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Adverse Effects of Mono Sodium Glutamate, Sodium Benzoate and Chlorophyllins on some Physiological Parameters in Male Albino Rats

Eman G.E. Helal*[1], Abrar W. Barayan[2], Mohamed A. Abdelaziz[3], Nahla S.A. EL-Shenawe[1]

[1]Department of Zoology, Faculty of Science (Girls), Al-Azhar University, Egypt,

[2]Ministry of Health, Maternity and Children Hospital, Saudi Arabia, Makkah,

[3]Physiology Department, Faculty of Medicine, (Boys), Al –Azhar University, Egypt

***Corresponding Author**: Eman Helal, **Email**: emanhelal@hotmail.com, **Mobile:** 00201001025364, orcid.org/0000-0003-0527-7028

ABSTRACT

Background: Chlorophyllin is known as natural green. Chlorophyll derivative is used as a food additive (food–coloring agent). Mono sodium glutamate (MSG), the sodium salt of amino acid glutamate, is a food additive that popularly used all over the world as "Flavor enhancer".

Aim of the work: This study aimed to determine the hazardous effects of sodium benzoate, chlorophyllin and mono sodium glutamate on some physiological parameters in male albino rats.

Materials and Methods: This study had been done on forty male albino rats. The Animals were divided into four groups; **Group I** (Control untreated group), **Group II** (Sodium benzoate-treated group), **Group III** (Chlorophyllin-treated group) and **Group IV** (Mono sodium glutamate-treated group). Blood samples were collected, sera were separated and used for estimation of some biochemical parameters (liver enzymes, kidney function, glucose, protein profile and lipid profile) and hormonal levels [testosterone, T3 (triiodothyronine) and T4 (thyroxine)].

Results: There was an increase in the activities of liver enzymes ASAT and ALAT as well as the levels of glucose, kidney function (urea and creatinine), lipid profile (TC, TG, VLDL and LDL), Insulin and HOMA-IR (insulin resistance) in the sodium benzoate- and mono sodium glutamate-treated groups. While chlorophyllin-treated group showed the same results except for glucose level, kidney function, insulin and HOMA-IR. In addition, there was an increase in the level of (T4) and (T3) in MSG group but these levels decreased in benzoate group. A drop in protein profile (total proteins, albumin), high-density lipoprotein (HDL) and testosterone hormone in benzoate and glutamate groups as compared to the control rats.

Conclusion: It could be concluded that some food additive like sodium benzoate and mono sodium glutamate have extreme effects on liver and kidney function, protein and lipid profiles as well as on thyroid and testosterone hormones. So, it is recommended to minimize the use of these additives to protect young children and mature people from these destructive effects.

Keywords: Food Additives, Thyroid hormones, Monosodium Glutamate, Sodium Benzoate, Chlorophyllins.

INTRODUCTION

Food additives are substances used in the food industry to maintain consistency, texture, taste, color, quality, alkalinity or acidity. There are many food additives that are widely located in two main categories depending on their purpose (i) safety and prevention of food degradation by bacteria, oxidation or chemical reactions. (ii) improving product taste and appearance. They are classified according to their functions like: preservatives, flavor or color agent [(1)].

Monosodium L-glutamate (MSG) is a common glutamic acid salt containing 78% glutamic acid and 22% sodium salt and water. MSG is the most common food additive that has been used as a flavor enhancer at home as well as in the food industry since 1907. Therefore, most canned foods and fast food such as flavored flakes, canned soups, ready meals, mutton meat, bottled soy or Eastern sauces, frozen and tested tuna containing variable concentrations of MSG [(2)].

Monosodium glutamate can produce symptoms such as numbness, weakness, irritability, sweating, dizziness and headache. In addition, MSG may cause or exacerbate many conditions, including asthma, urticaria, atopic dermatitis, arrhythmias, neuropathy and abdominal discomfort [(3)]. In animals, high doses of monosodium glutamate is neurotoxic because it destroys nerve cells in the hypothalamic nucleus through changes in the pituitary-adrenal axis. Moreover, excessive MSG administration may lead to liver and kidney damage.

