Journal of Ethnopharmacology 211 (2018) 340–347

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Anti-scorpion venom activity of *Thapsia garganica* methanolic extract: Histopathological and biochemical evidences

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ARTICLE INFO

Chemical compounds studied in this article:
Ethanol (Pubchem CID :702)
Methanol (Pubchem CID :887)
DPPH (Pubchem CID :2735032)
β –Carotene (Pubchem CID: 5280489)
linoleic acid (Pubchem CID: 5280450)
bleu de comasie (Pubchem CID: 101394904)
sodium carbonate solution(Pubchem CID :10340)
gallic acid (Pubchem CID: 370)
aluminum trichloride (Pubchem CID: 24012)
Catechin (Pubchem CID: 73160)
vaniline-MeOH (Pubchem CID: 102154025)
choridric acid (Pubchem CID: 28153)
Butylated hydroxytoluene (Pubchem CID :31404)
quercetin (Pubchem CID: 5280343)
chloroform (Pubchem CID: 6212)
phosphate (Pubchem CID: 8295)
potassium ferricyanide (Pubchem CID: 26250)
trichloroacetic acid (Pubchem CID: 6421)
ferric chloride (Pubchem CID: 68541)
Sodium Chloride (Pubchem CID: 5234)
fomaline (Pubchem CID: 712)
paraffin (Pubchem CID: 109453)
hematoxyline eosin (Pubchem CID: 86598188)

Keywords:
Thapsia garganica
B. occitanus
Antivenoms activity
Antioxidant
Histology

ABSTRACT

Ethnopharmacological relevance: *Thapsia garganica*, is a herbal medicine traditionally used as diuretic, emetic and purgative. It is also used as anti-scorpion venom in Morocco; however, its protective effects against scorpion venom remain elusive.

Aim of the study: The present study was undertaken to evaluate anti-venom activity of *T. garganica* in vivo through histological and biochemical studies.

Materials and methods: Methanolic leaves extract of *T. garganica* was evaluated for anti-venom activity against *buthus. occitanus* under *in vivo* conditions. Histopathological and biochemical changes in envenomed and treated mice were also examined. Phytochemical screening was conducted to estimate the major constituents whereas DPPH, β -Carotene–linoleic acid and reducing power assays were performed to evaluate the anti-oxidant activity of *T. garganica* extract.

Results: Methanolic leaves extract of *T. garganica* (2 g/kg) increased the survival time (> 18 h) of mice injected with lethal doses of *B. occitanus* venom, with remarkable recovery of histology damage. Furthermore *T. garganica* induced a significant decreased of biochemical markers of kidney, liver and heart function. Phytochemistry screening revealed the presence of phenolic compounds, flavonoids, tannins and steroids/terpenoids, which might explain the bioactivity of the extract. It was also shown that the extract has an exceptionally high anti-oxidant activity compared to well-known antioxidants used as standards.

Conclusion: The present study provides strong evidence that support the use of *T. garganica* as anti-scorpion

Abbreviations: B. occitanus, *Buthus occitanus*; LD50, Median lethal dose; LD99, Lethal Dose 99; IC 50, Concentration of 50% inhibition; LDH, Lactate deshydrogenase; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; CPK, Créatine phosphokinase; T. garganica, Thapsia garganica; DPPH, 1,1-diphenyl- 2-picrylhydrazyl; PCCM, Poison Control Center of Morocco; BHT, Butylated hydroxytoluene

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http://dx.doi.org/10.1016/j.jep.2017.10.003
Received 7 June 2017; Received in revised form 3 October 2017; Accepted 5 October 2017
Available online 06 October 2017
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venom in traditional medicine in Morocco. However, additional studies are required to isolate and identify the metabolites responsible for the activity.

1. Introduction

Scorpion envenomation is an important public health issue in many countries of the world, including North-Africa, the Middle East and South America (Al-sadoon and Jarrar, 2003; De Roodt et al., 2003; Patil, 2009). The estimated number of scorpion stings worldwide is over one million per year, resulting in massive cases of morbidity and mortality (Sofer et al., 2013; Coelho et al., 2014). In Morocco, scorpion envenomation is the leading cause of intoxication with a rate of 30–50% of all the poisoning cases reported to the Poison Control Center of Morocco (PCCM). The major causative species are from *Androctonus* and *Buthus* genus, of these, *Buthus occitanus* causes severe envenomation (Ismail, 1995).

Scorpion envenomation pathophysiology is characterized by a systemic response consisting of hypertension or hypotension, tachycardia, hypothermia, leucocytosis, hyperglycaemia, myocarditis, pancreatitis, respiratory distress and other physiological disturbances, both in humans and animals (D'Suze et al., 2003). Pathology induced by scorpion envenomation is generally due to a possible release of pro-inflammatory mediators of cardiorespiratory perturbations (Sofer et al., 1996) and/or a sympathetic and parasympathetic stimulation of the autonomic nervous system by neurotoxins (Sofer and Gueron, 1988; Ismail, 1995).

