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Alterations in Oxidative Stress and Antioxidantin Albino rats Treated with Individual and Combined Various Food Additives

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Abstract

Aim: food additives are substances of natural or synthetic origin, which are added to foods to serve a certain technological or sensory function. this work was aimed to investigate the effects of individual and combined treatment of artificial food additives on oxidative stress, antioxidant parameters in male juvenile albino rats. **Exparimental Design**:Thirty youngmale albino rats weighing around 90-110 gm. were divided into six groups (5 /cage); Group I: (Control untreated group), Group II: treated with MSG (300mg/kg), Group III: treated with Sodium benzoate (5mg /kg), Group IV: treat with Carmoisine (4mg/kg), Group V: treated with EDTA (2.5mg/kg), Group VI: treated with mixture of MSG (300/kg), Carmosine (4mg/kg), Sodium benzoate (5mg/kg) and EDTA (2.5 mg/ kg) simultaneously. All the treatments for 28 days. **Results:**The obtained results revealed elevation in oxidative stress markers malondialdehyde MDA and protein carbonyl PC in individually treated MSG and sodium benzoate and remarkable in mixture group. Significant reduction in catalase, total antioxidant capacity, and glutathione content were recorded in individually treated groups and pronounced in mixture group.**Concolusion**: food additives affect adversely on oxidative stress and antioxidant markers that may cause adverse health effects. It is advisable to limit the uses of commodities containing these additives.

Keywords: Food additives; Monosodium glutamate; Sodium benzoate; carmosine; EDTA; Oxidative stress.

Introduction

Food additives are substances which is use in food industry to keep consistency, texture, taste, color, quality, alkalinity or acidity **(Nwajei et al, 2015).**There are many of food additives which fall broadly into two main categories depending on their purpose (i) safety and prevention of degradation of food by bacteria, oxidation or chemical reactions or (ii) improvement of the taste, appearance or mouth-feel of the product **(Emerton and Choi, 2008).** They are classified according to their function such as: Preservatives, flavor, color and antioxidant **(El-Samrages, 2012).**

Food flavor: enhance flavor, include sweet, sour, bitter, and salty as monosodium glutamate (MSG) **(Tawfek et al, 2015).** MSG it is one of the principal food flavors. it has number 621 with Chemical formula $C_5H_8NNaO_4 \cdot H_2O$. It is White, practically odorless crystals or powder **(Husarova and Ostatnikova, 2013).**

Food preservatives: increase food safety and prolong shelf life by inhibition viral, bacterial, and fungal growt has sodium benzoate. **(Tawfek et al, 2015).**

Sodium benzoate It is the sodium salt of the non-essential aminoacid glutamic acid, sodium benzoate it has number E211. IT is the sodium salt of benzene carboxylic acid (benzoic acid) with the chemical formula $C_7H_5NaO_2$ **(Mpountoukas et al, 2008; Shahmohammadi et al, 2016).**

Food color: substance that promote color added to food, drink and candyas Carmoisine **(de Boer, 2014).** Carmoisine has E122, it is a synthetic red food dye from the azo dye group with the formula $C_{20}H_{12}N_2Na_2O_7S_2$. it is a red to maroon powder **(Amin, 2010).**

Food antioxidant: substance additional to fats and fat-containing to retard oxidation and thereby prolong their wholesomeness, palatability, and sometimes keeping time for example, Ethylenediamine Tetra Acetic Acid (EDTA) **(Dalton, 2002).** EDTA it has E385 and its salts are substituted diamine. It is a white, odorless, no hygroscopic crystalline powder and has formulas $C_{10}H_{16}N_2O_8$ **(Dalton, 2002).**

The goal of this study is to determine the oxidative stress and antioxidants levels after treatment with different artificial food additives in case of individual and combined exposure to male juvenile albino rats.

