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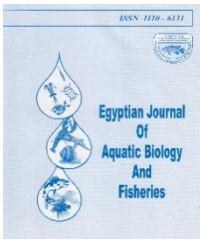

Antiproliferative and antioxidant activities of the edible crab *Callinectes sapidus* hepatopancreas and hemolymph extracts

Somaia M. Zakzok [1], Rabab M. Alkaradawe [2], Samya H. Mohammad [1], Mohamed M. Tawfik [1*]

[1]. Zoology Departement, Faculty of Science, Port Said University, Egypt.
[2]. Zoology Departement, Faculty of Science, El-Arish University, Egypt.
[*]**Corresponding Author: Tawfik@sci.psu.edu.eg**

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ABSTRACT

Crabs, decapods crustaceans, have been recognized as a rich natural source of unique antioxidant and therapeutic agents. *Callinectes sapidus* is an edible crab used as a popular food source in coastal cities like Port Said, Egypt. In the present study, *in vitro* cytotoxic and antioxidant properties of the hepatopancreas and hemolymph of *C. sapidus* were investigated on some human cell lines (HeLa, HepG 2, MCF-7, MDA-MB-231 and WI-38). The MTT results showed that both extracts possess selective cytotoxicity against HepG2, MCF-7 and HeLa. No cytotoxicity was observed on normal lung cells (WI-38). The hepatopancreas and hemolymph showed the highest antiproliferative activity against HeLa cells (IC_{50}: 0.39 and 2.01 mg/ml respectively). Flow cytometry revealed that both extracts can induce apoptosis in HeLa cells and cause cell cycle arrest in the G2/M phase after 48 h treatment. Hepatopancreas showed greater DPPH radical-scavenging activity with IC_{50} value of 38.99 µg/ml than hemolymph (IC_{50} value of 464 µg/ml). Both extracts at 2 mg/ml were effective to attenuate the perturbations in the tested parameters (superoxide dismutase, catalase, reduced glutathione and malondialdehyde) of H_2O_2-induced oxidative stress in WI-38 cells. Findings suggest that these blue crab extracts have protective effects on H_2O_2-induced lipid peroxidation and might be used for the development of new drugs against human cervical adenocarcinoma.

INTRODUCTION

A remarkable number of novel bioactive agents have been isolated from marine invertebrates such as sponges, molluscs, cnidarians and crustaceans due to the unique biological and chemical diversity of the marine environment **(Abou El-Ezz *et al.*, 2017; Blunt *et al.*, 2018)**. Among crustaceans, crabs represent a potential source of various natural antioxidants and anticancer molecules **(Datta *et al.*, 2016; Katran *et al.*, 2019)**. Crabs are continuously exposed to several stress factors, such as microbes, pathogens and changes in environmental and physiological conditions, leading to enhance oxidative

stress. Moreover, like invertebrates, crabs lack adaptive immune system. Therefore they have been assumed to exhibit a rich variation of effective innate immune responses and have developed antioxidant defense systems to protect themselves **(Sánchez-Salgado *et al.*, 2017; Huang *et al*., 2020).**

The hepatopancreas and hemolymph play crucial defensive and immunological roles in crabs **(Rőszer, 2014)**. Hepatopancreas is responsible for the elimination and detoxification of xenobiotics **(Ortega *et al.*, 2011)**. This digestive gland is the primary site for the synthesis and production of some immune response molecules such as lectins, hemocyanin, serine protease inhibitors, stress proteins, antimicrobial and apoptotic peptides **(Shi *et al.*, 2010; Li *et al.*, 2013; Wei et al., 2020)**. Similarly, hemolymph is an important defense line in crabs. It includes hemocytes, the main immune cells, which perform various immunological functions such as phagocytosis, encapsulation, lysis of foreign bodies and release of humoural proteins **(Parrinello *et al.*, 2015; Wu *et al.*, 2019)**. The hemolymph of crabs exhibits potent anticancer activities that have been investigated in *Atergatis roseus* and *Eriphia verrucosa* **(Salama and Mona, 2018)**, *Dromia dehanni* **(Priya and Ravichandr, 2015a; RethnaPriya *et al.*, 2019)** and *Calappa calappa* **(Priya and Ravichandr, 2015b)**.

The blue crab *Callinectes sapidus* (Crustacea: Decapoda: Portunidae) is widely distributed along the Mediterranean coast of Port Said, Egypt. This edible crab is one of the most valuable sources of nutrition for human consumption since it is enriched with proteins, minerals, vitamins and omega-3 fatty acids **(Küçükgülmez *et al.*, 2006)**. However, there is little attention that has been given to investigating or evaluating the biological activities of these blue crab extracts.

The current study was carried out to assess the potential antiproliferative activities of hepatopancreas and hemolymph extracts of *C. sapidus* against some human cell lines and investigate the cytoprotective properties of both extracts on H_2O_2-treated lung cells *in vitro*.

MATERIALS AND METHODS

Sample collection

Live blue crabs were collected in 2018 from the Mediterranean Sea in Port Said, Egypt. Samples were transported in tanks to the laboratory of the Zoology department, Faculty of Science, Port Said University. The crabs were cryoanesthetized on ice for 15 min.

Hemolymph and Hepatopancreas collection

Hemolymph was withdrawn from the base of the swimming legs of the blue crabs using fine sterile syringes. Then, hemolymph was mixed with sodium citrate buffer, pH 4.6 (3:1 v/v) as an anticoagulant to prevent hemocytes degranulation and coagulation

(Sivaperumal *et al.*, 2013). After that, the crabs were dissected in order to separate and collect the hepatopancreas. The hepatopancreas was then mixed with 50 mM Tris/HCl at pH 8.0 (4 mL/g), and homogenized on ice **(Asaro *et al.*, 2011)**. Both hepatopancreas homogenate and hemolymph were centrifuged at $1x10^4$ rpm at 4 °C, for 15 min. After centrifugation, the supernatant was collected, stored at -20 °C then lyophilized.

