

## Effects of Some Food Additives on Some Biochemical Parameters in Young Male Albino Rats and the Ameliorative Role of Royal Jelly

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### ABSTRACT

**Background:** the use of food additives is one of the most important problems in the human health nutrition field. Food additives are widely used for various purposes; including preservation, coloring, and sweetening, however, the physiological and biochemical changes may be produced.

**Aim of the work:** was to investigate the protective role of royal jelly (RJ) against abnormalities in metabolic biochemical parameters that induced by these food additives in male albino rats.

**Materials and Methods:** thirty young male albino rats with an average body weight 120-140 g were divided into three groups (10/cage); **Group I:** served as normal control group, **Group II:** rats orally administrated with the mixture which consists of sodium nitrite (NaNO<sub>2</sub> 0.1 mg/kg b.wt./ day), annatto (0.065 mg/kg b.wt./day) and monosodium glutamate (MSG 15 mg/kg b.wt./day) and **Group III:** rats orally administrated with the previous mixture and then orally administrated with royal jelly (14.28 mg/kg b.wt./day soluble in maize oil). At the end of the experiment, blood samples were collected for biochemical estimations which including levels of serum glucose, creatinine, urea, testosterone, thyroid hormones (T3 and T4), activities of AST and ALT, total protein (TP), albumin, total cholesterol, triglycerides, high-density lipoprotein (HDL-c) and low-density lipoprotein (LDL-c). **Results:** the present study showed marked elevation in levels of fasting blood glucose, activities of AST, ALT, levels of serum urea, creatinine, TC, TG, LDL-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 significant reduction in the body weight, serum total proteins, albumin, globulin, albumin/creatinine, testosterone and HDL-C concentrations in the group that administrated with the mixture which consists of (NaNO<sub>2</sub>, MSG and annatto) as compared to control rats. While administration with royal jelly significantly ameliorated the disturbed biochemical parameters and showed significant improvement in most of these parameters. **Conclusion:** it could be concluded that royal jelly offers a therapeutic advantage that minimizes the metabolic abnormalities and biochemical changes which induced by these food additives.

**Keywords:** Royal jelly, Testosterone, Food Additives, Biochemical parameters, Sodium nitrite.

### INTRODUCTION

Food additives are organic substances that are intentionally added to food in small quantities during production or processing to improve the organoleptic quality (color, flavor, appearance, taste and texture) of the food. One of these food additives is monosodium glutamate (MSG) which widely used as a flavor enhancing amino acid <sup>(1)</sup>. It is a common glutamic acid salt <sup>(2)</sup> that has 78% glutamic acid, 22% sodium salt and water. The excessive administration of MSG may lead to liver and kidney damages <sup>(3)</sup>. It is reported that rats exposed to MSG encounter many problems like learning difficulty, gonadaldysfunction, brain damage, depleted in some of the neurotransmitters like nor epinephrine, serotonin, dopamine and their metabolites in the hypothalamus region, an increase in the incidence of stomach cancer, oxidative stress in the hepatic tissue with degenerative changes in hepatocytes <sup>(4)</sup>.

Synthetic and natural dyes are also used in the food industry as additives to intensify, compensate or add color to a manufactured product, thereby maintaining the pleasant and attractive appearance that resembles the natural product. Annatto pigments one of the natural dyes that derived from *B. Orellana* and commonly used as food coloring agent that has great economic and commercial importance <sup>(5)</sup>. Many reports confirmed that *B. Orellana* is rich in flavonoids, tannins, saponins, steroids and alkaloids. In addition, Bixin and norbixin are the principle coloring constituents of annatto <sup>(6)</sup>.

On the other hand, sodium nitrite (NaNO<sub>2</sub>) is used as an antimicrobial agent, a preservative and a color fixative in meats and fish and also inhibits the growth of *Clostridium botulinum*, effectively control rancidity by inhibiting lipid oxidation, the microbiological safety of these

foods and extends their safe shelf-life leading to excellent storage stability<sup>(7)</sup>. Experimental studies have shown that a number of N-nitrosamines are carcinogenic in animal species. The toxic effects of nitrates and nitrites include impairment of reproductive function, hepatotoxicity, and methemoglobinemia. Instance, highly carcinogenic N-nitrosocompounds are produced when nitrite reacts with secondary amines and N-alkyl amides under acidic conditions *in vitro*<sup>(8)</sup>.