Although, serotherapy is generally accepted for scorpion envenomation treatment, it is not always available to millions of rural population in the third world, and its effectiveness remains very controversial. Thus, extensive researches for new active substances with an antagonistic effect against neurotropic activity of scorpion venom are needed (Possani, 2005).

Several medicinal plants are extensively used in scorpion envenomation treatment. Phytochemicals isolated from different plant species have been associated with multiple beneficial pharmacological properties such as reducing inflammation, myotoxicity, neurotoxicity and oxidative stress. While many studies have demonstrated the effectiveness of various plant extracts against scorpion venom in mice (Jiménez-Ferrer et al., 2005), and several plant species or phytochemicals have been pharmacologically investigated for anti-venom activity in south-America and south-Asia. However, little is known about antitoxin activity of Moroccan medicinal plants in scorpion envenomation treatment.

T. garganica is a plant widely used for its therapeutic benefits against scorpion stings in Morocco (Bellakhdar, 1978), however, its antivenom activity remain unclear. The aim of this study is evaluated anti-venom activity of *T. garganica* in vivo through histological and biochemical studies.

2. Materials and methods

2.1. Mice

Male Swiss Albino mice (20–22 g) were used. The animals were kept at a constant room temperature (25 °C), with a 12 h dark–light cycle and free access to food. All animals were treated according to the European decree, related to the ethical evaluation and authorization of projects using animals for experimental procedures, 1st February 2013, NOR: AGRG1238767A. Thus, all efforts were made to minimize the number and reduce the suffering of animals used.

2.2. Scorpions and venom extraction

Scorpions were collected from El-Jadida province in the southern region of Morocco. They were housed in well ventilated wooden cages with free access to food and water. The species was determined according to an appropriate identification key. Venom was obtained from mature *B. occitanus* scorpions by electrical stimulation of the telson as described by Ozkan et al. (2007). The venom was diluted by sterile double distilled water and the protein content of venom was determined according to the method of Bradford (1976). Until use, the diluted venom was stored at −20 °C.

2.3. Plant material and preparation of total extract

The leaves of *T. garganica* were collected from *Amizmiz* in the central part of Morocco. Taxonomic identification of the plant material was performed by Ouhammou A. at the University Cadi Ayyad Herbarium, where a voucher specimen (MARK10013) has been deposited. The plant name has been checked with http://www.theplantlist.org (Accessed: 1 June 2017). Leaves were dried under dark conditions at room. The dried material was individually processed; subsequently extraction was performed by percolation with ethanol. The extract was quantified and stored away from light at 0–4 °C.

2.4. Identification of secondary metabolites

Preliminary phytochemical screening was conducted according to the protocol described by Harborne (1998). The qualitative presence of the following secondary metabolites was analyzed: flavonoids, steroids, terpenes, tannins, coumarins, saponins, and alkaloids.

2.5. Determination of total phenolic compounds, tannins and flavonoids content in T. garganica extract

Total phenolic compound content was quantified using the Folin-Ciocalteau method (Singleton et al., 1999). Briefly, 20 µl of extract was mixed with 1.16 ml of distilled water and 100 µl of Folin-Ciocalteau reagent, 300 µl of sodium carbonate solution (Na_2CO_3) was then added. After incubation for 30 min at 40 °C. The absorbance was measured at 760 nm. Results were expressed as gallic acid equivalents per gram of extract (GAE/g DM).

Total flavonoids were estimated using the aluminum trichloride method (Ordon et al., 2006). Briefly, 0.5 ml of extract was mixed with 0.5 ml of 2% $AlCl_3$ ethanol solution. After incubation at room temperature for 1 h, the absorbance was measured at 420 nm. Results were expressed as catechin equivalents per gram of extract (CAT/g DM).

Tanins were quantified as follow, 100 µl of sample were added to 1 ml of (vaniline-MeOH) 4% and 0.5 ml of HCl. After 15 min of incubation at room temperature, the absorbance was measured at 550 nm (Xu and Chang, 2007). Results were expressed as catechin equivalents per gram of extract (CAT/g DM).

2.6. DPPH free radical-scavenging activity

The ability of methanolic extracts of *T. garganica* to scavenge the DPPH radical was estimated using the method described by Sahin et al. (2004). An aliquot of 50 µL of various sample concentrations was added to a volume of 2 ml of the DPPH methanolic solution (60 µM). The reaction mixture was well stirred and incubated for 20 min at room temperature in the dark. The absorbance of the extracts was recorded at

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