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DNA Damaging Effect of Selected Salted and Fermented Food Products against Chang Liver Cell

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ABSTRACT

DNA damaging effect of the salted and fermented food products (salted fishes, dried shrimps and shrimp pastes) collected from three different locations in Malacca namely Pantai Puteri, Batang Tiga and Kelemak on the DNA of the Chang liver cells were evaluated via Alkaline Comet Assay. Treatment at 62.5 mg/ml following 24 hours of incubation was used based on the preliminary cytotoxicity data. Percentage of damage to the DNA was calculated using software for scoring based on the tail moment and tail intensity (severity of the DNA damage). Hydrogen peroxide was used as positive control at 0.1 mM following 30 minutes of incubation in 4 C. The results showed that the methanol extracts of shrimp pastes and salted fish from Pantai Puteri, exhibited a higher DNA damage (shrimp pastes - TM – 8.33 ± 2.19; TI – 31.67 ± 5.84, salted fishes - TM – 2.25 ± 0.86; TI – 9.25 ± 1.55) and were expressed as (shrimp pastes) 56.66 ± 8.74% of DNA damage and methanol salted fish extracts from the same location showed 13.00 ± 2.84% of the DNA damage on Chang liver cells compared to the other extracts. Values for methanol extract of shrimp pastes from Pantai Puteri were comparable to the positive control – Hydrogen peroxide (TM – 9.50 ± 1.50; TI – 30.50 ± 2.50). On the other hand, aqueous salted fishes extract from Pantai Puteri (TM – 1.33 ± 0.42; TI – 8.67 ± 2.42) and shrimp pastes extracts from Kelemak (methanol extract – TM – 1.75 ± 0.15; TI –7.50 ± 0.50, aqueous extract - TM – 1.00 ± 0.00; TI – 5.00 ± 0.00) showed slightly high value for tail moment and tail intensity as compared to negative control (TM – 0.29 ± 0.05; TI – 2.50 ± 0.29). Values for methanol extracts of shrimp pastes from Pantai Puteri were comparable to the positive control (TM – 9.50 ± 1.50; TI – 30.50 ± 2.50). In conclusion, our results demonstrate genotoxic damage induced by few salted and fermented food extracts in Chang liver cell.

Key words: DNA damage, food toxicology, salted and fermented foods, Alkaline Comet Assay

ABSTRAK

Kesan kerosakan DNA produk makanan asin dan terproses (ikan masin, udang geragau dan belacan) yang diperoleh dari tiga tempat berbeza di Melaka

iaitu Pantai Puteri, Batang Tiga dan Kelemak terhadap DNA sel hepar Chang (genotoksisiti) dinilai dengan menggunakan asai komet beralkali. Rawatan pada kepekatan 62.5 mg/ml selama 24 jam digunakan berdasarkan ujian saringan sitotoksisiti yang dijalankan. Peratus kerosakan DNA dikira dengan menggunakan perisian untuk penilaian berasaskan momen ekor (TM) dan kepadatan ekor (TI) yang terbentuk (tahap kerosakan DNA). Hidrogen peroksida digunakan dalam kajian ini sebagai kawalan positif dan rawatan dilakukan pada kepekatan 0.1 mM selama 30 minit pada 4 C. Keputusan menunjukkan ekstrak metanol belacan dan ikan masin dari Pantai Puteri menyebabkan kerosakan DNA yang lebih tinggi (belacan - TM – 8.33 ± 2.19; TI – 31.67 ± 5.84, ikan masin - TM – 2.25 ± 0.86; TI – 9.25 ± 1.55) dan diwakili oleh 56.66 ± 8.74% peratus kerosakan DNA dan ekstrak ikan masin metanol dari tempat yang sama menunjukkan 13.00 ± 2.84% kerosakan DNA pada sel hepar Chang berbanding ekstrak lain. Nilai pada ektrak metanol belacan Pantai Puteri dibandingkan dengan kawalan positif (TM – 9.50 ± 1.50; TI – 30.50 ± 2.50). Sebaliknya, ekstrak ikan masin akuas dari Pantai Puteri (TM – 1.33 ± 0.42; TI – 8.67 ± 2.42) dan ekstrak belacan dari Kelemak (ekstrak metanol – TM – 1.75 ± 0.15; TI –7.50 ± 0.50, ekstrak akuas - TM – 1.00 ± 0.00; TI – 5.00 ± 0.00) menunjukkan nilai momen ekor dan kepadatan ekor yang lebih tinggi berbanding kawalan negatif (TM – 0.29 ± 0.05; TI – 2.50 ± 0.29). Kesimpulannya, keputusan kami menunjukkan keupayaan beberapa ekstrak makanan asin dan terproses yang digunakan yang dapat menyebabkan kerosakan genotoksik terhadap sel hepar Chang.