Materials and Methods

Treatments:

Monosodium glutamate (MSG): white colored substance used as flavor enhancer was gained from Sigma chemical Co (USA) the dose is ADI of MSG (300mg/kg b.wt /day) **(Geha et al, 2000).**

Sodium benzoate: white colored substance used as preservative and it was collected from chemical Co (USA) Sigma the dose is ADI of Sodium benzoate (5mg /kg b.wt /day) **(Shahmohammadi et al, 2016).**

Carmoisine: red color substance used as coloring food and it was gained from Star Aromatic Company for flavors and fragrance food colors (Egypt), the dose is ADI of Carmoisine (4mg/kg b.wt /day)**(Larsen et al, 2009)**. Ethylene diamine tetra acidic acid (EDTA): white colored substance used as antioxidant and it was obtained from central Drug house (India), the dose is ADI of EDTA (2.5mg/kg b.wt /day) **(Van et al, 2014)**. All chemicals were liquefied in distilled water.

Animals and Experimental Design:

Young male albino rats Rattus *Norvigecus* weighing around 90-110 g. thirty rats were obtained from the animal house of the King Fahd Medical Research Center (KFMRC), Jeddah, KSA.The animals were acclimatized according the lab conditions and allowed to adjust to the environment for two weeks before starting the experiment. All animals were treated according to the standard procedures laid down by OECD guidelines 407 repeated dose 28 days oral toxicity study in rodent. Animals were randomly divided into six groups of five rats each as follows: **Group I (control group):** untreated group each rat was given distilled water. **Group II**: rats were orally treated with ADI of MSG (300mg/kg b.wt /day). **Group III**: rats were orally treated with ADI of Sodium benzoate (5mg /kg b.wt /day). **Group IIV**: rats were orally treated with ADI of Carmoisine (4mg/kg b.wt /day). **Group V:** rats were orally treated with ADI of EDTA (2.5mg/kg b.wt /day). **GroupVI**: rats were orally treated with mixture of ADI of MSG (300/kg), ADI of Carmosine (4mg/kg), ADI of Sodium benzoate (5mg/kg) and ADI of EDTA (2.5 mg/ kg) simultaneously. All treatments were performed orally for 28 days.

Blood sample collection:

When 28 days treatments ended. Blood sample were taken from orbital vein by glass capillaries according to and collected in centrifuge tubes and centrifuged at 3600 rpm for 15 min. Serum samples were immediately stored at -20^{o}c in Eppendorf tubes till used for analysis of biochemical parameters. After that animals were sacrificed, and the samples of pancreas were enucleated for tissues examination studies.

Biochemical analyses:

Malondialdehyde (MDA) marker of lipid peroxidation was measured in serum of rats by method of **Ohkawa et al. (1979).** Activity of Protein carbonyl **(Yan et al., 1995)** and Catalase assay **(Aebi,1984)** and Total antioxidant capacity TCA **(Koracevic et al., 2001)** were measured in plasma respectively. Serum total GSH **(Hu and Dillard, 1994)** determined, and oxidized GSSG. All kits were obtained from Biodiagnostic company diagnostic and research reagents (Egypt)

Statistical analysis:

Using one-way analysis of variance (ANOVA) included in statistical package (SPSS) program, version 23. The gained data were analyzed. the demonstrated results were showed as Mean ± S.E of the mean.

Results

Presented data in Table (1) depicted that treatment with each of monosodium glutamate MSG 300 mg/kg and sodium benzoate 5mg/kg induced remarkable elevation in oxidative stress marks malondialdehyde MDA and protein carbonyl PC this elevation is significant as compared with control at $P < 0.05$. However, treatment with food coloring Carmosine 4mg/kg and ethylenediamine tetra acetic acid salt EDTA 2.5mg/kg induced significant decrease in MDA when compared with control and other groups. PC recorded nonsignificant change with the same treatments. On the other hand, rats treated with mixture of the previous fore mentioned food additives (MSG, sodium benzoate, Carmosine and EDTA) remarkable significant elevation in both examined parameters (MDA & PC) against control and all experimental groups at $P < 0.05$.

