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Protective and Antigenotoxic Potentials of *Lantana camara* Linn. Leaves

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Abstract
Peripheral blood micronucleus assay was used to evaluate the antigenotoxic and protective potentials of crude methanolic extract of air-dried leaves of *Lantana camara* (Linn.) against methyl methanesulfonate (MMS)-induced genotoxicity in albino mice. Mice pre-treated with MMS prior to extract treatments served as the antigenotoxic tests while those pre-treated with the extract before MMS treatment were the protective tests. The frequencies of micronuclei in the erythrocytes of the treated mice were scored 36 hours after the last treatment. Results demonstrated that the extract has possessed both the protective and antigenotoxic properties against MMS-induced genotoxicity in mice in a dose-dependent manner. The study indicated that the plant could be a potential source of cure against genotoxic-related problems.

Keywords: Micronucleus assay, *Lantana camara* Linn., methyl methanesulfonate, antigenotoxicity test, protective test

Introduction

Unknowingly, some of the natural substances in the environment are genotoxic that may bring damage to the genetic material (DNA, RNA) in any organisms including humans which usually leads to mutations in different cells of the body causing various problems and diseases to the host. Mutations can cause genetic aberrations which may lead to serious abnormalities such as cancer and birth defects, and in worst cases could be heritable (Shah, 2012). Interestingly, micronuclei present in cells have been utilized as biomarker of genotoxity. These micronuclei appear as small satellite nucleus surrounding the cell nucleus; these are actually chromosomes or fragments of chromosomes which are left behind after cell division (Gentile *et al.* 2012 & Heddle, 1973). Nowadays, peripheral blood micronucleus assay in mice is widely utilized in assessing the genotoxic potential of some substances against living systems (CSGMT, 1992).

Significance of the study

Presently, researches in ethnomedicinal plants as possible source of cure for various diseases are growing tremendously in the Philippines. *Lantana camara* Linn. is a well-known ethnomedicinal weed not only in Philippines but also in many countries which can cure myriads of common diseases including coughs and colds, pains, headaches and fever (Joy *et al.* 2012). This flowering plant is ornamental which belongs to family Verbenaceae. Lantadenes, the natural compounds present in all *L. camara* are probably accountable for almost all of its biological activities. Phenolics, alkaloids and terpenoids are some of the secondary metabolites found in the plant

which could account for some of its biological activities such as antibacterial, antifungal, anticancer, anti-inflammatory, anti-helminthic, antioxidant, and larvicidal activity (Kalita *et al.*, 1997). But to date there are no studies on the protective and antigenotoxic potentials of the crude methanolic extract from air-dried leaves of *L. camara* using peripheral blood micronucleus assay in mice. Thus, this study was conducted primarily to evaluate such potentials utilizing the assay.

Figure 1. *Lantara camara* Linn.

Methodology

Extract preparation

Fresh leaves of the *L. camara* collected from the 4th Street of Mindanao State University campus, Marawi City, Philippines were washed with distilled water, air-dried for 2 weeks and powderized using an electric grinder. Powdered leaves (250g) were soaked up to 1000 ml 95% methanol for 72 hours, and the supernatant was filtered by filter paper Whatman # 1. Methanol was evaporated to dryness using rotary evaporator at 115 rpm, 40°C. The extract was dissolved in Dimethyl sulfoxide (DMSO) and diluted with distilled water just before use to prepare 250 ppm and 500 ppm concentrations.

Treatment administration and research design

The mice were grouped into seven treatments (Table 1) with three replicates composing one mouse per replicate. Distilled water (T1) and DMSO (2) were used as negative controls. Methyl methanesulfonate (MMS) (Sigma Aldrich) was the mutagen used to induce mutations and DNA damage in mice (T3, positive control). To evaluate the antimutagenic potential of the extract against MMS–induced mutation, the mice were pre-treated with MMS 2 days prior to extract administration at two different concentrations, 250 ppm (T4) and 500 ppm (T5). To determine the protective potential of the extract against MMS–induced micronuclei formation, the mice were pre-treated with the leaf extract for 3 days at two different concentrations, 250 ppm (T6) and 500 ppm (T7), before MMS treatment. MMS was diluted with distilled water just before use to a final dose of 50mg/kg mouse body weight during treatment. MMS, extract, DMSO and distilled water were administered to mice once daily at 24-hour interval by oral gavage at 0.2mL/20g mouse body weight.

