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Effect of the aqueous crude extract of *Matricaria pubescens*: histopathological and biochemical study on scorpion venom given rats

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Abstract. Scorpion envenomation is a major public health problem, not only in terms of its frequency, but especially by its lethality. Immunotherapy is the only treatment that can neutralize scorpion toxins. Several medicinal plants offer an unlimited source of therapeutic potential with a wide spectrum of bioactivity. The present study was undertaken to evaluate the effects of *M. pubescens* crude extracts against scorpion venom activity through histological and biochemical studies. This study was conducted on 20 Albino rats which were divided into four groups (a control group, a group envenomated by a sublethal dose of *Androctonus australis hector* venom, an envenomated group treated with the aqueous extract of *Matricaria pubescens*, and the last envenomated group treated with the anti-venom serum). Metabolic and histopathological changes were examined in different treated groups. Our results clearly show that the venom of *Androctonus australis hector* induces a considerable disorder in the biochemical parameters and tissue structure of the different organs (liver, lung, heart and kidney). The treatment of the envenomated rats with the aqueous extract of the studied plant neutralizes almost all the observed disturbances. It can be concluded that the aqueous extract of *Matricaria pubescens* Owns evident effects against *Androctonus australis hector* venom activity with a better organization of certain damaged tissue structures and a relative normalization of biochemical parameters.

Key words: antivenomous activity, *Androctonus australis hector, Matricaria pubescens,* in vivo, scorpion envenomation.

Introduction

Scorpions are widely distributed throughout the world (Uawonggul et al. 2005). In Algeria, scorpion sting remains a serious public health problem due to its frequency and severity (Chippaux and Goyffon 2008). According to epidemiological data established by the Ministry of Health and Population, every year Algeria registers 30,000 to 50,000 cases of scorpion bites, including more than 150 cases of death, particularly during the heat season (Hellal et al. 2012). Immunotherapy is currently the only treatment capable of neutralizing scorpion toxins (Pepin-Covatta et al. 1996) despite unsatisfactory results in some cases.

Traditional treatments are available in many parts of the world, the most common of which is the use of herbal products.

However, according to the bibliography and to the best of our knowledge, the majority of medicinal plants used by people against scorpion venom activity, abundant in Algeria, have not been scientifically tested for this property. *Matricaria pubescens* is a medicinal plant widely used by the Algerian population against scorpion venom activity

Therefore, the aim of this work was to establish, scientifically, through an animal experiment, the anti-venom properties of crude aqueous extract of the medicinal plant *Matricaria pubescens* by studying their effect on biochemical parameters and the tissue structure of the different organs (liver, lung, kidney and heart) after an injection of the venom in rats and to compare their effect with an anti-venom serum.

Material and Methods

Abbreviations
Aah: *Androctonus australis hector*;
s.c.: subcutaneous;
i.p.: intraperitoneal;
ALT: Alanine aminotransferase;
AST: aspartate aminotransferase;
ALP: alkaline phosphate;
GGT gamma-glutamyl transférase.

Plant material
The aerial part of *Matricaria pubescens* plants, was collected from the village of Reguiba in El Oued. The plant sample was cleaned and then dried at room temperature away from the light, in order to preserve as much as possible, the integrity of its chemical composition.

Preparation of Plant Extract
The extract to be tested was obtained according to methods which have remained as close as possible to the traditional method of preparation. 100 g of *Matricaria pubescens* powder are homogenized with 250 ml of distilled water and macerated in a flask with continuous magnetic agitation for 15 hours. After maceration, the aqueous extract was recovered by filtration on Wattman paper N1 and then centrifuged at 3000 rotations per minute for 10 minutes. Supernatants were collected and stored at -20 ° C in sterile polyethylene vials hermetically closed.

Animals
Healthy female *Wistar albino* rats aged 8 weeks old weighting 160-180 g, were obtained at the Animal Service of the Pasteur Institute, Algeria. They were kept under standard environmental conditions at 25 °C with 12:12 h light–dark cycle in ventilated plastic cages. Animals have free access to water and food.

