Journal of Biosciences and Medicines, 2019, 7, 10-19
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Mutagenic Potential of Medicinal Polyherbal Preparations

Mohd Fuat Abd Razak

Department of Biological and Biomedical Sciences, Glasgow Caledonian University, Glasgow, UK
Email: fuat62@gmail.com

How to cite this paper: Razak, M.F.A. (2019) Mutagenic Potential of Medicinal Polyherbal Preparations. *Journal of Biosciences and Medicines*, **7**, 10-19.
https://doi.org/10.4236/jbm.2019.711002

Received: October 2, 2019
Accepted: October 21, 2019
Published: October 24, 2019

Abstract

Extracts of twelve medicinal polyherbal preparations in powder form sold at retail shops were tested for mutagenicity using Ames *in vitro* test. Five of the polyherbal preparations were found to be mutagenic ($p \leq 0.05$) at 250 μg/ml. Two mutagenic preparations comprised of traditionally used medicinal plants with no mutagenicity property being reported. However, one polyherbal preparation which contained one mutagenic plant was found not mutagenic. Under the conditions of this study, it can be concluded that some polyherbal preparations were potentially mutagenic and mutagenicity of polyherbal preparation cannot always be deduced from the mutagenicity status of each individual plant components of the polyherbal preparations.

Keywords

Ames Test, Mutagenic, Mutagenicity, Polyherbal, *Salmonella typhimurium* TA100

1. Introduction

It has been known that toxic components are present in many plants including vegetables [1] [2] [3]. Besides several reports on the cytotoxicity of some plants, several investigations have revealed that many plants used as food or in traditional medicine have mutagenic effects in *in vitro* assays [4] [5] [6] [7].

Some plants that expressed cytotoxic and mutagenic activities had shown correlation with the incidence of tumours and cancers [8]. Cancer is one of the major causes of morbidity and mortality. While all the factors that contribute to its onset are not fully known, it is clear that there are chemical agents that can induce cancer. These agents are called carcinogens. Most carcinogens induce cancer because they are mutagens.

The most definitive way to detect carcinogens is to inoculate a sample into animals and monitor for the development of tumours. This process is expensive, time consuming, and cumbersome. Therefore, a rapid, economical screening method is used to distinguish between compounds that might be carcinogenic and those that are likely to be proven harmless. The Ames test is a screening assay for carcinogens that uses bacteria to detect chemical mutagens. It is based on the premise that most carcinogens induce cancer because they are mutagens. The *Salmonella* mutagenicity test was designed to detect chemically induced mutagenesis [9]. If these agents are shown to be mutagenic for bacteria, they may also alter DNA in eukaryotic cells. The test is used world-wide as an initial screen to determine the mutagenic potential of chemicals and drugs and had been shown to correlate well with carcinogenicity test using rodent [10] [11].

Regulation of herbal products in many countries does not require mutagenicity testing although many herbal plants have been reported to be mutagenic. However, it has been reported that some polyherbal products which contains traditionally used herbs were found to be cytotoxic [12] and thus they might be mutagenic. Thus, the aim of this study was to evaluate the mutagenicity potential of some medicinal herbal preparations sold over-the-counter.

2. Materials and Methods

2.1. Herbal Samples

Twelves polyherbal preparations were purchased from retail shops. The products were packaged in either plastic bottles or plastic envelope, appropriately labelled with information on herbal composition and were at least 6 months before the expiration date. All products were in fine powder form approximately of 12 mesh particle size. **Table 1** shows the formulation of the polyherbal preparations and their intended uses.

