Original Article

Anti-neoplastic activities of *sepia officinalis* ink and *coelatura aegyptiaca* extracts against Ehrlich ascites carcinoma in Swiss albino mice

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Abstract: Objectives: With the development of sophisticated instruments for the isolation and elucidation of natural products structures from marine and freshwater organisms, major advances have been made in the discovery of aquatic derived therapeutics. Present investigations were carried out to evaluate cuttlefish (*Sepia officinalis*) ink extract (IE) and freshwater clam (*Coelatura aegyptiaca*) extract (CE) for their anticancer and antioxidant activities as compared to 5-flurouracil (5-Fu), in Ehrlich ascites carcinoma (EAC). Methods: Sixty female Swiss albino mice were divided into five groups (n = 12). All groups except group I received EAC cells (5 × 10⁶ cells/mouse i.p.) and this was taken as the 0th day. Group I served as saline control (5 ml/kg 0.9% NaCl w/v p.o). Group II served as EAC control. Rats of groups III, IV and V received IE, CE (200 mg/kg body weight i.p.), and reference drug (5-Fu, 20 mg/kg body weight i.p.), respectively. Results: The reduction in tumor volume, packed cell volume, tumor cell counts and increase in median survival time and percentage increase in life span in treated animals were observed. There was a significant increase in RBC count; Hb content in treated animals and reduction in total WBC count. There was a significant decrease in AST, ALT, ALP and liver MDA levels and increase in GSH, SOD and NO levels were observed in all treated animals. Conclusion: Both IE and CE were effective in inhibiting the tumor growth in ascitic tumor models. The biochemical, antioxidants and histopathological studies were also supported their antitumor properties.

Keywords: *Sepia officinalis*, *coelatura aegyptiaca*, Ehrlich ascites carcinoma, antitumor, oxidative stress

Introduction

Over the past few decades, cancer is one of the most prevalent diseases worldwide. The intensive studies on the transplantable tumors were taken into consideration in the last 2 to 3 decades [1]. The planned goal of the research was to improve new techniques especially for experimental tumors in animals that have been underlain at the basis of recent achievements in cancer therapy. Cancer chemoprevention can be defined as the prevention, inhibition or reversal of carcinogenesis by administration of one or more chemical entities, either as individual drugs or as naturally occurring constituents of the diet [2]. Despite of the important advances achieved over recent decades in the research and development of various cancer-static drugs, current antitumor chemotherapy still suffers from two major limitations, the first is the lack of selectivity of conventional chemotherapeutic agents for cancer tissues, bringing about unwanted side effects. The second is the acquisition by cancer cells of multiple-drug resistance [3]. Unwanted side effects of antitumor drugs could be overcome with agents capable of discriminating tumor cells from normal proliferative cells and the resistance is minimized using combined modality approach with different complementary mechanism of action [4]. At this point, the usage of natural sources is thought to have a great value for cancer control and programs destruction [5].

Almost 60% of drugs approved for cancer treatment are of natural origin. It is anticipated that the aquatic environment will become an invaluable source of novel compounds in the future [6]. Research into the pharmacological properties of marine natural products has led to the
discovery of many potently active agents considered worthy of clinical application. The marine environment is an exceptional reservoir of bioactive natural products, many of which exhibit structural/chemical features not found in terrestrial natural products [7]. Marine organisms have evolved biochemical and physiological mechanisms that include the production of bioactive compounds for such purposes as reproduction, communication, and protection against predation, infection and competition [8].

Products from freshwater and marine sources have recently become attractive as nutraceutical and functional foods and as a source material for the development of drugs and specific health foods [9-12]. Emerging evidence suggests that marine natural products, especially the secondary metabolites from marine organisms, are far more likely to yield anticancer drugs than terrestrial sources [13]. Furthermore, the new classes of anticancer drugs that have been isolated from marine organisms have been shown to possess cytotoxic activity against multiple tumor types [14-16].

In nature, animals are provided with their own protective response against their predators, likewise freshwater and marine mollusks are protected by their shells, but many of them are not fully protected by shells. Chemical defenses are used extensively by both shelled and shell-less mollusks. Caldwell [17] has proposed that the ink of cephalopods contain compounds that are capable of disrupting predator’s chemical senses. Squid ink is a multifunctional marine bioactive-material can promotes thromboxane production, kills cancer cells, and elevates leukocyte number [18]. Moreover, it has anti-oxidant [12, 19], anti-radiation, anti-retrovirus and anti-bacterial properties [20-22]. It has been discovered that squid ink could ameliorate chemotherapeutic injury induced by cyclophosphamide in model animals; mice or rats [20, 23, 24].

