Egypt. J. Chem. **Vol. 65,** No. 9 pp. 531 - 538 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Amelioratic Effect of Vitamin C against Hazards of Treating Male Albino Rats with Mixture of Food Additives (Sodium Nitrate+ Glycine + Fast Green)

Asmaa A.H.Mohamed[a], Eman G.E. Helal[a]*, Lilian N.Hanna[b], Mohamed A. Abdelaziz[c]

[a]*Department of Zoology, Faculty of Science (Girls), Al-Azhar University, Cairo, Egypt,11651*

[b] *Department of Medical Research, Nuclear Materials Authority, Cairo, Egypt, 11381*

[c]*Department of Physiology, Faculty of Medicine, (Boys), Al-Azhar University, Cairo, Egypt,* 11651*

Abstract

Background: Food additives are compounds that are purposely added to food in order to alter its features, maintain and enhance safety, maintain and increase nutrient value, and improve flavor, texture, and appearance of the food.
Objective: Sodium nitrate, quick green, and glycine, all known carcinogens, were tested on albino male rats in this investigation to check if vitamin C had any protective benefits against those toxins.
Materials and methods: Thirty male albino rats with an average weight of 120-140 g were used in this investigation. Three groupings of animals were formed; control, sodium nitrate + fast green + glycine-treated rats, and rats given the same mixture of food additives plus vitamin C. Samples were taken and the separated sera were used to estimate several biochemical parameters (kidney functions, liver enzymes, glucose, lipid profile as well as protein profile) and hormonal levels of triiodothyronine (T3), thyroxin (T4) and testosterone.
Results: body weight, total protein, albumin and testosterone hormone were decreased in mixture group. The glucose, HOMA-IR ratio, liver enzymes (ASAT, ALAT) and the kidney function (urea and creatinine), TG and TC were increased in mixture group. While HDL and testosterone were decreased in mixture-treated rats. After ingesting vitamin C, these findings, returned nearly to normal levels.
Conclusion: It was shown that vitamin C was able to counteract the negative effects of dietary additives on important physiological indicators in this investigation.

Keywords: Fast green (FG); glycine (GL); Sodium nitrate (SN); Vitamin C (VIT. C); food additives.

1. Introduction

Additives to food are substances other than food that are added to food for a technological purpose in the manufacturing, preparation, processing, packaging, treatment, transportation, as well as storage of this foods. Whether or not they have nutritional value, and the intentional addition of which to a food for this purpose results in it or its by-products are becoming directly or indirectly part of that food [1].

As a substitute for the yellowish light green SF, Fast Green FCF is recommended since its color is more dazzling and lower expected to go away. It is difficult for the digestive system to absorb Fast Green [2]. The European Union and certain other nations have banned its use as a food color. It can be employed at a dosage of up to 100 mg/kg in canned green peas and other vegetables, jellies, sauces, seafood, desserts, and dry pastry mixes. At the previously specified daily intake level of Fast Green FCF, there were no health problems associated with its use, according to a review by the World Health Organization (WHO) released in 2017 (Fast Green FCF's nutritional intake is estimated to be substantially greater than normal) [3].

As a protein-building amino acid, glycine is important. Interference with its release in the spinal cord (such as during a Clostridium tetany infection) can produce spastic paralysis owing to unrestrained muscle contractions because it is an inhibitory neurotransmitter [4]. Schizophrenia and memory improvement are two possible uses for glycine, as it is a key component in the transmission of chemical impulses in the brain. Research suggests that glycine

*Corresponding author e-mail: lilianhanna@yahoo.com.; (Lilian N.Hanna).
Receive Date: 20 November 2021, Revise Date: 21 January 2022, Accept Date: 30 January 2022
DOI: 10.21608/EJCHEM.2022.106840.4910

may be useful in cancer prevention since it appears to interfere with the blood flow required by some cancers. Instead, glycine serves as a flavoring agent in food chemistry. As a result of its complexation with metal ions, it also exhibits antimicrobial characteristics [5].