Table 1. The experimental set-up and treatment administration

TREATMENTS	ADMINISTRATION/DURATION
Control Tests	
T1 (negative)	Distilled water only, 5 days
T2 (negative)	DMSO only, 5 days
T3 (positive)	Distilled water, 3 days + MMS, 2 days
Antimutagenic Tests	
T4	MMS, 2 days + 250 ppm extract, 3 days
T5	MMS, 2 days + 500 ppm leaf extract, 3 days
Protective Tests	
T6	250 ppm leaf extract, 3 days + MMS, 2 days
T7	500 ppm leaf extract, 3 days + MMS, 2 days

Peripheral blood micronucleus assay (PBMA) using albino mice

Albino mice (*Mus musculus*) used in the study were 7–12 weeks old of either sex, with 10-30 grams body weights. PBMA outlined in CSGMT (1992) was followed in this study with minor modifications. Peripheral blood in all treatments was collected 36 hours after the last treatment. Blood was obtained by cutting the tails of the mice. A drop of blood was smeared into glass slide, air-dried, fixed by dipping once in 95% methanol for two seconds then air-dried again. The fixed blood samples were stained with Acridine Orange (Sigma Aldrich) at 0.1g/100mL dilution by distilled water, air-dried overnight, then finally covered with cover slip ready for micronuclei scoring. Five blood smears (slides) were prepared per replicate and 1000 erythrocytes per slide were scored blindly for micronuclei using a Ken-α-vision light microscope at 1000x magnification.

Statistical Tests

Kruskal Wallis and Tamhane tests at 1% and 5% levels of significance were the statistical tools used to analyze the results.

Results and discussion

Micronucleated erythrocytes were observed in all treatments (Figure 2). Expectedly, the positive control with MMS-treatment had the highest frequency of micronuclei (Figure 3) while the negative controls had the lower scores of micronuclei. The minimal number of micronuclei observed in the negative controls was probably due to spontaneous chromosomal aberrations in the mice erythrocytes (CSGMT, 1995). Both the protective and antigenotoxic tests had lower micronuclei counts than the positive control. Kruskal Wallis Test (Table 2) confirmed that the variations of the treatment means were highly significant at p value of 0.003. Furthermore, Tamhane Test demonstrated that the positive control having the highest score for micronucleated erythrocytes was significantly higher than the rest of the treatments. This result implied that the mutagen MMS used effectively induced micronuclei formation in the treated erythrocytes.

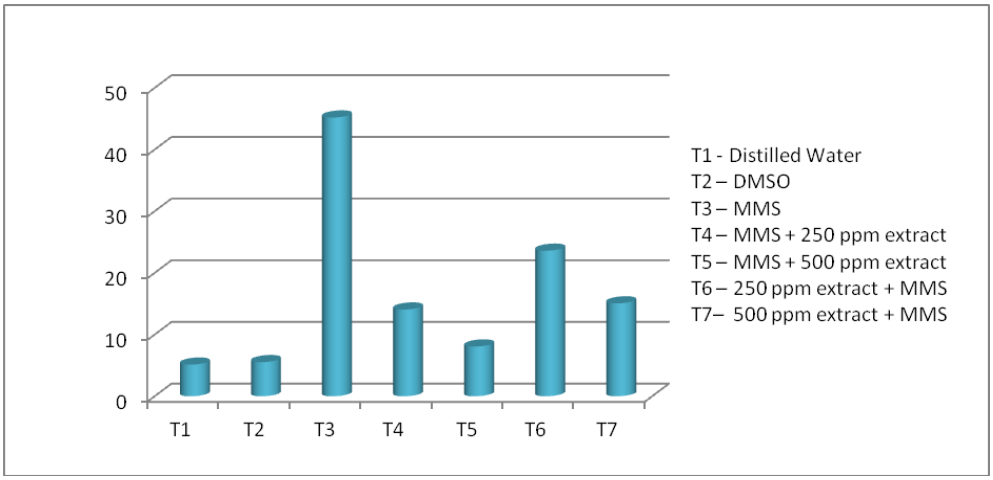

Figure 2. Mean frequency distribution of micronucleated erythrocytes in seven treatments

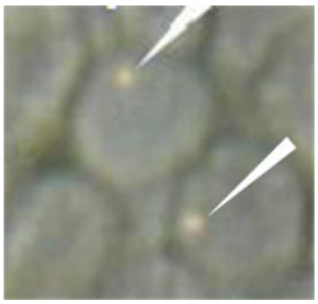

Figure 3. Photomicrograph of mice erythrocytes (1000x) showing MMS-induced micronuclei stained with acridine orange.

