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The effects of *Cryptelytrops albolabris, Calloselasma rhodostoma* and *Daboia siamensis* venoms on human cancer cells

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Abstract

Three snake venoms: *Cryptelytrops albolabris, Calloselasma rhodostoma and Daboia siamensis* were studied for cytotoxic activity and apoptosis on five cancer cell lines; BT474, SW620, KATO-III, Hep-G_2 and ChaGo. Cytotoxic effect was determined by MTT assay and flow cytometry has been used for apoptosis. Various concentrations of three venoms showed cytotoxic against cancer cells as time-dependent. The potent of cytotoxic from *C. albolabris* venom against cancer cells were: BT474 (2.96 ± 0.44 µg/ml), SW620 (3.32 ± 0.14 µg/ml), KATO-III (3.72 ± 0.11 µg/ml) and Hep-G_2 (3.74 ± 0.43 µg/ml), respectively. *C. rhodostoma* venom showed cytotoxic to BT474 (3.16 ± 0.69 µg/ml), SW620 (3.5 ± 0.01 µg/ml) and KATO-III (3.74 ± 0.37 µg/ml) while the venom of *D. siamensis* was highly toxic to only ChaGo (0.48 ± 0.04 µg/ml). Apoptotic cell death using PI staining was dose and time dependent.

INTRODUCTION

Cancer is one of the major causes of death in the world. The factors are concerned including genetics, feeding, environmental conditions and exposure to carcinogens. Cancer is uncontrolled cell proliferation and decrease apoptosis. Apoptosis is program cell death which is essential to cellular homeostasis of multi-cellular organisms. The induction of tumor cell apoptosis has been observed by difference mechanisms such as the morphology change, condensation of cytoplasm and chromatin, DNA fragmentation, and cell fragmentation into apoptotic bodies. The easiest characteristic of apoptotic cell is loss of permeable cells due to DNA fragmentation. Sub-G1 peak, low molecular weight fragmented is extracted from apoptotic cells. PI (Propidium iodide) staining is a simple assay for sub-G1 detection which is the marker of anticancer activity. Cytotoxic activity of cell needs to be investigated to assess the optimal concentration to cells. A simple method is MTT ([3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyl tetrazolium bromide]) assay to estimate the living cells after treatment with the venoms compared to without venoms. However, these compounds have the ability to induce apoptosis in cancer cells as potentially as anticancer agents [1]

There are many studies on natural biological anticancer, such as bacteria [2], bee venom [3] and also snake venoms: *Siam Russell viper* venom [4-5], *Trimeresurus jerdonii* venom [6] and *Bothrops jararacussu* venom [7]. Snake venom is a complex mixture of biological active polypeptides which used to kill and digest preys. Many components of snake venoms were used as biomedical agents. For example, Adinbitor from *Agkistrodon halys brevicaudus stejneger* venom [8] or Contortrostatin from *Agkistrodon contortrix contortrix* venom [9-10] could potentially inhibit angiogenesis of both *in vitro* and *in vivo*.

C. albolabris, *C. rhodostoma* and *D. siamensis* are considered as hematotoxic snakes. Venoms from these snakes cause hemorrhage, necrosis, edema and often changes in the blood coagulation system. The venom compounds have been investigated as pharmacologically active proteins or peptides. Viperidae (Russell's viper) venom caused cytotoxicity on EAC cell [5]. A heat stable 7.2 kDa protein toxin (drCT-I), from Indian russell's viper venom had anti-proliferative, cytotoxic and apoptotic activity[11]. In this study, venoms of *C. albolabris*, *C. rhodostoma,* and *D. siamensis* were used to screen for cytotoxic activity in human cancer cell lines using MTT assay. The apoptosis induction was also studied using PI staining and % sub-G1 was monitored by flow cytometry.

MATERIALS AND METHODS

Materials

Venoms were milked from *Cryptelytrops albolabris*, *Calloselasma rhodostom and, Daboia siamensis*, lyophilized and stored at 4 °C. RPMI 1640 medium, FBS (Fetal bovine serum), streptomycin, penicillin and MTT were purchased from Sigma (Sigma, St Louis, MO, USA). Other chemicals and solvents were analytical grade.

