Online ISSN: 2682-2628
Print ISSN: 2682-261X

IJCBR

International Journal of Cancer and Biomedical Research

https://jcbr.journals.ekb.eg

Evaluation of the anti-tumor and immunomodulatory effects of synthetic chalcones in a tumor mouse model

Mohamed M. Nasef, Mohamed Gaber, Asmaa A. El-Gamal, Mohamed L. Salem

EKB Egyptian Knowledge Bank بنك المعرفة المصري

PUBLISHED BY
EACR EGYPTIAN ASSOCIAN FOR CANCER RESEARCH
Since 2014

INTERNATIONAL JOURNAL OF CANCER AND BIOMEDICAL RESEARCH (IJCBR)

RESEARCH ARTICLE

Evaluation of the anti-tumor and immunomodulatory effects of synthetic chalcones in a tumor mouse model

Mohamed M. Nasef[1], Mohamed Gaber[2], Asmaa A. El-Gamal[1], Mohamed L. Salem[1]

[1]Zoology Department, Faculty of Science, Tanta University, Tanta, Egypt
[2]Department of Chemistry, Faculty of Science, Tanta University, Egypt

ABSTRACT

Introduction: Traditional treatment of cancer by using chemotherapy not only kills cancer cells but also normal cells. It also fails in the complete prevention of the spread of tumor cells. Chalcone have several biological activities, such as antioxidant, antibacterial, antifungal, anti-inflammatory, and anti-tumor. **Aim**: To compare the antitumor effects of chalcones in Ehrlich ascetic carcinoma (EAC) bearing mice. **Materials and Method**: Female Swiss albino mice were randomly divided into 6 groups as the following; Group 1 were served as a negative control, group 2 were inoculated with 2.5×10^5 (EAC) cells, group 3 were inoculated with 2.5×10^5 EAC cells and then treated with cisplatin (2 mg/kg), group 4 were inoculated with 2.5×10^5 EAC cells and then treated with chalcone 1 (0.3 ml gm/L), group 5 were inoculated with 2.5×10^5 EAC cells and then treated with chalcone II (0.3 ml gm/L). After 7 days, all groups of mice were sacrificed to measure the number and cell cycle of tumor cells as well as the number and activation of $CD8^+$ T cells. **Results**: Treatment of tumor-bearing mice with chalcone I, but not chalcone II induced decreases in the total number of live cells when compared to control tumor-bearing mice, coinciding with significant increases in G0, G1, S and G2 phases of EAC cells which were comparable to the effects of cisplatin. As compared to control tumor-bearing mice, tumor cells harvested form mice treated with chalcone I showed significant increases in the numbers of activated $CD8^+$ T cells. **Conclusion**: Chalcone I possesses anti-tumor effects by inducing tumor cells arrest and activation of T cells.

Keywords: Cancer, CD8 T cells, Chalcone; Chemotherapy; Cisplatin; Metal complex; Cell cycle, Tumor

Editor-in-Chief: Prof. M.L. Salem, PhD - Article DOI: 10.21608/jcbr.2019.65449

ARTICLE INFO

Article history
Received: July 20, 2019
Revised: September 10, 2019
Accepted: October 25, 2019

Correspondence to
Asmaa Al-Gamal
Zoology Department,
Faculty of Science
Tanta University,
Tanta, Egypt
Tel: +201274272624
Email: asmaa_elgamal90@yahoo.com

INTRODUCTION

The term "cancer" refers to a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body (Weinberg 1996). It is a complex disease strongly influenced by a number of factors including genetics, behavioral aspects such as smoking, poor diet and physical inactivity. At the cellular level, these factors impact on cell signaling leading to uncontrolled proliferation and cell migration with adverse consequences in the formation of tumors and metastases. Cancer treatment depends on the type of cancer, the stage of the cancer, age, health status, and additional personal characteristics. Treatments of cancer were primarily focused on surgery, chemotherapy, radiation, medicinal plants, hormone therapy, gene therapy and immunotherapy (Qi et al., 2011).

Although treatment with chemotherapy or radiation therapy kills cancer cells, these treatments associates with serious effect on normal cells and often fail in preventing the dissemination of the tumor cells (Parish 2003). Therefore, developing novel anticancer compounds with less toxic effects is of paramount significance (Salem et al., 2009; Fox et al., 2011).

Chalcones are precursors of flavonoids and which are abundant in edible plants. The chemical structure of chalcones consists of two aromatic rings joined by a three-carbon α, β-unsaturated carbonyl system. Chalcone

derivatives have several applications as fluorescent probes for sensing of DNA (Gaber et al., 2015). Chalcones exhibit several biological properties, including antibacterial and antifungal, anti-inflammatory, anti-parasitic activities, antitumoral, antimalarial, anti-HIV, antihyperglycemic as well as antioxidant activities (Yan et al., 2014). Naturally and synthetic chalcone derivatives are of interest as cytotoxic (anticancer) agents (Tseng et al., 2013) due to their photophysical properties (Gaber et al., 2008).