Royal jelly (RJ) is a thick, extremely nutrition, milky white, creamy liquid secreted by the hypopharyngeal and mandibular glands of the worker honeybees<sup>(9)</sup>. Royal jelly contains considerable amounts of protein, glucose, lipid, vitamins, minerals, aspartic acid, gelatin, sterols, phosphorous compounds, acetylcholine, nucleic acids and numerous trace ingredients which are all important in RJ's documented therapeutic and nutritional properties<sup>(10)</sup>. These ingredients are represented in 65% water, 12% crude protein, 10% monosaccharides and the remainder is an ether-soluble fraction of fatty acids<sup>(11)</sup>. Previous studies have shown that RJ has a number of physiological effects such as anti-inflammatory, anti-tumor, anti-metastatic effects, antiallergic, antioxidant activities, antibacterial, vasodilative and hypertensive activities, disinfectant action and anti-hypercholesterolemic activity<sup>(12)</sup>. Moreover, RJ has received particular attention because of its highly efficient antioxidant and free radical scavenging capacities that nominate its use for decreasing the toxic effects of chemical agents<sup>(13)</sup>.

Hence, this study aimed to investigate the effects of the mixture of different types of food additives (MSG, NaNO<sub>2</sub> and annatto pigment) on some biochemical parameters of male albino rats as well as the effect of royal jelly as antidote to these compounds and whether if it is able to improve these parameters and amelioration the toxicity induced by MSG and NaNO<sub>2</sub>.

## MATERIALS AND METHODS

Experimental animals were randomly divided into three groups (ten animals/each) as follows: **group I: Control**, untreated group, **group II:** rats were administered orally with the mixture of (sodium nitrite (0.1 mg/kg b.wt./day), annatto (0.065 mg/kg b.wt./day) and monosodium glutamate (15 mg/kg b.wt./day)) by gastric intubation daily, **group III:** rats were orally administered with the previous mixture and then with royal jelly (14.28 mg/kg b.wt./day soluble in maize oil) by gastric intubation daily. Body

weights were recorded every week. After 30 days of treatment, animals were weighed 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 an orbital vein and centrifuged at 5000 rpm for 10 min. The clear non-haemolysed supernatant sera were quickly separated and immediately stored at -20°C till used for further 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), high-density lipoprotein (HDL-c) and low-density lipoprotein (LDL-c) were also determined. Concentrations of testosterone and thyroid hormones (T3 and T4) were measured. All parameters were estimated by using kits of **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>(14)</sup>:

$$\text{Globulin (mg/dl)} = \text{total protein (mg/dl)} - \text{albumin (mg/dl)}$$

The ratio of serum albumin/ globulin was determined as albumin/globulin 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>(15)</sup> and **Norbert**<sup>(16)</sup> formulas, respectively as following:

$$\text{Friedewald's}^{(15)} \text{ equation: } \text{LDL (mg/dl)} = \text{TC} - \{\text{HDL} + [\text{TG}/5]\}.$$

$$\text{Norbert}^{(16)} \text{ equation: } \text{VLDL} = \text{TG}/5$$

### Statistical analysis

The results were expressed as Mean  $\pm$  SEM (standard error of the mean). Data were analyzed by one-way analysis of variance (ANOVA) and were performed using the Statistical Package for Social Sciences (SPSS) program, version 20. The Bonferroni test was used as a method to compare significance

between groups. The significance level was accepted at  $p$ -value  $<0.05$ .

## RESULTS

**Body weight change:** no significant change was noticed in the percentage of body weight change in both treated groups (**Table 1**).

**Glucose level:** there was a highly significant increase ( $p < 0.001$ ) in glucose level in the mixture group of food additives in contrast to control rats. While in the group of (mixture + RJ) there was an only significant increase ( $p < 0.05$ ) as compared to control animals. Percentage of change of mixture treated rats was 51% and using RJ reduced the percentage to 6% (**Table 1**).

