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CYTOTOXICITY AND GENOTOXICITY EVALUATION OF GADOLINIUM (III) CHLORIDE TOWARDS V79-4 FIBROBLAST CELL LINE

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Background:

Rare earth mineral are of the lanthanide series and has been widely used in the field of medical and clinical application. The most preferred rare earth minerals used are the elements gadolinium (Gd) which acts as magnets, superconductors and magnetic resonance imaging (MRI) contrast. With increasing production of waste from Gd, toxicity of this element and its Material Safety Data Sheet (MSDS) were of interest for the prediction and possible risk to human health.

Methods:

Cytotoxicity evaluation was conducted using MTT assay of $GdCl_3$. Two genotoxicity assay were used in the study: i.e. clastogenicity which employed the In vitro micronucleus assay of $GdCl_3$ without metabolic activation where two treatment time were used which were the short treatment (3 hours) and long treatment (24 hours) times. On the other hand, for the DNA damage evaluation the Alkaline Comet assay of $GdCl_3$ without metabolic activation were conducted with both time point.

Results:

Based on the MTT assay, $GdCl_3$ showed no cytotoxicity at the highest concentration (1mM) used. Hence, three analysable concentrations (0.25 mM, 0.5 mM & 1.0 mM) were selected for both genotoxicity assay. $GdCl_3$ showed no significant DNA damage. However, it induces significant ($p<0.05$) clastogenic effect towards the cell line in the concentration of 1.0 mM for both time points.

Conclusion: $GdCl_3$ does not induce cytotoxicity and DNA damage at concentration of 0.25 mM, 0.5 mM and 1.0 mM. However, $GdCl_3$ is a probable clastogen. Further studies are needed to investigate further effect of free gadolinium ion (Gd^{3+}) for risk assessment on human health.

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INVESTIGATION OF CYTOTOXICITY AND ANTI-OXIDANT EFFECTS OF *Clinacanthus nutans* AQUEOUS AND METHANOL EXTRACTS ON GLIOMA IN VITRO

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Background:
Glioma is a primary brain tumor derived from glial cells. Current standard therapies available are either invasive, non-specific or very aggressive, urging the need for an alternative treatment that is not only effective but is safer to use. *Clinacanthus nutans* has been utilized in different regions of South East Asia as a source of traditional medicine for treatment of several health-related diseases and has been reported to possess bioactive compounds that may act as potential anti-cancer agents. The objective of this study was to determine the cytotoxicity and anti-oxidant effects of *C. nutans* on human brain glioma cell line (DBTRG-05MG) and human normal glial cell line (SVG p12).

Methods:
Active compounds from *C. nutans* were extracted using two different solvents (aqueous and methanol). The effectiveness of both extracts were investigated through methods of MTT assay, Western blot (determination of apoptotic caspase 3 expression level) and radical scavenging assay (determination of anti-oxidant activity).

Results:
The findings showed that only the aqueous extract exhibited cytotoxic effects on the glioma cells (IC_{50} = 53.3 ± 2.1) with expression of apoptotic caspase 3 protein without affecting the glial cells, proving its selective cytotoxicity. On the other hand, the methanol extract showed no cytotoxicity effects on both cancerous and normal cells. Taken together, this showed that aqueous extract could be a more suitable anti-cancer agent compared to methanol extract. However, for the radical scavenging assay, methanol extract showed significant anti-oxidant activity (83.3% ± 2.1) as compared to aqueous extract (31.1% ± 1.3). Besides, the yield from the methanol extract (20.5%) was twice greater than the aqueous extract (11.1%).

Conclusion:
The findings in this study demonstrated that the usage of different solvents during extraction procedure could affect the quantity and quality of yield. In conclusion, *C. nutans* aqueous extract showed potential in inhibiting cancerous brain cell growth while *C. nutans* methanol extract proved to be a better candidate for obtaining higher yield of extract and antioxidant activity.

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CYTOTOXICITY AND GENOTOXICITY ASSESSMENT OF ISOLATED PHYTOCHEMICALS FROM Macaranga heynei ON THE HUMAN COLORECTAL ADENOCARCINOMA CELL LINE HT – 29 AND NORMAL COLON CELL LINE CCD – 18CO

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Background:

The plants from the genus *Macaranga* were widely used by the traditional healers as an alternative medicine for varieties of illness. Studies were also done to evaluate medicinal values contained in this genus such as anti-inflammatory, antioxidants and anticancer properties. As the most common cancer in Malaysia, colorectal adenocarcinoma is also one of the most drug resistant cancer that requires combination of chemotherapeutic drugs for its treatment modulation. Therefore, this study was conducted to determine the cytotoxicity and genotoxicity potentials of isolated phytochemicals from *Macaranga heynei* on the colorectal adenocarcinoma and normal colon cell lines.