Sodium benzoate is widely used as preservative for food and beverages. It is a common preservative in soft drinks because it prevents the growth of bacteria and fungi under the acidic conditions found in soft drinks. In addition, it is used in many foods including salads, soft drinks, jams, fruit juices, as well as in pharmaceutical industries to keep liquid medicines. It is usually chemically produced and added as preservatives in foods because of its antimicrobial function and its effect

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against yeast and mold. Sodium benzoate occurs naturally in many fruits such as apples, cranberries and plums and in spices such as cinnamon and cloves. The presence of sodium benzoate in these foods does not make them act as preservatives. Sodium benzoate has already a concern about cancer because when mixed with other additives, vitamin C, soft drinks, they form benzene and carcinogenic substance. It may also lead to mitochondrial DNA damage [(4)].

Chlorophyllin, a chlorophyll derivative that is used as a dietary supplement and in alternative medicine. As a food coloring agent, chlorophyllin is known as green 3 and has E E1, E141. The main food groups that contribute to the amount of food from the copper compounds of chlorophyll and chlorophyllin are sugary sweets, desserts, sauces, spices, cheese and soft drinks. Chlorophyll and chlorophyllin can form compound compositions with some cancer-causing chemicals such as aflatoxin B1 found in powders, many extracts of spices, herbs and higher plants or some heterogeneous amines found in cooked meat or polycyclic aromatic hydrocarbons in tobacco smoke [(5)].

The present study, therefore, aims to investigate and compare some of the toxic effects and biochemical changes induced by monosodium glutamate, chlorophyllin and sodium benzoate in male albino rats.

MATERIALS AND METHODS

Forty young male albino rats (weighing 120-140 g) were used in this study. Animals were housed in stainless steel cages, fed on rat chow and offered water *ad libitum*. The animals were divided into four equal groups (10 rats each) as follows: **The first group**: the control untreated group, **the second group:** orally administered with sodium benzoate (5 mg/k g b.wt./ day), **the third group**: orally administrated with monosodium glutamate (15 mg/kg b.wt./day) and **the fourth group:** orally administrated with chlorophyllin (15 mg/kg b.wt./day). Body weights were recorded every week. After 30 days of treatment, animals were weighed and then decapitated. Blood samples were collected for biochemical parameters. Blood samples were centrifuged for 10 min. at 5000 rpm and supernatant sera were separated for analysis without storage or delay.

Biochemical Examination:

In the present study, total protein (TP) and albumin concentration were estimated, then serum globulin concentrations were calculated according to the formula.

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

Aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) activities, creatinine, urea, glucose concentrations as well as lipid profile that including total cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C) were also determined. All parameters were estimated using **BioMerieux SA kits, France**. Both ratios of serum albumin/globulin and albumin/creatinine were determined. 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 [(6)] equation: LDL (mg/dl) = TC- {HDL + [TG/5]} and Norbert**'s** [(7)] equation: VLDL = TG/5

Determination of testosterone, thyroid hormones (T3 and T4) and serum insulin level:

Concentrations of testosterone and thyroid hormones (T3 and T4) were measured by an ELISA (Enzyme Linked Immunosorbent Assay) kit (U E Type). Insulin was measured Biovendor Research and Diagnostic product [(8)].

HOMA-IR:

The approximating equation for insulin resistance, in the early model, used a fasting plasma glucose sample. Then it was calculated using insulin-glucose product divided by a constant as follows:

HOMA –IR = fasting glucose mg/dl x Insulin μu/L /405

Fasting glucose in mass units mg/dl. **IR** is insulin resistance. Insulin is given in μu/L.

Statistical analysis

The results were expressed as Mean ± SEM. Data were analyzed by one way analysis of variance (ANOVA) and were performed using the Statistical Package (SPSS) program, version 25. The Bonferroni test was used as a method to compare significance between groups.

RESULTS

In the present study, rats showed many signs of behavioral variations like hyperactivity, nervous motion and became aggressive to each other.

Body weight: animals that received sodium benzoate showed insignificant changes in the percentage of body weight change from basal levels, while those administrated with mono sodium glutamate group showed a highly significant increases in the percentage of body weight ($P < 0.01$). Chlorophyllin group showed a highly significant decrease in the percentage of body weight ($p < 0.01$) as compared to control rats (Table 1).

