Evaluation of the mutagenic and antimutagenic activities of saponins from *Hedera colchica* K. Koch

Mshvildadze V.[1], De Méo M.[2], Dumenil G.[2], Dekanosidze G.[1], Kemertelidze E.[1], Elias R.[3], Balansard G.[3]

1. Institute of Pharmacochemistry, Academy of Science of Georgia 36, P.Sarajishvili St. 380059 Tbilisi (Georgia)
2. Laboratoire of Biogenotoxicity and Mutagenese Environnementale, (EA 1784)
3. Laboratoire of Pharmacognosie, Faculty of Pharmacy 27 Boulevard Jean Moulin 13385 Marseille cedex 5 (France).

Introduction

Hedera colchica K. Kock. (Araliaceae) is an endemic plant in Georgia. The leaves of this plant are used in traditional medicine as bronchospasmolitic, secretolytic and anti-inflammatory remedies. (Sakartvelos Floras, 1984; Rastitllnie and col., 1988)
The mutagenic and antimutagenic activities of 7 monodesmoside glycosides as well as the less polar glycoside fraction, were evaluated by using a modified version Ames test.

Material and methods

Extraction and Isolation

Leaves and berries of *Hedera colchica* were collected in the Bagdathi region of Georgia.
Powdered plant materials were extracted with MeH-H2O (80:20). After concentration, the aqueous layer was treated with CHCl3 then, with BuOH to obtain a crude extract of saponins. Pure compounds were isolated from the BuOH extract by the combination of different chromatographic technics. Structure elucidation were established using spectroscopic methods FAB-MS and 1H, 13C NMR (Mshvildadze and col., in press).

Salmonella mutagenicity test

• Tester strains
Salmonella typhimurium TA97a, TA98, TA100,TA102 and YG1041.

• Activation mixture S9mix
The liver homogenate (S9) was prepared from Sprague-Dawley rats treated with Araclor 1254 (500 mg/kg body weight). The protein concentration was 30.7 mg/ml (Lowry and col., 1951). The S9mix was a mixture of 4 % S9 and a solution of cofactors (Maron and col., 1983).

• Assay procedure
All samples were assessed for mutagenicity and antimutagenicity by a modified version the liquid incubation assay of the Ames test (Maron and col., 1983; De Méo and col., 1996).

• Determination of the mutagenic and antimutagenic activities
The mutagenic activity (MA) and the antimutagenic activity (AA) were calculated by non linear regression analysis using the Statgraphics Plus Software (STSC, Uniware, version 6.1) (De Méo and col., 1996).

Results

The mutagenic and the antimutagenic activities of tested saponins (Fig. 1) were evaluated by using the Ames test. Assessment of the mutagenic activities was performed using tester strains TA97a, TA98, TA100 and TA102 with and without the metabolic fraction S9mix. The antimutagenic activity was performed against two known mutagens: BaP (benzo(a)pyrene) and a complex mixture CSC (Cigarette Smoke Concentrate)

→ No mutagenic activity could be detected.

The antimutagenic activities, expressed as ID50 were evaluated against 2 known mutagenic compounds. Results are summarized in Tables I and II. Typical dose-response curves are included in Fig. 2. No antimutagenic activity could be detected against BaP. The saponosids 3 and 5 showed a weak antimutagenic activity against CSC (Table II).

References

SAKARTVELOS FLORA (1984) *Metsniereba*, Tbilisi, IX, 124.

RASTITLLNIE (1988) Resursi SSSR, Nauka, Leningrad Fam. Rutaceae-Elaegnaceae, 4, 62.

MSHVILDADZE V.D., FAVEL A., DELMAS F., ELIAS R., FAURE R., DEKANOSIDZE G.E., KEMERTELIDZE E.P., BALANSARD G. (in press) Antifungal and antiprotozoal activities of saponins from *Hedera colchica*, *Die Pharmazie*, Ref. NP9629.

LOWRY O.H., ROSEBROUGH N.J., FARR A.L., RANDALL R.J. (1951) Protein measu-rements with the folin-phenol reagent, *J. Biol. Chem.*, 193, 265-275.

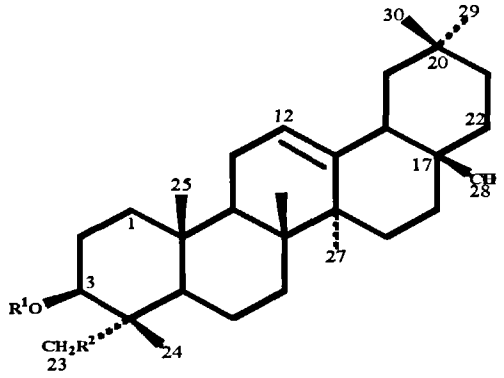
MARON D.M., AMES B.N. (1983) Revised methods for the Salmonella mutagenicity test, *Mutation Res.*, 113, 173-215.