Methods:

Six Isolated phytochemicals were screened for their IC_{50} values using Sulforhodamine B (SRB) assay for 48 hours of treatment time on both HT-29 and CCD-18Co cell lines. The IC_{50} of the most potent phytochemical was chosen for further testing on HT-29 cell line. The mode of cell death was determined using annexin V – FITC/PI flow cytometry assay with the time points of 4, 24 and 48 hours. Genotoxicity was assessed using the Alkaline Comet Assay with the time points of 30 minutes, 1, 4 and 24 hours.

Results:

Only one of the six phytochemicals known as "Laevifolin A" gave an IC_{50} values towards both cell lines. The IC_{50} value for HT-29 is 21.20µM. On the other hand, the IC_{50} value for CCD-18Co is 59.50µM. Although the mode of death is observed in HT-29 cells but there are no significant apoptotic activities ($p>0.05$) were seen even up to 48 hours of treatment. Genotoxicity assessment on HT-29 cell following 24 hours of treatment gave a significant ($p<0.05$) DNA damaging activities based on their tail moment and tail intensity values.

Conclusion:

"Laevifolin A" exhibits traits of anticancer agent via its cytotoxicity and genotoxicity potential on colorectal adenocarcinoma cell line. Further studies are needed to evaluate the mechanism of action such as induction of oxidative stress for its to exert its anticancer properties.

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ANTI-CANCER EFFECT OF *Moringa oleifera* LEAVES EXTRACT ON TRIPLE NEGATIVE BREAST CANCER CELL LINE (MDA-MB-231)

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Moringa oleifera had been used by old folks during the ancient times in many ways either in healthcare, medication and as foods. Many studies done showed that this plant had a significant therapeutic effect such as anti-microbials, anti-cancer, anti-diabetic, anti-inflammatory and antioxidants effects. This study was done to determine the anti-cancer effect of *M. oleifera* leaves extract on triple negative breast cancer cell lines which is MDA-MB-231. The anti-cancer features involved in this study was cytotoxic testing and apoptosis activity. Cytotoxic testing was determined by methyl thiazolyl tetrazolium (MTT) assay using plate reader while apoptosis activity was determined by Annexin V kit using flow cytometer. For cytotoxic testing of *M. oleifera*, the best IC_{50} were 12.834 µg/ml and 13.124 µg/ml for both 24 and 48 hours respectively. Thus, at those concentration, *M. oleifera* extract can inhibits the viability of cell by 50%. For apoptosis activity, the best concentration which induced the highest percentage for total apoptosis activity was at 50 µg/ml by 11.71%. Thus, it indicated that *M. oleifera* extract was able to initiate the migration of phosphatidyl serine on the extracellular membrane. The function of phosphatidyl serine in the apoptosis pathway is for cell signalling. In conclusion, *M. oleifera* extracts showed a significant cytotoxic effect and apoptosis activity. Thus, *M. oleifera* can be used as an alternatives medication which produced minimal side effects compared to current treatment which is chemotherapy.

Keywords: *Moringa oleifera*, Cytotoxic testing, Apoptosis activity, IC_{50}, MTT assay, Annexin V kit, Flow cytometer, MDA-MB-231.

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MicroRNA as a Potential Biomarker in Diagnosis and Prognosis of Hodgkin's Lymphoma: A Systematic Review

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Background:
Hodgkin's lymphoma (HL) is derived from germinal centre (GC) which shows somatically mutated clonally rearranged immunoglobulin genes. Research to provide a comprehensive study for HL targeted therapies is emerging throughout the years. However, there are still relapses cases being reported. MicroRNAs (miRNAs) were proven by many studies to have an important role in cancer pathogenesis. It is substantially expressed in most cancer types and the expression level is believed to be useful in diagnostic and prognostic predictions. Although there were specific miRNAs identified for HL in previous studies, its significance in clinical outcome is yet to be defined. To assess the true value of miRNA as biomarker in Hodgkin's lymphoma, we conducted a systematic review to collect and evaluate the current knowledge of miRNAs functioning as diagnostic and prognostic biomarkers in Hodgkin's lymphoma.

Methods:
A systematic literature search was conducted to retrieve relevant articles from different available databases including "PUBMED", "SCOPUS", "PROQUEST", "EMBASE" and "RESEARCH GATE" pertaining to the objective of the study, up to March 2018. The keywords used were 'Hodgkin's lymphoma', 'microRNA', 'prognostic biomarker' and 'diagnostic biomarker'. The literature search identified a total of 1362 articles from which a total of 101 articles resulted after reviewing the title relevant to the topic. From the total of 101 articles, duplicates were removed, abstracts and full text were reviewed and selected based on inclusion and exclusion criteria. The selected articles were assessed using Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) protocol of systematic review.

