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Micronucleus Assay on Crude Petchay (*Brassica rapa chinensis*) Extract: Preliminary Study on its Cancer Chemopreventive Potential

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Abstract

Crude *Brassica rapa chinensis* extracts were tested for mutagenic and/or anti-mutagenic properties as preliminary investigation for possible cancer chemopreventive potentials. Results from micronucleus assay involving human lymphocytes showed that the ratio between the normal cells and micronucleated cells or the mutated cells were low at those cultured cells with crude Petchay extract and Mitomycin-C. This suggests that glucosinolates in the crude Petchay extract are potential anti-mutagenic compounds in human lymphocytes. Also female lymphocytes had higher micronucleated cell count than those of males. These results suggest that glucosinolates may potentially prevent cancer from proliferating in the human body.

Keywords: micronucleus assay, brassica rapa chinensis, cancer chemoprevention, mutagenic

Introduction

Glucosinolates are S- and N-containing secondary metabolites that are abundantly found in plants belonging to the *Brassicacaea* (Cruciferae) genus and posses chemopreventive agents.

In the Philippines the popular Brassica plant that is generally included in the Filipino diet is "Petchay" *(Brassica rapa chinensis)* because it is abundant and relatively cheap in the local wet market.

Crude petchay extract was investigated for mutagenic and anti-mutagenic potential by comparing it against Mitomycin-C, a known mutagenic drug, through micronucleus assay in human lymphocytes. The mutagenic and anti-mutagenic potential of the crude petchay extract is associated with glucosinolates since their hydrolysis products are capable of modulating biotransformation of enzyme activity.

This research provides new information on crude petchay extract that adds to the growing scientific knowledge on them. Moreover, this could validate preconceived beliefs on the cancer chemoprevention of vegetables such as petchay. This also aims to substantiate the issue regarding differences in susceptibility of females and males to mutation.

Materials and Method

Collection of blood samples and Plant Source and Extraction

The blood samples that were used in this experiment were from four (4) volunteers, two males and two females. The Petchay used were obtained from the Masinag market in Antipolo. Both blood and plant samples were freshly obtained for every trial of the experiment.

Culture Tubes Preparation and Intervention

The medium was prepared by mixing 100mL of Roswell Park Memorial Institute medium (RPMI) with 20mL fetal bovine serum, 1.3mL Penstrepp and 1.3 mL L-glutamine. Each of the six culture tubes per trial and per human subject was filled with 4.5mL of the medium, 0.5mL of human blood and 0.1mL of Phytohemagglutinin. The tubes were mixed thoroughly and stored in the cell incubator at 37ºC. The six culture tubes were labeled F1 1-6, F2 1-6, M1 1-6, and M2 1-6 per trial. F1 and F2 correspond to the blood samples from the first and second female volunteer, respectively, while M1 and M2 correspond to the blood samples from the first and second male volunteers.

Harvesting

After 72 hours of incubation the culture tubes were removed from the incubator and were centrifuged for 10 minutes at 1000 revolution per minute (rpm) and 25^{o}C. Afterwards, the supernatants were removed with the use of Pasteur pipettes and 6.0mL of pre-warmed 0.075M HCl (prepared from 5.60 grams of KCl pellet dissolved in 1 liter of distilled water) portions were added, followed by thorough mixing. The solutions were immersed in a water bath for 20 minutes at 37^{o}C followed by the addition of 1.0mL of Carnoy's fixer (1 CH_3COOH: 3 CH_3OH) per culture tube. The tubes were again centrifuged for 10 minutes at 1000rpm and 25^{o}C then the supernatant again removed. The cells in each culture tube were re-suspended by the addition of 5mL Carnoy's fixer then again mixed and centrifuged. This process of centrifugation, removal of supernatant and addition of 5mL Carnoy's was repeated until a white precipitate was obtained per culture tube.

