Egyptian Journal of Zoology (EJZ), Vol. 79: 66-77 (June, 2023) p-ISSN: 1110-6344
Publisher: The Zoological Society of A. R. Egypt e-ISSN: 2682-3160
DOI: 10.21608/ejz.2022.147732.1084

RESEARCH ARTICLE

PROTECTIVE EFFECT OF GARLIC OIL AGAINST TARTRAZINE-INDUCED HAEMATO-IMMUNOTOXICITY IN RATS

Heba M. Abd El Latif*; Asmaa M. El-Morsy

Zoology Department, Faculty of Science, Menoufia University, Menoufia, Egypt

Article History:
Received: 29 June 2022
Revised: 10 August 2022
Accepted: 16 August 2022

Published Online:
30 September 2022

Keywords:
Blood biochemical
Complete blood count
Food azo dye
Garlic oil
Splenic antioxidants

***Correspondence:**
Heba Abd El Latif
Zoology Department
Faculty of Sciences
Menoufia University
Menoufia, Egypt
E-mail:
heba.abdellatif@science.menofia.edu.eg

ABSTRACT

Constituents of garlic oil are reported to possess immunomodulatory, antioxidant, and anticancer effects. Use of tartrazine as a food colouring-compound is associated with significant toxicity on the body. The present study was conducted to evaluate the protective effect of garlic oil on haemato-immunotoxicity induced by tartrazine. Male Wistar albino rats were treated orally with 40 mg garlic oil/kg body weight thirty minutes before the administration of 300 mg tartrazine/kg body weight for thirty consecutive days. Twenty-four hours after the last garlic oil and tartrazine dose, blood and spleen were harvested to analyse toxicity-related parameters. The tartrazine treatment induced significant changes in the body weight gain, relative spleen weight, haematological parameters, proliferation rate of splenocytes, and percentage of T-cell subtypes in the spleen, as detected by flow cytometric analysis, in male rats. Moreover, oxidative stress in the spleen tissue and hepatic and renal dysfunction were detected after exposure to tartrazine. Treatment with garlic oil showed significant reduction in overall toxicity in the tartrazine-treated rats. The antitoxic effects of garlic oil were associated with the induction of antioxidant mechanisms. In conclusion, garlic oil can lower tartrazine-induced haemato-immunotoxicity through its antioxidant activity.

INTRODUCTION

Food additives are compounds added to basic food for many purposes, so they are divided into five main groups according to their function: taste enhancers, preservatives, antioxidants, emulsifiers and stabilizers, and colouring agents[1]. Food dyes are the most employed group of food additives that enhance the visual appearance of foods making them attractive for consumers. Some of them are natural and others are synthetic dyes[2]. Synthetic dyes are very harmful to living things because they generally contain one or more azo group in their structure. These azo groups (-N=N-) are reduced in mammals by bacterial activity of lower part of gastrointestinal tract, into aromatic amines, which are known by their mutagenic and carcinogenic effects[3,4].

The tartrazine (Tz), trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-[(E)-(4-sulfonatophenyl) diazenyl]-1H-pyrazole-3-carboxylate, is man-made yellow azo dye used as food colouring compound. Amin and Al-Shehri[2] have documented the use of Tz in the manufacture of many products including food stuffs (sauces, jam, candy, mustard, noodles, etc.), non-food products

(crayons, hair products, soaps, etc.) and medications (antacids, vitamins, certain prescription medications, and medical capsules). Tz was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which elevates its acceptable daily intake (ADI) dose from 0-7 mg/kg body weight/day to 0-10 mg/kg body weight/day at their 2016 meeting[5]. The study of Rao and Sudershan[6] showed that the rate of exposure to Tz exceeds ADI especially in festive and wedding times. Amin *et al.*[7] reported that the consumption of Tz at higher doses leads to detrimental health effects. It has also been concluded that, Tz induced embryotoxic and teratogenic effects[8,9]. It also exerted significant adverse effects in the vital organs including brain, kidney, liver, and spleen[10-12]. Moreover, the findings of Abd-Elhakim *et al.*[13] indicated that Tz showed haematotoxic and immunotoxic effects following long-term exposure. Such toxic effects of Tz encourage the researchers to develop new strategies to reduce its adverse effects.

Garlic (*Allium sativum* L.; Alliaceae) belongs to a group of plants with high nutritional and medicinal value, its medicinal use dates back to ancient times[14]. Garlic and its components have been reviewed for their different biological activities[15]. Garlic oil (GO) has been documented to possess several organo-sulphur compounds including diallyl sulphide, diallyl disulphide, and diallyl trisulphides[16]. Along the same lines, previous studies revealed the beneficial and protective effects of GO including its ability to prevent cardiovascular diseases[17], and as immunomodulatory[18], anticancer[19], and anti-obesity agent[20]. Furthermore, the findings of Aly *et al.*[21] proposed that GO has the ability to reduce hepatocellular carcinoma development.

The dietary supplementation with nutritional antioxidants and immuno-modulators could reduce oxidative stress, limit organ damage, and improve vital functions of the body. From this point of view, the current work was planned to indicate the haemato-immunotoxic effects of Tz and to study the protective effect of GO against them.