Scorpion venom sample
The scorpion species used in this study is *Androctonus australis hector* (Aah) which is the most common in Algeria (Chippaux and Goyffon 2008). The venom was obtained by electrical excitation, the telson is subjected to low frequency discontinuous electrical stimulation allowing the ejection of the venom (Oukkache 2016). Lyophilized venom sample was stored at 4°C for future use.

Medicament
The anti-venom serum is obtained from Ben OMor Djilani Hospital in El Oued region. It is an anti-toxic serum, produced by the Pasteur Institute of Algeria, from horse plasma hyperimmune against Aah's

venom. Each 1ml of reconstituted serum, contains F (ab')$_2$ fragments of immunoglobulins, neutralized not less than 1 mg of dried Aah venom.

Animal treatment

The experiment was according to an approved protocol from the animal ethics committee according to guidelines of care and using animals in Algeria. After the adaptation period, rats were equalized with respect to body weight and randomly divided into four groups of seven rats each.

The first group serving as a control received 100µl of physiological water. Group II animals received an injection of 100 µl of a sublethal dose (0.5 µg/g rat) of Aah venom (Bessalem et al. 2003). Group III rats received an intraperitoneal (i.p.) injection of 500 µl aqueous *Matricaria Pubescens* (0.5 µg/g rat) 30 minutes after subcutaneous (s.c.) injection of a sublethal dose of Aah venom (0.5 µg/g rat). While group IV rats received an injection (i.p.) of 500 µl aqueous antivenom serum (20 µg/g) 30 minutes after subcutaneous injection (s.c.) of a sublethal dose of Aah venom (0.5 µg/g rat). The animals were sacrificed 24 hours after injection of the venom.

Biochemical analysis

Blood samples for biochemical assays were collected in tubes without anticoagulant and serum was obtained by centrifugation of the samples for 15 min at (3000 rotation per minute, 4 °C) for the determination of biochemical parameters: glycemia, urea, creatinine, Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphate (ALP), gamma-glutamyl transférase (GGT).

Histological analyses

A fresh piece of different organs (heart, liver, kidney and lung) from each rat was rapidly fixed in 10 % neutral formalin. The fixed tissues were then embedded in paraffin, sectioned (5 µm) with a rotary microtome and stained with haematoxylin and eosine. The liver sections were evaluated histologically with a camera attached to a light microscope (Nikon E400).

Statistical analysis

The effects of drug treatments were evaluated statistically using the one-way analysis of variance (one-way ANOVA) followed by the Dunnett post-hoc test to correct for multiple comparison treatments.

Results

Effect of different treatments on the glycemic and renal balance

The results of our study show a significant increase in the blood sugar level, urea and creatinine in the group treated with a sublethal dose of Aah venom (10µ g) compared to the control group, and a significant decrease in the blood sugar level and Urea in the other groups (treated with Matricaria extract and that treated with anti-venom serum) (Table 1). Regarding the creatinine level, we observed a significant decrease in the group treated with the plant extract and no significant decrease in the group treated with antivenom.

Effect of different treatments on biochemical parameters

The effects of a sublethal dose of Aah venom on enzyme activities of ALT, AST, ALPand GGT were studied (Table 1). Administration of a sublethal dose of Aah venom resulted in a significant increase in liver transaminases (ALT and AST), ALP, GGT. This increase is due to the tissue alterations caused by the venom resulting in the release of enzymes to the extracellular medium.

Measurement of the activities of these enzymes in rats in the group treated with Matricaria extract and the group treated with antivenom serum indicates a significant decrease in their serum levels 24 hours after injection (s.c.) of a sublethal dose of Aah venom.

Histological analyses

A histopathological study was performed on liver, heart, kidney and lung organs after envenomation of rats with a sublethal dose (0.5 µg/ g rat) of Aah scorpion venom.

