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AN INVESTIGATION ABOUT THE GENOTOXIC ACTIVITIES OF SOME HERBAL TEAS USED AS FOLK MEDICINE

HALK ARASINDA TEDAVİ AMACIYLA KULLANILAN BAZI BİTKİSEL ÇAYLARIN GENOTOKSIK AKTİVİTELERİNİN ARAŞTIRILMASI

BUKET ALPERTUNGA, GÜLDEN Z. OMURTAG, NALAN ÖZMENTEŞE

University of Marmara, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, 81010 Haydarpaşa, Istanbul, Turkey

In this research the genotoxic activities of some herbal teas used as folk medicine were investigated by the umu-test using a tester strain Salmonella typhimurium TA 1535/pSK 1002 carrying the umuC-lacZ fused gene with (+S9) and without (-S9) metabolic activation. The induction of umu gene expression was monitored by measuring the cellular β-galactosidase activity produced by fusion gene using a colorimetric method. Consequently, from 10 herbal teas used in this research, only two of them showed weak genotoxicity.

Bu çalışmada; halk arasında tedavi amacıyla kullanılan bazı bitkisel çayların genotoksik aktiviteleri umu-test sistemi ile incelendi. Bitkisel çayların umuC-lacZ segmentlerini içeren bir plazmid transfer edilmiş olan Salmonella typhimurium TA 1535/pSK 1002 suşundaki umu gen ifadesini indükleme yeteneğine bağlı β-galaktosidaz aktivitesi kolorimetrik bir yöntemle ölçüldü. Test hem metabolik aktivasyonsuz (-S9) hem de metabolik aktivasyon ilavesiyle (+S9) uygulandı. İncelenen 10 bitkisel çaydan iki tanesi zayıf mutajenik aktivite gösterdi.

Keywords :*Genotoxicity; umu test; herbal teas*

Anahtar kelimeler: *Genotoksisite; umu-test; bitkisel çaylar*

Introduction

Several short-term tests to detect environmental mutagens and carcinogens have been developed (1-7). Among them, the umu-test system is a simple, sensitive and rapid bacterial colorimetric assay to evaluate genotoxic activities of these chemicals. The umu-test is based on the ability of DNA-damaging agents, to induce umu gene

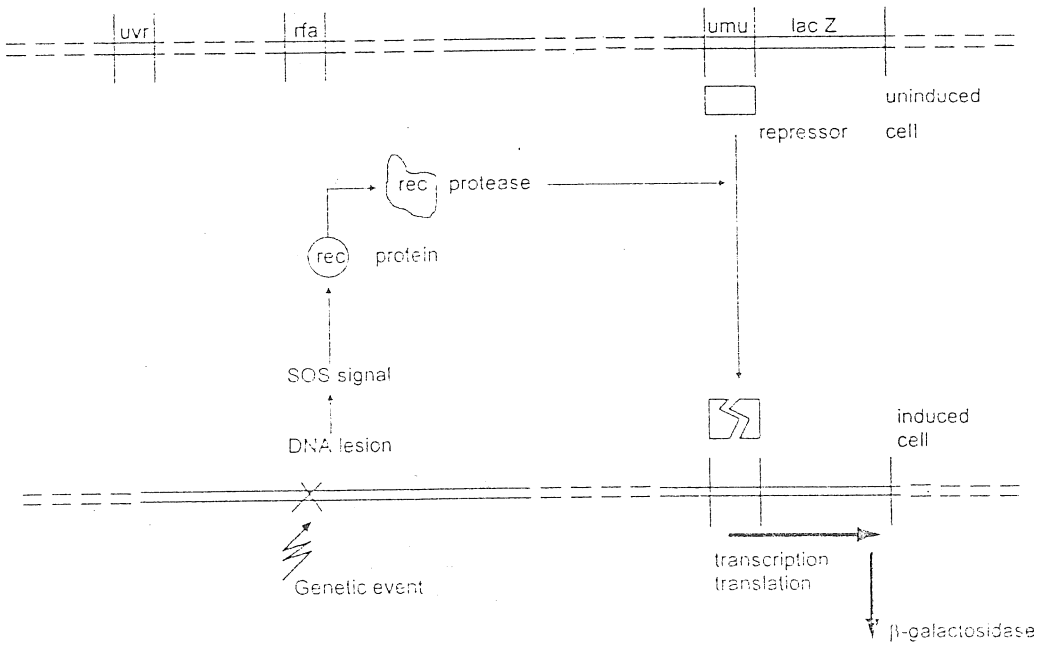


Fig.1. Principle of the umu-test

expression in a new tester strain *Salmonella typhimurium* TA 1535/pSK 1002 in which an umuC-lacZ fused gene had been introduced. The levels of umu gene expression can be monitored by measuring the cellular β-galactosidase activity produced by fusion gene (Fig.1) (8-15).

