Journal of Medicinal Plants Research Vol. 5(8), pp. 1345-1348, 18 April, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Mutagenic and antimutagenic activities of *Mitragyna speciosa* Korth extract using Ames test

A. R. Ghazali[1]*, R. Abdullah[2], N. Ramli [1], N. F. Rajab [1], M. S. Ahmad-Kamal[3] and N. A. Yahya[3]

[1]Biomedical Science Department, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia, 50 300, Kuala Kuala Lumpur, Malaysia.
[2]Environmental Health Programme, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia, 50 300, Kuala Kuala Lumpur, Malaysia.
[3]Herbal Medicine Research Centre, Institute of Medical Research (IMR), 50 300, Kuala Lumpur, Malaysia.

Accepted 6 December, 2010

***Mitragyna speciosa* Korth (Ketum) is a medicinal plant that has been used traditionally to enhance energy, cure illnesses, fever and diarrhea. Aqueous extract of *M. speciosa* was screened for the potential of mutagenic and antimutagenic activity using Ames test (Salmonella/microsome mutagenicity assay). Ames test involved the pre-incubation assay against *Salmonella typhimurium* TA 98 and TA 100 bacterial strains in the presence and absence of metabolic activator S9 system. Every extract was evaluated using two-fold value of the number of revertant's colony in negative control plate as cut-off point, to determine the mutagenicity effects. No mutagenic activity was found for frameshift mutation (TA98) and base-pair substitution (TA100) in all concentrations of *M. speciosa* in the presence and absence of metabolic activator S9 system. Inhibition percentage of revertant's colony was used to evaluate the antimutagenic activity of *M. speciosa* aqueous extract by simultaneous addition of mutagen. Significant antimutagenic activity ($p<0.001$) were observed in three concentrations of *M. speciosa* as compared to mutagenicity induced by 2-aminoanthracene for both TA 98 and TA 100 strain with the presence of metabolic activator S9 system. In conclusion, *M. speciosa* did not show any mutagenicity effects in both tester strains in the presence and absence of metabolic activator S9 system. However, *M. speciosa* showed strong antimutagenicity properties in both strains with the presence of metabolic activator S9 system.**

Key words: *Mitragyna speciosa* Korth, mutagenic, antimutagenic, Ames test.

INTRODUCTION

Since ancient time, several diseases have been treated by administration of plant extracts based on traditional medicine (Pezzuto, 1997). It is important to note that most of the traditional medicinal plants have never been the subject of exhaustive toxicological tests as required for modern pharmaceutical compounds. Based on their traditional use for long periods of time, they are often assumed to be safe. However, research has shown that a lot of plants which are used as food ingredients or in traditional medicine have *in vitro* mutagenic (Cardoso et al., 2006; Déciga-Campos et al., 2007; Mohd-Fuat et al., 2007) and antimutagenic properties (Calomme et al., 1996; Choi et al., 1997; Park et al., 2004; Kilani et al., 2005; Bouhlel et al., 2007). Thus, investigation of traditionally used medicinal plants is valuable as a source of potential chemotherapeutic drugs and as a measure of safety for the continuous use of medicinal plants. In the last few decades, much study has been directed at popular medicine, with the aim of identifying natural products with therapeutic properties (Hamburger and Hostettmann, 1991; Weisburger et al., 1996).

*Corresponding author. E-mail: rohi@medic.ukm.my. Tel: +603 92897618. Fax: +603 26938717.

Mitragyna speciosa Korth (Ketum), family Rubiciacceae, is found naturally in tropical and subtropical regions of Asia, especially in Thailand and Malaysia (Jansen and Prast, 1988). The leaf of *M. speciosa* has been used traditionally to increase work efficiency and enhance tolerance to hard work under a scorching sun (Suwanlert, 1975). It also used to cure fever, cough and treatment for diarrhea (Kumarnsit et al., 2007). From the leaves, mitragynine was obtained as the major constituent (66.2% base on the crude base extract) together with its analogues, paynanthneine (8.6%), speciogynine (6.6%), 7-hydroxymitragynine (2.0%) and speciociliatine (0.8%) (Takayama, 2004).

The aim of this study was to evaluate the mutagenic and antimutagenic potential of *M. speciosa* by studying their effect on two histidine requiring *Salmonella typhimurium* in the absence and presence of a liver metabolizing system.

METHODOLOGY

Sample

Sample of *M. speciosa* (Voucher No: RM 0108) was received and has been stored at the Institute of Medical Research, Kuala Lumpur. The material was extracted using the aqueous extraction. 100 g of dried powdered form of *M. speciosa* was dissolved in 2 L of distilled water. The plant material was boiled for 2 h and filtered. The supernatant was collected and dried using spray drier. The yield of 100 g crude extract of *M. speciosa* was 18±2 g of aqueous extract of *M. speciosa*. The sample was dissolved in sterile distilled water in 3 concentrations which are 50, 12.5 and 3.125 mg/ml.