Kata kunci: kerosakan DNA, toksikologi makanan, makanan asin dan terproses, Asai Komet Beralkali

INTRODUCTION

Diet plays a major role in cancer etiology and prevention. Doll and Peto (1981) had reported the first relationship between diet and cancer. Overall, there have been current available data to support direct relationship between cancer risk and intakes of total fat, alcohol, as well as between obesity and certain food preparation methods such as smoking, salting and pickling foods and high temperature cooking of meats (AICR 1997; Smith & Giovannucci 1999; US Department of Health and Human Service 1996; Ballard-Barbash 1999; Longnecker & Tseng 1999; Martinez et al. 1999; Zhou & Blackburn 1999; Combs & Clark 1999).

These days, many food components were reported to be potentially genotoxic (Manson & Benford 1999). Numerous types of genes are likely to be involved in human carcinogenesis such as genes that could influence the metabolic activation/detoxification, DNA repair, chromosome stability, activity of oncogenes or tumor suppressor genes, cell cycle control, signal transduction,

hormonal pathways, vitamin metabolism pathways, immune function and receptor or neurotransmitter action (Sinha & Caporaso 1999). Thus, to understand how nutrients and other diet-related factors can modulate the carcinogenic process through interactions with various genes is always essential.

Previous study showed genotoxicity effects and maximum chromatid damages caused from salted fishes and meat in India (Taj & Nagarajan 1994). In Japan, a novel type of chemical, 2-chloro-4-methylthiobutanoate was found in salted Japanese fish that act as a powerful direct-acting mutagen (Chen et al. 1996). Interestingly, this chemical induces DNA repair in gastric mucosa, similar to the effect of the classic gastric carcinogen N-methyl-N-Nitro-N-methylnitrosoguanidine (Furihata et al. 1996).

Genotoxicity refers to an adverse effect on the genetic material (DNA) of living cells. Various techniques for detecting DNA damage, as opposed to the biological effects (e.g. micronuclei, mutations, chromosomal aberrations) that results from DNA damage have been used to identify substances with genotoxic activity. However, the Comet Assay or Single Cell Electrophoresis Assay that were introduced by Ostling and Johanson (1984) and modified by Singh and co-workers (1988) have been widely used in the genotoxicological study. Comet assay is often used to identify agents with genotoxic activity. The term 'comet' is used to identify the individual cell migration patterns produced by this assay. Relative to other genotoxicity tests, the advantages of the assay includes its sensitivity for detecting low levels of DNA damage, the requirement for small number of cells, its flexibility, its ease of application and the short duration to complete a study (Tice et al. 2000).

In this study, human Chang liver cell was used to evaluate the toxicity effect of the selected salted and fermented food (salted fishes, dried shrimps and shrimp pastes) collected from three different locations in Malacca. The liver cell was chosen in this study because of its richness in the metabolizing enzymes such as cytochrome P_{450}.

Owing to this major concern, samples such as salted fishes, shrimp paste and dried anchovies are used to determine their toxicity and food composition. Previous study had shown that these food items contained saturated salts and that could also be linked to pathogenesis of cancer (Ghazali et al. 2005).

MATERIALS AND METHODS

EXTRACTION OF FOOD SAMPLES

All the food samples (salted fishes, shrimp pastes and dried shrimps) were collected from three different places in Malacca namely Pantai Puteri, Kelemak and Batang Tiga. These locations are been selected based on the referral from State Fisheries Department, Malacca. At each location, 1 kg of each of the samples were bought and brought to the lab to be processed.

The ground powder (200 g) of the food samples was soaked in 500 ml methanol (99.8%, Chemical Industries, Malaysia) for two days (Saha et al. 2004). Each of the mixtures was then filtered and evaporated using a rotary evaporator (Buchi Rotavapor R-114, Switzerland). As for the aqueous extraction, the ground powder (200 g) of the foods was soaked in 500 ml of distilled water for 24 hours, filtered and freeze-dried (Sakanaka et al. 2005). Both of the extracts were kept at 4°C in an air-tight jar prior to the bioassays.

REAGENT AND CELLS

Human Chang liver cells were obtained from ATCC (Rockville, MD) and cultured as described previously (Ghazali et al. 2005). Cells were grown as monolayers in T-25 cm^2 culture flask. Media were supplemented with 2.0 g/l sodium bicarbonate, antibiotics; 100 units of penicillin/ml, 100 µg of streptomycin/ml and 10% fetal bovine serum (FBS). Cell culture media and their supplements were purchased from Life Technologies, Gibco BRL Products (Rockville, MD). The cell cultures were maintained in a humidified atmosphere of 5% CO_2 at 37°C and were harvested when they reached 80% confluence to maintain exponential growth. The methanol (MetOH) and aqueous extracts of each sample were dissolved in 5% dimethyl Sulphoxide (DMSO) (Chemical Industries, Kuala Lumpur, Malaysia) and media RPMI-1640 (Flowlab, California, USA) to a final concentration of 10 mg/ml. These solutions were then filtered using sterile syringe filter 0.45 µm. Before Comet Assay were carried out, preliminary cytotoxicity measurement with the tetrazolium reduction assay (MTT) was performed to ensure the concentration that had been used in the comet assay does not induce excessive cytotoxicity because DNA damage is commonly associated with cell death.