Sodium nitrate ($NaNO_3$): Nitrates are a white substance that is easily dissolved in water and utilised as a preservative in cured meats and poultry products. Sodium nitrate should not be confused with sodium nitrite, a popular food ingredient and preservative. Gastric and esophageal cancers have been related to nitrosamines, which are generated in cured meats containing sodium nitrate and nitrite [6]. The use of sodium nitrate has been linked to an increased risk of colorectal cancer (CRC). Industrial-scale manufacture of fertilizers, pyrotechnics, food preservatives (especially meats), smoke bombs as well as solid rocket propellants is facilitated by this compound [6].

Ascorbic acid, or vitamin C, is a water-soluble nutrient. Vitamin C cannot be synthesised by the human body. Vitamin C is essential for our health, and we must get it through our food. Vitamin C serves a wide range of purposes: (i) A significant structural component of scar tissue, blood vessels and tendons and ligaments as well as bone synthesis of collagen. (ii) Norepinephrine, a neurotransmitter, is crucial to brain function and has been shown to influence mood, therefore its synthesis is essential. (iii) Protects proteins, lipids, carbohydrates and nucleic acid (DNA and RNA) against destruction by free radicals with a high degree of effectiveness. (iv) Antioxidants like vitamin E. (v) Fat is transported to the mitochondria, where it is converted to energy by the enzyme carnitine synthesized from vitamin C, endothelial nitric oxide (NO) essential for vascular relaxation can be protected and stimulated by vitamin C and erectile and testicular functioning appear to benefit from vitamin C in both healthy and unwell patients with reproductive issues [7].

Aim of work: We aimed to investigate how the combination of several dietary additives (FG, GL, and SN) affected male albino rats' biochemical parameters. Besides, to examine effects of vitamin C, as a medicinal agent, it may enhance these parameters and reduce the toxicity produced by SN and FG.

2. Materials and methods:

30 albino male rats (weighing 120-140 g) were employed. All the animals were housed in cages of steel with free access to water ad libidum. The rats were divided into three equal groups (ten rats each). Control untreated group was the first group. Second-group included rats that received a variety of dietary additives, including quick green (12.5 mg/kg body weight daily), glycine (15 mg/kg body weight daily) and sodium nitrate (10 mg/kg body weight daily). The third group included rats that received a daily dosage of vitamin C (7 mg/kg body weight) along with the preceding mixture of food additives administered orally. Every week, body mass indices were recorded. Anesthetized by inhalation anaesthesia with alcohol, chloroform, and ether in a 1:2:3 ratio, the animals were then weighed and killed after 30 days of therapy. After 10 minutes of centrifugation at 5000 rpm, serums was separated from clotting blood and biochemical characteristics analyzed without delay or storage.

Biochemical investigations:

Total protein and albumin concentration were measured in this study, and then the formula was used to compute serum globulin concentrations (SGC) [8]:

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

Total cholesterol (TC), LDL-C and HDL-C levels, as well as creatinine, urea and fasting blood glucose concentrations, alanine aminotransferase (ALT), Aspartate aminotransferase (AST) activities were also measured. Liaison from Diasorin Italy SA kits, France was used to estimate all parameters. T3 and T4 levels, testosterone and thyroid hormones were quickly measured utilizing Biovendor Research and Diagnostic product reff (Czech republic).

Blood albumin/globulin ratio was calculated. Even though serum LDL-C (low-density lipoprotein cholesterol) and VLDL-C (very low density lipoprotein cholesterol) were obtained, ratios of TC/HDL (risk factor 1) and LDL/HDL (risk factor 2) were computed (very low-density lipoprotein cholesterol) utilizing the Friedwald's [9] as well as Norbert [10] methods, correspondingly as follows:

Friedewald's [9] equation: LDL (mg/dl) = TC-{HDL + [TG/5]}.

Norbert [10] equation: VLDL = TG/5

Serum insulin levels and the HOMA-IR index were measured:

An enzyme-linked immunosorbent assay (ELISA) kit (U.E. Type) was used to quantify rat insulin quickly and accurately (Biovendor Research and Diagnostic product reff (Czech republic)[11].