Result:
The information that will be retrieved from each of selected papers are author, year of the journal, total samples and patients used for the study, type of expressed microRNAs, microRNA expression profiling in diagnostic and prognostic, sensitivity and specificity of the microRNAs (if available). This study is expected to cover as many articles pertaining to the role of microRNAs in the diagnosis and prognosis in HL published worldwide.

Conclusion:
By having a clear review of the potential of miRNAs as diagnostic and prognostic biomarkers, it is possible to help clinical experts to treat patients with Hodgkin's lymphoma based on individualized treatment which fulfil the treatment needs of each patients based on their predicted response.

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PHYTOCHEMICAL SCREENING AND TOXICOLOGICAL ANALYSIS OF POGONATUM CIRRATUM EXTRACTS ON CCL-119 HUMAN ACUTE LYMPHOBLASTIC LEUKEMIA CELL LINE

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Background:

Acute lymphoblastic leukemia (ALL), one of the most notorious cancers of the childhood is characterized by impaired differentiation, proliferation, and accumulation of lymphoid progenitor cells in the bone marrow and extramedullary sites. However, because of various harmful side effects associated with current treatment regimen of ALL, natural products has become the focus of many studies searching for alternative anticancer agents. Bryophyte mosses that possess medicinal values have been neglected for a long time. Thus, this study aims to investigate phytochemicals and cytotoxic activity of *Pogonatum cirratum subsp. microphyllum* extracts on CCL-119 human T-cell acute lymphoblastic leukemia cell line.

Method:

Pogonatum cirratum was extracted using three types of solvents: aqueous, methanol and petroleum ether. Each extracts were then subjected chemically to phytochemical screening of phenol, flavonoid, alkaloid, tannin, coumarin, saponin, terpenoid and glycoside. CCL-119 cells were treated with aqueous, methanol and petroleum ether extracts at different concentrations ranging from 0-200µg/ml for 48 hours. Cytotoxicity effect of the extracts against CCL-119 ALL cell line was assessed using MTT assay. Dexamethasone was used as positive control.

Results:

The percentage yield from 100g of plant in aqueous, methanol and petroleum ether extracts are 1.24%, 6.27% and 0.34% respectively. The tested phytochemicals from the extracts are selectively soluble in varying polarities of solvents used. Cytotoxicity effect is shown to be exerted by *Pogonatum cirratum* on CCL-119 cells with aqueous extract being the most potent followed by methanol and petroleum ether.

Conclusion:

This is the first report on cytotoxic effect of *Pogonatum cirratum* in cultured leukemia cells, which provides scientific basis for its usefulness as potential chemotherapeutic agent.

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INVESTIGATING THE EFFECT OF ANDROGRAPHOLIDE ON THE PROTEIN EXPRESSION OF HMGCR AND PPARɣ IN OESTROGEN RECEPTOR (ER) POSITIVE BREAST CANCER CELL LINE

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Background:

Luminal type A is a type of breast cancer that expresses high level of ER (Oestrogen receptor) and PR (Progesterone receptor), and the most common type of breast cancer among women worldwide. Despite having a good prognosis of treatment, the emergence of Tamoxifen resistance limits the progress. Nowadays, Andrographolide has been suggested as an alternative way to treat breast cancer. Therefore, we aim to study the effect of Andrographolide on the growth of ER-positive breast cancer cells as well as the expression of HMGCR and PPARɣ.

Methods:

ER-positive breast cancer cell lines, MCF-7 were treated with Andrographolide compounds at different dose and time points. The Trypan blue exclusion dye viability assay was used to monitor the effect of Andrographolide in inhibiting the proliferation of MCF-7 cells. Western blot analysis was conducted to observe the effect of Andrographolide on the protein expression of HMGCR and PPARɣ.

Results:

In dose dependent treatment, Andrographolide significantly decreases cell viability at 50 µM and 100 µM of concentration. In addition, treatment with Andrographolide significantly decreases cell viability at acute response (Treatment at 4 hours) and prolonged response at (Treatment at 24 and 48 hours). We also noted that increasing Andrographolide concentration resulted in HMGCR downregulation and PPARγ upregulation.

Conclusion:

The results show that Andrographolide inhibits the proliferation of ER-positive breast carcinoma by disrupting the mevalonate pathway through the inhibition of the HMGCR activity that is crucial for lipid metabolism and acts as a ligand to activate and enhance the PPARɣ signalling pathway protein. To conclude, our findings suggest that Andrographolide may have potential to act as an anti-cancer agent in combatting ER-positive breast cancer progression.

