

## Adverse Effects of Two Kinds of Food Additive Mixtures (Flavor Enhancer, Food Preservative or Food Coloring Agent) on Physiological Parameters in Young Male Albino Rats

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### ABSTRACT

**Background:** food additives are substances used in food industry in order to improve the food's taste, appearance by preserving its flavor and preventing it from souring. Food additives are added to the most junk and fast foods, especially food for kids.

**Aim of the work:** this work was aimed to investigate the adverse effects of some food additives on the biochemical parameters in addition to study the side effects of these food additives in male albino rats.

**Materials and Methods:** this study was performed on twenty four young male albino rats with an average 120-145 g body weight. Animals were divided into four groups (6 /cage); **Group I** (Control untreated group), **Group II** (administered orally with sodium nitrite (0.1 mg/kg b.wt./day) and annatto (0.065 mg/kg b.wt./day)), **Group III** (administered orally with sodium nitrite (0.1 mg/kg b.wt./day) and monosodium glutamate (MSG) (15 mg/kg b.wt./day)) and **Group IV** (administered orally with annatto (0.065 mg/kg b.wt./day) and monosodium glutamate (15 mg/kg b.wt./day)). Blood samples were collected on the last day of experiment, for biochemical estimations which included levels of serum glucose, total protein (TP), albumin, creatinine, urea, testosterone, thyroid hormones (T3 and T4), activities of AST and ALT in addition to lipid profile.

**Results:** showed marked elevation in levels of fasting glucose, activities of AST, ALT, urea, creatinine, total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-C), VLDL and ratios of TC/HDL-C and LDL-C/HDL-C (risk factors) as well as albumin / globulin ratio and serum thyroid hormones (T3 & T4) accompanied with marked decline in levels of serum total proteins, albumin, globulin, albumin/ creatinine ratio, testosterone and HDL-C in all treated groups in comparison to the control group. There was a significant reduction in the body weight in groups that received (NaNO<sub>2</sub> with annatto) and (MSG with NaNO<sub>2</sub>) while treated rats with (MSG and annatto) showed a significant increase in body weight as compared to control rats.

**Conclusion:** Due to the harmful effects of food additives, the use of these compounds must be limited as it resulted in a vehement disturbance in the biochemical and physiological parameters that was grievously pronounced on many hormones.

**Key Words:** Food Additives, Thyroid hormones, Monosodium Glutamate, Biochemical parameters, Coloring agent.

### INTRODUCTION

Food additives are substances intentionally added to food that changes its characteristics, maintain and improve safety (preservatives), improve or maintain nutrient value and also improve taste, texture and appearance<sup>(1)</sup>. Food additives are divided into five broad categories according to their function: 1) Taste enhancers, 2) Antioxidants, 3) Preservatives, 4) Stabilizers and emulsifier and 5) Coloring agents<sup>(2)</sup>. These additives may come from natural or synthetic origin<sup>(1)</sup>.

Synthetic dyes and textile finishing agents have come under severe criticism for their high environmental pollution effects at the stage of manufacturing as well as application<sup>(3)</sup>. Therefore,

there is a world-wide interest generated in the development of natural dyes<sup>(4)</sup>. Annatto pigment is one of the most important natural colorant agents that is derived from *Bixa orellana* L. (*B. orellana*) and used in food industries, textiles, cosmetic and pharmaceutical products<sup>(5)</sup>. *Bixa orellana* L. (*B. orellana*) is an evergreen tree belonging to family Bixaceae that growing in several tropical countries of the world<sup>(6)</sup> and recognized for its medicinal applications as an antioxidant, analgesic, wound healer, homeostatic, hypoglycemic, as an antidote in snake bite, cough treatment, and diuretic among others<sup>(7)</sup>. As far as, it is commonly used by urologists for prostate

cancer prevention<sup>(8)</sup>. Annatto dye has two major carotenoids including Bixin (fat-soluble pigment) and norbixin (water soluble pigment) that considered as the important food colourants<sup>(9)</sup>.

