International Journal of Scholarly Research in
Life Sciences
Journal homepage: https://srrjournals.com/ijsrls/
ISSN: 2961-3264 (Online)

(RESEARCH ARTICLE)

Evaluation of genotoxicity of dry powders of pomelo (*Citrus maxima*) flowers by micronucleus assay

Chien-Hsiang Ni [1, #], Yun-Xuan Chang [2, #], Tsung-Han Wu [2], Ya-Peng Wang [2], Chia-Chi Chen [2], Tzu-Yun Chi [2], Yen-Jung Lu [2], Pi-Hsin Chen [2], Ya-Ling Cyue [2], Shih-Yi Guo [2], Suz-Ching Ke [2], Yu-Ying Fang [2], Szu-Ping Sung [2], Chien-Chao Chiu [2], Ching-Feng Chiu [3], Hsuan-Wen Chiu [2], Wei-Huang Tsai [4], Yu-Hsing Lin [5] and Shao-Wen Hung [2, 6, *]

[1] *Department of Animal Science, Chinese Culture University, Taipei 111, Taiwan.*
[2] *Division of Animal Industry, Animal Technology Research Center, Agricultural Technology Research Institute, Hsinchu 300, Taiwan.*
[3] *Graduate Institute of Metabolism and Obesity Sciences, College of Nutrition, Taipei Medical University, Taipei 110, Taiwan.*
[4] *Ministry of Agriculture, Executive Yuan, Taipei 100, Taiwan.*
[5] *Department of Pet Healthcare, Yuanpei University of Medical Technology, Xiangshan, Hsinchu 300, Taiwan.*
[6] *Department of Nursing, Yuanpei University of Medical Technology, Hsinchu 300, Taiwan.*
 # Contributed equally to this work.

International Journal of Scholarly Research in Life Sciences, 2023, 02(01), 014–021

Publication history: Received on 07 July 2023; revised on 02 September 2023; accepted on 05 September 2023

Article DOI: https://doi.org/10.56781/ijsrls.2023.2.1.0031

Abstract

Plants have been used as traditional medicine or health products for several thousands of years. The present study was aimed to evaluate the genotoxicity of pomelo flower powders by micronucleus assay *In vivo*. During the *In vivo* genotoxicity-evaluated experiment, the experimental animal's clinical behavior, body weight (BW), food consumption, and the percentage of RET/RBCs (reticulocytes/red blood cells) and MN-RET/RETs (micronucleated reticulocytes/reticulocytes) after the treatments of pomelo (Citrus maxima) flower powders were evaluated. Both sexes ICR mice were treated three daily treatments by intraperitoneal injection of 2 mg/kg of mitomycin C (genotoxicity induction) or by oral route of 200 μL of PBS (the normal control group). Until 30th hours after the last treatment, K_2-EDTA-anticoagulated peripheral blood specimens were collected. These blood samples were processed for the microscopy-based analysis using Giemsa stain and the percentage of reticulocytes and micronucleated reticulocytes was determined. The results were shown that the experimental animal's clinical behaviors were normal in all groups. The BW and food consumption were no significant difference between all groups. RET/RBCs (%) in male or female ICR mice in the negative control group, the normal control group, the low dose of pomelo (*C. maxima*) flower powder group, the middle dose of pomelo (*C. maxima*) flower powder group, and the high dose of pomelo (*C. maxima*) flower powder group were respectively 8.8 ± 2.3 / 9.6 ± 2.6, 23.0 ± 2.5 / 22.4 ± 2.3, 23.4 ± 2.1 / 23.2 ± 3.8, 24.2 ± 3.6 / 23.0 ± 1.9, and 21.6 ± 3.2 / 21.6 ± 2.4; MN-RET/RETs (‰) in male or female ICR mice in the negative control group, the normal control group, the low dose of pomelo (*C. maxima*) flower powders group, the middle dose of pomelo (*C. maxima*) flower powder group, and the high dose of pomelo (*C. maxima*) flower powder group were 43.0 ± 12.5 / 39.4 ± 9.8, 2.6 ± 1.5 / 2.6 ± 1.5, 2.4 ± 1.1 / 2.2 ± 1.3, 2.2 ± 1.3 / 2.0 ± 1.2, and 1.8 ± 0.8 / 1.8 ± 0.8, respectively. Both RET/RBCs (%) and MN-RET/RETs (%) in male or female ICR mice in the negative control group were significantly difference than the other groups (p < 0.001). Taken all results together, pomelo (*C. maxima*) flower powders were without genotoxicity. Therefore, pomelo (*C. maxima*) flower powders were safety.