Bacterial strains

S. typhimurium strains TA 98 and TA 100 which are histidine-requiring mutant as previously described by Maron and Ames (1983). The genotypes of test strains were checked routinely for their histidine requirement, deep rough (rfa) character, UV sensitivity (uvrB mutation) and presence of the R factor plasmid. They were stored at -80°C. *S. typhimurium* TA 98 is frame shift strain which contain the his3052 mutation and *S. typhimurium* TA 100 contain the base-pair substitution mutation hisG46.

Mutagens

2-Nitrofluorene (2-NF), sodium azide (NaN_3) and 2-aminoanthracene (2-AA) were purchased from Sigma-Aldrich and dissolved in dimethyl-sulfoxide (DMSO).

Mutagenicity testing

The test is based on the plates incorporation method (Maron and Ames, 1983), using *S. typhimurium* test strains TA 98 and TA 100, with and without exogenous metabolic activation system, the S9 fraction in S9 mix. The S9 fraction from liver, Pooled from male rat (Sprague-Dawley) were bought from Sigma-Aldrich. The test strains from frozen cultures were grown overnight for 12 to 16 h in nutrient broth. Various concentrations of each extract dissolved in sterile distilled water were added to 2 ml of top agar, supplemented with 0.5 mM L-histidine and 0.5 mM D-biotine, mixed with 100 µl of bacterial culture (approximate cell density 2-5 x 108 cells/ml) and then poured on to a plate containing minimum agar medium. The plates were incubated at 37°C for 48 h and the his+ revertant colonies were counted. The influence of metabolic activation was tested by adding 500 µl of S9 mixture. Negative and positive control cultures gave number of revertants per plate that were within the normal limits found in the laboratory. The data were collected in mean±SEM in three plates (n=3).

Antimutagenicity testing

A modified plate incorporated procedure (Lee et al., 2000) was employed to determine the effect of *M. speciosa* extracts on 2-NF, NaN_3 and 2-AA induced mutagenicity. In brief, 0.5 ml of S9 mixture or phosphate buffer was distributed in sterilized capped tubes in an ice bath, then 0.1 ml of mutagen, 0.1 ml of plant extract and 0.1 ml of bacterial culture were added. After, it was gently mixed and preincubated at 37°C for 45 min, 2 ml of top agar supplemented with 0.5 mM L-histidine and 0.5 mM D-biotine were added to each tube and vortexed for 3 s. The mixture was overlaid on the minimal agar plates. The plates were incubated at 37°C for 48 h and the revertant colonies were counted. The inhibition rate of mutagenicity (%) was calculated by using equation from (Ong et al., 1986) and (Negi et al., 2003):

Inhibiton rate (%) = 1 - (T / M) x 100%

Where T, is the number of revertants per plate in the presence of mutagen and plant extract and M is the number of revertants per plate in positive control. No antimutagenic effect was considered to give a value smaller than 25%, a moderate effect value between 25 and 40% and a strong antimutagenicity value greater than 40%.

Statistical analysis

The results were expressed as mean±SEM of three independent experiments. The one-way ANOVA test was used to analyze the result and $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Table 1 showed the results of mutagenic evaluation assays in revertants/plate with and without the metabolic activation system S9. The absence of mutagenic activity can be seen for frame shift (TA 98) and base-pair (TA 100) in the presence and absence of S9 mix. The highest number of mean revertant/plate in TA 98 strain were observed at the dose of 12.5 mg/ml *M. speciosa* aqueous extract without the present of metabolic activation. At the same concentration, with the presence of metabolic activation, S9 also induced the highest mean/plate in TA 100. However, the mutation frequency for both strains did not change significantly when compared to spontaneous mutation frequency ($p>0.05$) as using one-way ANOVA test. The absence of a mutagenic response by *M. speciosa* against *S. typhimurium* bacterial strains in the Ames assay is a positive step forward in determining the safe use of this plant utilized in traditional medicine. However, plant extracts exhibiting a positive response

Table 1. Mutagenic acitivities of *M. speciosa* without metabolic activation S9 (-S9) and with metabolic activation S9 (+S9).