MATERIAL AND METHODS

Chemicals and reagents

Tz (FD&C yellow 5, E102) powder was obtained from Kamina Company (Cairo, Egypt) and dissolved in distilled water. GO was obtained from El-Captain Company for Oil and Herbs Extraction (Cairo, Egypt). Anti-mouse CD3 monoclonal antibody labelled with fluorescein isothiocyanate (FITC), anti-mouse CD4 monoclonal antibody labelled with allophycocyanin (APC) and anti-mouse CD8 monoclonal antibody labelled with phycoerythrin (PE) were obtained from BD Bioscience Company (San Jose, CA, USA).

Animals and experimental design

Twenty-four male Wistar albino rats (*Rattus norvegicus*), weighing about 200.0±20.0 g and their ages = 17.0±1.0 weeks, were purchased from animal house of National Research Centre (Dokki, Giza, Egypt) and kept under proper conditions with 12-hour dark/light cycle. Rats were acclimated 14 day before use in our study. They had free access to food and water *ad libitum.* Animals were used after consent of the Institutional Animal Ethical Committee, Menoufia University (approval ID: MUFS/F/PH/1/20). Rats were divided into four groups (n=6). Group I: control group received distilled water, group II: GO group received 40 mg GO/kg body weight[18], group III: Tz group received 300 mgTz/kg body weight (less than 15% of rat LD50)[12], and group IV: Tz+GO group received Tz 30 minutes after GO administration. All compounds were orally administered for 30 consecutive days.

Sampling

Rats were anesthetized using Halothane (Pharco, Alexandria, Egypt) 24 hour after the last treatment and then dissected; immediately blood samples were collected from the hepatic portal vein. Each sample

was divided into 2 tubes, the first was mixed with EDTA and the second was allowed to coagulate at room temperature for serum separation that kept at –80ºC in deep freezer for biochemical assays outlined below.

Body weight gain and relative spleen weight

Body weight of animals was documented at the beginning and on the last day of the experiment to calculate the percentage of body weight gain as follows:

Body weight gain (%) = [(Final body weight – Initial body weight) / Initial body weight] × 100.

Spleen was carefully removed, made free of adherents, and weighed to calculate its relative weight as follows:

Relative spleen weight = (spleen weight/body weight) × 100[12].

Evaluation of haematological parameters

The red blood cell (RBC) counts, haemoglobin content, haematocrit value, white blood cell (WBC) total and differential counts, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and platelet counts were performed manually using blood sample mixed with EDTA as described previously[22].

Analyses of the biochemical parameters in serum

The albumin, total protein, creatinine, and urea concentrations, as well as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined by means of spectrophotometer utilizing their respective kits obtained from Biodiagnostic Company (Giza, Egypt) according to the manufacturer's guides.

Oxidative stress biomarkers in spleen homogenate

Homogenates of spleen tissues were prepared for the detection of biomarkers of oxidative stress as mentioned by Elsayed *et al.*[23]. The measured oxidative stress biomarkers were malondialdehyde (MDA; catalogue number: MD 2529, Biodiagnostic, Cairo, Egypt), catalase (CAT, catalogue number: CA 2517, Biodiagnostic), superoxide dismutase (SOD; catalogue number: SD 2521, Biodiagnostic), and reduced glutathione (GSH; catalogue number: SD 2511, Biodiagnostic).

Splenic T cell phenotyping

Spleen cell suspension was prepared, viable cells were counted using trypan blue dye exclusion method, for determination of $CD3^+CD4^+$ T-helper and $CD3^+CD8^+$ T-cytotoxic cells by FACS flow cytometer (Becton Dickinson, Sunnyvale, CA, USA) with Cell Quest Software for data acquisition and analysis as mentioned by Ibrahim *et al.*[24].

Spleen cells proliferation assay

Spleen cells proliferative response to mitogen concanavalin A (Con A, Sigma, St Louis, MO, USA) was detected by a micro-tissue culture system as described previously by Ibrahim *et al.*[24]. Briefly, Spleen cells (5×10^5) from control or treated rats were cultured for 24 hour with Con A. Then, Cell Counting Kit-8 (Sigma) reagent (10 µL) was added. After three hours of incubation at 37 °C and 5% CO_2, the optical density was determined at 450 nm by microplate reader (SEAC srl, Calenzano, Italy).

Data evaluation and statistical analysis

All data sets were expressed as mean ± standard error of the mean. The data were statistically analysed by one-way ANOVA test followed by post-hoc analysis of group differences that was accomplished by the least significant differences (LSD) test; using statistical package of social sciences (IBM SPSS) statistics software for windows Version 22 (IBM corp., Armonk, NY, USA). Differences considered significant if $P<0.05$ and highly significant if $P<0.001$.

RESULTS

Effect of garlic oil on the changes in the body weight and the relative spleen weight in tartrazine-treated rats

Figures "1 and 2" summarized the effect of GO on changes in the percentage of body weight gain and the relative spleen weight induced by Tz. After thirty days of exposure to Tz, the mean weight gain and relative spleen weight decreased significantly (P<0.001) compared with the control group. In contrast, GO treatment modulated significantly (P<0.001) the decrease in the body weight gain (%) and relative spleen weight in Tz-treated rats.