Keywords: Genotoxicity; *In vivo*; Micronucleus assay; Pomelo flower powders; Reticulocytes

* Corresponding author: Shao-Wen Hung

1 Introduction

In recent years, factors such as changes in people's health concepts, changes in living habits, emphasis on dietary intake, the promotion of alternative medical concepts, and advances in life science and technology have promoted the vigorous development of today's health food industry. Due to the gradual aging of the world and the rising market demand in Association of Southeast Asian Nations countries, it is estimated that the functional food market is promising [1-2]. Plants have been used as traditional medicine or health products for several thousands of years. Plant medicine is still a mainstay of the world's population as they are easily available source for healthcare purposes. Pomelo (*Citrus maxima*) possesses the ethanobotanical, pharmacognostic, phytochemical, and pharmacological properties. The various parts of pomelo (*C. maxima*) are widely used. The leaves of pomelo (*C. maxima*) are treated in epilepsy, chorea, convulsive cough, and hemorrhage disease. Oil of pomelo (*C. maxima*) fresh leaves possess anti-dermatophytic and fungicidal activities. Flowers of pomelo (*C. maxima*) are used as sedative in the nervous affection. Fruits of pomelo (*C. maxima*) acts as cardiotonic in leprosy, asthma, cough, hiccough, mental aberration, epilepsy. Rind of pomelo (*C. maxima*) are used as anti-asthmatic, sedative in nervous affection, brain tonic, the attenuation of vomiting, griping of abdomen, diarrhea, headache, and eye troubles. Root and bark of pomelo (*C. maxima*) possess anti-microbial activity. Due to the various claims for functional activities of numerous diseases, many literatures reveal some notable pharmacological activities of pomelo (*C. maxima*) such as activity on the attenuation of central nervous system disorder, anti-diabetic property and the decrease of cholesterol, analgesic property, anti-inflammatory, hepatoprotective property, anti-oxidative property, cytotoxic activity, and many more medicinal and health values [3-7].

According to the research and investigation, Taiwan's agricultural science papers are cited more than other fields, showing Taiwan's agricultural science and technology has R&D energy that cannot be ignored. Recently, the industrialization of scientific and technological achievements has attracted the attention of the government and the public. At the same time, the demonstration of this global trend is also an opportunity for Taiwan's agricultural science and technology to once again demonstrate the miracle. Looking at the industrialization development of agricultural science and technology in Taiwan today, a considerable amount of resources and technologies have been accumulated in upstream R&D, but how to quickly transform technologies and resources into commodities through the industrialization development platform, and then form settlements and industries, is a topic worthy of attention. Therefore, it is an urgent need to establish a stable, accurate and rapid animal test evaluation system to evaluate the safety of healthy foods and functional foods as a pre-market screening tool [1, 8-11].

An increasing number of reports are shown that "functional foods" are containing some functional component benefits may be observed to enhance short-term well-being and increasingly considered healthful. Based on these findings, the study was aimed to upgrade the production capacity of high value-added agricultural products. For the quality assurance, toxicity assessment for the functional foods is necessary. There is an emerging requirement to apply *In vivo* genotoxicity assays to evaluate the carcinogenic potential of these functional foods. Based on these requirements, we suggested that the mammalian erythrocyte micronucleus assay is used for the detection of damage induced by the test substance to the chromosomes of erythroblasts [12-23]. In this study, we tried to institute a sensitive and stable method for quantifying the formation of micronuclei in erythrocytes *In vivo*. The objective of this study was to evaluate the safety of powders of pomelo (*C. maxima*) flowers via micronucleus assay *In vivo*. The resulting data will enable us to evaluate the potential toxicity assessment of agricultural functional products in the future.

2 Material and methods

2.1 Chemicals and Reagents

Mitomycin C (Cayman, CAS 50-07-7, Item No.11435), Giemsa staining solution kit (Baso corporation, WGO-020), phosphate-buffered saline (PBS; Sigma-Aldrich, Cat. No.P3813), saline (Taiwan Biotech Co., LTD, Cat. No. 100-120-1101), Zoletil 50 (Virbac, Carros, France), DPX mountant (Sigma-Aldrich, Cat. No. 44581).

2.2 Source of Dry Powders of Pomelo (*Citrus maxima*) Flowers

The dry powders of pomelo (*C. maxima*) flowers were provided from Hualien District Agricultural Research and Extension Station, Hualien, Taiwan.