MTT ASSAY
[3-(4,5-diemethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphenyl)-2H-tetrazolium] cytotoxicity assay (Mosmann 1983)

Human Chang liver cells were used to determine the cytotoxicity effect of each of the foods samples. The cells monolayers in exponential growth were harvested using 0.025% trypsin (10 mM, trypsin-EDTA 0.025% trypsin, 1mM EDTA.4Na) and single cell suspensions were obtained by repeated pipetting. The cells were counted with a hemacytometer and 100 µl of single cell suspensions at 5 x 10^4 cells per ml were loaded into the 96 well plates (Nunclon™, VWR International Inc. MD USA) and incubated for 24 hours at 37°C in a humidified atmosphere of 5% CO_2. For treatment, media was discarded and 200 µl of the test extracts for each samples were loaded into the 96 well plates ranging from high to low concentrations. Treatment at 500 µg/ml was used to determine their cytotoxic effect. After 72 hours incubation, 20 µl of the MTT (Sigma Chemical Co. St. Louis, MO USA) solution was added to each well of 96 well plates and incubated

for 4 hours at 37°C in a humidified atmosphere of 5% CO_2. At the end of the incubation period, the media was discarded and 100 µl of DMSO was added to solubilize the formazan crystals. The plates were incubated for 15 minutes to dissolve the entire crystals formed. The absorbance was measured at 570 nm with a microplate reader. The metabolic activity of the cells was measured after 72 hours of treatment where the MTT salt was reduced to a coloured formazan by the active viable cells with dehydrogenase enzyme.

THE COMET ASSAY (Singh et al. 1988)

Human Chang liver cells were used in this study and seeded at 5 x 10^4 cells per ml (2 ml each well) into 6 well plates (Nunclon™, VWR International Inc. MD) and incubated for 24 hours at 37°C in a humidified atmosphere of 5% CO_2. Media was discarded and MetOH and aqueous extracts of each sample at 62.5 µg/ml were added for 24 h treatment. Negative control contained the media itself without any extract. Hydrogen peroxide at 0.1 nM was used as the positive control. After 24 h treatment, the cells were washed using phosphate buffer saline (PBS) and trypsinized to detach the cells and transferred to three eppendorf tubes for each well. Cells were centrifuged at 2 500 rpm for 5 minutes to obtain the cell pellet. Supernatant was discarded and PBS was added in each of the eppendorf before centrifuged again at 2 500 rpm for 5 minutes to wash out any media residue. Frosted slides were prepared with a layer of normal melting agarose (Sigma Chemical Co. St. Louis, MO, USA). Cells were then suspended in low-melting point agarose (Sigma Chemical Co. St. Louis, MO, USA) maintained at 37°C and placed on the slides coated with normal melting agarose. After the agarose gel had solidified, the slides are placed generally for at least 1 hour in a lysis solution consisting of high salts and detergents (100 mM ethylenediaminetetraacetic acid (EDTA), 2.5 M sodium chloride, 10 mM Trizma base, adjusted to pH 10, with 1% Triton X-100 added just prior to use. Then, slides were incubated in alkaline (pH > 13) electrophoresis buffer for 20 minutes to produce single stranded DNA. The alkaline solution consists of 1 mM EDTA and 300 mM sodium hydroxide. After alkali unwinding, the single stranded DNA in the gel was electrophoresed under alkaline conditions at 25V and 300 mA for 20 minutes to produce comets. The alkaline buffer used during electrophoresis was the same (pH > 13) buffer used during alkali unwinding. Next, the alkali in the gels was then neutralized by rinsing the slides with a suitable buffer (Trizma at pH 7.5) three times for 5 minutes each. The slides were stained using fluorescent dye (ethidium bromide). Slides were analyzed using Leitz Laborlux Epifluoresence Microscope, Germany equipped with 515 barrier filter and 560 emmision filter. Analysis was conducted using software Comet Assay Analysis System, Kinetics, USA. Based on the tail moment and tail intensity, the cells were classified to without DNA damage (tail moment < 5, tail intensity < 10) and cells with DNA damage (tail moment > 5, tail intensity > 10).

STATISTICAL ANALYSIS

All the data were expressed as the mean ± standard error of the mean. ANOVA was used to measure significant differences between the means.