HOMA-IR:

A fasting plasma glucose sample was utilised to approximate insulin resistance in the early model. A constant was then used to split the insulin-glucose product:

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

Glucose levels in mg/dl of fasting blood.

IR stands for insulin resistance Insulin is measured and given micrograms per liter (u/L) [12].

The Animal Care and Use Committee of the Faculty of Science, Al-Azhar University, Cairo, Egypt, accepted this study's ethical procedures and

regulations. Al-Azhar University's Ethics Board authorized the study.

3. Statistical analysis:

The SPSS (Statistical Package for the Social Sciences) version 22 for Windows® was used to code, process, and to analyse the data (IBM SPSS Inc, Chicago, IL, USA). The Shapiro Walk test was used to examine the distribution of the data. Frequencies and percentages were used to depict qualitative data. For qualitative variables, a Chi square test (2) was used to calculate the difference between two or more groups. Mean SD was used to represent quantitative data (Standard deviation). To compare two independent groups of normally distributed variables, an independent samples t-test was performed (parametric data). It was judged significant when the $P \leq 0.05$.

4. Results:

Body weight: body weight showed highly significant decrease in mixture group compared to control group while body weight showed insignificant change when rats treated with mixture +VC were compared to control (Table 1). **Glucose level, insulin and HOMA-IR,** when mixture group was compared to control rats, there was a substantial (p 0.01) rise in glucose levels and the HOMA-IR ratio, but no change in insulin levels. In contrast, insulin, glucose levels, and the HOMA-IR ratio did not alter significantly in the group of combination + Vitamin C (Table 1).

Protein profile: While albumin/globulin ratio increased (p0.05) in the mixture-treated rats, total protein, albumin, and globulin decreased (p0.01) in comparison to the control group. The addition of Vitamin C restored these measures as compared to the control rats. **(Table 2)**

Liver functions: The ALT and AST activity of the mixture-treated animals increased significantly (p 0.001), whereas the Vitamin C-treated animals showed no change (p 0.05) compared to the control group, meaning that vit.C restored the changes in these enzymes to its normal level (Table 3).

Lipid profile: However, the HDL-C levels of the animals that received the mixture of food additives were significantly lower than the control group's levels (p 0.05) when compared to the TG and TC levels. VIT. C-treated rats, on the other hand, significantly improved all of these indicators (**Table 4)**.

Kidney functions: Rats fed a variety of dietary additives had a substantial rise in creatinine and urea levels (p 0.01), while the addition of vitamin C had no effect on the previous values (Table 5).

Hormones: There were insignificant change inT3 and T4 hormones in mixture- and vitamin C- treated groups. Testosterone hormone showed highly significant decrease in rats treated with mixture and significant decrease in rats treated with mixture and VC (Table 6).

Table (1): Fast green + glycine + sodium nitrate and combination + VC- treated rats were compared for changes in BWC, glucose, insulin, and HOMA-IR

Group	Control	FG+GL+SN	Mixture+ VC
BWC (g)	**35.78±0.59**	**27.74±0.40****	37.33±0.51
% of change		**-22.47 %**	4.33 %
Glucose (mg/dl)	**75.40±0.70**	77.22±0.35*	78.41±2.11
% of change		2.41 %	4%
Insulin (ng/dl)	**4.04±0.06**	4.19±0.09	4.66±0.24
% of change		3.71 %	15 %
HOMA-IR ng/dl)	**0.74±0.01**	0.75±0.01*	0.89±.04
% of change		6.75 %	20 %

FG=fast green; GL= glycine; SN=sodium nitrate; Mean Standard Error (SE) (standard error). As compared to the control group, P* 0.05, P** 0.01.

