International Food Research Journal 31(1): 149 - 156 (February 2024)
Journal homepage: *http://www.ifrj.upm.edu.my*

Abrogation of methyl methanesulphonate (MMS)-induced cytotoxic and genotoxic effects of tropical fruit juice mixture on fibroblast cells

[1]Ooi, T. C., [2]Abd Rahman, N., [2,3]Ibrahim, F. W., [1]Shahar, S. and [1,2]*Rajab, N. F.

[1]Center for Healthy Ageing and Wellness, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia
[2]Biomedical Science Programme, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia
[3]Center for Toxicology and Health Risk Studies, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

Article history

Received:
28 March 2022
Received in revised form:
1 June 2023
Accepted:
31 October 2023

Keywords

antigenotoxic, cytoprotective, methyl methanesulphonate, tropical fruit juice mixture, V79 cells

Abstract

Tropical fruit juice mixture consisting of pomegranate, guava, and roselle has been proven to possess high polyphenolic composition and antioxidant capacity. The present work aimed to evaluate the cytoprotective and antigenotoxic potentials of juice mixture in methyl methanesulphonate (MMS)-induced V79 Chinese hamster lung fibroblast cell line. MTT assay showed that the IC_{50} value of the juice mixture was 193.33 ± 46.40 µg/mL. Cells pretreated with 6, 12, and 25 µg/mL juice mixture showed significant increment in viability ($p < 0.05$) following induction with MMS. However, only cells co-treated with 6 and 12 µg/mL juice mixture showed protective effect ($p < 0.05$) against MMS-induced cytotoxicity under the co-treatment setting. Comet assay showed that the tail moment and percentage of DNA in tail in cells pretreated with the juice mixture significantly decreased compared with those in positive control groups. However, under the co-treatment setting, only 12 µg/mL juice mixture showed significant reduction ($p < 0.05$) in tail moment compared with MMS alone. In conclusion, the tropical fruit juice mixture can abrogate and protect cells from the cytotoxic and genotoxic effects of MMS, and has the potential to be developed as beneficial formulation for health preservation.

DOI
https://doi.org/10.47836/ifrj.31.1.13

© All Rights Reserved

Introduction

Genotoxicity is the ability of chemical agents to damage genetic information within a cell, thus causing mutations, which may lead to cancers. Changes within genetic materials can occur directly or indirectly, thereby affecting somatic or germ cells that may be passed on to the next generation (Basu, 2018). Damages to DNA caused by free radicals, ionising radiations, alkylating agents, and nanoparticles could be DNA strand breaks, DNA adducts, or other types of DNA-base modifications which are recognised and fixed by various DNA repair mechanisms (Chatterjee and Walker, 2017; Maluin *et al*., 2020; Siew *et al*., 2020).

Methyl methanesulphonate (MMS) is a carcinogenic alkylating agent commonly applied in genotoxic and antigenotoxic research. MMS modifies the guanine and adenine of DNA to 7-methylguanine and 3-methlyladenine, respectively, thus leading to DNA damage (Lundin *et al*., 2005). Repairs of the N-alkylation base in living cells occur through base excision repair (BER) and nucleotide excision repair (NER) mechanisms (Kondo *et al*., 2010). Therefore, an increase in the breakage of the DNA strand observed after treatment with MMS reflects the excision of damaged nucleotides, while the decrease in the migration of DNA reflects the ligation process (Viau *et al*., 2009).

Fruits are rich in vitamins, minerals, proteins, carbohydrates, fatty acids, fibres, carotenoids, and polyphenols (Wallace *et al*., 2020). Polyphenols are secondary metabolites of plants important for the growth and reproduction of certain plants, and contribute other characteristics, such as bitter and spicy tastes, colour, odours, and enabling oxidative balance in foods (Yordi *et al*., 2012). High intake of fruits, vegetables, and cereals rich in polyphenols can lower the risks of many chronic diseases, such as cancers, cardiovascular diseases, and

*Corresponding author.
Email: nfadilah@ukm.edu.my

neurodegenerative diseases (Cory *et al*., 2018; Koch, 2019). As a competent free radical scavenger, polyphenols play an important role in maintaining genomic stability, and reducing the effect of oxidising agents from endogenous or exogenous sources (Majidinia *et al*., 2019).

