PharmacologyOnLine

Archives • 2021 • vol.2 • 465-469

DNA AFFINITY SCREENING OF EXTRACTS THE *ANDROCTONUS MAURETANICUS*

Aıt-Mouh, Siham [1,2] ; Mohamed Abdoul-Latif, Fatouma[3] ; Ainane, Ayoub[1] ; AM, Amira[2] ; Mohamed, Jalludin[3] ; EL KOUALI, M'hamed[2], TALBI, Mohamed [2], Ainane, Tarik[1]*

[1]Superior School of Technology of Khenifra (EST-Khenifra), University of Sultan Moulay Slimane, BP 170, Khenifra 54000 Morocco.

[2]LCAPCM, Faculty of Sciences Ben M'sik, Hassan II University Casablanca, Morocco.

[3]Medicinal Research Institute, Center for Research and Study of Djibouti, BP 486, Djibouti.

*t.ainane@usms.ma

Abstract

The *Androctonus mauretanicus* is largely found in North Africa, especially in central and southern Morocco, and is one of the animals with unsuspected potential in the medical field. The aim of this study is to demonstrate the pharmacological potential of extracts from this genus of scorpion and its therapeutic effect against tumour diseases based on DNA affinity screening of four extracts from *Androctonus mauretanicus.* In this context, a rapid and efficient method was used for the preliminary determination of the interaction of crude extracts of *Androctonus mauretanicus* with DNA using HPLC to detect the presence or absence of interaction with low molecular weight DNA molecules, and to demonstrate the ability of anti-tumour agents to reduce the size of the DNA peak compared to Doxorubicin. The results obtained in this work show that Ether extract has a positive activity and strong interaction with DNA compared to that of methanol and chloroform extract while Petroleum ether extract showed a negative peak reduction. These results confirm the anti-tumour activity of the *Androctonus mauretanicus* components which bind perfectly to DNA.

Keywords: *DNA; extracts; Androctonus mauretanicus; antitumour activity.*

Introduction

Androctonus mauretanicus belongs to the genus Androctonus of fat-tailed scorpions in the family Buthidae. This genus, which is largely found in North Africa, is the most dangerous type of scorpion for humans, yet they are among the animals that have unsuspected potential in the medical field [1-3]. The pharmacological potential of scorpion extracts has been the subject of numerous studies [4-6], the study of the mechanism of action of the venom of the Buthidae family, with its variable and peptide-rich composition, shows a high affinity and selectivity of scorpion toxins to membrane ion channels, reminding us of the therapeutic effect of extracts of *Androctonus mauretanicus* against channelopathies in which ion channels play an important role in their development [7-8]. These diseases cover a wide variety of conditions among them tumour diseases which are the second most common cause of human mortality. In some tumour cells, the functional activity of membrane channels is involved in the regulation of proliferative, migratory and invasive properties of the cells and they are proposed as therapeutic targets [9]. To demonstrate the anti-tumour activity of *Androctonus mauretanicus* extracts, a comparative study was performed with Doxorubicin, an anthracycline chemotherapeutic antibiotic derived from the bacterium *Streptomyces peucetius*, and commonly used in the treatment of solid tumours by intercalating between DNA base pairs causing DNA strand breaks, inhibition of DNA and RNA synthesis or DNA damage and induction of apoptosis [10-11].

In this context, four extracts of *Androctonus mauretanicus* were screened for DNA affinity using an HPLC method to detect the presence or absence of interaction with low molecular weight DNA molecules, and to demonstrate the ability of anti-tumour agents to reduce the size of the DNA peak compared to Doxorubicin. This rapid and efficient method was developed by Petuzzo et al. (1991) [12] and optimised by Ainane et al. (2015) [13] for the preliminary determination of the interaction of pure compounds and crude extracts with DNA, using HPLC as an affinity probe and the ability of binding compounds to reduce DNA size.

In our work, four extracts of this scorpion were screened to implement a rapid and inexpensive preliminary assay to demonstrate the anti-tumour activity of *Androctonus mauretanicus.*

Materials and methods

Preparation of the extract

- The harvested *Androctonus mauretanicus* were cut into small pieces and placed in $CHCl_3$/EtOH (V/V) for extraction and filtration.
- The filtrates obtained were concentrated by evaporation under reduced pressure.
- The residual solution was extracted successively in four solvents: petroleum ether, ether, chloroform and methanol.

DNA affinity test

The calf thymus and Doxorubicin used were purchased from Acros organics.