2.3 Preparation of Mitomycin C

Weighing 0.75 mg of mitomycin C and add it to 3 mL of saline and mix it to a concentration of 250 μg/mL mitomycin C. To draw mitomycin C solution (2 mg/kg BW) with a 1 mL syringe for the subsequent intraperitoneal injection (the injection volume is adjusted according to the BW of mice).

2.4 Experimental Animals and Experimental Design

Adult male and female ICR mice [6 weeks old; 50 mice; body weight (BW) between 26-27 g] with specific pathogen-free conditions were used for this study, were purchased from BioLASCO Taiwan Co., Ltd. (Yilan, Taiwan). These ICR mice were fed with standard laboratory diet (No. 5001, LabDiet®; PMI Nutrition International, St. Louis, MO, USA) and distilled water ad libitum during the experimental period. The environment was maintained room temperature (24-27°C) and 60%-70% humidity with a photoperiod of 12-hr light/12-hr dark cycle. The study will begin after a week acclimation. The Institutional Animal Care and Use Committee (IACUC) of Agricultural Technology Research Institute inspected all animal experiments and this study comply with the guidelines of protocol IACUC-111046 approved by the IACUC ethics committee. The 25 ICR male mice and 25 ICR female mice were divided respectively into as the normal control group (n = 10; 5 male and 5 female mice), the negative control group (n = 10; 5 male and 5 female mice), the high dose (1,000 mg/kg BW) of pomelo (*C. maxima*) flower powder group (n = 10; 5 male and 5 female mice), the middle dose (500 mg/kg BW) of pomelo (*C. maxima*) flower powder group (n = 10; 5 male and 5 female mice), and the low dose (250 mg/kg BW) of pomelo (*C. maxima*) flower powder group (n = 10; 5 male and 5 female mice). The mitomycin C-induced genotoxicity were performed in the negative control group. The clinical behaviors, BW, food consumption, and blood smear exanimation were monitored and performed during the experiment.

2.5 Collection of peripheral blood from ICR mice

The ICR mice were anesthetized with Zoletil 50 and the blood collection site was cleaned with 70% alcohol. The blood was stored in anticoagulant tubes containing K_2-EDTA for use in the subsequent experiments. ICR mice in three doses of pomelo (*C. maxima*) flower powder groups were respectively administered three doses of Pomelo flower powders (the interval between each time must exceed 24 hours), and peripheral blood in all groups must be collected at 30th hours after the last administration of pomelo (*C. maxima*) flower powders.

2.6 Preparation of Blood Smear and Giemsa Staining with Light Microscopic Examination

Blood smear on the glass slides were prepared by a senior researcher. The air-dried blood smears were stained with 1 mL of Giemsa stain for 1-3 minutes, then add 2 mL of PBS (pH 7.2) or distilled water for 2-6 minutes. The slides were washed with the running water, air-dried and mounted by DPX mountant, and then placed under a light microscope (1,000× magnification) to observe and count the number of cells. At least, 1,000 reticulocytes were observed in each ICR mouse, the number of micronuclei was recorded, and the proportion of reticulocytes in total red blood cells was calculated.

2.7 Statistical Analysis

The data were expressed as mean ± SD. All comparisons were made by one-way ANOVA and all significant differences are reported at $^{***}p < 0.001$.

3 Results

3.1 Change of ICR Mouse's BW in All Groups

The pomelo (*C. maxima*) flower powders were provided via the oral administration in three pomelo (*C. maxima*) flower powder groups. In this study, the clinical behavior observation indexes of ICR mice in each group were normal during the experiments. During the experiments, the ICR mice in each group had smooth hair, normal hair color, and the normal activity. Moreover, all ICR mice were survival until the end of the experiments. The survival rate of all ICR mice was 100% (50/50) (data not shown). The BW of ICR mice was detected on day 0, day 7, day 8, day 9, and day 10. Day 0 was ICR mice entering the animal rooms. Day 7 to day 9 were genotoxicity induction. The day 9 to day 10 were three doses of Pomelo flower powders administration. During the experiments, the ICR mice' BW were no statistically significant difference between all groups.