Glucose level: there was significant increases in glucose level in sodium benzoate groups ($p < 0.05$), while mono sodium glutamate group showed a highly significant increases in glucose ($P < 0.01$). Chlorophyllin group showed insignificant changes in comparison with the

control group. There was a highly significant increases in insulin value and HOMA-IR ratio ($p < 0.01$) in sodium benzoate and MSG groups, while there was insignificant change in chlorophyllin as compared to control groups (Table 1).

Protein profile: the present study showed that there was a highly significant decrease in the total protein level and albumin ($p < 0.01$) in sodium benzoate and mono sodium glutamate groups. Chlorophyllin showed insignificant change in total protein and albumin. Meanwhile, the all treated groups recorded insignificant changes in globulin and albumin/globulin ratio as compared to the control group (Table 2).

Liver functions: ASAT and ALAT activities revealed a highly significant increase in benzoate and glutamate as compared to the control group ($p < 0.01$). Besides, chlorophyllin showed a significant increase in ASAT and ALAT value ($p < 0.05$) as shown in table (3).

Lipid profile: there was a highly significant increases in total cholesterol, triglycerides, LDLC, VLDL, LDL/HDL and TC/HDL ($p < 0.01$), while there was a highly significant decrease in HDL level in benzoate and glutamate groups as compared to control rats. Meanwhile, there was insignificant change in all lipid profile in chlorophyllin group as compare to control values (Table 4).

Kidney functions: there was a highly significant increase in urea value in sodium benzoate and MSG groups ($p < 0.01$), while there was insignificant change in urea and creatinine level in chlorophyllin group. Creatinine level revealed a significant increase in benzoate and glutamate groups ($p < 0.05$) in comparison to the control group (Table 5).

Hormones: Chlorophyllin group recorded insignificant change in testosterone level, T3 and T4 level as compared to control rats, while glutamate and benzoate groups showed significant decrease in testosterone level ($p < 0.05$). Glutamate recorded a highly significant increase in T3 and T4 level ($p < 0.01$), but benzoate recorded a highly significant decrease in T3 and T4 level ($p<0.01$) as compared to control rats. (Table 6).

Table (1): Percentage of body weight change from basal levels, glucose level, insulin and HOMA-IR in control, sodium benzoate-, MSG- and chlorophyllin-treated animals

Groups	control	Sodium benzoate	Mono Sodium glutamate	Chlorophyllin
% of body weight change from basal levels	35.78 ± 0.81	36.58 ± 0.83	46.92 ± 1.06**	30.21 ± 0.68**
% of change from control		2%	31%	-16%
Glucose (mg/dl)	75.41 ± 2.21	83.84 ± 2.03*	88.99 ± 2.19**	74.72 ± 1.26
% of change from control		11%	18%	-1%
Insulin (µg/dl)	4.04 ± 0.37	5.62 ± 0.22**	6.07 ± 0.27**	3.80 ± 0.18
% of change from control		39%	50%	-6%
HOM-IR	0.74 ± 0.07	1.15 ± 0.07**	1.33 ± 0.09**	0.69 ± 0.03
% of change from control		55%	80%	-7%

Values represent mean ± SE (standard error). (P* < 0.05, P** < 0.01 as compared to control group).

Table (2): Serum total protein (g/dl), albumin (g/dl), globulin and albumin globulin ratio in control, sodium benzoate- , MSG- and chlorophyllin-treated animals

Groups	Control	Sodium benzoate	Mono Sodium glutamate	Chlorophyllin
Total Protein (g/dl)	6.66 ± 0.34	4.94 ± 0.13**	4.83 ± 0.22**	6.90 ± 0.22
% of change		-26%	-27%	3%
Albumin (g/dl)	3.82 ± 0.25	2.55 ± 0.17**	2.48 ± 0.23**	3.64 ± 0.16
% of change		-33%	-35%	-5%
Globulin (g/dl)	2.83 ± 0.25	2.38 ± 0.07	2.35 ± 0.07	3.26 ± 0.12
% of change		-16%	-17%	15%
Albumin/Globulin	1.39 ± 0.14	1.07 ± 0.09	1.05 ± 0.12	1.11 ± 0.05
% of change		-23%	-24%	-20%

Values represent mean ± SE (standard error). (P*<0.05, P**<0.01 as compared to control group).