Extracts from plants have been used to be a major source for medicinal material. However, little information is available on their potential risk to health. Many mutagens and/or carcinogens of plant origin have been identified in the last decade (16-19). Evaluation of the mutagenic activity of these extracts is needed in order to estimate the long term risk to human health.

In this communication, we report about the genotoxic activities of some herbal teas used as folk medicine by "umu-test system".

Materials and Methods

Chemicals

The enzyme substrate o-nitrophenyl - β - D - galactopyranoside (ONPG), 4-nitroquinoline-1-oxide (NQO), 4 - nitro - o - phenylenediamine (NODP), 2-aminoanthracene (2-AA), 7,12 - dimethylbenzanthracene (DMBA), and nicotinamide adenine dinucleotide phosphate (NADP) were from Sigma. Bacto tryptone and yeast extract were from Difco. All the other chemicals were from Merck.

Bacterial strain

Salmonella typhimurium TA 1535/pSK 1002 carrying the umuC-lacZ fusion gene (kindly provided by Dr.Y.Oda of Osaka Prefectural Institute of Public Health) was used.

The bacteria were grown in Luria broth (LB medium) supplemented with 50 μg/ml ampicillin. The overnight culture of the tester bacterial strain was diluted 20-fold with TGA medium and was incubated at 37°C until the bacterial density reached an absorbance at 600 nm of 0.25 to 0.3.

Media, buffer and reagent

LB medium : Bacto tryptone 10 g, yeast extract 5 g, NaCl 10 g/l of distilled water. LB medium is supplemented with 50 μg/ml ampicillin.

TGA medium : Bacto tryptone 10 g, NaCl 5 g, glucose 2 g/l of distilled water.

Z buffer: $NaH_2PO_4.2H_2O$ 6.2 g, $Na_2HPO_4.7H_2O$ 16.1 g, NaCl 0.58 g, $MgSO_4.7H_2O$ 0.25 g, Sodium dodecyl sulfate 1 g, β-mercaptoethanol 2.7 ml/l of distilled water and adjusted to pH 7.

ONPG solution: 400 mg ONPG per 100 ml of phosphate buffer pH 7.

Compounds to be tested (NQO, NODP, 2-AA, DMBA) were dissolved in DMSO.

Activation mixture

The S9 microsome fraction was prepared from livers of male Sprague Dawley rats pretreated with sodium phenobarbital. The activation mixture was prepared according to Maron and Ames (6).

The S9 mix contained per 10 ml : 1ml of S9 fraction, 1 ml of 0.04 M NADP, 0.25 ml of 0.2 M G6P, 0.3 ml of 0.25 M $MgCl_2.6H_2O$, 0.3 ml of 1M KCl, 5 ml of 0.2 M Na_2HPO_4, 2 ml of distilled water.

Sample collection and preparation

The plants were bought from the herb dealers. The extracts of dried plant material were prepared in boiling water at a concentration range of 0.2%-20% which was advised by the herb dealer.

Umu-Test Procedure

The bacterial culture was subdivided into 2.4 ml portions in test tubes and 0.1 ml of the test compound was added to each tube. Then, either 0.5 ml of 0.1 M phosphate buffer pH 7.4 or S9 mix was added. After 2h of incubation at 37°C, the bacterial density was measured at 600 nm (OD_{600}). The level of β-galactosidase activity (units) was measured by the method of Miller (20) as follows:

Fractions(0.2 ml) of the culture were diluted with 1.8 ml of Z buffer and the bacterial cells were made permeable to the chromogenic substrate for β-galactosidase by adding 0.01 ml of chloroform and mixing vigorously. The enzyme reaction was initiated by the addition of 0.2 ml of ONPG solution at 28°C. After 15 min, the reaction was stopped by adding 1 ml of 1 M Na_2CO_3 and the absorbance of the mixture was measured at 420 nm (OD_{420}). In addition, each sample was measured at 550 nm (OD_{550}) in order to correct for light scattering due to cell fragments. The β-galactosidase activity (U) is calculated according to the formula of Miller (20) (Scheme 1).:

$$U = \frac{1000x\ (OD_{420} - 1.75\ OD_{550})}{15x0.1xOD_{600}}$$

The "induction factors" for a given concentration $I_{(c)}$ were calculated by dividing the β-galactosidase activity $U_{(c)}$ at a given concentration(c) with the background β-galactosidase activity $U_{(o)}$, $I_{(c)} = U_{(c)} / U_{(o)}$ (21).

Each assay with plant extract was included positive controls (NQO = 0.5 μg/ml, 2-AA = 4 μg/ml).

Results and Discussion

The umu-test procedure was applied to four positive controls, directly acting potent mutagens (NQO, NODP) and promutagens

Table 1. The values obtained with recommended positive controls NQO and NODP were tested in the absence of activation mixture, 2-AA and DMBA were tested in its presence as described in procedures

Compound (µg/ml)	β-galactosidase activity[a] (units)	Induction factor (I_c)
NQO		
O[b]	278±26	1
0.1	559±14	2.0
0.25	689±30	2.5
0.5	953±19	3.4
0.75	1041±57	3.7
1.0	1261±24	4.5
NODP		
O[b]	255±26	1
5	541±27	2.1
10	671±30	2.6
20	836±25	3.3
30	940±17	3.7
40	1203±31	4.7
2-AA		
O[b]	247±5	1
0.5	301±5	1.2
1	508±9	2.1
2	734±11	2.9
3	869±9	3.5
4	972±12	3.9
DMBA		
O[b]	234±11	1
2	391±7	1.7
4	453±9	1.9
8	548±11	2.3
10	661±12	2.8
12	746±4	3.2

[a]Mean ± SD (n=4)

[b]Solvent control, DMSO (100 µl per assay)

(2-AA, DMBA). The results obtained with these compounds using the conditions described are given in Table 1. Two fold increase in β-galactosidase activity above the control levels

Table 2. Herbal teas tested in **umu-test** system

	Contituents	Usages
I-	Lavandula sp. Anthemis sp. Hypericum sp. Artemisia sp.	Intestinal parasitic diseases
II-	Urtica sp.	Cell regenerator
III-	Rosa canina Cassia sp. Erica sp. Juniperus sp.	Slimming tea
IV-	Althaea sp. Rosa canina Juniperus sp. Cinnamomum sp. Pimpinella anisum Anthemis sp.	Antitussive
V-	Equisetum sp. Helichrysum sp. Foeniculum vulgare Petroselinum crispum	Kidney diseases, Diüretic:
VI-	Equisetum sp. Hypericum sp Vitex sp. Lythrum sp	Antipruritic
VII-	Lavandula sp.	Sinusitis
VIII-	Eucalyptus camaldulensis Rosa canina Anthemis sp. Althaea sp. Tilia sp.	Asthma, Bronchitis, Weakness of breathing
IX-	Viscum sp.	Hypotensive
X-	Foeniculum vulgare Rosa canina Juniperus sp.	Diabetic