Injection of a sublethal dose of Aah's scorpion venom to rats showed morphological changes and disorganization of the tissue structure of all organs studied.

The envenomation of rats with Aah venom showed degenerative changes in the tubular epithelium of the renal cortex. The proximal and distal tubules are swollen and cloudy. The lumens of the tubules are no longer distinguished. The glomeruli appear completely disorganized.

Histological examination of the lung sections showed thickening of the alveolar walls and infiltration of polynuclear cells after injection of a sublethal dose. Haemorrhage, disorganization and necrosis were found in all tissue structures.

Microscopic observation of the livers of rats poisoned with a sublethal dose indicates that most liver cells have lost their cytoplasmic membrane. Necrotic areas and hemorrhages were observed (Fig. 1). Heart tissue showed fragmentation of myocardial fibers and hemorrhages after venom injection.

The neutralizing capacity of a scorpionic antivenom serum widely used in the El Oued region and *Matricaria pubescens* aqueous extract after injection of a sublethal dose of Aah venom on the histopathological changes in the liver, heart, kidney and lung of rats was also analyzed (Fig. 2 and Fig. 3).

In Group III (Fig. 2), almost all tissue disturbances in-

Table 1. Plasma biochemical parameters in control and experimental rats.

Parameters	Group I	Group II	Group III	Group IV
Glucose (g/l)	0,84±0,086^b	2,19± 0,108^a	0,77± 0,081^b	0,81 ±0,08^b
Urea (g/l)	0,61± 0,119^b	1,41±0,0276^a	0,31±0,05^c	0,31±0,03^c
Creatinine (mg/l)	5,67±0,00047^b	12,82±0,00196^a	4,95±0,00009^b	11,10±0,0008^a
AST (IU/mL)	45,33 ±2,65^c	441.0± 7.6^a	61,450± 0,566^b	68± 1.2^b
ALT (IU/mL)	13,83±1.01^c	311± 4.75^a	27.5±2.74 ^{b}	56,50±8,58^b
ALP (IU/mL)	159,68 ± 9,91^b	271,5±10,6^a	38,00±4,38^c	95,5±11,9^c
GGT (IU/mL)	9,650± 0.602^b	15.25±0.456 ^{a}	8,67 ±1,52^b	9.5±1.64^b

Values are mean ± SD for groups of 7 animals each. Means that do not share the same letter are significantly different.

