Archives of Oncology and Cancer Therapy

ISSN: 2638-5074

Volume 2, Issue 1, 2019, PP: 16-29

Evaluation of the Antitoxic Effect of Levamisole or Taurine Against High Dose of Cyclophosphamide in Tumor-Bearing Mice

Faten R. Abdel Ghaffar [1], Heba M. Abd El latif [1], Mona S. Gouida[2], Ibrahim A. El-Elaimy[1], Hany M. Ibrahim[1]*

[1]Immunology & Physiology Unit, Zoology Department, Faculty of Science, Menoufia University, Shibin El Kom, Egypt.

[2]Department of Molecular Immunology, Flow Cytometry and Genetic Units, Mansoura Children Hospital Mansoura University, Mansoura, Egypt.

hany.mohamed@science.menofia.edu.eg

***Corresponding Author:** Hany M. Ibrahim, Ph.D., Immunology & Physiology Unit, Zoology Department, Faculty of Science, Menoufia University, Shibin El Kom, Egypt.*

Abstract

***Objective:** To evaluate the antitoxic effects of levamisole (Lms) or taurine (Tau) against high dose of CTX (200 mg/kg) in Ehrlich Ascites Carcinoma (EAC)-bearing mice.*

***Methods:** Thirty five female Swiss Albino mice were used in this study; sub-grouped into five groups: Control group (non-tumorized); EAC tumorized and non-treated group; EAC tumorized and mono-treated with CTX group; EAC tumorized and co-treated with CTX and Lms group; EAC tumorized and co-treated with CTX and Tau group.*

***Results:** Co-treatment of CTX with subcutaneous injection of Lms (10 mg/kg) or intragastric administration of Tau (640 mg/kg) resulted in a reduction of the tumor cell count, viability and proliferation accompanied by an elevation in the rate of tumor growth inhibition and apoptotic index. Furthermore, co-treatments of CTX with Lms or Tau restored the immune suppression induced by CTX mono-treatment. However, co-treatments of CTX with Lms or Tau partially normalized bone marrow nucleated cell count, viability, splenocytes proliferation, immune organ indices as well as hematological alterations; it was obvious that Tau co-treatment was much more effective than Lms as hepatic and renal protective agent.*

***Conclusion:** Data suggested that, both agents, Lms or Tau, exhibits strong potentiality to enhance sensitivity of EAC to chemotherapy and attenuate the toxicity induced by high dose of CTX in tumor-bearing mice.*

Keywords: *Cyclophosphamide toxicity, Hematological alterations, Immune suppression, Levamisole, Taurine.*

Introduction

Cancer is one of the leading causes of death among human beings worldwide. In spite of advancement of many treatment approaches, chemotherapy remains fundamental regimen for various forms of malignancy [1]. However, conventional chemotherapy has many disadvantages and severe side effects [2].

Cyclophosphamide (CTX), DNA alkylating agent, is a common anticancer drug widely used for the treatment of various types of malignant tumors, including breast cancer, malignant lymphoma, multiple myeloma and leukemia [3-5]. However, it was found that biological activities of CTX are dose dependent, it was reported that effector T-cells necessary for induction of immune response against tumor were depleted by high dose CTX [6, 7]. Moreover, damaging of normal cells during attack of malignant cells, lack of tumor specificity, is the critical adverse effect of using CTX. In particular, CTX inhibits the immune system and highly proliferative hematopoietic cells, leading to immune suppression and peripheral leucopenia [5]. Therefore, the need to use supplementary drugs to enhance immune functions and attenuate chemotherapy-induced myelosuppression has become essential to overcome this obstacle.

Immunostimulants are agents used to trigger the immune response in order to enhance the disease resistance. Several compounds that have been recorded to have immunostimulation properties mostly are derivatives or cellular components of animal, fungal or bacterial origin [8]. Among these agents, taurine (Tau: 2-aminoethanesulphonic acid), is a sulfur-containing β-amino acid extensively distributed in different cells of mammals [9]. Several studies and clinical applications demonstrated that Tau has extensive physiological effects, so it was known as an endogenous anti-injury material [10, 11]. Tau as a therapeutic tool against cancer is identified to have antioxidant properties by scavenging strong oxidant and cytotoxic agents [12]. Moreover, Tau has been used as an antipyretic and anti-inflammatory agent, to treat hepatic disease [13], diabetes [14, 15] and cardiovascular disease [16]. Many studies have revealed that Tau promote immunological functions and can be used as a good immunological adjuvant [17, 18]. Maher *et al.* reported that Tau can be utilized in reversing the lymphopenia associated with IL-2, thereby augmenting its immunotherapeutic potential [19].

Levamisole (Lms), the broad spectrum antinematodal drug, was also reported to have an immunomodulatory effect on different cancer cells, including colorectal, breast cancer, melanoma and leukemia [20]. It has a sufficient immunostimulant effect on CTX immunosuppressed rats [21].

Undiandeye *et al.* reported that Lms enhances the production of immune cells through significant increase in lymphocytes, monocytes and eosinophils following its administration [22]. Moreover, Lms stimulates T-cell differentiation and response to antigens, elicits cell-mediated cytotoxicity, lymphokine production and enhances phagocytosis [23]. Therefore, Lms may assist in the treatment of chronic infections and neoplastic diseases [24, 25].

In this study, Ehrich Ascites Carcinoma (EAC)-bearing mouse model was used to investigate the antitoxic effects of either Tau or Lms against high dose of chemotherapy (CTX) induced toxicity.

Materials and Methods

Animals

Swiss female albino mice (20-22 g) were procured from the holding company for biological products and vaccines (VACSERA), Cairo, Egypt. Mice were housed in clean and dry plastic cages in a controlled environment, (temperature 25 ± 2 °C and 12 hour dark/light cycle), with standard laboratory diet and water *ad libitum*. The study was conducted after consent of the Institutional Animal Ethical Committee, Menoufia University (approval ID: MUFS/F/HE/1/16).

Mouse Tumor Model

Ehrlich Ascites Carcinoma (EAC) cell line was obtained from The National Cancer Institute, Cairo University, Egypt. EAC tumor was maintained in the ascitic form by *in vivo* sequential passages in naïve female Swiss albino mice through bi-weekly intraperitoneal (i.p.) transplantations of 0.25 x 10^6 tumor cells/mouse. The ascitic fluid was collected by sterile syringe and diluted by PBS. Then tumor cell count was performed using the trypan blue dye exclusion method (cell viability was ≥ 95%) before injection into naïve female Swiss mice for experimentation.

Reagents

Cyclophosphamide (CTX) was obtained from Sigma (CA, USA), reconstituted with saline and diluted to the required dose (200 mg/kg) according to Salem et al. [26]. Levamisole (Lms) was purchased from ADWIA Company, Egypt and diluted to the required dose (10 mg/kg) according to Clarke et al. [27]. Taurine (Tau) was obtained from Sigma; it was reconstituted in sterile distilled water and diluted to the required dose (640 mg/kg) according to Zhao et al. [5]. All other chemicals and reagents were of the highest purity available.