Table (3): ALAT and ASAT activities in control, Sodium benzoate-, MSG- and Chlorophyllin- treated animals.

Groups	Control	Sodium benzoate	Mono Sodium glutamate	Chlorophyllin
ALAT (U/l)	22.98 ± 1.69	33.69 ± 1.97**	38.30 ± 2.00**	32.24 ± 2.40*
% of change		47%	67%	40%
ASAT (U/l)	51.71 ± 1.48	61.50 ± 2.40**	67.88 ± 2.00**	56.44 ± 0.57*
% of change		19%	31%	9%

Values represent mean ± SE (standard error). (P*<0.05, P**<0.01 as compared to control group

Table (4): Changes in total cholesterol (TC), triglyceride (TG), HDL-C, LDL-C, VLDL-C, LDL/HDL ratio and TC/HDL ratio in control, Sodium benzoate-, MSG- and Chlorophyllin- treated animals.

Groups	Control	Sodium benzoate	Mono Sodium glutamate	Chlorophyllin
Total Cholesterol (mg/dl)	80.00 ± 1.55	93.80 ± 3.47**	109.66 ± 2.07**	79.65 ± 1.85
% of change		17%	37%	-0.5%
Triglycerides (mg/dl)	75.78 ± 1.96	95.88 ± 2.20**	107.73 ± 3.63**	79.78 ± 1.94
% of change		27%	42%	5%
HDL-C (mg/dl)	43.75 ± 1.53	30.26 ± 1.49**	32.82 ± 1.31**	43.05 ± 1.38
% of change		-31%	-25%	-2%
LDL-C (mg/dl)	21.09 ± 1.89	44.37 ± 3.59**	55.29 ± 2.20**	20.64 ± 2.57
% of change		110%	162%	-2%
VLDL (mg/dl)	15.15 ± 0.39	19.17 ± 0.44**	21.54 ± 0.72**	15.95 ± 0.38
% of change		27%	42%	5%
LDL/HDL	0.484 ± 0.05	1.48 ± 0.16**	1.70 ± 0.12**	0.482 ± 0.07
% of change		208%	254%	-0.4%
TC/HDL	1.83 ± 0.06	3.12 ± 0.19**	3.36 ± 0.16**	1.85 ± 0.09
% of change		70%	84%	1%

Values represent mean ± SE (standard error). (P*<0.05, P**<0.01 as compared to control group).

Table (5): Serum creatinine and urea levels in control, Sodium benzoate-, MSG- and Chlorophyllin-treated animals

Groups	Control	Sodium benzoate	Mono Sodium glutamate	Chlorophyllin
Creatinine (mg/l)	0.91 ± 0.11	1.63 ± 0.24*	1.77 ± 0.29*	1.05 ± 0.17
% of change		79%	95%	16%
Urea (mg/dl)	30.34 ± 1.98	39.27 ± 1.43**	45.66 ± 2.51**	30.75 ± 2.15
% of change		29%	50%	1%

Values represent mean ± SE (standard error). (P*<0.05, P**<0.01 as compared to control group).

Table (6): Serum Testosterone, T3 and T4 levels in control, Sodium benzoate, MSG and Chlorophyllin treated animals.

Groups	Control	Sodium benzoate	Mono Sodium glutamate	Chlorophyllin
Testosterone (ng/dl)	57.30 ± 2.14	51.00 ± 1.38*	46.35 ± 2.72*	55.30 ± 0.76
% of change		-10%	-19%	-3%
T3 (ng/dl)	108.22 ± 2.27	94.67 ± 2.79**	125.70 ± 1.8**	109.28 ± 1.88
% of change		-12%	16%	1%
T4 (ng/dl)	4.57 ± 0.45	2.81 ± 0.15**	8.32 ± 0.83**	3.84 ± 0.21
% of change		-38%	82%	-16%

Values represent mean ± SE (standard error). (P*<0.05, P**<0.01 as compared to control group).

