International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.9, pp 261-270, 2016

Influence of Carmosine (E122) on oxidative stress status and the protective effect of vitamins C and E in male rats

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Abstract : Carmosine (E122) is a pigment structure of one of dyes type, azo (Azo food), which is widely used in ice cream, soft drinks, sauces, fish and meat as well as with desserts and others. The current study was conducted to verify the effects of different doses of the dye E122 on some calibrated physiological: (SOD, GST, GPx, MDA, NO and TAOC).The experiment included 48 rats were divided into eight equal groups (six animals in each group).
The results of the present study recorded, there are a significant decrease in (SOD, GST, Gpx and TAOC) as well as a significant increase in NO & MDA in the positive control group (G3) compared with negative control group. In the same time There are a significant increase in the SOD, GST, GPx and TAOC in the groups that treated with vitamin (C and E) alone , whereas there are significant decrease in the levels of (NO & MDA) compared with positive control group.
Key words : carmosine dye, oxidative stress , vitamin C, vitamin E , male rats.

Introduction:

Food additives have a very important role to meet the needs of the population growth during the preparation and presentation of food was plentiful, tasty and nutritious [(1)].Termination synthetic natural food additives can be used and flavoring or coloring materials [(2)]. Colorings provide the aesthetic appearance of food preferred by consumers [(3)]. Azo dyes have a wide range of applications in the field of textiles, leather, paper, food, pharmaceutical and cosmetic industry[(3)]. Azo dyes are described by azo groups (–N=N) bound to aromatic rings in their chemical structures [(2)].

Carmoisine is used as food colorants in different industries. Carmoisine sky and different as Azorubine, Food Red 3Azorubin S, brillantcarmoisine O, Acid Red 14, or CI 14720 is a Synthetic red food dye from a dye pigments. Usually comes Sodium salt. It is red to maroon powder. It has been used Where food is for the purposes of post-fermentation heat-treated Has a number of E122 carmoisine found in foods such as pudding Marzipan, Swiss roll, jam, preserves, yogurt, jellies, breadcrumbs, cheese blends. It is also present in oraldene mouthwash [(4)].It is used as a red colorant when solubility in water .The chemical composition of disodium 4-hydroxy-3-(4-sulfonat-1-naphthylazo) naphthalene-1sulphonate (chemical formula $C_{20}H_{12}N_2Na_2O_7S_2$ [(5)]. Classify the dye as a factor harmful to humans depends on its ability to generate and reductive cleave aromatic amines, which have a carcinogenic effect and can accumulate through the food chains to interact with body secretions such as saliva and secretions of the stomach. Azo dyes can be reduced to aromatic amines through the small intestine and perhaps by the reduction of azo mammals in the liver or intestine following ingestion of a wall [(6,7)] Carmoisine is nitrous derivatives can be reduced to aromatic amines by intestinal microflora and perhaps by mammalian azoreductase in the liver or in the intestinal wall following ingestion . Aromatic amine can be oxidation by P450 enzyme oxidase to N-hydroxy_derivatives [(8,9)] and this

leading to increase oxidative stress .

Oxidative stress is the result of an increased amount of reactive Oxygen species (ROS) and reactive nitrogen species (RNS) that could cause extensive injuries toCell structures by attacking DNA, proteins and lipid and it is one of the great cause of chronic diseases ([10,11]) .oxidative stress can be result from metabolism of carmoisine dye .ROS can neutralize the antioxidant defense systems including anti-oxidant enzyme, which is one of the first enzymes Antioxidants line of defense materials, such as superoxide dismutase (SOD), Glutathione –S- transferase (GST) and (SOD), glutathione peroxidase (GPx) [12] and the second type of defense again ROS and RNS are anti-oxidant non-enzymes are GSH, Vitamin C and vitamin E that play roles in the protection against oxidative stress.[12,13] Therefore the present study was aimed to investigate the effect of carmoisine dye as food coloring on the level of some serum biochemical parameters in male rats (SOD , GST ,GPx, MDA, NO and TAOC) and study the protective role of vitamins E & C against harmful effects of carmoisine dye.