Recent studies have shown that chalcones can induce anti-tumor effect in vitro. We therefore hypothesized that treatment of tumor bearing mice with chalcones will induce efficacious antitumor effects with less toxicity. Therefore, this study aimed to compare the antitumor and immunomodulatory effects of two different chalcones in vivo as compared to those of conventional anticancer therapies such as cisplatin.

MATERIALS AND METHODS

Mice

Female Swiss albino mice (10-12 weeks old, n=36 mice) were purchased from VACSERA (The Egyptian Company for Biological Products and Vaccines, Giza, Egypt). Mice weighing (22-25g) at the beginning of the experiment. Mice were handled and kept in a specific animal facility at the Faculty of Science, Tanta University, Egypt.

Maintenance of tumor cells line

Ehrlich ascites carcinoma (EAC) cell line was maintained in female Swiss albino mice and propagated in our laboratory by weekly intraperitoneal (i.p) inoculation of about 2.5×105 cells per mouse. The ascetic fluid was collected using a syringe and the tumor cells were counted using a Neubauer hemocytometer. The viable cells were determined using trypan blue dye exclusion assay (Salem et al. 2009).

Experimental protocol

Mice were divided into groups (n= 6/group) including one control group. Then allowed for one week for acclimatization and then they were randomly divided into six groups as follow. Group1 was served as a negative control. Group 2 was inoculated with 2.5×10^5 EAC cells. Group 3 was inoculated with 2.5×10^5 EAC cells and then inoculated with dimethyl sulfoxide (DMSO; diluted in distal water to 0.05%) three times every other day starting from day 1 post tumor inoculation. Group 4 was inoculated with 2.5×10^5 EAC cells and then treated with cisplatin (2 mg/kg) three times every other day starting from day one post tumor inoculation. Group 5 was inoculated with 2.5×10^5 EAC cells and then treated with chalcone 1 (with side chain CuCl2) (0.3 mg/L) three times every other day starting from day 1 post tumor inoculation. Group 6 was inoculated with 2.5×10^5 EAC cells and then treated with chalcone 2 (with side chain CuSo4) (0.3 ml gm/L) three times every other day starting from day 1 post tumor inoculation. Seven days later, all mice were sacrificed and blood was collected form the orbital plexus to determine the hematological parameters using VetScan HM2™ Hematology System, Abaxis® (Union City, CA). Using sterile 5 ml syringes, tumor ascites (cells) was withdrawn from the abdominal cavity to determine tumor volume, tumor cell count, life and dead cells. After counting the tumor cells from all different groups of mice, cells were processed to determine the tumor cell cycle at Flow Cytometry core Facility Unit, Tanta University Teaching Hospital, using Flow cytometer (BD CANTO), (BD Biosciences, San Jose, CA).

Counting splenic leukocytes

Mice form all groups were dissected to harvest spleen for immunological phenotyping. Spleen cell suspensions were prepared and counted using a hemocytometer with trypan blue dye exclusion as described previously (Salem et al. 2009). Mice were sacrificed by cervical dislocation. The spleen was placed into PBS at room temperature and squeezed between two frosted glass slides to prepare the single cell suspension. The cell suspension of the spleen was transferred into 50 mL falcon tube and washed twice with 10 mL of PBS by centrifugation at 1500 rpm for 5 min at room temperature. To remove erythrocytes, the cell pellet was re-suspended in 5 mL of ACK lysing buffer, incubated for 5 min at room temperature, and then diluted with 45 mL of PBS; the cell suspension was centrifuged at

1500 rpm for 5 minutes at room temperature, and the supernatant was discarded. The cell pellet was re-suspended in PBS and filtered through 45 µm cell strainer. Cells were counted using a hemocytometer with trypan blue dye exclusion as described briefly pervious.

Phenotypic analysis of splenic T cells

Spleen cells were prepared as described previously and 50 µl of $10x10^4$ cells suspension were added to 25 µl of diluted FITC and Pecp.Cy7 conjugated mAbs to each tube.Cells were mixed gently and the tubes were then incubated for 20 min at 4ºC. Cells were washed twice with staining buffer by centrifugation at 300 x g for 5 min at 4^{o}C. The cell pellets were re-suspended in 200 µl flow cytometry staining buffer and transferred into 12x75mm flow acquisition tubes containing 200 µl staining buffer. Cell suspensions were kept on ice until analyzed by flow cytometry or 200 µl of PFA was added to each tube, then data were acquired by flow cytometey. Cells were then acquired on a FACS CaliburTM (BD Bio-sciences, San Jose, CA) and analyzed using FlowJo software (BD Biosciences).

Statistical analysis

Numerical data obtained from each experiment were expressed as mean ± SE and the statistical differences between experimental and control groups were assessed using One Way Analysis of Variance (ANOVA) and Student's t-test. Graph Pad Prism (Graph Pad Software, Inc., San Diego, CA) was used to analyze P values. P values ≤ 0.05 were considered statistically significant.

RESULTS

Antitumor effect of chalcones

EAC-bearing mice treated with cisplatin showed a significant reduction in the total tumor cell count as compared to control group. As shown in Figure 1, treatment with Ch1 significantly decreased the tumor numbers. However, treatment with Ch2 did not show any significant change in the tumor numbers when compared to the EAC-bearing mice alone (Figure 1).

