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Aqueous leaves extract of *Artemisia campestris* inhibition of the scorpion venom induced hypertension

Ben Nasr Hamed*, Hammami T. Serria, Mahmoudi Lobna and Zeghal Khaled

Laboratory of Pharmacology, Faculty of Medicine of Sfax, Street of Magida Boulila, 3029 Sfax- Tunisia.

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The boiled leaves of *Artemisia campestris* (Asteraceae) was used as a folk-medicine against ophidian and scorpion envenoming in rural and nomad populations, but its bona fide mechanisms are still unknown. In this report, the effect of the aqueous dry leaves' extract of this plant on hemodynamic variations induced by *Buthus occitanus tunetanus* venom was assayed in pregnant and non pregnant rats. Our results showed that the venom induced hypertension magnitude was much important in pregnant rats (maximal of 156% of baseline) than in cycling ones (maximal of 143.9% of baseline). When injected alone, the aqueous leaves extract of *A. campestris* induced a progressive significant diminishing of the mean arterial pressure both in 72.4 ± 7.6% and out of pregnancy (90.4 ± 4.4%). This effect did completely abolish the venom induced hypertensive shock, when envenomed rats were pretreated with the extract. It was concluded that aqueous extract of *A. campestris* leaves prevents the induced hypertensive phase induced by the scorpion venom, probably through adrenergic pathway. The potential of *A. campestris* water-boiled extract to revert hypertension is sought to be useful for anti-hypertensive drugs development; and worth much merit to be investigated.

Key words: *Artemisia campestris*, scorpion envenomation, blood pressure, pregnancy, rat.

INTRODUCTION

Harmful hemodynamic perturbations are a common injury following scorpion stings, especially in severe cases. In association to respiratory distress, the induced cardio-vascular disorders are the prominent cause of death, in such intoxication. They usually manifest into different grades, benign, moderate and severe to fatal (Goyffon, 2002). The scorpion induced hemodynamic disorders usually manifests by a rapid and transient hypertensive phase, which is followed by a more lasting hypotensive one (Gueron and Yaron, 1970; Tarasiuk et al., 1998). The mechanism of cardiovascular anomalies had been in the long run debated. However, the largely admitted explanation is that the venom induces a hyper-stimulation of both cholinergic and adrenergic neurons, through its action on presynaptic cell membranes. It was proved that the intravenous injection of *Buthus occitanus* venom induces 30 to 40 fold increases in plasma epinephrine and nor-epinephrine; and causes a significant augmentation of blood pressure, total peripheral, renal and muscular resistances, in association to cardiac output decrease; and arrhythmias (Zeghal et al., 2000). Furthermore, scorpion envenoming results into rennin and aldosterone plasma levels' elevation that is consistent with autonomic nervous system hyperstimulation (Gueron et al., 1992). The hypo-oxygenic statute consequent to pulmonary edema could also contribute in the observed alterations. Other authors suggested a direct effect of scorpion toxins on the electrophysiological statute of cardiomyocytes

*Corresponding author. E-mail: hmedbnasr@gmail.com. Tel: (216) 74 247 036. Fax: (216) 74 246 217.

(Gueron and Yaron, 1970). Because of its important worldwide frequency, reaching above one million stung human each year (Chippaux and Goyffon, 2008), various preventive and curative anti-scorpionism strategies were envisaged. At emergency to care-medical units, the treatment of scorpion poisoning is concentrated on resuscitating the damaged vital functions, essentially those of the cardiovascular and respiratory systems. The anti-venin sera becomes lesser administrated, as long as the elapsed time to emergency is overdue. In fact, scorpion toxins are of rapid bio-distribution and tissular fixation (within few hours); which render antibodies incapable of their neutralization (Goyffon, 2002; Gueron and Yaron, 1970). In folk medicine, medical plants had occupied a great place. Their effects could console envenomed patients (Abubakar et al., 2000; Alam and Gomes, 2003; Melo et al., 2007). In such issue, the boiled dried leaves of *Artemisia campestris* (Asteracae family) have been used for long time to counteract ophidian and scorpion envenomations in rural and bedouin populations in our country. Artemisia shrub contains several bioactive substances that could prevent hypertension and cardio-vascular disorder (Ben-Nasr et al., 2013). Nevertheless, at our knowledge, no concise assays had been carried out to reveal such remedy's effects on these injuries.