Likewise, one of the principal food flavors is Monosodium glutamate (MSG) which is commonly consumed as a flavor enhancer or food additive improves the palatability of meals<sup>(10)</sup>. MSG is the sodium salt of the non-essential amino acid glutamic acid<sup>(11)</sup>. Glutamic acid is transformed into alanine in intestinal mucosa and lactate in the liver<sup>(12)</sup>. Natural food contains two different forms of glutamate, free and bound forms, and some food (e.g. tomatoes, mushrooms) contains a high amount of free glutamate<sup>(13)</sup>. It is estimated that 650,000 tons of MSG are used worldwide each year<sup>(14)</sup>. In Western countries MSG is usually consumed in processed and packaged products. Therefore, most of canned and fast food as flavored chips, canned soups, prepared meals, marinated meats, bottled soy or oriental sauces, freezing foods and tinned tuna containing variable concentrations of MSG<sup>(15)</sup>. Monosodium glutamate (MSG) treatment is able to produce metabolic changes, which can further result in severe bodily disturbances<sup>(16)</sup>. Several previous studies reported hazards and toxic effects for MSG administration in human and animals<sup>(17)</sup>. MSG could produce symptoms such as numbness, weakness, flushing, sweating, dizziness and headaches<sup>(18)</sup>. In addition to these MSG symptoms, ingestion of MSG has been alleged to cause or exacerbate numerous conditions, including asthma, urticaria, atopic dermatitis, ventricular arrhythmia, neuropathy and abdominal discomfort<sup>(18)</sup>. As far as, MSG administration may be supposed to cause lethal damage to many organs including; male and female genital organs, liver and kidney<sup>(19)</sup>.

Moreover, one of the most common food preservatives is sodium nitrite ( $\text{NaNO}_2$ ), which is used as an antimicrobial agent and preservative in meats and fish<sup>(20)</sup>. The addition of  $\text{NaNO}_2$  as a food additive may react with food amines in the stomach and produce nitrosamines or large numbers of free radicals. Such products may increase lipid peroxidation which can create many hazards to the different body organs<sup>(21)</sup>. Sodium nitrite has been reported to cause adverse health effects due to increased oxidative stress that could be harmful to different organs. The reactive nitrogen species that are produced by exposure to nitrite have many toxic effects including hepatotoxicity, nephrotoxicity and dysregulation

of inflammatory responses, tissue injury and hyperlipidemic effects<sup>(20)</sup>.

It has been noticed that our children often eat food containing preservatives, food flavor and may be containing colorants at the same time. So, this study is planned to test the interaction of these classes of food additives with each other in addition to follow up the biological effects of these mixtures on young male albino rats.

## MATERIALS AND METHODS

### Plant material and extracts

*B. orellana* was purchased from natural stores and had sanitary registration. Seeds and leaves were chopped and soaked in absolute methanol (1:2, w/v), and stored without sunlight for 10 days at room temperature then filtered through filter paper. After that, the extract was stored in a glass container in refrigerator until used.

### Drugs and chemicals:

**Monosodium glutamate:** white colored substance used as flavor enhancer and it was liquefied in distilled water.

**Sodium nitrite:** white colored substance used as preservative and it was liquefied in distilled water.

### Animals

Twenty four young male albino rats of Sprague Dawley strain, weighing around 120-145 g were used in this experiment. Animals were housed in stainless steel cages, fed on rat chow and offered water ad libitum. They were obtained from the animal house of National Research Institute, El-Giza, Egypt.

### Experimental design:

Experimental animals were randomly divided into four groups of six animals each, as follows:

**Group I (Control group):** untreated group.

**Group II:** Rats were administered orally with sodium nitrite (0.1 mg/kg b.wt./ day) and annatto (0.065 mg/kg b.wt./day) by gastric intubation daily.

**Group III:** Rats were injected orally with sodium nitrite (0.1 mg/kg b.wt./ day) and mono sodium glutamate (15 mg/kg b.wt./day) by gastric intubation daily.