Protein estimation

After lyophilization, crude extracts were weighed and dissolved in phosphate buffer saline (PBS). The total protein concentration of both extracts was determined using the method of **Lowry *et al.* (1951)**. Bovine serum albumin (BSA) was used as a standard. The extracts were used in experiments based on their protein concentrations.

Cells and cell cultures

Human cervical carcinoma (HeLa), human hepatocellular carcinoma (HepG2), human breast cancer cell lines (MCF-7 and MDA-MB-231) and normal human lung cells (WI-38) were obtained from the Holding company for biological products and vaccines (VACSERA), Giza, Egypt. Cells were propagated in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% antibiotic (penicillin), then incubated in 5% CO_2 at 37°C for growth. After that, the cells were seeded on 96 well plates at a concentration of $1x10^4$ cells/well (100 µl/well) for 24 h. After seeding, cells were treated with serial concentrations of hepatopancreas and hemolymph extracts (from 0.125 to 8 mg/ml) and incubated at 37 °C and 5% CO_2 for 48 h.

Cell proliferation by MTT assay

The antiproliferative effects of hepatopancreas and hemolymph were evaluated using the 3-[4,5-methylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay **(Mosmann, 1983)**. Briefly, after treatment with both extracts for 48 h, fresh medium containing MTT dye was added to cells (10 µl/well) and incubated at 37°C for 4 h to allow the formation of formazan crystals in viable cells only. In order to solubilize these crystals, 100 µl of Dimethyl sulfoxide (DMSO) was added to each well, then the plates were centrifuged for 5 minutes at 4000 rpm. The absorbance was measured at 560 nm using a Bio-Tek microplate reader ELISA. The experiment was performed in triplicates. The percentage of cell viability was calculated according to the following formula:

$$\text{Cell viability (\%)} = (A_T / A_C) \times 100.$$

Where A_T denotes the absorbance of treated cells and A_C denotes the absorbance of the control (untreated cells).

The IC_{50} (the concentration that inhibits the growth of 50% of cells) values of each sample were determined from a plot of dose-response curve between dose concentration on X-axis and cell inhibition percentage on the Y-axis.

Analysis of Cellular DNA Content Using Propidium Iodide

Flow cytometry is used to analyze cell cycle progression by measuring the percentage of cellular DNA content **(Pozarowski and Darzynkiewicz, 2004)**. HeLa cells were seeded at a density of 1×10^4 into a 6-well plate and incubated with IC_{50} concentrations of each extract at 37 °C in 5% CO_2 atmosphere for 48 h. Cells without any treatment were used as a control. Then, cells were resuspended in PBS (500 μl) prior to being fixed at 70% cold ethanol for at least 2h at –20°C. The fixed cells were washed twice with PBS, then treated with 500 μl PI/RNase (4:1 propidium iodide/Ribonuclease A) and incubated for 30 min in the dark at room temperature. The cellular DNA content was measured using a flow cytometer (FACSCalibur, Becton Dickinson, USA).

Annexin V/PI double staining assay

$1x10^4$ HeLa cells were seeded into a 6-well plate and treated with an IC_{50} concentration of hepatopancreas and hemolymph extracts. After 48 h of incubation, the cells were centrifuged (1000 rpm for 5 min), resuspended in 500 ml 1X binding buffer and stained with 5 μl of PI and 5μl of Annexin-V-FITC (Annexin V-FITC Apoptosis Detection Kit). Finally, the cells were incubated in the dark for 5 min at room temperature. The cells were analyzed and the percentage of total apoptotic and necrotic cells was calculated using a BD FACSCalibur flow cytometer.

DPPH radical scavenging assay

Free radical scavenging activities of both extracts were determined by the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay as described by **Brand-Williams *et al.* (1995)**. In brief, 1 ml of DPPH methanolic solution (0.1 mM, in 95% methanol) was mixed with 3 ml of varying concentrations of each extracts or standard (3.9 – 1000 µg/ml) and incubated for 30 min at room temperature. Ascorbic acid (Vitamin C) was used as a standard (positive control), and absorbance at 517 nm was measured with a spectrophotometer (UV-VIS Milton Roy). The DPPH scavenging effect (%) was calculated according to the following formula:

DPPH scavenging effect (%) = $[(A_B - A_S) / A_B] \times 100$.

Where A_B was the Absorbance of blank and A_S was the Absorbance in presence of samples or standard.

The IC_{50} values (the concentration of extracts with the ability to decrease 50% of the initial DPPH concentration) were determined using the Log dose inhibition curve.

Effect of hepatopancreas and hemolymph extracts on H_2O_2 -induced oxidative stress in WI-38 cells

Normal human lung WI-38 cells were seeded in culture plates at a density of $1x10^6$ cells/ml. After 24 h, the cells were washed twice and divided into 6 groups. The control group was represented by cells without any treatment. Hepatopancreas and hemolymph extracts (2 mg/ml in PBS) were added to some cells (2^{nd} and 3^{rd} groups).

Oxidative stress was induced by treating the cells with freshly prepared hydrogen peroxide (H_2O_2) with a final concentration of 500 μM in a FBS-free DMEM medium (4^{th} group). The other cells were subjected to equal volumes of each extracts and H_2O_2 (5^{th} and 6^{th} groups). All cells were incubated for 24 h at 37 °C in a 5% CO_2 atmosphere. After incubation, the activities of superoxide dismutase (SOD), catalase (CAT), the content of reduced glutathione (GSH) and malondialdehyde (MDA) were measured by commercial colorimetric assay kits obtained from Bio-diagnostic Co., Giza, Egypt.