Effect of chalcones on the number of viable and dead tumor cells

Treatment with ch1 increased the number of dead cells and decreased the number of viable cells when compared to mice bearing tumor with no treatment. Treatment with ch2, however, did not show a significant reduction in the number of the viable and dead tumor cells as compared to their control (Figure 2).

Tumor cell cycle analysis

As shown in Figure 3, there was a significant increase in the G0 phase in the tumor cells harvested from either cisplatin or Ch1 treated mice as compared to control tumor bearing mice, or mice treated either with DMSO or Ch2. The G1, S and G2 phases in the tumor cells harvested from groups of mice which was treated with cisplatin or with Ch1 showed a significant decrease as shown in Table 1.

Effect of chalcones on the number and activation of $CD8^+$ T cells

As shown in Figure 5, the early activation marker CD69 was analyzed on CD8 T cells in the PBL of all groups. The results showed that the levels of the co-stimulatory molecules CD69 were declined in all groups of mice inoculated with EAC-cells. As shown in Table 2, the lowest expression of CD69 marker was reported on the $CD8^+$ T cells harvested form the group of mice inoculated with EAC-cells and treated with Ch1.

Effect of chalcones on total and differential white blood cells count (WBCs)

At the indicated time points, mice were bled from the orbital sinus to harvest peripheral blood. The total and differential number of leukocytes in peripheral blood was enumerated Inoculation of mice with EAC-cells led to an increase in the total numbers of white blood cells when compared to the control group. However, treatment with cisplatin or with Ch1 led to a decrease in the total number of WBCs when compared to the control groups (Figure 6). As compared to the control group, the lymphocytes percentage did not show any significant changes among different groups (Figure 7).

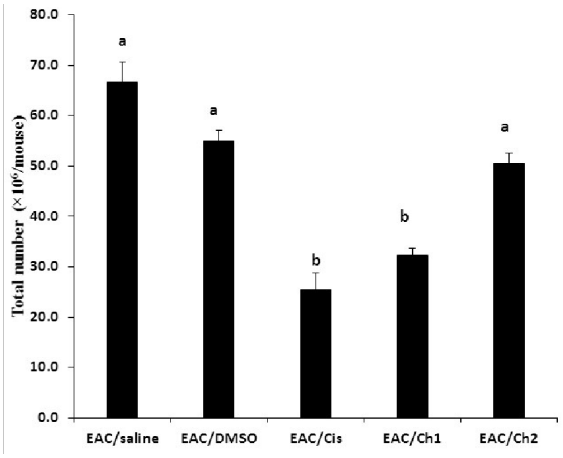


Figure 1. Total tumor cells count in different groups. Mice were challenged i.p with EAC cells on day 0 then treated with saline, DMSO, cis, Ch1 or Ch2 on day 1,3 and 5, Mice were sacrificed on day 7 for counting tumor cells from ascetic fluid.

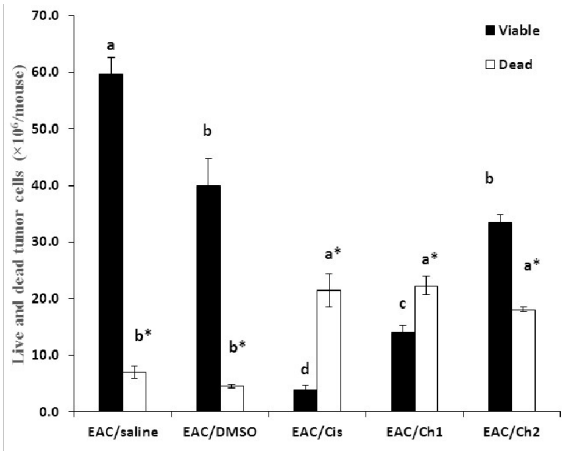
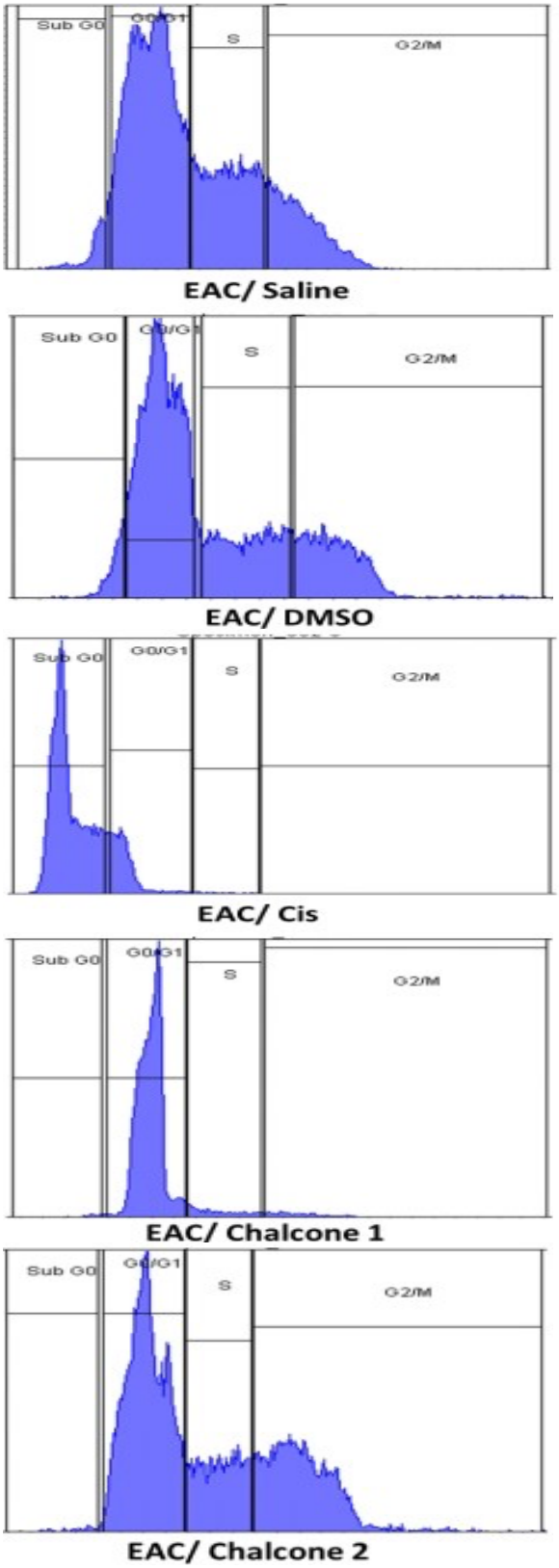