**Group IV:** Rats were administered orally with annatto (0.065 mg/kg b.wt./day) and mono sodium glutamate (15 mg/kg b.wt./day) by gastric intubation daily. All the administrations were performed orally and daily between 8.00 am and 10.00a.m. Body weights were recorded every week. After 30 days of treatment, animals were weighted and then decapitated.

**Blood sample collection:** At the end of the experimental period, the overnight fasted animals

(12- 16h) were sacrificed under diethyl ether anesthesia. Blood samples were taken from orbital vein and centrifuged at 5000 rpm for 10 min. The clear non-haemolysed sera were quickly separated and immediately stored at -20°C till used for analysis of biochemical parameters.

#### Biochemical analyses:

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), Creatinine, urea, glucose concentrations as well as lipid profile that including total cholesterol(TC), triglycerides (TG) and high density lipoprotein-cholesterol (HDL-C) were determined. Concentrations of testosterone and thyroid hormones (T3 and T4) were also measured. All parameters were estimated by using **BioMerieux SA, France**.

In the present study total protein (TP) and albumin concentrations were estimated, then serum globulin concentrations were calculated according to the formula<sup>(22)</sup>:

**Globulin (g/dl) = total protein (g/dl) –albumin (g/dl)**

The ratios of serum albumin/ globulin and albumin/creatinine were determined as albumin / globulin level and serum albumin / creatinine level. However, ratios of TC/HDL (risk factor 1) and LDL/HDL (risk factor 2) were also calculated after calculation of serum LDL-C (low density

lipoprotein cholesterol) and VLDL (very low density lipoprotein cholesterol) using the **Friedwald's** formula<sup>(23)</sup>.

Friedewald's equation: LDL (mg/dl) = TC- {HDL-C + [TG/5]}.

VLDL = TG/5

Risk 1 = TC / HDL-C

Risk 2 = LDL-C / HDL-C

#### Statistical analysis

The results were expressed as Mean ± S.E of the mean. Data were analyzed by one way analysis of variance (ANOVA) and were performed using the Statistical Package (SPSS) program, version 20. The post-hoc test was used as a method to compare significance between groups.

## RESULTS

**Body weight and glucose level:** the percent change in the body weight was significantly decreased in treated rats that received NaNO<sub>2</sub> with annatto and MSG with NaNO<sub>2</sub> while treated rats with MSG and annatto showed a significant increase in body weight as compared to control rats (**Table 1**). On the other hand, glucose level revealed a highly significant increase ( $p < 0.001$ ) in rats treated with all combinations of food additives, in contrast to control ones (**Table 1**).

**Table (1): Percentage of body weight change and glucose level in control, NaNO<sub>2</sub>, annatto and MSG treated animals.**

Parameter	Control	NaNO <sub>2</sub> +Annatto	NaNO <sub>2</sub> +MSG	Annatto+ MSG
% of body weight	35.34 ± 0.31	20.25 ± 1.14**	25.32 ± 2.7*	41.47 ± 1.38**
Glucose(mg/dl)	66.61 ± 1.3	94.91 ± 0.9**	96.99 ± 0.4**	93.37 ± 0.8**

Values represent mean ±S.E (standard error). (P\* < 0.05, P\*\* < 0.001 as compared to control group).

Protein profile: our results showed highly significant decrease ( $p < 0.001$ ) in serum total proteins, albumin and globulin levels except for albumin level of annatto and MSG treated group that was showed a significant decrease ( $p < 0.05$ ). As far as, Albumin/Creatinine ratios showed highly significant decrease ( $p < 0.001$ )

relative to the corresponding controls while A/G ratio values showed significant increase ( $p < 0.05$ ) in two groups: NaNO<sub>2</sub>+MSG group and annatto + MSG group as compared to control rats, while NaNO<sub>2</sub>+annatto group has no significant change (Table 2).