RESULTS

Cell cytotoxicity was evaluated using MTT assay. Treatments at 500 mg/ml with six serial dilutions ranged from 500 mg/ml to 15.625 mg/ml were used. Table 1 and Table 2 showed percentage of cell viability for all the extracts at different concentrations from different locations. At maximum concentration (500 mg/ml), all the extracts showed 10%-30% cell death. Dried shrimp extracts from both area (Batang Tiga and Kelemak) did not show high percentage of cell death with maximum percentage of cell death observed is by aqueous dried shrimp extract from Kelemak (2.29 ± 3.00%). Aqueous shrimp pastes extract from Pantai Puteri caused highest percentage of cell death with 45.26 ± 9.86% followed with methanol shrimp pastes extract from Kelemak with 25.83 ± 12.75%. Generally, all the extracts exhibited reducing trend in percentage of cell viability at different concentrations. Alkaline comet assay was used to evaluate the genotoxicity of every foods extracts by observing the cell's DNA damage. Figure 1 showed the bar chart of the DNA percentage of cells according to the DNA damage scoring categories for each treatment. Basically, all the extracts did not show any severe DNA damage as compared to the negative control except for methanol shrimp pastes and salted fishes extracts from Pantai Puteri. Methanol shrimp pastes extract from Pantai Puteri caused 56.66 ± 8.74 % of DNA damage (tail moment > 5) and methanol salted fish extracts from the same location showed 13.00 ± 2.84% (tail moment > 5) of the DNA damage on Chang liver cells. The differences is significantly higher compared to negative control ($p < 0.05$). Tail moment is defined by the product of the distance between the head and the tail by the proportion of DNA in the tail, was used to evaluate the extent of DNA migration (Olive et al. 1990) while tail intensity refers to percentage of DNA at the tail of the comet. Figure 2 showed tail moment and Figure 3 showed tail intensity for each of the sample used in this study. Methanol extracts of shrimp pastes and salted fish from Pantai Puteri, exhibited higher DNA damage (TM – 8.33 ± 2.19; TI – 31.67 ± 5.84, TM – 2.25 ± 0.86; TI – 9.25 ± 1.55, respectively), than the other extracts. Values for methanol extract of shrimp pastes from Pantai Puteri were comparable to the positive control – Hydrogen peroxide (TM – 9.50 ± 1.50; TI – 30.50 ± 2.50). On the other hand, aqueous salted fishes extract from Pantai Puteri (TM – 1.33 ± 0.42 ; TI – 8.67 ± 2.42) and shrimp pastes extracts from Kelemak (methanol extract – TM – 1.75 ± 0.15 ; TI –7.50 ± 0.50, aqueous extract - TM – 1.00 ± 0.00 ; TI – 5.00 ± 0.00) showed slightly high value for tail moment and tail intensity as compared to negative control (TM – 0.29 ± 0.05 ; TI – 2.50 ± 0.29).

TABLE 1. Percentage of cells viability (%) for samples from Kelemak and Pantai Puteri following different concentrations (mean ± SEM), n (triplicates and three different independent experiments) = 3

Treatment Concentrations (µg/ml)	Sample							
	Kelemak				Pantai Puteri			
	Dried Shrimps		Shrimp Pastes		Shrimp Pastes		Salted Fishes	
	MetOH (%)	Aqueous (%)	MetOH (%)	Aqueous (%)	MetOH (%)	Aqueous (%)	MetOH (%)	Aqueous (%)
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
15.625	84.17 ± 7.41	83.42 ± 7.78	86.23 ± 7.54	71.95 ± 5.60	72.00 ± 7.00	94.49 ± 7.90	81.62 ± 3.16	89.2 ± 2.48
31.25	76.25 ± 5.94	76.37 ± 8.31	75.44 ± 5.97	72.74 ± 6.64	76.04 ± 4.83	99.77 ± 5.17	80.91 ± 1.92	87.83 ± 1.15
62.5	64.63 ± 5.87	97.71 ± 3.00	81.34 ± 4.08	76.38 ± 4.96	71.36 ± 9.32	91.22 ± 6.84	89.07 ± 5.13	87.41 ± 1.93
125	79.11 ± 9.97	89.3 ± 4.92	86.58 ± 6.82	68.30 ± 3.53	80.94 ± 6.40	93.43 ± 5.74	82.77 ± 5.32	97.46 ± 7.37
250	79.87 ± 11.18	84.95 ± 3.33	76.16 ± 8.00	77.01 ± 8.51	87.96 ± 3.00	76.89 ± 12.26	82.1 ± 7.43	82.1 ± 0.89
500	73.02 ± 4.57	82.57 ± 6.16	74.17 ± 12.75	62.47 ± 6.45	86.79 ± 10.70	54.74 ± 9.86	73.45 ± 7.76	83.24 ± 3.44

TABLE 2. Percentage of cells viability (%) for samples from Batang Tiga following different concentrations (mean ± SEM), n (triplicates and three different independent experiments) = 3

