



Short communication

Investigation on the genotoxicity of extracts from *Cleome amblyocarpa* Barr. and Murb, an important Tunisian medicinal plantH. Edziri^a, M. Mastouri^b, M. Aouni^a, R. Anthonissen^c, L. Verschaeve^{c,d,*}^a Laboratoire des Maladies transmissibles et des Substances Biologiquement Actives, Faculté de Pharmacie, 5000 Monastir, Tunisie^b Laboratoire de Microbiologie CHU Fattouma Bouguiba, 5000 Monastir, Tunisie^c Scientific Institute of Public Health, D.O. Public Health and Surveillance, Laboratory of Toxicology, Brussels, Belgium^d University of Antwerp, Dept. Biomedical Sciences, Antwerp, Belgium

ARTICLE INFO

Article history:

Received 5 June 2012

Received in revised form 3 October 2012

Accepted 15 October 2012

Available online 20 November 2012

Edited by GI Stafford

Keywords:

Cleome amblyocarpa

Neutral red uptake

Comet assay

(Geno)toxicity

Water extract

ABSTRACT

We conducted an investigation of the *in vitro* toxic and genotoxic properties of *Cleome amblyocarpa* Barr. and Murb, which is an important medicinal plant in Tunisia where it is widely used against colic and diabetes. In this study we investigated methanol extracts of the whole plant with the neutral red uptake test for assessing its *in vitro* toxicity and the alkaline comet assay for genotoxicity. It appeared that the extract was not genotoxic in subtoxic concentrations. However, the neutral red uptake test revealed that it was highly toxic and therefore some caution, especially regarding dosage and frequency of use, is warranted.

© 2012 SAAB. Published by Elsevier B.V. All rights reserved.

1. Introduction

As part of our ongoing effort to establish the safety of herbal products we conducted a preliminary investigation of the *in vitro* toxic and genotoxic properties of *Cleome amblyocarpa*, which is an important Tunisian medicinal plant. *Cleome* species are generally used in folk medicine as stomachics, rubefacients and in the treatment of scabies, rheumatic fever and inflammation (Boulos, 1983, 1999; Chopra et al., 1972; Ghazanfar, 1994). *C. amblyocarpa* is commonly used as a bechic and a sedative and is also used mixed with *Juniperus phoenicea* to ease pain, *Hammada scoparia* for headaches, and *Artemisia herba alba* for nausea, gastralgia, vomiting and colic. It is known that the plant causes nervous disorders in animals (Le Floch, 1983). This plant is of particular importance in Tunisia where it is used against colic and diabetes.

We here report on the neutral red uptake (NRU) test and the alkaline comet assay for *in vitro* toxicity and genotoxicity evaluation. Both tests are well known and considered important tools in (geno)toxicity testing.

2. Materials and methods

C. amblyocarpa was collected in 2009 in Kerker (Tunisian centre). A voucher specimen (C.A.02.02) was deposited in the biological laboratory of the Faculty of Pharmacy of Monastir. Methanol extracts were prepared according to standard methods (Edziri et al., 2012) and subjected to the neutral red uptake (NRU) cytotoxicity assay and the alkaline comet assay to assess genotoxicity. Tests were performed as described elsewhere (Verschaeve et al., *in press*). Statistical analysis between control and exposed cells was performed with the non-parametric Mann-Whitney U test.

3. Results and discussion

Tests were performed in C3A cells that are derived from HepG2 human carcinoma cells. This cell line was chosen as the cells have the essential structural, biochemical and growth features of normal human liver cells and have conserved both phase I and phase II metabolic capacities (Kelly, 1994). We have conducted several NRU tests using different concentration ranges as shown in Fig. 1. It can be seen that the results indicate that the NI₅₀ (concentration inducing a 50% lethality) is approximately 5 µg/ml. The extract is therefore even more toxic (× 10) than our positive control (SDS) where the NI₅₀ was found to be 57.7 µg/ml. It is also much more toxic than some other commonly used medicinal plants and commercial plant preparations (e.g., Ndhlala et al., 2010; Edziri et al.,

* Corresponding author at: Scientific Institute of Public Health, D.O. Public Health and Surveillance, Laboratory of Toxicology, Brussels, Belgium. Tel.: +32 2 642 54 36; fax: +32 2 642 52 24.

E-mail address: luc.verschaeve@wiv-isp.be (L. Verschaeve).

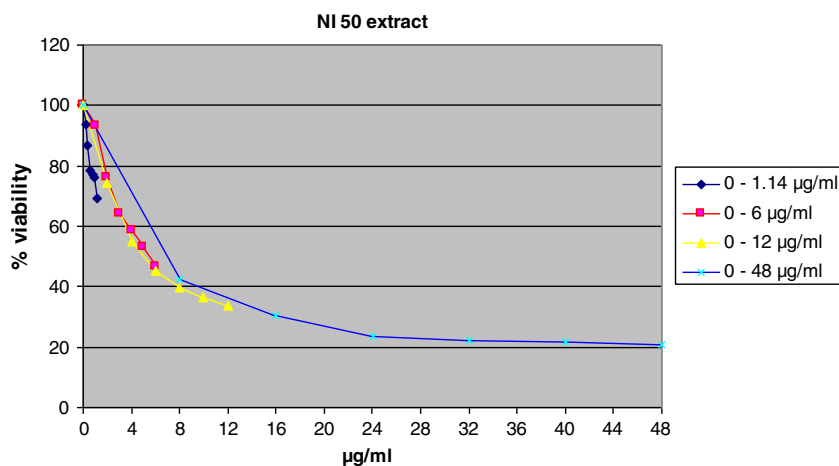


Fig. 1. Results of the NRU-test as performed with the *Cleome amblyocarpa* extract in 4 different experiments using different concentration ranges.