**Table (1): Percentage of body weight change and glucose level in control, Mixture (consists of sodium nitrite+ annatto+ monosodium glutamate) and mixture+ Royal jelly treated animals.**

Parameter	Control	Mixture	Mixture +Royal Jelly
% of body weight change	35.34 ± 0.3	32.8 ± 3.6	35.4 ± 2.7
Glucose (mg/dl)	66.6 ± 1.3	100.5 ± 1.4**	70.5 ± 0.8*
% of change		51%	6%

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

**Protein profile:** our results recorded a highly significant decrease ( $p < 0.001$ ) in serum total protein, albumin and globulin, and highly significant increase ( $p < 0.001$ ) in A/G ratio in the mixture group relative to the corresponding control group. In addition, royal jelly treated animals showed no significant change in total protein and albumin, while there was a

significant decrease ( $p < 0.05$ ) in globulin and a highly significant increase in A/G ratio as compared to control rats. Percentages of change in mixture treated rats of total protein, albumin, globulin and albumin/globulin are (-59%, -50%, -73%, 104%) respectively, and after using SCE these ratios were (-6%, 6%, -26%, 46%) respectively (**Table 2**).

**Table (2): Serum total protein (g/dl), albumin (g/dl), globulin, albumin/globulin ratio in control, Mixture (consists of sodium nitrite+ annatto+ monosodium glutamate) and mixture+ Royal Jelly treated animals.**

Groups	Control	Mixture	Mixture +Royal Jelly
Total Protein(g/dl)	6.28 ± 0.4	2.6 ± 0.2**	5.4 ± 0.3
% of change		-59%	-6%
Albumin(g/dl)	3.86 ± 0.29	1.94 ± 0.17**	4.1 ± 0.28
% of change		-50%	6%
Globulin (g/dl)	2.43 ± 0.1	0.65 ± 0.1**	1.25 ± 0.2*
% of change		-73%	-26%
Albumin/Globulin	1.58 ± 0.08	3.23 ± 0.7**	3.5 ± 0.5**
% of change		104%	46%

Values represent mean ±SE (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 AST and ALT enzymes were observed in rats treated with the mixture when compared to control rats. On the other hand, treatment of this mixture group with

royal jelly has an only significant increase in these activities. Percentages of change of ALT and AST in the mixture group are (156% and 125%); using RJ reduced it to (23% and 10%) respectively (**Tables 3**).

**Table (3): ALT and AST activities in control, Mixture (consists of sodium nitrite+ annatto+ monosodium glutamate) and mixture+ Royal Jelly treated animals.**

Groups	Control	Mixture	Mixture +Royal Jelly
ALT (U/l)	20 ± 0.88	51.1 ± 1.4**	24.6 ± 1.5*
% of change		156%	23%
AST (U/l)	50 ± 1.06	112.4 ± 1.4**	55.2 ± 1.5*

% of change	125%	10%
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Values represent mean  $\pm$ SE (standard error). ( $P^* < 0.05$ ,  $P^{**} < 0.001$  as compared to control group).

**Lipid profile:** the present results revealed 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 rats treated with food additives as compared to control rats. Treatment with royal jelly produced significantly increase ( $p < 0.05$ ) in the total cholesterol, triglycerides, LDL, VLDL

levels, TC/HDL and LDL/HDL values while the HDL value showed no significant change in comparison with control group. Percentages of change in lipid profile (TC, TG, HDL-C, LDL-C, VLDL, LDL/HDL, TC/HDL) of mixture treated rats were: (55%, 94%, -71%, 294%, 94%, 1245%, 431%) these percentages were decreased to (12%, 12%, -0.04%, 47%, 12%, 53%, 18%) after giving rats RJ (**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, Mixture (consists of sodium nitrite+ annatto+ monosodium glutamate) and mixture+ Royal Jelly treated animals.**