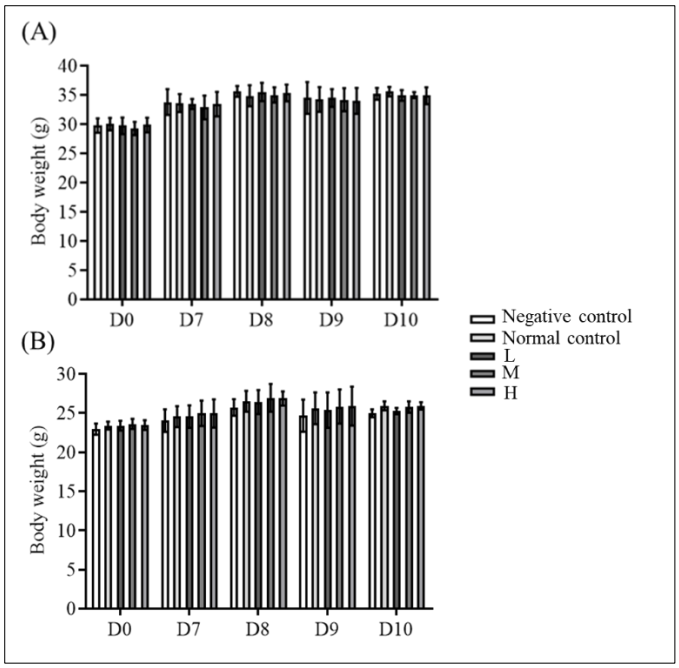


Figure 1 Change of BW before and after the treatments of three doses of pomelo (*C. maxima*) flower powders. (A) Male ICR mice. (B) Female ICR mice. All data are expressed as mean ± SD. 'H' is high dose (1,000 mg/kg BW) of pomelo (*C. maxima*) flower powder group. 'M' is middle dose (500 mg/kg BW) of pomelo (*C. maxima*) flower powder group. 'L' is low dose (250 mg/kg BW) of pomelo (*C. maxima*) flower powder group

3.2 Change of ICR Mice' Food Consumption in All Groups

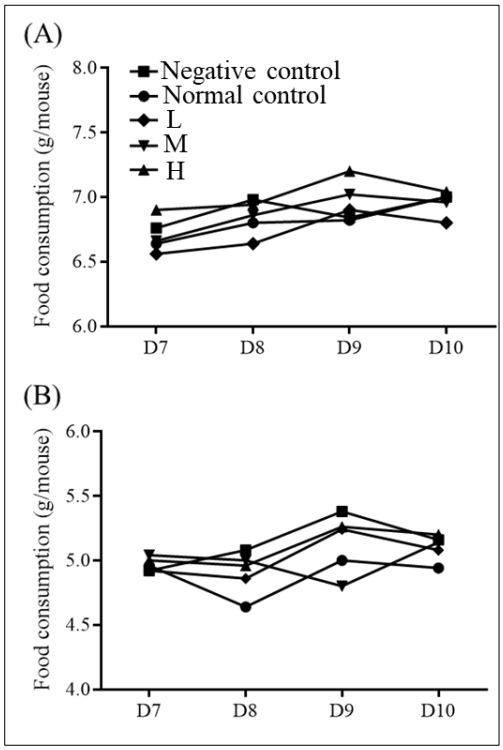
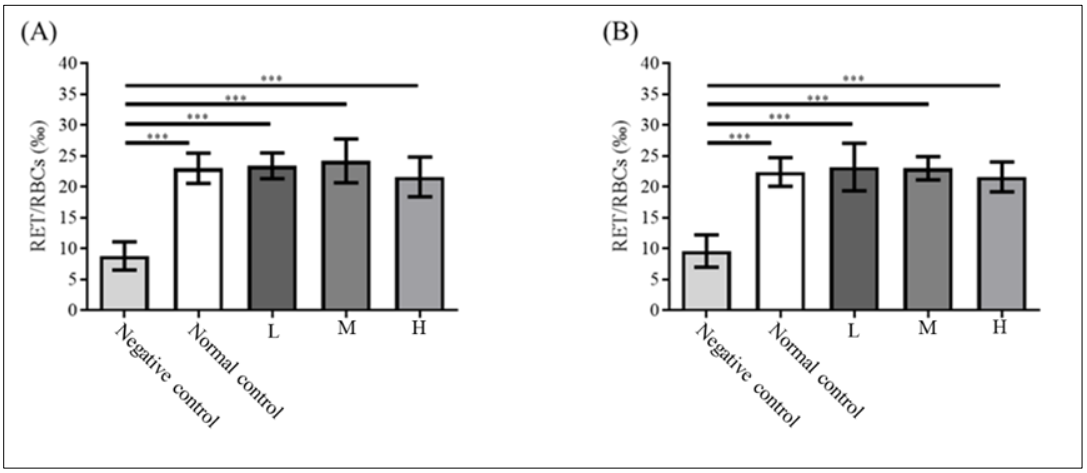