2.2. Herbal Extracts

Since all of the herbal preparations were in fine powder form, it could be extracted directly with some solvents. There is no universal solvent to extract all plant constituents. However, methanol has been reported to be able to extract a wide range of plant constituents and combination of methanol with chloroform had been used in extraction of metabolites, DNAs, RNAs and proteins from plant simultaneously [13]. The herbal samples in this study were extracted according to the method described by [14]. Sample (20 g) was mixed with 75 ml of methanol/chloroform (1:1) in a screw-capped bottle and stirred with magnetic bar on a stirrer plate for 24 h. The extract was filtered through Whatman No. 1 filter paper and evaporated to dryness using vacuum evaporator at 40˚C. A portion of the dried extract was reconstituted in dimethyl sulfoxide (DMSO) to 250 μg/ml.

2.3. Mutagenicity Test

The most definitive way to detect carcinogens is to inoculate the test sample into

Table 1. Herbal formulations and intended usage of some polyherbal preparations.

Herbal preparation	Constituents	Plant parts used[a]	Percentage/amount (mg) in formulation	Intended use
A	*Curcuma*	Rhizoma	25%	Beauty cares; promoting liveliness and youthfulness.
	Curcumae	Herba	15	
	Archihilleae	Fructus	10	
	Coptici	Semen	10	
	Colae	Cortex	5	
	Alyxiae	Folium	4	
	Baeckeae	Semen	8	
	Parkiae,	Cortex	6	
	Parameriae	Fructus	6	
	Anisi	Herba	5	
	Phyllanthi	n.i	6	
B	*Curcumae*	Rhizoma	30%	To improve appetite and digestion
	Languati,	Rhizoma	15	
	Zingiberis	Rhizoma	15	
	Zingiberis aromatica	Rhizoma	15	
	Coriandri	Fructus	15	
C	*Hirtae*	Herba	10%	Cure influenza cough
	Kaempferiae	Rhizoma	15	
	Zingiberis	Rhizoma	12	
	Curcumae domestica	Rhizoma	20	
	Amomi	Fructus	5	
	Anisi	Fructus	15	
	Glycyrrhizae	Radix	10	
D	*Retrofracti*	Fructus	5%	Built a firm and attractive burst line
	Myristicae	Semen	5	
	Curcumae	Rhizoma	20	
	Curcumae domesticate	Rhizoma	5	
	Languatis	Rhizoma	15	
	Zingiberis aromatica	Rhizoma	15	
E	*Baeckeae folium*	Folium	15%	Regulate menstruation and alleviates pain during menstruation
	Coptisi	Fructus	15	
	Phyllanthi herba	Herba	25	
	Gallae	n.i	5	
	Coriandri	Fructus	10	
	Piperis nigri	Fructus	10	
	Curcumae rhizome	Rhizoma	30	

Continued

F	*Eurycoma longifolia*	Radix	40%	Increase passion in women
	Curcumae spp.	Rhizoma	10	
	Mel	Whole	50	
G	*Zingiber minus*	Rhizoma	5.6 mg	For smooth menstrual flow; to relieve joints pain.
	Euginea caryophyllata	Flos	11.1	
	Piper nigrum	Fructus	11.1	
	Illium verum	Flos	11.1	
	Carum copticum	Semen	16.7	
	Astragalus membraneceus	Radix	22.2	
	Angelica sinensis	Rhizoma	100	
H	*Eurycoma longifolia*	Radix	50%	To increase sexual stamina energy in man
	Cistanche deserticola	Herba	50	
I	*Eurycoma longifolia*	n.i	30.4%	For energy, increase sexual stamina and men's health.
	Tacca palmate	n.i	21.4	
	Zingiberis aromaticae	n.i	17.9	
	Zingiberis officinale	n.i	14.3	
	Helminthoctachys zeylanica	n.i	16	
J	*Astragalus membraceus*	Radix	10%	Promote blood circulation; to clear phlegm, relieving cough and pain
	Cinnamomum cassia	Ramulus	10	
	Ephedra sinica	Herba	10	
	Prumus armeniaca	Semen	10	
	Schisandra chinensis	Fructus	10	
	Perilla fruitescens	Fructus	10	
	Lepidium apetalum	Semen	10	
	Trichosanthes kiriozuli	Fructus	10	
	Aster tataricus	Radix	10	
	Glycyrhiza uralensi	Radix	10	
K	*Spina gleditsiae*	Spina	100 mg	For general health
	Angelica esinensis	Radix	75	
	Salvia emiltiorrhizae	Radix	75	
	Ziziphi jujubae	Fructus	20	
	Cordyceps sinensis	n.i	50	
	Dictamni dasycarpl	Cordex	30	
	Rhodlolasecra	Radix	50	
	Schizonepetae spp.	herba	50	
L	*Rehmanniae glutinosa*	Radix	69 mg	For general health
	Paeonia suffruticosa	Cordex	23	