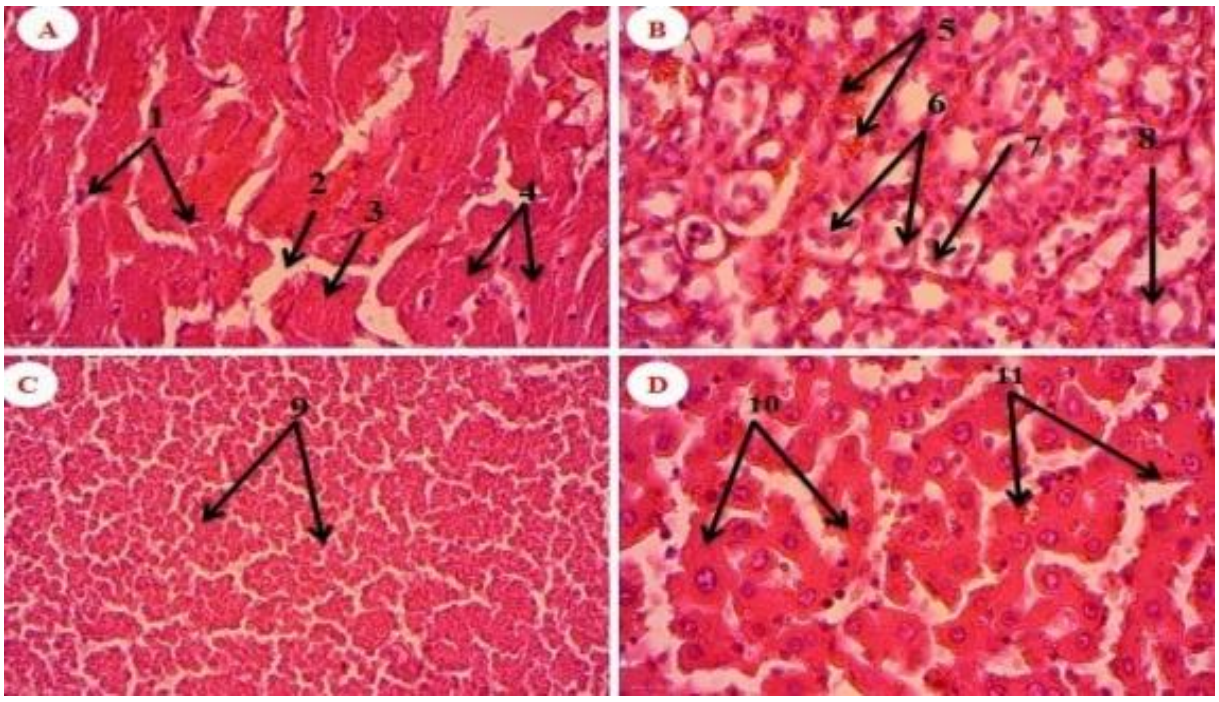

Figure 1. Representative organs tissues collected from envenomed rats A: Heart, B: Kidney, C: Lung D: Liver.

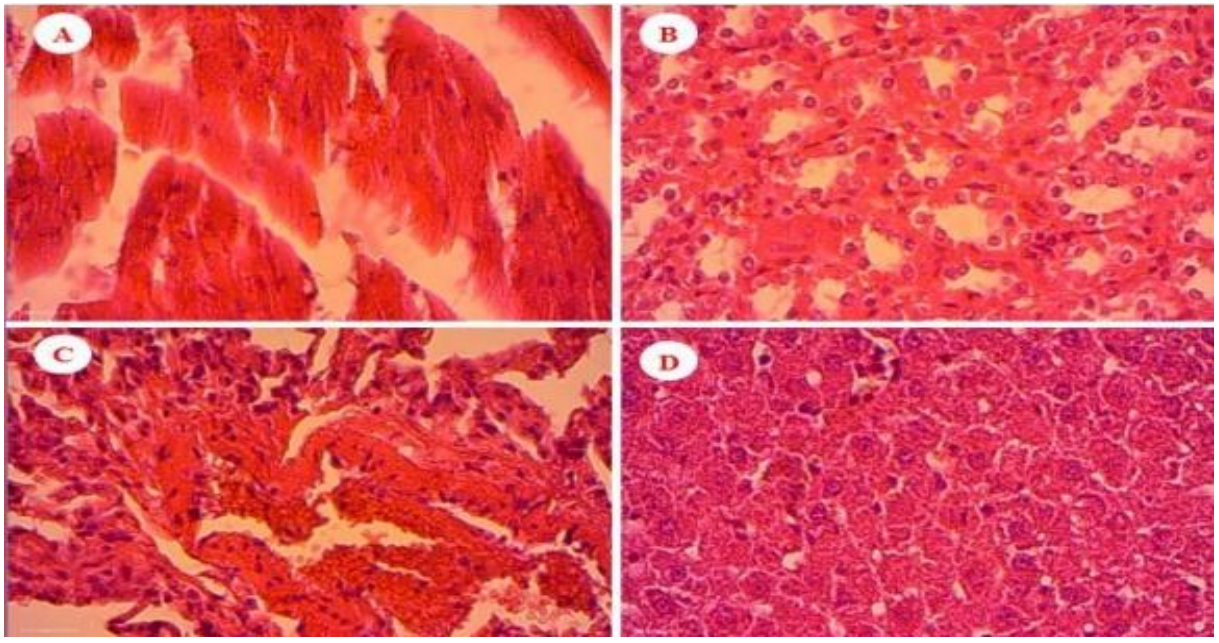

Figure 2. Representative organs tissues collected from envenomed rats treated with antivenom serum A: Heart, B: Kidney, C: Lung D: Liver.

duced by a sublethal dose of Aah venom were neutralized. Slight hemorrhage and less necrosis were observed in the liver, and very little tissue disorganization was observed in the lungs and kidneys. Myocardial fibers were unchanged (compared to the control).