Figure 2 Change of food consumption after the treatments of three doses of Pomelo (*C. maxima*) flower powders. (A) Male ICR mice. (B) Female ICR mice. All data are expressed as mean ± SD. 'H' is high dose (1,000 mg/kg BW) of Pomelo (*C. maxima*) flower powder group. 'M' is middle dose (500 mg/kg BW) of Pomelo (*C. maxima*) flower powder group. 'L' is low dose (250 mg/kg BW) of Pomelo (*C. maxima*) flower powder group

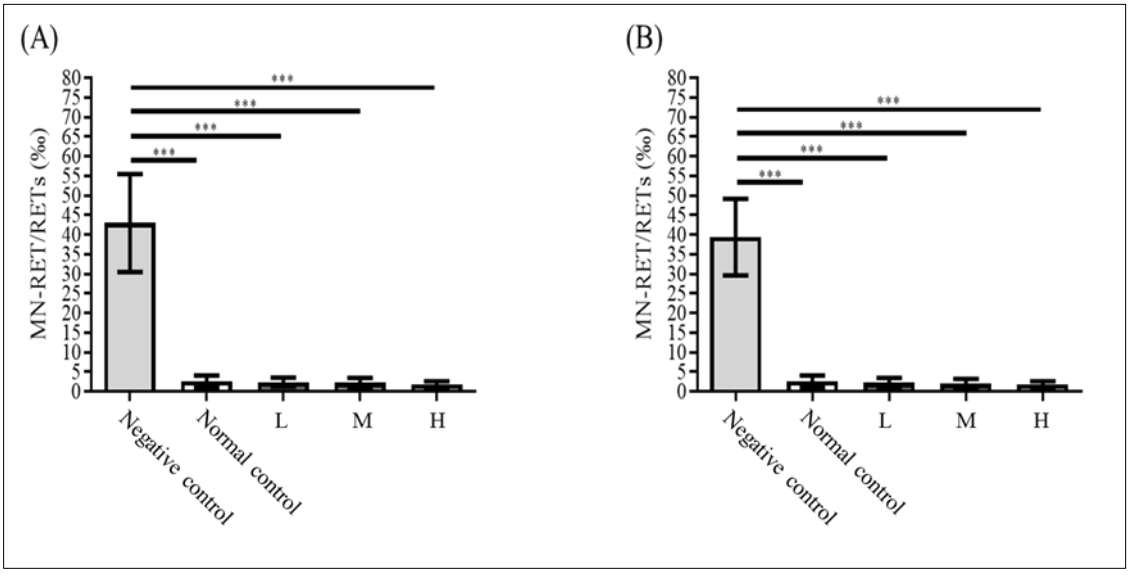
In this study, the ICR mice' food consumption in each group were monitored during the experiments. The food consumption of mice was detected on day 0, day 7, day 8, day 9, and day 10. The day 0 was mice entering the animal room. The day 7 to day 9 were genotoxicity induction. The day 9 to day 10 were three doses of pomelo (*C. maxima*) flower powder administration. During the experiments, three doses of pomelo (*C. maxima*) flower powders did not cause a decrease in appetite and abrupt decrease in food intake (Figure 2).

3.3 Change of RET/RBCs and MN-RET/RETs Percentages in All Groups



All data are expressed as mean ± SD. All significant differences compared to negative control group were reported at $^{***}p < 0.001$

Figure 3 Change of RET/RBCs and MN-RET/RETs after the treatments of three doses of pomelo (*C. maxima*) flower powders. (A) RET/RBCs (‰) in male ICR mice. (B) RET/RBCs (‰) in female ICR mice. 'H' is high dose (1,000 mg/kg BW) of Pomelo (*C. maxima*) flower powder group. 'M' is middle dose (500 mg/kg BW) of Pomelo (*C. maxima*) flower powder group. 'L' is low dose (250 mg/kg BW) of Pomelo (*C. maxima*) flower powder group.



All data are expressed as mean ± SD. All significant differences compared to negative control group were reported at $^{***}p < 0.001$

Figure 4 Change of RET/RBCs and MN-RET/RETs after the treatments of three doses of Pomelo flower powders. (A) MN-RET/RETs (‰) in male ICR mice. (B) MN-RET/RETs (‰) in female ICR mice. 'H' is high dose (1,000 mg/kg BW) of pomelo (*C. maxima*) flower powder group. 'M' is middle dose (500 mg/kg BW) of pomelo (*C. maxima*) flower powder group. 'L' is low dose (250 mg/kg BW) of pomelo (*C. maxima*) flower powder group.