Continued

Paeonia alba	Radix	23
Alisma orientalis	Rhizoma	23
Chrysanthemum morifolium	Flos	23
Lycium barbarum	Fructus	23
Tribulus terestris	Fructus	23
Dioscorea opposita	Rhizoma	30
Holoiotidis diversicolor	Concha	30
Cornus officinalis	Fructus	30
Angelica sinensis	Radix	23
Mel	Whole	166

[a]Plant parts used: n.i, not indicated; Radix, the root; Rhizoma, rhizome or a creeping horizontal stem generally bearing roots on its underside; Flos, the flowers, Fructus, the fruit or berry; Semen, the seed usually removed from the fruit and may or may not contain the seed coat; Herba, the aerial parts or the aboveground parts of plants which may include the flower, leaf, and the stem; Cordex, the bark collected from the root, stem, or branches; Concha; Spina.

animals such as rodent and monitor for the development of tumours. This process is expensive, time consuming, and cumbersome. The bacterial *Salmonella* mutagenicity test has been used world-wide as an initial screen to determine the mutagenic potential of chemicals and drugs and had been shown to correlate well with carcinogenicity test using rodent [10] [11].

2.3.1. Muta-Chromoplate Kit

A commercial kit, the Muta-Chromplate (Environmental Biodetection Products Incorporation, EBPI, Ontario, Canada), was used to evaluate the mutagenicity of the herbal extracts. This test kit was based on the validated Ames bacterial reverse-mutation test [9] but was performed entirely in liquid culture.

2.3.2. Chemicals

The following chemicals were provided by EBPI: Davis-Mingioli salt (5.5 times concentrated), D-glucose (40%, w/v), bromocressol purple (2 mg/ml), D-biotin (0.1 mg/ml), and L-histidine (0.1 mg/ml). A standard mutagen provided by the manufacturer was sodium azide (NaN_3, 0.5 μg/100 μl). All chemicals were kept at 2˚C ± 1˚C prior to use.

2.3.3. Preparation of Reagent Mixture

The solutions provided by EBPI were mixed aseptically in a sterile bottle as follows: Davis-Mingioli salt, 21.62 ml; D-glucose, 4.75 ml; bromocressol purple, 2.38 ml; D-biotin, 1.19 ml; and L-histidine, 0.06 ml.

2.3.4. Test Bacterial Strain

Salmonella typhimurium TA100 were purchased from Environmental Biodetection Products Incorporation (EBPI, Ontario, Canada). The bacterium was maintained on Nutrient agar at 4˚C. The bacteria was streaked for single colonies on

Nutrient agar plate and incubated at 37˚C for 48 h. Several single colonies were picked using inoculating loop and inoculated into Nutrient broth and incubated at 37˚C for 18 h before the test was carried out.

2.3.5. Mutagenicity Assay

Reagent mixture, herbal extract, sterile distilled water and standard mutagen were mixed in 4 treatment bottles at the amount indicated in Table 2. An overnight culture broth of *S. typhimurium* (5 µl) was inoculated into the bottles and mixed thoroughly with vortex mixer. The content of each bottle was poured into a multi-channel reagent boat and 200 µl aliquots of the mixture were dispensed into all wells of a 96-well microtitration plate using a multi-channel pipette. The plates were placed in a plastic bag to prevent evaporation and then incubated in an incubator (Gallenkamp) at 37˚C for 4 days. Each polyherbal extracts, background and standard mutagen were tested in duplicates.

