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Welcome to the Int J Cancer and Biomedical Research (IJCBR)**!**

It is with great pleasure that I write this editorial to welcome you to the IJCBR. This journal provides a platform for publication of original and reviews research articles, short communications, letter to editor, thesis abstract, conference report, and case studies. These types of publication are directed at the interface of the fields of cancer and biomedical research.

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Mohamed L. Salem,

Editor in Chief

INTERNATIONAL JOURNAL OF CANCER AND BIOMEDICAL RESEARCH (IJCBR)

RESEARCH ARTICLE

In vivo and *in vitro* antitumor effects of *Helix desertorum* hemolymph by inducing cell cycle arrest and apoptosis

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ABSTRACT

Background: Natural products, those extracted from invertebrate animals have medical importance as therapeutic agents especially for cancer treatment. **Aim of the Work:** The current study was conducted to evaluate the antitumor effect of the hemolymph of the desert snail, *Helix desertorum* (HD-H) on tumor Ehrlich ascetic carcinoma cells (EAC) cell line *in vitro* and *in vivo*. **Materials and Methods:** *Helix desertorum* (HD-H) was collected from their suitable habitats, identified, and then their hemolymph was collected, to evaluate their antitumor effect. 40 female albino mice were divided into five groups (n=8) as the following: group 1 (Gp1) control mice were inoculated intraperitoneal (i.p) with 1x106 EAC cells/mouseat day 0, Gp2 (EAC/Cis): Mice were injected with EAC-cells as in Gp1 and at day 2 were injected with cisplatin (40 mg/Kg), Gp3 was inoculated with EAC and then treated with splenocytes activated with HD-H, Gp4was inoculated with EAC and then treated with splenocytes activated with IL-2/Con A, and Gp5 was inoculated with EAC and then treated with splenocytes without activation. All groups were sacrificed to evaluate the tumor profile, hematological and biochemical. **Results:** The results showed that treatment with HD-H led to decrease tumor volume, their cell counts, increase the percentage of the apoptotic cells and arresting the cancer cell cycle. Moreover, treatment with HD-H improved the hematological and biochemical parameters on tumor-bearing mice. **Conclusion:** In conclusion, HD-H has potential in vitro and in vivo antitumor effects against EAC-cells. Further study is recommended to evaluate the potential efficacy of HD-H as a potential anticancer

Keywords: Anticancer, Apoptosis, Cell cycle, *Helix desertorum* (HD-H), Hemolymph.

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INTRODUCTION

Natural products are promising sources for having new anticancer agents. Approximately, half of the currently used anticancer drugs in the markets are derived from natural products due to their chemical diversity (Da Rocha et al., 2001). The terrestrial and marine invertebrates have been less explored to produce safe and novel antitumor compounds (Pomponi, 1999). The chemical and biological diversity of the terrestrial and marine invertebrates are immeasurable and therefore is an extraordinary resource for the discovery of new anticancer drugs (Leng et al., 2005).

In recent years, different marine organisms provided growing interest as potential sources for new anticancer agents against several types of cancers due to their chemical constituents with active antitumor activities (Khalifa et *al*., 2019; Wali et *al.*, 2019). Till now, thousands of bioactive compounds have been isolated and characterized from marine sources; however, hundreds of new compounds are still being discovered every year (Newman and Cragg, 2004). Currently, several drugs are derived from marine natural products (Schwarzenberg and Vollmar, 2013). Some bioactive compounds extracted from marine sources were isolated, identified, and approved as anticancer drugs (Jimeno et al., 2004). Natural products extracted from terrestrial and marine animals are currently in clinical trials as new drug candidates (Newman and cragg, 2004; Khalifa et al., 2019). This is reflected in the number of marine-derived compounds undergoing preclinical and early clinical development (Schwartsmann et al., 2005). Over the past

decade, several new experimental anticancer agents derived from marine sources have entered preclinical and clinical evaluations. Thousands of new compounds from marine and terrestrial invertebrates have been described and have entered clinical trials (Cragg et al., 1997). For instance, Cytarabine is considered the first marine-derived anticancer agent be developed for clinical use, which is isolated from a marine sponge that can be used for the treatment of leukemia and lymphoma (Schwartsmann et al., 2001; Hansen and Andersen, 2016).

Hemolymph is the body fluid found in snails; mainly composed of plasma, which is a protein in nature. This hemolymph not only provides nutrients and metabolites but also plays a key role in the response of snails to invading organisms (Martin et al., 2007).Multitudes of proteins whose concentrations change in hemolymph have been identified. For instance, lectins, selectin, galectin, C-type lectin, and fibrinogen-related proteins (FREPs), which are considered to play a promising role in pathogen recognition mechanisms (Vergote et al., 2005; Prasopdee et al., 2015). The present study aimed to address the impact of snails' hemolymph extracts as an anticancer agent, both *in vitro* and *in vivo* approaches. To conduct this aim, *Helix desertorum* snail's hemolymph was used to investigate its antitumor activity against *Ehrlisch ascetic* carcinoma (EAC) cell line.