The HPLC instrument used was the Agilent/HP 1100 Series with a UV absorption detector (G1314A UV Detector) and a ZORBAX RP-18 (5µm) 4.6 x 200 mm column.

The DNA solutions (0.1 mg/ml) prepared in water (H_2O) are dispensed into sterile 1ml vials and stored at -20°C.

The solutions of the used extracts dissolved beforehand in MeOH (1.0 mg/ml) are centrifuged and filtered in order to avoid the injection of solids in the column. The mobile phase used is H_2O - MeOH (8:2) for a duration of 5 minutes and a gradient towards pure MeOh in 5 minutes, before injection the column was rinsed with MeOh for 20 minutes and an equilibration time of 10 minutes was respected, the injected volume is 20 µl with a flow rate of 1 ml/min, pre-mixing of the sample with the DNA solution and incubation of the mixture at room temperature for 5-30 min are necessary before injection.

Free DNA was eluted in approximately 1.8 min, an injection of the same DNA used for sample incubation is required to compare the size of the eluted peak with that of the incubated sample. All samples were tested according to the described methodology and the extracts were tested three times. To confirm the effectiveness of this method,

Doxoribicin was used as a positive control for DNA binding [13].

Results and discussion

A study of color and yield in relation to the initial quantity was carried out directly after the extraction step on the residual solutions obtained in the four solvents of increasing polarity: petroleum ether, ether, chloroform and methanol. (Table 1)

The prepared DNA (calf thymus) solution was injected onto a C18 grafted silica HPLC column filled with a mixture of H_2O/MeOH solvent (8:2), the peak corresponding to the DNA is detected by UV (254 nm). (Figure 1)

Doxoribicin standard and tested extracts previously diluted in methanol (1 mg/mL) and co-injected with DNA (20 µL) were eluted for 10 minutes with the eluent. A decrease in DNA peak area after injection of the test material reflects an interaction with DNA as shown in Figure 1, the peak area of the DNA chromatogram alone was decreased after injection of the Doxoribicin stadard. The rate results of the area decrease of DNA peaks after injection of the tested extracts of *Androctonus mauretanicus* and Doxorubicin are shown in Table 2. Petroleum ether extract shows a negative response. Methanol and chloroform extract elicit a positive response with 16.7% inhibition for Chloroform and 37.8% for Methanol. The ether extract elicits a strong interaction with DNA, this extract reduces the DNA peak by 80.8% more than Doxorubicin which has an inhibition rate of 66.2%. This result confirms that the components of *Androctonus mauretanicus* bind to DNA. In conclusion, the area of the DNA peaks after injection of the tested product reflects a strong interaction with DNA that exceeds even that of Doxoribicin, which confirms the anti-tumor activity of the *Androctonus mauretanicus* extracts [14-16].

Conclusion

The study of the antitumor activity of *Androctonus mauretanicus* extracts based on an HPLC system and a simple and efficient method shows that ether extract elicits a strong interaction with DNA, this extract reduces the DNA peak by 80,8 % more than Doxorubicin which has an inhibition rate of 66.2 %, methanol and chloroform extract elicit a positive response with an inhibition of 16.7 % for Chloroform and 37.8 % for Methanol while Petroleum ether extract does not reduce the DNA surface. These results confirm the anti-tumour activity of the components of *Androctonus mauretanicus* which bind perfectly to DNA.

References

1. Ward, M. J., Ellsworth, S. A., & Nystrom, G. S. (2018). A global accounting of medically significant scorpions: Epidemiology, major toxins, and comparative resources in harmless counterparts. Toxicon, 151, 137-155.
2. Sadílek, D., Nguyen, P., Koç, H., Kovařík, F., Yağmur, E. A., & Šťáhlavský, F. (2015). Molecular cytogenetics of Androctonus scorpions: an oasis of calm in the turbulent karyotype evolution of the diverse family Buthidae. Biological Journal of the Linnean Society, 115(1), 69-76.
3. Ward, M. J., Ellsworth, S. A., & Nystrom, G. S. (2018). A global accounting of medically significant scorpions: Epidemiology, major toxins, and comparative resources in harmless counterparts. Toxicon, 151, 137-155.
4. Majeed, I., Rizwan, K., Ashar, A., Rasheed, T., Amarowicz, R., Kausar, H., Marceanu, L. G. (2021). A Comprehensive Review of the Ethnotraditional Uses and Biological and Pharmacological Potential of the Genus Mimosa. International Journal of Molecular Sciences, 22(14), 7463, 1-43
5. Butt, S. A., Tahir, H. M., Ali, S., Tariq, M., Hassan, A., Summer, M., ... & Khan, S. Y. (2021). Evaluation of anti-scorpion (Hottentota tamulus) venom potential of native plants extracts using mice model. Toxin Reviews, 1-11.
6. Srairi-Abid, N., Othman, H., Aissaoui, D., & BenAissa, R. (2019). Anti-tumoral effect of scorpion peptides: Emerging new cellular targets and signaling pathways. Cell Calcium, 80, 160-174.
7. Tahir, H. M., Tobassum, S., Ali, S., Zahid, M. T., Arshad, M., Gardner, Q. A., & Ahsan, M. M. (2021). Characterization of Venom Components of Two Medically Important Scorpion Species, Hottentota tamulus (Fabricius, 1798) and Androctonus finitimus

