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Surabaya, 27 – 29 Nopember 2014

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Kata Pengantar

Perkembangan IPTEK bidang kesehatan gigi dalam beberapa dasawarsa terakhir ini sangat cepat akibat tuntutan masyarakat yang berkembang. Selain itu, masyarakat selalu menuntut untuk mendapatkan pelayanan kesehatan gigi yang sempurna. Seorang dokter gigi saat ini tidak bisa menghindar dari persaingan yang semakin ketat, oleh karena itu harus terus menerus meningkatkan profesionalismenya, salah satunya dengan terus menerus menambah informasi ilmiah terbaru. Informasi ini selalu diperlukan demi tercapainya profesionalisme dokter gigi yang handal yang siap bersaing di pasar bebas. Pada era globalisasi saat ini, akan membuat persaingan dunia usaha yang sangat ketat dengan kompetisi yang terbuka. Hal tersebut akan membuat pelanggan (pasien) dengan mudah membanding-bandingkan kualitas pelayanan antara dokter gigi satu dengan yang lain. Oleh karena itu, secara tidak langsung akan memaksa dokter gigi untuk mengembangkan model dan strategi pelayanan yang tepat dan bermutu.

Untuk mengantisipasi hal tersebut, Ikatan Konservasi Gigi Indonesia terus berusaha untuk meningkatkan kualitas dokter gigi Indonesia khusus dalam bidang konservasi gigi dengan cara mengadakan seminar ilmiah secara berkala. Temu Ilmiah Nasional IKORGI (TINI III) ini diharapkan dapat digunakan sebagai sarana untuk alih teknologi ilmu kedokteran gigi mutakhir dalam upaya meningkatkan profesionalisme dokter gigi di era persaingan global. TINI III ini diharapkan dapat menambah pengetahuan dokter gigi sehingga dapat melahirkan dokter gigi dan dokter gigi spesialis konservasi gigi yang sukses dan mampu melayani masyarakat secara optimal serta diharapkan dapat digunakan untuk alih pengetahuan dan teknologi baik di bidang ilmu manajemen kesehatan maupun ilmu kedokteran gigi mutakhir.

Selamat mengikuti seminar, sampai jumpa di Temu Ilmiah Nasional Ikatan Konservasi Gigi Indonesia III yang akan datang.

Surabaya, 27-29 Nopember 2014

Ari Subiyanto,drg.,SpKG(K).,MKes
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Cytoxicity Test of Diadema Setosum Shell Extract Againts Fibroblast Culture Cell

(Uji Sitotoksisitas Ekstrak Cangkang Landak Laut (Diadema Setosum) Terhadap Kultur Sel Fibroblas)

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ABSTRACT
Background:
Calcium is one of the basic ingredients of calcium hydroxide which is used in the field of dentistry as pulp capping material. Utilization of shells to be used as biomaterials Diadema setosum new to the field of dentistry, because one of the ingredients in Diadema setosum shells is calcium. ***Purpose:*** *To find out the cytotoxicity of Diadema setosum shell extract against fibroblast cell cultures.* ***Material and Methods:*** *This research was carried out by using post test only control group design. Fibroblasts cultured in 96 wells were divided into a control group of cells (n=6), media control (n=6) and treatment (n=6). Treatment groups were given various doses extract with concentration of shells Diadema setosum 125 µg, 250 µg, 500 µg, 1000 µg, 2000 µg. Optical density was read with an ELISA reader and calculated the percentage of viability. The cell viability data were analyzed by One-way ANOVA statistical test and LSD.* ***Result:*** *Data indicate decreased cell viability in all treatment groups, level of concentration of the extract by Diadema setosum shell, that is 125 µg(83.7%), 250 µg(81.9%), 500 µg(66.25%), 1000 µg(57.08%), 2000 µg(42.95%). There is a significant difference (p=0.000) in all treatment groups after analyzed by using One-way ANOVA.* ***Conclusion:*** *Diadema setosum shell extract do not have toxic effects on cultured fibroblast cells at concentrations 125 µg, 250 µg, 500 µg, 1000 µg and has toxic effects on cultured fibroblast cells at the highest concentration, that is 2000 µg.*

Keywords : *Diadema setosum, fibroblast cell culture, cytotoxicity*

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INTRODUCTION

Pulp as one of the formative organs of tooth reacts to hot and cold stimuli which are referred as pain. That pain sensation acts as a warning sign of unusual situation in pulp as well as a protective reaction. Stimuli that induce protective reactions and are capable of damaging the pulp are bacteria (in the event of caries), mechanical stimuli (traumas, fractures, cavity preparations and attritions), and chemical stimuli, such as acidic food and toxic dental materials. While mild pulp injury does not cause significant change, a severe one will result in local inflammation called pulpitis.