Materials and methods:

Chemical utilized in the experiment:

E122 dye was obtained from the local Iraqi market imported from Ajanata Chemical Industries (India)

Experimental animals: This study was conducted in the animal house of the Department of Life Sciences / College of Education / University of Al-Qadisiyah.Wister rats of the both sexes (180-200) gram was purchased from the Animal House of the college of Science / university of Babylon. They are maintained under standard conditions (temperature 20-25 °C) at least 2 weeks prior to the study, so that animals could acclimatize to the new environment. The animals were housed in sanitized polypropylene cages (50x35x15) cm with stainless steel grill top, bedded with rice husk containing sterile conditions. They had free access to standard pellet diet and water was provided ad libitum.

Experience Design: The animals are divided into eight different groups in each 6 animals as follows :

Control negative group(C) administered drinking water only for two months.

The first treatment group(G1) administered vitamin C (50 mg / kg BW) for two months .The second treatment (G2)group administered vitamin E (15 mg / kg BW) for two months.Third treatment group (positive group) (G3) administered E122 dye (250 mg / kg BW) for two months.The fourth treatment group (G4) administered Vitamin C (50mg / kg BW) for two months then administered E122 dye (250 mg / kg BW) for two months .fifth treatment group (G5) administered Vitamin E (15mg/kg BW) for two months then administered E122 dye (250 mg / kg BW) for two months. Sixth-treatment group(G6) administered E122 dye (250 mg / kg BW) for two months, then administered vitamin E (15 mg / kg BW) for two months . Seven-treatment group (G7) administered E122 dye (250 mg / kg BW) for two months, then administered vitamin C (50 mg / kg BW) for two months .

Blood sampling:

At the end of the experimental period, blood samples were collected directly from the heart to obtain hemolysis free clear serum. The blood samples were used for the preparation of serum after centrifugation at 1500rpm for 10 min and then kept in clean Eppendorf tubes at -20 °C until analysis of the some biochemical parameters.

Biochemical parameters

Determination of superoxide dismutase (SOD)

The method is based on the SOD ability to inhibit the Epinephrine oxidation to adrenochrome. Absorbance was determined at length 480 nm [14]

Determination of Glutathione-S-Transferase (GST)

The activity of GST was determined depending on the conjugation of GSH with 1-chloro-2,4-dinitrobenzene (CDNB) a hydrophilic substrate was observed spectrophotometrically at 340 nm to measure the activity GST [15]

Determination of Glutathione peroxidase (GPx)

The principle of this method is that the rate of glutathione oxidation by H_2O_2 as catalyzed by the GPx present in the supernatant is determined. The color that develops is read against a reagent blank at the range 420 nm on a spectrophotometer [16]

Determination of Lipid Peroxidation in Serum (Malondialdehyde)

The measuring MDA is based on the reaction with thiobarbituric acid (TBA), for assay by the Spectrophotometric method. MDA reacts with thiobarbituric acid under high temperature (90-100^0 C) and acidic condition, The reaction yields a pink MDA-TBA. The absorbent of the sample read at 532 nm [17]

Measurement of nitric oxide

A spectrophotometric determination using a reaction between nitrite, sulfanilamide and N-(1-naphthyl)ethylene diaminedihydro chloride to produce a pink colored complex which is measured by its absorbance at 548nm [18]

Determination of Total Antioxidant capacity.

Determination by using Elabscience Biotechnology Kit [19]

Statistical analysis

The results are expressed as mean ± SEM. The data were analyzed for statistical significance using SPSS software (version 19) and one-way analysis of variance, (ANOVA) followed by LSD test. Values of $p < 0.05$ were considered statistically significant.

Results and discussion:

Food colors including carmosine are usually added to different commercial food products in order to make them appear more attractive and to achieve the desired color. [20] For safety reasons, there have been recent reductions in the number of permitted food colors. At the same time they are still being used over the world because number of considerations as their low price , effectiveness & their stability. Moreover , the food processing industry uses all types of food colors , but to minimize potential toxicity the amounts of permitted synthetic colors used are strictly limited ([21,22]). The use of certain dyes has been banned as they are well known for their toxicity in experimental animals as consumption of Metanil yellow could lead to degenerative changes in the stomach, ileum, rectum, kidney, liver, ovary & testis. Rhodamine B was shown to cause retardation of growth, hemolysis of red blood cells and degenerative changes in liver & kidney. Sudan dyes were found to be toxic to the liver & produce kidney lesions. Malachite green damage to organs like liver, kidney, heart and spleen as well as lesions of skin, eyes, lungs & bones. All the above colors are also mutagenic and most of them have been identified as potential carcinogens.[7,23,24].So that, to evaluate the toxic influences of food color (carmoisine) on the biological system by measure the activity of some biochemical indicators in experimental rats .