ICR mice' peripheral anticoagulant blood were collected at 30th hours after the third administration, respectively. Blood samples were prepared for blood smears and processed with Giemsa staining. After blood sample processing, they were examined by using light microscope. The percentages of RET/RBCs and MN-RET/RETs were evaluated. The results showed that the male and female mice in the negative control group were induced genotoxicity. The percentage of RET/RBCs in the negative control group was significantly decrease than the other groups ($p < 0.001$) (Figure 3A-B). Except for the negative control group, there were no significant difference for the comparison of the other groups ($p > 0.05$) (Figure 3A-B). RET/RBCs (‰) in male / female ICR mice in the negative control group, the normal control group, the low dose of pomelo (*C. maxima*) flower powder group, the middle dose of pomelo (*C. maxima*) flower powder group, and the high dose of pomelo (*C. maxima*) flower powder group were respectively 8.8 ± 2.3 / 9.6 ± 2.6, 23.0 ± 2.5 / 22.4 ± 2.3, 23.4 ± 2.1 / 23.2 ± 3.8, 24.2 ± 3.6 / 23.0 ± 1.9, and 21.6 ± 3.2 / 21.6 ± 2.4 (Figure 3A-B). The percentage of MN-RET/RETs in the negative control group was significantly increase than the other groups ($p < 0.001$) (Figure 4A-B). Except for the negative control group, there were no significant difference for the comparison of the other groups ($p > 0.05$) (Figure 4A-B). MN-RET/RETs (‰) in male or female ICR mice in the negative control group, the normal control group, the low dose of pomelo (*C. maxima*) flower powder group, the middle dose of pomelo (*C. maxima*) flower powder group, and the high dose of pomelo flower powder group were 43.0 ± 12.5 / 39.4 ± 9.8, 2.6 ± 1.5 / 2.6 ± 1.5, 2.4 ± 1.1 / 2.2 ± 1.3, 2.2 ± 1.3 / 2.0 ± 1.2, and 1.8 ± 0.8 / 1.8 ± 0.8, respectively (Figure 4A-B). Taken all results together, pomelo (*C. maxima*) flower powders were without genotoxicity. Therefore, pomelo (*C. maxima*) flower powders were safety.

4 Discussion

In order to evaluate the safety of health food, Taiwan government has formulated a method for evaluating the safety of health food [22-23]. Among health food safety assessment methods, genotoxicity test methods can be divided into *In vivo* and *in vitro* tests, the purpose of which is to detect the genetic damage and extent directly or indirectly caused by the test substances, including the microbial gene mutation assays, the *in vitro* genotoxicity analysis of mammalian cells, and the genotoxicity analysis of animals *In vivo* [24-25]. *In vivo* genotoxicity assays in animals generally were used chromosomal damage assays of rodent hematopoietic cells, including bone marrow cell micronucleus assays, chromosomal abnormalities assays or peripheral blood micronucleus assays. Due to the high sensitivity of the micronucleus assays, this method is the most commonly used method for testing toxic drug-induced chromosomal aberrations *In vivo* [15-27].

Pomelo (*C. maxima*) are a perennial shrub and generally used eaten as fruit. They are rich in vitamin C and also contains high amount of polyphenolic compound like hesperidin, naringin, caffeic acid, ferulic acid, and vanillic acid. Their bark and root contain β-sitosterol, acridone alkaloid. The leaves contain essential oil and unripe fruits contain limonin, nerolol, nerolyl acetate, and geraniol. Pomelo (*C. maxima*) shows various pharmacological activities which has been studied. Currently, the aim of this study focused on the safety of pomelo (*C. maxima*). In this study, ICR mice' BW and food consumption were no significant difference between all groups. RET/RBCs (‰) in male or female ICR mice respectively in the negative control group, the normal control group, the low dose of pomelo (*C. maxima*) flower powder group, the middle dose of pomelo (*C. maxima*) flower powder, and the high dose of pomelo (*C. maxima*) flower powder group were 8.8 ± 2.3 / 9.6 ± 2.6, 23.0 ± 2.5 / 22.4 ± 2.3, 23.4 ± 2.1 / 23.2 ± 3.8, 24.2 ± 3.6 / 23.0 ± 1.9, and 21.6 ± 3.2 / 21.6 ± 2.4. In additionally, MN-RET/RETs (‰) in male or female ICR mice in the negative control group, the normal control group, the low dose of pomelo (*C. maxima*) flower powder group, the middle dose of pomelo (*C. maxima*) flower powder group, and the high dose of pomelo (*C. maxima*) flower powder group were 43.0 ± 12.5 / 39.4 ± 9.8, 2.6 ± 1.5 / 2.6 ± 1.5, 2.4 ± 1.1 / 2.2 ± 1.3, 2.2 ± 1.3 / 2.0 ± 1.2, and 1.8 ± 0.8 / 1.8 ± 0.8, respectively. Both RET/RBCs (‰) and MN-RET/RETs (‰) in the negative control group were significantly difference than other groups ($p < 0.001$). Among pomelo (*C. maxima*) flower powder groups, there were not significant differences ($p > 0.05$). Taken all results together, pomelo (*C. maxima*) flower powders were without genotoxicity. Therefore, pomelo (*C. maxima*) flower powders were safety.

