Evaluation of the Genotoxicity and Cytotoxicity of *Pereskia Bleo's* Extract

Deborah David Henry, Zainon Mohd Noor, Noriham Abdullah, Nooraain Hashim, Azizah Abdul Hamid, Normah Ismail, Wan Rozianoor Mohd Hassan and Mohd Faiz Foong Abdullah
Department of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), Shah Alam, 40450 Selangor Darul Ehsan

Abstract: *Pereskia bleo is commonly called 'Jarum Tujuh Bilah' in Malaysia. It is also one of the plants that are recognized for its antioxidant activity in which claimed to protect human against neurological diseases such as Alzheimer's and Parkinson diseases. Although P.bleo is widely used in Malaysia, there is still little information provided on the safety and toxicity of P. bleo extracts. A series of standard test were done to evaluate the genotoxicity and toxicity of P.bleo extracts. These tests included the mutagenicity test performed based on OECD guidelines section #471 that was proposed by Maron and Ames (1983), in vitro micronucleus test subjected to OECD guidelines #487using Chinese hamster lung cell (V79) and neutral red uptake cytotoxicity assay using normal mouse fibroblast cell (NIH/3T3) as per OECD guidelines section #129. P. bleo extract was tested on histidine-dependent mutant of Salmonella typhimurium TA98, TA100, TA1535 and TA1537 strains with and without S9 mix. The result showed a slight mutagenic activity on strain TA98 (with metabolic activation) at concentration of 0.62 mg/ml. Apart from that, P. bleo extract also showed no growth of the revertant colonies at all at the concentration of 50 mg/ml, 16.67 mg/ml and 5.56 mg/ml when treated on all tester strains with and without the presence of rat liver enzyme which indicates some antimicrobial activities. In the micronucleus test, P. bleo extract at the concentration of 50.0 µg/ml (with and without rat liver enzymes), 25 µg/ml (without rat liver enzyme), and 12.5 µg/ml (without rat liver enzyme) induced and increase in the formation of micronucleus when screened enzyme using V79 cell. In addition, the results also demonstrated some cytotoxic effect when P.bleo extract was tested against NIH/3T3 cells. Based on the result obtained, P. bleo extract exhibits genotoxic and cytotoxic activity. Therefore, further detailed experiments will be needed to examine the ingredient responsible for causing the genotoxicity and cytotoxicity of P. bleo.*

Keywords: *Mutageniciy, genotoxicity, cytotoxicity, Pereskia bleo.*

1. Introduction

P. bleo or famously known as 'Jarum Tujuh Bilah" by the locals and it belongs to the Cactaceae family. *P. bleo* was originated from South America and was brought and developed to several tropical countries for medicinal purposes. It has a spiny shrub and it could grow to around 2- 8m tall. The leaves had a spiral shape and glossy surface [1]

Medicinal plants are natural product which responsible for the production of bioactive compounds that has medicinal value and help to cure or prevent ailments [2][3][4]. Since some of the medicinal plants were lack in safety and quality information nowadays, the needs to find scientific evidence on the safety consumption of these medicinal plants is very crucial.

P. bleo was claimed to have anti-cancer, anti-rheumatic and anti-inflammatory properties [5]. The hexane and methanol extracts of *P. bleo* showed highly and moderately antibacterial activity when screened against Gram-negative bacteria [6]. In addition, a study was conducted and stated that the Ethyl acetate extract of P. bleo leaf gives high antioxidant and bioactive compound [7].

However, there is limited information on the genotoxicity and cytotoxicity study from the plant extract of P. bleo. A study showed that they have investigated the anti- cancer, antibacterial, antioxidant and cytotoxic activity of P. bleo but so far there is no study conducted on the P.bleo mutagenicity and genotoxicity. Thus an investigation into the mutagenicity and genotoxicity of the P. bleo extract can be beneficial in contributing information on the safe consumption of this plant.

http://dx.doi.org/10.17758/ER615007

2. Materials and methods

2.1. Chemical and raw materials

P. bleo extracts was obtained from the planted MARDI Jalan Kebun Klang. The leaves were washed, air-dried and grinded using liquid nitrogen into a fine powder. The leaves in powder form was freeze dried, extracted with ethanol (1:10) and filtered using a vacuum pump. The aqueous ethanolic extracts were then separated using a rotary evaporator (45ºC-60°C), freeze dried once again and finally stored at -20°C freezer.