Table (2): Albumin, Total protein, globulin and albumin/ globulin ratio in control, mixture (fast green+ glycine +sodium nitrate) and mixture+ VC

Group	Control	FG+GL+SN	Mixture +VC
Total protein (g/dl)	6.18±0.08	4.45±0.15**	6.27±0.10
% of change		-27.99 %	1.45 %
Albumin (g/dl)	3.44±0.03	2.73±0.14**	3.52±0.07
% of change		-20.63 %	2.32 %
Globulin (g/dl)	2.74±0.11	1.67±0.12**	2.75±0.13
% of change		-39.05 %	0.36 %
Albumin/Globulin	1.26±0.06	1.67±0.17*	1.29±0.08
% of change		32.53 %	2.38 %

FG=fast green; GL= glycine; SN=sodium nitrate Mean Standard Error (SE) is shown in the values (standard error). Comparing the experimental group to the control group, the results show a P* of 0.05 and a P** of 0.01.

Table (3): Alanine transaminase, aspartic transaminase, in control, mixture (fast green +glycine +sodium nitrate) and mixture+ VC animals

Group	Control	FG+GL+SN	Mixture+ VC
ALAT (i.u/l)	22.98±0.47	42.96±0.69**	24.00±0.70
%of change		86.94 %	4.43 %
ASAT (i.u./l)	51.71±0.31	65.00±0.70**	52.00±0.70
%of change		25.70 %	0.56 %

FG=fast green; GL= glycine; SN=sodium nitrate Mean Standard Error (SE) is shown in the values (standard error). Comparing the experimental group to the control group, the results show a P* of 0.05 and a P** of 0.01.

Table (4): Cholesterol (TC), triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), LDL/HDL and TC/HDL in control, mixture (fast green+ glycine +sodium nitrate) and mixture+ VC

Group	Control	FG+GL+SN	Mixture+ VC
Cholesterol (mg/dl)	80.00±1.14	83.00±0.70*	80.00±0.70
% of change		3.75 %	0.00 %
Triglycerides (mg/dl)	75.78±0.36	78.20±0.58*	75.20±0.80
% of change		3.19 %	-0.76 %
HDL (mg/dl)	43.75±0.53	40.40±0.50*	43.09±0.36
% of change		-7.65 %	-1.50 %
LDL (mg/dl)	21.09±0.78	26.96±0.85**	21.87±0.96
% of change		27.83 %	3.69 %
VLDL (mg/dl)	15.15±0.07	15.64±0.11	15.04±0.16
% of change		3.23 %	-0.72 %
LDL/HDL	0.48±0.01	0.66±0.02**	0.50±0.02
% of change		37.50 %	4.16 %
TC/HDL	1.82±0.01	2.06±0.03**	1.85±0.02
% of change		13.18 %	1.64 %

FG=fast green; GL= glycine; SN=sodium nitrate; Mean Standard Error (SE) is shown in the values (standard error). Comparing the experimental group to the control group, the results show a P* of 0.05 and a P** of 0.01.

Table (5): Creatinine and urea in control, mixture (fast green +glycine+ sodium nitrate) and mixture + VC

Group	Control	FG+GL+SN	Mixture + VC
Creatinine (mg/dl)	0.91±0.05	1.60±0.11**	1.15±.10
%of change		75.82 %	26 %
Urea (mg/dl)	30.34±0.53	42.06±0.33**	34.52±1.65
%of change		38.62 %	14 %

FG=fast green; GL= glycine; SN=sodium nitrate; Mean Standard Error (SE) is shown in the values (standard error). Comparing the experimental group to the control group, the results show a P* of 0.05 and a P** of 0.01

Table (6): Tri-iodothyronin (T_3), thyroxine (T_4), testosterone hormones in control, mixture (fast green +glycine+ sodium nitrate) and mixture+ VC.

Group	Control	FG+GL+SN	Mixture+ VC
T_3 hormone (ng/dl)	108.22±0.83	109.74±0.40	109.50±0.90
% of change		1.40 %	3.03 %
T_4 hormone (ng/dl)	4.57±0.15	4.29±0.12	4.79±0.12
% of change		-6.12 %	4.81 %
Testosterone hormone (ng/dl)	57.30±0.42	41.50±0.50**	55.20±0.66*
% of change		-27.57 %	-3.66 %