DE MÉO M.P., LAGET M., DI GIORGIO C., GUIRAUD H., BOTTA A., CASTAGNARO M., DUMENIL G. (1996) Optimisation of the Salmonella/mammalian microsome assay for urine mutagenesis by experimental designs, *Mutation Res.*, 340, 51-65.

Compound	R1	R2	Part of plant	Name
1	Glc	OH	Berries	Colchiside 4
2	Ara	OH	Berries	d-hederin
3	Glc2-1Glc	H	Berries	Colchiside 7
4	Glc2-1Glc	OH	Berries	Colchiside 6
5	Glu	H	Berries	Colchiside 9
7	Ara [Glc4-1]2-1Rha	H	Leaves	Hederacolchiside A1
8	Ara [Glc4-1]2-1Rha	OH	Leaves	Hederacolchiside A

Glc: Glucose Ara : Arabinose Glu: ac. Glucuronique Rha: Rhamnose

Figure 1. Tested saponins: Crude extract of saponins (n° 6) and 7 monodesmosides (n° 1 to 8) were tested for their mutagenic and antimutagenic activities.



The effect of 3 and 5 on the mutagenic activity of CSC (2.5 µg) was determined in the Ames test with tester strain YG1041 + S9mix. The mutagenic activities of CSC were 844 ± 14. Saponosid 3 showed a synergistic effect up to 20 µg/plate.

Figure 2. Antimutagenic activities of Colchiside 7 (3) and Colchiside 9 (5) against Cigarette Smoke Concentrate (CSC).

Table I. Antimutagenicity of the 14 saponosids against BaP (0.5 µg) using TA100+S9mix

Compound N°	Dose/Plate	Rev/Plate
BaP	**0.5 µg**	**1186 ± 27**
Colchiside 4	20 µg	1100 ± 78
	30 µg	1260 ± 61
	40 µg	1189 ± 68
	50 µg	1181 ± 57
d-hederin	20 µg	1171 ± 131
	30 µg	1043 ± 69
	40 µg	1247 ± 26
	50 µg	1183 ± 146
Colchiside 7	20 µg	924 ± 86
	30 µg	873 ± 35
	40 µg	892 ± 64
	50 µg	1182 ± 197
Colchiside 6	20 µg	1089 ± 109
	30 µg	939 ± 33
	40 µg	1030 ± 62
	50 µg	1090 ± 5
Colchiside 9	20 µg	1370 ± 21
	30 µg	1324 ± 105
	40 µg	1274 ± 52
	50 µg	1368 ± 50
BuOH ext.	20 µg	1117 ± 21
	30 µg	973 ± 17
	40 µg	979 ± 17
	50 µg	1122 ± 37
Hederacolchiside A1	20 µg	1000 ± 118
	30 µg	857 ± 9
	40 µg	1031 ± 21
	50 µg	1193 ± 64
Hederacolchiside A	20 µg	908 ± 82
	30 µg	843 ± 12
	40 µg	897 ± 17
	50 µg	977 ± 5

Table II. Antimutagenicity of the 14 saponosids against cigarette smoke concentrate (2.5 µg) using YG1041+S9mix

Compound N°	Dose/Plate	Rev/Plate
CSC	**2.5 µg**	**844 ± 14**
Colchiside 4	20 µg	928 ± 57
	30 µg	997 ± 64
	40 µg	932 ± 39
	50 µg	915 ± 95
d-hederin	20 µg	777 ± 59
	30 µg	981 ± 67
	40 µg	859 ± 55
	50 µg	821 ± 34
Colchiside 7	20 µg	968 ± 16
	30 µg	907 ± 3
	40 µg	810 ± 42
	50 µg	756 ± 4
Colchiside 6	20 µg	867 ± 19
	30 µg	876 ± 73
	40 µg	821 ± 36
	50 µg	858 ± 19
Colchiside 9	20 µg	761 ± 8
	30 µg	720 ± 62
	40 µg	697 ± 11
	50 µg	625 ± 30
BuOH ext.	20 µg	826 ± 44
	30 µg	894 ± 85
	40 µg	866 ± 30
	50 µg	844 ± 56
Hederacolchiside A1	20 µg	873 ± 6
	30 µg	906 ± 20
	40 µg	820 ± 65
	50 µg	797 ± 44
Hederacolchiside A	20 µg	828 ± 73
	30 µg	906 ± 20
	40 µg	820 ± 65
	50 µg	797 ± 44