Influence of carmosine(E 122) on the Activity of Superoxide dismutase(SOD)

The result of the present study recorded a significant decrease ($p<0.05$) in the activity of SOD in G3 that giving E122 when compared with the negative control group. Administration of vitamins (C and E) alone (G1,G2)leads to an increase in levels of SOD activity compared with G3 and leads to restore the SOD level near to negative control value. At the same time, when the administration of vitamins two months before giving dye caused no significant increase in the level of SOD enzyme activity compared with G3. But it caused a significant decrease in enzyme compared to the negative control , while giving vitamins under study

two months after giving dye (G6,G7) lead to a significant increase compared to the G3 (Table 1) (Figure -1)

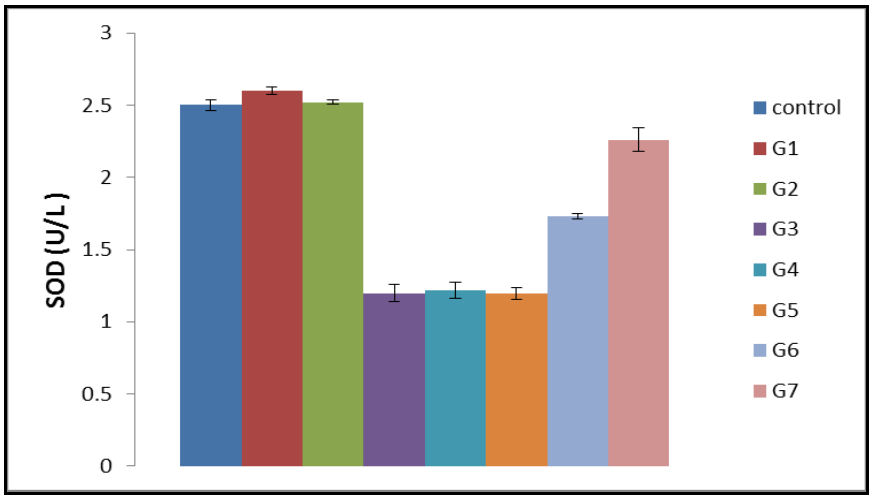


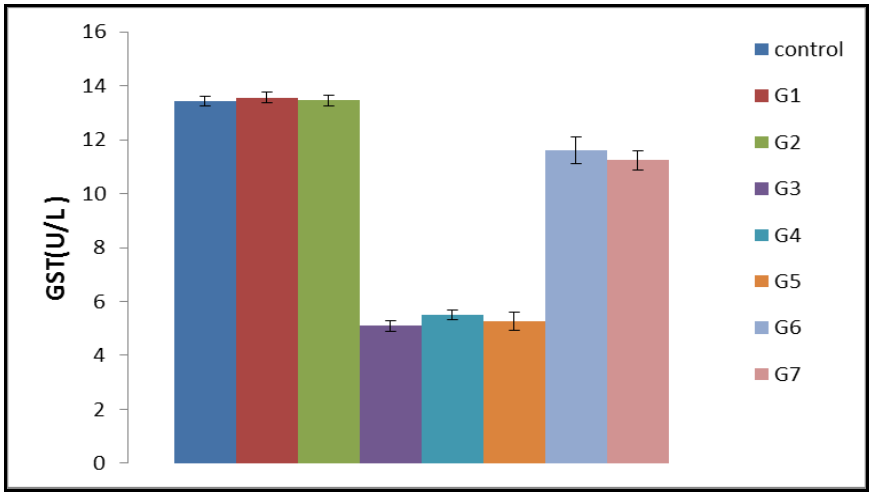
Figure (1) Effect of E122 Dye with & without vitamins on the Activity of SOD (U/L) in Rats.