**Table (2): Serum total protein (g/dl), albumin (g/dl), globulin, albumin/globulin ratio and albumin/creatinine ratio in control, NaNO<sub>2</sub>, annatto and MSG treated animals.**

Parameter	Control	NaNO <sub>2</sub> +Annatto	NaNO <sub>2</sub> +MSG	Annatto+ MSG
T.P (g/dl)	6.3 ± 0.4	3.43 ± 0.2**	2.81 ± 0.3**	3.91 ± 0.2**
Albumin (g/dl)	3.9 ± 0.3	2.43 ± 0.14**	1.9 ± 0.14**	2.9 ± 0.1*
Globulin (g/dl)	2.4 ± 0.1	0.9 ± 0.03**	0.8 ± 0.15**	0.96 ± 0.08**
Albumin /Globulin	1.58 ± 0.1	2.5 ± 0.14	2.8 ± 0.5*	3.1 ± 0.2*
Albumin/Creatinine	7.9 ± 1.1	2.9 ± 0.3**	2.3 ± 0.3**	3.5 ± 0.3**

Values represent mean ±S.E (standard error). (P\* < 0.05, P\*\* < 0.001 as compared to control group).

**Liver functions:** a highly significant increase ( $p<0.001$ ) in the activities of the AST and ALT enzymes were observed in all rat groups treated with different food additive combinations for 30 days as compared to control rats (Table 3).

**Table (3): ALT and AST activities in control, NaNO<sub>2</sub>, annatto and MSG treated animals.**

Parameter	Control	NaNO <sub>2</sub> +Annatto	NaNO <sub>2</sub> +MSG	Annatto+MSG
AST (u/l)	262.7 ± 1.1	301.1 ± 0.95**	305.7 ± 0.3**	293.6 ± 0.96**
ALT (u/l)	69.8 ± 0.9	93.52 ± 1.02**	95.99 ± 0.7**	92.98 ± 1.02**

Values represent mean ±S.E (standard error). (P\* $<0.05$ , P\*\* $<0.001$  as compared to control group).

**Lipid profile:** the present results showed highly significant increase ( $p<0.001$ ) in TC, TG, LDL, VLDL, ratios of TC/HDL (risk ratio 1) and LDL/HDL (risk ratio 2) accompanied with marked decline ( $p<0.001$ ) in HDL in treated rats as compared to control (Table 4).

**Table (4): Changes in total cholesterol (TC), triglyceride (TG), HDL-C, LDL-C, vLDL-C, LDL/HDL ratio and TC/HDL ratio in control, NaNO<sub>2</sub>, annatto and MSG treated animals.**

Parameter	Control	NaNO <sub>2</sub> +Annatto	NaNO <sub>2</sub> +MSG	Annatto+MSG
Total cholesterol (mg/dl)	55.02 ± 1.2	88.64 ± 1.04**	88.7 ± 1.6**	86.5 ± 1.6**
Triglycerides (mg/dl)	49.6 ± 0.8	108.5 ± 1.2**	110.9 ± 1.3**	104.8 ± 1.5**
HDL (mg/dl)	37.97 ± 1.2	11.5 ± 0.9**	7.7 ± 0.9**	14.5 ± 1.02**
LDL	7 ± 1.1	55.5 ± 1.9**	58.9 ± 2.8**	51.1 ± 3.1**
VLDL	9.8 ± 0.4	21.7 ± 0.5**	22.2 ± 0.5**	20.96 ± 0.5**
LDL/HDL	0.18 ± 0.02	4.95 ± 0.5**	8.04 ± 0.9**	3.7 ± 0.5**
Total cholesterol/HDL	1.4 ± 0.02	7.9 ± 0.6**	12.06 ± 1.2**	6.2 ± 0.6**

Values represent mean ±S.E (standard error). (P\* $<0.05$ , P\*\* $<0.001$  as compared to control group).

**Kidney functions:** the recorded results of renal function parameters including urea and creatinine showed highly significant increase ( $p<0.001$ ) in all food additive combinations treated groups in comparison with the control group (Table 5).