MATERIALS AND METHODS

Chemicals and reagents

Crystal violet and trypan blue dyes were purchased from Sigma (St. Louis, Mo., USA). Phosphate buffer saline (PBS) was obtained from (Verviers, Belgium). RPMI-1640 media were purchased from Biochrom (Berlin, Germany). L-glutamine, gentamycin and trypsin-EDTA were purchased from Life Technologies (Paisley, Scotland). Penicillin streptomycin, concanvalin A (Con A) and brefeldin A were purchased from Sigma (Sigma Chemical Co., Munich, Germany). Interleukin-2 (IL-2) was obtained from R&D Systems (Minneapolis, Minnesota, USA). Phosphate buffer saline (PBS) was obtained from (Verviers, Belgium). FACS (fluorescence-activated cell sorting) was purchased from (BD Biosciences, San Jose, CA, USA). Sheath fluid was purchased from (Luminex Crop, Austin, TX, USA) and cisplatin (Cis-diammine dichloro platinumII) purchased from Sigma-Aldrich (St Quentin Fallavier, France). The FITC Annexin V apoptosis (Apoptosis Detection Kit II; Cat. No 556570; BD Bioscience, USA).

Snails

Helix desertorum snails were collected from their desert habitats. Live snails used in the present experiments were collected during the period from December-January 2018 from El-Alamen, Western desert, Egypt. Snails with the initial body weight of 11–15 g, were kept in the plastic box with dimensions (50 cm x 50cm x25cm) and containing the ventilation openings, under proper zoohygienic conditions. Every other day, the box was washed, and a fresh standard diet was provided. Snails were fed two or three times weekly with cabbage leaves. Some collected snails were used for description and identification, while others were used for further studies.

Hemolymph collection

Hemolymph was collected from each snail (about 1.0 ml per sample) with a 22-gaugehypodermic needle from the posterior adductor muscle sinus as described by Auffr et and Oubella (1995), then centrifuged immediately at 800 g in a cooling centrifuge for 15 minutes at 4 ˚C to remove all debris. Supernatant which refers as clean hemolymph was extensively dialyzed against invertebrate isotonic (50 mμ Tris-buffer saline (pH 8.5) before immediate use or storage at -80˚C (Oubella et al., 1996).

In-vitro antitumor assessment of snail'shemolymph:

Ehrlich Ascities Carcinoma (EAC) cell line was used to assess the in vitro antitumor activity of the hemolymph isolated from *Helix desertorum*. This tumor grows aggressively as ascites if injected intraperitoneal (i.p). The EAC cell line was purchased from The National Cancer Institute, Cairo University, Egypt. Cell line maintenance: The cell line was maintained by serial transplantation (i.p.) at 2.5 x 10^6 viable tumor cells in 0.3 ml of saline. EAC cell line was

cultured according to the standard protocol of cell culture. Cells were cultured in a 6-well plate in RPMI-1640 medium with L-glutamine and supplemented with 10% fetal bovine serum (FBS) and 1% of the penicillin-streptomycin solution. Cells were seeded with a density of ($1x10^6$ cells/well), and maintained at 37 ˚C in 5% CO_2/95% air atmosphere, with 95% humidity in CO_2 incubator.

Briefly, cells were cultured in 6-well plate in RPMI-1640 medium with L-glutamine and supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution. Cells were seeded with a density of ($1x10^6$ cells/well) and maintained at 37 ˚C in 5% CO2atmosphericair, with 95% humidity in CO_2 incubator. Sets of subconfluently plates were treated with different concentrations of hemolymph. Cells were let to grow at 37˚C in CO_2 incubator. After 48 h, cells were harvested by trypsinization using trypsin-EDTA, where old media was decanted, cells were washed with sterile saline (0.9%) and 0.5-1 ml of trypsin-EDTA solution was added to the cell monolayer. After incubation for 3-5 min, cells were collected for counting or other investigations and these procedures were repeated at 72 h.

In vivo antitumor assessment of adoptively transferred *Helix desertorum* hemolymph (HD-H) stimulated splenocytes

Mice and Ehrlich ascites carcinoma (EAC) tumor cells inoculation

Female Swiss albino mice (20 ± 2 g) were obtained from National Research Center (NRC, Cairo, Egypt). Animals were housed in cages (6/cage), in 12 h/12 h dark/light cycle under laboratory conditions of temperature and humidity. Mice were kept for a week before starting the experiment for acclimatization. Handling of mice was according to the ethical guidelines approved by the animal care and use committee, Faculty of Science, Tanta University (ACUC-SCI-TU), Egypt. The EAC cells were collected from the tumor-bearing mice purchased from the National Cancer Institute (NCI, Cairo, Egypt). The viable and dead cells were counted using the trypan blue method (The chamber of hemocytometer and a cover slip was cleaned with 70% alcohol and distilled water. A volume of 10µl of EAC suspension was added to 90 µl of trypan blue (Sigma Chemical Co. USA) (0.4% in distilled water), mixed well and left for 3 minutes. 10µl of the mixture was pipetted into the hemocytometer chambers. The cells were counted under a microscope using trypan blue, in all four outer quadrants of the hemocytometer and the number of cells was divided by four to get the cells in one quadrant. The number of EAC/ (N) ml was calculated as follows: number of EAC / (N) ml=cells in one quadrant x dilution factor x 10000 x N (number of ml(s) of the suspension), tumor cells were adjusted at 1×10^6 cells/mouse for (i.p) inoculation.