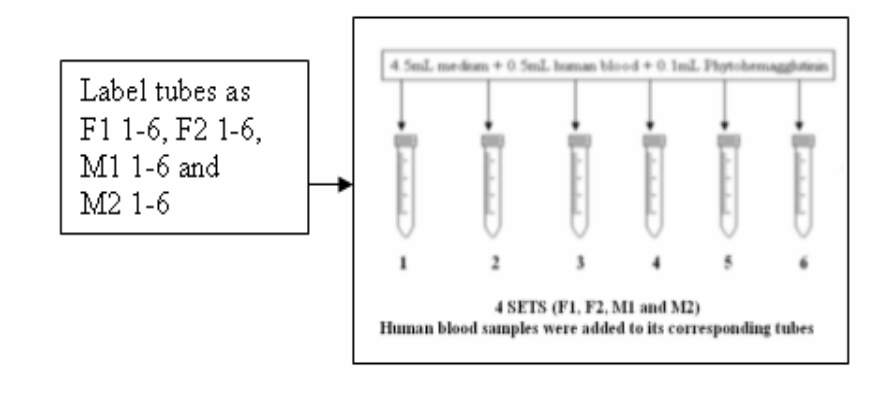

Figure 1 Schematic diagram of culture tube preparation

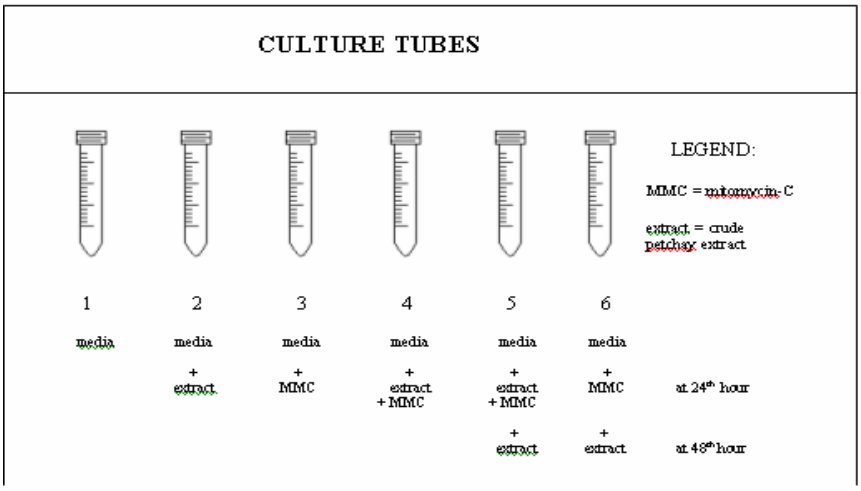

Figure 2 Content of culture tubes and their time of interventions

Slide Preparation and Scoring

After harvesting the samples were prepared onto slides with their respective labels and were kept for scoring. For a cell to be counted as a micronucleated cell it should possess the following:

1. It is colored violet with the same intensity as the normal cells.
2. Its size is about 1/3 of a normal cell
3. It is located between two normal cells and still contained in the cytoplasm.

Results and Discussion

The experimentation for this research required three trials for the four sets of culture tubes (one per subject), which consists of six different treatments. The number of normal cells and micronucleated cells that were obtained from all culture tubes in three trials are shown on the Table 1.

All Tube 1's had no micronucleated cells. Tube 2's had micronucleated cells ranging from 0-4. This result implies that crude Petchay extract is a weak mutagen. Tube 3's generally had the highest number of micronucleated cells among all the cultured cell samples ranging from 4-25. This also was an expected result because Mitomycin-C is a strong mutagen. Tube 4's also had small numbers of micronucleated cells ranging from 0-5. This is interesting because even with the presence of Mitomycin-C the number of micronucleated cells were very small. Recalling the result from culture tubes numbered as 3, Mitomycin-C produced high number of micronucleated cells. The crude Petchay extract may have affected the results of the #4 tubes. Thus, one of two scenarios may be working here:

1. Crude Petchay extract totally disallowed the Mitomycin-C from producing micronucleated cells at the onset so that it was the only one that was producing micronucleated cells and not the mutagenic drug.
2. Mitomycin-C was able to produce micronucleated cells. However, the crude Petchay extract eventually lowers the ratio of micronucleated cells to normal cells.