Egyptian freshwater mussel (Coelatura aegyptiaca) is a Molluscan bivalve that belonging to Unionoidae common in the Egypt along the River Nile from Assiut (Upper Egypt) to Damietta branches (Lower Egypt) [25]. Soliman [11] investigated the antioxidant activity of Coelatura aegyptiaca extract and its ability to alleviate the hepatic oxidative stress induced by monosodium glutamate in male rats.

Oxidative damage has been implicated in cell injury, including possible participation in the formation and promotion of cancer. Unlike normal cells, cancer cells can live in a redox environment where the elevated reactive oxygen species (ROS), which have been indicated as vital signaling molecules [26]. Epidemiologic studies have suggested that some antioxidants agent as well dietary constituents with antioxidant properties may be acting as naturally occurring cancer preventing agents and may explain some of the differences in cancer incidence seen in populations with varying dietary intake [27]. Many cancer patients who are undergoing therapy take antioxidant supplements in an effort to alleviate treatment toxicity and improve long-term outcome [28]. Our previous study with crude extracts from Sepia officinalis ink (IE) and Coelatura aegyptiaca (CE) had shown their in vitro antioxidant and analgesic activities as well as their cytotoxic activity against hepatocellular carcinoma (HepG2) cell lines [12]. Accordingly, the present study extending to evaluate the in vivo antioxidant status and anticancer activity of both aqueous crude extracts from Sepia officinalis ink (IE) and Coelatura aegyptiaca (CE) against Ehrlich Ascites Carcinoma (EAC) tumor model in female Swiss albino mice.

Materials and methods

Preparation of cuttlefish ink extract (IE)

Fresh cuttlefish (Sepia officinalis) were purchased directly from a fishmonger and rapidly transferred to the laboratory where they were dissected and the ink was collected and diluted immediately with an equal volume of distilled water and mixed sufficiently. The admixture collected immediately, concentrated and lyophilized to a black residue using a lyophilizer (LABCONCO lyophilizer, shell freeze system, USA).

Preparation of crude freshwater clam extract (CE)

Freshwater clam; Coelatura aegyptiaca were collected from the River Nile at Giza Governorate, Egypt. The crude extract was prepared as follows: fresh clam 1 kg was extracted in a boiler with one liter of distilled water for 30 min. 3 times. After filtration, the filtrate obtained was then concentrated and dried using a lyoph-
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Animals

Female Swiss albino (Mus musculus) mice (25-30 g) were used in all experiments. The animals were obtained from a closed random-bred colony at the animal's house, National Research Center. The used mice for any one experiment were selected from mice of similar age (± 1 week) and weight (± 2 g). Animals were housed in polycarbonate boxes with steel-wire tops (not more than five animals per cage) and bedded with wood shavings. Ambient temperature was controlled at 22 ± 3°C with a relative humidity of 50 ± 15% and a 12-h light/dark photoperiod. Food and water were provided ad libitum.

Drugs and chemicals

All drugs, chemicals and solvents were purchased from local firms (Egypt) and they were of highest purity and analytical grade.

Ehrlich ascitic tumor

Ehrlich ascites tumors were kindly provided by the Egyptian National Cancer Research Center, Cairo University. The parent line was kindly supplied by the National Cancer Institute, Cairo University, Egypt. EAC was maintained in BALB/c mice in the ascites form by serial transplantation. Ascitic fluid was withdrawn under aseptic conditions from tumor-bearing mice by needle aspiration from the peritoneal cavity. Ascitic tumor cell counts were done in a Neubauer hemocytometer by using a Trypan blue dye exclusion method. The animals used for the experiment received, i.p., 0.2 ml of a suspension containing 5 × 10^6 Ehrlich tumor cells. The preparation of the suspension containing tumor cells was prepared according to Dagli et al. [29].