Figure 2. Viable and dead tumor cells in different groups mice were inoculated as described in the legend of Figure 1 to count viable and dead tumor cells using trypan blue assay.

The percentage of monocytes was increased in the group of mice inoculated with EAC cells and treated either with cis or with Ch1. The percentage of monocytes did not change in the group of mice inoculated with EAC-cells and treated with Ch2 when compared to the control group (Figure 8).

Effect of chalcones on granulocytes percentage

Treatment of tumor bearing mice with cisplatin or with Ch2 led to a decrease in the percentage of granulocytes when compared to control group. However, tumor bearing mice treated with ch1 did not show a significant change in granulocytes (Figure 9).



Nuclear DNA content PI-PE cell cycle analysis

Figure 3. Representative cell cycle analysis in different groups stained with PI. Mice were inoculated with EAC and treated as described in the legend of Fig 1, then ascetic fluid was harvested on day 7 and cell cycle of EAC was analyzed by flow cytometry.

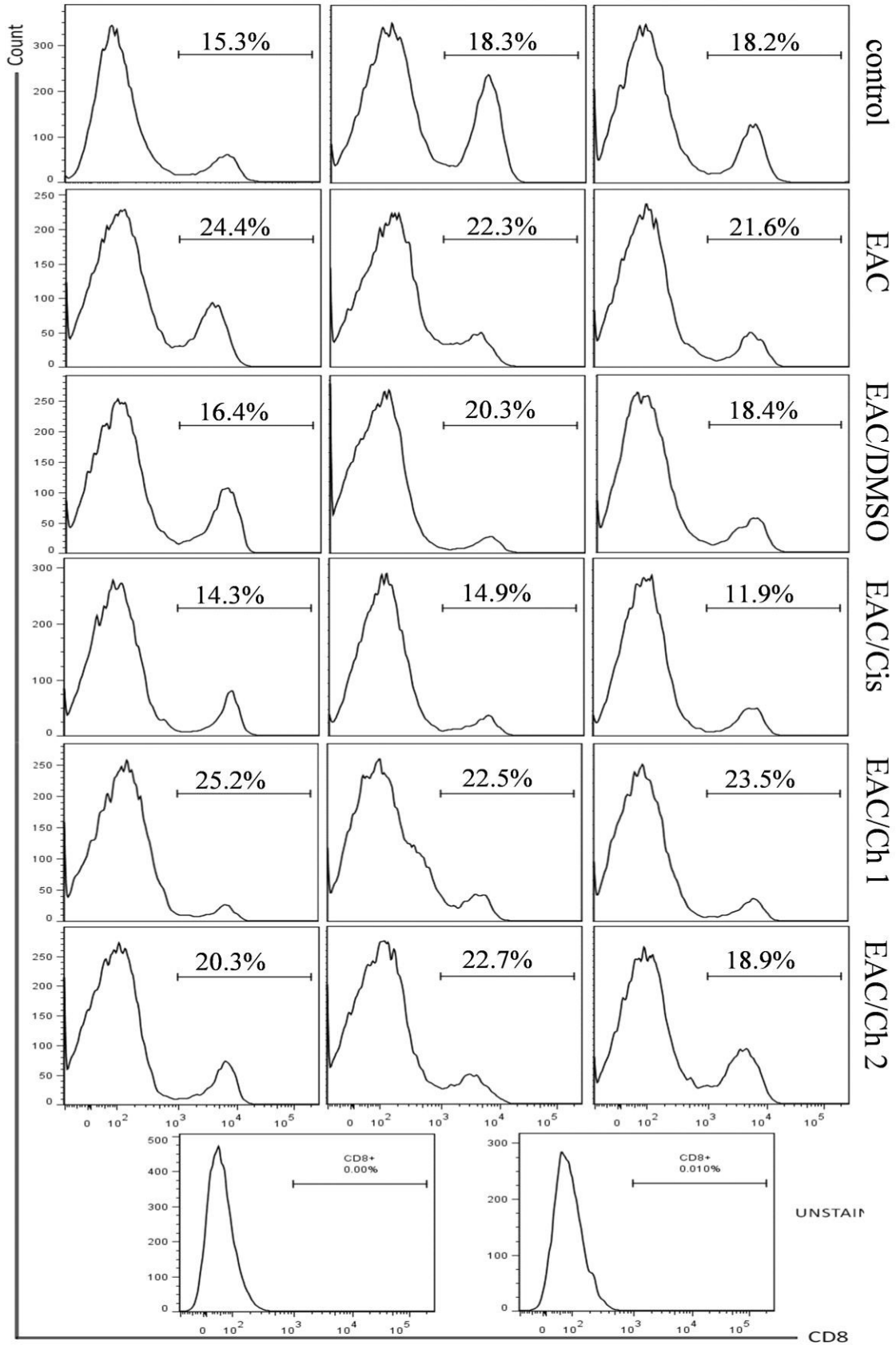


Figure 4. The percentage of CD8 T cells in PBL of different groups. Mice were inoculated with EAC and treated as described in the legend of Fig 1. Then spleen cells were harvested on day 7 and stained for immunophenotyping of $CD8^+$ T cells by flow cytometry.

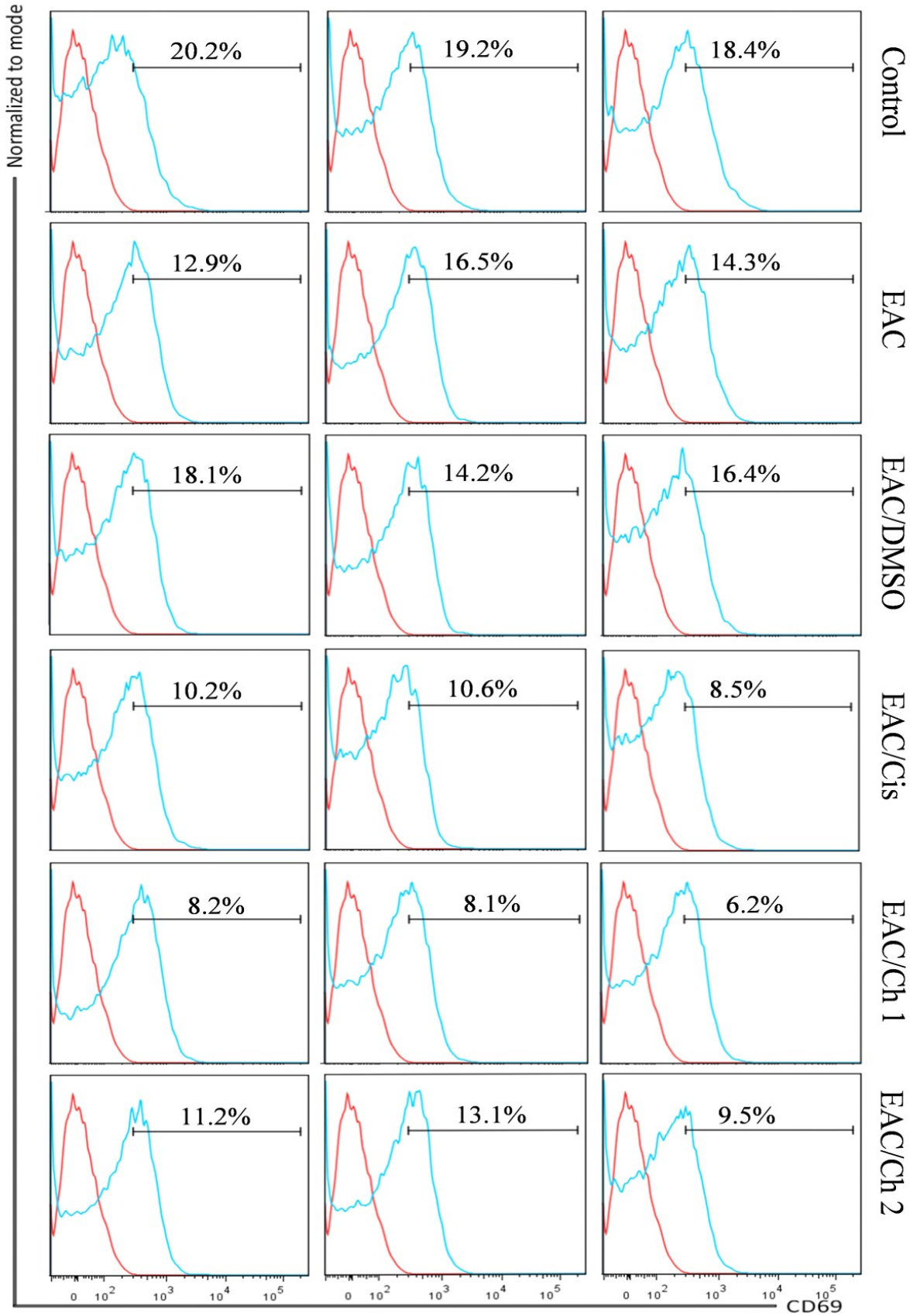


Figure 5. The expression of the early- activation molecule CD69 on CD8 T cells. Mice were inoculated with EAC and treated as described in the legend of Figure 1. Then spleen cells were harvested on day 7 and stained for activation of $CD8^+$ T cells by flow cytometry after staining with anti-CD69 mAb.