Groups	Control	Mixture	Mixture +Royal Jelly
Total Cholesterol(mg/dl)	85.02 $\pm$ 1.2	131.88 $\pm$ 1.8**	94.99 $\pm$ 1.1*
% of change		55%	12%
Triglycerides(mg/dl)	79.6 $\pm$ 0.8	154.41 $\pm$ 1.5**	89.17 $\pm$ 1.1*
% of change		94%	12%
HDL-C (mg/dl)	46.98 $\pm$ 1.2	13.79 $\pm$ 0.9**	44.7 $\pm$ 1.2
% of change		-71%	-0.04%
LDL-C (mg/dl)	22.12 $\pm$ 1.5	87.21 $\pm$ 1.9**	32.45 $\pm$ 2.3*
% of change		294%	47%
VLDL (mg/dl)	15.92 $\pm$ 0.37	30.88 $\pm$ 0.5**	17.83 $\pm$ 0.4*
% of change		94%	12%
LDL/HDL	0.47 $\pm$ 0.02	6.32 $\pm$ 3.5**	0.72 $\pm$ 0.1*
% of change		1245%	53%
TC/HDL	1.8 $\pm$ 0.02	9.56 $\pm$ 4.7**	2.12 $\pm$ 0.1*
% of change		431%	18%

Values represent mean  $\pm$ SE (standard error). ( $P^* < 0.05$ ,  $P^{**} < 0.001$  as compared to control group).

**Kidney functions:** the recorded results of biochemical parameters of renal function including urea and creatinine showed a highly significant increase ( $p < 0.001$ ) mixture treated group in comparison with the control group.

Moreover, treatment with royal jelly produced no significant change in the serum urea and creatinine when compared with control group (**Table 5**).

**Table (5): Serum creatinine and urea levels in control, Mixture (consists of sodium nitrite+ annatto+ monosodium glutamate) and mixture+ Royal Jelly treated animals.**

Groups	Control	Mixture	Mixture +Royal Jelly
Creatinine(mg/l)	0.51 $\pm$ 0.04	1.1 $\pm$ 0.1**	0.59 $\pm$ 0.03
Urea(mg/dl)	34.44 $\pm$ 0.6	58.3 $\pm$ 1.3**	37.3 $\pm$ 1.6

Values represent mean  $\pm$ 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 received the mixture of food additive when compared to control rats. On the other hand, a highly significant decrease ( $P < 0.001$ ) was recorded in serum Testosterone level in mixture group when compared to control group. After 30 days of treatment with

royal jelly, there was no significant change in the levels of both T3 and T4, as well as a significant decrease ( $p < 0.05$ ) in serum Testosterone level occurred when compared to control group. These levels of T3, T4 and testosterone showed a high percentage of change in mixture treated rats (50%, 133% and -36%) respectively, and after royal jelly

treatments, it recorded (1.2%, 12% and -7%)

when compared to control group.

**Table (6): Serum Testosterone, T3 and T4 levels in control, Mixture (consists of sodium nitrite+ annatto+ monosodium glutamate) and mixture+ Royal Jelly treated animals.**

Groups	Control	Mixture	Mixture +Royal Jelly
<b>Testosterone(ng/dl)</b>	60.6 ± 1	38.6 ± 0.6**	56.66 ± 1.2*
<b>% of change</b>		-36%	-7%
<b>T3(ng/dl)</b>	115.41±1.1	172.63 ± 1.3**	116.76 ± 1.1
<b>% of change</b>		50%	1.2%
<b>T4(µg/dl)</b>	5.578 ± 0.19	13.02 ± 1.9**	6.26 ± 0.2
<b>% of change</b>		133%	12%

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

## DISCUSSION

The present results revealed a significant decrease in body weight gain after one month of treatment with the mixture which consists of (sodium nitrite+ annatto+ monosodium glutamate). This is in accordance with another previous study<sup>(17)</sup>. This result may be attributed to an increase in the sodium nitrite level in the body that lead to an increased rate of the catabolic process because of NaNO<sub>2</sub> reaction with food amines in the stomach and production of free radicals and nitrosamines that cause oxidative stress and thus leading to lipid peroxidation<sup>(18)</sup>. Furthermore, 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 impairing the growth of rats<sup>(19)</sup>. On the other hand, RJ supplementation significantly ameliorated the body weight reduction in the mixture treated animals. This effect could be attributed to RJ antioxidant properties and its increasing activity of antioxidant defense systems in free radicals scavenging because of its higher content of peptides and essential amino acids<sup>(20)</sup>. Moreover, experimental studies showed that RJ exerts protective effects on different tissues, including anti-inflammatory effects<sup>(21)</sup>.