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Metabolic role of glucose and insulin on endometrial cancer cells proliferation

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Background:

Endometrial cancer is rated as the 4th most common cancer among women in Malaysia. Type II diabetes mellitus, a metabolic disorder characterized by hyperglycemia, has recently been shown to double the risk for endometrial cancer. Altered cellular metabolism is an emerging hallmark of cancer, and drugs targeting cancer metabolism are rigorously being developed, particularly for breast and lung cancer. However, how hyperglycemia affects endometrial cancer cell metabolism and hence progression is still not well understood. Thus, this project aimed to investigate how conditions mimicking type II diabetes mellitus can alter the metabolic phenotype of endometrial cancer and subsequently affects its biology.

Methods:

Cell proliferation of endometrial cancer cells (ECC-1) treated with different glucose concentration (0.625mM to 40mM) was assessed with MTT assay. The metabolic phenotype of ECC-1 cells treated with condition mimicking type II diabetes mellitus was subsequently profiled by measuring the expression of key metabolic genes that involved in glucose metabolism via Real-time Polymerase Chain Reaction (qPCR).

Results:

ECC-1 cell proliferation increased with increasing glucose concentration. Highest percentage of proliferation is observed when ECC-1 was treated with 10mM of glucose. In 1950, Otto Warburg discovered that cancer cells have altered metabolism, with increased preference for the more oxygenefficient substrate, glucose, to fuel tumour growth. In agreement with this, ECC-1 cells treated with high glucose (25mM) showed an increase glucose uptake by upregulating the expression of glucose transporter 6 (GLUT6) by 2.7 folds. ECC-1 cells also favoured glycolytic metabolism in which there is an upregulation of key glycolytic enzyme, lactate dehydrogenase (LDH). Increased glycolytic flux is accompanied by a reduction in mitochondrial oxidative metabolism, as PDK4, the key enzyme that limits the entry of glucose into the mitochondria, is upregulated. Indeed, lack of oxygen is a hallmark of fast growing cancer cells, and it appears that ECC-1 cells promote anaerobic glucose metabolism to sustain cell growth. This effect of high glucose on endometrial cancer cell proliferation and metabolic alteration is independent of the fatty acids uptake as the gene expression of fatty acids transporter (CD36) is downregulated.

Conclusion:

In conclusion, under conditions mimicking hyperglycemia, ECC-1 cells have altered metabolism associated with increased anaerobic metabolism, which potentially promotes endometrial cancer cell proliferation.

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THE EFFECT OF Clinacanthus nutans EXTRACTS ON THE PROLIFERATION AND MIGRATION OF BREAST CANCER CELL LINES

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Background:

Breast cancer is the most frequent cancer in women (28.8% of all cancers), the second most common in the world with an estimated 1.67 million new cases diagnosed in 2012. Genetic alteration cause abnormal regulation of a single cell, which then proliferate, leading to the outgrowth of a population of clonally derived tumour cells. The ability of a cancer cell to undergo migration and invasion allows it to change position within the tissues and spread to distant organs. Current treatment do cures but they give unwanted effects as they not only kill fast-growing cancer cells, but also kill or slow the growth of healthy cells. Previous study shows that *Clinacanthus nutans* (CN) extracts possess antioxidant properties against cultured cancer cell lines, thus it could be used as an alternate adjunctive regimen for cancer prevention. Therefore, in this study, we aimed to investigate whether CN extracts possess anti-cancer effects on breast cancer cells proliferation and migration ability by using MDA-MB-231 and MCF-7 cell lines as models.

Methods:

Clinacanthus nutans were extracted using methanol and aqueous extract by reflux and filter paper method. Proliferative effect of CN-methanol and CN-aqueous extracts on breast cancer cells; MCF-7 and MDA-MB-231 proliferation were studied using trypan blue exclusion assay. Migratory ability of CN-methanol and CN-aqueous extracts on both breast cancer modelswere studied using scratch wound migration assay. Data were analysed using Microsoft Excel 2010.

Results:

Percentage yield of CN-methanol and CN-aqueous extract were 29.00% and 52.84% respectively. Both extracts shows no significant different towards breast cancer cell proliferation with trypan blue exclusion assay. Similarly, with scratch wound migration assay, no significant effect was observed on the migratory ability of MDA-MB-231 and MCF-7.

Conclusion:

In the in vitro model, CN-methanol and CN-aqueous extract have no significant effect on MDAMB-231 and MCF-7 cell proliferation and migration.