Table 1. The percentage of G0, G1, S, G2 phases in the cell cycle of tumor cells harvested from different groups.

Phase	G0	G1	S	G2
EAC/salin	3.2	53.7	24.8	15.5
EAC/DEMSO	4.6	51.7	21	18.8
EAC/Cis	78.3	16.8	1	0.1
EAC/Ch1	55	33.3	6	4.1
EAC/Ch2	3	48.9	18.8	30.3

Table 2. The percentage of CD8 % and CD 69% in all groups

Groups	% CD8	% CD69
Control	17.30 ± 6.13	19.37 ± 6.67
EAC/saline	22.87 ± 2.59	14.57 ± 4.16
EAC/DEMSO	18.47 ± 3.48	16.26 ± 3.91
EAC/cis	13.7 ± 5.47	9.72 ± 2.13
EAC/Ch1	23.7 ± 7.27	7.54 ± 2.12
EAC/Ch2	20.63 ± 6.9	11.25 ± 0.85

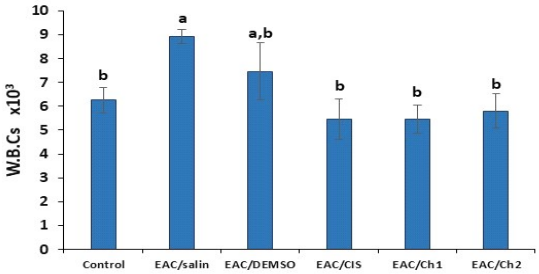


Figure 6. Total white blood cells count (W.B.Cs) in different groups. Mice were inoculated with EAC and treated as described in the legend of Figure 1. Then total number of leukocytes in the peripheral blood was counted on day 7.

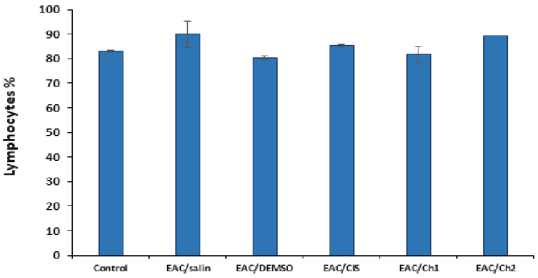


Figure 7. Percentage of lymphocytes in different groups. Mice were inoculated with EAC and treated as described in the legend of Figure 1. Then relative numbers of lymphocytes in the peripheral blood were counted on day 7.

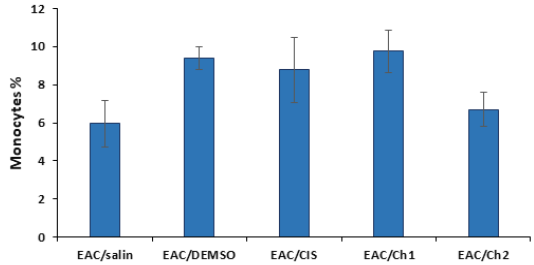


Figure 8. Percentage of monocytes in different groups. Mice were inoculated with EAC and treated as described in the legend of Figure 1. Then relative numbers of monocytes in the peripheral blood were counted on day 7.

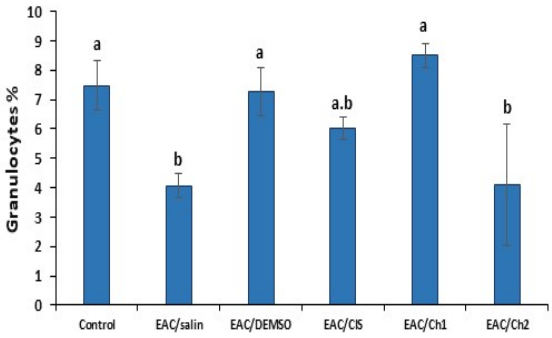


Figure 9. Percentage of granulocytes in different groups. Mice were inoculated with EAC and treated as described in the legend of Figure 1. Then, relative numbers of granulocytes in the peripheral blood were counted on day 7.

DISCUSSION

The present study investigated the antitumor activity of two types of chalcones 1 and 2. The obtained data showed that treatment with Ch1 decreased the tumor volume. However, the treatment with Ch2 did not show any significant change in the tumor volumes when compared to the EAC bearing mice. Furthermore, treatment with Ch1 increased the number of dead tumor cells and decreased the number of live tumor cells. Ch2 did not show any significant effects on the tumor cell number. This anticancer activity of Ch1 might be due to molecular alteration such as induction of apoptosis, DNA and mitochondrial damage, inhibition of angiogenesis, tubulin inhibition, kinases inhibition, and also drug efflux protein activities. This is in agreement with the previous studies demonstrated that chalcone are traditionally employed for therapeutically purposes as a potent anticancer (Batovska et al., 2010). Other study also reported that hybrid chalcones formulated by chemically linking to other prominent anticancer scaffolds such as benzodiazepines, benzothiazoles and imidazolones have demonstrated synergistic or additive pharmacological activities (Babu et al., 2013).