(Pocock, 1897). Journal of the Kansas Entomological Society, 93(3), 230-245.
8. Martin-Eauclaire, M. F., Adi-Bessalem, S., Hammoudi-Triki, D., Laraba-Djebari, F., & Bougis, P. E. (2019). Serotherapy against voltage-gated sodium channel-targeting α-toxins from androctonus scorpion venom. Toxins, 11(2), 63.
9. Amirgholami, N., Karampour, N. S., Ghadiri, A., & Pipelzadeh, M. H. (2020). A. crassicauda, M. eupeus and H. lepturus scorpion venoms initiate a strong in vivo anticancer immune response in CT26-tumor mice model. Toxicon, 180, 31-38.
10. Dardevet, L., Rani, D., Aziz, T. A. E., Bazin, I., Sabatier, J. M., Fadl, M., De Waard, M. (2015). Chlorotoxin: a helpful natural scorpion peptide to diagnose glioma and fight tumor invasion. Toxins, 7(4), 1079-1101.
11. Ortiz, E., Gurrola, G. B., Schwartz, E. F., & Possani, L. D. (2015). Scorpion venom components as potential candidates for drug development. Toxicon, 93, 125-135.
12. Pezzuto, J. M., Che, C. T., McPherson, D. D., Zhu, J. P., Topcu, G., Erdelmeier, C. A., & Cordell, G. A. (1991). DNA as an affinity probe useful in the detection and isolation of biologically active natural products. Journal of Natural Products, 54(6), 1522-1530.
13. Ainane, T., Abourriche, A., Oukkache, N., Lamdini, H., Hammouti, B., & Bennamara, A. (2015) DNA affinity screening of extracts tunicate Cynthia squamulata from the Atlantic coast. Der Pharma Chemica, 7(10), 45-49.
14. Wu, Y., Liu, M., Pei, W., Zhao, Y., Wang, D., Liu, T., ... & Han, J. (2020). Thermodynamics, in vitro release and cytotoxity studies on doxorubicin–toluidine blue O combination drugs co-loaded in aptamer-tethered DNA nanostructures. Journal of Molecular Liquids, 320, 114390.
15. Li, D., Xu, Y., Fan, L., Shen, B., Ding, X., Yuan, R., ... & Chen, W. (2020). Target-driven rolling walker based electrochemical biosensor for ultrasensitive detection of circulating tumor DNA using doxorubicin@ tetrahedron-Au tags. Biosensors and Bioelectronics, 148, 111826.
16. Kang, J. H., Kim, K. R., Lee, H., Ahn, D. R., & Ko, Y. T. (2017). In vitro and in vivo behavior of DNA tetrahedrons as tumor-targeting nanocarriers for doxorubicin delivery. Colloids and Surfaces B: Biointerfaces, 157, 424-431.

Table 1. The different extracts of *Androctonus mauretanicus*with yield and color

Extract	Color	Yield (%)
Hexane	Dark red	2.39
Ether	Black – Brown	3.74
Chloroform	Green	1.08
Methanol	Brown	6.12
Marc (*)	Brown	85.66

Table 2. Results of screening for DNA interaction of extracts of *Androctonus mauretanicus* and Doxorubicin.

Extract	DNA peakreduced (%)
Petroleum ether	0.00
Ether	80.8
Chloroform	16.7
Methanol	37.8
Doxorubicin	66.2

Figure 1. DNA chromatogram peak. (a) DNA alone, (b) DNA + Doxorubicin.