SOD provide major protection in oxidative stress injury by participating in the cellular system of defense against oxidative damage the levels of free radicals are neutrized by antioxidant enzyme and nonenzyme –scavengers.[25] Carmoisine especially those containing azo dyes and aromatic amine structure are genotoxic and cytotoxic compound because they are metabolized by intestinal flora & produced ROS which increase oxidative stress and decrease in SOD activity [7], but use of vitamins leading to increase in SOD activity

This result may be returned to the use of vitamins (C and E) played salient biological role in depress of dye toxicity through inhibiting the formation of free radical [27], vitamin (C and E) can participate in the redox mechanism of the cell and neutralize ROS[26].

Influence of carmosine (E 122) on the activity of Glutathione –S-transfrase (GST)

The results illustrated that administration of E122 dye in group (G3) leads to cause a significant decrease in level of GST activity when compared with the negative control group. At the same time administration of vitamins (C and E) causes an increase in level of GST activity compared with the positive control group and this result become closer with a negative control values. On the other hand, when giving vitamins two months before the administration of dye, this revealed non- significant increase in the level of GST enzyme activity compared with positive control. But it caused a significant decrease compared to the negative control while giving vitamins under study two months after giving dye cause a significant increase compared to the positive control. (Table1) (Figure -2) Shown the results of GST activity.



Figure(2) Effect of E122 dye with & without vitamins on the activity of GST (U/L) in the rats.

This enzyme is detoxifying enzyme that catalyzed the conjugation of compounds or free radical that result from metabolism of the dye by binding with thiol group of GSH to conversion to less toxic compounds [27] The enzyme protects cells against toxicants by conjugating the thiol group of the glutathione to electrophilic xenobiotics and increased oxidative stress result from metabolism dyes lead to decrease GST[7] but when administration of vitamin (C and E) the oxidative stress was decrease through inhibiting formation of free radical [28]

Influence of carmosine(E 122) on the activity of Glutathione Peroxidase(Gpx):

In regards to glutathione peroxidase activity, the results of the present study showed a significant decrease in the level of enzyme activity compared with the negative control group. Administration of vitamins(C and E) leads to an increase in the level of GPx activity compared with the positive control group and this lead to restored the GPx level near to negative control value. At the same time , when giving vitamins E and C for two months before administration of the dye this lead to caused significant increase in the level of GPx enzyme activity compared with positive control group. But it caused a significant decrease compared to the negative control group.(Table 1) (Figure -3)

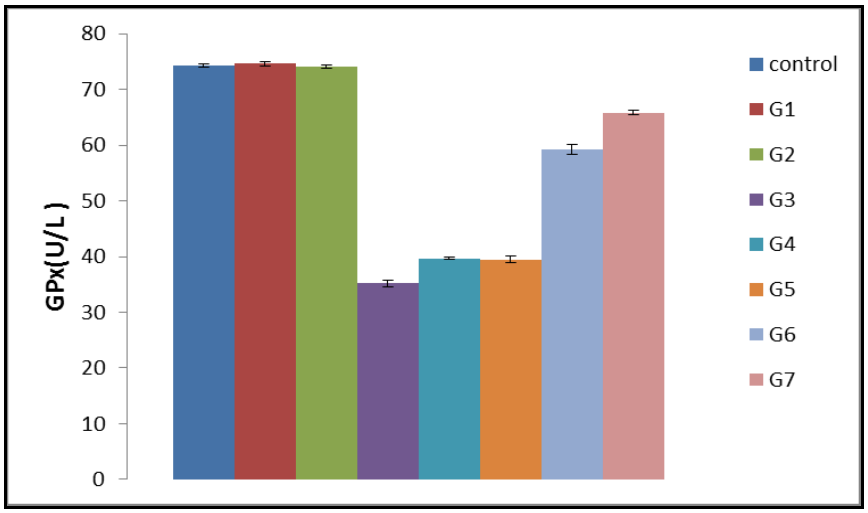


Figure (3) Effect of E122 dye with & without vitamins on the activity of GST (U/L) in the rats.

This results may be due to Gpx prevent free radical damage[29] .It is act as detoxification by conjugated with compound foreigner and reduce lipid peroxidation and conversion to less toxic compound while when used the dye leading to increase oxidative stress through formation free radical from metabolism of dye leaded to decrease activity of GPx [29,30,31] were as the ademenstration of vitamins (C and E) leading to balance reactive oxygen species (ROS) and decrease oxidative stress, then the GPx will be increased [28,29] .