Data showed that there was significant increase in the G0 phase in the tumor cells harvested from either cisplatin or Ch1 treated mice. Also, in group of mice treated either with DMSO or Ch1, the G1, S and G2 phases in tumor cells harvested form groups of mice treated with cis

or with ch1 showed significant decrease as compared to tumor bearing mice alone. This finding was in line with previous studies demonstrated the mechanism of anticancer activity of chalcone (Boumendjel et al., 2008; Batovska et al., 2010).

Treatment of cells with chalcones can decrease cell viability, which led cells to rapid necrosis or activate apoptosis. Chalcone can potentially induce cytotoxicity and apoptosis in prostate cancer cell line (Szliszka et al., 2010). It has been reported that the prenylated chalcone isolated from Ashitaba, which called (ashitaba chalcone) could induce expressions of caspase-3 and Bax protein and inhibit hepatocarcinoma cellular proliferation in mice (Meng et al., 2011). Chalcone was observed to inhibit growth of skin tumor cells in a two-stage carcinogenesis test in mouse skin using DMBA and TPA as the inhibitor and promoter, respectively (Akihisa et al., 2006).

The cytotoxicity of chalcone derivatives such as 2'hydroxy2,3,4',6'tetramethoxychalcone showed strong inhibitory activity against U937 tumor cell lines (Ducki, 2009). This chalcone was also a selective cytotoxic agent in human lung cancer cells such as chalcone epoxides, i.e., epoxides of 1,3diarylpropenones, are simple derivatives of the widely diverse chalcones, which are biosynthesized by plants. Chalcone epoxides (α, β-epoxyketones) have been found to inhibit human pancreatic cancer cellular proliferation in vitro (Ducki, 2009). Treatment of BxPC-3 cells with chalcone resulted in rapid induction of apoptosis. Cell cycle control at G1 is typically abnormal in pancreatic cancer cells; the compounds do not appear to be arresting growth by restoring normal G1 checkpoint control (Han., et al., 2010). Chalcones, aromatic ketones and enones, are known for their anticancer effects. Although parent chalcones consist of two aromatic rings joined by a three-carbon α,β-unsaturated carbonyl system, various synthetic compounds possessing heterocyclic rings like pyrazole, indole are well known and proved to be effective anticancer agents (Sharma et al., 2013).

In addition, there is evidence indicating that chalcone derivatives demonstrated preferential inhibition against the growth of human cancer cell lines MDA-MB-231 (estrogen receptor-negative) and MCF7 (estrogen receptor-positive) over normal breast epithelial cell lines, at low concentrations (Chen et al., 2010). Meanwhile, parasitic in (5,7-dihydroxy-6-methyl-4-phenyl-8(3-phenyltrans-acryloyl)-3,4-dihydro-1benzopyran-2-one), a chalcone isolated from Cyclosorus parasiticus leaves was documented to exert antiproliferative activity against human cancer cell lines, especially toward the HepG2 hepatocellular carcinoma cell line (Wei et al., 2013). Previous studies reported that chalcone derivatives inhibit phospholipase A2, cyclooxygenase (COX), lipoxygenases, proinflammatory cytokines production, neutrophil chemotaxis, phagocytosis, and production of reactive oxygen species (Bukhari et al., 2014).

The results of the present study clearly demonstrate the tumor inhibitory activity of chalcones against EAC. The reliable criteria for evaluating an anticancer drug are prolongation of lifespan of the animal and decrease in white blood cells count of blood. Our results have shown an increase in lifespan accompanied by a reduction in white blood cells count in chalcone treated mice. Our flow cytometry data showed that the highest percentage of $CD8^+$ T cells was found in the peripheral blood (PBL) of the group of mice which inoculated with EAC-cells and treated with Ch1. These cells also showed significant activation as indicated by the higher expression level of CD69. Indeed, Ch induced significant effect on increasing the life span of ascities tumour bearing animals and also found to reduce the viable EAC cells in animal models. These results clearly demonstrate the antitumour effect of chalcones against EAC. During the process of cancer chemotherapy, the major problems are myelosuppression and anaemia (Hirsch, 2006).

CONCLUSION

In the present study, the antitumor activity and immunomodulatory effect of synthetic chalcones were investigated in the tumor bearing mice. The study was conducted on two types of Ch1 and Ch2. The data showed that Ch1 was more potent anticancer agent than Ch2 against EAC cells in vivo. This anti-tumor

effects are mediated by induction of tumor cell arrest and activation of $CD8^+$ T cells.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

Akihisa T, Tokuda H, Hasegawa D, Ukiya M, Kimura Y, Enjo F, Suzuki T and Nishino H (2006). Chalcones and Other Compounds from the Exudates of Angelica k eiskei and Their Cancer Chemopreventive Effects. Journal of natural products, 69(1): 38-42.