The chemical used in Ames test includes positive controls; sodium azide, [3-chloro-7-methoxy-9-(3-[chloro-ethyl] aminopropylamino) acridine dihydrochloride], daunomycin and 2-aminoanthracene, Salmonella thyphimurium mutants strain (TA98, TA100, TA1535, TA1537) was supplied by Moltox, molecular toxicology, Inc (USA). Dimethyl sulfoxide (DMSO) as negative control supplied by Amresco, USA. In In vitro micronucleus test, the chemicals used were chinese hamster lung cells (V79) supplied by ATCC cell lines, Dulbecco's Modified Eagle's Medium (DMEM) by Lonza, USA, fetal bovine serum (FBS) by MP Bio, USA, Sodium pyruvate by Sigma-Aldrich, USA, Sodium bicarbonate by Amresco, USA, penicillin-streptomycin-amphotericin b by Lonza, USA, mitomycin c as the positive control supplied by Moltox, USA. While for Neutral red cytotoxicity test the chemicals used were mouse fibrobast cell (NIH/3T3) supplied by ATCC cell lines, Sodium dodecyl sulfate (SDS) by Sigma, USA as the positive control and acridine orange by Sigma-Aldrich, USA.

2.2. Cell culture

The in vitro micronucleus test was performed by using V79 cell, which was purchased from ATCC cell lines. The cells were cultured in DMEM supplemented with 10% of FBS, sodium pyruvate, penicillin-streptomycin-amphotericin b mixture and sodium bicarbonate.

2.3. Bacterial reverse mutation test (Ames Test)

The Ames test was done based from OECD guideline section 471 (Updated Guidelines, 21th July 1997). Basically, this test allows for the detection of mutations by reverting the mutations that are present in the Salmonella typhimurium test strains. This allows the mutated test strains to gain back its functional ability to produce the essential amino acid which is histidine. Therefore, the mutated Salmonella typhimurium strains are able to grow in the absence of histidine and were transformed into histidine independent bacteria. The test method involved the preparation of fresh bacterial culture of Salmonella typhimurium which was procured from Moltox,USA. The most commonly used Salmonella strain were TA1535, TA100, TA98, and TA1537. These strains were genetically modified in the histidine operon. The test was performed with different concentrations with the highest dose is 50000 µg/ml, 16670 µg/ml, 5560 µg/ml, 1850 µg/ml, and 620 µg/ml . The leaves extract were exposed to the bacteria with and without metabolic activation.The negative control was dimethyl sulfoxide (DMSO).The positive controls were sodium azide, [3-chloro-7-methoxy-9-(3-[chloro-ethyl] aminopropylamino) acridine dihydrochloride], daunomycin and 2-aminoanthracene and was supplied by Moltox,USA.

2.4. Neutral red cytotoxicity assay

The test was based on Borenfreund & Puerner (1985) shows only live cells can take up neutral red (NR) dye, while dead cells do not absorb the dye. Cytotoxicity neutral red assay gives a quantitative estimation of the number of Neutral Red Dye in lysosomes of viable cells in a culture. This test basically prove only viable cells have the capability to penetrate and bind to the neutral red dye in the lysosomes. The normal mouse fibroblast cells (NIH/3T3) were seeded in 96-well tissue culture plates together with the plant and fruit extracts and was incubated for 3 hours at 37ºC with a medium containing neutral red. Then, the cells were washed and the dye was extracted from each well and fixed. Lastly, a spectrophotometer was used to read the absorbance at 540nm. Sodium dodecyl sulfate was used as the positive control for its mutagenic activity towards the mouse fibroblast cells.

2.5. *In vitro* micronucleus test

The In vitro mammalian cell micronucleus test was done based from the OECD guideline section 487 (Updated Guidelines, adopted 22nd July 2010). This test allows for the detection of micronuclei in the cells that have undergone cell division, it was usually formed during the metaphase or anaphase stage. The test substances

are not mutagenic when cells have the ability to finish nuclear division after being exposed to the test samples. V79 cells were seeded in 5 mL of fresh complete culture medium (DMEM) and grown for 24 hrs. The V79 cells were treated with P. bleo with concentration of 50 µg/ml, 25 µg/ml and 12.5 µg/ml and incubated for 3 hours. Upon finishing the treatment, the cells we washed by using PBS-EDTA and the cell suspension was gently transferred to a centrifuge tube. The cell suspensions prepared were centrifuged at 1500 r.p.m around 5 min, supernatant discarded .Then, 0.075M KCI was slowly added onto the cells pellet and was gently mixed. The cells were then centrifuged (1500 r.p.m. for 5 min) and fixed in Carnoy's fixative (3:1 methanol: acetic acid) for 30 min. Finally, the cells were resuspended in a small volume of 20 µl and dropped on to cleaned slides and dried using slide warmer. Acridine orange was added as the staining agent and the slides were covered with a coverslip and observed under a fluorescent microscope. A total of 1000 micronueleated and non-micronucleated cells were observed from each concentration and controls.