On the other side, the present investigation revealed that rats treated with a mixture of food additives had significantly increased fasting blood glucose level in comparison to control rats. The elevation of glucose level can be explained by stimulation of glycogenolysis and gluconeogenesis by the liver with temporary loss of endocrine functions of pancreas leading to

hyperglycemia<sup>(22)</sup>. This may be caused as a result of MSG toxicity which leading to increased gluconeogenesis from glutamate and glutamine. Although, the abnormal glucose tolerance could be attributed to decreased cellular insulin sensitivity even under conditions of hyperinsulinemia observed in animals treated with MSG<sup>(23)</sup>. Under conditions of hyperinsulinemia, cells could switch to pathways that favor gluconeogenesis to compensate for the increased insulin release<sup>(24)</sup>. Moreover, other findings suggested nitrite-stimulation of gluconeogenesis and glucose shift from tissue to blood or an impairment of glucose mobilization. Furthermore, nitroso-compounds can alter the antioxidant system causing a disturbance in the metabolic processes leading to hyperglycemia<sup>(25)</sup>. While the treatment of mixture treated rats with RJ showed a significant decrease in blood glucose levels. Also, these findings were in agreement with previous reports<sup>(26)</sup>. These reports suggest that RJ supplementation ameliorates hyperglycemia and insulin resistance associated with type 2 diabetes<sup>(27)</sup>. In addition, RJ has been recognized for having hypoglycemic properties<sup>(28)</sup>. This may be attributed to the presence of hypoglycemic compounds which are responsible for antioxidant activity such as polyphenols and phenols in major protein structures<sup>(29)</sup>.

In the present investigation, oral administration with food additives mixture showed a significantly marked decline in the serum total proteins, albumin, and globulin while A/G ratio recorded increasing its levels in relative to the corresponding controls. This may be explained by stimulation of thyroid and adrenal glands by

NaNO<sub>2</sub> and MSG which lead to a blocked protein synthesis, fast break down, increased the rate of free amino acids and decreased protein turnover<sup>(30)</sup>. Also, reductions of globulin in the group treated with MSG, NaNO<sub>2</sub>, and annatto can reflect the liver condition that causing hepatotoxicity and liver damage that lead to the inability of the liver to produce proteins and causing utilization of amino acids for the oxidation or gluconeogenesis<sup>(31)</sup>.

However, daily treatment with royal jelly recorded increasing in serum total proteins, albumin and globulin levels as well as A/G ratio. These results are inconsistent with other previous study indicating the hepatoprotective properties of RJ<sup>(32)</sup>. Therefore, These findings could be attributed to the anti-inflammatory and antioxidant influence of RJ acting as scavengers of reactive oxygen species (ROS) and possess significant hepatoprotective effect<sup>(33)</sup>.

Moreover, the present study recorded that rats consumed the mixture exhibited a highly significant increase in serum ALT and AST activities when compared to control one. The elevation in the activities of these enzymes reflects a state of hepatocyte injury<sup>(34)</sup>. In addition, 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>(35)</sup>. Otherwise, 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>(36)</sup>. On the other hand, we found that RJ improved serum biomarkers (ALT and AST) levels of liver damage. This may be attributed to its protective effect against hepatocyte damage probably by its high antioxidative and scavenging activities, thus ameliorated the toxic effects of chemical agents<sup>(37)</sup>.

This study revealed that rats orally administrated with food additives NaNO<sub>2</sub>, annatto and MSG showed a 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. This may be attributed to the mobilization of free fatty acids from the adipose tissue to the bloodstream and increase the level of acetyl CoA, leading to an increase in the synthesis of cholesterol or due to peroxidation of cell membrane lipids<sup>(38)</sup>. Increasing in LDL and VLDL levels is increase the risk of cardiovascular diseases<sup>(39)</sup>. 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 of cholesterol concentration in the present study may be an indication of membrane structure and function disruption, thus influence its fluidity, permeability, the activity of associated enzymes and transport system<sup>(40)</sup>. However, MSG was seen to increase hepatic lipid catabolism via up-regulation of oxidative genes. It was especially 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 the liver of rats on MSG diets<sup>(41)</sup>.