Hemolytic activity assay

Hemolytic activities of both extracts were assayed on human erythrocytes as described by **Malagoli (2007)**. Fresh blood samples collected in tubes containing anticoagulant as EDTA (3:1 v/v) were centrifuged at 2000 rpm for 5 min. Briefly, a suspension of red blood cells in sterile PBS was incubated with various serial concentrations of each extract (0.25 - 8 mg/ml) for 1 h at room temperature. After incubation, the cells were centrifuged at $1x10^4$ rpm for 5 min and the supernatant was used to measure the absorbance of the liberated hemoglobin at 570 nm. Sterile phosphate buffer saline and 10% Triton X-100 were used as a negative control (0% hemolysis) and positive control (100% hemolysis), respectively. The experiment was conducted in triplicate. The hemolysis percentage for each sample was calculated by the following equation:

$$\text{Hemolysis (\%)} = 100 \times [(A_S - A_{N.C}) / (A_{P.C} - A_{N.C}].$$

Where A_S is the sample absorbance, $A_{N.C}$ is the negative control absorbance, and $A_{P.C}$ is the positive control absorbance.

SDS-polyacrylamide gel electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is used to separate proteins of both samples according to their molecular weights. According to **Laemmli (1970)**, SDS-PAGE was performed at a total acrylamide concentration of 12%. In the presence of the sample buffer (70 mM Tris–HCl, pH 6.8, 11.4% glycerol, 3% SDS, 0.01% bromophenol blue, and 5% b-mercaptoethanol), the samples were boiled at 95° for 5 min. Protein samples were immediately loaded into wells, and electrophoresis was carried out at 200 V for 35 – 40 min. After separation, the gel was stained by Coomassie Brilliant Blue (CBB G-250) in order to visualize protein bands.

Statistical analysis

Statistical analysis was carried out using SPSS 22.0. The data was expressed as a mean value ± SE and data was analyzed using a Student's t-test and a One-way ANOVA followed by the Tukey's test. When the p-value was 0.05, a statistically significant difference in meaning was reported.

RESULTS

Cell proliferation by MTT assay

The viability of four human cancer cell lines (HeLa, MCF-7, MDA-MB-231 and HepG2) was inhibited in a dose-dependent manner after being treated with hepatopancreas and hemolymph extracts of the blue crab *C. sapidus* for 48 h (Figure 1). Hepatopancreas possessed more cytotoxic activity than hemolymph against all tested cancer cells. Growth of more than 90% of HeLa, MCF-7 and HepG2 cells were inhibited after being treated with hepatopancreas at 8 mg/ml while hemolymph inhibited only about 75% of the growth of the same cells at the same concentration. Low or no cytotoxicity was detected on normal lung cells (WI-38) after treatment with both extracts. It is clear that HeLa cells were the most susceptible cancer cell line to both extracts. Therefore, cell cycle arrest and apoptotic analysis were carried out on these cells only.

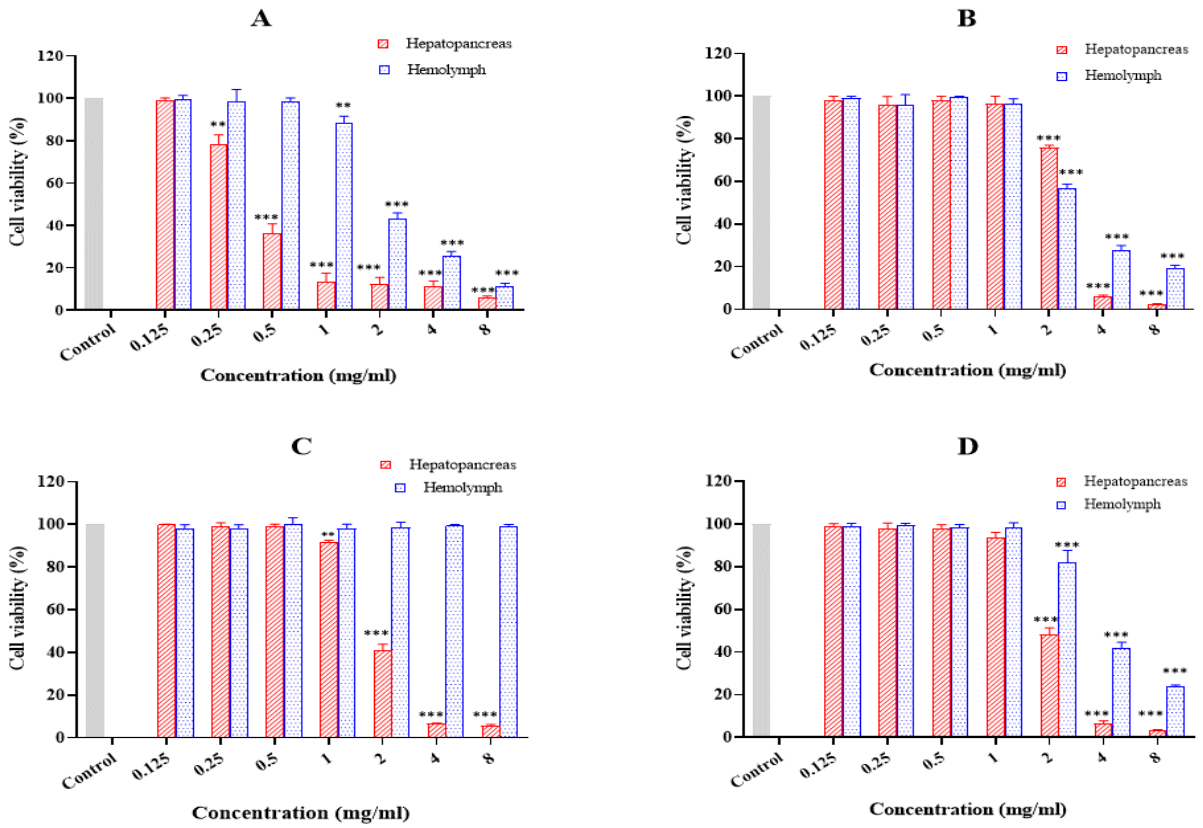

Figure 1. Effects of hepatopancreas and hemolymph extracts of *C. sapidus* on cell proliferation of different human cell lines at different concentrations. A: Cervical (HeLa), B: breast (MCF-7), C: breast (MDA-MB-231) and D: liver (HepG2) cell lines. Non-treated cells are used as a control. Bars sharing superscript of stars (*) differ significantly as compared against control (** $P < 0.01$, *** $P < 0.001$) using a One-way ANOVA followed by the Tukey's test.