Experimental Design

Thirty five female Swiss albino mice were divided into five groups (n=7/group). The weight of the animals in each group was recorded at the beginning and at the end of the experiment. All the animals in each group except group (I) received EAC cells (0.25 x 10^6 cells/mouse i.p.) on day 0 according to Abdel Salam et al. [28]. Group (I) served as normal control (0.2 ml saline/mouse i.p.) and group (II) served as EAC control. Twenty four hours after EAC transplantation, group (III), (IV) and (V) received CTX i.p. at a dose of 200 mg/kg. Group (IV) injected subcutaneously with two doses of Lms (10 mg/kg) 48-hour before and after EAC transplantation. Finally, group (V) received eight

doses of Tau (640 mg/kg, intragastric) 24-hour after EAC transplantation for eight consecutive days.

Sampling and Cell Preparation

Blood samples were collected, on day 9, using orbital bleeding and divided into two tubes, one was mixed with EDTA and the other was permitted to clot. Serum samples were separated at 4000 rpm for 20 minutes, and stored at -80 °C until use [29]. Then, mice were sacrificed by cervical dislocation and EAC cells were harvested according to Salem et al. [30]. In addition, thymus, spleen and femur bone were also harvested for the subsequent analysis.

Spleen cell suspension was prepared according to Ibrahim et al. [31]. Moreover, bone marrow cell suspension was collected from the femur bone and prepared according to Zhu et al. [32]. The count and viability of EAC, bone marrow nucleated cells and spleen cells were determined by trypan blue dye exclusion method [33]. Percent of tumor growth inhibition was calculated according to Jaganathan et al. [34] as the following: Tumor inhibition rate (%) = [(Average number of tumor cells of control group - Average number of tumor cells of treated group) / Average number of tumor cells of control group] × 100. The spleen and thymus indices were determined according to Zhao et al. [5] using the following formula: organ index = organ weight (g) / Body weight (g).

Hematological and Biochemical Evaluation

Hematological parameters [hemoglobin content, hematocrit value, red blood cells (RBCs) count, white blood cells (WBCs) total and relative differential counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count] were performed manually using blood sample mixed with EDTA as previously described [35]. The serum concentration of aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, total protein and albumin were determined using Spinreact kits (Spain) according to the manufactures' instructions of kits. The serum globulin level was calculated by subtracting the obtained serum albumin concentration from the obtained serum total protein concentration [36].

Apoptosis Rate of Tumor Cells

Double staining with Annexin V–FITC and propidium iodide (PI) was performed using FITC Annexin V apoptosis detection kit (Abcam, Canada) [37]. Briefly, tumor cells were collected in PBS and washed, then, the cells were stained with Annexin V–FITC and PI for 15 minutes in the dark at 25°C. The cells were analyzed using a flow cytometer (BD FACS Canto II; BD Biosciences) and the percentage of cells undergoing apoptosis were evaluated by BD FACS DIVA™ software (BD Biosciences).

Phenotypic Analysis of Splenocytes by Flow Cytometry

Spleen cells were stained with anti-mouse mAbs against CD3 (FITC labeled, clone: 17A2), CD4 (APC labeled, clone: RM4-5), CD8 (PE.Cy5 labeled, clone: 53-6.7), CD25 (PE labeled, clone: PC61), CD11b (APC labeled, clone: M1/70) and Ly6G (FITC labeled, clone: RB6-8C5) for 30 minutes at 4°C in the dark through two panels. Then cells were washed twice and re-suspended in 0.3 ml of 0.5 % bovine serum albumin and 0.02 % sodium azide solution [28]. Surface marker expression was analyzed with BD FACS Canto II flow cytometer using BD FACS DIVA™ software.

Proliferation Assay

Tumor cells and splenocytes proliferative responses to mitogen Con A were determined by a micro-tissue culture system as described previously [38]. Cells (2.5 x 10^6/ml) were suspended in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), 100 IU/ml penicillin and 100 µg/ml streptomycin. Then the cells were cultured with Con A and after 24 hours, ten microliters of cell counting kit-8 (Sigma) reagent was added. Using a microplate ELISA reader (Seac, Radim Company, Italy), the optical density was determined at 450 nm. after 3 hours of incubation at 37 °C in 5% CO_2. The percentage of growth of tumor cells or splenocytes was calculated by dividing each value by the average mean of the EAC or normal control group, respectively, multiplied by 100.

Statistical Analysis

For statistical analysis, the statistical package of social science (SPSS) software for Windows, version 22 was utilized. One way analysis of variance (ANOVA) followed by least significant difference (LSD) for post hoc analysis was used for multiple comparisons. Statistical significance was considered when $P < 0.05$.

Results

Effect of Mono- and Co-Treatments on Tumor Growth, Proliferation and Apoptosis

CTX induced a significant ($P < 0.05$) reduction in tumor cells count, viability and proliferation accompanied by a significant increase in the rates of tumor cell apoptosis compared to EAC group. The results revealed that co-treatment of CTX with Lms or Tau significantly decreased ($P < 0.05$) the percentage of tumor cells viability and induced its apoptosis compared to CTX-treated mice. While, co-treatment of Lms and CTX decreased the tumor cells count and proliferation without any significant difference with CTX-treated group, tumor cells count and proliferation were significantly decreased ($P < 0.05$) in mice co-treated with CTX and Tau when compared with CTX treated animals (Table 1). On the level of tumor growth inhibition percentage, CTX (200 mg/kg) achieved 81.19%, while, higher rates of inhibition were achieved after co-treatments of (CTX and Lms), (CTX and Tau), 88.49% and 95.23%, respectively.

Table 1. *Effect of mono- and co-treatments on tumor status (tumor cell count, viability, growth inhibition rate, proliferation and apoptosis rates).*

	Tumor cell count (10^6)	Tumor cell viability (%)	Tumor cell proliferation (%)	Tumor cell apoptosis rate (%)	Tumor inhibition rate (%)
EAC	42.25 ± 3.30	98.90 ± 0.48	100.00 ± 10.63	1.44 ± 0.16	0
CTX	7.90 ± 0.75 *	55.42 ± 1.92 *	69.40 ± 7.70 *	18.90 ± 1.05 *	81.19
Lms+CTX	4.83 ± 0.40 *	44.45 ± 3.10 *#	68.87 ± 8.41 *	22.40 ± 0.65 *#	88.49
Tau+CTX	2.00 ± 0.56 *#	37.09 ± 7.29 *#	53.04 ± 8.15 *#	30.33 ±1.05 *#	95.23

Data were expressed as mean ± SD, n = 7. * denotes $P < 0.05$, statistically compared with EAC control group and # denotes $P < 0.05$, statistically compared with CTX-treated mice. EAC: Ehrlich Ascites carcinoma; CTX: Cyclophosphamide; Lms: levamisole; Tau: taurine.

Effect of Mono- and Co-Treatments on Phenotypic Analysis of Splenocytes

CTX treatment induced non-significant decrease in the percentage of $CD3^+CD4^+$ cells compared to EAC group. Significant ($P < 0.05$) depletion in the percentages of $CD3^+CD8^+$, $CD3^-CD8^+$, $CD4^+CD25^+$ and $CD11b^+Ly6G^+$ cells was observed after CTX mono-treatment when compared with EAC bearing animals. In addition, co-treatments of CTX with Lms or Tau enforced the effect on $CD11b^+Ly6G^+$ cells and showed highly significant ($P < 0.05$) decrease in their percentage compared to CTX-treated mice. In contrast, the percentages of $CD3^+CD4^+$, $CD3^+CD8^+$ and $CD4^+CD25^+$ cells were significantly increased ($P < 0.05$) after co-treatments of CTX with Lms or Tau when compared to CTX-treated mice, (Table 2 and Fig. 1). Altogether, partial restoration was achieved by co-treatment of CTX with Lms or Tau compared to CTX mono-treatment.