Ethical statement

Experimental protocols and procedures used in this study were approved by the Cairo University, Faculty of Science Institutional Animal Care and Use Committee (IACUC) (Egypt), (CUPS/F/14/14). All the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

Treatment schedule

Sixty female Swiss albino mice were divided into five groups (n = 12). All groups except group I received EAC cells (5 × 10^6 cells/mouse i.p.) and this was taken as the 0th day. Group I served as saline control (5 ml/kg 0.9% NaCl w/v p.o). Group II served as EAC control. Twenty-four hours after EAC transplantation, rats of groups III, IV and V received ink extract (IE), clam extract(CE) (200 mg/kg body weight i.p.) [12], and reference drug 5-fluorouracil (5-Fu, 20 mg/kg body weight i.p.), respectively, daily for nine consecutive days [30]. Twenty four hours of last dose and 18 h of fasting, six animals of each group were sacrificed by cervical dislocation for measurement of tumor and biochemical parameters, and the rest were kept with food and water ad libitum to check the survival time of EAC-tumor bearing mice. The antitumor activity of the IE and CE were measured in EAC animals with respect to the following parameters:

Ehrlich ascitic volume (EAV): The ascitic fluid was collected from the peritoneal cavity, and volume was measured by taking it in a graduated centrifuge tube.

Packed cell volume (PCV) of ascitic fluid: PCV was determined by centrifuging the ascitic fluid in a hematocrit tube using microhematocrit centrifuge and special tube reader.

Mean survival time (MST) (days) = first death +last death/2.

Percentage Increase in Life Span (ILS): The effect of IE, CE, and 5-Fu on percentage increases in life span were calculated on the basis of mortality of the experimental mice [30].

ILS (%) = (mean survival time of treated group/mean survival time of control group-1) × 100.

Tumor cell count: The ascitic fluid was taken in a WBC pipette and diluted to 100 times. Then a drop of the diluted cell suspension was placed on the Neubauer's counting chamber, and the numbers of cells in the 64 small squares were counted.

Viable/Nonviable tumor cell count: The viability and non viability of the cell were checked by trypan blue assay. The cells were stained with trypan blue (0.4% in normal saline) dye. The cells
that did not take up the dye were viable, and those that took the dye were nonviable. These viable and nonviable cells were counted.

Hematological and biochemical parameters

Blood was collected into prelabelled EDTA-treated tubes and centrifuge tubes for estimation of hematological and biochemical parameters, respectively. Hematological parameters (Hemoglobin, RBC, WBC, WBC, Differential count of WBC and PCV) were measured. blood samples kept at room temperature for 1 h and centrifuged at 3000 rpm for 20 min to obtain clear serum. Serum was analyzed for serum amino-transferase enzyme activities (AST & ALT) [31], and alkaline phosphatase (ALP) activity [32].

Liver oxidative stress markers

Liver was quickly removed, cleaned with saline. Part of liver tissues was homogenized (10% w/v) in ice-cold sodium-potassium phosphate buffer (0.01 M, pH 7.4), and centrifuged at 9000 rpm for 15 min at 4°C. The supernatant obtained was used for lipid peroxidation evaluation which was measured by the formation of malondialdehyde (MDA) [33], glutathione reduced (GSH) [34], nitric oxide (NO) [35], and superoxide dismutase (SOD) [36], using test kit (Bio-Diagnostic-Egypt).

Histopathology of liver tissues

Liver slices were taken from each lobe of the liver. After fixation in 10% formalin, embedded in paraffin, section, and stained with hematoxylin and eosin (HE) for histological examination using standard techniques. After hematoxylin-eosin staining, the slides were observed and photos were taken using optical microscope.

Statistical analysis

Results were expressed as mean ± standard error (S.E). All data obtained were analyzed by ANOVA followed by Student’s t test at 95% confidence level. Values of $P < 0.05$ were considered as statistically significant. All computations were performed using SPSS version 15.0 software.

Results

Effect of ink extract (IE), clam extract (CE) and 5-fluorouracil (5-Fu) on ascitic Ehrlich tumor

Total Ehrlich ascitic volume (EAV) and Packed cell volume (PCV) of the ascitic fluid were significantly lower ($P < 0.05$) in mice treated with IE, CE and 5-Fu daily for nine consecutive days, as compared to EAC-bearing mice (Figures 1, 2).