Analysis of Cellular DNA Content Using Propidium Iodide

Hepatopancreas and hemolymph extracts induce cell cycle arrest at the G2/M phase after 48 h treatment of HeLa cells (Figure 2). Both extracts induced a statistically significant increase in the percentage of the cells at the G2/M phase from 8.25% in untreated cells to 31.02% and 28.66% in the treated cells with hepatopancreas and hemolymph, respectively ($P < 0.001$). Also, both extracts significantly increase the pre-G1 population of cells treated with hepatopancreas (18.21%) and hemolymph (12.37%) ($P < 0.001$). While the population of cells at G0-G1 and S phases significantly declined after being treated with both extracts ($P < 0.05$).

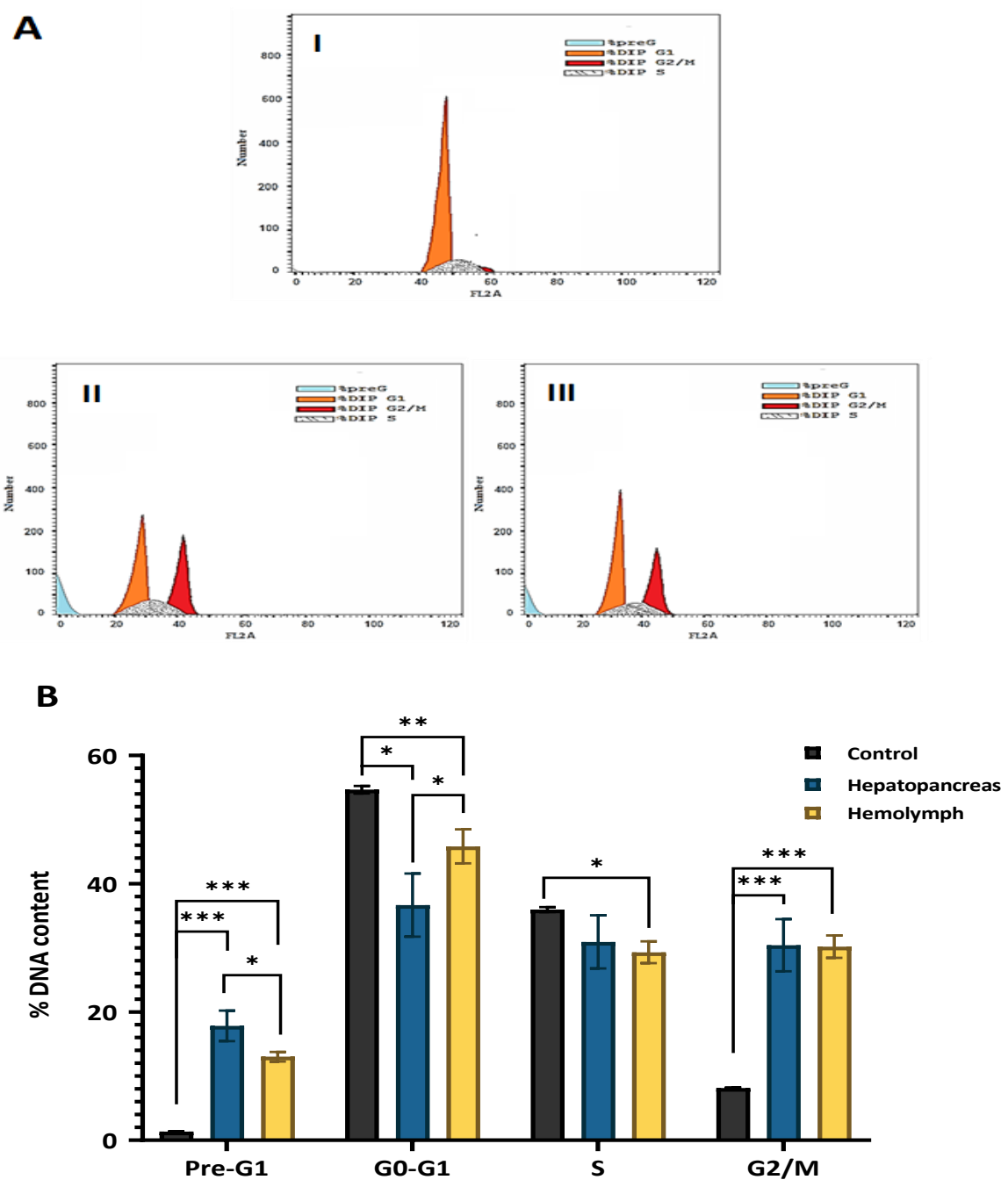

Figure 2. Cell cycle distributions of HeLa cells treated with IC_{50} values of hepatopancreas and hemolymph extracts for 48 h. (A) Representative profiles of cell cycle distribution in HeLa cells before and after treatments: I) Untreated HeLa cells, II) HeLa cells treated with hepatopancreas extract and III) HeLa cells treated with hemolymph extract. (B) The percentages of cell populations in pre-G1, G0-G1, S and G2/M phases. Bars sharing superscript of stars (*) differ significantly (*P < 0.05, ** P < 0.01, *** P < 0.001) using a One-way ANOVA followed by the Tukey's test.

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Annexin V/PI double staining assay

Hepatopancreas and hemolymph extracts induced apoptotic cell death in HeLa cells (Figure 3). After 48 h treatment with IC_{50} of both extracts, a significant increase in early and late apoptotic cells population was observed as compared to control cells ($P < 0.001$). The percentage of total apoptotic cell was significantly increased from 0.75% in non-treated cells to 16.02% and 10.66% in treated cells with hepatopancreas and hemolymph extracts respectively ($P < 0.001$).