Influence of carmosine(E 122) on the level of Malondialdehyde (MDA)

In regarding to MDA level , the results of the present study showed administration of E122 dye to rats cause significant increase in the level of MDA activity compared with negative control group .administration of vitamins (C and E) leads to decrease in level of MDA activity compared with the positive control group and this lead to restored the enzyme level near to negative control value. At the same time , when giving vitamins C & E two months prior administration of dye this caused significant decrease in the level of MDA compared with control positive, but it caused a significant increase compared to the negative control while giving vitamins under study (C and E) two months after giving dye induce significant decrease compared to the positive control (table 2) (figure 4).

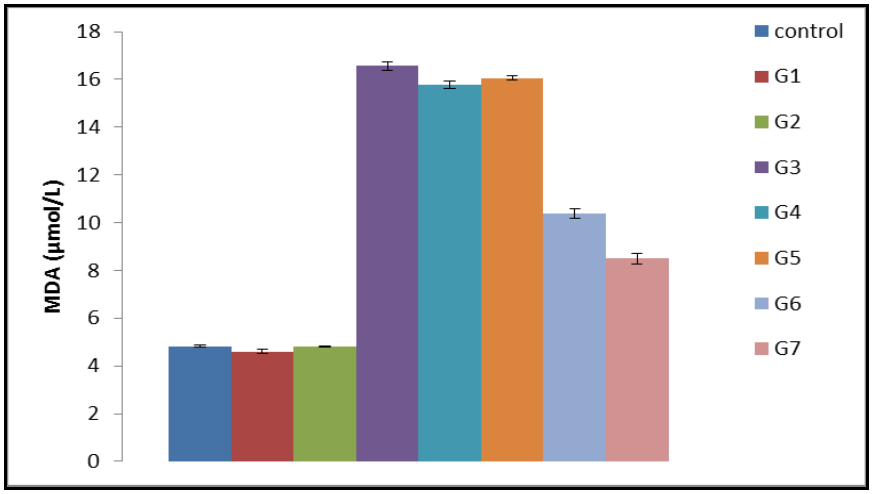


Figure (4) The Effect of E122 dye with & without vitamins on the levels of MDA(µmol/L) in the rats.

The lipid peroxidation is major indicator of oxidative damage initiated by ROS and cause impairment of membrane cell function . MDA level is increase as a product of lipid peroxidation.[32] In the study the increase in the MDA level by carmoisine dye is related to the azo group in dye food colorant, they is metabolized aromatic amine by intestinal bacteria & the aromatic amine can be generate ROS as part of their metabolized by interaction between amine group with nitrite or nitrate containing food[7] . the metabolized of nitrosamine can be produced superoxide , hydroxyl radical and H_2O_2 and increase oxidative stress as the result of the ROS genesis , the MDA levels were increased as product of lipid peroxidation happen by ROS work on lipid [33].

The present study revealed that Use of vitamins (E and C) lead to decrease in the MDA level & this result may be related to the role of the vitamins through decrease oxidative stress and reduction of lipid peroxidation during inhibition of free radical formation[34].

Influence of carmosine(E 122) on the of level Nitric Oxide

The results showed that giving of E122 dye to rats induce significant increase ($P<0.05$) in the level of NO when compared with negative control rats . Administration of vitamins (C and E) alone leads to decrease in $NO^{\cdot}$ level compared with the positive control rats and this result near from negative control values . At the same time , giving of vitamins two months prior to give dye caused significant decrease in the level of NO compared with positive control rats, but it caused a significant increase compared to the negative control. on the other hand, giving of vitamins (C and E) two months after giving of dye produce a significant decrease in the level of NO compared to the positive control but in the same time cause a significant increase compared with negative control (table 2)(figure 5).