3. Results and Discussion

In Ames test, the number of the revertant bacteria also exceeded two fold of the negative control at this concentration. P. bleo plant extracts as in Table 3, showed a slight mutagenic activity on strain TA98 (with metabolic activation) at concentration of 0.62 mg/ml. Apart from that, the results in Table 3 mostly shows no growth of the revertant colonies at the concentration of 50 mg/ml, 16.67 mg/ml and 5.56 mg/ml when treated on all four S.thyphimurium strains with and without the presence of rat liver enzyme. It had been reported that P. bleo that was extracted using ethyl acetate had showed some antimicrobial activity against Pseudomonas aeruginosa [8].

In neutral red cytotoxcity assay, P.bleo demonstrated some cytotoxic effect when screened against NIH/3T3 cells. The IC50 shown by P.bleo was at 0.018192 mg/ml. It was reported in a study that neutral red cytotoxicity assay using P. bleo extracts gave an IC50 value of 0.02 mg/ml [9]. Sri et al., 2008 also stated in their study that the ethyl acetate fraction from P. bleo showed remarkable cytotoxic activity when screened against Human Nasopharyngeal Epidermoid Carcinoma cell line (KB cell).

While for in vitro micronucleus assay P. Bleo extract has the ability to induce the formation of micronucleus when screened against V79 cells without the presence of metabolic activation. The formation of micronucleus also indicates the presence of DNA damage which caused cell death as referred to the cytotoxic result in this experiment.

TABLE I The response of *P. bleo* in the *Salmonella typhimurium* reverse mutation assay. Results represent mean number of revertant colonies/3 replicate plates ± standard deviation

Concentration (mg/ml)	**TA98**	**TA100**	**TA1535**	**TA1537**
Without metabolic activation				
Negative control	10±2	129±11	14±2	9±1
0.62	10±1	151±17	4±2	39±31
1.85	13±5	39±7	1±1	0±0
5.56	15±3	0±0	0±0	0±0
16.67	7±2	0±0	0±0	0±0
50	0±0	0±0	0±0	0±0
Positive control	148±45	938±380	637±38	5003±182
With metabolic activation (10% S9-mix)				
Negative control	16±5	117±9	20±2	18±7
0.62	34±8	96±10	12±4	15±2
1.85	22±8	90±7	4±1	10±3
5.56	1±1	1±1	0±0	0±0
16.67	11±2	0±1	1±1	0±0
50	4±1	0±1	1±1	0±0
Positive control	1188±424	1927±81	108±12	362±63

Negative control used was DMSO and if the test sample is two fold increase when compared to the spontaneous reversion rate it is considered to be mutagenic. Daun=Daunomycin; 2-AA= 2-aminoanthracene; ICR- 191= [3-chloro-7-methoxy-9-(3-[chloro-ethyl] aminopropylamino) acridine dihydrochloride]; SA=Sodium azide

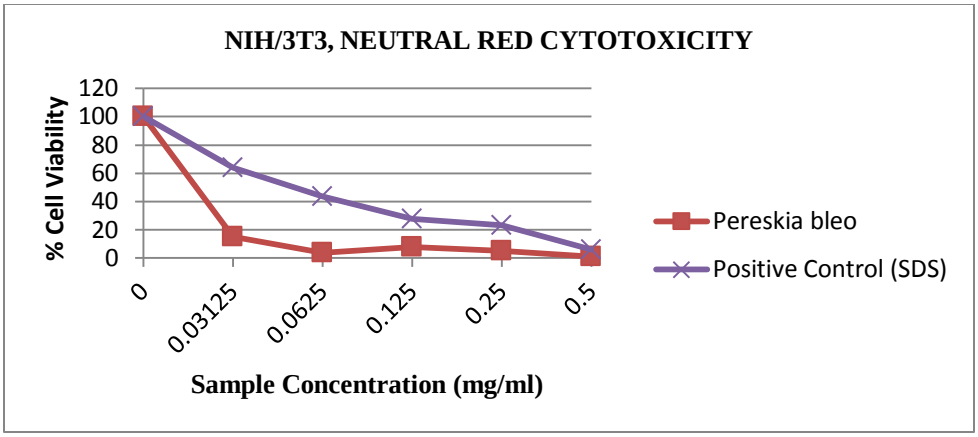

Fig. 1: The percentage of viability versus the sample concentration

TABLE II: Effect of *A.bilimbi*, *C.caudatus* and *P. bleo* on micronucleus induction towards V79 cells

