CANCER IN THE PHILIPPINES

1988 Philippine Cancer Control Program started [1]

"Cancer can largely be prevented by a public health effort"[1]

1. Buban CE. Colorectal cancer curable if detected early. Philippine Daily Inquirer [Internet]. 2013 Sept 20. Available from: http://business.inquirer.net/143697/colorectal-cancer-curable-if-detected-early

COLORECTAL CANCER

3rd leading cause of morbidity and mortality in the **PHILIPPINES**[1]

4th most common cancer in the **WORLD**[2]

1. Buban CE. Colorectal cancer curable if detected early. Philippine Daily Inquirer [Internet]. 2013 Sept 20. Available from: http://business.inquirer.net/143697/colorectal-cancer-curable-if-detected-early
2. Cappell M. The pathophysiology clinical presentation, and diagnosis of colon cancer and adenomatous polyps. Elsevier Saunders [Internet]. The Medical Clinics of North America, Volume 89, p. 1; 2005. Available from: https://www.med.upenn.edu/gastro/documents/MedClinNAcolonicpolyps.pd

IT'S A FACT!

1 out of **100** Filipinos will develop **Colon CA**[1]

Diagnosis at **60** years old[1]

40% 5 year survival rate[1]

1. Buban CE. Colorectal cancer curable if detected early. Philippine Daily Inquirer [Internet]. 2013 Sept 20. Available from: http://business.inquirer.net/143697/colorectal-cancer-curable-if-detected-early

APITHERAPY THEN…

Image from Pinterest: Sumerian stele of winged bee goddess

Image from A. Dürer, 1514: Eros, Venus and the bees

Image from China Daily: Apitherapy treatment in a hospital in Zhengzhou, Henan Province

Image from apitherapy.org: Dry venom preparation

…AND NOW.

Apis mellifera

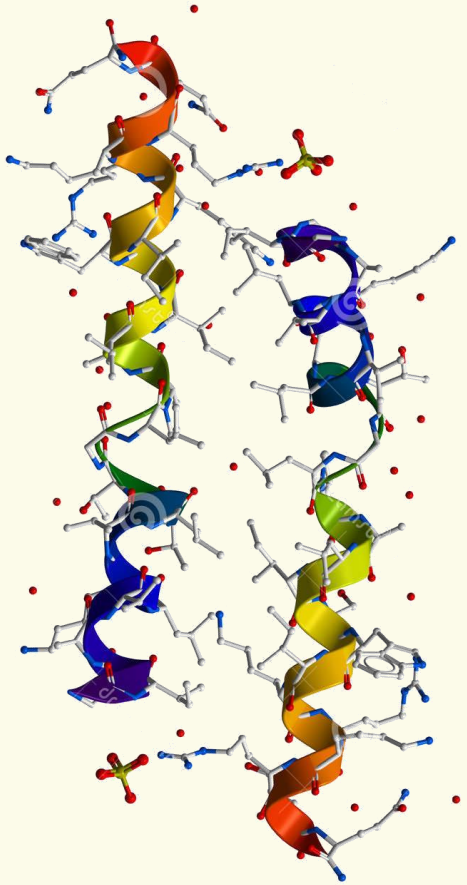
preferably grown in bee farms in the Philippines[3]

Image from Flickr.com

3. Hadley, D. Honey Bee- habits and traits of the honey bee, Apis mellifera [Internet]. [Place unknown]: About.com; 2014. Available from: http://insects.about.com/od/antsbeeswasps/p/A_ mellifera.htm

MELLITIN

- Principal component of bee venom
  - anti-cancer
  - anti-bacterial
  - anti-fungal
  - anti-viral properties[4]
- At 2.8 mg/kg body weight LD50, it is safe for human treatment[4]
- Minimal side effects[4]



4. Bogdanov S. Bee venom: composition, health, medicine: a review. Bee product science [Internet]. Feb 2014. Available from: http://www.bee-hexagon.net/files/file/fileE/Health/ VenomBook Review.pdf




IN VITRO ANTI-PROLIFERATIVE EFFECTS OF BEE (*Apis mellifera*) VENOM IN HCT 116 COLON CANCER CELL LINES

Bangero J, Calise DK, de la Peña LJ, Delos Reyes F, Documento E, Durana V, Faculin AK, Ong PKM, Pueblo RL, Roldan NA

West Visayas State University | College of Medicine

SIGNIFICANCE OF THE STUDY

- Colon cancer patients
- Healthcare providers
- Pharmaceutical companies
- Beekeepers
- Future researchers

GENERAL OBJECTIVE

To determine the in vitro anti-proliferative effects of bee venom in HCT116 colon cancer cell lines using Doxorubicin as positive control and Dimethylsulfoxide (DMSO) as negative control

SPECIFIC OBJECTIVES

- To determine the effects of the different concentrations of bee venom on:
    a. Cell number
    b. Cell morphology
    c. Cell viability
    d. Percent cell lysis

SPECIFIC OBJECTIVES

- To identify the most effective concentration of bee venom in which 50% growth of colon cancer cells is inhibited