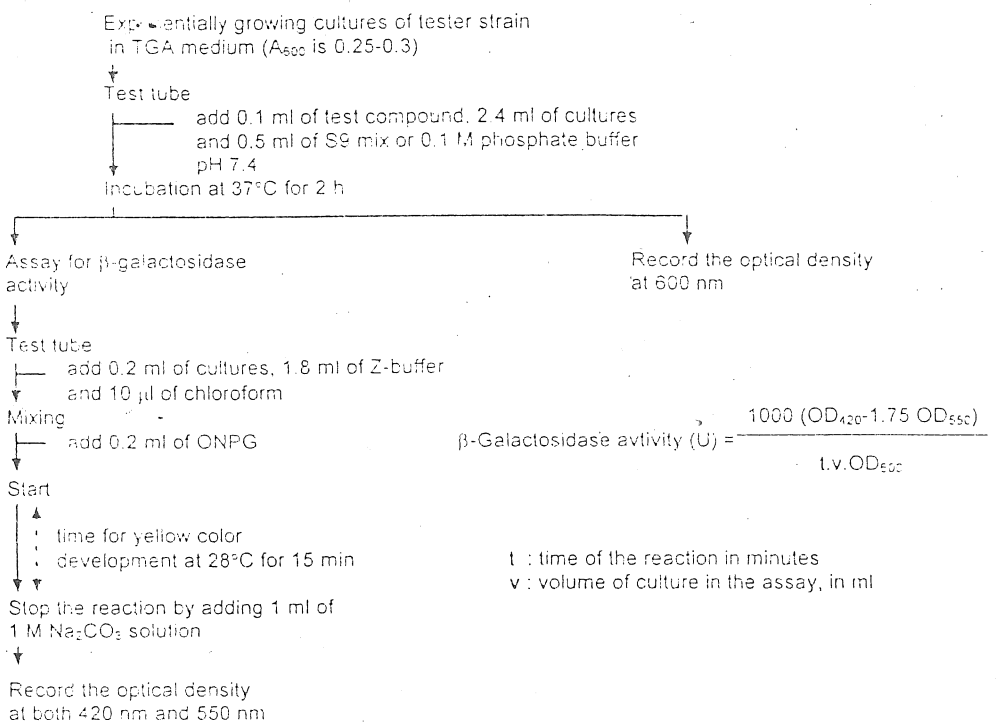
was defined to be positive. The potent mutagens induced 6-fold, intermediate mutagens 3-fold and weak mutagens 2-fold umu gene expression over the background level in the assay conditions (9). The background level of umu gene expression in the absence of substrate were found 234-278 units for the DMSO control and 89-257 units for the water control as determined by β-galactosidase activity.

Table 3. The genotoxicity of herbal tea extracts by the umu-test with and without S9 mix.

Sample	Concentration (µg/ml)	β-galactosidase activity[a] (Units)		Induction factor(I_c)	
		-S9	+S9	-S9	+S9
I	O[b]	107±4	127±4	1	1
	10	109±5		1	
	25	101±11		<1	
	50	107±12		1	
	75	116±10	223±8	1.1	1.8
	100	106±6	240±9	<1	1.9
II	0	107±4	127±4	1	1
	10	102±4		<1	
	25	100±5		<1	
	50	99±5		<1	
	75	107±7	242±4	1	1.9
	100	105±9	259±8	<1	2.0
III	0	116±5	142±8	1	1
	10	118±9		1	
	25	138±7		1.2	
	50	169±2		1.5	
	75	267±9	304±11	2.3	2.1
	100	208±3	293±9	1.8	2.0
IV	0	116±5	134±3	1	1
	10	206±11		1.8	
	25	260±17		2.2	
	50	328±9		2.8	
	75	396±12	417±4	3.4	3.1
	100	429±11	441±6	3.7	3.3
V	0	125±6	246±8	1	1
	10	122±4		<1	
	25	120±11		<1	
	50	125±7		1	
	75	123±7	328±9	<1	1.3
	100	111±6	250±6	<1	1
VI	0	156±8	134±3	1	1
	10	138±12		<1	
	25	148±10		<1	
	50	145±8		<1	
	75	151±13	213±4	<1	1.6
	100	143±10	242±4	<1	1.8
VII	0	156±8	257±9	1	1
	10	164±7		1.1	
	25	157±8		1	
	50	154±11		<1	
	75	152±13	239±3	<1	<1
	100	155±11	247±4	<1	<1
VIII	0	89±3	141±3	1	1
	10	86±2		<1	
	25	88±3		<1	
	50	87±2		<1	
	75	92±2	164±4	1	1.2
	100	88±3	187±5	<1	1.3
IX	0	89±3	246±8	1	1
	10	91±3		1	
	25	98±2		1.1	
	50	96±2		1.1	
	75	93±2	265±7	1	1.1
	100	94±2	281±8	1.1	1.1
X	0	134±11	257±9	1	1
	10	123±7		<1	
	25	134±9		1	
	50	132±5		<1	
	75	136±5	266±5	1	1
	100	134±7	293±7	1	1.1

[a]Mean ± SD (n=4)

[b]Solvent control, dist. water (100 µl per assay)



Schema 1-Flow chart for the umu-test

Herbal teas used in this study were mixtures of several plants except Sample II, Sample VII and Sample IX (Table 2). Their extracts were prepared according the recommendations of the herb dealers.