The effect of IE, CE and 5-Fu on the survival of tumor bearing mice is shown in Figure 3. The mean survival time (MST) for the EAC-control group was 19.5 ± 0.47 days, whereas it was 28.5 ± 1.27, 31.1 ± 1.52 and 32.1 ± 0.85 days for the groups treated with IE (200 mg/kg i.p), CE (200 mg/kg i.p) and 5-Fu (20 mg/kg i.p), respectively. The highest increase in the lifespan (ILS%) of tumor-bearing mice treated with 5-Fu was found to be 64.62% as compared to the control group whereas it was 59.49% for CE and 46.15% for IE (Figure 4).

The viable tumor cell count of mice was found significantly decreased ($P < 0.05$) in all the
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**Figure 3.** Effect of *Sepia officinalis* ink extract (IE), *Coelatura aegyptiaca* extract (CE) and 5-fluorouracil (5-Fu) on mean survival time (MST) of EAC bearing mice. *:* significant ($P < 0.05$) as compared with EAC group.

**Figure 4.** Effect of *Sepia officinalis* ink extract (IE), *Coelatura aegyptiaca* extract (CE) and 5-fluorouracil (5-Fu) on percentage increase of life span (%ILS) of EAC bearing mice.

**Figure 5.** Effect of *Sepia officinalis* ink extract (IE), *Coelatura aegyptiaca* extract (CE) and 5-fluorouracil (5-Fu) on viable and non-viable tumor cell count of EAC bearing mice. *:* significant ($P < 0.05$) as compared with EAC group.

Treatment groups, as compared to EAC group (**Figure 5**). On the other hand, significant increase ($P < 0.05$) in the non viable tumor cell count was noticed after nine days of post treatment with IE, CE and 5-Fu (**Figure 5**).

**Effect of ink extract (IE), clam extract (CE) and 5-fluorouracil (5-Fu) on hematological parameters**

Hemoglobin content (Hb), The total count (TC) of RBC, packed cell volume (PCV %), WBC, and differential count of WBC of different groups of mice are shown in **Table 1**. It is evident that, there was a significant ($P < 0.05$) alterations in the hematological parameters of tumor bearing mice. A general decrease was noticed in Hb, RBCs, PCV, lymphocytes and monocytes, accompanied by an increase in WBCs, especially neutrophils (granulocyte). At the same time interval, IE, CE and 5-Fu treatments changed the altered parameters significantly ($P < 0.05$) to near normal (**Table 1**).

**Effect of ink extract (IE), clam extract (CE) and 5-fluorouracil (5-Fu) on biochemical parameters**

Serum activities of AST, ALT and ALP of EAC bearing mice were significantly increased ($P < 0.05$), as compared to control mice. Interestingly, post treatment with IE, CE and 5-Fu at the selected doses was found ameliorating the alterations in the previous enzymes towards the control values (**Table 2**).

**Effect of ink extract (IE), clam extract (CE) and 5-fluorouracil (5-Fu) on liver oxidative stress markers**

The levels of lipid peroxidation which measured by the formation of malondialdehyde (MDA) in liver tissues were significantly ($P < 0.05$) increased in EAC group, as compared to control mice (**Table 3**). On the other hand, inoculation with EAC caused significant decrease ($P < 0.05$) in the levels of NO, SOD and GSH of mice, as compared to the corresponding ones of control mice. However, administration of IE, CE and 5-Fu to the EAC bearing mice decreased MDA levels, and increased levels of NO, SOD and
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**Table 1.** Effect of *Sepia officinalis* ink extract (IE) and *Coelatura aegyptiaca* extract (CE) on the hematological parameters of EAC bearing mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Saline control</th>
<th>EAC control (5 × 10⁶ cells/mouse)</th>
<th>IE (200 mg/kg) + EAC</th>
<th>CE (200 mg/kg) + EAC</th>
<th>5-Fu (20 mg/kg) + EAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/L)</td>
<td>143.50 ± 10.77</td>
<td>99.66 ± 4.30a</td>
<td>104.00 ± 4.50</td>
<td>111.00 ± 3.90b</td>
<td>119.00 ± 2.01b</td>
</tr>
<tr>
<td>RBCs x 10¹²/L</td>
<td>7.81 ± 0.19</td>
<td>4.59 ± 0.34a</td>
<td>7.12 ± 0.28b</td>
<td>5.88 ± 0.16b</td>
<td>7.19 ± 0.16b</td>
</tr>
<tr>
<td>PCV %</td>
<td>29.28 ± 1.77</td>
<td>24.63 ± 0.89a</td>
<td>29.64 ± 0.96b</td>
<td>26.11 ± 0.70b</td>
<td>26.47 ± 0.57b</td>
</tr>
<tr>
<td>WBCs x 10⁹/L</td>
<td>3.40 ± 0.07</td>
<td>6.47 ± 0.09b</td>
<td>2.86 ± 0.47b</td>
<td>3.06 ± 0.39b</td>
<td>1.87 ± 0.25b</td>
</tr>
<tr>
<td>Lymphocyte %</td>
<td>90.20 ± 1.70</td>
<td>73.38 ± 1.60a</td>
<td>81.03 ± 3.60b</td>
<td>88.24 ± 0.91b</td>
<td>74.93 ± 1.03</td>
</tr>
<tr>
<td>Monocyte %</td>
<td>6.40 ± 0.38</td>
<td>5.60 ± 0.19</td>
<td>6.16 ± 0.62</td>
<td>6.31 ± 0.51</td>
<td>5.96 ± 0.15</td>
</tr>
<tr>
<td>Neutrophils %</td>
<td>5.49 ± 0.63</td>
<td>15.30 ± 0.70a</td>
<td>8.86 ± 1.73b</td>
<td>5.45 ± 0.39b</td>
<td>19.08 ± 0.89b</td>
</tr>
</tbody>
</table>