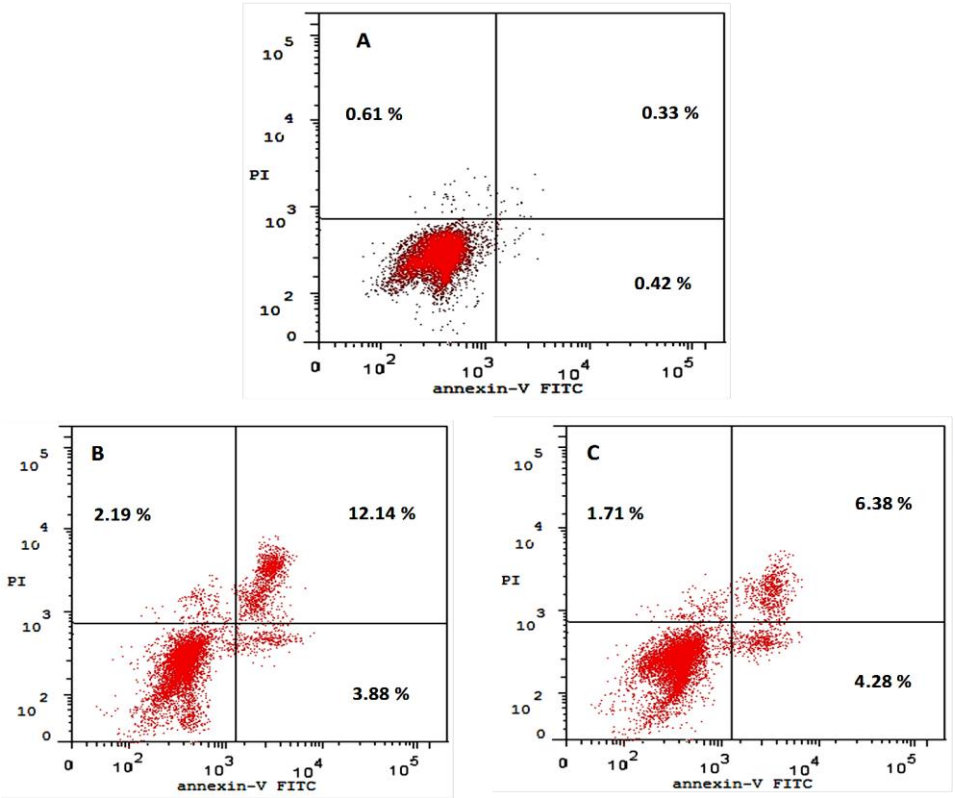

Figure 3. The contour diagram of Annexin V/PI Flow Cytometry shows the apoptotic potential effects of the hepatopancreas and hemolymph of the blue crab, *C. sapidus***.** A) Untreated HeLa cells, B) HeLa cells after 48 h treatment with IC_{50} of hepatopancreas extract and C) HeLa cells after 48 h treatment with IC_{50} of hemolymph extract. Lower left quadrants show viable cells (An -, PI -), whereas the lower right quadrants represent early apoptotic cells (An +, PI -). Necrotic cells (An -, PI +) are found in the upper left quadrants, while late apoptotic cells (An +, PI +) are found in the upper right quadrants.

DPPH radical scavenging assay

Hepatopancreas and hemolymph extracts of *C. sapidus* showed a potent radical scavenging activity in a dose-dependent manner (Figure 4). Overall, hepatopancreas extracts displayed higher antioxidant properties with a significantly lower IC_{50} value of 38.99 µg/ml than that of hemolymph (IC_{50} value= 464 µg/ml; $P < 0.001$). Hepatopancreas extract exhibited similar radical-scavenging activities as those of ascorbic acid, particularly at higher concentrations.

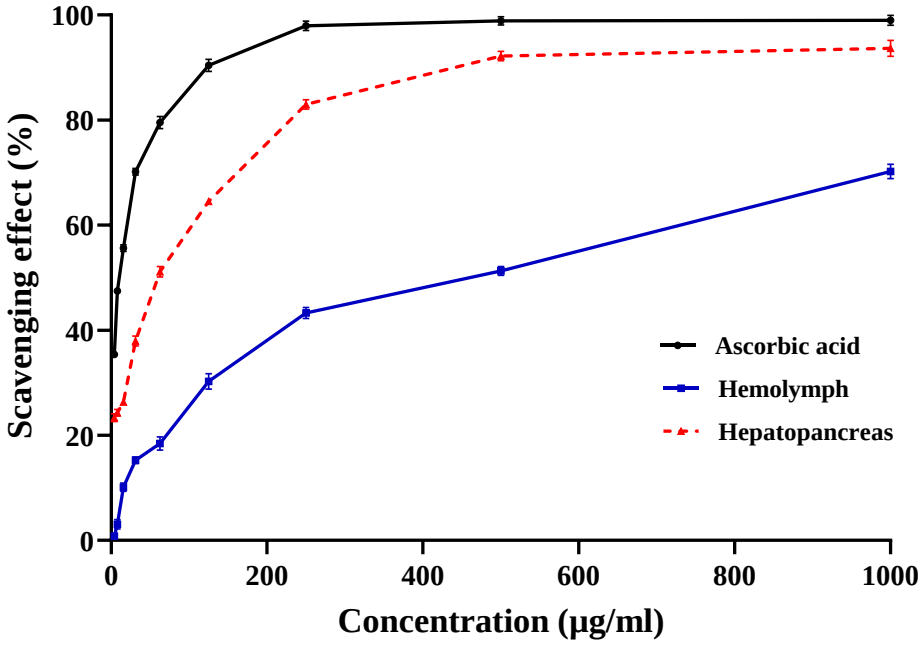

Figure 4. DPPH radical-scavenging activity of the hepatopancreas and hemolymph extracts comparing with the standard (Ascorbic acid).