Table 1 Ratio of normal cells to micronucleated cells per culture tube for each sample set in three trials.

		F1	F2	M1	M2
Trial 1					
Tube #	CONTENTS				
1	media	4507: 0	4943: 0	8478: 0	4712: 0
2	media + extract	4100: 0	3937: 0	4862: 0	3627: 0
3	media + MMC	2584:12	3075: 5	5324:10	5939:12
4	Media + extract + MMC	5333: 3	4983: 0	5940: 1	4302: 5
5	media + extract&MMc@ 24th hour + extract@48th hour	4253: 3	3231: 2	5459: 7	4844: 2
6	media + MMC@28th hour + extract@48t h hour	3334: 2	3910: 1	4557: 3	3515: 3
Trial 2					
Tube #	CONTENTS				
1	media	2969: 0	1963: 0	3544: 0	2975: 0
2	media + extract	897:4	4394: 0	3478: 1	1194: 0
3	media + MMC	1365:25	1224: 7	2103: 5	1305: 9
4	Media + extract + MMC	1036: 0	1787: 3	1893: 0	1542: 2
5	**Trial 2**			1465: 2	2225: 2
6	media + MMC@28th hour + extract@48t h hour	1667: 0	3124: 2	1445: 1	1209: 1
Trial 3					
Tube #	CONTENTS				
1	media	1984: 0	2316: 0	2561: 0	1841: 0
2	media + extract	1225: 1	2104: 0	2950: 2	2789: 1
3	media + MMC	1041: 4	2230: 7	2007: 6	2239: 9
4	media + extract + MMC	1111: 0	1653: 1	2002: 0	1796: 1
5	media + extract&MMc@ 24th hour + extract@48th hour	1461: 1	1698: 2	1791: 1	956: 1
6	media + MMC@28th hour + extract@48t h hour	1073: 1	1770: 2	2637: 1	1747: 0

Tube 5's all had micronucleated cells ranging from 1-7. The second intervention of crude extract may have caused the number of micronucleated cells to increase.

Tube 6's had micronucleated cells ranging from 0-3. The results of tubes 5's and 6's are almost the same therefore the possible differences affected by time of intervention seem to be irrelevant.

These data all seem to consistently validate the second scenario. That is, the crude Petchay extract significantly lowers the ratio of micronucleated cells/normal cells when present in a culture with a strong mutagen in this case Mitomycin-C.

According to the results of culture tubes numbered as 6, the ratio of micronucleated cells to normal cells is lower than that of the results of culture tubes numbered as 3. Both cultures were intervened with Mitomycin-C after the 24th hour but the results are very much different because of the intervention of crude Petchay extract after the 48th hour for the culture tubes numbered as 6. Micronucleation is not reversible and so crude Petchay extract could not have turned the micronucleated cells produced due to the Mitomycin-C into normal cells again. Rather it may have increased the number of normal cells while keeping the production of micronucleated cells very low once it was introduced to the culture. The proponents also aimed to know the difference on the pattern of mutagenicity between females and males.

Table 2 separates the average counts and ratio of the female and male subjects per culture tube in three trials.

Most of the ratios for females are higher than the ratios of male except for Trial 1 culture tubes 4, 5, 6; Trial 2 culture tube 6 and Trial 3culture tubes 2 and 3. 66.7 % of all samples showed that females have higher ratio of micronucleated cells to normal cells.