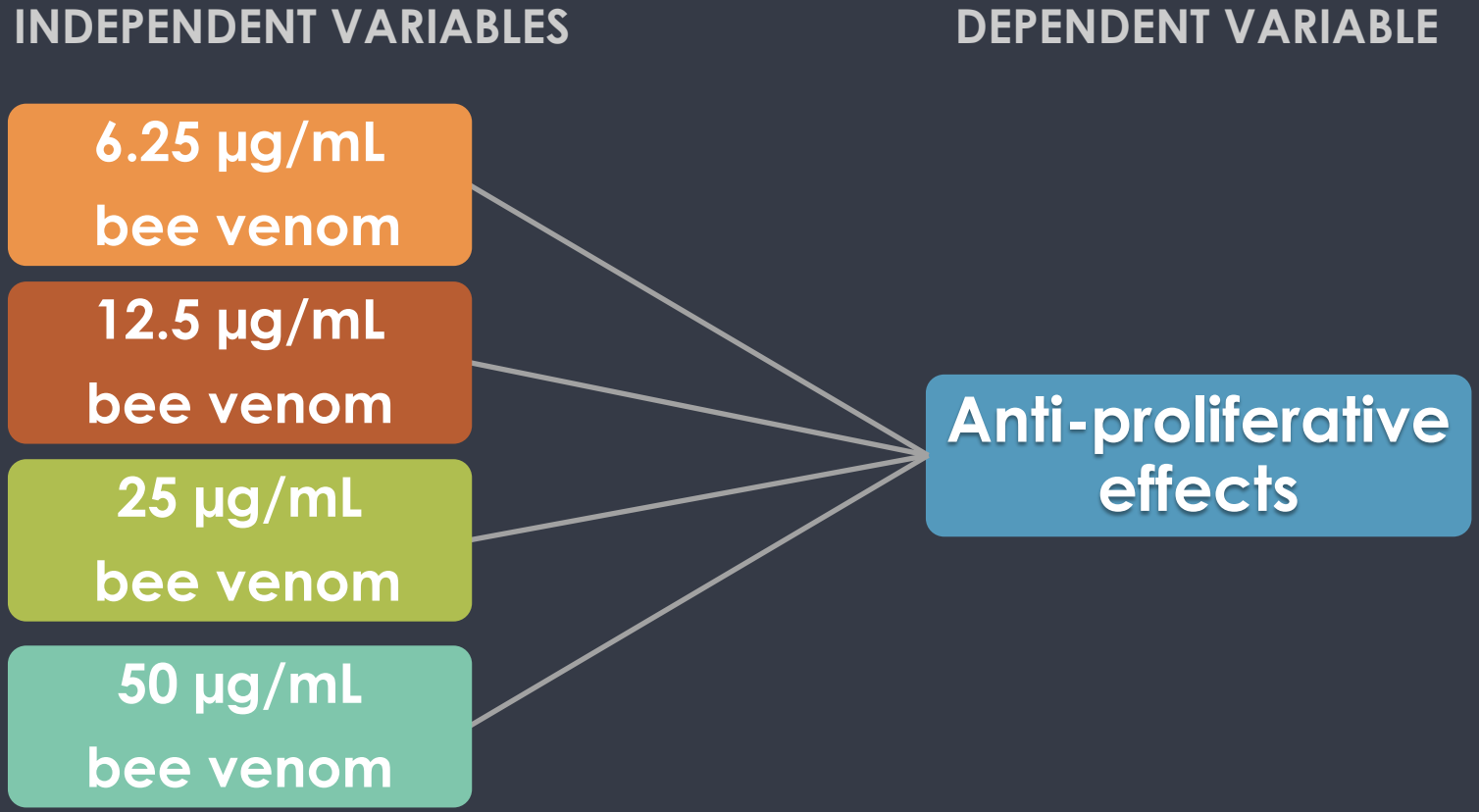


Figure 1. Relationship between various concentrations of bee venom and dependent variables.

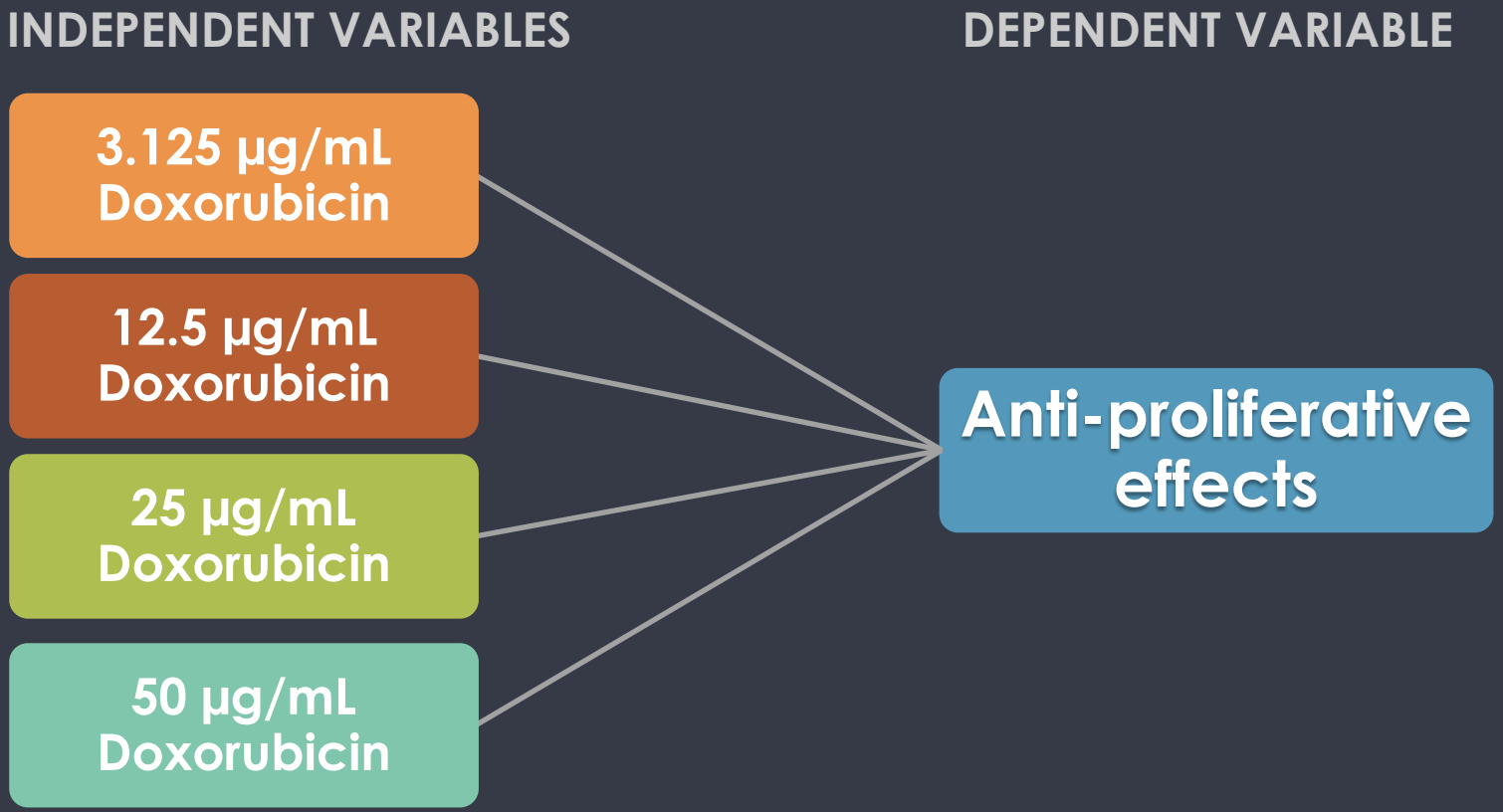


Figure 2. Relationship between various concentrations of doxorubicin and dependent variables.