Hence the scope of this work was to delineate the effect of the aqueous extract of *A. campestris* leaves on the scorpion venom induced-hemodynamic perturbations. Our experimental animal model did include, in addition to normal (non pregnant), the gestation statute which was assigned as critical by clinicians.

MATERIALS AND METHODS

Animals

Three to four months old, virgin female white Wistar rats (from the Pasteur Institute of Tunis- Tunisia) were used. They were elevated six per cage, at constant environmental conditions (temperature of 23 to 25°C, air humidity ≈ 40%, and diurnal cycle of 14 h of light/10 h of obscurity). Animals had received water and standard food pellets (SICO Sfax- Tunisia), free *ad libitum*. After one week of acclimatization to these conditions, females were allowed to an overnight mating with mature males from the same pool, in order to obtain dated pregnancies. The first gestation day coincides with when spermatozoa were cheeked, in the daily vaginal smear. In such conditions, pregnancy lasted for 22 ± 1 day.

Scorpion venom

Buthus occitanus tunetanus (Bot), from the Buthidae family, is one of the most incriminated scorpion envenomations in Tunisia. Its crude venom was kindly offered by the Pasteur Institute of Tunis (Tunisia). It was obtained by telson electrical milking and extracted dealing with Miranda and al method (1970) (Miranda et al., 1970). Briefly, after electrical milking, it was water extracted; freeze dried, rehydrated and stored at -20°C until further use. At the day of experiment, crude scorpion venom was appropriately diluted with a 0.9% saline physiologic solution, in order to obtain a concentration of protein content of 1 mg/ml.

Artemisia campestris' leaves extract

20 g of dried leaves of *A. campestris* were boiled for 15 min in 100 ml of bi-distilled water. After cooling, the aqueous phase was twice filtrated using filter paper of 0.22 µm diameter pores (Millipore XX15047005). The prepared extract was daily prepared and warmed (37°C) before use.

Experimental design

To carry out our experiments, agitated (stressed before anesthesia) animals were excluded. Six groups of either pregnant rats or not were used. Rats were anaesthetized by intra-peritoneal injection of 60 mg/kg body weight (BW) of thiopental (Sandoz Gmbh, Kundall, Austria); on the day corresponding to the 22nd of gestation (9:00 to 10:00). After anesthesia, the right carotid artery was catheterized using a heparinated polyvinyl catheter attached to a pressure transducer (Pression/calculator; Goldinstrument). Records of the systemic mean arterial pressure (MAP) were carried out using a thermal array corder WR700 (Graphtec-Ankersmit) on polygraph paper. The calibration of the apparatus was kept constant during all recordings at an arbitrary unit for blood pressure leveling. After animals' stabilization, 10 min recordings were considered as a baseline. Thereafter, each group of rats was submitted to one of the three following treatments:

1. Intra-peritoneal injection of a 0.5 ml/animal of aqueous *A. campestris* leaves extract (EXT).
2. Sub-cutaneous injection of 1 mg (protein content)/ml^{-1} kg^{-1} of *B. occitanus tunetanus* crude venom (BOT).
3. Or injection of the leaves extract and 15 min later the venom was administrated (EXT+BOT).

The study of mean arterial pressure variation was evaluated by calculating its percentage relatively to the baseline value, each two minutes-recording interval. The elapsed time from the last injection to the appearance of the first hypertensive spike (determined once the variation percent exceeds 120% of baseline); and which separating the first and the latest ones were measured. Moreover, the number of hypertensive bursts was determined. Once the experiment was finished (three hours of recordings, or by animal death), rats' necropsy was performed in order to determine maternal organs (heart, liver, lungs and kidneys) and the utero-fetal unit parameters (the total gravid uteri weights and number of fetuses).