**Table (5): Serum creatinine and urea levels in control, NaNO<sub>2</sub>, annatto and MSG treated animals.**

Parameter	Control	NaNO <sub>2</sub> +Annatto	NaNO <sub>2</sub> +MSG	Annatto+MSG
Creatinine (mg/l)	0.5 ± 0.03	0.9 ± 0.04**	0.9 ± 0.04**	0.85 ± 0.05**
Urea (mg/l)	34.4 ± 0.6	51.8 ± 0.9**	53.8 ± 1.5**	50.2 ± 0.99**

Values represent mean ±S.E (standard error). (P\* $<0.05$ , P\*\* $<0.001$  as compared to control group).

**Hormones:** a highly significant increase ( $p<0.001$ ) in the levels of both T3 and T4 was observed in rats which received different food additive types when compared to control rats (Table 6). On the other hand, a highly significant decrease ( $P<0.001$ ) was recorded in serum testosterone level in all treated groups after 30 days of treatment as compared to control group (Table 6).

**Table (6): Serum Testosterone, T3 and T4 levels in control, NaNO<sub>2</sub>, annatto and MSG treated animals.**

Parameter	Control	NaNO <sub>2</sub> +Annatto	NaNO <sub>2</sub> +MSG	Annatto+MSG
Testosterone (ng/dl)	60.6 ± 0.97	42.1 ± 0.8**	40.5 ± 0.9**	43.3 ± 0.7**
T3 (ng/dl)	94.4 ± 1.1	134.9 ± 1.6**	143.5 ± 1.9**	126.1 ± 1.97**
T4 (ug/dl)	5.6 ± 0.2	37.3 ± 1.5**	44.8 ± 1.5**	29.3 ± 1.2**

Values represent mean ±S.E (standard error). (P\* $<0.05$ , P\*\* $<0.001$  as compared to control group).

## DISCUSSION

In the present work, rats treated with mixture of NaNO<sub>2</sub> and annatto and mixture of NaNO<sub>2</sub> and MSG showed highly significant decrease in body weight and less weight gain than the control group throughout the experimental periods. This reduction may be attributed to increased the level of sodium nitrite in the body that lead to increased the rate of catabolic process due to reaction of NaNO<sub>2</sub> with food amines in the stomach producing nitrosamines and free radicals that may be caused an oxidative stress and thus leading to lipid peroxidation<sup>(24)</sup>. The results of this study are in line with the observation of Kostogry's *et al.*<sup>(25)</sup>. In general, the possible causes of the reduction in body weight were either a reduction of food and water intake or an increase in protein catabolism or disturbance in hormonal balance that impairing the growth of rats<sup>(26)</sup>. On the other side, the present investigation revealed that rats treated by MSG accompanied with annatto for 30 days had significantly higher body weight gain as compared to control ones. This may be attributed to MSG intake that could induce an increase in energy intake which could lead to obesity or alter the levels of carbohydrates, lipids and proteins in rats<sup>(27)</sup>.

Furthermore, oral administration with the mixtures of food additives MSG, NaNO<sub>2</sub> and annatto to young male albino rats for 30 days was found to significantly increase fasting blood glucose level in comparison to control rats<sup>(28)</sup>. This elevation of glucose levels can be explained by stimulation of glycogenolysis by the liver with the temporary loss of endocrine functions of pancreas that leads to hyperglycemia<sup>(29)</sup>. In the MSG given groups, it is believed that these effects may be caused as result of MSG toxicity which leading to increased gluconeogenesis from glutamate and glutamine. It has been also suggested a possible deterioration of glucose tolerance in rats following MSG administration that could be attributed to decreased cellular insulin sensitivity even under conditions of hyperinsulinemia observed in animals treated with MSG<sup>(30)</sup>. Under conditions of hyperinsulinemia, cells could switch to pathways that favor gluconeogenesis to compensate for the increased insulin release<sup>(31)</sup>. In addition, other findings suggested nitrite-stimulation of gluconeogenesis and glucose shift from tissue to blood or an impairment of glucose mobilization. Furthermore, it is previously confirmed that nitroso-compounds can alter the antioxidant system causing disturbance in the metabolic processes leading to hyperglycemia<sup>(32)</sup>.