Treatment Concentrations (µg/ml)	Sample					
	Batang Tiga					
	Dried Shrimps		Shrimp Pastes		Shrimp Pastes	
	MetOH (%)	Aqueous (%)	MetOH (%)	Aqueous (%)	MetOH (%)	Aqueous (%)
0	100.00	100.00	100.00	100.00	100.00	100.00
15.625	75.98 ± 7.27	72.50 ± 5.02	94.57 ± 9.39	78.58 ± 6.83	88.29 ± 2.56	86.14 ± 3.50
31.25	86.81 ± 5.32	84.32 ± 4.80	96.78 ± 5.57	86.97 ± 4.78	91.23 ± 4.60	86.62 ± 1.30
62.5	89.55 ± 3.35	85.28 ± 3.70	85.24 ± 7.92	84.46 ± 1.00	79.28 ± 4.60	96.36 ± 6.36
125	83.14 ± 1.67	83.79 ± 3.09	97.58 ± 11.87	89.22 ± 4.56	83.55 ± 1.98	79.44 ± 4.58
250	86.45 ± 6.51	76.54 ± 8.22	95.98 ± 0.68	85.73 ± 8.77	80.98 ± 1.55	82.69 ± 3.52
500	83.67 ± 2.21	86.48 ± 5.90	94.50 ± 9.65	75.15 ± 14.27	78.66 ± 1.49	86.14 ± 7.71

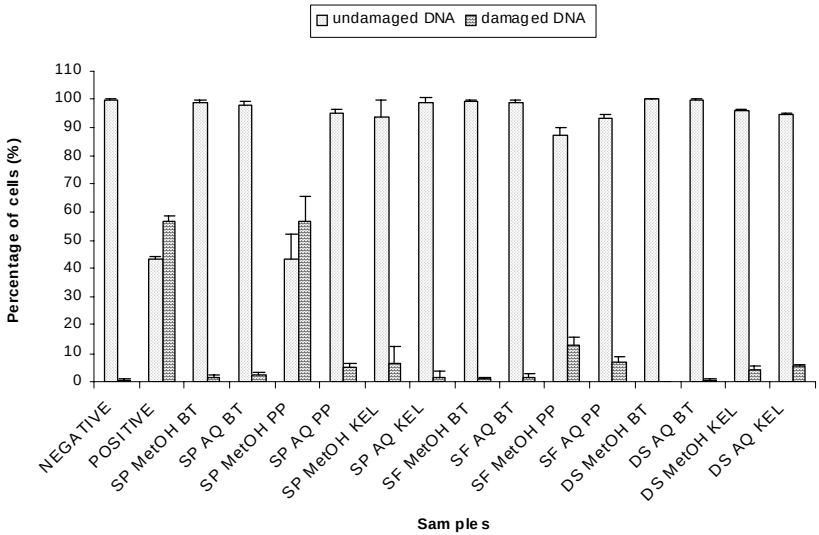

FIGURE 1. Percentage of cells based on the Comet Assay scoring of its DNA damage induced by the Malaysian food items (mean ± SD) (n = 50 cells, experiments = 3)

Note:
Malaysian Food Items - SP – Shrimp Pastes, DS – Dried shrimp, SF – Salted Fishes.
Areas from Malacca - BT – Batang Tiga, KEL – Kelemak, PP – Pantai Puteri
Extracts - AQ – aqueous, MetOH – Methanol

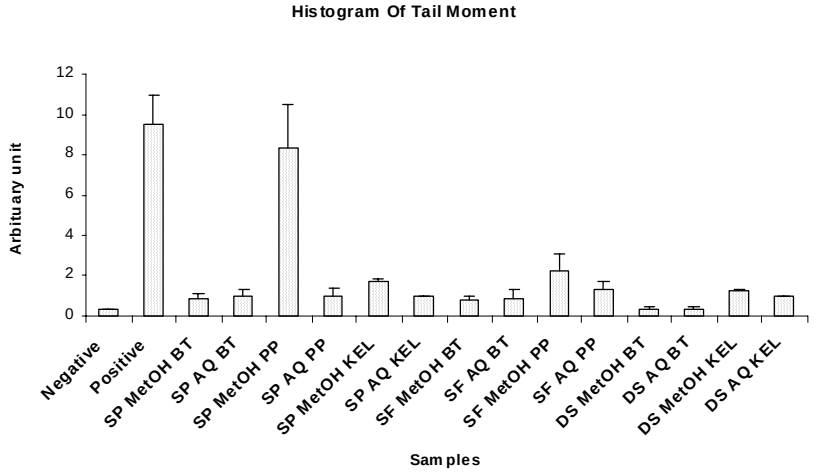

FIGURE 2. Bar Chart of tail moment based on the Comet Assay scoring of its DNA damage induced by the Malaysian food items (mean ± SD) (n = 50 cells, experiments = 3). * significant compared to negative control $p < 0.05$