Strain	Concentration	Mean ± SEM (-S9)	Mean ± SEM (+S9)
TA 98	Positive control	643 ± 32	497 ± 67
TA 98	Negative control	11 ± 2	18 ± 4
TA 98	3.125 mg/ml	10 ± 2	18 ± 2
TA 98	12.5 mg/ml	12 ± 3	17 ± 1
TA 98	50 mg/ml	11 ± 3	20 ± 5
TA 100	Positive control	1463 ± 123	299 ± 23
TA 100	Negative control	14 ± 1	72 ± 20
TA 100	3.125 mg/ml	12 ± 2	79 ± 16
TA 100	12.5 mg/ml	10 ± 1	80 ± 17
TA 100	50 mg/ml	5 ± 3	67 ± 11

Negative control = sterile distilled water, positive control; strain TA 98 (-S9 = 2-NF, +S9 = 2-AA), strain TA 100 (-S9 = NaN_3, +S9 = 2-AA).

Table 2. Antimutagenic acitivities of *M. speciosa* without metabolic activation S9 (-S9) and with metabolic activation S9 (+S9).

Strain	Concentration	Mean ± SEM (-S9)	Inhibition rate (%)	Mean ± SEM (+S9)	Inhibition rate (%)
TA 98	Positive control	643 ± 32	-	497 ± 67	-
TA 98	Negative control	20 ± 5	-	33 ± 8	-
TA 98	3.125 mg/ml	679 ± 148	0	*104 ± 13	79.07
TA 98	12.5 mg/ml	572 ± 54	11.05	*67 ± 16	86.63
TA 98	50 mg/ml	417 ± 37	35.12	*43 ± 9	91.35
TA 100	Positive control	1463 ± 123	-	299 ± 23	-
TA 100	Negative control	20 ± 2	-	72 ± 20	-
TA 100	3.125 mg/ml	1346 ± 91	8	*97 ± 18	67.77
TA 100	12.5 mg/ml	1179 ± 117	19.39	*87 ± 14	71.03
TA 100	50 mg/ml	1313 ±107	10.27	*91 ± 16	69.73

Negative control = sterile distilled water, positive control; strain TA 98 (-S9 = 2-NF, +S9 = 2-AA), strain TA 100 (-S9 = NaN_3, +S9 = 2-AA).
*Significance compare to control at $p<0.001$.

and hence a mutagenic effect need to be extensively investigated, to determine their possible genotoxicity to humans as their safe use in traditional medicine is questionable. The absence of mutagenic effect cannot be considered as safe to be used in all natural products, since other medicinal plants assayed with Ames test, with or without the S9, have resulted positive for mutagenicity (Kul et al., 1987; Sohni et al., 1995; Diana, 1997). Table 2 showed the response of the interaction between the mutagens (2-NF, NaN_3 and 2-AA) and the different concentration of *M. speciosa* with and without metabolic activation of S9 system. The extracts were unable to inhibit the mutagenic activity in the absence of S9 mix. However, in the presence of S9 mix, all concentration of *M. speciosa*, induced strong antimutagenic activity. All the test (TA 98 and TA 100) presented signs of significant inhibition of the induced mutagenic effect in the presence of S9 mix with ($P<0.001$) using one-way ANOVA test as compared to positive control. Over 25 alkaloids have been isolated from *M. speciosa* leaves. Mitragynine is the major alkaloid found in this plant. Mitragynine has been demonstrated to produce an antinoceceptive effect through the action of supraspinal opiod receptors and descending noradrenergic and serotonergic systems (Matsumoto et al., 1997).

Antimutagenic properties elicited by plant species have a full range of prospective applications in human health. Herbal remedies and phytotherapy drugs, containing active principles are currently developed to protect against electrophile (e.g free radical) attack on DNA and its widespread outcomes such as ageing and cancer. The occurrence rate of cancer is increasing worldwide and the determination of chemopreventive or chemoprophylaxis compound is important in the effort to reduce the risk of cancer (Kundu et al., 2005). Antimutagenicity determination of plant extracts is important in the discovery of new

effective anticarcinogenic treatments. A plant extract indicating antimutagenicity is not necessarily an anticarcinogen; however, it is an indication of possible candidates for such purposes.

Based on this *in vitro* evaluation of the plant, further investigation on phytochemical screening and extensive cytotoxic and genotoxic testing should be done, to determine its safe use as a traditional medicine. This plant should be gazetted for research purposes, only to reduce the risk of misuse by irresponsible persons.

CONCLUSION

No indication of any mutagenic activity in both strains of bacteria, TA 98 and TA 100, with and without the S9 metabolic activation system of aqueous extract of *M. speciosa*. However, in all concentrations of *M. speciosa* aqueous extract, strong antimutagenic activities were seen in the presence of S9 metabolic activation system.

ACKNOWLEDGEMENT

The Institute of Medical Research Kuala Lumpur is thanked for providing an adequate sample of *M. speciosa* used in this research.