Table 2. *Effect of co-treatments of cyclophosphamide with levamisole or taurine on phenotypic analysis of splenocytes in tumor-bearing mice.*

	$CD3^+CD4^+$ (%)	$CD3^+CD8^+$ (%)	$CD3^-CD8^+$ (%)	$CD4^+CD25^+$ (%)	$CD11b^+Ly6G^+$ (%)
Control	52.91 ± 5.80	19.36 ± 1.68	13.03 ± 2.31	1.06 ± 0.15	1.63 ± 0.56
EAC	22.46 ± 1.92	11.56 ± 1.26	7.13 ± 0.70	1.76 ± 0.25	14.03 ± 1.28
CTX	18.80 ± 1.20	7.56 ± 1.00 *	3.60 ± 0.45 *	0.01 ± 0.002 *	10.73 ± 0.77 *
Lms+CTX	28.16 ± 2.81 *#	15.31 ± 1.57 *#	5.56 ± 1.72	0.55 ± 0.25 *#	5.95 ± 1.45 *#
Tau+CTX	37.28 ± 3.46 *#	14.56± 1.33 #	3.30 ± 0.55 *	0.73 ± 0.25 *#	4.91 ± 0.85 *#

Data were expressed as mean ± SD, n = 5. * denotes $P < 0.05$, statistically compared with EAC control group and # denotes $P < 0.05$, statistically compared with CTX-treated mice. EAC: Ehrlich Ascites carcinoma; CTX: Cyclophosphamide; Lms: levamisole; Tau: taurine.

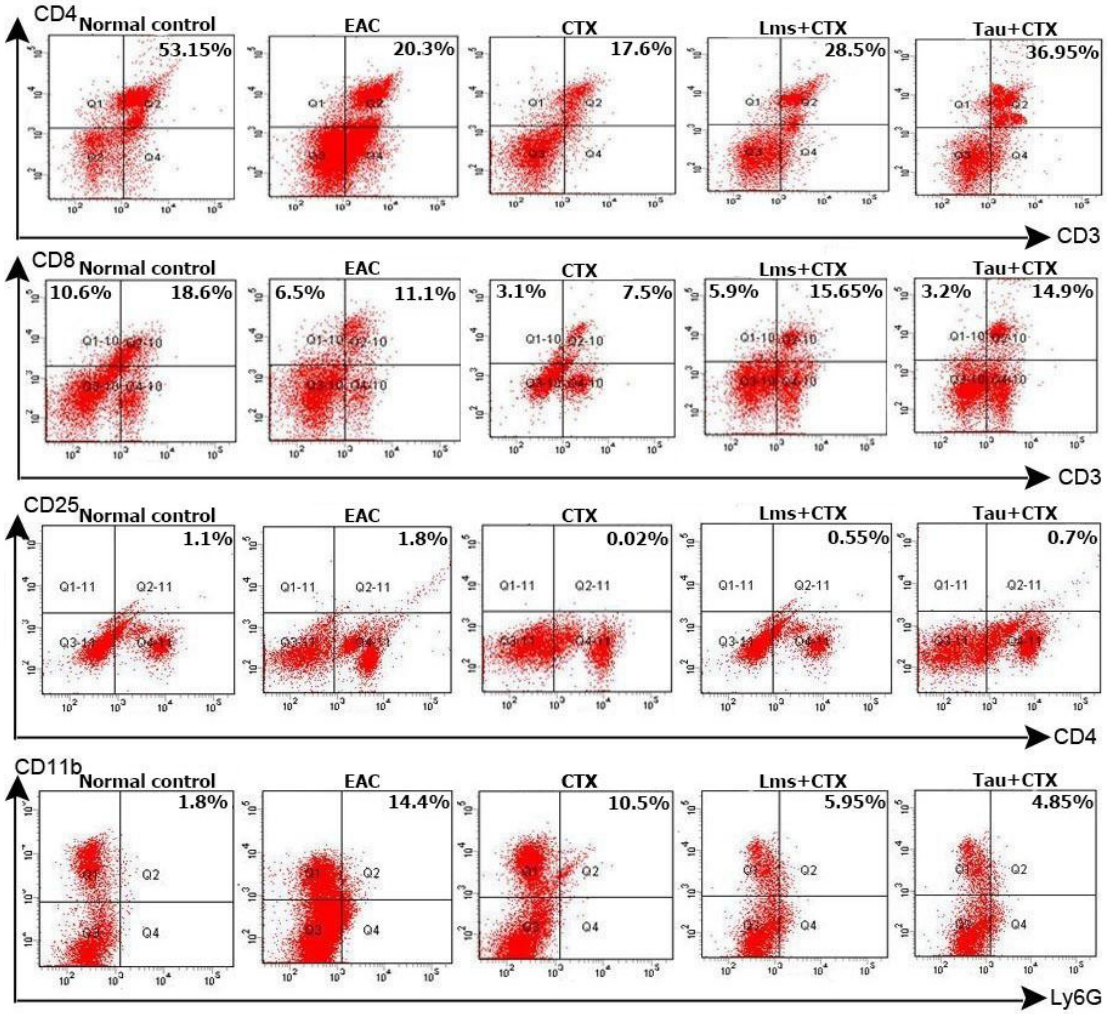

Figure 1. *Flowcytometric dot plot representation of splenocytes phenotypic analysis. Numbers correspond to the percentage of corresponding flourochrome labelled cells. Data are representative of five separate experiments. EAC: Ehrlich Ascites Carcinoma; CTX: cyclophosphamide; Lms: levamisole; Tau: taurine.*

Effect of Mono- and Co-Treatments on Bone Marrow (Bm) Cellularity, Spleen Proliferation and Immune Organ Indices

The effect of mono- and co-treatments on bone marrow (BM) cellularity and spleen cells proliferation was demonstrated in Table (3). Results revealed that CTX mono-treatment resulted in significant ($P < 0.05$) decrease in BM nucleated cells count, viability and spleen cells proliferation as compared to EAC group. Co-treatment of CTX with Lms induced significant ($P < 0.05$) partial restoration in BM nucleated cells count compared to CTX mono-treatment. In the same manner, co-treatment of CTX with Tau induced marked significant ($P < 0.05$) restoration of BM nucleated cells viability compared to CTX mono-treatment. Furthermore, compared with CTX group the proliferation rates of splenocytes were elevated after co-treatments of CTX with Lms or Tau. This elevation was significant ($P < 0.05$) only in co-treatment of CTX with Tau.

Mono-treatment of EAC-bearing mice with CTX (200 mg/kg), significantly ($P < 0.05$) decreased body weight, thymus and spleen indices compared with EAC group (Table 4). Intragastric administration of Tau or subcutaneous injection of Lms with CTX in tumor bearing mice significantly ($P < 0.05$) increased body weight and spleen index when compared with CTX treated group. Without any significant difference, co-treatments of CTX with Lms or Tau increased and tended to improve thymus index as compared to CTX mono-treatment. The above data suggest the antitoxic effect of Lms or Tau against CTX toxicity.