Pulp capping is one of the methods used to alleviate inflammation in exposed pulp, with calcium hydroxide as the material of choice. This is because calcium hydroxide has excellent biocompatibility with high pH and

bacteriostatic property. In addition, this material is also believed to stimulate new odontoblast cells differentiation which then form the reparative dentin.

One of the sea creatures whose potential has not really been tapped into is sea urchin/echinoid (or 'landak laut/bulu babi' as it is usually called in Indonesia) . Its shell is coated by stable black liquid pigment. The shells be included as endoskeleton because their bodies are covered by epitelial layers. The skeleton is called as test that structured by calcium carbonat which are produced on the oral area.

In dentistry, the high level of calcium inside the shell of sea urchin might be used as a pulp capping material. The pulp capping in the market are all synthetics. Nowadays, there are many researches that use natural materials as dental materials substitude. For that reason, a further experiment is needed to test the calcium inside the sea creatures as pulp capping materials. However, we have to test the cytoxicity test to fullfilled the material biocompatibility's requirement before we apply it on human being.

Based on that explanation, writer wants to do a research about cytoxicity test of diadema setosum shell extract againts fibroblast culture cell. This research is done by using a spesific type of sea urchin called Diadema setosum because they are easily obtained and they are animals under supervision.

MATERIALS DAN METHODS

This is an analitic experimental laboratorium research. 2 Kilos of spineless Diadema setosum were cleaned using ice water and the organs were taken out. After it was cleaned, we dried out the shells using freeze dry method. Next, we extract 250mg of diadema setosum using ethanol solvent with macetarion method. The result was paste and devided into 7 groups, which are the control cell group, media control, concentration of 125 µg, 250 µg, 500 µg, 1000 µg, 2000 µg. Diadema setosum extract was diluted into *α-MEM* medium solvent to get the treatment group. After that, we did cytoxicity test on fibroblast cell cultures and read the optical density in spectophotometry using the ELISA Reader with 620nm wavelength. Count the cells viability percentage mean of optical density sampel on each sample with various concentration againts cell control.

RESULTS

Cell viability results based on the cytoxicity experimental test of Diadema setosum shell against fibroblast cell cultures are presents on Table 1 bellow.

Sampel	**Control cells**	**125**	**250**	**500**	**1000**	**2000**
I	0	10.6	13.1	34.6	42	62.9
II	0	12	15.9	33.2	40.3	60.6
III	0	18.4	20.5	38.3	42.5	55.1
IV	0	12.7	12.5	23.1	38.1	50.9
V	0	22.3	21.2	33.7	52	52.7
VI	0	21.4	25.4	39.6	42.6	60.2

Tabel 1. Cell viability results

Based on the cell viability calculation results, we can calculate the mean and standard deviation score. Next, we tested the normality test (Shapiro-Wilk test) to find the data distribution. On this research, the data distribution is normal ($p>0.05$). After that, we do the homogenity test (Levene's test) and get 0.035 as the results which shows that the data is not homogen and we need to transform the data.

We get a homogen data ($p>0.05$) after the data was being transformed and next we do ANOVA test and get $F=116.946$ as the result. $P=0.000$ ($p<0.05$) means there was a significant cell viability difference between the treatment groups. For that reason we need to find the cell viability significant comparison using the LSD test. The LSD test result show that there was a significant cell viability between the control group and all of the treatment groups ($p>0.05$). There were no significant difference on the 125 µg and 250 µg ($p>0.05$) which is $p=0.522$.

DISCUSSION

Calculation is then done on the optical density results from the ELISA Reader to measure fibroblast cell viability in varying concentrations of *Diadema setosum* shell extract. The results were as follow: there occurred 57.05% cell death in 2000 µg concentration as compared to cell control (100%), whereas it was 42.92% in 1000 µg concentration, 33.75% in 500 µg concentration, 18.05% in 250 µg concentration, and finally 16.3% in 125 µg concentration.