All values are means ± SE (n = 6). a: significant (P < 0.05) as compared with saline control group. b: significant (P < 0.05) as compared with EAC group.

**Table 2.** Effect of *Sepia officinalis* ink extract (IE) and *Coelatura aegyptiaca* extract (CE) on aspartate (AST), alanine (ALT) aminotransaminases, and alkaline phosphatase (ALP) activities of EAC bearing mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Saline control</th>
<th>EAC control (5 × 10⁶ cells/mouse)</th>
<th>IE (200 mg/kg) + EAC</th>
<th>CE (200 mg/kg) + EAC</th>
<th>5-Fu (20 mg/kg) + EAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/ml)</td>
<td>57.34 ± 2.41</td>
<td>84.60 ± 1.66a</td>
<td>71.95 ± 3.77b</td>
<td>69.79 ± 0.91b</td>
<td>59.39 ± 3.97b</td>
</tr>
<tr>
<td>ALT (U/ml)</td>
<td>42.52 ± 1.73</td>
<td>59.84 ± 1.96a</td>
<td>45.58 ± 2.25b</td>
<td>46.24 ± 2.15b</td>
<td>44.90 ± 1.56b</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>2.45 ± 0.13</td>
<td>6.20 ± 0.49a</td>
<td>2.26 ± 0.12b</td>
<td>2.11 ± 0.11b</td>
<td>2.39 ± 0.28b</td>
</tr>
</tbody>
</table>

All values are means ± SE (n = 6). a: significant (P < 0.05) as compared with saline control group. b: significant (P < 0.05) as compared with EAC group.

**Table 3.** Effect of *Sepia officinalis* ink extract (IE) and *Coelatura aegyptiaca* extract (CE) on the levels of malondialdehyde (MDA), nitric oxide (NO), glutathione reduced (GSH) and superoxide dismutase (SOD) activity of EAC bearing mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Saline control</th>
<th>EAC control (5 × 10⁶ cells/mouse)</th>
<th>IE (200 mg/kg) + EAC</th>
<th>CE (200 mg/kg) + EAC</th>
<th>5-Fu (20 mg/kg) + EAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmole/mg protein)</td>
<td>0.12 ± 0.01</td>
<td>0.20 ± 0.012a</td>
<td>0.15 ± 0.01</td>
<td>0.13 ± 0.01b</td>
<td>0.12 ± 0.01b</td>
</tr>
<tr>
<td>NO (µmole/mg protein)</td>
<td>0.21 ± 0.02</td>
<td>0.10 ± 0.03a</td>
<td>0.14 ± 0.01b</td>
<td>0.18 ± 0.01b</td>
<td>0.14 ± 0.01b</td>
</tr>
<tr>
<td>GSH (mg/mg protein)</td>
<td>0.05 ± 0.01</td>
<td>0.02 ± 0.01a</td>
<td>0.06 ± 0.01b</td>
<td>0.05 ± 0.01b</td>
<td>0.06 ± 0.01b</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>1.22 ± 0.04</td>
<td>0.63 ± 0.02a</td>
<td>0.94 ± 0.02b</td>
<td>0.99 ± 0.07b</td>
<td>0.93 ± 0.03b</td>
</tr>
</tbody>
</table>

All values are means ± SE (n = 6). a: significant (P < 0.05) as compared with saline control group. b: significant (P < 0.05) as compared with EAC group.