The tests were performed without and with the addition of activation system (S9 mix). The results obtained are given in Table 3.

As shown in Table 3, weak umu gene expression was observed with Sample III and Sample IV without and with S9 mix. With Sample III, induction of umu gene expression could be detected only within the concentration ranges of 75-100 µl/ml and the induction factors were 2.3, 2.1 and 2.0 respectively.

With Sample IV the level of umu gene expression was increased with the amount of extract in the medium. The inducibility without S9 mix within the concentration ranges of 25-100 µl/ml were 2.2, 2.8, 3.4, 3.7 and with S9 mix within the concentration ranges of 75-100 µl/ml 3.1, 3.3 respectively. Maximum β-galactosidase activity was found to be 429±11 units (approximately 3.7 times as high as the background level).

The other 8 Samples showed negative results by the umu-test both in the presence and absence of S9 mix.

As medicinal plants contain various chemical substances with different structures, in vitro mutagenicity testing of plant extracts should also be involved in evaluation of the genotoxicity of these chemicals.

Further investigation is needed to decide whether these specific extracts and extracts from other medicinal plants can be used without hazard to human health.

References

1. Delgado-Rodriguez, A., Ortiz-Marttelo, R., Graf, U., Villalobos-Pietrini, R., Gómez-Arroyo, S.: Mutation Res. 341, 235 (1995)
2. De Méo, M., Vanelle, P., Bernadini, E., Laget, M., Maldonado, J., Jentzer, O., Crozet, M.P., Dumenil, G.: Environ. Mol. Mutagen 19, 167 (1992)
3. Hamasaki, T., Sato, T., Nagase, H., Kito, H.: Mutation Res. 280, 195 (1992)
4. Ariza, R.R., Serrano, A., Pueyo, C.: Environ. Mol. Mutagen 19, 14 (1992)

5. Nestmann, E.R., Brillinger, R.L., Mc Pherson, M.F., Maus, K.L.: Environ. Mol. Mutagen, 10, 169 (1987)
6. Maron, D.M., Ames, B.N.: Mutation Res. 113, 173 (1983)
7. Quillardet, P., De Belecombe, C., Hofnung M.: Mutation Res. 147, 79 (1985)
8. Oda, Y., Nakamura, S., Oki, I., Kato, T., Shinagawa, H.: Mutation Res. 147, 219 (1985)
9. Nakamura, S., Oda, Y., Shimada, T., Oki, I., Sugimoto, K.: Mutation Res. 192, 239 (1987)
10. Shimada, T., Nakamura, S.: Biochem.Pharmac. 36(12), 1979 (1987)
11. Sakagami, Y., Yamazaki, H., Ogasawara, N., Yokoyama, H., Ose, Y., Sato, T.: Mutation Res. 209, 155 (1988)
12. Yamaguchi, T.: Mutation Res. 224, 493 (1989)
13. Nakamura, S., Oda, Y., Ugawa, M.: Mutation Res. 229, 11 (1990)
14. Oda, Y., Shimada, T., Watanabe, M., Ishidate Jr. M., Nohmi, T.: Mutation Res. 272, 91 (1992)
15. Oda, Y., Yamazaki, H., Watanabe, M., Nohmi, T., Shimada, T.: Environ. Mol. Mutagen. 21, 357 (1993)
16. Schimmer, O., Krüger, A., Paulini, H., Haefele, F.:Pharmazie 49(6), 148 (1994)
17. Paulini, H., Eileret, U., Schimmer, O.:Mutagenesis 2, 271 (1987)
18. Schimmer, O., Häfele, F., Krüger, A.: Mutation Res. 206, 201 (1988)
19. Czeczot, H., Tudek, B., Kusztelak, J., Szymczyk, T., Dobrowalska, B., Glikowska, G., Malinowski, J., Strzelecka, H.: Mutation Res. 240, 209 (1990)
20. Miller, J.H. : Experiments in Molecular Genetics pp 352, Cold Spring Harbor Laboratory, 1972
21. Eder, E. Favre, A., Stichtmann, C., Deininger, C.: Toxicol. Lett. 48, 225 (1989)

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