Table 2. Kruskal Wallis and Tamhane Tests on the treatment means of micronucleated erythrocytes

Treatments	R1*	R2*	R3*	Mean	Tamhane Test**	Kruskal Wallis Test
T1 (Distillled Water)	5.4	4.6	5.4	5.133	A	Chi-square value 22.025 p value = 0.003***
T2 (DMSO)	5.6	5.2	5.8	5.533	A	
T3 (MMS)	44.6	46	45.0	45.2	D	
T4 (MMS + 250 ppm extract)	12.4	14.6	15.2	14.066	B	
T5 (MMS + 500 ppm extract)	8.4	8.2	7.6	8.066	A	
T6 (250 ppm extract + MMS)	23.6	23.8	23.4	23.6	C	
T7 (500 ppm extract + MMS)	14.6	15.2	15.4	15.066	B	

*values of the replicates are means of the five blood smears
**means having the same letters are not significantly different at $\alpha \leq 0.05$
***highly significance at p value ≤ 0.01

For antigenotoxic evaluation of the extract, mice were pre-treated with MMS prior to extract treatments. This test was designed to induce first the micronuclei formation in mice by MMS but it should reverse the mutation after the treatment of the extract thus, antimutagenic (CSGMT, 1992). Indeed the results conformed to the premise; T4 and T5 yielded remarkably lower frequencies of micronuclei (Figure 2 and Table 2), significantly lower than the positive control T3 as confirmed by Tamhane test demonstrating the antimutagenic properties of the extract. Somehow the treatment reversed the mutagenic effect of MMS on micronuclei formation. Tamhane test further demonstrated that the effect of extract in reducing MMS-micronuclei worked in dose-dependent manner, i.e. the higher the dose, the lower the number of micronuclei. In fact the micronuclei count at higher dose of 500 ppm (T5), was not statistically different from the negative controls.

For protective evaluation, mice were pre-treated with the extract prior to MMS treatment. This design should inhibit or reduce the formation of micronuclei in mice erythrocytes when treated with MMS, thus protective (CSGMT, 1992). Interestingly, the data corroborated the argument; much lower counts of micronuclei in T6 and T7 than that of the positive control T3 were observed (Figure 2) and the reduced counts were statistically significant by Tamhane test which decreased as the dose was reduced (Table 2). Though the reduced counts of micronuclei were significantly higher than the negative controls, nevertheless, the counts were lower significantly than the positive control implying that the extract effectively reduced the ability of the MMS to induce micronuclei formation in mice thus demonstrating its protective property.

The mutagen MMS as positive control showed the highest count of micronuclei. MMS is commonly used in experimental research with chemicals such as catalyst for chemical synthesis (Merck 1989; and IARC 1974 & 1999). MMS, being a monofunctional alkylating agent is known to cause damage to DNA because it can directly interact with DNA forming O-alkylated and N-alkylated DNA bases (Sylianco, 1998). Figure 4 shows the metabolic activation of MMS in the formation of the alkylated DNA. If repair is not immediately carried out when alkylation occurs, destabilization of the DNA base pair follows leading to chromosome breakage.

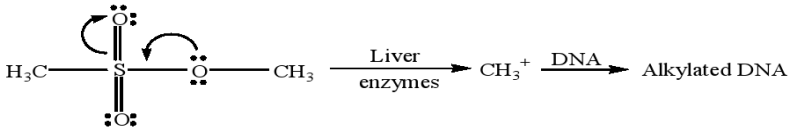

Figure 4. Metabolic activation of methyl methanesulfonate

Figure 4. Metabolic activation of methyl methanesulfonate

Major phytochemical groups present in the *L. camara* Linn., which include proteins, carbohydrates, oligosaccharides, glycosides, iridoid glycosides, phenolic compounds, phenyl ethanoid, flavonoids, alkaloids, steroids, sesquiterpenoides, triterpens, tannins, quinines, and saponins were suspected for its antimutagenic and protective potentials against MMS – induced mutagenicity in mice erythrocytes. There are several mechanisms by which chemopreventive agents can bring about antimutagenic effects, i.e. inhibiting tumour progression, carcinogen activation, replication and modulation of DNA metabolism and repair; promoting apoptosis and detoxification of carcinogenic agents; or control of gene expression (Kumar *et al.* 2008).