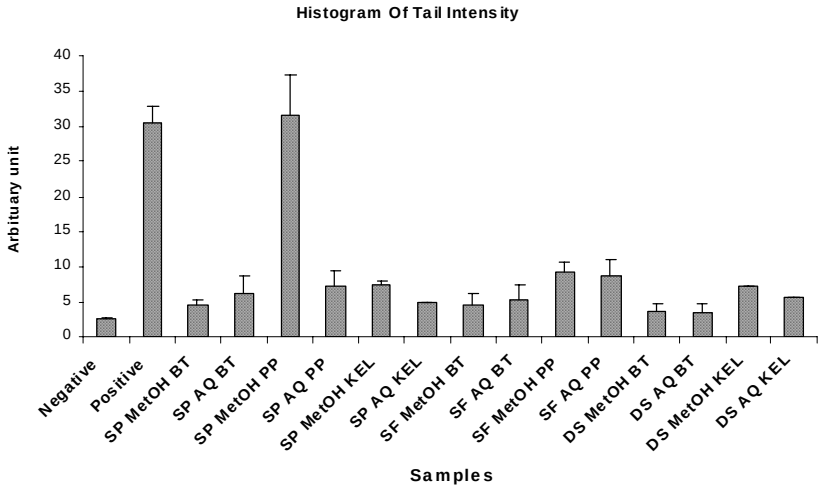

FIGURE 3. Bar Chart of tail intensity based on the Comet Assay scoring of its DNA damage induced by the Malaysian food items (mean ± SD) (n = 50 cells, experiments = 3). * significant compared to negative control $p < 0.05$

Dried shrimps extract from Batang Tiga caused the least damage to the DNA of the cell (methanol extract – TM – 0.322 ± 0.13; TI – 3.56 ± 1.09, aqueous extract – TM – 0.35 ± 0.11; TI – 3.49 ± 1.21) followed by salted fishes extract from the same area (methanol extract – TM – 0.75 ± 0.27; TI – 4.55 ± 1.62, aqueous extract – TM – 0.84 ± 0.48; TI – 5.30 ± 2.18). Figure 4 and 5 showed captured images of the cells and there was no DNA damage in the cells for the negative control and the methanol extracts of dried shrimps from Batang Tiga. Cells were intact and comet tail was not observed in the slides. However, image in Figure 6 showed cells with DNA damage following treatment of hydrogen peroxide (positive control) at 0.1 mM for 30 minutes at 4ºC. Comet tail was observed in the slide and DNA of the cells lysed (Figure 6).

DISCUSSIONS

It is recommended in the Comet Assay Guidelines to avoid the testing of doses that decrease the viability by more than 30% (Anderson et al. 1998; Henderson et al. 1998; Tice et al. 2000). In order to fulfill these requirements and to be able to discern between cytotoxicity and genotoxicity, a short incubation time of 24 h at 37ºC had been applied.

Basically, because DNA damage is associated with cell death, it is critical that the highest dose tested does not induce excessive cytotoxicity and that cytotoxicity be evaluated concurrently with each comet experiment. In this study,

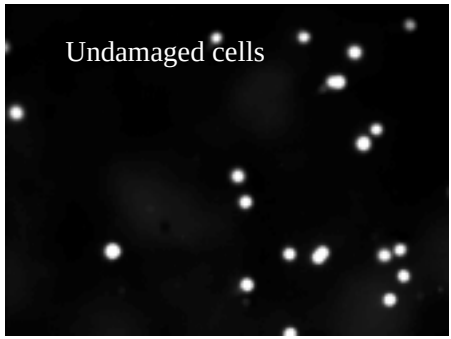

FIGURE 4. Chang liver cells processed in the Comet Assay following negative treatment (10X magnification)

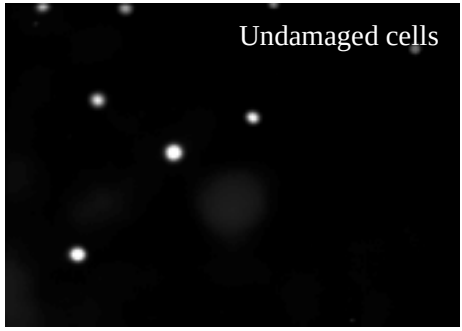

FIGURE 5. Chang liver cells processed in the Comet Assay following treatment with MetOH dried shrimps extracts from Batang Tiga for 24 hours (10X magnification)

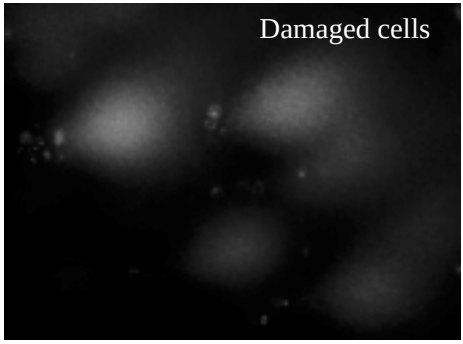

FIGURE 6. Chang liver cells processed in the Comet Assay following treatment with 0.1 μM Hydrogen Peroxide for 30 minutes at 4^{o}C (20X) magnification

preliminary cytotoxicity measurement with the tetrazolium reduction assay (MTT) was performed. The concentration of 62.5 µg/ml was used in the Comet assay based on the graph of cells viability as shown in Table 1 & 2 which showed less than 30% cell death at this point.

In this study, DNA damage was evaluated by using Alkaline Comet assay as it is one of the simple, rapid, visual and sensitive technique for measuring and analysing DNA breakage in mammalian cells (Ostling & Johanson 1984; Singh et al. 1988; Olive et al. 1990). Based on the findings, all the extracts did not showed any severe DNA damage except for methanol extracts of salted fishes and shrimp pastes, both from Pantai Puteri.