Table (1): Effect Of MSG, Sodium Benzoate, Carmosine, EDTA And Their Mixture on Oxidative Stress markers in Serum of Experimental Animals.

Parameters groups	Lipid peroxide (nmol/ml)	PC (nmol/ml)
Control	**4.49 ±0.17**	**4.11 ± 0.16**
MSG (300mg/Kg)	**6.96 ± 0.15^{a}**	**8.20 ± 0.415^{a}**
Sodium benzoate (5mg /kg)	**5.90 ± 0.17^{a}**	**4.41 ± 0.21^{ab}**
Carmosine (4mg/kg)	**2.93 ± 0.16^{ab}**	**4.15 ± 0.23^{abc}**
EDTA (2.5mg/kg)	**3.99 ±0.27^{abcde}**	**4.49 ± 0.15^{abcd}**
Mixture	**5.64 ±0.18^{abcde}**	**7.56 ± 0.44^{abcd}**

All groups were demonstrated as mean ± SE for 5 rats
a significance difference against control at $P < 0.05$.
b significance difference against MSG group at $P < 0.05$
c significance difference against Sodium benzoate group at $P < 0.05$
d significance difference against Carmosine group at $P < 0.05$
e significance difference against EDTA at $P < 0.05$

Demonstrated data in Table (2) declared reduction in the activity of catalase enzyme in serum of all treated groups pronounced reduction in catalase activity was recorded in Carmosine, EDTA and mixture groups. This reduction was significant against control and other groups at $P < 0.05$. main while, total antioxidant capacity (TAC) level in serum of MSG, Carmosine and mixture treated groups recorded significant decrease versus control and between groups. Furthermore, significant depletion in total glutathione content (GSH) was recorded all through the experimental groups pronounced in mixture treated group significant against control and between groups at $P < 0.05$. remarkable elevation in serum oxidized glutathione GSSG in most of the treated groups pronounced in mixture group. significant elevation in GSSG was recorded against control and other groups at $P < 0.05$. reduction in GSH levels in serum of treated rats with each of MSG, sodium benzoate, Carmosine, EDTA and their mixture was declared. In table (2) concomitant with elevation of GSSG.

Difference the ratio of GSSG/GSH level reflect the pronounced reduction in total reduced glutathione as previously demonstrated.

Table (2): Effect Of MSG, Sodium Benzoate, Carmosine, EDTA And Their Mixture on Antioxidant Markers in Serum of Albino Rats

Parameters groups	Catalase (U/L)	TAC (mmol/ml)	total GSH (mmol/ml)	GSSG (mmol/ml)	reduced GSH (mmol/ml)
Control	276.72±9.07	0.20±0.003	9.22±0.11	0.65±0.03	8.62±0.12
MSG (300mg/Kg)	240.07±12.06	0.02±0.001[a]	4.51±0.27[a]	1.31±0.06[a]	3.43±0.38[a]
Sodium benzoate (5mg /kg)	232.82±11.10	0.19±0.002[b]	8.32±0.50[b]	0.81±0.04[b]	7.51±0.48[ab]
Carmosine (4mg/kg)	71.911±3.63[abc]	0.02±0.001[ac]	7.75±0.43[ab]	0.51±0.04[bc]	7.16±0.43[ab]
EDTA (2.5mg/kg)	126.90±46.65[abc]	0.205±0.003[bd]	7.85±0.08[ab]	0.77±0.05[bd]	7.04±0.09[ab]
Mixture	215.75±4.72[ade]	0.11±0.01[bcd]	5.67±0.34[abcde]	1.32±0.10[acde]	4.31±0.27[acde]

All groups were demonstrated as mean ± SE for 5 rats
a significance difference against control at $P < 0.05$
b significance difference against MSG group at $P < 0.05$
c significance difference against Sodium benzoate group at $P < 0.05$
d significance difference against Carmosine group at $P < 0.05$
e significance difference versus EDTA at $P < 0.05$