On the other hand, oral administration food additives MSG, NaNO<sub>2</sub> and annatto for 30 days showed significant marked decline in the serum total proteins, albumin, globulin and albumin/creatinine ratio nevertheless A/G ratio recorded an increase in its levels in relative to the corresponding controls<sup>(33)</sup>. This may be due to stimulation of thyroid and adrenal glands by NaNO<sub>2</sub> and MSG which lead to a blocked protein synthesis, fast break down, increased rate of free amino acids and decreased protein turnover<sup>(34)</sup>. Yousef *et al.*<sup>(35)</sup> indicated to the inhibitory effect of some food additives on the biosynthesis of protein and albumin which in turn reflects impairment in liver functions. Furthermore, reductions of protein profile in all treated groups can reflect the liver condition that causing hepatotoxicity and liver damage that lead to inability of liver to produce proteins and causing utilization of amino acids for the oxidation or gluconeogenesis<sup>(36)</sup>.

Moreover, the present study recorded that rats consumed the mixtures of both food flavor and food colorant as well as food preservation exhibited a highly significant increase in serum ALT and AST activities when compared to control. This may be attributed to the excessive hepatocellular damage caused by the toxic effect of these agents. As a result of cellular damage, several enzymes like ALT and AST beach out into the serum and hence their level indicate the type and extent of damage inflicted that reflects a state of hepatocyte injury<sup>(37)</sup>. In addition, the significant elevation of serum aminotransferases may be attributed to what mentioned that under pathological conditions, the parenchymal cells of hepatic lobules fail to carry out vital functions, which usually results in disturbed or imbalanced intermediary metabolism. It is reported that MSG causes changes in the liver parenchyma of mice around central vein, dilated sinusoids, inflammatory cells and nuclei were pyknotic<sup>(12)</sup>. However, the sodium moiety in monosodium glutamate could easily dissociate to yield free glutamate. The diminution of glutamate produces ammonium ion (NH<sup>4+</sup>) that could be toxic unless detoxified in the liver via the reactions of the urea cycle. Thus, the possible ammonium ions overload that may occur as a result of an increased level of glutamate following MSG intake could damage the liver, consequently releasing the ALT enzyme. This increase could also be explained by free radical production which reacts with polyunsaturated fatty acids of cell membrane

leading to oxidative stress which induces impairment of mitochondrial and plasma membranes resulting in enzyme leakage<sup>(38)</sup>.

Remarkably, this study also revealed that rats orally administrated with food additives NaNO<sub>2</sub> and annatto, MSG and NaNO<sub>2</sub> and MSG and annatto showed significant increase in total cholesterol, triglycerides, LDL-C and vLDL-C levels, while HDL-C concentration showed a reduction in its level when compared with control rats. These changes may be attributed to the mobilization of free fatty acids from the adipose tissue to the blood stream and increase level of acetyl CoA, leading to increase in the synthesis of cholesterol or due to peroxidation of cell membrane lipids<sup>(39)</sup>. It is worth to be mentioned an increase in LDL and vLDL levels are increase in the risk of cardiovascular diseases<sup>(40)</sup>. Although, the possible explanation of these observed increments may reside in the direct or indirect action of these food additives on lipid metabolism or lipid peroxidation. Increasing effect in cholesterol concentration in the present study may be an indication of membrane structure and function disruption, thus influence its fluidity, permeability, activity of associated enzymes and transport system<sup>(41)</sup>. However, MSG was seen to increase hepatic lipid catabolism via up regulation of oxidative genes. It was specially seen to activate genes involved in bile acid pathway including key regulatory enzyme, cholesterol-7- $\alpha$  hydroxylase (CYP7A1). Lipid mobilization and storage processes were shown to be affected in liver of rats on MSG diets<sup>(20)</sup>.