Experimental design

($15x10^6$ /15 ml) cells of splenocytes were cultured using complete RPMI 1640 supplemented with 2% human serum FPS, 2 mM L-glutamine, 50 unit/ml penicillin, and 50 ng/ml streptomycin in the presence of 5µg/ml concanavalin A (Con A) and 50ng/ml interleukin-2 (IL-2) in 3 different flasks then 100 µg/ml of HD-H was added to the flasks. Then cultured cells were incubated at CO2 incubator at 37˚C in atmospheric pressure of 5% CO_2 for 72 hrs. Brefeldin A was added to the cultured cells 3 hours before harvesting.

After harvesting in previously inoculated 40 female Swiss albino mice i.p with 1x10ˆ6 EAC cells/mouse and divided into 5 groups (n=8) as following:

- Group 1 (EAC group): Negative control mice were injected i.p with 1 x106 EAC cells at day0.
- Group 2 (EAC/Cis): Mice were injected with EAC-cells as in Gp1 and at day 2 were injected with cisplatin (4mg/Kg) i.p as a reference drug (positive control).
- Group 3(EAC/Cis/activated spleenocytes with HD-H): inoculated with EAC and Cisplatin as in group 2 and then treated with 100µg/Kg HD-H at day 3.
- Group 4 (EAC/Cis/activated spleenocytes): inoculated with EAC and Cisplatin as in group 2 and then treated with activated splenocytes.

- Group 5 (EAC/Cis/fresh splenocytes): inoculated with EAC and Cisplatin as in group 2 and then treated with fresh splenocytes.

At day 10, all mice were bled *via* the orbital plexus to collect blood and sera for hematological and biochemical assessments.

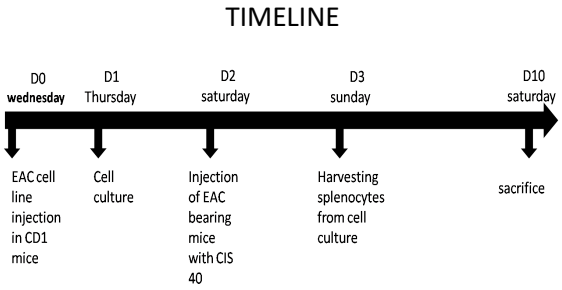

Figure 1. Timeline sketch showing EAC cell line injection and treatment protocol in mice groups.

Counting of tumor cells and splenocytes

To count the tumor cells, EAC cells suspension was collected and diluted with PBS, and 10µl was added to 90 µl of trypan blue. Then 10µl of the mixture was pipetted into the hemocytometer chambers. The cells were counted under a microscope using trypan blue; the number of EAC/ (N) ml was calculated as follows:

- NO of EAC / (N) ml=cells in one quadrant x dilution factor x 10000x N (number of ml(s) of the suspension).
- To count splenocytes, the spleen was collected and placed into Petri dish and crashed gently by glass slides. Splenocytes were filtered and washed twice with PBS, then by ACK buffer and adjusted for the count.

Statistical analysis

Data were presented as means ± SD and were analyzed using one–way analysis of variance (ANOVA) followed by Dunnet test at $p < 0.05$ (statistically significant).

RESULTS

In vitro effect of *Helix desertorum* hemolymph on EAC proliferation capacity post 48 h treatment

The results as compared to EAC-cells with no treatment showed that all conditions of EAC-cells, which treated with Cis alone or with different concentrations of HD-H after 48 hours showed a low rate of proliferation. The effect of different concentrations of HD-H after 48 hours was close to the effect of treatment with 40 or 20 µg/ml of Cis at the same time points (Figure 2).

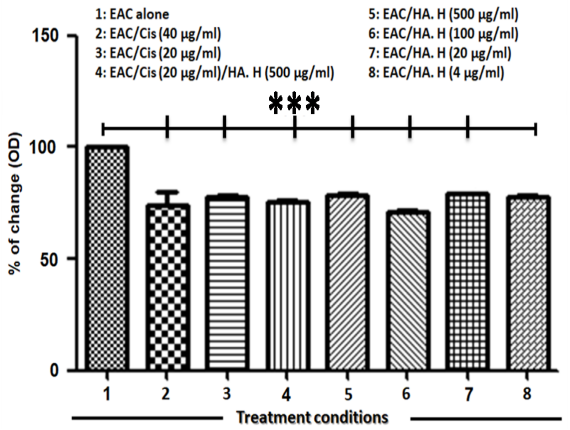

Figure 2. The cytotoxic effect of Helix desertorum (HD-H) hemolymph on EAC-cells 48 hours post-treatment in vitro. *** refers to significant differences from the control (group1) when $P < 0.001$.