Treatment	Micronuelated cells (%)
Without metabolic activation	
Negative control	0.4
P. bleo (12.5 µg/ml)	1.1
P. bleo (25.0 µg/ml)	1.2
P. bleo (50.0 µg/ml)	4.5
Positive control	4.6
With metabolic activation (10% of S9 mix)	
Negative control	1.5
P. bleo (12.5 µg/ml)	1.3
P. bleo (25.0 µg/ml)	1.5
P. bleo (50.0 µg/ml)	2.4
Positive control	2.5

4. Conclusion

A standard test was done to assess the mutagenicity, genotoxicity and cytotoxicity of P. *bleo* in order to establish the profiles of *P. bleo* extract. These results showed that *P. bleo* may exhibit a mutagenic, genotoxic and cytotoxic effect based on the bacterial reverse mutation assay (Ames test), neutral red cytotoxicity assay and *in vitro* micronucleus assay. Thus, it is very important to study the genotoxic mechanism of *P. bleo* extracts. Therefore, further detailed experiments will be needed to examine the ingredient responsible for causing the genotoxicity and cytotoxicity of *P. bleo.*

5. Acknowledgment

The authors would like to acknowledge the funding support from Ministry of Science and Technology (MOSTI) and Agro Biotechnology Institute (ABI) (100-RMI/MOSTI 16/6/2 (1/2011).

6. References

[1] C. Wiart, *Medicinal plants of the Asia-Pacific: Drugs for the future*, World scientific Publishing, 2006, pp. 98-99 http://dx.doi.org/10.1142/9789812707260

[2] Firenzuoli F., & Gori L. (2007). Herbal Medicine Today: Clinical and Research Issues. *Evidence-Based Complementary and Alternative Medicine, 4*, 37-40. http://dx.doi.org/10.1093/ecam/nem096

[3] Ha H., Lee,J. K., Lee H. Y., Seo C. S., Lee M. Y., Huh J. I., & Shin H. K. (2011). Genotoxicity assessment of a herbal formula, Ojeok-san. *Journal of Ethnopharmacology*, *135*(2), 586-589. http://dx.doi.org/10.1016/j.jep.2011.03.024

[4] Roy A., Geetha R. V., & Lakshmi T. (2011). Averrhoa bilimbi Linn-Nature's Drug store-A pharmacological review. *International Journal of Drug Development and Research, 3*(3), 101-106.

[5] Tan, M. L., Sulaiman, S. F., Najimuddin, N., Samian, M. R., & Muhammad, T. S. T. (2005). Methanolic extract of Pereskia bleo (Kunth) DC. (Cactaceae) induces apoptosis in breast carcinoma, T47-D cell line. *Journal of Ethnopharmacology, 96*(1–2), 287-294. doi: 10.1016/j.jep.2004.09.025
http://dx.doi.org/10.1016/j.jep.2004.09.025

[6] Wahab S.I.A, Mohan S.M., Al-Zubairi A.S, Elhassan M.M., & Ibrahim M. Y. Biological acivities of Pereskia bleo extracts (2009). *International Journal of Pharmacology* 5(1): 71-75.

[7] Hassanbaglou, B., Hamid, A. A., Roheeyati, A., Saleh, N. M., Abdulamir, A. S., Khatib, A., & Sabu, M. Antioxidant activity of different extracts from leaves of Pereskia bleo (Cactaceae).

[8] Koshy, P., Sri, N. A. M., Wirakarnian, S., Sim, K. S., Saravana, K., Hong, S. L., Lee, G. S., &Syarifah N. S. R. (2009). Antimicrobial Activity of Some Medicinal Plants from Malaysia. *American Journal of Applied Sciences* 6 (8): 1613-1617.
http://dx.doi.org/10.3844/ajassp.2009.1613.1617

[9] Sri, N. A. M., Norhanom, A. W., Hashim, Y.,Sim, K. S., Hong, S. L., Lee, G. S., &Syarifah N. S. R. (2008). Cytotoxic Activity of Pereskia bleo (Cactaceae) against selected Human Cell lines. *International Journal of Cancer Research* 4 (1): 20-27.
http://dx.doi.org/10.3923/ijcr.2008.20.27