Table 3. *Effect of co-treatments of cyclophosphamide with levamisole or taurine on splenocytes proliferation and bone marrow nucleated cells count and viability in EAC-bearing mice.*

	Splenocytes proliferation (%)	Bone marrow nucleated cell count(10^6)	Bone marrow nucleated cell viability (%)
Control	100 ± 3.34	7.02 ± 0.84	98.91 ± 2.17
EAC	101.75 ± 4.52	11.9 ±1.94	97.33 ±1.86
CTX	76.63 ± 1.63 *	2.75 ± 0.25 *	90.52 ± 3.49 *
Lms+CTX	78.61 ± 6.02 *	5.05 ± 0.59 *#	92.32 ± 2.53 *
Tau+CTX	86.46 ± 4.05 *#	4.35 ± 0.55 *	96.63 ± 0.85 #

Data were expressed as mean ± SD, n = 7. * denotes $P < 0.05$, statistically compared with EAC control group and # denotes $P < 0.05$, statistically compared with CTX-treated mice. EAC: Ehrlich Ascites carcinoma; CTX: Cyclophosphamide; Lms: levamisole; Tau: taurine.

Table 4. *Effect of mono- and co-treatments on body weight, thymus and spleen indices in EAC-bearing mice.*

	Body weight (g)	Thymus index (10^{-3})	Spleen index (10^{-3})
Control	21.58 ± 0.25	1.97 ± 0.27	5.29 ± 0.98
EAC	25.8 ± 0.71	3.81 ± 0.61	7.39 ± 0.53
CTX	17.72 ± 0.27 *	0.97 ± 0. 16 *	3.21 ± 0.94 *
Lms+CTX	22.07 ± 0.17 *#	1.37 ± 0.21 *	6.98 ± 0.40 #
Tau+CTX	22.47 ± 0.23 *#	1.46 ± 0.19 *	6.75 ± 0.30 #

Data were expressed as mean ± SD, n = 7. * denotes $P < 0.05$, statistically compared with EAC control group and # denotes $P < 0.05$, statistically compared with CTX-treated mice. EAC: Ehrlich Ascites carcinoma; CTX: Cyclophosphamide; Lms: levamisole; Tau: taurine.

Hematological and Biochemical Evaluations

Table (5) demonstrates the hematological changes observed after treating EAC-bearing mice with CTX and combinations of CTX with Lms or Tau. The results revealed that CTX significantly ($P < 0.05$) reduced total RBCs, WBCs, platelets count and relative granulocytes count, when compared with EAC bearing animals. On the other hand, CTX mono-treatment induced significant ($P < 0.05$) increase in the relative lymphocyte count, MCV and MCH as compared to EAC group. Co-treatments of CTX with Lms or Tau resulted in significant ($P < 0.05$) reduction in total WBCs, relative granulocytes count, MCV and MCH in comparison with CTX mono-treated group. In contrary, these co-treatments induced significant ($P < 0.05$) increase in RBCs count, hemoglobin, hematocrit and relative lymphocyte count as compared to CTX mono-treated group, while no significant difference was observed on platelet count after co-treatments. Altogether, co-treatments of CTX with Lms or Tau tend to retrieve many hematological changes induced by CTX mono-treatment.

Tumor growth was associated with alteration in liver and kidney functions as revealed by an observable rise in serum ALT, AST and creatinine levels (Table 6). After CTX mono-treatment significant increase ($P < 0.05$) was recorded in serum ALT and creatinine levels as compared to EAC group. Obtained data suggested that, co-treatment of CTX with Lms enhanced liver and kidney toxicity as evidenced by increased creatinine, ALT and AST levels compared to CTX mono-treated group. On the other hand, co-treatment of CTX with Tau showed a significant ($P < 0.05$) decrease in ALT, AST and creatinine serum levels when compared to CTX mono-treatment.

Finally, CTX mono-treatment resulted in significant ($P < 0.05$) decrease in total protein and globulin when compared to EAC-bearing mice (Table 6). Co-treatment of CTX with Lms showed significant ($P < 0.05$) increase in total protein and globulin compared to CTX group. Moreover, co-treatment of CTX with Tau tended to achieve a significant ($P < 0.05$) improvement in serum total protein, albumin and globulin levels when compared to CTX mono-treated group.

Table 5. *Hematological alterations of EAC-bearing mice co-treated with cyclophosphamide and levamisole or taurine.*

	Control	EAC	CTX	Lms+CTX	Tau+CTX
Hemoglobin (g/dl)	12.80 ± 0.26	9.35 ± 0.41	8.42 ± 1.37	10.02 ± 0.18 #	11.07 ± 1.15 *#
RBCs (10^6/mm^3)	6.50 ± 0.20	4.32 ± 0.38	2.65 ± 0.34 *	4.52 ± 0.41 #	4.60 ± 0.43 #
Hematocrit (%)	39.00 ± 1.00	28.00 ± 2.16	25.25 ± 4.57	29.75 ± 0.95 #	32.50 ± 2.64 *#
MCV (fl)	60.00 ± 0.30	64.82 ± 2.27	97.68 ± 27.80 *	66.22 ± 7.25 #	70.71 ± 1.12 #
MCH (pg)	19.69 ± 0.25	21.68 ± 1.08	32.56 ± 8.79 *	22.30 ± 2.23 #	24.06 ± 0.41 #
MCHC (g%)	32.82 ± 0.29	33.46 ± 1.26	33.44 ± 0.62	33.70 ± 0.49	34.03 ± 0.75
Platelets (10^3/mm^3)	631.66 ±50.08	642.5 ± 52.04	507.50 ± 32.27 *	516.25 ± 51.37 *	527.5 ± 38.62 *
WBCs (10^3/mm^3)	7.96 ± 0.80	12.83 ± 0.43	10.75 ± 0.36 *	9.91 ± 0.16 *#	8.92 ± 0.47 *#
Granulocytes (%)	30.66 ± 3.05	64.25 ± 3.86	52.25 ± 1.70 *	41 ± 2.94 *#	42.5 ± 3 *#
Lymphocytes (%)	64.66 ± 2.30	31.00 ± 4.08	42.75 ± 1.50 *	54.25 ± 3.40 *#	52.25 ± 3.2 *#
Monocytes (%)	4.66 ± 1.15	4.75 ± 0.50	5.00 ± 0.81	4.75 ± 0.95	5.25 ± 0.95

Data were expressed as mean ± SD, n = 7. * denotes $P < 0.05$, statistically compared with EAC control group and # denotes $P < 0.05$, statistically compared with CTX-treated group. EAC: Ehrlich Ascites Carcinoma; CTX: cyclophosphamide; Lms: levamisole; Tau: taurine; RBCs: red blood cells; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBCs: white blood cells.