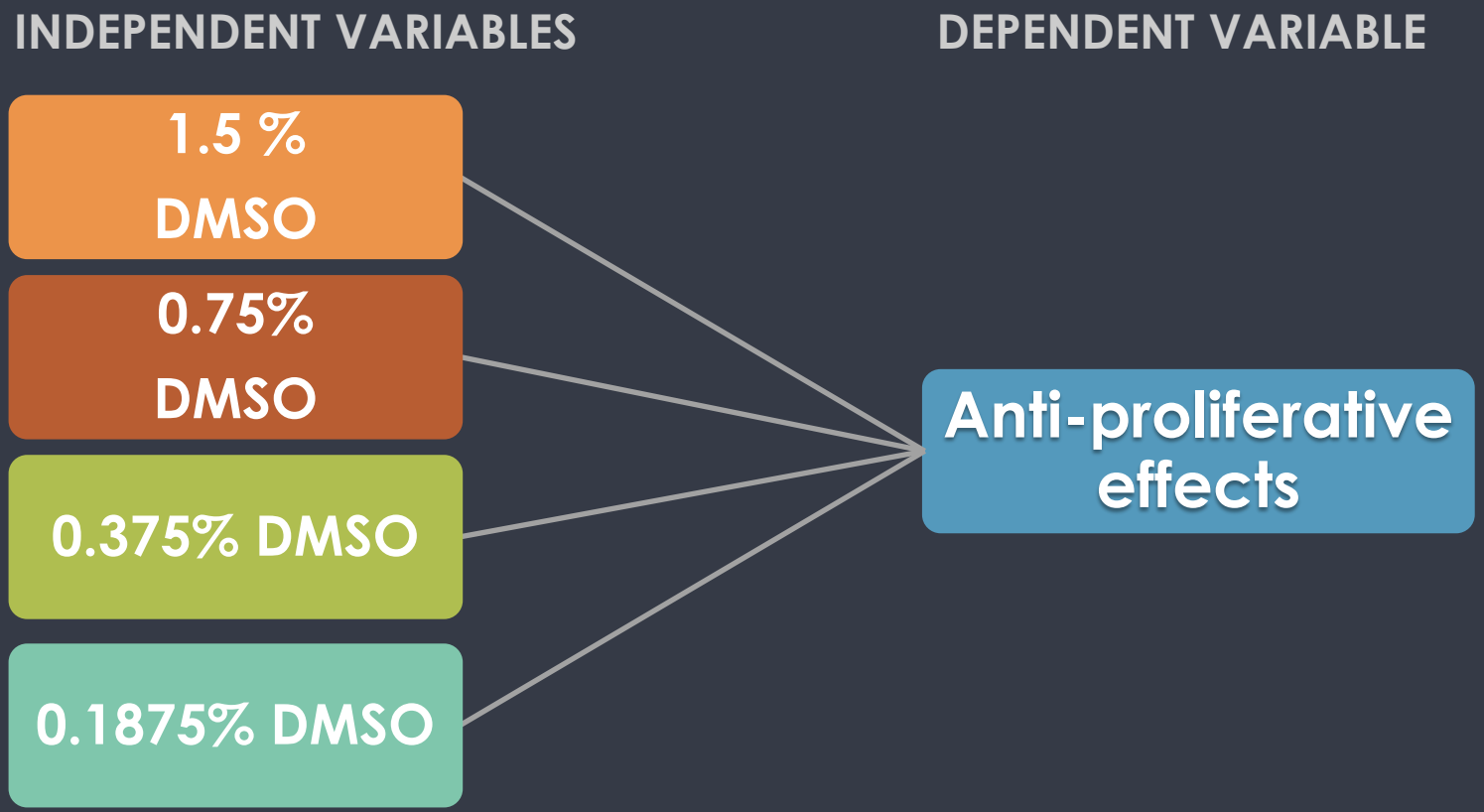


Figure 3. Relationship between various concentrations of DMSO and dependent variables.

STUDY DESIGN

Completely Randomized Design (CRD) in two replications with three trials per treatment

STUDY SETTING

University of the Philippines Diliman, QC

Private Laboratory, Novaliches, QC

WVSU College of Medicine Clinical Laboratory, Iloilo City

STUDY PERIOD

Research Proposal	Experiment and data collection	Data Processing and Analysis	Final Manuscript
• June 2014 to March 2015	• April 6-10, 2015	• April to May 2015	• June 2015 to February 2016

MANEUVERS

1

PRELIMINARY ACTIVITIES

Procurement of dried lyophilized bee venom powder from Apitoxin Corporation

Storage of bee venom at -20˚C in SEAFDEC Aquaculture Department

2 MEDIA PREPARATION

University of the Philippines - Diliman

3

PLATING OF CELLS AND INCUBATION

$6x10^4$ cells/mL in sterile 96-well microtiter plates

RPMI 1640 medium at 37°C with 5% CO_2

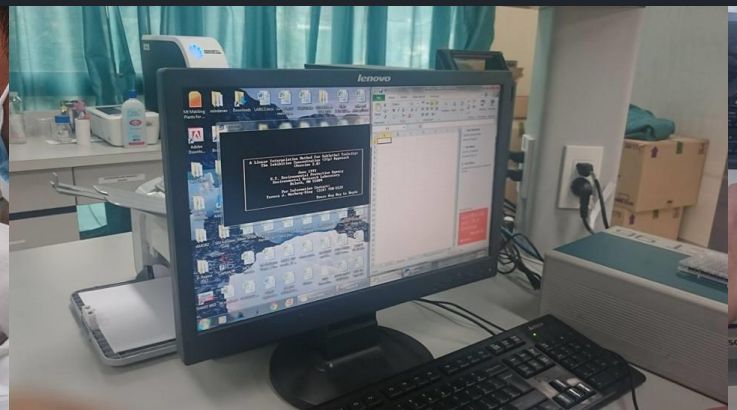
4 BEE VENOM TREATMENT

- Positive Control: Doxorubicin
- Negative Control: DMSO
- Incubated for 72 h at 37°C with 5% CO_2