In vitro apoptotic activity of *Helix desertorum* hemolymph on EAC-cells upon 48 hours treatment

As compared to the untreated EAC-cells, cells that were treated with Cis (40 or 20 µg/ml) post 48 hours showed a significant increase in the percentage of apoptotic cells. Also, EAC-cells that were treated with Cis (20 µg/ml)/*Helix desertorum* hemolymph (500 µg/ml) showed an increase in the percentage of apoptotic EAC-cells when compared to the untreated cells post 48 hours. Interestingly, the percentage of apoptotic cells post-treatment with Cis (20 µg/ml)/*H. desertorum* hemolymph (500 µg/ml) or *H. desertorum* hemolymph(100 µg/ml) was higher than the apoptotic percentage of cells post Cis 40 or 20 µg/ml post 48 of the treatment (Figure 3).

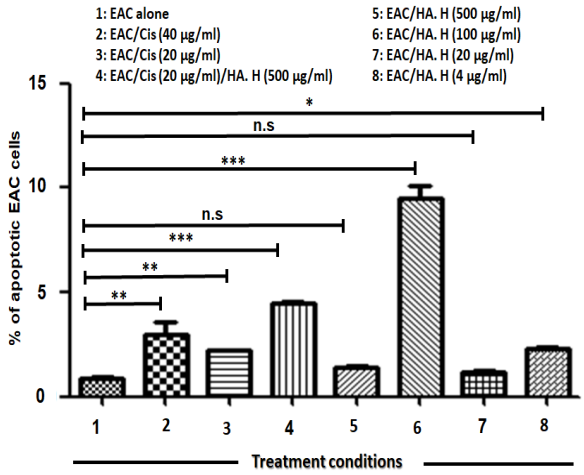

Figure 3. The percentages of apoptotic EAC cells post-treatment with Helix desertorum hemolymph for 48. n.s. refers to non-significant difference between the treatment and the control when P ≥ 0.001. *, ** &*** refers to significant differences from the control (group1) when P <0.05, 0.01 and 0.001, respectively.

The *in vitro* effect of *Helix desertorum* hemolymph on EAC-cell cycle post 48 hours treatment

The results showed that the percentage of G0/G1 phase increased post 48 hours treatment with Cis (20 µg/ml)/*Helix desertorum* hemolymph (500 µg/ml) and *Helix desertorum* hemolymph alone (100 µg/ml), when compared with non-treated EAC-cells. Consistent with the increase in G0/G1 phase post-treatment with Cis (20 µg/ml)/ *Helix desertorum* hemolymph (500 µg/ml) and *Helix desertorum* hemolymph (100 µg/ml) alone, the percentages of S-phase were decreased when compared to non-treated EAC-cells (Figure 4A and B). Treatment with *Helix desertorum* hemolymph(100µg/ml) alone showed a significant increase in the percentages of G2/M cell cycle phases when compared to those percentages of untreated EAC-cells post 48 hours of the treatments. Treatment with 40 or 20 µg/ml of Cis for 48 hours did not show any significant changes in the percentages of G2/M, when compared to the non-treated EAC-cells (Figure 4C).