GSH of mice, as compared to EAC mice (Table 3).

**Effect of ink extract (IE), clam extract (CE) and 5-fluorouracil (5-Fu) on liver histopathological examinations**

**Figure 6A** showed the normal histological structure of the central vein and the surrounding hepatocytes of normal mice liver sections. However, the liver sections of EAC bearing mice have inflammatory cells infiltration in the portal area with dilatation and congestion in the portal vein and hepatocytes degeneration (Figure 6B). In addition, focal necrosis with inflammatory cells infiltration were detected in the hepatic parenchyma (Figure 6C). On the other hand, treatment with IE, CE and 5-Fu to the EAC bearing mice decrease to some extent the histopathological alterations caused by EAC (Figure 6D-F).

**Discussion**

The Ehrlich tumor was initially described as a spontaneous murine mammary adenocarcinoma. It is a rapidly growing carcinoma with very aggressive behavior and is able to grow in...
Figure 6. Haematoxylin and eosin stained liver sections from normal mice liver section (A) showing the normal histological structure of the central vein (CV) and the surrounding hepatocytes (H). Liver sections of EAC bearing mice (B, C) have inflammatory cells infiltration (m) in the portal area with dilatation and congestion in the portal vein and hepatocytes degeneration (D). Liver sections of treatment with IE, CE and 5-Fu to the EAC bearing mice (D-F).

almost all strains of mice [37]. In ascitic form it has been used as a transplantable tumor model to investigate the antitumor effect of several substances [37]. The search for selective and less toxic molecules for cancer treatment is an ongoing process. Rapid scientific advances in recent years have enhanced our understanding of the biology of cancer. Consequently, several novel targets have been identified [3]. The main advantage of using natural or dietary compounds as anti-cancer remedy is that they seem to have low toxicity and show very few adverse side effects [38]. Marine organisms are rich source for natural products. Many compounds that are derived from these organisms have generated interest both as
The major problems encountered in cancer were diagnostic, especially for malignant tumors. The potential diagnosis, prognosis, monitoring the change in hematological parameters. The positive effect of the compound on EAC-tumor cell growth. The potency of any anticancer agent has been judged by measuring i) reduction in viable tumor cell count, ii) reduction in ascites tumor volume and iii) increase of mean survival time of the EAC-bearing mice. Treatment with cuttlefish (Sepia officinalis) ink extract (IE), Coelatura aegyptiaca extract (CE) and the standard drug 5-fluorouracil (5-Fu) reduced ascites tumor volume, packed cell volume of the ascites fluid, viable tumor cell count and brought about a marked increase in mean survival time and the lifespan percentage of tumor-bearing mice. Hertog et al. [41] has suggested that an increase in the lifespan of ascites bearing animals by 25% can be considered as indication of significant anticancer activity of the drug. This observation suggests the effectiveness of IE and CE extracts against EAC cells in mice as it increased the lifespan by almost 46.15 and 59.49%, respectively. Inflammation is increasingly recognized as an essential component of tumor development [42]. Viewed in conjunction with the report of El-Mowafy et al. [43], our previous research has clearly demonstrated that, the potent anti-inflammatory activities of both IE and CE extracts which anticipated to exert cytotoxic effects against HepG2 cell lines in vitro [12]. A reduction in the number of ascitic Ehrlich tumor cells may indicate either an effect of the IE and CE extracts on peritoneal macrophages or other components of the immune system [44], therefore increasing their capacity of killing the tumor cells, or a direct effect on tumor cell growth.

The anti-inflammatory and antineoplastic activities of IE and CE extracts which anticipated to exert cytotoxic effects against HepG2 cell lines in vitro [12]. A reduction in the number of ascitic Ehrlich tumor cells may indicate either an effect of the IE and CE extracts on peritoneal macrophages or other components of the immune system [44], therefore increasing their capacity of killing the tumor cells, or a direct effect on tumor cell growth.