5

METHYL THIAZOL TETRAZOLIUM (MTT) ASSAY

- UP Diliman Biology Department Protocol
- 20 µL MTT at 5 mg/mL PBS
- Incubated for 2 to 4 h at 37˚C with 5% CO_2
- Absorbance measured at 570 nm with a microtiter plate reader



6

MANUAL CELL COUNTING

- Neubauer counting chamber
- 1:20 dilution
- Estimates of the number per well was made

7 CYTOPATHOLOGY

- Hematoxylin & Eosin (H&E) staining
- Final labelling of the finished slides

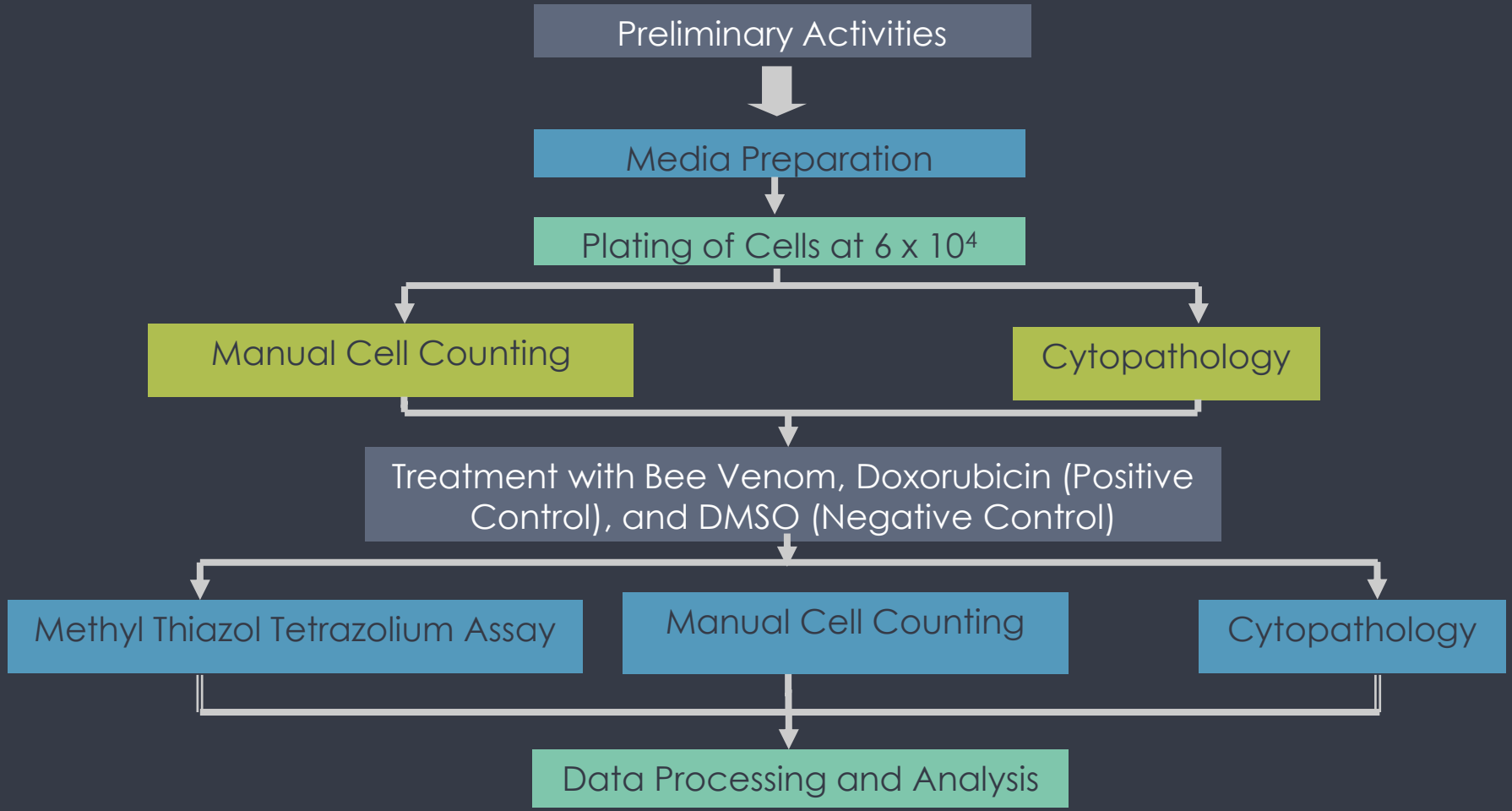
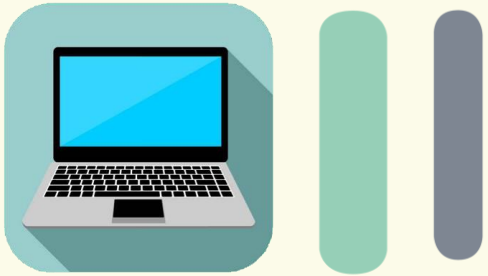


Figure 4. Flow chart that illustrates the manner in which the research methods ensued.