Babu B, Konduru K, Nakanishi N, Hayashi W, Ahmed S and Mitrasinovic M (2013). Experimental and theoretical advances in functional understanding of flavonoids as anti-tumor agents. Anti-Cancer Agents in Medicinal Chemistry. Formerly Current Medicinal Chemistry-Anti-Cancer Agents, 13(2): 307-332.

Batovska, D. I., & Todorova, I. T. (2010). Trends in utilization of the pharmacological potential of chalcones. Current Clinical Pharmacology, 5(1), 1-29.

Boumendjel A, Boccard J, Carrupt PA, Nicolle E,

Blanc M, Geze A, Choisnard L, Wouessidjewe D, Matera E.L and Dumontet C (2008). Antimitotic and antiproliferative activities of chalcones: forward structure–activity relationship. Journal of medicinal chemistry, 51(7): 2307-2310.

Bukhari S.N, Tajuddin Y, Benedict V.J, Lam K.W, Jantan I, Jalil J and Jasamai M (2014). Synthesis and evaluation of chalcone derivatives as inhibitors of neutrophils' chemotaxis, phagocytosis and production of reactive oxygen species. Chemical biology & drug design, 83(2): 198-206.

Chen ZH, Zheng CJ, Sun LP and Piao HR (2010). Synthesis of new chalcone derivatives containing a rhodanine-3-acetic acid moiety with potential anti-bacterial activity. European Journal of Medicinal Chemistry, 45(12): 5739-5743.

Ducki S (2009). Antimitotic chalcones and related compounds as inhibitors of tubulin assembly. Anti-cancer agents in medicinal chemistry, 9(3): 336.

Fox BA, Schendel DJ, Butterfield LH, Aamdal S, Allison JP, Ascierto PA, Atkins MB, Bartunkova J, Bergmann L, Berinstein N and Bonorino CC (2011). Defining the critical hurdles in cancer immunotherapy. Journal of translational medicine, 9(1): 214.

Gaber M, Al-Daly S, Fayed T, and El-Sayed Y (2015). Synthesis, spectral, thermal studies and electrical conductivity of Co (II) and Ni (II) complexes 3-[4dimethylaminophenyl]-1-(2-pyridyl) prop-2-en-1-one (DMAPP). Journal of Luminescence, 157: 1-9.

Gaber M, Fayed T.A, El-Daly S.A and El-Sayed Y.S (2008). Spectral properties and inclusion of a hetero-chalcone analogue in organized media of micellar solutions and beta-cyclodextrin. Photochemical & Photobiological Sciences, 7(2): 257-262.

Han H, Zhao Y, Cuthbertson T, Hartman R.F and Rose S.D (2010). Cell cycle arrest and apoptosis induction by an anticancer chalcone epoxide. Archiv der Pharmazie, 343(8): 429-439.

Hirsch J (2006). An anniversary for cancer chemotherapy. Jama, 296(12): 15181520.

Meng Y, Zhong JY and Sun H (2011). Effects of Ashitaba chalcone on caspase-3 and Bax protein expressions of mouse hepatocarcinoma cells. Carcinogenesis, Teratogenesis and Mutagenesis, 23(1): 50-53.

Parish CR (2003). Cancer immunotherapy: the past, the present and the future. Immunology and cell biology, 81(2): 106113.

Qi X, Chang Z, Song J, Gao G and Shen Z (2011). Adenovirus-mediated p53 gene therapy reverses resistance of breast cancer cells to adriamycin. Anti-cancer drugs, 22(6): 556-562.

Salem ML, Díaz-Montero CM, Al-Khami AA, ElNaggar SA, Naga O, Montero AJ, Khafagy A and Cole DJ (2009). 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). The Journal of Immunology, 182(4): 2030-2040.

Sharma PL, Mehta A, Prabhakar M, Kumar P and Deshmukh R (2013). Excitotoxicity: bridge to various triggers in neurodegenerative disorders. European journal of pharmacology, 698(1-3): 6-18.

Szliszka E, Czuba Z, Mazur B, Sedek L, Paradysz A and Krol W (2010). Chalcones enhance TRAIL-induced apoptosis in prostate cancer cells. International journal of molecular sciences, 11(1): 1-13.

Tseng CH, Chen YL, Hsu CY, Chen TC, Cheng CM, Tso HC, Lu YJ and Tzeng CC (2013). Synthesis and anti-proliferative evaluation of 3phenylquinolinylchalcone derivatives against non-small cell lung cancers and breast cancers. European journal of medicinal chemistry, 59: 274-282.

Wei H, Zhang X, Wu G, Yang X, Pan S, Wang Y and Ruan J (2013). Chalcone derivatives from the fern Cyclosorus parasiticus and their anti-proliferative activity. Food and chemical toxicology, 60: 147-152.

Weinberg, R. A. (1996). How cancer arises. Scientific American, 275(3), 62-70.

Yan CG, and Gao H and Sun J (2014). Selective synthesis of functionalized spiro [indoline-3, 2'-pyridines] and spiro [indoline-3, 4'-pyridines] by Lewis's acid catalyzed reactions of acetylenedicarboxylate, arylamines, and isatins. The Journal of organic chemistry, 79(9): 4131-4136.