FG=fast green; GL= glycine; SN=sodium nitrate

5. Discussion:

It was shown that rats administered with the combination had a considerable drop in body weight,

since rapid green has a big impact on body weight. Synthetic food colourants have been linked to a decrease in body weight [13]. Nitrate's effect on eating behavior's neurological system might possibly lead to weight reduction through nitrate's ability to restrict food intake [14]. A reduction in growth hormone receptors in the liver was discovered to induce a deficiency of plasma somatomedins [14], which affects the growth of body. We found that rats treated with the combination had a considerable rise in glucose and HOMA-IR ratio. When the pancreas loses its endocrine activities, the liver stimulates glycogenolysis and gluconeogenesis, resulting in rise in blood glucose levels. It was shown that the activity of amylase and phosphorylase was increased, leading to the release of glucose from glycogen, which resulted in a rise in blood glucose levels [15]. In addition, the impaired glucose tolerance found in mice treated with sodium nitrate might be ascribed to a lower cellular insulin sensitivity even in the presence of hyperinsulinemia. Gluconeogenesis can compensate for increased insulin secretion in the presence of hyperinsulinemia [15]. Other research showed that nitrate had stimulated gluconeogenesis and increased glucose transport from tissues to the circulation, or that it hampered glucose mobilization [16]. Calcium levels are reduced by nitrate opening potassium channels, which close voltage-controlled calcium channels. Hyperglycemia can be triggered by the release of insulin by calcium, and calcium channel blockers have been shown to cause insulin resistance [16].

Blood glucose, insulin and HOMA-IR are all indications of diabetes mellitus in mixture-treated rats, and vitamin C improved their condition. These actions may be attributed to the regeneration of -cells and the improvement of insulin sensitivity, which lowers the blood sugar levels by suppressing hepatic glucose synthesis and by enhancing the adipose tissue's glucose absorption and metabolism [17]. Our findings indicated that the total protein level in mixture-treated rats was significantly reduced. Fast green increases amino acid deamination as a result of the presence of some hazardous substances [18]. The decrease is due to the reduction of serum globulin level which markedly decline at the same time. The decrease may be an indication of the delayed depression effect of colorant used on immunoglobulin production. The body's defensive mechanism is designed to keep it safe from harmful substances. Nitric oxide and peroxy nitrite, which oxidise proteins and lipoproteins, may be responsible for sodium nitrate's drop in total protein content. No doubt that nitrate has a direct effect on the liver, either by necrotic alterations to the plasma membrane or by inhibiting the oxidative phosphorylation process at first and then the availability of energy source for the synthesis of protein and other metabolic activities [19]. Vitamin C, on the other hand, had a substantial effect on blood total protein levels. Oxidative stress, which damages the liver, which is the body's primary location for protein synthesis, can be prevented by vitamins [20].

Our results stated that the observed elevation in serum ASAT and ALAT activities in response to the administration of the mixture due to hepatic potency of the fast green substance resulting in destructive changes in the hepatic cell. Hepatotoxicity indicators, such as the activity of liver enzymes, have long been employed in clinical trials. Liver cell injury and cellular degeneration or destruction may be a result of increased permeability of the plasma membrane or necrosis in the presence of ALAT and ASAT in the blood of patients treated with these additions. Bloodstream enzymes that typically reside in the cytoplasm of a damaged liver cell membrane might leak out into the bloodstream. When ASAT and ALAT levels are elevated, it suggests that amino acids are being used for oxidation or gluconeogenesis. Toxicity-induced liver damage and necrosis can be detected using these tests [21]. **Amin *et al.*** [22] and **Mekkawy *et al.*** [23] reported that those artificial dyes such as (fast green) can cause considerable elevations in serum ALT and AST whether used in low or high dosages. In addition, elevated ALT and AST activities in rats provided by food azo dyes (fast green) revealed that both the hepatic cellular and mitochondrial membranes were damaged in rats exposed to food azo dyes.

Due to the production of the free radical ONOO- from nitric oxide, sodium nitrate treatment resulted in a rise in ASAT and ALAT levels in rats. There are several ways in which NO and oxygen radicals might combine further to form various oxidants and nitro compounds such as peroxynitrite, which in turn could cause liver damage and cell death [24]. With VIT C, the animals' ASAT and ALAT levels were brought back into normal range. Vitamin C's antioxidant properties may be to blame. Vitamin C's anti-inflammatory properties may be attributed to its ability to suppress the production of free radicals and/or its ability to scavenge free radicals, according to a prior study [25].