Statistical analysis

Mann-Whitney test was used to compare the checked morphometric parameters between the different groups of rat. The mean of the recorded MAP variations at each time-interval of 10 min had been considered for comparison between the studied groups and to determine its chronological progression following each treatment, using uni-variate generalized linear model. Statistical package for social sciences (SPSS) for Windows.11.0 program was used to accomplish the statistical analysis.

RESULTS

The statistical analysis showed a significant difference in the lungs absolute weight, between cycling rats receiving the venom (1.47 ± 0.07 g) and those treated by the leaves extract (1.33 ± 0.05 g). Other morphometric parameters were comparable either in pregnant rat groups, or

Table 1. Morphometric parameters of non pregnant rats' organs [(weight (g)].

Treatment		BW	H	L	Lv	RK	LK
	Mean	179.4	0.78	1.33	6.77	0.74	0.69
EXT+BOT	SD	9.7	0.07	0.28	1.11	0.07	0.08
	N	5	5	5	5	5	5
	Mean	180	0.73	*1.47	7.05	0.71	0.70
BOT	SD	10.2	0,01	0.07	0.77	0.08	0.07
	N	3	3	3	3	3	3
	Mean	194.2	0.75	*1.33	6.85	0.77	0.71
EXT	SD	14.9	0.02	0.05	1.38	0.11	0.1
	N	4	4	4	4	4	4

BW: Body weight; H: heart, L: lung, Lv: liver, RK and LK: right and left kidney, respectively. (*) significant difference between the two groups, at p ≤ 0.05.

Table 2. Morphometric parameters of pregnant rats' organs.

Treatment		BW	H	L	Lv	RK	LK	G. Ut	NF
	Mean	261	0.71	1.09	10.1	0.74	0.69	47	10.40
EXT+BOT	SD	5.3	0.03	0.10	1.17	0.04	0.06	8.14	1.67
	N	5	5	5	5	5	5	5	5
	Mean	261.6	0.74	1.29	11.2	0.81	0.76	46.86	7.33
BOT	SD	43.4	0.11	0.19	0.26	0.07	0.08	19	3.51
	N	3	3	3	3	3	3	3	3
	Mean	266.3	0.79	1.38	9.50	0.81	0.77	42.81	9.00
EXT	SD	17	0.06	0.19	0.23	0.01	0.01	3.12	0.00
	N	3	3	3	3	3	3	3	3

BW: body weight; G. Ut: gravid uteri, H: heart, L: lung, Lv: liver, RK, LK: right and left kidney, respectively; and NF: number of fetuses per rat. There was no significant differences between the studied groups (treated with *A. campestris* extract (EXT), *B. occitanus tunetanus* venom (BOT), or both (EXT+BOT).

in cycling ones (Table 1 and 2). Hypertensive spikes appeared earlier in pregnant (33.3 ± 2.1 min) rats than in non pregnant ones (62.6 ± 1 min), following the scorpion venom injection. The mean recorded blood pressure increased to reach 119 ± 2.7% at 70 min and 131 ± 3.1% of baseline at 50 min after the venom injection in non pregnant and pregnant rats, respectively. The maximal recorded individual's blood pressure augmentations were of 156.2 and 143.9%, respectively gravid and non gravid rats. Further, the number of hypertensive bursts (14 ± 4 and 3 ± 2, respectively) and their duration (33.3 ± 14 and 21.3 ± 15, respectively) were significantly greater after envenomation in pregnant rats than in cycling ones (Figure 1).

The intra-peritoneal injection of the filtrated boiled-extract of *A. campestris* induced a significant and progressive diminishing of the mean arterial blood pressure by about 30% (72.4 ± 7.6%) at 140 min and 10% (90.4 ± 4.4 %) at 130 min, respectively in pregnant and cycling rats. In exception of the group of normal rats receiving the aqueous extract followed by the venom injection, mean arterial pressure variations were negatively correlated to the time progression. Figure 2 presents the dependent time evolution of MAP. It clearly reveals the absence of hypertensive bursts in both pregnant and cycling rats injected with 1000 µg/ml/kg of the venom following the pretreatment with the aqueous extract of *A. campestris* leaves (EXTBOT).