In this study, the protective effect of the crude methanolic leaf extract of *L. camara* is probably due to the antioxidant property of natural compounds in the extract that traps free radicals, and

interacts with the mutagen. Related studies on garlic, curcumin and saffron conducted by Goud *et al.*, (1993) Oda *et al.* (1995) and Premkumar *et al.* (2004) mentioned that the antimutagens may work by changing the activation and detoxification of the mutagens; by modulating the metabolism of xenobiotics through absorption; or by inhibiting the functions of superactive oxygen species. In desmutagenic manner, the antimutagens in the extract may directly interact with MMS without affecting the DNA molecules. The bio-active compounds may inactivate the MMS by reacting directly with the latter's methyl cation. Another mode of action is possibly through the interaction of the DNA's nucleophilic sites with the bioactive compounds present in the extract hence the mutagen could no longer bind to these sites.

Conclusion

The study significantly illustrates that *L. camara* methanolic leaf extract has antimutagenic and protective properties against the MMS-induced mutagenicity using PBMA in albino mice. The extract worked in dose-dependent manner in which the dosage is inversely proportional to the MMS-induction of micronuclei formation. Further studies should be conducted to exploit the potential of *L. camara* Linn. as possible source of cure against genotoxic-related problems.

References

CSGMT (The Collaborative Study Group for the Micronucleus Test). (1992). Micronucleus test with mouse peripheral blood erythrocytes by acridine orange supravital staining: The summary report of the 5th collaborative study by CSGMT/JEMS.MMS. *Mutation Research, 278*, 83-89.

Gentile, N., **Mañas, F**., **Bosch, B**., **Peralta, L**., **Gorla, N**., **Aiassa. D**. (2012). Micronucleus assay as a biomarker of genotoxicity in the occupational exposure to agrochemicals in rural workers. ***Bulletin of Environmental Contamination and Toxicology,*** *88(6), 816-22.*

Goud, V. K., Polasa, K., Krishnaswamy, K. (1993). Effect of turmeric on xenobiotic metabolizing enzymes. *Plant Foods for Human Nutrition, 44(1)*, 87-92.

Heddle, J. A. (1973). A Rapid In Vivo Test for Chromosomal Damage. *Mutation Research, 18*, 187-190.

IARC (**International Agency for Research on Cancer**). (1974). Methyl methanesulfonate. In Some Anti-thyroid and Related Substances, Nitrofurans and Industrial Chemicals. *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, 7,* 253-260.

Joy, J. M., Vamsi, S., Satish, C., Nagaveni, K. (2012). Lantana camara Linn: *A REVIEW. International Journal of Phytotherapy, 2(2),* 66-73.

Kalita, S., Kumar, G., Karthik, L., Rao, K. V. B. (2012). A Review on Medicinal Properties of Lantana camara Linn. ***Research Journal of Pharmacy and Technology****, 5(6)*, 711-715.

Kumar, M. S., Maneemegalai, S. (2008). Evaluation of larvicidal effect of Lantana camara Linn. against mosquito species Aedes aegypti and Culex quinquefasciatus. *Advances in Biological Research, 2,* 39-43.

Merck. (1989). *The Merck Index*, 11th ed. Rahway, NJ: Merck & Company, Inc.

Oda, H., Nakatsuru, Y., Shimizu, S., Yamazaki, Y., Nikaido, O., Ishikawa, T. (1995). Quantitative detection of ultraviolet light-induced photoproducts in mouse skin by immunohistochemistry. *Japan Journal on Cancer Research*, *86(11)*, 1041-8.

Premkumar, A., Kreitman R. J., Kindler, H., Willingham, M. C., Pastan, I. (2004). Phase I study of SS1P, a recombinant anti-mesothelin immunotoxin given as a bolus I.V. infusion to patients with mesothelin-expressing mesothelioma, ovarian, and pancreatic cancers. *Clinical Cancer, 13(17),* 5144–5149.

Shah, S. U. 2012. Importance of Genotoxicity & S2A guidelines for genotoxicity testing for pharmaceuticals. *IOSR Journal of Pharmacy and Biological Sciences*, *1(2),* 43-54.

Sylianco, C. Y. L. 1998. Philippine Science Encyclopedia: Pharmaceutical and Chemical Sciences, National Research Council of the Philippine.