REFERENCES

Bouhlel I, Mansour H (2007).Screening of antimutagenicity via antioxidant activity in different extracts from the leaves of Acacia salicina from the center of Tunisia. Environ. Toxicol. Pharmacol., 23(1): 56-63.

Calomme M, Pieters L (1996). Inhibition of bacterial mutagenesis by Citrus flavonoids. Planta medica, 62: 222-226.

Cardoso C, Cólus I (2006). Mutagenic activity promoted by amentoflavone and methanolic extract of Byrsonima crassa Niedenzu. Toxicol., 225(1): 55-63.

Choi J, Lee H (1997). *In vitro* antimutagenic effects of anthraquinone aglycones and naphthopyrone glycosides from Cassia tora. Planta. Med., 63(1): 11-14.

Déciga-Campos M, Rivero-Cruz I (2007). Acute toxicity and mutagenic activity of Mexican plants used in traditional medicine. J. Ethnopharmacol., 110(2): 334-342.

Diana AN (1997). Modulating effects of flavonoids on food mutagens in human blood and sperm samples in the comet assay. Teratogen. Carcinogen. Mutagen, 17(2): 45-58.

Hamburger M, Hostettmann K (1991). 7. Bioactivity in plants: the link between phytochemistry and medicine. Phytochemistry, 30(12): 3864-3874.

Jansen K, Prast C (1988). Psychoactive properties of mitragynine (kratom). J. Psychoactive Drugs, 20(4): 455-457.

Kilani S, Ben AR (2005). Investigation of extracts from (Tunisian) Cyperus rotundus as antimutagens and radical scavengers. Environ. Toxicol. Pharmacol., 20(3): 478-484.

Kul L, Kook KHY (1987). Effect of bacterial growth-inhibiting ingredients on the Ames mutagenicity of medicinal herbs. Mutat. Res. Lett., 192(2): 99-104.

Kumarnsit E, Keawpradub N (2007). Effect of Mitragyna speciosa aqueous extract on ethanol withdrawal symptoms in mice. Fitoterapia, 78(3): 182-185.

Kundu J, Mossanda K (2005). Inhibitory effects of the extracts of Sutherlandia frutescens (L.) R. Br. and Harpagophytum procumbens DC. on phorbol ester-induced COX-2 expression in mouse skin: AP-1 and CREB as potential upstream targets. Cancer let., 218(1): 21-31.

Lee K, Sohn I (2000). Essential moiety for antimutagenic and cytotoxic activity of hederagenin monodesmosides and bisdesmosides isolated from the stem bark of Kalopanax pictus. Planta Medica, 66(4): 329-332.

Maron D, Ames B (1983). Revised methods for the Salmonella mutagenicity test. Mutat. Res./Environ. Mutagen. Relat. Subj., 113(3-4): 173-215.

Matsumoto K, Mizowaki M (1997). Suppressive effect of mitragynine on the 5-methoxy-N, N-dimethyltryptamine-induced head-twitch response in mice. Pharmacol. Biochem. Behavior, 57(1-2): 319-323.

Mohd-Fuat A, Kofi E (2007). Mutagenic and cytotoxic properties of three herbal plants from Southeast Asia. Trop. Biomed., 24(2): 49.

Negi P, Jayaprakasha G, Jena BS (2003). "Antioxidant and antimutagenic activities of pomegranate peel extracts." Food Chem., 80(3): 393-397.

Ong T, Whong WZ, Stewart JD, Brockman HE (1986). "Chlorophyllin: a potent antimutagen against environmental and dietary complex mixtures." Mutat. Res. Lett., 173(2): 111-115.

Park K, Jung G, Lee K, Choi J (2004). "Antimutagenic activity of flavonoids from the heartwood of Rhus verniciflua." J. Ethnopharmacol., 90(1): 73-79.

Pezzuto J (1997). "Plant-derived anticancer agents." Biochem. Pharmacol., 53(2): 121-133.

Sohni Y, Davis C, Champs A, Kale P (1995). "Frameshift mutations in salmonella induced by the extracts of medicinal herbs Lannea edulis (sond.) engl. and Monotes glaber sprague." Environ. Mol. Mutagen., 25(1): 77-82.

Suwanlert S (1975). "A study of kratom eaters in Thailand." Bull. Narcotics, 27(3): 21-28.

Takayama H (2004). "Chemistry and pharmacology of analgesic indole alkaloids from the rubiaceous plant, Mitragyna speciosa." Chem. Pharm. Bull., 52(8): 916-928.

Weisburger JH, Hara Y, Dolan L, Luo F, Pittmana B, Zang E (1996). "Tea polyphenols as inhibitors of mutagenicity of major classes of carcinogens." Mutat. Res./Genet. Toxicol., 371(1-2): 57-63.