Table 2 Average female and male normal cell count, micronucleated cell count and micronucleated cell/normal cell ratio

		F1	F2	M1	M2
Trial 1					
Tube #	CONTENTS				
1	media	4507:0	4943:0	8478:0	4712:0
2	media + extract	4100:0	3937:0	4862:0	3627:0
3	media + MMC	2584:12	3075:5	5324:10	5939:12
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6	media + MMC@28th hour + extract@48th hour	1667:0	3124:2	1445:1	1209:1
Trial 3					
Tube #	CONTENTS				
1	media	1984:0	2316:0	2561:0	1841:0
2	media + extract	1225:1	2104:0	2950:2	2789:1
3	media + MMC	1041:4	2230:7	2007:6	2239:9
4	media + extract + MMC	1111:0	1653:1	2002:0	1796:1
5	media + extract&MMc@24th hour + extract@48th hour	1461:1	1698:2	1791:1	956:1
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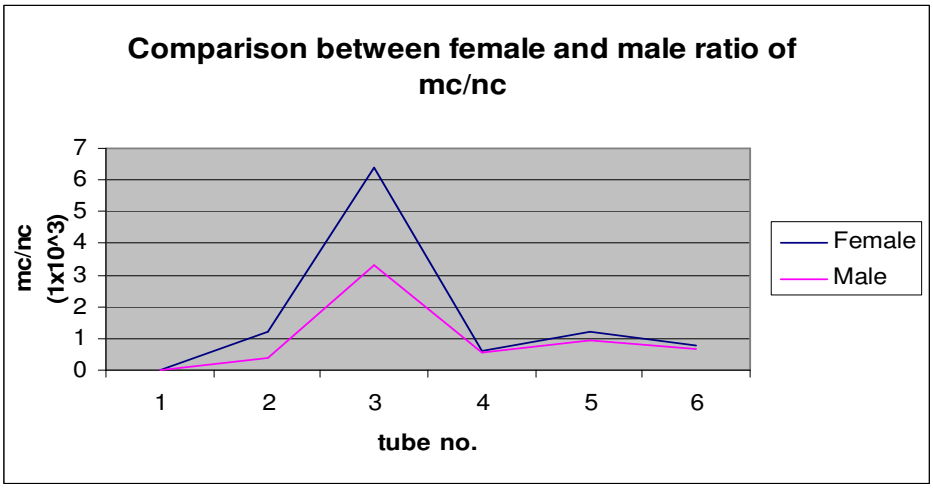

Figure 3 Comparison between female and male ratio of micronucleated cells over normal cells

Table 3 Average ratio of the number of micronucleated cells over the number of normal cells for the three trials.

Tube #	Female (1x10^3)	Male (1x10^3)
1	0	0
2	1.21	0.375
3	6.42	3.28
4	0.607	0.565
5	1.24	0.926
6	0.793	0.651

In examining table 3 and the figure 3 it can be inferred that females have higher chance of acquiring mutations than males since all average ratio of females are higher than that of the average ratio of males.

Time and dose dependency can also be measured in this experiment and next table will show the results. Tube 4 vs 5 – dosage dependent and Tube 4 vs 6 – time dependent.

Taking into account time-dependency it can be observed that the ratio of tubes numbered as 6 are higher than those of the tubes numbered as 4. Crude petchay extract was cultured for 48 hours in tubes numbered as 4 while in tubes numbered as 6 it was only for 24 hours. Therefore the shorter time crude petchay extract is introduced into the matrix, the more micronucleated cells are obtained.

In observing the amount of crude petchay extract introduced to the culture tubes, culture tube numbered as 6 had the greatest amount of the crude petchay extract that resulted to the increase in the ratio of micronucleus over normal cells in the culture.

Conclusions

Crude Petchay extract have exhibited low mutagenic potential when alone but when mixed with a strong mutagen like Mitomycin-C, it shows evidence that it acts as an anti-mutagen and lowers the mutagenic ratio produced by Mitomycin-C. However, the longer time the extract is present or the more cycles it undergoes the more potent it becomes. This seems to be achieved by means of generating relatively large numbers of normal cells and at the same time generating less micronucleated cells, since micronucleation is not reversible. In terms of time, the ratio decreases as introduction of the extract is delayed while in terms of dosage, the ratio increases as the dosage is increased. The average ratio of micronucleated cells/normal cells is higher in females than males, which suggests that acquiring mutations are highly probable in females. This is due to the fact that females exhibit aggressive cell cycle and is susceptible to cancer. The results indicate that Petchay may act as an anti-mutagen and having it in our regular diet may help us prevent having cancer.

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