DISCUSSION

Usually, scorpion stings lead to two hemodynamic events: (i) a prolonged hypotensive phase, which could or not be preceded by (ii) a transient hypertensive one (Goyffon, 2002; Gueron and Yaron, 1970; Tarasiuk et al.,

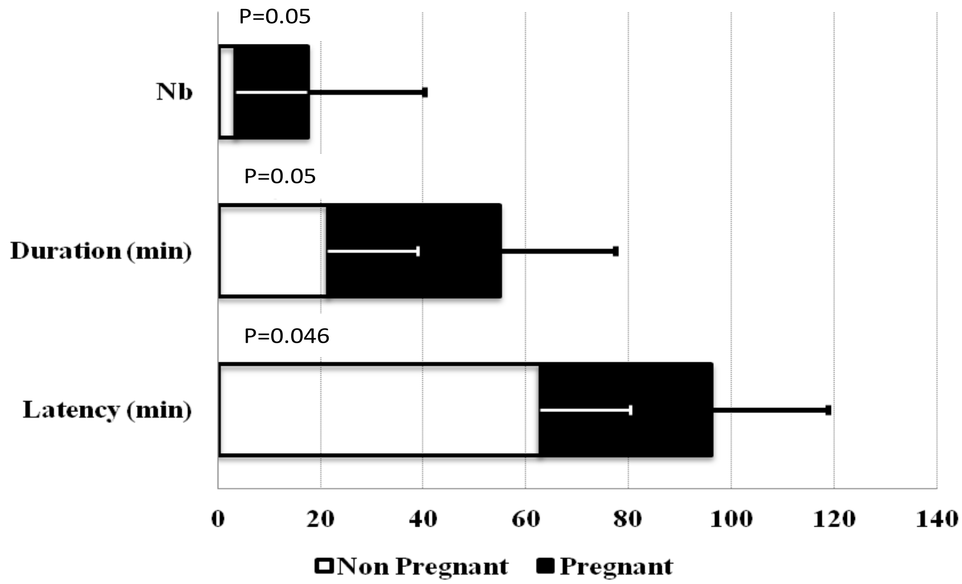

Figure 1. Characterization of the venom–induced hypertensive phase (Nb: number of hypertensive spikes, the latency to the first hypertensive spike and the duration of the phase). Bars represent the mean values in each group of rat (pregnant and non pregnant).

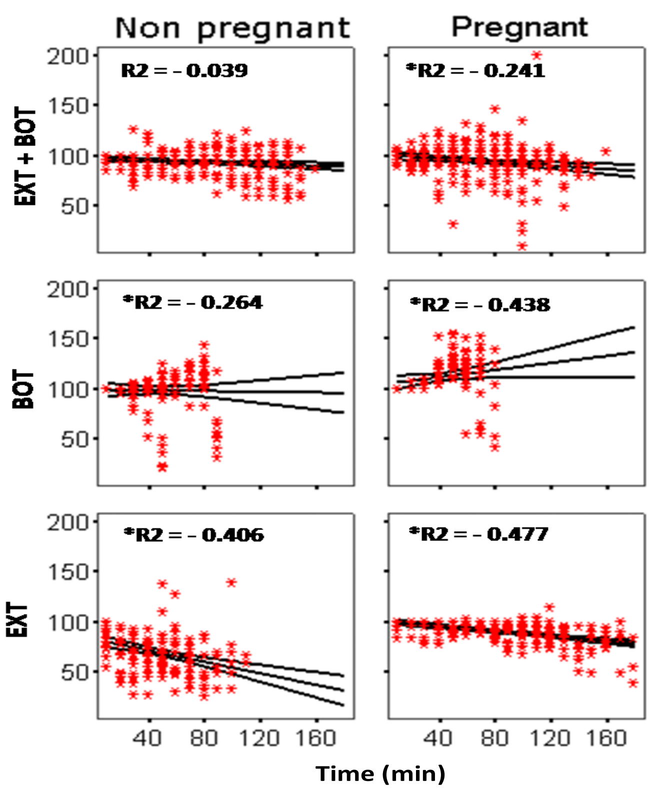

Figure 2. Mean arterial pressure (MAP%) variations in venom (BOT), *Artemisia campestris* extract (EXT), or both (EXT+BOT) treated pregnant and non pregnant rats. the curves represent the mean values in each of the used group. Symbols represent individual recorded MAP% at each 10 min time–intervals, and lines show the spearman-linear correlations and their margins at 95% of significance. *R2 designates the significant correlations.