Tropical fruits, such as pomegranate (*Punica granatum* L.), guava (*Psidium guajava* L.), and roselle (*Hibiscus sabdariffa* L.) have high phenolic contents and potent antioxidant activities (Jiménez-Escrig *et al*., 2001; Wu *et al*., 2018; Fahmy *et al*., 2020). Pomegranate, guava, and roselle juices individually contain various phytochemical contents, and their combination may provide beneficial activity towards health (Abdul Malek *et al*., 2017). A previous study reported that tropical fruit juice mixture consisting of pomegranate concentrate with guava and roselle extract protected Wistar male rats from β-amyloid-induced brain tissue damage in the CA1 region of the hippocampus (Ooi *et al*., 2020). A randomised controlled trial among middle-aged women showed that such tropical fruit juice mixture has the potential to improve cognitive function in various domains, such as learning, memory, and processing speed (Rosli *et al*., 2021). However, limited information is known about the cytoprotective and antigenotoxic potential of this mixed fruit juice. The present work thus aimed to evaluate the cytoprotective and antigenotoxicity potential of this polyphenol-rich tropical fruit juice mixture in MMS-induced V79 Chinese hamster lung fibroblast line.

Materials and methods

Preparation of tropical fruit juice mixture

Pomegranate (The Passionate Pomegranate Company, South Africa), white guava (Heng Hwa Trading, Malaysia), and calyx of roselle variety UKMR-1 were used to produce a tropical fruit juice mixture. The fruit was washed, and then dried at room temperature. The net weight of the fruit was recorded before cutting into pieces. The cut fruit was then placed into a blender for juice preparation. The juice was filtered using muslin cloth to remove fibres. This process was repeated with the two other fruits. The three different fruit juices were then mixed based on the formulation that has been proved to yield high antioxidant capacity, which was 50% distilled water, 32% pomegranate juice, 9% white guava juice, and 9% roselle juice. The fruit juice mixture was stored at -20°C overnight, and freeze-dried for 4 d to obtain juice powder. The powder was reconstituted with DMSO as solvent to prepare a stock solution with a concentration of 60 mg/mL.

Cell culture

V79 Chinese hamster lung fibroblast cells (ATCC, Rockville, MD) were used to evaluate the cytotoxicity and genotoxicity of the tropical fruit juice mixture since this cell line has stable genome numbers that can prevent genetic drift following subculture; it is also listed as one of the mammalian cell lines that are commonly used for *in vitro* genetic toxicology testing based on the OECD guidelines (OECD, 2016). In general, V79 cells were grown as a monolayer in culture flasks using Dulbecco's modified Eagle minimal essential medium (DMEM) (Invitrogen Cooperation, UK), supplemented with 10% foetal bovine serum (PAA Laboratories GmbH, Australia), in an incubator at 37°C with 5% CO_2. Under these conditions, the average cell cycle time was 14 h.

Determination of cell viability

The cytotoxic effects of the tropical fruit juice mixture on V79 cells were determined by MTT assay as previously described (Siew *et al*., 2020). The cell monolayers in exponential growth were harvested, and 5×10^4 cells/mL in 200 µL were placed into each well of 96-well plates (Nunclon™, VWR International Inc., MD). The plates were incubated for 24 h at 37°C in 5% CO_2. The medium was discarded, and 200 µL of the treatment medium containing various concentrations of juice mixture (0 - 300 µg/mL) were loaded into the 96-well plates, and incubated for another 24 h.

The protective effect of the juice mixture against MMS-induced cytotoxicity was also investigated. V79 cells were treated with 200 µL of the treatment medium containing various concentrations of juice mixture (0 - 200 µg/mL) in two different settings. The concentrations used were chosen based on the results of the cytotoxicity evaluation. The IC_{20} (116.67 ± 17.91 µM) and IC_{50} values (400.00 ± 49.21 µM) of MMS were used to induce the cytotoxic effects on V79 cells. In the first setting, V79 cells were pretreated with the juice mixture for 24 h before being induced with MMS for another 24 h. In the second setting, V79 cells were co-treated with treatment medium containing juice mixture and MMS (co-treatment) for 24 h.