DNA damage of the cells could be due to the high amount of salt in the samples. With reference to Malaysian Food Act (2004), the amount of salt is higher than the standard value which is 15% for all processed shrimp pastes. Salt analysis was conducted in this study using Volhard method and showed that shrimp paste contained highest percentage of salt with 28% for Batang Tiga, 26% for Pantai Puteri and 20% for Kelemak (Figure 7). Lowest percentage of salt is showed by dried shrimps from Batang Tiga and Kelemak with 4% and 5%, respectively.

The difference in the amount could be one of the reasons for the difference in the genotoxic effect caused by the food extracts. High salt content can cause the death of the cells in mechanisms such as osmosis or alteration to the homeostasis of the cells (Cohen & Roe 1997). A bacterium named *H. pylori* was found in the stomach and can increase the risk of gastric cancer because of the damaging effect on the gastric mucosa, leading to an increase in regenerative

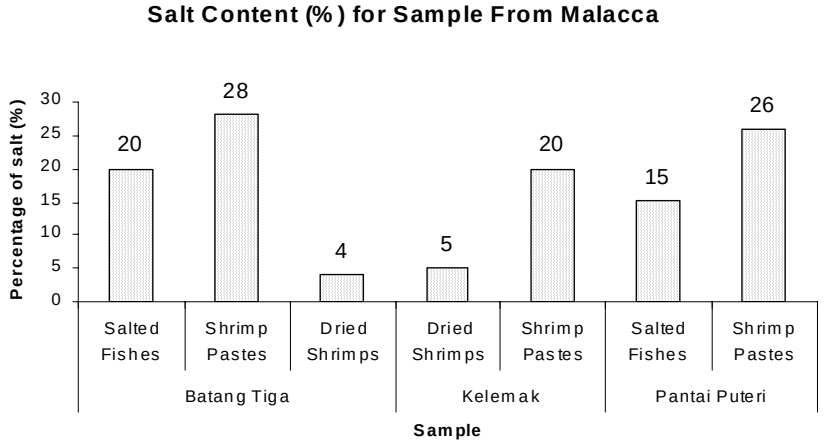

FIGURE 7. Percentage of salt for samples from Malacca. Values above are the data (%) for salt content in every sample

cell duplication (Weisburger 2000). Similarly, salt has the same effect in the stomach as the bacteria (Tsugane et al. 1994; Fox et al. 1999).

Findings showed that high concentrations of salt might also increase the incidence of gastric cancer in laboratory animals where it was attributed by a marked and sustained regenerative response in the gastric mucosa of those laboratory animals (Cohen & Roe 1997). The mitogenic response would also favour the progression of the cells towards neoplasia. In addition, high salt concentrations are also capable of inducing chromosomal damage through indirect genotoxic action. It is most likely that the chromosomal changes observed from the salted foods are due to the clastogenic compounds present (IARC 1993a). Moreover, extracts of salted foods were found to be mutagenic and clastogenic in *in vitro* system and not causing any direct DNA damage (IARC 1993a, b)

CONCLUSIONS

This study showed that there are few extracts tested caused genotoxic effect towards Chang liver cells. This indicated that those food items might be potential genotoxic agents. Further in depth research should be carried out to determine more of their toxicological profiles that will affect human health.

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REFERENCES

American Institute for Cancer Research (AICR). 1997. *Food, nutrition and the prevention of cance: a global perspective*. Washington: American Institute for Cancer Research.

Anderson, D., Yu, T-W & McGregor, D.B. 1998. Comet assay responses as indicators of carcinogenic exposure. *Mutagen.* 13: 539-555.

Ballard-Barbash, R. 1999. Energy balance, antropometry and cancer. In D. Heber, G.L. Blackburn and V.L.W. Go. (eds.), *Nutritional Oncology*, pp. 137-151. San Diego: Academic Press.

Chen, W., Weisburger, J.H., Fiala, E.S., Spratt, T.E., Carmella, S.G. Chen, D. & Hecht, S.S. 1996. Gastric carcinogens : 2-chloro-4-methylthiobutanoic acid, a novel mutagen in salted, pickled Sanma Hiraki fish or similarly treated methionine. *Chemical Research Toxicology* 9: 58-66.

Combs, G.F. Jr. & Clark, L.C. 1999. Selenium and cancer, In D. Heber, G.L. Blackburn and V.L.W. Go. (eds.), *Nutritional Oncology*, pp. 215-222. San Diego: Academic Press.

Cohen, A.J. & Roe, F.J.C. 1997. Evaluation of the aetiological role of dietary salt exposure in gastric and other cancers in humans. *Food and Chemical Toxicology* 35: 271-293.

Doll. R. & Peto. R.. 1981. *The Causes of Cancer*. Oxford: Oxford Press.

Fox, J.G., Dangler, D.A., Taylor, N.S., King, A., Koh, T.J & Wang, T.C. 1999. High-salt diet induces gastric epithelial hyperplasia and parietal cell loss, and enhances *Helicobacter pylori* colonization in C57BL/6 mice. *Cancer Research* 59: 4823-4828.