The neutralization of sublethal dose-induced histopathological effects by the antivenom serum shows that the antivenom serum is more effective in different organs studied (Fig. 2).

Discussion

Scorpion poisoning is a major health problem in the world, including Algeria. To date, antisera are the only therapeutic agents available to treat victims of scorpionic envenomation. While several Algerian medicinal plants offer an unlimited source of therapeutic potential with a broad spectrum of bioactivity.

According to the results obtained the administration of a

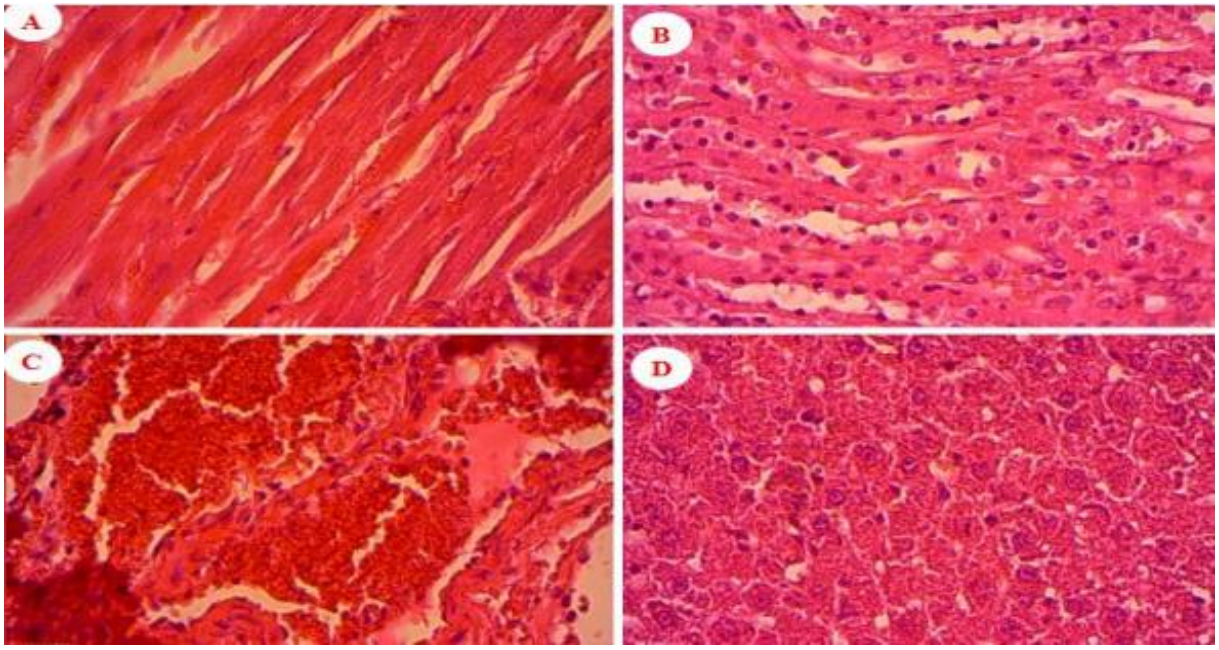

Figure 3. Representative organs tissues collected from envenomed rats treated with *Matricaria pubescens* extract. A: Heart, B: Kidney, C: Lung D: Liver.

subletal dose (0.5 µl/ g rat) of venom in rats caused metabolic disturbances resulting in a significant increase in the biochemical parameters studied in the serum, which are good markers of renal and hepatic impairment. However, the increased levels of transaminases (ALT and AST), ALP, GGT, blood glucose, urea, and creatinine in the blood may be due to the release of neurotransmitters and/or tissue damage at the tissue level (Bessalem et al. 2003).