Each well was added with 20 µL of the 3-(4, 5-dimethylthiazol -2-yl)-2-5-diphenyl tetrazolium bromide (MTT) solution [5 mg/mL dissolved in phosphate-buffered solution (PBS)] (Sigma-Aldrich Company, USA), and reincubated for 4 h at 37°C. The medium was discarded, and 200 µL of DMSO was added to dissolve the formazan crystals. The plate was shaken gently for 5 min, and the absorbance was recorded at 570 nm using a microplate reader.

Alkaline comet assay

Alkaline comet assay was carried out using previously reported method with slight modifications (Siew *et al*., 2020). V79 cells were harvested, and 3 mL of cells at the concentration of 5×10^4 cells/mL were seeded into each well in 6-well plates (Nunclon™, VWR International Inc., MD). The cells were incubated for 24 h before: (i) pretreated with medium containing the juice mixture for 1 h followed by MMS induction for 1 h; and (ii) co-treated with medium containing the juice mixture and MMS (co-treatment) for 1 h. The concentrations used to study the antigenotoxic effects of the juice mixture were 12 and 200 µg/mL. The IC_{20} (100 µM) and IC_{50} (400 µM) values of MMS were used to induce DNA damage in V79 cells. Cells induced with MMS only served as the positive control, while cells cultivated in complete cell culture medium only served as the negative control.

The cells were inspected visually under a microscope to observe for any qualitative/morphological death-related changes, such as cell shrinkage, cellular rounding, blebbing of the cytoplasmic membrane, and chromatin condensation before harvest to avoid false-positive results due to DNA damage associated with cytotoxicity. The cells were harvested and washed with PBS after treatment. The cell pellets obtained were mixed thoroughly with 0.6% low melting point agarose (Sigma-Aldrich, USA), and laid on hardened 0.6% normal melting agarose (Sigma-Aldrich, USA). The agarose was allowed to solidify, and placed in a chilled lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, and 1% Triton-X) for lysis to occur. The slides were incubated in an electrophoresis buffer (0.3 N NaOH, 1 mM EDTA) for 20 min to facilitate DNA unwinding. Electrophoresis was performed under 25 V and 300 mA for 20 min. The slides were rinsed three times with neutralising buffer (400 mM Tris) before staining with 10 µg/mL ethidium bromide solution (Sigma-Aldrich, USA). The slides were kept overnight before observation under a fluorescence microscope (Olympus, Japan). The assay was run in triplicate, and DNA damage scoring was performed on a total of 150 cells (50 cells per technical replicate) for each independent experiment using Comet ScoreTM software version 1.5 (Tritek Corp., USA).

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS) version 22.0 (SPSS Inc. USA). One-way ANOVA followed by *post hoc* Tukey's test was used for data analysis, and values at $p < 0.05$ indicated significant differences. All the experimental results were presented as mean ± standard error of the mean (SEM).

Results

Cytotoxicity evaluation of tropical fruit juice mixture

The cytotoxicity of the tropical fruit juice mixture was evaluated by MTT assay (Figure 1). No significant differences were observed among cells treated with 9, 19, 37, or 75 µg/mL of the juice mixture and the negative control. By contrast, the viability of V79 cells treated with 150 and 300 µg/mL of the juice mixture was reduced significantly compared with that of the negative control, indicating that two concentrations were cytotoxic to the cells. Based on the cell viability curve plotted in Figure 1, the IC_{50} value of the juice mixture was 193.33 ± 46.40 µg/mL.