In the present work, treatment of rats with the mixture resulted in obvious changes in the lipid profile causing significant increase in total cholesterol, triglycerides, and highly significant increase LDL-C, TC/HDL and LDL/HDL, while HDL was decreased. This because nitrate affected directly the liver which plays an important active role on cholesterol metabolism and increased cholesterol levels [26]. Accumulation of acetyl CoA causes a rise in the production of cholesterol by mobilisation of adipose-derived free fatty acids or peroxidation of membrane lipids within cells [26]. Increasing in LDL increases the risk of cardiovascular diseases. 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. Cholesterol may be a sign that membrane structure and function have been compromised, which might affect fluidity, permeability and the activity of enzymes that are connected with it [27]. LDL oxidation can be prevented by the water-soluble antioxidant vitamin C, which regulates lipids [28].

Fast green improved protein catabolism and rapid amino acid deamination for gluconeogenesis may explain why urea and creatinine levels increased in mixture-treated rats. Degradation of purines or an increase in uric acid levels as a result of excessive production or inability to excrete might explain the rises in uric acid levels [29]. Renal dysfunction is indicated by higher urea and creatinine values. The oxidative stress generated by treatment with SN may be to blame. Sodium nitrate induces renal tissue necrosis and the degradation of the epithelium lining renal tubules as a result of these pathological alterations. The findings of **El-Ezaby *et al.*** [30] are in agreement with these findings of renal dysfunction. Kidney biochemical markers improved significantly when VC was added to the bloodstream. Vitamin C's modulator effects induced oxidative damage to rats' livers and kidneys. As renal failure progresses, the levels of urea and creatinine in the blood vary [31]. In the mixed group, there was no significant change in thyroid hormones T3 and T4 due to the daily ingestion of sodium nitrate, which reduces T3 and T4 and fast green, which increases T3 and T4. That's why we've found that T3 and T4 levels are close to those of the control rats.

When we tested the combination group, we found that testosterone levels were significantly reduced. In rats, fast green has several detrimental consequences on sperm and steroid production. A lack of testosterone in the testis is linked to a decrease in Leydig cell activity or a decrease in testosterone capture in the testis. It is a known fact that testosterone is a crucial element governing testicular growth and sperm death [32]. Neurogenic functional alterations in the hypothalamus of glycine-treated rats reduced the levels of LH, FSH, and testosterone. Glycine was shown to kill hypothalamic neurons in rats, according to one study. The hypothalamic-pituitary-testis regulatory axis, which governs the steroidogenesis of testicular Leydig cells, can be disrupted by such neuronal losses in the hypothalamus [33]. Serum testosterone levels will fall as a result of this. The testosterone hormone value and level were significantly improved in VIT. C animals compared to the mixture-treated animals. Testicular steroid dehydrogenase was induced by ascorbic acid and testosterone levels were elevated, according to Biswas *et al.* [34]. FSH and LH are released from the anterior pituitary gland by autocrine action via nitric oxide when ascorbic acid is taken, according to **Karanth *et al.*** [35]. Testosterone is released by Leydig cells in response to LH. **Sönmez *et al.*** [36] demonstrated that ascorbic acid-treated rats had a considerably higher plasma testosterone level than control animals. It's possible that ascorbic acid, which is found in citrus fruits, stimulates the release of LH.

6. Conclusion:

Most physiological indices, including testosterone hormone, kidney, liver enzymes, protein as well as lipid profiles, are severely harmed by food additives. It's best to avoid using them in most dishes, especially for children. Our recommendation is to use VIT C, which has great therapeutic benefits against the abnormalities caused by dietary additive ingestion and restores these abnormalities to their normal values.