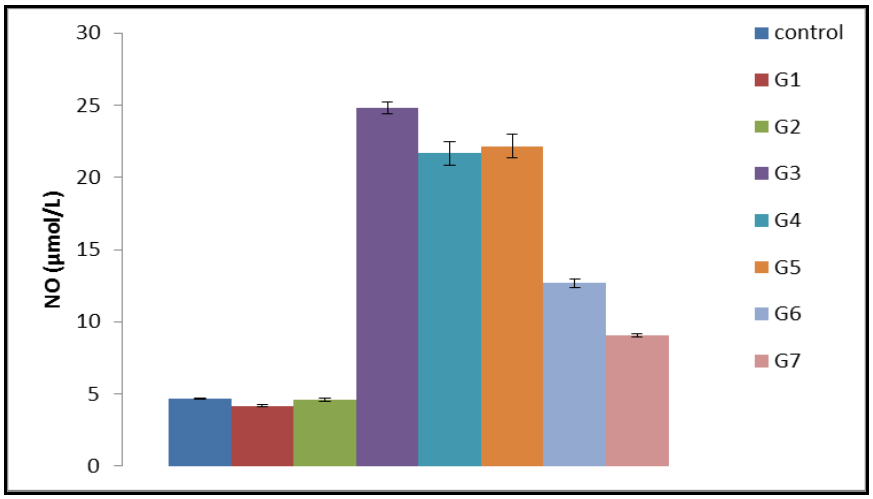


Figure (5) the effect of E122 dye with & without vitamins on the levels of NO(µmol/L) in the rats

The increases of NO levels in the rats group that treated with dye may be related to increase oxidative stress result from exposure to the carmoisine dye when metabolism by (NOS) through the interaction of amino group with nitrite or nitrate- contain food and metabolism of nitrosamine increased oxidative stress. Free radical production by nitric oxide synthase (NOS)during the metabolism of azo dye ,the NO reacting with superoxide leading to peroxynitrite anion [35,36] but NO was decreased by vitamins (C and E) groups because the their role as antioxidant activity through inhibition free radical formation or through redox mechanism of the cell and neutralize reactive oxygen space (ROS). The primary role of vitamins C and E are to neutralize free radical . it can work both inside and outside the cell to combat free radical damage , the free radical will required out an electron to regain their stability , vitamin C and E are a prime source of electrons , therefor vitamins can be donated to free radical like superoxide and hydroxyl radical and inhibited their reactivity.[34]

Influence of carmosine(E 122) on the level of Total Antioxidant Capacity (TAOC)

The results showed that giving of E122 dye to rats lead to cause a significant decrease in TAOC levels compared with negative control while the administration of vitamins (C and E) leads to an increase in TAOC level compared with the positive control and this result near from negative control values. At the same time , when administration of vitamins two months prior to giving of dye caused significant increases in the level of TAOC compared with positive control , but it caused a significant decrease in the parameter compared to the negative control while giving vitamins (C and E) after two months of giving dye induce a significant increase compared to the positive control but decrease a significantly when compared with the negative control (Table 2)(Figure 6)

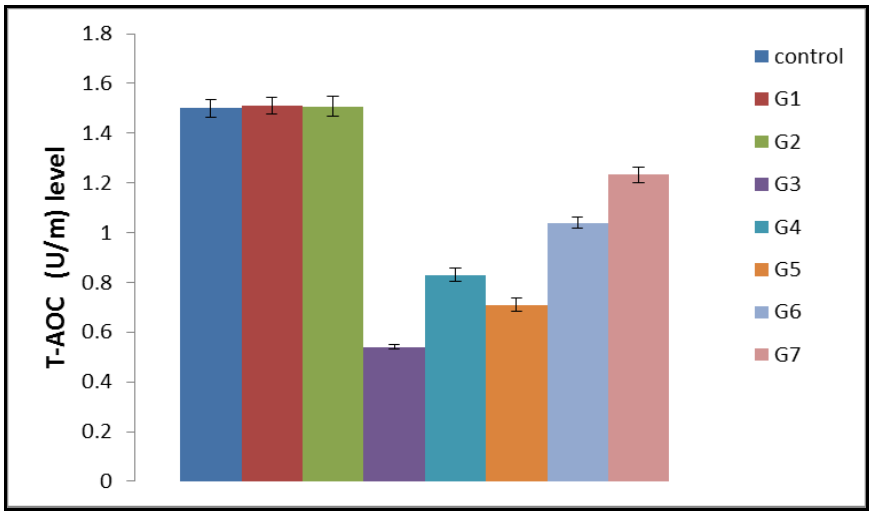


Figure (6) Effect of E122 Dye with & without Vitamins on the Levels of TAOC(U/ml) in the Rats