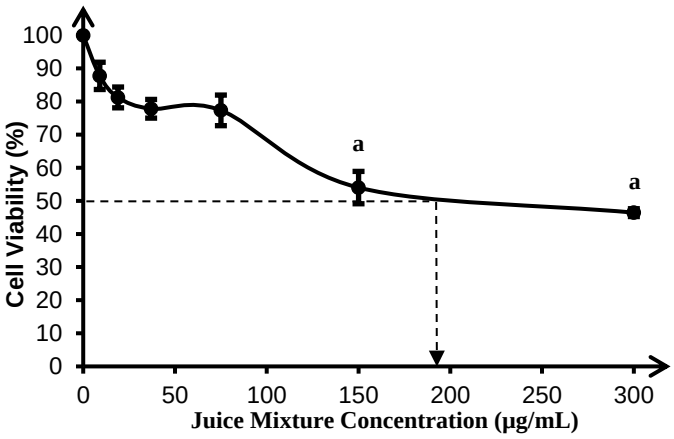

Figure 1. Mean percentage of V79 cell viability after treatment with juice mixture ranging from 0 to 300 µg/mL. Results are mean ± SEM of three independent experiments, with three technical replicates performed for each experiment. ([a]) Significant difference from negative control group ($p < 0.05$).

Cytoprotective effects of tropical fruit juice mixture on MMS-induced V79 cells

Table 1 shows the cytoprotective effects of the juice mixture against MMS-induced cytotoxicity on V79 cells under the pre- or co-treatment setting. The pre-treatment with the juice mixture for 24 h at the concentrations of 6, 12, and 25 µg/mL showed significant protection ($p < 0.05$) against MMS-induced cytotoxicity in V79 cells challenged with the IC_{50} value of MMS. However, no significant difference in cell viability was detected among cells pretreated with various concentrations of the juice mixture compared with cells induced with the IC_{20} value of MMS. In the co-treatment setting, only cells treated with 6 and 12 µg/mL of the juice mixture showed protective effects against MMS-induced cytotoxicity in V79 cells challenged with the IC_{20} or IC_{50} value of MMS ($p < 0.05$).

Table 1. Cytoprotective effects of juice mixture against MMS-induced cytotoxicity in V79 cells under pre- or co-treatment setting.

Group	Pretreatment		Co-treatment	
	MMS IC_{20}	**MMS IC_{50}**	**MMS IC_{20}**	**MMS IC_{50}**
Negative control	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
MMS (positive control)	81.47 ± 2.67	50.73 ± 4.01	57.60 ± 1.95	54.86 ± 0.81
MMS + 6 µg/mL juice	95.52 ± 6.86[a]	88.29 ± 6.73[b]	107.08 ± 5.02[b]	75.58 ± 3.37[b]
MMS + 12 µg/mL juice	92.80 ± 3.9[a]	95.61 ± 7.59[b]	107.75 ± 7.59[b]	76.65 ± 1.09[b]
MMS + 25 µg/mL juice	91.93 ± 2.61[a]	86.22 ± 6.68[b]	82.62 ± 4.82	57.38 ±5.93
MMS + 50 µg/mL juice	84.54 ± 6.45[a]	80.24 ± 7.53	75.08 ± 4.27[a]	55.40 ± 6.13
MMS + 100 µg/mL juice	77.18 ± 6.88[a]	62.45 ± 8.34	69.68 ± 6.21[a]	43.32 ± 1.87
MMS + 200 µg/mL juice	68.73 ± 4.67[b]	53.20 ± 8.27[a]	61.58 ± 5.91[a]	37.93 ± 5.47

MMS, methyl methanesulfonate. Results are mean ± SEM of three independent experiments, with three technical replicates performed for each experiment. ([a]) Significant difference from negative control group ($p < 0.05$). ([b]) Significant difference from positive control group ($p < 0.05$).