DATA PROCESSING AND ANALYSIS

- SPSS v. 20.0
- "icpin software"
- p value < 0.05



- Mean
- Median Rank
- Paired *t*-test
- Analysis of Variance (ANOVA)
- Duncan's Multiple Range Test (DMRT)
- Orthogonal Contrast

  Kruskall-Wallis Test

ETHICAL CONSIDERATIONS

- HCT 116 colon cancer cell lines grown in vitro
- Laboratory personnel supervised procedures
- No human participation
- Proposal submitted to UBERRC for review and was approved

RESULTS AND DISCUSSION

Anti-proliferative effects conferred by the purified bee venom

Table 1. Cell number before and after treatment

Treatment	Concentration	Mean Number of Cells (per µL)		p - value
		Before Treatment	After Treatment	
Bee Venom (µg/mL)	6.25	7600	318.50	<0.001*
	12.5	7600	177.83	<0.001*
	25	7600	144.67	<0.001*
	50	7600	140.67	<0.001*
Doxorubicin (µg/mL)	3.125	7600	548.17	<0.001*
	6.25	7600	344.50	<0.001*
	12.5	7600	314.83	<0.001*
	25	7600	240.67	<0.001*
DMSO (%)	0.1875	7600	4726.00	<0.001*
	0.375	7600	5192.67	0.001*
	0.75	7600	4674.17	<0.001*
	1.5	7600	5292.50	0.002*

*Significant at $p<0.05$; Number of cells before treatment was based on estimate

Table 2. ANOVA for the Comparison of Cell Number after Treatment

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	354903161.486	11	32263923.771	175.450	<0.001*
Within Groups	11033515.167	60	183891.919		
Total	365936676.653	71			

*Significant at p<0.05

Table 3. DMRT Post-Hoc Analysis for Number of Cells per µL after Treatment

Treatment	Concentration	Subsets		
		1	2	3
Bee venom	50	140.670		
Bee venom	25	144.670		
Bee venom	12.5	177.830		
Doxorubicin	25	240.670		
Doxorubicin	12.5	314.830		
Bee venom	6.25	318.500		
Doxorubicin	6.25	344.500		
Doxorubicin	0.3125	548.170		
DMSO	0.1875		4674.170	
DMSO	0.375		4726.000	
DMSO	0.75		5192.670	5192.670
DMSO	1.5			5292.500
p-value	0.1875	0.167	0.051	0.688

*Significant at $p<0.05$

Table 4. Orthogonal Contrast for Number of Cells per µL after Treatment

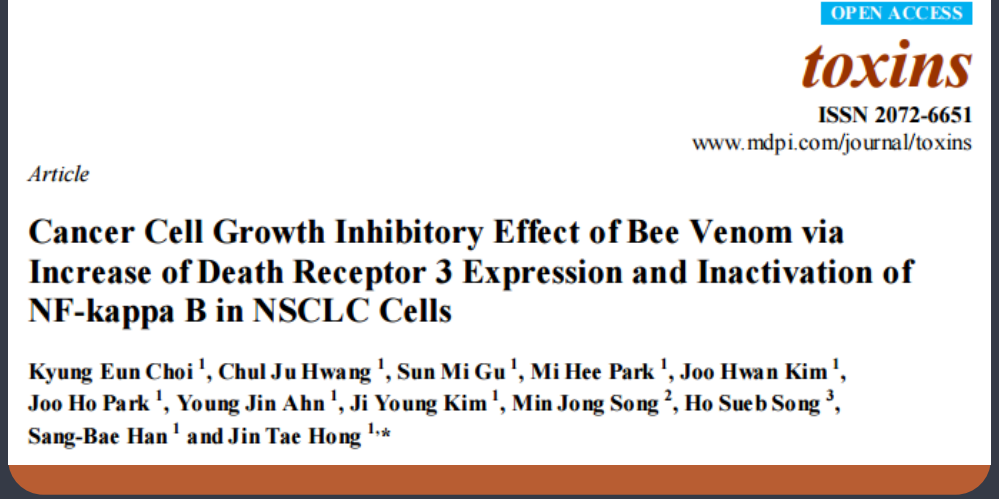
Contrast	Value of Contrast	Std. Error	T	df	p-value
DOX-DMSO	-18437.17	605.257	-30.462	17.757	<0.001*
DOX-BV	666.50	58.562	11.381	28.115	<0.001*
DMSO-BV	19103.67	604.816	31.586	17.706	<0.001*

*Significant at $p<0.05$

Table 5. ANOVA for the Comparison of Cell Number after Treatment between Bee Venom and Doxorubicin

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	770821.979	7	110117.426	42.812	<0.001*
Within Groups	102885.500	40	2572.138		
Total	873707.479	47			

*Significant at $p<0.05$



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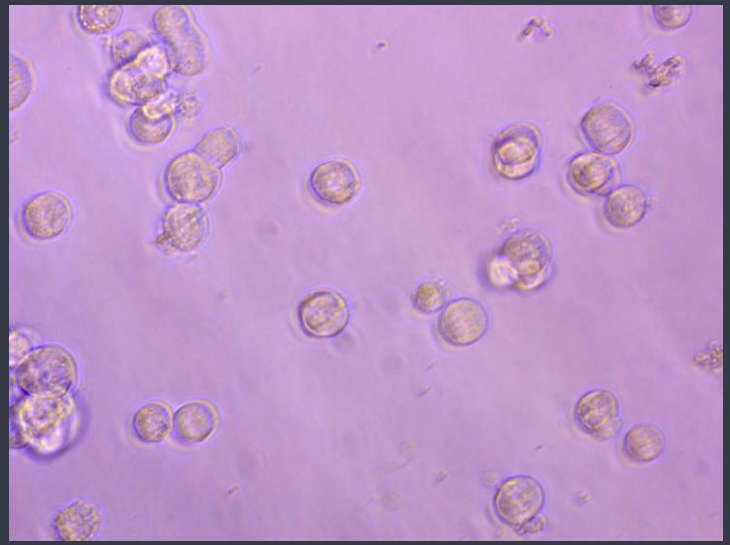
toxins
ISSN 2072-6651
www.mdpi.com/journal/toxins

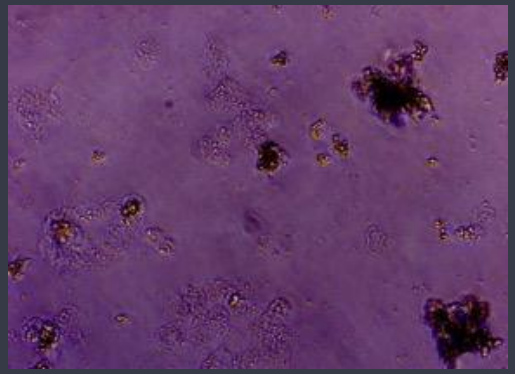
Article

Cancer Cell Growth Inhibitory Effect of Bee Venom via Increase of Death Receptor 3 Expression and Inactivation of NF-kappa B in NSCLC Cells