2.3.6. Interpretation of Results and Statistical Analysis

After incubation, the "blank" plate was observed first and the rest of the plates were read only when all wells in the blank plate were purple indicating the assay was not contaminated. The "background", "standard" and "test" plates were scored visually and all yellow, partially yellow or turbid wells were scored as positive while purple wells were scored negative. Numbers of all positive wells were recorded. The "background" plate (no herbal extract or standard mutagen added) showed the level of spontaneous or background mutation of the test bacteria. The extract was considered toxic to the test strain if all wells in the test plate were purple.

The mutagenicity of the sample was determined by comparing the number of wells scored as positive in the background plate to the number of positive wells in the treatment plate [15]. Statistical differences were determined using the table for analysis of results provided by the manufacturer (EBPI, Canada) based on statistical analysis by [16].

3. Results

Table 3 shows the results of mutagenicity testing using S. *typhimurium* strain TA100. Out of 12 samples tested at 250 µg/ml, 5 extracts were found to be

Table 2. Set-up of the mutagenicity assay.

Treatment	Volume added (ml)				
	Standard mutagen (NaN_3)	Herbal extract	Reagent mixture	Water	*Salmonella* strain TA100
Blank	-	-	2.5	17.5	-
Background	-	-	2.5	17.5	0.005
Standard mutagen	0.1	-	2.5	17.4	0.005
Test sample	-	0.005	2.5	17.5	0.005

-, not added.

Table 3. Mutagenic activity of the polyherbal extracts in the Ames test using *S. typhimurium* TA100. All extracts were tested at 250 μg/ml.

Herbal preparation	Number of positive wells per 96 wells		Results	
	Replicate 1	Replicate 2	P ≤ 0.05	P ≤ 0.01
Control	12	14		
NaN_3 (0.025 μg/ml)	96	93	+	+
A	35	30	+	+
B	14	18	–	–
C	40	46	+	+
D	11	14	–	–
E	25	27	+	–
F	12	8	–	–
G	36	30	+	+
H	27	28	+	–
I	11	14	–	–
J	18	24	–	–
K	16	12	–	–
L	17	13	–	–

+, significant increase in the number of positive wells compared to the control; –, no significant effect observed in the number of positive wells compared to the related control (background).

potentially mutagenic. Extracts of "A", "C" and "G" were significantly mutagenic at p ≤ 0.01 while "E" and "H" were mutagenic at p ≤ 0.05. Extract "H" contained 2 herbs while "A", "C", "E" and "G" comprised of 7 - 11 herbs.

At concentration 250 μg/ml, extracts of polyherbal preparation from samples of "B", "D", "F", "I", "J", "K" and "L" were not potentially mutagenic in this experimental condition. Chemical constituents in extracts of "D", "F", "I" and "B" had no mutagenic activity at all as number of positive wells was almost similar to the control wells (contained growth medium only).

4. Discussions

Seven out of 12 polyherbal preparations were found to be non-mutagenic at test concentration of 250 μg/ml. The mutagenicity of these extracts could not be definitely ruled out until test using higher extract concentration is carried out as mutagenicity was reported to increase with the increase of the extract concentration [17].

Polyherbal preparations "B", "D", "F", "J" and "L" comprised several different herbs. There were little or no reports of mutagenicity of any of these plants and combination of these herbs as found in the current study was not mutagenic.

Polyherbal preparation "I" comprised *of Eurycoma longifolia*, *Tacca palmate*, *Zingiberis aromaticae*, *Zingiberis officinale*, and *Helminthoctachys zeylanica*. *Tacca palmate* was reported to be mutagenic [18]. However, combination of *T.*

palmate (30%) with four other herbs (70%) rendered the product to be non-mutagenic.