Antigenotoxic effects of tropical fruit juice mixture on MMS-induced V79 cells

The differential protective effects of low (12 µg/mL) and high concentration (200 µg/mL) of the tropical fruit juice mixture against MMS-induced genotoxicity were investigated. The concentration of 12 µg/mL was chosen to represent low-dose supplementation because it was the most effective in protecting V79 cells against MMS-induced cytotoxicity. The IC_{20} or IC_{50} value of MMS was used as the positive control to induce DNA strand breaks in V79 cells. As shown in Table 2, supplementation with the juice mixture showed antigenotoxic activity in MMS-induced V79 cells. The tail moment and the percentage of DNA in tail in cells pretreated with the juice mixture were significantly lower than those in the positive controls (IC_{20} and IC_{50} value of MMS). The co-treatment of cells with 12 µg/mL juice mixture with MMS showed a significant reduction of in tail moment scores compared with cells induced with the IC_{20} or IC_{50} value of MMS. In cells co-treated with 200 µg/mL juice mixture, the tail moment scores significantly decreased in cells induced with the IC_{20} value of MMS but not in cells induced with the IC_{50} value of MMS in comparison with their respective positive control groups. No significant differences in the percentage of DNA in tail were detected among cells co-treated with 12 and 200 µg/mL juice mixture and MMS.

Discussion

In our previous work, we successfully developed a tropical fruit juice mixture that consisted of pomegranate concentrate with guava and roselle extracts, and reported its high polyphenolic contents and antioxidant capacity (Abdul Malek *et al*., 2017; Ooi *et al*., 2020). The polyphenols abundant in the juice mixture were ellagic acid, gallic acid, catechin, epicatechin, *p*-coumaric acid, chlorogenic acid, procyanidin B_2, and kuromanin chloride (Abdul Malek *et al*., 2017). In the present work, we used the same formulation of the juice mixture to investigate its protective capacity and abrogating effect on the mutagenicity of MMS.

Table 2. Antigenotoxic effects of juice mixture against MMS-induced genotoxicity in V79 cells under pre- or co-treatment setting.

Group	Percentage of DNA in tail (%)		Tail moment	
	Pretreatment	Co-treatment	Pretreatment	Co-treatment
Control	4.03 ± 0.38	4.30 ± 0.60	0.02 ± 0.03	0.33 ± 0.05
MMS IC_{20}	49.60 ± 0.96	17.13 ± 1.03	57.60 ± 1.95	8.16 ± 0.77
MMS IC_{20} + 12 µg/mL juice	24.89 ± 0.66*	15.75 ± 0.95	14.01 ± 0.68*	2.22 ± 0.16*
MMS IC_{20} + 200 µg/mL juice	23.46 ± 0.85*	15.32 ± 1.11	16.60 ± 1.02*	6.08 ± 0.51*
MMS IC_{50}	77.66 ± 0.91	47.44 ± 1.18	122.56 ± 3.93	29.04 ± 1.26
MMS IC_{50} + 12 µg/mL juice	60.75 ± 0.92*	46.69 ± 1.59	73.53 ± 1.54*	19.19 ± 1.34*
MMS IC_{50} + 200 µg/mL juice	67.92 ± 0.90*	44.62 ± 1.21	87.30 ± 87.30*	26.24 ± 1.55

MMS, methyl methanesulfonate. Results are mean ± SEM of three independent experiments, with three technical replicates performed for each experiment. (*) Significant difference from positive control group ($p < 0.05$).

In general, the juice mixture at lower concentrations (≤ 25 µg/mL) was found to protect V79 cells from MMS-induced cytotoxicity. However, at higher concentrations, the cytotoxic effects of MMS on V79 cells were augmented. In the absence of MMS induction, the viability of V79 cells was reduced with increasing concentration of the juice mixture. Exogenous antioxidants, such as polyphenols, that are present at sufficiently high levels, can act as a pro-oxidant, thereby producing free radicals and causing oxidative stress in cells (Bouayed and Bohn, 2010; Babich *et al*., 2011). Some polyphenolic antioxidants, mainly flavonoids and phenolic acids, such as protocatechuic, gallic, and ellagic acids may induce pro-oxidant condition *in vitro* at high doses or in the presence of metal ion (Yordi *et al*., 2012). Therefore, the pro-oxidant and antioxidant potentials of phenolic compounds may be responsible for the protective and cytotoxic activity of the juice mixture. The results from the comet assay showed that a high dose of the juice mixture (200 µg/mL) did not protect cells from MMS-induced DNA damage. This finding partly explains why the juice mixture was unable to protect V79 cells from MMS-induced cytotoxicity given that extensive DNA damage can cause cell death as a consequence of a cytotoxic event.