2011). *C. amblyocarpa* should therefore be considered as a potent *in vitro* toxic compound. Use of this plant in medicinal preparations should for this reason, especially if confirmed by *in vivo* investigations, be subjected to strict conditions, in particular regarding dosage. Strong *in vivo* toxicity of this plant was already reported. It causes fever, rapid pulse, dilation of pupils, hot and dry flushed skin, headache, dry mouth, and difficulty in swallowing, burning of the throat, hallucinations, and convulsions (Sharawy and Alshammari, 2009).

We tested 0–1 µg/ml concentrations of the plant extract with the alkaline comet assay that detects single and double strand DNA breaks as well as alkali labile sites. These concentrations were based on the results of the NRU test and were chosen in such a way that viability was always higher than 70%. Table 1 clearly shows that the percentage of DNA in the 'comet tail', which is indicative of the degree of DNA damage, was not significantly different from that of the unexposed control cells (mean and median values were almost identical in all cases). Regarding the alkaline comet assay it should be noted that the % DNA in the comet tail can theoretically be between 0% and 100% (apoptotic) with most cells having low % tail DNA in the control cultures and a shift to more cells with more substantial DNA damage for increasing concentrations of a genotoxic compound. Although most cells have more or less similar (and low) DNA damage levels some have excess damage (also in the unexposed controls). Inclusion of these (up to 70% damage) results in high standard deviations which are therefore not unusual and normal.

4. Conclusions

In conclusion our data clearly show that the *C. amblyocarpa* extract is toxic but does not show any genotoxicity based on the results of the alkaline comet assay.

Because of its widespread use and high toxicity further studies should be encouraged.

Table 1

Summary of the comet assay results obtained with subtoxic doses of the *Cleome amblyocarpa* extract (EMS: ethyl methane sulfonate = positive control).

	0 µg/ml	0.25 µg/ml	0.5 µg/ml	0.75 µg/ml	1 µg/ml	0.6 mM EMS
% DNA in comet tail	2.54	2.52	2.52	1.75	2.13	15.51
SD	5.79	4.13	3.63	3.09	3.34	9.87
Median	0	0	0.42	0	0	13.54
p-Level	/	0.579	0.224	0.636	0.577	<0.01

References

- Boulos, L., 1983. Medicinal Plants of North Africa. Reference Publication Inc., Michigan, USA, p. 52.
- Boulos, L., 1999. Flora of Egypt. Al Hadara Publishing, Cairo, Egypt, p. 177.
- Chopra, R.N., Chopra, I.C., Handa, K.L., Kapur, L.D., 1972. Chopra's Indigenous Drugs of India. Dhur & Sons, Private Limited, Calcutta, India, p. 321. 669.
- Edziri, H., Mastouri, M., Mahjoub, A., Anthonissen, R., Mertens, B., Cammaerts, S., Gevaert, L., Verschaeve, L., 2011. Toxic and mutagenic properties of extracts from Tunisian traditional medicinal plants investigated by the neutral red uptake-, VITOTOX- and alkaline comet assays. South African Journal of Botany 77, 703–710.
- Edziri, H., Mastouri, M., Aouni, M., Verschaeve, L., 2012. Polyphenols content, antioxidant and antiviral activities of leaf extracts of *Marrubium deserti* growing in Tunisia. South African Journal of Botany 80, 104–109.
- Ghazanfar, S.A., 1994. Handbook of Arabian Medicinal Plants. CRC Press, Boca Raton–London–Tokyo, p. 76.
- Kelly, J.H. 1994. Permanent human hepatocyte cell line and its use in a liver assist device (LAD). US Patent Nr. 5,290,68.
- Le Floch, E., 1983. Contribution à une étude ethnobotanique de la flore tunisienne. Programme Flore et Végétation tunisienne. Ministère de l'Enseignement Supérieur et de la Recherche. 387 pp.
- Ndhlala, A.R., Anthonissen, R., Stafford, G.I., Finnie, J.F., Verschaeve, L., Van Staden, J., 2010. In vitro cytotoxic and mutagenic evaluation of thirteen commercial herbal mixtures sold in KwaZulu-Natal, South Africa. South African Journal of Botany 76, 132–138.
- Sharawy, S.M., Alshammari, A.M., 2009. Checklist of poisonous plants and animals in Aja Mountain, Ha'il Region, Saudi Arabia. Australian Journal of Basic and Applied Sciences 3, 2217–2225.
- Verschaeve, L., Mertens, B., Nhlala, A.R., Anthonissen, R., Gorissen, B., Van Staden, J. in press. Investigation of the genotoxicity of water extracts from *Hypoxis* species and a commercially available hypoxis preparation. Phytoter. Res., <http://dx.doi.org/10.1002/ptr.4732>.