Antioxidative effect of hepatopancreas and hemolymph extracts on H_2O_2 -induced oxidative stress in WI-38 cells

The activities of antioxidant enzymes (SOD and CAT) in H_2O_2 -treated cells were significantly depleted as compared to control cells (4.38 ± 0.01 to 2.33 ± 0.25 units of SOD/mg protein and 2.31 ± 0.09 to 0.58 ± 0.1 units of CAT/mg protein, $P < 0.001$). Hepatopancreas and hemolymph extracts restored and induced SOD (4.05 ± 0.06 and 3.89 ± 0.23U/mg protein) and CAT (1.69 ± 0.14 and 1.68 ± 0.15 U/mg protein) activities (Figure 5A and B). Figure (5C) shows that both extracts significantly increased levels of GSH in cells from 0.13 ± 0.006 in cells exposed to H_2O_2 to 0.25 ± 0.03 nmol/ml and 0.23 ± 0.02 nmol/ml for hepatopancreas and hemolymph, respectively ($P < 0.05$). A remarkably depletions in oxidative stress biomarker, MDA levels, from 30.43 ± 1.02 in H_2O_2 -treated cells to 16.38 ± 0.98 and 18.03 ± 1.08 nmol/ml were observed in cells treated with hepatopancreas and hemolymph extracts, respectively (Figure 5D).

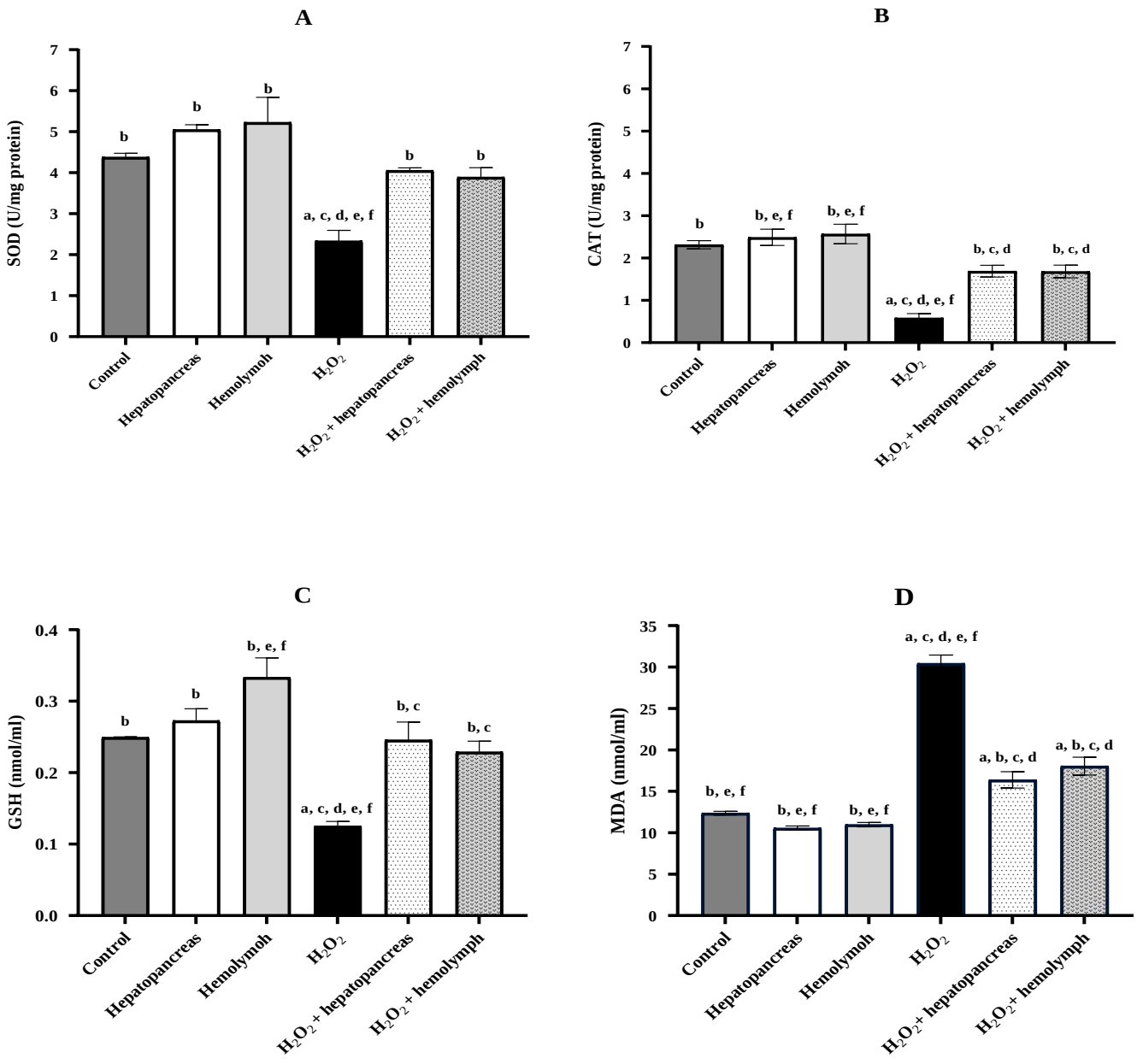

Figure 5. Antioxidant effect of hepatopancreas and hemolymph extracts from *C. sapidus* on normal lung cells (WI-38). Activities of SOD (A), CAT (B) and levels of GSH (C) and MDA (D) were assayed in all cell groups. Bars with different letters (a-f) differ significantly by a $P < 0.05$ (a versus control, b versus H_2O_2, c versus hepatopancreas, d versus hemolymph, e versus H_2O_2 + hepatopancreas and f versus H_2O_2 + hemolymph) using a One-way ANOVA followed by the Tukey's test.

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Hemolytic activity assay

Hemolytic activities of hepatopancreas and hemolymph were evaluated on erythrocytes. Both extracts displayed low or no hemolytic activity on erythrocytes (Figure 6), as compared to negative and positive controls.