In the present work, pretreatment with the juice mixture protected V79 cells from MMS-induced genotoxicity, as determined using the comet assay. In the pretreatment setting, the major constituents of orange juice, such as ascorbic acid and phenolic compounds, may compete with DNA as the target of the alkylation process, thereby reducing the effect of genotoxic alkylating agents (Franke *et al*., 2005b). In the co-treatment setting, phenolic compounds and ascorbic acid can influence the kinetics of DNA repair. Besides competing with DNA as the target for alkylation, ascorbic acid plays an important role in the regulation of DNA repair enzymes (Sram *et al*., 2012). Hence, the results of the present work indicated that the tropical fruit juice mixture might exert antigenotoxic effects through similar mechanisms reported previously (Franke *et al*., 2005b).

In the co-treatment setting, a significant reduction in tail moment was only observed in cells treated with a low dose of the juice mixture. As mentioned earlier, the tropical fruit juice mixture has pro-oxidant activities at high doses or in the presence of metal ions, leading to an increase in the production of ROS, and consequently DNA damage (Yordi *et al*., 2012). Hence, we cannot exclude the postulation that DNA damage detected in cells treated with high doses of polyphenols was partly caused by the polyphenols itself, and not solely due to the genotoxic effects of MMS. Similar to our previous work, the effects of ascorbic acid were investigated compared with various genotoxic agents, including MMS; ascorbic acid at lower doses significantly reduced DNA damage (Franke *et al*., 2005a). Ascorbic acid at higher doses may act as pro-oxidant, and cause cell death (Kaźmierczak-Barańska *et al*., 2020).

The juice mixture contained different phenolic compounds, and its ability to protect the biological system is expected to vary. The mechanisms underlying the antigenotoxic effects of different polyphenols vary due to variations in their chemical

constituent (Majidinia *et al*., 2019). Punicalagin of ellagitannin group is the main contributor to the antioxidant activity of pomegranate juice, and hydrolysed to smaller phenolic compounds, such as ellagic acid (Gil *et al*., 2000). Polyphenols, such as ellagic acid, can affect the expression of genes, such as those associated with DNA repair (Aiyer *et al*., 2008). Tannins and flavonoid-rich extracts of *Myrtus communis* also have potential antigenotoxic effects possibly by inhibiting the activation of microsomal enzymes or acting as a scavenger of electrophilic metabolites from the mutagen, thereby protecting the DNA strand from damages caused by the metabolite (Hayder *et al*., 2004). This was consistent with the findings on the constituent of the juice mixture observed in the present work. Furthermore, Rodrigues *et al*. (2009) conducted comet and micronucleus assays, and reported the antigenotoxic effect of *Baccharis trimera* that is rich in flavonoids, tannins, and anthraquinone against oxidative DNA damages caused by hydrogen peroxide. Smith *et al*. (2004) reported the antimutagenic activities of anthocyanin-rich berry juice against mutation caused by the direct action of activated MMS and the carcinogenic metabolite of benzo-alpha-pyrene.

Although the present work demonstrated that tropical fruit juice mixture possesses cytoprotective and antigenotoxic effects, it has several limitations. Firstly, the protective effects of tropical fruit juice mixture were only tested on a single mammalian cell line (V79 cell line) *in vitro*. Determining the significance of the findings in an *in vitro* model has certain restrictions compared with using an *in vivo* model. The effects of tropical fruit juice mixture alone on DNA damage should also be tested in the future to confirm its antigenotoxic properties for safe use. Moreover, the mechanisms underlying the cytoprotective and antigenotoxic effects of tropical fruit juice mixture remain unexplored, and should be investigated before human consumption.

Conclusion

The tropical fruit juice mixture developed was abundant in different polyphenolic compounds which could serve as antigenotoxic and cytoprotective agents against MMS-induced damage in V79 cells. The present work, although preliminary, has the potential to highlight the capability of the tropical fruit juice mixture in abrogating genotoxicity caused by a known mutagenic agent.