Administration of RJ to mixture treated rats caused a reduction in the levels of triglyceride (TG), total cholesterol (TC), LDL, VLDL and ratios of TC/HDL and LDL/HDL with marked elevation of HDL. Our results are in agreement with pervious study<sup>(42)</sup>. The improvement in lipid profile by RJ treatment may be due to RJ exhibited antihypercholesterolemic and antioxidant properties<sup>(43)</sup>. On the other hand, our results demonstrated that the daily intake of food additives mixture exhibited an increase in serum creatinine and urea levels when compared with the control group<sup>(44)</sup>. Otherwise, it is believed that the significant elevation in urea and creatinine levels is closely related to the impairment of renal function. 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>(45)</sup>. Increased concentration of xanthine oxidase was observed 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>(46)</sup>.

In addition, royal jelly supplementation induced significant improvements in kidney functions

including the serum urea and creatinine levels in the mixture treated rats. This could be due to its protective role against oxidative damages due to its antioxidant potency and free radical scavenging capacity<sup>(47)</sup>. Moreover, in the present study, it was noticed that the administration of food additives mixture to rats showed increased in the levels of serum 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 the neurotoxic effect of MSG as it destructs neurons in the hypothalamic nuclei through their changes in the hypothalamo-pituitary-adrenal axis (HPA)<sup>(48)</sup>. Also, the altered concentrations of thyroid metabolism hormones are associated with elevated total cholesterol levels, increased LDL-cholesterol and lower HDL-cholesterol concentrations<sup>(49)</sup>. Thus, these changes in thyroid hormones might result 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>(50)</sup>. Subsequently, it was documented that MSG-induced oxidative stress in experimental animals, MSG causes endocrine disorder<sup>(51)</sup>. When rats treated with the royal jelly for one month the levels of T3 and T4 were significantly decreased. So, royal jelly ameliorates this effect due to its antioxidant property by blocking the generation of free radicals<sup>(20)</sup>.

In the current study, rats treated with a mixture of food additive showed that there is a reduction in testosterone hormone when compared with control rats. Our findings are in agreement with another study that was recorded a significantly lower serum testosterone in the MSG-treated groups at Days 14 and 28 of MSG administration<sup>(52)</sup>. At the same time, our results are in agreement with AlYoussef and Al-Gayyar who showed that sodium nitrite resulted in significant reduction in serum testosterone concentration<sup>(53)</sup>. Therefore, the reduction in serum testosterone levels may result 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 other authors who stated that administration of monosodium glutamate destroyed neurons of the hypothalamus in rats and mice<sup>(54)</sup>.

Also, this result may be due to a reduce in gonadotrophin-releasing hormone (GnRH) associated with the lesions of the arcuate nucleus

of the hypothalamus that occurs in animals given MSG<sup>(55)</sup>. Such neuronal losses in the hypothalamus can result in disruption of the hypothalamic-pituitary-testes regulatory axis. However, our results demonstrated that treatment with royal jelly produced a significant increase in the serum testosterone. Royal jelly is known as asexual tonic and used for the treatment of impotence infertility. Furthermore, testosterone could be elevated as a result of exogenously supplied by royal jelly, so it contains testosterone in amount 0.012g/g fresh weight. On the other hand, elevation of testosterone level could be attributed to zinc found in royal jelly. So zinc deficiency causes low testosterone level, while zinc supplementation can raise testosterone level and increase fertility<sup>(56)</sup>.

## CONCLUSION

Our findings revealed that royal jelly supplementation ameliorates the metabolic alterations caused by food additives through inhibition of the oxidative stress and reducing lipid peroxidation. Although, based on the results of the present study, royal jelly may be beneficial in normalized glucose levels and minimize the toxic effects of food additives on liver and kidney functions. These effects are associated with the powerful antioxidant properties of royal jelly as it produces beneficial effects against metabolic disorders.

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