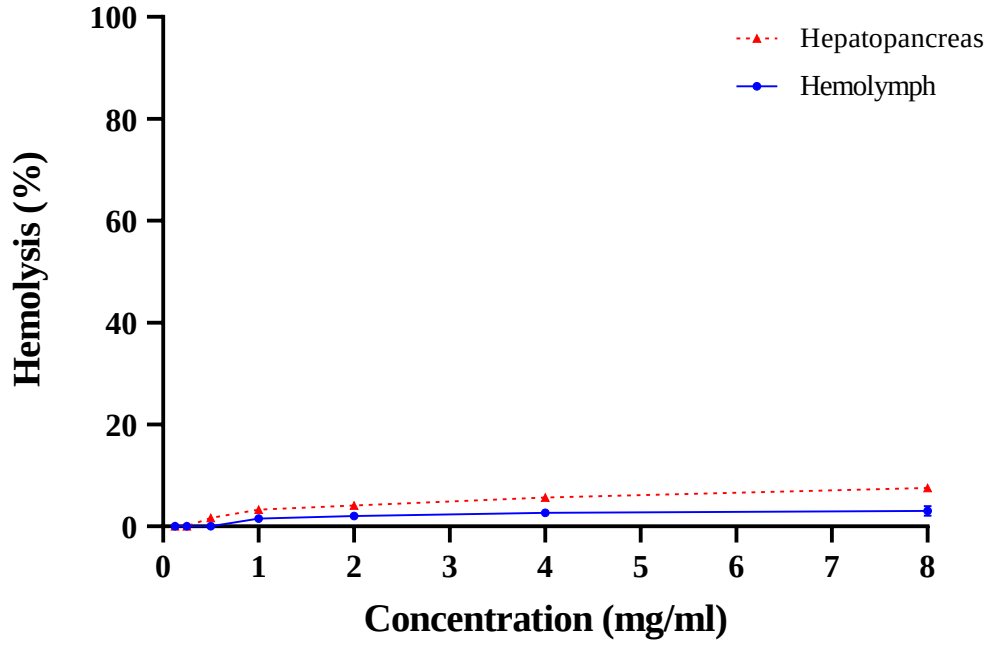

Figure 6. The hemolytic activities of hepatopancreas and hemolymph extracts on erythrocytes.

SDS-polyacrylamide gel electrophoresis

Quantitative analysis of proteins from the hepatopancreas and hemolymph of the blue crab *C. sapidus* was performed using SDS-PAGE (Figure 7). The SDS profile represented protein bands found in both extracts. Hemolymph showed an increased number of bands than hepatopancreas. In hemolymph, the results revealed the presence of proteins in the range of between 10 and ~130 kDa (lane 2). Meanwhile, about eight protein bands with molecular masses of 10, ~ 17, ~ 20, 25, ~ 34, ~ 50, ~ 66 and ~ 90 kDa were detected in the hepatopancreas (lane3). Several bands with molecular weights of 10, 17, 35, 50, 70 and 95 KDa were observed commonly in both samples.

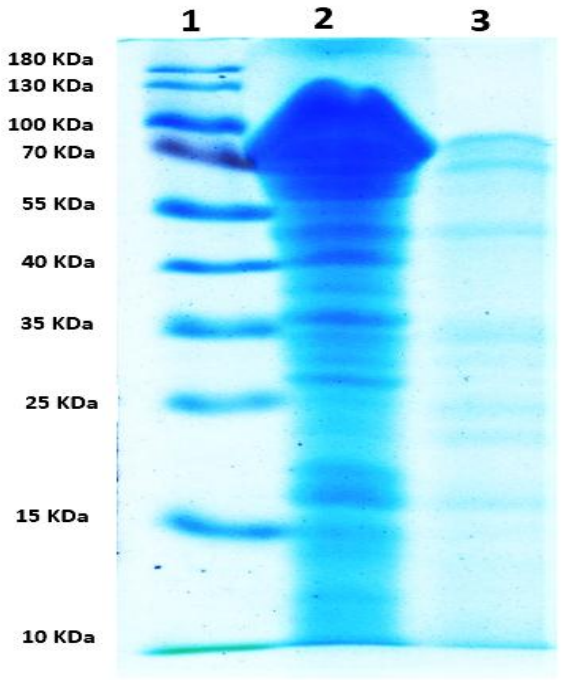

Figure 7. The SDS-PAGE gel shows the protein profile in the hemolymph and hepatopancreas extracts of *C. sapidus.* Lane 1: marker, Lane 2: protein extracts from the hemolymph and Lane 3: protein extracts from the hepatopancreas.

DISCUSSION

Edible crabs are one of the most valuable sources of human nutrition. Among the benefits of these creatures, they possess structurally diverse bioactive components including some anticancer and antioxidant agents **(Jiang *et al.*, 2017; Mohamed *et al.*, 2017; Katran *et al.*, 2019)**. Accordingly, they used as a natural source in pharmaceutical applications.

In the present study, both hepatopancreas and hemolymph extracts of *C. sapidus* showed a relative degree of cytotoxicity against all tested cancer cell lines in a dose-dependent manner with some significant preference for hepatopancreas. This variation in the cytotoxicity between two extracts on the same tested cancer cell lines can be attributed to the significant difference in their antioxidant properties and protein profiles. In most invertebrates, several immune proteins are often synthesized in hepatopancreas then released to the hemolymph upon immune-stimulation **(Rőszer, 2014)**. However, limited studies have examined the cytotoxic properties of hepatopancreas extracted from crabs against cancer cell lines. **Kuznetsov *et al.* (2012)** found that a protease inhibitor (66 KDa) from *Paralithodes camtschatica* slowed down cell spreading and suppressed the growth of HeLa cells. SDS-PAGE gel analysis in the present study revealed the presence of a protein band with a similar molecular weight of PC inhibitor that may interpret the antiproliferative activity of the hepatopancreas extract against HeLa cells.

The anticancer activities of protease inhibitors are related to their ability to inhibit the function of proteases that play an important role in cancer development, progression and metastasis **(Castro Guillén *et al.*, 2010)**.