The reducing levels of TAOC may be related to increase oxidative stress that results from production of free radical by the exposure to the dye through oxidative stress that increased formation of reactive oxygen species in rats lead to decreased total antioxidant capacity [7,34] but when administration of vitamins (C and E) orally plays important role in decrease oxidative stress through inhibiting formation of free radical, so this lead to increase TAOC. Vitamin C is considered as the most important water-soluble antioxidant in extracellular fluids due to its hydrophilic properties. It is capable of neutralizing ROS in the aqueous phase before the initiation of lipid peroxidation. At the same time Vitamin E is the major lipid soluble antioxidant and the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation and LDL from oxidative attack. Vitamin C has been cited for its capability in regenerating vit.E and thus restores its original function in the antioxidant network this leading to increase to T-AOC. [28]

Table(1) influence of carmosine (E122) dye on the activity of SOD , GST and GPX in the rats

Groups	Parameters								
	SOD (U/L)			GST (U/L)			GPX (U/L)		
	Mean ± SE	Lower	Upper	Mean ± SE	Lower	Upper	Mean ± SE	Lower	Upper
Control	2.5 ± 0.04 [a]	2.39	2.63	13.43 ± 0.16a	13.02	13.84	74.28 ± 0.23a	73.68	74.88
G1	2.6± 0.029 [a]	2.53	2.68	13.566 ± 0.21 [a]	13	14.12	74.66 ± 0.39[a]	73.64	75.68
G2	2.52 ± 0.016 [a]	2.47	2.56	13.45 ± 0.2a	12.87	14.02	74.08 ± 0.32[a]	73.25	74.91
G3	1.196 ± 0.06 [b]	1.02	1.367	5.1 ± 0.2 [b]	4.66	5.7	74.28 ± 0..23[a]	33.77	36.56
G4	1.22 ± 0.055 [b]	1.08	1.366	5.5 ± 0.18 [b]	5.08	6	74.66 ± 0.39[a]	39.12	40.2
G5	1.197 ±0.04 [b]	1.06	1.31	5.27 ± 0.35 [b]	4.36	6.1	74.08 ± 0.32[a]	37.92	41.04
G6	1.73 ± 0.02 [c]	1.67	1.78	11.6 ± 0.5 [c]	10.305	12.9	74.28 ± 0..23[a]	56.92	61.4
G7	2.26 ± 0.08 [d]	2.21	2.31	11.23 ± 0.36 [c]	10.302	12.16	74.66 ± 0.39[a]	64.8	66.86

Similar letters denote non-significant differences (p>0.05)
Different letters denote to significant differences (p<0.05)

Table(2) influence of carmosine (E122) dye on the level of MDA ,NO and T-AOC in the rats

Groups	Parameters								
	MDA (µmol/L)			NO (µmol/L)			T-AOC (U/ml)		
	Mean ± SE	Lower	Upper	Mean ± SE	Lower	Upper	Mean ± SE	Lower	Upper
Control	4.82 ± 0.05a	4.69	4.95	4.65 ± 0.05[a]	4.5	4.79	1.5 ± 0.036 a	1.4	1.59
G1	4.6 ±0.09[a]	4.35	4.8	4.18 ± 0.06 [a]	4.02	4.33	1.51 ±0.035[a]	1.42	1.69
G2	4.82 ± 0.02[a]	4.76	4.87	4.6 ± 0.08 [a]	4.4	4.86	1.51 ± 0.04 [a]	1.4	1.61
G3	16.56 ± 0.18[b]	16.08	17.05	24.83± 0.4[b]	23.8	25.86	0.54 ± 0.01[b]	0.5	0.58
G4	15.78 ± 0.16[c]	15.36	16.2	21.66 ±0.8[c]	19.6	23.73	0.83 ± 0.028 [c]	0.75	0.9
G5	16.05 ± 0.095[c]	15.8	16.29	22.166 ± 0.79[c]	20.12	24.2	0.71 ± 0.026 [d]	0.65	0.78
G6	10.4 ±0.2[d]	9.9	10.93	12.66 ± 0.33[d]	11.8	13.5	1.04 ± 0.023 [f]	0.98	1.1
G7	8.5 ± 0.23[f]	7.92	9.11	9.03 ± 0.1[f]	8.77	9.2	1.233 ± 0.03 [g]	1.14	1.31

Similar letters denote non-significant differences (p>0.05)
Different letters denote to significant differences (p<0.05)

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