These results show that *Diadema setosum* shell extract concentration of 1000 µg, 500 µg, 250 µg, and 125 µg do not exhibit cytotoxicity since the fibroblast cell death caused was still below 50%. On the other hand, there was more than 50% cell death in 2000 µg concentration (57.05%) and hence this particular shell extract concentration is considered cytotoxic to fibroblast cells. Sea urchin shells and spikes contain toxic active substances like polyhydroxy and apelastroside A and B and the increase of these substances dissolved in the cell media results in its increasing cytotoxicity property. Polyhydroxy compound contains highly polar phenol groups which form polar bonds with cells' lipoprotein, resulting in the substance accumulation and followed with cell membrane lipid disintegration. This disintegration disrupts cell permeability and causes fibroblast cells to swell and finally burst, resulting in the eventual cell death. Therefore high phenol concentration is more cytotoxic than substituted lower ones and this explains the cell death that exceeds 50% in shell extract concentration of 2000 µg.

On the other hand, *Diadema setosum* shell also contains advantageous phenol compound like *polyhydroxyl naphtoquinone* which has the same composition as *echinochrome A*. Phenol compound acts as antioxidant that scavenges ROS (*Reactive Oxygen Species*), which are pathogenesis causing endogenous and/or exogenous free radicals. These free radicals oxidise cell membrane lipoprotein and cause tissue damage, eventually leading to cell death. Whereas antioxidant is needed by the body to stabilise and stop the formation of free radicals as well as preventing damage caused by the oxidative stresses on protein, lipid, and normal cells.

Diadema setosum shell extract also contains calcium and magnesium minerals. In this study, we found 0.048% b/b calcium ions in the shell extract as compared to the 21.6% b/b measured in shell without the extraction process where the use of the ethanol containing substance may possibly cause the decrease in the calcium ions composition. The human body contains as much calcium as 2% of its total body weight and calcium itself has an important role in the organism's physiological and biochemical processes. Calcium ions are involved in the basic processes in the body like regulation of the cell membrane electric potential, DNA synthesis, enzyme activities, photosensory and chemosensory transductions, neurotransmitters release, membrane permeability, and intercellular communication. Calcium ion is one of the various *second messengers* that mediate cellular responses for a variety of stimuly like proliferation, movement, secretion, and cell neurotransmission. It enters the cell by diffusing through calcium *channels* on the plasma membrane, where the calcium ions move from the region with high concentration to the low concentration.

Beside intracellularly, calcium ions are also found in the tooth enamel and dentine in the form of big and compact hydroxyapatite crystals ($Ca_{10}(PO_4)_6(OH)_2$) which together with carbonate, magnesium, sodium, potassium, and other ions are embedded in strong, almost insoluble protein fibers and constitutes enamel. These crystallized salts make enamel even harder than dentine, but dentine contains calcium salts which makes it very resistant to compression and collagen fibers which contributes to dentine's higher tensile strength.

CONCLUSION

From this study, we found variable cytotoxicity results with *MTT assay* using *Diadema setosum* in differing concentrations,

where concentrations of 125 µg, 250 µg, 500 µg, and 1000 µg do not show cytotoxicity as evident in cell death below 50% as compared to 2000 µg which therefore translates to this concentration being cytotoxic. The percentages of the cell death with respect to the concentrations are 125 µg (16.3%), 250 µg (18.05%), 500 µg (33.75%), 1000 µg (42.917%), and 2000 µg (57.05%).

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CYTOTOXICITY TEST OF DIADEMA SETOSUM SHELL EXTRACT AGAINTS FIBROBLAST CULTURE CELL

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BACKGROUND

Calcium is one of content of sea urchin shells. Calcium is basic ingredient of calcium hydroxide which is used in dentistry as pulp capping material. Sea urchin shells have possibility to be used as a new biomaterial in dentistry, but previously had a cytotoxicity assay test to determine toxic effects before applied. The research purpose is to find out the cytotoxicity of Diadema setosum shell extract against fibroblast cell cultures.

MATERIAL & METHODS

(Diadema Setosum)

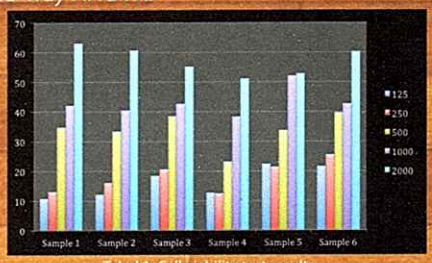
(Freeze Dry Result of Diadema Setosum Shell)

(Etanol Extract Result of Diadema Setosum Shell)

(Cytotoxicity Test of Diadema Setosum Shell using MTT Assay and ELISA Reader)