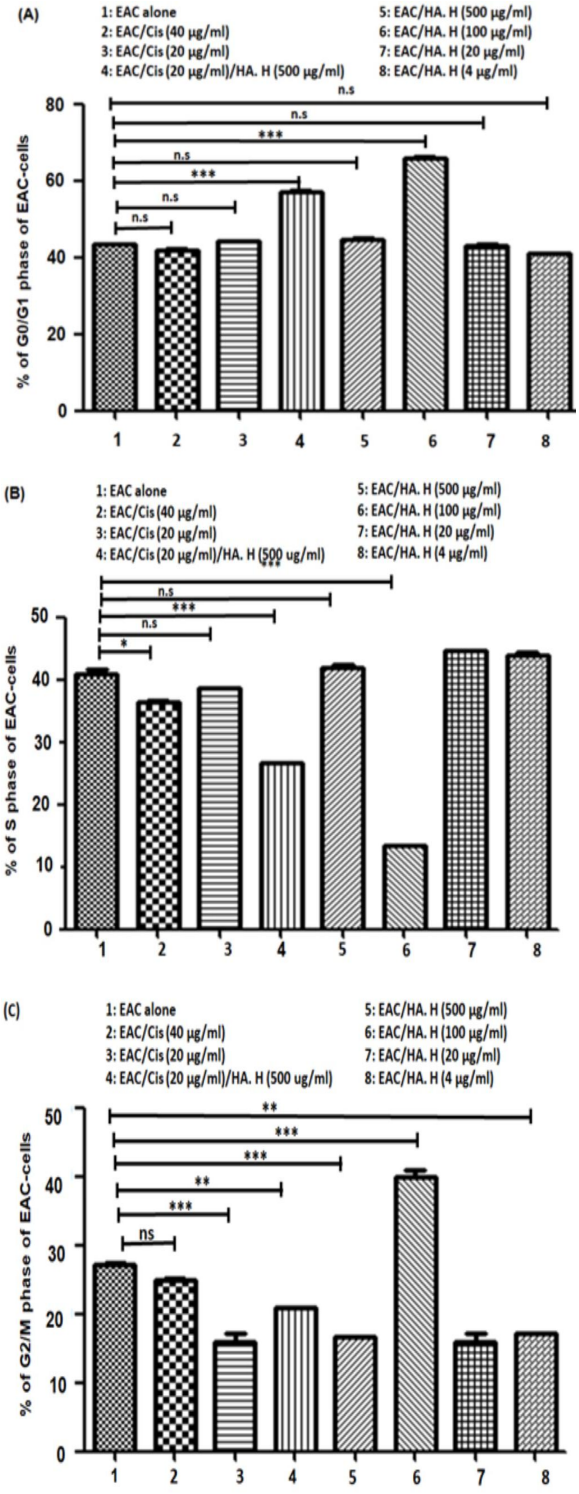

Figure 4 (A–C). Percentages of EAC-cell cycle phases after treatment with Helix desertorum hemolymph (HD-H): (A) G0/G1 phases; (B) S phases and (C) G2/M phases for 48 hours. n.s. refers to non-significant difference between the treatment and the control when P ≥ 0.001. *, ** & *** refers to significant differences from the control (group1) when P < 0.05, 0.01 and 0.001, respectively.

Effect of adoptively transferred *Helix desertorum* hemolymph (HD-H) stimulated splenocytes on both EAC-cells and splenocytes count in EAC-bearing mice

As compared to EAC-bearing mice alone, 10 days post Cis (40 mg) injection, treatment with preconditioned splenocytes *in vitro* HD-H, Il-2/Con-A alone or with non-activated splenocytes led to a significant decrease in EAC-cells count (Figure5 A). All treated groups of mice showed a significant increase in splenocytes count followed by the EAC-bearing mice treated with Cis (40 mg) when compared to EAC-bearing mice alone (Figure 5B).

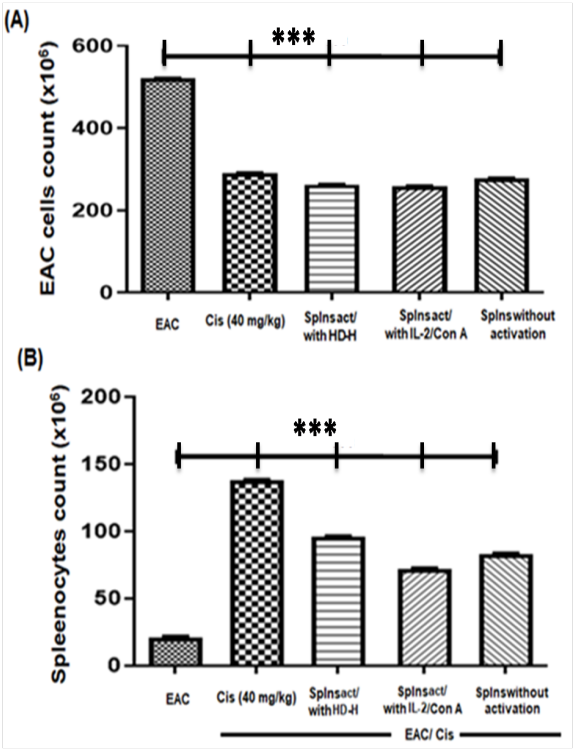

Figure 5 (A and B). The count of EAC-cells (A) and splenocytes (B) in groups of EAC-bearing mice treated with splenocytes activated with HD-H. *** refers to significant differences from the control (group1) when P < 0.001.