On the other hand, our results demonstrated that the daily intake of food additives (MSG and NaNO<sub>2</sub>), (NaNO<sub>2</sub> and annatto) and (MSG and annatto) exhibited an increase in serum creatinine and urea levels when compared with the control group. It is believed that the significant elevation in urea and creatinine levels is closely related to the impairment of renal function<sup>(42)</sup>. Increased concentrations of creatinine and total urea in blood during renal diseases or renal damage may be due to high activities of xanthine oxidase, lipid peroxidation, and increased triacylglycerol and cholesterol levels, as well as impairment of the urea cycle enzyme activities<sup>(43)</sup>. Increased concentration of xanthine oxidase was previously reported in rats injected with MSG. Therefore; these impairments could also be attributed to the changes in the threshold of tubular reabsorption, renal blood flow and glomerular filtration rate (GFR)<sup>(35)</sup>.

Moreover, in the present study, it was found that the oral administration of food additive mixtures MSG, NaNO<sub>2</sub> and annatto to rats showed increased in concentrations of thyroid metabolism hormones (T3 and T4) when compared with control rats. This observation could be attributed to stimulation of thyroid gland and adrenal glands by MSG and NaNO<sub>2</sub> or may be due to neurotoxic effect of MSG as it destructs neurons in the hypothalamic nuclei through their changes in the hypothalamo-pituitary-adrenal axis (HPA)<sup>(44)</sup>. According to Duntas and Luboshitzky<sup>(45)</sup> altered concentrations of thyroid metabolism hormones are usually associated with elevated total cholesterol levels, increased LDL-cholesterol and lower HDL-cholesterol concentrations<sup>(45)</sup>. Thus, these changes in thyroid hormones might be resulted from alteration in the pituitary – thyroid axis and this might play a role in children hyperactivity probably through affecting higher centers in the brain<sup>(46)</sup>. It was documented that MSG causes endocrine disorder as a result for induced oxidative stress in experimental animals<sup>(47)</sup>. Noteworthy, the current study reported that rats treated with (NaNO<sub>2</sub> and annatto), (MSG and NaNO<sub>2</sub>) and (MSG and annatto) showed that there is revealed a highly significant reduction in testosterone hormone levels as compared with control level. Therefore, this reduction in serum testosterone levels may be resulted from disruption of the hypothalamic-pituitary-testes regulatory axis that controls testosterone production by testicular Leydig cells. This proposition is supported by the reports of previous authors who stated that administration of monosodium glutamate destroyed neurons of the hypothalamus in rats and mice<sup>(48)</sup>. Moreover, this result may be due to reduce gonadotrophin-releasing hormone (GnRH) associated with the lesions on the arcuate nucleus of the hypothalamus that occurs in animals given MSG<sup>(49)</sup>. Such neuronal losses in the hypothalamus can result in disruption of the hypothalamic-pituitary-testes regulatory axis. Also, our findings are in agreement with Ochiogu *et al*. who recorded a significant lower serum testosterone in the MSG-treated groups at Days 14 and 28 of MSG administration<sup>(50)</sup>. At the same time, this result agreement with Alyoussef and Al-Gayyar who showed that sodium nitrite resulted in significant reduction in serum testosterone concentration<sup>(51)</sup>.

**In conclusion**, our study has demonstrated that oral administration of mixtures of different types of food additive resulted in a vehement disturbance in all studied biochemical parameters

including glucose, total protein (TP), albumin, creatinine, urea, activities of AST and ALT in addition to lipid profile by inducing metabolic alterations. The most grievous effects of these food additives reside in its hormonal effect that was pronounced on testosterone and thyroid hormones that can lead to fertility problems and hyperthyroidism, respectively. So, due to the hazardous effect of food additives, it is recommended to limit their uses.

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