Similar previous studies have reported the antitumor effects of hemolymph in other crabs like *Atergatis roseus* & *Eriphia verrucosa* **(Salama and Mona, 2018)**, *Scylla serrata* **(Pramanik *et al.*, 2010)** and *Dromia dehaani* **(Priya and Ravichandr, 2015a; RethnaPriya *et al.*, 2019)**. These antiproliferative activities of hemolymph were attributed to glycoproteins such as lectins and hemocyanin with molecular weights of ~75 KDa. Remarkably, a protein band with an apparent molecular weight similar to that of the above glycoproteins was found in the hemolymph extract of *C. sapidus*. Hemocyanin and lectins possess a wide spectrum of biological effects such as antiviral, antiparasitic and antitumor activities **(Zhang *et al.*, 2004; Rizvi *et al.*, 2007; Guo *et al.*, 2011; Cheung *et al.*, 2015)**. Marine lectins can recognize and bind to specific carbohydrate structures or motifs on the cell membranes of cancer cells that lead to growth inhibition and induction of the apoptotic pathway in these cells **(Cheung *et al.*, 2015)**.

The present study showed that both extracts exhibited selective antiproliferative activities against cancer cells only, with no cytotoxicity against erythrocytes and normal lung cells. The selectivity of these molecules may be attributed to the fundamental differences in membrane lipid asymmetry between normal and cancerous cells **(Hoskin and Ramamoorthy, 2008)**. Neutrally charged phospholipids such as phosphatidylcholines and sphingomyelins are located in the outer leaflet of normal cell membranes. While the outer leaflet of cancer cell membranes is characterized by the presence of negative phospholipids such as phosphatidylserines and those facilitate specific recognition of tumor cells by cationic peptides **(Zalba and Ten Hagen, 2017; Bernardes and Fialho, 2018; Wang *et al.*, 2017)**. The hemolymph of crabs is rich in naturally occurring cationic peptides such as callinectin which was previously isolated from the same blue crab *Callinectes sapidus* **(Noga *et al.*, 2011)**. Such peptides exhibit a broad spectrum of cytotoxicity against bacterial and cancer cells with no or limited activity against normal healthy cells **(Fredrick and Ravichandran, 2012; Bernardes and Fialho, 2018; Lei *et al.*, 2019)**. The electrostatic attraction between callinectin and negatively charged phospholipids of cancer cells may participate in the selective antiproliferative activities of both extracts. The selectivity of these molecules may provide useful leads to overcome the cancer cell resistance to chemotherapy and may help to reduce toxicity and risk factors associated with the use of traditional anticancer drugs due to a lack of specificity for cancer cells **(Alfarouk *et al.*, 2015; Oun *et al.*, 2018)**.

Cervical cancer is one of the leading causes of death in women worldwide, with the African continent having the highest incidence and mortality **(Khazaei *et al.*, 2019)**. Based on the findings of flow cytometry analysis, the IC_{50} of both extracts caused G2/M

cell cycle arrest of HeLa cells by significantly increasing the accumulation of cell populations in this phase with a pronounced increase in the pre-G1 populations (dead cells). After 48 h of treatment, Annexin V and PI double staining demonstrated that both extracts induced apoptosis of HeLa cells. Interestingly, the protein electrophoretic profiles of both extracts showed a protein band with a 17 KDa molecular size, similar to the small classes of lectins isolated from marine invertebrates. The antiproliferative activities of these lectins may be related to the loss of membrane integrity and late-stage apoptosis in cancer cells **(Fujii *et al.*, 2012)**.

In the current study, hepatopancreas extract was found to possess higher antioxidant activity than hemolymph. This indicated the ability of both extracts to decrease oxidative stress by scavenging ROS. DPPH is a stable free radical at room temperature that can be used to assess *in vitro* antioxidant properties of any compound **(Kedare and Singh, 2011)**. The ability of the extract to scavenge DPPH radical was determined by the decline in the absorbance as a result of a conversion of DPPH solution (diphenylpicrylhydrazyl) to the reduced form (Diphenylpicrylhydrazine) after receiving a hydrogen atom from extract **(Molyneux, 2003)**. The antioxidant properties of both extracts on DPPH radical scavenging is thought to be due to their hydrogen donating ability. These results of the present study are consistent with previous studies wherein the hemolymph of *Ocypoda macrocera* **(Sivaperumal *et al.*, 2013)**, *Liagore rubromaculata* **(Priya *et al.*, 2014)** and *Scylla serrate* **(Sujeetha *et al.*, 2015)** have been reported to possess radical scavenging activities.

Oxidative stress is caused in cells and tissues as a result of the imbalance between the accumulation of reactive oxygen species (ROS) and the production of antioxidants that detoxify these reactive products **(Pizzino *et al.*, 2017)**. The increased production of ROS and cellular oxidative stress in cells has long been associated with an elevated metabolic rate and gene mutations **(Liou and Storz, 2010; Bhardwaj and He, 2020)**. H_2O_2 is one of the major ROS that induces oxidative stress in cells that leads to DNA damage **(Coyle and Kader, 2007)**. In the current study, H_2O_2 induced lipid peroxidation in WI-38 cells, leading to increase the accumulation of the oxidative stress biomarker (MDA). It also decreased GSH levels and the activities of SOD and CAT in cells. The present results show that hepatopancreas and hemolymph extracts alone or as co-treatment with H_2O_2 significantly enhanced intracellular antioxidant parameters (CAT, SOD and GSH) and reduced lipid peroxidation (MDA content) in normal human lung cells. This study agreed with previous studies that proved the ability of compounds isolated from marine invertebrates to exert cytoprotective activities on cells by inducing activities of SOD, CAT and increasing GSH levels **(Roel *et al.*, 2015)**. These antioxidants may be the cause of the antiproliferative activities of both extracts. Potent antioxidants may decelerate the growth of cancer cells, decrease persistent oxidative stress, or inhibit cancer progression **(Ilghami *et al.*, 2019)**.

CONCLUSION

The present study revealed for the first time the cytoprotective and inhibitory properties of the hepatopancreas and hemolymph of the edible blue crab *Callinectes sapidus*. Both extracts could be effectively used as potential sources for anticancer drugs discovery because of their selective antiproliferative effects. The results indicated that both extracts inhibit growth and proliferation of HeLa cells through induction of apoptosis and cell cycle arrest at G2/M phase. This study showed that the hepatopancreas extract exhibited more antioxidant, antiproliferative activities and apoptosis induction than hemolymph.

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