Early, late apoptotic cells percentages and necrotic EAC-cells upon the treatments with the adoptively transferred *Helix desertorum* hemolymph (HD-H) stimulated splenocytes in EAC-bearing mice

The results showed that post 10 days of Cis (40 mg) injection, different treatments protocols of Cis/splenocytes activated with HD-H, Cis/splenocytes activated with IL-2 and Con-A or Cis/splenocytes without activation led to a significant increase in the percentage of apoptotic cells compared to the group of mice which inoculated with EAC-cells (Figure 6A). As compared to the EAC-bearing mice, treatment with Cis (40 mg) injection, treatment with Cis/preconditioned splenocytes*In vitro* HD-H, Il-2/Con-A alone or Cis/ non-activated splenocytes led to a significant increase in the number of early apoptotic cells when compared to mice group which inoculated with EAC-cells alone (Figure 6B). All treated groups showed an increase in the percentages of late apoptotic cells compared to the group of mice inoculated with EAC-cells alone (Figure 7A). As compared to the mice inoculated with EAC-cells, groups of mice treated with Cis(40 mg), Cis/preconditioned splenocytes *In vitro* with HD-H, or treated with Cis/non-activated splenocytes showed a significant decrease in the number of necrotic cells. However, treatment with Cis/splenocytes activated with IL-2 and Con-A cause a significant increase in the number of necrotic cells as compared with the control group (Figure 7B).

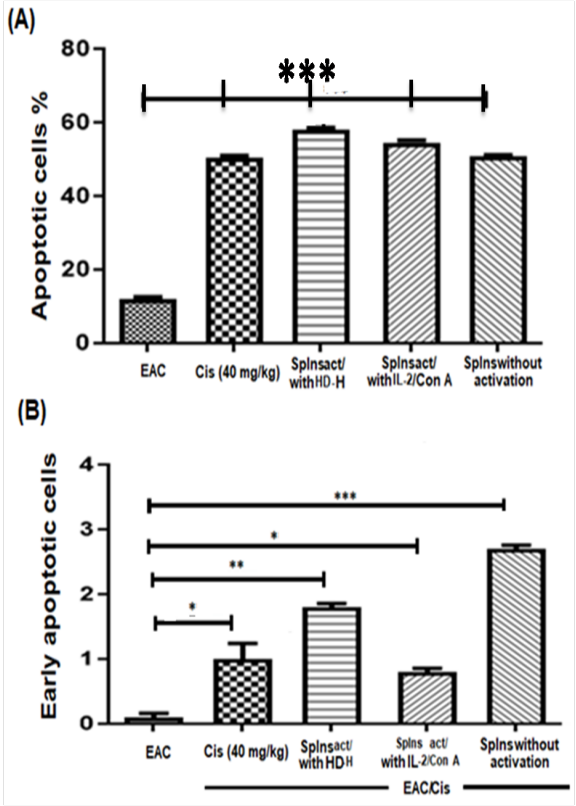

Figure 6 (A and B). Apoptotic cells percentages (A) and early apoptotic EAC-cells (B) in EAC-bearing mice treated with splenocytes activated with HD-H. *, ** &*** refer to significant difference from the control (group1) when P < 0.05, 0.01 and 0.001, respectively.

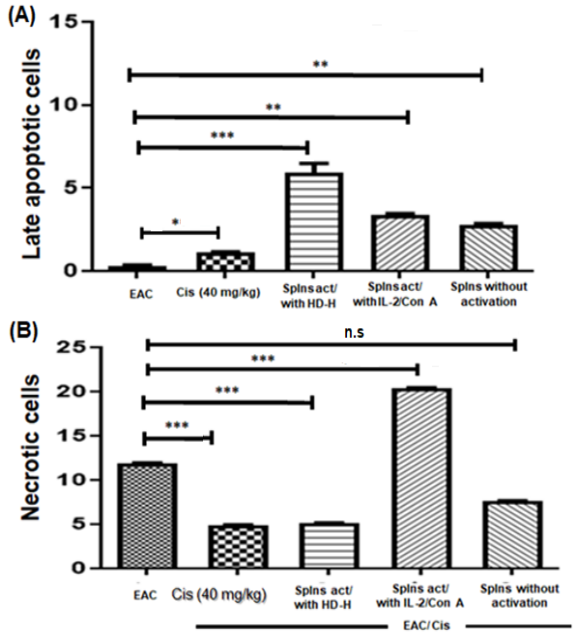

Figure 7 (A and B). Late apoptotic EAC-cells (A) and necrotic cells (B) in EAC-bearing mice treated with splenocytes activated with HD-H. n.s. refers to non-significant difference between the treatment and the control when P ≥ 0.001. *, ** & *** refers to significant differences from the control (group1) when P < 0.05, 0.01 and 0.001, respectively.

EAC-cell cycle G0/G1, S and G2/M phases percentages upon the treatments with adoptively transferred *Helix desertorum* hemolymph (HD-H) stimulated splenocytes in EAC-bearing mice

The data of EAC-cells cycle analysis showed that Cis (40 mg) injection, different treatments conditions of Cis/splenocytes activated with HD-H, Cis/splenocytes activated with IL-2 and Con-A or Cis/splenocytes without activation after 10 days of EAC-cells inoculation led to a significant decrease in the percentage of G0/G1 phase compared to the group of mice, which inoculated with EAC-cells. A similar pattern was obtained in the data of S-phase percentages (Figure 8A and B). As compared to the mice inoculated with EAC-cells, groups of mice treated with Cis (40 mg), Cis/preconditioned splenocytes*In vitro* with HD-H or treated with Cis/non-activated splenocytes showed a significant decrease in the percentage of G2/M phase. However, treatment with Cis/non-activated splenocytes led to a non-significant decrease in the percentage of G2/M phase (Figure 9).