7. Reference:

1. **Tawfek N, Amin H, Abdalla A *et al.* (2015).** Adverse effects of some food additives in adult male albino rats. Current Science International, 4 (4). 525-537
2. **Helal EG, Zaahkouk SA, Mekkawy HA (2000).** Effect of Journal of some food colorants (synthetic and natural products) of young albino rats. I–Liver and kidney functions. The Egyptian Hospital Medicine, 1: 103-113. 7
3. **Amin KA, Hameid H A, Elsttar, AA (2010).** Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food and Chemical Toxicology, 48 (10). 2994-2999.
4. **Drauz, Karlheinz; Grayson, Ian; Kleemann, Axel; Krimmer, Hans-Peter; Leuchtenberger, Wolfgang and Weckbecker, Christoph (2007).** "Amino Acids". Ullmann's Encyclopedia of Industrial Chemistry. Weinheim: Wiley-VCH.
5. **Meléndez-Hevia 9 E, De Paz-Lugo P, Cornish-Bowden A, Cárdenas M L (2009).** A weak link in metabolism: the metabolic Capacity for glycine biosynthesis does not satisfy the need for collagen synthesis. Journal of Biosciences, 34 (6). 853–72.
6. **Jakszyn, Paula, Gonzalez, Carlos A (2006).** Nitrosamine and related food intake and gastric and oesophageal cancer risk: a systematic review of the epidemiological evidence. World Journal of Gastroenterology, 12 (27). 4296–4303
7. **Kehinde OS, Christianah O I,Oyetunji OA (2018).** Ascorbic acid and sodium benzoate synergistically aggravates testicular dysfunction in adult Wistar rats.International Journal of Physiology, Pathophysiology and Pharmacology, 10 (1). 39-46.