Most scorpion venom compounds are peptides with different types of biological activity (Possani et al. 2000). In addition, Rodriguez de la Vega and Possani (2005) have been shown that the neurotoxic effects reported in cases of scorpionic envenomation are mainly related to the deterioration of Na^+ channels function. The effects of these toxins on the ion channels could promote an alteration that could lead to an abnormal release of neurotransmitters (Nencioni et al. 2003); known to be involved in the control of several physiological processes.

Studies conducted with the venom of *Leiurus quinquestriatus* have shown that the turgidity of hepatocytes would be a consequence of the increased flow of Ca^{2+} ions which would activate phospholipases (Bertke & Atkins1961).

Confirmation of the biochemical results is made by the histopathological study, which shows considerable histological disorders and changes in the tissue structures of the different organs (heart, liver, kidney, and lung) in the envenomed rats.

These biochemical and histopathological alterations found in our study have also been reported in several studies with highly toxic venoms. Currently, injection of venom from *Hypopygus lepturus*, *Hotentotta gentili*, *Tityus serrulatus*, *Androctonus amoreuxi*, *Androctonus liouvilei* and *Androctonus mauritanucus* venoms induces biochemical and histopathological changes are similar to those found in our study (Corrêa et al. 1997; Heidarpour et al. 2012; El Hidan e al 2015a; El Hidan et al. 2015b).

In our study, it is clearly established that treatment by peritoneal injection of the aqueous extract of *Matricaria pubescens* within a relatively short period of time (30 minutes), presents evident effects against scorpion venom activity through relative normalization of biochemical parameters. The histopathological study carried out on the organs of envenomed rats treated with the aqueous extract of the plant shows less significant tissue alterations in the organs studied. These alterations are totally absent at the level of the organs studied in the rats treated with the antivenom serum.

The efficacy of immunotherapy has been shown in several experimental and clinical envenomation studies (Revelo et al. 1996; Pepin-Covatta et al. 1996; Rezende et al. 1998). Numerous studies have shown that anti-venom agents consisting of $F(ab')_2$ fragments administered rapidly and intravenously are capable of neutralizing almost all circulating venom and preventing the initiation of scorpion venom-induced effects (Bessalem et al. 2003).

Therefore, it can be concluded that *Matricaria pubescens* aqueous extract is capable of neutralizing the effects of the venom of *Androctonus australis hector* with a better organization of certain damaged tissue structures and a relative normalization of biochemical parameters. This may be due to the inactivation or precipitation of active venom components by the plant extract, which can be attributed to certain bioactive components present in our extract. Numerous studies have demonstrated *in vivo* the efficacy of various plant extracts against scorpion venom (Jiménez-Ferrera et al. 2005; Kale et al. 2013; Gomes et al. 2016; Bouimejaa et al.2018).

Different active phytochemical compounds have been found in our plant extracts such as saponins, terpenoids, coumarins, flavonoids, tannins and steroids (Gherboudj et al. 2012; Cherif et al. 2017; Makhloufi et al. 2014; Makhloufi et al. 2012). Most of these compounds have protein binding

and enzyme inhibition properties and/or antioxidant activities that protect the different organs against venom toxins and enzymes.

Conclusion

The results of this study will certainly shed light on the benefic effects of crude extract of *Matricaria pubescens* against scorpionic envenomation. However, further studies are needed to determine the bioactive molecules of the plant extracts and their mode of action. In addition, exploration of these phyto-constituents with anti-venomous effects would help to develop complementary medicine for scorpionic envenomation treatments.

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