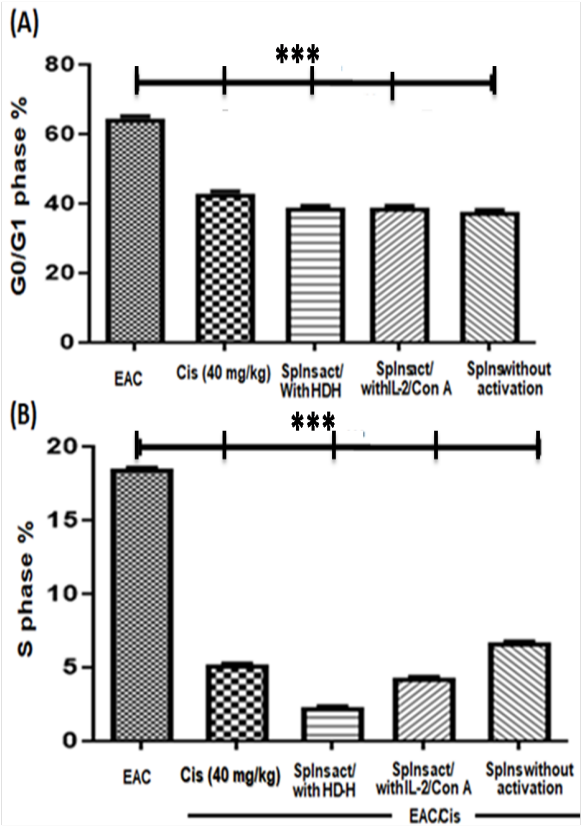

Figure 8 (A and B). Shows the percentages of EAC-cells cycle phases (A) G0/G1 phase and (B) S phase in EAC-bearing mice treated with splenocytes activated with HD-H.***refers to significant differences between the treatments and the control (group1) when P < 0.001.

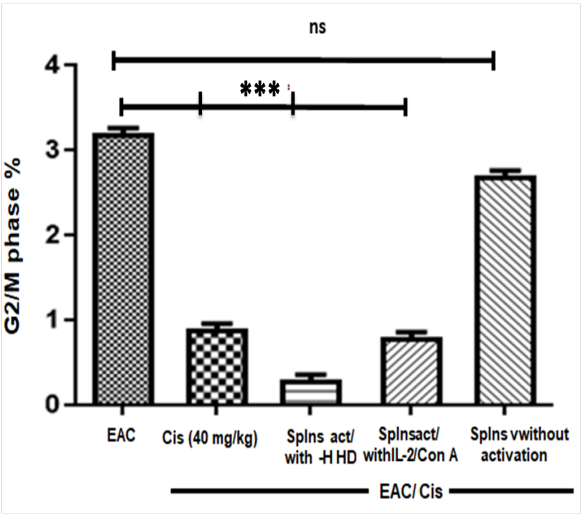

Figure 9. The percentages of EAC-cellsG2/M phase in EAC-bearing mice treated with splenocytes activated with HD-H. n.s. refers to the non-significant difference between the treatment and the control when P ≥ 0.001. *** refers to significant differences from the control (group1) when P < 0.001.

DISCUSSION

Finding new natural products as new chemotherapeutic agents are well recognized nowadays and profound development has been achieved by researchers to deal with different molecular pathways of tumors.

Anticancer compounds have characteristics of multi-function, high sensitivity, and stability (Leng et al., 2005). The hemolymph of the land snail, Helix lucorum and the marine snail Rapanavenosa exhibited potential anticancer activities against human bladder carcinoma cells (T-24) (Dolashka et al., 2015). Furthermore, tissue extracts of some garden snails including Helix aspersa and Actinia equina were shown to possess anticancer activity against breast cancer cells leukemia-derived cells (Teerasak et al., 2016; El Ouar et al., 2017). Isolated hemocyanins from the terrestrial snail Helix pomatia and the marine snail Rapanathomasiana showed promising anti-proliferative effects on colon carcinoma (Gesheva et al., 2014).