Cell cultures

All cell lines and medium used in this work are shown in Table 1. Medium was supplemented with 5% FBS (Fetal bovine serum) and 1 mM glutamine, 100U/ml streptomycin and 100U/ml penicillin. Cells were incubated in 37°C incubating chamber with 5% Co_2.

Cytotoxic study by MTT assay

The cytotoxic effect of venoms against several human cancer cell lines was determined using CH-liver as normal cell. Doxorubicin was used as positive control. Briefly, cell was seeded in 96 well plates (NUNC, Denmark) at an approximate concentration of $1x10^6$ cells/ml, incubated at 37 °C with 5%

Table1. Tumor and cell lines

Cell line	Name	Medium	Sources
KATO-III	Gastric carcinoma	RPMI 1640-5% FBS	Human ATCC No.HTB103
SW620	Colon carcinoma	RPMI 1640-5% FBS	Human ATCC No.CCL227
BT474	Duetol carcinoma	RPMI 1640-5% FBS	Human ATCC No.HTB20
Hep-G_2	Liver hepatoblastoma	RPMI 1640-5% FBS	Human ATCC No.HB8065
ChaGo	Lung undifferentiated	RPMI 1640-5% FBS	Rabson, AS. National Cancer Institute, USA
CH-liver	Human liver cell	RPMI 1640-5% FBS	Human ATCC No.CCL13

Table 2. Cytotoxic activity of three snake venoms

	Cytotoxic IC50 (µg/ml)			
Cell type	*Cryptelytrops albolabris*	*Calloselasma rhodostoma*	*Daboia siamensis*	Doxorubicin
KATO-III	3.72 ± 0.11	3.74 ± 0.37	82.00 ± 6.00	5.24 ± 1.14
Hep-G2	3.74 ± 0.43	7.81 ± 0.79	91.91 ± 2.06	0.71 ± 0.14
SW620	3.32 ± 0.14	3.50 ± 0.01	81.00 ± 7.00	0.82 ± 0.01
BT474	2.96 ± 0.44	3.16 ± 0.69	33.69 ± 0.45	5.43 ± 0.43
ChaGo	7.68 ± 0.41	3.88 ± 0.41	0.48 ± 0.04	1.31 ± 0.36
CH-liver	5.28 ± 0.68	4.63 ± 1.18	71.88 ± 7.65	4.54 ± 0.18

CO2 for 24 hours and treated with various concentrations of each venom (10, 1, 0.1, 0.01 and 0.001 µg/ml) for 72 hours. Then, cell viability was observed by incubating with 10 µl of MTT (5 mg/ml) for 4 hours and dimethyl sulfoxide (DMSO) was added to dissolve the crystal. The experiments were measured at 540 nm and untreated was set to 100% viability.