Furihata, C., Oka. M., Amin, S., Krzeminski and Weisburger, J.H. 1996. Effect of 2-chloro-4-methylthiobutanoic acid in a rapid bioassay for gastric carcinogens. *Cancer Letters* 108: 129-135.

Ghazali, A.R., Rajab, N.F., Sharif, R., Yaakob, T. A. & Arshad, F. 2005. The genotoxicological evaluation of several local raw foods extracts on Chang liver cells by Single Cell Electrophoresis Assay. *Environmental Mutagen Research* 27: 165-170.

Henderson, L., Wolfreys, A., Fedyk, J., Bourner, C. & Windebank, S. 1998. The ability of the comet assay to discriminate between genotoxins and cytotoxins. *Mutagenesis* 13: 89-94.

IARC. 1993a. Salted fish. Monographs on the evaluation of carcinogens risks to humans, Vol. 56. *Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins*, pp 41-82. Lyon : International Agency for Research on Cancer.

IARC. 1993b. Pickled vegetables. Monographs on the evaluation of carcinogens risks to humans Vol. 56. *Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins*, pp 83-113. Lyon: International Agency for Research on Cancer.

Longnecker, M.P. & Tseng, M. 1999. Alcohol and cancer. In D. Heber, G.L. Blackburn and V.L.W. Go, (eds.), *Nutritional Oncology*, pp. 277-298. San Diego: Academic Press.

Malaysian Food Act. 2004. *Food Act 1983 (Act 281) & Regulations*. Kuala Lumpur: International Law Book Services.

Manson, M. & Benford, D.J. 1999. Factors influencing the carcinogenocity of food chemicals. *Toxicology.* 134: 93-108.

Martinez, M. E., Marshall, J.R. & Alberts, D.S. 1999. Dietary fiber, carbohydrates and cancer. In D. Heber, G.L. Blackburn and V.L.W. Go (eds.), *Nutritional Oncology*, pp. 185-194. San Diego: Academic Press.

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival : application to proliferation and cytotoxicity assays. *Journal Immunology Method.* 65: 55-63.

Olive, P.L., Banath, J.P., & Durrand, R.E. 1990. Heterogenecity in radiation-induced DNA damage and repair in tumor and normal cells measured using the comet assay. *Radiation Research* 122: 86-94.

Ostling, O. & Johanson, K.J. 1984. Microelectrophoretic study of radiation-induced DNA damage in individual mammalian cells. *Biochemical and Biophysics Research Communication* 123: 291-298.

Saha, K., Lajis, N.H., Israf, D. A., Hamzah, A.S., Khozirah, S., Khamis, S. & Syahida, A. 2004. Evaluation of antioxidant and nitric oxide inhibitory activities of selested Malaysian medicinal plants. *Journal of Ethnopharmacology* 92: 263-267.

Sakanaka, S., Tachinaba, Y. & Okada, Y. 2005. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea. *Food Chemisry* 89: 569-575.

Sinha, R. & Caporaso., N. 1999. Diet, genetic susceptibility and human cancer etiology, *Journal of. Nutrition* 129: 556-559.

Singh, N.P., McCoy, M.T., Tice, R.R. & Schneider, E.L. 1988. A simple technique for quantification of low levels of DNA damage in individual cells. *Experiment Cell Research* 175(1): 184-191.

Smith-Warner, E. & Giovannucci. 1999. Fruit and vegetable intake and cancer. In D. Heber, G.L. Blackburn and V.L.W. Go (eds.), *Nutritional Oncology*, pp, 153-193. San Diego: Academic Press.

Taj, S. & Nagarajan, B. 1994. Induction of genotoxicity by salted deep fried fish and mutton. *Mutation Research/Genetic Toxicology* 322 (1): 45-54.

Tice, R.R., E. Agurell, D. Anderson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J.-C. Ryu & Y. F. Sasaki. 2000. Single cell gel/comet assay : Guidelines for in vitro and in vivo genetic toxicology testing. *Environmental and Molecular Mutagenesis* 35: 206-221.

Tsugane, S., Tei, Y., Takahashi, T., Watanabe, S. & Sugano, K. 1994. Salty food intake and risk of *Helicobacter pylori* infection. *Japan Journal of Cancer Research* 85: 474-478.

US Department of Health and Human Service. 1996. *Physical Activity and Health: A Report of the Surgeon General*. Atlanta, USA: US Department of Health and Human Services, Centers for Disease Control and Prevention, National Centers for Chronic Disease Prevention and Health Promotion.

Weisburger, H.J. 2000. Eat to live, not live to eat. *Nutrition* 16 (9): 767-773.

Zhou, J-R. & Blackburn, G.L. 1999. Dietary lipid modulation of immune response in tumor genesis. In D. Heber, G.L. Blackburn and V.L.W. Go (eds,). *Nutritional Oncology*, pp. 195-213. San Diego: Academic Press.

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