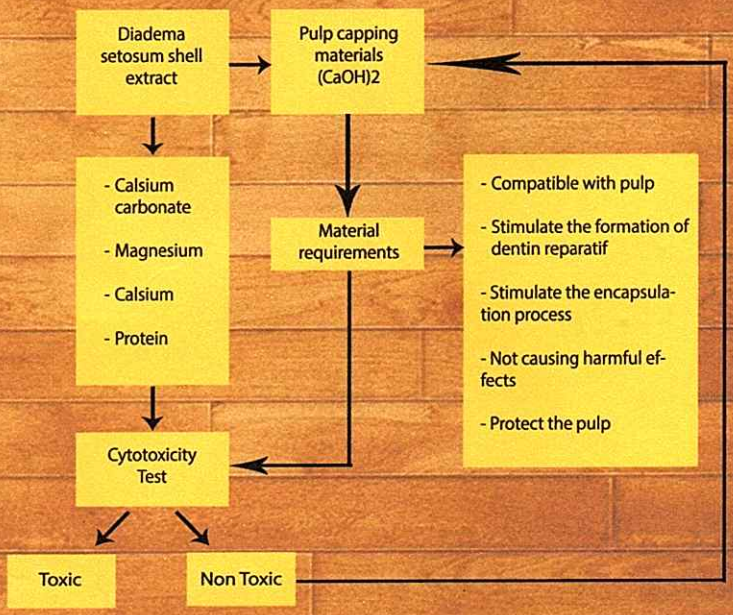
RESULTS

Data indicate decreased cell viability in all treatment groups, level of concentration of the extract by Diadema setosum shell, that is 125 µg(83.7%), 250 µg(81.9%), 500 µg(66.25%), 1000 µg(57.08%), 2000 µg(42.95%). There is a significant difference (p=0.000) in all treatment groups after analyzed by using One-way ANOVA.



Tabel 1. Cell viability test results

DISCUSSION



Calculation of viability cell based optical density value from ELISA Reader shown at high concentration (2000 g) occured increasing death cell (57,05%).

The dead cell caused by toxicity content dissolved of extract. The extract content polyhydroxil and apelasterosida A & B had known has toxicity it caused permeability cell disruption and swelling cell then caused dead cell.

Differentiation about the calcium result of extract and without extraction caused by etanol content of materials. Etanol could decreased calclum of materials.

CONCLUSION

Diadema setosum shell extract has toxicity effect of fibroblast cell at the highest concentration from this research.

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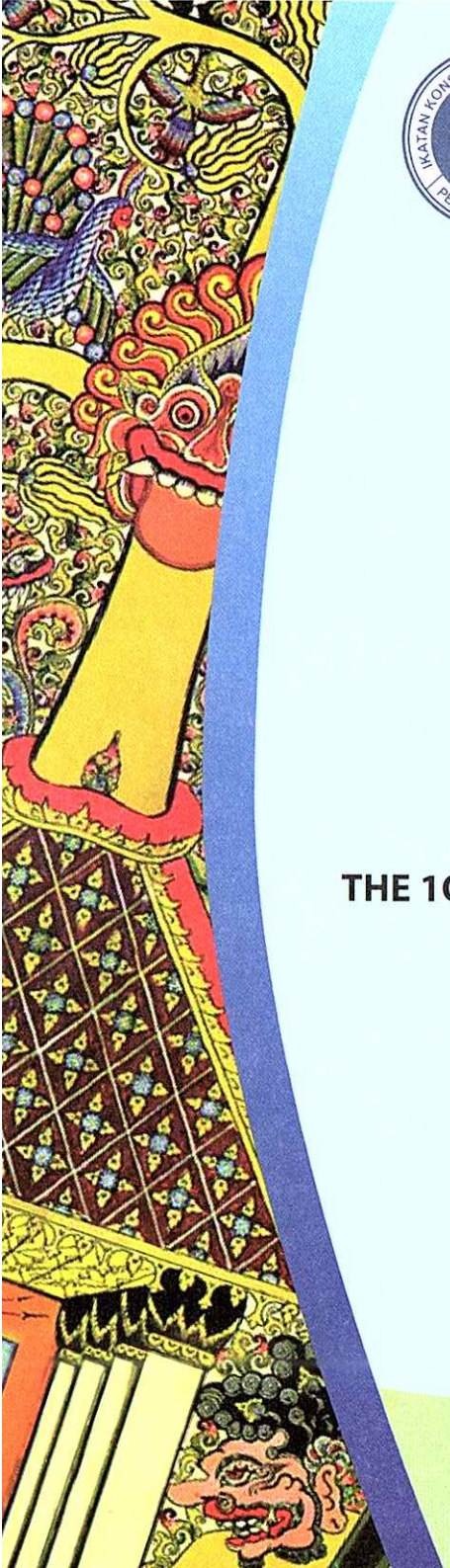
TNI III



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