1998; Zeghal et al., 2000). The real mechanism of these perturbations is still debated. In medical care units, the resuscitation of these injuries, in association to those of the respiratory system, represents the essential treatment of envenomation. Far from medical centers, in rural and nomad populations, where the accident frequently occurred, the boiled water-infusion of *A. campestris* leaves constitutes a folkloric remedy against both ophidian and scorpion poisonings. In this context, we had proposed to divulge this aqueous extract effect on the scorpion venom induced hemodynamic alterations, in an animal model. Our experimental design did include the pregnancy statute which is described as critical case by clinicians.

The yellow scorpion's (*B. occitanus tunetanus*) venom induced a higher increase of the arterial blood pressure in pregnant rats than in non pregnant ones. In fact, during pregnancy, various physiological modifications did occur to adapt the maternal organism for the reception of the fetal unit and supplying it in essential metabolites. The gestation cardiovascular changes during pregnancy manifest in an augmentation of the cardiac output, myocardial contractility resulting from placentation and progesterone effects, and of peripheral vascular dilatation as a consequence of vascular resistance decrease (Girling, 2004). These modifications might explain the difference in physiological and pathological hemodynamic between pregnant and non-pregnant animals.

The administration of the leaves extract, alone, induced a progressive significant diminution in MAP in both pregnant and non pregnant rats. Similarly, our recent investigation indicated that the oral administration of this extract effect to healthy volunteers induced a lowering of both the diastolic and systolic pressures, within one hour after the treatment (Data not shown). Such effect completely abolished the hypertensive phase induced by the scorpion venom, when the extract was administered as a pretreatment. The hypotensive effect of A. *campestris* was similarly observed when using other medicinal plants (Tingo et al., 2000; Mehlsen et al., 2002; Fatehi et al., 2004). Tingo et al. (2000) mentioned that the aqueous extract of *A. vulgaris* re-established the hypertension induced by noradrenalin (Tingo et al., 2000), which is a potent α- adrenergic vasoconstrictor (Imai et al., 1978; Datta and Magder, 1999). The probable mode of action of *Artemisia* extracts on the cardiovascular system has been discussed, and might involve an inhibitory effect of the epinephrine induced hypertension. Five different flavonoids (jaceosidine, eupafolin, leuteolin, quercetin and apigenin) and three coumarins (aesculetin, 7-methyl ether and scopoletin) extracted from *A. vulgaris* exhibited potent capacities to restrain the brain monoamine oxidase that plays important role in different neurotransmitters metabolism (Lee et al., 2000). Furher chemical compounds, such as estragon (from *A. anomala*), are ligand of neurotransmitters receptors and could inhibit their signaling pathway (Luedtke et al., 2003). Because of the prominent role of epinephrine and nor-epinephrine in the venom induced blood pressure increase (Zeghal et al., 2000), it could be envisaged that the *A. campestris* extract inhibits either nor- and epinephrine secretion, or their signaling pathway, to induce vasorelaxation and hypotension (Ben-Nasr et al., 2013).

The pulmonary edema did usually occur after scorpion envenomation (De Matos et al., 1997), which could contribute to the observed lungs absolute weight difference between non pregnant rats receiving the venom and those treated with the extract. Such observation speculates an anti-edematous effect of the vegetal extract that merits further studies to be disclosed. In contrary, lungs weights were comparable in pregnant rats, between all treatments; probably because of the physiological edematous statute of gestation (Reynolds, 2003).

Conclusion

The aqueous extract of *A. campestris* antagonizes the hypertensive effect of *B. occitanus tunetanus* venom, via a mechanism probably involving an adrenergic action. Further studies are foreseen to determine if this effect will extend the latest hemodynamic choc, or in contrary will prevent this cardiac choc attributed to the adrenergic discharge.

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Conflict of Interests

The author(s) have not declared any conflict of interests.

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