Acknowledgement

The present work was financially supported by the Universiti Kebangsaan Malaysia through the Dana Modal Insan (grant no.: MI-2019-012) and the Ministry of Higher Education Malaysia through the Fundamental Research Grant Scheme (grant no.: FRGS/1/2013/SKK01/UKM/02/3). Authors thank Prof. Dr. Michael Fenech, Distinguished Professor, Faculty of Health Sciences, Universiti Kebangsaan Malaysia for reviewing and proofreading this manuscript.

References

Abdul Malek, S. N. A., Haron, H., Wan Mustafa, W. A. and Shahar, S. 2017. Physicochemical properties, total phenolic and antioxidant activity of mixed tropical fruit juice, TP 3 in 1™. Journal of Agricultural Science 9(13): 50.

Aiyer, H. S., Vadhanam, M. V., Stoyanova, R., Caprio, G. D., Clapper, M. L. and Gupta, R. C. 2008. Dietary berries and ellagic acid prevent oxidative DNA damage and modulate expression of DNA repair genes. International Journal of Molecular Sciences 9(3): 327-341.

Babich, H., Schuck, A. G., Weisburg, J. H. and Zuckerbraun, H. L. 2011. Research strategies in the study of the pro-oxidant nature of polyphenol nutraceuticals. Journal of Toxicology 2011: 467305.

Basu, A. K. 2018. DNA damage, mutagenesis and cancer. International Journal of Molecular Sciences 19(4): 970.

Bouayed, J. and Bohn, T. 2010. Exogenous antioxidants - Double-edged swords in cellular redox state: Health beneficial effects at physiologic doses versus deleterious effects at high doses. Oxidative Medicine and Cellular Longevity 3(4): 228-237.

Chatterjee, N. and Walker, G. C. 2017. Mechanisms of DNA damage, repair, and mutagenesis. Environmental and Molecular Mutagenesis 58(5): 235-263.

Cory, H., Passarelli, S., Szeto, J., Tamez, M. and Mattei, J. 2018. The role of polyphenols in human health and food systems: A mini review. Frontiers in Nutrition 5: 87.

Fahmy, H., Hegazi, N., El-Shamy, S. and Farag, M. A. 2020. Pomegranate juice as a functional food: A comprehensive review of its

polyphenols, therapeutic merits, and recent patents. Food and Function 11(7): 5768-5781.

Franke, S. I. R., Prá, D., Da Silva, J., Erdtmann, B. and Henriques, J. A. P. 2005a. Possible repair action of vitamin C on DNA damage induced by methyl methanesulfonate, cyclophosphamide, $FeSO_4$ and $CuSO_4$ in mouse blood cells *in vivo*. Mutation Research - Genetic Toxicology and Environmental Mutagenesis 583(1): 75-84.

Franke, S. I. R., Prá, D., Erdtmann, B., Henriques, J. A. P. and Da Silva, J. 2005b. Influence of orange juice over the genotoxicity induced by alkylating agents: An *in vivo* analysis. Mutagenesis 20(4): 279-283.

Gil, M. I., Tomas-Barberan, F. A., Hess-Pierce, B., Holcroft, D. M. and Kader, A. A. 2000. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. Journal of Agricultural and Food Chemistry 48(10): 4581-4589.

Hayder, N., Abdelwahed, A., Kilani, S., Ben Ammar, R., Mahmoud, A., Ghedira, K. and Chekir-Ghedira, L. 2004. Anti-genotoxic and free-radical scavenging activities of extracts from (Tunisian) *Myrtus communis*. Mutation Research - Genetic Toxicology and Environmental Mutagenesis 564(1): 89-95.

Jiménez-Escrig, A., Rincón, M., Pulido, R. and Saura-Calixto, F. 2001. Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. Journal of Agricultural and Food Chemistry 49(11): 5489-5493.

Kaźmierczak-Barańska, J., Boguszewska, K., Adamus-Grabicka, A. and Karwowski, B. T. 2020. Two faces of vitamin C—Antioxidative and pro-oxidative agent. Nutrients 12(5): 1501.

Koch, W. 2019. Dietary polyphenols-important non-nutrients in the prevention of chronic noncommunicable diseases. A systematic review. Nutrients,11(5): 1039.