Table 6. *Biochemical alterations of EAC-bearing mice co-treated with cyclophosphamide and levamisole or taurine.*

	Control	EAC	CTX	Lms+CTX	Tau+CTX
AST (U/L)	60.0 ± 5.56	145.33 ± 10.11	153.66 ± 9.29	154.66 ± 5.50	85.66 ± 8.14 *#
ALT (U/L)	15.0 ± 3.46	37.33 ± 5.68	60.00 ± 1.73 *	72.66 ± 7.09 *#	21.33 ± 1.15 *#
Creatinine (mg/dL)	0.91 ± 0.08	1.56 ± 0.11	1.97 ± 0.24 *	2.04 ± 0.24 *	1.27 ± 0.25 #
Total protein (g/dL)	6.73 ± 0.30	4.50 ± 0.55	3.29 ± 0.27 *	4.40 ± 0.25 #	5.12 ± 0.20 #
Albumin (g/dL)	4.13 ± 0.34	2.03 ± 0.53	2.10 ± 0.41	2.28 ± 0.12	3.11 ± 0.14 *#
Globulin (g/dL)	2.60 ± 0.05	2.47 ± 0.54	1.19 ± 0.47 *	2.12 ± 0.30 #	2.01 ± 0.09 #

Data were expressed as mean ± SD, n = 7. * denotes $P < 0.05$, statistically compared with EAC control group and # denotes $P < 0.05$, statistically compared with CTX-treated group. EAC: Ehrlich Ascites Carcinoma; CTX: cyclophosphamide; Lms: levamisole; Tau: taurine; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

Discussion

In the present study, Tau or Lms enhance sensitivity of EAC to the high dose of CTX by reducing the tumor cell viability and proliferation, increasing the rate of tumor inhibition and apoptosis. These data indicate that co-treatment of CTX with Tau or Lms could be an effective therapy against tumor. These results are consistent with Zhang et al. who demonstrated that Tau inhibited the cell proliferation, induced cell apoptosis in human colon cancer cells and could inhibit tumor growth of these cells in nude mice xenografts [11]. In the same manner, Tau enhanced the tumor inhibition effect of CTX in S_{180}-bearing mice [5]. The effect of Tau on tumor inhibition, tumor cell proliferation and viability may be rendered to the induction of apoptosis through its ability to enhance the expression of B-cell lymphoma-2-associated X (Bax), and p53-upregulated modulator of apoptosis (PUMA), in addition to its ability to inhibit the expression of the antiapoptotic protein, B-cell lymphoma-2 (Bcl-2) [11, 39].

In addition, Lms antitumor effect was established as it significantly reduced the growth of WEHI-164 tumor cells via NC-1.1+ cells [27]. Other investigators recorded the inhibitory effect of Lms and its derivative on tumor growth and proliferation [40-42]. Furthermore, a new Lms derivative (4a) was also reported to induce extrinsic pathway of apoptosis in cancer cells and inhibit tumor progression in a murine

model [41]. The antitumor effect of Lms might be attributed to the induction of apoptosis, which may hint at Lms angiostatic role, thereby impairing tumor nutrition and growth [43].

A growing tumor often establishes itself through the induction of severe immunosuppression, which almost abolishes effective immune responses of the host against such tumor [44]. In the present study, neoplastic condition due to EAC injection significantly decreased effector T-cells ($CD3^+CD4^+$ and $CD3^+CD8^+$) and mostly $CD3^-CD8^+$ natural killer (NK) cells, while resulted in significant increase in $CD4^+CD25^+$ T-reg cells and $CD11b^+Ly6G^+$ myloid derived suppressor cells (MDSC) which suppress the immune response against tumor. Chemotherapeutic treatment with high dose, 200 mg/kg, CTX unspecifically depleted the different examined effector immune cells. These results are in the same line with Bhattacharyya et al. who recorded the inhibitory effect of EAC on $CD4^+$ and $CD8^+$ T-cells percentages that accompanied by increased level of $CD4^+CD25^+$ T-reg cells [44]. In addition, previous studies demonstrated similar alterations induced by CTX treatment (200 mg/kg) on $CD4^+$ and $CD8^+$ T-cells, NK cells, T-reg cells and MDSCs in EAC bearing mice [28,45]. In contrast, co-treatment of CTX with Lms or Tau alleviated the immunosuppressive effect of high dose CTX through modulating the percentages of $CD3^+CD4^+$, $CD3^+CD8^+$, $CD4^+CD25^+$ and $CD11b^+Ly6G^+$ cells toward the normal values.

It was reported that Tau can enhance the functions of leukocytes after chemotherapy of CTX in Lewis lung carcinoma bearing mice [46]. In the same manner, there was high elevation in percentage of $CD8^+$ cells of hepatocellular carcinoma patients treated with a combination of Tau and curcumin [47]. Tau was reported to augment the immunetherapeutic potential of IL-2 against melanoma by enhancing lymphocyte cytotoxicity, increasing survival and reducing tumor burden [48]. Moreover, Tau was demonstrated to induce antitumor activity and reduce the adverse toxicity of doxorubicin chemotherapy through reducing doxorubicin efflux from tumor cells, thereby increasing its concentration in tumor cells and decreases its concentration in normal tissue [49], in turn, Tau may control the toxicity of chemotherapy on the immune cells in the present study.

Consistent with the current study, Gomi *et al.* reported that Lms administration increased the inhibition of tumor growth through the enhancement of tumor-specific cytotoxic T-cells function in Meth 1-bearing mice [50]. In the same way, another study suggested that co-treatment of 5-fluorouracil and Lms has several stimulatory effects on the immune system, which enhances the outcome of colon carcinoma patients [51]. Moreover, Chen *et al.* reported that Lms could drive the immune response toward T helper 1 development through the activation of dendritic cells or T-cell aspects and this phenomenon could be effective against cancer [52].

Bone marrow, spleen and thymus are mainly involved in immune response. Thymus and spleen indices are important to evaluate the immune state of the host [5]. In the present study, splenocytes proliferation, thymus and spleen indices were decreased after the administration of CTX. Compared with CTX mono-treatment, in the current study, splenocytes proliferation was improved and the indices were increased after co-treatment of CTX with Tau or Lms, suggesting that Tau and Lms could improve the immunodepression induced by CTX. These findings are consistent with previous studies which reported that relative organ weights of thymus and spleen were significantly enhanced by Lms or Tau administration [53, 54]. Furthermore, Zhao *et al.* demonstrated that co-treatment of CTX with Tau elevated thymus and spleen indices, when compared with CTX alone, in S_{180}-bearing mice [5].

The count of bone marrow (BM) nucleated cells is an index which directly reflects hematopoiesis. A large number of BM nucleated cells represent a large number of immature blood cells [55,56]. In the current study, the count and viability of BM nucleated cells were markedly suppressed after CTX mono-treatment. Similarly, it was reported that CTX (200 mg/kg) induced a rapid decrease in BM nucleated cells and lymphopenia in BM, spleen and peripheral blood [57, 58]. In the present study co-treatment of CTX with Lms or Tau showed partial recovery in BM count and viability. These results suggest that, Lms and Tau could improve the myelosuppression induced by CTX. Tau can promote hematopoietic recovery through up regulating the count of BM nucleated cells and splenocytes proliferation that were decreased after the administration of CTX [5]. Furthermore, Lms

administration obviously enhanced BM cellularity, total leukocyte count and proliferation of splenocytes [54].

In order to evaluate the overall well-being and the effects of the therapy on the host, hematological alterations were recorded during the therapy of malignant tumors [59]. In the current study, mono-treatment with CTX caused a sharp reduction in RBCs count, WBCs count, platelet count, hemoglobin content, hematocrit value, relative granulocytes count, induced anemia and increased the relative lymphocyte count, which might be attributed to the observed myelosuppression in this study. On the other hand, co-treatments of CTX with Lms or Tau mostly improved all hematological abnormalities induced by CTX mono-treatment and this effect, according to the current results, may be rendered to their abilities to partially recover BM alterations.

Consistent with the obtained results, previous reports demonstrated that CTX treatment caused a decrease in the number of WBCs, RBCs, bone marrow cell count and hemoglobin content [1, 60]. Moreover, the improving effects of Lms or Tau on hematological profile were previously recorded [21, 46, 61, 62]. The improving properties of these agents, Lms and Tau, might be attributed to stimulating and immunomodulatory activity of Lms [63, 64] or may partially contributed to the enhanced stability of red blood cells, antioxidant and antiinflamatory properties of Tau [65, 66].