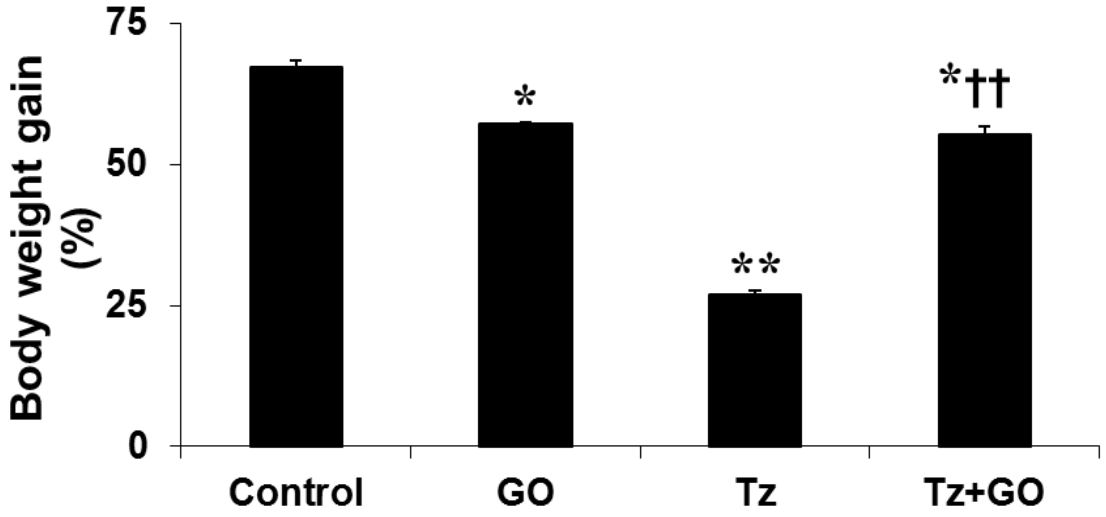

Figure 1: Effect of garlic oil (GO) on the body weight gain (%) of rats treated with tartrazine (Tz). Data are expressed as mean ± standard error of the mean (n=6). *, **: Significant (P<0.05) and highly significant (P<0.001) compared with the control group, respectively; ††: highly significant (P<0.001) compared with the tartrazine group.

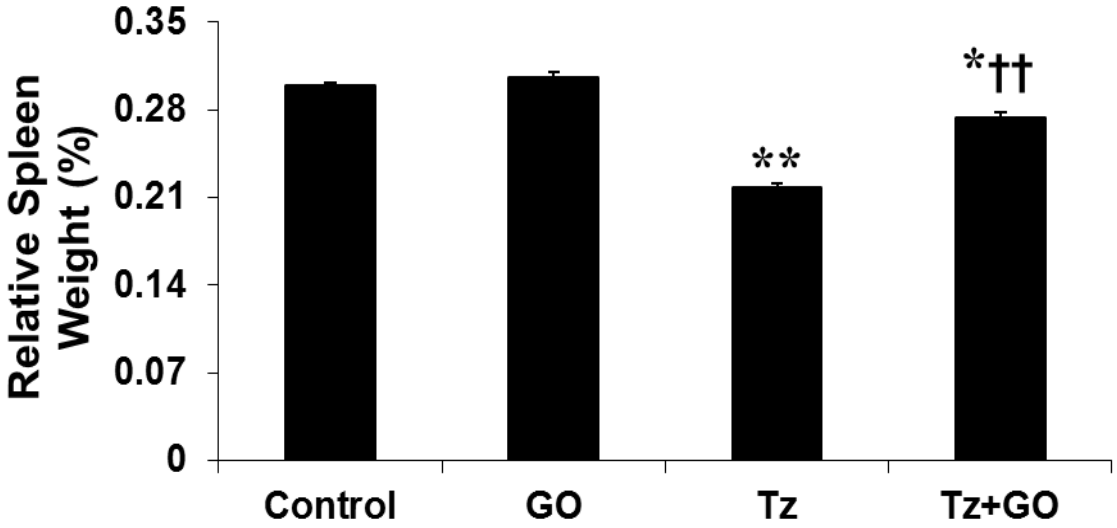

Figure 2: Effect of garlic oil (GO) on the relative spleen weight of rats treated with tartrazine (Tz). Data are expressed as mean ± standard error of the mean (n=6). *, **: Significant (P<0.05) and highly significant (P<0.001) compared with the control group, respectively; ††: highly significant (P<0.001) compared with the tartrazine group.

Effect of garlic oil on the changes in the haematological parameters of tartrazine-treated rats

After the treatment period, almost all the haematological parameters were altered significantly (P<0.05-0.001) in Tz-treated rats compared with the control animals (Table 1). The erythrogram results revealed that the RBCs count, haemoglobin content, haematocrit value, MCV, and platelet count were reduced significantly (P<0.05), while MCHC increased significantly (P<0.05) in response to Tz, indicating hyperchromic microcytic anaemia and thrombocytopenia. In contrast, they were significantly modulated (P<0.05-0.001) upon GO treatment in the Tz-treated group (Table 1). Moreover, the leukogram results revealed that, Tz administration induced significant increases (P<0.001) in the total WBCs count, absolute monocyte and granulocyte counts, associated with a significant decrease (P<0.05) in the absolute lymphocyte count as compared with the control group (Table 2). On the other hand, treatment with GO modulated significantly (P<0.05-0.001) the changes in leukogram induced by Tz (Table 2).

Table 1: Effect of garlic oil (GO) on the erythrogram and platelet count of rats treated with tartrazine (Tz).