DISCUSSION

After administration of all food additives used (sodium benzoate, chlorophyllin and monosodium glutamate) so the goal of this study was to assess the side effects of treatment with these food additives on some physiological parameters in male albino rats. Increased percentage of body weight in MSG may be due to the palatability of food and disruption of the hypothalamic signaling cascade of leptin action, which cause the link between monosodium glutamate and obesity and its effect on energy balance [(10)]. However, chlorophyllin group recorded a highly significant decrease. Similar finding was also recorded in **Abou El- Zahab *et al.*** [(11)] who stated that synthetic food colorants cause a reduction in body weight. Treatment with benzoate did not alter the body weight when compared to control as reported by **Kehinde *et al.*** [(12)].

Our results showed a highly significant elevation in blood glucose level, insulin level and insulin resistance (HOMA-IR) in rats orally administrated with sodium benzoate and MSG. 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 [(13)]**.** The increased blood glucose level following MSG administration was attributed to increased gluconeogenesis from glutamate and glutamine. It has been suggested that a possible deterioration of glucose tolerance in rats following MSG administration. The abnormal glucose tolerance could be attributed to decreased cellular insulin sensitivity even under conditions of hyperinsulinemia observed in animals treated with MSG [(14)]. Under conditions of hyperinsulinemia, cells could switch to pathways that favor gluconeogenesis to compensate for the increased insulin release [(15)]. **Yukio *et al.*** [(16)] recoded that glutamate alone does not stimulate insulin secretion. The activation of glutamate dehydrogenase enzyme GDH), stimulates the conversion of glutamate to α-ketoglutarate and play more important role in insulin secretion. GDH enhances glutamate oxidation and increases ATP production by providing the TCA cycle with substrate (α-ketoglutarate) and therefore stimulates insulin secretion.

The presence of hyperinsulinemia/hyperglycemia in MSG-treated rats was detected also by **Hugues *et al.*** [(17)]. They found that glutamate did not lower blood glucose level despite the rise in insulin secretion to a very higher value. They suggested that glutamate caused insulin resistance. In an insulin-resistant person, normal levels of insulin do not have the same effect in controlling blood glucose levels.

During the compensated phase on insulin resistance, insulin levels are higher, and blood glucose levels are still maintained. If compensatory insulin secretion fails, then either fasting (impaired fasting glucose) or postprandial (impaired glucose tolerance) glucose concentrations increase. Insulin itself leads to a kind of insulin resistance, a higher level of insulin than usual that can lead to a kind of positive feedback, increases the need for insulin and causes down regulation of GLUT4 receptor. Insulin resistance in glutamate-treated group could also be due to changes in insulin binding or postreceptor insulin effects in target tissues [(14)]. Also, there was an impairment in glucose adipose tissue uptake in MSG-treated rats due to decrease in GLUT4 in fat cell [(14)] and there was a significant reduction in GLUT4 in skeletal muscle, cardiac muscle and brown adipose tissue in mice. **Machado *et al.*** [(18)] **and Macho *et al.*** [(14)] reported that there was a decrease in the number of insulin receptors in target tissues in MSG-treated rats. They found lower values of binding capacity of insulin receptor in adipocytes, skeletal muscle and liver tissues. This lowering could be due to down regulation of insulin receptor by hyperinsulinemia.

The present study showed that benzoate decreased serum albumin and serum total proteins. This was related to increased oxidative stress leading to poor protein synthesis in different tissues of the treated rats [(19)].

A drop in total protein, albumin levels in MSG-treated groups were determined by our results. **Yousef *et al.*** [(20)] reported an inhibitory effect of some food additives on the biosynthesis of protein and albumin, which in turn indicated that the liver is unable to perform its functions. This might be attributed to decrease protein synthesis or especially albumin through its effect on the liver by inhibiting oxidative phosphorylation process [(21)] or due to the alternation of synthetic function of the liver by MSG.

The present study revealed that rats consumed MSG and sodium benzoate exhibited a highly significant increase in serum ALT and AST, activities when compared to control rats. This agreed with **Tawfek *et al.*** [(4)] who reported an increase in both serum AST and ALT of rats and attributed this to the changes in liver function and hepatocellular impairment, which subsequently caused the release of greater than normal levels of intracellular enzymes into the blood [(22)]. MSG could dissociate easily to

release free glutamate. The diminution of glutamate produces ammonium ion ($NH4^+$) 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 impairment of mitochondrial and plasma membranes resulting in enzyme leakage [23].