The positive effect of the compound on EAC-bearing mice has further been verified by monitoring the change in hematological parameters. The major problems encountered in cancer chemotherapy are myeloid-suppressor and anemia due to reduction in RBC or Hb content [45]. In the present study EAC bearing mice have decreased Hb, RBC and PCV which may be due to the suppressive effect of EAC on bone marrow erythropoiesis [46]. This is probably due to the deficiency of iron of haemolytic or myelopathic condition [47]. Treatment with IE, CE, and 5-Fu resulted in appreciable improvements Hb content, RBC and PCV more or less to normal levels. These observations assume great significance as anemia is a common complication in cancer and the situation aggravates further during chemotherapy since a majority of antineoplastic agents exert suppressive effects on erythropoiesis [45, 48] and thereby limiting the use of these drugs. The reversal of Hb content, RBC and PCV towards the normal values clearly indicates that both IE and CE extracts possessed protective action on the haemopoietic system. It was reported that, induction of Hb by the tested compounds as observed herein, could have a broad implication with respect to the antitumor efficacy of the extract if one considers the fact that high Hb level has been found to possess an inhibitory influence on tumor growth [49, 50]. Furthermore, induction of Hb following IE and CE extracts treatment may be due to enhancement of iron level which may be improving hemopoietic function in EAC mice. In consonance with the present findings, Wang et al. [51] showed that squid ink melanin is an effective source of iron supplement for treatment of iron deficiency anemia (IDA) rats and might be exploited as a new iron fortifier.

On the other hand, the granulocytic leucocytosis that was observed in EAC mice may be due to the acute inflammatory response or stress as a result of the proliferation of Ehrlich cells [52]. Interestingly, IE, CE and 5-Fu administration realized an effective role in treatment of EAC tumor which was manifested in restoring the WBC count towards normal level. One of the major criteria for judging clinically effective antineoplastic agents is that it should be able to prolong the survival and decrease the leukocyte count of blood of tumor bearing animals [53].

Biochemical marker enzymes are used to screen particularly cancer conditions for differential diagnosis, prognosis, monitoring the progress and for assessing the response to.
Anti-Neoplastic activities of IE and CE

therapy [54]. These enzymes are more unique and their activity changes reflect the effect of proliferation of cells with growth potential and its metabolic turnover. The rise in their activities is shown to be a good correlation with the number of transformed cells in cancer conditions [55]. Furthermore, Abu-sinna et al. [56] suggest that, the consumption of free amino acids in building the protein of rapidly dividing tumor cells might result in the disturbance of the enzyme activity in the liver. Borentian et al. [57] have reported that the presence of adenocarcinoma in liver increases the activities of AST and ALP in circulation and the subsequent surgical removal of carcinoma resulted in a drastic decrease of these enzyme activities to near normal. In this study, increase in pathophysiological marker enzyme levels (AST, ALT and ALP) upon EAC induction might due to disturbance in the transport function and the leakage of the enzyme. This is indicative of the onset of hepatocellular damage due to liver dysfunction and disturbance of the biosynthesis of these enzymes, with alteration in the permeability of liver membrane. Treatment with IE and CE extracts significantly decreased the levels of serum AST, ALT and ALP activities in EAC-treated mice indicating maintenance of functional integrity of hepatic cell membrane. The mentioned results were further supported by the histopathological examination of mice bearing EAC and/or various treatment groups. There was a diminishing in pathological structure, to a great degree, towards normal intact histological structure. The findings of Soliman [11] indicated that administration of CE decreased lipid peroxidation, improved antioxidant status and thereby prevent the damaging to the liver and leakage of its enzymes (AST and ALT).

Oxidative stress act as a pivotal trigger of cancer initiation/progression [58]. The direct and indirect roles of reactive oxygen species (ROS) in the process of tumor genesis have been increasingly characterized to introduce new dogmas and concepts. Accordingly, the old view that ROS can merely cause cellular damage has now been revolutionized. Thus, it has become evident that, in response to oxidative stress, a few cells may escape damage/apoptosis to emerge as neoplasms with oncogenic mutations that ultimately serve as immortalized cancer cells [59]. Cancer chemotherapy using antioxidant formulations is an exciting pharmaceutical research involving the use of either natural or synthetic components to delay inhibit or reverse the development of cancer in normal or preneoplastic conditions [60]. Under conditions of excessive oxidative stress, however antioxidants are depleted and ROS can damage cellular components and interfere with critical cellular activity [61].