	Control	GO	Tz	Tz+GO
RBCs count (10^6/mm^3)	6.31±0.14	6.15±0.13	4.23±0.12**	5.66±0.20*††
Haemoglobin content (g/dL)	12.93±0.17	13.21±0.20	8.01±0.12**	11.86±0.23*††
Haematocrit value (%)	39.00±0.44	39.66±0.66	23.00±0.73**	36.50±0.61*††
MCV (pg)	61.95±1.90	64.61±1.51	54.42±1.61*	64.84±2.61††
MCH (fL)	20.55±0.70	21.54±0.57	18.98±0.39	21.08±0.88†
MCHC (g%)	33.16±0.15	33.32±0.22	34.95±0.63*	32.50±0.13††
Platelet count (10^5/mm^3)	4.36±0.12	4.50±0.10	3.16±0.07**	4.11±0.09††

Data are expressed as mean ± standard error of the mean (n=6). *, **: Significant (P<0.05) and highly significant (P<0.001) compared with the control group, respectively; †, ††: significant (P<0.05) and highly significant (P<0.001) compared with the tartrazine group, respectively; RBCs: red blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration.

Table 2: Effect of garlic oil (GO) on the leukogram and spleen T-cell subtyping of rats treated with tartrazine (Tz).

	Control	GO	Tz	Tz+GO
WBCs (10^3/mm^3)	9.01±0.16	8.85±0.12	10.43±0.12**	9.33±0.13††
Lymphocytes (10^3/mm^3)	6.17±0.14	6.04±0.14	5.49±0.13*	5.89±0.11†
Monocytes (10^3/mm^3)	0.91±0.02	0.85±0.03	1.66±0.05**	1.08±0.04*†
Granulocytes (10^3/mm^3)	1.92±0.06	1.94±0.06	3.26±0.06**	2.41±0.10**††
$CD3^+CD4^+$ (%)	56.66±0.91	52.83±0.74	25.33±0.61**	47.16±0.70*††
$CD3^+CD8^+$ (%)	23.66±0.91	22.16±0.83	14.16±0.47**	19.5±0.42*††

Data are expressed as mean ± standard error of the mean (n=6). *, ****:** Significant (P<0.05) and highly significant (P<0.001) compared with the control group, respectively; †, ††: significant (P<0.05) and highly significant (P<0.001) compared with the tartrazine group, respectively; WBCs: white blood cells.

The immunomodulatory and antioxidant effects of garlic oil on spleen cells of tartrazine-treated rats

The percentage of splenocytes proliferation was reduced significantly (P<0.001) in Tz-treated rats when compared with the control rats. Administration of GO to Tz-treated rats was able to modulate significantly (P<0.001) the changes in splenocytes proliferation (Figure 3). In addition, a significant reduction (P<0.001) in $CD3^+CD4^+$ and $CD3^+CD8^+$ subsets of spleen cells were recorded in Tz-treated rats, as compared with the control rats (Table 2). Co-administration of GO with Tz modulated significantly (P<0.001) the reductions in both spleen T-helper and T-cytotoxic cell subtypes (Table 2).

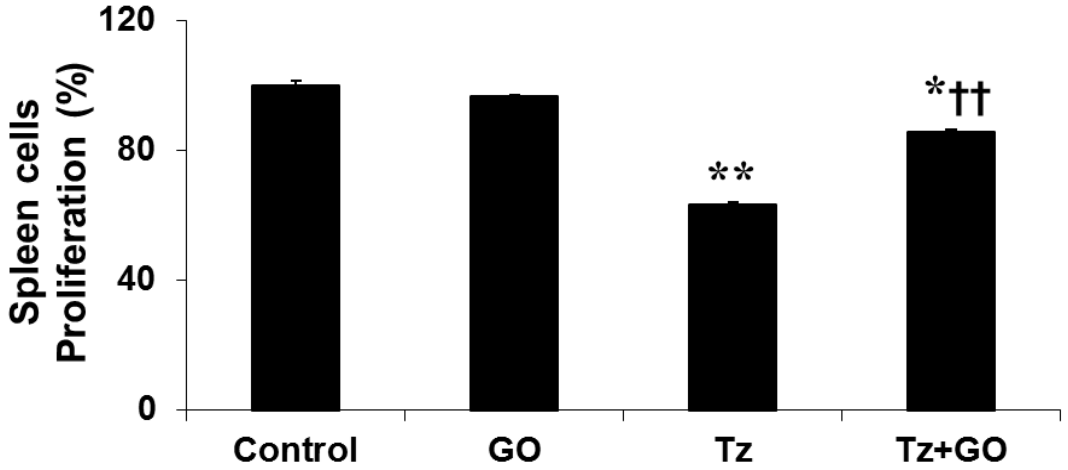

Figure 3: Effect of garlic oil (GO) on the spleen cells proliferation of rats treated with tartrazine (Tz). Data are expressed as mean ± standard error of the mean (n=6). *, **: Significant (P<0.05) and highly significant (P<0.001) compared with the control group, respectively; ††: highly significant (P<0.001) compared with the tartrazine group.

The effects of Tz and GO on the lipid peroxidation and antioxidant defence system of the spleen tissue were summarized in Table "3". The Tz treatment resulted in a significant (P<0.001) increase in MDA level and a significant (P<0.001) decrease in GSH level and the activities of CAT and SOD when compared with the control group. The effect of GO was very helpful to significantly (P<0.001) decrease the MDA level and increase the level of GSH and the activities of CAT and SOD in Tz-treated group (Table 3).