Hepatic and renal dysfunction is the most common regimen related toxicity reported in patients treated with CTX [67, 68]. Hepatic activation of CTX leads to the formation of toxic metabolites causing damage to the liver tissues. Due to such damage, the enzymes such as ALT and AST leak from the damaged liver to the serum [1, 69], as found in this study in CTX treated tumor-bearing mice, suggesting hepatic injury. While, co-treatment of CTX with Lms failed to control the hepatic and renal toxicity, co-treatment with Tau succeeded to partially restore the normal activity of serum ALT, AST and creatinine, which indicates the partial protective capability of Tau against liver and kidney toxicities induced by CTX in the current study. Previous study reported that combined treatment with chlorambucil and Lms caused an elevation in ALT, AST and creatinine which confirms the current results [36]. Islambulchilar *et al.* reported that Tau oral administration, in young adults with acute lymphoblastic leukemia, improved liver and kidney functions through its antioxidant ability [70].

Finally, the present study showed a significant decrease in total protein, albumin and globulin levels during CTX mono-treatment. Co-treatments of CTX with Lms or Tau tend to improve these parameters. These results agree with a previous study recorded that CTX resulted in a significant decrease of total protein, albumin, α-globulin, and β-globulin compared with the control group [21]. This result may be attributed to decreased protein synthesis as a result of liver toxicity and immunosuppression caused by CTX [21, 71]. It was demonstrated that Lms significantly increased the total protein and gamma globins levels in a murine model, which confirms the present data [72]. Moreover, Tau was reported to be applicable as a protective agent for proteinuria and albuminuria associated with nephrotic syndrome through its antioxidant properties [73, 74].

Conclusion

The present study demonstrated that Tau and Lms exhibited antitoxic effect against CTX induced toxicity in EAC-bearing mice. Moreover, these agents enhanced the sensitivity of the tumor to chemotherapy (high dose of CTX) through inducing tumor anti-proliferative properties, apoptosis and recruiting effective immune response against tumor. The obtained data suggest the possible use of levamisole or taurine during chemotherapy of different types of cancer.

References

[1] Basu A, Bhattacharjee A, Baral R, Biswas J, Samanta A, Bhattacharya S. Vanadium (III)-l-cysteine enhances the sensitivity of murine breast adenocarcinoma cells to cyclophosphamide by promoting apoptosis and blocking angiogenesis. Tumour Biol. 2017; 39(5): 1-17.

[2] Salem ML, Shoukry NM, Teleb WK, Abdel-Daim MM, Abdel-Rahman MA. In vitro and in vivo antitumor effects of the Egyptian scorpion *Androctonus amoreuxi* venom in an Ehrlich ascites tumor model. Springerplus. 2016b; 5: 570. DOI 10.1186/s40064-016-2269-3.

[3] Bass KK, Mastrangelo MJ. Immunopotentiation with low-dose cyclophosphamide in the active

specific immunotherapy of cancer. Cancer Immunol. Immunother. 1998; 47(1): 1-12.

[4] Brode S, Cooke A. Immune-potentiating effects of the chemotherapeutic drug cyclophosphamide. Crit. Rev. immunol. 2008; 28(2): 109-126.

[5] Zhao N, Wang L, Mou HY, Liang M, Yue W. Synergism and attenuation effects of taurine on cyclophosphamide. Chinese J. Cancer. 2009; 28(3): 244-248.

[6] Castano AP, Mroz P, Wu MX, Hamblin MR. Photodynamic therapy plus low-dose cyclophosphamide generates antitumor immunity in a mouse model. Proc. Natl. Acad. Sci. USA. 2008; 105(14): 5495-5500.

[7] Sistigu A, Viaud S, Chaput N, Bracci L, Proietti E, Zitvogel L. Immunomodulatory effects of cyclophosphamide and implementations for vaccine design. Semin. Immunopathol. 2011; 33(4): 369-383.

[8] Rajasekar T, Usharani J, Sakthivel M, Deivasigamani B. Immunostimulatory effects of *Cardiospermum halicacubum* against *Vibrio parahaemolyticus* on tiger shrimp *Penaeus monodon*. J. Chem. Pharm. Res. 2011; 3(5): 501-513.

[9] El-Sayed WM, Al-Kahtani MA, Abdel-Moneim AM. Prophylactic and therapeutic effects of taurine against aluminum-induced acute hepatotoxicity in mice. J. Hazard Mater. 2011; 192(2): 880-886.

[10] Schaffer SW, Jong CJ, Ramila KC, Azuma J. Physiological roles of taurine in heart and muscle. J. Biomed. Sci. 2010; 24(17 Suppl): 1:S2. Doi: 10.1186/1423-0127-17-S1-S2.

[11] Zhang X, Tu S, Wang Y, Xu B, Wan F. Mechanism of taurine-induced apoptosis in human colon cancer cells. Acta. Biochim. Biophys. Sin. 2014; 46(4): 261-272.

[12] Mates JM, Segura JA, Alonso FJ, Marquez J. Sulphur-containing non enzymatic antioxidants: therapeutic tools against cancer. Front Biosci. 2012; 4: 722-748.

[13] Megaraj V, Iida T, Jungsuwadee P, Hofmann AF, Vore M. Hepatobiliary disposition of 3 alpha, 6 alpha, 7 alpha, 12 alpha-tetrahydroxy-cholanoyl taurine: a substrate for multiple canalicular transporters. Drug Metab. Dispos. 2010; 38(10): 1723-1730.

[14] Das J, Vasan V, Sil PC. Taurine exerts hypoglycemic effect in alloxan-induced diabetic rats, improves insulin-mediated glucose transport signaling pathway in heart and ameliorates cardiac oxidative stress and apoptosis. Toxicol. Appl. Pharmacol. 2012; 258(2): 296-308.

[15] EI Ashry FE, Mahmoud MF, EI Maraghy NN, Ahmed AF. Effect of Cordyceps sinensis and taurine either alone or in combination on streptozotocin induced diabetes. Food Chem. Toxicol. 2012; 50(3-4): 1159-1165.

[16] Yin Y, Wen K, Wu Y, Kang Y, Lou J. Inhibition of sodium current by taurine magnesium coordination compound prevents cesium chloride-induced arrhythmias. Biol. Trace Elem. Res. 2012; 146(2): 192-198.

[17] Sadzuka Y, Matsuura M, Sonobe T. The effect of taurine, a novel biochemical modulator, on the antitumor activity of doxorubicin. Biol. Pharm. Bull. 2009; 32(9): 1584-1587.

[18] Schuller-Levis GB, Park E. Taurine and its chloramine: modulators of immunity. Neurochem. Res. 2004; 29(1): 117-126.

[19] Maher SG, Condron CE, Bouchier-Hayes DJ, Toomey DM. Taurine attenuates CD3/interleukin-2-induced T cell apoptosis in an in vitro model of activation-induced cell death (AICD). Clin. Exp. Immunol. 2005; 139(2): 279-286.

[20] Stevenson HC, Green I, Hamilton JM, Calabro BA, Parkinson DR. Levamisole: known effects on the immune system, clinical results, and future applications to the treatment of cancer. J. Clin. Oncol. 1991; 9(11): 2052-2066.

[21] Mohamed WAM. Can lactoferrin modulate the immunostimulant activity of levamisole in rats immunosuppressed by cyclophosphamide? J. Clin. Exp. Invest. 2014; 5(1): 48-53.