Kondo, N., Takahashi, A., Ono, K. and Ohnishi, T. 2010. DNA damage induced by alkylating agents and repair pathways. Journal of Nucleic Acids 2010: 543531.

Lundin, C., North, M., Erixon, K., Walters, K., Jenssen, D., Goldman, A. S. H. and Helleday, T. 2005. Methyl methanesulfonate (MMS) produces heat-labile DNA damage but no detectable *in vivo* DNA double-strand breaks. Nucleic Acids Research 33(12): 3799-3811.

Majidinia, M., Bishayee, A. and Yousefi, B. 2019. Polyphenols: Major regulators of key components of DNA damage response in cancer. DNA Repair 82: 102679.

Maluin, F. N., Hussein, M. Z., Yusof, N. A., Idris, A. S., Daim, L. D. J., Sarian, M. N., … and Fakurazi, S. 2020. Cytoprotection, genoprotection, and dermal exposure assessment of chitosan-based agronanofungicides. Pharmaceutics 12(6): 497.

Organisation for Economic Co-operation and Development (OECD). 2016. OECD guidelines for the testing of chemicals, section 4: Health effects. Test no. 487: *In vitro* mammalian cell micronucleus test. France: OECD.

Ooi, T. C., Ahmad Munawar, M., Mohd Rosli, N. H., Abdul Malek, S. N. A., Rosli, H., Ibrahim, F. W., … and Rajab, N. F. 2020. Neuroprotection of tropical fruit juice mixture *via* the reduction of iNOS expression and CRH level in β-amyloid-induced rats model of Alzheimer's disease. Evidence-Based Complementary and Alternative Medicine 2020: 5126457.

Rodrigues, C. R. F., Dias, J. H., de Mello, R. N., Richter, M. F., Picada, J. N. and Ferraz, A. B. F. 2009. Genotoxic and antigenotoxic properties of *Baccharis trimera* in mice. Journal of Ethnopharmacology 125(1): 97-101.

Rosli, H., Shahar, S., Rajab, N. F., Che Din, N. and Haron, H. 2021. The effects of polyphenols-rich tropical fruit juice on cognitive function and metabolomics profile – A randomized controlled trial in middle-aged women. Nutritional Neuroscience 22(8): 1577-1593.

Siew, E. L., Farris, A. F., Rashid, N., Chan, K. M. and Rajab, N. F. 2020. *In vitro* toxicological assessment of gadolinium (III) chloride in V79-4 fibroblasts. Genes and Environment 42(1): 22.

Smith, S. H., Tate, P. L., Huang, G., Magee, J. B., Meepagala, K. M., Wedge, D. E. and Larcom, L. L. 2004. Antimutagenic activity of berry extracts. Journal of Medicinal Food 7(4): 450-455.

Sram, R. J., Binkova, B. and Rossner, P. 2012. Vitamin C for DNA damage prevention. Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis 733(1-2): 39-49.

Viau, C. M., Guecheva, T. N., Sousa, F. G., Pungartnik, C., Brendel, M., Saffi, J. and Henriques, J. A. 2009. SnCl2-induced DNA damage and repair inhibition of MMS-caused lesions in V79 Chinese hamster fibroblasts. Archives of Toxicology 83(8): 769-775.

Wallace, T. C., Bailey, R. L., Blumberg, J. B., Burton-Freeman, B., Chen, C. Y. O., Crowe-White, K. M., … and Wang, D. D. 2020. Fruits, vegetables, and health: A comprehensive narrative, umbrella review of the science and recommendations for enhanced public policy to improve intake. Critical Reviews in Food Science and Nutrition, 60(13): 2174-2211.

Wu, H.-Y., Yang, K.-M. and Chiang, P.-Y. 2018. Roselle anthocyanins: Antioxidant properties and stability to heat and pH. Molecules 23(6): 1357.

Yordi, E. G., Pérez, E. M., Matos, M. J. and Villares, E. U. 2012. Antioxidant and pro-oxidant effects of polyphenolic compounds and structure-activity relationship evidence. In Bouayed, J. and Bohn, T. (eds). Nutrition, Well-Being and Health, p. 23-48. United Kingdom: IntechOpen.