Table 3: Effect of garlic oil (GO) on the lipid peroxidation and antioxidant defence system in spleen tissue of rats treated with tartrazine (Tz).

	Control	GO	Tz	Tz+GO
MDA (nmol/g tissue)	144.88±1.08	141.02±1.34	259.51±3.77**	179.83±1.40*††
GSH (mg/g tissue)	125.73±0.47	126.80±1.12	76.41±0.77**	112.36±1.33*††
SOD activity (U/g tissue)	30.40±0.24	31.15±0.19	21.15±0.14**	28.08±0.20*††
CAT activity (U/g tissue)	3.52±0.07	3.27±0.05	2.35±0.05**	3.11±0.06††

Data are expressed as mean ± standard error of the mean (n=6). *, **: Significant (P<0.05) and highly significant (P<0.001) compared with the control group, respectively; ††: highly significant (P<0.001) compared with the tartrazine group; MDA: malondialdehyde, GSH: reduced glutathione, SOD: superoxide dismutase; CAT: catalase.

The effects of the garlic oil on the biochemical parameters in serum of tartrazine-treated rats

Table "4" showed that Tz induced a significant (P<0.001) increase in serum ALT and AST activities (indicating tissue damage including liver injury), and total protein, albumin, urea, and creatinine levels (indicating dehydration and kidney dysfunction) in comparison with the control rats. On the other hand, GO administration induced a significant (P<0.001) modulation in the above serum biochemical parameters in the Tz-treated rats (Table 4).

Table 4: Effect of garlic oil (GO) on serum biochemical analyses related to liver and kidney functions of rats treated with tartrazine (Tz).

	Control	GO	Tz	Tz+GO
ALT activity (U/L)	8.45±0.14	8.00±0.11	17.00±0.26**	9.85±0.20*††
AST activity (U/L)	49.31±0.19	47.90±0.45	59.50±0.31**	50.98±0.30††
Total protein (g/dL)	5.55±0.09	5.22±0.15	7.05±0.15**	6.00±0.17*††
Albumin (g/dL)	3.45±0.13	3.25±0.20	4.93±0.16**	3.69±0.11*††
Urea (mg/dL)	15.20±0.22	14.70±0.28	30.21±0.26**	17.01±0.41††
Creatinine (mg/dL)	1.17±0.06	1.07±0.04	2.10±0.06**	1.09±0.03††

Data are expressed as mean ± standard error of the mean (n=6). *, ****:** Significant (P<0.05) and highly significant (P<0.001) compared with the control group, respectively; ††: highly significant (P<0.001) compared with the tartrazine group; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

DISCUSSION

Recently, the excessive use of synthetic azo dyes such as Tz has led to a strong trend towards examining their harmful effects on the body and the possibility of reducing these damages using natural antioxidant and therapeutic compounds. Perceiving plant foods as beneficial diet is advised by the folklore of many cultures over centuries. The present study identified the therapeutic effects of GO on the toxicity induced by Tz, on different physiological parameters in rats. Thirty days of exposure to Tz (300 mg/kg body weight) induced a significant decrease in the percentage of the body weight gain and relative spleen weight, which is considered as a primary sign of toxicity resulting from the use of Tz. These findings are in concordance with Amin *et al*.[7] and El Golli *et al*.[12] who reported a reduction in the body weight gain of rats exposed to Tz. The relative spleen weight was also reported to be reduced after mice treatment with Tz[25]. Abd El-Wahab and Moram[26] documented that food colorants, when administered through the mouth, might bind to active intestinal bacteria, and decrease their number leading to inhibition of food absorption and decrease body weight. Furthermore, Amin *et al*.[7] documented that the ingestion of food colour or parenteral, may reaches into the gut promptly or through the bile. Therefore, it could be subjected to the digestive enzymes, gastric acids, or microbiota.

Obviously, our results demonstrated that the Tz treatment of male rats resulted in anaemia, thrompocytopoenia, and leucocytosis. Similar findings were recorded by previous studies[13,27,28]. These haematological changes could be attributed to the oxidative stress induced by Tz in RBCs and bone marrow as indicated by Barhoma *et al*.[28] who reported an increase in MDA level and a decrease in GSH level of RBCs, which could shorten their life span due to oxidation of their membrane phospholipids. The effect of Tz on bone marrow may affect

the function of haemopoietic stem cells of bone marrow, most probably, through mediating different signalling pathways that affect their hematopoietic function as recorded previously[29-31]. The treatment of rats with Tz induced atrophy in gastric glands and ulceration of its mucosal lining[32,33], thus it may affect the absorption of vitamins and minerals essential for erythropoiesis.

Interestingly, the co-administration of Tz and GO in the present study resulted in a significant reduction of Tz toxicity on the body weight gain, relative spleen weight, and haematological parameters. Hassouna *et al*.[18] and Mahmoud *et al*.[34] also recorded that GO, as a natural antioxidant, can modulate the haemato-toxicity induced by diazinon pesticide and zinc oxide nanoparticles in experimental animals, respectively. In addition, Zhang *et al*.[14] documented that GO treatment significantly recovered the body weight gain in a rat model of hepatocarcinoma.