Sodium benzoate caused disruption of liver function as revealed by significant elevation of serum ALT and AST. In blood plasma, sodium benzoate has a binding affinity for plasma proteins where it is carried out to different tissues. In the liver, it is metabolized by conjugation with glycine, resulting in the formation of hippuric acid [24].

The significant increase in the activities of serum AST and ALT in rats treated with chlorophyllin may be due to the heptic potency of these colour resulting in destructive changes in the hepatic cells. The colour was administered orally and, hence, they reach the liver first through the portal vein. The effect of the colorants on the liver is in accordance with **Helal *et al.*** [25]**.**

This study, also revealed that rats orally administrated with food additives containing benzoate and MSG 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 to control rats. Similar results were obtained from **Tawfek *et al.*** [4] **and Helal *et al.*** [2]**.** These changes might 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 [26]. 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 might be an indication of membrane structure and function disruption, thus influence its fluidity, permeability, activity of associated enzymes and transport system [20]. 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-α hydroxylase (CYP7A1). Lipid mobilization and storage processes were shown to be affected in liver of rats on MSG diets [27]. The effect of sodium benzoate on lipid profile and their increasing effect in cholesterol concentration in the present study might be an indication of membrane structure and function disruption, thus influence its fluidity, permeability, activity of associated enzymes and transport system [28]**. .**

Our results showed a highly significant elevation in urea and creatinine levels, which is in agreement with [2,4]. This might indicate that MSG and sodium benzoate could impair kidney function that may be due to the effect of these food additives metabolites on the kidney tissues. Also, serum urea and creatinine increased when the ability of the kidney to filter fluid within the body declines. However, MSG and sodium benzoate might have either interfered with creatinine metabolism leading to increased synthesis or the tissues might have compromised all or part of its functional capacity of tubular excretion [3].

The decrease in testosterone hormone in the MSG-treated group agreed with **Helal *et al.*** [2]**, Burde *et al.*** [29] **and Bodnár *et al.*** [30] studies who reported that this might be resulting 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 and disrupted the hypothalamic-pituitary-testes regulatory axis, and these results also agreed with **Ochiogu *et al.*** [31]**.**

The results of this study showed significant reduction in testosterone in rats that were exposed to benzoate, which is in agreement with **Kehinde *et al.*** [32]. The possibility of the low levels of plasma Follicle stimulating hormone; FSH, Luteinizing hormone; LH and testosterone concentration following benzoate exposure in this study might be due to increased oxidative stress. Oxidative stress may suppress the sensitivity of the gonadotrophic cells to gonadotropin-releasing hormone and, therefore, may prevent gonadotropin secretion **(Kamel and Kubajak)** [33]**.**

Our results of this study showed significant reduction in thyroid hormones: triiodothyronine (T3) and thyroxine (T4) in rats exposed to benzoate as compared to control rats. This result agreed with **Sabr** [34] **and Ali** [35] who suggested that loss of thyroid hormones, most probably caused by loss of thyroxin-binding globulin along with T4 bound to it, thus stimulating TSH production. found that

hypothyroidism, thyroid hormone levels were very low, which suggests the possible direct involvement of free radical scavengers and lipid peroxidation. Increased glutathione peroxidase activity could be a compensatory mechanism in response to increased oxidative stress. These results agreed with the present study suggesting that hypothyroidism syndrome is associated with an overall increase in oxidative stress due to treatment with sodium benzoate. Other than the decrease in thyroid function due to oxidative stress, loss of thyroid-hormone-binding proteins in urine could be another cause of the decrease in serum T3 and T4 levels .

Our study showed an increase in the thyroid hormones (T3 and T4) in the glutamate group compared to the control. These changes in thyroid hormones could result from alteration in the pituitary–thyroid axis because of the stressor effect of the chemical component. This was in accordance with **Helal *et al*. [(2)].**

CONCLUSION

Sodium benzoate, monosodium glutamate and chlorophyllin adversely affect and alter different biochemical parameters, especially those related to vital organs e.g. liver and kidney and that chlorophyllin has the least adverse effects in comparison with the other food additives.

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