8. **Young DS (2001).** Effects of disease on Clinical Lab. Tests.4thed.AACC. WB Saunders Company, Philadelphia, Pp: 147–148.
9. **Friedewald WT, Levy RI, Fredrickson DS (1972).** Estimation of the concentratin of low density lipoprotein cholesterolinplasma, without use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.
10. **Norbert WT (1995).** Clinical guidetolaboratorytests. 3rded.Saunders W.B., Company, Philadelphia. 244-249
11. **Reeves WG (1983).** Insulin antibody determination: The oretical and practical. Diabetologia, 24 (6). 399-403
12. **Matthews DR, Hosker JP, Rudenski AS, Treacher DF *et al.* (1985).** Homeostasis model assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentration inman. Diabetologia, 28 (7). 412-419.
13. **Helal EG, Barayan AW, Abdelaziz MA, EL-Shenawe N S (2019).** Adverse Effects of Two Kinds of Food Additive Mixtures (Sodium benzoate+ Monosodium glutamate, Monosodium glutamate + Chlorophyllin and Sodium benzoate + Chlorophyllin) on Some Physiological Parameters in Male Albino Rats. Egyptian Journal of Hospital Medicine, 75 (4). 27362744
14. **Al-Ayed, M. I. (2000).** Toxicity of drinking water with different nitrate levels. J. Egypt. Ger. Soc. Zool., 31 (A). 197-209.
15. **Shelpov V, Chekulaev V, Pasha-Zade G (1991).** Factors within the body determining the glycogen reserves in the tissues of rats. Biomed Sci., 2: 111-20.
16. **16-Boukerche S, Aouacheri W, Saka S (1997).** Toxicological effects of nitrate: biological study in human and animal. Ann de Biol Clin., 65, 385-91. 31
17. **Helal E G, Abd-Elwahab S M, Mohammad A A (2013).** Comparison between the effect of ozone and vitamin C intreatment of diabetes mellitus. The Egyptian Journal of Hospital Medicine,31 (762). 1-14.
18. **Amin, K., I.H. Hameid and A. AbdElsttar, (2010).** Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food and - Chemical Toxicology, 48 (6). 2994-2999.
19. **El-Wakf AM, Elhabiby EM, El-kholy WM, *et al.* (2011).** Use of tumeric and curcumin to alleviate adverse reproductive outcomes of water nitrate pollution in male rats. Nature and Science, 9. 229-39999.
20. **Tawfik M S,Al-Badr N (2012).** Adverse effects of monosodium glutamate on liver and kidney functions in adult rats and potential protective effect of vitamins C and E. Food and Nutrition Sciences, 3 (05). 651-659.
21. **Amin KA, Abdel Hameid H, Abd Elsttar AH (2010).** Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young malerats. Food. Chem. Toxicol., 48 (10). 2994-2999.
22. **Mekkawy HA, M O Ali A M El-Zawahry (1998).** Toxic effect of syntheti and natural food dyes on renal and hepatic functions in rats. Toxicology Letters 95 Supplement 1. 155.
23. **Ahmed M (2016).** Effect of some Food Additives Consumption on the Body Weight and Toxicity and the Possible Ameliorative Role of Green Tea Extract.Sciences, 6 (04). 716-730.
24. **Dosuky MA, Zaghlol DA, Ouies SM *et al.* (2018).** Effects of Monosodium Glutamate on the Liver of Male Adult Albino Rat and the Possible Protective Role of Vitamin C (Light and Electron Microscopic Study Med. JCairoUniv., 86 (7). 3407-3418.
25. **Barja G, Lopez Torres M, Perez-Campo R *et al.* (1994).** Dietary vitamin c decreases endogenous protein oxidative damage, malondialdehyde, and lipid peroxidation and maintains fatty acid unsaturation in the guinea pig liver. Free Radicals Biology and Medicine, 17 (2). 105-115.
26. **Husain A, Sawaya W, Al-Omair A, Al-Zenki S, Al-Amiri H, Ahmed N.(2006).** Estimates of dietary exposure of children to artificial food colours in Kuwait. Food Addit Contam., 23 (3). 245-51.
27. **Abu Aita N, Mohammed F. (2014).** Effect of marjoram oil on the clinicopathological, cytogenetic and histopathological alterations induced by sodium nitrite toxicity in rats. Glob Vet., 12 (5). 606-16.
28. **El Mashad G M, ElSayed H M, Nosair N A (2016).** Effect of vitamin C supplementation on lipid profile, serum uric acid, and ascorbic acid in children on hemodialysis. Saudi Journal of Kidney Diseases and Transplantation, 27 (6). 1148-1154.
29. **Helal EGE, Zaahkouk SAM, Mekkawy HA. (2000).** Effect of some food colorants (synthetic and natural products) of young albino rats. Egypt J Hosp Med., 1 (1). 103-13.
30. **El-Ezaby M, Hamide N, Abd El-Maksoud M, Shaheen E, Embashi M (2018).** Effect of some food additives on lipid profile, kidney function and liver function of adult male albino rats. J Basic and Environmental Sciences, 5. 52-59
31. **Tawfik M S, Al-Badr N (2012).** Adverse effects of monosodium glutamate on liver and kidney

functions in adult rats and potential protective effect of vitamins C and E.Food and Nutrition Sciences, 3 (05). 651-659.

32. **Bodnár I, Göõz P, Okamura H, Tóth B, Vecsernyé M, Halász B, *et al.* (2001).** Effect of neonatal treatment with monosodium glutamate on dopaminergic and L-DOPA-ergic neurons of the medial basal hypothalamus and on prolactin and MSH secretion of rats. Brain Res Bull., 55 (6). 767-74.
33. **Singh J, O'Neill C, Handelsman D.(1995).** Induction of spermatogenesis by androgens in gonadotropin-deficient (hpg) mice. Endocrinology, 136 (12). 5311-21.
34. **Biswas N M, Chaudhuri A, Sarkar M *et al.* (1996).** Effect of ascorbic acid on in vitro synthesis of testosterone in rat testis.Indian Journal of Experimental Biology, 4 (6). 612-613.29.
35. **Karanth S, Wen H Y, Walczewska A *et al.* (2001).** Ascorbic acid stimulates gonadotropin release by autocrine action by means of NO.Proceedings of the National Academy of Sciences, 98 (20). 11783-11788.30.
36. **Sönmez M, Türk G,Yüce A (2005).** The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats.Theriogenology, 63 (7). 2063-2072