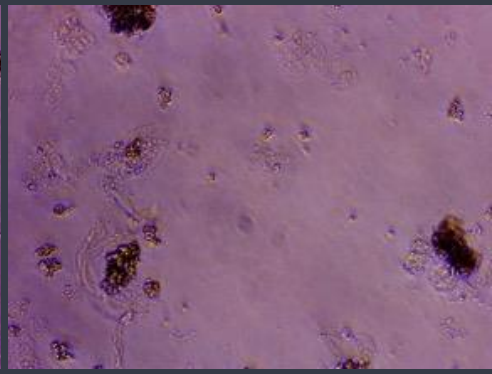
Kyung Eun Choi [1], Chul Ju Hwang [1], Sun Mi Gu [1], Mi Hee Park [1], Joo Hwan Kim [1], Joo Ho Park [1], Young Jin Ahn [1], Ji Young Kim [1], Min Jong Song [2], Ho Sueb Song [3], Sang-Bae Han [1] and Jin Tae Hong [1,*]

- Asian honey bee (*Apis cerana)* venom showed significant decrease in the number of A549 and NCI-H460 lung cancer cells in a concentration-dependent manner
- No effect in normal LL24 lung cancer cells

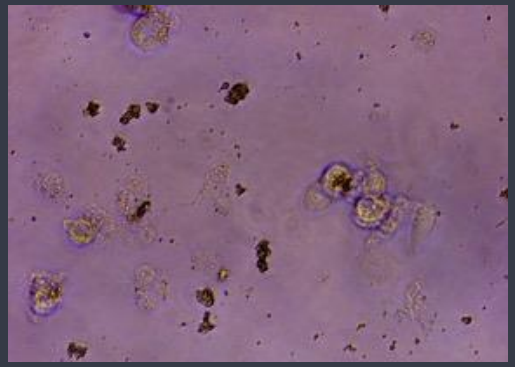




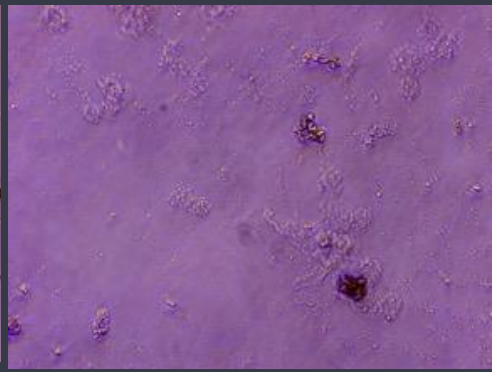
6.25 µg/mL
bee venom



12.5 µg/mL
bee venom



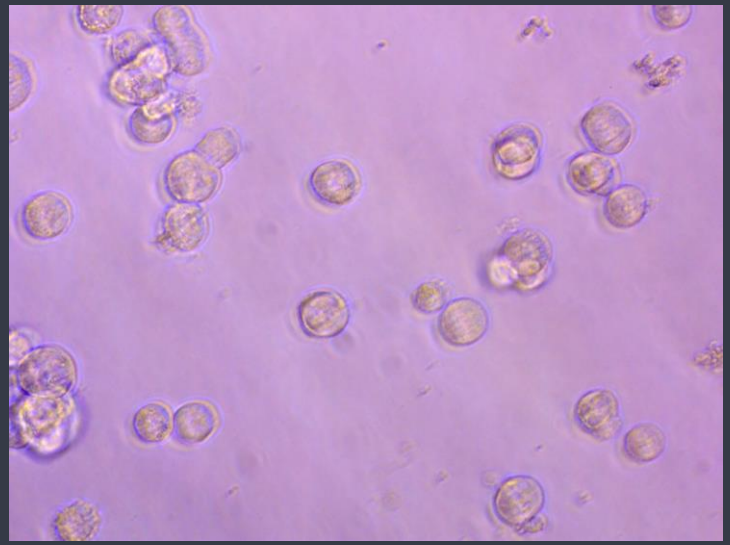
25 µg/mL
bee venom

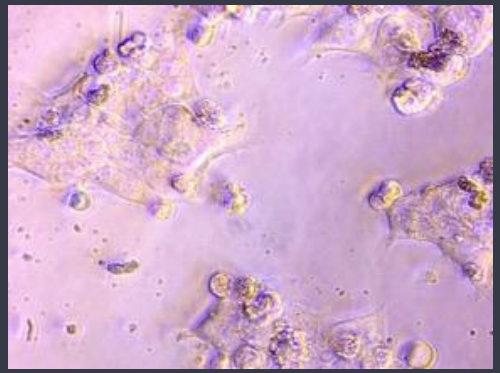


50 µg/mL
bee venom

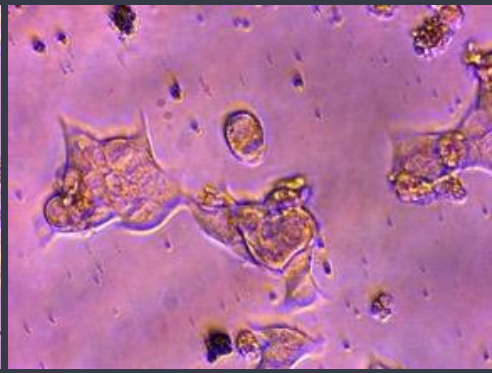
BEFORE TREATMENT

AFTER TREATMENT

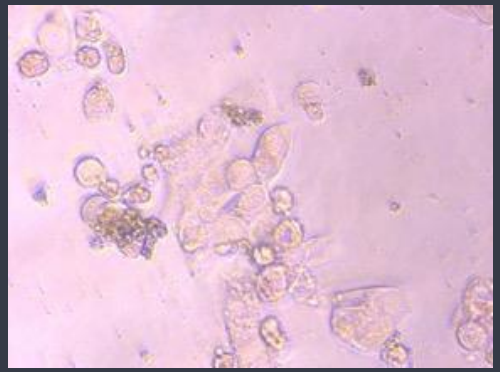




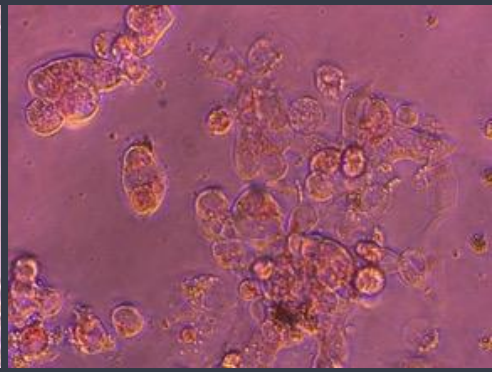
