Rahiem et al., 1999)*.

From the above mentiond results, it is clear that the administration of food colour (ponceau 4R) to rats caused many disturbance in different biochemical parameters of rats.

Finally, it is recommended that the use of ponceau 4R as food colour must be limited due to hazardous effect to the childs and human health. It is well recommended the use of vitamin E as antioxidant to prevent the toxic effect of food colours.

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دراسة بعض المعايير الكيميائية علي ذكور الجرذان البيضاء الغير بالغة المعالجة بمادة البونسوا 4R وفيتامين هـ .

إيمان جمال الدين عزت هلال * محمود أحمد القصاص * سمير عطية زعقوق *
أماني فوزي محمود ** وهشام جمال عبد الواحد *
*قسم علم الحيوان – كلية العلوم - جامعة الأزهر(بنات) **كلية الاقتصاد المنزلي بجدة .

تهدف الدراسة الحالية لمعرفة التأثير الضار لمادة البونسوا 4R إحدى المواد الملونة التي تضاف إلي الأغذية والمشروبات والأدوية علي الجرذان الصغيرة وكذلك تأثير فيتامين هـ كواحد من مضادات الأكسدة الطبيعية.

ولقد تم تقسيم عدد أربعين جرذا من الذكور البيضاء غير البالغة إلي أربع مجموعات المجموعة الأولي تم استخدامها كمجموعة ضابطة طبيعية ، المجموعة الثانية تم معالجتها بفيتامين هـ (150مجم /كجم) ، المجموعة الثالثة تم معالجتها بمادة البونسوا 4R (0.19 مجم/ كجم) ، أما المجموعة الرابعة فتم معالجتها بمادة البونسوا 4R بالإضافة إلي فيتامين هـ.

وبعد ثلاث شهور من بدأ المعالجة تم ذبح نصف كل مجموعة وترك الباقي لمدة ثلاثة شهور إضافية مع نفس المعالجة.

ولقد أظهرت النتائج نقصاً ذا دلاله إحصائية في مستوي الدهون والبروتينات والزلال (الألبيومين) والكولين استريز و A/G و الجلوكوز وكذلك سجلت انخفاضاً في محتوي الدم من الجلوبيولين بينما أظهرت زيادة ذات دلالة إحصائية في مستوي الدم من الكولسترول ونشاط AST في المجموعة المعالجة بمادة البونسوا 4R عند مقارنتها بالمجموعة الضابطة بينما لم يتغير مستوى نشاط انزيم ALT.

وقد أظهرت الدراسة أن المعالجة بفيتامين هـ وكذلك المعالجة بفيتامين هـ والبونسوا 4R معا ليس له تأثير علي أي من المعايير السابقة بالمقارنة بالمجموعة الضابطة.

و قد أوضحت الدراسة الدور الفعال لفيتامين E فى تقليل التأثيرات الضاره لهذة المادة الملونة.