5 Conclusion

The purpose of this study was to induce genotoxicity in ICR mice with mitomycin C and evaluate whether pomelo (*C. maxima*) flower powders were safe. Taken these results together, we successfully established the phenomenon of micronucleus in peripheral blood cells induced by mitomycin C in ICR mice. After detecting via this genotoxicity mouse

platform, we have demonstrated that pomelo (*C. maxima*) flower powders were no genotoxicity. In the future, we hope this genotoxicity mouse platform will provide to detect the genotoxicity in the test samples.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The Institutional Animal Care and Use Committee (IACUC) of Agricultural Technology Research Institute inspected all animal experiments and this study comply with the guidelines of protocol IACUC-111046 approved by the IACUC ethics committee.

References

[1] Vijaylakshmi P, Radha R. An overview: Citrus maxima. J. Phytopharmacol. 2015; 4: 263-267.

[2] Ling W, Kaliaperumal K, Huang M, Liang Y, Ouyang Z, Zhou Z, Jiang Y, Zhang J. pomelo seed oil: natural insecticide against cowpea aphid. Front Plant Sci. 2022; 13: 1048814.

[3] Chen Q, Liang J, Liu Q, Dong K, Yue L, Wei P, Luo Y, Liu Q, Li N, Tang B, Alshehri AA, Hamdy MS, Jiang Z, Sun X. Co nanoparticle-decorated pomelo-peel-derived carbon enabled high-efficiency electrocatalytic nitrate reduction to ammonia. Chem. Commun. (Camb). 2022; 58: 4259-4262.

[4] Qiu Y, Yang J, Ma L, Song M, Liu G. Limonin isolated from pomelo seed antagonizes αβ25-35-mediated neuron injury via PI3K/AKT signaling pathway by regulating cell apoptosis. Front Nutr. 2022; 9: 879028.

[5] Hu W, Chen M, Wang W, Huang F, Tian X, Xie L. Pomelo peel essential oil ameliorates cerebral ischemia-reperfusion injury through regulating redox homeostasis in rats and SH-SY5Y cells. Oxid. Med. Cell Longev. 2022; 2022: 8279851.

[6] He C, Li H, Hong J, Xiong H, Ni H, Zheng M. Characterization and functionality of cellulose from pomelo fruitlets by different extraction methods. Polymers (Basel). 2022; 14: 518.

[7] Okawa M, Kinjo J, Nohara T, Ono M. DPPH (1, 1-Diphenyl-2-Picrylhydrazyl) radical scavenging activity of flavonoids obtained from some medicinal plants. Boil. Pharm. Bull. 2001; 24: 1202-1205.

[8] Bryce SM, Bemis JC, Avlasevich SL, Dertinger SD. *In vitro* micronucleus assay scored by flow cytometry provides a comprehensive evaluation of cytogenetic damage and cytotoxicity. Mutat. Res. 2007; 630: 78-91.

[9] Hara M, Nakagawa S, Fujioka E, Ayukawa E. Detection of micronuclei in peripheral blood of mitomycin C-treated mice using supravital staining with acridine orange. Mutat. Res. 1992; 278: 175-179.

[10] Recio L. Comparison of flow cytometry- and microscopy-based methods for measuring micronucleated reticulocyte frequencies in rodents treated with nongenotoxic and genotoxic chemicals. Mutat. Res. 2008; 649: 101-113.

[11] Tometsko AM, Torous DK, Dertinger SD. Analysis of micronucleated cells by flow cytometry. 1. Achieving high resolution with a malaria model. Mutat. Res. 1993a; 292: 129-135.