[22] Undiandeye UJ, Oderinde BS, El-Yuguda A, Baba SS. Immunostimulatory effect of levamisole on

the immune response of goats to *Peste des petits ruminants* (PPR) vaccination. World J. Vaccines. 2014; 4: 88-95.

[23] Ganguly S. Efficacy of levamisole as non-specific immunomodulator: A Review. Annal Biomed Nat Products. 2014; 1(1): 22-23.

[24] Ganguly S. Immunostimulatory and growth promoting effects of levamisole, a potential anthelminitic drug: A Review. Asian J. Medi. Pharm. Sci. 2013; 1(1): 30-31.

[25] Prasad B, Prasad A, Tiwary BK, Ganguly S. Studies on immunomodulatory effects of *Ocimum sanctum* and levamisole in broiler chicks vaccinated against Newcastle disease. J. Immunol. Immunopathol. 2012; 14(1): 14-21.

[26] Salem ML, Kadima AN, El-Naggar SA, Rubinstein MP, Chen Y, Gillanders WE. *et al.,* Defining the ability of cyclophosphamide preconditioning to enhance the antigen-specific $CD8^+$ T-cell response to peptide vaccination: creation of a beneficial host microenvironment involving type I IFNs and myeloid cells. J. Immunother. 2007; 30(1): 40-53.

[27] Clarke GR, Burton RC, Cheng Smart Y. The antitumor effects of levamisole in mice are mediated by NC-1.1+ cells. Cancer Immunol. Immunother. 1997; 45(2): 115-118.

[28] Abdel Salam SG, Salem ML, Nassef M, Abdu SH, El-Adl R. Efficacy of combined administration of chemoimmunotherapy with bone marrow cells or granulocyte-colony stimulating factor-mobilized stem cells on expansion of myeloid and stem cells. Clin. Cancer Investig. J. 2017; 6(1): 73-80.

[29] Alekseeva LA, Mironova NL, Brenner EV, Kurilshikov AM, Patutina OA, Zenkova MA. Alteration of the exDNA profile in blood serum of LLC-bearing mice under the decrease of tumour invasion potential by bovine pancreatic DNase I treatment. PLoS ONE. 2017; 12(2): e0171988. Doi:10.1371/journal.pone.0171988.

[30] Salem ML, Attia ZI, Galal SM. Acute inflammation induces immunomodulatory effects on myloid cells associated with anti-tumour responses in a tumour mouse model. J. Adv. Res. 2016a; 7: 243-253.

[31] Ibrahim HM, Xuan X, Nishikawa Y. *Toxoplasma gondii* cyclophilin 18 regulates the proliferation and migration of murine macrophages and spleen cells. Clin. Vaccine Immunol. 2010; 17(9): 1322-1329.

[32] Zhu H, Guo ZK, Jiang XX, Li H, Wang XY, Yao HY. *et al.,* A protocol for isolation and culture of mesenchymal stem cells from mouse compact bone. Nat. Protoc. 2010; 5(3): 550-560.

[33] Lutz MB, Kukutsch N, Ogilvie AL, Rössner S, Koch F, Romani N. *et al.,* An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. J. Immunol. Methods. 1999; 223(1): 77-92.

[34] Jaganathan SK, Mondhe D, Wani ZA, Pal HC, Mandal M. Effect of honey and eugenol on Ehrlich ascites and solid carcinoma. J. Biomed. Biotechnol. 2010; 2010: Doi: 10.1155/2010/989163.

[35] Dacie SJV, lewis SM. Practical Hematology. 6th ed. Churchill Livingstone. UK. 1984; 24-45.

[36] Salem FS, Badr MOT, Neamat-Allah ANF. Biochemical and pathological studies on the effects of levamisole and chlorambucil on Ehrlich ascites carcinoma-bearing mice. Vet. Ital. 2011; 47(1): 89-95.

[37] Badr BM, Moustafa NA, Saad-Eldien HM, Mohamed AO, Ibrahim HM, El-Elaimy IA. *et al.,* Increased levels of type 1 interferon in a type 1 diabetic mouse model induce the elimination of B cells from the periphery by apoptosis and increase their retention in the spleen. Cell Physiol. Biochem. 2015; 35(1): 137-147. DOI: 10.1159/000369682.

[38] Hassouna I, Ibrahim H, Abdel Ghaffar F, El-Elaimy I, Abdel Latif H. Simultaneous administration of hesperidin or garlic oil modulates diazinon-induced hemato- and immunotoxicity in rats. Immunopharmacol. Immunotoxicol. 2015; 37(5): 442-449.

[39] Zhang X, Sheng J, Zhang C, Zhao F. Taurine induces apoptosis in pulmonary artery smooth muscle cells. Zhongguo Zhong Yao Za Zhi. 2012; 37(5): 654-657.

[40] Ibrahim HM, Abdel Ghaffar FR, El-Elaimy IA, Gouida MS, Abd El latif HM. Antitumor and immune-modulatory efficacy of dual-treatment based on levamisole and/or taurine in Ehrlich ascites carcinoma-bearing mice. Biomed. Pharmacother. 2018; 106: 43-49.

[41] Hegde M, Karki SS, Thomas E, Kumar S, Panjamurthy K, Ranganatha SR. *et al.,* Novel levamisole derivative induces extrinsic pathway of apoptosis in cancer cells and inhibits tumor progression in mice. PLoS One. 2012; 7(9): e43632. Doi: 10.1371/journal.pone.0043632.

[42] Ramanadham M, Nageshwari B. Anti-proliferative effect of levamisole on human myeloma cell lines in vitro. J. Immunotoxicology. 2010; 7(4): 327-332.

[43] Artwohl M, Holzenbein T, Wagner L, Freudenthaler A, Waldhausl W, Baumgartner-Parzer SM. Levamisole induced apoptosis in cultured vascular endothelial cells. Br. J. Pharmacol. 2000; 131(8): 1577-1583.

[44] Bhattacharyya S, Md Sakib Hossain D, Mohanty S, Sankar Sen G, Chattopadhyay S, Banerjee S. *et al.,* Curcumin reverses T cell-mediated adaptive immune dysfunctions in tumor-bearing hosts. Cell Mol. Immunol. 2010; 7(4): 306-315.

[45] Salem ML, Al-Khami AA, El-Nagaar SA, Zidan AA, Al-Sharkawi IM, Marcela Díaz-Montero C. *et al.,* Kinetics of rebounding of lymphoid and myeloid cells in mouse peripheral blood, spleen and bone marrow after treatment with cyclophosphamide. Cell Immunol. 2012; 276(1-2): 67-74.

[46] Wang L, Zhao N, Zhang F, Yue W, Liang M. Effect of taurine on leucocyte function. Eur. J. Pharmacol. 2009; 616(1-3): 275-280.

[47] Abdeen SH, EL-Houseini ME, EL-Sherbiny M, Tabashy R, Salah A. Ex vivo assessment of the protective effect of curcumin and taurine against human hepatocarcinogenesis. J. Basic. Appl. Zool. 2013; 66(4): 180-187.

[48] Finnegan N, Toomey D, Condron C, Redmond HP, Da Costa M, Bouchier-Hayes D. Potentiation of the therapeutic index of IL-2 immunotherapy by combination with taurine in a syngeneic murine tumour model. Irish. J. Med. Sci. 2002; 171(2); 85-88.