3.125 µg/mL
Doxorubicin



6.25 µg/mL
Doxorubicin



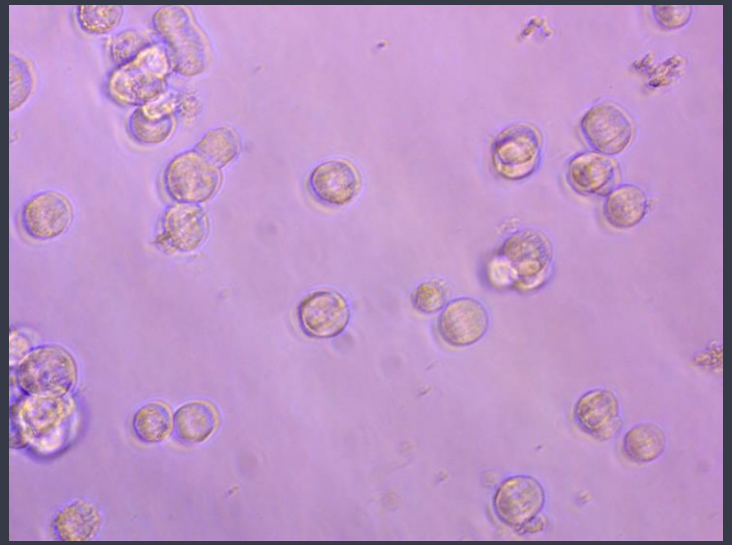
12.5 µg/mL
Doxorubicin

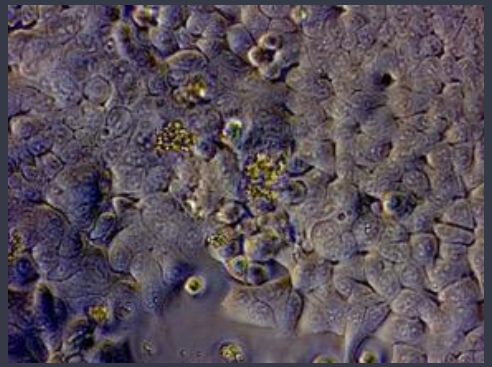


25 µg/mL
Doxorubicin

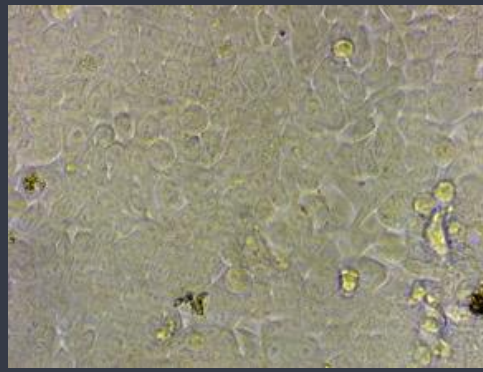
BEFORE TREATMENT

AFTER TREATMENT

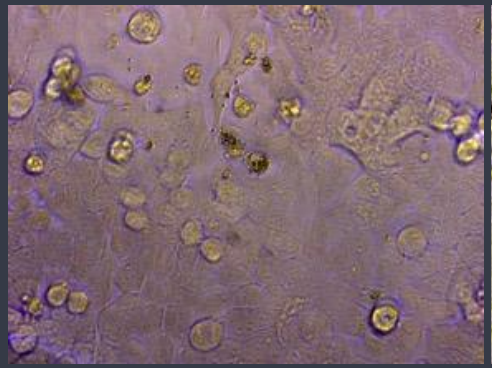




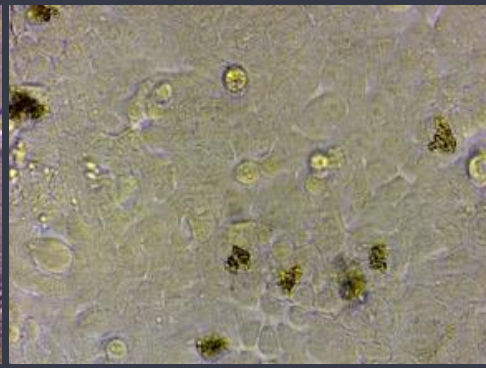
0.1875% DMSO



0.375% DMSO



0.75% DMSO



1.5% DMSO

BEFORE TREATMENT

AFTER TREATMENT

Table 6. Cell Morphology after Treatment with Bee Venom, Doxorubicin and DMSO

Treatment	Concentration	
Bee Venom	6.25	Non-intact
(μg/mL)	12.5	Non-intact
	25	Non-intact
	50	Non-intact
Doxorubicin	3.125	Non-intact
(μg/mL)	6.25	Non-intact
	12.5	Non-intact
	25	Non-intact
DMSO	0.1875	Intact
(%)	0.375	Intact
	0.75	Intact
	1.5	Non-intact

Bee venom brought about cellular degeneration described morphologically as decreased cell count and non-intact morphology.