Spleen is the largest secondary hemato-poietic organ in the body. It plays vital immunological functions, in addition to its role in removal of the old blood cells and haematopoiesis process[35]. From this point of view, the ability of GO to treat Tz-induced toxicity on the spleen was determined. As recorded in our results, Tz inhibited the rate of splenocytes proliferation, reduced the percentage of $CD3^+CD4^+$ and $CD3^+$ $CD8^+$ subsets of spleen cells. Moreover, Tz induced oxidative stress in spleen tissue that was obvious in the current study by suppressing the antioxidant defence mechanisms and increasing the lipid peroxidation in spleen tissue. This oxidative stress stem on the generation of semiquinone radicals and aromatic amines from Tz metabolism that in turn produces superoxide radicals, hydroxyl radicals, and H_2O_2, which possibly weaken the cellular antioxidant defence mechanisms and cause a wide variety of oxidative stress-related conditions[36]. Many aromatic amines are not degradable or are very slowly degraded and induce wide range of damages[37] that may explain the immunotoxic effects induced by Tz on the spleen tissue.

Notably, treatment with GO exhibited a significant improvement in the per-centage of proliferating splenocytes, splenic $CD3^+CD4^+$ and $CD3^+CD8^+$ subsets, and splenic antioxidant defence system. In the same line, Hassouna *et al*.[18] confirmed that GO can alleviate diazinon-induced hemato-immunotoxicity in rats. In addition, Nasr[38] reported that aged garlic extract (another garlic preparation) alleviated the cisplatin-induced haematotoxicity by decreasing its oxidative stress on blood cells.

The results of the present work showed that Tz induced hepato- and renal-toxicity as it increased serum ALT activity, as well as urea and creatinine levels. In other studies, it was observed that excessive consumption of Tz could produce adverse effects on liver and kidneys[7,27,39]. Balta *et al*.[27] concluded that Tz did not only produce changes in the liver or kidney parameters, but also the impact of this dye became more dangerous when higher doses were applied, because it can induce oxidative stress by formation of free radicals.

GO has a protective effect on the liver and kidney, as shown in the present study through improving serum bio-chemical indices regarding the liver and kidney injury/dysfunction, respectively, induced by TZ. The hepatoprotective effect of GO was reported previously by Abdel-Naim *et al*.[40] who attributed this effect to the fact that GO contains numerous organosulphur compounds. These ingredients have been shown to possess antioxidant activity and to protect against experimentally induced liver damage[41]. In addition, Marsoul *et al*.[42] concluded that GO decreased the elevated serum urea and creatinine levels in cyclosporine-treated rats, thus it showed a protective effect against nephrotoxicity.

In conclusion, our data proved that Tz caused significant toxicity in blood, spleen, liver, and kidney due to its oxidative stress as evidenced by altered biochemical

parameters. The combination of GO and Tz exhibited protective effects against Tz-induced haemato-immunotoxicity, as well as decreasing the liver injury and improving the kidney functions in Tz-treated rats.

FUNDING SOURCE DISCLOSURE
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST
The authors declared that they have no conflict of interest.

REFERENCES
[1] Abdel-Reheim, E., S.; Abdel-Hafeez, H. A.; Mahmoud, B. M. *et al*. (2014). Effect of food additive (monosodium glutamate and sodium nitrite) on some biochemical parameters in albino rats. Int J Bioassays, 3(08): 3260-3273.
[2] Amin, K. A. and Al-Shehri, F. S. (2018). Toxicological and safety assessment of tartrazine as a synthetic food additive on health biomarkers: a review. Afr J Biotechnol, 17(6): 139-149.
[3] Rafii, F.; Fraeankalin, W. and Cerniglia, C. E. (1990). Azoreductase activity of anaerobic bacteria isolated from human intestinal microflora. Appl Environ Microbiol, 56(7): 2146-2151.
[4] Lopez-de-Alba, P. L.; Lopez-Martinez, L.; Cerdá, V. *et al*. (2001). Simultaneous determination of tartrazine, sunset yellow and allura red in commercial soft drinks by multivariate spectral analysis. Quimica Analitica, 20(2): 63-72.
[5] WHO and JECFA (2016). Evaluation of Certain Food Additives. Eighty-Second Report of the Joint FAO/ WHO Expert Committee on Food Additives. World Health Organization, Geneva, Switzerland (https://apps.who.int/iris/handle/10665/250277).
[6] Rao, P. and Sudershan, R. V. (2008). Risk assessment of synthetic food colours: a case study in Hyderabad, India. Int J Food Safety Nutr Public Health, 1(1): 68-87.
[7] Amin, K. A.; Abdel Hameid, H. II and Abd Elsttar, A. H. (2010). Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress bio-markers in young male rats. Food Chem Toxicol, 48(10): 2994-2999.
[8] Hashem, M. M.; Abd-Elhakim, Y. M.; Abo-EL-Sooud, K. *et al*. (2019). Embryotoxic and teratogenic effects of tartrazine in rats. Toxicol Res, 35: 75-81.
[9] El-Borm, H. T.; Badawy, G. M.; Hassab El-Nabi, S. *et al*. (2020). Toxicity of sunset yellow FCF and tartrazine dyes on DNA and cell cycle of liver and kidneys of the chick embryo: the alleviative effects of curcumin. Egyptian Journal of Zoology, 74: 43-55.
[10] Gao, Y.; Li, C.; Shen, J. *et al*. (2011). Effect of food azo dye tartrazine on learning and memory functions in mice and rats, and the possible mechanisms involved. J Food Science, 76(6): 125-129.
[11] Tawfek, N. S.; Amin, H. M.; Abdalla, A. A. *et al*. (2015). Adverse effects of some food additives in adult male albino rats. Curr Sci Int, 4(4): 525-537.
[12] El Golli, N.; Bini-Dhouib, I.; Jrad, A. *et al*. (2016). Toxicity induced after subchronic administration of the synthetic food dye tartrazine in adult rats, role of oxidative stress. Recent Adv Biol Med, 2: 20-28.
[13] Abd-Elhakim, Y. M.; Hashem, M. M. and El-Metwally, A. E. (2018). Comparative haemato-immunotoxic impacts of long-term exposure to tartrazine and chlorophyll in rats. Int Immunopharmacol, 63: 145-154.
[14] Zhang, C.-L.; Zeng, T.; Zhao, X.-L. *et al*. (2012). Protective effects of garlic oil on hepatocarcinoma induced