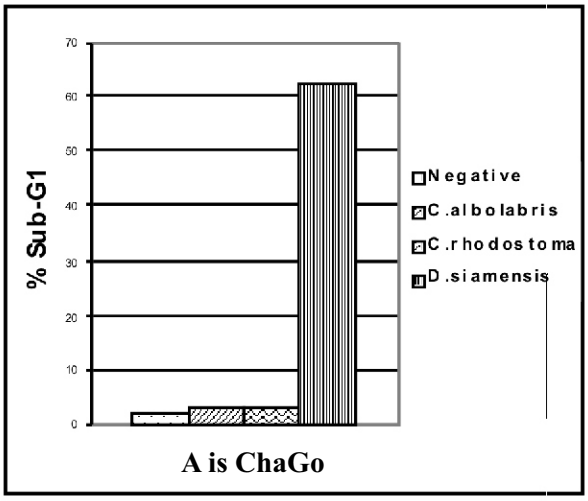
Sub-G1 analysis

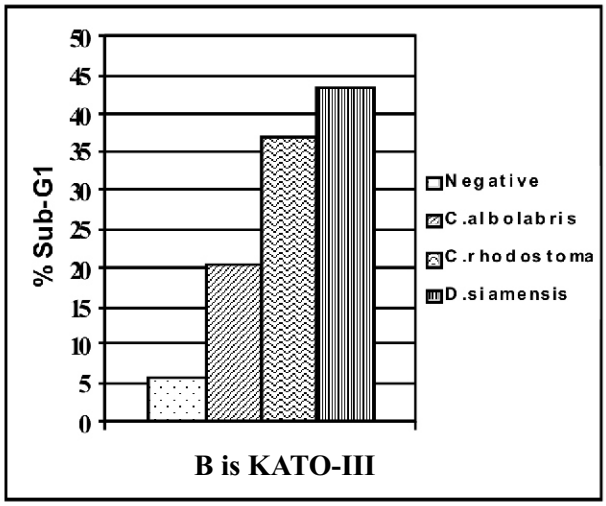
Each cell line was seeded in 24 well plates and incubated at 37 °C for 24 hours. The optimal concentration of venom was added to the cells, harvested and washed with PBS 7.2 for 3 times. Cell was stained with 0.25 µg propidium iodide (Sigma, St Louis, MO, USA) and sub-G1 was determined by flow cytometry.

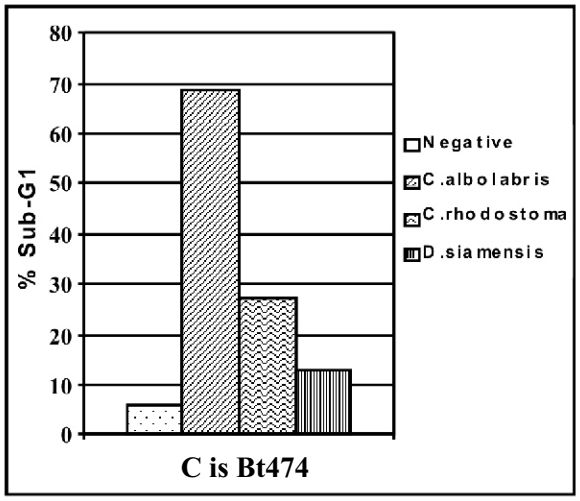
RESULTS

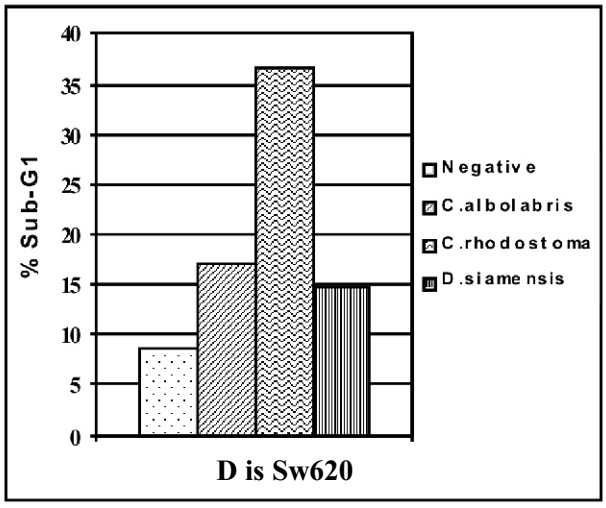
Cytotoxic studies by MTT assay

C. albolabris venom had a great toxic to most cell lines including KATO-III, Hep-G2, SW620 and BT474. *C. rhodostoma* was also toxic to the same cells accepted Hep-G2 and also potent to ChaGo cell. On the other hand, Doxorubicin affected on three cells; Hep-G2, SW620, and ChaGo cells. However, *D. siamensis* venom could affect only on ChaGo cell and it was greater than doxorubicin, a drug cancer. Doxorubicin was very sensitive to Hep-G2, SW620 and ChaGo. All venoms and doxorubicin were toxic to CH liver cell and normal cell. The IC_{50} of the venoms to each cell is in Table2.