Lipid peroxidation (LPO), an autocatalytic free radical chain propagating reaction, is known to be associated with pathological conditions of a cell [62]. Increased lipid peroxidation would cause degeneration of tissues. Lipid peroxide formed in the primary site would be transferred through the circulation and provoke damage by propagating the process of lipid peroxidation [63]. It was also reported that the presence of tumors in the human body or in experimental animals was known to affect many functions of the vital organs, especially in the liver, even when the site of the tumor does not interfere directly with organ function [64]. Malondialdehyde (MDA), the end product of lipid peroxidation was reported to be higher in carcinomatous tissue than in non-diseased organs [65]. Glutathione reduced (GSH), an important non-protein thiol, plays a significant role in protecting cells from neoplastic process. In addition, GSH plays a role as an endogenous antioxidant molecule that is found particularly in high concentration in liver and is known to have key function in the protective process [63]. The present study confirmed the finding of Sreelatha et al. [66] and Tohamy et al. [67] who suggested that enhancement of lipid peroxidation in EAC-bearing mice is a consequence of depletion of GSH to certain critical levels.

Superoxide dismutase (SOD) are a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide [68]. The amount of SOD is organ specific and it is abundant in hepatic tissue [69]. Sun et al. [70] reported a decrease in SOD activity in EAC bearing mice which might be due to loss of Mn SOD activity in EAC cells and the loss of mitochondria, leading to a decrease in total SOD activity in the liver. The inhibition of SOD activity as a result of tumor growth was also reported [71]. Similar findings were observed in the present investigation with EAC bearing mice. Nitric oxide (NO) is a molecule that easily passes through the cell membrane, and it is synthesized from the amino acid L-arginine in many cells of the body.
Nitric oxide synthase (NOS) is involved in the formation of NO, and it is a deoxygenase that is dependent on NADPH [72]. The discovery of the generation of NO by mammalian tissues and the elucidation of some of its biological roles in cancer has thrown new light onto many areas of tumor biology research. Although initial findings suggested that the immune-cell generated NO is cytostatic or cytotoxic for tumor cells, later findings have shown that NO can also possess apparently contradictory activity leading to increased tumor growth. The present study confirmed the finding of Nishikawa et al. [73], who reported that rate of NO generation, was low one week after inoculation. Furthermore, in accord with our results, Xu et al. [74], have reported that at (relatively) low concentrations of NO, (for example, at concentrations measurable in many different types of clinical cancer samples), tumor growth and proliferation is promoted.

Over two-third of cancer relation death could be prevented through the life-style modification and minimize cancer risk through antioxidant input [75]. The lowering of lipid peroxidation (MDA) and increase in levels of GSH, SOD and NO in the IE, CE and 5-Fu groups in the present work indicate their potential as an inhibitor of EAC induced intracellular oxidative stress and suggesting that their hepatoprotection mechanism may be due to their antioxidant effect [12]. Background researches showed that melanin of squid ink, like superoxide dismutase, can catalyze $O_2^-$ to $H_2O_2$, and thus avoid the free radical chain reaction triggered by $O_2^-$. [76]. Zhang et al. [77] reported that squid ink elevated SOD activity in the liver and kidney of mice in a dose-dependent manner. Squid ink is a mixture containing melanin, protein, carbohydrate and lipid [19]. Melanin are efficient free radical scavengers and antioxidants [78]. Taurine is a sulfur containing amino acid which has been previously found to exhibit antioxidant properties [79, 80]. Viewed in conjunction with the report of Derby et al. [81] and Soliman [11], both squid ink extract (IE) and freshwater mussel extract of C. aegyptiaca (CE), contain considerable amounts of taurine. Moreover, Soliman [11] have shown the presence of high levels of precursor amino acids of GSH (glycine, glutamine and cysteine) in the freshwater mussel extract of C. aegyptiaca.

In conclusion, the present study serve to extend the growing number of earlier investigations on therapeutic products from freshwater and marine sources as potent antineoplastic agents and confirm that both squid ink extract and freshwater mussel extract of C. aegyptiaca decreased lipid peroxidation, improved antioxidant status, and thereby act as a potential therapeutic complement in the treatment of different pathologies that may be related to an imbalance of the cellular oxidoreductive status associated with liver injury following tumor inoculation.

Disclosure of conflict of interest

None.

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Antioxidant properties of Rajgira (Amaranthuspaniculatus)