by N-nitrosodiethylamine in rats. Int J Biol Sci, 8(3): 363-374.

[15] Bayan, L.; Koulivand, P. H. and Gorji, A. (2014). Garlic: a review of potential therapeutic effects. Avicenna J Phytomed, 4: 1-14.

[16] Gao, C.; Jiang, X.; Wang, H. *et al*. (2013). Drug metabolism and pharmacokinetics of organosulfur compounds from garlic. J Drug Metab Toxicol, 4(5): 159 (DOI: 10.4172/ 2157-7609.1000159).

[17] Banerjee, S. K. and Maulik, S. K. (2002). Effect of garlic on cardiovascular disorders: a review. Nut J, 1: 4 (DOI: 10.1186/1475-2891-1-4).

[18] Hassouna, I.; Ibrahim, H.; Abdel Gaffar, F. *et al*. (2015). Simultaneous administration of hesperidin or garlic oil modulates diazinon-induced hemato- and immunotoxicity in rats. Immunopharmacol Immunotoxicol, 37(5): 442-449.

[19] Tsubura, A.; Lai, Y.-C.; Kuwata, M. *et al*. (2011). Anticancer effects of garlic and garlic-derived compounds for breast cancer control. Anticancer Agents Med Chem, 11(3): 249-253.

[20] Zhang, Y.; Xu, L.; Ding, M. *et al*. (2019). Anti-obesity effect of garlic oil on obese rats via Shenque point administration. J Ethnopharmacol, 231: 486-493.

[21] Aly, S. M.; Fetaih, H. A.; Hassanin, A. A. I. *et al*. (2019). Protective effects of garlic and cinnamon oils on hepatocellular carcinoma in albino rats. Anal Cell Pathol (Amst), 2019: 9895485 (DOI: 10.1155/2019/ 9895485).

[22] Dacie, S. J. V. and Lewis, S. M. (1984). Practical Haematology (6^{th} Edition). Churchill Livingstone, London, UK.

[23] Elsayed, A.; Elkomy, A.; Elkammar, R. *et al*. (2021). Synergistic protective effects of lycopene and N-acetyl-cysteine against cisplatin-induced hepatorenal toxicity in rats. Sci Rep, 11: 13979 (DOI: 10.1038/s41598-021-93196-7).

[24] Ibrahim, H. M.; Abdel Ghaffar, F. R.; El-Elaimy, I. A. *et al*. (2018). Antitumor and immune-modulatory efficacy of dual-treatment based on levamisole and/or taurine in Ehrlich ascites carcinoma-bearing mice. Biomed Pharmacother, 106: 43-49.

[25] Guendouz, M.; Mehedi, N.; Zaoui, C. *et al*. (2013). Immune response after tartrazine subchronic ingestion in swiss albino mice. Int J Pharm Pharm Sci, 5(2): 584-592.

[26] Abd El-Wahab, H. M. F. and Moram, G. S. (2013). Toxic effects of some synthetic food colorants and/or flavor additives on male rats. Toxicol Ind Health, 29(2): 224-232.

[27] Balta, I.; Sevastre, B.; Mireşan, V. *et al*. (2019). Protective effect of blackthorn fruits (*Prunus spinosa*) against tartrazine toxicity development in albino Wistar rats. BMC Chem, 13: 104 (DOI: 10.1186/s13065-019-0610-y).

[28] Barhoma, R. A.; Elshamy, A. M. and Amer, A. I. (2019). Study of the protective effect of *Nigella Sativa* oil on tartrazine- induced hematological disorders in rats. Med J Cairo Unvi, 87(7): 4661-4670.

[29] Lu, W. Y. and Zhao, M. F. (2012). Effect of oxidative stress on bone marrow mesenchymal stem cells. Zhongguo Yi Xue Ke Xue Yuan Xue Bao, 34: 90-94.

[30] AL-Shinnawy, M. S. and ELkattann, N. A. (2013). Assessment of the changes in some diagnostic parameters in male albino rats fed on an Azo Dye. IJESE, 4: 85-92.

[31] Denu, R. A. and Hematti, P. (2016). Effects of oxidative stress on mesenchymal stem cell biology. Oxid Med Cell Longev, 2016: 2989076 (DOI: 10.1155/2016/2989076).