Zingiber minus, *Eugenia caryophyllata*, *Piper nigrum*, *Illicium verum*, *Carum copticum*, *Astragalus membranaceus* and *Angelica sinensis* were plants in the formulation of "G". There were little or no reports of mutagenicity of any of these plants. Instead of being mutagenic, *Astragalus membranaceus* was reported to have antimutagenic property [19] and was used as a natural herbal medicine in East Asia for preventing severe side effects of chemotherapy in patients with cancer. However, combination of these plants as in "G" was found to be mutagenic. Similarly, there were little or no reports of mutagenicity of both *E. longifolia* and *Cistanche deserticola* which were the component of "H" but combination of these plants was also mutagenic. These results suggest that the mutagenicity of prepations of "G" and "H" could be due to the synergistic effects of various phytochemicals in the polyherbal preparation.

The idea of formulating polyherbal medicine is to take advantage of synergistic effect of various plants chemical constituents that will increase the effectiveness of the medicine. Use of varieties of herbs in a medicinal herbal formulation is thought to be able to minimise toxicity of the formulation [20]. However, in the presence of diverse phytochemicals in a polyherbal formulation, some phytochemicals may activate promutagens amongst the phytochemicals or some weak mutagens may acts synergistically enhancing the mutagenic effect [21].

Results of this study show that mutagenicity of a polyherbal preparation cannot be deduced from the information of mutagenicity of individual components of the polyherbal preparation and combination of non-mutagenic plants is not necessarily produce a non-mutagenic herbal medicine

5. Conclusion

Combination of traditionally used herbs or non-mutagenic herbs may produce a mutagenic product most probably through synergistic effect of various phytochemicals combination in the polyherbal extract.

Funding

This research work was sponsored by the Public Service Department of Malaysia through the Federal Government Training Scholarship Program.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

[1] Van der Hoven, J.C. (1985) Occurrence and Detection of Natural Mutagens and Modifying Factors in Food Products. *Princess Takamatsu Symposium*, **16**, 119-137.

[2] Guil, J.L., Rodriguez-Garcia, I. and Torija, E. (1997) Nutritional and Toxic Factors

in Selected Wild Edible Plants. *Plant Foods for Human Nutrition*, **51**, 99-107. https://doi.org/10.1023/A:1007988815888

[3] Yen, G.C., Chen, H.F. and Peng, H.H. (2001) Evaluation of the Cytotoxicity, Mutagenicity and Antimutagenicity of Emerging Edible Plants. *Food and Chemical Toxicology*, **39**, 1045-1053. https://doi.org/10.1016/S0278-6915(01)00053-9

[4] Morimoto, I., Watanabe, F., Osawa, T., Okitsu, T. and Kada, T. (1982) Mutagenicity Screening of Crude Drugs with *Bacillus subtilisrecassay* and Salmonella/Microsome Reversion Assay. *Mutation Research*, **97**, 81-102. https://doi.org/10.1016/0165-1161(82)90007-3

[5] Riazuddin, S., Malik, M.M. and Nasim, A. (1987) Mutagenicity Testing of Some Medicinal Herbs. *Environmental and Molecular Mutagenesis*, **10**, 141-148. https://doi.org/10.1002/em.2850100204

[6] Yin, X.-J., Liu, D.-X., Hecknan, W. and Yu, Z. (1991) A Study on the Mutagenicity of 102 Raw Pharmaceuticals Used in Chinese Traditional Medicine. *Mutation Research*, **260**, 73-82. https://doi.org/10.1016/0165-1218(91)90082-W

[7] Schimmer, O., Kruger, A., Paulini, H. and Haefele, F. (1994) An Evaluation of 55 Commercial Plant Extracts in the Ames Mutagenicity Test. *Pharmazie*, **49**, 448-451.