Toxicology and Applied Pharmacology

Volume 258, Issue 1, 1 January 2012, Pages 72–81

Anti-cancer effect of bee venom toxin and melittin in ovarian cancer cells through induction of death receptors and inhibition of JAK2/STAT3 pathway

Miran Jo[a], Mi Hee Park[a], Pushpa Saranya Kollipara[a], Byeong Jun An[b], Ho Sueb Song[b], Sang Bae Han[a], Jang Heub Kim[c], Min Jong Song[c,*], Jin Tae Hong[a,*]

Journal of Chemical and Pharmaceutical Research, 2015, 7(2):1-5

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Investigating the effect of bee venom on human colon cancer cells (HT-29) and hepatic cells (HepG2) in comparison to L929 cells

Khozeimeh F.[a], Golestannejad Z.[a], Doostmohammadi M.[b*] and Gavanj S.[b]

OPEN ACCESS

toxins

ISSN 2072-6651

www.mdpi.com/journal/toxins

Article

Cancer Cell Growth Inhibitory Effect of Bee Venom via Increase of Death Receptor 3 Expression and Inactivation of NF-kappa B in NSCLC Cells

Kyung Eun Choi [1], Chul Ju Hwang [1], Sun Mi Gu [1], Mi Hee Park [1], Joo Hwan Kim [1], Joo Ho Park [1], Young Jin Ahn [1], Ji Young Kim [1], Min Jong Song [2], Ho Sueb Song [3], Sang-Bae Han [1] and Jin Tae Hong [1,*]

The Prostate

Original Article

Anti-cancer effect of bee venom in prostate cancer cells through activation of caspase pathway via inactivation of NF-κB†

Mi Hee Park[1], Myoung Suk Choi[1], Dong Hoon Kwak[1], Ki-Wan Oh[1], Do Young Yoon[2], Sang Bae Han[1], Ho Sueb Song[3], Min Jong Song[4,*] and Jin Tae Hong[1,*]

Article first published online: 17 NOV 2010

DOI: 10.1002/pros.21296

Issue

The Prostate

Volume 71, Issue 8, pages 801–812, 1 June 2011

Table 7. Descriptive Summary of MTT Assay Absorbance Readings

Treatment	Concentration (µg/mL)	Mean Absorbance Readings	Std. Deviation
DMSO		1.135	0.330
Doxorubicin	3.125	0.220	0.078
	6.25	0.213	0.075
	12.5	0.214	0.073
	25	0.179	0.054
Bee venom	6.25	0.233	0.117
	12.5	0.122	0.091
	25	0.096	0.011
	50	0.094	0.014

Table 8. ANOVA for the Comparison of MTT Assay Absorbance Readings

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	7.564	8	0.946	49.266	<0.001*
Within Groups	1.152	60	0.019		
Total	8.716	68			

*Significant at $p<0.05$

Table 9. DMRT Post-Hoc Analysis for MTT Assay Absorbance Readings

Treatment	Concentration (µg/mL)	Subset	
		1	2
Bee venom	50	0.09444	
Bee venom	25	0.09633	
Bee venom	12.5	0.12167	
Doxorubicin	25	0.17933	
Doxorubicin	6.25	0.21333	
Doxorubicin	12.5	0.21367	
Doxorubicin	0.3125	0.21950	
Bee venom	6.25	0.23267	
DMSO			1.13489
p-value		0.107	1.000

*Significant at $p<0.05$

Table 10. Orthogonal Contrast between Treatments

Contrast	Value of Contrast	Std. Error	t	df	p-value
DOX-BEE	0.28072	0.076196	3.684	34.195	0.001*

*Significant at p<0.05

Table 11. ANOVA for the Comparison of MTT Assay Absorbance Readings between Bee Venom and Doxorubicin

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	0.193	7	0.028	5.146	<0.001*
Within Groups	0.278	52	0.005		
Total	0.471	59			

*Significant at p<0.05

Table 12. Post-Hoc Analysis for MTT Assay Absorbance Readings using DMRT

Treatment	Concentration (µg/mL)	Subset		
		1	2	3
Bee venom	50	0.09444		
Bee venom	25	0.09633		
Bee venom	12.5	0.12167	0.12167	
Doxorubicin	25		0.17933	0.17933
Doxorubicin	6.25			0.21333
Doxorubicin	12.5			0.21367
Doxorubicin	3.125			0.21950
Bee venom	6.25			0.23267
p-value		0.511	0.141	0.22600

*Significant at $p<0.05$

Amount of cytotoxicity against HT-29 human colon cancer cells and L929 fibroblast cells enhanced as concentration of bee venom increases

Table 13. Testing Differences in the Average Percent Lysis

Treatment Group	Mean Percent Lysis (%)	Test Statistic	p-value
Bee Venom	97.43	8.769	0.012*
Doxo	95.24		
DMSO	34.59		

*Significant at $p<0.05$

Table 14. Post Hoc Analysis

Treatment 1	Treatment 2	Test Statistic	p-value
Bee Venom	Doxo	3.0	0.239
Bee Venom	DMSO	7.5	0.003*
Doxo	DMSO	4.5	0.078

*Significant at $p<0.05$

Table 15. Mean IC_{50} Results

Treatment Group	Mean IC_{50} (ug/mL)
Bee Venom	3.920
Doxo	1.937

CONCLUSION

- Significant *in vitro* anti-proliferative effects
- Decrease in cell number in a dose-dependent manner
- 6.25 µg/mL bee venom,12.5 µg/mL and 25 µg/mL Doxorubicin have the same effect

- Decrease in absorbance as concentration increases indicating a decrease in cell viability
- Comparable effects in cell viability exhibited by bee venom at 6.25 µg/mL and all concentrations of doxorubicin

- Bee venom had the most non-intact cells, increasing in number as the concentration increased
- Mean percent cell lysis showed bee venom as the most effective treatment
- IC_{50} of 3.920 µg/mL

RECOMMENDATIONS

Further studies about the effects of bee venom against cancer cells and normal colon cells

Other forms of microscopy such as electron and fluorescent microscopes

- Biochemical markers of apoptosis such as peptide annexin; DAPI and TUNEL staining assays and Western blot as alternative assays
- Fluorescence in situ hybridization (FISH) and other fluorescent techniques

Flow cytometry for better accuracy in cell counting

Adjunctive effects of bee venom with other medical procedures, drugs or additives

Animal studies as potential anti-cancer agent

THANK YOU!

Grupo Nuebe, Medicine II-B

IN VITRO ANTI-PROLIFERATIVE EFFECTS OF BEE (*Apis mellifera*) VENOM IN HCT 116 COLON CANCER CELL LINES

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