[32] Sarkar, R. and Ghosh, A. R. (2012). Metanil yellow – an azo dye induced histopathological and ultruasrtctrual

changes in albino rat (*Rattus norvegicus*). BioScan, 7: 427-432.

[33] Al-Seeni, M. N.; El Rabey, H. A.; Al-Hamed, A. M. *et al*. (2017). *Nigella sativa* oil protects against tartrazine toxicity in male rats. Toxicol Rep, 5: 146-155.

[34] Mahmoud, H. J.; Ghareeb, O. A. and Mahmood, Y. H. (2022). The role of garlic oil in improving disturbances in blood parameters caused by zinc oxide nanoparticles. J Med Chem Sci, 5: 76-81.

[35] Lewis, S. M.; Williams, A. and Eisenbarth, S. C. (2019). Structure and function of the immune system in the spleen. Sci Immunol, 4(33): eaau6085 (DOI: 10.1126/sciimmunol.aau6085).

[36] Demirkol, O.; Zhang, X. and Ercal, N. (2012). Oxidative effects of tartrazine (CAS No. 1934-21-0) and new coccin (CAS No. 2611-82-7) azo dyes on CHO cells. J Verbr Lebensm, 7(3): 229-236.

[37] Khalid, A.; Arshad, M. and Crowley, D. E. (2009). Biodegradation potential of pure and mixed bacterial cultures for removal of 4-nitroaniline from textile dye wastewater. Water Res, 43(4): 1110-1116.

[38] Nasr, A. Y. (2014). Protective effect of aged garlic extract against the oxidative stress induced by cisplatin on blood cells parameters and hepatic antioxidant enzymes in rats. Toxicol Rep, 1: 682-691.

[39] Mekkawy, H. A.; Ali, M. O. and El-Zawahry, A. M. (1998). OP3A18 - Toxic effect of synthetic and natural food dyes on renal and hepatic functions in rats. Toxicol Lett, 95(1): 155 (DOI: 10.1016/S0378-4274(98) 80621-8).

[40] Abdel-Naim, A. B.; Khalifaa, A. E. and Ahmed, S. H. (2002). Protective effects of garlic oil against liver damage induced by combined administration of ethanol and carbon tetrachloride in rats. Egypt J Hosp Med, 6: 27-36.

[41] Wu, C. C.; Sheen, L. Y.; Chen, H. W. *et al*. (2001). Effects of organosulfur compounds from garlic oil on the antioxidation system in rat liver and red blood cells. Food Chem Toxicol, 39(6): 563-569.

[42] Marsoul, R. D.; Abbood, R. M., and Abbas, M. T. (2016). Effect of garlic oil on cyclosporine induced renal toxicity in rats. IJPPR, 5(2): 209-221.

How to cite this article:
Abd El Latif, H. M. and El-Morsy, A. M. (2023). Protective effect of garlic oil against tartrazine-induced haemato-immunotoxicity in rats. Egyptian Journal of Zoology, 79: 66-77 (DOI: 10.21608/ ejz.2022.147732.1084).

التأثير الوقائي لزيت الثوم ضد السُمية الدموية-المناعية المحدثة بالتارترازين في الجِرذان

هبه محمد عبداللطيف، أسماء محمد المرسي

قسم علم الحيوان، كلية العلوم، جامعة المنوفية، المنوفية، جمهورية مصر العربية

أوضحت دراسات سابقة أن مكونات زيت الثوم لها تأثيرات معدلة للمناعة، ومضادة للأكسدة، ومضادة للسرطان. يرتبط استخدام التارترازين كملون غذائي بسُمية كبيرة على الجسم. أجريت هذه الدراسة لتقييم التأثير الوقائي لزيت الثوم على السُمية الدموية-المناعية المحدثة بالتارترازين. عوملت ذكور الجِرذان المهقاء من سلالة "Wistar" عن طريق الفم بزيت الثوم (40 مجم/كجم من وزن الجسم) وذلك قبل المعاملة بالتارترازين (300 مجم/ كجم وزن الجسم) بثلاثين دقيقة ولمدة ثلاثين يومًا متتالية. بعد أربع وعشرين ساعة من آخر جرعة لزيت الثوم والتارترازين، تم جمع عينات الدم والطحال لإجراء القياسات المرتبطة بالسُمية. تسببت المعاملة بالتارترازين في تغيرات ذات دلالة إحصائية في وزن الجسم، ووزن الطحال النسبي، ومعايير الدم، ومعدل تكاثر خلايا الطحال، والنسبة المئوية للطرز الفرعية من الخلايا التائية للطحال والتي تم الكشف عنها بواسطة تحليل "التدفق الخلوي"، في ذكور الجِرذان. علاوة على ذلك، تم الكشف عن الإجهاد التأكسدي في نسيج الطحال والخلل في وظائف الكبد والكُلى بعد التعرض لمركب التارترازين. أظهرت المعاملة بزيت الثوم انخفاض ذو دلالة إحصائية في السُمية الكُلية في الجرذان المعاملة بالتارترازين. وارتبطت التأثيرات المضادة للسموم لزيت الثوم بزيادة تفعيل آليات مضادات الأكسدة. الخلاصة أن تناول زيت الثوم يمكن أن يقلل من السُمية الدموية-المناعية التي يسببها التارترازين من خلال نشاطه المضاد للأكسدة.