[12] Tometsko AM, Dertinger SD, Torous DK. 1993b. Analysis of micronucleated cells by flow cytometry. 2. Evaluating the accuracy of high-speed scoring. Mutat. Res. 1993b; 292: 137-143.

[13] Witt KL, Livanos E, Kissling GE, Torous DK, Caspary W, Tice RR, Tometsko AM, Dertinger SD, Torous DK. Analysis of micronucleated cells by flow cytometry. 2. Evaluating the accuracy of high-speed scoring. Mutat. Res. 1993; 292: 137-143.

[14] Garriott ML, Piper CE, Kokkino AJ. A simplified protocol for the mouse bone marrow micronucleus assay. J. Appl. Toxicol. 1988; 8:141-144.

[15] Sato S, Inui N, Ikeda Y, Hiraga Y. A comparison of intraperitoneal injection and oral gavage in the micronucleus test with mitomycin C in mice. Mutat. Res. 1989; 223: 387-390.

[16] Hayashi M, MacGregor JT, Gatehouse DG, Adler I-D, Blakey DH, Dertinger SD, Krishna G, Morita T, Russo A, Sutou S. *In vivo* rodent erythrocyte micronucleus assay. II. Some aspects of protocol design including repeated treatments, integration with toxicity testing, and automated scoring. Environ. Mol. Mutagen. 2000; 35: 234-252.

[17] Hayashi M, Tice RR, MacGregor JT, Anderson D, Blakey DH, Kirsh-Volders M, Oleson FBJr, Pacchierotti F, Romagna F, Shimada H, Sutou S, Vannier B. *In vivo* rodent erythrocyte micronucleus assay. Mutat. Res. 1994; 312: 293-304.

[18] OE G, Karp NA, Ng BL, Jackson SP, Adams DJ, McIntyre RE. A high -throughput *In vivo* micronucleus assay for genome instability screening in mice. Nat. Protoc. 2015; 10: 205-215.

[19] Krishna G, Hayashi M. *In vivo* rodent micronucleus assay: protocol, conduct and data interpretation. Mutat. Res. 2000; 455: 155-166.

[20] Mavournin KH, Blakey DH, Cimino MC, Salamone MF, Heddle JA. The *In vivo* micronucleus assay in mammalian bone marrow and peripheral blood. Mutat Res. 1990; 239: 29-80.

[21] OECD. Test No. 487: *In vivo* mammalian cell micronucleus test. In series: OECD guidelines for the testing of chemicals, Section 4. OECD Publishing, Paris. 2014.

[22] Lin JW, Chen CC, Chiu CC, Yang CY, Hung SW. A study to establish a mouse genotoxicity model by mitomycin C induction. J. Chin. Soc. Anim. Sci. 2017; 46: 193-207.

[23] Lin YH, Chang YX, Chi TY, Chen HY, Hung YC, Lin CY, Chen GH, Wang YP, Huang PM, Wu TH, Lu YJ, Chiu CC, Chiu CF, Chiu HW, Tsai WH, Chen CC, Hung SW. 2022. Evaluation of genotoxicity of ethanolic extracts of *Platostoma palustre* by micronucleus assay. Int. J. Sci. Technol. Res. Arch. 5: 133-139.

[24] Qi H, Huang D, Jing J, Ran M, Jing T, Zhao M, Zhang C, Sun X, Sami R, Benajiba N. Transforming waste into value: pomelo-peel-based nitrogen-doped carbon dots for the highly selective detection of tetracycline. RSC Adv. 2022; 12: 7574-7583.

[25] Babaoğlu AS, Ainiwaer T, Özkan H, Karakaya M. Grapefruit and pomelo peel extracts as natural antioxidants for improved storage stability of Turkey patties during refrigerated storage. J. Food Sci. Technol. 2022; 59: 4067-4074.

[26] Wei Y, Fakudze S, Zhang Y, Song M, Xue T, Xie R, Chen J. Low-temperature hydrothermal liquefaction of pomelo peel for production of 5-hydroxymethylfurfural-rich bio-oil using ionic liquid loaded ZSM-5. Bioresour Technol. 2022; 352: 127050.

[27] Gao K, Liu T, Cao L, Liu Y, Zhang Q, Ruan R, Feng S, Wu X. Feasibility of pomelo peel dietary fiber as natural functional emulsifier for preparation of Pickering-type emulsion. J. Sci. Food Agric. 2022; 102: 4491-4499.