[49] Sugiyama T, Kubodera M, Inoue C, Sadzuka Y. Enhancing effects of unique amino acids, taurine and theanine, on the antitumor activity of doxorubicin. Cancer Res. 2004; 64(7 Suppl.): 128-129.

[50] Gomi K, Morimoto M, Nomoto K. Cytotoxic T-Cell-mediated antitumor effect of levamisole against murine syngeneic fibrosarcoma. Cancer Res. 1982; 42: 4197-4202.

[51] Holcombe RE, Stewart RM, Betzing KW, Kannanl K. Alteration in lymphocyte phenotype associated with administration of adjuvant levamisole and 5-fluorouracil. Cancer Immunol. Immunother. 1994; 38(6): 394-398.

[52] Chen LY, Lin YL, Chiang BL. Levamisole enhances immune response by affecting the activation and maturation of human monocyte-derived dendritic cells. Clin. Exp. Immunol. 2008; 151(1): 174-181.

[53] Dong JF, Zheng XQ, Rui HB. Effect of taurine on immune function in mice with T-cell lymphoma during chemotherapy. Asian Pac. J. Trop. Med. 2017; 10(11): 1090-1094.

[54] Yuvaraj N, Ramnath V. Physiological responses in mice to levamisole. Indian J. Anim. Sci. 2004; 74: 135-138.

[55] Feng YY, Niu YB, Zhong YG, Shao J. Clinical observation of zhenqi fuzheng granule used in adjunctive therapy of augmentation and maintenance chemotherapy after relief of acute leukemia. Zhong Guo Zhong Xi Yi Jie He Za Zhi. 2003; 23(11): 868-869.

[56] Gai Y, Gao RL, Niu YP. Effect of Panax notoginsenosides on the proliferation of

hematopoietic progenitor cells in mice with immune-mediated aplastic anemia. J. Zhong Guo Zhong Xi Yi Jie He Za Zhi. 2003; 23(9): 680-683.

[57] Salem ML, Abdel Salam SGR, Nassef M, Hammad S, El Adl R. Immunoenhancing properties of the anti-tumor effects of adoptively transferred T cells with chemotherapeutic cyclophosphamide by co-administration of bone marrow cells. J. Basic Appl. Zool. 2015; 72: 96-103.

[58] Salem ML, Díaz-Montero CM, Al-Khami AA, El-Naggar SA, Naga O, Montero AJ. *et al.,* Recovery from cyclophosphamide-induced lymphopenia results in expansion of immature dendritic cells which can mediate enhanced prime-boost vaccination antitumor responses in vivo when stimulated with the TLR3 agonist poly(I:C). J. Immunol. 2009; 182(4): 2030-2040.

[59] Gangar SR, Koul A. Effects of *Azadirachta indica* on certain hematological parameters during benzo(a)pyrene induced murine forestomach tumorigenesis. Eur. Rev. Med. Pharmacol. Sci. 2010; 14(12): 1055-1072.

[60] Jang SE, Joh EH, Lee HY, Ahn YT, Lee JH, Huh CS. *et al., Lactobacillus plantarum* HY7712 ameliorates cyclophosphamide-induced immunosuppression in mice. J. Microbiol. Biotechnol. 2013; 23(3): 414-421.

[61] Vijitjaroen K, Punjaruk W, Wyss JM, Roysommuti S. Perinatal taurine exposure alters hematological and chemical properties of blood in adult male rats. *In* Taurine 9, Advances in Experimental Medicine and Biology, J. Marcinkiewicz, and S. Schaffer (eds.). Springer International Publishing, Switzerland, 2015, pp. 157-166. DOI 10.1007/978-3-319-15126-7_14.

[62] Zia R, Sandhu MA, Ahmad T. Haematological and serum biochemical profiles of buffalo heifers as influenced by levamisole. Comp. Clin. Path. 2003; 12(3): 147-150.

[63] Aly S, Abd-Allah O, Mahmoud A, Gafer H. Efficiency of levamisole in improving the immune response of catfish (*Clarias Gariepenus*) to *Aeromonas hydrophila* vaccine: clinico-pathological studies. Mediterr. Aquacul. J. 2011; 1(1): 8-17.

[64] El-Boshy ME, El-Deean NN. Comparative study on the effect of fucoidan and levamisole on some selective biochemical and hematological parameters in heat stress rabbits. Int. J. Pharm. Med. Res. 2013; 1(1): 1-5.

[65] Gossai D, Lau-Cam CA. The effects of taurine, hypotaurine, and taurine homologs on erythrocyte morphology, membrane fluidity and cytoskeletal spectrin alterations due to diabetes, alcoholism and diabetes-alcoholism in the rat. Adv. Exp. Med. Biol. 2009; 643: 369-379.

[66] Sirdah MM, Abushahla AK, Al-Sarraj HA. Effect of the addition of the antioxidant taurine on the complete blood count of whole blood stored at room temperature and at 4 masculine C for up to 7 days. Rev. Bras. Hematol. Hemoter. 2013; 35: 44-51.

[67] Badr OM, Sakr SA, Abd-Eltawab HM. Ameliorative effect of ginger extract against pathological alterations induced in mice bearing solid tumors. J. Biosci. Appl. Res. 2016; 2(3): 185-196.

[68] Basu A, Bhattacharjee A, Roy SS, Ghosh P, Chakraborty P, Das I. *et al.,* Vanadium as a chemoprotectant: effect of vanadium(III)-L-cysteine complex against cyclophosphamide-induced hepatotoxicity and genotoxicity in Swiss albino mice. J. Biol. Inorg. Chem. 2014; 19(6): 981-996.

[69] Roy SS, Chakraborty P, Bhattacharya S. Intervention in cyclophosphamide induced oxidative stress and DNA damage by a flavonyl-thiazolidinedione based organoselenocyanate and evaluation of its efficacy during adjuvant therapy in tumor bearing mice. Eur. J. Med. Chem. 2014; 73: 195-209.

[70] Islambulchilar M, Asvadi I, Sanaat Z, Esfahani A, Sattari M. Effect of taurine on attenuating chemotherapy-induced adverse effects in acute lymphoblastic leukemia. J. Can. Res. Ther. 2015; 11(2): 426-432.

[71] Senthilkumar S, Devaki T, Manohar BM, Babu MS. Effect of squalene on cyclophosphamide-induced toxicity. Clin. Chim. Acta. 2006; 364(1-2): 335-342.

[72] Sadigh-Eteghad S, khayat-Nuri H, Abadi N, Ghavami S, Golabi M, Shanebandi D. Synergetic effects of oral administration of levamisole and *Echinacea purpurea* on immune response in Wistar rat. Res. Vet. Sc. 2011; 91(1): 82-85.

[73] Ezekiel I, Ayo JO, Tanko Y, Magaji RA. Effect of taurine on acute restraint stress induced biochemical alterations in Wistar rats. Int. J. Sci. Engineer Res. 2015; 6(5): 958-965.

[74] Venkatesan N, Venkatesan P, Karthikeyan J, Arumugam V. Protection by taurine against adriamycin-induced proteinuria and hyperlipidemia in rats. Proc. Soc. Exp. Biol. Med. 1997; 215(2): 158-164.

Citation: **Faten R. Abdel Ghaffar, Heba M. Abd El latif, Hany M. Ibrahim, et al.** *Evaluation of the Antitoxic Effect of Levamisole or Taurine Against High Dose of Cyclophosphamide in Tumor-Bearing Mice. Archives of Oncology and Cancer Therapy. 2019; 2(1): 16-29.*