The results of this study showed that the cytotoxic effect ofdifferent concentrations of HD-H on EAC in vitro, 48 hours post-treatment did not show any significant changes when compared to their control. Our data werein agreement with the previous studies that showed the antitumor effects of Helix aspersa hemolymph against bladder cancer (CAL-29), ovarian cancer (FraWü), acute monocytic leukemia (THP-1), prostate cancer (DU-145), glioma cancer (LN-18), and Burkitt's lymphoma (Patra and Muthuraman, 2013; Matusiewicz et al., 2018). Treatment with Cis (40 mg/Kg) post-EAC-cells inoculation led to a significant decrease in the number of tumor cells when compared to EAC-group. Also, treatment with HD-H (100 µg/Kg) post-EAC-cells inoculation led to a decrease in the tumor cells count, but not that much as Cis treated group. Compared to tumor-bearing mice alone, treatment with Cis (40 mg/Kg) injection, HD-H post-EAC-cells inoculation increased the number of splenocytes significantly, and the number of splenocytes in EAC-bearing mice treated with Cis (40 mg/Kg) was higher than this number in mice bearing tumor and treated with HD-H (100 µg/Kg). The present data werein agreement with the previous studies, which demonstrated increased splenocytes count in tumor-bearing mice treated with chemotherapeutic agents (Merritt et al., 2003; Park et al., 2009; Rosa et al., 2013; Zhu et al., 2019; Hashem et al., 2020).

Apoptosis is a vital homeostatic mechanism to equilibrate the cell division and death, and as a consequence, it is employed by the host to sustain the proper number of cells in the tissue. Apoptotic induction of tumors is a common phenomenon by anticancer treatment and has been recognized as an effective strategy for the development of an anticancer drug. The results of the current study showed that the number of EAC-apoptotic cells post-treatment with Cis (40 mg/Kg) was higher than those of mice inoculated with EAC-cells alone. Treatment with HD-H (100 µg/Kg) post EAC inoculation showed an increase in the number of apoptotic cells. As compared to EAC-bearing mice, treatment with Cis or HD-H post tumor inoculation increased the percentages of early apoptotic EAC-cells. Treated groups with Cis, HD-H or SH-H showed an increase in the percentages of the late apoptotic EAC-cells, however, the percentage of these cells was higher in HD-H (100 µg/Kg) than those in the group of mice inoculated with EAC-cells and treated with Cis (40 mg/Kg). Our results were in consistent with previous studies that investigated the number of apoptotic cells in tumor-bearing mice treated with chemotherapeutic agents (Zhivotovsky et al., 2006; Donaghy et al., 2010; Patra and Muthuraman, 2013; Matusiewicz et al., 2018; Zhu et al., 2019; Hashem et al., 2020).

Upon the treatment of EAC-bearing mice with Cis (40 mg/Kg), the percentages of G0/G1 phase of the EAC-cell cycle were decreased while treatment with HD-H (100 µg/Kg) did not show significant changes in the percentages of this phase compared to EAC-bearing mice alone. Treatment of EAC-mice with Cis alone or with HD-H (100 µg/Kg) post-EAC-inoculation decreased the percentages of S phase of the EAC-cells. A similar pattern was found in the G2/M phase as in the S phase post-treatment with Cis (40 mg/Kg), HD-H (100µg/Kg). These findings were in consistent with previous studies that investigated the analysis of tumor cell cycle upon treatment with chemotherapeutic agents (Simmons et al.,

2005; Sala-Vila et al., 2010; Donaghy et al., 2010; Rosa et al., 2013; Zhu et al., 2019; Hashem et al., 2020).

CONCLUSION

In conclusion, hemolymph of *Helix desertorum* (HD-H) hasa potential antitumor effect against EAC-cells *in vivo.* Further study is recommended to evaluate the potential efficacy of the hemolymph of HD-H as potential anticancer agents on other tumor models.

CONFLICT OF INTEREST

Authors declare that they have no conflicts of interest.

FUDING

There is no financial support for this study.

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Egyptian Association for Cancer Research (EACR)
http://eacr.tanta.edu.eg/

EACR is an NGO society that was declared by the Ministry of Social Solidarity (Egypt) No. 1938 in 19/11/2014 based on the initiative of Prof. Mohamed Labib Salem, the current Chairman of EACR. EACR aims primarily to assist researchers, in particular young researchers in the field of cancer research through workshops, seminars and conferences. Its first international annual conference entitled "Anti-Cancer Drug Discovery" was successfully organized in April 2019 (http://acdd.tanta.edu.eg). Additionally, EACR aims to raise the awareness of the society about the importance of scientific research in the field of cancer research in prediction, early diagnosis and treatment of cancer. EACR is also keen to outreach the scientific community with periodicals and news on cancer research including peer-reviewed scientific journals for the publication of cutting-edge research. The official scientific journal of EACR is "International Journal of Cancer and biomedical Research (IJCBR: https://jcbr.journals.ekb.eg) was successfully issued in 2017 and has been sponsored by the Egyptian Knowledge Bank (EKB: www.ekb.eg).

EACR Chairman,
Prof. Mohamed Labib Salem, PhD
Professor of Immunology
Faculty of Science, Tanta Universiy, Egypt

ABOUT JOURNAL

International Journal of Cancer and Biomedical Research (IJCBR), a publication of the Egyptian Association for Cancer Research (EACR), is a peer-reviewed online journal published quarterly. The journal allows free access (Open Access) to its contents and permits authors to self-archive a final accepted version of the articles on any OAI-compliant institutional / subject-based repository.

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