[8] Ames, B.N. (1983) Dietary Carcinogenesis and Anticarcinogens. *Science*, **221**, 1256-1264. https://doi.org/10.1126/science.6351251

[9] Ames, B.N., McCann, J. and Yamasaki, E. (1975) Methods for Detecting Carcinogens and Mutagens with Salmonella/Mammalian-Microsome Mutagenicity Test. *Mutation Research*, **31**, 347-364. https://doi.org/10.1016/0165-1161(75)90046-1

[10] McCann, J., Choi, E., Yamasaki, E. and Ames, B.N. (1975) Detection of Carcinogens in the Salmonella/Microsome Test. Assay of 300 Chemicals. *Proceedings of the National Academy of Sciences of the United States of America*, **72**, 5135-5139. https://doi.org/10.1073/pnas.72.12.5135

[11] Zeiger, E., Hasemann, J.K., Shelby, M.D., Margolin, B.H. and Tennant, R.W. (1990) Evaluation of Four *in Vitro* Genetic Toxicity Tests for Predicting Rodent Carcinogenicity: Confirmation of Earlier Results with 41 Additional Chemicals. *Environmental Molecular Mutagenesis*, **16**, 1-14. https://doi.org/10.1002/em.2850160502

[12] Mohd Fuat, A.R., Aidoo, K.E., Calvert, T.W. and Candlish, A.A.G. (2006) Mycoflora, Cytotoxicity and DNA Interaction of Polyherbal Products from Malaysia. *Pharmaceutical Biology*, **44**, 23-31. https://doi.org/10.1080/13880200500530500

[13] Valledo, L., Monica, E., Monica, M., Ella, N., Marıa, J.C. and Wolfram, W. (2014) A Universal Protocol for the Combined Isolation of Metabolites, DNA, Long RNAs, Small RNAs, and Proteins from Plants and Microorganisms. *The Plant Journal*, **79**, 173-180. https://doi.org/10.1111/tpj.12546

[14] Prozesky, E.A., Meyer, J.J.M. and Louw, A.I. (2001) *In Vitro* Antiplasmodial Activity and Cytotoxicity of Ethnobotanically Selected South African Plants. *Journal of Ethnopharmacology*, **76**, 239-245. https://doi.org/10.1016/S0378-8741(01)00245-8

[15] Zeiger, E. and Mortelmans, K. (1999) The *Salmonella* (Ames) Test for Mutagenicity. *Current Protocols in Toxicology*, **3**, 1-29. https://doi.org/10.1002/0471140856.tx0301s00

[16] Gilbert, R.I. (1980) The Analysis of Fluctuation Tests. *Mutation Research*, **74**, 283-289. https://doi.org/10.1016/0165-1161(80)90168-5

[17] De Carvalho, M.C.R.D., Barca, F.N.T.V., Lima, L.F.A. and De Medeiros, S.R.B. (2003) Evaluation of Mutagenic Activity in an Extract of Pepper Tree Stem Bark (*Schinus terebinthifolius* Raddi). *Environmental and Molecular Mutagenesis*, **42**,

185-191. https://doi.org/10.1002/em.10183

[18] Mohd-Fuat, A.R., Aidoo, K.E. and Candlish, A.G.G. (2007) Mutagenic and Cytotoxic Properties of Three Herbal Plants from Southeast Asia. *Tropical Biomedicine*, **24**, 49-59.

[19] Cui, R., He, J., Wang, B., Zhang, F., Chen, G., Yin, S. and Shen, H. (2003) Suppressive Effects of *Astragalus membranaceus* Bunge on Chemical Hepatocarcinogenesis in Rats. *Cancer Chemotherapy and Pharmacology*, **51**, 75-80. https://doi.org/10.1007/s00280-002-0532-5

[20] Caesar, L.K. and Cech, N.B. (2019) Synergy and Antagonism in Natural Product Extracts: When 1 + 1 Does Not Equal 2. *Natural Product Reports*, **36**, 869-888. https://doi.org/10.1039/C9NP00011A

[21] Sarkar, D., Talukder, G. and Sharma, A. (1996) Plant Extracts as Modulators of Genotoxic Effects. *The Botanical Review*, **62**, 275-300. https://doi.org/10.1007/BF02856614