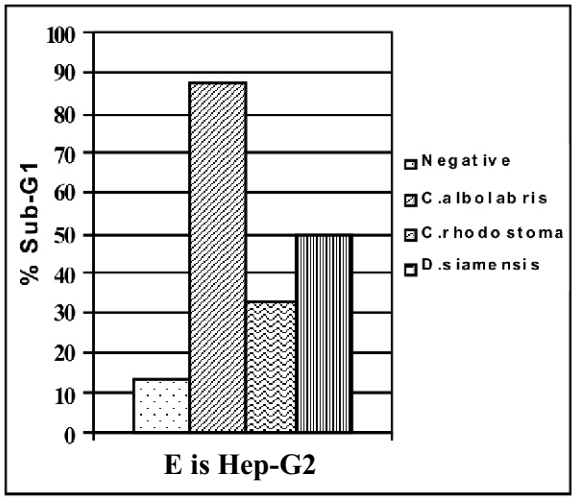


Figure1. The percentage Sub-G1; the cells were treated with three snake venoms and untreated as a negative control.

A: ChaGo cell was treated with 0.2 µg/ml three venoms and untreated as a negative control.

B: KATO-III cell was treated with 5 µg/ml three snake venoms and untreated as a negative control.

C: BT474 cell was treated with 3 µg/ml three snake venoms and untreated as a negative control.

D: SW620 cell was treated with 3 µg/ml three snake venoms and untreated as a negative control.

E: Hep-G2 cell was treated with 5 µg/ml three snake venoms and untreated as a negative control.

Sub-G1 analysis

The lowest concentration of *D. siamensis* venom showed the highest apoptotic activity to ChaGo cell (0.2µg/ml) when compared with *C. rhodostoma* and *C. albolabris* venoms. Sub-G1 of negative control, the untreated cell, was closed to zero (Figure1A). For KATO-III cell, the high effects were *D. siamensi*, *C. rhodostoma* and *C. albolabris* respectively (Figure1B). *C. albolabris* venom showed higher toxic activity on BT474 cell than *C. rhodostoma* venom and *D. siamensis* venom at the same concentration, 3µg/ml (Figure1C). On SW620 cell at 3 µg/ml of *C. rhodostoma* venom were more toxic than *C. albolabris* and *D. siamensi* venoms while the negative control had close to 5% (Figure1D). *C. albolabris* venom at 5µg/ml showed the highest activity against $HepG_2$ cell followed by *D. siamensi* and *C. rhodostoma* venoms. (Figure1E)

DISCUSSION

Snake venoms have long been studied for many effects against various human cancer cells. Previous study, for example, King cobra venom had cytotoxic effect on some human cancer cells by concentration dependent (Unpublished). In this report, *C. albolabris* and *C. rhodostoma* venoms showed similar cytotoxic effects on all cancer cells and also CH-liver as normal cell. Doxorubicin, the anti-cancer drug, showed greater potency on Hep-G_2, SW620 and ChaGo cells than *C. albolabris* and *C. rhodostoma* venoms. However, both venoms showed higher potency on KATO-III and BT474 cells than the anti-cancer drug which similar to the effect of king cobra venom. *D. siamensis* venom was more effective than the anti-cancer drug on only ChaGo cell. ChaGo is an undifferentiated cell and not mature lung cancer cell. It might be easier to destroy than the mature cancer cell. *D. siamensis* venom also had cytotoxic effect and inhibited cell migration on SK-MEL-28; skin melanoma cancer [12].

D. siamensis venom not only had a direct cytotoxic effect on ChaGo cell but also had a high sub-G1 at concentration 0.2 µg/ml. The results indicated that *D. siamensis* venom was an appropriate agent for further biomedical study. The venom may attack at lysosomes in plasma membrane. For example, the cytotoxins from cobra venom injured cells at lysosomes in plasma membrane of human lung adenocarcinoma A549 and promyelocytic leukaemia HL60 cells [13]. The lysosomes in plasma membrane may be the venom killing targets on cancer cells. However, *C. albolabris* and *C. rhodostoma* venoms also had cytotoxic effects and apoptosis on five cancer cells as mentioned above. Only *C. albolabris* venom had high percent sub-G1 to Hep-G_2 and BT474 cells. On the other hand, *C. rhodostoma* venom had potent on SW620 and KATO-III.

CONCLUSION

D. siamensis, *C. rhodostoma* and *C. albolabris* venoms are the mixture components. The result of these venoms on the cells might come from one or more proteins and have been indicated to be biomedical agents. However, purification of bioactive compounds and their mechanism are required for further study.

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