Discussion

Artificial additives of food have an important role in different food commodities. The purpose of using these additives is keeping the shelf life of food longer, progress the taste, texture, constituents and color as well as prevent the deterioration of food by bacteria present in containers **(Houghton, 2002).** Side effects of using these chemicals are allergies, stomach pains, vomiting, relaxing problems, hives furthermore skin rashes. The current work examines the effect of individual and combined administration of different food additives on induction of the free radicals and effect on antioxidant enzymes and the consequence effect on pancreatic enzymes and insulin hormone. The results revealed that treatment with each of monosodium glutamate and sodium benzoate induced remarkable elevation in oxidative stress markers malondialdehyde MDA and protein carbonyl PC. concomitant with these findings reduction in some measured antioxidant markers Catalase, total antioxidant capacity as well as total glutathione content and reduced glutathione. elevation in oxidized glutathione was also reported. These results run parallel with **(Selvakumar et al., 2006). (Okwudiri et al, 2012; Onyema et al., 2006)** who reported that male Wister rats treated with MSG at a dose of 4g/kg body weight for ten days showed elevation in lipid peroxidation may be refer to a direct effect of propagation of ROS resulting from MSG treatment. Also, **Villagarcía et al, (2016)** recorded significant increase in protein carbonyl in newborn male pups of Wistar rats treated with 4mg/g BW MSGthey also reported reduction in GSH and antioxidant enzymes in MSG treated Wister rats. Meanwhile, **Khoshnoud, et al; (2017)** reported significant elevation in MDA and reduction in GSH levels in mice administrated different concentrations of Sodium benzoate (0.56, 1.125, and 2.25 mg/mL) for 4 weeks.

On the other hand, our treatment with food color Carmosine and antioxidant EDTA induced decrease in serum MDA and non-significant changes in protein carbonyl. **Elgazar, (2013)** agree with our findings color and antioxidant food additives did not induce elevation in oxidative stress markers and in total glutathione and antioxidant enzymes. On the other hand, the results declared that treatment with mixture of MSG, sodium benzoate, Carmosine and EDTA induced remarkable significant elevation in oxidative stress examined parameters (MDA & PC) in addition to the reduction in antioxidant enzymes and GSH. Meanwhile, **Helal et al., (2017)** reported induction in oxidative stress biomarkers after oral administration with the mixtures of food additives MSG, NaNO2 and annatto to young male albino rats for 30 days. additives to food produces significant alterations in antioxidant enzyme activity. whereas, **Sivaramakrishnan et al. (2008)** explained that Glutathione is important to preserve the reduced state of cells and to abolish all the damage effects of oxidative stress. GSH may be key in A large number

living exercises including those detoxifications about endogenous and exogenous mixes. Over production in ROS is the results of increased consumption of food additives and reduction of antioxidants, such as glutathione **(Chaves et al, 2008).** The major intracellular antioxidant is the reduced form (GSH), glutathione (glycyl-cysteinyl-γ-glutamate) and a major and detoxifying agent **(Reid and Jahoor, 2001).** oxidation of GSH into glutathione disulfide (GSSG) is the result of ROS. The intracellular glutathione concentration is the final result of a balance between GSH generation (via de novo synthesis and recycling from GSSG by glutathione reductase) and the combined rate of GSH consumption by ROS and excretion of the resulting GSSG **(Griffith, 1999).** Under normal conditions 95% of the intracellular glutathione is present in its reduced GSH form **(Reid and Jahoor, 2001).** The oxidized GSSG form can either be recycled to GSH or removed from the intracellular environment by certain transportation mechanism that explain the increase in serum GSSG and reduction in GSH recorded in the present study.

Conclusion

Our study has demonstrated that oral administration of individual and mixtures of different types of food additive resulted in a vehement disturbance in antioxidant enzymes system that stimulate oxidative stress. So, due to the hazardous effect of food additives, it is recommended to limit their uses.

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